City of Austin, Watershed Protection Dept.
2021 Annual Report
U.S. Fish and Wildlife Service Scientific Permit (TE-833851)

Reporting period: November 19, 2020 through November 18, 2021
This report documents activities involving Barton Springs, Austin Blind, and Jollyville Plateau salamanders (*Eurycea sosorum*, *E. waterlooensis*, and *E. tonkawae*, respectively) and karst invertebrates.

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General Annual Reporting Requirements for Barton Springs and Austin Blind salamanders

1) Precise locations of previously undocumented surveyed areas
None.

2) Dates of surveys conducted
Please see # 4, below.

3) Survey methods
Barton Springs and Austin Blind salamander counts were conducted quarterly throughout the year at Parthenia, Eliza, Old Mill (Sunken Gardens) and Upper Barton springs. For each survey, the date, weather, type of flow (base flow or storm flow) and aquifer discharge are recorded by the U.S. Geological Survey station at Parthenia Spring. Additionally, discharge and water quality measurements are taken at each of the other springs. Photographs of substrate are taken to measure relative sediment cover and embeddedness and rough estimates of fish abundance (by species) within or near the habitat are made before each survey. Each site was searched using a drive survey method where all non-embedded substrate is searched, except for at Old Mill Spring, where a timed survey is used due to the low abundance of salamanders at that site. Every individual salamander found was identified to species and categorized by an estimate of total length (<25 mm, 25–50 mm, >50mm) or measured from photographs. Photographic capture-recapture surveys were performed at all sites except Parthenia Spring. Salamanders are captured using small handheld dip nets or basters, photographed, and released as soon as possible, usually within 1–4 hours. The total number of salamanders of each species and size class found were recorded, although we only present the totals below.

4) Survey results
Salamander counts from 2021 surveys are presented in Table 1 below. Tallies include the number of individuals captured plus the estimated number of those missed. Surveys in Barton Springs Pool were performed by visual count only, without capturing salamanders. Environmental data are presented in Table 2 and fish data in Figure 2.

Figure 1: One salamander egg (to the right of and below the clam shell) was found while conducting a quarterly survey in Barton Springs Pool on November 18, 2021.

Table 1. Barton Springs and Austin Blind salamander counts from 2021 surveys. Tallies include individuals captured and photographed (sites at Eliza, Old Mill and Upper Barton) plus individuals observed but not captured (all sites).

Site	**Date**	**Number *E. sosorum***	**Number *E. waterlooensis***
Barton Spring	2/24/2021	163	0
Barton Spring	7/1/2021	9	0
Barton Spring	9/2/2021	32	0
Barton Spring	11/18/2021	44	0
Eliza Spring	3/2/2021	159	0
Eliza Spring	3/5/2021	138	0
Eliza Spring	3/10/2021	194	0
Eliza Spring	5/11/2021	274	0
Eliza Spring	5/13/2021	265	0
Eliza Spring	5/20/2021	220	0
Eliza Spring	8/20/2021	73	0
Eliza Spring	8/23/2021	58	0
Eliza Spring	8/26/2021	68	0
Eliza Spring	11/1/2021	54	0
Eliza Spring	11/5/2021	41	0
Eliza Spring	11/8/2021	44	0
Eliza Stream (outflow from Eliza Spring)	3/2/2021	101	0
Eliza Stream (outflow from Eliza Spring)	3/5/2021	81	0

Site	Date	Number *E. sosorum*	Number *E. waterlooensis*
Eliza Stream (outflow from Eliza Spring)	3/10/2021	155	0
Eliza Stream (outflow from Eliza Spring)	5/11/2021	153	0
Eliza Stream (outflow from Eliza Spring)	5/13/2021	98	0
Eliza Stream (outflow from Eliza Spring)	5/20/2021	96	0
Eliza Stream (outflow from Eliza Spring)	8/20/2021	125	0
Eliza Stream (outflow from Eliza Spring)	8/23/2021	125	0
Eliza Stream (outflow from Eliza Spring)	8/26/2021	123	0
Eliza Stream (outflow from Eliza Spring)	11/1/2021	91	0
Eliza Stream (outflow from Eliza Spring)	11/5/2021	80	0
Eliza Stream (outflow from Eliza Spring)	11/8/2021	76	0
Old Mill (Sunken Gardens) Spring	2/23/2021	1	0
Old Mill (Sunken Gardens) Spring	6/4/2021	4	0
Old Mill (Sunken Gardens) Spring	8/30/2021	3	0
Old Mill (Sunken Gardens) Spring	11/9/2021	5	0
Old Mill Stream (outflow from Sunken Gardens Pool)	2/23/2021	0	0
Old Mill Stream (outflow from Sunken Gardens Pool)	8/30/2021	3	0
Old Mill Stream (outflow from Sunken Gardens Pool)	11/9/2021	0	0
Upper Barton Spring	7/2/2021	1	0
Upper Barton Spring	8/30/2021	3	0
Upper Barton Spring	11/9/2021	6	0

Table 2. Environmental data for Barton Springs and Austin Blind salamander surveys from 2021. Data for Barton Springs Pool (Parthenia Spring) are recorded by the **USGS**.

Site	Date	Flow (ft^3/s)	Conductivity ($\mu S/cm$)	Dissolved oxygen (mg/l)	pH	Water Temp (°C)
Eliza Spring	Mar-21	3.62	702	5.50	7.12	21.19
Eliza Spring	May-21	6.71	657	5.64	6.93	21.57
Eliza Spring	Aug-21	12.87	668	5.86	6.93	22.77
Eliza Spring	Nov-21	13.21	663	5.65	7.00	22.20
Old Mill (Sunken Gardens) Spring	Feb-21	2.72	793	5.26	6.91	21.48
Old Mill (Sunken Gardens) Spring	Jun-21	N/A	717	5.36	6.99	22.02
Old Mill (Sunken Gardens) Spring	Aug-21	6.65	739	5.25	7.03	22.18
Old Mill (Sunken Gardens) Spring	Nov-21	8.82	726	5.73	7.02	21.61
Upper Barton Spring	Feb-20	Dry				
Upper Barton Spring	Jul-21	1.0	639	6.68	6.94	21.72
Upper Barton Spring	Aug-21	0.87	643	6.72	7.02	21.91
Upper Barton Spring	Nov-21	1.85	632	N/A	6.95	N/A

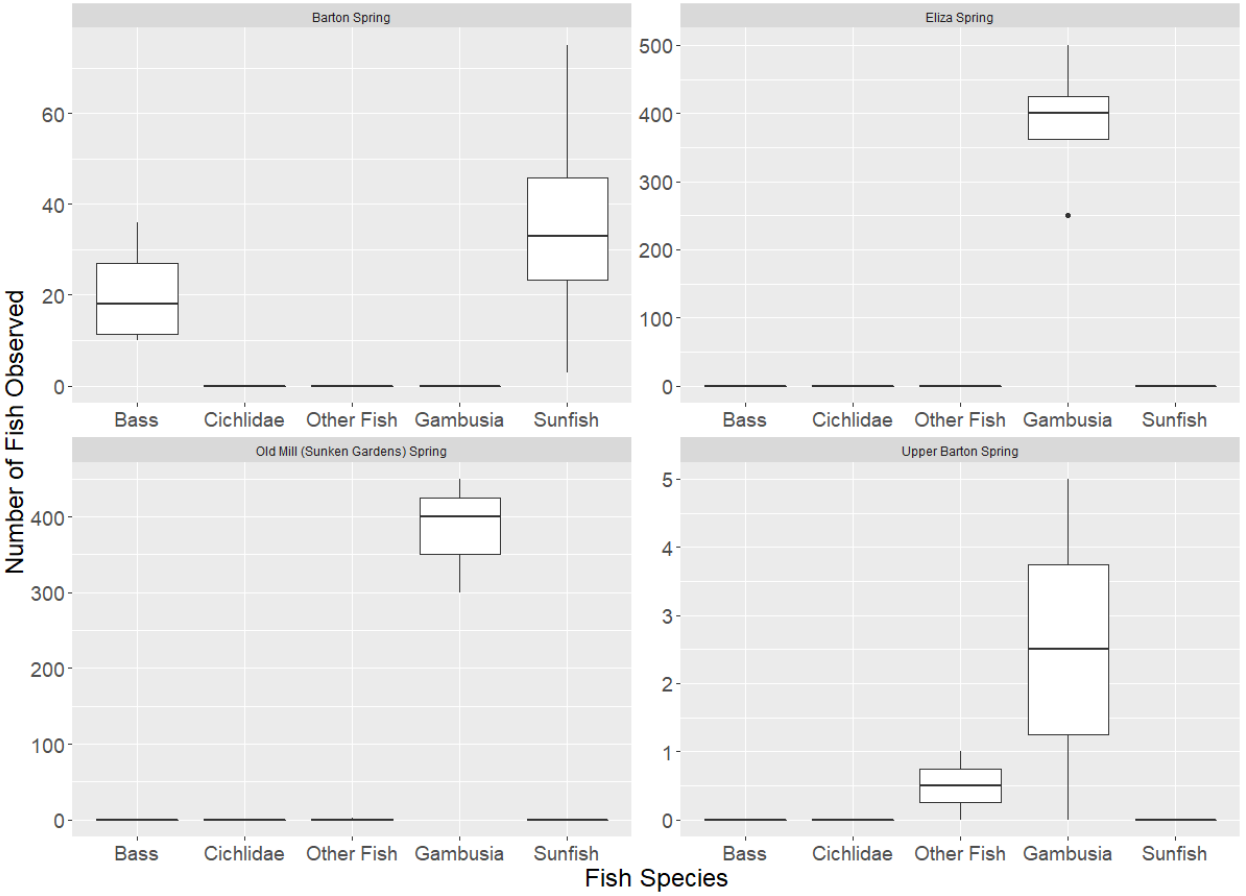

Figure 2: Box plots of fish counted during quarterly surveys. No fish were present in Old Mill Stream. "Other Fish" found at Old Mill were stonerollers and "Other Fish" found at Upper Barton Spring were ciprinids, likely stonerollers.

5) Species ID by Taxonomist
Not applicable.

6) Number of salamanders collected from the wild
Salamanders collected from the wild (salvaged from surveys, drift nets, or collected alive for captive propagation) are presented in Table 3, below.

7) Results of species identifications
See Table 1, above.

8) Number of salamanders handled and marked with elastomers
None.

9) Observations of abnormal behavior or condition of salamanders handled/marked
We periodically encounter animals with injuries while doing surveys. Sometimes injuries are caused by our survey activities, but it is not always obvious when this occurs. Sometimes injuries are obviously caused by other animals, possibly crayfish, that are abundant in their habitat. Figure 3 shows one example of any injury likely caused by a predator.

Figure 3. Injured *Eurycea sosorum* found during a survey this year in Eliza stream.

Table 3. Salamanders collected from the wild. Salvaged individuals were killed or injured during surveys, or otherwise found dead. Individuals that were collected alive were done in accordance with the City's captive population management plan.

Species	Date	Site	Number Collected	Disposition	Method of Collection	Notes
Eurycea sosorum	3/10/2021	Eliza Spring	3	Alive at captive breeding (ASCC)	capture-mark-recapture	3 juveniles < 1.5"TL
Eurycea waterlooensis	3/12/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	1 < 1" TL mortality ~5/5/2021
Eurycea waterlooensis	3/12/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL, Mortality ~4/17/2021
Eurycea waterlooensis	3/19/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL mortality ~6/7/2021
Eurycea sosorum	5/11/2021	Eliza Spring	1	Preserved in 95%+ EtOH	capture-mark-recapture	<1" TL When getting photographed the juvenile appeared to be dead or almost dead
Eurycea sosorum	5/13/2021	Eliza Spring	1	Preserved in 95%+ EtOH	capture-mark-recapture	<1" TL Looks like it was injured. When getting photographed the juvenile appeared to be dead.
Eurycea sosorum	5/25/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in Net #3
Eurycea sosorum	7/1/2021	Barton Springs Pool	4	Alive at captive breeding (ASCC)	net	all 1-2" TL
Eurycea sosorum	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found caught in drift net #11 which is a floor vent. It actually had it's head stuck in the mesh on the side of the "tee" in the PVC. It could have been alive but after a while did not seem alive and would have been very hard to remove from the mesh without injuring it. Changed drift net design after this.
Eurycea sosorum	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in Net #5 which is a floor vent.
Eurycea waterlooensis	8/4/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL. Found in Net # 3 which is a side vent. It was not doing well when found, close to dead, but took to CB to see if it would recover. It later was dead and preserved.
Eurycea waterlooensis	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	It looks like waterlooensis but might be sosorum? <1" TL. Found dead in Net #8 (downstream) in the initial part of the vent net along the seam.

Species	Date	Site	Number Collected	Disposition	Method of Collection	Notes
Eurycea waterlooensis	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in the morning in Net #8 (downstream). Ruben Tovar took specimen to his lab and took a tail tip and ran qPCR. Returned specimen to us and his qPCR confirmed it should be waterlooensis.
Eurycea sosorum	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	1"-2" TL. Found dead in Net #9. Only time we have found a larger individual dead in the drift nets. Looked possibly like it had been for a little bit, partially decaying maybe? Ruben Tovar took specimen to his lab and took a tail tip and ran qPCR. Returned specimen to us and his qPCR confirmed it should be sosorum.
Eurycea sosorum	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in Net #5. Ruben Tovar took specimen to his lab and took a tail tip and ran qPCR. Returned specimen to us and his qPCR confirmed it should be sosorum.
Eurycea sosorum	8/4/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in Net #9 with the dead larger sosorum. Ruben Tovar took specimen to his lab and took a tail tip and ran qPCR. Returned specimen to us. His qPCR confirmed it should be sosorum.
Eurycea waterlooensis	8/6/2021	Eliza Spring	1	Alive at captive breeding (ASCC)	drift net	<1" TL; collected from vent #8 upstream net
Eurycea waterlooensis	8/6/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1"TL; collected barely alive from drift net, died within ~an hour. collected from upwelling #5.
Eurycea waterlooensis	8/6/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL; collected from side vent #9; collected barely alive from drift net and died ~an hour later.
Eurycea waterlooensis	8/10/2021	Eliza Spring	1	Alive at captive breeding (ASCC)	drift net	<1" TL; collected from vent #2
Eurycea waterlooensis	8/11/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL; collected from vent #2; PM collection. On 8/14, salamander ventral side up, heart beating but maybe swollen; on 8/16, salamander dead.
Eurycea waterlooensis	8/11/2021	Eliza Spring	1	Alive at captive breeding (ASCC)	drift net	<1"TL; collected from vent #2, AM collection.

