Salado Salamander Monitoring Final Report 2023

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Acknowledgements
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Executive Summary

Monitoring of the Salado salamander (*Eurycea chisholmensis*) concluded in January of 2024 finalizing the ninth year of monitoring at the Salado Downtown Spring Complex (DSC) and at Robertson Springs in Bell County (Figure 1). One Salado salamander was detected during 2023 at the DSC in Anderson Spring. There was no observable flow at Robertson Springs throughout 2023. Flow at Side Spring in the DSC has been variable due to infrequent rainfall and habitat changes, however higher flow rates similar to 2018 have not returned and no salamanders have been detected there this year. All salamanders were captured during active searches. This was the second lowest average year for discharge on Salado Creek during our monitoring period since 2015 beating out 2022 for the spot. In 2014, the average discharge of Salado Creek was 9.07 ft^3/s while in 2023 it is 10.43 ft^3/s with the data available from USGS (Gauge #08104300 accessed 1/22/2024).

Monitoring continued at Solana Ranch Spring #1 (SR1), providing a fifth year of quarterly data. A total of 121 detections, made up of 68 individual salamanders (determined through photographic analysis) were documented over the 2023 seasonal monitoring period. A total of nine juveniles were collected in April at SR1.

An additional spring site known as Kings Garden on the Tres Palacios Tract was added to the overall monitoring program for the Salado salamander in 2022. This site was visited four times during 2023 and during each visit salamanders were detected. A total of 64 detections, made up of 44 individual salamanders (determined through photographic analysis) were documented over the seasonal monitoring period. Recaptures were documented this year as the sample size of the population has increased. A major highlight this year was the addition of a new site to the documented population of Salado salamanders. This was at Keeta Spring.

Introduction

The Salado salamander (*Eurycea chisholmensis*) was first described as a species in 2000 (Chippindale et al. 2000). Although the salamander had been discovered earlier and was in a collection kept at Baylor University by B.C. Brown, no formal description had been made. In addition, collecting individuals from this population proved to be difficult (Chippindale et al. 2000). Due to the limited knowledge about the species (population density, life history patterns), potential threats (dewatering and urbanization), and limited geographical range, this species was listed as threatened by the U.S. Fish and Wildlife Service (USFWS) on February 21, 2014.

Critical habitat was designated in 2021 and more information can be found at *http://www.fws.gov/southwest/es/austintexas*.

The Salado salamander is the most northern population of fully aquatic *Eurycea* in Texas. The species is highly restricted geographically and is hypothesized to have a very low population within Central Texas (Norris et al. 2012). Nice et al. (2021) presented an analysis on the effective population size, showing that the northern populations (i.e. DSC, Robertson, Solana) have a lower effective population size compared to sampled populations in the southern group of Salado salamanders (Cowan Creek Spring and Twin Springs).

Over nine years of monitoring by the TXWFCO, we have added two new Salado salamander locations, one at Anderson Spring in the DSC and one at Keeta Spring further upstream along Salado Creek in proximity of Kings Garden. There have been three peer-reviewed publications relating to the Salado salamander (Diaz et al. 2020; Nice et al. 2021; Diaz et al. 2023). In addition, five peer-reviewed publications describing the aquifer community and species present in this northern section of the Edwards Aquifer have come from the Salado salamander work (Okan Külköylüoğlu et al. 2017; Gibson et al. 2020; Alvear, Dominique et al. 2020a; Alvear, Dominique et al. 2020b; Perez et al. 2023). This information will be valuable and hopefully aid in management decisions as the Village of Salado, Bell County and the northern portion of Williamson County continue to expand their conservation into the future.

Before monitoring by TXFWCO, an active research or monitoring program had not been established for this species. In addition, the known community structure of aquifer-dwelling species in the northern segment of the aquifer was not well studied. Due to these gaps in scientific knowledge of the species and the aquifer, the TXFWCO has been collecting data on habitat associations, reproduction, seasonality, surface densities, and the aquifer community with the intent of creating a long-term data set for the species within its known geographical range.

Methods

Sampling was conducted quarterly this year at the DSC, Robertson Springs, Kings Garden, and SR1 (Figure 1). The DSC consists of Big Boiling, Side Spring, and Anderson Spring. Keeta Spring was sampled three times when schedules with the landowner and monitoring staff overlapped. Sampling at Kings Garden Spring was sampled quarterly and followed the same methods as Solana listed below. Timed searches were used at Robertson, while Side and Anderson spring were searched entirely due to the small area of the springs.

Solana Ranch Spring #1 was sampled from the spring orifice to a location where the spring run fans out and enters the main channel. Areas where the water emerged from under the gravel and cobble were searched. Another smaller spring adjacent to the main spring was also entirely searched (from spring run to spring orifice) each visit. Sampling at Kings Garden was done from the spring orifice down to a pool approximately 20 feet from the opening. The pool creates a shift from a cobble and gravel run to silt substrates, which appear to be present due to the slower flowing water in the pool.

All springs were actively searched by uniformly turning over rocks and sifting through vegetation and debris. During timed searches all mesohabitats were searched for salamanders. Salamanders were captured using small aquarium nets. Captured salamanders were placed into mesh bags and kept in the spring run for processing (see below).

