

Salado Salamander Monitoring Final Report 2019



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

Jennifer Bronson Warren
Texas Parks and Wildlife Department
Waco, Texas



Table of Contents

Acknowledgements	2
Executive Summary	3
Introduction	4
Methods	4
Results	6
Discussion	8

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Executive Summary

Monitoring of the Salado salamander (*Eurycea chisholmensis*) concluded in December of 2019 finalizing the fifth 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. We detected 44 Salado salamanders this year at these locations. Most salamanders were detected at Robertson Springs ($n = 27$), in the Ludwigia spring zone ($n = 15$). Within the DSC, Side Spring produced the most salamanders over the course of the year ($n = 11$). Twenty-six salamanders were captured using drift nets this year, while the remaining 18 salamanders were captured during active searches.

Other monitoring this year included quarterly monitoring at Solana Ranch Spring #1 (SRS1), where we detected 148 salamanders. In addition to quarterly sampling, a surface population estimate was determined using mark-recapture methods. This provided not only information about the surface population at SRS1, but valuable data regarding the validity of the head photo identification method.

Introduction

The Salado salamander (*Eurycea chisholmensis*) was first described as a species 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 on February 21, 2014. The USFWS is in the process of designating critical habitat for this species.

The Salado salamander is highly restricted geographically and is hypothesized to have a very low population within Central Texas (Norris et al. 2012). It has been proposed recently, that a much more streamlined phylogenetic hypothesis may apply to Central Texas *Eurycea*, (Forstner et al. 2012) and that the additional *Eurycea* within the Central Texas area had not been analyzed in context with congeners, but that is not the case. A peer-reviewed publication by Pyron and Weins (2011) genetically examined all Spelerpines, a subfamily under the family Plethodontidae, which included all *Eurycea* (*E. chisholmensis*, *E. naufragia*, and *E. tonkawae*). Pyron and Weins (2011) suggests that the phylogenetic analysis by Chippendale et al. (2004) was appropriate and that indeed these are distinct species. In addition, a recent study, funded through a section six 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 TXFWCO there was no active research or monitoring program that was working with this species. The TXFWCO proposes to conduct long term monitoring of the species within its known geographical range. A long-term data set will eventually provide a statistically valid sample size to base future management decisions.

Methods

Sampling was conducted monthly in 2019 at the DSC and at Robertson Springs (Figure 1). At Big Boiling and Robertson springs timed searches were conducted, while Side Spring and Anderson Spring were searched entirely due to their small areas. Salamanders were searched for in all mesohabitats. Passive sampling was conducted using drift nets with 250 µm mesh at a number of locations at Robertson and Anderson springs. Nets were set in place for a minimum of seven days following active searching. To account for the effort of netting, each net has days

counted individually. For example, if three nets were set out for three days, that would be nine days of drift netting. When collected, salamanders were photographed and released. All measurements were acquired using Image J software.

As in the 2018 report, the overall dataset has been updated to include 2019 detections within the running long-term data set for substrate, vegetation, and lengths. For length data, salamanders were 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. Finally, associated substrate and vegetation percentages were updated to reflect the new collections.

Following initial visits to SRS1 in 2017 and 2018 the searches were constrained to the main run and areas where water is exposed (Figure 2). SRS1 was visited in November of 2018 to prepare for the quarterly monitoring in 2019 with the intent of conducting a population estimate using open models. We used a drive survey technique starting at the bottom of the search area while moving toward the spring orifice to look for salamanders. When salamanders were collected the process was similar to the monthly monitoring at Robertson and the DSC with sampling from the spring orifice to the location where the spring run intersects with the main channel, about a 10.8 m long run. In addition, there is another smaller spring adjacent to the main spring which was searched each visit as well. These springs were actively searched by turning over rocks and debris. Salamanders collected were placed into mesh bags and kept in the spring run until processing.

Quarterly monitoring at SRS1 was in February, May, August and November of 2019. The return trip on February 26, 2019 did not have sufficient recaptures to provide confidence that open season mark recapture models would be appropriate for this type of data. Therefore, SRS1 was revisited the following two weeks into March to collect sufficient data for a closed model to estimate surface population of salamanders at SRS1.

Processing salamanders began by anesthetizing two or three individuals at a time using a solution of one part baking soda and one part MS-222 (tricaine) dissolved in the local water. Once sedated salamanders were removed from the sedation tray and placed on a moist towel to be marked. Salamanders were marked with visible implant elastomer (VIE); (Northwest Marine Technology Inc., Shaw Island, Washington). Salamanders had the potential to be marked in two of four available spots on the dorsal side of the body (Figure 3). Color combinations were

determined using all available options from six different colors. Photographs of the salamander were collected to determine total length and to compare recapture rates based solely on head photographs and marked individuals. Once the salamander had been processed, it was returned to the mesh bag and left in the spring water until revived; then returned to the spring.