Species	Date	Site	Number Collected	Disposition	Method of Collection	Notes
Eurycea waterlooensis	8/13/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL; collected from side vent #2; collected dead and mangled after net out for ~2.5 hours.
Eurycea waterlooensis	8/13/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1"TL; collected from floor vent #5; could see some blood in heart but not flowing and heart not beating.
Eurycea sosorum	8/19/2021	Zara Well	1	Alive at captive breeding (ASCC)		On 8/19/21, we received a call from Rachel with Zara saying that they caught a salamander in their bottle trap in their well (~100' below the water surface) and Paige Najvar wants them to give it to us. So, we picked it up and set it up at ASCC. Salamander ~1.75" TL.
Eurycea sosorum	8/19/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	<1" TL, tiny, may even still have some of its yolk sac. Sal collected with major injuries to lower abdomen, placed in tank and died 8/20/21. Found in vent #18.
Eurycea waterlooensis	8/19/2021	Eliza Spring	1	Preserved in 95%+ EtOH	drift net	<1" TL. Found dead in Net #9. Checked net after about 2 hours.
Eurycea sp.	8/26/2021	Eliza Spring	1	Alive at captive breeding (ASCC)		1-2" TL, collected during survey
Eurycea sosorum	8/26/2021	Eliza Spring	1	Preserved in 95%+ EtOH	capture-mark-recapture	~1" TL. While conducting the survey it was found in the amphitheater pool and looked dead already. Collected and photographed and then preserved.
Eurycea sosorum	9/2/2021	Barton Springs Pool	3	Alive at captive breeding (ASCC)	net	2 @ ~1.25"TL 1 @ ~1.5"TL
Eurycea waterlooensis	10/26/2021	Eliza	1		drift net	<1/2" TL salamander found alive in drift net, died within ~30 min. Gave specimen to Ruben Tovar for research project.
Eurycea sosorum	10/28/2021	Eliza Spring	1	Preserved in 70% EtOH	drift net	~11mm TL. Specimen was not noticed when collecting samples from drift nets but later noticed by Texas State staff when sorting the sample in the lab. Texas State Schwartz lab kept the specimen.
Eurycea sosorum	11/5/2021	Eliza Spring	1	Preserved in 70% EtOH	capture-mark-recapture	<1" TL. Found dead when photographing individuals for survey. Collected in amphitheater.

10) Results of any mark-recapture work

We performed capture-recapture surveys at three sites in 2021 using photographic identification methods (Bendik et al. 2013). Not enough recaptures were made at Upper Barton or Old Mill springs, so we are unable to calculate estimates of abundance at those sites. One individual was recaptured between August and November at Upper Barton, but no recaptures were recorded at Old Mill.

We conducted robust-design mark-recapture sampling at Eliza Spring in March, May, August, and November. Abundance estimates are provided in Table 4 and Figures 4 and 5. Estimates were generated from a hierarchical closed-population model using parameter-expanded data augmentation, modified from Kéry and Schaub[1]. The model included site, size-class and temporal effects for each sampling period; parameters were estimated using Bayesian analysis in MultiBUGS. Estimates were updated compared to our prior report using a slightly different model, which excluded individual random effects and explicitly modeled site effects on detection. This new approach resulted in some differences in the abundance estimates.

Results from the first four years of capture-recapture data at Eliza Spring have now been published in a peer-reviewed journal. In this article, we examined the probably and frequency of movement of *E. sosorum* between the surface and underground habitats. The abstract is reproduced below.

> Movement behavior is an important aspect of animal ecology but is challenging to study in species that are unobservable for some portion of their lives, such as those inhabiting subterranean environments. Using four years of robust-design capture-recapture data, we examined the probability of movement into subterranean habitat by a population of endangered Barton Springs salamanders (*Eurycea sosorum*), a species that inhabits both surface and subterranean groundwater habitats. We tested the effects of environmental variables and body size on survival and temporary emigration, using the latter as a measure of subterranean habitat use. Based on 2,046 observations of 1,578 individuals, we found that temporary emigration was higher for larger salamanders, 79% of which temporarily emigrated into subterranean habitat between primary sampling intervals, on average. Body size was a better predictor of temporary emigration and survival compared to environmental covariates, although coefficients from lower ranked models suggested turbidity and dissolved oxygen may influence salamander movement between the surface and subsurface. Surface population dynamics are partly driven by movement below ground and therefore surface abundance estimates represent a fraction of the superpopulation. As such, while surface habitat management remains an important conservation strategy for this species, periodic declines in apparent surface abundance do not necessarily indicate declines of the superpopulation associated with the spring habitat.

[1] Kéry, M., and M. Schaub. 2012. Bayesian Population Analysis using WinBUGS. Academic Press.

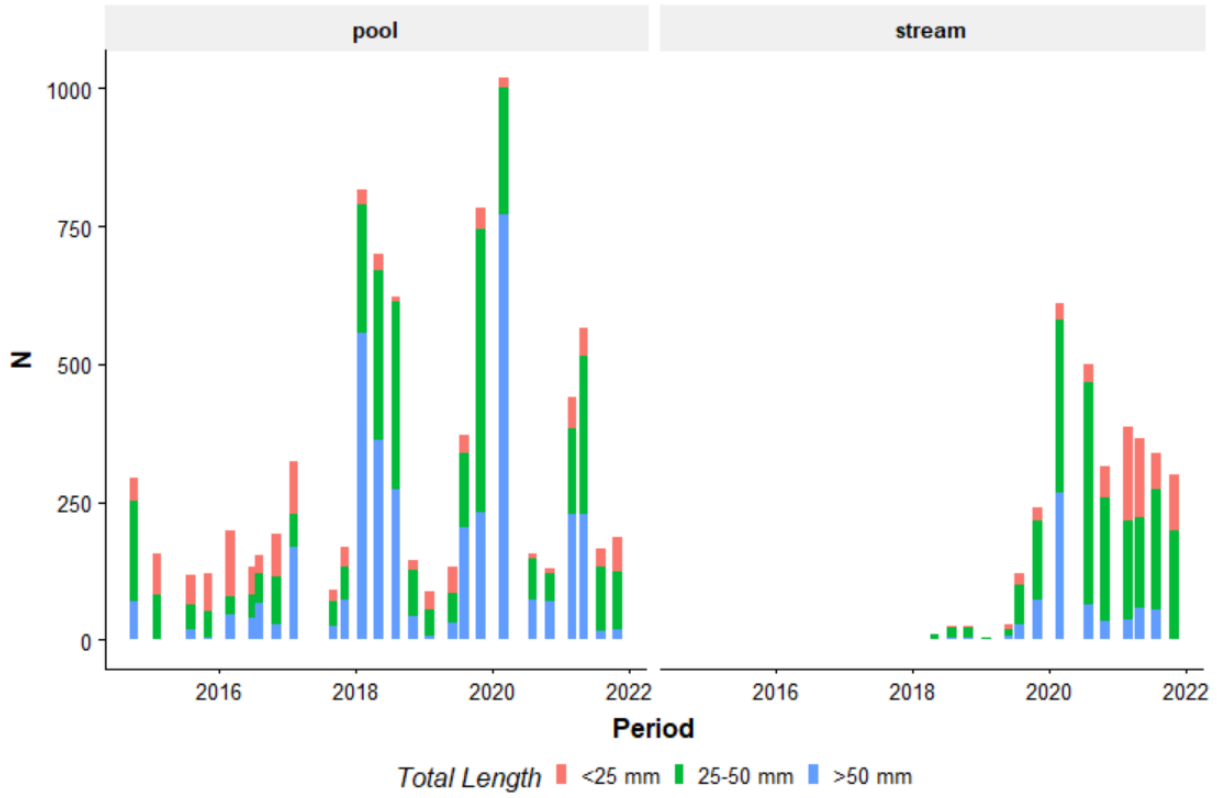

Figure 4. Estimates of abundance ($\widehat{N}$) based on size classes at Eliza Spring from October 2014 through November 2021 within the spring pool and stream.

Table 4. Estimates of abundance ($\widehat{N}$) based on size class and the 95% credence interval (CI) for capture-recapture surveys at Eliza Spring (spring pool and stream) from November 2020 through November 2021.

Period	Site	$\widehat{N}$ <25 mm (95% CI)	$\widehat{N}$ 25–50 mm (95% CI)	$\widehat{N}$ >50 mm (95% CI)
Nov 2020	Spring pool	70 (58, 85)	50 (42, 61)	10 (7, 14)
Nov 2020	Stream	33 (24, 45)	223 (184, 275)	57 (44, 74)
Mar 2021	Spring pool	228 (208, 253)	154 (142, 170)	56 (49, 65)
Mar 2021	Stream	38 (29, 49)	178 (153, 208)	170 (146, 200)
May 2021	Spring pool	228 (211, 247)	285 (269, 303)	52 (46, 60)
May 2021	Stream	58 (48, 71)	164 (144, 188)	143 (124, 166)
Aug 2021	Spring pool	17 (14, 22)	115 (105, 128)	32 (27, 39)
Aug 2021	Stream	55 (44, 69)	215 (186, 252)	65 (53, 81)
Nov 2021	Spring pool	20 (15, 27)	103 (88, 123)	61 (50, 76)
Nov 2021	Stream	0 (0, 0)	196 (157, 249)	101 (79, 130)

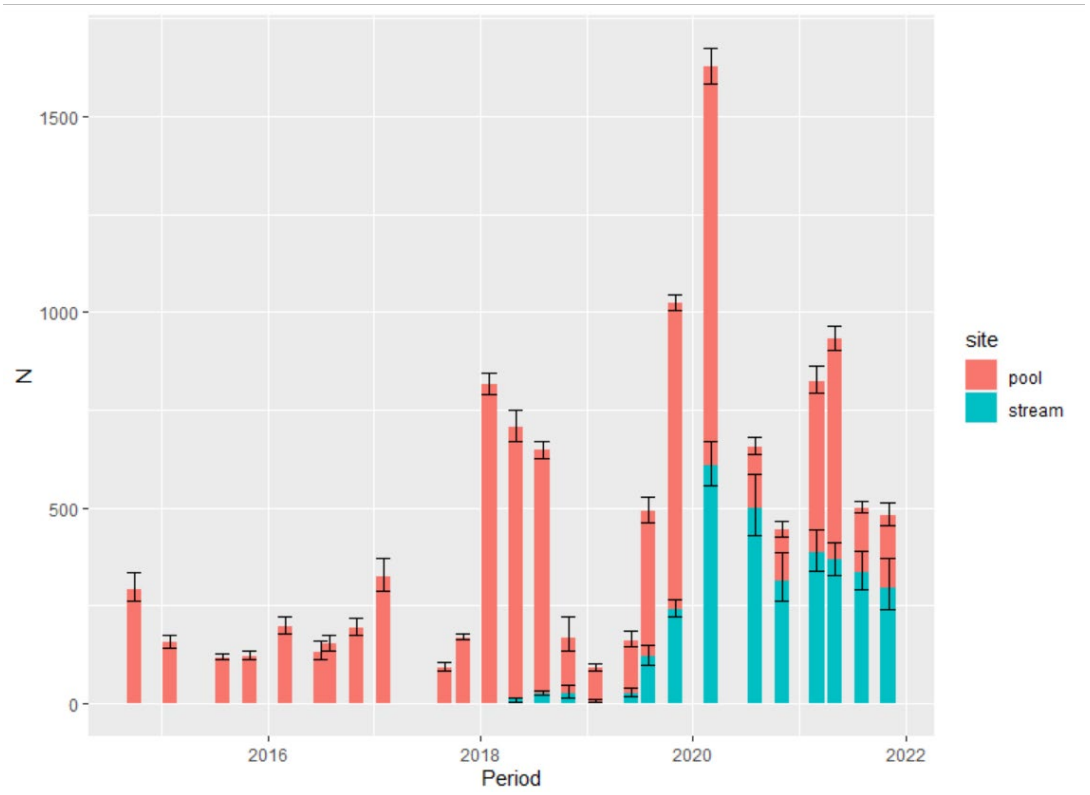

Figure 5. Estimates of abundance ($\widehat{N}$) by location at Eliza Spring from October 2014 through November 2021. Error bars represent 95% credence intervals.

11) Results of genetic research conducted as a result of tail-clipping
No tail-tips were collected in 2021 for genetics research.

12) Results of any research or management activities authorized by this permit and approved through the submission of study plans to the CPI Branch of the Austin ESFO

a. City of Austin monitors water quality at Barton Springs complex under this permit to meet the requirements of the Habitat Conservation Plan contained in the USFWS l0(a)(l)(B) permit PRT-839031and the Texas Pollutant Discharge Elimination System permit WQ0004705000 (EPA NPDES TXS000401). Tested parameters include total suspended solids, volatile suspended solids, N03+N02-N, NH3-N, Ortho-P, temperature, dissolved oxygen, pH, conductivity, and turbidity. Quarterly sampling includes monthly parameters plus alkalinity, Ca, Na, K, Mg, Cl, S04, F, As, Cu, Fe, Pb, Ni, Zn. TPDES annual sampling includes the above plus hardness, Ag, Cd, Cr, Hg, TOC, TPH, total polycyclic aromatic hydrocarbons, bromacil, organophosphate pesticides, chlorinated herbicides, volatiles, and semi-volatiles. Monthly sampling is performed at Barton Springs Pool only while all springs are sampled quarterly and for the more comprehensive annual sample (Parthenia, Eliza, Upper Barton, Old Mill, Backdoor and Cold springs). Additionally, the City of Austin in cooperation with the United States Geological Survey maintains continuous monitoring for spring discharge and physiochemical parameters at Barton Springs.

b. U.S. Geological Survey deploys and maintains water quality sampling equipment in Parthenia

Spring. Equipment was serviced by USGS dive teams.

c. Sampling has been completed for the Barton Springs Salamander Conservation (BSSCF) project "Microbiome characterization of captive and wild Barton Springs and Austin Blind Salamanders (*Eurycea*) and associated habitats (Tarleton State University)." Please see the attached progress report below.

d. Sampling has been completed for the BSSCF Fund Trophic Ecology Project with Texas State University. Drift net samples for aquatic invertebrates were collected as part of this study. Collections took place for nine days at Eliza Spring from 03/8/2021 to 03/19/2021, nine days from 05/5/2021 to 05/25/2021, and eight days from 10/19/2021 to 10/28/2021 for a total of 26 trap days. Collections took place for seven days at Old Mill Spring from 03/9/2021 to 03/19/2021 and four days from 05/6/2021 to 05/12/2021 for a total of 11 trap days. During drift net sampling, a number of salamanders (*E. waterlooensis* and *E. sosorum*) were captured in the nets. Live salamanders were released or transferred to the City of Austin's captive breeding facility. Dead salamanders were retained for stable isotope studies or for museum specimens. From Eliza Spring, 11 alive juvenile *E. sosorum* were found in the nets and released, three dead juvenile *E. sosorum* were collected from the nets, and four alive juvenile *E. waterlooensis* were collected for the captive breeding facility (Figure 6). More information on sampling dates can be found in Table 5. No salamanders were found in the nets at Old Mill Spring.

