

Salado Salamander Monitoring, Bell County Final Report 2021



Peter H. Diaz
Texas Fish and Wildlife Conservation Office
San Marcos Texas

Jennifer Bronson Warren
Texas Parks and Wildlife
Waco Texas

Justin Crow
San Marcos Aquatic Resources Center
San Marcos Texas



Table of Contents

Acknowledgements.....	2
Executive Summary.....	3
Introduction.....	3
Methods.....	4
Results.....	5
Robertson Springs and Downtown Spring Complex.....	5
Solana Ranch Spring #1.....	7
Aquifer Invertebrates.....	8
Stream Flow and Well Height Data.....	8
Discussion.....	9
Literature Cited.....	23



Salamander #211 from September of 2017 (top), February of 2019 (left) and August of 2021 (right) identified from photographic analysis.

Acknowledgements

We appreciate the support from Clearwater Underground Water Conservation District for funding the monitoring in 2021.

Executive Summary

Monitoring of the Salado salamander concluded in December of 2021 finalizing the seventh 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. A total of 6 Salado salamanders were detected this year at the DSC and Robertson springs. Four were collected from Side Spring (DSC) in May and two from drift nets at Robertson Springs in the summer. Collections at Robertson Springs were low during 2021. The springs were dry for around nine months and once the springs started to flow, the presence of beaver dams along the spring run flooded the cobble runs and potentially decreased the flow at the spring heads.

Monitoring continued at Solana Ranch Spring #1 (SR1) and providing a third year of quarterly count data. Quarterly monitoring producing a total of 108 detections producing 75 new individuals, and 33 recaptured salamanders from this year and previous sampling (determined through photographic analysis). One individual that was recaptured (#211) was first documented in September of 2017 and recaptured in October of 2021. Once again, the majority of salamanders captured at Solana were adults.

Introduction

The Salado salamander (*Eurycea chisholmensis*) was first described in 2000 (Chippendale 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 (Chippendale 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. The USFWS designated critical habitat areas in 2021. Information regarding critical habitat can be found at <http://www.fws.gov/southwest/es/austintexas>.

The Salado salamander 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). Pyron and

Weins (2011) conducted an overarching genetic analysis of Amphibia and their analysis also suggests that the original phylogenetic analysis by Chippendale et al. (2004) was appropriate and that indeed the Salado and Georgetown salamanders are distinct species. Finally, a recent genetic study of the entire Edwards Plateau, funded through a Section 6 grant (#443022) by Dr. Hillis of the University of Texas, confirms the species designation was indeed scientifically valid (Devitt et al. 2019).

Before monitoring by Texas Fish and Wildlife Conservation Office (TXFWCO), there was no active research or monitoring program in place for this species. The TXFWCO has been conducting long term monitoring of the species within Bell County. A long-term data set will eventually provide a statistically valid sample size for future management decisions.

Methods

Sampling was conducted quarterly this year at the DSC, Robertson Springs, and SR1 (Figure 1). Timed searches were used at Robertson Springs, while Side and Anderson springs (both in the DSC) 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 and enters the main channel. Any areas where the water emerged from under gravel and cobble piles were searched. A smaller spring, adjacent to the main spring, was also searched quarterly from the orifice to the main channel. All springs were actively searched by turning over rocks and debris. Captured salamanders were placed into mesh bags and kept in the spring run for processing. During timed searches, all mesohabitats were searched for salamanders. Basic water quality parameters were measured using a Hydrotech compact DS5 (Hydrotech ZS Consulting, Round Rock, Texas).

At several locations, drift nets with 250 μm mesh were used for passive sampling at Robertson Springs and SR1. The drift nets were placed over the spring orifice for a minimum of seven days as part of the monitoring regime. Aquatic invertebrates captured in the drift nets 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. Salamanders were considered adults if

they measured over 25 mm (Bowles et al. 2006). 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 to be used 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 (size classes, associated substrate and vegetation percentages) has been updated to include the 2021 collections. Data was grouped into seasonal blocks for a size distribution analysis. The relative abundance of the salamanders was calculated for each season based upon size classes. Size classes are from 0-19, 20-29, 30-39, 40-49, 50-59, 60-69 mm.

The salamanders from SR1 were examined by creating a probability from the 2019 - 2021 capture history of each salamander. In 2019, 87 individuals were detected from six sampling events. In 2020, 101 individuals from five sampling events were detected. Then in 2021, 107 individuals were detected from four sampling events. Each time a salamander was captured and identified, it received a 1 for that sampling event, 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. By examining average probabilities of the capture history for the salamanders, there might be some insight into the effort of sampling between years.

Water level and flow data were collected from the Cemetery Well (Monitor well #5804628) and from the USGS gauge on the Salado Creek (USGS #08104300) from 2014 to 2021. 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 Springs, and to identify preliminary trends associated with the salamander collections.

Results

Robertson Springs and Downtown Spring Complex

In 2021, a total of 6 salamanders were detected at Robertson Springs and the DSC (Table 1). Of these 6, two were juveniles (< 25 mm total length; Bowles et al 2006) and 4 were adults.

Most salamanders were captured from the DSC at Side Spring (n = 4). Robertson Springs produced two salamanders, both from the Headwaters zone (Figure 2). Spring flow at Robertson Springs had not returned to the Headwater, Middle, or Ludwigia zones by January 12, 2021. However, beginning in May four or five major rain events produced enough rain to begin flows at Robertson Springs (Figure 3). By June 6th of 2021, flow at the Robertson Springs complex began to issue forth from the main spring zones. Drift netting captured both salamanders at Robertson Springs in July and September of 2021. No orifices were drift netted for passive sampling at the DSC in 2021. Drift netting at the DSC is complicated because the public use the area and the equipment is likely to be tampered with or removed. Water quality data is presented in Table 4.

A total of 177 Salado salamanders have been captured since 2015. Three salamanders do not have associated substrate or vegetation data, leaving 174 salamanders to examine the substrate and vegetation associations. A total of 67 (38%) salamanders have been captured in drift nets, presumably leaving the aquifer. Of the remaining 107 salamanders caught on the surface, 70 (65%) have been caught in gravel as the primary substrate, and 28 (26%) have been caught in cobble as the primary substrate (Table 2). Data from past habitat sampling at Robertson Springs has shown around 50% of the substrate to be silt when no beaver dams are present and up to 90% silt when inundated due to dams (Diaz et al. 2016). Salamanders have been captured in many types of vegetation, but 43 (41%) have been shown to associate with watercress (*Nasturtium* sp.), and 38 (36%) have been captured in areas with no vegetation.