If a salamander was captured during any survey the primary substrate and vegetation were documented. If a salamander was captured in the drift net placed over an orifice, a designation of cave conduit was applied for substrate. All captured salamanders had two sets of photographs taken. First, photographs alongside a ruler were taken to determine total length of the salamander (mm) using the program ImageJ (Schneider et al. 2012). Following that, a close-up photograph of the head was taken and analyzed with the program WildID (Bolger et al. 2012) to determine if any individuals were recaptures (Bendik et al. 2013).

Drift nets with 250 µm mesh were positioned over the spring opening and used for passive sampling at Robertson and SR1 when spring flow was available. Nets were left in place for seven days to passively collect organisms as part of the monitoring regime. Aquatic invertebrates captured during this sampling were taken back to the lab, sorted, identified, and enumerated. Most taxa were photographed using a dissecting scope with certain taxa sent to experts for identification.

Due to low surface densities encountered at the sites over the years, the data have been collapsed and examined cumulatively. As in previous reports the overall dataset has been updated to include the 2023 collections. Data was grouped into seasonal blocks for a size distribution analysis. The relative abundance of salamanders was calculated for each season separated into size classes. Size classes are from 0-19, 20-29, 30-39, 40-49, 50-59, 60-69 mm; 1, 2, 3, etc. respectively. Associated substrate and vegetation percentages were updated to reflect the new collections.

Solana Ranch Spring #1 statistical analysis included probability of capture from quarterly data collected from 2020 – 2023. The probability calculations marked each time a salamander was captured and identified as “1”, therefore the capture history of a salamander for 2019 may resemble 101001 (six number places for six events, 0 = not detected, 1 = detected). For this example, the probability is the sum of the captures divided by the number of events, therefore, 0.5. Examining the average probabilities of capture history provides some insight into the effort of sampling between years.

Water level and flow data was collected from the Cemetery Well (Monitor well #5804628) and from the USGS gauge on the Salado Creek (USGS #08104300) from 2014 to 2022. This data was plotted with the total collection of salamanders from each year of sampling since 2015. This analysis was conducted to determine if there is an indicator for the issuance of spring flow at Robertson, and to identify preliminary trends associated with the salamander collections.

Results

Robertson and Downtown Spring Complex

No spring flow has been detected at Robertson Spring Complex since approximately June of 2022. The last collection of a salamander there was in April of 2022 from Middle (Bathtub) Spring. The detection of the salamanders at Robertson from all known springs had been decreasing since the end of 2019; with three in 2020 and two in 2021. The DSC flow has been continuous except for Critchfield. The flow at Side Spring has been decreasing and sediment has been filing in the spring run. Side Spring is also being cut back into the bank.

A total of 181 Salado salamanders have been captured since 2015 from all Robertson and DSC sampling locations. Only three of these salamanders do not have associated substrate or vegetation data, leaving 178 salamanders to examine with substrate and vegetation associations. A total of 67 (38%) salamanders were captured in drift nets, presumably leaving the aquifer. Of the remaining 111 salamanders caught on the surface, 73 (65%) were caught in gravel as the primary substrate, and 28 (25%) were caught in cobble as the primary substrate (Table 2). Other substrates included boulder, sand and silt. Data from past habitat sampling at Robertson Springs has shown around 50% of the substrate to be silt (Diaz et al. 2016). Salamanders have been captured in different types of vegetation, but 47 (43%) were associated with watercress (*Nasturtium* sp.), and 43 (39%) were captured in areas with no vegetation.

From the 181 total individuals detected, 174 were used to examine the temporal shift in size for surface populations at the DSC and Robertson Springs. The updated temporal shift in surface population size classes displays a classic ecological progression from smaller to larger, over the course of the year (Figure 3). In spring, the majority of salamanders captured were in the smallest size class ranging from 10 to 19 mm. The spring trend line shows (dashed blue line) a minimal bimodal hump, with a smaller hump in the fifth size class. In summer (solid green line), the smallest size class is still prevalent by one salamander. However, the second hump in the third size class is comparable. During fall (dot and dash purple line), the community is dominated by the fourth size class. The winter trend line (dotted red) is similar to the fall line except the initial hump of the line is in the first size class rather than the second size class as in fall. Overall, more salamanders have been detected in spring, with the fewest detected in winter, and juveniles are more prevalent in spring with adults more dominant in fall and winter.

Solana Ranch Spring #1

A total of 121 salamanders were captured at SR1 during 2023 monitoring. After removing recaptures of individual adult salamanders, the capture history shows that 68 individual adult salamanders were detected and photographed during 2023. The number of recaptures from the previous year was similar to other calculated years. However just as in 2022, the actual number of new individuals was lower compared to previous years (Table 3). Probabilities for recapture are listed in Table 3 and are similar between sampling events from the last three years.

Nine of the 121 salamanders were considered juveniles (<25 mm). Reviewing salamanders capture data dating back to 2017, the majority of the surface captures were adults (91%). The size average, based on the 608 salamanders detected since 2017, is 52.72 mm. The largest Salado salamander (87 mm) captured to date was in October 2020.

The temporal shifts in size class follow the same trends as the DSC and Robertson springs, but the overall population exhibits larger salamanders on the surface year-round (Figure 4). During the fall there have been no documented occurrences of salamanders in the first or second size class. This type of graph when compared to individual graphs from the other springs in the monitoring area highlight the permanence of the spring at Solana Ranch by exhibiting most of the salamander community at size classes 4 – 6 throughout the year (Figure 5).

