2020 ACTIVITIES REPORT FOR THE LANDS MANAGED BY THE WILLIAMSON COUNTY CONSERVATION FOUNDATION IN WILLIAMSON COUNTY, TEXAS

JUNE 2021

PREPARED FOR

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SWCA Project No. 52052

June 2021

EXECUTIVE SUMMARY

The purpose of this Activities Report is twofold: 1) To detail Williamson County Conservation Foundation (WCCF) activities performed during fiscal year 2020 related to federally protected species under the Endangered Species Act (ESA) in managed preserves; and 2) To document participation within the Williamson County Regional Habitat Conservation Plan (RHCP) for organizations seeking incidental take coverage for covered activities. The RHCP provides incidental take coverage for the following federally listed endangered species: the Inner Space Caverns mold beetle (*Batrisodes texanus*), the Bone Cave harvestman (*Texella reyesi*), and the golden-cheeked warbler (*Setophaga chrysoparia*). A fourth species, the black-capped vireo (*Vireo atricapilla*), was originally covered under the RHCP; however, this species was delisted in 2018. The RHCP also allocates funding for research of three *Eurycea* salamander species known to occur in Williamson County, all of which are federally listed as threatened.

Previous recommendations from the 2019 Activities Report and corresponding actions undertaken in 2020 are summarized in Table ES1. Proposed recommendations for 2021 and for consideration by the Adaptive Management Committee are summarized in Table ES2.

Genera	I Recommendations for Al	l Preserves – 2020	Actions Undertaken
Increase eradic	ation efforts of invasive plant	s across specific preserves.	On June 8, 2020, five large tree of heaven (<i>Ailanthus altissima</i>) trees and approximately 100 saplings were treated with herbicide at the Twin Springs Karst fauna Area (KFA).
	on County non-profit organiza veys within WCCF-administe	ations for recurring vegetation ared preserves.	Continuing to work with Williamson County Master Naturalists and Williamson County Chapter of the Native Plant Society of Texas (NPSOT) to develop vegetative inventories.
Final allocation	n for collected black-capped	vireo habitat impact funds.	Black-capped vireo impact funds remain unallocated but available.
	Preserve-Specific Recomr	nendations	
Preserve	Cave	2020 Recommendation	- Actions Undertaken
	_	Upgrade fence along southern boundary.	None.
Twin Springs	_	Continue control and management of tree of heaven infestation near Twin Springs.	On June 8, 2020, five large tree of heaven trees and approximately 100 saplings were treated with herbicide at the Twin Springs KFA.
	. <u></u>		
	-	Continue shaded fuel break program.	Approximately 1,700 feet of shaded fuel break was created at the northwest corner.
	– Potential New Feature		

Table ES1. Previous (2020) Recommendations and Corresponding Actions Undertaken

P	reserve-Specific Recom	mendations	
Preserve	Cave	2020 Recommendation	Actions Undertaken
Cobbs Cavern	_	Continue feral hog trapping as needed.	None.
Millennium	_	None.	-
Wilco	-	None.	_
Chaos Cave	_	Additional shaded fuel break.	An additional 1,000 feet of shaded fuel break was created at the northwest corner in January 2020.
Big Oak Cave	Big Oak	Continue to work with local law enforcement to minimize impacts from homeless encampment.	The WCCF has continued coordinating with law enforcement to address homeless camping problem within the preserve, which increases and decreases month-to-month.
Priscilla's Well	Yearwood Gold Mine	Excavate to determine if covered species present and install gate to preclude falling hazard to trespassers.	None.
	_	Excavate new karst feature.	None.
	Duckworth Bat	Fuel load reduction.	None.
Woodland Park	Duckworth Bat	Remove trash in dedicated effort.	None.
	Pemmican	Complete preserve border fence.	None.
Karankawa Cave	Pemmican	Replace silt fence with permanent rock wall or cobble filter.	Material for rock gabions was dropped off within the preserve at the end of 2020.
Coffin Cave	_	None.	_
Beck Commons	-	None.	_
Shaman Cave	_	None.	
Bat Well Cave	_	None.	_
Snowmelt Cave	_	None.	_

Table ES2. Current (2021) Recommendations for the Adaptive Management Committee

General Recommendations for All Preserves – 2021	Carry Over from Last Year?
Increase invasive plant eradication efforts across specific preserves.	Yes
Contact Williamson County non-profit organizations for recurring vegetation surveys within WCCF- administered preserves.	Yes
Final allocation for collected black-capped vireo habitat impact funds.	Yes

	Preserve-Spe	cific Recommendations	Carry Over	
Preserve	Preserve-Specific Recommendations Cave Recommendation - Upgrade fence along southern boundary. - Continue control and management of tree of heaven infestation near Twin Springs. - Continue shaded fuel break program. Potentially New Feature Excavate and gate new feature washing open near Sunless City Cave. - None. Cobbs Cavern Continue feral hog trapping as needed. - Perform golden-cheeked warbler surveys during 2022 breeding season. - Additional shaded fuel break. - Additional shaded fuel break. - Continue work with local law enforcement to minimize impacts from homeless encampment. Yearwood Gold Mine Excavate to determine if covered species are present and install gate to preclude falling hazard to trespassers.		from Last Year?	
	_	Upgrade fence along southern boundary.	Yes	
Turin Orninga	_		Yes	
Twin Springs	_	Continue shaded fuel break program.	Yes	
	Potentially New Feature		Yes	
Beck	-	None.	-	
Cobbs Cavern	Cobbs Cavern	Continue feral hog trapping as needed.	Yes	
Millennium	-		No	
Wilco	_		No	
Chaos Cave	-	Additional shaded fuel break.	Yes	
Big Oak Cave	Big Oak		Yes	
Priscilla's Well	Yearwood Gold Mine		Yes	
	_	Excavate new karst feature.	Yes	
	Duckworth Bat	Fuel load reduction.	Yes	
Woodland Park	Duckworth Bat	Remove trash in dedicated effort.	Yes	
Karankawa Cave	Pemmican	Complete preserve border fence.	Yes	
Coffin Cave	=	None.	_	
Beck Commons	_	None.	_	
Shaman Cave	_	None.	_	
Bat Well Cave	at Well Cave – None.		_	
Snowmelt Cave	_	None.	_	
Hidden Springs	_	None.	_	

As required by the USFWS-issued incidental take permit, ecological monitoring of karst preserves is performed annually. A summary of the 2020 annual biota survey for federally listed karst invertebrates documented within WCCF caves is shown in Table ES3. Biologists collected several additional *Batrisodes reyesi* (not protected, no common name) on the Beck Preserve. This species is a troglobite previously only known west of the Beck Preserve until 2017; therefore, collecting additional specimens is important for gaining a more thorough understanding of rare karst invertebrate habitat and the ability for the WCCF to protect such habitat.

Preserve	Monitored Cave Name	Known <i>Texella</i> Location?	Known <i>Batrisodes</i> Location?	<i>Texella</i> Identified in 2020?	Blind <i>Batrisodes</i> Identified in 2020 <i>?</i>
T · O ·	Sunless City	Yes	Yes	Yes	No
Twin Springs	Whitney West	Yes	No	Not monitored due	to COVID-19
	Beck Bat	Yes	No	Yes	Yes*
	Beck Pride	Yes	No	Yes	_
	Beck Crevice	Yes	No	Sealed and not	monitored [‡]
Beck	Beck Horse	Yes	No	Yes	_
	Beck Tex-2	Yes	No	Not monitored due	to COVID-19
	Beck Salamander	No	No	Not monitored due	to COVID-19
	Show Side	Yes	Yes	Yes	Yes
Cobbs Cavern	Wild Side	Yes	Yes	No	No
	Millennium	Yes	No	No	-
Millennium	Through Trip	No	No	-	_
	Little Demon	Yes	No	No	_
	Mongo	Yes	No	Yes	_
	Wilco	Yes	No	Not monitored due to COVID-1	
	Rock Ridge	Yes	No	Not monitored due	to COVID-19
Wilco	Wild West	Yes	No	No longer monitored due to rattlesnakes	
	Prospector	No	No	Not monitored due to COVID-	
	Venture	No	No	Not monitored due	to COVID-19
	Chaos	Yes	No	Yes	_
Chaos Cave	Poison Ivy	Yes	No	Not monitored due	to COVID-19
	Under the Fence	Yes	No	No longer monitore	ed (no habitat)
	Priscilla's	Yes	Yes	Not monitored due	to COVID-19
Priscilla's Well	Priscilla's Well	Yes	No	Not monitored due	to COVID-19
Weedland Deuk	Duckworth Bat	Yes	No	Yes	_
Woodland Park	Cat	Yes	No	Yes	_
	Angostura	No	No	No longer monitore	ed (no habitat)
	Armadon	No	No	-	_
Karankawa Cave	Karankawa	Yes	Yes	No	No
	Pemmican	Yes	No	Yes	_
	Polaris	Yes	No	No	-
	Snake Dancer	No	No	_	_

Table ES3. Annual Biota Survey Summary for Monitored Caves Shown Alongside DocumentedLocations Where Federally Listed Karst Invertebrates have been Previously Found

Preserve	Monitored Cave Name	Known <i>Texella</i> Location?	Known <i>Batrisodes</i> Location?	<i>Texella</i> Identified in 2020?	Blind <i>Batrisodes</i> Identified in 2020?
	War Party	Yes	No	Yes	_
Coffin Cave	Coffin	Yes	Yes	Not monitored (too warm)	
Beck Commons	Beck Sewer	Yes	No	Yes	_
Chaman Caus	Shaman	Yes	Yes	Yes	Yes
Shaman Cave	Powwow	Yes	Yes	Yes	Yes
Bat Well	Bat Well	No	No	_	_
Snowmelt	Snowmelt	Yes	No	No	_

* Batrisodes reyesi confirmed by Dr. Donald Chandler. Species is not federally protected.

[‡] Considered a smaller, secondary entrance to Beck Bat Cave and not monitored.

The WCCF was busy with additional activities during 2020, including publication of multiple *Eurycea* salamander papers and acquiring the largest WCCF managed preserve to date. The WCCF acquired the approximately 935.0-acre Hidden Springs Preserve in the northwestern portion of Williamson County and into Burnett County in 2020. The WCCF will send biologists to Hidden Springs during the 2021 golden-cheeked warbler (GCWA) breeding season to determine the number of breeding individuals that utilize this area. Afterwards, the WCCF intends to add approximately 240 acres of habitat within the Hidden Springs Preserve to its reserve of GCWA habitat credits for the species with an application to the USFWS that will be submitted in 2021. Initial surveys at Hidden Springs have shown the area to be occupied by breeding GCWA.

The WCCF is also actively engaged with multiple research programs dedicated to better understanding rare species in Williamson County. The WCCF actively funded *Eurycea* salamander monitoring and recapture efforts at many spring locations throughout Williamson County in 2020 (see Sections 19.1–19.6 for details). Dr. Chris Maupin of Texas A&M University continued to recreate the hydroclimate in the Southern Great Plains using oxygen isotope records from Cobbs Cavern and other locations around central Texas. Dr. Maupin anticipates three high-impact journal submissions regarding this research in 2021 (see Section 19.7 for details).

Table ES4 summarizes actual impacts to covered federally listed species habitat that occurred in 2020 versus the RHCP anticipated impacts to habitat. Table ES4 also portrays covered species habitat mitigation available for use. During 2020, the WCCF recorded no cave Zone A (50–345 feet from cave footprint) intrusions and recorded one intrusion to cave Zone B (within 50 feet of cave footprint) at a location with presumed endangered species presence (see SWCA et al. 2008 for details regarding cave impact zones). Approximately 129.8 acres of golden-cheeked warbler habitat were impacted during the same period. Table ES4 gives a brief overview of covered species impacts under the RHCP versus available mitigation, including karst fauna areas (KFAs).

Covered Species		Anticipated pacts	Actual Ir	npacts	Mitigation			
	Per Year Impacted Habitat (Caves)	Impacted Habitat Through 2020 (Caves)	2020 Impacted Caves	Impacted Caves Through 2020	2020 KFAs Added	Total KFAs	2020 Non-KFA Preserves Added	Total Non-KFA Preserves
KARST INVERTEBRATES	Impact Zone A: 5	Impact Zone A: 60	Impact Zone A: 0	Impact Zone A: 31	0	5	1	10
	Impact Zone B: 2	Impact Zone B: 24	Impact Zone B: 1	Impact Zone B: 5	0	5	1	10
BIRDS	Per Year Impacted Habitat (Credits)	Impacted Habitat Through 2020 (Credits)			Remaining Available			
Golden-cheeked Warbler	200.0	2,400.0	100.3	919.0	0	1,115.5	196.	6
Black-capped Vireo	142.2	1,706.4	DELISTED	22.5	0	0	-22.	5

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 - Jones, R. Z.C. Adcock, and K. White. 2020. *Eurycea naufragia* (Georgetown Salamander) predation. *Herpetological Review* 51:291–292.
 - Adcock, Z.C., A. Parandhaman, W.W. Keitt, and M.R.J. Forstner. 2020. *Eurycea tonkawae* (Jollyville Plateau Salamander) response to spring drying. *Herpetological Review* 51:808–809.

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Adcock, Z.C., M.E. Adcock, B.E. Hall, and M.R.J. Forstner. Modification of a water hyacinth sieve and description of Hubbard rakes for sampling small, aquatic salamanders. *Amphibian & Reptile Conservation*.

Appendix D. Salamander Manuscripts Submitted in 2020 and Currently In Review

McAllister, C.T., Z.C. Adcock, A. Villamizar-Gomez, R.M. Jones, and M.R.J. Forstner. A new host record for *Clinostomum* cf. *marginatum* (Trematoda: Digenea: Clinostomidae) from the endemic Salado Salamander, *Eurycea chisholmensis* (Caudata: Plethodontidae), from the Edwards Plateau, Texas, U.S.A. *Comparative Parasitology*.

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1 INTRODUCTION

Williamson County (the County) and the Williamson County Conservation Foundation (WCCF) were issued an incidental take permit by the U.S. Fish and Wildlife Service (USFWS) in October 2008 to authorize take of four federally protected species listed under the Endangered Species Act (ESA) arising from a variety of covered land development activities in properties voluntarily enrolled in the Williamson County Regional Habitat Conservation Plan (RHCP). The RHCP was prepared by the County with funding and technical assistance from the USFWS (SWCA Environmental Consultants [SWCA] et al. 2008). The RHCP supports an incidental take permit that authorizes the take of the Bone Cave harvestman (*Texella reyesi*), the Inner Space Caverns mold beetle (*Batrisodes texanus*¹), the golden-cheeked warbler (GCWA) (*Setophaga chrysoparia*), and the black-capped vireo (BCVI) (*Vireo atricapilla*²), collectively defined as the Covered Species. Take is defined under the ESA as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct" (Chapter 16 United States Code Section 1532(19)).

The WCCF preserve system (Figure 1) is intended to sustain high-quality habitat for species already on the endangered species list and to proactively conserve habitat thus precluding the need to list other species. As part of the WCCF preserve system, karst fauna areas (KFA) have been identified and consist of preserves that meet certain USFWS (2012) criteria and are thought to contribute to recovery of endangered karst invertebrates. Karst preserves do not always meet KFA criteria or have not always been reviewed by USFWS as potentially qualifying for KFA designation.

Additional species discussed within the RCHP as potential benefactors for conservation activities performed by the WCCF include 20 additional troglobitic cave invertebrates and up to three species of spring-adapted *Eurycea* salamanders, including the Salado salamander (*Eurycea chisholmensis*), the Georgetown salamander (*Eurycea naufragia*), and the Jollyville Plateau salamander (*Eurycea tonkawae*). See the RHCP for a complete list of permitted and additional species covered under this plan (SWCA et al. 2008).

This Yearly Activities Report describes the activities performed during fiscal year 2020 (including RHCP participation and monthly maintenance around caves), annual biota monitoring as required by the USFWS-issued incidental take permit, and other activities within the WCCF-administered preserves.

Due to the poor scientific understanding of habitat dynamics associated with terrestrial karst invertebrates, monitoring is particularly important for these species. Monitoring data are the best and only available measure of preserve performance. Ecological monitoring of karst preserves is performed annually by personnel holding a valid Section 10(a)(1)(A) scientific permit issued by the USFWS. Monitoring forms are completed during each monitoring trip.

