City of Austin, Watershed Protection Dept.
2020 Annual Report
U.S. Fish and Wildlife Service Scientific Permit (TE-833851)

Reporting period: November 1, 2019 through November 18, 2020
This report documents activities involving Barton Springs, Austin Blind, and Jollyville Plateau salamanders (*Eurycea sosorum*, *E. waterlooensis*, and *E. tonkawae*, respectively) and karst invertebrates that are authorized under the above permit for 2020.

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. 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.

   Due to the COVID-19 pandemic safety protocols, Eliza spring drive surveys were cancelled in May and modified thereafter to allow for safe distancing, but this did not affect our results.

4) Survey results
   Salamander counts from 2020 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 is presented in Table 2.

Table 1. Barton Springs and Austin Blind salamander counts from 2020 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 Springs Pool (Parthenia Spring)	3/5/2020	318	0
Barton Springs Pool (Parthenia Spring)	6/3/2020	118	0
Barton Springs Pool (Parthenia Spring)	8/20/2020	83	0
Barton Springs Pool (Parthenia Spring)	11/19/2020	59	0
Eliza Spring	2/24/2020	410	1
Eliza Spring	2/27/2020	334	1
Eliza Spring	3/3/2020	541	2
Eliza Spring	8/12/2020	61	1
Eliza Spring	8/14/2020	51	1
Eliza Spring	8/18/2020	45	3
Eliza Spring	11/2/2020	46	0
Eliza Spring	11/4/2020	48	0
Eliza Spring	11/6/2020	41	0
Eliza Stream (outflow from Eliza Spring)	2/24/2020	228	0
Eliza Stream (outflow from Eliza Spring)	2/28/2020	118	0
Eliza Stream (outflow from Eliza Spring)	3/4/2020	217	1
Eliza Stream (outflow from Eliza Spring)	8/12/2020	129	0
Eliza Stream (outflow from Eliza Spring)	8/14/2020	127	0
Eliza Stream (outflow from Eliza Spring)	8/18/2020	144	0
Eliza Stream (outflow from Eliza Spring)	11/2/2020	91	1
Eliza Stream (outflow from Eliza Spring)	11/4/2020	75	0
Eliza Stream (outflow from Eliza Spring)	11/6/2020	82	1
Old Mill (Sunken Gardens) Spring	3/9/2020	6	0
Old Mill (Sunken Gardens) Spring	6/23/2020	3	0
Old Mill (Sunken Gardens) Spring	9/3/2020	7	0
Old Mill (Sunken Gardens) Spring	11/10/2020	0	1
Old Mill Stream (outflow from Sunken Gardens Pool)	3/9/2020	2	0
Old Mill Stream (outflow from Sunken Gardens Pool)	6/23/2020	0	0
Old Mill Stream (outflow from Sunken Gardens Pool)	9/3/2020	2	0
Old Mill Stream (outflow from Sunken Gardens Pool)	11/10/2020	2	0
Upper Barton Spring	2/21/2020	0	0
Upper Barton Spring	6/17/2020	6	0

Table 2. Environmental data for Barton Springs and Austin Blind salamander surveys from 2020. No data on record for Feb/Mar survey. Data for Barton Springs Pool (Parthenia Spring) are recorded by the USGS.

Site	Date	Flow (cfs)	Conductivity (uS/cm)	D.O. (MG/L)	pH	Water Temp (°C)
Eliza Spring	Jun-20	10.13	639	5.75	7.06	23.18
Eliza Spring	Aug-20	3.99	659	5.46	7.18	21.56
Eliza Spring	Nov-20	3.73	684	5.31	7.04	21.08
Old Mill (Sunken Gardens) Spring	Mar-20	4.47	773	5.23	7.00	21.40
Old Mill (Sunken Gardens) Spring	Jun-20	8.38	733	5.13	6.94	21.82
Old Mill (Sunken Gardens) Spring	Sep-20	2.96	762	4.65	7.30	22.81
Old Mill (Sunken Gardens) Spring	Nov-20	1.66	800	4.56	7.08	21.75
Upper Barton Spring	Feb-20	0.054	641	7.90	7.08	21.29
Upper Barton Spring	Jun-20	1.13	636	6.57	7.19	21.78
Upper Barton Spring	Sep-20	Dry				
Upper Barton Spring	Nov-20	Dry				

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.

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	Deposition	Notes
Eurycea waterlooensis	1/17/20	Eliza Spring	1	Alive at captive breeding (ASCC)	Found alive in drift net; <25 mm; COA CB# A94
Eurycea waterlooensis	1/29/20	Eliza Spring	1	No specimen (degraded)	Found alive in drift net; <25 mm TL; mortality ~2/20/20
Eurycea waterlooensis	2/24/20	Eliza Spring	1	Preserved in 70% EtOH	Collected during survey; <25 mm TL; mortality 4/9/2020
Eurycea waterlooensis	2/27/20	Eliza Spring	1	Preserved in 70% EtOH	Collected during survey; <25 mm TL; mortality 3/17/2020.
Eurycea sosorum	3/3/20	Barton Springs Pool	9	Preserved in 95%+ EtOH	Found dead in drift nets; <25 mm; Donated to Schwartz Team for isotope work
Eurycea sosorum	3/3/20	Eliza Spring	1	Alive at captive breeding (ASCC)	Collected during survey; <25 mm TL; mortality 3/4/2020
Eurycea sosorum	3/3/20	Eliza Spring	4	Alive at captive breeding (ASCC)	Collected during survey; COA CB group #1056-1059
Eurycea sosorum	3/5/20	Barton Springs Pool	2	Preserved in 95%+ EtOH	Found dead in drift nets; <25 mm; Donated to Schwartz Team for isotope work
Eurycea waterlooensis	3/9/20	Barton Springs Pool	1	No specimen (degraded)	Found alive in drift net; <25 mm TL; mortality ~5/5/2020
Eurycea sosorum	3/11/20	Barton Springs Pool	5	Preserved in 95%+ EtOH	Found dead in drift nets; <25 mm; Donated to Schwartz Team for isotope work
Eurycea sosorum	3/12/20	Barton Springs Pool	4	Preserved in 95%+ EtOH	Found dead in drift nets; <25 mm; Donated to Schwartz Team for isotope work
Eurycea waterlooensis	3/12/20	Barton Springs Pool	1	No specimen (degraded)	Found alive in drift net; <25 mm TL; collected with injury, spinal problem; COA CB #A95; mortality by 10/5/2020
Eurycea waterlooensis	8/12/20	Eliza Spring	1	No specimen (degraded)	Collected during survey; ~17mm TL; mortality by 10/11/2020
Eurycea sosorum	8/12/20	Eliza Spring	1	Preserved in 95%+ EtOH	Collected in stream alive during survey. Appears to have been injured by a rock in basket while waiting to be released.
Eurycea waterlooensis	8/14/20	Eliza Spring	1	Alive at captive breeding (ASCC)	Collected during survey. Head shape appears as *E. waterlooensis*, but the "eyes" seem large (but don't seem to have the lens). 16mm TL

