U.S. Fish and Wildlife Service Research Permit
Annual Report 2015 for Permit TE52824B (Salado Salamander)

Submitted By:
Stephanie S. Wong, graduate student
Dr. Joe C. Yelderman Jr., Ph.D., P.G.

Baylor University, Department of Geology
One Bear Place #97354
Waco TX 76798

On behalf of:
Clearwater Underground Water Conservation District
700 Kennedy Court
Belton TX 76513

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BAYLOR UNIVERSITY

Clearwater Underground Water Conservation District
Every drop counts!

Background to research activities

This report describes research undertaken to better understand habitat of the Salado Salamander (*Eurycea chisolmensis*), the Salado Springs complex. All our work has been at the same site, therefore we did not need to decontaminate boots and other field gear between sampling. All our work was regarding water flow and quality, and did not involve the Salado salamander directly. No salamanders were encountered through the course of our research. All activities were conducted by Stephanie Wong and Joe Yelderman, or by field assistants under their supervision. Care was taken to minimize disturbances and to replace any cobbles or substrate that were disturbed.

Detailed accounts of our research activities in 2015 are described below. Locations for these activities are shown in Figure 1. Augering shallow piezometers in the alluvial point bar took place on April 2; work related to the dye trace test took place on April 13, 18, 19, and 27; and water samples were collected for radon-222 analysis on April 3, May 14, 17, and 26, July 27-30, and September 17-22.

Figure 1: Map showing precise locations of spring outlets, augering sites, and locations for dye trace monitoring and water quality sampling

Dye trace test

On April 18, 2015, a dye trace test using a single injection point and one fluorescent dye (uranine) was conducted to investigate relatively short groundwater flowpaths between the Stagecoach Inn Cave Well and springs in the Salado Springs complex. The trace tested the hypothesis that fractures like the one observable in the Stagecoach Inn Cave support specific groundwater flowpaths directly to specific springs and do not affect other springs in the area. All spring outlets, as well as other groundwater and surface water sites, were monitored along Salado Creek (Figure 2). Both passive and active sampling were employed to detect the presence or absence of dye at each monitor site. At 8:45 am on April 18, 2015, 74 g of uranine dye were introduced into the Stagecoach Inn Cave Well. Detection sites were sampled until 7 pm of the same day. Charcoal receptors were collected and replaced at 7 pm, collected and replaced again at 3 pm on April 19, 2015, and then collected on April 27, 2015.