From the 177 total individuals detected, 169 were used to examine the temporal shift in size for surface populations at the DSC and Robertson Springs. The updated temporal shift in size of the surface population shows a classic size progression from smaller to larger, over the course of the year (Figure 4). Size class trends remain the same throughout the year. In spring, most salamanders captured were in the smallest size class ranging from 10 to 19 mm. The line for spring is minimally expressing a bimodal hump, with a smaller hump in the fifth size class. In summer, the smallest size class is still distinct, however, the third size class is represented the most and constitutes the second hump. During fall, the bimodal hump resembles the inverse of the spring line as the 4th size class is the most expressed. The winter line is similar to the fall line except the initial hump of the line is in the first size class other than the second size class as in

fall. Overall, the most salamanders have been detected in spring, with the least detected in winter (Figure 5).

There appears to be a trend with juvenile salamanders and their appearance on the surface. While juvenile salamanders have been captured throughout the year, there is some clustering from spring to summer, indicating young of the year being detected on the surface (Figure 6). To test for this we ran a chi-squared test with the software package R, where we assume that juvenile salamanders should be present equally over the course of the year. This would be the case if there is no breeding season. To estimate the age of the juvenile salamanders, we used growth curves from the San Marcos Aquatic Resource Center that show a salamander under 20 mm is about a month old (unpublished data). Therefore, using these assumptions a chi-squared test was run and a significant difference was detected with season and juvenile salamanders under 20 mm in total length which are hypothesized to be around 30 days old ($\chi^2 = 37$; $df = 3$; $p = <0.0001$).

Solana Ranch Spring #1

A total of 123 salamanders were captured at SR1 during 2021. After removing recaptures of individual adult salamanders ($n=33$), the capture history shows that 75 new individual adult salamanders were detected and photographed during 2021 (Table 3). Monitoring data from 2021 identified one recaptured individual (salamander #211) from September 2017. The average capture probability for detecting an individual at SR1 this year increased to 0.30 compared to 0.24 from 2020.

Only one salamander was considered a juvenile (<25 mm). Reviewing the salamanders capture data at SR1, dating back to 2017, the majority of the surface population were adults (91%). The size average, based on the 440 salamanders detected since 2017, is 51.39 mm. The largest Salado salamander (87 mm), captured to date, was captured at SR1 in October 2020. The largest salamander captured in 2021 was 72.87 mm.

The temporal shifts in size class follow the same trends as the DSC and Robertson Springs data, but the overall population exhibits larger salamanders on the surface year-round (Figure 7). During the fall there have been no documented occurrences of salamanders in the first or second size class.

Cannibalism was documented in 2019 during the mark and recapture work. One individual salamander was caught with three eyes at first glance (Photo 1). Following a closer

inspection, a juvenile salamander head was seen sticking out of the larger salamander's mouth. The juvenile salamander appeared incapacitated and was left in place.

Aquifer Invertebrates

Drift net sampling at some of springs within the Robertson Springs and Anderson Springs (DSC) has been conducted to examine surface recruitment and detect juveniles exiting the aquifer. It has also provided a detailed data set of the karst invertebrates present at each spring opening or complex. Sampling the springs with drift nets has shown large range extensions for a number of aquifer-dwelling taxa (Alvear et al. 2020^a) and provided samples of undescribed species (Alvear et al. 2020^b, Gibson et al. 2021) present within this section of the Northern Edwards Aquifer.

Based on samples collected since 2015, we have a basic understanding of the stygofaunal (aquifer-dwelling taxa) community structure at the springs studied in Bell County. Drift net sampling examined over 2,100 days (2015 to June of 2018), collected over 4,500 aquifer dwelling individuals (Table 5). The majority of the community is comprised of *Stygobromus* spp. (45%; Amphipoda; three species) and aquifer snails (32%; five species). The two most abundant families of snails are in the family Hydrobiidae (*Phreatodrobia conica*; 14%) and then the family Lithoglyphidae (*Phreatocerus taylori*; 9%). The isopod, *Lirceolus* spp. totaled 13% of the collected individuals with three species present in the community (Schwartz et al. 2020). All of these stygofauna are within the size range, during all life stages, to be prey items for salamanders. Two studies suggest that there are increases in other aquifer snails, although a different family, during the summer (Johnson et al. 2019) and spring (Diaz et al. 2020). In addition to the aquifer invertebrates, many terrestrial karst invertebrates have been captured over the monitoring period. Most interesting from the terrestrial group include specimens of *Cicurina* sp., *Speodesmus* sp., and *Lymantes nadineae* (Photo 2). In the 2015 report, *Myrmecodesmus reddelli*, was stated as being present at Robertson Springs. Paul Marek, a millipede expert at Virginia Tech, was sent the specimens and in May of 2019 a determination was made that the *Myrmecodesmus* in question is indeed, *M. formicarius*, **not** *Myrmecodesmus reddelli*, which would have been a large range extension.

Stream Flow and Well Height Data

Although there have been varying levels of sampling 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 potential total at year end. Only when flows return to the springs at the Robertson property do the probabilities of capturing a salamander return. The USGS gauge on Salado Creek (#08104300) and Cemetery Well levels were plotted to see if there is a connection between well level and spring flow at Robertson Springs (Figure 8). In 2020, flows from the productive spring zones at Robertson Springs began to fade out in early June and the flows did not return at the end of the year as they have in the past. . In 2021, spring flow returned in early June reducing the amount of time the salamanders were available for detection at the surface. Figure 9 provides a visual representation between the Cemetery Well water level, flows from Salado Creek below the spring confluence (USGS #08104300) and yearly salamander detection totals. This graph suggest that the Cemetery Well levels, spring flows, and salamander detections reflect each other.

Discussion

The collection of salamanders in 2021 was even lower than 2020 although spring flow returned at Robertson Springs. The DSC produced four salamanders which is low, but still within the range of what would be expected from this particular spring. In 2017, Side Spring produced six salamanders which is the second lowest year for collections with Side Spring having an average of 8.4 salamanders per year detected. Side Spring has produced more salamanders than other springs associated with the DSC.

The lack of salamander collections at Robertson Springs was initially surprising given the return of spring flow. However, once the springs began to flow, the area was colonized by at least one beaver. During 2016 and 2017, beaver dams were actively removed from the spring run at Robertson Springs monthly. This was done to provide optimal habitat for the salamanders to colonize, once out of the aquifer. The presence of the dams causes the spring run to rise into the spring zones submerging the available orifice present (Photo 3). This not only puts predation pressures on the salamanders from fish and crayfish, but in theory would decrease the flow of water exiting the orifice, and in turn, the reduced water pressures may cause less salamanders to be pushed from the aquifer.

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. This life history pattern in the northern salamander group seems unique and could be facilitated by the shallowing of the

aquifer as the limestone tends to decrease in depth as the aquifer moves north. This type of shallowing of the limestone could cause the influx of recharge supplying allochthonous material to areas more rapidly 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*) both 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. However, 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 the gravid females observed by the two other authors and the observation of the 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 to be a peak in Salado salamander gravidity in December, the juveniles would be on the surface and 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 a 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 of those observations collected in the Salado salamander. 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 salamander data despite the smaller sample size.