Table 3, continued.

Species	Date	Site	Number Collected	Deposition	Notes
Eurycea waterlooensis	8/18/20	Eliza Spring	2	Preserved in 70% EtOH	Collected during survey; all <25mm TL, found dead 9/30/2020
Eurycea sosorum	8/18/20	Eliza Spring	3	Alive at captive breeding (ASCC)	Collected during survey; sizes 32mm TL, 35mmTL, 27mm TL
Eurycea waterlooensis	8/18/20	Eliza Spring	1	Preserved in 70% EtOH	Collected during survey; <25 mm TL; mortality 8/19/2020
Eurycea sosorum	9/16/20	Eliza Spring	1	No Specimen (only part of body found)	Found dead in drift net; <25 mm
Eurycea sosorum	11/2/20	Eliza Spring	1	Preserved in 95%+ EtOH	Collected during survey; <25 mm TL
Eurycea waterlooensis	11/2/20	Eliza Spring	1	Alive at captive breeding (ASCC)	Collected during survey; >50 mm TL
Eurycea sosorum	11/6/20	Eliza Spring	3	Alive at captive breeding (ASCC)	Collected during survey; 2 <1" TL, 1 ~1.25"TL
Eurycea sosorum	11/6/20	Eliza Spring	1	Preserved in 70% EtOH	Collected during survey; <25 mm TL but missing much of tail
Eurycea waterlooensis	11/6/20	Eliza Spring	1	Alive at captive breeding (ASCC)	Collected during survey; <25 mm TL
Eurycea waterlooensis	11/10/20	Old Mill Spring	1	Preserved in 70% EtOH	Collected during survey; <25 mm TL at collection; mortality 11/18/2020

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
None.

10) Results of any mark-recapture work
We conducted capture-recapture surveys at three sites in 2020 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.

We conducted robust-design mark-recapture sampling at Eliza Spring in February, August, and November. Abundance estimates are provided in Table 4 and Figures 1 and 2.

Table 4. Estimates of abundance ($\hat{N}$) based on size class and the 95% credence interval (CI) for capture-recapture surveys at Eliza Spring from November 2019 through November 2020.

Period	$\hat{N}$<25 mm (95% CI)	$\hat{N}$ 25–50 mm (95% CI)	$\hat{N}$ >50 mm (95% CI)	Total $\hat{N}$
19-Nov	350 (324, 380)	749 (705, 801)	66 (58, 76)	1165
20-Mar	1517 (1359, 1695)	755 (669, 851)	64 (52, 79)	2336
20-Aug	244 (191, 304)	800 (628, 986)	70 (52, 92)	1114
20-Nov	89 (68, 115)	233 (186, 293)	59 (45, 78)	381

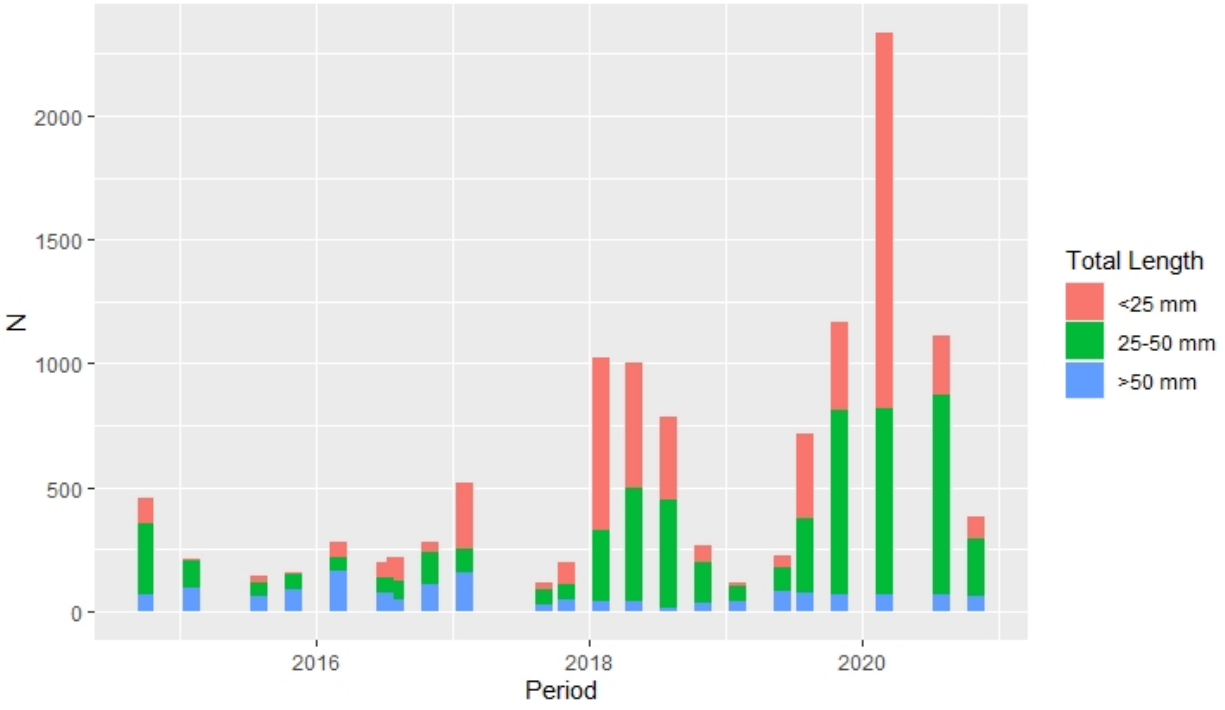

Figure 1. Estimates of abundance ($\hat{\hat{N}}$) based on size classes at Eliza Spring from October 2014 through November 2020.