For further details on species descriptions and preserve details, refer to *Preserve Descriptions of Land Maintained by the Williamson County Conservation Foundation Under the Williamson County Regional Habitat Conservation Plan* (Preserve Descriptions) (Van Kampen-Lewis and White 2020a).

¹ Chandler and Reddell (2001) split the listed Inner Space Caverns mold beetle (*Batrisodes texanus*) into two species, *B. texanus* and *B. cryptotexanus*, but the USFWS (2018a) does not officially recognize the split. Species identified as *B. cryptotexanus* are known from 15 caves, all in Williamson County (Chandler and Reddell 2001). Both species are federally listed endangered and are protected under the Endangered Species Act.

² The black-capped vireo was delisted on April 16, 2018, due to recovery (USFWS 2018b).

1.1 COVID-19 Impacts to WCCF Activities

The COVID-19 pandemic did have some impact on WCCF activities in 2020, notably on cave monitoring. A safety protocol was implemented by SWCA and Cambrian Environmental (Cambrian) that minimized contact between the two companies, thereby minimizing potential spread of the virus among individuals exerting significant energy in confined spaces. As a result, surveyors focused their efforts on those caves with the best habitat for karst invertebrates and adequate space for social distancing. Surveys in small caves with high atmospheric exchange that have traditionally not yielded endangered species counts were omitted to reduce unnecessary contact among surveyors. A total of 10 caves were excluded from survey efforts in 2020 (Table ES3). SWCA and Cambrian personnel also split up by company to perform biota surveys at preserves and caves that are large enough to warrant biota surveys but small enough to only require a few people from a single company to monitor. Other activities that often require close contact in confined spaces, such as karst feature excavation, were postponed until a later date, likely in 2021.

However, some caves managed by the WCCF are large enough that both SWCA and Cambrian were able to perform biota surveys simultaneously to ensure efficiency and effectiveness. In most cases, these caves were large enough that the respective companies could travel in opposite directions and/or survey different areas of the cave while remaining socially distant inside the cave. The following features are examples of caves large enough to allow for inter-company survey, with a quick note regarding potential team contact:

- Cobbs Cavern: This cave is approximately 1 mile long with the entrance essentially bisecting the cave in half. Each company could easily maintain distance by traveling in opposite directions. Dr. Chris Maupin of Texas A&M University also accompanied the team periodically visiting the Wild Side portion of the cave to collect data from his climate monitoring equipment. Dr. Maupin is typically accompanied by two undergraduate students; however, he visited the cave without students in 2020.
- Beck Sewer Cave: This cave is similarly bisected by the entrance and each company was able to easily maintain distance by traveling in opposite directions.
- Coffin Cave: This is a large cave with a single entrance and very little ability to separate each company; however, there is enough room in the cave that allowed surveyors to maintain safe distance between individuals. Moreover, the vertical nature of the entrance (~40-foot rope climb) means that there are safety-related benefits to having simultaneous ingress and egress.
- Bat Well Cave: This is a large cave with a single entrance and very little ability to separate each company; however, there is enough room in the cave that allowed surveyors to maintain significant distance between individuals. Moreover, the vertical nature of the entrance (~20-foot rope climb) means that there are safety-related benefits to having simultaneous ingress and egress. Additionally, the tightest squeezes are in two separate chambers, which allowed each company to maintain distance while surveying stream passages for *Eurycea* salamanders.

Additionally, staff from both companies followed these COVID-19 safety protocols in 2020:

- While on the surface, staff from both companies maintained social distance (6 feet) from one another and wore masks.
- Masks were always worn by personnel on the surface and when inside a cave.
- Aspirators to collect invertebrates were not used due to the requirement for mask removal and rapid inhalation of potential pathogens.

- Entry into tight squeezes by multiple staff required a 2-minute "cool-down period," whereby staff waited 2 minutes prior to entry into the squeeze after the previous entrant in order to allow some dispersal of potential exhaled pathogens.
- SWCA and Cambrian staff avoided inter-company vehicle travel.
- When inter-company cave visits could not be avoided, all personnel received a forehead temperature reading prior to cave entry. This technique is known to detect elevated temperatures of infected individuals who may not yet feel ill. Personnel with an elevated temperature would have been asked to leave the project area if so detected.
- Finally, personnel also answered the following questions prior to cave entry and would have been precluded from entering the cave if answering "yes" to any of these questions:

1. Have you tested positive for COVID-19 or had close contact with or cared for someone that has been diagnosed or is pending test results due to symptoms for COVID-19 in the last 14 days?

2. Have you experienced any cold or flu-like symptoms or cared for someone with these symptoms in the last 14 days?

3. Do you currently have an elevated temperature of more than 100.4? (Thermometer will be provided upon request.)

4. Have you traveled by air or been on a cruise ship in the last 14 days?

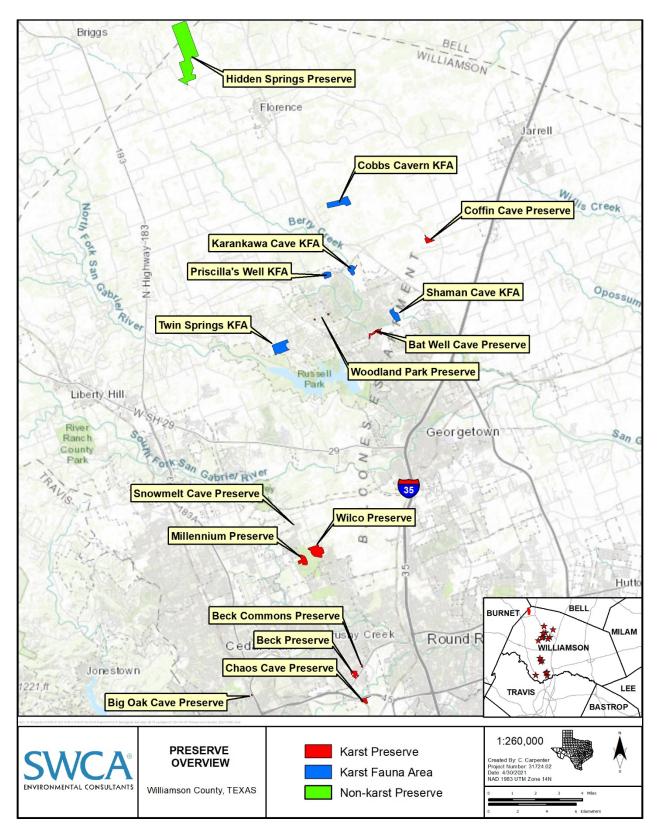


Figure 1. Location map of lands managed by the Williamson County Conservation Foundation.

1.2 Adaptive Management Issues

Adaptive management is an iterative process that allows preserve management flexibility as new data are collected and is a core tenet of the RHCP (SWCA et al. 2008). Adaptive management issues are presented to the Adaptive Management Committee (AMC) within each Yearly Activities Report and actions are investigated by the group during subsequent meetings (see SWCA et al. 2008 for AMC description). The AMC makes recommendations to the RHCP Administrator, who then presents recommendations to the WCCF Board. The WCCF Board votes on action funding and implementation.

The AMC reviewed the previous annual reports (i.e., Management Plan, Preserve Descriptions, Activities Report) after they were sent out for review on May 5, 2020. A teleconference with the AMC to discuss various issues occurred on May 8, 2020.

Each preserve is unique with respect to ecological conditions present within the surface and subsurface environment. Therefore, individual preserves receive AMC recommendations within their specific section. However, the following are some blanket comments that address several issues throughout most or all of the preserves:

- Invasive plant species within preserves are an ongoing issue and eradication efforts are planned for 2021. These species are often fast-growing and can quickly dominate large tracts of land. In addition to crowding and diverting resources from native plants, many invertebrate and vertebrate species will not utilize habitat dominated with unfamiliar plants. Common non-native plants in central Texas that require reduction management within the preserves include privet (*Ligustrum* spp.), Chinaberry (*Melia azedarach*), tree of heaven (*Ailanthus altissima*), sacred bamboo (*Nandina domestica*), King Ranch bluestem (*Bothriochloa ischaemum* var. *songarica*), and Bermudagrass (*Cynodon dactylon*). Identified invasive plants around cave entrances are generally removed by the WCCF's Trail and Preserve Steward. However, the areas adjacent to cave entrances constitute a limited area as compared with the overall preserves. Invasive species are generally found throughout preserves and are only removed when they encroach on cave entrance habitat. Removing individual plant species across the landscape would require large-scale, expensive hand removal. The AMC may wish to consider which set of tools is appropriate for WCCF-administered lands and which preserves could potentially benefit.
- Continuing vegetation surveys are useful tools for assessing overall preserve health. The AMC may wish to considering having the Williamson County Master Gardeners, the Williamson County Chapter of the Native Plant Society of Texas (NPSOT), or another qualified organization perform vegetation surveys within each WCCF-administered preserve on a recurring basis.
- The AMC may wish to consider final allocation for BCVI collected participation funds to cover habitat impacts across 22.5 acres.

2 METHODS

Recurring annual activities at each preserve are generally broken into two categories: monthly maintenance activities and biota surveys. Caves are generally surveyed once per year, while *Eurycea* localities may be surveyed monthly. The WCCF's Trail and Preserve Steward (Mark Pettigrew) conducts monthly maintenance activities around each cave's portal under the WCCF's care. Monthly maintenance activities are restricted to the general vicinity around each cave's entrance and do not involve cave ingress. This report includes the monthly maintenance notes under each preserve's designated section. Biota surveys involve annual physical entry within the caves to document encountered organisms. This report also includes the results from each preserve's biota surveys.

The WCCF performs additional activities that may only occur once or less often than annually. For example, GCWA surveys occur every other year at the Twin Springs KFA and did not occur in 2020.

2.1 Maintenance Activities

The Trail and Preserve Steward inspects general site conditions monthly for noticeable changes that have occurred since the prior inspection. The Trail and Preserve Steward repairs fences and signs; documents damage caused by storms, fire, humans, or mammals (i.e., feral hogs, cattle, deer); and removes trash.

The Trail and Preserve Steward's monthly inspections generally examine a 200-foot area around each cave for noticeable changes to the immediate environment. The Trail and Preserve Steward examines cave gates and locks for damage from natural metal fatigue or from human attempts to gain access. The Trail and Preserve Steward generally fixes noted damage quickly and records such activities in the preserve notebook. He also lubricates locks if needed and notes invasive plant encroachment around caves for removal efforts.

The RHCP notes red imported fire ant (RIFA) (*Solenopsis invicta*) presence around cave entrances, which is thought by USFWS to have negative effects to the cave ecosystem and associated karst invertebrates. The Trail and Preserve Steward documents RIFA mounds within 10 meters (m) (33 feet) and 50 m (164 feet) from cave entrances with listed karst invertebrates during monthly inspections. He treats mounds with boiling water biennially regardless of mound density and conducts additional treatments if a RIFA mound is located within 10 m of the cave entrance or if mound density exceeds 80 mounds within 50 m (164 feet) of the cave entrance. The RHCP contains additional details regarding RIFA control (SWCA et al. 2008).

The Trail and Preserve Steward records activities at each cave, and these notes are presented in the preserve sections of this report (Sections 3 through 18).

The RHCP Management Plan (Van Kampen-Lewis and White 2020b) describes all activities to be completed within each preserve in greater detail.

2.2 Annual Biota Surveys

Monitored caves are generally surveyed in the same manner. Biologists record climate (i.e., temperature and humidity) within the deepest accessible cave location. Cave size dictates the number of biologists within each cave and the survey duration. Large caves require greater person-hours³ of survey effort. Small caves may only allow for one surveyor and may not require as much time to record species composition when compared to larger caves. Biologists investigate rock, wall, ceiling, and floor surfaces for invertebrate and vertebrate habitation. Biologists also flip rocks (which are then returned to original position) and examine void spaces to ensure thorough cave investigation. If a species is observed, a biologist records the species (if known), estimated quantities of each species, and notable cave characteristics. Collected specimens are preserved in 100% non-denatured alcohol and sent to Mr. James Reddell at the Texas Memorial Museum or other invertebrate specialists, such as Dr. Donald S. Chandler at the University of New Hampshire.

³ A person-hour is the survey time spent per person. For example, two surveyors spending one-half hour within a cave would total one person-hour of survey time.

3 TWIN SPRINGS KARST FAUNA AREA

Preserves are listed in this report in chronological order as they are managed by the WCCF. As such, Twin Springs is the first preserve managed by the WCCF and the first preserve where annual activities are discussed within this report.

The Twin Springs KFA is located adjacent to U.S. Army Corps of Engineers-owned land on the north side of Lake Georgetown, west of Russell Park Road and south of the end of Twin Springs Road. It is composed of three contiguous management areas that are managed collectively as the 172.2-acre Twin Springs KFA.

3.1 Maintenance Activities

One of the cave gates within the KFA, specifically the Whitney West Cave gate, was extended during 2016 to fully cover the cave entrance in an effort to reduce unauthorized access potential; however, trespassers were able to dig around the gate to gain access to the cave during 2017. The cave gate was not fixed due to current excavation efforts at Whitney West Cave; however, it will be fixed after excavation efforts are concluded. Whitney West Cave excavation efforts are intended to locate an additional Inner Space Caverns mold beetle locality and will continue into the foreseeable future. On June 8, 2020, five large tree of heaven trees and approximately 100 saplings were treated with herbicide. Approximately 1,700 feet of shaded fuel break was created within the northwestern corner of the Twin Springs KFA during 2020.

Two caves are associated with this KFA: Sunless City Cave and Whitney West Cave. Vandalism was detected at Sunless City Cave in May of 2020 when a pile of vegetation was dumped at the cave entrance. The Trail and Preserve Steward contacted the landowner and requested that they not dump near the cave entrance. Whitney West Cave was not surveyed in 2020 due to the COVID-19 pandemic. Notes from the monthly cave inspections are provided in Table 1.

	Vandalism,	Damage to	Gate	Off-Trail	RIFA Mounds	1. Comments
	Trash Dumping and Unauthorized	Vegetation, Pet Issues and Feral Animals	Inspection / Lock Lubrication	Activity	Within 10 m of Cave Entrance	2. Tasks Completed
	Entry					3. Tasks Outstanding
January						
Sunless City	No	No	Yes/No	Νο	None.	1. Trash and human feces near parking lot.
Cave						Picked up trash and feces.
						3. None.
						1. None.
Whitney West Cave	No	No	Yes/No	No	None.	2. None.
	NO	INU	169/100	NO		 Need to extend gate.

Table 1. Preserve Maintenance Triggers within the Twin Springs KFA

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
February						
Sunless City Cave	No	No	Yes/No	No	None.	None.
Whitney West Cave	No	No	Yes/No	No	None.	1. None. 2. None. 3. Need to extend gate.
March						
Sunless City Cave	No	No	Yes/No	No	None.	None.
Whitney West Cave	No	No	Yes/No	No	None.	 None. None. Need to extend gate.
April						
Sunless City Cave	No	No	Yes/Yes	No	None.	None.
Whitney West Cave	No	No	Yes/Yes	No	None.	 None. None. Need to extend gate.
Мау						
Sunless City Cave	Yes	No	Yes/No	No	None.	 Vegetation dumped near cave entrance. Contacted neighbor, asket them not to dump vegetation near the cave. None.
Whitney West Cave	No	No	Yes/No	No	None.	 None. None. Need to extend gate.
June						-
Sunless City Cave	No	No	Yes/No	No	None.	None.
Whitney West Cave	No	No	Yes/No	No	None.	 None. None. Need to extend gate.
July						
Sunless City Cave	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed
	Entry					3. Tasks Outstanding
						1. None.
Whitney West	No	No	Yes/Yes	No	None.	2. None.
Cave						 Need to extend gate.
August						
Sunless City Cave	No	No	Yes/No	No	None.	None.
						1. None.
Whitney West	No	No	Yes/No	No	None.	2. None.
Cave	110		100/110	110	None.	3. Need to extend gate.
September						
Sunless City Cave	No	No	Yes/No	No	None.	 Trash in parking lot. Erosion around the edge of the gate. Picked up the trash. Repaired erosion around the cave gate.
						3. Erosion around the gat needs repair.
						1. None.
Whitney West	No	No No	Yes/No	No	None.	2. None.
Cave						 Need to extend gate.
October						
Sunless City						1. Trash in parking lot. Erosion around the edge of the gate.
Cave	No	No	Yes/Yes	No	None.	2. Picked up the trash. Repaired erosion around the cave gate.
						3. None.
						1. None.
Whitney West Cave	No	No	Yes/Yes	No	None.	 None. Need to extend gate.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
November						-
Sunless City Cave	No	No	Yes/No	No	None.	 Trash in parking lot. Picked up trash. None.
Whitney West Cave	No	No	Yes/No	No	None.	1. None. 2. None. 3. Need to extend gate.
December						
Sunless City Cave	No	No	Yes/No	No	None.	None.
Whitney West Cave	No	No	Yes/No	No	None.	 None. None. Need to extend gate.