Kings Garden and Pecan Springs

Initially we visited Kings Garden, Pipe, and Pecan springs in northern Williamson County on April 4, 2022. No salamanders were detected at Pipe or Pecan springs during surveys. Salamander have been found at Kings Garden Spring and since our initial visit in April 2022, however salamanders have been know at this location for some time.

Kings Garden is located in a genetic hole for *Eurycea* based on historical sampling. Other reports have shown a small genetic shift highlighting species variability from Robertson springs and associated sites to the north of Solana Ranch (Nice 2021). The goal now is to monitor Kings Garden Spring quarterly with active searches while sampling aquifer taxa using the passive sampling techniques previously described following the surveys. This will be done with the help of Texas Parks and Wildlife Department over the next couple of years.

Kings Garden Spring run consists of two branches. The branch termed “secondary” was searched for salamanders in April of 2022, however, none were detected. Cobble and gravel substrates were present in the run although most were embedded or covered in silt. The main branch had a noticeable orifice where water was emerging and flowing over cobble and gravel substrates. The flow continued down over a small riffle covered in watercress, and aquatic moss with cobble and gravel substrate. The salamanders were detected in this riffle that was supported by laminar flow. Downstream of the riffle section was a larger silt dominated shallow pond. The silted pond flowed into another riffle that feeds into a more stream like habitat (narrow with flow). This second riffle below the silted pond also has salamanders.

In 2023 Kings Garden was sampled in April, July, October and December. Sampling in 2023 was conducted in the main branch along the first riffle below the orifice. A total of 64 detections were made during the quarterly sampling events including seven juvenile salamanders. Of that following post processing with Wild ID, a total of 44 individual salamanders were detected. The most salamanders were captured in April (n = 25). A total of 12 recaptures were documented in 2023. Seven recaptures were made from salamanders collected 2022, while the remaining five recaptures were all from new salamanders observed in 2023. In 2024, the second riffle will be searched to determine if salamanders are moving across the silted pool that also contains *Gambusia*.

Our interest in Pipe and Pecan springs would involve restoration work at Pecan Spring. These springs were modified and now have no apparent orifice where water was discharging. Historically, each spring did have flow producing a slow spring run. The restoration should consider altering flow velocity. The low flow coming from either of these springs may allow the accumulation of silt within the interstitial spaces causing embedded cover objects. An elevational gradient from orifice to the end of a spring run could be used to combat the low flow issue. At Pecan Spring my recommendation would be to just remove the blocks at the end of the pool and let time and a good rain clean the area. A recent conversation with Tim Brown about the structure at Pecan Spring was interesting as he mentioned the blocks at the end of the pool were limestone, there weren't quarries back in the time period that the Texas Historic Commission would be interested in; however the rest of the structure, probably so.

Keeta Spring

The new Keeta Spring location was visited three times in 2023 and one individual was captured in July. The individual was clipped for genetics and returned. There have been a total of two individuals collected from this new location just south and east of Kings Garden.

Stream Flow and Well Height Data

During this study, assessments of a potential connection between surface salamander population densities and rainfall, stream flow and/or well gauge height were examined. The Cemetery Well (#5804628) has shown the best connection between water level (gauge height) and surface population densities. Recently the available data from the Cemetery Well has been sporadic. From 2015 to 2019, the data was reported monthly. Gaps in the well data began to appear during 2020. For 2020, only data from March to December is available. In 2021, only data from January and February are available. Finally in 2022, the data reporting increased and includes data from February to December. So far in 2023, only January data has been made available.

We compared Cemetery Well (feet below surface (fbs)), Salado Creek flow (USGS, ft^3/s), and the number of captured salamanders per year (Figure 6). The goal is be to predict when Robertson Springs would be flowing based off the Cemetery Well. We understand the Cemetery Well has an inverse relationship with salamander abundance at Robertson Springs

(Figure 7). So, as the feet below surface decrease we observe more salamanders at Robertson Springs. Salamanders were captured at Robertson Spring when levels at the Cemetery Well ranged from 12 to 75 feet below the surface. Although there have been varying levels of effort over the years, our data demonstrates that if the springs are dry no salamanders will be detected at the surface. Once the springs on the Robertson property go dry, a large percentage of salamanders are removed from the overall potential total at year end. Only when flows return to Robertson springs do the probabilities of capturing a salamander return. This year, there was no spring flow from any of the spring outlets resulting in zero salamanders captured (Table 1).

Discussion

The low number of encounters with Salado salamanders in 2023 was once again due to the lack of rain and an ongoing drought beginning around the end of 2019. The lack of rain has led to the lack of spring flow and the dewatering of Robertson Springs. During the last drought in 2014, a pool of water remained from Ludwigia Spring down to the confluence of Salado Creek. In comparison, as of January 2023 no springs were flowing at Robertson and the run was dry to the confluence of Salado Creek.

The Cemetery Well water level was examined to determine if it correlated to Robertson Spring flow reductions or ceasing of flows. When well water level was compared to salamander surface population data, it showed a negative correlation to “feet below the surface” data. Although a relationship was shown it is not predictive enough with the salamander data to be useful at this time. The effects of time spent underground for surface species have been documented and correlates to loss of tail width during long periods without surface interaction (Bendik and Gluesenkamp 2013).