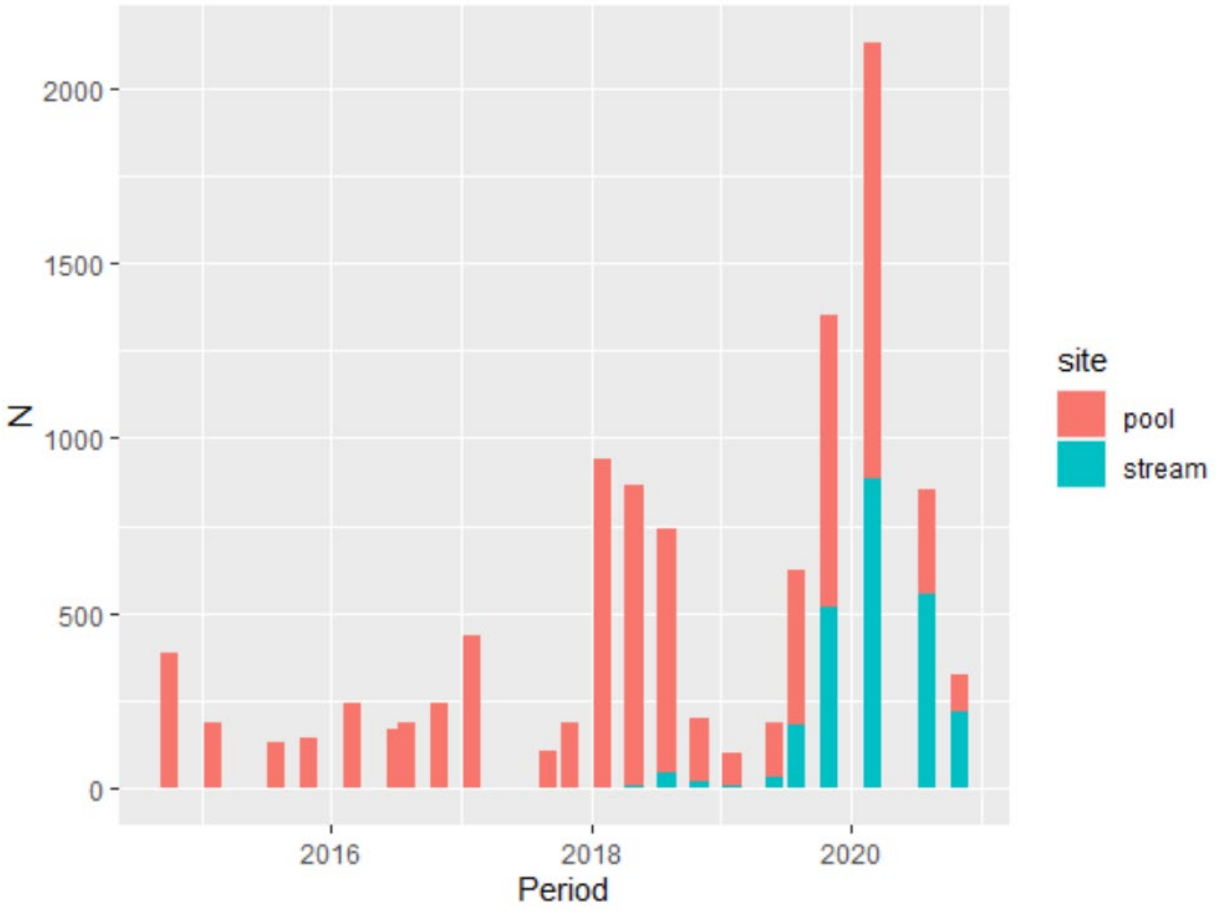

Figure 2. Estimates of abundance (*N*) by location at Eliza Spring from October 2014 through November 2020. Both locations were modeled as independent sites, so total abundance estimates may differ slightly from Fig. 1.

11) Results of genetic research conducted as a result of tail-clipping
No tail-tips were collected in 2020 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 in the 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). Permitted staff collect water samples from each spring in the Barton Springs complex. On an approximately monthly frequency, 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 biweekly 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, oil and grease, total polycyclic aromatic hydrocarbons, bromacil, organophosphate pesticides, chlorinated herbicides, volatiles, and semi-volatiles. 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. City of Austin staff collect sediment samples at the four Barton springs sites for testing to meet requirements of the City's TPDES permit. Samples were collected on 04/23/2019 at all four spring sites (Eliza, Old Mill, Upper Barton, and Barton Springs Pool), and on 07/25/2019 at Barton Springs Pool only. Due to restrictions related to the use of shared SCUBA equipment and the risk of COVID-19 contamination, samples were not collected during 2020, as of 11/19/2020. The project manager is scheduled to meet with staff to discuss the possibility of obtaining future samples on 12/02/2020.

d. BSSCF Trophic Ecology Project (Texas State University). Drift samples for aquatic invertebrates were collected as part of this study. Collections took place for 7 days at Eliza Spring from 01/16/2020 to 01/30/2020, and 4 days from 09/14/2020 to 09/18/2020; 4 days at Old Mill Spring from 09/15/2020 to 09/18/2020; 8 days at Barton Springs Pool from 03/03/2020 to 03/12/2020; 7 days at Upper Barton from 01/14/2020 to 01/28/2020 and 4 days from 09/15/2020 to 09/18/2020. During drift 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 refugia. Dead salamanders were retained for stable isotope studies. From Eliza Spring, 22 alive juvenile *E. sosorum* were released, 1 dead juvenile *E. sosorum* was collected, and 2 alive juvenile *E. waterlooensis* were collected for the refugia as seen in Figure 2. From the pool, 23 alive juvenile *E. sosorum* were released, 22 dead juvenile *E. sosorum* were collected, and 2 alive juvenile *E. waterlooensis* were collected for the refugia. Please see the attached progress report for a summary of all other activities performed.

