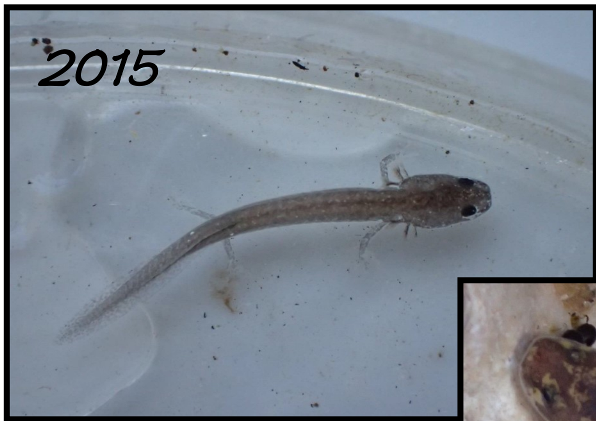
# Salado Salamander Monitoring Final Reports





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# Final Report
# 2015

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Figure 1. Meter tape shown alongside of Big Boiling spring.

# Executive Summary

The Texas Fish and Wildlife Conservation Office (TXFWCO) began systematic monitoring at the Salado Spring Complex and the Robertson springs in Bell County under federal permit TE676811-9 and state permit SPR-0111-003 on March 24, 2015. Systematic surveys were conducted in March, June, September, and December of 2015. In addition to systematic surveys, opportunistic surveys were conducted to increase documentation of salamanders within the system. The TXFWCO conducted 17 surveys of the springs over the course of 2015 (12 systematic and five opportunistic events) to monitor the salamander population, resulting in the capture of seven Salado salamanders. Six of the seven salamanders were juveniles ranging in length from 14-17 mm. One adult salamander was collected at the Robertson springs, measuring 50 mm in length.

A single season occupancy model was populated for Big Boiling spring based on the data collected during the April 2015 sampling event. The model suggests that salamanders are present within the spring, however, are very difficult to detect. Salamanders were captured at both Robertson and Anderson springs, but not within the methodology of the systematic sampling, and therefore no probability of detection was calculated for those sites. If probabilities of detection were calculated using this capture data, they would be similar to Big Boiling.

Habitat associations were documented with each salamander captured, and it is suggested due to the low sample size that the salamanders associated with cobble and gravel substrates, and vegetation types such as *Ludwigia sp*, filamentous algae and detritus. Estimates of abundance for adults and juveniles within these springs would be low given the lack of individuals captured during the sampling events. There are likely a number of reasons for the theoretical low surface population densities. First, being that this is the northern most edge of the *Eurycea sp.* distribution within the Edwards Plateau, densities may be low due to historical changes in temperature and rainfall over the course of the geologic period that have curtailed the species to this small range. Another might be that the available habitat within the spring systems is not conducive to life history patterns known from other species along the Edwards Plateau. Finally, that the subterranean ecosystem is sufficient to sustain this population and the need for juveniles or adults to migrate within the aquifer looking for food or a mate and eventually being purged from the aquifer may not be strong.

Goals proposed for 2016 will be: to conduct habitat association surveys for the salamanders, explore areas within the creek that may provide available habitat for the salamander, and examine the survival of surface salamanders and the migration of subsurface salamanders to the surface, in regards to the frequency of salamanders coming to the surface. The TXFWCO will continue to explore the possibility of bringing salamanders back to the San Marcos Aquatic Resource Center to undergo life history studies and provide a refugium for these rare salamanders.

## 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 designated the downtown spring complex, the Robertson estate spring, and a few sites upstream in the Salado creek watershed, as critical habitat.

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*, including the ones in question at the time (*E. chisholmensis, E. naufragia, and E. tonkawae*), suggest 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, shows the species designation was indeed scientifically valid (Hillis et al. 2015).

Although sporadic sampling for Salado salamander has occurred, no active research or monitoring program had been established to gather data about this particular 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. In 2015, the TXFWCO sampled and collected data to determine distribution and abundance of the salamander within its range and examine the status of historical occurrences.

## Methods

Transect surveys were conducted at Big Boiling, Anderson/ Benedict, and at Robertson springs to monitor the Salado salamander within the springs. Meter tape was used to identify transects along the spring runs, which were considered sites (Figure 1). Sampling began by starting downstream and moving up towards the spring opening. At each transect the dominant substrate and vegetation were recorded. Sampling for salamanders was conducted in two ways in order to maximize efforts and minimize the potential for injuring a salamander. First, in areas that are in suboptimal habitat (mud/silt or detritus), a ½ meter wide modified dip net was dragged along the bottom perpendicular to the edge of the bank collecting substrate and debris, across the entire channel, from bank to bank. Material collected in the net was examined either in the net or in a tray with a sieve. In optimal habitat (cobble, gravel), a visual survey was conducted along the transect, prior to doing sweeps with the dip net. If a salamander was found, the salamander was photographed and returned to the area where captured. All salamanders captured were reported to Texas Parks and Wildlife Department in the form of the Texas Natural Diversity Database, so the information is available for other researchers or studies.

Mesohabitat surveys were conducted by the TXFWCO at Little Bubbly, the side spring and Critchfield springs. Here, I define mesohabitat as visually distinct habitat (Pardo and Armitage 1997) within a system (e.g. riffle, pool, etc). Available habitat types were identified and then searched, using the modified net technique. Given the smaller size and homogenous nature of the habitat of the side spring off of Little Bubbly, the entire area was sampled using smaller aquarium nets.

Passive sampling was also conducted in an attempt to collect salamanders exiting the subterranean environment. This was accomplished using drift nets placed over the spring orifices (Figure 2). When the samples are examined, the entire sample is sorted in the field to look for salamanders. If a salamander was present the salamander was removed, photographed, then returned to the area where it was captured. After this initial search, the entire sample is preserved and stored in 95% EtOH, and taken back to the lab where the sample is sorted and enumerated under a compound microscope. Capture rates for salamanders and prey densities are calculated as *x* per day.

Figure 2. Drift nets placed on spring orifices to collect salamanders and examine prey densities

Water quality data was collected at each site during the survey using a Hydrolab compact DS 5. Water quality measurements are collected from each spring and averaged for each site. Data collected included temperature, dissolved oxygen, pH, conductivity, nitrates and total dissolved solids.

The program Presence was used to calculate occupancy models for Big Boiling. Two single season models were run in the Presence program. The first model kept detection constant throughout the three surveys while the other model allowed the probabilities of detection to vary between surveys. Akaike information criterion (AIC) was used to evaluate the models and select the appropriate model. The AIC scores are a way of selecting a model due to parsimony. Akaike information criterion does not show how correct the model is in reality, but demonstrate which model has a best fit given the collected data. The model produces three main results: a naïve occupancy (which is a frequency of occurrence for salamanders that site); a calculated occupancy score (which is a modified frequency of occurrence considering in the probability of non-detection within that sampling event); a probability of detection (stating the chance of collecting a salamander at that site with these particular methods). These scores were calculated for each of the sampling events when possible. Scores will be compared to determine inferences about the sampling technique and the time of sampling throughout the year in relation to occupancy and detection.

# Results

<u>Salamanders</u>

*Big Boiling*

During the first sampling event (March/April), two salamanders were captured, but only one counts for the occupancy model. This is due to the fact that the salamander captured on the 1st of April was outside of the transect and captured opportunistically. Therefore, the model was populated with only one capture. A 3 X 12 matrix was created to run in the program Presence. Two single season models were run in the Presence program. The first model kept detection constant throughout the three surveys while the other model allowed the probabilities of detection to vary between surveys. Results from the Presence program show AIC scores of 13.14 for the constant model and 14.88 for the model which allowed detection to vary. The AIC scores are a way of selecting a model due to parsimony. Akaike information criterion does not show how correct the model is in reality, but demonstrate which model has a best fit given the collected data. In this case, the best fit model is the constant model, however, the model allowing detection to vary was still significant due to the close range in AIC scores. For Big Boiling, the constant model, the naïve occupancy score was 0.083, with a calculated occupancy score of 1.0, and a probability of detection of 0.027. What theses scores refer to are the chances of detecting a salamander within Big Boiling during a systematic survey using these collection techniques. The naïve occupancy is a frequency of occurrence for that site. The occupancy score is stating that the salamander is present within the system, while the probability of detection is stating that the chance of collecting a salamander is extremely low. Other surveys were not successful in detecting salamanders within Big Boiling.

Another salamander was captured during an opportunistic event on May the 14th, 2015. This salamander was captured within filamentous algae and on substrates of gravel and sand. All three salamanders captured at Big Boiling were juvenile salamanders with lengths of 15 (2) and 17 mm (Table 1). Salamanders were captured at distances of 4, 6, and 10 meters away from the spring opening.

Table 1.  Salamanders captured from 2015 sampling events.

| Location | Date | Transect (m) | Size (mm) | Primary Substrate | Secondary Substrate | Vegetation |
|---|---|---|---|---|---|---|
| **Big Boiling** | 4/1/2015 | 6 | 15 | Cobble | Gravel | *Ludwigia* |
| **Big Boiling** | 4/8/2015 | 4 | 17 | Gravel | Sand | None |
| **Big Boiling** | 5/14/2015 | 10 | 15 | Gravel | Sand | FA |
| **Robertson Spring** | 7/16/2015 | Drift Net 0 | 14 | NA | NA | NA |
| **Anderson Spring** | 9/11/2015 | 46 | 17 | Gravel | Cobble | FA |
| **Robertson Spring** | 10/2/2015 | Drift Net 25 | 15 | NA | NA | NA |
| **Robertson Spring** | 10/2/2015 | 27 | 50 | Silt | Gravel | Detritus |

*Little Bubbly*

Due to the intermittent flow from Little Bubbly, mesohabitat surveys were conducted.  In addition bottle traps were placed within the orifices.  No salamanders were detected at this site.

*Anderson/Benedict*

During the third visit to (September 11, 2015) Anderson/Benedict spring site, a salamander was collected opportunistically near meter 47.  This is the first detection of a salamander at this spring site.  This salamander was opportunistically collected at the orifice of Anderson spring in gravel and cobble substrates (Figure 3).  Since the salamander was captured outside of the framework for the occupancy study an occupancy score was not calculated.  However, if a score were to be calculated, it would be very similar to the score calculated for Big Boiling, very low.  Passive sampling was conducted using bottle traps at the fissure where Benedict begins, but no salamanders were collected.

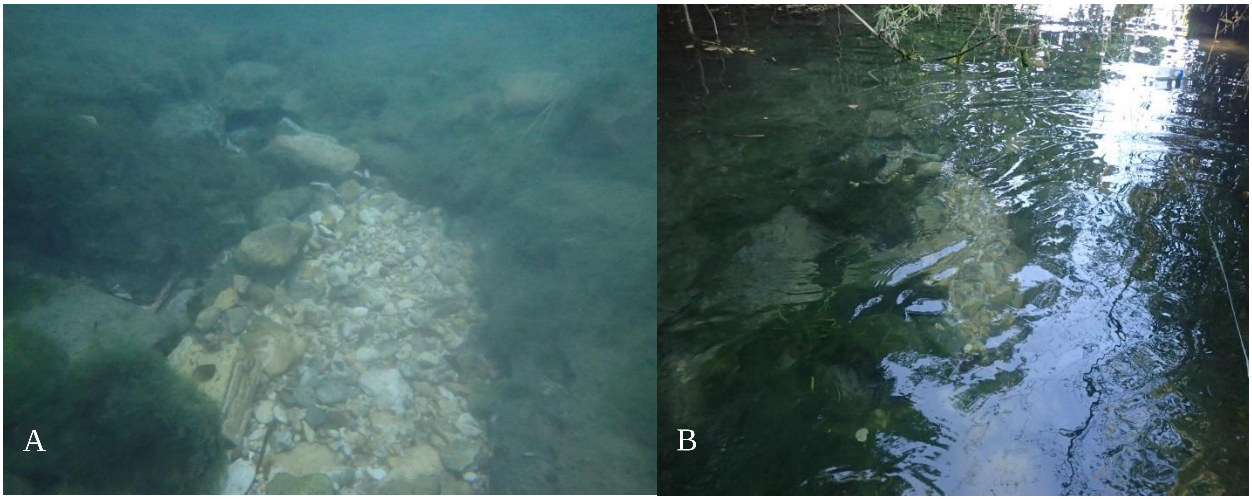


Figure 3.  Site at Benedict springs where a salamander was collected.  Figure A is the view below the water surface, and figure B is the view above the water surface.

*Critchfield*

This spring site was sampled using the mesohabitat approach from the area above the dam (just above the Benedict fissure) to the spring opening. No salamanders were detected.

*Robertson*

Systematic sampling at Robertson detected no salamanders. However, passive sampling with drift nets was successful at this site. Two salamanders were captured from two different orifices. The first salamander captured was from a spring we have been calling beetle spring, due to a new species predatory diving beetle found there (Figure 4A). The second salamander was captured in what has been called the middle spring (Figure 4B; right arrow). The only adult salamander (~50 mm) captured was collected at Robertson spring during opportunistic sampling. The salamander was captured using an aquarium net almost at the interface of the spring run and the terrestrial environment, at the top end of a series of springs in grass and silt with flowing water moving through just above the middle spring, where the salamander was captured in the drift net at meter 25 (Figure 4B; left arrow).


Figure 4. Photos from Robertson springs. Figure A is a photograph of beetle spring, where a salamander was caught in the drift net. Figure B is a photograph of middle spring where another salamander was captured with a drift net (arrow pointing to the right). The other arrow pointing to the left shows where the only adult was captured at Robertson.

Habitat Availability

Big Boiling and Robertson springs were assessed to determine the percentages of available substrates within each site. Big Boiling was shown to have over 50% gravel substrates and cobble being the second most available substrate (Table 2). Robertson spring was initially composed mainly of silt and mud substrates (Figure 5A).

However, with the removal of a beaver dam, the substrates have begun to shift in proportions (Figure 5B; Table 2). The Anderson/Benedict site showed changes in substrates over time due to the scouring effects of high flow events. Initially, the upper area by the Benedict fissure was covered with an aquatic plant (Figure 6A). After rains in June the site became scoured and the vegetation was washed away due to the high flows (Figure 6B).

Figure 5. Robertson spring before and a week after the removal of a beaver dam.

Table 2. Habitat availability at selected springs within the study area.

| | | Big Boiling | | Robertson July 2015 | | Robertson December 2015 | |
|---|---|---|---|---|---|---|---|
| **Substrate** | Number | Count | Percentage | Count | Percentage | Count | Percentage |
| **Mud/silt** | 1 | 1 | 0.88 | 149 | 92.55 | 130 | 64.36 |
| **Sand** | 2 | 10 | 8.77 | 0 | 0.00 | 5 | 2.48 |
| **Gravel** | 3 | 65 | 57.02 | 8 | 4.97 | 12 | 5.94 |
| **Cobble** | 4 | 18 | 15.79 | 2 | 1.24 | 7 | 3.47 |
| **Boulder** | 5 | 4 | 3.51 | 2 | 1.24 | 0 | 0.00 |
| **Bedrock** | 6 | 16 | 14.04 | 0 | 0.00 | 29 | 14.36 |

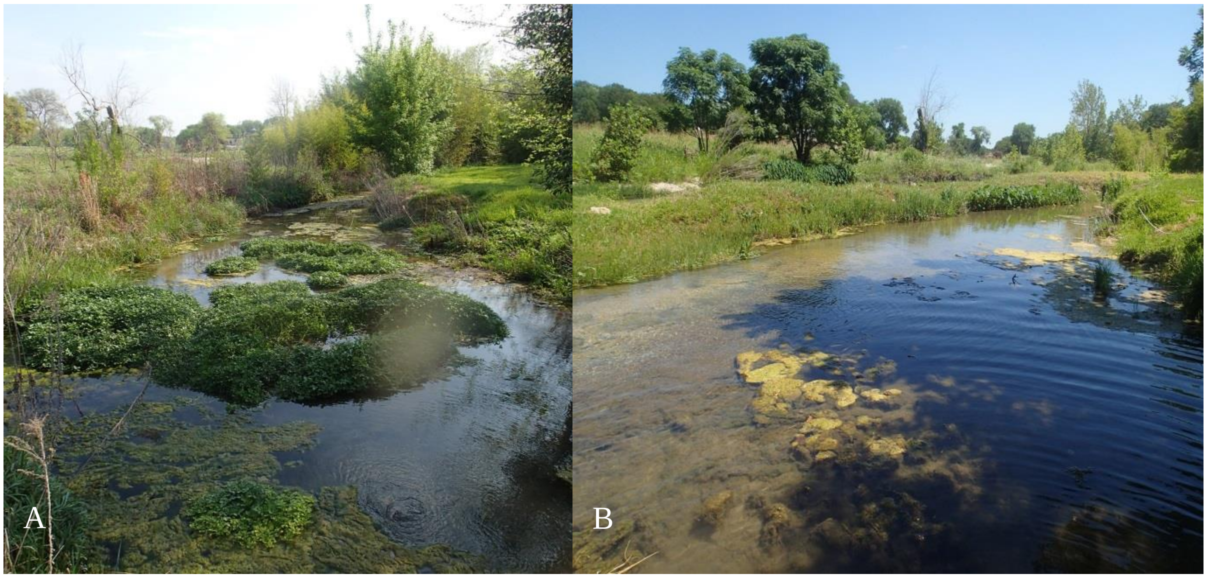

Figure 6. Benedict springs and habitat changes due to scouring events.

Surface Recruitment

Drift nets were left in place at beetle spring and middle spring, two of the largest spring openings at the Robertson spring site. The drift nets were left in place for 28 and 30 days, respectively, but checked weekly. One salamander was captured from each site, making the rate at which salamanders may potentially be populating the surface is around 0.03 salamanders per day, or about one salamander per 30 days.

Water Quality

Water quality data was collected two different ways during this study. A HydroTech hydrolab sonde was used to collect basic water quality parameters on each visit. The values have been averaged and are presented in Table 3. No values exceeded any ecological limits for salamanders taken from the dissolved oxygen, temperature, pH, conductivity or turbidity, althoug high levels of nitrates were present within the system.

Table 3. Average water quality data collected over 2015.

| | Benedict/Anderson | Big Boiling | Robertson | Side Spring off Little | Critchfield |
|---|---|---|---|---|---|
| Temperature | 20.36 | 20.78 | 20.60 | 20.73 | 20.79 |
| Dissolved Oxygen | 7.04 | 7.65 | 7.66 | 7.52 | 6.87 |
| Nitrates | 3.05 | 3.08 | 3.50 | 3.13 | NA |
| pH | 6.41 | 7.08 | 7.10 | 7.13 | 7.12 |
| Conductivity | 586.92 | 578.03 | 563.94 | 576.79 | 581.90 |
| Total Dissolved Solids | 0.3759 | 0.3701 | 0.3619 | 0.3694 | 0.3721 |

The second type of water quality sampling included the placement of a PVC container with a semipermeable membrane inside to collect contaminants at two sites (Stage Coach Inn Cave and Robertson Spring). Each sample was targeted to gather data on contaminants within the site over a period of 45 to 50 days. These results are presented below in Table 4. Overall, the Robertson site had more contaminants by number and by the amount. Differences between the Robertson 2014 and 2015 sample may be due to the amount of water that was passing the sampler in 2015 compared to the dryer 2014 year and the mobilization of the sediments in the wetter year. Compared to quartiles from data collected in 2013 and 2014 from known salamander sites using the same methods there are elevated levels of contaminants in the form of organochlorides, polybrominated diphenyl ethers (flame retardants), total number of contaminants, and the total amount (pg/L).

Table 4. List of contaminants sampled for in 2015 at Robertson spring and Stage Coach Inn cave along with results from 2014 sampling at Robertson spring. The last three columns show water quality data collected using the same methods in 2013 and 2014 from 23 other springs with historical salamander presence shown in quartiles.

| Contaminant | Stage Coach Inn Cave 2015 | Robertson Spring 2015 | Robertson Spring 2014 | 1st Quartile | 2nd Quartile | 3rd Quartile |
|---|---|---|---|---|---|---|
| **Organochlorines (#)** | 13 | 11 | 5 | 4 | 7 | 11 |
| **Polychlorinated biphenlys (#)** | 0 | 0 | 0 | 0 | 0 | 1 |
| **Polybrominated diphenyl ethers (#)** | 9 | 9 | 0 | 0 | 0 | 1 |
| **Polycyclic aromatic hydrocarbons (#)** | 2 | 6 | 2 | 2 | 7 | 14.5 |
| **Organochlorines (pg/L)** | 339.6 | 628.1 | 75.9 | 88 | 302 | 707 |
| **Polybrominated diphenyl ethers (pg/L)** | 162.3 | 898.1 | 0 | 0 | 0 | 15 |
| **Polycyclic aromatic hydrocarbons (pg/L)** | 12.8 | 197 | 324 | 321 | 1188 | 2741 |
| **Impervious Cover (%)** | 6.25 | 6.25 | 6.25 | 6 | 17 | 23 |
| | | | | | | |
| **Total Number of Contaminants** | 24 | 26 | 7 | 12 | 19 | 32 |
| **Total Amount (pg/L)** | 514.70 | 1723.20 | 399.90 | 208 | 563 | 2262 |

Prey Base

To examine the prey base of the subterranean environment, drift net samples were taken back to the lab and identified. Future samples will be used to calculate prey density per hour. These estimates will be compared to other sites where *Eurycea* salamanders are present at. Initially, the prey base of these springs within the study area appear to be robust and diverse, due to the amount of inverts collected given the time the net has been on the spring. Many of the species collected are known from the Edwards Aquifer area, however, few of the species may be new to science (Figure 7A and 7F). For example, the predatory diving beetle collected at Robertson springs is definitely new (Figure 7A). In addition, a potentially new species of *Phreatodrobia* (Hydrobiidae) has been collected (Pers comm. Dr. Hershler). Range extensions for other Hydrobiidae species include *Phreatoceras taylori* (previously only recorded from Real county), *Phreatodrobia micra* (previously only recorded from Hays, Comal, and Kendall counties) (Figure 7B). In addition, other troglobites have been recorded, alluding to open areas within this cave system (Figure 7C; 7D; 7E). *Myrmecodesmus reddelli* (Figure 7D) is one of those species and has only been recorded from Bexar, Kendall, and Guadalupe counties. A full list of prey items is listed in Table A2.

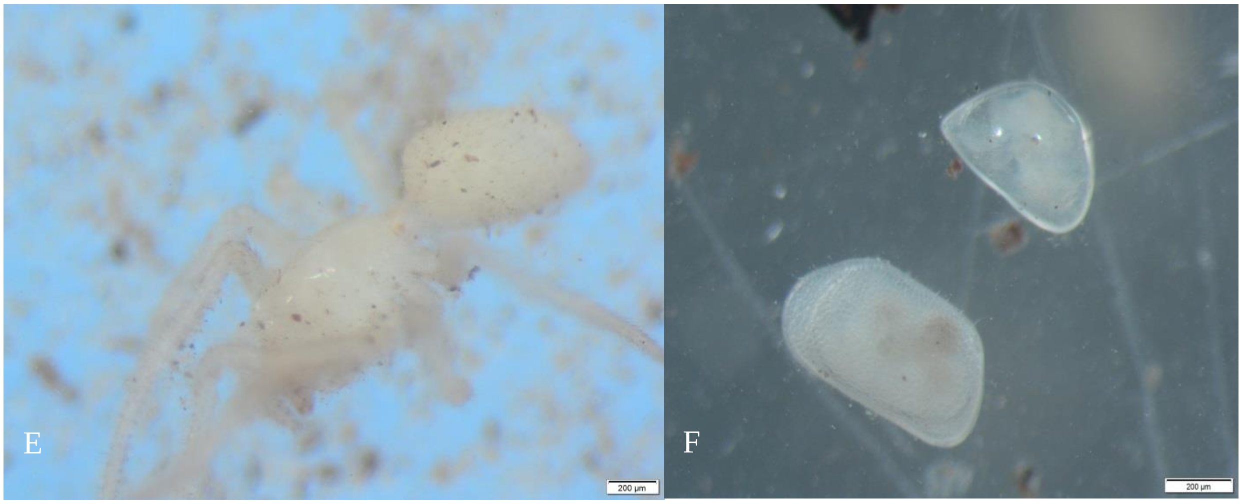


Figure 7. Invertebrates captured from drift nets at Robertson springs.