To analyze the data collected from the three events over three weeks, the program Mark was used (White 2005). Two basic models were run using closed population models in the Higgins' p and c framework (Higgins 1989). The first model kept the probability (p) of encountering and marking an individual salamander the same for each sampling period, the second model allowed p to vary between events. Model selection was based upon the corrected Akaike's Information Criterion for small sample sizes (AICc; Hurvich and Tsai 1989). The AIC score is a way of ranking models using parsimony (Akaike 1973). To examine our sampling efficiency the p for the entire period of sampling from the three events was calculated using the formula $1 - ((1-p_1)(1-p_2)(1-p_3))$.

In addition to the regular monitoring sites, three other locations were sampled this year for the Salado salamander. To minimize the time spent at a spring that may not be productive regarding the Salado salamander, passive sampling methods with a drift net were used. Nets were set in the same fashion as the routine monitoring sites and left in place for a week. In addition to nets, at seep areas mop heads and rags were used to sample.

Results

A total of 44 salamanders were detected in 2019 from the DSC and Robertson springs. Of these 44, 19 were juveniles (<30 mm) and 25 were adults (Table 1). Salamanders were detected during each monthly visit except for October and November. The highest salamander producing springs were Robertson Springs in the Ludwigia Spring zone (n = 15), followed by Side Spring (n = 11) at the DSC. Of the 44 salamanders captured, 26 were captured passively using drift nets deployed over a combined 585 days of drift netting. Ludwigia Spring zone, within Robertson Springs, had the most detections of salamanders using the drift nets (n = 11), followed by Middle Spring (n = 6), Anderson Spring at the DSC (n = 4), Headwaters Spring zone (n = 3, Robertson), and one from Creek Spring (Robertson). From a monitoring perspective, the passive sampling using drift nets during monitoring events in 2019 (n = 21) was more productive when compared to the active searching (n = 11) component of the monitoring (Table 2).

Some salamanders escaped without a photo, therefore, a total of 151 Salado salamanders that have been captured and measured since 2015 were used for statistical analysis. A classic size progression from smaller to larger salamanders, over the course of the year has been demonstrated (Figure 6). In winter, all the size classes were present. In spring, the smallest size class was dominant, with the second largest percentage in the second size class (20 -29 mm). In summer, there appear to be two peaks in size class, initially it is the first size class (10-19 mm) followed by a slightly lower peak in the third size class (30 – 39 mm) (Table 3). In the fall, the largest number of individuals observed were within the fourth size class (40 – 49 mm). Overall, the most salamanders were detected in the spring than are detected in winter (Figure 7; Table 3).

A total of 154 Salado salamanders have been used to examine the substrate and vegetation associations. Of the 154 salamanders, 91 were detected on the surface (59%), while 62 (40%) were captured in drift nets, presumably from the aquifer. The 91 salamanders detected on the surface, 58 (63%) were captured in gravel and 24 (26%) were captured in cobble as the primary substrate (Table 4). Salamanders were detected in many types of vegetation, but 35 (38%) of the 73 salamanders captured in vegetation were shown to associate with watercress (*Nasturtium* sp.), while 33 (36%) have been captured in areas with no vegetation.

Drift net sampling at a number of springs within the Robertson property and at Anderson Springs was conducted to examine recruitment of the salamanders to the surface. Surface recruitment of salamanders at Robertson Springs from the aquifer to the surface habitat has been calculated at 0.03 salamanders per day. This rate was calculated using data from a total 2,091 days of drift net sampling from 2015 to 2019. These activities have also provided a detailed data set of the karst invertebrates present at each spring opening or complex. Some of the determinations have been coming back about the karst invertebrates captured during this sampling (Table 5).

A total of 148 detections were observed at SRS1 during 2019. Twenty-two salamanders were considered juveniles (<30 mm), therefore, 122 were adults. After removing recaptures of individuals, the data shows that 73 individual salamanders were detected and photographed during 2019. Four recaptures go back to September of 2017. Reviewing the salamanders captured at SRS1 dating back to 2017, 165 were adults (85%). The overall size average based on the 194 salamanders detected is 48.34 mm. In addition, the largest Salado salamander within this data set has come from SRS1 at 75.29 mm.