The temporal shifts in size class for the Salado salamander appear to echo other research for the northern group of *Eurycea* sp. indicating a Fall/Winter breeding season and the increase of juveniles at the surface in spring (Pierce et al. 2014). This breeding season in the northern salamander group appears unique and could be facilitated by the shallowing of the aquifer as the limestone generally decreases in depth as the aquifer moves north. This shallowing of the limestone in this northern segment could cause the influx of recharge water into inhabited areas more quickly than in deeper portions of the aquifer to the south.

Other research by Bendik et al. (2017) on the Jollyville Plateau salamander (*E. tonkawae*) and Pierce et al. (2014) on the Georgetown salamander (*E. naufragia*) showed a peak time for gravidity in December, with Pierce et al. (2014) showing an additional peak in February or March for the Georgetown salamander. Gravidity has not been observed in the Salado salamander in the number of observations necessary to elucidate any trends. What would be expected is to see a lag time between gravid females observed by the two mentioned authors and the observation of salamanders in the first size class. Growth curves in captive San Marcos salamanders show that it takes about 60 days to reach around 15 mm. Therefore, if there was a peak in Salado salamander gravidity in December, the juveniles would be on the spring surface and measure up to about 15 mm at the earliest in late February. The Salado salamander seasonal dynamics graph shows the largest percentages of juveniles occur during spring, which runs from March to May. In other words, we might hypothesize that there is some peak in gravidity for the Salado salamander sometime in December or January, although undetected. This section of the work on breeding seasonality has recently been published (Diaz et al. 2023; Herp Review).

Insights into why the surface densities of these salamanders are historically small (Norris et al. 2012), with estimates by the author that surface populations are around 10 salamanders at the DSC and Robertson Springs sites, could be based on nine years of monitoring observations. The hydroperiod of the springs (i.e. the duration of discharge over time) and proximity to larger order streams, (i.e. ecological disturbance) may play a large part of influencing surface densities at historic Salado salamander sites (Robertson Springs and DSC). Salado Creek's hydroperiod includes large pulses of water after large rain events in the watershed. These pulses cause Salado Creek to rise high enough that it floods the spring outlets at the DSC and at Robertson Springs. The flood waters also bring or remove sediment, gravel and cobble changing the habitat substrate and even depth over the spring orifices as seen at Side Spring (DSC).

The spring flows in the DSC appear to be stable except for Little Bubbly Springs which has been intermittent during the study. However, Robertson Springs has a large fluctuation in hydroperiod and was not flowing in 2015 and resumed discharging at many of the orifices in 2016. In 2017, the discharge began to decline again and ceased to flow in 2018. Flow returned to the springs at the beginning of 2019. In 2020, the flows began to subside in May and by August no salamander producing mapped spring zones were flowing. Flows were sporadic in early 2021 but by May 2021 the springs were dry. The springs began to flow again in June of 2021 and flowed till around June of 2022. However, flow ceased for all springs in the Robertson springs complex between July 2022 and January 2024. In addition, Robertson and the DSC springs are at the known northern fringe of *Eurycea* distribution in Texas and the Edwards Aquifer. In comparison, the surface population present at SR1, just south of Salado, over the last eight years have always been detectable and consistent with regards to count data. Solana Ranch Spring #1 has had a consistent hydroperiod, is not near a larger order stream or river, and is south of the known northern locations for these salamanders.

These factors may be a large part of why the surface densities are low at the historic Salado salamander sites. In addition, the small surface recruitment of salamanders seen at Robertson and Anderson springs (DSC), based on the drift net sampling data, suggest that the populations at these sites may be slow to recover from natural disturbances like a flood or cessation in flows. Given that surface densities are low but appear to be consistent given the flows over the last eight years (2015- 2022), it has been suggested that a large proportion of the Salado salamander population is below the surface within the aquifer (Nice et al. 2021). The first genetic analysis for the Salado salamander was completed in 2021 and expanded the known range. A second round of genetic collections began in 2023 with the goal to estimate an effective population density at the sites previously assessed and new sites will be included in the genetic analysis providing more information about the species range. This type of analysis can be woven into part of the monitoring program for the Habitat Conservation Plan in development for the area.

The views expressed in this paper are the authors and do not necessarily reflect the view of the U.S. Fish and Wildlife Service or Texas Parks and Wildlife Department.

Table 1. Number of Salado salamanders collected during quarterly monitoring using active and passive sampling techniques 2023.

Season	Robertson	Downtown Spring Complex	Solana Ranch Spring #1	Kings Garden
Spring	0	1	57	25
Summer	0	0	14	8
Fall	0	0	30	13
Winter	0	0	20	18

Table 2. Habitat associations of the Salado salamander determined by 177 salamanders collected from 2015 to 2023 at the Downtown Springs Complex (DSC) and Robertson Springs.

	#	%
Cave Conduit	67	37.85
Substrate		
Silt	3	2.73
Sand	2	1.82
Gravel	73	66.36
Cobble	28	25.45
Boulder	4	3.64
Vegetation		
***Sagittaria* sp.**	1	0.93
***Nasturtium* sp.**	47	43.52
Filamentous Algae	4	3.70
***Ludwigia* sp.**	3	2.78
***Amblystegium* sp.**	5	4.63
***Hydrocotyle* sp.**	2	1.85
none	43	39.81
Organic Debris	2	1.85
Grass	1	0.93

Table 3. History of quarterly monitoring data from Solana Spring Ranch #1 (SR1). "Recaps Previous Years" are individuals that were captured more than once between sampling years.