Based on the seven years of monitoring we have developed a hypothesis as to why the surface densities of these salamanders are historically small (Norris et al. 2012). 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 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. It 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 in 2021 did not resume until June unlike years past when flows would return within the first quarter in the year then slow or stop in the summer months and begin flowing again in the fall. 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 five years have always been detectable and consistent with regards to count data. SR1 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. In addition, the small surface recruitment of salamanders seen at Robertson, DSC, and Anderson springs, based on the drift net data, suggest that the populations at these sites may be slow to recover from natural disturbances like a flood or cessation in flows.

Human disturbances have also been noted at the DSC. These cryptic salamanders require cover objects and associate with cobble and large gravel substrates. The movement of gravel and cobble in Salado Creek and at Big Boiling and Side springs, can cause the lack of cover objects in the pool area, reduced vegetative cover, and deepening of the spring riffles and spring orifices from artificial dams (human-made). These pressures are at the highest in the warmer months and add to the natural changes to the springs after flood events. In addition, the runoff flowing into Big Boiling during heavy rain events could be modified to prevent this surface flow entering the spring. Human pressure and salamanders have co-existed over the years at the DSC and other

sites along the Edwards Plateau. However, these impacts should be documented and made apparent to management within the area. Other spring locations to the south, such as Barton Springs and Landa Lake, have similar situations where there are state and federally listed species present with heavy anthropogenic activity.

Given that surface densities are low but appear to be consistent given the flows over the last five years (2015- 2019), it is likely that a large proportion of the Salado salamander population is below the surface within the aquifer. In addition, if there is a catastrophic event that affects the aquifer, a long cessation in flows, or there is a need to simply examine changes in the next ten years based on population density, this genetic analysis can be repeated and genetic bottle neck events or recalculation of site population estimates can be reexamined with more certainty.

Nice et al. (2021) examined the genetic structure of accessible populations of the Salado salamander in Bell and Williamson counties. This analysis from 175 Salado salamanders revealed a homogenization of the genetic diversity in the northern group of Salado salamanders sampled at the DSC and Robertson Springs, with minute genetic drift to the south at SR1 (Nice et al. 2021). Due to the homogenization of the genetics from the sampled sites certain questions asked of the data were unanswerable, such as flow paths of genes or unique alleles present at a specific location. However, the data did reveal no level of genetic mixing between the southern and northern groups of the Salado salamanders. The management value of this research means that none of the northern sites are genetically unique, therefore no site is a cornerstone to the conservation of this species. This however does not mean that the known sites are not of ecological importance in terms of resiliency and redundancy for the species given its small geographic range. In addition, the data suggests that the population sizes at each site are large enough to maintain stable populations over the near future (Nice et al. 2021).

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 data using active and passive sampling techniques in Bell County, TX. (NS = not sampled).

Season	Robertson Springs	Downtown Spring Complex	Solana Ranch Spring #1
Winter	0	0	8
Spring	0	2	65
Summer	2	0	30
Fall	0	0	19

Table 2. Habitat associations of the Salado salamander determined by 168 salamanders collected from 2015 to 2021 at the Downtown Springs Complex and Robertson springs.

	#	%
Cave Conduit	67	38.51
Substrate		
Silt	3	2.80
Sand	2	1.87
Gravel	70	65.42
Cobble	28	26.17
Boulder	4	3.74
Vegetation		
<i>Sagittaria</i> sp.	1	0.92
<i>Nasturtium</i> sp.	47	43.12
Filamentous Algae	4	3.67
<i>Ludwigia</i> sp.	3	2.75
<i>Amblystegium</i> sp.	5	4.59
<i>Hydrocotyle</i> sp.	2	1.83
none	40	36.70
Organic Debris	5	4.59
Grass	2	1.83

Table 3. Data collected from quarterly monitoring at Solana Ranch Spring #1 on individual salamanders collected from each year. Recaptures are salamanders captured again during the yearly monitoring although from a previous year of monitoring.

Solana	2019	2020	2021
Recaptures	12	15	33
New Individuals	75	86	75
Total Individuals	87	101	108
Average Prob	0.2	0.24	0.3

Table 4. Water Quality collected during 2021 from Salado salamander monitoring sites. LB = Little Bubbly, BB = Big Boiling, HW = Headwaters, Temperature (°C), Conductivity (µS/cm), DO (mg/L), pH (s.u.).

Site	Location	Date	Temp	Cond	DO	pH	Sallies
Robertson	HW	1/12/2021	20.49	585.5	7.60	7.11	0
LB		1/12/2021	19.12	610.3	7.35	6.89	0
Side		1/12/2021	20.57	604.5	7.89	7.03	0
BB		1/12/2021	20.78	604.2	7.77	7.08	0
Stagecoach		5/19/2021	20.81	593.4	7.70	6.61	0
Anderson		5/19/2021	20.4	609.8	6.28	6.71	0
Side		5/19/2021	20.86	608.1	7.55	6.90	4
Robertson		5/19/2021	Main	Springs	Dry	-	0
Solana	Side	5/25/2021	20.33	409.0	7.37	6.75	9
Solana	Main	5/25/2021	20.26	409.5	8.01	6.85	57
Stagecoach		5/25/2021	20.88	595.9	7.59	6.67	0
Robertson	HW	6/30/2021	20.99	581.3	13.06	6.81	0
Anderson		7/23/2021	21.32	604.6	7.12	6.67	0
Side		7/23/2021	21.38	594.8	NA	6.48	0
Stagecoach		7/23/2021	20.42	592.3	7.58	6.65	0
Robertson	HW	7/23/2021	21.01	578.1	11.88	6.78	1
Solana	Side	8/18/2021	21.13	487.1	7.24	6.63	3
Solana	Main	8/18/2021	20.89	487.3	7.74	6.71	27
Anderson		10/7/2021	20.95	583.3	7.14	6.72	0
Side		10/7/2021	20.99	585.7	7.54	6.57	0
Stagecoach		10/7/2021	20.91	583.0	7.29	6.82	0
Robertson	Middle	10/7/2021	21.16	579.5	7.53	6.40	0
Solana		10/12/2021	21.10	465.4	7.05	7.20	19
Solana		12/14/2021	20.69	485.3	6.44	7.53	8
Anderson		12/15/2021	20.91	596.1	7.04	6.65	0
Stagecoach		12/15/2021	20.92	594.6	7.2	6.67	0
Robertson	HW	12/15/2021	20.91	591.2	7.45	6.92	0
Robertson	Middle	12/15/2021	20.94	590.8	7.48	6.97	0
Hidden Spring	Side	12/15/2021	20.95	803.5	4.94	6.78	0

Table 5. Aquifer invertebrates collected from the Downtown Spring Complex and Robertson Springs. The *Lirceolus* sp. with an asterisk does not designate a new species, but the lack of species determination at that site.