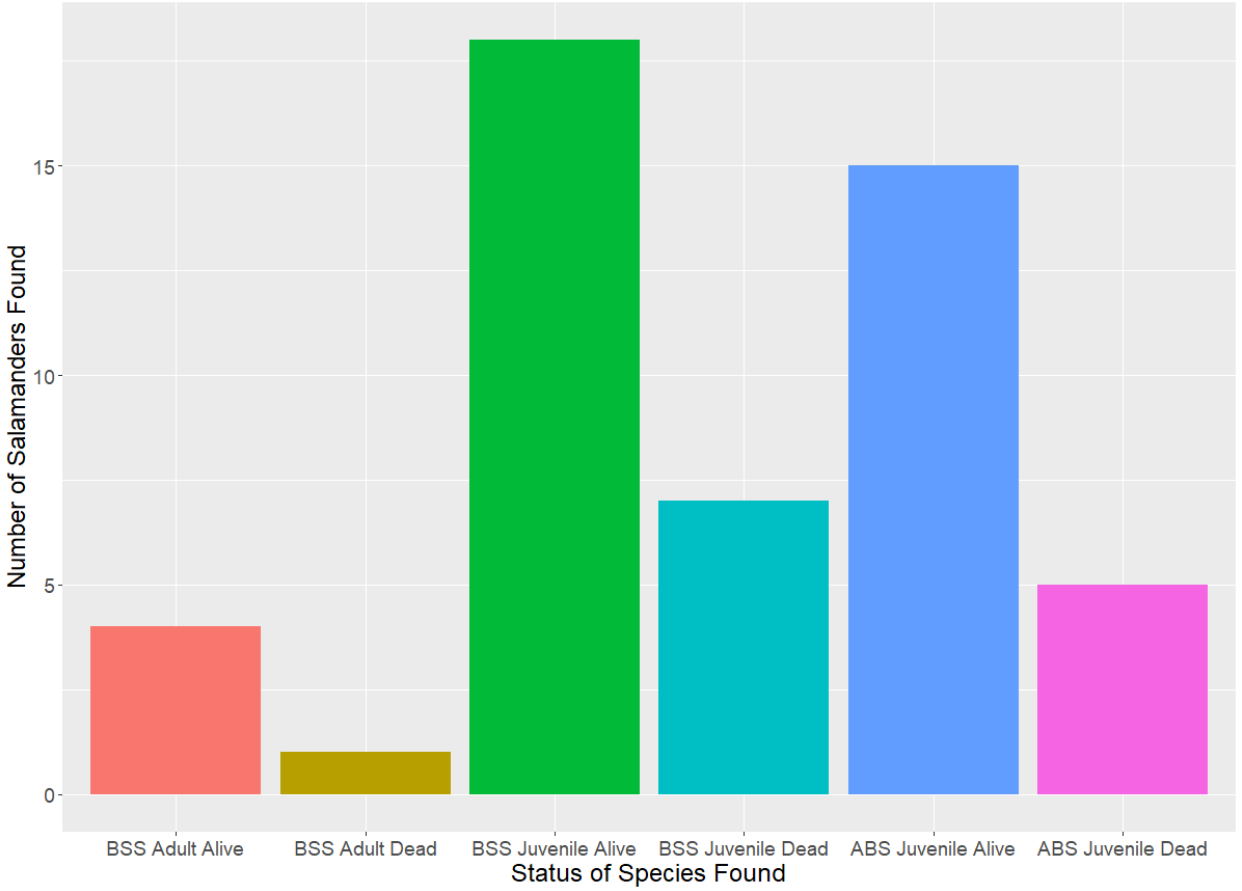

Figure 6. Total number of salamanders (BSS - *E. sosorum*, ABS - *E. waterlooensis*) and their status (found alive or dead) during the Eliza Spring drift net sampling events in 2021.

e. The City worked with Ruben Tovar and Tom Devitt from the University of Texas on more drift net sampling for a project they are doing on evolutionary development of groundwater salamanders in central Texas. The project focused on collecting *E. waterlooensis* for their study. While assisting with this project City staff also worked on collecting *E. waterlooensis* to add to the refugia. Sampling took place mostly during the month of August for a total of 16 sample days. Live salamanders were released or transferred to the City of Austin's captive breeding facility. Dead salamanders were retained for museum specimens or further research. During these sampling dates, four adult *E. sosorum* were found alive and released, one adult *E. sosorum* was found dead and preserved, seven juvenile *E. sosorum* were found alive, four dead juvenile *E. sosorum* were preserved, 11 alive juvenile *E. waterlooensis* were collected for the captive breeding facility or the University of Texas, and five dead juvenile *E. waterlooensis* were preserved (Figure 6). Not all of the alive *E. waterlooensis* that were transferred survived. A list of all drift net sampling that took place in 2021 can be found in Table 5.

Table 5: Drift net surveys for 2021.

Date	Site	Project	Sampling Type	Number of Nets	How often nets checked	BSS Adult Alive	BSS Adult Alive	BSS Juv Alive	BSS Juv Dead	ABS Juv Alive	ABS Juv Dead	Notes
3/8/2021	Eliza Spring	Texas State	drift net	2	Nets put out in morning and checked in evening	0	0	0	0	0	0	
3/9/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening	0	0	1	0	0	0	BSS maybe injured in net but released
3/9/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/10/2021	Eliza Spring	Texas State	drift net	2	Evening only	0	0	0	0	0	0	
3/11/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening	0	0	2	0	0	0	Both BSS released
3/11/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/12/2021	Eliza Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	2	0	2	0	Both BSS released and both ABS taken to Captive Breeding
3/12/2021	Old Mill Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
3/16/2021	Eliza Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/16/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/17/2021	Eliza Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/17/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/18/2021	Eliza Spring	Texas State	drift net	2	Morning only	0	0	1	0	0	0	BSS released
3/18/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
3/19/2021	Eliza Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	1	0	ABS taken to Captive Breeding

Date	Site	Project	Sampling Type	Number of Nets	How often nets checked	BSS Adult Alive	BSS Adult Alive	BSS Juv Alive	BSS Juv Dead	ABS Juv Alive	ABS Juv Dead	Notes
3/19/2021	Old Mill Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/5/2021	Eliza Spring	Texas State	drift net	2	Nets put out in morning and checked in evening	0	0	0	0	0	0	
5/6/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening	0	0	0	0	0	0	
5/6/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
5/7/2021	Eliza Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/7/2021	Old Mill Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/11/2021	Eliza Spring	Texas State	drift net	2	Nets put out in morning and checked in evening	0	0	0	0	0	0	
5/11/2021	Old Mill Spring	Texas State	drift net	2	Morning only	0	0	0	0	0	0	
5/12/2021	Eliza Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/12/2021	Old Mill Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/19/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening (Nets put out in afternoon on 5/18)	0	0	0	0	0	0	
5/20/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening	0	0	2	0	0	0	Both BSS released
5/21/2021	Eliza Spring	Texas State	drift net	2	Morning only and then pulled nets	0	0	0	0	0	0	
5/25/2021	Eliza Spring	Texas State	drift net	2	Morning and Evening (Nets put out in afternoon on 5/24)	0	0	0	1	0	0	We kept specimen and placed in 95% Ethanol
5/26/2021	Eliza Spring	COA	drift net	2	Morning and Evening	0	0	0	0	0	0	
5/27/2021	Eliza Spring	COA	drift net	2	Morning and Evening	1	0	0	0	0	0	Adult BSS released

Date	Site	Project	Sampling Type	Number of Nets	How often nets checked	BSS Adult Alive	BSS Adult Alive	BSS Juv Alive	BSS Juv Dead	ABS Juv Alive	ABS Juv Dead	Notes
5/28/2021	Eliza Spring	COA	drift net	2	Morning and Afternoon and then pulled the nets	0	0	0	0	0	0	
8/3/2021	Eliza Spring	University of Texas	drift net	6	Nets put out in late morning and checked in afternoon	0	0	0	0	0	0	
8/4/2021	Eliza Spring	University of Texas	drift net	6	Morning and Afternoon and then pulled the nets	0	1	1	4	1	2	1 alive BSS released, 1 ABS taken to Captive Breeding but later died and preserved
8/6/2021	Eliza Spring	University of Texas	drift net	6	Nets set at 9:30am and checked at 11:40am, 2:00pm, and 3:50pm and then pulled out	0	0	0	0	3	0	All 3 ABS were taken to Captive Breeding and 2 died shortly later and were preserved.
8/10/2021	Eliza Spring	University of Texas	drift net	11	Nets set at 8:50am and checked at 11:00am, 1:30pm, 3:30pm, and 5:30pm and left nets overnight	0	0	1	0	3	0	1 BSS was released. 1 ABS was not doing well and decided it wouldn't make it so Ruben Tovar put in paxgene fixative and took specimen. 1 ABS doing well and taken to Captive Breeding. 1 ABS doing well and Ruben took to his lab.
8/11/2021	Eliza Spring	University of Texas	drift net	13	Nets checked at 8:10am, 11:00am, 1:40pm, 3:30pm, 4:30pm and then pulled the nets	2	0	3	0	4	0	2 adult BSS released. 3 BSS juveniles released. 2 ABS taken to Captive Breeding. 1 ABS initially taken to Captive Breeding but wasn't going to make it so Ruben put it in fixative and is taking specimen. 1 ABS given to Ruben.
8/13/2021	Eliza Spring	University of Texas	drift net	12	Nets put out on 8/12 at 4:30 pm and then checked at 7:30am, 10:30am, 12:25pm and then pulled the nets	1	0	0	0	0	2	1 adult BSS released. 2 dead ABS preserved.
8/16/2021	Eliza Spring	COA	drift net	7	Nets put out at 9:00am and and some checked once and some checked twice and then pulled the nets	0	0	0	0	0	0	Nets 1, 2, 14, 18, 19 were out by 9am and checked at 11:30 and 2:00pm. Nets 9 and 11 were put out at 12:45pm and checked at 1:45pm.
8/18/2021	Eliza Spring	University of Texas	drift net	14	Nets put out at 9:30am and checked at 11:30am, 1:30pm, and 3:00pm and then pulled the nets	0	0	0	0	0	0	
8/19/2021	Eliza Spring	University of Texas	drift net	12	Nets put out at 11:45am and checked at 1:30pm, 3:30pm, 6:00pm. Some nets were left overnight and the rest pulled	0	0	1	0	0	1	BSS injured and taken to CB but later died and ABS specimen preserved

Date	Site	Project	Sampling Type	Number of Nets	How often nets checked	BSS Adult Alive	BSS Adult Alive	BSS Juv Alive	BSS Juv Dead	ABS Juv Alive	ABS Juv Dead	Notes
8/20/2021	Eliza Spring	COA	drift net	7	Morning and Afternoon and then pulled the nets	0	0	1	0	0	0	BSS released
8/23/2021	Eliza Spring	COA	drift net	7	Nets put out at 10:00am and checked around 3:45pm and then pulled the nets	0	0	0	0	0		
8/26/2021	Eliza Spring	COA	drift net	4	Nets put out at 9:30am and checked around 3:00pm and then pulled the nets	0	0	0	0	0	0	
9/2/2021	Eliza Spring	COA	drift net	6	Nets put out at 8:15am and checked at 10:00am and 12:30pm and then pulled the nets	0	0	0	0	0	0	
10/19/2021	Eliza Spring	Texas State	drift net	3	Nets put out in morning and checked in evening	0	0	0	0	0	0	
10/20/2021	Eliza Spring	Texas State	drift net	3	Morning and Evening	0	0	0	0	0	0	
10/21/2021	Eliza Spring	Texas State	drift net	3	Morning and Evening	0	0	0	0	0	0	
10/22/2021	Eliza Spring	Texas State	drift net	3	Morning only and then pulled nets	0	0	0	0	0	0	
10/25/2021	Eliza Spring	Texas State	drift net	3	Nets put out in morning and checked in evening	0	0	0	1	0	0	BSS not preserved
10/26/2021	Eliza Spring	Texas State	drift net	3	Morning and Evening	0	0	2	0	1	0	2 BSS were released, the ABS was alive but dies ~30 minutes later and specimen was give to Ruben Tovar (UT)
10/27/2021	Eliza Spring	Texas State	drift net	3	Morning and Evening	0	0	0	0	0	0	
10/28/2021	Eliza Spring	Texas State	drift net	3	Morning only and then pulled nets	0	0	1	1	0	0	Alive BSS were released, the other dead BSS was not initially noticed but later found by Texas State when sorting sample

Captive Breeding Annual Reporting Requirements

1) The number of *Eurycea sosorum, E. waterlooensis,* and *E. tonkawae* held at the captive breeding facility (including the number of wild-caught and captive-bred individuals from each spring site collected) (Table 6).

Table 6. Inventory of salamanders in the captive breeding program. WC = wild caught, CB = captive bred, F1 = first generation, F2 = second generation.

Species	**Spring of Origin**	**WC**	**CB F1>6 mo.**	**CB F2>6 mo.**
Eurycea sosorum	Parthenia	14	20	15
	Old Mill	4	45	40
	Eliza	39	55	4
	UBS	0	0	2
	Zara Well	1	0	0
Total		**58**	**120**	**61**
E. sosorum from SMARC[1]	Eliza	0	NA[2]	**102[2]**
E. waterlooensis	Parthenia	0	NA[3]	NA[3]
	Old Mill	5	NA[3]	NA[3]
	Eliza	11	NA[3]	NA[3]
	UBS	0	NA[3]	NA[3]
Total		**16[4]**	**22**	**11**
E. sosorum/waterlooensis hybrids (per genetics)	various	**3**	**8**	**0**
E. tonkawae	Bull Creek	1	3	0
	McDonald Well	0	3	0
	Testudo Tube	1	0	0
Total		**2**	**6**	**0**

[1]San Marcos Aquatic Resources Center (U.S. Fish and Wildlife Service facility); salamanders for experiment.
[2]Includes F1s and F2s but the exact number of each is not known.
[3] *E. waterlooensis* have not been separated and bred according to spring site of origin because the species is primarily aquifer-dwelling.
[4]Genetics will be conducted on select individuals to confirm species ID.