## Discussion

The Salado salamander appears to have survived the recent drought. Certain aspects of *Eurycea* the life history such as cryptic behavior, generalist predation (Diaz et al. 2010), laying of eggs within submerged habitat (Fries 2002), and the ability to reenter the aquifer (Bendik and Gluesenkamp 2012), have allowed them to persist. Although the duration of the recent drought was not as long as the 1950's drought (Figure 8), there are more anthropogenic stressors present within the landscape at present. These stressors may exacerbate the effects of the drought and potentially cause genetic shifts within local populations.

While these salamanders persist within the area, the lack of adults present within the populated sites is disconcerting. Sites within the downtown spring complex are subject to many types of disturbance (natural and unnatural) and with a high frequency of occurrence during wet years. Something like the Intermediate Disturbance Hypothesis (Connel 1978) may explain the lack of large surface populations or adults within these springs, however, the lack of adults at Robertson spring compared to other known localities for the Salado salamander (Cowen, Twin Springs, Solana Ranch) may highlight the lack of viable habitat for the completion of all life stages at these sites. Removing the beaver dam at Robertson spring is underway and may provide more insight within the next year at that site. Another hypothesis acknowledges that these sites are on the edge of known *Eurycea* distribution in Texas. Coupled with the recent information about how often the surface population receives new individuals (~1 per 30 days) could account for the small surface population densities.

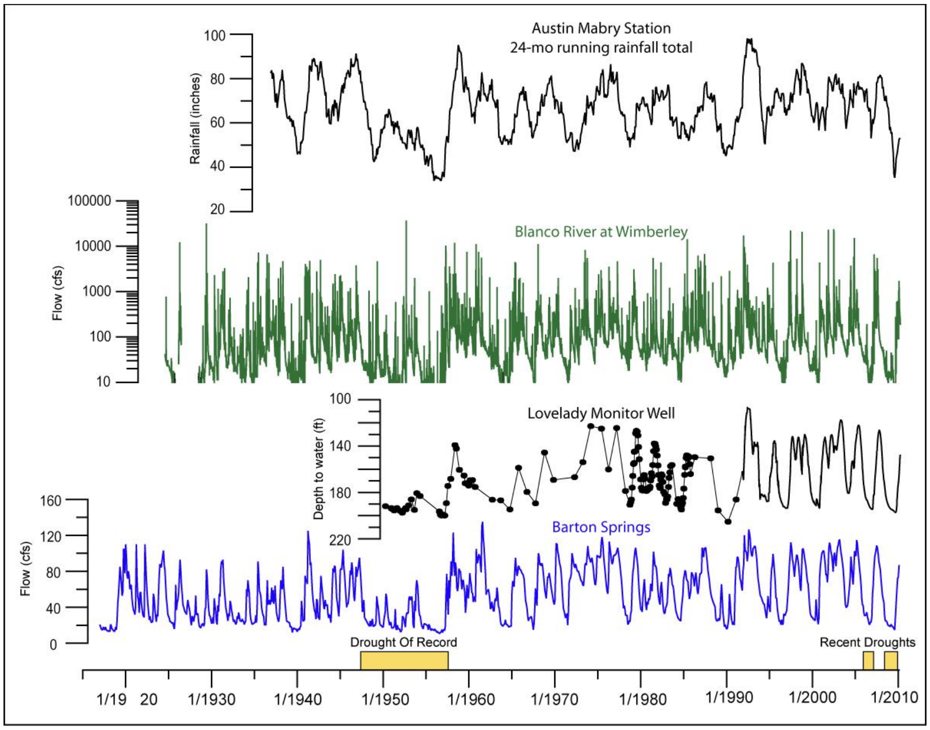


Figure 8. Graphs showing the drought of record in the 1950's and the recent drought. Taken from Smith and Hunt (2010).

Recent work by Hillis et al. 2015 has shown that the Salado salamander is present within Williamson County (*In press* Hillis et al.; Figure 9). These new findings double the known localities for this species, and allow for comparison between this study site and of sites with larger populations. The Twin Springs population size has been estimated to be around 119 (Pierce et al. 2014). Based on some of this work, it could be assumed that the Salado populations at both Robertson and Big Boiling are smaller than the Twin Springs sites (e.g. Robertson N < 119). The second assumption is that these salamanders just haven't been detected at the sites. This second assumption has a low probability of reliability due to the recent surveys at these sites.

Future efforts will include the monitoring of the habitat within Robertson and quantification of the substrate and aquatic vegetation due to the beaver dam removal. More sampling of the orifices will be done to examine the microhabitat of spring orifice associations with salamander presence. Data collection will shift from transect surveys to quadrat surveys with a focus on habitat associations within each site. Thought should be given to a genetic study of the Salado salamanders at each site and their contributions to the species overall. In addition, population estimates at each site could be done with this type of genetic work similar to Lucas et al. 2009.  Finally, another year of data from the semipermeable membrane devices may be useful due to the variation seen within the current dataset.

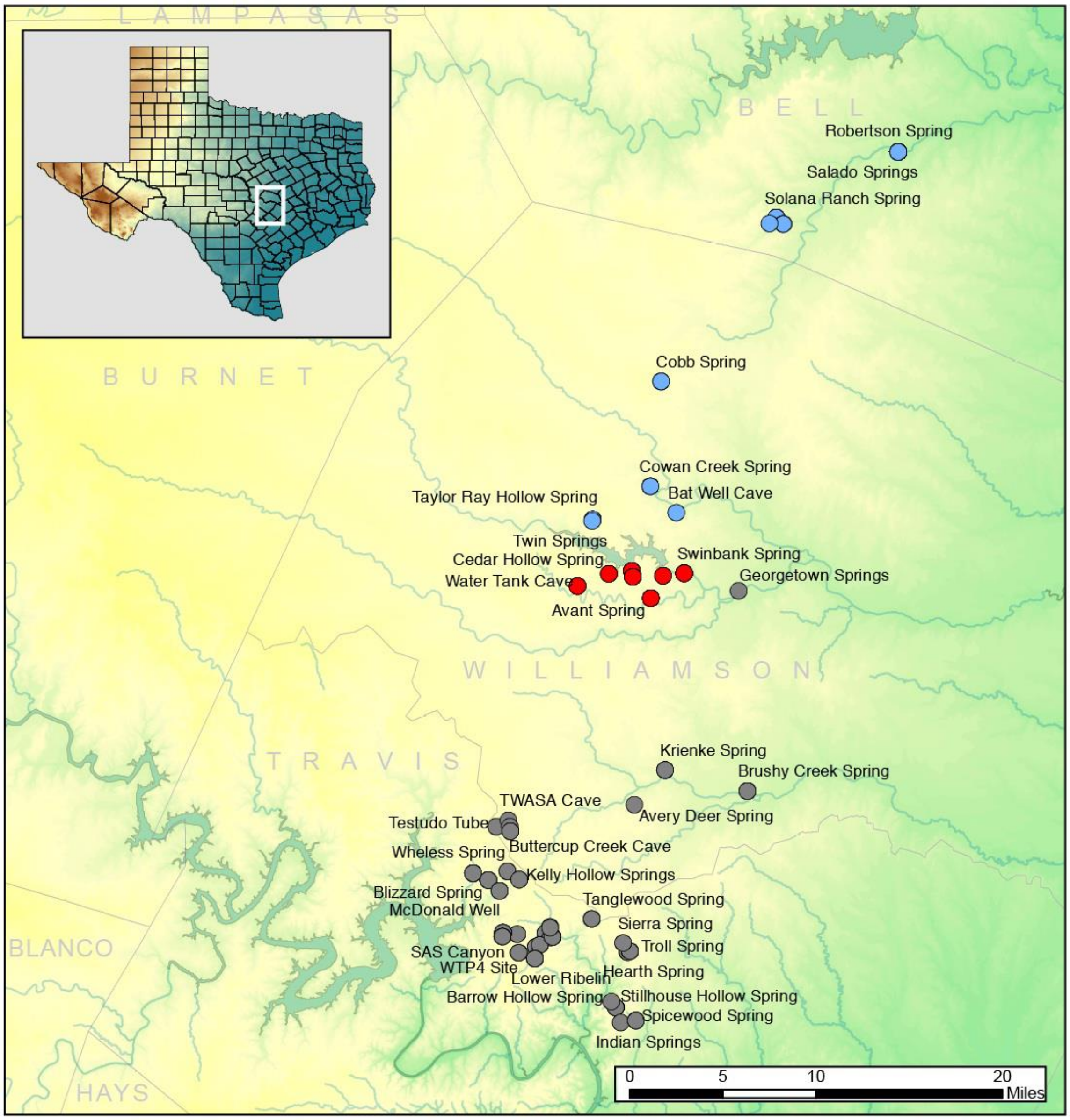


Figure 9.  Map from Hillis et al. 2015 final report from a section six grant showing the newly revised Eurycea species distribution for the three recently listed species.

## Acknowledgments

Thanks goes to Chris Harper (Austin Ecological Field Service) and Brendan Witt (Partners for Fish and Wildlife) for help with sampling and habitat restoration at the site.

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

# Appendix

A1: List of contaminants from 2015 sampling season

| CERC Site # | | | Site 1 | Site 2 |
|---|---|---|---|---|
| Site Identification | MDL | MQL | Stagecoach Inn Cave | Robertson #2 |
| **Organochlorine Pesticides** | pg/L | pg/L | pg/L | pg/L |
| Trifluralin | 0.14 | 12 | **14** | **24** |
| Hexachlorobenzene (HCB) | 0.51 | 2.6 | <0.51 [b] | <0.51 |
| Pentachloroanisole (PCA) | 0.53 | 2.6 | <0.53 | <0.53 |
| Tefluthrin | 0.93 | 4.6 | <0.93 | <0.93 |
| a-Benzenehexachloride (a-BHC) | 4.7 | 23 | <4.7 | <4.7 |
| Lindane | 6.8 | 34 | <6.8 | <6.8 |
| b-Benzenehexachloride (b-BHC) | 4.7 | 23 | <4.7 | <4.7 |
| Heptachlor | 0.59 | 2.9 | <0.59 | <0.59 |
| d-Benzenehexachloride (d-BHC) | 2.5 | 13 | <2.5 | <2.5 |
| Dacthal | 1.9 | 9.5 | **13** | *5.5* |
| Chlorpyrifos | 0.52 | 57 | **34** | **270** |
| Oxychlordane | 0.53 | 2.6 | *1.0* | **210** |
| Heptachlor Epoxide | 1.2 | 6.0 | **8.1** | <1.2 |
| trans-Chlordane | 0.54 | 2.7 | *2.1* | **11** |
| trans-Nonachlor | 0.89 | 2.9 | **4.7** | **27** |
| o,p'-DDE | 0.52 | 2.6 | <0.52 | <0.52 |
| cis-Chlordane | 0.54 | 2.7 | <0.54 | <0.54 |
| Endosulfan | 22 | 110 | <22 | <22 |
| p,p'-DDE | 0.55 | 2.7 | <0.55 | <0.55 |
| Dieldrin | 1.0 | 5.2 | *1.4* | <1.0 |
| o,p'-DDD | 0.54 | 2.7 | 0 | **17** |
| Endrin | 1.0 | 5.0 | **9.7** | **46** |
| cis-Nonachlor | 0.56 | 2.8 | *1.5* | **5.1** |
| o,p'-DDT | 0.52 | 3.0 | **3.9** | **4.4** |
| p,p'-DDD | 0.51 | 2.6 | <0.51 | <0.51 |
| Endosulfan-II | 46 | 230 | **240** | <46 |
| p,p'-DDT | 0.53 | 4.1 | **6.2** | **8.1** |
| Endosulfan Sulfate | 32 | 160 | <32 | <32 |
| Methoxychlor | 10 | 52 | <10 | <10 |
| Mirex | 0.77 | 3.8 | <0.77 | <0.77 |
| cis-Permethrin | 3.8 | 19 | <3.8 | <3.8 |

| | | | | |
|---|---|---|---|---|
| trans-Permethrin | 1.6 | 8.2 | <1.5 | <1.5 |
| **PCBs** | | | | |
| Total PCBs | 120 | 590 | <120 | <120 |
| **PBDEs** | | | | |
| PBDE-28 | 0.52 | 2.6 | **3.4** | **7.3** |
| PBDE-47 | 0.72 | 33 | *30* | **120** |
| PBDE-66 | 0.72 | 3.6 | *1.5* | **4.8** |
| PBDE-85 | 1.3 | 10 | *6.7* | **48** |
| PBDE-99 | 1.3 | 37 | *35* | **120** |
| PBDE-100 | 1.3 | 6.9 | *5.7* | **21** |
| PBDE-153 | 2.6 | 47 | *16* | **250** |
| PBDE-154 | 2.6 | 24 | *13* | **47** |
| PBDE-183 | 4.9 | 25 | **51** | **280** |
| **PAHs** | pg/L | pg/L | pg/L | pg/L |
| Naphthalene | 140 | 680 | <140 [a] | <140 |
| Acenaphthylene | 28 | 140 | <28 | <28 |
| Acenaphthene | 21 | 100 | <21 | <21 |
| Fluorene | 15 | 75 | <15 | <15 |
| Phenanthrene | 13 | 98 | <13 | *29* |
| Anthracene | 11 | 57 | <11 | <11 |
| Fluoranthene | 5.8 | 32 | *5.8* | *21* |
| Pyrene | 5.6 | 33 | *7.0* | *23* |
| Benz[a]anthracene | 5.2 | 26 | <5.2 | <5.2 |
| Chrysene | 5.2 | 26 | <5.2 | **27** |
| Benzo[b]fluoranthene | 5.1 | 26 | <5.1 | <5.1 |
| Benzo[k]fluoranthene | 5.7 | 28 | <5.7 | <5.7 |
| Benzo[a]pyrene | 5.9 | 29 | <5.9 | **60** |
| Indeno[1,2,3-c,d]pyrene | 7.1 | 35 | <7.1 | <7.1 |
| Dibenz[a,h]anthracene | 6.3 | 32 | <6.3 | <6.3 |
| Benzo[g,h,i]perylene | 7.7 | 39 | <7.7 | <7.7 |
| Benzo[b]thiophene | 530 | 2600 | <530 | <530 |
| 2-methylnaphthalene | 47 | 230 | <47 | <47 |
| 1-methylnaphthalene | 47 | 230 | <47 | <47 |
| Biphenyl | 42 | 210 | <42 | <42 |
| 1-ethylnaphthalene | 15 | 74 | <15 | <15 |
| 1,2-dimethylnaphthalene | 19 | 95 | <19 | <19 |
| 4-methylbiphenyl | 17 | 87 | <17 | <17 |
| 2,3,5-trimethylnaphthalene | 7.3 | 36 | <7.3 | <7.3 |
| 1-methylfluorene | 6.8 | 34 | <6.8 | <6.8 |
| Dibenzothiophene | 15 | 75 | <15 | <15 |
| 2-methylphenanthrene | 7.4 | 37 | <7.4 | <7.4 |

| | | | | |
|---|---|---|---|---|
| 9-methylanthracene | 6.3 | 32 | <6.3 | <6.3 |
| 3,6-dimethylphenanthrene | 5.3 | 27 | <5.3 | <5.3 |
| 2-methylfluoranthene | 5.3 | 26 | <5.3 | <5.3 |
| Benzo[b]naphtho[2,1-d]thiophene | 5.5 | 27 | <5.3 | <5.3 |
| Benzo[e]pyrene | 6.0 | 30 | <6.0 | <6.0 |
| Perylene | 5.4 | 27 | <5.4 | **37** |

Table A2. Potential prey items collected from drift nets.

| Potential Prey Items<br>Order | Family | Genus | Robertson Springs | Big Boiling Spring |
|---|---|---|---|---|
| **Trichoptera** | **Polycentropodidae** | ***Polycentropus sp.*** | X | |
| **Trichoptera** | **Heliocopsychidae** | ***Heliocopsyche sp.*** | | X |
| **Coleoptera** | **Elmidae** | ***Microcylloepus sp.*** | X | X |
| **Coleoptera** | **Elmidae** | ***Stenelmis sp.*** | X | |
| **Coleoptera** | **Dytiscidae** | ***Sanfilippodytes sp.*** | X | |
| **Coleoptera** | **Dytiscidae** | **Blind Hydroporinae** | X | |
| **Polydesmida** | **Pyrgodesmidae** | ***Myrmecodesmus reddelli*** | X | |
| **Blind Collembola** | | | | X |
| **Blind Dipluran** | | | | X |
| **Isopoda** | **Asellidae** | ***Lirceolus sp.*** | X | X |
| **Isopoda** | **Asellidae** | ***Caecidotea reddeli*** | X | X |
| **Bathynellacea** | **Parabathynellidae** | ***Texanobathynella*** | | X |
| **Amphipoda** | **Crangonyctidae** | ***Stygobromus russeli*** | X | X |
| **Amphipoda** | **Crangonyctidae** | ***Stygobromus bifricatus*** | X | X |
| **Amphipoda** | **Crangonyctidae** | ***Stygobromus n. sp.*** | X | X |
| **Amphipoda** | **Bogidiellidae** | ***Parabogidiella americana*** | | X |
| **Nymphophilinae** | **Hydrobiidae** | ***Phreatoceras taylori*** | X | X |
| **Nymphophilinae** | **Hydrobiidae** | ***Phreatodrobia micra*** | X | |
| **Subterranean Ostracoda** | | | X | |
| **Cyclopoid** | | | X | |
| **Annelida** | | | X | |

# Final Report
# 2016

# Salado Salamander Monitoring Final Report 2016


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Figure 1. Flowing water, cobble substrates and the aquatic plant, *Justicia americana* in the Robertson spring run.

## Summary

The Texas Fish and Wildlife Conservation Office (TXFWCO) completed the 2016 monitoring at the Salado Spring Complex and the Robertson Springs in Bell County under federal permit TE676811-9 and state permit SPR-0111-003. The TXFWCO created a map of the springs in early 2016 as basis to conduct surveys in a stratified random fashion. Surveys for salamanders were completed in January, April, June, September and October. Transect surveys, time search surveys, and quadrat surveys were completed this year to examine different sampling techniques and their efficiency and productivity. A total of 20 visits were conducted in 2016. A total of 27 salamanders, 11 juveniles (< 30 mm) and 16 adults, were collected and documented. Only seven salamanders (one adult) were captured in 2015.

Data collected from the different types of surveys at Robertson Springs clearly show that timed searches provided more detections of salamanders, likely due to low salamander population densities present at the sites. However, the quadrat searches provide valuable information as to available habitat, water chemistry differences at each spring site as well as between the spring run and spring source sites. In addition, drift netting at certain springs was productive in capturing salamanders without large amounts of personnel time.

A total of 34 salamanders were captured from the all combined sampling events. A modified Wentworth scale was used to describe substrate. The designation of “cave conduit” was applied to salamanders caught within a drift net. The two dominant substrates were gravel and “cave conduit” with 19 and 11 occurrences respectively (Table 2). These are some of the first captures of Salado salamanders from cave conduit type areas. The drift net captures are strong evidence for the presence of a large proportion of the Salado salamander population being present subsurface. Salado salamanders are more often captured in the lower section, particularly *Ludwigia* spring (subset of Robertson Springs), suggesting a more stable hydroperiod (duration a body of water has water present) for the lower springs compared to the upper section of the spring run which drys out periodically.

The goals for 2017 are: to continue habitat association surveys using quadrats surveys for the salamanders, begin work on a refugium within the downtown complex, collect genetic material when possible from the downtown spring complex, and continue to explore different methods to capture salamanders at different locations. The creek was not examined for potential transient or permanent resident salamanders in 2016, but will be attempted in 2017.

## Methods

At the beginning of the year, a map was made of the survey areas in order to facilitate random stratified sampling of the spring run at Robertson Springs. Data was collected on a Trimble Nomad with an XT Pro receiver. This data was then post processed using Pathfinder Office. Accuracy of collected data points is presented in Table 2. Data was collected in WGS 1984 datum. Primary and secondary substrates were categorized using a modified Wentworth scale (Table 1). Flow was assessed and given a categorical value ranging from one to four. All data collected is presented in Table 3.

Table 1. Modified Wentworth scale used to quantify substrates at spring opening during the mapping event.

| Code | Classification | Size (mm) |
|---|---|---|
| 0 | Organics | Organic Debris |
| 1 | Clay | <0.004 |
| 2 | Silt | 0.004 - 0.062 |
| 3 | Sand | 0.062 - 1.0 |
| 4 | Course Sand | 1.0 - 2.0 |
| 5 | Very Small Gravel | 2.0 - 4.0 |
| 6 | Small Gravel | 4.0 - 8.0 |
| 7 | Medium Gravel | 8.0 - 16.0 |
| 8 | Large Gravel | 16 -32 |
| 9 | Rubble | 32 - 64 |
| 10 | Small Cobble | 64 - 128 |
| 11 | Large Cobble | 128 - 256 |
| 12 | Small Boulder | 256 - 512 |
| 13 | Medium Boulder | 512 - 1024 |
| 14 | Large Boulder | >1024 |
| 15 | Bedrock | Solid Substrate |

In order to determine the efficiency and productivity of each method, sampling for Salado salamanders was conducted using three different methods: transect surveys, timed searches, and quadrat sampling in conjunction with drift netting orifices along the springs runs during all methods. Timed searches were conducted with at least three people for a minimum of 30 minutes, providing a total of ~1.5 people hours. The timed searches were conducted at Big Boiling, Anderson / Benedict, and at Robertson springs. Surveys at Critchfield Spring and the side spring were conducted using either mesohabitat or surveying the entire area, respectively.

Due to the higher probability of encountering salamanders (based on previous data), only quadrat and transect surveys were conducted at Robertson Springs. Transect surveys were conducted in the same method as the previous year, by running a meter tape and sampling along the tape every x number of meters (dependent upon site) and then sampling across the entire stretch of the transect from bank to bank. Quadrat surveys were conducted using a random stratified design and a ½ meter quadrat. The spring run was divided into spring areas and run (or mixed zone) areas. A spring area was defined as the area where the water emerging from the orifice does not mix with the spring run water. Spring areas were identified on the map and their areas were quantified. A total of 36 quadrats were sampled from the spring areas. The amount of effort expended per spring was derived from the area of the spring, therefore, springs with a larger wetted area were surveyed more often. In addition, ten surveys were added to the spring run to examine differences between spring areas and the spring run with respect to habitat and water chemistry. At each quadrat depth, flow, temperature, conductivity, pH, dissolved oxygen, total dissolved solids, substrate, and percent vegetation were recorded. Data collected from quadrat surveys were z-scored and analyzed using principal component analysis in R using the “princomp” function. Associated with the quadrat search discharge was measured at the outflow of the spring run. Discharge was collected by dividing the outflow area by 25 and then taking a reading evenly across the mouth of the outflow.