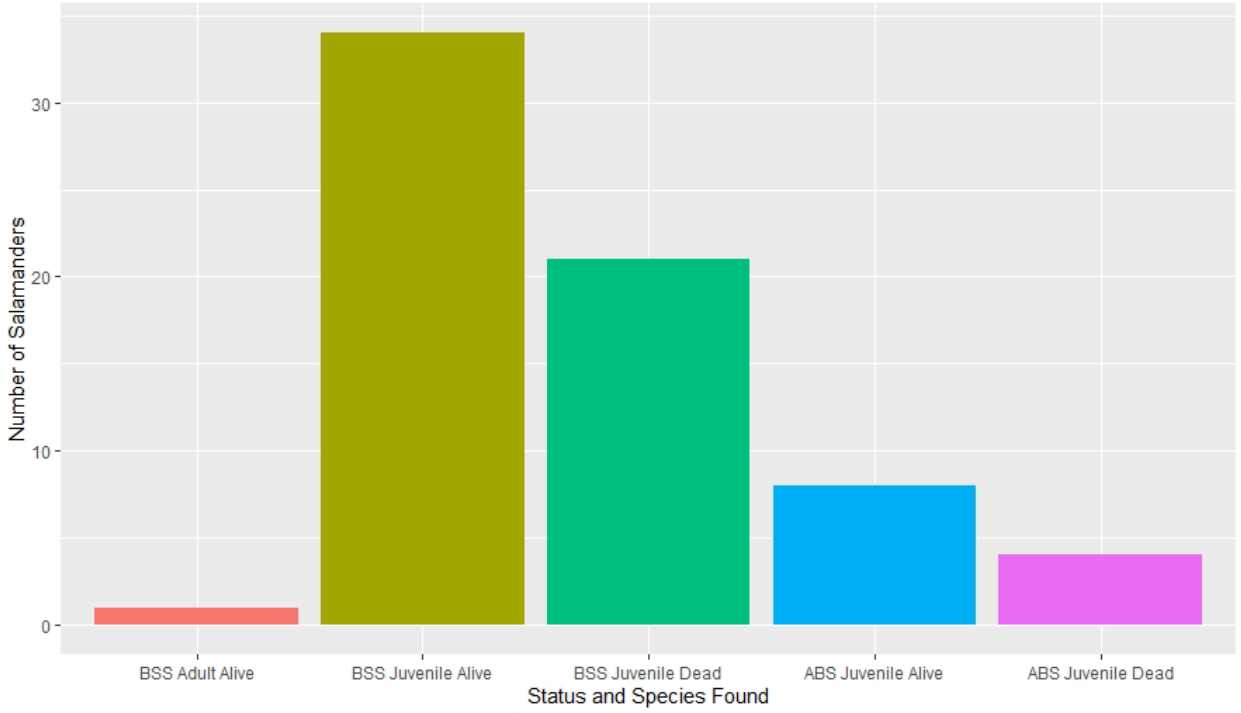

Figure 2. Total number of salamanders (BSS - *E. sosorum*, ABS - *E. waterlooensis*) and their status (found alive or dead) during the Eliza Spring drift net sampling events in 2020.

e. BSSCF Microbiome characterization of captive and wild Barton Springs and Austin Blind Salamanders (*Eurycea*) and associated habitats (Tarleton State University). Due to the COVID-19 pandemic the study was postponed until Sept. of 2020. Please see the attached progress report below. The next sampling event is scheduled for mid-December of 2020.

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 5).

Table 5. 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	7	22	15
	Old Mill	5	52	40
	Eliza	37	59	5
	UBS	0	0	2
Total		**49**	**133**	**62**
E. sosorum from SMARC[1]	Eliza	0	NA[2]	**94[2]**
E. waterlooensis	Parthenia	0	NA[3]	NA[3]
	Old Mill	5	NA[3]	NA[3]
	Eliza	8	NA[3]	NA[3]
	UBS	0	NA[3]	NA[3]
Total		**13**	**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.

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

In 2020, 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 sperm transfer, and possibly multiple transfers. Oviposition data are presented in Table 6. In addition, a study to describe courtship behavior was continued in early 2020 but then put on hold due to the pandemic. Ten pairs of Austin Blind salamanders were videotaped to score courtship behaviors. Of these, three pairs engaged in tail-straddle walk. These trials will be scored further to characterize courtship behavior.

Table 6. Ovipositions in captivity 12/01/19–11/18/20. 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.

Estimated Oviposition Date	Tank ID	Clutch Size	No. Hatched
	Eurycea sosorum		
12/28/2019	E (C253)	8	5
01/06/2020	OM (C005)	11	8
01/17/2020	E (C178-2)	15	Did not develop
01/27/2020	OMF1 (C052)	9	Did not develop (female not housed with male)
01/30/2020	E (C304)	30	Culled all but 5
02/04/2020	OMF2 (C184)	27	Did not develop (female not housed with male)
02/10/2020	BSPF2 (C311)	19	NA[1]
02/28/2020	OMF1 (C228)	9	Did not develop (female not housed with male)
03/06/2020	E (C297)	14	14
03/22/2020	SMARC F1/F2	25	Did not develop
04/07/2020	SMARC F1/F2	57 (2 salamanders oviposited)	Did not develop
	E. waterlooensis		
03/12/2020	F1 (CW15-3)	19	Did not develop
03/16/2020	F1 (courtship tank and then CW38)	16	Did not develop
	E. tonkawae		
03/11/2020	F1 (J07B)	36	Did not develop

[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 7.