2) Number of observations of courtship behavior, spermatophores, spermatophore depositions, sperm transfers, and ovipositions.

In 2021, courtship behavior was observed in both wild-caught and captive-bred salamanders at the captive breeding facility. In general, salamanders are not disturbed by City staff during courtship. Because salamanders can store sperm, observed courtship behavior does not necessarily result in immediate egg-laying. Each oviposition with viable offspring represents at least one spermatophore transfer, and possibly multiple transfers. Oviposition data are presented in Table 7.

Table 7. Ovipositions in captivity 11/19/20–11/18/21. Tank ID indicates spring site of origin, reproductive group, and wild-caught or captive-bred status. Individuals in reproductive groups are recorded in order to follow actual or potential dams and sires. BSP denotes groups from Parthenia Spring, E, groups from Eliza Spring, OM, groups from Old Mill Spring, UBS, groups from Upper Barton Spring, and F1/F2, first/second generation captive-bred salamanders. SMARC=salamanders from San Marcos Aquatic Resources Center. TS=denotes Toe study tanks containing salamanders from SMARC.

Estimated Oviposition Date	**Tank ID**	**Clutch Size**	**No. Hatched**	**Comments**
	Eurycea sosorum			
11/19/2020	OMF2 (C184)	22	NA	Did not develop (female not housed with males)
12/03/2020	EF1 (C317)	17	NA	Culled[1]
12/04/2020	OMF1 (C052)	1	0	Decomposed
12/27/2020	PF2 (C311)	7	0	Eggs not found later
12/27/2020	EF1 (C317B)	14	NA	Culled[1]
12/30/2020	OMF2 (C315)	15	NA	Culled[1]
12/30/2020	OMF2 (C315B)	10	NA	Did not develop (females not housed with males)
01/17/2021	PF2 (C311)	13	NA	Culled[1]
02/08/2021	EF1 (C317)	17	NA	Culled[1]
03/12/2021	PF2 (C311)	12	NA	Culled[1]
04/10/2021	PF1 (C285)	12	1	
08/29/2021	SMARC F1/F2 (TS Tank 6)	26	15	
08/29/2021	SMARC F1/F2 (TS Tank 3)	14	3	
09/29/2021	SMARC F1/F2 (TS Tank 5)	1	1	
10/06/2021	SMARC F1/F2 (TS Tank 2)	5	2	
10/15/2021	SMARC F1/F2 (TS Tank 6)	12	0	
10/20/2021	SMARC F1/F2 (TS Tank 7)	29	9	
10/21/2021	SMARC F1/F2 (TS Tank 6)	36	11	
10/25/2021	SMARC F1/F2 (TS Tank 1)	36	15	
10/25/2021	SMARC F1/F2 (TS Tank 3)	19	12	
10/27/2021	SMARC F1/F2 (TS Tank 2)	10	0	Fungus, eggs discarded
11/08/2021	SMARC F1/F2 (TS Tank 1)	15	TBD	
11/11/2021	SMARC F1/F2 (TS Tank 3)	16	TBD	
11/11/2021	SMARC F1/F2 (TS Tank 5)	29	TBD	
11/15/2021	SMARC F1/F2 (TS Tank 5)	27	TBD	
11/15/2021	SMARC F1/F2 (TS Tank 8)	21	TBD	
11/18/2021	SMARC F1/F2 (TS Tank 3)	24	TBD	

Estimated Oviposition Date	Tank ID	Clutch Size	No. Hatched	Comments
	E. waterlooensis			
12/09/2020	F1 (CW15-3)	30	NA	Did not develop (female not housed with male)

[1]Eggs preserved to manage the population size and genetic diversity (prevent a disproportionate number of offspring produced from a single reproductive group, or to minimize inbreeding)

3) Information on clutch sizes (range, mean, and standard deviation) and hatching success (range, mean, and standard deviation) are shown in Table 8.

Table 8. Salamander clutch size and hatching success from 11/19/20–11/18/21.

	Min-Max Range	Mean	Standard Deviation
Eurycea sosorum			
Clutch Size	1-36 (N=27)	17.0	9.32
No. Hatched	0-15 (N=13)	5.3	6.09
% Hatched	0-100 (N=13)	30.3	30.48
E. waterlooensis			
Clutch Size	(N=1)	30	0
No. Hatched	NA	NA	NA
% Hatched	NA	NA	NA

4) Salamander mortalities, including age and cause of death, if known are shown in Table 9.

Table 9. Salamander mortalities from 11/19/20–11/18/21.

Species	Wild-Caught or Captive-Bred	Age[1] (years)	No. Mortalities	Cause of Death (health condition observed)
Eurycea sosorum				
	CB, WC	1-2	3, 1	Unknown
	CB	7.5–10	4	Unknown, age ≥ 7.6 years (50% survivorship)[2]
	CB	10–14	8	1 with edema, others unknown cause, age > 7.6 years (50% survivorship)[2]
	WC	20	1	Unknown, age > 7.6 years (50% survivorship)[2]
	CB transferred from SMARC (2020-2021)	~4-9	7	Unknown
E. waterlooensis	WC	<1	6	Unknown, small fragile size < 1" TL, possibly injured from drift net collection

[1]Age of wild-caught salamanders is estimated based on size at collection, with a maximum estimated age of 1.5 years for salamanders > 2 inches total length at collection. [2]Chamberlain DA. 2019. Barton Springs Salamander (*Eurycea sosorum*) and Austin Blind Salamander (*Eurycea waterlooensis*) captive breeding population management plan. City of Austin Watershed Protection Department. SR-20-03.

5) Information on Obvious Health Conditions or Behavioral Aberrations

In 2021, there were no unusual health conditions or behavioral aberrations observed.

6) Special Projects

In preparation for the study on the effects on salamander toe reduction of two levels of pH and two concentrations of calcium, we evaluated the captive-raised salamanders obtained from SMARC in 2020 to be utilized in the experiment. We deemed 37 unsuitable as test subjects, so we returned those salamanders to SMARC, our project collaborator, and received 52 more captive-raised (see attached Receipt for Donation of Fish and Wildlife Specimens). We began the year-long experiment in July 2021 and a report will be sent after completion.

Between December 2020 and June 2021, 20 *Eurycea sosorum* and 20 *E. waterlooensis* were sampled as part of the BSSCF microbiome project awarded to researchers at Tarleton University. In addition, 70 *E. sosorum* and 30 *E. waterlooensis* fecal pellets were collected for the project. Please see the attached progress report.

On August 19, 2021, we accepted a 1.5" TL *Eurycea sosorum* collected by Zara Environmental, LLC from the Zara well (1707 FM 1626, Manchaca, TX 78652) at a water depth of 100'. Current plans are to incorporate this salamander into our breeding population. Please see attached Receipt for Donation of Fish or Wildlife Specimens.

General Annual Reporting Requirements for Jollyville Plateau Salamanders

Two research projects are ongoing on Jollyville Plateau salamanders (*Eurycea tonkawae*) (JPS), CoA WPD project 545: JPS Occupancy and CoA WPD project 637: Old Lampasas Dam JPS Monitoring. Please see attached summary report for 2021.

All raw tabular data are publicly available from this link.

Personnel

Sampling personnel included Nathan F. Bendik (COA), Sarah Donelson (COA), Radmon Rice (COA), Angelita Rodriguez (COA), Blake Sissel (Travis County), Staryn Wagner (COA), Matthew Westbrook (COA), and Crystal Datri (COA contractor).

Locations

Please refer to Figure 8 for locations sampled this year.

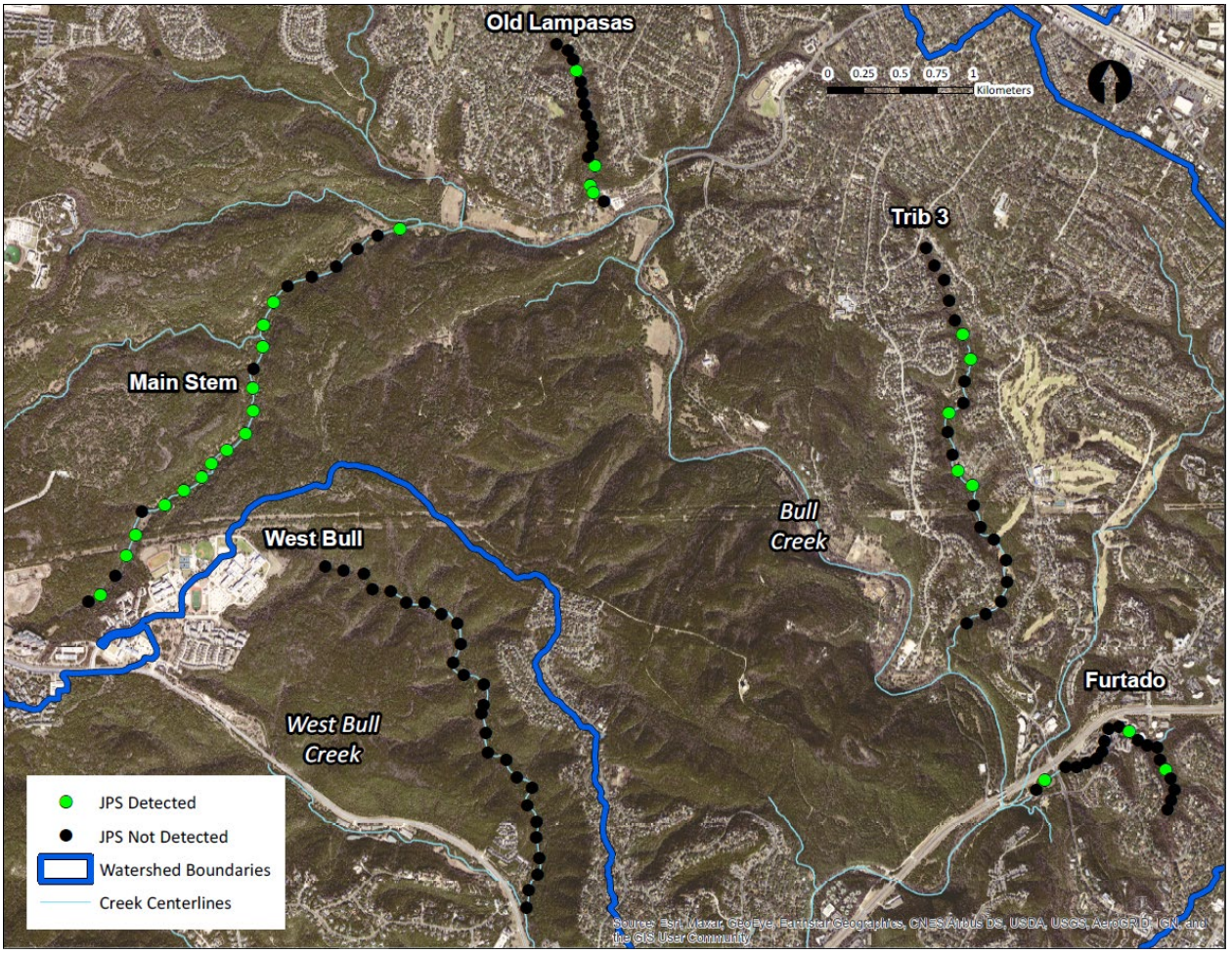

Figure 7. Summary of uncorrected site occupancy data for *Eurycea tonkawae* surveyed in 2021.

Survey Results

Table 10. Sum of all *Eurycea tonkawae* salamanders observed (by size class) at each site during the reporting period. Zero-observations were excluded, but the locations of these sites are shown in Figure 8. For survey dates and other ancillary information, please follow the raw data link.

Site Name	Latitude	Longitude	Parameter	Total Observed
Bull Creek @ JPS Occupancy Site 057	30.41905178	-97.81252801	SALS NOT PHOTGRAPHED >2 INCHES	1
Bull Creek @ JPS Occupancy Site 057	30.41905178	-97.81252801	SALS NOT PHOTGRAPHED 1-2 INCHES	2
Bull Creek @ JPS Occupancy Site 063	30.41467703	-97.82164751	SALS NOT PHOTGRAPHED >2 INCHES	1
Bull Creek @ JPS Occupancy Site 064	30.41326648	-97.82239327	SALS NOT PHOTGRAPHED >2 INCHES	2
Bull Creek @ JPS Occupancy Site 065	30.41191116	-97.82248208	SALS NOT PHOTGRAPHED >2 INCHES	2
Bull Creek @ JPS Occupancy Site 065	30.41191116	-97.82248208	SALS NOT PHOTGRAPHED 1-2 INCHES	1
Bull Creek @ JPS Occupancy Site 067	30.40940879	-97.82318604	SALS NOT PHOTGRAPHED >2 INCHES	3
Bull Creek @ JPS Occupancy Site 068	30.40799397	-97.82329324	SALS NOT PHOTGRAPHED <1 INCH	5
Bull Creek @ JPS Occupancy Site 068	30.40799397	-97.82329324	SALS NOT PHOTGRAPHED >2 INCHES	8
Bull Creek @ JPS Occupancy Site 068	30.40799397	-97.82329324	SALS NOT PHOTGRAPHED 1-2 INCHES	4
Bull Creek @ JPS Occupancy Site 069	30.40660967	-97.82389487	SALS NOT PHOTGRAPHED 1-2 INCHES	1
Bull Creek @ JPS Occupancy Site 070	30.40554726	-97.82520528	SALS NOT PHOTGRAPHED >2 INCHES	2
Bull Creek @ JPS Occupancy Site 070	30.40554726	-97.82520528	SALS NOT PHOTGRAPHED 1-2 INCHES	3
Bull Creek @ JPS Occupancy Site 071	30.40476092	-97.82635729	SALS NOT PHOTGRAPHED <1 INCH	3
Bull Creek @ JPS Occupancy Site 071	30.40476092	-97.82635729	SALS NOT PHOTGRAPHED >2 INCHES	11
Bull Creek @ JPS Occupancy Site 071	30.40476092	-97.82635729	SALS NOT PHOTGRAPHED 1-2 INCHES	31
Bull Creek @ JPS Occupancy Site 072	30.40398331	-97.82706936	SALS NOT PHOTGRAPHED 1-2 INCHES	3
Bull Creek @ JPS Occupancy Site 073	30.40314823	-97.82835348	SALS NOT PHOTGRAPHED >2 INCHES	12
Bull Creek @ JPS Occupancy Site 073	30.40314823	-97.82835348	SALS NOT PHOTGRAPHED 1-2 INCHES	7
Bull Creek @ JPS Occupancy Site 074	30.40233974	-97.82976742	SALS NOT PHOTGRAPHED >2 INCHES	9
Bull Creek @ JPS Occupancy Site 074	30.40233974	-97.82976742	SALS NOT PHOTGRAPHED 1-2 INCHES	6
Bull Creek @ JPS Occupancy Site 076	30.40049247	-97.83187449	SALS NOT PHOTGRAPHED 1-2 INCHES	1
Bull Creek @ JPS Occupancy Site 077	30.39922964	-97.83258077	SALS NOT PHOTGRAPHED <1 INCH	1
Bull Creek @ JPS Occupancy Site 077	30.39922964	-97.83258077	SALS NOT PHOTGRAPHED >2 INCHES	2
Bull Creek @ JPS Occupancy Site 077	30.39922964	-97.83258077	SALS NOT PHOTGRAPHED 1-2 INCHES	4