If a salamander was found, it was photographed and returned to the area where captured. All salamanders captured were reported to Texas Parks and Wildlife Department in the Texas Natural Diversity Database, allowing for the capture to be recorded and the data made available for other researchers or studies. All measurements were acquired using Image J software. Additionally, The software Wild ID was used to determine if any salamanders were recaptures.

The passive sampling is an important component for the monitoring of these salamanders due to the small surface population present at most sites. Drift nets were placed over the spring orifice (Figure 2), left in place and checked weekly. Nets were set on October 26, 2016 at Anderson and Beetle springs at the Robertson property and left indefinitely to collect salamanders for genetic material. When the nets were examined, the entire sample was stored in 95% EtOH and taken back to the lab where the contents were sorted and enumerated under a compound microscope. Rates for salamanders and prey densities were calculated as *x* per day.

Following the removal of a beaver dam during late 2015and into early 2016, available habitat was quantified by gridding out the spring run. Habitat was measured by running meter tape along the length of the spring run for 100 meters. At every five meters, transects were sampled and quantified using a 1/3 $m^2$ quadrat. Substrate was identified along transect within the first quadrat (0-0.3m) and then alternated every 0.3 m thereafter. A minimum of 165 measurements of substrates were made for each available habitat determination.

Figure 2. Drift nets placed on spring orifices to collect salamanders and examine prey densities

Figure 2.  Drift nets placed on spring orifices to collect salamanders and examine prey densities

Water quality data was collected at each site during the course of a survey using a Hydrotech compact DS 5 meter. Water quality measurements were collected from each spring and averaged for each site. Measured parameters included: temperature, dissolved oxygen, pH, conductivity, and total dissolved solids.  To examine contaminant loads present at Robertson Springs and the downtown complex, passive samplers were used collect data.  The samplers collect organochlorines, polybrominated diphenyl ethers, polychlorinated biphenyl, and polycyclic aromatic hydrocarbons.  These samplers were left in place for 34 (Robertson Sp.) and 37 (Stagecoach Inn cave) days.

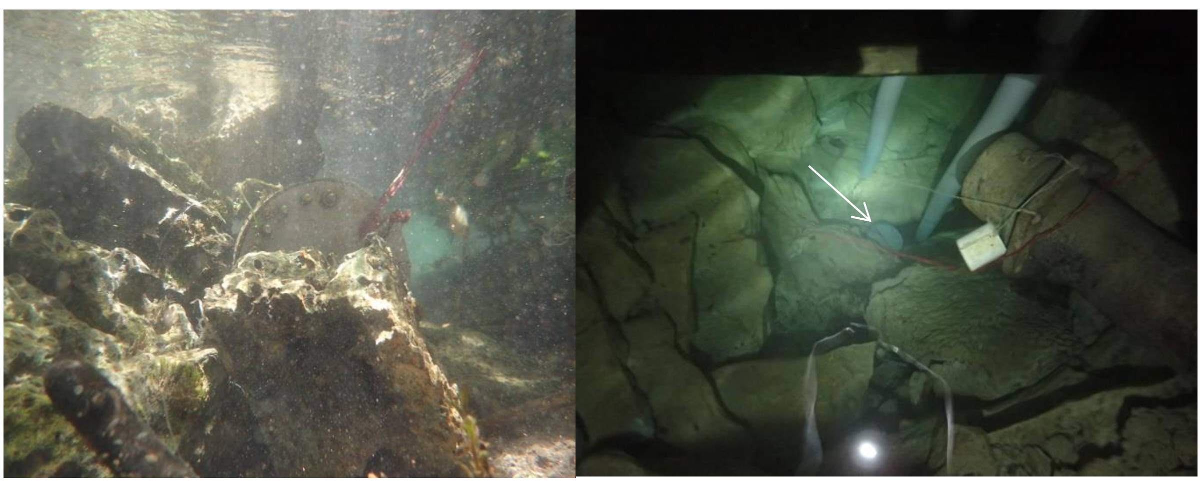
Figure 3.  Passive water samplers left in place at Robertson Spring and Stagecoach Inn cave to collect contaminants.

## Results

Mapping

Mapping of Robertson Springs was conducted on February 4th 2016, and 31 spring openings were identified (Figure 4). There were three types of spring openings present: seeps (alluvial), orifice and upwellings. The orifice type were the most common. The most common substrates encountered at spring orifices were gravel (n = 18) followed by silt (n = 9). Following post processing, the GIS data was most in the 0.5-1 m range for the accuracy of collected spatial data (Table 2).

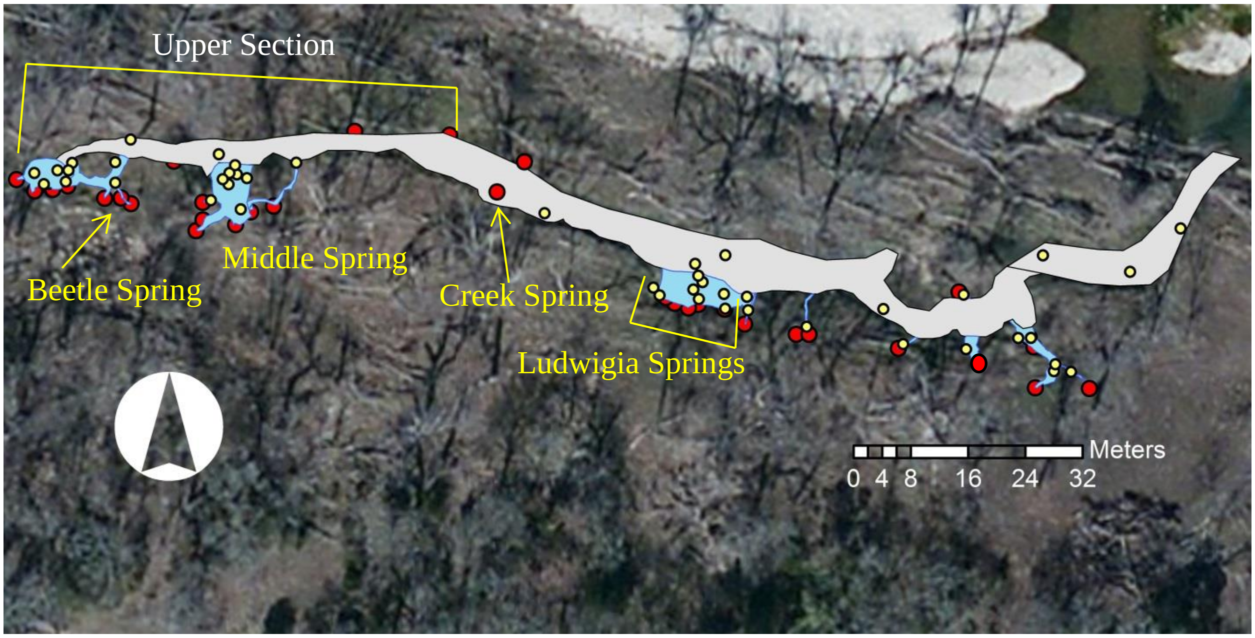


Figure 4. Map of Robertson Springs showing spring areas and spring openings. Red dots are spring openings. Yellow dots are quadrat sites. Light blue areas are spring areas. The purple is the spring run.

Table 2. Output from Pathfinder Office following post processing of data collected from February 4th 2016 at Robertson Springs. Showing accuracy of spatial data collected.

| Range | Percentage |
|---|---|
| 0-5cm | - |
| 5-15cm | - |
| 15-30cm | 3.53% |
| 30-50cm | 33.27% |
| 0.5-1m | 41.54% |
| 1-2m | 20.58% |
| 2-5m | 1.08% |
| >5m | - |

Table 3. Data from Robertson Springs mapping event on February 4th 2016.

| Number | Location | Spring Type | Primary Substrate | Secondary Substrate | Flow |
|---|---|---|---|---|---|
| **1** | Robertson | orifice | 2 silt | 5 gravel very small | 1 |
| **2** | Robertson | orifice | 2 silt | 1 clay | 1 |
| **3** | Robertson | orifice | 2 silt | 1 clay | 2 |
| **4** | Robertson | orifice | 6 gravel small | 8 gravel large | 3 |
| **5** | Robertson | orifice | 6 gravel small | 1 clay | 3 |
| **6** | Robertson | orifice | 6 gravel small | 5 gravel very small | 2 |
| **7** | Robertson | orifice | 6 gravel small | 5 gravel very small | 2 |
| **8** | Robertson | orifice | 5 gravel very small | 7 gravel medium | 3 |
| **9** | Robertson | orifice | 5 gravel very small | 7 gravel medium | 2 |
| **10** | Robertson | orifice | 5 gravel very small | 7 gravel medium | 2 |
| **11** | Robertson | orifice | 5 gravel very small | 7 gravel medium | 1 |
| **12** | Robertson | orifice | 5 gravel very small | 7 gravel medium | 3 |
| **13** | Robertson | upwelling | 5 gravel very small | 3 sand | 4 |
| **14** | Robertson | upwelling | 2 silt | 3 sand | 2 |
| **15** | Robertson | alluvial | 2 silt | 1 clay | 1 |
| **16** | Robertson | alluvial | 2 silt | 0 organics | 1 |
| **17** | Robertson | upwelling | 8 gravel large | 5 gravel very small | 1 |
| **18** | Robertson | orifice | 5 gravel very small | 6 gravel small | 1 |
| **19** | Robertson | orifice | 0 organics | 2 silt | 1 |
| **20** | Robertson | seep | 0 organics | 2 silt | 1 |
| **21** | Robertson | seep | 0 organics | 2 silt | 1 |
| **22** | Robertson | upwelling | 7 gravel medium | 10 cobble small | 4 |
| **23** | Robertson | orifice | 2 silt | 6 gravel small | 2 |
| **24** | Robertson | orifice | 2 silt | 5 gravel very small | 2 |
| **25** | Robertson | orifice | 2 silt | 5 gravel very small | 2 |
| **26** | Robertson | orifice | 7 gravel medium | 10 cobble small | 3 |
| **27** | Robertson | orifice | 7 gravel medium | 10 cobble small | 3 |
| **28** | Robertson | orifice | 7 gravel medium | 10 cobble small | 2 |
| **29** | Robertson | seep | 7 gravel medium | 10 cobble small | 2 |
| **30** | Robertson | orifice | 7 gravel medium | 5 gravel very small | 3 |
| **31** | Robertson | orifice | 7 gravel medium | 5 gravel very small | 4 |

Salamanders

Downtown Spring Complex

No salamanders were captured this year at the downtown spring complex either by active searching or by passive sampling. The drift net on Anderson Spring was set on October 26, 2016 and has not been disturbed, however, it has not captured any salamanders.

Table 4. Dates and time searched for Big Boiling, Anderson/Benedict, Critchfiled and the side springs.

| | **Big Boiling** | **Anderson/Benedict** | **Critchfield** | **Side Spring** |
|---|---|---|---|---|
| **Date** | Minutes | Minutes | Mesohabitat | All |
| **4/14/2016** | 120 | 150 | | |
| **6/7/2016** | 80 | 150 | Searched | Searched |
| **6/27/2016** | 75 | - | | |
| **9/7/2016** | 120 | 150 | Searched | Searched |
| **10/26/2016** | 90 | 150 | | Searched |
| | | | | |
| **Salamanders** | 0 | 0 | 0 | 0 |

*Robertson*

Neither transect nor quadrat surveys at Robertson Springs detected salamanders, though timed surveys and passive sampling with drift nets was successful at this site (Table 5). A total of 27 salamanders were collected and documented (Table 6). There were 11 juveniles (< 30 mm) and 16 adults captured in 2016. Only seven salamanders were captured (one adult) in 2015. The dominant substrate at the sites of salamander collection consisted of gravel and "cave conduit" (drift nets). Salamanders collected on the surface tended to be associated with watercress (*Nasturtium* sp.). Fifteen salamanders out of the 27 were collected within this vegetation.

Although the quadrat sampling did not result in the capture of any salamanders, the benefits were seen from a statistical point of view due to the random stratified sampling design regarding abiotic parameters (substrate, depth, flow, etc). Results from the first quadat event are not shown since it was not stratified, just a random sample of the entire system. Distinctions were observed between the spring run and the spring areas and between sampling events in June and September of 2016 (Figure 5A). The discharge for in June was 6.15 m/sec and for September, 4.67 m/sec. The principal component analysis explains the mechanisms for the separation of these mesohabitats. The analysis explains 57% of the variance by principal component axis two (PCII) (Table 8). Principal component axis one (PC I) explains 35% of the variance and PC II explains the other 22%. Principal component axis I has a gradient from negative loadings for conductivity, total dissolved solids and pH to positive loadings for flow (although very low loading for flow). Principal component axis II has a gradient from negative loadings for flow and pH to positive loadings for vegetation and temperature. Therefore, sites along the PC I axis that are on the left side (negative) of the axis have lower conductivity, pH, total dissolved solids and lower flow than sites present on the right side (positive) of PC I. Although other parameters were measured such as mud and silt these parameters were not significant enough to have loadings on either the first and second PC axis (Table 7). The changes in discharge may explain the separation of sites by time period (Figure 5B).

Table 5. Sampling events at Robertson springs.

| Date | Survey Type | Time (min) | Salamanders |
|---|---|---|---|
| **1/29/2016** | Transect | - | 0 |
| **2/4/2016** | Timed | 120 | 2 |
| **2/4/2016** | Drift Net | 2280 | 1 |
| **2/26/2016** | Transect | - | 0 |
| **3/1/2016** | Drift Net | 10080 | 3 |
| **3/1/2016** | Timed | 165 | 1 |
| **3/24/2016** | Quadrat | - | 0 |
| **3/24/2016** | Timed | 150 | 6 |
| **3/31/2016** | Drift Net | 10080 | 3 |
| **6/7/2016** | Quadrat | - | 0 |
| **6/7/2016** | Timed | 150 | 2 |
| **8/4/2016** | Drift Net | 7200 | 0 |
| **8/12/2016** | Drift Net | 4320 | 1 |
| **8/18/2016** | Drift Net | 8640 | 1 |
| **9/8/2016** | Quadrat | - | 0 |
| **10/27/2016** | Timed | 180 | 1 |

Table 6. Salamanders captured from 2016 sampling events.

| Location | Date | Location | Size (mm) | Primary Substrate | Secondary Substrate | Vegetation |
|---|---|---|---|---|---|---|
| **Robertson Spring** | 2/4/2016 | 130 meters | 14 | Silt | Silt | Sagittaria |
| **Robertson Spring** | 2/4/2016 | Ludwigia Sp | 16 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 2/4/2016 | Ludwigia Sp | 31 | Gravel | Sand | Watercress |
| **Robertson Spring** | 3/1/2016 | Ludwigia Sp | 55.5 | Gravel | Gravel | - |
| **Robertson Spring** | 3/1/2016 | Creek Spring | 43.6 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 3/1/2016 | Creek Spring | 42.2 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 3/1/2016 | Creek Spring | 11.72 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 3/24/2016 | Ludwigia Sp | 23.15 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/24/2016 | Ludwigia Sp | 30.93 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/24/2016 | Ludwigia Sp | 22.95 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/24/2016 | Ludwigia Sp | 18.29 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/24/2016 | Ludwigia Sp | 18.67 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/31/2016 | Ludwigia Sp | 33.28 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/31/2016 | Ludwigia Sp | 20.09 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/31/2016 | Ludwigia Sp | 37.16 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 3/31/2016 | Beetle Spring | 17.22 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 3/31/2016 | Creek Spring | 32.23 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 3/31/2016 | Ludwigia Upper | 49.91 | Cave Conduit | Cave Conduit | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Robertson Spring** | 6/7/2016 | Ludwigia Sp | 12.69 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 6/7/2016 | Ludwigia Sp | 33.84 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 8/9/2016 | Ludwigia Sp | 21.61 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 8/9/2016 | Ludwigia Sp | 37.55 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 8/9/2016 | Ludwigia Sp | 40 | Gravel | Gravel | Watercress |
| **Robertson Spring** | 8/12/2016 | Ludwigia Sp | 60.4 | Boulder | Gravel | - |
| **Robertson Spring** | 8/12/2016 | Ludwigia Upper | 53.72 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 8/18/2016 | Creek Spring | 35 | Cave Conduit | Cave Conduit | - |
| **Robertson Spring** | 10/27/2016 | Ludwigia Sp | 25.05 | Gravel | Gravel | Watercress |

Table 7. Loadings from principal component analysis taken from quadrat sampling from Robertson Springs examining the spring run and spring areas.

| | PC I | PC II |
|---|---|---|
| Temp | -0.322 | 0.372 |
| DO | | 0.395 |
| cond | -0.538 | -0.189 |
| ph | -0.495 | -0.205 |
| tds | -0.538 | -0.188 |
| Mud/silt | | 0.132 |
| Sand | | |
| Gravel | | |
| Cobble | 0.125 | |
| Bedrock | | |
| Vegetation | -0.146 | 0.394 |
| Depth | | 0.483 |
| Flow | 0.112 | -0.415 |

Table 8. Proportion of variance explained by principal component analysis from random stratified sampling of mesohabitats along Robertson Springs.

| | PC I | PC II |
|---|---|---|
| Standard deviation | 1.73 | 1.37 |
| Proportion of Variance | 0.35 | 0.22 |
| Cumulative Proportion | 0.35 | 0.57 |

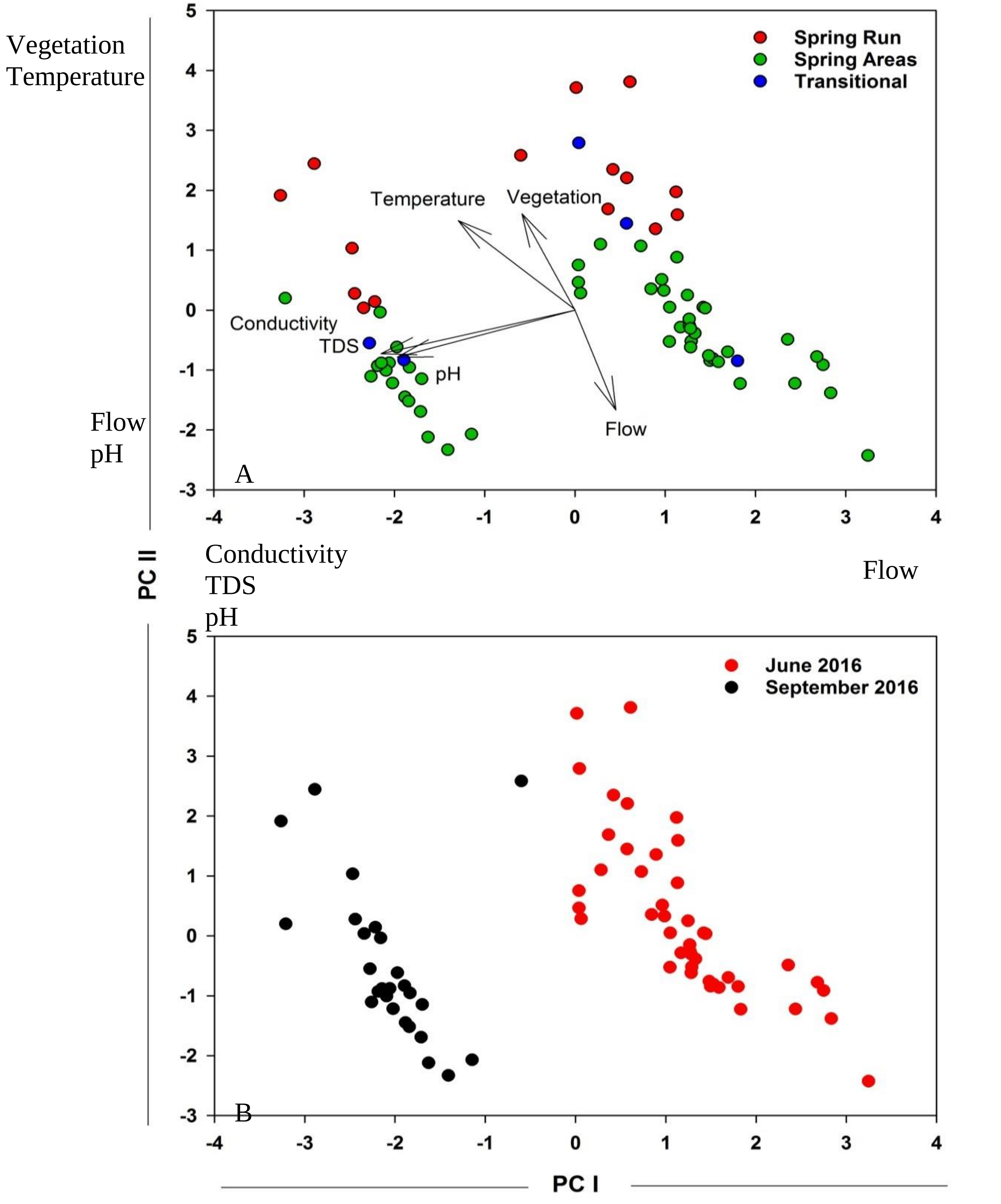


Figure 5. Principal component analysis results from stratified random sampling using quadrats along Robertson Springs run and spring areas from June and September 2016. Figure 5A shows the results of the PCA and figure 5B shows the separation of sites by sampling event.

Habitat Availability

The habitat at Robertson Springs was the primary focus in 2016 (Figure 6). Habitat was quantified four times during the year (Table 9). The substrates present seem to be reaching equilibrium as shown by the last two sampling events. Sand substrates have increased fairly consistently over the course of the year. This may be due to the types of sediment being dislodged from the associated subterranean environment and drifting out of the spring sources.

Table 9. Habitat availability at Robertson springs following removal of a beaver dam.

| | 12/10/2015 | 12/17/2015 | 1/29/2016 | 2/26/2016 | 6/27/2016 | 9/21/2016 |
|---|---|---|---|---|---|---|
| **Days** | Before Dam | 7 | 21 | 77 | 189 | 273 |
| **Mud/Silt** | 92.55 | 64.36 | 68.48 | 58.56 | 55.62 | 47.93 |
| **Sand** | 0.00 | 2.48 | 6.06 | 4.42 | 7.69 | 14.79 |
| **Gravel** | 4.97 | 5.94 | 10.30 | 17.13 | 20.12 | 20.71 |
| **Cobble** | 1.24 | 3.47 | 3.03 | 4.97 | 6.51 | 3.55 |
| **Boulder** | 1.24 | 0.00 | 2.42 | 0.55 | 3.55 | 2.96 |
| **Bedrock** | 0.00 | 14.36 | 9.70 | 14.36 | 6.51 | 7.96 |
| **Sum of Rocks** | 7.45 | 9.41 | 15.76 | 22.65 | 36.69 | 35.76 |

Figure 6. Robertson Spring before and after the removal of a beaver dam.

Surface Recruitment

Drift nets were set 23 times at Robertson springs over the course of the year. Springs were divided into an upper section and a lower section. Any springs above Creek Spring were designated within the upper section (Figure 4). There were ten sampling events at the upper section and 13 in the lower section. The total average recruitment for the entire spring run was 0.078 salamanders per day. The upper section had a rate of 0.021 salamanders per day, while the lower section had 0.122 salamanders per day. Creek spring had the highest rate for any of the springs sampled (Table 10). Creek spring also was rated as the highest discharge during the mapping event.