* 80% Fence intact. Need new fence along southeast corner.

3.2 Karst Biota Surveys

Biologists performed karst biota surveys on December 10, 2020, within Sunless City Cave. Due to the ongoing COVID-19 pandemic, biologists did not survey Whitney West Cave. Biologists detected three Bone Cave harvestmen and no Inner Space Caverns mold beetles within Sunless City Cave (Table 2). Biologists collected no specimens from the Twin Springs KFA in 2020. Karst biota survey results are summarized in Table 2. Endangered species are highlighted in table cells throughout this report.

		Insic	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
December 10, 2	020	-	-	-			-
Twin Springs	Sunless City	77.1	74.8	1.5	S. Van Kampen-Lewis, L. Rome, C. Crawford	Texella reyesi	3
						Ceuthophilus secretus	dozens
						Cicurina varians	2
						Cicurina vibora	13
						Leiobunum townsendi	6
						Lithobiomorpha	1
						Scutigeridae	2
						Rhadine subterranea	1
						Collembola	dozens
						Helicodiscus sp.	4
						Annelid	10
						Surface Caterpillar	1
Twin Springs	Whitney West				*Not surveyed in 2020*		

Table 2. Karst Biota Survey Results at Twin Springs KFA

A potential karst feature appears to be naturally washing open within approximately 50 feet of the designated parking lot at the northeastern corner of Twin Springs KFA. It does not appear that runoff from the parking lot is entering this feature. This feature was noticeable during multiple previous karst biota surveys and has since become more defined. The potential exists for a third cave containing listed troglobites to occur within the Twin Springs KFA. Photograph 1 depicts the feature as it appeared during the 2018 karst biota survey. It was similar in appearance during the 2020 biota survey.



Photograph 1. Potential new karst feature as it appeared during the 2018 karst biota survey.

Sunless City Cave is the only cave in the KFA with documented sightings of both the Bone Cave harvestman and the Inner Space Caverns mold beetle. However, Bone Cave harvestman detection inside Sunless City Cave has been inconsistent over time, with a notable detection spike in 2014 and again in 2017, as compared to 2016, 2019, and 2020 where biologists documented only two individuals each year (Figure 2). Potential cave flooding (as evidenced by flood debris within the cave) may decrease the detectable Bone Cave harvestman population during heavy rainfall events.

Climate readings within Sunless City Cave are not static when viewed across time. Relative humidity appears variable, with an overall declining trend line from near 100% in 2009 down to 66.9% in 2017, and then back up to saturation in 2018 (Figure 3). Temperature has fluctuated between just over 60 degrees Fahrenheit (°F) and nearly 80°F over the nine survey periods, with 2009, 2016, and 2017 as the warmest documented survey years (see Figure 3). Two of the warmest years (2009, 2016) also coincide with Inner Space Caverns mold beetle appearances. Due to equipment malfunction, 2019 was omitted from the climate chart. Temperature and humidity within Sunless City Cave were well within typical parameters encountered over previous years.

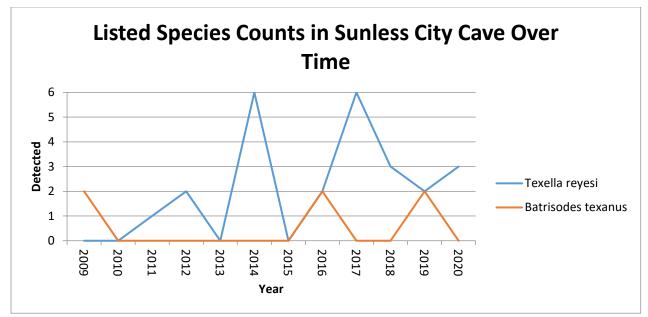


Figure 2. Endangered karst invertebrate species detection within the Twin Springs KFA.

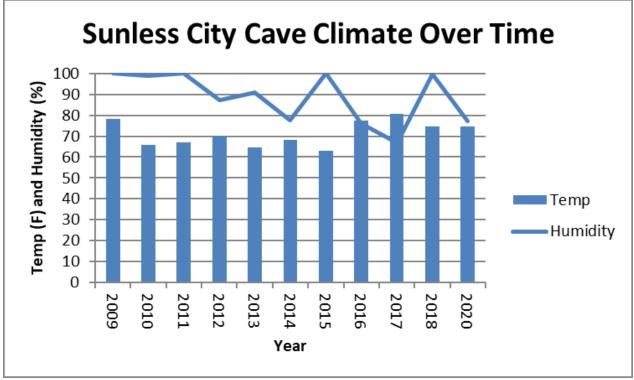


Figure 3. Climate readings within Sunless City Cave.

3.3 Adaptive Management Issues

It is recommended that the AMC consider the following specific issues at the Twin Springs KFA.

- A new residential development near the southern Twin Springs KFA boundary may increase foot traffic to the area. The AMC may wish to consider allocating funding for fence upgrades along the southern boundary to help reduce the potential for trespassing; discussions with the developer are occurring now. This recommendation is carried over from last year.
- An infestation of the invasive tree of heaven currently occupies a large area just downstream from Twin Spring. The AMC may wish to consider continuing the control and management of this invasive species.
- Twin Springs KFA is a large natural area with significant fuel load directly adjacent to several low-density residential development. As such, the AMC may wish to continue the shaded fuel break program around the perimeter of Twin Springs KFA.
- A new karst feature is washing open near the Twin Springs KFA parking lot (see Photograph 1). The Sunless City portal is similar in appearance to this new karst feature, which could indicate another vertical cave feature within the KFA. This feature may contain cave passage inhabited by the Inner Space Caverns mold beetle and would be considered the second such cave within the KFA to have this species. The new feature may invite curious onlookers to get too close to an open or nearly open pit, which could cause safety concerns to the public. We recommend excavating the feature to reveal cave extent and biotic presence and installing a cave gate to protect preserve users. This recommendation is carried over from last year.

4 BECK PRESERVE

The Beck Preserve is approximately 44.5 acres in size. The preserve is located in the City of Round Rock, southwest of the intersection of Ranch-to-Market 620 and Great Oaks Drive.

4.1 Maintenance Activities

In 2020, maintenance activities were routine, with most notable actions restricted to RIFA eradication efforts.

There are seven caves associated with this preserve: Beck Bat, Beck Creek, Beck Crevice, Beck Horse, Beck Pride, Beck Salamander, and Beck Tex-2 Caves. The Beck Crevice Cave continues to be covered with large rocks and is no longer surveyed due to its connection with the larger, deeper Beck Bat Cave. Notes from the 2020 monthly cave inspections are provided in Table 3.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Beck Bat Cave	No	No	Yes/No	No	None	None.

Table 3. Preserve Maintenance Triggers within the Beck Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection /	Off-Trail Activity	RIFA Mounds Within 10 m of	1. Comments
			Lock Lubrication	, . . ,	Cave Entrance	2. Tasks Completed
	Entry					3. Tasks Outstanding
Beck Creek Cave	No	No	Yes/No	No	None	None.
Beck Crevice	Ne	Na	NI- /NI-	Na	Nere	1. Rocks still intact over gate
Cave	No	No	No/No	No	None	2. None.
						3. None.
Beck Horse Cave	No	No	Yes/No	No	None	None.
Beck Pride Cave	No	No	Yes/No	No	1×3 m S.W. (not active)	None.
Beck Salamander Cave	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
February						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	None	None.
Beck Crevice Cave	No	No	No/No	No	None	1. Rocks still intact over gate 2. None. 3. None.
Beck Horse Cave	No	No	Yes/No	No	None	None.
Beck Pride Cave	No	No	Yes/No	No	None.	None.
Beck Salamander Cave	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
March						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	None	None.
Beck Crevice Cave	No	No	No/No	No	None	1. Rocks still intact over gate 2. None. 3. None.
Beck Horse Cave	No	No	Yes/No	No	None	None.
Beck Pride Cave	No	No	Yes/No	No	None	None.
Beck Salamander Cave	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
April						
Beck Bat Cave	No	No	Yes/Yes	No	None	None.
Beck Creek Cave	No	No	Yes/Yes	No	None	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection /	Off-Trail Activity	RIFA Mounds Within 10 m of	1. Comments
			Lock Lubrication		Cave Entrance	2. Tasks Completed
	Entry					3. Tasks Outstanding
Beck Crevice Cave	No	No	No/No	No	None	 Rocks still intact over gate None. None.
Beck Horse Cave	No	No	Yes/Yes	No	1×5 m S. (Not active).	None.
Beck Pride Cave	No	No	Yes/Yes	No	None	None.
Beck Salamander Cave	No	No	Yes/Yes	No	1×10 m N. (Not active).	None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	None	None.
Мау						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	None	None.
Beck Crevice Cave	No	No	No/No	No	None	1. Rocks still intact over gate 2. None. 3. None.
Beck Horse Cave	No	No	Yes/No	No	Old mound no longer active.	None.
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to long grass).	None.
Beck Salamander Cave	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.
June						
Beck Bat Cave	No	No	Yes/No	No	None.	None.
Beck Creek Cave	No	No	Yes/No	No	None.	None.
Beck Crevice Cave	Yes	No	No/No	No	None.	 Rocks still intact over gate Evidence of digging 15 feet north of the cave entrance. Hole filled in 3. None.
Beck Horse Cave	No	No	Yes/No	No	None.	None.
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to long grass)	None.
Beck Salamander Cave	No	No	Yes/No	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed
	Entry					3. Tasks Outstanding
July	-		-	-	-	•
Beck Bat Cave	No	No	Yes/Yes	No	None.	None.
Beck Creek Cave	No	No	Yes/Yes	No	None.	None.
Beck Crevice Cave	No	No	No/No	No	None.	None.
Beck Horse Cave	No	No	Yes/Yes	No	None.	None.
Beck Pride Cave	No	No	Yes/Yes	No	None (Difficult to monitor due to long grass)	None.
Beck Salamander Cave	No	No	Yes/Yes	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	None.	None.
August						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	None	None.
Beck Crevice Cave	No	No	No/No	No	None	None.
Beck Horse Cave	No	No	Yes/No	No	None.	None.
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to long grass)	None.
Beck Salamander Cave	No	No	Yes/No	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.
September						
Beck Bat Cave	No	Yes	Yes/No	No	None.	1. Cat in cave entrance. 2. None. 3. None.
Beck Creek Cave	No	No	Yes/No	No	None.	None.
Beck Crevice Cave	No	No	No/No	No	None.	None.
Beck Horse Cave	No	No	Yes/No	No	None.	None.
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to long grass).	None.
Beck Salamander Cave	No	No	Yes/No	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
October	-	-	-	-	-	-
Beck Bat Cave	No	No	Yes/Yes	No	None.	 Trash around the perimeter. Picked up trash. None.
Beck Creek Cave	No	No	Yes/Yes	No	None.	None.
Beck Crevice Cave	No	No	No/No	No	1×5 m W.	1. Too cold to treat RIFA. 2. None. 3. None.
Beck Horse Cave	No	No	Yes/Yes	No	None.	None.
Beck Pride Cave	No	No	Yes/Yes	No	None (Difficult to monitor due to long grass).	None.
Beck Salamander Cave	No	No	Yes/Yes	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	None.	None.
November						
Beck Bat Cave	Yes	No	Yes/No	No	None.	 Mattress dumped along roadside. Picked up and removed. None.
Beck Creek Cave	No	No	Yes/No	No	None.	None.
Beck Crevice Cave	No	No	Yes/No	No	1×4 m S.W.	1. Treated RIFA. 2. None. 3. None.
Beck Horse Cave	No	No	Yes/No	No	None.	None.
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to long grass).	None.
Beck Salamander Cave	No	No	Yes/No	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.
December						
Beck Bat Cave	No	No	Yes/No	No	None.	None.
Beck Creek Cave	No	No	Yes/No	No	None.	None.
Beck Crevice Cave	No	No	Yes/No	No	None.	None.
Beck Horse Cave	No	No	Yes/No	No	None.	None.

Preserve/Cave*	Vandalism,	Damage to	Gate	Off-Trail	RIFA Mounds	1. Comments
	Trash Dumping Vegetation, Inspection / Activ and Pet Issues and Lock Unauthorized Feral Animals Lubrication		Activity	Within 10 m of Cave Entrance	2. Tasks Completed	
	Entry					3. Tasks Outstanding
Beck Pride Cave	No	No	Yes/No	No	None (Difficult to monitor due to tall grass).	None.
Beck Salamander Cave	No	No	Yes/No	No	None.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None.	None.

* No fence.

4.2 Karst Biota Surveys

Biologists performed karst biota surveys on October 14, 2020. As of 2017, the Beck Preserve is now a known locality for an unlisted troglobitic mold beetle (*Batrisodes reyesi*). Biologists collected one additional specimen in Beck Bat Cave, increasing the number of individuals known from the Beck Preserve. Collections yielding new species in 2017 indicate the Beck Preserve is a unique place that may have additional species not previously known from the area.

Biologists also continue to focus collection efforts to locate the endangered Tooth Cave spider (*Tayshaneta myopica*) within the caves associated with this preserve. The species is documented from Goat Cave, approximately 0.5 mile northeast of the Beck Preserve. The proximity of these locations indicates a high probability the Tooth Cave spider inhabits the Beck Preserve. Documentation of this species in the Beck Preserve would increase the preserve's conservation value and may be valuable to the County if Endangered Species Act compliance issues arise for the Tooth Cave spider.

Table 4 lists the karst invertebrate specimen collections made at the Beck Preserve in 2020.

Cave	Таха	Quantity
	Batrisodes reyesi	1
	Batrisodes uncicornis	2
Deals Dat	Anillinus sp.	1
Beck Bat	Pseudoscorpion	2
	Speodesmus bicornorus	2
	Helicodiscus eigenmanii	1
	Batrisodes uncicornis	5
Beck Horse	Rhadine subterranea	1
	Batrisodes uncicornis	3
Beck Pride	Pseudoscorpion	1

Table 4. Collected Specimens at the Beck Preserve during 2020

Biologists detected eight Bone Cave harvestmen within Beck Bat Cave, six Bone Cave harvestmen in Beck Horse Cave, and three Bone Cave harvestmen in Beck Pride Cave. Beck Salamander Cave and Beck

Tex-2 Cave were not surveyed in 2020 due to the COVID-19 pandemic. Karst biota survey results are summarized in Table 5. Photograph 2 depicts several collected specimens from 2020.



Photograph 2. Invertebrates collected from Beck Preserve viewed beneath a microscope from left to right: *Batrisodes reyesi*, troglobitic Pseudoscorpion, *Batrisodes uncicornis* (Photograph credit: Isaac Lord).