Taxa	Downtown Spring Complex	Robertson	Gault Site	Hidden Spring
Blind Dytiscidae sp. nov.		X		
<i>Caecidotea reddelli</i>	X	X	X	X
<i>Caecidotea bilineata</i>		X		
<i>Lirceolus</i> sp.*	X		X	X
<i>Lirceolus bisetus</i>		X		
<i>Lirceolus hardeni</i> *		X		
<i>Lirceolus pilus</i>		X		
Microcerberidae		X		
<i>Parabogidiella americana</i>	X	X		
<i>Stygobromus bakeri</i>	X	X		
<i>Stygobromus bifurcatus</i>	X	X		X
<i>Stygobromus russelli</i>	X	X		X
<i>Texanobathynella bowmani</i> cf	X	X	X	
Bathynellacea Type II		X		
<i>Phreatoceras taylori</i>	X	X	X	X
<i>Phreatodrobia conica</i>	X	X	X	X
<i>Phreatodrobia micra</i>	X	X		
<i>Phreatodrobia nugax</i>	X	X	X	
<i>Sphalloplana mohria</i>	X	X		X
<i>Schornikovdona bellensis</i>	X	X	X	X
<i>Uchidastygacarus</i> sp.	X	X		

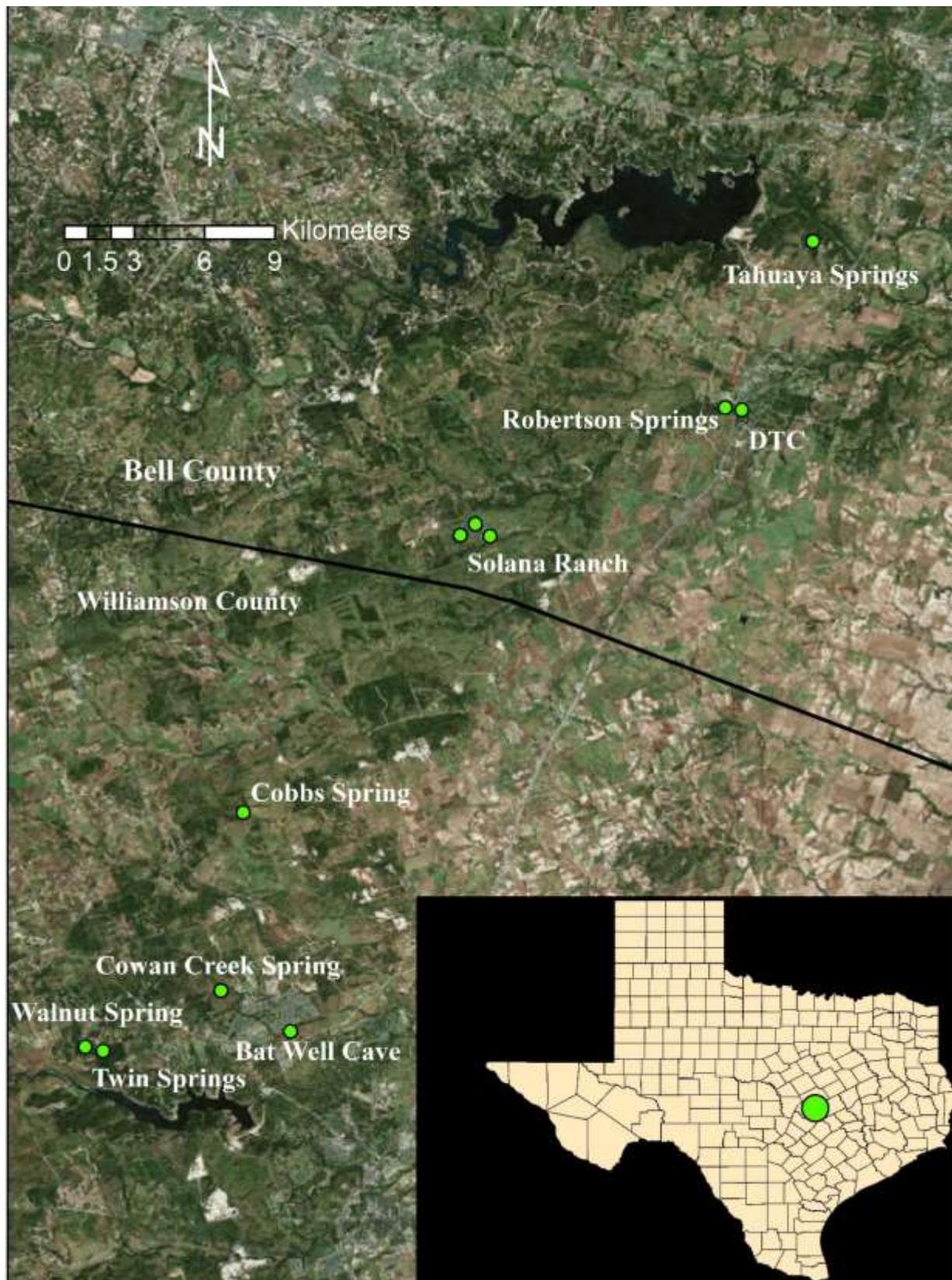


Figure 1. Study area for Salado salamander monitoring conducted from 2015 to 2020.

