Salado Salamander Monitoring
Final Report 2022

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Executive Summary

Monitoring of the Salado salamander (*Eurycea chisholmensis*) concluded in January of 2023 finalizing the eighth year of monitoring by the Texas Fish and Wildlife Conservation Office (TXFWCO) at the Salado Downtown Spring Complex (DSC) and at Robertson Springs in Bell County (Figure 1). A total of three Salado salamanders were detected this year at Robertson and the DSC. A single salamander was collected at Anderson, Big Boiling, and from Middle Spring on the Robertson Ranch. Robertson Springs ceased flow by August and did not begin to flow again for the remainder of the year. Flow at Side Spring in the DSC continued to decrease until the flow began from below the orifice through the alluvial sediments. 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.

Monitoring continued at Solana Ranch Spring #1 (SR1), providing a fourth year of quarterly data. A total of 114 detections, made up of 58 individual salamanders (determined through photographic analysis) were documented over the seasonal monitoring period. Over 90% of the of salamanders captured at SR1 were adults.

An additional spring site known as Kings Garden on the Tres Palacios Tract was added to the overall monitoring program for the Salado salamander. This site was visited three times during 2022 and during each visit salamanders were detected (n=31). No recaptures were documented. Sampling protocols followed the TXFWCO Salado salamander monitoring protocol.

Over eight years of monitoring by the TXWFCO, we have added one new Salado salamander location at Anderson Spring in the DSC. At the time there were only a few sites where the salamander had been documented. 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 press). In addition, four 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). This information will be valuable and 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.

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).

Before monitoring by TXFWCO, there was no active research or monitoring program in place 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, and SR1 (Figure 1). The DSC consists of Big Boiling, Side Spring, and Anderson Spring. Sampling at Kings Garden Spring was done when time was available, but 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 pile 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 to a pool. 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, 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.

Drift nets with 250 µm mesh were 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.

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).

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 2022 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 – 2022 (n=553). 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

A total of three salamanders were detected at Robertson and the DSC (Table 1). Of these three, two were juveniles (< 25 mm total length; Bowles et al 2006) and one was marginally an adult, 25.58 mm, well within the margin of error from photographs. Two were captured from the DSC,at Big Boiling Spring (n = 1) and Anderson Spring (n = 1). Robertson Springs produced one salamander from Middle Spring (Figure 2). By August of 2022 Robertson Springs complex was completely dry to the confluence of Salado Creek. Spring flows at Robertson had still not returned by the last sampling event in January of 2023. Drift netting captured zero salamanders at Robertson Springs. Drift net sampling was not used at the DSC in 2022.

A total of 179 Salado salamanders have been captured since 2015. Three salamanders do not have associated substrate or vegetation data, leaving 176 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 109 salamanders caught on the surface, 73 (66%) 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 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 179 total individuals detected, 172 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 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.

Solana Ranch Spring #1

A total of 116 salamanders were captured at SR1 during 2022 monitoring. After removing recaptures of individual adult salamanders, the capture history shows that 58 individual adult salamanders were detected and photographed during 2022. The number of recaptures from the previous year were similar to 2021. However 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.

Five of the 116 salamanders were considered juveniles (<25 mm). Reviewing salamanders capture data dating back to 2017, the majority of the surface captures were adults (92%). The size average, based on the 558 salamanders detected since 2017, is 52.22 mm. The largest Salado salamander (87 mm) captured to date was ein October 2020.

The temporal shifts in size class follow the same trends as the DSC and Robertson data, 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).

Stream Flow and Well Height Data

This analysis shows the tracking of the Cemetery Well with the capture of salamanders (Figure 6). The Cemetery Well has an inverse relationship with salamander abundance at Robertson Springs (Figure 7). 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, if the springs are dry no salamanders will be surfacing. 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 the springs at the Robertson property do the probabilities of capturing a salamander return. This year, the flows did not return at the end of the year as they have in the past and flows from the productive spring zones at Robertson began to noticeably recede in early June.

Discussion

The low number of encounters with Salado salamanders in 2022 was due to the lack of rain and an ongoing drought beginning around the end of 2019. The lack of rain has caused 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. Salamander data from Robertson was shown to have a negative correlation with the “feet below the surface” data collected from the well. Although a relationship was shown it is not predictive enough with the salamander data to be useful at this time. Effects of time spent underground for surface species has been documented and was shown to have 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 season for breeding (Pierce et al. 2014). This pulse 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 type of shallowing of the limestone could cause the influx of recharge water into inhabited areas more quickly than in deeper portions of the aquifer.