During the population estimate sampling at SRS1, a total of 38 individual adult salamanders were detected and marked from the three events in 2019. A total of 11 juveniles (< 28 mm) were detected although not marked. The average size of salamanders, including juveniles, from the three events was 45.63 mm. During the first event, 29 salamanders were detected and marked. The second event had 23 detections, with 17 recaptures and six new marks. The third event had 17 captures with 14 recaptures and three new marks (Table 6). Ten salamanders were captured during each event. Seventeen salamanders were only detected once. Ninety percent of the detections were within the main pool which stretches out about 2 m from the orifice.

The two population models had almost identical outputs for estimates of N, however, the model allowing p to vary between events had the lowest AICc score. There was a change in AICc of 2.95 between the two models (Table 7). Therefore, the model with p allowed to vary was selected with the estimate of 41 (38-49) salamanders within the searched area. The p for the entire period of sampling was 0.92.

There were issues identifying individuals with the VIE tags. During the three week period, two salamanders shed tags entirely. Four other salamanders had tags that were not complete (the elastomer had broken up) or were difficult to detect. Therefore, around 20% of the salamanders with VIE tags would have lost data. However, head photos were able to match salamanders from previous events that had issues with the tags.

Three other springs were examined this year for Salado salamanders. They were Hidden, Gault, and Brinegar springs. No salamanders were detected at these springs during 2019. Passive sampling of the springs will continue into 2020.

Discussion

This year was the second most productive year in salamander detections with 42 compared to 45 in 2017. We detected salamanders each month until October; then no detections occurred until December. There were no detections at Big Boiling Springs this year, which is similar to 2016 when no salamanders were detected. This year had the highest average discharge on Salado Creek while monitoring was occurring ($117.9 \text{ m}^3/\text{s}$; Figure 9). This average was followed by 2016 discharge in Salado Creek ($89.1 \text{ m}^3/\text{s}$).

With the addition of the 44 salamanders detected this year, the sample size of the seasonal dynamics graph (Figure 6) has increased from 107 to 151 salamanders. 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, is 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 collection and then anecdotally examining the congruence of the patterns within the data sets to provide evidence of those observations collected in the Salado. For example, our substrate and diet data collected from 2015 to 2018 mentioned above 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 given the smaller sample size of salamanders.

Insights into why the surface densities of these salamanders are historically small (Norris et al. 2012), with estimates of the author around 10 salamanders at the DSC and Robertson Springs sites, could be based on five 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 in surface densities at historic Salado salamander sites (Robertson Springs and DSC). Salado Creek's hydroperiod includes large pulses of water after large rain events locally and upstream 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 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 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 SRS1, just south of Salado, over the last three visits has 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 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 five years (2015- 2019), it is likely that a large proportion of the Salado salamander population is below the surface within the aquifer. The ongoing genetics project, mentioned earlier, is likely to provide insights into the subterranean population densities when it is completed. 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.

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

Table 1. Collections of Salado salamanders from 2019 timed monitoring, passive drift netting, and opportunistic collections. DSC = Downtown Complex; CC = Cave conduit; LN = little net, by hand; DN = drift net; HW = Headwaters.