		Inside Cave					
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
October 14, 2020	•	-	-				-
Beck	Beck Bat	88.5	73.2	3	S. Van Kampen-Lewis, L. Rome, I. Lord	Texella reyesi	8
						Ceuthophilus cunicularis	9
						Ceuthophilus secretus	dozens
						Cicurina buwata	2
						Cicurina varians	9
						<i>Eidmannella</i> sp.	4
						Pseudoscorpion	2
						Cambala speobia	dozens
						Speodesmus bicornourus	7
						Lithobiomorpha	2
						Scutigeridae	2
						Anillinus sp.	7
						Batrisodes reyesi	1
						Batrisodes uncicornis	3
						Perimyotis subflavus	9
						Helicodiscus sp.	12
						Collembola	dozens
						Isopod	4
						Tick	1
						Gnat	1

Table 5. Karst Biota Survey Results at Beck Preserve

		Insid	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Numbe
Beck	Beck Horse	73.5	73.1	2.5	S. Van Kampen-Lewis, L. Rome, I. Lord	Texella reyesi	6
						Ceuthophilus cunicularis	1
						Ceuthophilus secretus	dozen
						Cicurina buwata	1
						Cicurina varians	4
						Cambala speobia	18
						Speodesmus bicornourus	2
						Surface Millipede	3
						Batrisodes uncicornis	6
						Rhadine subterranea	9
						Surface Beetle	1
						Helicodiscus sp.	15
						Assassin Bug	1
						Collembola	doze
						Moth	6
Beck	Beck Pride	76.6	82.7	2.25	S. Van Kampen-Lewis, L. Rome, I. Lord	Texella reyesi	3
						Ceuthophilus cunicularis	4
						Ceuthophilus secretus	4
						Cicurina varians	1
						Tartarocreagris sp.	2
						Cambala speobia	20
						Speodesmus bicornourus	8
						Batrisodes uncicornis	5
						Helicodiscus sp.	11
						Collembola	3
Beck	Beck Tex-2				*Not surveyed in 2020*		
Beck	Beck Salamander				*Not surveyed in 2020*		

The Bone Cave harvestman is the only endangered karst invertebrate currently documented within the Beck Preserve. This species has been previously documented within Beck Bat Cave, Beck Pride Cave, Beck Horse Cave, Beck Crevice Cave, and Beck Tex-2 Cave. Beck Crevice Cave is known to contain the Bone Cave harvestman but, as stated above, this cave is no longer monitored. Figure 4 shows Bone Cave harvestman detection during annual biota surveys since 2009. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 4. Surveys were not conducted in Beck Tex-2 Cave in 2020 due to the COVID-19 pandemic.

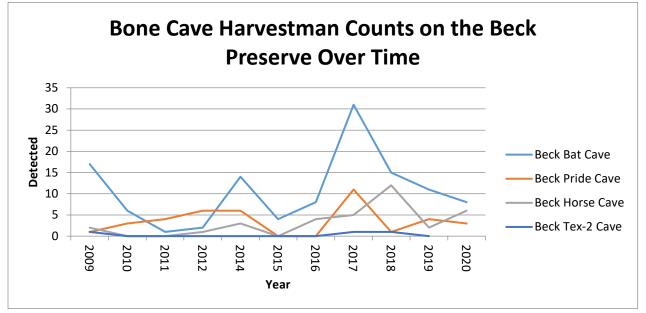


Figure 4. Endangered karst invertebrate species detection within the Beck Preserve.

The climate within the Beck Preserve caves known to contain the Bone Cave harvestman is variable from year to year (Figure 5). The three largest caves (Beck Pride, Beck Bat, and Beck Horse) have the most stable climatic conditions, but even these caves exhibit significant temperature and humidity fluctuations throughout the biota survey time frame. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 5. Equipment malfunctions in 2019 precluded recording climate data in Beck Tex-2 and Beck Pride Caves. Due to the COVID-19 pandemic, climatic data was not collected in Beck Tex-2 Cave in 2020.

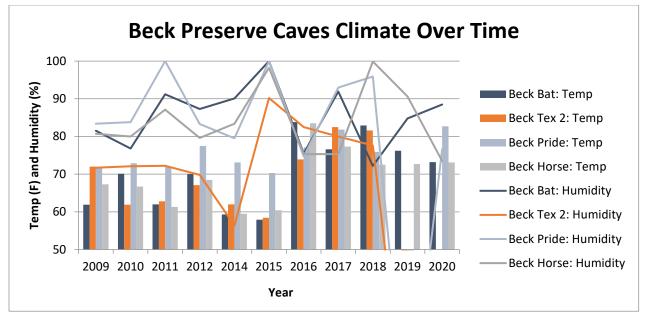


Figure 5. Climate readings within caves of the Beck Preserve.

4.3 Adaptive Management Issues

There are currently no adaptive management considerations for the Beck Preserve.

5 COBBS CAVERN KARST FAUNA AREA

The 163.0-acre Cobbs Cavern KFA lies within the 1,670.0-acre Cobbs Ranch, located north of State Highway 195, approximately 5 miles northwest of the City of Georgetown.

Cobbs Cavern has two distinct sides. The eastern third was physically modified and used as a show cave open to the general public in the late 1960s and early 1970s (i.e., "Show Side"), whereas the remainder of the cave is relatively unaltered by human activity compared with the Show Side and is labeled the "Wild Side." Biologists surveyed each area separately in 2020 due to Cobbs Cavern's large size.

5.1 Maintenance Activities

Salamander biologists working at Cobbs Spring have noted a large feral hog presence for many years. It appears that the feral hog population may have grown large enough to encroach upon Cobbs Cavern as evidenced by hog damage and hog sightings during biota surveys.

There is one cave associated with this preserve: Cobbs Cavern. The Cobbs Cavern KFA is an easement situated within a larger private property. As such, the KFA depends on fencing around the exterior of the larger private property to reduce the likelihood of trespassing. The Trail and Preserve Steward only looks for signs of broken fencing when trespassing or vandalism have occurred. Due to the COVID-19 pandemic, the preserve was not checked between March and August of 2020. Notes from the monthly cave inspections are provided in Table 6.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Cobbs Cavern	No	No	Yes/No	No	None.	None.
February						
Cobbs Cavern	No	No	Yes/No	No	None.	None.
March						
Not monitored due	e to COVID-19					
April						
Not monitored due	e to COVID-19					
Мау						
Not monitored due	e to COVID-19					
June						
Not monitored due	e to COVID-19					
July						
Not monitored due	e to COVID-19					
August						
Not monitored due	e to COVID-19					
September						
Cobbs Cavern	No	No	Yes/No	No	1×4 m S., 1×5 m S.W., 1×4 m N.	None.
October						
Cobbs Cavern	No	No	Yes/No	No	1×4 m S., 1×5 m S.W., 1×4 m N.	1. Too cold to treat RIFA. 2. None. 3. None.
November						
Cobbs Cavern	No	No	Yes/No	No	1×4 m S., 1×5 m S.W., 1×4 m N.	1. Treated RIFA. 2. None. 3. None.
December						

Table 6. Preserve Maintenance Triggers within the Cobbs Cavern KFA

* Fence around entire perimeter. Privately owned.

5.2 Karst Biota Surveys

Both sides of Cobbs Cavern were surveyed on September 16, 2020, and biologists detected no Bone Cave harvestmen or Inner Space Caverns mold beetles on the Wild Side; however, biologists detected 13 Bone Cave harvestmen and two Inner Space Caverns mold beetles on the Show Side. Biologists collected two

Tayshaneta anopica specimens during this survey of the Wild Side. *Rhadine noctivaga* (ground beetles) were found on both the Wild and Show Sides of Cobbs Cavern in 2020 but were not documented in previous years. Karst biota survey results are summarized in Table 7.

		Inside Cave					
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
September 16, 2	2020	-	-	-	-	-	-
Cobbs Cavern	Cobbs Cavern – Wild Side	82.3	71.9	7.5	S. Van Kampen-Lewis, B. Dilly, L.	Ceuthophilus secretus	22
					Rome	Cicurina vibora	1
						Tayshaneta anopica	4
						Speodesmus bicornorus	1
						Rhadine noctivaga	1
						Perimyotis subflavus	1
Cobbs Cavern	Cobbs Cavern – Show Side	85.9	72.8	1.67	H. Beatty, C. Crawford, R. Jones	Texella reyesi	13
						Batrisodes texanus	2
						Ceuthophilus cunicularis	7
						Cicurina varians	1
						Cicurina vibora	11
						Cambala speobia	1
						Speodesmus bicornorus	14
						Scutigeridae	1
						Rhadine noctivaga	4
						Perimyotis subflavus	1
						Helicodiscus sp.	1
						Collembola	12

Table 7. Karst Biota Survey Results at Cobbs Cavern KFA

Both the Bone Cave harvestman and Inner Space Caverns mold beetle have been regularly detected within Cobbs Cavern since it was established as a KFA in 2010. Photograph 3 shows biologists surveying for endangered karst invertebrates. Typically, few mold beetles are observed during the annual biota survey; however, the number of Bone Cave harvestmen detected on these same surveys can fluctuate significantly (Figure 6). Biologists detected 13 Bone Cave harvestmen within Cobbs Cavern during 2020. Species detection in 2019 tied with 2018 as an all-time high (14 individual documented), which is counter to a downward trend during the previous 2 years. Such fluctuations appear normal, and it is unclear if these fluctuations represent natural population variation over time or detection ability that fluctuates between years. Biologists identified two Inner Space Caverns mold beetles in Cobbs Cavern in 2020. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 6.



Photograph 3. Biologists discussing endangered karst invertebrate detection within Cobbs Cavern Show Side during a 2019 biota survey.

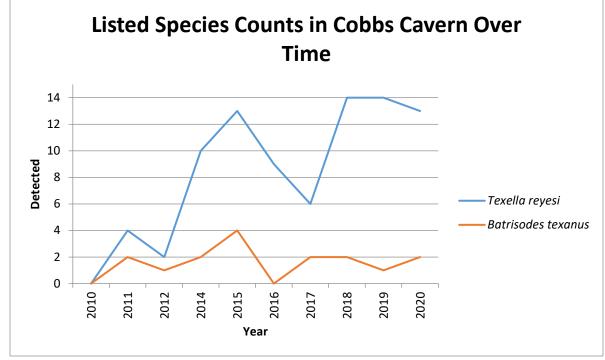


Figure 6. Endangered karst invertebrate species detection within the Cobbs Cavern KFA.

Cobbs Cavern is a very large feature and maintains a stable climate, as seen in Figure 7, where humidity at the far end of the Show Side fluctuates between 74% and 100%. The temperature was nearly 71°F during the most recent 2020 reading. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 7.

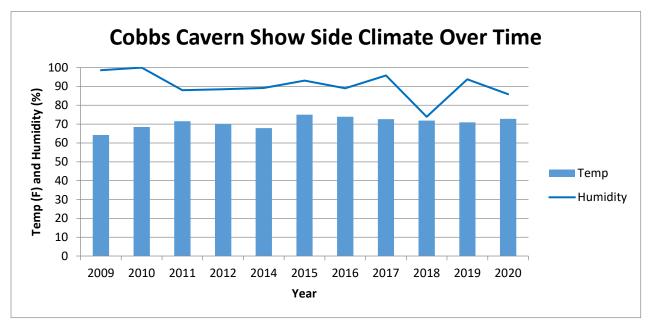


Figure 7. Climate readings from handheld device during biota surveys within Cobbs Cavern Show Side.

Dr. Chris Maupin placed two Onset HOBO (brand name) dataloggers at two separate locations within the Cobbs Cavern Wild Side for several years (2018 - 2020). Each logger collected thousands of data points. The climate in this area is stable due to its long distance from sources of surface climate input. These conditions, coupled with the scant organic input from the surface, creates the ideal environment for the type of speleothem generation that enables high-quality data for Dr. Maupin's research. Dr. Maupin's 2020 study summary is provided in Section 19.7 of this report. Photograph 4 shows just one of many different types of limestone formations afforded by Cobbs Cavern's stable climate.



Photograph 4. Helictites are uncommon cave structures that are found deep inside Cobbs Cavern Wild Side (Photograph credit: Ryan Jones).

5.3 Adaptive Management Issues

The AMC, in consultation with the Lyda family (Cobbs Cavern easement owner), should consider the following specific issues:

• Feral hogs are actively rooting around the entrance to Cobbs Cavern and may facilitate RIFA infestation due to the insect's preference for founding colonies at disturbed sites. The feral hog presence at Cobbs Spring and now Cobbs Cavern may require a continued eradication program. The AMC may wish to consider hiring a feral hog specialist to remove the offending animals. This recommendation is carried over from last year.

6 MILLENNIUM PRESERVE

The approximately 74.4-acre Millennium Preserve is within the Southwest Williamson County Regional Park (SWWCRP) located northeast of the intersection of Farm-to-Market 1431 and County Road 175 near Leander, Texas. This preserve is currently under review by USFWS for full KFA status.

6.1 Maintenance Activities

There are six caves associated with this preserve: Cap, Fence Trail, Knuckle, Little Demon, Millennium, and Through Trip Caves. One of the caves in this preserve (Through Trip Cave) represents potentially high-quality troglobite habitat where Bone Cave harvestman has never been detected. It is hypothesized that the cave's dual entrances allow significant influence from surface climate conditions, which dries out the cave beyond the Bone Cave harvestman's capabilities to survive. However, it is very likely the species is found within adjacent interstitial spaces, where humidity is higher than ambient cave conditions. Biologists placed cotton cloth covers on the cave entrances in 2017 to restrict airflow but allow water and *Ceuthophilus* crickets to naturally access Through Trip Cave as under normal conditions. The cotton cloth covers were left in place during 2018 and 2019 and have remained in place for 2020. There are plans to fill in the south entrance of Through Trip Cave with rocks and dirt to enhance Bone Cave harvestman habitat with even more restricted airflow. Approximately 60 feet of fence was replaced within the Millennium Preserve during 2020. Notes from the 2020 monthly cave inspections are provided in Table 8.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Cap Cave	No	No	No Gate	No	None.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	 None. Welded new hinges on gate. None.
Millennium Cave	No	No	Yes/No	No	None.	 None. Welded new hinges on gate. None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
February						
Cap Cave	No	No	No Gate	No	1×3 m E.	None.

Table 8. Preserve Maintenance Triggers within the Millennium Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
March						
Cap Cave	No	No	No Gate	No	1×3 m E. (Not very active).	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment. None. None.
April						
Cap Cave	No	No	No Gate	No	1×3 m E.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/Yes	No	None.	None.
Millennium Cave	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	 Comments Tasks Completed Tasks Outstanding
Through Trip North	Yes	No	No/No	No	None.	 Cave gate tarp and wood cover were removed. Put tarp and wood back over gate. None
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Мау						
Cap Cave	No	No	No Gate	No	None.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	Yes	No	No/No	No	None.	 Cave gate tarp and wood cover were removed. Put tarp and wood back over gate. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
June						
Cap Cave	No	No	No Gate	No	1×8 m E.	 None. Treated RIFA with boiling water. None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
July						
Cap Cave	No	No	No Gate	No	None.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/Yes	No	None.	None.
Millennium Cave	No	No	Yes/Yes	No	None.	None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
August						
Cap Cave	No	No	No Gate	No	None.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
September						
Cap Cave	No	No	No Gate	No	None.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	 Comments Tasks Completed Tasks Outstanding
Millennium Cave	No	No	Yes/No	No	None.	 Fence beside the cave needs repair. Fence repaired. None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
October						
Cap Cave	No	No	No Gate	No	1×2 m S.E.	 Too cold to treat RIFA. None. None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/Yes	No	None.	None.
Millennium Cave	No	No	Yes/Yes	No	None.	 Fence beside the cave needs repair. Fence repaired. None.
Through Trip North	No	No	No/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	No/No	No	1×5 m S.	 Cave gate covered to encourage a moist environment Too cold to treat RIFA. None. None.
November						
Cap Cave	No	No	No Gate	No	1×3 m S.E.	1. Treated RIFA. 2. None. 3. None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	1×4 m S.W.	1. Treated RIFA. 2. None. 3. None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Millennium Cave	No	No	Yes/No	No	1×3 m S.E.	1. Treated RIFA. 2. None. 3. None.
Through Trip North	No	No	Yes/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	Yes/No	No	1×5 m S.	 Cave gate covered to encourage a moist environment Cover was removed. Treated RIFA. Replaced cover.
						3. None.
December						
Cap Cave	No	No	No Gate	No	2×3 m E.	None.
Fence Trail Cave	No	No	No Gate	No	None.	None.
Knuckle Cave	No	No	No Gate	No	None.	None.
Little Demon Cave	No	No	Yes/No	No	None.	None.
Millennium Cave	No	No	Yes/No	No	None.	None.
Through Trip North	No	No	Yes/No	No	None.	 Cave gate covered to encourage a moist environment None. None.
Through Trip South	No	No	Yes/No	No	None.	 Cave gate covered to encourage a moist environment Replaced cover. None.

* No fence. Within park boundary.

6.2 Karst Biota Surveys

Biologists performed biota surveys on October 2, 2020. No Bone Cave harvestmen were found within Millennium Cave, Through Trip Cave, or Little Demon Cave, even though cotton cloth cave covers were in place to reduce airflow and elevate humidity within the caves. Additionally, biologists collected no invertebrates from Millennium Preserve in 2020. Karst biota survey results are summarized in Table 9.