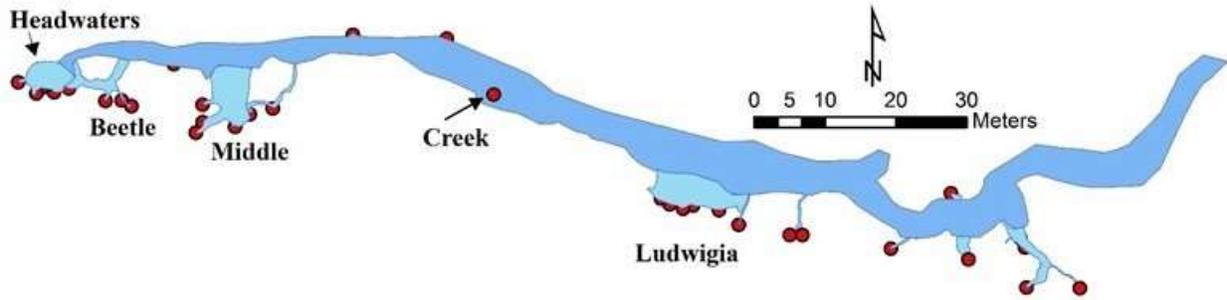
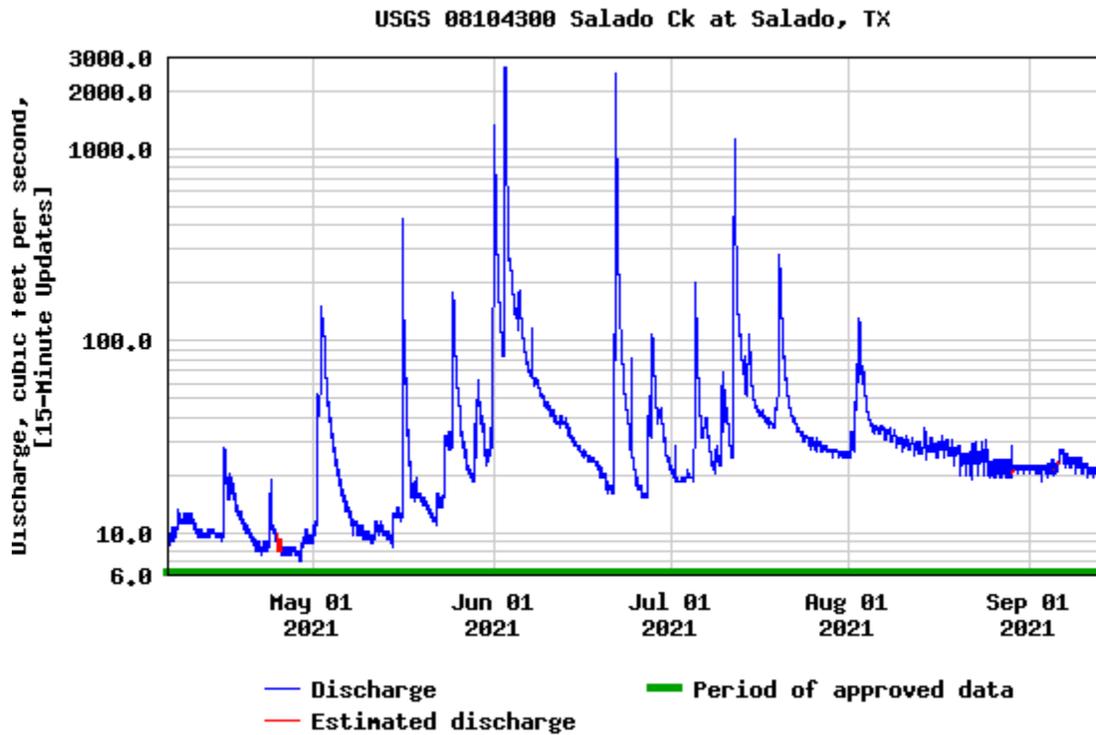


Figure 2. Map of Robertson Springs showing spring zones mapped in 2016 during optimal flow conditions at the site. Light blue zones are spring zones, red dots are orifice, and the blue is the spring run terminating into Salado Creek (top right of image).



Graph courtesy of the U.S. Geological Survey

Figure 3. Hydrograph of Salado Creek showing the rain events causing Robertson Springs to flow in early June and the Downtown Springs Complex sites to flood.

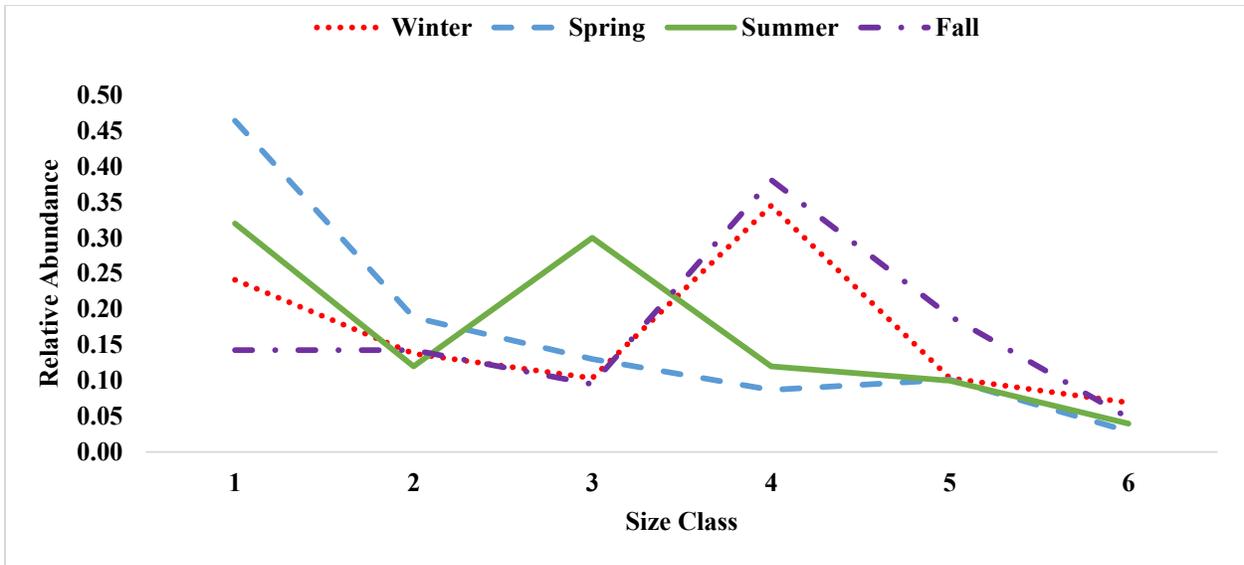


Figure 4. Relative abundance of Salado salamanders reflecting the dominant size class captured from the Downtown Spring Complex and Robertson Springs by season from 2015 to 2021 for 169 salamanders. Size classes: 1 = 10 - 19.99 mm; 2 = 20 - 29.99 mm; etc.

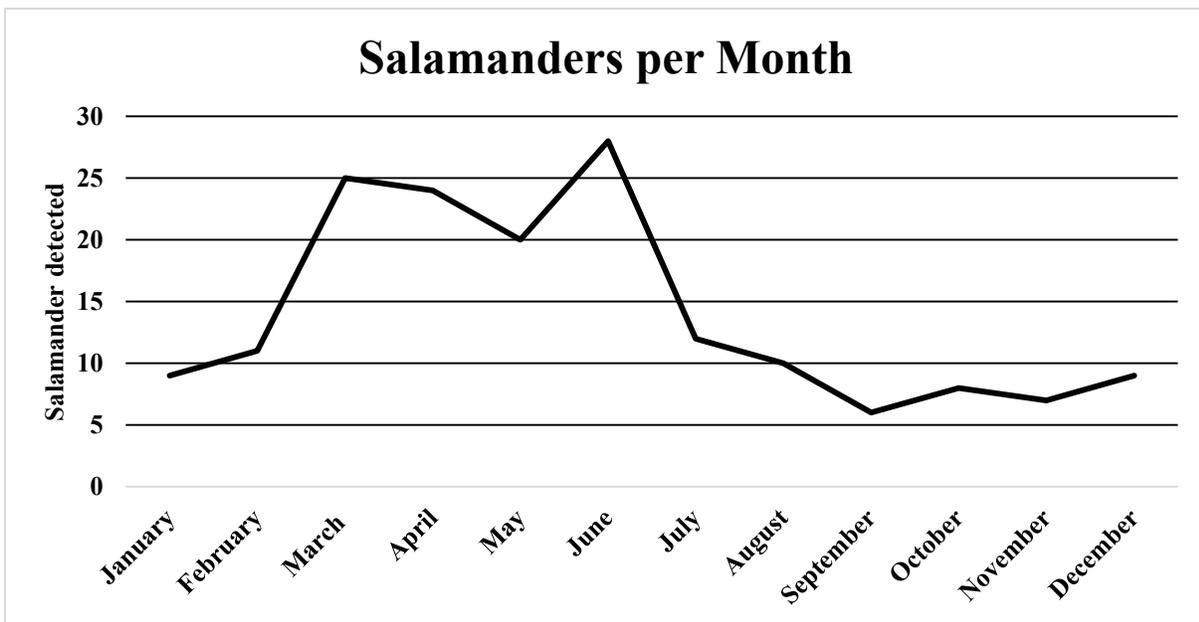


Figure 5. Pooled collections by month of 169 Salado salamanders collected from 2015 to 2021.