Spring	Location	Date	Size (mm)	Primary Substrate	Vegetation	Method
Ludwigia	Robertson	1/9/2019	46.61	CC	Cave	DN
Mid Spring	Robertson	1/9/2019	43.43	CC	Cave	DN
Mid Spring	Robertson	1/9/2019	48.08	CC	Cave	DN
Mid Spring	Robertson	1/9/2019	54.93	CC	Cave	DN
Mid Spring	Robertson	1/16/2019	62.02	CC	Cave	DN
Creek	Robertson	1/16/2019	54.34	CC	Cave	DN
HW	Robertson	1/31/2019	30.9	CC	Cave	DN
Mid Spring	Robertson	2/6/2019	25.32	CC	Cave	DN
Side Spring	DSC	2/22/2019	43.04	Gravel	None	LN
Side Spring	DSC	2/22/2019	61.04	Gravel	Watercress	LN
Side Spring	DSC	2/22/2019	57.18	Gravel	None	LN
Side Spring	DSC	3/1/2019	55.15	Gravel	Watercress	LN
Side Spring	DSC	3/1/2019	44.62	Gravel	Watercress	LN
Ludwigia	Robertson	3/1/2019	16.69	CC	Cave	DN
Ludwigia	Robertson	3/1/2019	15.16	CC	Cave	DN
Anderson	DSC	3/28/2019	57.39	Gravel	none	LN
Side Spring	DSC	3/28/2019	60.52	Gravel	Watercress	LN
Ludwigia	Robertson	3/28/2019	29.52	Gravel	Ludwigia	LN
Mid Spring	Robertson	3/28/2019	56.55	Gravel	none	LN
Side Spring	DSC	4/4/2019	54.95	Gravel	none	LN
Anderson	DSC	4/4/2019	16.25	CC	Cave	DN
HW	Robertson	4/4/2019	16.54	CC	Cave	DN
HW	Robertson	4/4/2019	13.43	CC	Cave	DN
Side Spring	DSC	4/5/2019	63.45	Gravel	Watercress	LN
Side Spring	DSC	4/5/2019	53.42	Gravel	none	LN
Ludwigia	Robertson	4/29/2019	30.36	Gravel	none	LN
Ludwigia	Robertson	4/29/2019	27.83	Gravel	Ludwigia	LN
Ludwigia	Robertson	5/6/2019	17.82	CC	Cave	DN
Ludwigia	Robertson	5/6/2019	43.36	CC	Cave	DN
Ludwigia	Robertson	5/28/2019	28.5	Cobble	Amblystegium	LN
Mid Spring	Robertson	5/28/2019	32.73	Silt	Detritus	LN
Ludwigia	Robertson	6/3/2019	16.07	CC	Cave	DN
Ludwigia	Robertson	6/3/2019	16.1	CC	Cave	DN
Ludwigia	Robertson	6/3/2019	53.26	CC	Cave	DN
Ludwigia	Robertson	6/3/2019	54.31	CC	Cave	DN
Anderson	DSC	7/2/2019	18.51	CC	Cave	DN
Ludwigia	Robertson	8/6/2019	15.06	CC	Cave	DN
Ludwigia	Robertson	8/6/2019	17.01	CC	Cave	DN

Spring	Location	Date	Size (mm)	Primary Substrate	Vegetation	Method
Anderson	DSC	8/27/2019	16.82	CC	Cave	DN
Anderson	DSC	8/27/2019	14.57	CC	Cave	DN
Side Spring	DSC	9/30/2019	41.96	Cobble	none	LN
Mid Spring	Robertson	9/30/2019	33	CC	Cave	DN

Table 2. Data collected from the 2019 timed monitoring events at the Downtown Complex and Robertson springs. Other collections of Salado salamanders occurred during opportunistic sampling events.

Month	Active Searching	Passive Drift Netting
January	0	2
February	3	4
March	4	4
April	2	2
May	2	3
June	0	1
July	0	2
August	0	2
September	0	1
October	0	0
November	0	0
December	0	2

Table 3. Cumulative Salado salamander data collected from 2015 to 2019 used to create population dynamics graph.

Size Class	Winter	Spring	Summer	Fall
1	5	32	16	3
2	4	12	5	2
3	3	7	12	2
4	6	6	5	8
5	3	6	2	4
6	2	2	1	1
Size Class	Winter	Spring	Summer	Fall
1	0.22	0.49	0.39	0.15
2	0.17	0.18	0.12	0.10
3	0.13	0.11	0.29	0.10
4	0.26	0.09	0.12	0.40
5	0.13	0.09	0.05	0.20
6	0.09	0.03	0.02	0.05
Totals	12	45	32	18

Table 4. Habitat associations of the Salado salamander determined by 154 salamanders collected from 2015 to 2019 at the downtown spring complex (DSC) and Robertson springs. Substrate and vegetation percentages were calculated only using surface collections.

	#	%
Cave Conduit	63	40.90
Substrate	#	%
Silt	3	3.30
Sand	2	2.20
Gravel	58	63.74
Cobble	24	26.37
Boulder	4	4.40
Vegetation	#	%
<i>Sagittaria</i> sp.	1	1.10
<i>Nasturtium</i> sp.	35	38.46
Filamentous Algae	4	4.40
<i>Ludwigia</i> sp.	3	3.30
<i>Amblystegium</i> sp.	5	5.49
<i>Hydrocotyle</i> sp.	2	2.20
Leaves	3	3.30
None	34	37.36
Grass	2	2.20
Organic Debris	2	2.20

Table 5. Species determination by experts from Bell County, TX.

Species	Class/Order	Expert
<i>Schornikovdona bellensis</i>	Ostracoda	Okan Kulkoyluoglu
<i>Pygmarrhopalites</i> sp.	Collembola	Felipe Soto-Adames
<i>Pseudosinella violenta</i>	Collembola	Felipe Soto-Adames
Undescribed Dytiscidae	Coleoptera	Kelly Miller
<i>Uchidastygacarus</i> sp.	Acari	Ian Smith
Chernetidae	Pseudoscorpions	Charles Stephen
Chthoniidae	Pseudoscorpions	Charles Stephen
<i>Cicurina</i> sp. (juvenile)	Araneae	Marshal Hedin
<i>Speodesmus</i> sp.	Polydesmida	Paul Marek
<i>Lymanthes nadineae</i>	Coleoptera	Roberts Anderson

Table 6. Data collected for the mark recapture study at Solana Ranch Spring #1.