		Inside Cave							
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species			
October 2, 20	20		I	Γ			I		
Millennium	Through Trip	74.2	69.4	1.5	S. Van Kampen-Lewis, M. Heimbuch, I. Lord	Ceuthophilus cunicularis	2		
						Ceuthophilus secretus	hundreds		
						<i>Agyneta</i> sp.	3		
						Cicurina varians	dozens		
						Leiobunum townsendi	dozens		
						Cambala speobia	10		
						Scutigeridae	6		
						Batrisodes uncicornis	1		
						Staphyllinidae	2		
						Dung Beetle	1		
						Eleutherodactylus sp.	1		
						Incilius nebulifer	2		
						Rana berlandieri	1		
						Arenivaga sp.	8		
						Helicodiscus sp.	2		
						Assassin Bug	5		
						Collembola	dozens		
						Dark Springtail	6		
						Isopod	1		
						Mouse	1		
Millennium	Millennium	83.4	71.3	1.5	S. Van Kampen-Lewis, M. Heimbuch, I. Lord	Ceuthophilus cunicularis	3		
						Ceuthophilus secretus	dozens		
						<i>Agyneta</i> sp.	6		
						Cicurina browni	1		
						Cicurina varians	dozens		

Table 9. Karst Biota Survey Results at Millennium Preserve

		Insic	de Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	
						<i>Eidmanella</i> sp.	2
						Leiobunum townsendi	thousands
						Cambala speobia	dozens
						Speodesmus bicornourus	1
						Lithobiomorpha	2
						Batrisodes uncicornis	1
						Rhadine subterranea	1
						Staphylinidae	4
						Eleutherodactylus sp.	1
						Incilius nebulifer	2
						<i>Helicodiscus</i> sp.	13
						<i>Texoreddellia</i> sp.	1
						Collembola	thousands
						Isopod	dozens
Millennium	Little Demon	85.6	75.9	1	S. Van Kampen-Lewis, M. Heimbuch, I. Lord	Ceuthophilus cunicularis	1
						Ceuthophilus secretus	dozens
						Cicurina varians	8
						Cryptachea porteri	4
						<i>Eidmanella</i> sp.	1
						Leiobunum townsendi	dozens
						Cambala speobia	24
						Lithobiomorph	3
						Scutigeridae	3
						Batrisodes uncicornis	3
						Staphylinidae	1
						Eleutherodactylus sp.	5
						Gasrophryne olivacea	1

		Insic	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	
						Helicodiscus sp.	3
						Annelid	3
						Collembola	thousands
						Gnat	2
						Isopod	3
						Mite	1
						Wasp	1

Few Bone Cave harvestman individuals are typically detected during biota surveys within Little Demon and Millennium Caves (Figure 8). Biologists detected no Bone Cave harvestmen in either cave during 2020.

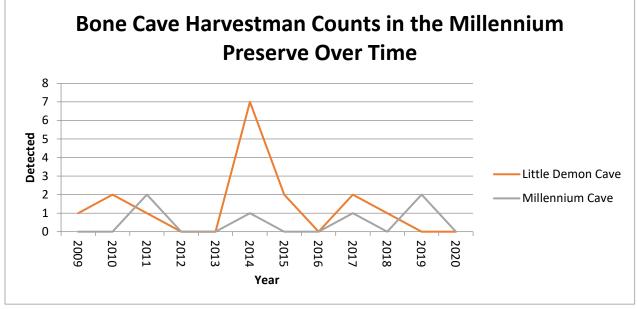


Figure 8. Endangered karst invertebrate species detection within the Millennium Preserve.

The humidity in Little Demon and Millennium Caves typically stays above 80% and the temperature regularly fluctuates between 60°F and 75°F (Figure 9). Note that biologists were forced to leave Millennium Cave prior to obtaining climate readings in 2018 due to aggressive rattlesnakes.

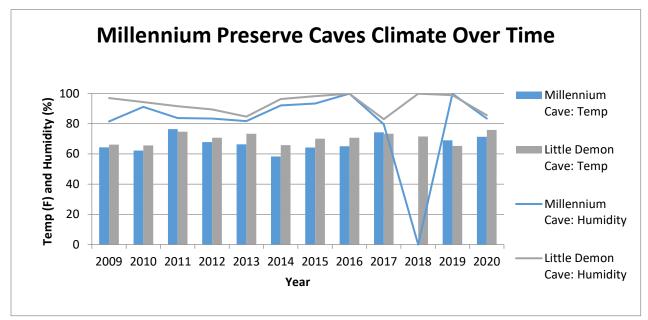


Figure 9. Climate readings from Millennium Preserve caves with recent documented Bone Cave harvestman sightings.

6.3 Adaptive Management Issues

In 2020, breeding GCWAs were observed and documented approximately 1,000 feet from the Millennium and Wilco Preserve (both contained within SWWCRP) on multiple occasions. Potential GCWA breeding habitat may have developed within these two preserves since the SWWCRP was established in 2003, and the AMC may wish to consider performing GCWA surveys in these two preserved during the 2022 breeding season.

7 WILCO PRESERVE

The 152.5-acre Wilco Preserve is located within the SWWCRP near the northeast of the intersection of Farm-to-Market 1431 and County Road 175 near Leander, Texas. This preserve is currently under review by USFWS for full KFA status.

7.1 Maintenance Activities

There are nine caves associated with this preserve: Choya, Mongo, Popping Rock, Prairie, Prospector, Rock Ridge, Venture, Wilco, and Wild West Caves. The Trail and Preserve Steward did not notice vandalism to Wilco Preserve caves during 2020. Repeated RIFA colony exterminations were documented at multiple caves throughout the year. Notes from the 2020 monthly cave inspections are provided in Table 10.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	No	None.	None.
Prospector Cave	No	No	Yes/No	No	None.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
February						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.

Table 10. Preserve Maintenance Triggers within the Wilco Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Prairie Cave	No	No	Yes/No	No	1×5 m N. (Not very active).	None.
Prospector Cave	No	No	Yes/No	No	None.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
March						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	Νο	1×5 m N. (Not very active).	None.
Prospector Cave	No	No	Yes/No	No	None.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	Yes	Yes/No	No	None.	 Unauthorized trimming of vegetation None. None.
Wild West Cave	No	No	Yes/No	No	None.	 Rattlesnake next to cave gate. None. None.
April						
Choya Cave	No	No	Yes/Yes	No	None.	None.
Mongo Cave	No	No	Yes/Yes	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/Yes	No	1×5 m N., 2×3 m N.	None.
Prospector Cave	No	No	Yes/Yes	No	1×4 m S.	None.
Rock Ridge Cave	No	No	Yes/Yes	No	None.	None.
Venture Cave	No	No	Yes/Yes	No	None.	None.
Wilco Cave	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Wild West Cave	No	No	Yes/Yes	No	None.	None.
Мау						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	No	1×5 m N., 2×3 m N.	None.
Prospector Cave	No	No	Yes/No	No	Old mound not active.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
June						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	No	1×5 m N. 2×3 m N.	 None. Treated RIFA with boiling water. None.
Prospector Cave	No	No	Yes/No	No	1×3 m S.E.	 None. Treated RIFA with boiling water. None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
July						
Choya Cave	No	No	Yes/Yes	No	None.	None.
Mongo Cave	No	No	Yes/Yes	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/Yes	No	None.	None.
Prospector Cave	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Rock Ridge Cave	No	No	Yes/Yes	No	None.	None.
Venture Cave	No	No	Yes/Yes	No	None.	None.
Wilco Cave	No	No	Yes/Yes	No	None.	None.
Wild West Cave	No	No	Yes/Yes	No	None.	None.
August						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	No	None.	None.
Prospector Cave	No	No	Yes/No	No	None.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
September						
Choya Cave	No	No	Yes/No	No	None.	None.
Mongo Cave	No	No	Yes/No	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.
Prairie Cave	No	No	Yes/No	No	None.	 Erosion to the side of the cave gate needs repair. None. None.
Prospector Cave	No	No	Yes/No	No	None.	None.
Rock Ridge Cave	No	No	Yes/No	No	None.	None.
Venture Cave	No	No	Yes/No	No	None.	None.
Wilco Cave	No	No	Yes/No	No	None.	None.
Wild West Cave	No	No	Yes/No	No	None.	None.
October						
Choya Cave	No	No	Yes/Yes	No	None.	None.
Mongo Cave	No	No	Yes/Yes	No	None.	None.
Poppin Rock Cave	No	No	No Gate	No	None.	None.

Preserve/Cave*	Vandalism,	Damage to	Gate	Off-Trail	RIFA	1. Comments	
	Trash Dumping and	Vegetation, Pet Issues and	Inspection / Lock	Activity	Mounds Within 10 m	2. Tasks Completed	
	Unauthorized Entry	Feral Animals	Lubrication		of Cave Entrance	3. Tasks Outstanding	
						1. Erosion to the side of the cave gate needs repair. Too cold to trea RIFA.	
Prairie Cave	No	No	Yes/Yes	No	1×10 m N.	2. Repaired erosion to cave gate with concrete around edges.	
_						3. None.	
Prospector Cave	No	No	Yes/Yes	No	None.	None.	
Rock Ridge Cave	No	No	Yes/Yes	No	None.	None.	
Venture Cave	No	No	Yes/Yes	No	None.	None.	
Wilco Cave	No	No	Yes/Yes	No	None.	None.	
Wild West Cave	No	No	Yes/Yes	No	None.	None.	
November							
Choya Cave	No	No	Yes/No	No	None.	None.	
Mongo Cave	No	No	Yes/No	No	None.	None.	
Poppin Rock Cave	No	No	No Gate	No	None.	None.	
Prairie Cave	No	No	Yes/No	No	None.	None.	
Prospector Cave	No	No	Yes/No	No	None.	None.	
Rock Ridge Cave	No	No	Yes/No	No	None.	None.	
Venture Cave	No	No	Yes/No	No	None.	None.	
Wilco Cave	No	No	Yes/No	No	None.	None.	
Wild West Cave	No	No	Yes/No	No	None.	None.	
December							
Choya Cave	No	No	Yes/No	No	None.	None.	
Mongo Cave	No	No	Yes/No	No	None.	None.	
Poppin Rock Cave	No	No	No Gate	No	None.	None.	
Prairie Cave	No	No	Yes/No	No	None.	None.	
Prospector Cave	No	No	Yes/No	No	None.	None.	
Rock Ridge Cave	No	No	Yes/No	No	None.	None.	
Venture Cave	No	No	Yes/No	No	None.	None.	
Wilco Cave	No	No	Yes/No	No	None.	None.	
Wild West Cave	No	No	Yes/No	No	None.	None.	

* No fence. Within park boundary.

7.2 Karst Biota Surveys

Biologists performed karst biota surveys within Mongo Cave on December 10, 2020. Biologists detected two Bone Cave harvestmen within Mongo Cave. The other caves in the preserve were not surveyed in 2020 due to the COVID-19 pandemic.

Biologists collected no invertebrates from the Wilco Preserve in 2020.

Large rattlesnakes are consistently observed within the Wilco Preserve caves during annual biota surveys and by the Trail and Preserve Steward during the monthly maintenance activities (Photograph 5).

Karst biota survey results are summarized in Table 11.



Photograph 5. Large western diamondback rattlesnake at entrance to Wild West Cave in 2016.

		Insi	de Cave				
Preserve	Cave	RH (%) Temp (°F)		Person- Hours	Surveyors	Species	Number
December 10, 2020				-			-
Wilco	Mongo	97.3	72.6	2	S. Van Kampen-Lewis, L. Rome, C. Crawford	Texella reyesi	2
						Ceuthophilus cunicularis	3
						Ceuthophilus secretus	Hundreds
						Cryptachaea porteri	7
						Cicurina browni	9
						Cicurina varians	10
						Cambala speobia	6
						Scutigeridae	4
						Rhadine subterranea	3
						Eleutherodactylus sp.	1
						Collembola	Dozens
						Isopod	Hundreds
						Tick	1

Table 11. Karst Biota Survey Results at Wilco Preserve

Mongo Cave is the only feature within the Wilco Preserve with regularly documented Bone Cave harvestman occurrence, even though the species was historically known from several other caves (see Van Kampen-Lewis and White 2020a for Wilco Cave inhabitant descriptions). This species has been detected every year in Mongo Cave since biota surveys began in 2010 (Figure 10). Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 10.

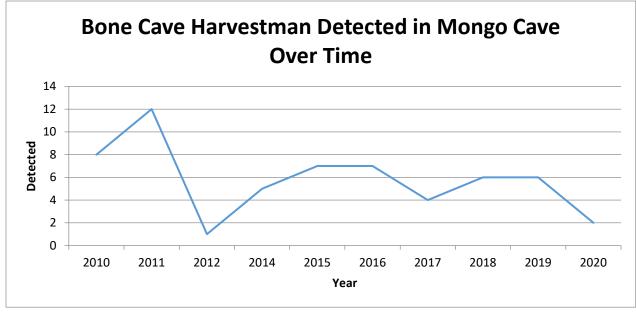


Figure 10. Endangered karst invertebrate species detection within the Wilco Preserve.

Mongo Cave has a fairly stable climate, with humidity typically reading at or above 90%, including in 2020. Detected humidity in 2020 (97.3%) was typical for the cave. Temperature typically ranges between 65°F and 80°F and the temperature in 2020 was 72.6°F (Figure 11). Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 11.

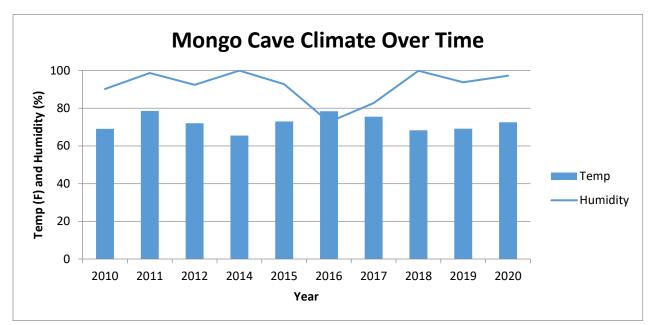


Figure 11. Climate readings within Mongo Cave.

7.3 Adaptive Management Issues

In 2020, breeding GCWAs were observed and documented approximately 1,000 feet from the Millennium and Wilco Preserve (both contained within SWWCRP) on multiple occasions. Potential GCWA breeding habitat may have developed within these two preserves since the SWWCRP was established in 2003, and the AMC may wish to consider performing GCWA surveys in these two preserved during the 2022 breeding season.

8 CHAOS CAVE PRESERVE

The 30.0-acre Chaos Cave Preserve is located in Austin, bounded by State Highway 45 to the north, by a rail line to the southwest, and by undeveloped portions of the Robinson Ranch to the east and west.

8.1 Maintenance Activities

There are three caves associated with this preserve: Chaos, Poison Ivy, and Under the Fence Caves. An additional 1,000 feet of shaded fuel break was created at the northwestern corner in January 2020. The Trail and Preserve Steward did not note additional activities in 2020 beyond the basic monthly maintenance inspections. Notes from the monthly cave inspections are provided in Table 12.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
February						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
March						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
April						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	 Vulture in cave None. None.
Under the Fence Cave	No	No	No gate	No	None.	None.
Мау						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
June						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
July						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.

Table 12. Preserve Maintenance Triggers within the Chaos Cave Preserve

	-	-			-	-
Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
September						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
October						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
November						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.
December						
Chaos Cave	No	No	No gate	No	None.	None.
Poison Ivy Cave	No	No	No gate	No	None.	None.
Under the Fence Cave	No	No	No gate	No	None.	None.

* Fence around entire perimeter.

8.2 Karst Biota Surveys

Biota surveys at the Chaos Cave Preserve occurred on October 1, 2020. Under the Fence Cave is such a small feature with strong influence from surface conditions that no Bone Cave harvestmen have been located since preserve inception in 2008. As such, this cave is no longer surveyed. Due to the COVID-19 pandemic, high atmospheric exchange, and size of Poison Ivy Cave, no survey was performed in 2020 for Poison Ivey Cave. Chaos Cave is much larger; therefore, this cave was surveyed in 2020, and biologists observed two Bone Cave harvestmen during the biota survey. Additionally, biologists collected two *Batrisodes uncicornis*, six *Eidmanella* sp., and one possible *Tayshaneta* sp. from Chaos Cave.