Table 7. Salamander clutch size and hatching success from 12/01/19–11/18/20.

	Range	Mean	Standard Deviation
Eurycea sosorum			
Clutch Size	8-30 (N=12)	18.7	8.67
No. Hatched	5-14 (N=3)	9.0	4.58
% Hatched	62-100 (N=3)	78.4	19.38
E. waterlooensis			
Clutch Size	16-19 (N=2)	17.5	2.12
No. Hatched	0 (N=2)	0	0.00
% Hatched	0 (N=2)	0	0.00
E. tonkawae			
Clutch Size	36 (N=1)	36	0
No. Hatched	0 (N=1)	0	0
% Hatched	0 (N=1)	0	0

4) Salamander mortalities, including age and cause of death, if known are shown in Table 8.

Table 8. Salamander mortalities from 12/01/19–11/18/20.

Species	Wild-Caught or Captive-Bred	Age (years)	No. Mortalities	Cause of Death (health condition observed)
Eurycea sosorum	WC	< 1	1	Unknown, small, fragile size < 1" TL
	CB	< 3	6	Unknown
	CB	8–10	15	> 7.6 years (50% survivorship)[2]
	CB	10–12	10	> 7.6 years (50% survivorship)[2]
	CB	12–14	13	> 7.6 years (50% survivorship)[2]
	CB	14–15	3	> 7.6 years (50% survivorship)[2]
	CB transferred from SMARC 3/2020	~3-8	6	Unknown
E. waterlooensis	WC	<1	14	Unknown, small fragile size < 1" TL; 1 with spinal injury at collection with drift net
	CB	2	1	Unknown
E. tonkawae	WC	14–15[1]	4	> 7.6 years (50% survivorship, based on *E. sosorum*)[2]
	CB	6–9	2	Unknown

[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 2020, there were no unusual health conditions or behavioral aberrations observed.

6) Special Projects

In January 2020, we shipped 25 captive-raised (14 F1 and 11 F2) *Eurycea sosorum* to the University of Tennessee for a study on Bsal conducted by UTIA Amphibian Disease Laboratory. The salamanders were products of 9 reproductive groups and ranged in age from approximately 1 to 9 years. This study was approved by USFWS and results should be obtained from the research group. We received the following information from Matt Gray:

> *E. sosorum* was exposed to one of four doses (5 x 103-6 zoospores per mL; n = 4 per dose + five controls) of *Batrachochytrium salamandrivorans* (Bsal) at the University of Tennessee, and their survival and infection status monitored for 60 days. Skin swabs and quantitative polymerase chain reaction (qPCR) were used to test for Bsal infection. We discovered that 25% and 60% of individuals became infected at the two highest doses. Individuals that were infected did not alter their behavior (i.e., feeding activity or cover object use) nor experience mortality. These results provide evidence that *E. sosorum* is a suitable host for Bsal; however, they may be able to tolerate infection unlike several other *Eurycea* species (e.g., *E. wilderae*, *E. longicauda*) that develop clinical chytridiomycosis. All animals were preserved in 10% formalin for histological

examination and remain at the University of Tennessee.

In March 2020, 100 captive-raised *Eurycea sosorum* were transferred from SMARC (San Marcos Aquatic Resources Center) to ASCC (Austin Salamander Conservation Center) for an experiment to be conducted on the effects on feet of select pH levels and calcium concentrations. This project was postponed due to the pandemic but we anticipate a start date in 2021.

In September 2020, five *Eurycea sosorum* and five *E. waterlooensis* were sampled as part of the BSSCF microbiome project awarded to researchers at Tarleton University. In addition, 10 fecal pellets from each species were collected for the project. Please see the attached progress report below.

The captive breeding program provides support and salamanders for the public display tank at the Splash! Into the Edwards Aquifer Educational Exhibit. Due to the pandemic, the Splash! Exhibit was closed and these salamanders have been maintained at ASCC since March 2020.

General Annual Reporting Requirements for Jollyville Plateau Salamanders

No research activities were performed on Jollyville Plateau salamanders (*Eurycea tonkawae*) due to the COVID-19 pandemic.

General Annual Reporting Requirements for San Marcos Salamanders

No research activities were performed on San Marcos salamanders (*Eurycea nana*) or within San Marcos Salamander habitat.

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 9).

Table 9. Caves entered by City of Austin staff. ES = endangered species. KZ = karst zone.