	2/22/2018	3/5/2019	3/12/2019
Detected	29	23	17
Recaptures		17	14
New marks	29	6	3

Table 7. Output from closed population estimates using Higgins' p and c models for the salamanders captured at Solana Ranch Spring #1 in Bell County TX in 2019. Mt = model with p varying between events, Mo = model with constant p.

Model	AICc	Δ AICc	SE	N	Lower	Upper
Mt	146.18	0	2.30	41.14	38.86	49.37
Mo	149.14	2.95	2.57	41.80	39.14	50.66

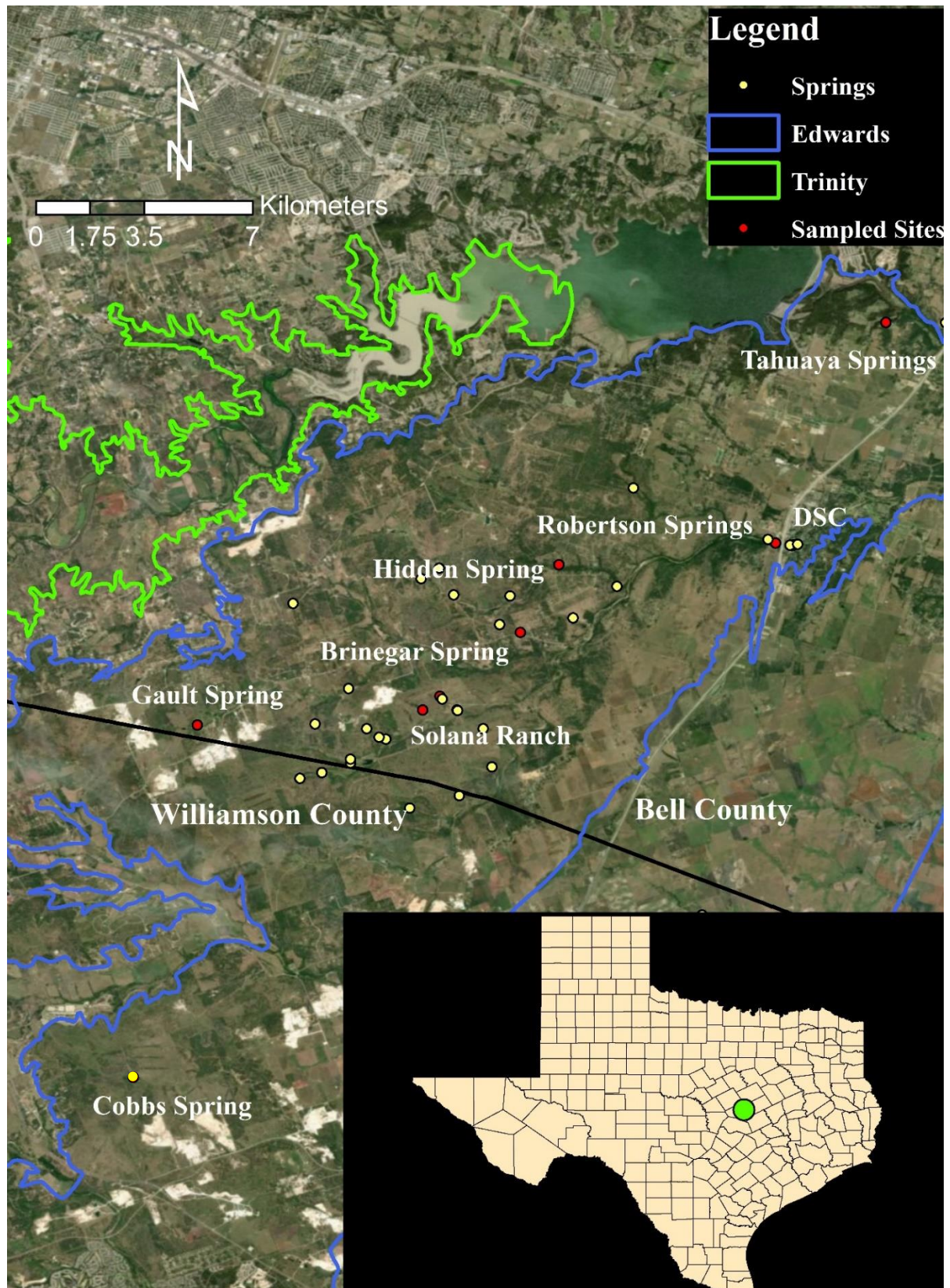


Figure 1. Known geographical range for Salado salamander and monitoring sites used in 2019. Yellow dots show mapped springs and red dots are areas sampled during this study.