	2020	2021	2022	2023
Recaps Previous Years	17	36	43	41
Recaps for Year	16	10	13	11
New Individuals	83	74	59	61
Naive Probability of recap	23	29	31	29

Table 4. Water quality collected during monitoring in 2023.

Spring	Date	Temperature °C	Dissolved Oxygen mg/L	pH	Conductivity µs/cm
Keeta	4/7/2023	19.8	6.23	7.09	503
Solana	4/12/2023	20.15	8.95	7.03	NA
Kings Garden	4/13/2023	20.50	7.68	7.31	NA
Stagecoach	4/18/2023	20.96	7.84	7.12	585
Anderson	4/18/2023	20.92	7.76	7.07	587
Big Boiling	4/18/2023	20.96	7.77	7.11	585
Side	4/18/2023	20.94	7.85	7.19	585

Anderson	7/18/2023	20.98	7.62	7.19	539.5
Big Boiling	7/18/2023	20.98	7.62	7.24	538.9
Side	7/18/2023	22.35	7.52	7.31	544.1
Solana	7/19/2023	20.94	7.81	6.75	429.2
Keeta	7/19/2023	20.98	6.52	6.88	526.6
Kings Garden	7/31/2023	21.24	7.49	7.1	375.4
Anderson	10/10/2023	21.94	7.13	7.1	563.1
Big Boiling	10/10/2023	21.17	7.27	7.2	566.4
Side	10/10/2023	NA	Damp		
Kings Garden	10/11/2023	23.47	4.31	6.89	555.9
Solana	10/13/2023	21.84	7.28	7.32	424
Solana	10/24/2023	21.5	7.66	7.31	357.7
Keeta	10/13/2023	21.6	3.8	7.06	598
Solana	12/19/2023	21.05	7.7	7.42	493.8
Kings Garden	12/20/2023	21.08	7.11	7.57	635.1

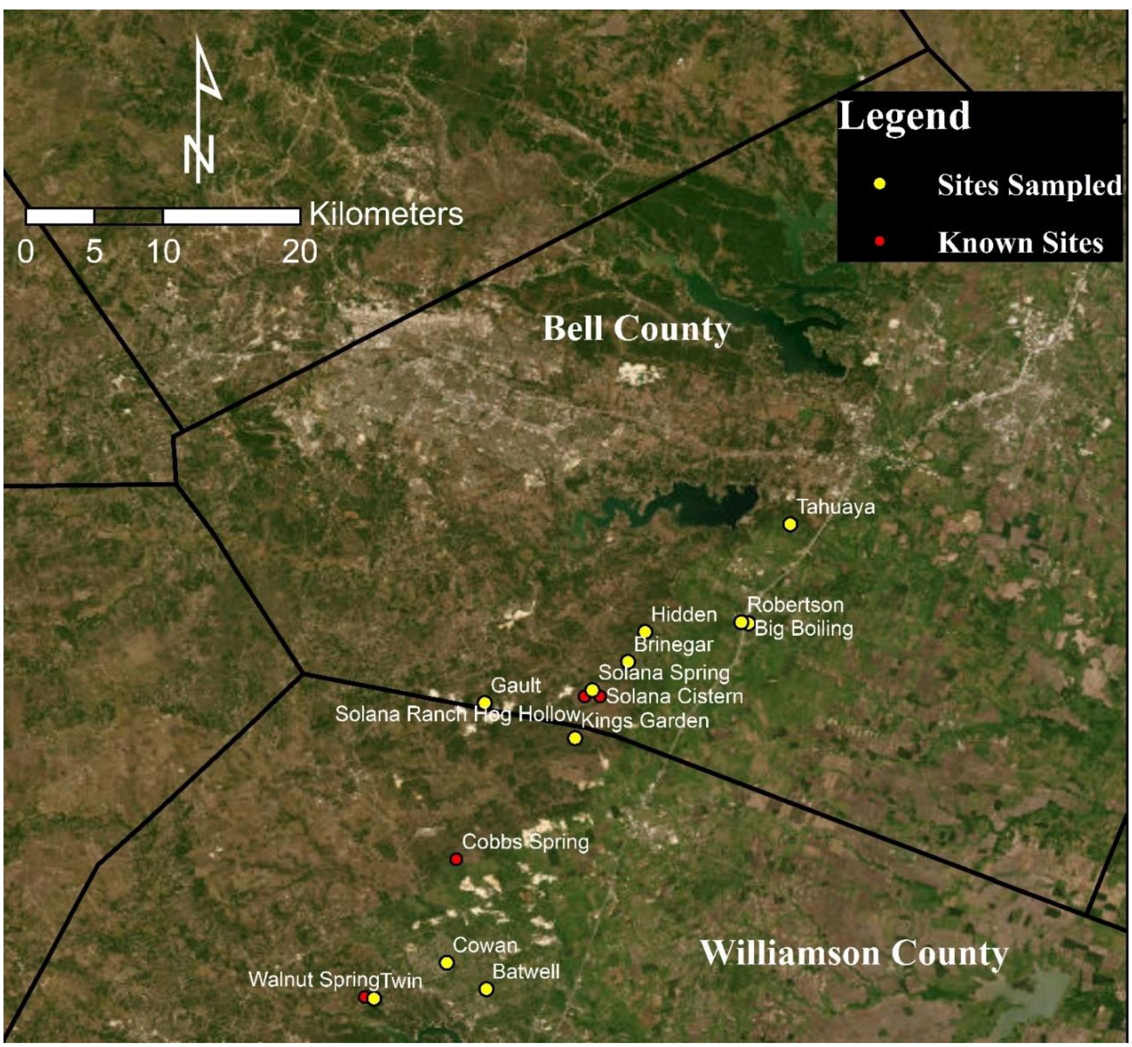

Figure 1. Study area for Salado salamander monitoring or searches conducted from 2015 to 2023.