Site Name	Latitude	Longitude	Parameter	Total Observed
Bull Creek @ JPS Occupancy Site 079	30.39680263	-97.83449818	SALS NOT PHOTGRAPHED <1 INCH	1
Bull Creek @ JPS Occupancy Site 079	30.39680263	-97.83449818	SALS NOT PHOTGRAPHED >2 INCHES	1
Bull Creek @ JPS Occupancy Site 079	30.39680263	-97.83449818	SALS NOT PHOTGRAPHED 1-2 INCHES	4
Bull Creek @ JPS Occupancy Site 080	30.39639576	-97.83533319	SALS NOT PHOTGRAPHED >2 INCHES	1
JPS Occupancy site 267	30.421467	-97.79885	E. TONKAWAE SALS PHOTOGRAPHED	12
JPS Occupancy site 267	30.421467	-97.79885	SALS NOT PHOTGRAPHED <1 INCH	1
JPS Occupancy site 269	30.42103395	-97.7986367	SALS NOT PHOTGRAPHED 1-2 INCHES	1
JPS Occupancy site 279	30.42859304	-97.79965527	SALS NOT PHOTGRAPHED >2 INCHES	1
JPS Occupancy Site 302@ Furtado Creek	30.386884	-97.761282	SALS NOT PHOTGRAPHED >2 INCHES	1
JPS Occupancy Site 307@ Furtado Creek	30.384531	-97.758714	SALS NOT PHOTGRAPHED 1-2 INCHES	1
JPS Occupancy Site 320@ Trib 3 Bull Creek	30.402363	-97.772059	SALS NOT PHOTGRAPHED >2 INCHES	2
JPS Occupancy Site 321@ Trib 3 Bull Creek	30.403279	-97.773057	SALS NOT PHOTGRAPHED >2 INCHES	1
JPS Occupancy Site 324@ Trib 3 Bull Creek	30.40686	-97.773622	SALS NOT PHOTGRAPHED <1 INCH	2
JPS Occupancy Site 324@ Trib 3 Bull Creek	30.40686	-97.773622	SALS NOT PHOTGRAPHED >2 INCHES	4
JPS Occupancy Site 324@ Trib 3 Bull Creek	30.40686	-97.773622	SALS NOT PHOTGRAPHED 1-2 INCHES	2
JPS Occupancy Site 327@ Trib 3 Bull Creek	30.410195	-97.771979	SALS NOT PHOTGRAPHED >2 INCHES	1
JPS Occupancy Site 327@ Trib 3 Bull Creek	30.410195	-97.771979	SALS NOT PHOTGRAPHED 1-2 INCHES	1
JPS Occupancy Site 328@ Trib 3 Bull Creek	30.411699	-97.772497	SALS NOT PHOTGRAPHED >2 INCHES	6
JPS Occupancy Site 328@ Trib 3 Bull Creek	30.411699	-97.772497	SALS NOT PHOTGRAPHED 1-2 INCHES	2
JPS Occupancy Site 335@ Furtado Creek	30.38398	-97.7674	SALS NOT PHOTGRAPHED 1-2 INCHES	1

General Annual Reporting Requirements for Karst Invertebrates

During hydrogeological work and void inspections, City of Austin permitted staff entered several caves that may harbor protected karst invertebrates (Table 11).

Table 11. Caves entered by City of Austin staff. ES = endangered species. KZ = karst zone.

Date	Cave or Property	Purpose	Habitat Observations	Karst Zone	Personnel
4/7/2021	Barton Creek Greenbelt	Reconnaissance looking for dye injection sites between Barton Springs and Loop 360	Walked by outside of Airmen's Cave. Did not enter any caves, observed a few sinkholes in the bed of Barton Creek.	2	Alan Andrews, Michael Markowski, Radmon Rice, Lindsey Sydow, Scott Hiers
4/16/2021	6709 Havenbrook Cove	Inspection of karst voids/caves encountered during pool construction	Karst void encountered while excavating for residential swimming pool, potential for endangered species due to Karst Zone 2	2	Michael Markowski, Alan Andrews
4/27/2021	4417 Westlake Dr	Inspection of karst voids/caves encountered during construction	Void encountered during parking garage excavation, low potential for endangered species due to Karst Zone 4	4b	Alan Andrews, Michael Markowski
5/7/2021	Gaines SW Parkway Cave	Assessing suitability for dye trace study	Cave in creek bed.	3a	Alan Andrews, Michael Markowski, Lindsey Sydow, Radmon Rice
5/13/2021	8112 ½ Mauai Drive	Responding to citizen call about two small voids being found in stormwater retention pond.	Air found to be blowing air from feature. Crew used heavy machinery to investigate possible connection to larger cave opening. No connection to cave found.	3a	Alan Andrews, Lindsey Sydow
6/21/2021	10616 Old Manchaca Rd	Inspection of karst voids/caves encountered during construction	Type C void, (small amount of water flow) possibly associated with later line, encountered during WQ pond excavation low potential for endangered species due to Karst Zone 4	4b	Michael Markowski, Alan Andrews, Lindsey Sydow
8/27/2021	2309 N FM 620 Rd	Inspection of karst voids/caves encountered during construction	Void in Glen Rose formation encountered during excavation for apartment building, located in sidewall of elevator shaft. Dry.	4b	Lindsey Sydow
9/28/2021	Red Fox Rd	Inspection of karst voids/caves encountered during construction	Horizontal karst void encountered along bedding plane inside walls during trenching, high potential for endangered species due to Karst Zone 1	4b	Michael Markowski, Alan Andrews
11/9/2021	TxDOT Pond at Southwest Parkway and Gaines Creek	TxDOT performing excavation of pond sidewall failure areas in known faulted area near proposed dye injection site. Went to observe excavation	First excavation did not hit bedrock, second excavation reached openings that were slightly blowing. Located less than 100 feet from known karst features in Gaines Creek	3a	Alan Andrews, Scott Hiers, Lindsey Sydow
11/17/2021	McNeil High School	Inspection of karst voids/caves encountered during construction	Two voids encountered at bottom of pier drillings along bedding plane, high potential for endangered species due to Karst Zone 1	1	Michael Markowski

Jollyville Plateau Salamander Occupancy Study Update

Project 545, WPD ERM

Nathan F. Bendik, Matthew Westbrook, Sarah Donelson, and Blake Sissel (Travis County)

Occupancy studies use information from detection/nondection data of species to make inferences about the probability of a species occupying a certain area. Because a species will not always be encountered when it is present, these studies rely on a sequence of surveys to estimate probabilities of detection, which are used to adjust estimates of occupancy (MacKenzie et al., 2006). The advantage of using this method to determine the status of threatened and endangered salamander populations is that it can easily be applied over a wide range of habitats and a large area to make inferences on a larger scale than is possible with more focused, spring-specific surveys of abundance. The objective of the current study was to expand our inference from the initial occupancy study performed in Bull Creek (Bendik et al., 2016) to other tributaries throughout the range of *Eurycea tonkawae* so that we may better understand its distribution and habitat throughout its range. The study plan describes a five-year period with which to map out new sites along accesible major tributaries with previously documented *E. tonkawae* localities within their watersheds. This research report describes the results from the first four years of this study. The sampling methodology and analystic methods used can be accessed through the project plan (QAPP for project 545) or from the final publication from the original study (Bendik et al., 2016).

Figure 1 shows all sites mapped and surveyed from 2013 through 2021, which includes areas within the Bull Creek, West Bull Creek, Cypress Creek and Long Hollow watersheds. Figure 2 shows the raw detect/nondetect data for the tributaries added in 2021: West Bull Creek, Old Lampasas (Bull Creek), Furtado Creek (Bull Creek), and Trib 3 (Bull Creek). West Bull Creek had no occupied sites despite having persistant flow, suitable habitat, and a known Jollyville Plateau salamander (JPS) locality within the basin at Ivanhoe spring.

Using data from 2017–2021 we compared several different models of occupancy using either environmental variables or tributary groupings as functions of occupancy using the package `unmarked` (Fiske and Chandler 2011) in program R (R Core Team 2020). Our top a priori model for detection was water depth (Table 1). Groupings by year were not practical, as most tributaries were only surveyed during a single year. We constructed four different models a priori for occupancy, as a function of 1) tributary, 2) water quality parameters (mean temperature, mean conductivity, mean pH, mean dissolved oxygen, and standard deviation of temperature), 3) the best covariates from the initial study ($CaC0_3$ deposition categorical variable, maidenhair fern presence, temperature SD), and 4) cover variables (rock cover, leaf cover, algae cover, dominant substrate). We did not exclude environmental variables if they were correlated (Figure 3 and Figure 4). We first assessed goodness-of-fit using the MacKenzie and Bailey goodness-of-fit test, which did not indicate any problems with fit (MacKenzie & Bailey, 2004).

Using depth as the model for detection we found that tributary was the top model for occupancy. This model outperformed all others based on AICc rankings (Table 2). Prior to 2021, where we sampled 5 tributaries, our model with temperature variation as a function of occupancy was the highest ranked model. Note that it remains the top environmental covariate in our model for the 2017-2021 data set. The increase in temperature variation for the 2021 field work could be the result of several severe

storms in late April and May which produced a total of 9 to 15 inches of rain across the study area, delaying fieldwork and increasing baseflow in the latter half of the sampling period.

Tributary specific variation in occupancy varies greatly across the study area (Table 3). Of the observed occupied sites, the highest occupancy occurs in one of the least urbanized areas of their range (within the Balcones Canyonland Preserve) and the lowest occupancy occurs within the most urbanized catchment along Furtado Creek. However, using impervious cover percentage as a metric for urbanization we found no significant effect of urbanization on estimated occupancy (Figure 5), which is consistent with Bendik et al. (2016).

Looking at just the 2021 results, higher specific conductivity led to a lower predicted occupancy of JPS in streams (Table 4). This effect was not as pronounced prior to 2021. Tributaries in the Balcones Canyonland Preserve have lower conductivity than other urbanized streams sampled this year (Figure 6). Although our sample size is relatively small, increases in urbanization have been highly correlated with increases in specific conductance for various levels of urbanization in Austin, the U.S., and Australia (Bowles et al., 2006; Gregory & Calhoun, 2006; Walsh et al., 2005).

Table 1. Model selection results for detection of *Eurycea tonkawae* in streams.

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
Depth	13	367.42	0.00	0.79	0.79	-169.59
Constant	12	370.71	3.29	0.15	0.94	-172.40
Tributary	22	372.61	5.20	0.06	1	-161.00

Table 2. Model selection results for occupancy of *Eurycea tonkawae* in streams.

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
Tributary	13	367.42	0.00	1	1	-169.59
Temperature variation	4	389.62	22.21	0	1	-190.70
Rock/algae cover	8	390.27	22.85	0	1	-186.71
Best covariates 2013-16	7	393.19	25.77	0	1	-189.26
All water quality	7	394.48	27.06	0	1	-189.91

Table 3. Occupancy estimates by tributary.

Tributary	Year	Occupancy Est.	SE
Mainstem (Bull Creek)	2021	0.87	0.11
Trib 6 (Bull Creek)	2019	0.53	0.11
SAS (Cypress Creek)	2017	0.36	0.15
Old Lampasas (Bull Creek)	2021	0.36	0.21
Trib 3 (Bull Creek)	2021	0.3	0.11
Snowden (Cypress Creek)	2018	0.21	0.14
SAS (Cypress Creek)	2018	0.2	0.11
Trib 5 (Bull Creek)	2019	0.2	0.09
Furtado (Bull Creek)	2021	0.13	0.09
Long Hollow Creek	2018	0	0
West Bull Creek	2021	0	0

Table 4. Occupancy predictions by water quality parameters for 2021 surveys.