Figure 2. Area sampled at Solana Ranch Spring #1 for population estimate and monitoring during 2019.



Figure 3. Marking locations for Solana Ranch mark-recapture study.



USGS 08104300 Salado Ck at Salado, TX

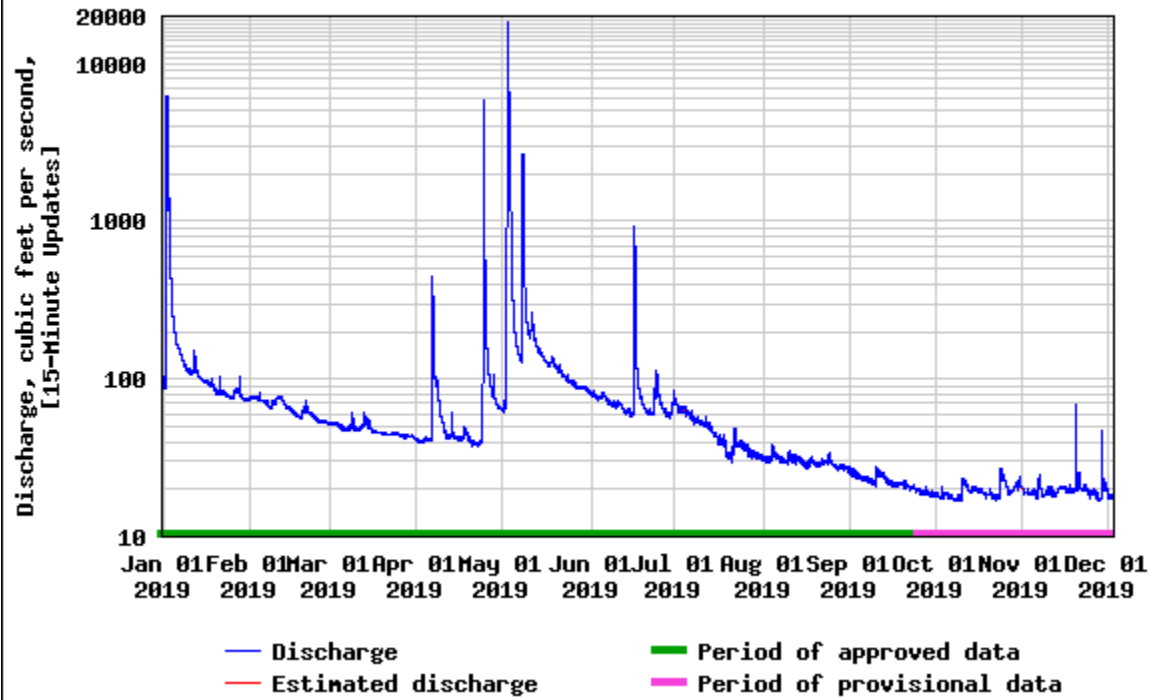


Figure 4. Hydrograph of Salado Creek for 2019.