Table 10. Salamander rates from individual springs sampled using drift nets at Robertson Springs.

| | Upper Spring | Beetle Spring | Mid Spring | Creek Spring | Ludwigia Springs--Upper | Ludwigia Springs--Mid | Ludwigia Springs--Lower |
|---|---|---|---|---|---|---|---|
| **Days Set** | 11 | 46 | 44 | 21 | 16 | 12 | 16 |
| **Number of Sallies** | 0 | 3 | 1 | 5 | 2 | 0 | 0 |
| **Rate** | 0 | 0.065 | 0.022 | 0.238 | 0.125 | 0 | 0 |

Water Quality

Water quality was measured two different ways during this study. Using the HydroTech sonde basic water quality parameters were collected at the time of each visit. The values have been averaged and are presented in Table 11. There were no values exceeding any ecological limits set by federal or state organizations taken from the dissolved oxygen, temperature, pH, conductivity or turbidity.

Table 11. Average water quality data collected over 2016.

| | Benedict/Anderson | Big Boiling | Robertson | Side Spring off Little | Critchfield |
|---|---|---|---|---|---|
| Temperature | 20.85 | 20.78 | 20.70 | 20.79 | 20.71 |
| Dissolved Oxygen | 6.92 | 7.57 | 7.40 | 7.37 | 7.31 |
| pH | 6.95 | 7.07 | 7.06 | 7.02 | 6.77 |
| Conductivity | 579.23 | 578.47 | 563.05 | 569.40 | 567.40 |
| Total Dissolved Solids | 0.36 | 0.37 | 0.36 | 0.36 | 0.36 |

The second type of water quality sampling included the placement of a semipermeable membrane device to collect contaminants at two sites (Stage Coach Inn Cave Conduit and Robertson Springs). These results are presented in Table 12. A more detail list is provided in the appendix (A1). There was an issue this year with the Robertson Springs site. The polycyclic aromatic hydrocarbons are likely degraded within this sample. If the sampler is exposed to sunlight then the more volatile chemicals have the chance to degrade either to below the detection limit, or to lower levels then present within the system. Although this may be the case, the PAHs this year were higher than any other year, and were similar between Stagecoach Inn cave and the Robertson Springs sample (426 pg/L and 470 pg/L respectively). The Stagecoach Inn cave sample was not influenced by solar degradation due to the fact that it was within a cave. Both sites sampled during 2016 had many overlapping and similar amounts of contaminants. Both samples are still within the second quartile based on the relationship between contaminant loadings and impervious cover scores from other salamander sites across the Edwards Plateau. Again this year, none of the individual contaminants detected are above any state or federal standards for freshwater.

Table 12. List of contaminants sampled for in 2014, 2015 and 2016 in the study area along with quartiles from other springs within the Edwards Aquifer Zone at salamander sites. The table is broken into two major parts. The upper section displays the number of contaminants present by category, while the lower section displays the amount within each category in pg/L.

| **Contaminant** | **Stage Coach Inn Cave 2016** | **Robertson Spring 2016** | **Stage Coach Inn Cave 2015** | **Robertson Spring 2015** | **Robertson Spring 2014** | **1st Quartile** | **2nd Quartile** | **3rd Quartile** |
|---|---|---|---|---|---|---|---|---|
| Organochlorines (#) | 11 | 8 | 13 | 11 | 5 | 4 | 7 | 11 |
| Polychlorinated biphenlys (#) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Polybrominated diphenyl ethers (#) | 5 | 6 | 9 | 9 | 0 | 0 | 0 | 1 |
| Polycyclic aromatic hydrocarbons (#) | 3 | 3 | 2 | 6 | 2 | 2 | 7 | 14.5 |
| | | | | | | | | |
| Organochlorines (pg/L) | 175.5 | 139.3 | 339.6 | 628.1 | 75.9 | 88 | 302 | 707 |
| Polybrominated diphenyl ethers (pg/L) | 102.6 | 113.9 | 162.3 | 898.1 | 0 | 0 | 0 | 15 |
| Polycyclic aromatic hydrocarbons (pg/L) | 426 | 470 | 12.8 | 197 | 324 | 321 | 1188 | 2741 |
| Impervious Cover (%) | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6 | 17 | 23 |
| | | | | | | | | |
| Total Number of Contaminants | 19 | 17 | 24 | 26 | 7 | 12 | 19 | 32 |
| Total Amount (pg/L) | 704.19 | 723.25 | 514.7 | 1723.2 | 399.9 | 208 | 563 | 2262 |

Prey Base

All invertebrates captured within the drift nets were taken back to the lab for sorting and enumeration. Most of the samples have been completed. While all orifices had the presence of stygobionts (cave adapted organisms that live in the aquatic area of caves) within the samples, Creek spring had the highest count of stygobionts within the samples. This year the blind dytiscid was sent off to Kelly Miller (University of New Mexico) for description and publication. The hope is to name the blind Dytiscidae after the matriarch of the Robertson family, Ruth. Also, the Ostracoda (seed shrimp) is in review now for publication and has been proposed as a new genus and species (*Schornikovcandona bellensis*). This species was named after a scientist who has contributed to the Ostracoda field throughout his life. This seed shrimp has also been collected at the downtown spring complex.

Figure 7. Pictures of *Schornikovcandona bellensis* (upper left) and the blind dytiscidae collected from the Salado area. The upper right and the lower left are photos of the larvae.

# Discussion

Mapping of the spring proved useful for surveys and understanding the contributions of spring orifices to the overall discharge. Mapping will be conducted again in 2017 and when changes to the system occur, such as a lowering of the water table. Throughout the year other openings appeared, however, these were low in discharge and mainly would have been designated as seeps.

The quadrat surveys will continue throughout 2017, to attempt to detect if the salamanders are colonizing the springs following the beaver dam removal. Although no salamanders were captured within the frame work of the quadrat sampling, more salamanders overall were collected at Robertson Springs. The habitat appears to be stabilizing naturally now that the beaver dam has been removed. This may provide less disturbance throughout the year within their optimal habitat.

The changes in discharge appear to have affected the distribution of sites within multivariate space (Figure 5B). It is unknown at this time how this may effect salamander densities as none were detected during either event. The discharge decreased between the two events and probably affected the flow of the springs.

At this time, Ludwigia springs seems to be the best potential surface habitat for the salamanders to colonize. Within Ludwigia springs, there are five major spring openings. The upper section of these springs have proven to be the most productive for capturing salamanders. It is unknown if these springs follow the same path underground. I would speculate that they are originated from different flow paths all discharging into Salado Creek. Monitoring of these sites will continue.

Creek spring has been the most productive when sampling with drift nets. In addition to more salamanders captured there the flow was categorized as a four, the highest rating, for Robertson during the mapping event. More stygobionts have been captured from this spring. Given the lower elevation in relationship to the other springs, this spring has the potential to be sampled during drought years with traps or nets placed within the orifice. This spring appears to be an offshoot of a larger conduit or cave system that is heading towards Salado creek.

Three years of contaminant data has been collected from Robertson and two years from Stagecoach Inn. The data has been fairly consistent and has shown the low levels of contaminants within the springs, although higher for the amount of impervious cover present. The reason for higher averages is due to more organochlorines and PBDEs present within the Salado area than other springs around the Edwards Plateau. Sampling of contaminants should be postponed until other changes within the area occur such as increases in impervious cover or an event such as a toxic spill.

Future efforts will include continued monitoring at the downtown complex and at Robertson Springs. Habitat availability and quadrat sampling will be conducted at Cowen Springs in Williamson County and compared to Robertson Springs. These sampling events will be conducted within the same week to assure similar conditions. Genetic material will continue to be collected for future population genetics. Habitat restoration will also be a major focus in the upcoming year at Critchfield Springs.

# Appendix

A1: List of contaminants from 2016 sampling season. Highlighted area may be lower than present at site due to solar degredation.

| CERC Site # | | | Site 1 | Site 2 |
|---|---|---|---|---|
| Site Identification | MDL | MQL | Stagecoach Inn Cave | Robertson #2 |
| **Organochlorine Pesticides** | pg/L | pg/L | pg/L | pg/L |
| Trifluralin | 0.10 | 0.52 | **15** [a] | **52** |
| Hexachlorobenzene (HCB) | 0.36 | 1.8 | <0.36 [b] | <0.36 |
| Pentachloroanisole (PCA) | 0.38 | 1.9 | <0.38 | *0.89* [c] |
| Tefluthrin | 0.60 | 3.0 | <0.60 | <0.60 |
| alpha-Benzenehexachloride (a-BHC) | 4.7 | 23 | <4.7 | <4.7 |
| Lindane | 6.8 | 34 | *31* | *27* |
| beta-Benzenehexachloride (b-BHC) | 4.7 | 23 | *18* | *18* |
| Heptachlor | 0.45 | 2.3 | <0.45 | <0.45 |
| delta-Benzenehexachloride (d-BHC) | 2.5 | 13 | <2.5 | <2.5 |
| Dacthal | 1.8 | 9.2 | **12** | <1.8 |
| Chlorpyrifos | 0.52 | 2.6 | **83** | **25** |
| Oxychlordane | 0.38 | 1.9 | **6.8** | *0.54* |
| Heptachlor Epoxide | 1.1 | 5.6 | <1.1 | <1.1 |
| trans-Chlordane | 0.40 | 2.0 | **5.3** | *1.5* |
| trans-Nonachlor | 0.39 | 2.0 | <0.39 | <0.39 |
| o,p'-DDE | 0.37 | 1.9 | <0.37 | <0.37 |
| cis-Chlordane | 0.40 | 2.0 | **2.3** | <0.40 |
| Endosulfan | 22 | 110 | <22 | <22 |
| p,p'-DDE | 0.37 | 1.8 | **4.9** | **6.2** |
| Dieldrin | 0.95 | 4.8 | **4.8** | <0.95 |
| o,p'-DDD | 0.36 | 1.8 | **3.9** | <0.36 |
| Endrin | 0.91 | 4.5 | <0.91 | **6.1** |
| cis-Nonachlor | 0.37 | 1.9 | <0.37 | <0.37 |
| o,p'-DDT | 0.37 | 1.9 | *0.95* | <0.37 |
| p,p'-DDD | 0.36 | 1.8 | *0.85* | <0.36 |
| Endosulfan-II | 46 | 230 | <46 | <46 |
| p,p'-DDT | 0.39 | 1.9 | *1.7* | **3.0** |
| Endosulfan Sulfate | 32 | 160 | <32 | <32 |
| p,p'-Methoxychlor | 9.4 | 17 | <9.4 | <9.4 |
| Mirex | 0.50 | 2.5 | <0.50 | <0.50 |
| cis-Permethrin | 2.5 | 12 | <2.5 | <2.5 |
| trans-Permethrin | 1.1 | 5.3 | <1.1 | <1.1 |

| | | | | |
|---|---|---|---|---|
| **PCBs** | | | | |
| Total PCBs | 79 | 390 | <79 | <79 |
| **PBDEs** | | | | |
| PBDE-28 | 0.36 | 1.8 | 0 | **2.5** |
| PBDE-47 | 0.47 | 2.3 | **8.1** | **5.7** |
| PBDE-66 | 0.47 | 2.3 | <0.47 | *0.81* |
| PBDE-85 | 0.83 | 4.2 | *1.8* | **4.4** |
| PBDE-99 | 0.83 | 4.2 | **7.1** | **4.5** |
| PBDE-100 | 0.83 | 4.2 | *0.89* | <0.83 |
| PBDE-153 | 1.7 | 8.3 | *7.8* | **17** |
| PBDE-154 | 1.7 | 8.3 | <1.7 | <1.7 |
| PBDE-183 | 3.2 | 16 | **77** | **79** |

| CERC Site # | | | Site 1 | Site 2 |
|---|---|---|---|---|
| Site Identification | MDL | MQL | Stagecoach Inn Cave | Robertson #2 |
| **PAHs** | pg/L | pg/L | pg/L | pg/L |
| Naphthalene | 140 | 680 | <140 [a] | *270* [b] |
| Acenaphthylene | 28 | 140 | <28 | <28 |
| Acenaphthene | 20 | 100 | <20 | <20 |
| Fluorene | 14 | 72 | <14 | <14 |
| Phenanthrene | 12 | 62 | <12 | <12 |
| Anthracene | 11 | 53 | <11 | <11 |
| Fluoranthene | 4.5 | 23 | <4.5 | <4.5 |
| Pyrene | 4.2 | 21 | <4.2 | <4.2 |
| Benz[a]anthracene | 3.6 | 18 | <3.6 | <3.6 |
| Chrysene | 3.7 | 18 | <3.7 | <3.7 |
| Benzo[b]fluoranthene | 3.6 | 18 | <3.6 | <3.6 |
| Benzo[k]fluoranthene | 3.7 | 19 | <3.7 | <3.7 |
| Benzo[a]pyrene | 3.9 | 20 | <3.9 | <3.9 |
| Indeno[1,2,3-cd]pyrene | 4.6 | 23 | <4.6 | <4.6 |
| Dibenzo[a,h]anthracene | 4.1 | 21 | <4.1 | <4.1 |
| Benzo[g,h,I]perylene | 5.0 | 25 | <5.0 | <5.0 |
| Benzo[b]thiophene | 530 | 2600 | <530 | <530 |
| 2-methylnaphthalene | 47 | 230 | *91* | *130* |
| 1-methylnaphthalene | 47 | 230 | *55* | *70* |
| Biphenyl | 42 | 210 | <42 | <42 |
| 1-ethylnaphthalene | 14 | 71 | <14 | <14 |
| 1,2-dimethylnaphthalene | 18 | 92 | <18 | <18 |
| 4-methylbiphenyl | 17 | 85 | **280** | **270** |
| 2,3,5-trimethylnaphthalene | 6.1 | 30 | <6.1 | <6.1 |

| | | | | |
|---|---|---|---|---|
| 1-methylfluorene | 5.6 | 28 | <5.6 | <5.6 |
| Dibenzothiophene | 14 | 72 | <14 | <14 |
| 2-methylphenanthrene | 6.2 | 31 | <6.2 | <6.2 |
| 9-methylanthracene | 5.0 | 25 | <5.0 | <5.0 |
| 3,6-dimethylphenanthrene | 3.9 | 20 | <3.9 | <3.9 |
| 2-methylfluoranthene | 3.8 | 19 | <3.8 | <3.8 |
| Benzo[b]naphtho[2,1-d]thiophene | 4.1 | 21 | <4.1 | <4.1 |
| Benzo[e]pyrene | 4.0 | 20 | <4.0 | <4.0 |
| Perylene | 3.7 | 18 | <3.7 | <3.7 |

# Final Report
# 2017

# Salado Salamander Monitoring Final Report 2017

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Texas Fish and Wildlife Conservation Office

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# Summary

The Texas Fish and Wildlife Conservation Office (TXFWCO) completed the 2017 monitoring schedule at the Salado Downtown Spring Complex and the Robertson Springs in Bell County under federal permit TE676811-9 and state permit SPR-0111-003. A total of 46 Salado salamanders were detected from Robertson Springs and the Downtown Spring Complex (DSC) in 2017. Most salamanders were captured, however, a few escaped after a visual observation before a photo or length could be taken. Of the remaining salamanders, 18 were considered adults (>30 mm) and 23 were considered juvenile. Seventeen salamanders were caught in drift nets and two salamanders were caught during the quadrat sampling. The remaining 27 were caught by actively searching. Salamanders were collected from eight different locations within the study area. The most salamanders captured this year by active searching and drift netting was from Robertson Springs, and within the Robertson Springs complex from five spring areas (Figure 1). Following Robertson Springs, Anderson Spring had 11, Big Boiling Spring had seven, and Side Spring had six salamanders detected.

There was a shift during 2017 from the Ludwigia spring zone producing more salamanders to Middle spring zone producing more this year as flows from Ludwigia began to subside. Flows at Robertson Springs were still strong at the beginning of the year, however, by July the flow at some of the spring orifices had dwindled or stopped entirely. Due to the loss of spring flow at some of these sites Robertson Springs was remapped in order to determine which spring outlets within the complex were more consistent. By July of 2017, seven individual springs had stopped flowing and the wetted area of the spring zones had shrunk compared to the areas in early 2016 (Figure 3).

Timed searches were conducted in February, May, June, September, and November. Quadrat searches were conducted in April and July. In addition to the DSC and the Robertson Springs sites, Solana Ranch was sampled in September, and Cowen Spring located in Sun City (Williamson County; Figure 1) was included in the quadrat sampling regime. Solana Ranch was added in order to collect genetic material for a future population genetics project. Cowen Spring was added to compare habitat and surface salamander counts with Robertson Spring.

# Methods

A combination of timed searches and drift netting were conducted this year to document the occurrence of Salado salamanders within the study area. Sampling was conducted at three locations within the DSC (Anderson/Benedict, Big Boiling, and Side Spring). Little Bubbly was dry for most of the year, although it was searched when the spring was flowing in February. Critchfield Spring was also searched in February, but not examined the remaining part of the year. Sampling at Robertson Springs was conducted within the entire spring run. For each event at Anderson/Benedict, Big Boiling, and Robertson springs, the area was searched for approximately an hour usually with at least three people (Table 1). Drift netting was conducted this year at six different locations over the course of the year. There was one location at the DSC (Anderson Spring) and five nets set at Robertson Springs (Table 2). When a salamander was found, it was photographed and returned to the area where captured. All measurements were acquired using Image J software. Additionally, the software Wild ID was used to determine if any salamanders were recaptures using photographed head shots of the salamanders.

Quadrat searches were conducted at two locations twice in 2017. Cowen Spring is located within Sun City (Williamson County) and is not part of the normal monitoring schedule (Figure 1). Sampling at Cowen Springs was conducted to compare habitat and salamander surface counts between Cowen Spring and Robertson Springs, which has a more sporadic hydroperiod. We mapped Cowen Springs in March of 2017 (Figure 4, Table 4). From the Robertson and Cowen spring maps a stratified random study design for the quadrat searches was created. Quadrat searches were done in April and July at each location. Quadrat searches at Cowen and Robertson springs were conducted within five days of each other. Random points were selected within ArcGIS using XTools. Quadrat surveys were conducted using a ½ meter quadrat actively searched for 2.5 minutes. Depth ($1/10^{th}$ ft), flow (ft/s), temperature (C°), dissolved oxygen (mg/L), pH, conductivity (µs/cm), and total dissolved solids (g/L) were collected at each quadrat. The spring run was divided into spring areas and run (or mixed zone) areas. A spring area was defined as the area where the water coming out of the orifice does not mix with the spring run water. Spring areas were identified on the map and the total area was calculated for each. A total of 26 quadrats were sampled in April and a total of 35 quadrats were sampled in July at Cowen Spring. At Robertson Springs 41 quadrats were selected and were sampled each. The effort per spring was derived from the area of the spring, therefore, springs with a larger wetted area were surveyed more than smaller spring zones. In addition, ten surveys were added to the spring runs to examine differences between spring areas and spring run with respect to habitat and water chemistry. Data collected from quadrat surveys were analyzed using principal component analysis (PCA). Prior to analysis all data was z-scored.

Following a quadrat search, substrate was quantified by gridding out the spring zones and runs. Habitat at Robertson Springs was measured by running meter tape along the length of the spring run for 100 meters. Every five meters transects were created. The search area was quantified using a 1/3 $m^2$ quadrat. Substrate was identified every other 1/3 of a meter along each transect. At Cowen Spring the area was divided into four sections due to the non-linear fashion of the spring. The four sections were the backwater spring area, the Cowen Spring cobble and gravel run, the Cowen Creek mixing zone, and the main spring (Figure 4). Transects were created every two meters at Cowen Spring to maximize the amount of data collected as the spring area is much smaller than Robertson Springs.

Examination of the overall data set going back to 2015 will be completed in order to examine habitat associations and size distributions. Data will be grouped into quarterly blocks for size distribution analysis. The relative abundance of the salamanders will be calculated for each quarter based upon size classes. Size classes are from 0-19, 20-29, 30-39, 40-49, 50-59, 60-69 mm.

## Results

A total of 46 Salado salamanders were detected from Robertson Springs and the DSC in 2017. Most salamanders were captured, however, a few escaped after a visual observation before a photo or length could be taken. Of the remaining salamanders 18 were considered adults (>30 mm) and 23 were considered juvenile. A total of 17 salamanders were captured using drift nets in the span of 1,359 days. Anderson Spring had the most drift net captures (n = 11) and catch per unit (CPUE) of 0.0251 salamanders per day, although the net was on the longest. Upper Ludwigia had a similar CPUE of 0.0204 salamanders per day. Drift net sampling at Upper Ludwigia was stopped when the spring dried up.

The remaining 27 salamanders were caught by actively searching, and were collected from eight different locations within Robertson Springs and DSC. The most salamanders captured this year by active searching was from Robertson Springs. Within Robertson Springs salamanders were captured from five spring areas (Figure 2). Water chemistry within the sampling area was within ranges of historical values (Table 3).

*Downtown Spring Complex*

In 2017, 25 salamanders were captured at the DSC. Timed searches yielded seven salamanders, and drift netting at Anderson Spring yielded 10 salamanders. One adult salamander was captured during the timed searches at Anderson Spring. This was the first adult captured at Anderson Spring. Other adults were captured from Side Spring over 2017, while no adults were captured within Big Boiling. Seven salamanders were captured at Big Boiling Spring, with 4 caught in May. The remaining salamanders were captured opportunistically during weekly net checking events. No recaptures were documented during 2017 from the DSC.

*Robertson Springs*

Twenty one salamanders were captured at Robertson Springs in 2017. Timed searches yielded seven salamanders. Seven salamanders were captured using drift nets and 7 salamanders were captured during opportunistic searches during weekly net checks. The Middle Spring zone yielded the most salamanders (n = 10). Ludwigia spring had seven, Creek, Beetle, and the Headwater springs all produced one salamander in 2017. As the year progressed, the flow at Robertson decreased. Salamander detections within Ludwigia decreased with flow.

The flow at Robertson Springs have been on the decline since the end of 2016. In June of 2016, the maximum flow of 6.15 m/sec was recorded, and the flow has steadily been decreasing. In April of 2017 the flow was 3.2 m/sec. Then in July, the flow was 1.11 m/sec, and finally in September the flow was 0.85 m/sec. Although salamanders have been captured as spring flows decrease, the main location yielding salamanders within Robertson Springs shifted to Middle Spring as the flow decreased. The flow at Middle Spring is still strong, however, the wetted area and the other smaller springs within the Middle Spring zone have dried up. The Headwater and Beetle springs stopped having detectable flow by August 31, 2017, but water is still flowing from the headwater section.

*Quadrat Searches*

Cowen and Robertson springs were selected for quadrat searches due to the potentially higher densities of salamanders. Quadrat sampling was conducted in April and July. A total of 101 quadrats were sampled and used for the PCA, with 54 quadrats from Cowen Spring and 47 from Robertson Springs. A total of 13 salamanders were detected using the quadrat method: 11

from Cowen Spring and two from Robertson Springs. This was the first collection of salamanders from Robertson Springs within sampled quadrats. In addition, one of the salamanders detected at Robertson Springs was found within a spring run quadrat, out of a spring zone.

Results from the PCA show a separation between the Robertson Springs and Cowen Spring sites along PC axis I (Figure 5). This division is driven by positive loadings of conductivity, total dissolved solids and temperature to negative loadings of flow and cobble (Table 5, Table 10). Therefore, at Robertson Springs there is higher flow, more cobble, with sand and mud/silt (MS) type habitats, and at Cowen Spring, conductivity, total dissolved solids, and temperature are higher with more gravel present. Robertson spring sites and spring run sites are more spatially separated than Cowen creek sites and Cowen spring sites. This indicates a larger change in habitat types between Robertson spring and run sites, than between Cowen creek and spring sites. The gradient along PC axis II shifts from positive loadings of pH, depth, and bedrock to negative loadings from gravel and flow.