Occupancy (logit-scale):	Estimate	SE	z	P(>|z|)
(Intercept)	-0.28	4.49	-0.061	0.95
Temp	0.22	0.24	0.89	0.37
DO	0.08	0.18	0.45	0.65
SpC	-0.0061	0.0027	-2.2	0.025
SD.Temp	-0.36	0.22	-1.6	0.11

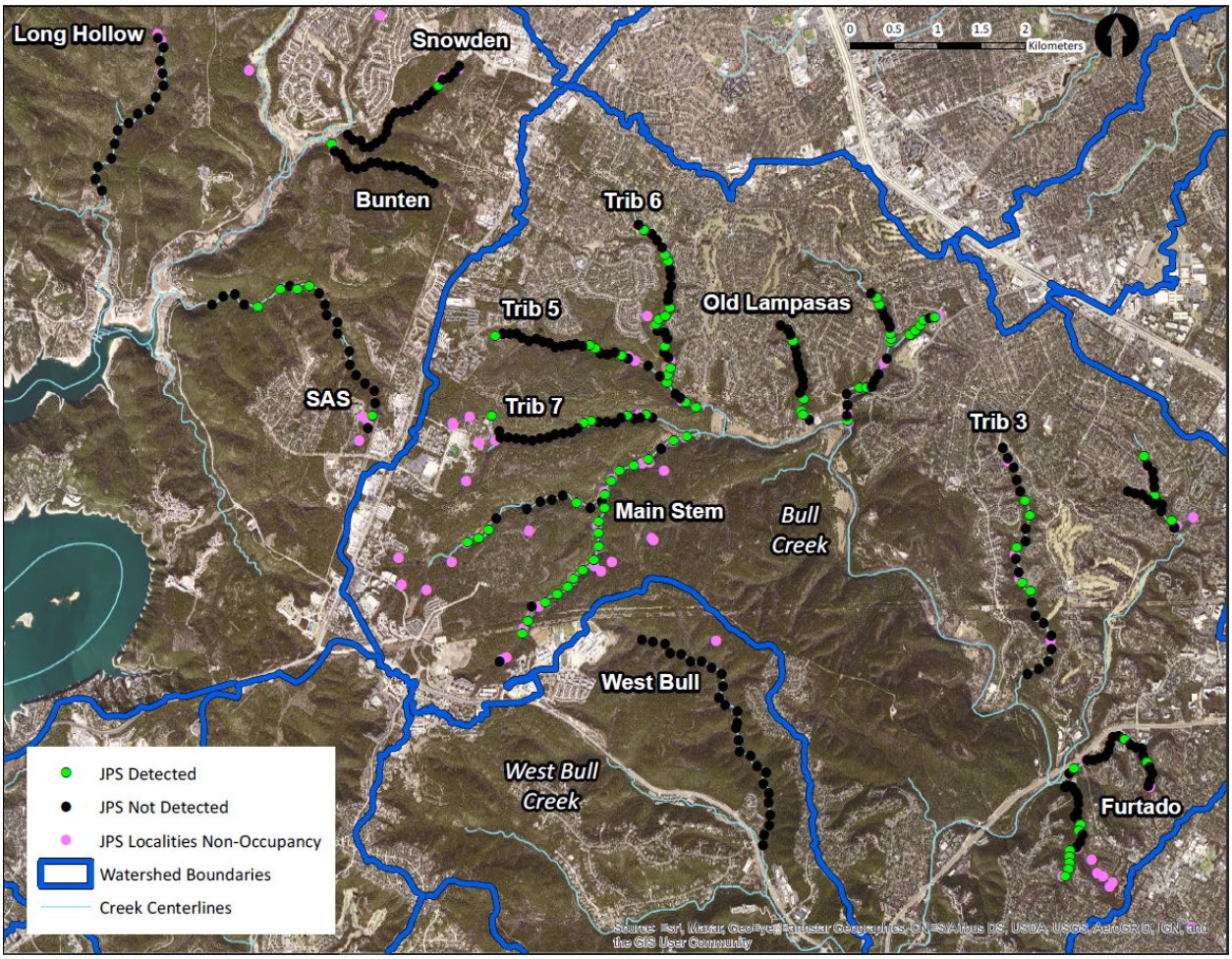

Figure 1. Summary of uncorrected site occupancy data for *Eurycea tonkawae* surveyed between 2013 and 2021. Data are raw sums of positive observations from all surveys and sites and as such, effort is not equivalent across this period. Note that the original names of Trib 5 and Trib 6 were actually the reverse of what they are noted here and in the City's water sampling database and in previous reports. For consistency with previous records, we maintain this error.

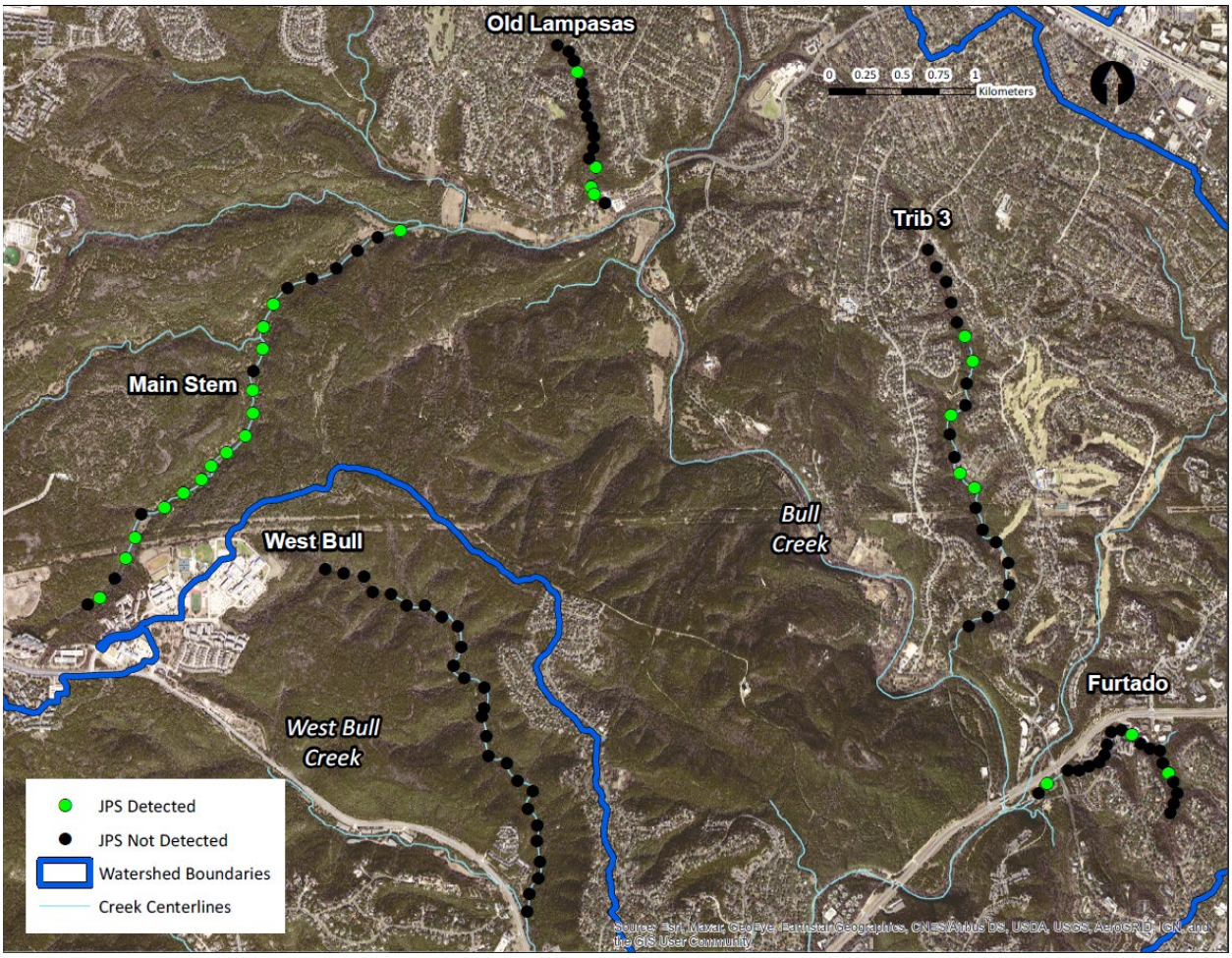

Figure 2. Summary of uncorrected site occupancy data for *Eurycea tonkawae* surveyed in 2021. Data are raw sums of positive observations. Table 3 shows the occupancy estimate for each tributary group.

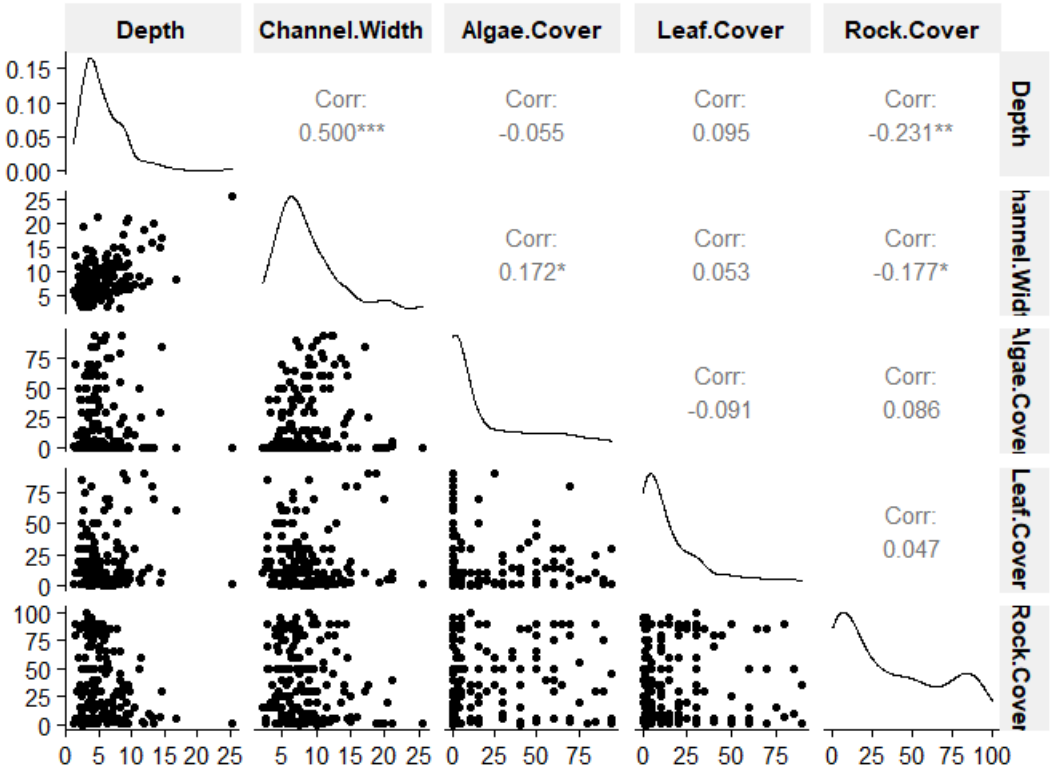

Figure 3. Pairs scatterplot (left), Pearson correlation statistics (right), and density plot (diagonal) for habitat covariates. An asterisk indicates P values below 0.05 based on a t-test, indicating a statistically significant correlation for $\alpha = 0.05$.

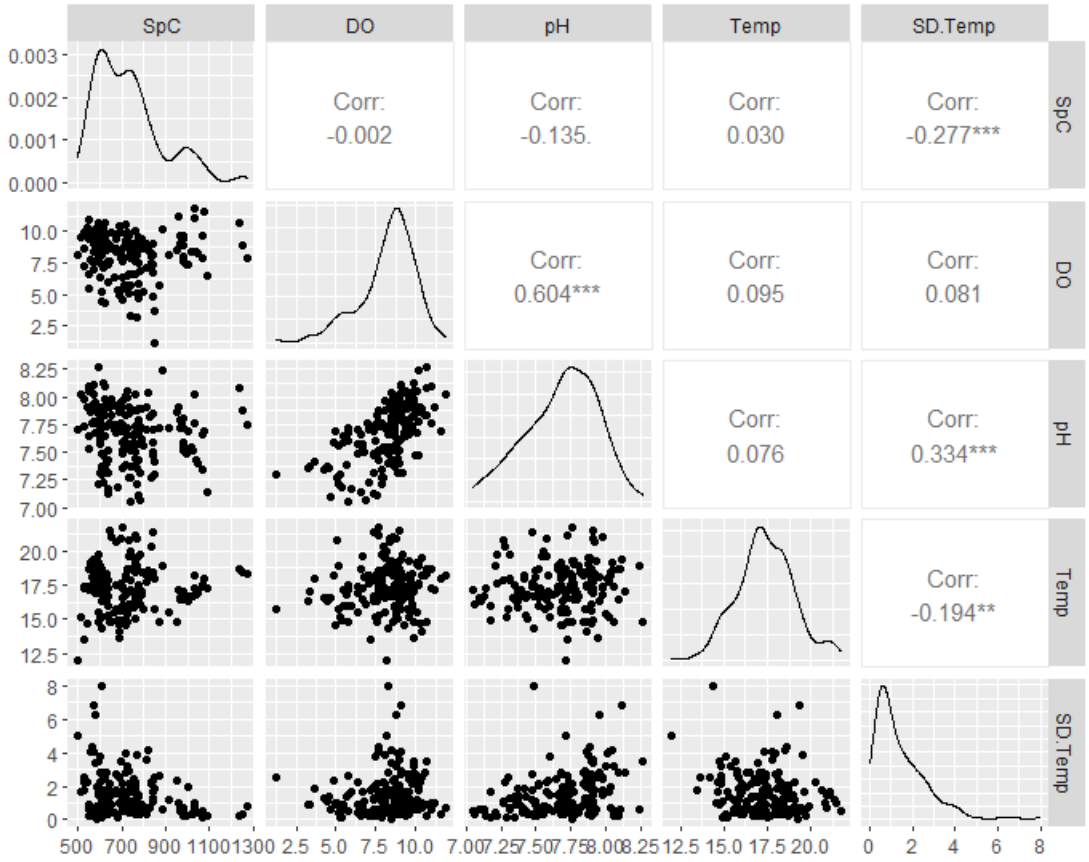

Figure 4. Pairs scatterplot (left), Pearson correlation statistics (right), and density plot (diagonal) for water quality covariates. Each value was calculated as the mean (or SD for SD.Temp) measurement during three visits. An asterisk indicates P values below 0.05 based on a t-test, indicating a statistically significant correlation for $\alpha = 0.05$.

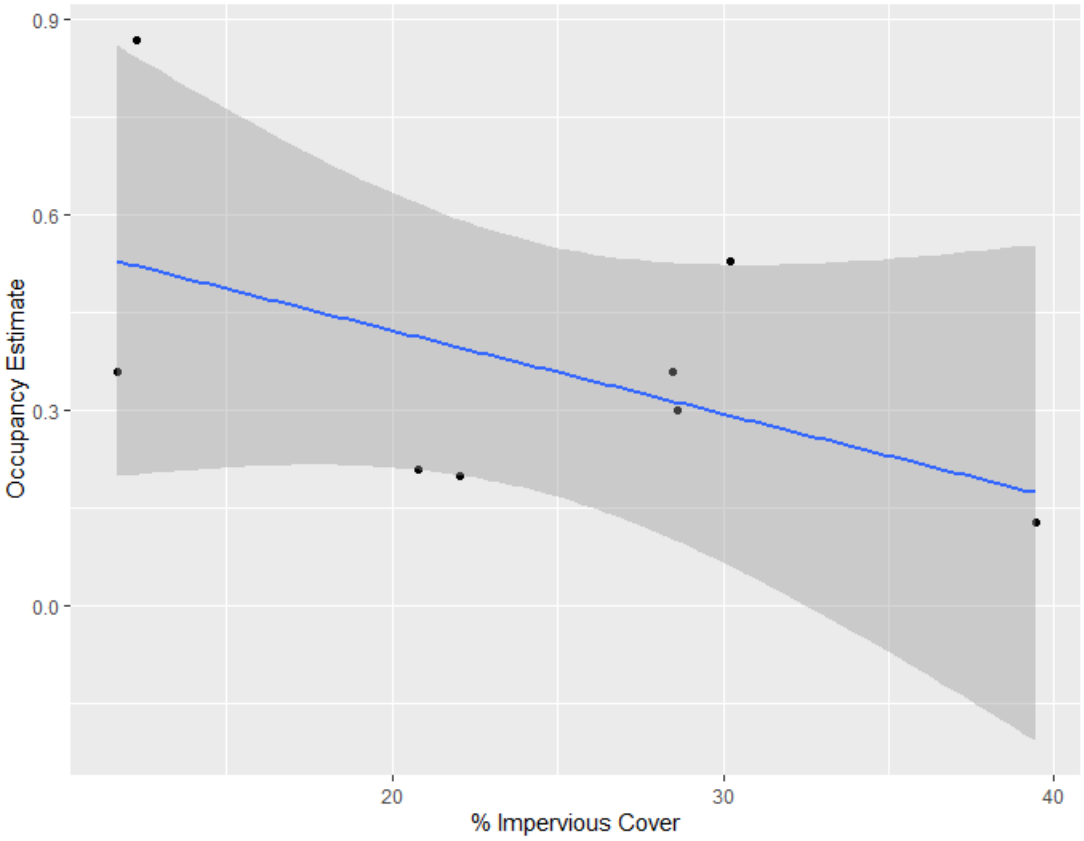

Figure 5. Plot of occupancy estimates and percent impervious cover for occupied tributaries (we excluded both West Bull Creek and Long Hollow Creeks, where no salamanders were observed; note that these are each low impervious cover watersheds within the Balcones Canyonlands Preserve).