Date	Cave or Property	Purpose	Habitat Observations	Karst Zone	Personnel
3/12/2020	Griffis Parmer Apartments Detention Pond	Inspect repairs to pond lining where water was previously leaking into subsurface	Excavations to bedrock, no obvious habitat observed	1	Lindsey Sydow
6/8/2020	Apple Campus Construction Site	Inspection of karst voids/caves encountered during construction	2 karst voids encountered in bedding plane while drilling piers, contained water when observed, habitat difficult to observe due to inaccessibility down-hole; high potential for ES due to KZ 1	1	Lindsey Sydow
6/23/2020	12505 Presque Cove	Inspection of karst voids/caves encountered during construction	Karst void encountered while excavating for residential swimming pool. Low potential for ES (KZ 3), but within 100 ft of KZ 1.	3	Michael Markowski, Scott Hiers, Lindsey Sydow, Radmon Rice
7/21/2020	Apple Campus Construction Site	Inspection of karst voids/caves encountered during construction	1 karst void encountered while excavating to grade, contained some debris and no visible speleothem development; high potential for ES due to KZ 1	1	Lindsey Sydow
7/23/2020	Property just south of 6715 Ranch Rd 620	Verify locations and types of karst features for development review	Verify recharge potential for solution cavity and verify other karst features onsite. High potential for ES (KZ 1).	1	M. Markowski, S. Hiers, L. Sydow, R. Rice
8/11/2020	McNeil High School Renovations (Void #1)	Inspection of karst voids/caves encountered during construction	Karst voids encountered along bedding plane in side walls during trenching, some speleothem development, high potential for ES due to KZ 1	1	Lindsey Sydow and Michael Markowski
9/11/2020	McNeil High School Renovations (Void #2)	Inspection of karst voids/caves encountered during construction	Karst voids encountered along bedding plane in side walls during trenching, some speleothem development, high potential for ES due to KZ 1	1	Michael Markowski
9/25/2020	Anderson Mill and FM 620	Inspection of karst dissolution fracture prior to nearby development	Previously observed dissolution fracture, will have protective buffer when surrounding area developed, potential for ES due to KZ 2	2	Lindsey Sydow
9/28/2020	1201 Dusky Thrush Trail	Inspection of karst voids/caves encountered during construction	Karst voids encountered along bedding plane in side walls during trenching, some speleothem development, high potential for ES due to KZ 1	1	Radmon Rice
10/14/2020	Circle C Park and Hielscher/Mary Gay Maxwell WQPL	Participate in dye tracing study	Participate is dye tracing study at four caves: GCC Anomaly Sink, Escarpment Sink, Injection Sink DT, and Hielscher Well. Low potential for ES (KZ 3), but within 100 ft of KZ 1.	3	Michael Markowski, Lindsey Sydow, Radmon Rice
10/30/2020	9301 Cedar Crest Drive	Inspection of karst voids/caves encountered during construction	Karst voids encountered during construction of a pool along bottom of pool excavation, some speleothem development, high potential for ES due to KZ 1	1	Radmon Rice
11/20/2020	McNeil High School Renovations (Void #3)	Inspection of karst voids/caves encountered during construction	Karst voids encountered along bedding plane in side walls during trenching, some speleothem development, high potential for ES due to KZ 1	1	Michael Markowski

Food Webs and Trophic Structure of the Groundwater Ecosystem Supporting Barton Springs and Austin Blind Salamanders

September 11, 2020 – Interim Progress Report; End of Year 1

Microscope photographs of an undescribed species of 'winged' ostracod ('seed shrimp') found in samples from Eliza Spring. The 'wings' may be unique among all known freshwater ostracods. Many other undescribed species of groundwater invertebrates were also found in samples. Photos: Benjamin Schwartz

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Executive Summary:

Covid-19-related prohibitions on performing field research at Texas State University caused unexpected delays in progress towards completion of the contracted research in mid-2020. However, since field research activities were approved in late June, we have worked hard and are nearly 'caught up' with the initially proposed work-schedule.

In late 2019 and early 2020, all sampling sites were selected and invertebrate sampling was completed at all the spring sites: Old Mill, Upper Barton, Eliza, and Barton Springs. In 2020, most wells and recharge sites have been visited and, at sites where possible due to well construction, sampled for invertebrates. Water, and microbe sampling has begun at a subset of wells. Collaboration with City of Austin staff in late 2019 allowed tissue sampling from salamanders to be completed. Work is currently underway to finish sampling at the remaining groundwater and recharge sites as soon as possible so that sample processing and preparation, and subsequent analyses at external labs, can be completed in late 2020 and early 2021.

Aside from the delays associated with Covid-19, no major, unexpected problems have been encountered and we anticipate no problems in completing the contracted work by the specified project end-date.

Progress details per task:

Unanticipated delays: Research restrictions associated with the Covid-19 pandemic caused delays when University faculty and staff were prohibited from performing field research from March until early July 2020. Despite these delays, and since field research was approved, work on the contracted field research and sampling has nearly all been completed on-schedule. The remaining Year 1 fieldwork is planned for completion in September or early October of 2020. A minority of wells previously identified for biological sampling have proven inappropriate due to obstructions or well construction. Alternative wells or alternative sampling methods are being evaluated.

Task 3.1.1: 90% complete. Early in the project (Fall 2019) we consulted and/or coordinated with the Barton Springs Edwards Aquifer Conservation District (BSEACD), City of Austin (CoA), and the United States Geological Survey (USGS) to select groundwater and surface water sites for sampling. USGS was included in these discussions to coordinate sampling (where possible) with separate but complimentary COA-funded research that the USGS is working on.

Task 3.1.2: ~90% of this task has been completed. Two recharge sites have not been flowing during most of spring and summer 2020 (drought) but will be sampled in the middle of September after recent rains have resulted in streamflows. On closer inspection, a few of the selected wells were determined to be unsuitable for sampling (obstruction in borehole, too small a diameter for bottle-trapping, etc), and some substitutes have been made, or are being investigated, to keep the total number of sampled sites as high as possible.

Task 3.1.3: 10% complete (scheduled for Y2). Samples for stable isotopes have been collected at some sites, and will be shipped to the lab at UC Davis as soon as all samples have been collected. This is anticipated to occur during Fall of 2020.

Task 3.1.4: ~50% complete. Some analyses of water samples have been completed, while others are in the queue for analysis after recent collections. All relevant field-parameters and field-analyses are being performed on-site at the time of water sample collection.

Task 3.1.5: 0% complete (scheduled for Y2). Most microbial samples have been collected and preserved in the field, and the remaining samples will be collected in Fall 2020. As soon as all these samples are collected, they will be analyzed by Dr. Engel, who is ready to receive them and process them in Year 2.

Task 3.1.6: ~25% complete. Most invertebrate samples from the major spring openings and most of the freshwater wells have been sorted and identified to a useful taxonomic level. Tail-clips from both salamander species have also been collected by CoA employees and transferred to TX State for isotopic analyses. This is the first step in the process. After all tissue samples are collected, they will be sent to the stable isotope lab at UC Davis. After the data return, the work on modeling and/or describing food resources, aquifer ecology, and ecosystem function will be completed in Year 2.