Salamander occurrences were plotted in three of the quadrats within the graph. Only the lower left quadrat does not have a salamander occurrence. The lower left quadrat had the highest average values of flow present within the data set (Table 9). The upper left quadrat within the graph has two points that represent the detections at Robertson. On the positive side of the graph is where the Cowen Spring detections of the salamanders were plotted.

Sites were segregated by quadrat results from the PCA analysis to examine abiotic associations of observed salamanders. Abiotic values were averaged to provide trends from each quadrat. The most salamanders were captured in the lower right quadrat (n = 6). The lower right quadrat had the lowest average of dissolved oxygen, the highest conductivity, the lowest pH, the most gravel present, and an average of 0.42 ft/sec flow (Table 9).

Substrate types were assessed at each spring location to help understand any potential connection between salamander presence and absence. A total of 488 quadrats were examined at Robertson Springs (302) and Cowen Spring (186) (Table 6). Mud/silt substrates dominated at Robertson Springs (+50%), while gravel substrates were dominant at Cowen Spring (+40%).

*Habitat Associations*

A total of 81 salamanders have been captured since 2015. Most salamanders have been captured from cave conduits (n = 29; 35.80%; drift nets) followed by gravel substrates (n = 27;

33.33%), then cobble (n = 17; 20.99%) (Table 7). The most frequently associated aquatic vegetation was *Nasturtium* sp. (Watercress) with 25 salamanders (52.08%) detected within, followed by no vegetation (n = 10; 20.83%). The average flow where salamanders were detected (n = 11) during quadrat searches was 0.309 ft/sec. The average depth in the quadrats where salamanders were detected (n = 11) was 0.29 inches.

The relative abundance of salamanders examined quarterly showed that juvenile salamanders are present within the first and second quarters with the population shifting to larger salamanders over the third and fourth quarters (Figure 6 and Table 8).

*Solana Ranch*

Sampling for genetic material was accomplished on September the 5$^{th}$, 2017. A total of 15 salamanders were collected at Solana Ranch Spring #1, from about a two $m^2$ area with cobble and gravel substrates with *Amblystegium* sp. (aquatic moss). Salamanders were photographed and returned to the location where they were collected. The average size of the salamanders collected was 50.76 mm. This is larger than the average size of salamanders collected from 2015 to 2017 from Robertson Springs and DSC (n = 75; 28.13 mm) and from Cowen (n = 10; 36.38 mm). All salamanders encountered at the Solana Ranch Spring #1 were adults.

## Discussion

Monitoring in 2017 provided the highest number of detections within the entire study from the last three years. Determining the mechanisms for the increased detections is not possible, but a number of hypothesis could be examined. First, the hydroperiod of the springs along Robertson Springs has been constant since 2015, creating breakthrough within the subterranean environment allowing salamanders to populate the surface environment more freely. Second, the removal of the beaver dam from Robertson Springs in late 2015 lowered the water levels within the spring zones and spring runs, created a change in substrate dominance, and a subsequent colonization of salamanders was seen within Robertson Springs. Third, as the flows have been decreasing the amount of wetted area has decreased, thereby, causing a crowding effect and consolidating the salamanders within a smaller area potentially making them easier to collect.

The monitoring at Cowen Spring was extremely useful as a comparison to Robertson Springs. In 2016, 43 salamanders were detected at Cowen Spring during regular monitoring (Cambrian Environmental 2016). In the 2017 July event at Cowen there were 10 salamanders detected using the random quadrat approach. In 2016, Robertson Spring had 27 salamanders detected. This year there were 25 salamanders collected from the DSC and 21 collected at Robertson Springs. Although the numbers at Cowen Spring are higher, the collections within the DSC and Robertson Springs are higher than previously expected.

The potential mechanisms for why more salamanders are consistently found at Cowen Spring compared to Robertson Springs are more apparent following the monitoring events this year. As with Robertson Springs, Cowen Spring is adjacent to a creek, however, the depth within Cowen Spring is too shallow to allow large bodied predatory fish within the spring run. Habitat analysis showed that Cowen contained substantially more gravel than at Robertson Springs. Cowen Spring has large contiguous gravel and cobble sections. Within Robertson Springs the patches of gravel and cobble are separated by silt areas and a deeper main spring run. Finally, the hydroperiod is more consistent within Cowen Spring than at Robertson Springs, which has shown large fluctuations overtime.

Larger salamanders on average were found at the Solana Ranch Spring #1 than at Cowen or at Robertson springs. The spring at the Solana Ranch is on the edge of a hill that flows into a small creek. One reason for the average larger size of salamanders is that the Solana Ranch Spring #1 site may not be as susceptible to flooding as the Cowen and Robertson spring sites. This may provide time more time for salamanders to colonize the surface between disturbances. This spring was fenced off and no intrusions were allowed within the spring. The larger size of salamanders and the small area in which the salamanders were found indicate that the surface population at Solana Ranch is much more stable than at Cowen or Robertson springs.

Brune (2002) believed that the primary recharge for Robertson Springs and DSC was located in Williamson County adjacent to I-35 within Salado Creek, where there are large faults. If that is the case, genetic material from salamanders within these southern areas may be mixing with the populations present within Robertson Springs or the DSC. In addition, this would show that the populations within the study area are coupled with potential deleterious effects to water quality and quantity from the south.

Genetic analysis has been proposed for the 2018 monitoring season to examine genetic flow, population size, and the population size needed to maintain genetic diversity within captive programs. This type of work will solidify some of the hypothesis regarding gene flow and subsurface population sizes. More site visits to other springs in the area should be done to verify that the Robertson Springs and DSC sites are the most northern Salado salamander sites.

Understanding the life cycle of the Salado salamander is important in order to better manage the species. The results from the cumulative work over the last three years shows that most juvenile salamanders were captured in the first and second quarters. No gravidity was observed during the 2017 season. Bendik et al. (2017) showed that the largest proportion of the Jollyville Plateau salamanders were gravid during December. Pierce et al. (2014), found that there were two peaks within the population of Georgetown salamanders over the course of a year that had eggs present. One of these peaks was present in winter (around December) and the other around February or March. These results may explain why salamanders within the first and second quarter of our surveys are within the smaller size classes.

Habitat associations of the surface population seem to be similar to other Eurycea within the Edwards Plateau, with optimal habitat being cobble and gravel substrates. Surface population densities appear to be small, due to the absence of recaptures. In addition, 1/3 of the captures were collected from drift nets within our study area. These results show a potentially larger population of Salado salamanders present within the subterranean environment and low surface recruitment at the Robertson Springs site. One hypothesis is that the southern portions of the Salado salamander populations are robust and well established, therefore driving the juvenile salamanders into our study area to forage for food or find mates.

Overall, the Salado salamander population within the DSC and Robertson Springs appear to be stable although low in surface densities. Data suggests that salamanders are being driven from the aquifer in low densities (Diaz Final Report 2016, unpublished report). Based on these data, the reason for low surface densities may not have to do with available habitat or other anthropogenic stressors, but could be due to this species being on the fringe of *Eurycea* distribution within Texas.

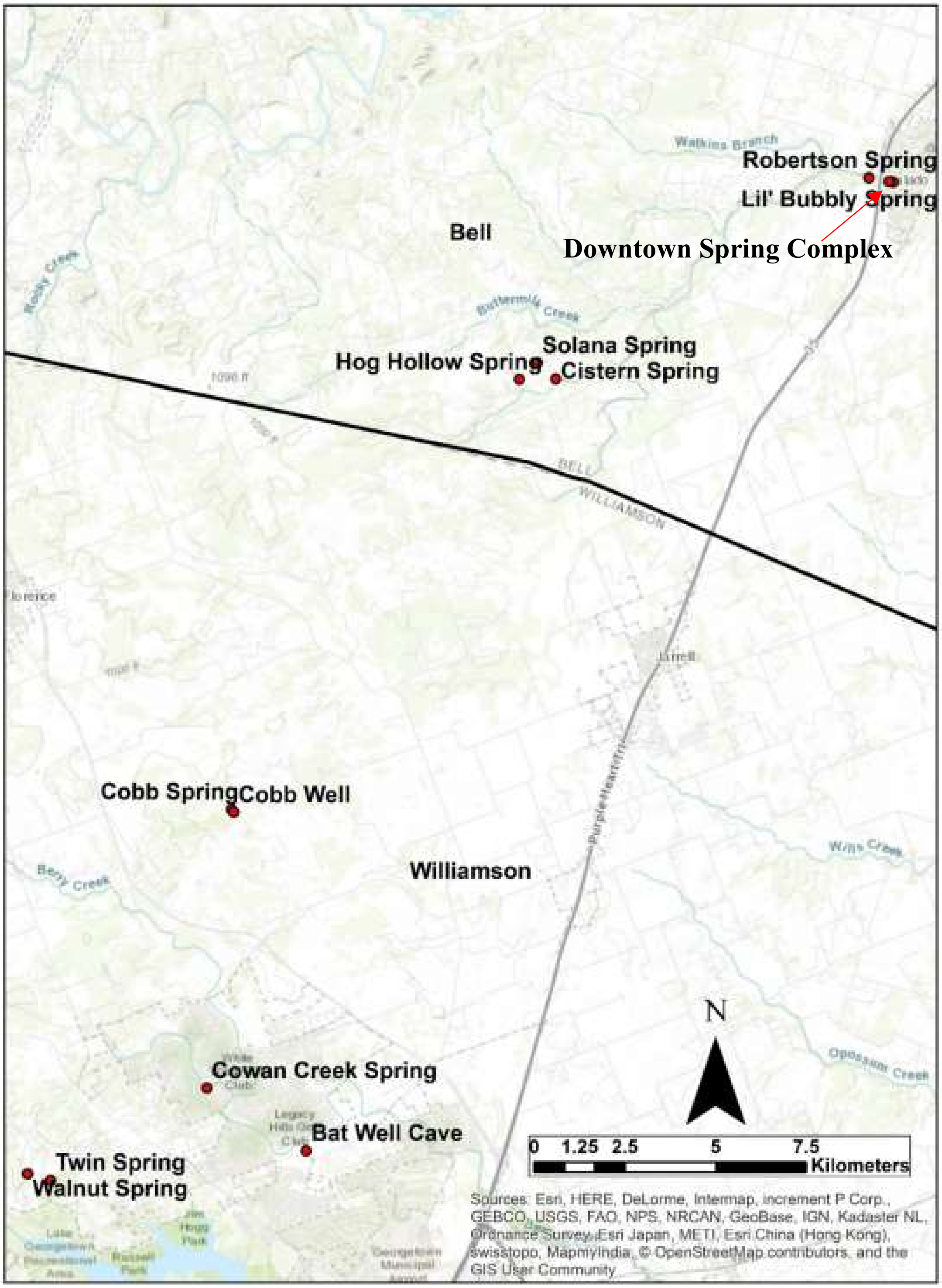


Figure 1. Map of *Eurycea* (salamanders) within the northern portion of the Edwards Aquifer.

Figure 2. Map of Robertson Springs taken from July of 2017. Yellow dots show locations where Salado salamanders have been capture. Red dots are other spring orifice. Light blue areas are the spring zones.

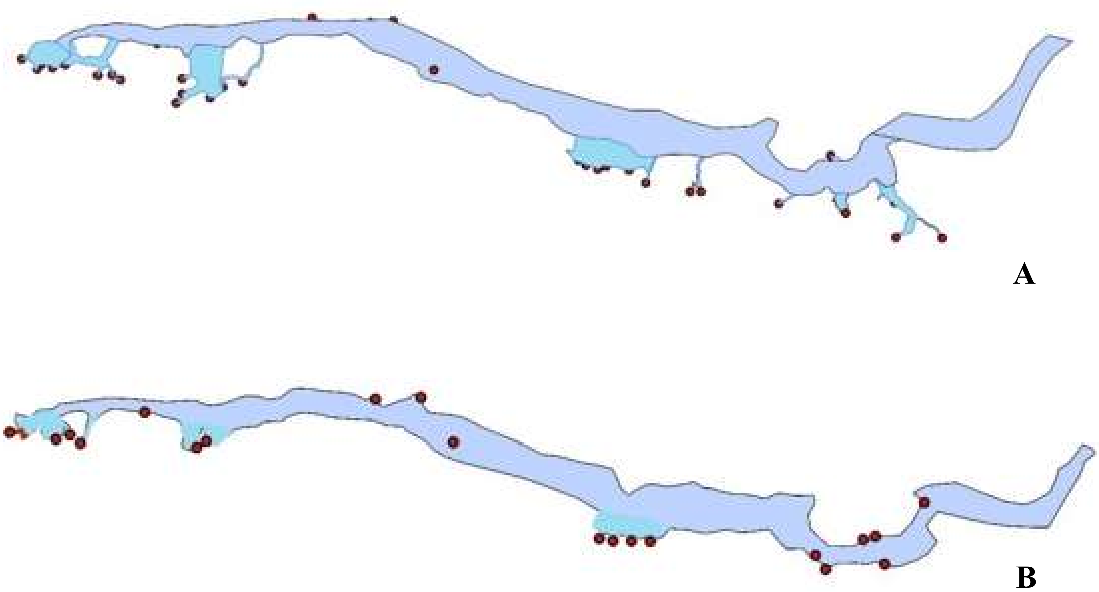


Figure 3. Mapping data collected at Robertson Springs in February of 2016 (A) and in July of 2017 (B). By July 2017, the wetted area of the spring zones has decreased and the overall number of spring orifice have decreased.

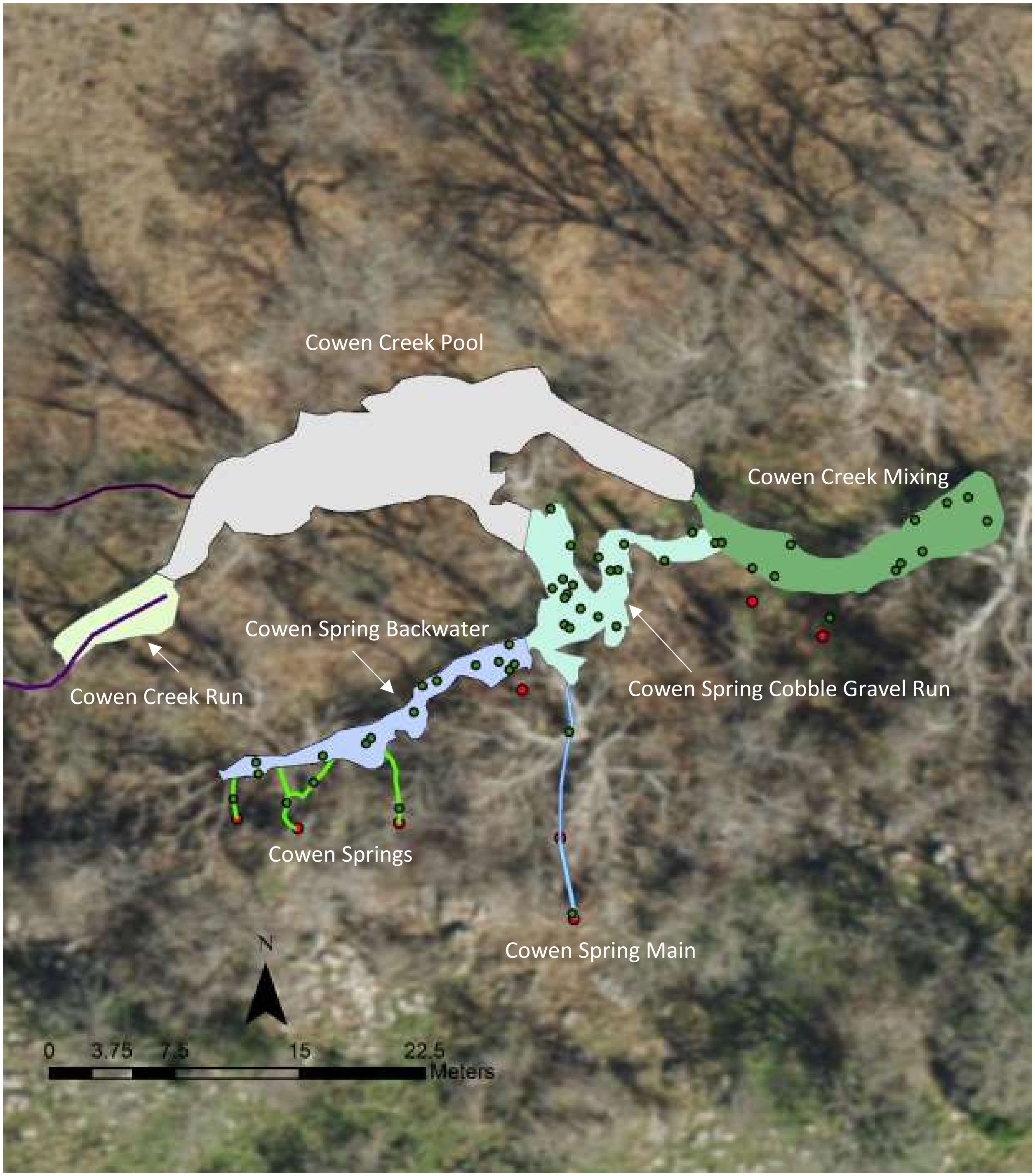

Figure 4. Map of Cowen Springs collected on March 23, 2017. The map is divided into zones of the creek and zones within the spring area. Green dots are randomly generated sampling locations and red dots are spring orifices.

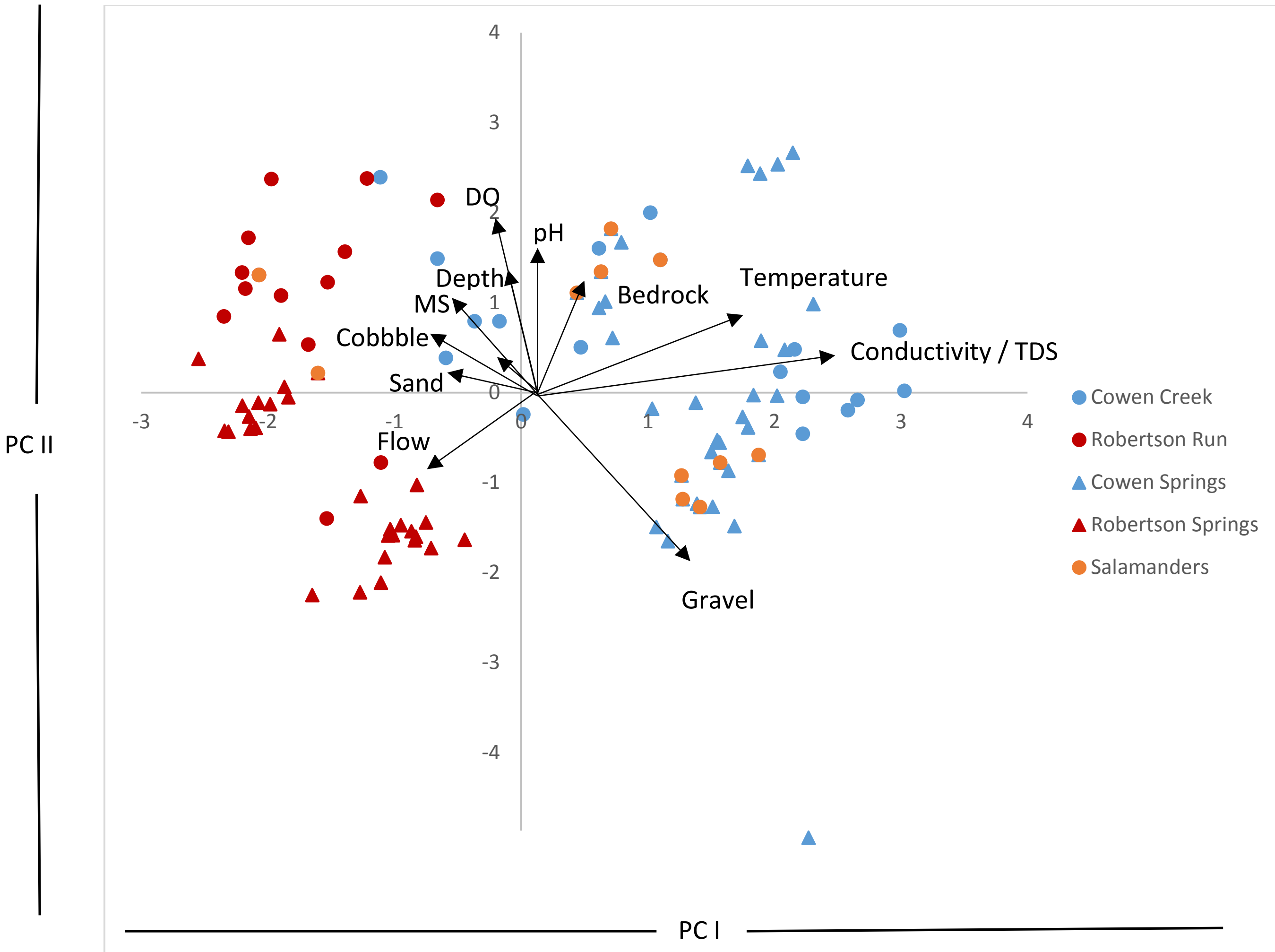


Figure 5. Results from principal components analysis at Cowen Spring and Robertson Springs with data collected during 2017. Abbreviations: DO = dissolved oxygen; TDS = total dissolved solids; MS = mud/silt.

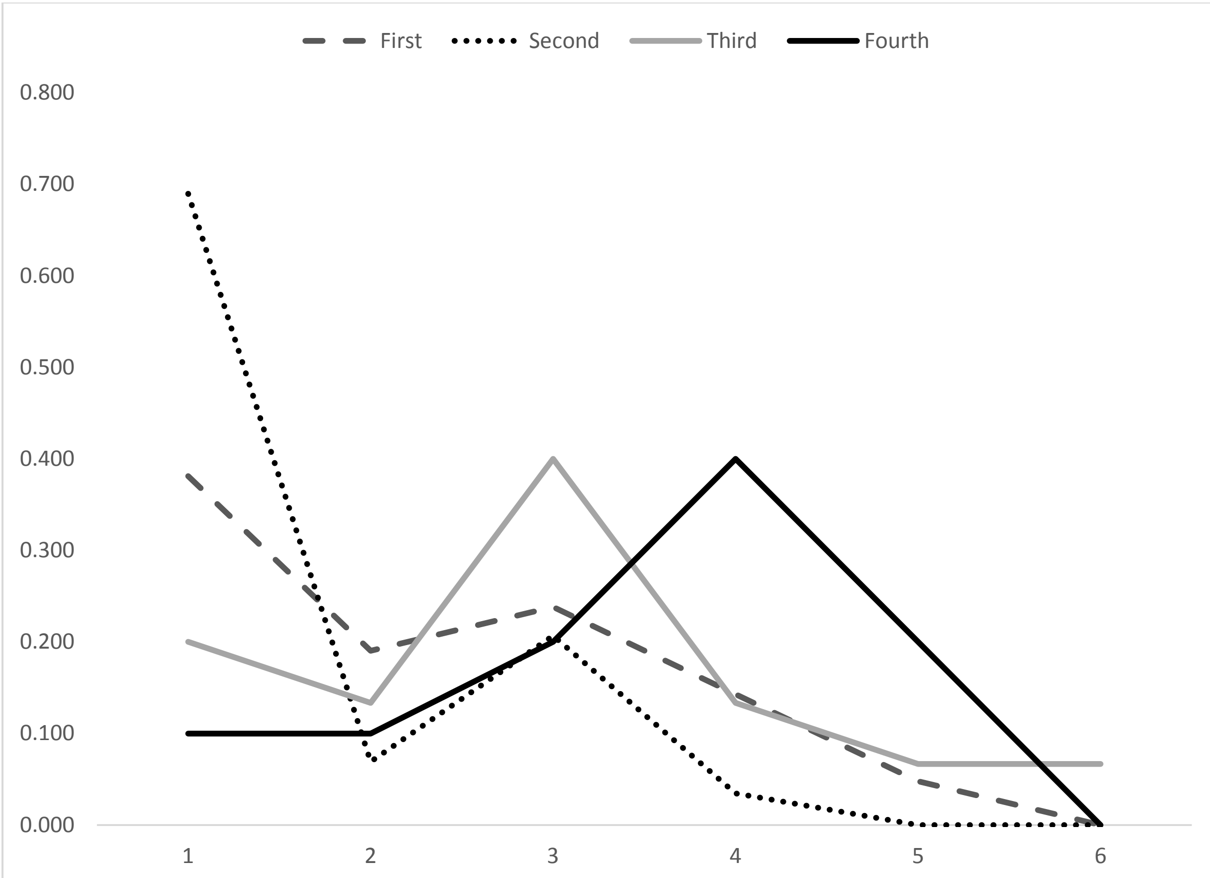

Figure 6. Relative abundance of size class for 75 Salado salamanders captured quarterly from 2015 - 2017 (1 = 10 - 19 mm; 2 = 20 - 29 mm; etc.).