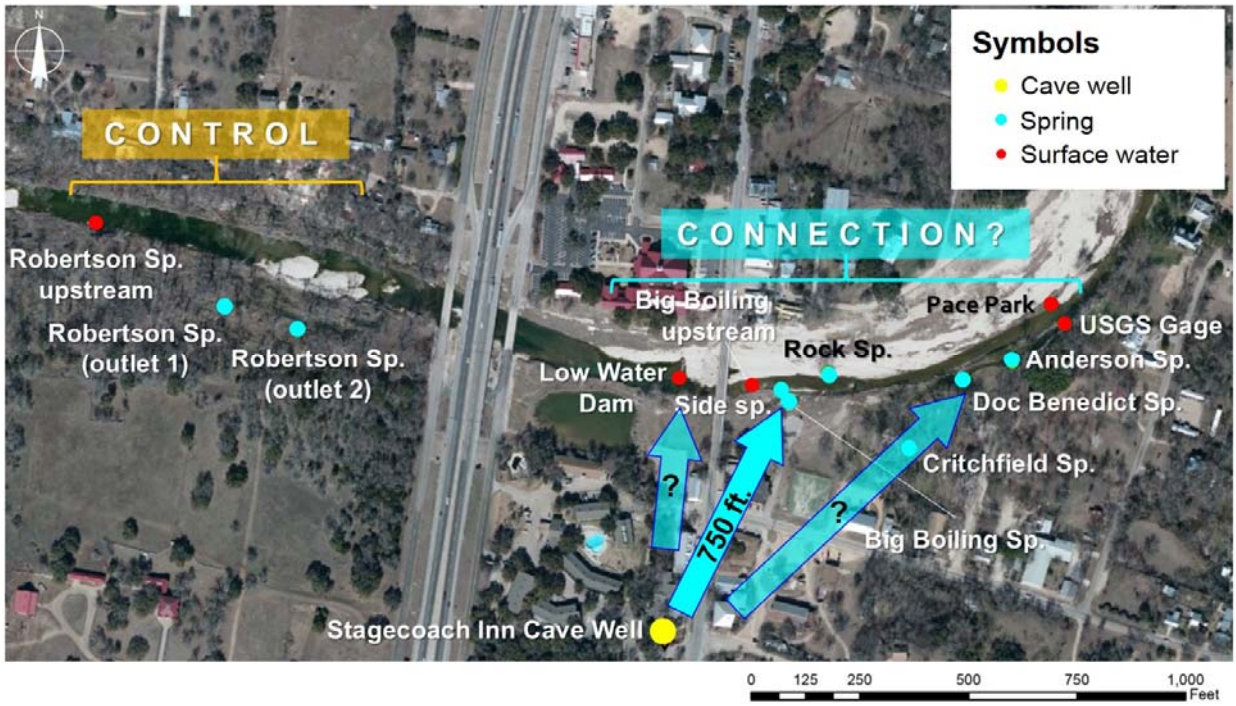

Figure 2: Conceptual overview of the Salado Springs dye trace test on April 18, 2015.

Monitoring sites

A series of groundwater and surface water monitoring sites were selected, including all the named springs in the Salado Springs complex: Robertson Springs, Big Boiling Spring, Little Bubbly Spring, Critchfield Spring, Doc Benedict Spring, and Anderson Spring. All the named springs were flowing and were monitored except Little Bubbly. Control sites included two outlets of Robertson Springs, Salado Creek upstream of Robertson Springs, immediately above the low-water dam between Main Street and Interstate Highway 35, and Salado Creek between the Main Street Bridge and Side Spring. In addition to the named springs of the complex, a groundwater seep ("Side Spring") and a groundwater discharge point on the north bank ("Rock Spring") were monitored. The USGS flow gage and Pace Park were also used as surface water monitoring sites.

Sampling

Sampling for presence of dye included both passive (charcoal receptors) and active (automated and manual grab sampling) methods. Charcoal receptors were placed at all monitoring sites a week before the test to assess

background concentrations of the tracer material and then also placed at each site during the test. During the test, one field assistant was assigned to manually sample each monitoring site. Big Boiling, Anderson, and Rock springs were sampled at 15 minute intervals; while Side Spring and all sites downstream of Big Boiling Spring were sampled at 1 hour intervals. Grab samples were also collected every time a charcoal receptor was collected. A control blank and field blank were collected every field day as quality control.

Lab preparation

An elution process was performed to analyze the level of dye picked up by the activated charcoal indicators. The packets of charcoal were air-dried and opened, and enough charcoal to fill the bottom of a plastic two-ounce Solo cup was removed from the packet. Fifteen milliliters of eluent, made up of a solution of 95% isopropyl alcohol and 5% potassium hydroxide, was added to the charcoal. After an hour of elution time, the eluate was poured into 10 ml glass vials for analysis.

Sample analysis

All samples were analyzed as continuous scans on a Perkin-Elmer LS-50B Luminescence Spectrometer. The resulting spectra are emission referenced with $\Delta\lambda$ of 15 nm, scan speed of 750 nm/min, scanning range from 401 nm to 650 nm, and a 6.0 nm slit. Samples that contained dye concentrations exceeding the analysis limit of the fluorimeter were first analyzed as-is. Following the initial analysis, the samples were diluted to a level that could be detected by the fluorimeter. The spectra produced were then normalized to 100% for comparison with the rest of the data.