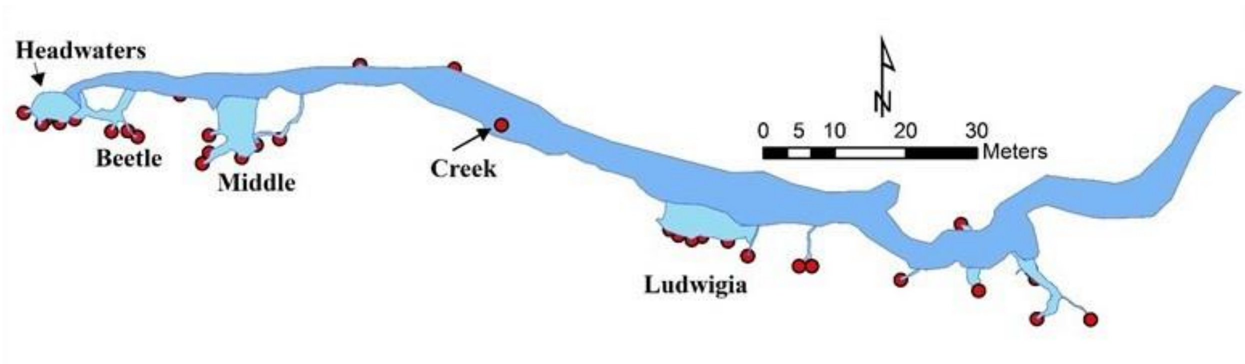

Figure 2. Map of Robertson Springs showing spring zones mapped in 2016 during optimal flow conditions at the site. Light blue zones are spring zones, red dots are orifice, and the blue is the spring run terminating into Salado Creek. From July of 2022 to January 2024 the system was dry to the creek.

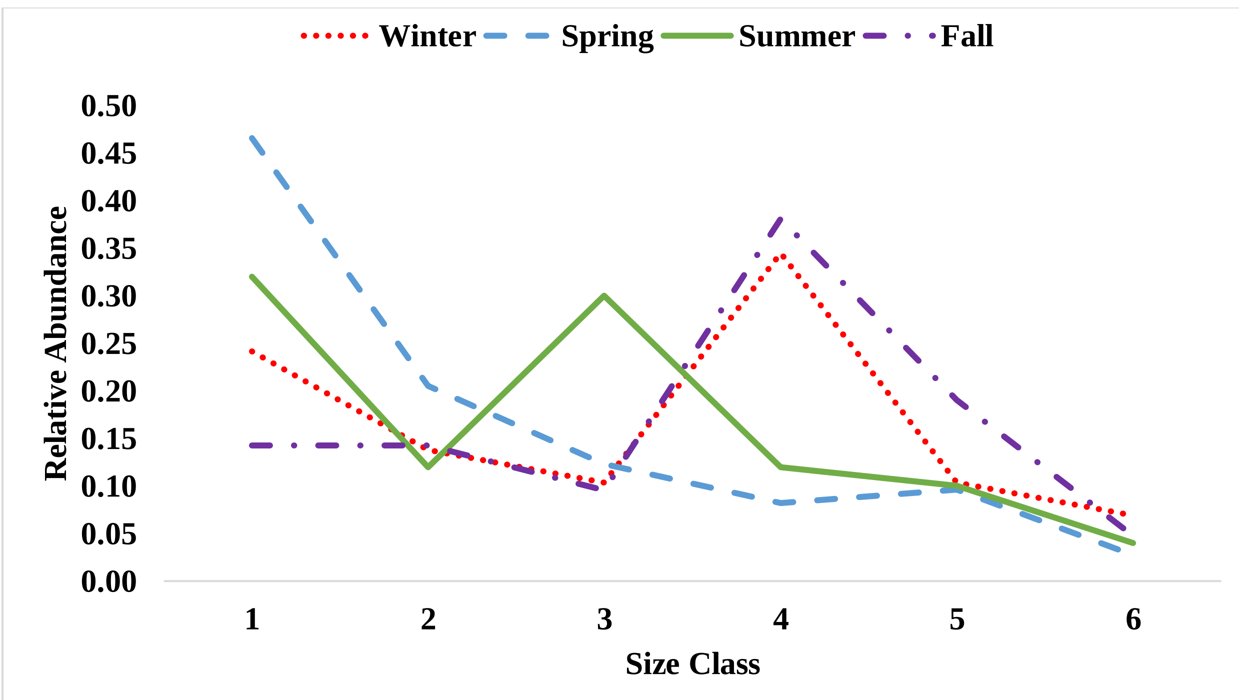

Figure 3. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Downtown Spring Complex (DSC) and Robertson Springs by season from 2015 to 2023 for 173 salamanders. Size classes range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