Table 1. Timed searches conducted within the 2017 field season.

| Location | Date | People | Time (min) | Total Time | Salamanders |
|---|---|---|---|---|---|
| **Anderson** | 9-Feb | 4 | 26 | 104 | 0 |
| **Critchfield** | 9-Feb | 4 | Mesohabitat | NA | 0 |
| **Big Boiling** | 9-Feb | 4 | 45 | 180 | 1 |
| **Little Bubbly** | 9-Feb | 4 | 10 | 40 | 0 |
| **Side Spring** | 9-Feb | 4 | All | NA | 0 |
| **Robertson** | 10-Feb | 4 | 85 | 340 | 1 |
| **Anderson** | 4-May | 3 | 50 | 150 | 1 |
| **Big Boiling** | 4-May | 3 | 40 | 120 | 1 |
| **Side Spring** | 4-May | 3 | All | 15 | 0 |
| **Robertson** | 4-May | 3 | 80 | 240 | 1 |
| **Anderson** | 15-Jun | 3 | 60 | 180 | 1 |
| **Big Boiling** | 15-Jun | 3 | 55 | 165 | 1 |
| **Side Spring** | 15-Jun | 3 | All | NA | 1 |
| **Robertson** | 15-Jun | 3 | 96 | 288 | 3 |
| **Anderson** | 21-Sep | 3 | 45 | 135 | 0 |
| **Big Boiling** | 21-Sep | 3 | 36 | 108 | 0 |
| **Side Spring** | 21-Sep | 3 | All | NA | 0 |
| **Robertson** | 21-Sep | 3 | 85 | 255 | 0 |
| **Anderson** | 28-Nov | 1 | 50 | 50 | 0 |
| **Big Boiling** | 28-Nov | 1 | 30 | 30 | 0 |
| **Side Spring** | 28-Nov | 1 | All | NA | 1 |
| **Robertson** | 29-Nov | 1 | 90 | 90 | 2 |

Table 2. Drift netting conducted during the 2017 field season.

| Spring | Location | Date Set | Date Removed | Salamanders | Days | CPUE/day |
|---|---|---|---|---|---|---|
| **Anderson** | DT Complex | 10/26/2016 | 11/28/2017 | 10 | 398 | 0.0251 |
| **Headwater** | Robertson | 1/20/2017 | 8/31/2017 | 1 | 223 | 0.0044 |
| **Beetle** | Robertson | 1/20/2017 | 8/31/2017 | 1 | 223 | 0.0044 |
| **Upper Ludwigia** | Robertson | 1/20/2017 | 8/4/2017 | 4 | 196 | 0.0204 |
| **Creek** | Robertson | 5/11/2017 | 11/29/2017 | 1 | 202 | 0.0049 |
| **Middle** | Robertson | 7/21/2017 | 11/22/2017 | 0 | 117 | 0 |

Table 3. Water chemistry collected during timed searched monitoring.

| Location | Date | Temperature | pH | Conductivity | DO | TDS |
|---|---|---|---|---|---|---|
| **Anderson** | 9-Feb | 20.43 | 7.14 | 595.80 | 7.58 | 0.3815 |
| **Big Boiling** | 9-Feb | 20.79 | 7.09 | 591.80 | 7.63 | 0.3786 |
| **Little Bubbly** | 9-Feb | 19.66 | 7.35 | 589.20 | 9.30 | 0.3775 |
| **Side Spring** | 9-Feb | 20.44 | 7.23 | 595.20 | 7.55 | 0.3804 |
| **Robertson** | 9-Feb | 28.81 | NA | NA | 7.83 | NA |
| **Anderson** | 4-May | 20.72 | 6.91 | 587.40 | 7.31 | 0.376 |
| **Big Boiling** | 4-May | 20.83 | 6.98 | 582.60 | 7.58 | 0.3733 |
| **Side Spring** | 4-May | 20.84 | 7.00 | 582.90 | 7.61 | 0.3731 |
| **Robertson** | 4-May | 20.81 | 7.05 | 578.10 | 7.75 | 0.3701 |
| **Anderson** | 21-Sep | 20.95 | 6.88 | 590.00 | 7.19 | 0.3000 |
| **Big Boiling** | 21-Sep | 20.89 | 6.98 | 589.40 | 7.71 | 0.3771 |
| **Side Spring** | 21-Sep | 21.02 | 7.00 | 589.40 | 7.70 | 0.3772 |
| **Robertson** | 21-Sep | 20.94 | 7.96 | 579.00 | NA | 0.3705 |
| **Anderson** | 29-Nov | 20.89 | 7.00 | 580.40 | 7.79 | 0.3715 |
| **Big Boiling** | 29-Nov | 20.86 | 7.05 | 585.40 | 7.60 | 0.3751 |
| **Side Spring** | 29-Nov | 20.84 | 7.05 | 580.70 | 7.81 | 0.3715 |
| **Robertson** | 29-Nov | 20.87 | 7.16 | 578.00 | 7.99 | 0.3000 |

Table 4. Results from post processing of mapping data collected July 17, 2017 from Robertson Springs and from March 23, 2017 at Cowen Springs.

| Post Process Data | Robertson | Cowen |
|---|---|---|
| **0-5cm** | - | - |
| **5-15cm** | - | - |
| **15-30cm** | 0.06% | 0.93% |
| **30-50cm** | 38.54% | 40.88% |
| **0.5-1m** | 45.40% | 45.48% |
| **1-2m** | 14.81% | 12.15% |
| **2-5m** | 1.19% | 0.56% |
| **>5m** | - | - |

Table 5. Loadings from principal components analysis

| Variable | PC I | PC II |
|---|---|---|
| **Temperature** | 0.386 | 0.210 |
| **Dissolved Oxygen** | -0.075 | 0.470 |
| **Conductivity** | 0.556 | 0.107 |
| **pH** | 0.006 | 0.378 |
| **Total dissolved solids** | 0.555 | 0.106 |
| **Mud/Silt** | -0.151 | 0.247 |
| **Sand** | -0.151 | 0.048 |
| **Gravel** | 0.289 | -0.474 |
| **Cobble** | -0.199 | 0.159 |
| **Boulder** | -0.074 | 0.093 |
| **Bedrock** | 0.088 | 0.297 |
| **Depth** | -0.052 | 0.336 |
| **Flow** | -0.207 | -0.216 |

Table 6. Results from habitat assessment at Robertson and Cowen springs.

| Robertson | | | | | Cowen | | | |
|---|---|---|---|---|---|---|---|---|
| | | April | | July | | April | | July |
| Substrate | Count | Percentage | Count | Percentage | Count | Percentage | Count | Percentage |
| **Mud/silt** | 92 | 58.23 | 77 | 53.47 | 9 | 13.84 | 26 | 21.48 |
| **Sand** | 10 | 6.33 | 14 | 9.72 | 3 | 4.62 | 1 | 0.82 |
| **Gravel** | 31 | 19.62 | 30 | 20.83 | 31 | 47.69 | 50 | 41.32 |
| **Cobble** | 5 | 3.16 | 9 | 6.25 | 15 | 23.08 | 29 | 23.96 |
| **Boulder** | 7 | 4.43 | 3 | 2.08 | 4 | 6.15 | 6 | 4.95 |
| **Bedrock** | 13 | 8.23 | 11 | 7.64 | 3 | 4.62 | 9 | 7.43 |
| **Total** | 158 | | 144 | | 65 | | 121 | |
| **Rocks** | | 27.22 | | 29.17 | | 76.92 | | 70.25 |

Table 7. Results from captures of Salado salamanders from 2015 – 2017.

| Substrate | Count | Percentage |
|---|---|---|
| **Silt** | 2 | 2.47 |
| **Sand** | 2 | 2.47 |
| **Gravel** | 27 | 33.33 |
| **Cobble** | 17 | 20.99 |
| **Boulder** | 4 | 4.94 |
| **Cave Conduit** | 29 | 35.80 |
| Vegetation | Count | Percentage |
| ***Sagittaria* sp.** | 1 | 2.08 |
| ***Nasturtium* sp.** | 25 | 52.08 |
| **Filamentous Algae** | 3 | 6.25 |
| ***Ludwigia* sp.** | 1 | 2.08 |
| ***Amblystegium* sp.** | 3 | 6.25 |
| ***Hydrocotyle* sp.** | 2 | 4.17 |
| **None** | 10 | 20.83 |
| ***Eleocharis* sp.** | 1 | 2.08 |
| **Organic Debris** | 2 | 4.16 |

Table 8. Count and relative abundance data by size class for salamanders captured from 2015 to 2017.

| Size Class | First | Second | Third | Fourth |
|---|---|---|---|---|
| **1** | 8 | 20 | 3 | 1 |
| **2** | 4 | 2 | 2 | 1 |
| **3** | 5 | 6 | 6 | 2 |
| **4** | 3 | 1 | 2 | 4 |
| **5** | 1 | 0 | 1 | 2 |
| **6** | 0 | 0 | 1 | 0 |
| **Sum** | 21 | 29 | 15 | 10 |
| | | | | |
| **1** | 0.381 | 0.690 | 0.200 | 0.100 |
| **2** | 0.190 | 0.069 | 0.133 | 0.100 |
| **3** | 0.238 | 0.207 | 0.400 | 0.200 |
| **4** | 0.143 | 0.034 | 0.133 | 0.400 |
| **5** | 0.048 | 0.000 | 0.067 | 0.200 |
| **6** | 0.000 | 0.000 | 0.067 | 0.000 |

Table 9. Average results from principle component analysis segregated by multivariate space (quadrats) and the number of salamanders captured within each section of multivariate space. Abbreviations: LL = lower left; UPL = upper left; LRT = lower right; UPRT = upper right; °C = temperature; DO = dissolved oxygen; µs/cm = conductivity; TDS = total dissolved solids; MS = mud/silt; GR = gravel; COB = cobble; BO = boulder; BED = bedrock; 1/10 = depth in tenths of feet.

| Quadrat | °C | DO | µs/cm | pH | TDS | MS | Sand | GR | COB | BO | BED | Depth | Flow | Salamanders |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **LL** | 20.89 | 8.01 | 579.10 | 7.07 | 0.37 | 0.00 | 0.04 | 0.68 | 0.29 | 0.00 | 0.00 | 0.20 | 0.61 | 0 |
| **UPL** | 20.94 | 8.52 | 604.88 | 7.17 | 0.39 | 0.25 | 0.13 | 0.17 | 0.25 | 0.13 | 0.08 | 0.51 | 0.53 | 2 |
| **LRT** | 21.30 | 7.92 | 765.39 | 7.03 | 0.49 | 0.00 | 0.00 | 0.96 | 0.00 | 0.00 | 0.04 | 0.29 | 0.42 | 6 |
| **UPRT** | 21.73 | 8.38 | 763.75 | 7.14 | 0.49 | 0.09 | 0.00 | 0.36 | 0.27 | 0.09 | 0.18 | 0.36 | 0.17 | 5 |

Table 10. Average results from principle component analysis taken from 101 quadrats in 2017 segregated by site. Abbreviations: °C = temperature; DO = dissolved oxygen; µs/cm = conductivity; TDS = total dissolved solids; MS = mud/silt; GR = gravel; COB = cobble; BO = boulder; BED = bedrock; 1/10 = depth in tenths of feet.

| Site | °C | DO | µs/cm | pH | TDS | MS | Sand | GR | COB | BO | BED | 1/10 | Flow | Salamanders |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Robertson** | 20.96 | 8.14 | 579.04 | 7.09 | 0.37 | 0.13 | 0.09 | 0.43 | 0.26 | 0.06 | 0.04 | 0.32 | 0.53 | 2 |
| **Cowen** | 21.41 | 8.23 | 758.98 | 7.11 | 0.49 | 0.04 | 0.00 | 0.69 | 0.15 | 0.04 | 0.09 | 0.34 | 0.36 | 11 |

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# Final Report
# 2018

# Salado Salamander Monitoring Final Report 2018


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# Acknowledgements


We would like to thank the staff at Camp Tahuaya, in particular Ranger Dennis, and the Longhorn Council of the Boy Scouts of America, along with the Williamson County Conservation Foundation for access to the springs. We appreciate the assistance of Justin Crow from the San Marcos Aquatic Resources Center for help with collections. We appreciate the support from Bell County, the Village of Salado, and Clearwater Underground Water Conservation District for funding this work.

# Executive Summary

Monitoring of the Salado salamander (*Eurycea chisholmensis*) concluded in December of 2018 finalizing the fourth 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 32 Salado salamanders were detected this year. Almost all salamanders were detected at the DSC (n = 24). Within the DSC, Side Spring produced the most salamanders over the course of the year. Only eight detections were documented at Robertson Springs during 2018, due to the loss of discharge from springs on the property. When discharged returned to Robertson Springs, salamanders were detected from three different spring zones: five in the Middle Springs zone, one in the Headwater Spring zone, and two in the the Ludwigia Spring zone (one in the upper spring and one in the middle spring). Ten salamanders were captured using drift nets this year, while the remaining salamanders were captured during active searches. In addition to the above mentioned collections at Robertson Springs, two salamanders were collected in drift nets from Anderson Spring in the DSC.

In addition to the regular monthly monitoring at the historic locations, monthly monitoring was added at Tahuaya Springs, just north of the Village of Salado. Tahuaya Spring has been sporadically searched for salamanders in the past, but no regular monitoring has occurred up to this point. Tahuaya Springs was monitored to collect data, either in support or opposition, to the currently established known northern range of the Salado salamander, which Tahuaya is north of. Drift nets were deployed to passively sample the spring orifice within the area. Four nets were used to sample the springs for a total of 89 days during the year. The spring run was searched on a number of occasions. No salamanders were detected during the efforts at Tahuaya Springs this year. We appreciate the cooperation from the staff at Camp Tahuaya and the Longhorn Council of the Boy Scouts of America for access to Tahuaya Springs.

Additional work associated with monitoring in 2018 included the collection of genetic material for a population genetics project in collaboration with Dr. Chris Nice and Corina Mier Y Turan at Texas State University. Material was collected from Anderson, Big Boiling, Side, Robertson, Solana, Cowan, and Twin springs over the course of 2018 with the aid of Justin Crow from the San Marcos Aquatic Resources Center. An attempt to collect genetic material was done at Batwell Cave as well, however no salamanders were detected there. A total of 183 samples

were collected for genetic analysis. Results from the population genetics project will be available by December of 2019.

## 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 designated the downtown spring complex, the Robertson estate spring, and a few sites upstream in the Salado creek watershed, as critical habitat.

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*, including the ones in question at the time (*E. chisholmensis, E. naufragia, and E. tonkawae*), suggest 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, shows 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 particular 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. This program began in 2015 and is in fifth year of monitoring.

## Methods

Sampling was conducted monthly in 2018 at the DSC and at Robertson Springs (Figure 1). Timed searches were conducted at Big Boiling and Robertson springs, while Side Spring and Anderson Spring were searched entirely due to their small areas. During timed searches, all mesohabitats were searched for salamanders. Passive sampling was conducted using drift nets with 250 µm mesh at a number of locations at Robertson, Anderson and Tahuaya springs. Nets were set in place for seven days. Aquatic invertebrates captured during drift netting were taken back to the lab sorted and identified. Aquatic invertebrate data presented will include analysis of certain spring communities from 2015 to 2018. Most taxa were photographed using a dissecting scope. Certain taxa with questions about taxonomic placement were sent to experts for species identification. Other passive sampling techniques were deployed when necessary due to the lack of discharge at spring locations. When discharge became negligible, but water still remained bottle traps and mop heads were used to passively collect salamanders. When salamanders were found, they were photographed and taken to the San Marcos Aquatic Resources Center for study. All measurements were acquired using Image J software.

As in the 2017 report, the overall dataset has been updated to include the 2018 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.

Other monitoring at Cowan and Twin springs was conducted in coordination with Cambrian Environmental, as these sites are part of the Cambrians regular monthly monitoring regime. The springs were searched thoroughly, and salamanders were collected, tail clipped, photographed and returned to the spring. Cowan and Twin springs were searched monthly from February through July as part of the genetics project. Passive monitoring techniques to collect salamander genetic material from Bat Well Cave took place from March 27 to April 12 of 2018, using drift nets placed within the cave. Sampling was conducted at Solana Ranch Spring #1 in September of 2017 and again in April of 2018, to gather additional genetic material. At the beginning of the genetics project 18 salamanders were collected and preserved from the field. To mitigate the loss of these salamanders and maximize the data gained from them, gut contents were examined from these 18 individuals.

## Results

A total of 32 salamanders were detected in 2018 during all collections. Of these 32, 21 were juveniles (<30 mm) and 11 were adults (Table 1). A total of 15 salamanders were detected during monthly monitoring at the DSC and Robertson springs. Most salamanders were captured this year from the DSC at Side Spring (n = 11). A total of 10 salamanders were captured passively using drift nets deployed over a combined 585 days of drift netting. Middle Spring within Robertson Springs had the most detections of salamanders using the drift nets (n = 5), followed by Anderson Spring (n = 2), Ludwigia Spring zone (n = 2), and one from the Headwaters Spring. Bottle traps and mop heads were used as the spring discharge decreased at Robertson Springs in Creek Spring and at Big Boiling Spring, however, no salamanders were captured using these methods.

Most salamanders were captured in the first half of the year during regular monthly monitoring (Table 2) with all the detections occurring within the DSC. Robertson Springs continued a downward trend in discharge from the end of 2017 to October 2018. By April, only four mapped springs were flowing along with a seep that had taken the place of the headwaters which were now below Middle Spring (Figure 2). All springs above the new seeping headwaters were now dry, including Beetle and Middle springs. By July, discharge from all mapped springs had completely stopped. This cessation in discharge lasted until early October, 2018. On October 17, 2018 nets were deployed to detect salamanders leaving the aquifer to recolonize the surface habitat and were left in place for the duration of the year. During that time, eight salamanders were captured at Robertson Springs. Middle Spring was the most productive in producing salamanders to the surface (n = 5; over 80 days of netting).

Some salamanders escaped without a photo, therefore, a total of 107 Salado salamanders that have been captured and measured since 2015 were used for the updated seasonal dynamics. A classic size progression from smaller to larger salamanders, over the course of the year is shown (Figure 3). In winter, the majority of salamanders captured were in the smallest size class ranging from 10 to 19 mm. In spring, the smallest size class was still dominant, however, the measurable population had spread out to include representation in the fourth and fifth size classes. In summer, the most prevalent size class was in the 30 – 39 mm size class with all other size classes including at least one capture (Table 7). 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 4; Table 7).

A total of 113 Salado salamanders have been detected since 2015. One salamander, captured in April of 2018, does not have substrate or vegetation data recorded, so 112 salamanders were used to examine the substrate and vegetation associations. A total of 73 salamanders were detected on the surface (67%), while 39 (36%) were captured in drift nets, presumably from the aquifer. Of the 73 salamanders detected on the surface, 43 (58%) have been captured in gravel as the primary substrate and 22 (30%) have been captured in cobble as the primary substrate (Table 3). Salamanders were detected in many types of vegetation, but 31 (42%) of the 73 were shown to associate with watercress (*Nasturtium* sp.) and 25 (34%) have been captured in areas with no vegetation.

Examination of the gut contents from 18 Salado salamanders showed similarities to other Eurycea from across the Edwards Plateau (Diaz 2010). Only one salamander out of the total 18 had an empty digestive tract. A total of 162 aquatic invertebrates were identified from the 18 salamanders. On average nine aquatic invertebrates were present within the gut tract. The Class Ostracoda (seed shrimp), represented by at least six genera, were the most common taxa encountered within the gut tract of the salamanders (Table 4). Following Ostracoda in abundance were Copepods and then the amphipod *Hyalella* sp. (Figure 5).

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. These activities have also provided a detailed data set of the karst invertebrates present at each spring opening or complex. Surface recruitment of salamanders at Robertson Springs from the aquifer to the surface habitat has been calculated at 0.029 salamanders per day, derived from drift netting data from major springs within the Robertson property and at Anderson Spring. This rate was calculated using data from a total 1,302 days of drift net sampling from 2015 to 2018. Surface taxa were collected from the drift nets and include three genra of riffle beetles (Elmidae), *Microcylloepus* sp., *Stenelmis* sp., and *Heterelmis* sp., which are considered good indicators of water quality. Robertson Springs, in general has more diversity with regards to aquifer taxa than Anderson Spring at the DSC (Table 5). Within Robertson Springs, Creek and Ludwigia springs contain the most diversity of aquifer taxa.

Collection for the population genetics project during 2018 was very productive at most sites. Sampling sizes (~ 30 individuals) were met for genetic material collected at Cowan and Twin springs on the Williamson County Conservation Foundation land. Collections at Solana Ranch were also successful from Solana Spring #1 yielding 29 salamanders. Collections at all historic Salado salamander locations were accomplished in 2018 within ranges of the maximum genetic target of 30 salamanders per site. Two sites that would have been of interest to the population genetics project were not represented in this data set by genetic material due to lack of access (Cobbs Spring) or the inability to capture any animals (Bat Well Cave). A total of 183 genetic samples were collected from seven locations (Table 6). Most of these are going to be unique collections, however, there may be a few repeat captures. This collection is the largest data set of genetic material taken from the Salado salamander's range (Table 6). Recently, DNA was extracted from 180 tail clipped tissue samples of salamanders. Reduced representation genomic libraries for all 180 samples were prepared in the Nice lab at Texas State University. These libraries were then combined and an aliquot was shipped to the Genomic Sequencing and Analysis Facility at the University of Texas, Austin where size selection of fragments and one lane of Illumina HiSeq 2500 sequencing was performed. This produced 250,208,691 sequence reads. Processing and assembly of these reads is under way currently. We expect to have preliminary results describing patterns of genetic variation within and among populations before May, 2019. We expect to produce a final report by our deadline in December, 2019.