Task 3.1.7: Ongoing. This progress status report satisfies the requirements of this task for the end of Year 1.

General Information:

As sorting of samples collected from the spring openings has progressed, a wide variety of invertebrates have been preliminarily identified to a reasonable taxonomic level. In the future, we will continue to refine taxonomic identifications in our lab as well as sending many of these specimens to taxonomic

experts for positive ID and/or description. These include groups of stygobites that were not previously documented at these sites and/or are unidentified/undescribed species. Many of these are macro- and micro-invertebrates. To date, apparently groundwater-obligate organisms collected include: Microcerberidae, Lirceolus, and Cirolanides (Isopoda), aquatic mites (Hydrachnidia), cyclopoid and harpacticoid copepods (Hexanauplia), ostracods (Ostracoda), Ingolfiellids (Ingolfiellida), Parabogidiella and Stygobromus (Amphipoda), snails (Cochliopidae), Bathynellacea, and worms (Annelida).

These preliminary data illustrate that aquifer biodiversity in the Barton Springs segment of the Edwards Aquifer is almost certainly much higher than has previously been assumed or documented, and that it may comparable in diversity to the better-studied San Antonio segment to the south. Although describing diversity and organisms (alpha taxonomy) is not a task or goal of this contracted work, the results and curated specimens from this study can be used to support future research in this area and provide a first glimpse into what the true aquifer biodiversity in the Barton Springs segment may be.

Conclusion:

Progress on the contracted work is slightly behind schedule (Task 3.1.2), but all remaining tasks are on-target to be completed by the dates proposed; some were partly completed in Y1 but others are scheduled for completion in Y2.

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

Initial pilot trip to collect Barton Spring Salamander microbiome samples was November 18, 2019. Tarleton State University work team arrived at the Eliza Springs site at 10:00 am and collected samples until 5:00 pm. Salamanders were captured by city officials then noninvasively swabbed with sterile rayon swabs on the dorsal and ventral sides. Swabbing and sample collection took an average of five minutes per salamander. Four salamanders were placed in sterile 250 mL cups that were left in environmental water for one hour to allow scat samples to be collected. Two water and two substrate samples were collected at two separate sites at the outside edge of the amphitheater with sterile collection bottles. Gloves were changed between samples and sterile instruments were used to minimize cross-contamination.

Captive samples were collected from four salamanders, including some with limb deformities, where single feet or toes were swabbed. Two substrate samples were collected from salamander cages. Water samples were prepared in a large jug before arrival. The team arrived at the captive facility at 12:00 pm and worked until 1:00 pm to allow for adequate time at the Eliza Spring site. Time constraints prevented captive scat collection. All salamander samples were collected with the same noninvasive technique as wild counterparts.

Water was filtered for DNA extraction according to EPA method 1609.1 using membrane filters and a bead beater. DNA was extracted from swabs, filtered water, and substrate samples using Brady et al. (2011) protocol. The V4 region of prokaryotic 16S rRNA was amplified using established Earth Microbiome Project primers with amplification tags for Illumina sequencing technology. Profiling of fungal communities was achieved by amplification of the ITS region established by Earth Microbiome Project. After amplification samples were cleaned up using AMPure beads then reamplified with Illumina Index primers.

A total of 39 samples were collected from the trip with 26 originating from the wild population and 13 from the captive population. The 39 DNA extractions were amplified as training samples associated with the project. A total of 9 samples from the 39 collected successfully amplified and produced sequencing libraries (nine 16S libraries and nine ITS libraries). The original DNA extractions are stored and could be used to re-create sequencing libraries and re-sequence, however, the pilot samples are not included in the project budget, and the cost associated with library preparation and sequencing is not trivial. The successfully sequenced samples included 7 of the 26 wild population samples and 2 of the 13 captive population samples. Our conclusions about the pilot trip sampling effort are as follows:

1. Sequencing libraries can be successfully created from skin swab, water, fecal, and streambed samples.
2. Thorough swabbing of each salamander will be required for library prep. None of the samples from single foot or toe swabs, or lightly swabbed salamanders produced satisfactory results.

3. All samples will have water microbes present. The swabs used for sampling readily absorb water microbes and amplification and sequencing of them appears unavoidable in fecal, skin, and streambed samples.

Following quality filtering and removal of singleton sequences, there were 87,174 DNA sequences generated for the 9 successfully constructed 16S libraries, or about 9,686 DNA sequences/sample. The sequences were distributed among 456 combined genera from bacteria and archaea. Sample type could be easily distinguished by composition (Fig. 1), but meaningful statistical comparisons are not possible with the small number of libraries produced. The dominant 16S sequences belonged to Proteobacteria, accounting for 78.6% of the total pool. The next most abundant phyla were Firmicutes at 8.1%, Actinobacteria at 5.1%, Bacteroidetes at 4.1%, and Cyanobacteria at 2.2%. Other phyla accounted for less than 0.5% of the sample population when all samples were combined. Fungal ITS libraries are not shown, but the same 9 samples amplified for both 16S and ITS library construction.