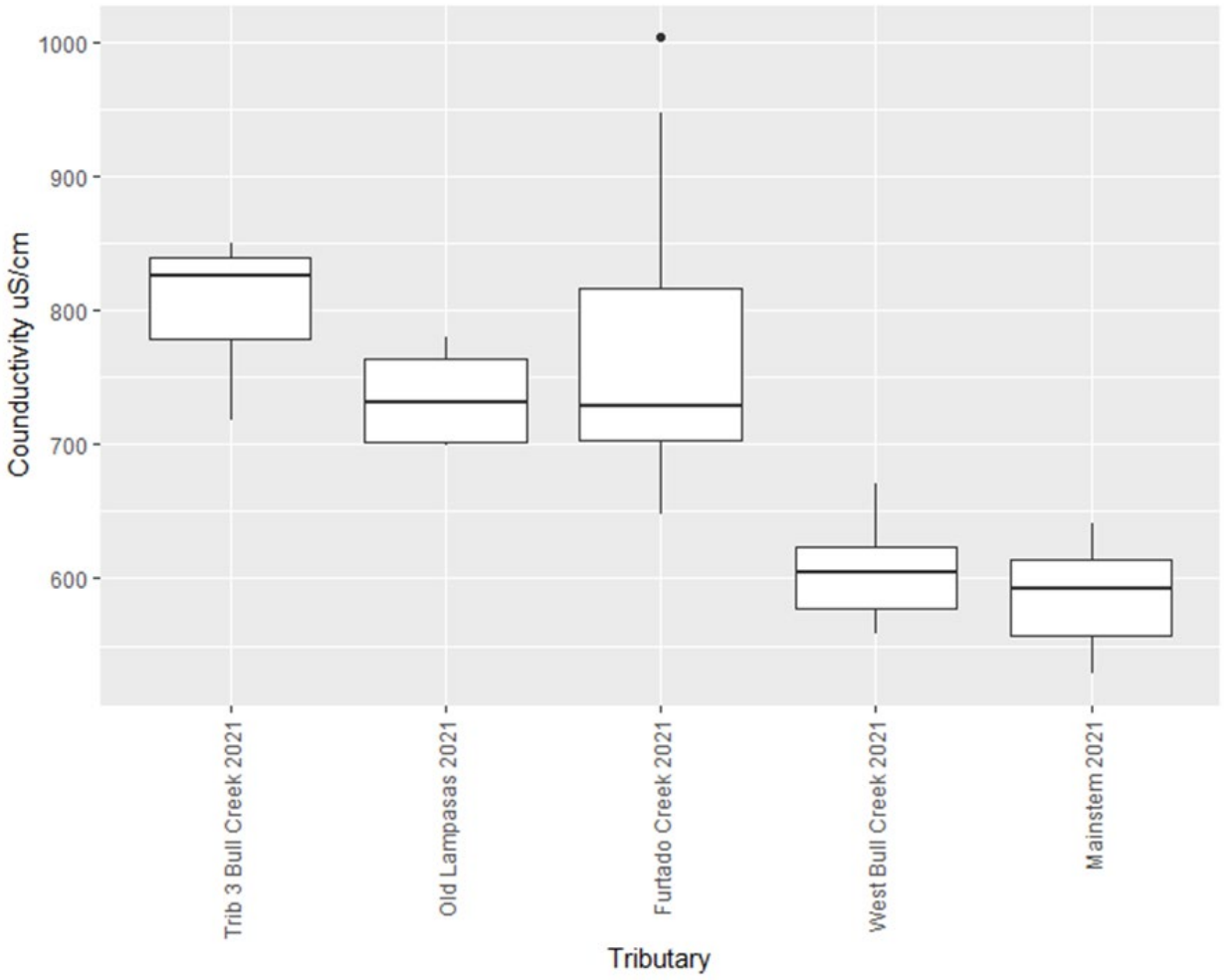

Figure 6. Box and whisker plot of conductivity per tributary. The first three tributaries are considered urbanized watersheds, the last two are within the Balcones Canyonland Preserve with minimal urbanization.

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Microbiome characterization of captive and wild Barton Springs and Austin Blind Salamanders (*Eurycea*) and associated habitats

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Barton Springs Salamander Conservation Fund Progress Report #3 - 9.01.21

As of the third reporting period for the BSSCF grant, Tarleton State University (TSU) scientists have collected a total of 740 samples over five collections for microbiome analysis (Tables 1 and 2). Our focus was on salamander skin and fecal samples and associated environmental samples in the main springs area (Parthenia and Eliza outflows) as well as the captive facility, with ancillary samples of Old Mills spring and Spillar Ranch spring. Salamanders were found at every site except for Spillar Ranch, nonetheless, we continued to collect environmental samples from this site. Collection 1 on 9/3/2020 - 9/4/2020 and Collection 2 on 12/10/2020 - 12/11/2020 were successfully sequenced and are now awaiting further analysis. We have processed Collection 3, and these samples are currently awaiting sequencing. We expect sequencing to be completed by the end of September 2021. Library preparations for Collections 4 and 5 are expected to be sequenced by the end of October. In addition, all water samples are currently being prepped for micro-and macromolecule analysis, which we expect to be completed by the end of September. We anticipate selecting a subset of water samples for high-depth polymer contamination analysis. In addition to sequence analyses, we will further process all salamander samples for a chytrid qPCR assay to determine the presence or absence of this fungus among samples.

Preliminary community structure analyses of the first collection are shown using a bar graph that grouped samples by sample type and displayed strong community similarities between sample locations (Fig. 1). Initial Shannon Diversity indices indicated differences in microbial diversity between sampling categories with the greatest diversity from substrate samples and lowest from fecal samples (Fig. 2). Although substrate had the highest microbial diversity, substrate samples also indicated the lowest variance in microbial assemblages, as indicated from a plot of principal coordinate analysis (PCOA) scores separated by sample type and location (Fig. 3). Samples also showed relatively high levels of segregation of microbial community similarity across different sampling locations (Fig. 4), and between captive and wild salamanders (Fig. 5). Community structure was further analyzed by comparing the 12 most reoccurring microbial OTUs within the four sample types (Fig. 6) and a heat map indicated the prevalence of a single microbial order across all samples (Fig. 7). The major phylum found in all samples was Proteobacterium, which had 56% prevalence across all samples, compared with Gammaproteobacteria (34%) and Alphaproteobacteria (10%) (Fig 8.).

Table 1: Summary of all samples collected for microbiome and water quality analyses by collection date.

Collection Date	Site	Water	Fecal	Skin	Substrate
9/3/2020 - 9/4/2020 (16S)	Old Mill	4	0	6	5
	Captive	3	22	10	0
	Parthenia	4	1	17	5
	Eliza	4	5	10	5
	Spillar	5	0	0	5
9/3/2020 - 9/4/202 (ITS)	Old Mill	4	0	6	5
	Captive	3	22	10	0
	Parthenia	4	1	17	5
	Eliza	4	3	10	5
	Spillar	5	0	0	5
12/10/2020 - 12/11/2020 (16S)	Old Mill	5	0	0	4
	Captive	3	32	15	0
	Parthenia	2	4	8	3
	Eliza	2	2	9	5
	Spillar	4	0	0	2
12/10/2020 - 12/11/2020 (ITS)	Old Mill	4	0	0	3
	Captive	0	35	19	0
	Parthenia	2	4	4	5
	Eliza	0	1	2	5
	Spillar	0	0	0	5
3/4/2021 - 3/5/2021 (16S & ITS)	Old Mill	4	0	0	5
	Captive	2	24	20	0
	Parthenia	5	4	11	5
	Eliza	4	9	21	5
	Spillar	3	0	0	5
6/3/2021 - 6/4/2021, 7/1/2021 (16S & ITS)	Old Mill	4	0	3	5
	Captive	3	35	20	0
	Parthenia	5	0	5	5
	Eliza	4	0	16	5
	Spillar	4	0	0	5

Table 2: Summary of samples for each category.

CaptiveWater	WildWater	CaptiveFecal	WildFecal	CaptiveSkin	WildSkin	TOTAL SAMPLES
19	114	238	34	134	201	740

Figure 1: Stacked bar graph of microbial abundance including both species of salamander and all samples collected from Collection 1.

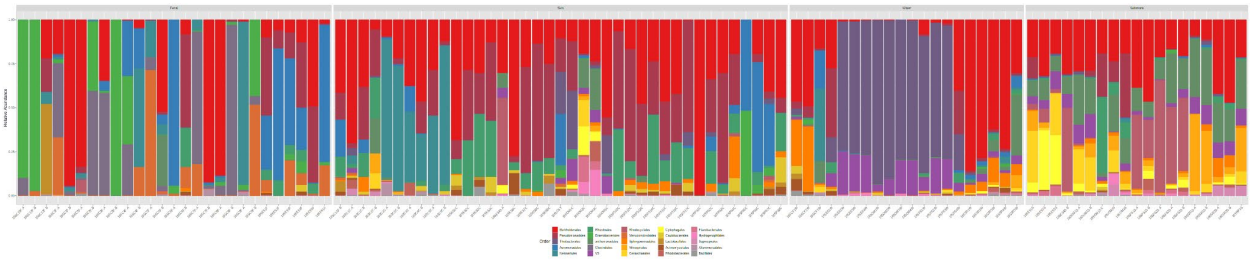


Figure 2: Box plot of alpha diversity, indicating Shannon diversity indices for microbiota among sample types.