# Discussion

Collections of the Salado salamander decreased from the previous year but was comparable to the levels detected in 2016 when discharge was beginning to return to Robertson Springs following the drought. Although collections from Robertson Springs did not happen until October, the collections at the DSC were consistent with past efforts in 2017. Twenty five salamanders were detected in 2017 and 24 were detected in 2018 from the DSC. During drought conditions or times where Robertson Springs is not flowing, it is still be possible to collect and detect salamanders within the DSC. For example, in 2015, four salamanders were detected at the DSC, when the Edwards Aquifer area was just coming out of a severe drought.

With the addition of the 32 salamanders detected this year the sample size of the population dynamics graph (Figure 3) has increased from 75 to 107 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 population 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. The calculation for this graph has changed a bit from the 2017 analysis to make it easier to understand and make more sense ecologically. In 2017, the graph was divided by quarters of the year, in 2018 the graph has been changed to represent seasons.

Diet and 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, Diaz 2010). 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. 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), maybe around 10 salmanders at the DSC and Robertson Spring sites could be based on four 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 Spring 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. However, Robertson Springs has a large fluctuation in hydroperiod and was not flowing in 2015. In 2016 the springs began to discharge water from a number of orifices. In 2017 the discharge began to decline and ceased to flow in 2018. 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 Solana Ranch Spring #1, 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 three years (2015- 2018), 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. These results will be compared to sites within the Barton Springs and San Marcos salamander populations. 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.

Table 1. Collections of Salado salamanders from 2018 timed monitoring and opportunistic collections. DSC = Downtown Complex; CC = Cave conduit; LN = little net, by hand; DN = drift net.

| # | Spring | Location | Date | Total Length (mm) | Primary Substrate | Vegetation | Method |
|---|---|---|---|---|---|---|---|
| **1** | Side Spring | DSC | 1/19/2018 | 17.11 | Gravel | Watercress | LN |
| **2** | Big Boiling | DSC | 2/26/2018 | 22.96 | Cobble | Leaves | LN |
| **3** | Big Boiling | DSC | 2/26/2018 | 23.21 | Cobble | Leaves | LN |
| **4** | Side Spring | DSC | 3/12/2018 | 19.27 | Cobble | none | LN |
| **5** | Anderson | DSC | 3/20/2018 | 18.92 | CC | Cave | DN |
| **6** | Side Spring | DSC | 4/12/2018 | 22.79 | Gravel | Watercress | LN |
| **7** | Big Boiling | DSC | 4/12/2018 | 19.53 | Gravel | none | LN |
| **8** | Anderson | DSC | 4/16/2018 | 17.07 | CC | Cave | DN |
| **9** | Side Spring | DSC | 4/16/2018 | 19.6 | Gravel | Watercress | LN |
| **10** | Side Spring | DSC | 4/16/2018 | 22.92 | Gravel | Watercress | LN |
| **11** | Side Spring | DSC | 4/16/2018 | 35.9 | Gravel | Watercress | LN |
| **12** | Side Spring | DSC | 4/16/2018 | 29.19 | Gravel | Watercress | LN |
| **13** | Big Boiling | DSC | 4/16/2018 | 16.46 | NA | NA | LN |
| **14** | Big Boiling | DSC | 5/16/2018 | 19.06 | Gravel | none | LN |
| **15** | Anderson | DSC | 5/16/2018 | 20.17 | Gravel | none | LN |
| **16** | Anderson | DSC | 5/31/2018 | 18.53 | Gravel | none | LN |
| **17** | Anderson | DSC | 6/6/2018 | 24.73 | Cobble | none | LN |
| **18** | Side Spring | DSC | 6/21/2018 | 54.37 | Gravel | none | LN |
| **19** | Side Spring | DSC | 6/21/2018 | 29.84 | Gravel | none | LN |
| **20** | Side Spring | DSC | 6/21/2018 | 18.57 | Gravel | none | LN |
| **21** | Anderson | DSC | 6/28/2018 | 23.99 | Gravel | none | LN |
| **22** | Side Spring | DSC | 6/28/2018 | 46.35 | Gravel | none | LN |
| **23** | Anderson | DSC | 9/24/2018 | 42.93 | Gravel | none | LN |
| **24** | Anderson | DSC | 9/24/2018 | 27.83 | Cobble | none | LN |
| **25** | Ludwigia Upper | Robertson | 10/30/2018 | 60.11 | CC | Cave | DN |
| **26** | Middle Spring | Robertson | 10/30/2018 | 45.24 | CC | Cave | DN |
| **27** | Middle Spring | Robertson | 10/30/2018 | 12.05 | CC | Cave | DN |
| **28** | Middle Spring | Robertson | 11/7/2018 | 46.99 | CC | Cave | DN |
| **29** | Middle Spring | Robertson | 11/15/2018 | 45.56 | CC | Cave | DN |
| **30** | Ludwigia Middle | Robertson | 12/19/2018 | 31.96 | CC | Cave | DN |
| **31** | Middle Spring | Robertson | 12/19/2018 | 47.39 | CC | Cave | DN |
| **32** | New Upper HW | Robertson | 12/19/2018 | 49.03 | CC | Cave | DN |

Table 2. Data collected from the 2018 timed monitoring events at the Downtown Complex and Robertson springs. Other collections of Salado salamanders occurred during opportunistic sampling events.

| Month | Salamanders | Spring |
|---|---|---|
| **January** | 1 | Side |
| **February** | 2 | Big Boiling |
| **March** | 1 | Anderson |
| **April** | 5 | Anderson and Side |
| **May** | 1 | Anderson |
| **June** | 3 | Side |
| **July** | 0 | - |
| **August** | 0 | - |
| **September** | 2 | Anderson |
| **October** | 0 | - |
| **November** | 0 | - |
| **December** | 0 | - |

Table 3. Habitat associations of the Salado salamander determined by 112 salamanders collected from 2015 to 2018 at the downtown springs complex (DSC) and Robertson springs. Substrate and vegetation percentages were calculated only using surface collections.

| | # | % |
|---|---|---|
| **Cave Conduit** | 39 | 36.11 |
| **Substrate** | **#** | **%** |
| **Silt** | 2 | 2.74 |
| **Sand** | 2 | 2.74 |
| **Gravel** | 43 | 58.90 |
| **Cobble** | 22 | 30.14 |
| **Boulder** | 4 | 5.48 |
| **Vegetation** | **#** | **%** |
| ***Sagittaria* sp.** | 1 | 1.37 |
| ***Nasturtium* sp.** | 31 | 42.47 |
| **Filamentous Algae** | 4 | 5.48 |
| ***Ludwigia* sp.** | 1 | 1.37 |
| ***Amblystegium* sp.** | 4 | 5.48 |
| ***Hydrocotyle* sp.** | 2 | 2.74 |
| **none** | 25 | 34.25 |
| **Organic Debris** | 4 | 5.48 |
| **Grass** | 1 | 1.37 |

Table 4. Items collected during gut contents analysis of 18 Salado salamanders collected from 2016 to 2018.

| Class/Order | Family | Genus | Totals | Composition | Frequency |
|---|---|---|---|---|---|
| **Ephemeroptera** | Caenidae | *Caenis sp.* | 1 | 0.62 | 0.06 |
| **Ephemeroptera** | Baetidae | *Callibaetis sp.* | 1 | 0.62 | 0.06 |
| **Trichoptera** | Leptoceridae | *Nectopsyche sp.* | 1 | 0.62 | 0.06 |
| **Diptera** | Chironomidae | Tanypodinae | 2 | 1.23 | 0.11 |
| **Amphipoda** | Hyalellidae | *Hyalella sp.* | 13 | 8.02 | 0.33 |
| **Mesogastropoda** | Pleuroceridae | *Elimia sp.* | 8 | 4.94 | 0.28 |
| **Mesogastropoda** | Hydrobiidae | Phreatodrobia nugax | 7 | 4.32 | 0.11 |
| **Copepoda** | | | 22 | 13.58 | 0.28 |
| **Cladocera** | | | 1 | 0.62 | 0.06 |
| **Ostracoda** | | *Stenocypris sp.* | 26 | 16.05 | 0.28 |
| **Ostracoda** | | Cypria -- Green | 66 | 40.74 | 0.61 |
| **Ostracoda** | | Cypria – Red | 4 | 2.47 | 0.11 |
| **Ostracoda** | | *S. bellensis* | 3 | 1.85 | 0.06 |
| **Ostracoda** | | Subterranean | 2 | 1.23 | 0.11 |
| **Ostracoda** | | Other | 2 | 1.23 | 0.11 |
| **Detris** | | | 3 | 1.85 | 0.17 |
| **All Ostracoda Combined** | | | 103 | 63.58 | 0.83 |

Table 5. Presence data of aquifer taxa collected from drift nets at springs in the Salado area from 2015 to 2017. LSL – Ludwigia spring lower, LSM – Ludwigia spring middle , LSU – Ludwigia spring upper.

| | Anderson | Beetle | Middle | Creek | LSL | LSM | LSU | Headwaters |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| **Blind Collembola** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| ***Folsomoides sp.*** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| **Blind Dytiscidae** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Curculionidae blind** | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| **Subterranean Ostracoda** | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| ***S. bellensis*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| ***Uchidastygacarus sp.*** | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| **Mite long appendages** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Big O Mite** | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| **Texanobathynella** | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| **Microcerberidae** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Phreatidobid sp.*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***P. nugax*** | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| ***P. micra*** | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***P. taylori*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***M. comal/P. integra*** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Lirceolus pilus*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***Caecidotea reddelli*** | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| ***Stygobromus sp.*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ***Parabogidiella americana*** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Totals** | 12 | 13 | 9 | 16 | 15 | 11 | 8 | 9 |

Table 6. Sites where Salado salamanders were collected during 2017 and 2018 for the Salado salamander population genetics project.

| Site | Sample Size |
|---|---|
| **Anderson** | 17 |
| **Big Boiling** | 11 |
| **Side Spring** | 14 |
| **Robertson** | 16 |
| **Solana Ranch** | 29 |
| **Cowen** | 58 |
| **Twin** | 38 |

Table 7. Cumulative Salado salamander data collected from 2015 to 2018 used to create population dynamics graph.

| Size Class | Winter | Spring | Summer | Fall |
|---|---|---|---|---|
| **1** | 5 | 26 | 9 | 3 |
| **2** | 3 | 9 | 5 | 2 |
| **3** | 2 | 5 | 12 | 1 |
| **4** | 2 | 4 | 3 | 7 |
| **5** | 0 | 1 | 2 | 4 |
| **6** | 0 | 0 | 1 | 1 |
| **Size Class** | Winter | Spring | Summer | Fall |
| **1** | 0.416667 | 0.577778 | 0.28125 | 0.166667 |
| **2** | 0.25 | 0.2 | 0.15625 | 0.111111 |
| **3** | 0.166667 | 0.111111 | 0.375 | 0.055556 |
| **4** | 0.166667 | 0.088889 | 0.09375 | 0.388889 |
| **5** | 0 | 0.022222 | 0.0625 | 0.222222 |
| **6** | 0 | 0 | 0.03125 | 0.055556 |
| **Totals** | 12 | 45 | 32 | 18 |

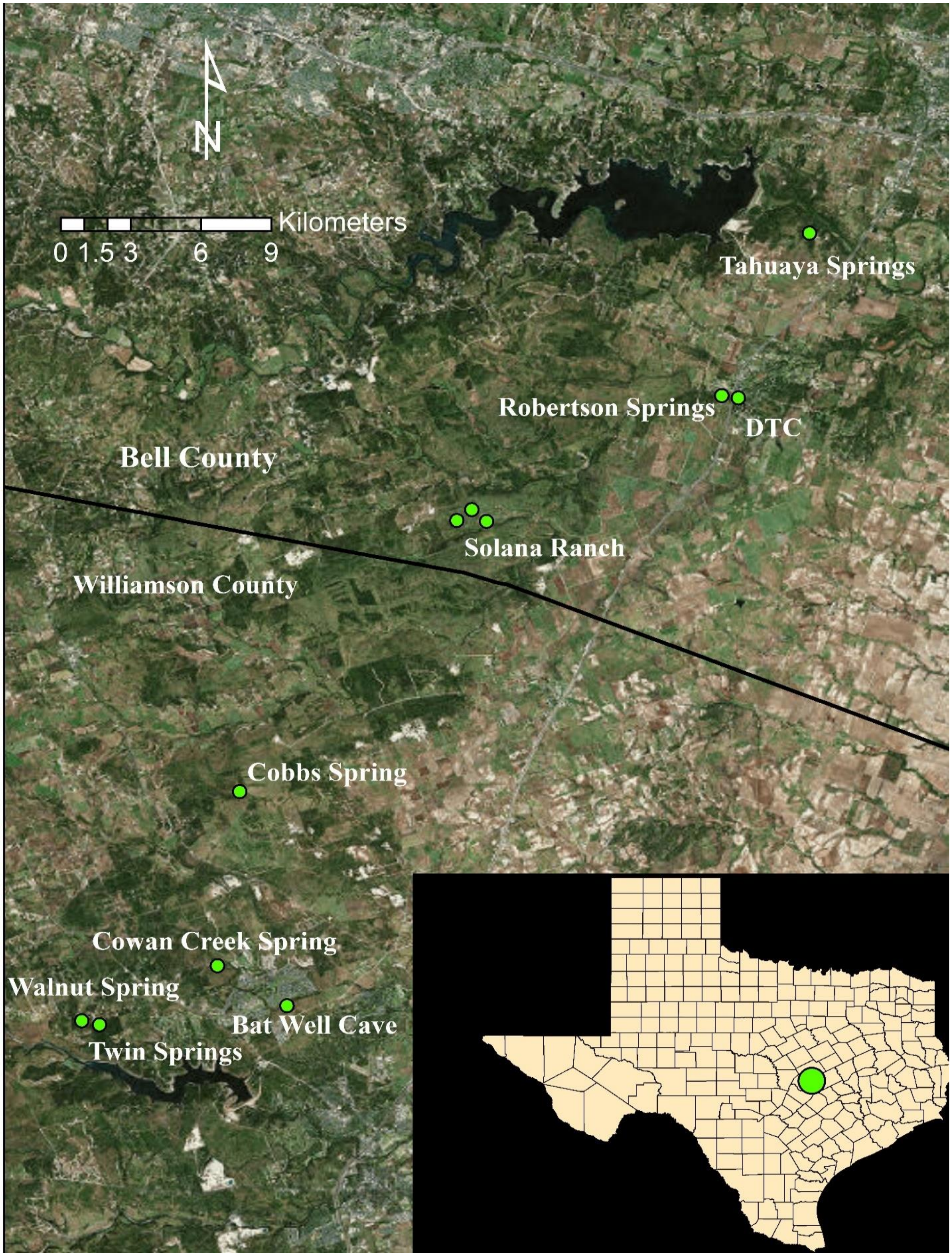


Figure 1. Known geographical range for Salado salamander and monitoring sites used in 2018.

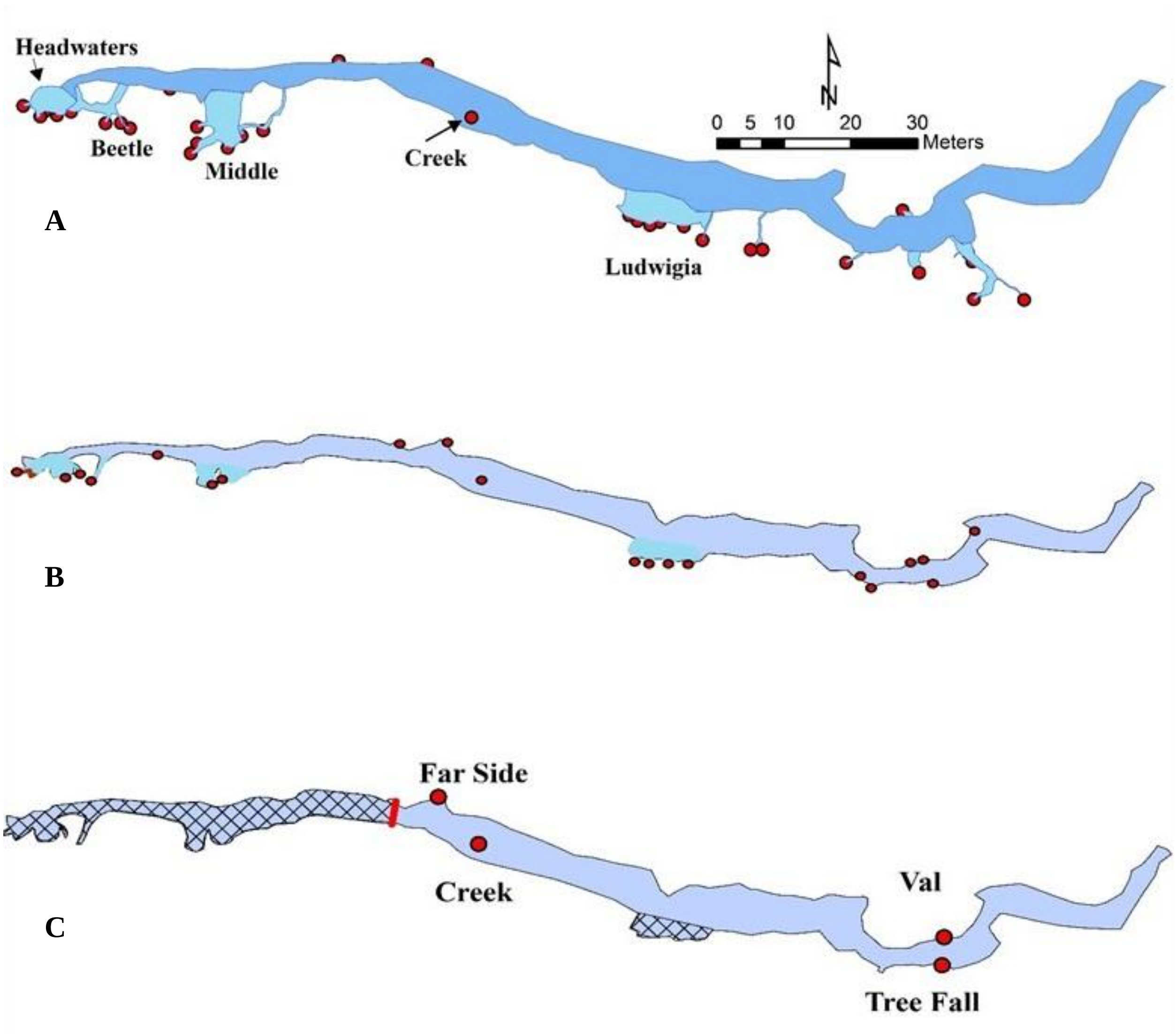


Figure 2. Robertson Springs starting in March of 2016 (A) into July of 2017 (B) and finally in April of 2018 (C). Hashed areas are places where there was no longer water. Red dots or lines are spring locations. Lighter blue sections are considered spring zones.

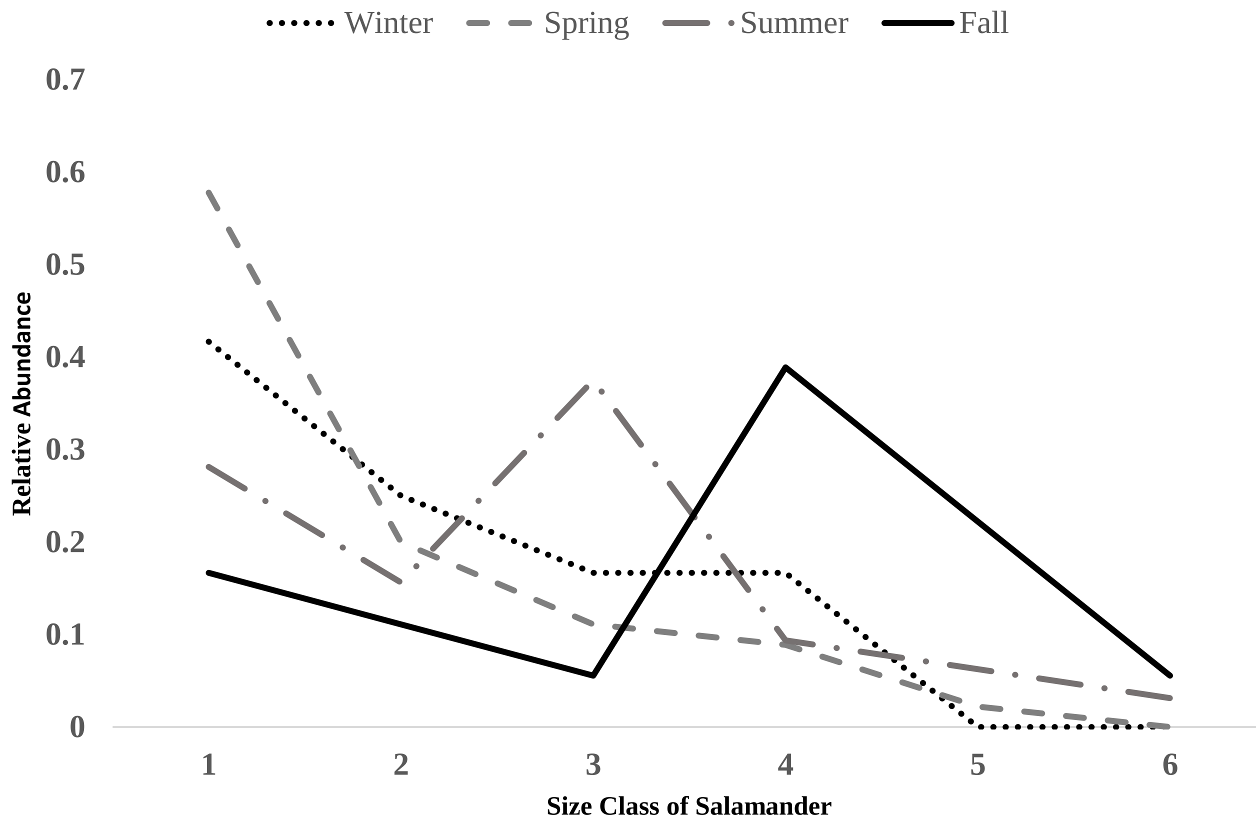


Figure 3. Relative abundance of size class for 107 Salado salamanders captured quarterly from 2015 - 2018 (1 = 10 - 19 mm; 2 = 20 - 29 mm; etc.).

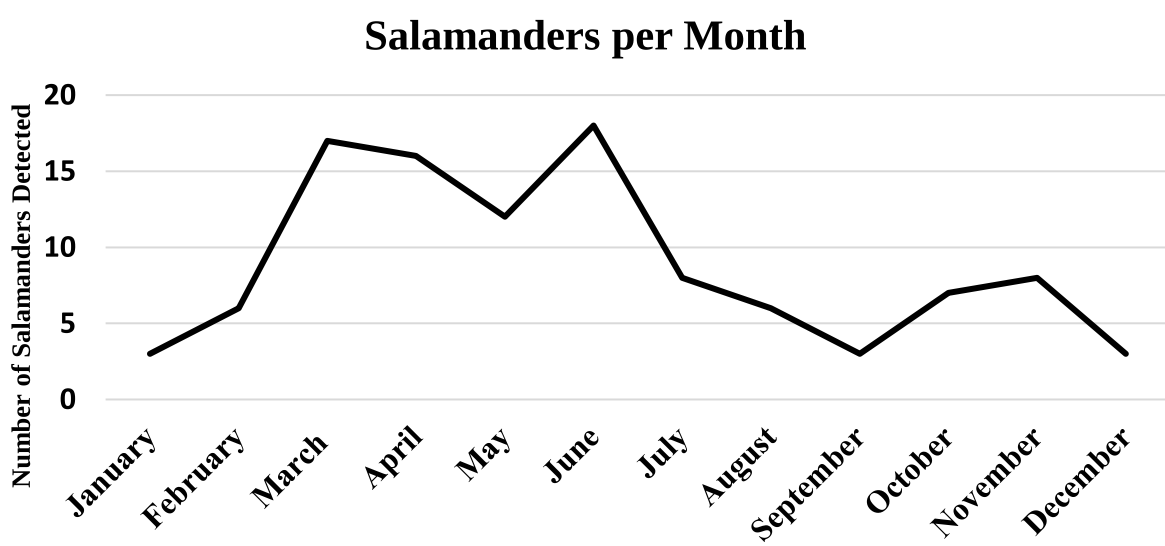


Figure 4. A cumulative depiction of when Salado salamanders are being caught using data from 2015 to 2018.