All spectra were fitted using Fityk (version 0.9.8) curve fitting and data analysis program (Wojdyr, 2010). The spectra were fitted using Pearson Type VII functions. Peak fluorescence intensity values were converted to dye concentration in parts per billion (ppb) by creating a linear regression with the peak intensities of standards and their corresponding concentrations. The peak concentration for samples with quantifiable detections were plotted against time elapsed to construct breakthrough curves.

Results

Dye was detected at all monitoring sites downstream of Big Boiling Spring, and was not detected at any upstream monitoring sites (Figure 3). Peak concentrations and detection times for all monitoring sites are summarized in Table 1. Seventy-four grams of uranine dye was introduced to the Stagecoach Inn Cave Well. Visually, uranine dye was detected at Big Boiling, Side, Doc Benedict, and Anderson springs.

Groundwater velocities were estimated by dividing the distance between the injection site and each spring by the first and peak detection times for uranine. The average groundwater velocity between the injection site and Big Boiling Spring was determined to be 0.0676 m/s. The average groundwater velocity from the injection site to Anderson Spring and Rock Spring were 0.0526 m/s and 0.0429 m/s respectively. The first and peak detection times, and calculated groundwater velocities for the traces are summarized in Table 2.

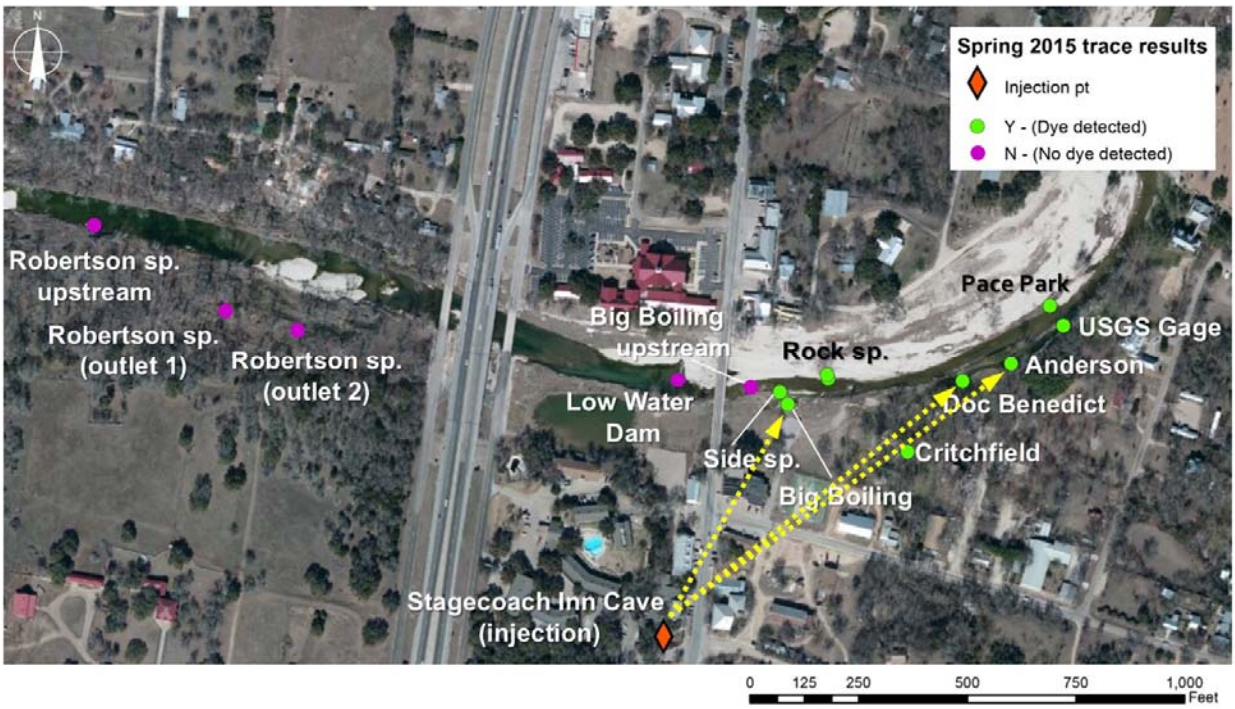

Figure 3: Results of the spring 2015 dye trace test. Purple dots indicate locations of no dye detection. Green dots indicate spring and creek locations where uranine was detected. Arrows represent confirmations of groundwater flow between the injection point at Stagecoach Inn Cave Well and a spring.