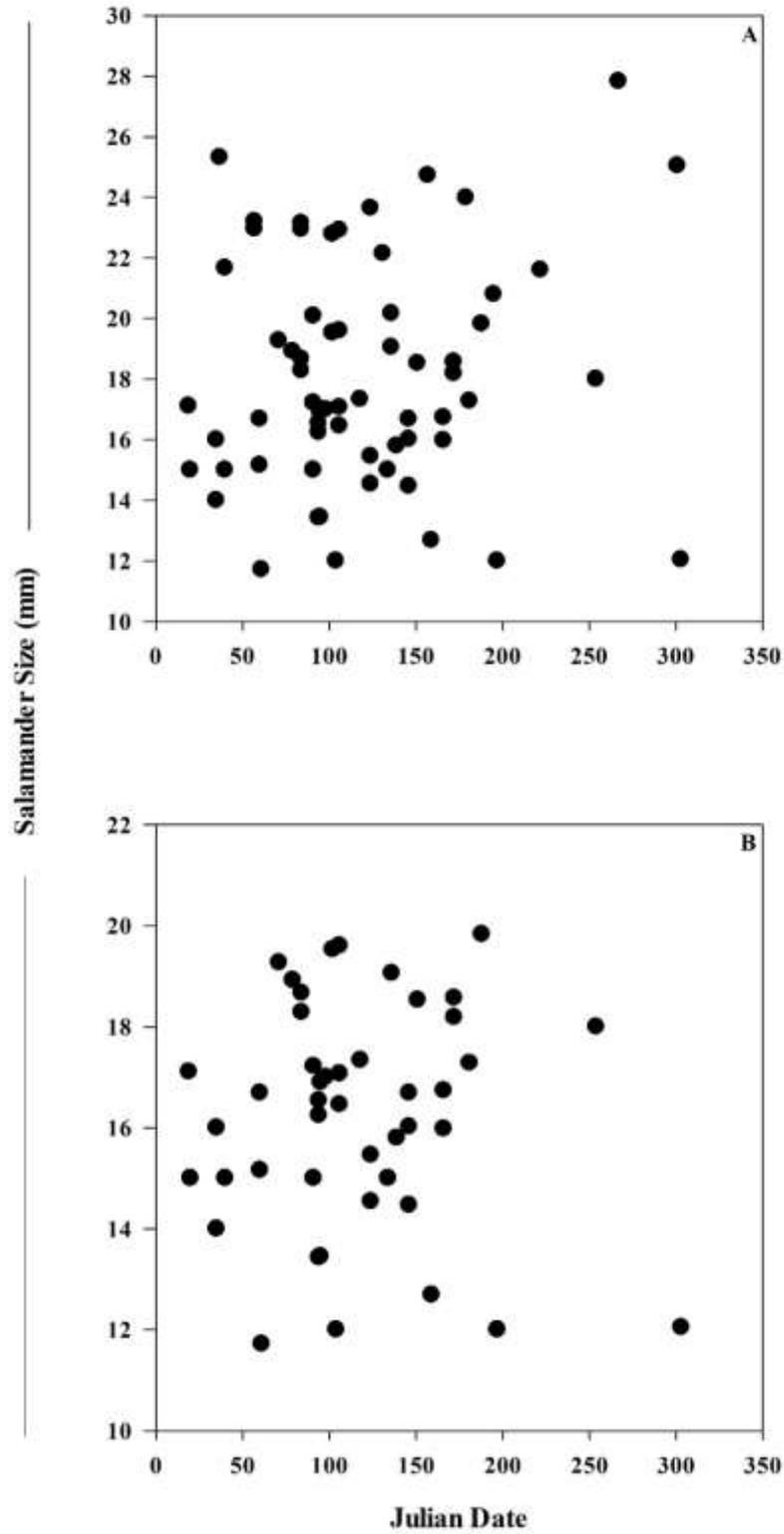


Figure 6. Timing of the capture of juvenile salamanders (< 25 mm) at the Downtown Spring Complex and Robertson Springs. Panel A is all juvenile salamanders (n = 72) and Panel B is only salamanders below 20 mm (n = 56) estimated to be around 55 days old.