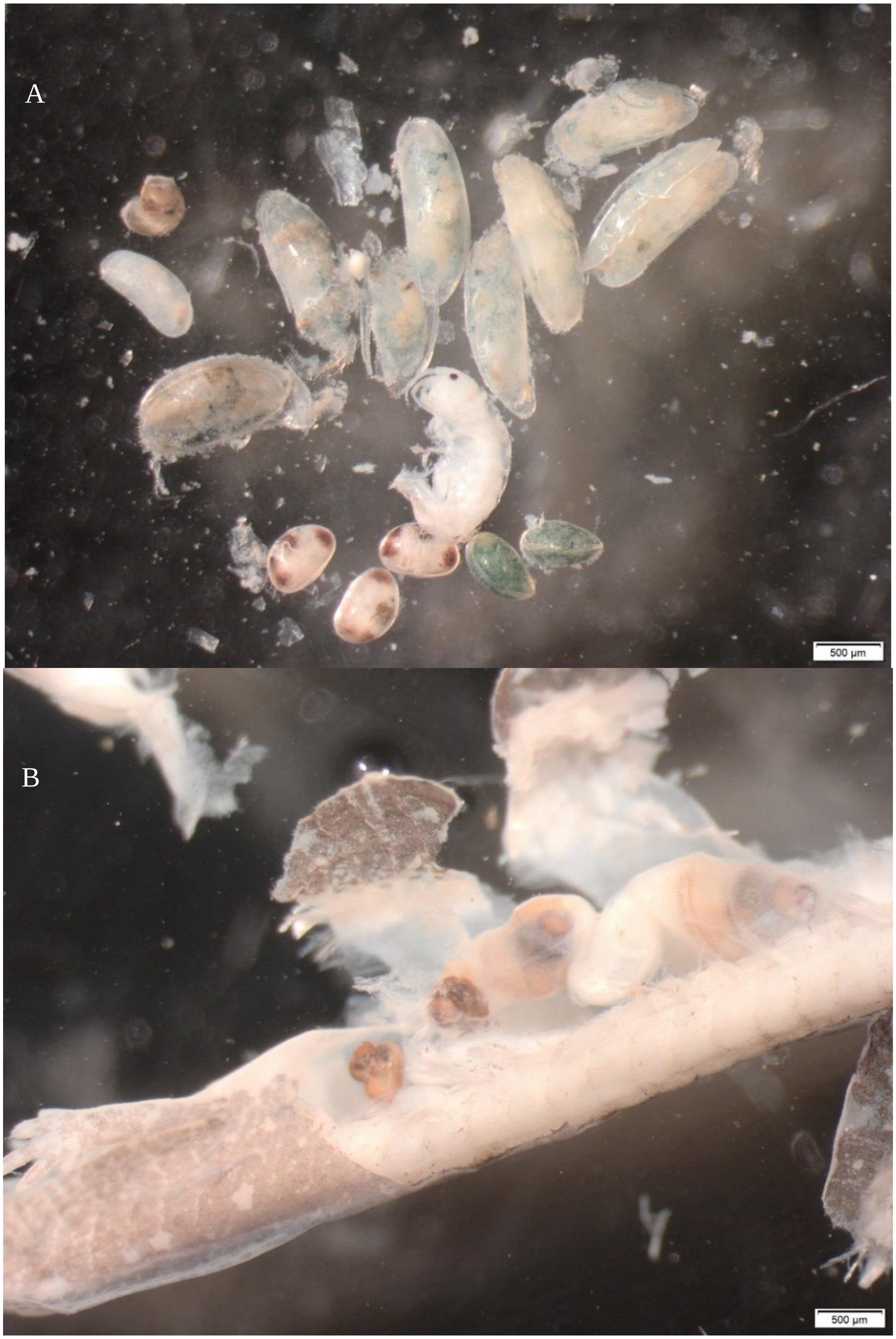


Figure 5. Gut contents of the Salado salamander. Photograph A shows dietary items pulled from a Salado salamander. Photograph B shows gastropods in the digestive tract of a Salado salamander.

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# Final Report
# 2019

# Salado Salamander Monitoring Final Report 2019


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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 designated 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 $1 - ((1-p1)(1-p2)(1-p3))$.

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 $m^3$/s; Figure 9). This average was followed by 2016 discharge in Salado Creek (89.1 $m^3$/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.

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** | **#** | **%** |
| ***Sagittaria* sp.** | 1 | 1.10 |
| ***Nasturtium* sp.** | 35 | 38.46 |
| **Filamentous Algae** | 4 | 4.40 |
| ***Ludwigia* sp.** | 3 | 3.30 |
| ***Amblystegium* sp.** | 5 | 5.49 |
| ***Hydrocotyle* 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 |
|---|---|---|
| ***Schornikovdona bellensis*** | Ostracoda | Okan Kulkoyluoglu |
| ***Pygmarrhopalites sp.*** | Collembola | Felipe Soto-Adames |
| ***Pseudosinella violenta*** | Collembola | Felipe Soto-Adames |
| **Undescribed Dytiscidae** | Coleoptera | Kelly Miller |
| **Uchidastygacarus sp.** | Acari | Ian Smith |
| **Chernetidae** | Pseudoscorpions | Charles Stephen |
| **Chthoniidae** | Pseudoscorpions | Charles Stephen |
| ***Cicurina* sp. (juvenile)** | Araneae | Marshal Hedin |
| **Speodesmus sp.** | Polydesmida | Paul Marek |
| **Lymantes nadineae** | 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 |

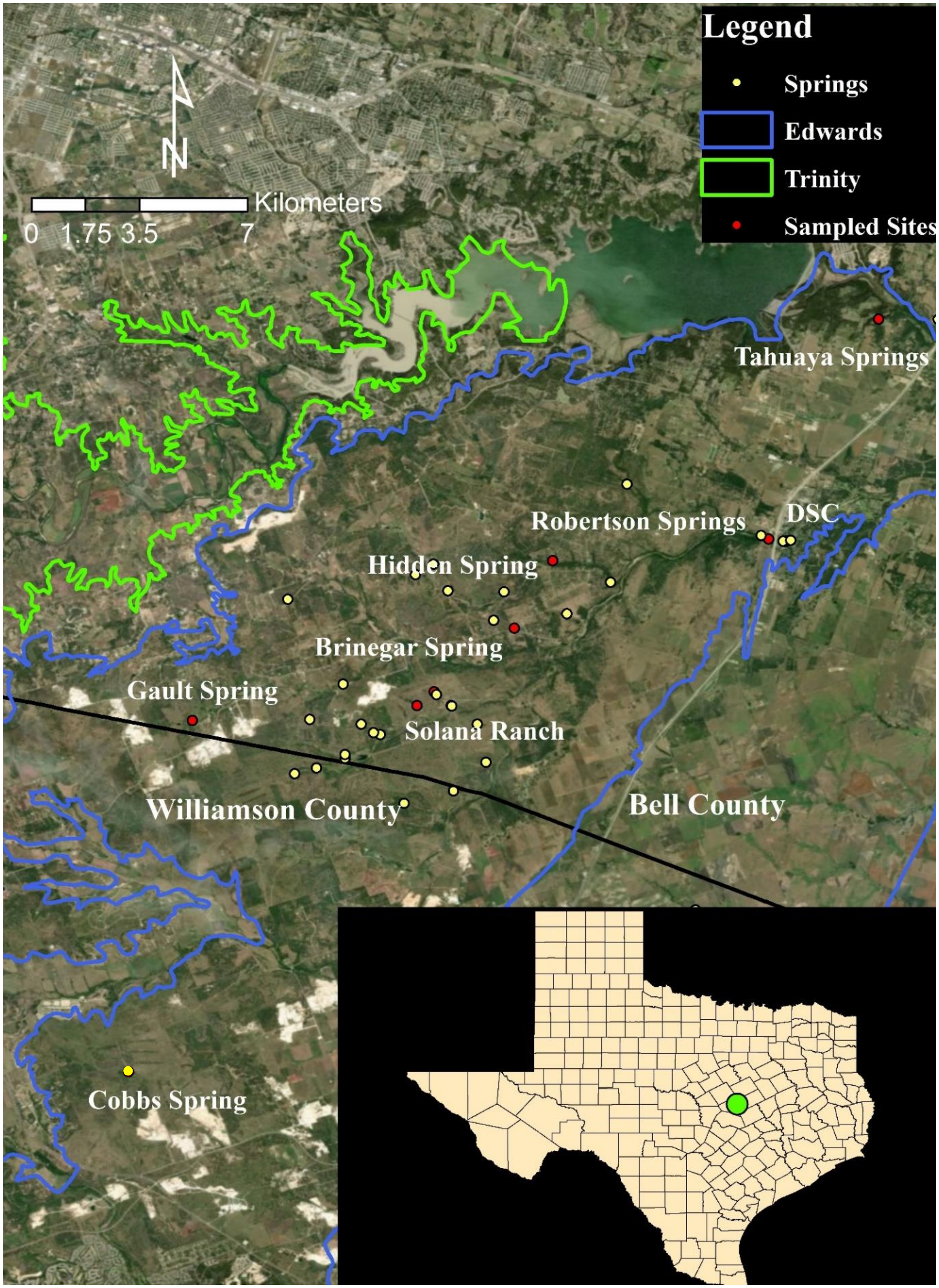


Figure 1. Known geographical range for Salado salamander and monitoring sites used in 2019. Yellow dots show mapped springs and red dots are areas sampled during this study.


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.

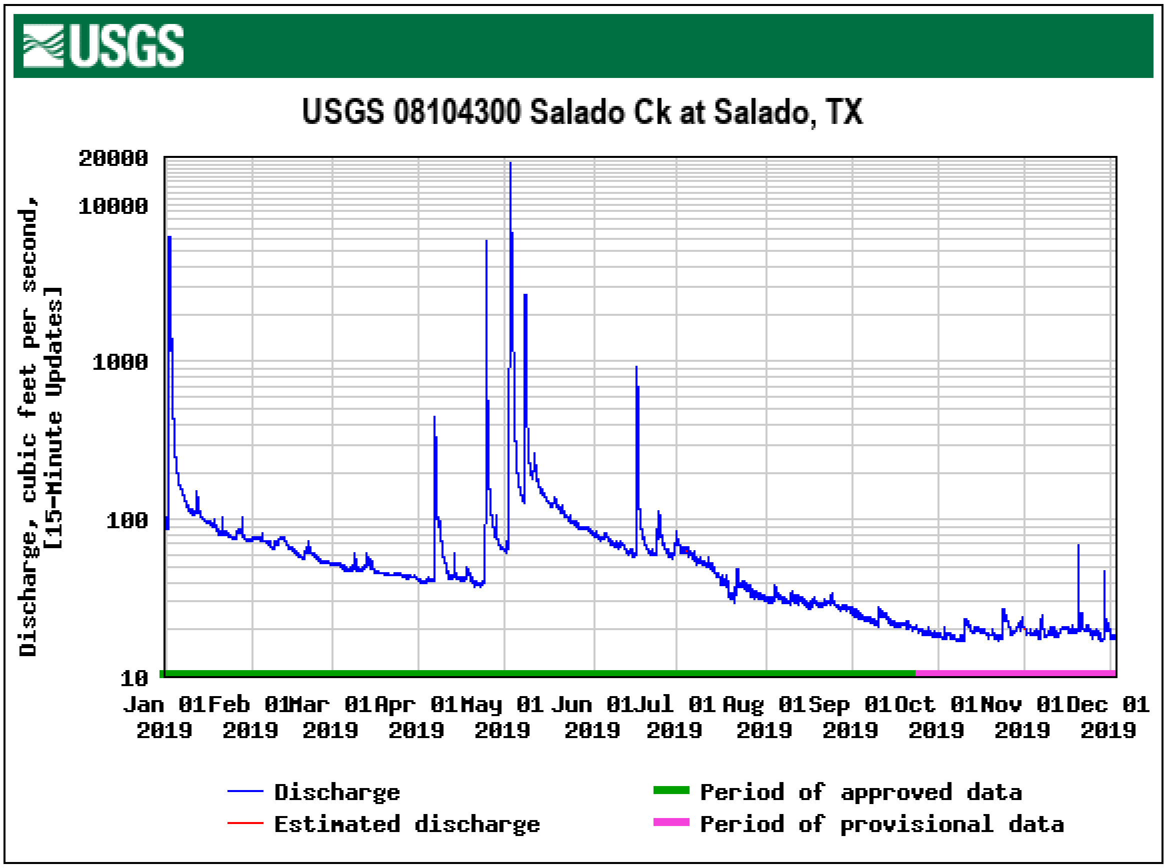


Figure 4. Hydrograph of Salado Creek for 2019.

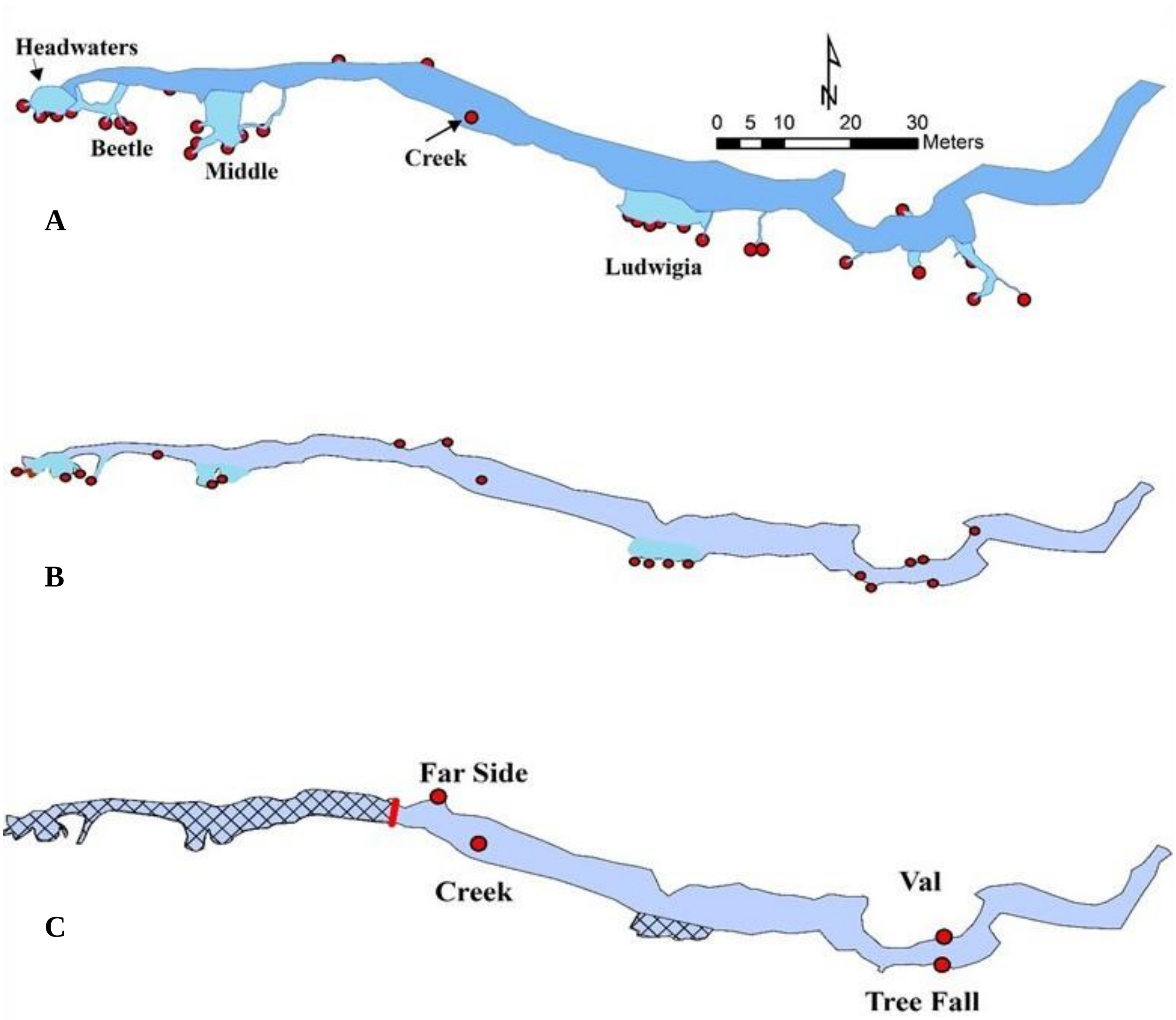


Figure 5. Robertson Springs starting in March of 2016 (A) into July of 2017 (B) and finally in April of 2018 (C). Hashed areas are places where there was no longer water. Red dots or lines are spring locations. Lighter blue sections are considered spring zones.

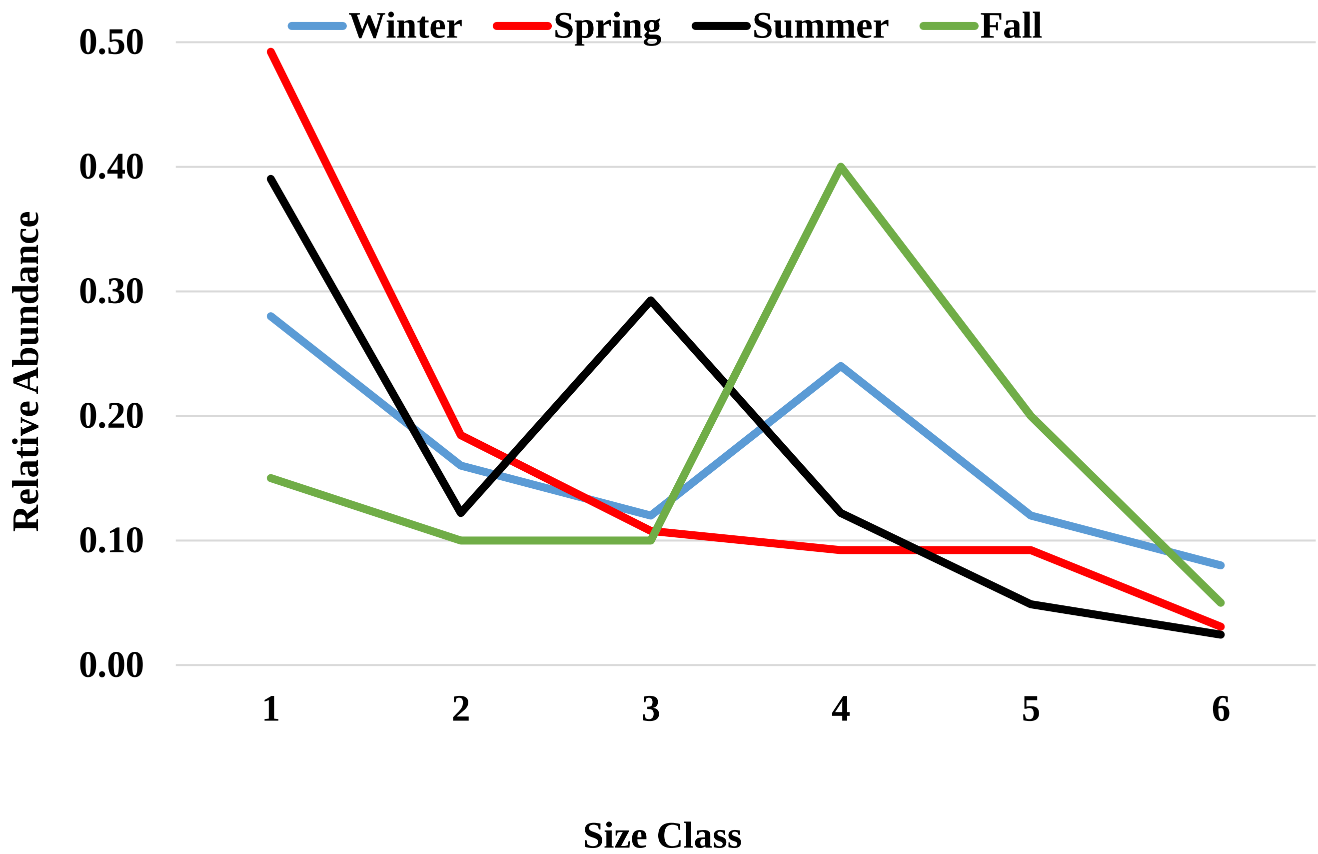


Figure 6. Relative abundance of size class for 151 Salado salamanders captured quarterly from 2015 - 2019 (1 = 10 - 19 mm; 2 = 20 - 29 mm; etc.).

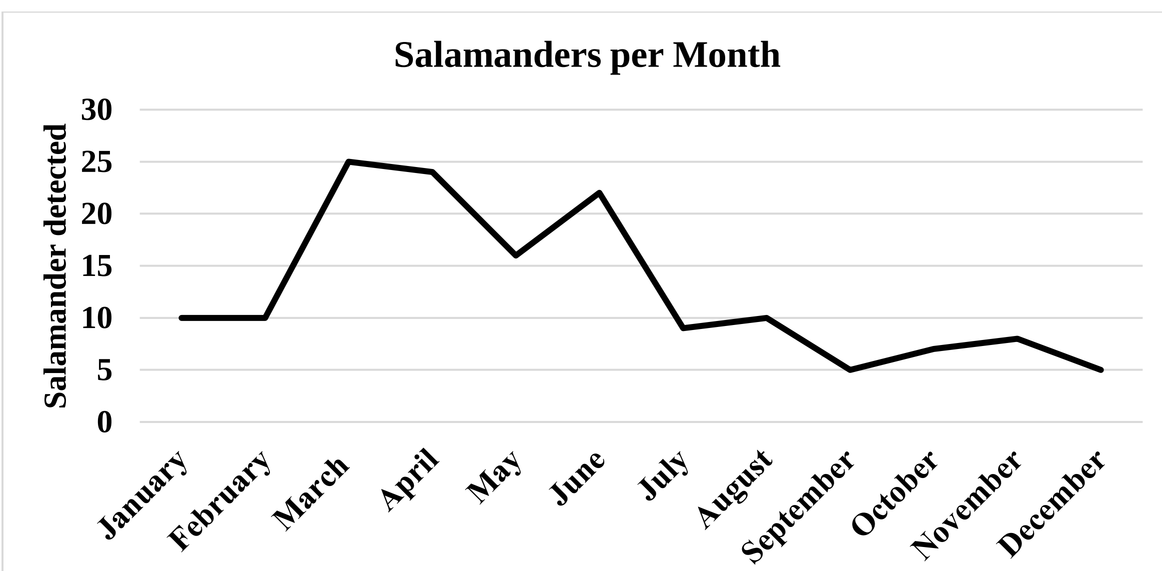


Figure 7. A cumulative depiction of when Salado salamanders are being caught using data from 2015 to 2019.

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