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.

Habitat associations, given the smaller data set collected for the Salado salamander, compared to the other species to the south, are consistent with their reports of habitat associations taken from larger sample sizes with more robust surface populations present (Bowles et al. 2006; Diaz et al. 2015). Due to the small surface populations at the monitoring sites, examining the data is statistically challenging, however, thinking about observed versus expected may be one way to look at the overall Salado salamander data set. Observed would be the data set for the Salado salamander (e.g. habitat associations). Expected would be the larger established and published data sets with more years of data collection and then anecdotally examining the congruence of the patterns within the two data sets to provide evidence for observations collected in the Salado. For example, substrate and diet data collected from 2015 to 2018 mentioned in the results is congruent with what is known and published about other southern salamander species (Bowles et al. 2006; Diaz et al. 2015). This published evidence does provide some further validity to the Salado data despite the smaller sample size of salamanders.

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 eight 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. 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, 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 a second round of genetic collections begin in 2023 with the goal to estimate an effective population density at the sites previously assessed. Additionally, new sites will be included in the genetic analysis. This type of analysis can be woven into part of the monitoring program for the Habitat Conservation Plan in development for the area.

Table 1. Number of Salado salamanders collected during quarterly monitoring using active and passive sampling techniques 2022. (NS = not sampled)

Season	Robertson	Downtown Spring Complex	Solana Ranch Spring #1	Kings Garden
Spring	1	2	47	11
Summer	0	0	26	20
Fall	0	0	28	NS
Winter	0	0	20	NS

Table 2. Habitat associations of the Salado salamander determined by 177 salamanders collected from 2015 to 2022 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
Recaps Previous Years	15	33	34
Recaps for Year	18	11	14
New Individuals	83	75	58
Adult Totals	116	119	106
Probability of recap	23	29	30