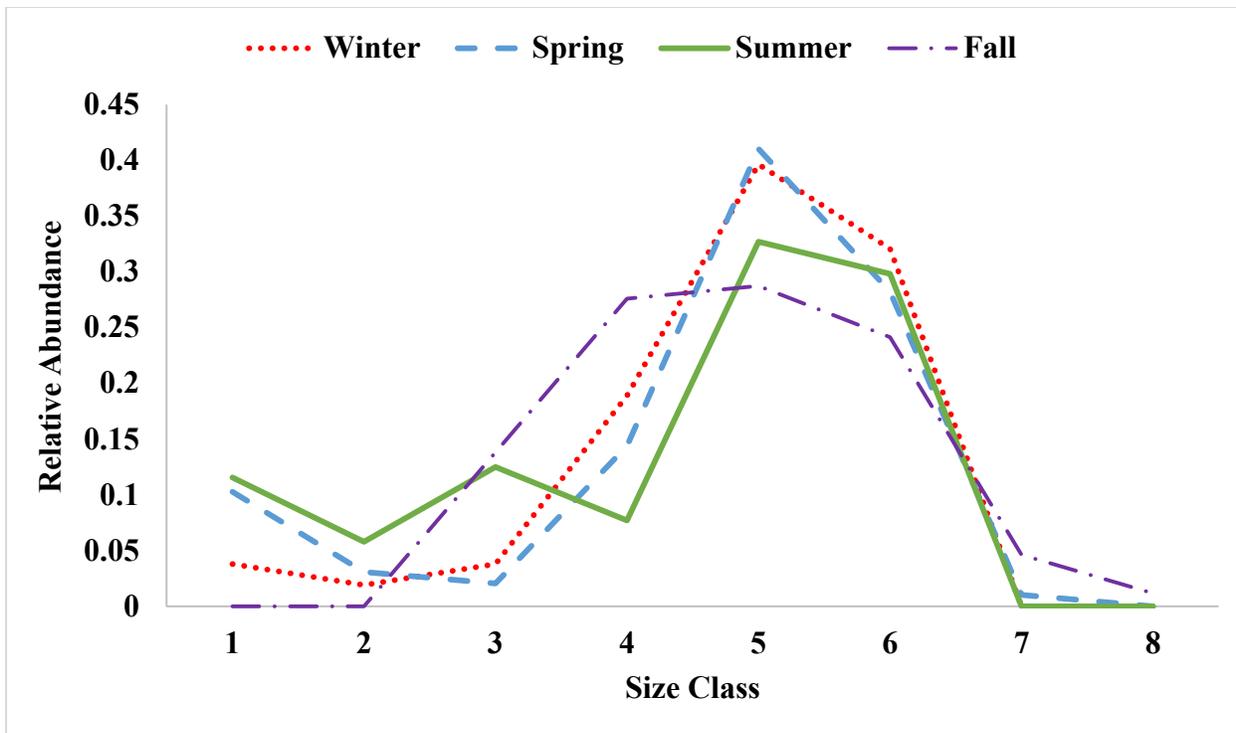


Figure 7. Relative abundance of Salado salamanders reflecting the temporal shift captured from sampling at Solana Ranch Spring #1 by season from 439 salamander detections (2015 – 2021). Size classes range from 10 -19.99 = 1; 20 -29.99 = 2; etc.

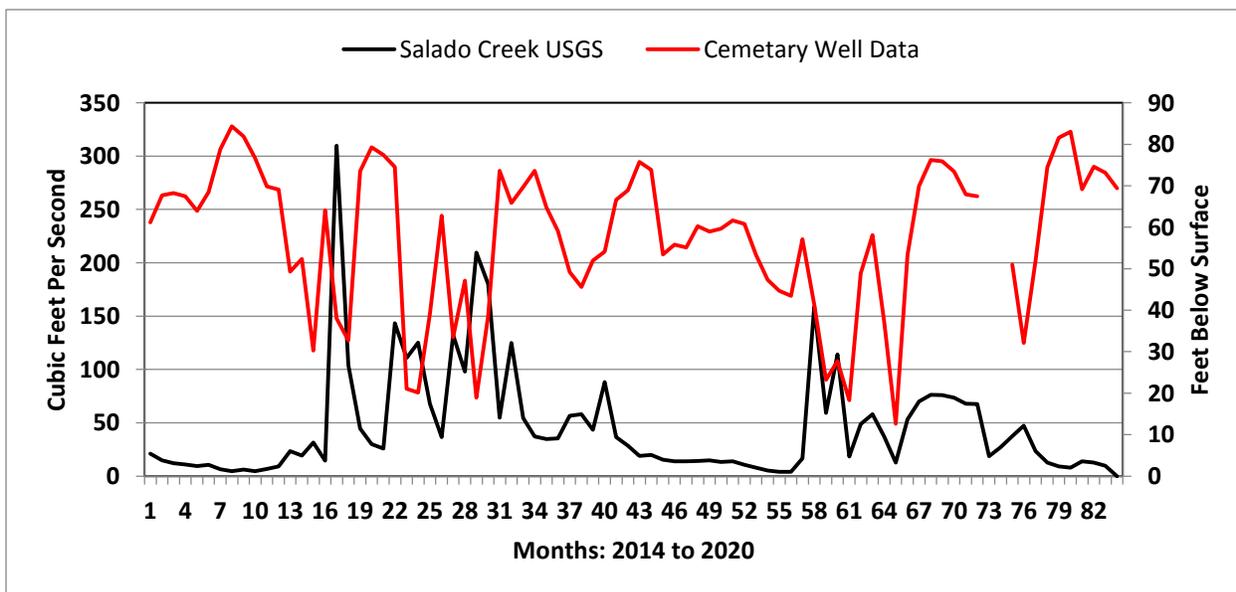


Figure 8. Data from the Cemetery Well (Monitor well #5804628) and from the USGS gauge on the Salado Creek (USGS #08104300) displayed monthly from 2014 to 2020.

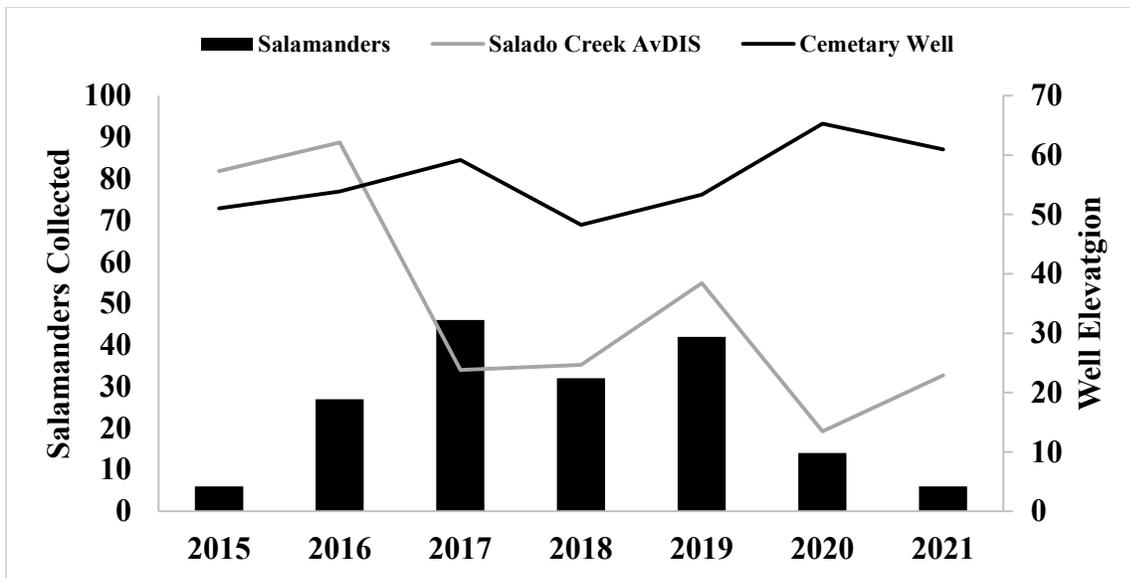


Figure 9. Data collected from the Cemetery Well (Monitor well #5804628) and from the USGS gauge on the Salado Creek (USGS #08104300) plotted with the total collection of salamanders from each year sampled at the Downtown Springs Complex and Robertson springs.



Photo 1. Salamander captured during a mark and recapture event showing evidence of cannibalism at Solana Ranch Spring #1.



Photo 2. Terrestrial karst invertebrates captured while drift netting springs in Bell County, TX. Top left is a blind spider in the genus *Cicurina*. The top right image is of a blind weevil, *Lymantes nadineae*. The bottom photo is of a blind millipede in the genus *Speodesmus*.