The Chaos Cave Preserve is approximately 1.25 miles from Goat Cave, which is the northernmost locality for the Tooth Cave spider. There is a possibility that this species inhabits the Chaos Cave Preserve and biologists are targeting collections to confirm as such. Much like the Beck Preserve, documenting the Tooth Cave spider in the Chaos Cave Preserve would increase the preserve's conservation value and may be important to the County if Endangered Species Act compliance issues arise for the species.

Karst biota survey results are summarized in Table 13.

		Insid	de Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
October 1, 202	20	-				-	-
Chaos	Chaos Cave	99.6	69.8	2.5	S. Van Kampen-Lewis, M. Heimbuch, I.	Texella reyesi	2
					Lord	Tayshaneta sp. (?)	1
						Ceuthophilus cunicularis	2
						Ceuthophilus secretus	dozens
						Cicurina buwata	2
						Cicurina varians	20
						<i>Eidmanella</i> sp.	9
						Leiobunum townsendi	hundreds
						Cambala speobia	dozens
						Scutigeridae	1
						Lithobiomorpha	9
						Batrisodes uncicornis	1
						Eleutherodactylus sp.	1
						Scincella lateralis	1
						Helicodiscus sp.	3
						Annelid	1
						Collembola	hundreds
						Dark Springtail	dozens
						Gnat	2

Table 13. Karst Biota Survey Results at Chaos Cave Preserve

Chaos Cave is the only feature on the Chaos Preserve with regularly documented Bone Cave harvestman occurrence since biota surveys began in 2009. With nine documented Bone Cave harvestmen each year, 2009 and 2018 are tied for the highest count of this species (Figure 12). Species detection has not consistent across all surveys of this cave, and biologists have highly variable counts from year to year (Figure 12).

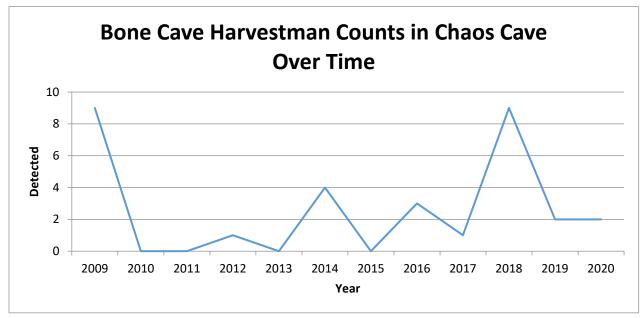
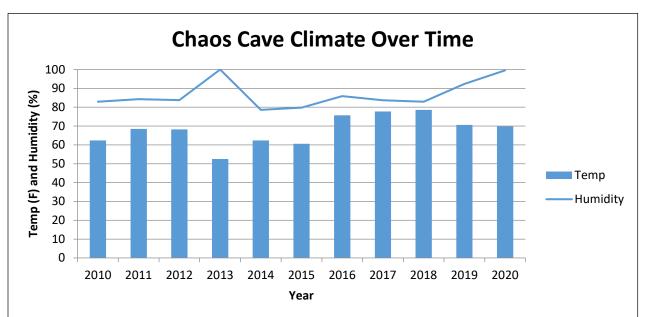


Figure 12. Endangered karst invertebrate species detection within the Chaos Cave Preserve.



The humidity within Chaos Cave is fairly stable and is usually over 80%. Temperature readings fluctuate from approximately 50°F to nearly 78°F (Figure 13). Climate readings were not collected in 2009.

Figure 13. Climate readings within Chaos Cave.

8.3 Adaptive Management Issues

The AMC should consider the following specific issue at the Chaos Cave Preserve.

• Chaos Cave Preserve is a large natural area with significant fuel load. Therefore, the AMC may wish to continue the shaded fuel break program around the perimeter.

9 BIG OAK CAVE PRESERVE

The Big Oak Cave Preserve spans approximately 10.0 acres within an approximately 0.5-mile-long median area between U.S. Highway 183A and the old U.S. 183 facilities.

9.1 Maintenance Activities

There is one cave associated with this preserve: Big Oak Cave. The unauthorized homeless camp problems that occurred in 2017 and 2019 were again a problem during 2020. As such, "No Entry" signs were placed around the preserve. However, unauthorized camping still occurred in November and December 2020, and latrines were dug next to the caves by the unauthorized campers. Trash also increased in 2020 and was present more often in the later months of the year. Notes from the monthly cave inspections are provided in Table 14.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January	-	-	-	-	-	
Big Oak Cave 1	No	No	Yes/No	No	None.	 Trash around perimeter. Homeless camp has gone. None. Picked up trash
Big Oak Cave 2	No	No	Yes/No	No	None.	 Trash around perimeter. Homeless camp has gone. None. Picked up trash
February						
Big Oak Cave 1	No	No	Yes/No	No	None.	 Trash around perimeter. None. Picked up trash.
Big Oak Cave 2	No	No	Yes/No	No	None.	 Trash around perimeter. None. Picked up trash.
March						
Big Oak Cave 1	No	No	Yes/No	No	None.	None.

Table 14. Preserve Maintenance Triggers within the Big Oak Cave Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Big Oak Cave 2	No	No	Yes/No	No	None.	None.
April						
Big Oak Cave 1	No	No	Yes/Yes	No	None.	None.
Big Oak Cave 2	No	No	Yes/No	No	None.	 1. Vulture and eggs in cave. 2. None. 3. None.
Мау						
Big Oak Cave 1	No	No	Yes/No	No	None.	None.
Big Oak Cave 2	No	No	Yes/No	No	None.	None.
June						
Big Oak Cave 1	No	No	Yes/No	No	None.	None.
Big Oak Cave 2	No	No	Yes/No	No	1×2 m S. (Mound became non-active before treatment).	None.
July						
Big Oak Cave 1	No	No	Yes/No	No	None.	None.
Big Oak Cave 2	No	No	Yes/No	No	None.	None.
August						
Big Oak Cave 1	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Big Oak Cave 2	No	No	Yes/No	No	None.	None.
September						
Big Oak Cave 1	No	No	Yes/No	No	None.	1. Trash around perimeter. 2. Picked up trash.
						3. None.
Big Oak Cave 2	No	No	Yes/No	No	None.	None.
October						
Big Oak Cave 1	No	No	Yes/Yes	No	None.	 Trash around perimeter. Picked up trash. None.
Big Oak Cave 2	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
November Big Oak Cave 1	Yes	No	Yes/No	No	None.	 Excessive trash around perimeter from neighboring homeless camp. Asked homeless to pick up trash.
Big Oak Cave 2	Yes	No	Yes/No	No	None.	 Recheck next month. Excessive trash around perimeter from neighboring homeless camp. Asked homeless to pick up trash. Recheck next month.
December						
Big Oak Cave 1	Yes	No	Yes/No	No	None.	 Excessive trash around perimeter from neighboring homeless camp. None. Recheck next month.
Big Oak Cave 2	Yes	No	Yes/No	No	None.	 Excessive trash around perimeter from neighboring homeless camp. None. Recheck next month.

* No fence.

9.2 Karst Biota Surveys

Annual monitoring efforts no longer occur at this location because surveys were only required within the Big Oak Cave Preserve for 4 years (2008 - 2011). Repeated surveys during this period failed to yield *Rhadine persephone* (endangered karst invertebrate) sightings, likely due to limited accessible cave extent that is almost entirely exposed to surface atmospheric conditions.

9.3 Adaptive Management Issues

The AMC should consider the following specific issue at the Big Oak Cave Preserve:

• Continue working with local law enforcement to minimize impacts from unauthorized camping by unauthorized, homeless people.

10 PRISCILLA'S WELL KARST FAUNA AREA

The Priscilla's Well KFA is a 51.5-acre tract between Phase III of Ronald W. Reagan Boulevard and a residential portion of Sun City.

10.1 Maintenance Activities

There are two caves associated with this preserve: Priscilla's and Priscilla's Well Caves. The Priscilla's Well KFA warranted no unusual or extraneous activities in 2020 beyond the basic monthly maintenance inspections. Notes from the monthly cave inspections are provided in Table 15.

Table 15. Preserve Maintenance Triggers within the Priscilla's Well KFA

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage To Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
February						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
March						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
April						
Priscilla's Cave	No	No	Yes/Yes	No	None.	None.
Priscilla's Well Cave	No	No	Yes/Yes	No	None.	None.
Мау						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
June						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
July						
Priscilla's Cave	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage To Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Priscilla's Well Cave	No	No	Yes/Yes	No	None.	None.
August						
Priscilla's Cave	No	No	Yes/No	No	None.	None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
September						
Priscilla's Cave	No	No	Yes/No	No	None.	 Mammal entrance has eroded and needs repair. None. None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
October						
Priscilla's Cave	No	No	Yes/Yes	No	None.	 Mammal entrance has eroded and needs repair. None. None.
Priscilla's Well Cave	No	No	Yes/Yes	No	None.	 None. Trash dumped on roadway. Picked up trash. None.
November						
Priscilla's Cave	No	No	Yes/No	No	None.	 Mammal entrance has eroded and needs repair. None. None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.
December						
Priscilla's Cave	No	No	Yes/No	No	None.	 Mammal entrance has eroded and needs repair. None. None.
Priscilla's Well Cave	No	No	Yes/No	No	None.	None.

* Fenced area intact. Need to install fence on south side of property line.

10.2 Karst Biota Surveys

Karst biota surveys were not performed in Priscilla's Well KFA in 2020 due to the COVID-19 pandemic. Priscilla's Cave is open to atmospheric exchange and listed species are rarely found within it. Priscilla's Well Cave has a long, tiny entrance with little to no atmospheric exchange, so biologists made the conscious decision not to survey in 2020 to limit COVID-19 exposure in exceedingly tight spaces. Karst biota survey results are summarized in Table 16.

	•						
		Insic	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
	-		-	**Priscilla's Well	Cave not surveyed in 2020**		
				Priscilla's Ca	ve not surveyed in 2020		

Table 16. Karst Biota Survey Results at Priscilla's Well KFA

Priscilla's Cave has only had a single Bone Cave harvestman detection since WCCF-sponsored biota surveys began in 2010. This species was only previously detected in 2011, and none have been located since that survey (Figure 14). Note that in previous annual reports, the 2011 occurrence was attributed to Priscilla's Well Cave. This is erroneous, and the 2011 occurrence should have been attributed to Priscilla's Cave. Access to the full Priscilla's Well Cave extent was blocked until the 2017 survey. Previous surveys within Priscilla's Well Cave have only been performed within the entrance due to blocked passage, and it is unlikely the Bone Cave harvestman was located under these conditions.

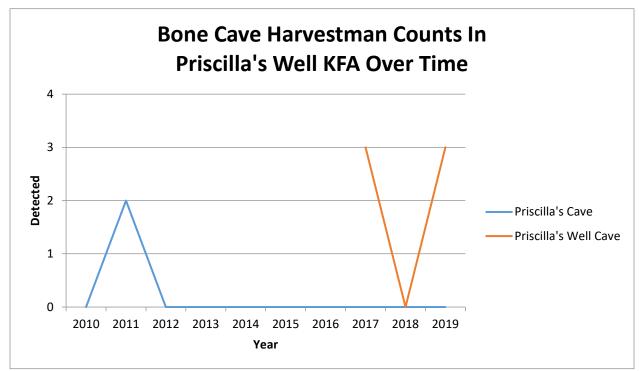


Figure 14. Endangered karst invertebrate species detection within the Priscilla's Well KFA.

10.3 Adaptive Management Issues

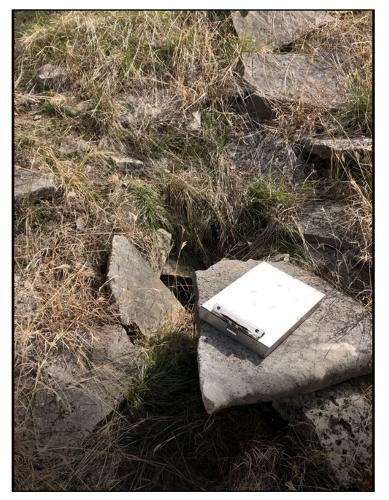
A new housing development is under construction immediately west of the Priscilla's Well KFA. The KFA itself is untouched by the development but increased human activity may occur at the Priscilla's Well KFA.

The AMC should consider the following specific issues at the Priscilla's Well KFA:

- Yearwood Gold Mine Cave is within Priscilla's Well KFA and may be inhabited by the Dragonfly Cave mold beetle due to its location within the species' range and proximity to Priscilla's Well Cave, a documented habitat for the species. The AMC may wish to consider excavating Yearwood Gold Mine Cave to determine if the listed karst invertebrate exists within this feature. The AMC may wish to consider adding a cave gate to keep people and livestock away from a fairly deep pit that may be a falling hazard (Photograph 6).
- A significantly large feature is piping soil into the subsurface and may represent a biologically important new cave (Photograph 7) within Pricilla's Well KFA. The AMC may wish to authorize full excavation and biota surveys to determine if this feature is inhabited by the Bone Cave harvestman and/or the Dragonfly Cave mold beetle.



Photograph 6. View of Yearwood Gold Mine during 2019 biota survey.



Photograph 7. View of potentially large new karst feature washing open at Priscilla's Well KFA as seen during the 2019 biota survey.

11 WOODLAND PARK PRESERVE

Woodland Park Preserve is composed of two conservation areas located in the Woodland Park subdivision, approximately 1.4 miles northeast of the Twin Springs KFA and immediately south of Sun City.

11.1 Maintenance Activities

There are two caves associated with this preserve: Cat and Duckworth Bat Caves. The Woodland Park Preserve warranted no unusual or extraneous activities in 2020 beyond the basic monthly maintenance tasks. Notes from the monthly cave inspections are provided in Table 17.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
February						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
March						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
April						
Cat Cave	No	No	Yes/Yes	No	None.	None.
Duckworth Bat Cave	No	No	Yes/Yes	No	None.	None.
Мау						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
June						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
July						
Cat Cave	No	No	Yes/Yes	No	None.	None.
Duckworth Bat Cave	No	No	Yes/Yes	No	None.	None.
August						
Cat Cave	No	No	Yes/No	No	None.	None.

Table 17. Preserve Maintenance Triggers within the Woodland Park Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave	1. Comments 2. Tasks Completed 3. Tasks
	Entry	Animais			Entrance	Outstanding
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
September						
Cat Cave	No	No	Yes/No	No	None	 Erosion around the gate. Repaired erosion around the cave gate. None. Erosion around the gate needs repair.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
October						
Cat Cave	No	No	Yes/Yes	No	None.	 Erosion around the gate. Repaired erosion around the cave gate. None.
Duckworth Bat Cave	No	No	Yes/Yes	No	None.	None.
November						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.
December						
Cat Cave	No	No	Yes/No	No	None.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None.	None.

* No fence.

11.2 Karst Biota Survey

The most recent karst biota surveys with the Woodland Park Preserve occurred on October 29, 2020. Cat Cave is open to atmospheric exchange and was thought to be a cold trap. However, Mr. James Reddell gave the WCCF biologists a cave map in early 2019, which indicated a much larger feature than previously known. In 2020, much of the new area was explored, revealing 12 Bone Cave Harvestmen, but biologists stopped before entering the final chamber due to low oxygen levels in the cave. Duckworth Bat Cave is a larger feature within the Woodland Park Preserve and appears to have less atmospheric exchange than Cat Cave. Similarly, Mr. Reddell provided a cave map for Duckworth Bat Cave depicting a much larger feature than previously known. In 2020, an attempt was made to get into the more disparate chambers, but they were too narrow to enter and will require further planning to survey in 2021. Biologists detected two Bone Cave harvestmen during the most recent biota survey of Duckworth Bat Cave. Biologists did not collect any specimens from Duckworth Bat Cave but did locate a *Rhadine noctivaga* ground beetle which were not seen in previous biota surveys at this cave. Karst biota survey results are summarized in Table 18.