Discussion

Results of the dye tracer test at Salado Springs confirmed a previous tracer test (Mahler et al, 1998) and the potential that anecdotal stories might be true regarding flowpaths between the Stagecoach Inn Cave Well and Big Boiling Spring. The test showed that groundwater flows freely between the injection point in the Stagecoach Inn Cave Well and the major springs along Salado Creek in the downtown area, demonstrating excellent communication between groundwater in all the flowing springs in the study area. The tracer tests revealed a spring system where the series of major springs in the downtown Salado area (with the exception of Robertson Spring) were interconnected to each other under flow conditions like those on April 18, 2015.

Flow was hypothesized to be toward Salado Creek with a downstream component and this appears correct as no dye was detected in the three upstream sites but was detected in all the downstream sites on both tracer tests. The dye reached Big Boiling Spring first and the amount was greater than at other sites except for Anderson Spring. Anderson Spring had a very strong showing of dye that appeared to be related to its strong discharge flow rate. Dye reached Anderson Spring later than Big Boiling Spring presumably because of a greater distance from the injection point. Results of the tracer test suggest that on this localized scale of several hundred meters, the springs are interconnected hydrogeologically and act as one system interacting with Salado Creek.

Table 1. Peak uranine concentrations and detection times for grab samples collected along Salado Creek on April 15, 2015. No dye detection at a given site is indicated by "ND" ("No detect").

Site	Peak concentration (ppb)	Peak time (hh:ss)
Robertson Spring	ND	--
Low water dam lake	ND	--
Salado Creek *(Main St. bridge to Side Spring)*	ND	--
Little Bubbly Spring	Dry spring	--
Side Spring	>1000 intensity units†	9:46
Big Boiling Spring	>1000 intensity units†	10:00
Critchfield Spring	8.14*	15:40
Doc Benedict Spring	>1000 intensity units†	10:44
Anderson Spring	>1000 intensity units†	11:35
USGS gage	10.96*	14:53
Rock Spring *(North bank)*	>1000 intensity units†	11:30
Pace Park	16.54*	15:02

** indicates peak concentrations that were also first detections.*
†denotes a sample with dye concentration that exceeded the detection limit of the fluorimeter. These samples are being re-analyzed.

Table 2. Groundwater velocity determined at Big Boiling, Anderson, and Rock springs at first and peak detection times. The injection point is 747 feet (228 meters) from Big Boiling Spring, 1258 feet (384 meters) from Anderson Spring, and 869 feet (265 meters) from Rock Spring.

	Big Boiling		Anderson		Rock	
	Time (h)	Velocity (m/s)	Time (h)	Velocity (m/s)	Time (h)	Velocity (m/s)
First detection	0.75	0.0844	1.58	0.0675	1.25	0.0589
Peak detection	1.25	0.0507	2.83	0.0377	2.75	0.0268
Average velocity	--	*0.0676*	--	*0.0526*	--	*0.0429*

Water quality: Radon-222

All the named springs in the Salado Springs complex were sampled to characterize the radon concentration of groundwater and monitor for seasonal change. Water was also collected at Main Street Bridge to characterize the radon concentration of surface water entering the Salado Springs system at downtown Salado. The complex was sampled in its entirety over several-day campaigns during May, July, and September 2015.