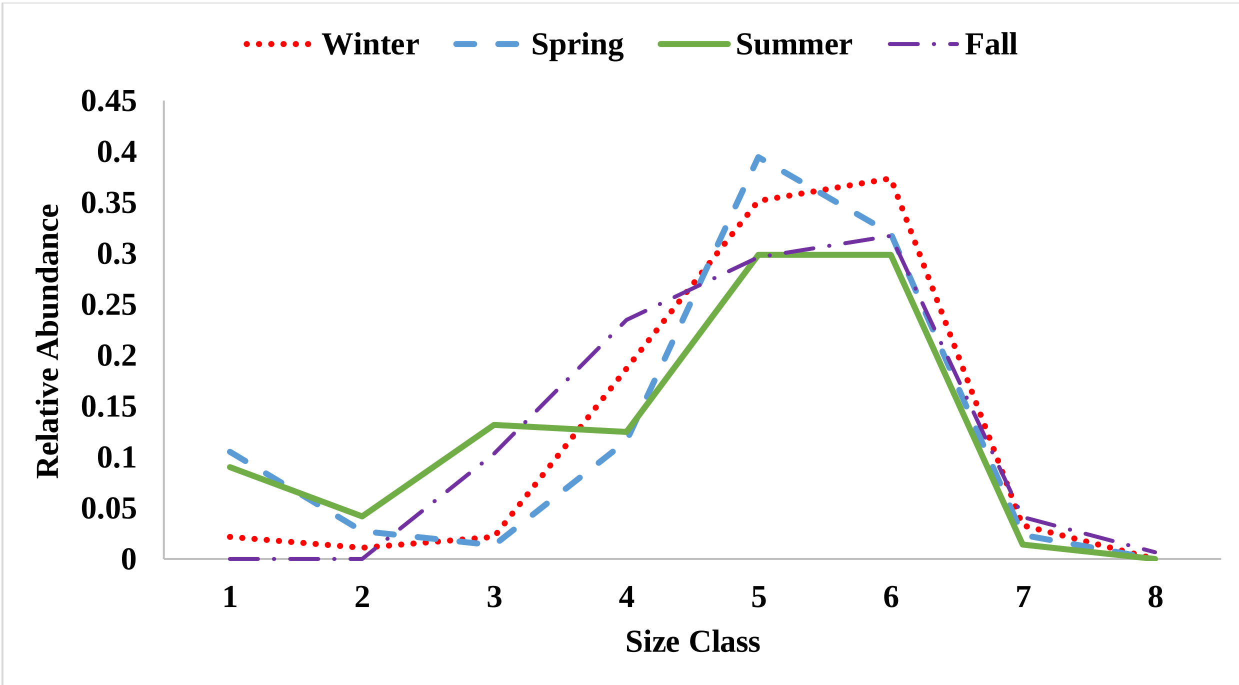

Figure 4. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Solana Ranch Spring #1 by season from 2015 to 2023 for 674 salamander observations. Size classes range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

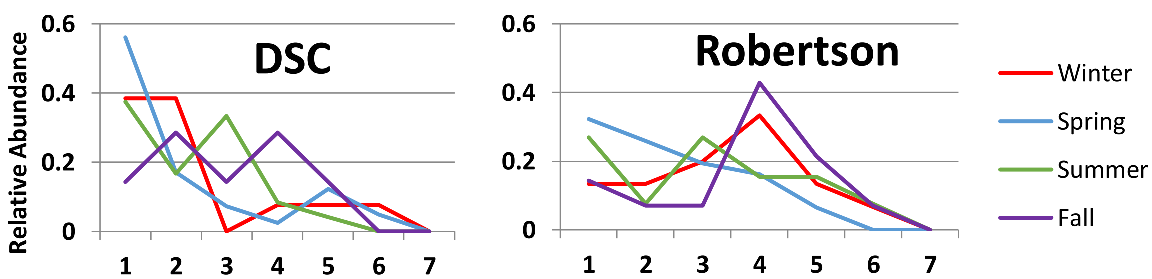

Figure 5. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Downtown Spring Complex (DSC) and Robertson Springs by season from 2015 to 2023. Salamander observations; 86 from Robertson Springs and 85 from the DSC. Size classes (x-axis) range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