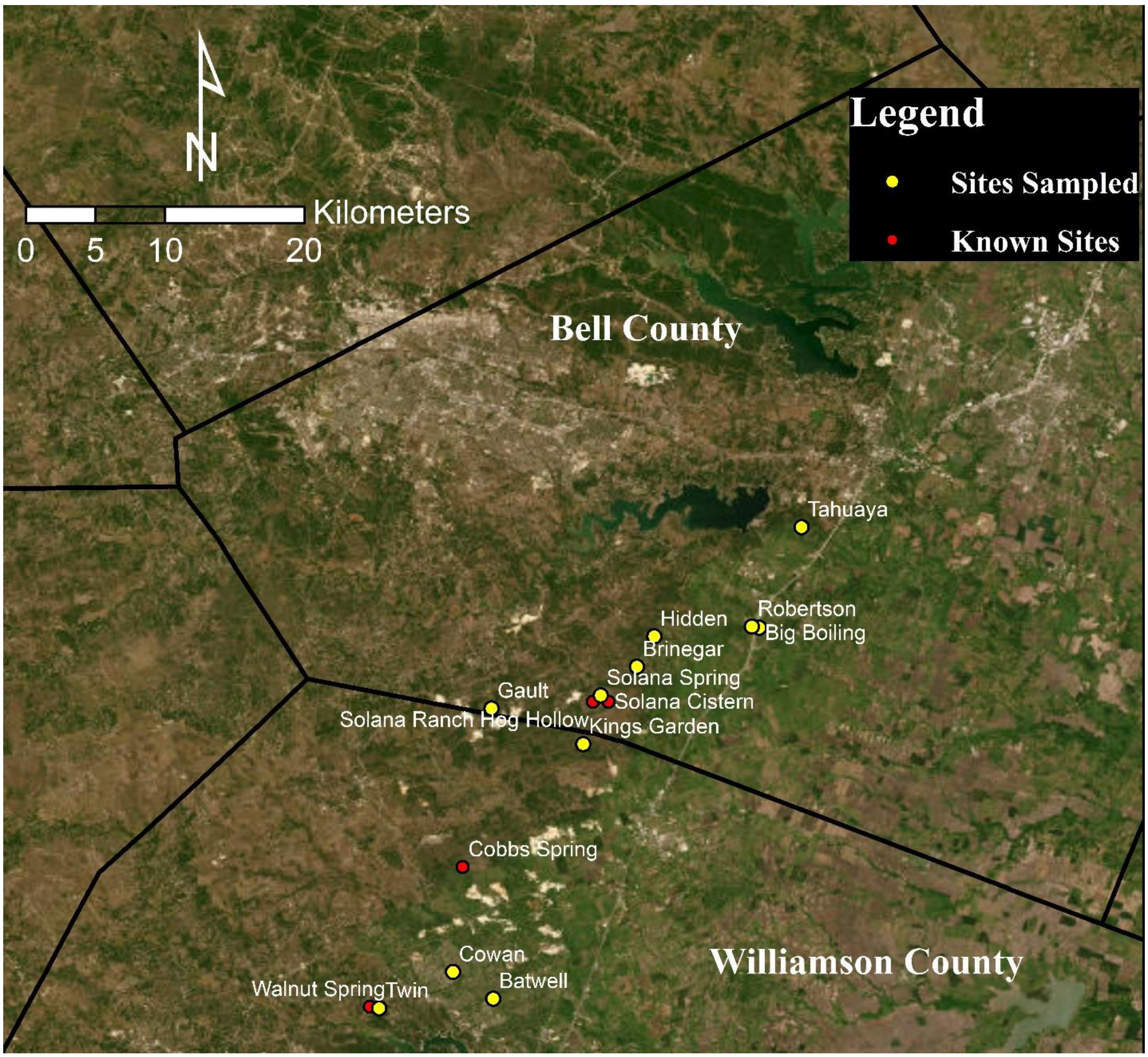

Figure 1. Study area for Salado salamander monitoring or searches conducted from 2015 to 2020.

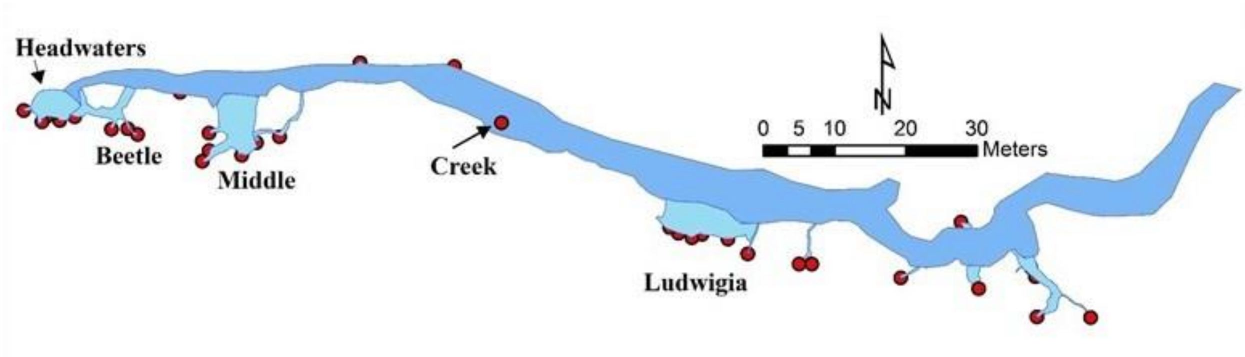

Figure 2. Map of Robertson Springs showing spring zones mapped in 2016 during optimal flow conditions at the site. Light blue zones are spring zones, red dots are orifice, and the blue is the spring run terminating into Salado Creek.

