Salado Salamander Monitoring Final Report 2015



Peter Diaz and Mike Montagne Texas Fish and Wildlife Conservation Office



Randy Gibson San Marcos Aquatic Resource Center



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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

Salamanders

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.

| 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 |

Table 1. Salamanders captured from 2015 sampling events.

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.

| | | Big | Big Boiling | | Robertson July 2015 | | 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 |

| Table 2. | Habitat availabili | ty at selected | springs | within | the study | area. |
|----------|--------------------|----------------|---------|--------|-----------|-------|
| | | | | | | |

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.

| | 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 |
| рН | 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 |

Table 3. Average water quality data collected over 2015.

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 | Robertson Spring | Robertson | 1 st Quartile | 2 nd Quartile | 3 rd Quartile |
|-----------------------------------------|-----------------|------------------|-----------|-----------------------------|-----------------------------|-----------------------------|
| | 2015 | 2015 | 2014 | Quartic | Quartic | Quartic |
| 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.

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Literature Cited

- Bendik, N. & A. G. Gluesenkamp. 2012. Body length shrinkage in an endangered amphibian is associated with drought. Journal of Zoology 290 (1): 35-41.
- Chippindale, P. T., A. H. Price, J. J. Wiens, & D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. Heretological Monographs 14:1-80.
- Chippindale, P. T., R. M. Bonett, A. S. Baldwin, & J. J. Wiens. 2004. Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. Evolution 58:2809–2822.
- Connell, J. H. 1978. Diversity of tropical rainforests and coral reefs. Science 199:1304–1310.
- Diaz, P. H. 2010. Diet and mesohabitat association of the threatened San Marcos salamander (*Eurycea nana*). MS Thesis. Texas State University.
- Forstner, M. 2012. An evaluation of the existing scientific evidence for the currently proposed hyperdiversity of salamanders (*Eurycea* sp.) in central Texas. Unpublished report prepared for the Texas Salamander Coalition. June, 2012, 28 pp.
- Fries, J. N. 2002. Upwelling flow velocity preferences of captive adult San Marcos salamanders. North American Journal of Aquaculture 64:113-116.
- Hillis, D. M., D. C. Cannatella, T. J. Devitt, & A. M. Wright. 2015. Genomic assessment of taxonomic status of central Texas *Eurycea* salamanders. Section Six Grant Number 443022. *In Press*
- Lucas, L. K., Z. Gompert, J. R. Ott, & C. C. Nice. 2009. Geographic and genetic isolation in spring-associated Eurycea salamanders endemic to the Edwards Plateau region of Texas. Conservation Genetics 10:1309-1319.
- Norris, C, A. Gluesenkamp, J. Singhurst, & D. Bradsby. 2012. A biological and hydrological assessment of the Salado Springs complex, Bell County, Texas. Texas Parks and Wildlife Department Unpublished Report.
- Pardo, I, and P. D. Armitage. 1997. Species assemblages as descriptors of mesohabitats. Hydrobiologia 344:111-128.
- Pierce, B. A., K. D. McEntire, & A. E. Wall. 2014. Population size, movement, and reproduction of the Georgetown Salamander, *Eurycea naufragia*. Herpetological Conservation and Biology 9:137-145.
- Pyron, R. A., Wiens J. J. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61(2): 543-583. <u>http://dx.doi.org/10.1016/j.ympev.2011.06.012</u>
- Smith, B. A. and B. B. Hunt. 2010. A comparison of the 1950s drought of record and the 2009 drought, Barton Springs segment of the Edwards Aquifer, Central Texas. Gulf Coast Association of Geological Societies Transactions 60:611-622.

***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

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|-------------------------------|------|------|---------------------|--------------|
| 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 |

A1: List of contaminants from 2015 sampling season

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