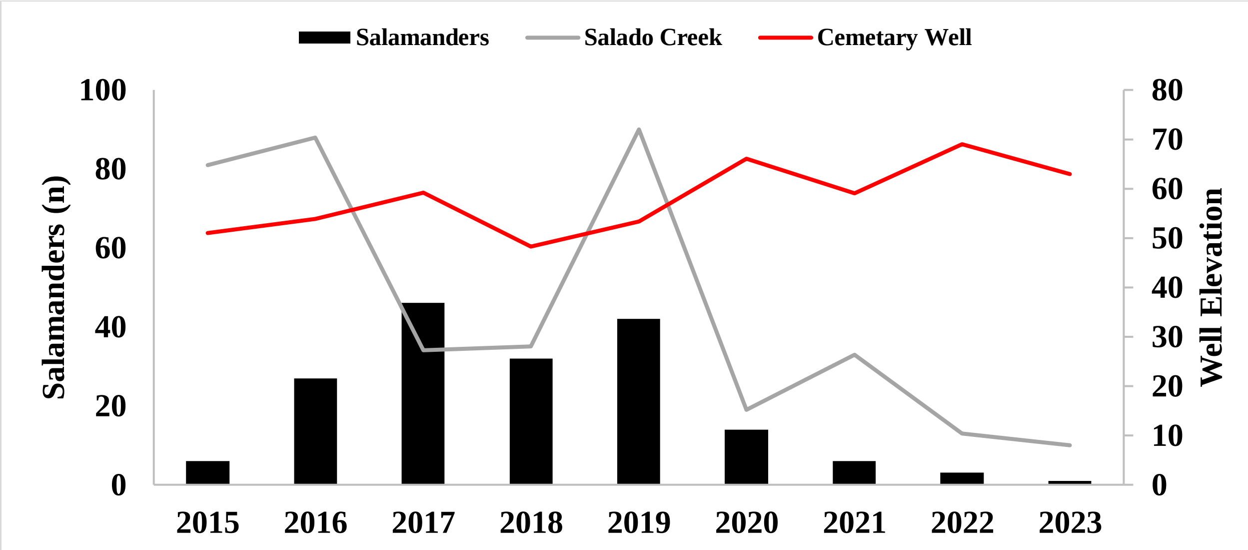

Figure 6. Data collected from the Cemetery Well (Monitor well #5804628; feet below surface) and from the USGS gauge on the Salado Creek (USGS #08104300; ft^3/sec) plotted with the total collection of salamanders (n) from each year sampled at the Downtown Spring Complex (DSC) and Robertson Springs.

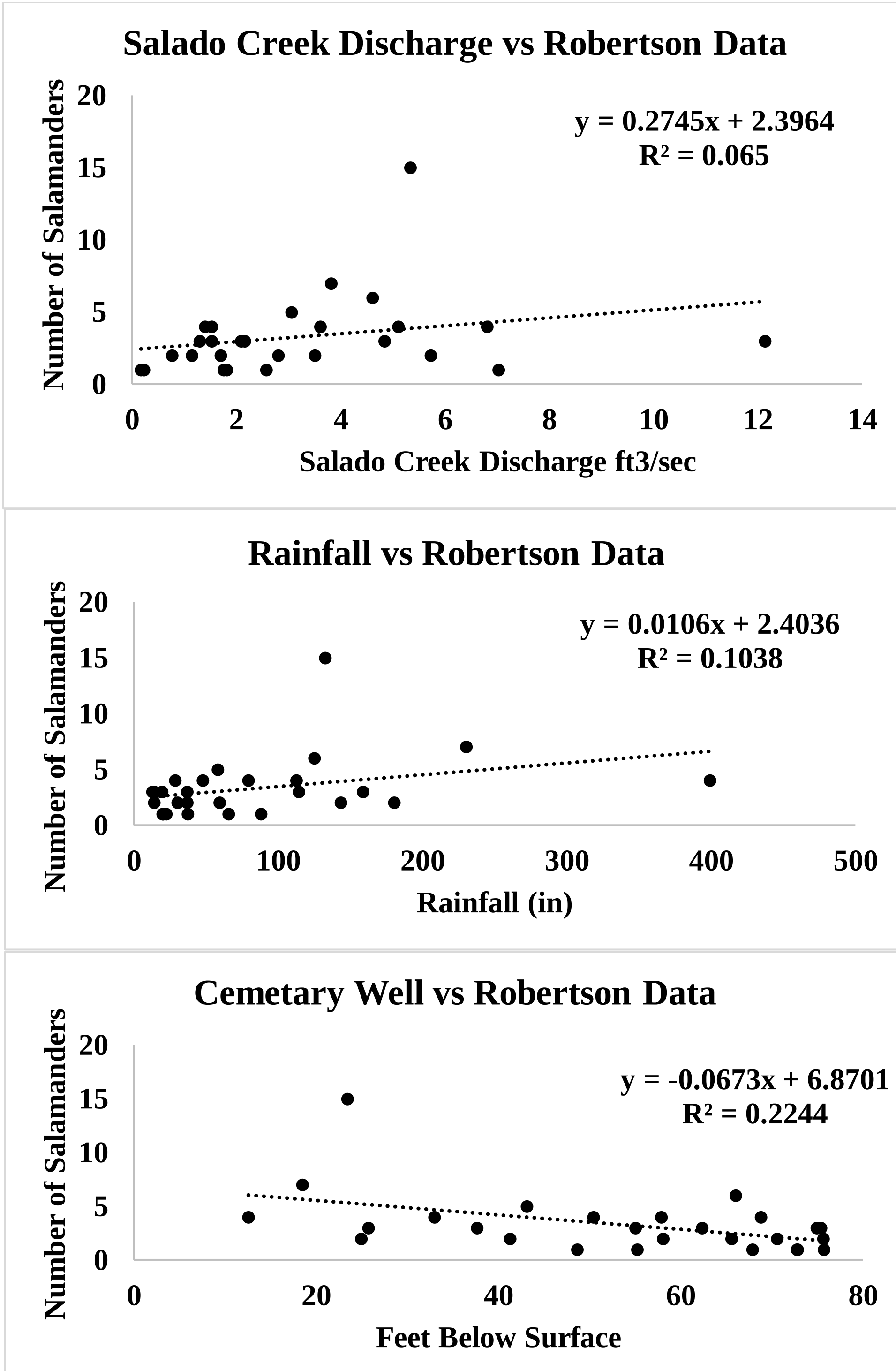

Figure 7. Relationships between Salado salamander capture data from Robertson Springs (2015 to January 2023) and predictors of abundance data.

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