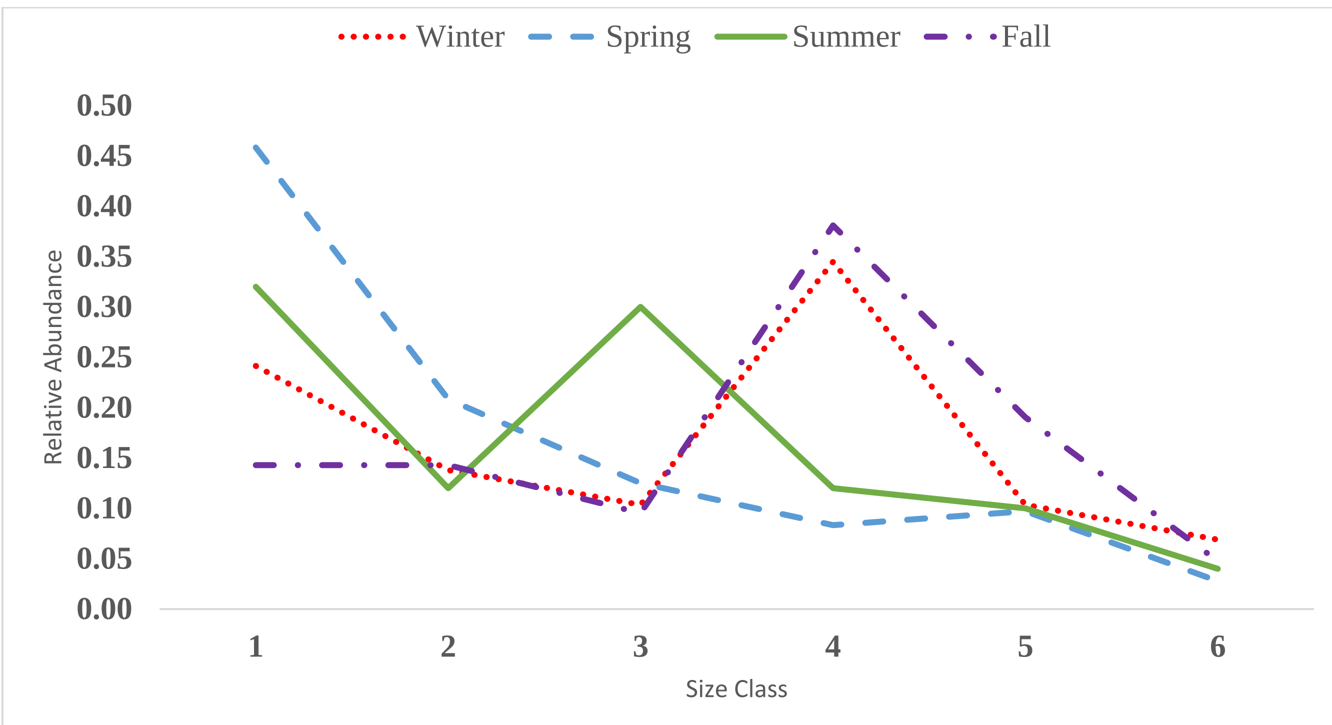

Figure 3. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Downtown Spring Complex (DSC) and Robertson Springs by season from 2015 to 2022 for 172 salamanders. Size classes range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