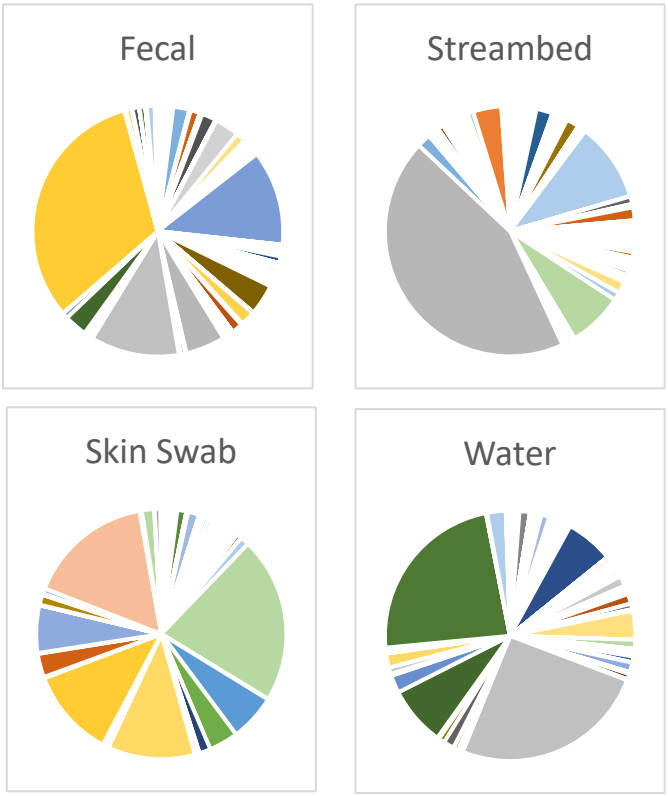

Fig. 1. Sample composition from 16S sequencing of archaea and bacteria. Pie wedges represent a total of 456 genera that were identified between the 9 samples producing 87,174 total DNA sequences.

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

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In preparation for the Fall 2020 sampling, cutaneous swabbing was optimized using three different amphibians. Amphibians were swabbed in 30 second increments with the maximum time allotted at two minutes. Cotton swabs were rotated continuously while swabbing to ensure all parts of the swab made contact with the mucosal layer. The maximum time an amphibian could be thoroughly swabbed while causing minimal stress was thirty seconds to one minute. All amphibian samples successfully amplified for 16S and ITS.

In collaboration with City of Austin scientists, Tarleton State University scientists collected a total of 109 samples from September 3, 2020 to September 4, 2020 on the first collection trip. Salamanders were found at every site except for Spillar Ranch. Four water and five substrate samples were taken from every site where salamanders were caught or where they are usually found. Water was taken from the inflow and outflow supply hoses at the captive facility. Six salamanders were found at Old Mill, five at Eliza Springs, and seventeen at Parthenia Pool. We hope to capture and sample a larger number of salamanders on future trips to acquire sample numbers closer to those envisioned in the proposal. Salamanders were swabbed for 20-30 seconds each. Fecal pellets were found at Eliza Springs site and the captive facility.

Site name	Old Mill	Spillar Ranch	Parthenia Pool	Eliza Springs	Captive Facility
Fecal samples	0	0	0	5	21
Skin samples	6	0	17	10	10
Water Samples	4	5	4	4	3
Substrate samples	5	5	5	5	0
Species found	*Eurycea sosorum*	N/A	*Eurycea sosorum*	*Eurycea sosorum*	*Eurycea sosorum* and *Eurycea waterlooensis*

The Tarleton State University graduate students are conducting sequencing library prep at Texas A&M AgriLife Research and Extension Center in Stephenville on all samples. Amplicon PCR produced 100% PCR amplification success on 16S DNA targets. Cleanup and indexing of 16S libraries is proceeding and will be followed by ITS library prep for fungal analysis, followed by normalization of libraries and pooling for sequencing. We currently project a shipping date of October 21, 2020 for all sequencing libraries with a one to two week turn around at the sequencing facility in College Station, TX. The following figures displays that (A.) all samples have successfully had the 16S region amplified and are ready for downstream processing and (B.) cleanup and indexing of 16S libraries and ITS library prep.

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

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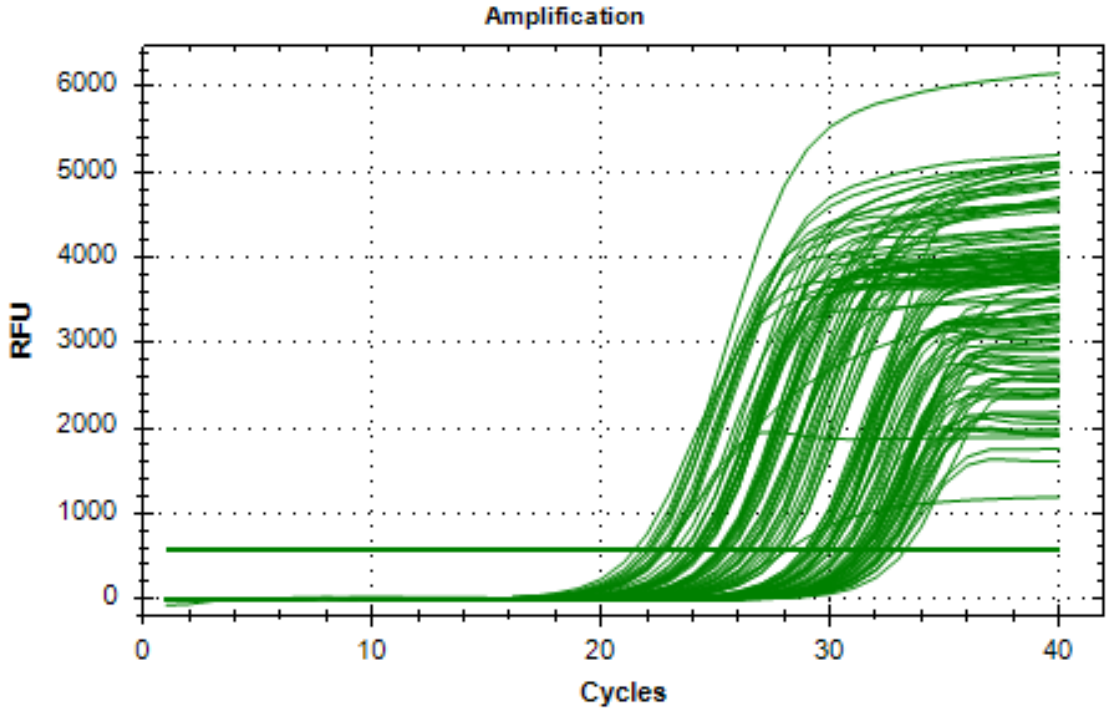
A.



B.