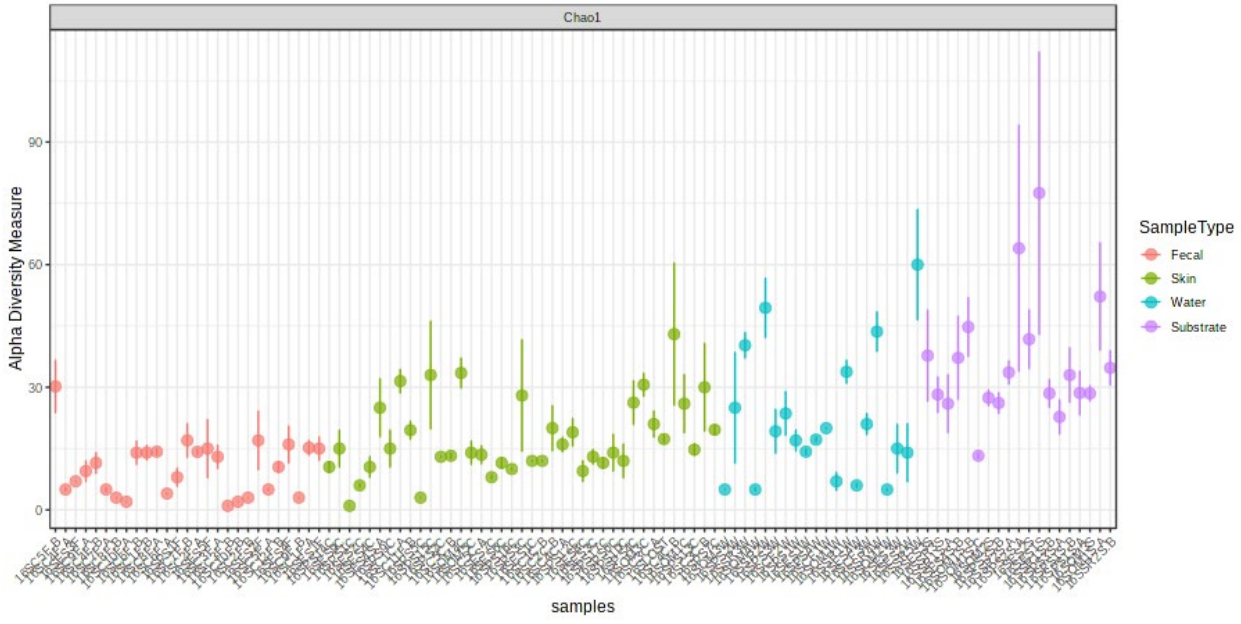
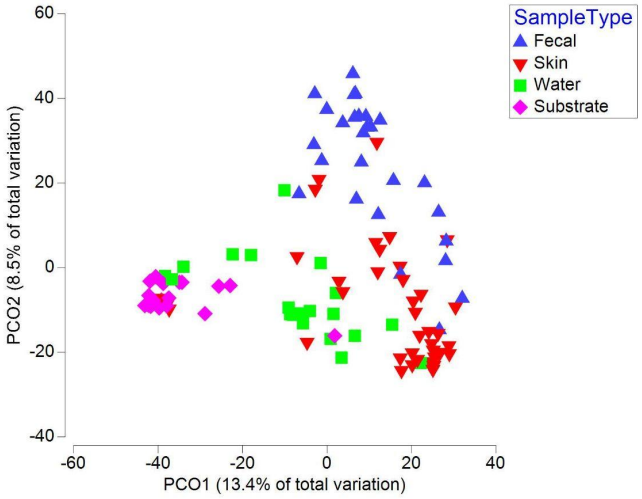
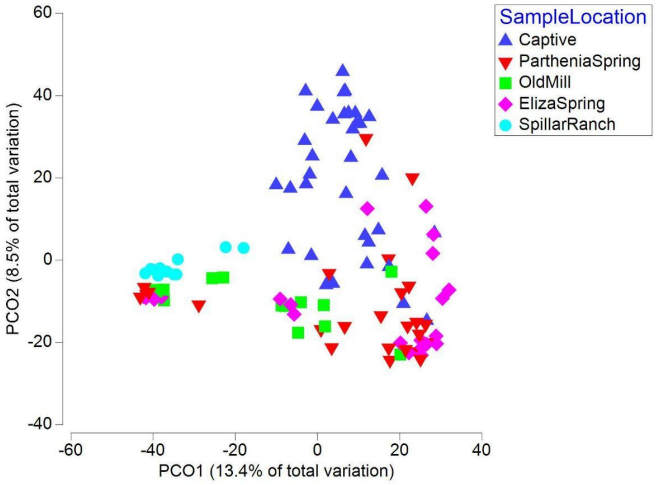
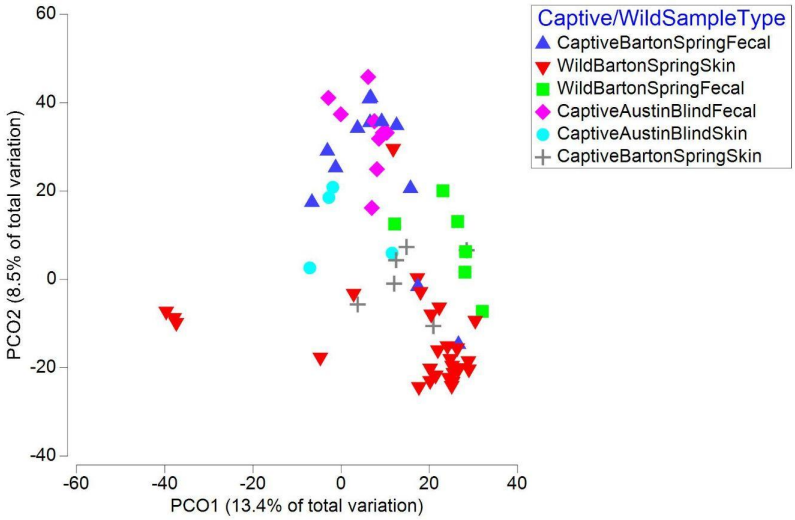
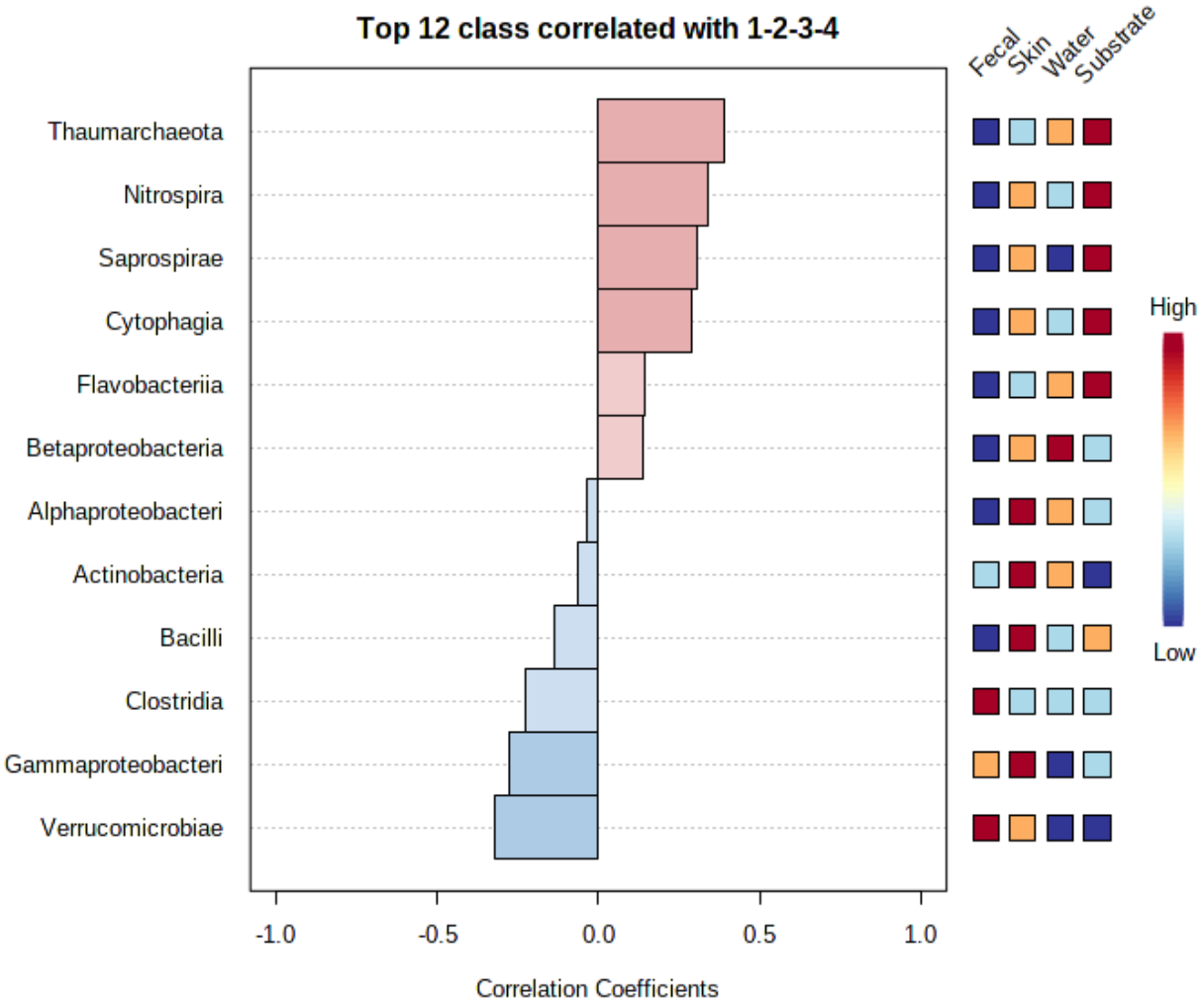


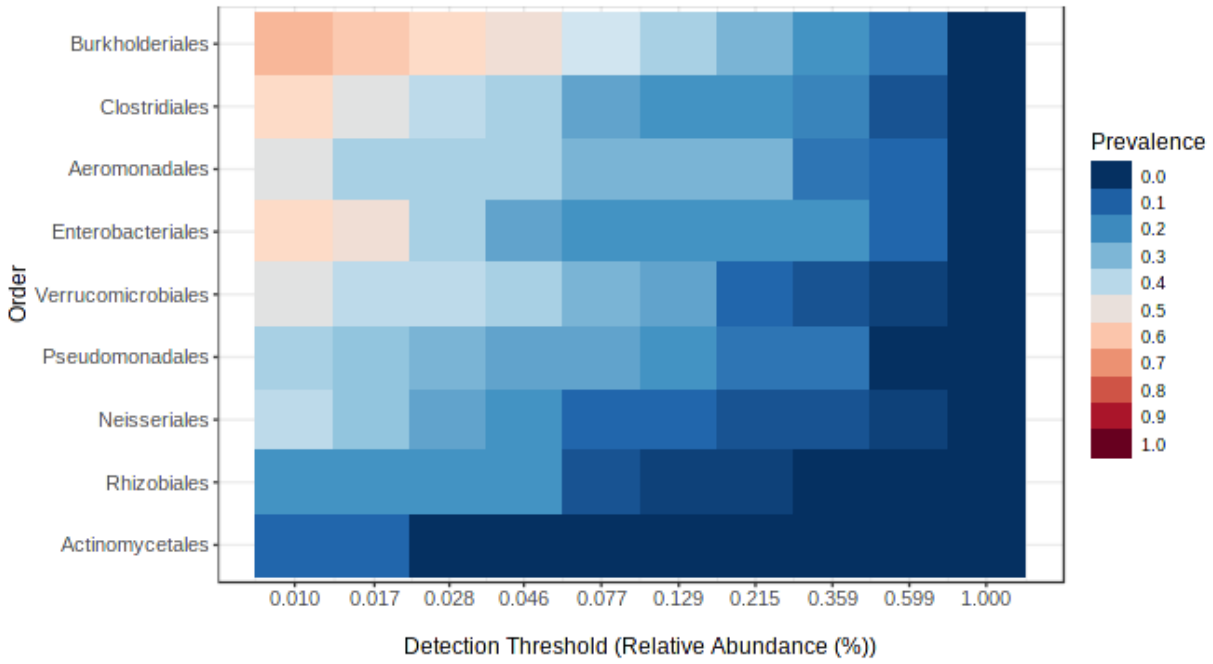
Figure 3: Beta diversity PCOA plot of samples comparing the four different sample types.

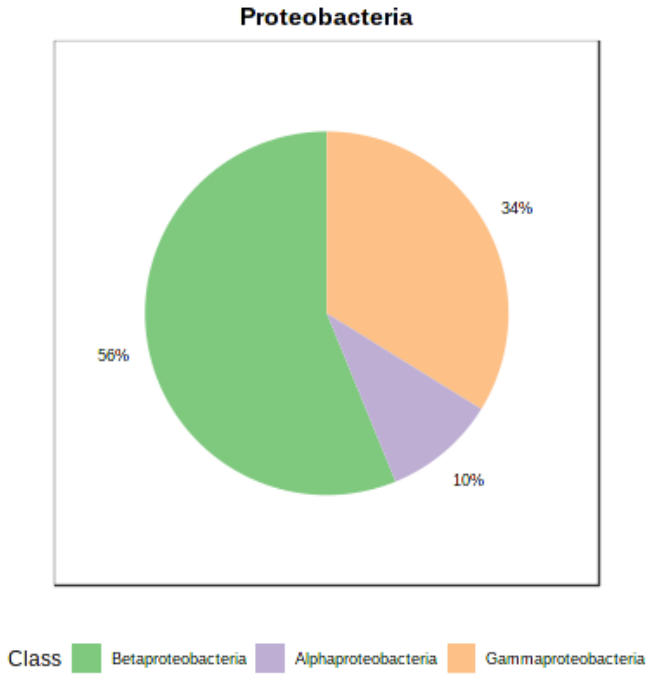







Top 12 class correlated with 1-2-3-4
Fecal
Skin
Water
Substrate
Thaumarchaeota
Nitrospira
Saprospirae
Cytophagia
Flavobacteriia
Betaproteobacteria
Alphaproteobacteri
Actinobacteria
Bacilli
Clostridia
Gammaproteobacteri
Verrucomicrobiae
High
Low
-1.0
-0.5
0.0
0.5
1.0
Correlation Coefficients


Burkholderiales
Clostridiales
Aeromonadales
Enterobacteriales
Verrucomicrobiales
Pseudomonadales
Neisseriales
Rhizobiales
Actinomycetales
Order
0.010
0.017
0.028
0.046
0.077
0.129
0.215
0.359
0.599
1.000
Detection Threshold (Relative Abundance (%))
Prevalence
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0


Proteobacteria
34%
56%
10%
Class
Betaproteobacteria
Alphaproteobacteria
Gammaproteobacteria

TEXAS PARKS & WILDLIFE

Receipt for Donation of Fish or Wildlife Specimens

Source *(Please place an "X" in one box and provide a valid permit number and the permit period of validity.)*

☐ Educational _____ ☒ Scientific SPR-0616-153

☐ Zoological _____ ☐ Rehabilitation _____

Permit Effective Period: 6/23/2019 through 6/23/2022

Permittee Name: Kenneth Ostrand Daytime Telephone: (512) 353-0011 ext. 223

Facility Name: San Marcos Aquatic Resources Center AZA Accredited? ☐ Yes ☐ No

Address: 500 East McCarty Lane City: San Marcos State: TX Zip: 78666

Destination *(Please place an "X" in one box and provide a valid permit number and the permit period of validity.)*

☐ Educational _____ ☒ Scientific _____ ☐ Zoological _____

Permit Effective Period: _____ through _____

Permittee Name: Dee Ann Chamberlain Daytime Telephone: ( )

Facility Name: City of Austin Watershed Protection Dept. AZA Accredited? ☐ Yes ☐ No

Address: 301 Nature Center Dr. City: Austin State: TX Zip: 78746

Specimens: (**Live** refers to live healthy specimens; **NR** refers to live specimens deemed as non-releasable; **Dead** refers to non-living specimens to be used for research, as voucher specimens or preserved/mounted specimens for display.)

Common Name	Scientific Name	Quantity	Live	N/R	Dead
Barton Springs Salamander	Eurycea sosorum	52 (F1)	☑	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

Source Signature: I certify that I, (place an "X" in one) ☐ permittee or ☐ sub-permittee, am authorized by my permit to donate the above referenced specimens to other Scientific, Educational and/or Zoological permit holders who are authorized to receive such specimens.

Signature of Donor: Justin Crow **Date:** 6-09-2021

Destination Signature: I certify that I, (place an "X" in one) ☐ permittee or ☒ sub-permittee, am authorized by my permit to receive the above referenced specimens.

Signature of Recipient: Dee Ann Chamberlain **Date:** 6/15/2021

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Receipt for Donation of Fish or Wildlife Specimens

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☐ Educational _____ ☒ Scientific TE850774

☐ Zoological _____ ☐ Rehabilitation _____

Permit Effective Period: _____ through _____

Permittee Name: Peter Sprouse Daytime Telephone: ( )

Facility Name: Zara Environmental AZA Accredited? ☐ Yes ☒ No

Address: 1707 FM 1626 City: Manchaca State: TX Zip: 78610

Destination *(Please place an "X" in one box and provide a valid permit number and the permit period of validity.)*

☐ Educational _____ ☒ Scientific SPR-0520-065 ☐ Zoological _____

Permit Effective Period: 5/27/2020 through 5/26/2023

Permittee Name: Nathan Bendik Daytime Telephone: ( )

Facility Name: Austin Salamander Conservation Center AZA Accredited? ☐ Yes ☐ No

Address: 301 Nature Center Dr. City: Austin State: TX Zip: 78746

Specimens: (**Live** refers to live healthy specimens; **NR** refers to live specimens deemed as non-releasable; **Dead** refers to non-living specimens to be used for research, as voucher specimens or preserved/mounted specimens for display.)

Common Name	Scientific Name	Quantity	Live	N/R	Dead
Barton Springs Salamander	Eurycea sosorum	1	☑	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

Source Signature: I certify that I, (place an "X" in one) ☐ permittee or ☐ sub-permittee, am authorized by my permit to donate the above referenced specimens to other Scientific, Educational and/or Zoological permit holders who are authorized to receive such specimens.

Signature of Donor: Peter Sprouse **Date:** 8/19/2021

Destination Signature: I certify that I, (place an "X" in one) ☐ permittee or ☐ sub-permittee, am authorized by my permit to receive the above referenced specimens.

Signature of Recipient: Dustin Chandler **Date:** 8/19/2021

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PWD 1143 - W7000 (11/06)