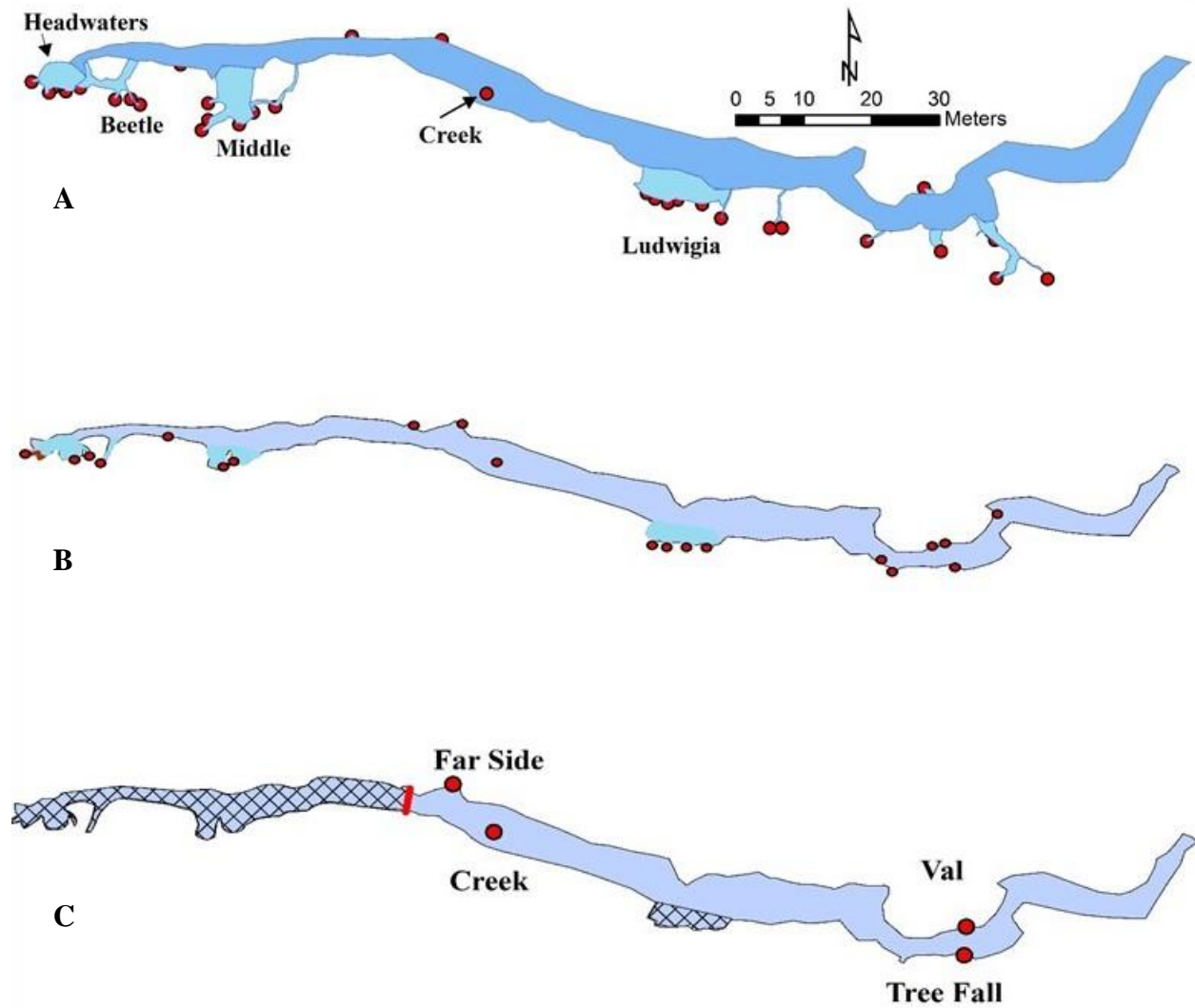


Figure 5. Robertson Springs starting in March of 2016 (A) into July of 2017 (B) and finally in April of 2018 (C). Hashed areas are places where there was no longer water. Red dots or lines are spring locations. Lighter blue sections are considered spring zones.