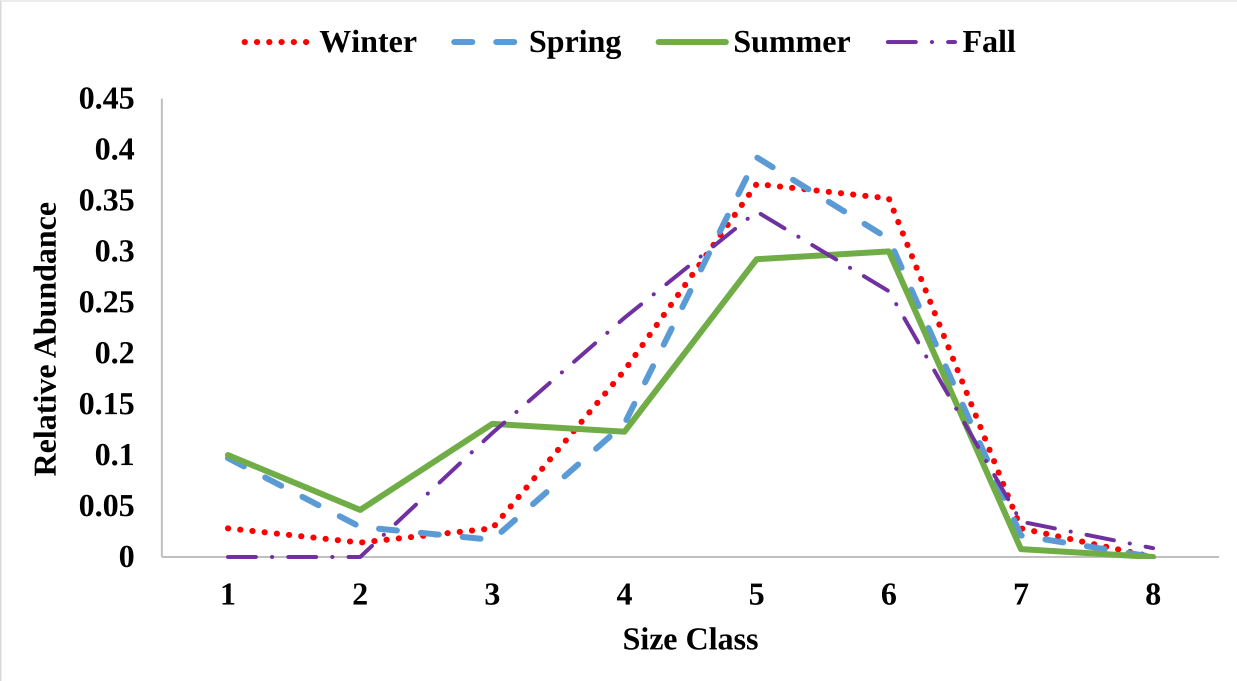

Figure 4. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Solana Ranch Spring #1 by season from 2015 to 2022 for 553 salamander observations. Size classes range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

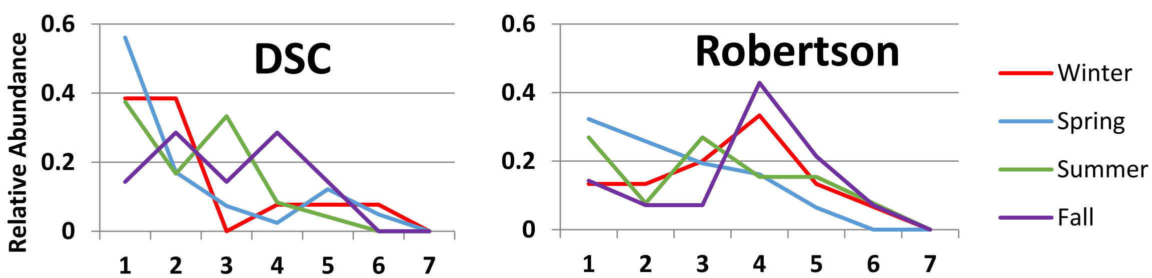

Figure 5. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Downtown Spring Complex (DSC) and Robertson Springs by season from 2015 to 2022. Salamander observations; 86 from Robertson Springs and 85 from the DSC. Size classes (x-axis) range from 10 - 19.99 mm = 1; 20 - 29.99 mm = 2; etc.

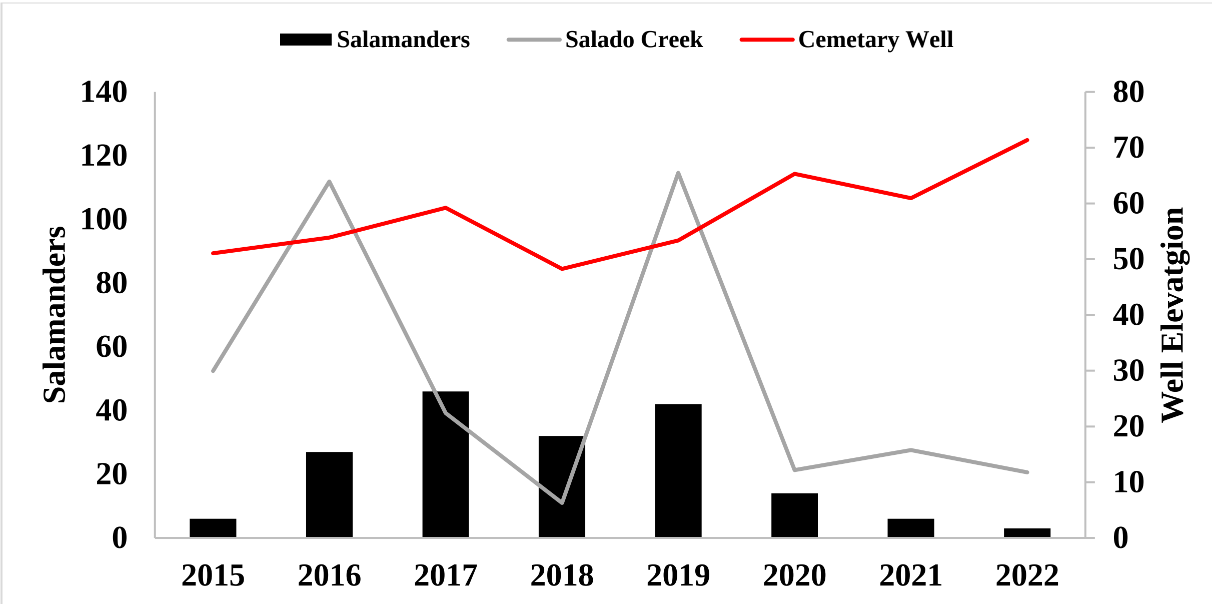

Figure 6. Data collected from the Cemetery Well (Monitor well #5804628) and from the USGS gauge on the Salado Creek (USGS #08104300) plotted with the total collection of salamanders from each year sampled at the Downtown Spring Complex (DSC) and Robertson Springs.