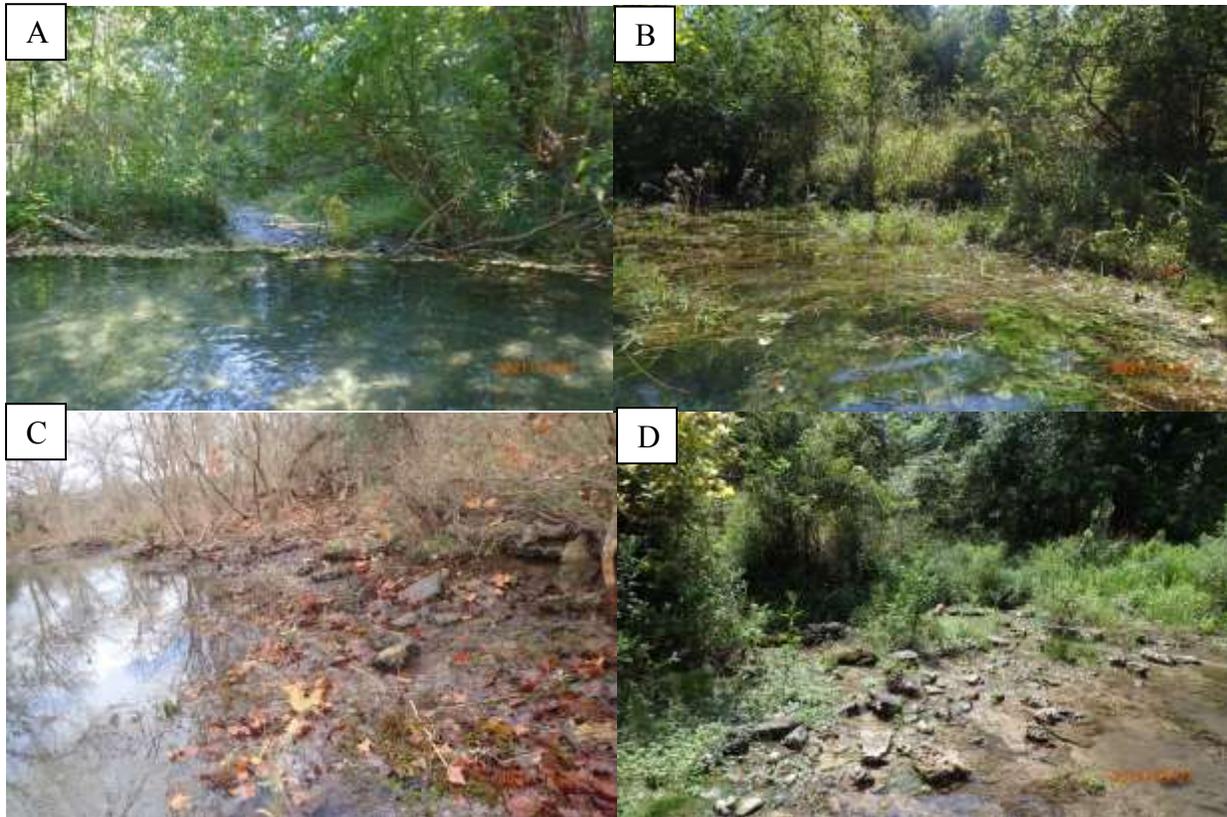


Photo 3. Stream habitat at different times at the Beaver Spring zone, Robertson Springs. Photo A) beaver dam downstream of Beaver Spring zone, October 2021; B) Beaver Spring zone submerged due to the dam, October 2021; C) Beaver Spring zone after the beaver dam was removed, October 2021; D) Beaver Spring zone in 2019 unhampered by any beaver dams.

Literature Cited

- Alvear, Dominique et al. 2020^a. Expanding the Known Ranges of the Phreatic Snails (Mollusca, Gastropoda, Cochliopidae) of Texas, USA. *Freshwater Mollusk Biology and Conservation* 23: 1-17.
- Alvear D, Diaz PH, Gibson JR, Jones M, Perez KE. 2020^b. An unusually sculptured new species of *Phreatodrobia* Hershler & Longley (Mollusca: Caenogastropoda: Cochliopidae) from central Texas. *Zootaxa*
- Bendik NF, Morrison TA, Gluesenkamp AG, Sanders MS, O'Donnell LJ. 2013b. Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. *PLoS ONE* 8:e59424 DOI 10.1371/journal.pone.0059424.
- Bolger, D.T., T.A. Morrison, B. Vance, D. Lee, & H. Farid. 2012. A computer-assisted system for photographic mark-recapture analysis. *Methods in Ecology and Evolution* 3:813-822.
- Bowles, B. D., M. S. Sanders, R. S. Hansen. 2006. Ecology of the Jollyville Plateau salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization. *Hydrobiologia* 553: 111-120.

- Chippindale, P. T., A. H. Price, J. J. Wiens, & D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. *Herpetological Monographs* 14:1-80.
- Chippindale, P. T., R. M. Bonett, A. S. Baldwin, & J. J. Wiens. 2004. Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution* 58:2809–2822.
- Diaz, P., M. Montagne, J.R. Gibson. 2016. Salado Salamander Monitoring Final Report 2016. Texas Fish and Wildlife Conservation Office. U.S. Fish and Wildlife Service, San Marcos, Texas.
- Diaz PH, Alvear D, Perez KE. 2020. Mesohabitat associations of the Devil Tryonia (*Tryonia diaboli*) (Gastropoda: Truncatelloidea: Cochliopidae). *Freshwater Mollusk Biology and Conservation* 23: 18-24.
- Forstner, M. 2012. An evaluation of the existing scientific evidence for the currently proposed hyperdiversity of salamanders (*Eurycea* sp.) in central Texas. Unpublished report prepared for the Texas Salamander Coalition. June, 2012, 28 pp.
- Gibson J R, Hutchins B T, Krejca J K, Diaz P H, Sprouse P S. 2021. *Stygobromus bakeri*, a new species of groundwater amphipod (Amphipoda, Crangonyctidae) associated with the Trinity and Edwards aquifers of central Texas, USA. Checklist dataset <https://doi.org/10.3897/subtbiol.38.61787>.
- Johnson, W. P., M. J. Butler, J. I. Sanchez, and B. E. Wadlington. 2019. Development of monitoring techniques for endangered spring endemic invertebrates: An assessment of abundance. *Natural Areas Journal* 39:150–168.
- Nice, C.C., Fordyce, J.A., Sotola, V.A. Crow, J. Diaz, P.H. 2021. Geographic patterns of genomic variation in the threatened Salado salamander, *Eurycea chisholmensis*. *Conservation Genetics* 22:811-821. <https://doi.org/10.1007/s10592-021-01364-z>
- Pyron, R. A., Wiens J. J. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61(2):543-583. <http://dx.doi.org/10.1016/j.ympev.2011.06.012>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. "NIH Image to ImageJ: 25 years of image analysis". *Nature Methods* 9, 671-675.
- Schwartz, B., C. C. Nice, W. Coleman, W.H. Nowlin. 2020. Status assessment and ecological characterization of the Texas Troglotic Water slater (*Lirceolus smithii*). Texas Parks and Wildlife Department Report. 26pp.