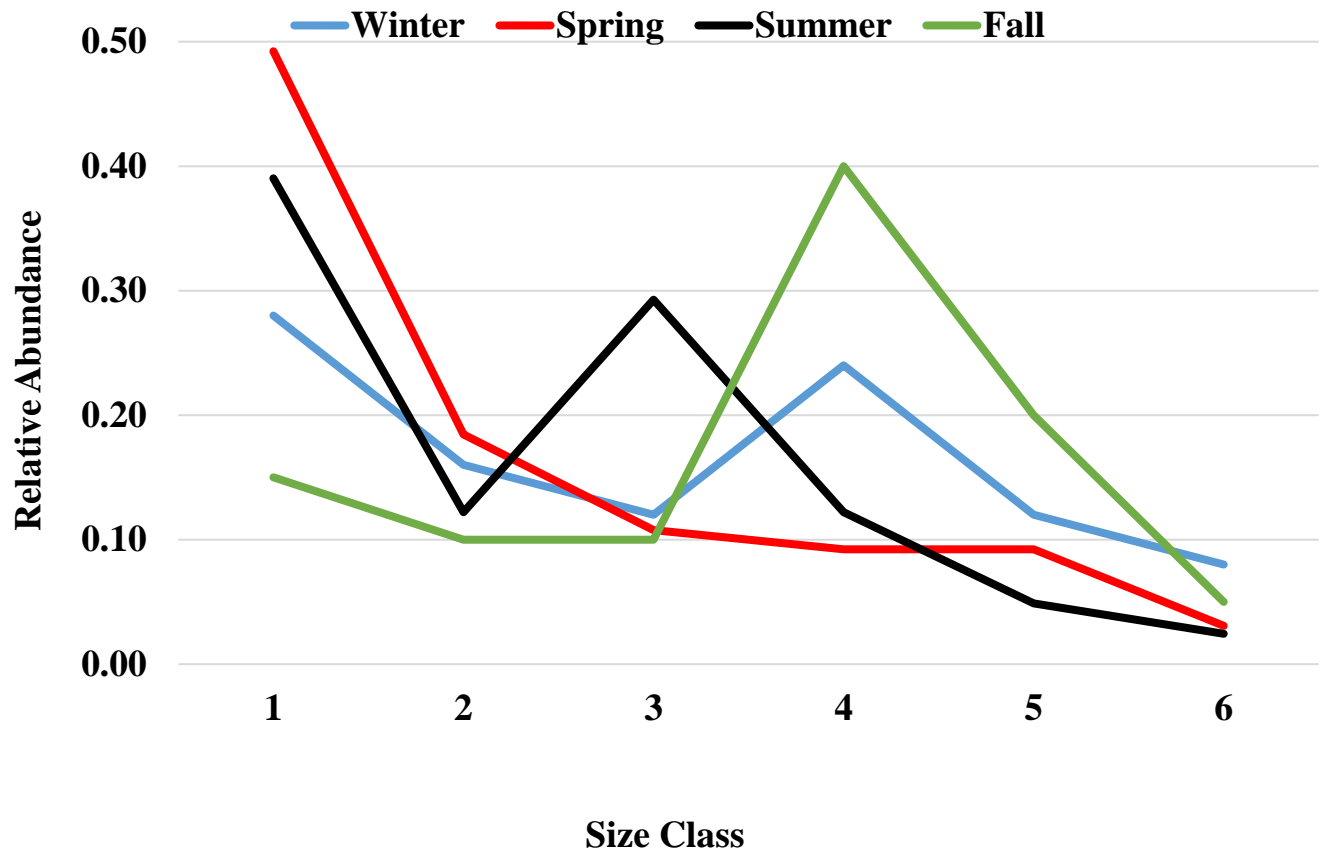


Figure 6. Relative abundance of size class for 151 Salado salamanders captured quarterly from 2015 - 2019 (1 = 10 - 19 mm; 2 = 20 - 29 mm; etc.).

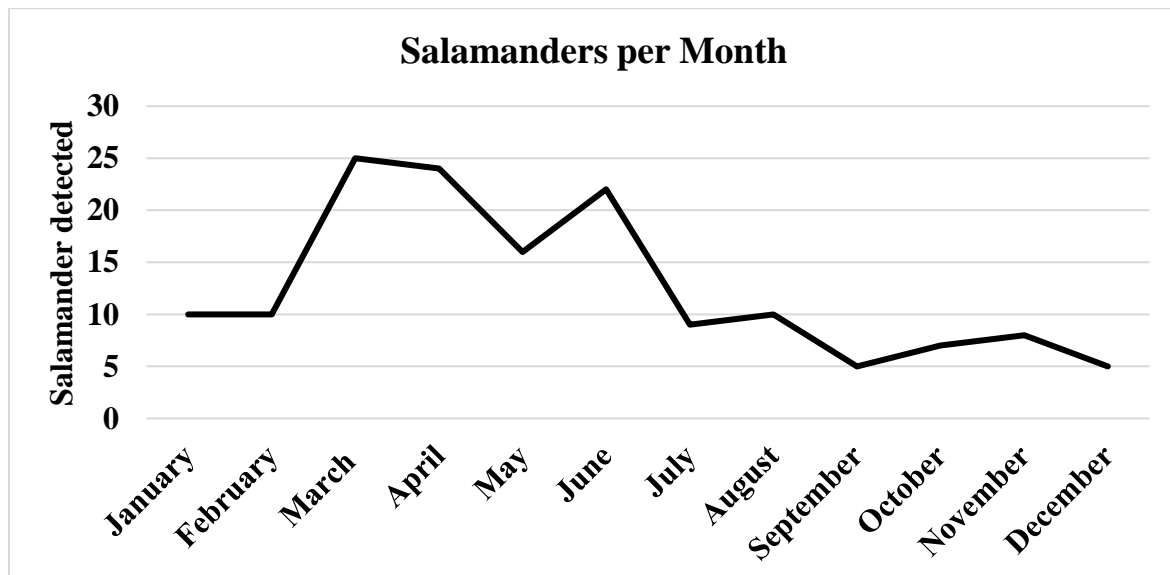


Figure 7. A cumulative depiction of when Salado salamanders are being caught using data from 2015 to 2019.

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