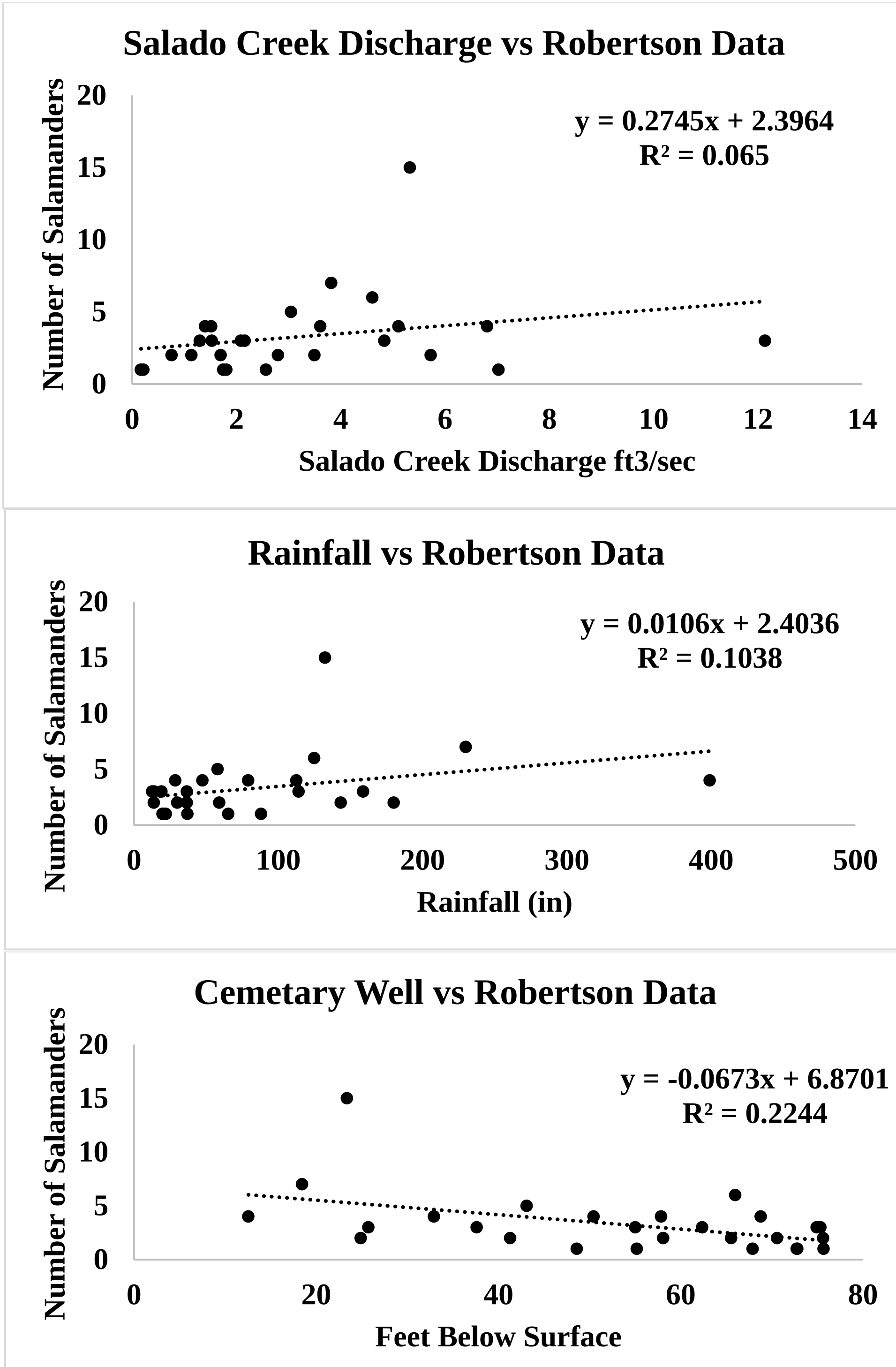

Figure 7. Relationships between Salado salamander capture data from Robertson Springs (2015 to January 2023) and predictors of abundance data.

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