Methodology

Water samples analyzed for Rn-222 were collected using air-dried 250 ml glass bottles with septum caps. Each bottle was triple-rinsed with sample water before sample collection. Where possible, samples were collected by completely immersing the bottles in the stream or spring discharge and, after the bottle had filled completely, capped underwater to avoid aerial exposure. Samples were collected with no headspace. For stream samples, water was collected from the thalweg of the channel. Spring samples were collected as close to the point of discharge as possible. After collection, water samples were placed in a cooler for insulation from temperature fluctuations and protection during transport.

Water samples were analyzed within 24 hours of collection to minimize loss of Rn-222 through radioactive decay. The activity of dissolved Rn-222 in each sample was measured using a RAD7 unit equipped with a RAD H_2O radon-in-water accessory (DURRIDGE Company, Inc., Billerica, Massachusetts). RAD7 results were corrected to account for radon activity decline due to radioactive decay from the time of sampling to analysis. The decay correction factor (DCF) was determined for each sample. Between every sample, the RAD7 was purged for a minimum of 15 minutes to flush the instrument of residual radon and lower the internal relative humidity to 6% or less. Also, a blank was measured between every sample to keep track of background radon levels.

Results

Average radon-222 concentrations for groundwater and surface water are summarized in Table 3 for all sampling campaigns. Radon-222 concentrations in groundwater samples were consistently greater than those of surface water, which agrees with published research. Generally, a 2-4 times difference in surface and groundwater radon-222 concentrations have been reported (Burnett et al., 2010). Radon-222 concentrations just above and below the low water dam shows the effect of aeration to expedite diffusion of radon into the atmosphere, resulting in a lower concentration immediately below the low water dam. Low radon-222 content at the Main Street Bridge is indicative of surface water which has had opportunity to de-gas its radon-222, while water sampled from a spring orifice would not have had time for gas exchange with the atmosphere (Cook et al., 2003). The radon-222 concentration for Salado Creek just downstream from the Big Boiling Spring confluence is an intermediate value between Main Street Bridge and Big Boiling Spring, suggesting a mixing of surface and groundwater at that location. Variations in groundwater radon-222 concentrations likely reflect differences in flowpath through the aquifer and degree of water-rock interaction.

Table 3. Average radon-222 concentrations in pCi/L for groundwater and surface water in the Salado Springs complex for sampling campaigns in 2014 and 2015.

	May 14-26, 2015	July 27-30, 2015	September 17-22, 2015
Groundwater	257.25	244.56	262.10
Surface water	n/m*	124.87	167.60

**n/m = not measured*

Piezometer installation

Piezometers were used to investigate groundwater flow in the gravel point bar on the north bank of Salado Creek. Five shallow piezometers were placed in the alluvial point bar on April 2, 2015 (Figure 1). They were excavated by hand with glove and a trowel, and we were careful to look for aquatic organisms. Water samples were collected from each piezometer using a smaller bailer on April 3, 2015 and analyzed for radon-222. Results are shown in Table 4. The piezometers were buried and/or washed away in the flooding in May 2015. We have not replaced them to date.

Table 4. Concentrations of Rn-222 in the alluvial point bar at Salado Creek.

Piezometer	Rn-222 concentration (pCi/L)
1	46.48
2A	125.94
2B	69.48
2C	170.51
3A	259.31

References

- Burnett, W.C., R.N. Peterson, I.R. Santos, and R.W. Hicks, 2010. Use of automated radon measurements for rapid assessment of groundwater flow into Florida streams. Journal of Hydrology, 380(2010): 298-304.

- Cook, P.G., G. Favreau, J.C. Dighton, and S. Tickrell, 2003. Determining natural groundwater influx to a tropical river using radon, chlorofluorocarbons and ionic environmental tracers. Journal of hydrology, 277(1-2): 74-88.

- Mahler, B.J., P.C. Bennet, M. Zimmerman, 1998, Lanthanide-labeled clay: a new method for tracing sediment transport in karst. Ground Water, 36(5): 835-843.

- Wojdyr, M. 2010. Fityk: a general-purpose peak fitting program. Journal of Applied Crystallography, 2010 (43): 1126-1128.