		Insid	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
October 29, 202	0	<u>-</u>					2
Woodland Park	Cat	99.9	71.6	4.17	S. Van Kampen-Lewis, C. Crawford, K. White, I.	Texella reyesi	12
					Lord, B. Frou	Ceuthophilus secretus	dozens
						Cryptachaea porteri	dozens
						Cicurina varians	2
						Cicurina vibora	dozens
						<i>Tayshaneta</i> sp.	1
						Cambala speobia	dozens
						Speodesmus bicornourus	1
						Scutigeridae	2
						Staphyllinidae	7
						Isopod	dozens
Woodland Park	Duckworth Bat	97.2	67.4	5.83	S. Van Kampen-Lewis, C. Crawford, K. White, I.	Texella reyesi	2
					Lord, B. Frou	Ceuthophilus cunicularis	3
						Ceuthophilus secretus	dozens
						<i>Eidmanella</i> sp.	4
						Cicurina varians	dozens
						Cicurina vibora	1
						Leiobunum townsendi	5
						Speodesmus bicornourus	1
						Lithobiomorpha	1
						Surface Millipede	1
						Rhadine noctivaga	1
						Staphylinidae	7
						Rana berlandieri	2
						Arenivaga sp.	2
						Collembola	dozens
						Isopod	9

Table 18. Karst Biota Survey Results at Woodland Park Preserve

Biologists observed two Bone Cave harvestmen during the 2020 biota survey (Figure 15) of Duckworth Bat Cave. Access to the greater Cat Cave extent yielded 12 Bone Cave harvestmen in 2020. Therefore, Cat Cave is now added to Figure 15, as biologists are now able to access high-quality troglobite habitat for 2 years in a row. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 15.

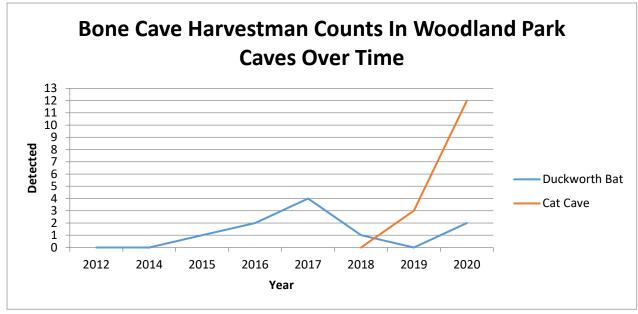


Figure 15. Endangered karst invertebrate species detection within the Woodland Park Preserve.

The Duckworth Bat Cave climate has been variable since monitoring began in 2012 (Figure 16). Temperature during biota surveys has ranged from 65°F to 84.1°F, while relative humidity has ranged from 61% to 100%. Due to new access to deeper karst habitat, Cat Cave is now added to Figure 16. Karst biota surveys were not performed in 2013; therefore, 2013 has been omitted from Figure 16.

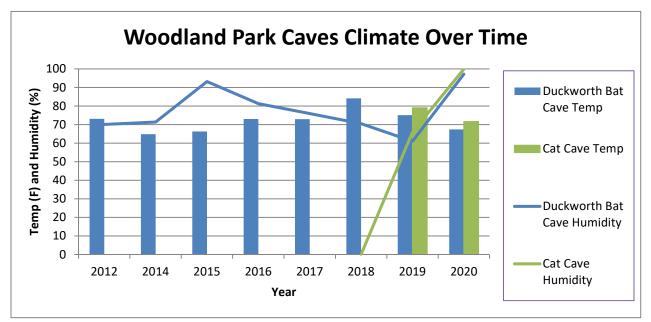


Figure 16. Climate readings within Woodland Park caves.

11.3 Adaptive Management Issues

There are currently no adaptive management considerations for the Woodland Park Preserve.

12 KARANKAWA CAVE KARST FAUNA AREA

The Karankawa Cave KFA consists of approximately 61.7 acres of private land located south of State Highway 195, approximately 7 miles northwest of the City of Georgetown in Williamson County, Texas.

12.1 Maintenance Activities

There are eight caves associated with this preserve: Angostura, Armadon, Karankawa, Pemmican, Polaris, Quahadi, Snake Dancer, and War Party Caves. A cave gate was installed on Karankawa Cave and material for rock gabions were delivered in December 2020. The Karankawa Cave KFA experienced no additional activities in 2020 beyond the basic monthly maintenance tasks and attending to RIFA mounds. Notes from the monthly cave inspections are provided in Table 19.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January	-	-	-	-	-	-
Angostura Cave	No	No	Yes/No	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
February						
Angostura Cave	No	No	Yes/No	No	1×4 m N.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.

Table 19. Preserve Maintenance Triggers within the Karankawa Cave KFA

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Angostura Cave	No	No	Yes/No	No	1×1 m N., 2×4 m N., 1×8 m N. (Too cold to treat)	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
April						
Angostura Cave	No	No	Yes/Yes	No	1×1 m N., 2×4 m N., 1×8 m N.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	1×4 m E., 1×8 m W.	None.
Pemmican Cave	No	No	Yes/Yes	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	1×3 m E.	None.
War Party Cave	No	No	No gate	No	None.	None.
Мау						
Angostura Cave	No	No	Yes/No	No	2×4 m N., 1×8 m N. (Not active, too hot).	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	1×4 m E., 1×8 m W. (Not active, too hot).	 Installed cave gate. None. None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Angostura Cave	No	No	Yes/No	No	2×4 m N.,1×8 m N. (Not active).	 None. Treated RIFA with boiling water. None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	No gate	No	1×4 m E., 1×8 m W. (Not active).	 None. Treated RIFA with boiling water. None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
July						
Angostura Cave	No	No	Yes/Yes	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/Yes	No	None.	None.
Pemmican Cave	No	No	Yes/Yes	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
August						
Angostura Cave	No	No	Yes/No	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/No	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
September						
Angostura Cave	No	No	Yes/No	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/No	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
October						
Angostura Cave	No	No	Yes/Yes	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/Yes	No	None.	None.
Pemmican Cave	No	No	Yes/Yes	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
November						
Angostura Cave	No	No	Yes/No	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/No	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.
December						
Angostura Cave	No	No	Yes/No	No	None.	None.
Armadon Cave	No	No	No gate	No	None.	None.
Karankawa Cave	No	No	Yes/No	No	None.	None.
Pemmican Cave	No	No	Yes/No	No	None.	None.
Polaris Cave	No	No	No gate	No	None.	None.
Quahadi Cave	No	No	No gate	No	None.	None.
Snake Dancer Cave	No	No	No gate	No	None.	None.
War Party Cave	No	No	No gate	No	None.	None.

* Northwest and south fences intact, need rest of preserve fenced around property line.

12.2 Karst Biota Survey

Karst biota surveys were completed on October 20, 2020. Biologists detected one Bone Cave harvestman in War Party Cave and one in Pemmican Cave. Biologists did not detect the Dragonfly Cave mold beetle within the Karankawa Cave KFA in 2020. One *Rhadine noctivaga* was collected from Pemmican Cave.

Biologists are no longer entering Angostura Cave. This feature is not a known locality for Bone Cave harvestman or Dragonfly Cave mold beetle. Furthermore, the humanly accessible portion of the cave is too small and exposed to surface conditions to maintain troglobitic habitat. Therefore, biologists chose to focus their efforts on more valuable features.

Biologists also noted significant cattle trails and cattle dropping within the southern portion of the KFA during their biota surveys.

Karst biota survey results are summarized in Table 20.

		Insic	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
December 15, 2	020	-				-	-
Karankawa	War Party	99.9	65.6	0.5	S. Van Kampen-Lewis, I. Lord	Texella reyesi	1
						Ceuthophilus cunicularis	1
						Ceuthophilus secretus	Hundreds
						Cicurina varians	11
						Cicurina vibora	1
						Pseudouronectes reddelli	2
						Cambala speobia	1
						Lithobiomorpha	1
						Staphyllinidae	1
						Eleutherodactylus sp.	4
						Helicodiscus sp.	1
						Assassin Bug	7
						Collembola	Thousands
						Moth	2
Karankawa	Karankawa	99.9	59.9	1	S. Van Kampen-Lewis, K. White, I. Lord	Ceuthophilus cunicularis	4
						Ceuthophilus secretus	10
						Leiobunum townsendi	1
						Pseudouronectes reddelli	6
						Cambala speobia	2
						Perimyotis subflavus	3
						Helicodiscus sp.	4
						Collembola	3
						Gnat	1
						Isopod	3
Karankawa	Pemmican	97.1	62.0	2.25	C. Crawford, H. Beatty, Andrew M.	Texella reyesi	1
						Ceuthophilus secretus	Hundreds
						Cryptachaea porteri	2
						Cicurina vibora	12
						Pseudouronectes reddelli	3
						Cambala speobia	3

Table 20. Karst Biota Survey Results at Karankawa Cave KFA

		Insic	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
						Scutigeridae	1
						Rhadine noctivaga	1
						Arenivaga sp.	1
						Assassin Bug	9
						Collembola	Hundreds
						Red Ant	Thousands
Karankawa	Polaris	98.9	65.4	1	C. Crawford, H. Beatty, Andrew M.	Ceuthophilus secretus.	Dozens
						Cryptachaea porteri	8
						Cicurina varians	6
						Cicurina vibora	6
						Leiobunum townsendi	1
						Pseudouronectes reddelli	7
						Cambala speobia	1
						Speodesmus bicornourus	5
						Eleutherodactylus sp.	2
						Assassin Bug	1
						Collembola	Dozens

Bone Cave harvestman detection has been sporadic within Karankawa Cave KFA, with only Karankawa Cave consistently containing more than five detected individuals (Figure 17). The 2018 biota survey represents an above-average detection year for Bone Cave harvestman in Pemmican Cave, with a more typical count in 2020. Pemmican Cave was not surveyed in 2014; therefore, data have been omitted for that cave in 2014. Snake Dancer Cave has been omitted from Figure 17 because the Bone Cave harvestman has not been located in Snake Dancer Cave since regular biota surveys began in 2014. Snake Dancer Cave is a small feature with significant exposure to atmospheric conditions. Note that rattlesnakes precluded full surveys in Karankawa and War Party Caves in 2019.

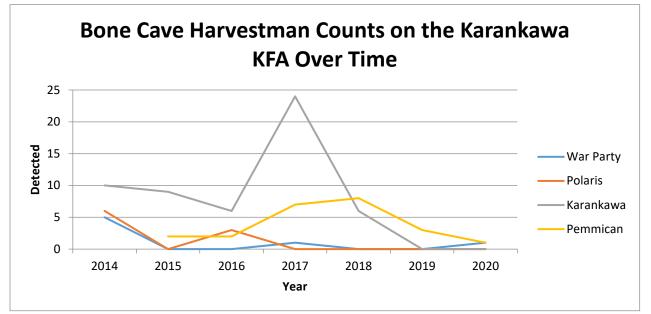


Figure 17. Endangered karst invertebrate species detection within the Karankawa Cave KFA.

Temperature and humidity readings in the Karankawa Cave KFA caves appear to fluctuate significantly (Figure 18). For example, relative humidity within War Party Cave ranges from 63.0% to 99.9%, while temperature ranges from 56.1°F to 75.5°F. Note that the humidity within War Party Cave in 2020 was measured as 99.9%, but is obscured in Figure 18 by humidity reading in Karankawa Cave which was also 99.9%. Pemmican Cave was not surveyed in 2014; therefore, data have been omitted for that cave in 2014. Climate readings were not taken within Polaris Cave during 2016; as a result, the humidity trend line is distorted and the temperature bar has been omitted for that cave in 2016 (see Figure 18). Finally, rattlesnakes precluded surveys in War Party and Karankawa Caves during 2019, further distorting Figure 18.

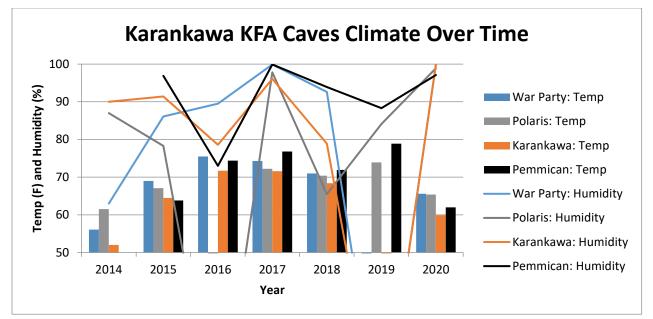


Figure 18. Climate readings within the Karankawa Cave KFA.

12.3 Adaptive Management Issues

The AMC should consider the following specific issues at the Karankawa Cave KFA:

- The AMC may wish to consider fully fencing the Karankawa Cave KFA due repeated presence of unauthorized cattle grazing and unauthorized trespassing of hunters.
- Pemmican Cave is directly adjacent to and downslope from the ranch road used to access the Karankawa Cave KFA interior. Because it is downslope, leaf litter and debris wash into the cave during rain events. Current stormwater management includes an artificial silt fence to filter water moving towards the cave (Photograph 8). The AMC should consider installing a permanent rockwall or cobble filter dam to continue stormwater filtration indefinitely. This recommendation is carried over from last year.



Photograph 8. Pemmican Cave silt fence filters stormwater entering the subsurface.

13 COFFIN CAVE PRESERVE

Coffin Cave Preserve is approximately 0.4 mile west from the Ronald Reagan Boulevard and County Road 234 intersection and occupies approximately 38.0 acres.

13.1 Maintenance Activities

There is one cave associated with this preserve: Coffin Cave. A large, heavy-duty, steel A-frame gate was constructed atop the Coffin Cave entrance in early 2017. This feature allowed biota survey crews to rappel into the nearly 40-foot shaft that leads to the greater cave. The A-frame is considered "overbuilt" for safety and is rated to hold approximately 1.5 tons (Photograph 9).

The Trail and Preserve Steward installed a game camera at the front gate of the Coffin Cave Preserve on July 18, 2018, to monitor for potential trespassing. The game camera was taken down on September 6, 2019, so that a theft-deterring mount could be installed. During this period, three trucks were recorded stopping at the front gate; however, no one was observed exiting the vehicles and no additional trespassing was detected. Vegetation was dumped in the preserve during the months of September and October of 2020. The game camera was re-installed on September 28, 2020, as a deterrent and no dumping was detected in the months following it. Notes from the monthly cave inspections are provided in Table 21.



Photograph 9. Finalized steel A-frame being used to rappel into Coffin Cave.

Table 21. Preserve Maintenance Triggers within the Coffin Cave Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Coffin	No	No	Yes/No	No	None.	None.
February						
Coffin	No	No	Yes/Yes	No	None.	None.
March						
Coffin	No	No	Yes/No	No	None.	None.
April						
Coffin	No	No	Yes/Yes	No	2×3 m S.	None.
May						
Coffin	No	No	Yes/No	No	Old mounds not active	None.
June						
Coffin	No	No	Yes/No	No	None.	None.
July						
Coffin	No	No	Yes/Yes	No	None.	None.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
August						
Coffin	No	No	Yes/No	No	None.	None.
September						
Coffin	Yes	No	Yes/No	No	None.	 Brush dumped at entrance. Removed brush. Installed game camera 9/28/20.
						3. Will need to install game camera.
October						
0	N	N I.		NI-	News	1. Game camera still installed.
Coffin	Yes	No	Yes/Yes	No	None.	2. None.
						3. None.
November						
Coffin	No	No	Yes/No	No	None.	 Game camera still installed. None. None.
December						
Coffin	No	No	Yes/No	No	None.	 Game camera still installed. Reviewed game camera footage, nothing to report. None.

* Fence around entire perimeter.

13.2 Karst Biota Survey

The Coffin Cave portal is within the streambed of an ephemeral stream, which pours water directly into the cave during rain events. Photograph 10 depicts the stream during dry conditions. The lack of atmospheric exchange with the surface environment also means that air within Coffin Cave is typically bad (high carbon dioxide) and biologists must wait for cold fronts to move through and provide atmospheric mixing within the cave. However, a combination of scheduling conflicts and poor weather conditions (no cold extended cold snaps to remove bad air) precluded a 2020 survey within Coffin Cave. Biologists completed a Coffin Cave survey on January 29, 2021, and sampling results will be included in the next activities report.



Photograph 10. Biologists examining Coffin Cave prior to cave gate construction in March 2016, directly in front of streambed leading to cave portal.

13.3 Adaptive Management Issues

There are currently no adaptive management considerations for the Coffin Cave Preserve.

14 BECK COMMONS PRESERVE

The Beck Commons Preserve occupies 5.0 acres and is approximately 0.3 mile north of the Beck Preserve, just off Ranch-to-Market 620.

14.1 Maintenance Activities

There are two caves associated with this preserve: Beck Sewer and Beck Trash Caves. The Trail and Preserve Steward repeatedly noted trash along the Beck Commons Preserve perimeter as a recurring problem. However, the fence appears to prevent most trash from entering the preserve. In March 2020, vegetation was dumped within the preserve but was not noted for other months. Notes from the monthly cave inspections are provided in Table 22.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
February						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
March						
Beck Sewer Cave	Yes	No	Yes/No	No	None.	 Vegetation dumped at preserve. Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash None.
April						
Beck Sewer Cave	No	No	Yes/Yes	No	None.	None.
Beck Trash Cave	No	No	Yes/Yes	No	None.	None.
Мау						
Beck Sewer Cave	No	No	Yes/No	No	None.	None.
Beck Trash Cave	No	No	Yes/No	No	None.	None.
June						
Beck Sewer Cave	No	No	Yes/No	No	2×2 m S.W. (Mound became non- active before treatment	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	None.

Table 22. Preserve Maintenance Triggers within the Beck Commons Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
July	-	-	-		-	-
Beck Sewer Cave	No	No	Yes/Yes	No	None.	None.
Beck Trash Cave	No	No	Yes/Yes	No	None.	None.
August						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	1×5 m S.W.	 Treated RIFA with boiling water. None. None.
September						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	 Raccoon under gate None. None.
October						
Beck Sewer Cave	No	No	Yes/Yes	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/Yes	No	None.	None.
November						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.
Beck Trash Cave	No	No	Yes/No	No	None.	None.
December						
Beck Sewer Cave	No	No	Yes/No	No	None.	 Trimmed vegetation around cave to provide easier access. None. None.
Beck Trash Cave	No	No	Yes/No	No	None.	 Trash around perimeter. Picked up trash. None.

* Fence intact around entire perimeter.

14.2 Karst Biota Survey

Biologists surveyed the entire Beck Sewer Cave in two trips. The upper portion of the cave was surveyed on October 1, 2020, and the lower on November 5, 2020. Eight Bone Cave harvestmen were detected in the upper segment and nine were found in the lower segment. It is unclear why the count fell from a record 43 detected individuals in 2017 and may simply be natural variation after a particularly productive year. However, an intense "flash drought" developed over central Texas during the second half of 2020 and may be the reason for the reduced Bone Cave harvestman count in Beck Sewer Cave compared to other years. Additionally, biologists collected two *Cambala speobia*, two *Batrisodes uncicornis*, two *Anapistula* sp., and one *Helicodiscus* sp. from the upper portion and two *Anapistula* sp. from the lower portion in 2020. Karst biota survey results are summarized in Table 23.

		Inside Cave					
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
October 1, 2020	-	-	-		-		-
Beck Commons	Beck Sewer – Upper	88.8	78.6	3	S. Van Kampen-Lewis, M. Heimbuch, I.	Texella reyesi	8
					Lord	Ceuthophilus secretus	18
						Anapistula sp.	18
						Cryptachaea porteri	dozens
						Cicurina browni	1
						Cicurina varians	3
						Leiobunum townsendi	dozens
						Cambala speobia	2
						Speodesmus bicornorus	6
						Batrisodes uncicornis	2
						Eleutherodactylus sp.	1
						Helicodiscus sp.	7
			Collembola	11			
						Gnat	dozens
						Isopod	2
						Mosquito	dozens
						Tick	1
November 5, 202	20						
Beck Commons	Beck Sewer – Lower	99.5	76.5	11.66	S. Van Kampen-Lewis, K. White, C.	Texella reyesi	9
					Crawford, H. Beatty, I. Lord	Ceuthophilus secretus	14
						Ceuthophilus cunicularis	5
						Anapistula sp.	2
						Cryptachaea porteri	4
						Cicurina varians	5
						Pale Surface Spider	4
						Tiny Spider	1
			Cambala speobia	4			
			Speodesmus bicornorus	2			
						Collembola	12
						Gnat	1

Table 23. Karst Biota Survey Results at Beck Commons Preserve

		Insid	le Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
						Isopod	4

Biologists located the Bone Cave harvestman during the two 2020 biota surveys of Beck Sewer Cave and documented 17 individuals in 2020 (Figure 19).

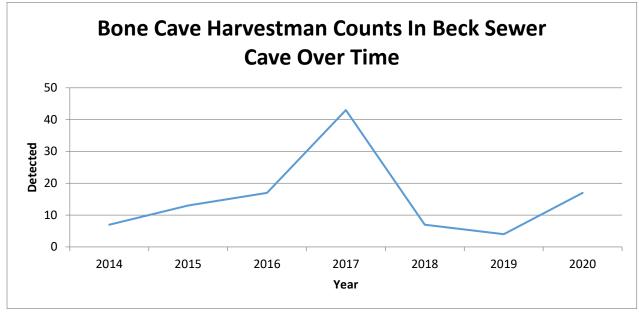
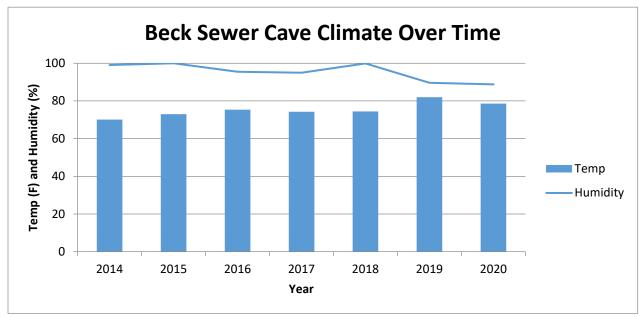


Figure 19. Endangered karst invertebrate species detection within Beck Sewer Cave.



The climate readings within Beck Sewer Cave indicate stable climatic conditions. Humidity is generally close to 100% and temperatures range from 70°F to 82°F (Figure 20).

Figure 20. Climate readings within Beck Sewer Cave.

14.3 Adaptive Management Issues

The AMC should consider the following specific issue at the Beck Commons Preserve:

• The Beck Trash Cave entrance is full of human-derived debris, much of which may have historic value. The AMC may wish to consider organizing a dedicated trash collecting effort. This recommendation is carried over from last year.

15 SHAMAN CAVE KARST FAUNA AREA

The Shaman Cave KFA occupies approximately 79.0 acres and is located approximately 1,800 feet north of Shell Road between Williams Drive (Farm-to-Market 2338) and State Highway 195 in Georgetown, Texas.

15.1 Maintenance Activities

There are two caves associated with this preserve that receive regular maintenance activities: Shaman and Powwow Caves. The Shaman Cave KFA warranted no unusual or extraneous activities in 2020 beyond the basic monthly maintenance tasks. Notes from the monthly cave inspections are provided in Table 24.

Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
No	No	Yes/No	No	1×1 m (not very active).	 Gate needs an external lock and new hinges. None. Lock and hinges.
No	Νο	Yes/No	No	None.	 Gate needs an external lock and new hinges. None. Lock and hinges.
No	No	Yes/Yes	No	None.	 None. Installed new hinges and lock on gate. None.
No	No	Yes/Yes	No	1×4 m W. (Not very active).	 None. Installed new hinges and lock on gate. None.
	Trash Dumping and Unauthorized Entry No No	Trash Dumping and UnauthorizedVegetation, Pet Issues and Feral AnimalsNoNoNoNoNoNoNoNo	Trash Dumping and UnauthorizedVegetation, Pet Issues and Feral AnimalsInspection / Lock LubricationNoNoYes/NoNoNoYes/NoNoNoYes/NoNoNoYes/No	Trash Dumping and Unauthorized EntryVegetation, Pet Issues and Feral AnimalsInspection / Lock LubricationActivityNoNoYes/NoNoNoNoYes/NoNoNoNoYes/NoNoNoNoYes/YesNo	Trash Dumping and Unauthorized Entry Vegetation, Pet Issues and Feral Animals Inspection / Lock Lubrication Activity More Within 10 m of Cave Entrance No No Yes/No No 1×1 m (not very active). No No Yes/No No 1×1 m (not very active). No No Yes/No No None. No No Yes/Yes No None.

Table 24. Preserve Maintenance Triggers within the Shaman Cave KFA

Preserve/Cave	Vandalism,	Damage to	Gate	Off-Trail	RIFA Mounds	1. Comments
	Trash Dumping and Unauthorized	Vegetation, Pet Issues and Feral Animals	Inspection / Lock Lubrication	Activity	Within 10 m of Cave Entrance	2. Tasks Completed
	Entry					3. Tasks Outstanding
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	1×4 m W. (Not very active).	None.
April						
Shaman Cave	No	No	Yes/Yes	No	1×0 m	None.
Powwow Cave	No	No	Yes/Yes	No	None.	None.
Мау						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.
June						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.
July						
Shaman Cave	No	No	Yes/Yes	No	None.	None.
Powwow Cave	No	No	Yes/Yes	No	None.	None.
August						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.
September						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.
October						
Shaman Cave	No	No	Yes/Yes	No	None.	None.
Powwow Cave	No	No	Yes/Yes	No	None.	None.
November						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.
December						
Shaman Cave	No	No	Yes/No	No	None.	None.
Powwow Cave	No	No	Yes/No	No	None.	None.

* No fence.

15.2 Karst Biota Survey

A karst biota survey at the Shaman Cave KFA occurred on October 20, 2020. Biologists detected 13 Bone Cave harvestmen (Photograph 11) and two potential Dragonfly Cave mold beetles in Shaman Cave. These individuals were not collected, so they may have been the similarly looking but common, *Batrisodes uncicornis*. They also detected 22 Bone Cave harvestmen and one potential Dragonfly Cave mold beetle from

Powwow Cave. Biologists collected one Geophilomorph centipede, one *Rhadine noctivaga*, and one potential Dragonfly Cave mold beetle from Shaman Cave and no invertebrates from Powwow Cave. Karst biota survey results are summarized in Table 25.



Photograph 11. Juvenile Bone Cave harvestman on gloved finger in Shaman Cave.

		Insi	ide Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
October 20, 2020	-	-	-	-			-
Shaman	Powwow	75.9	79.8	2.3	S. Van Kampen-Lewis, K. White, L. Rome, I. Lord	Texella reyesi	22
						Batrisodes cryptotexanus	1
						Ceuthophilus cunicularis	2
						Ceuthophilus secretus	dozens
						Cicurina varians	3
						Cicurina vibora	2
						Pseudouroctonus reddelli	1
						Cambala speobia	dozens
						Speodesmus bicornorus	23
						Anillinus sp.	2
						Rhadine noctivaga	4
						Assassin Bug	1
						Collembola	hundreds
						Gnat	1
						Red Ant	dozens
Shaman	Shaman	80.5	80.7	4.5	S. Van Kampen-Lewis, K. White, L. Rome, C. Crawford, I. Lord, B. Frou	Texella reyesi	13
						Batrisodes cryptotexanus	2
						Ceuthophilus cunicularis	2
						Ceuthophilus secretus	dozens
						Cicurina varians	2
						Cicurina vibora	3
						Pseudouroctonus reddelli	3
						Cambala speobia	8
						Speodesmus bicornorus	4
						Geophilomorph	1
						Anillinus sp.	1
						Rhadine noctivaga	5
						Eleutherodactylus sp.	2
						Perimyotis subflavus	2
						Helicodiscus sp.	1

Table 25. Karst Biota Survey Results at Shaman Cave KFA

		Inside Cave					
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
						Assassin Bug	2
						Collembola	dozens

The Shaman Cave KFA continues to be a very productive ecosystem with consistently high numbers of both Bone Cave harvestmen and Dragonfly Cave mold beetles (Figure 21). Both monitored caves are deep enough to provide excellent troglobitic habitat that is insulated from surface conditions.

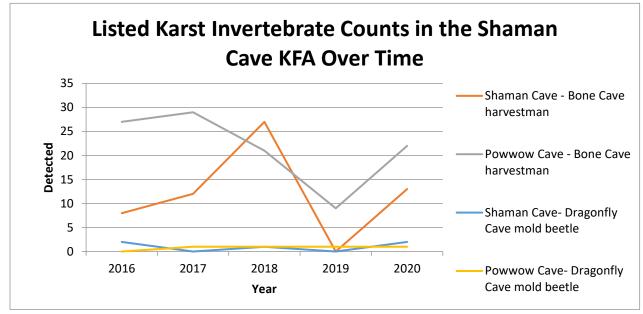


Figure 21. Endangered karst invertebrate species detection within the Shaman Cave KFA.

The climate readings within Shaman and Powwow Caves indicate stable climatic conditions. Humidity was lower and temperature was warmer than normal in 2020. (Figure 22). Abundant moisture in both caves is readily apparent and may help explain why large numbers of listed karst invertebrates are consistently detected each year. However, an intense "flash drought" developed over central Texas during the second half of 2019 and may be the reason for the reduced humidity in Powwow Cave compared to other years. The lowered humidity was the likely cause for reduced Bone Cave harvestman detections in Powwow Cave compared to previous surveys as well.

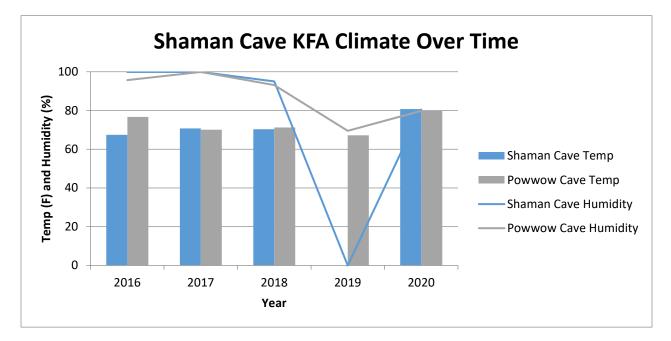


Figure 22. Climate readings within the Shaman Cave KFA.

15.3 Adaptive Management Issues

The Shaman Cave KFA currently presents no adaptive management considerations for the AMC.

16 BAT WELL CAVE PRESERVE

The Bat Well Cave Preserve occupies approximately 46.3 acres and is located along Berry Creek, partially within Sun City (north of Berry Creek) and adjacent to Georgetown Village development south of Berry Creek.

16.1 Maintenance Activities

There are two caves associated with this preserve: Bat Well and Berry Creek Caves. In 2020, the old cave gate atop Berry Creek Cave was removed and replaced with a new gate that excludes the first flush of water in Berry Creek. The new gate will keep out unauthorized users while facilitating cleaner water recharging into Berry Creek Cave. Regular maintenance activities did not occur at the Bat Well Cave Preserve until May 2020 following an acquisition procedure. Notes from the monthly cave inspections are provided in Table 26.

Preserve/Cave	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks
	-					Outstanding
January **Not mo	onitored**					
February **Not m	nonitored**					
March **Not mon	itored**					
April **Not monite	ored**					
May **Not monito	ored**					
Bat Well Cave	No	No	Yes/No	Νο	None.	1. Needs new larger gate installed. 2. None.
Dat Well Cave	NO	NU	Tes/NO	NO	None.	3. Needs new larger gate installed.
Berry Creek Cave	No	No	Yes/No	No	None.	 Cave gate underwater. None. None.
June						
Bat Well Cave	No	No	Yes/No	No	None.	 Needs new larger gate installed. None. Needs new larger gate installed.
Berry Creek Cave	No	No	Yes/No	No	None.	1. Cave gate underwater. 2. None. 3. None.
July						
Bat Well Cave	No	No	Yes/No	No	None.	 Needs new larger gate installed. None. Needs new larger gate installed.
Berry Creek Cave	Yes	No	Yes/Yes	No	None.	1. Cave gate exposed, lock broken off, gate tabs bent so it cannot open. 2. Put on new lock. 3. None.
August						
Bat Well Cave	No	No	Yes/No	No	None.	 Needs new larger gate installed. None. Needs new larger

Table 26. Preserve Maintenance Triggers within the Bat Well Cave Preserve KFA

Preserve/Cave	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks
Berry Creek Cave	Yes	No	Yes/No	No	None.	Outstanding None.
September	-	-		-		-
Bat Well Cave	No	No	Yes/No	No	None.	 Needs new larger gate installed. None.
						 Needs new larger gate installed.
Berry Creek Cave	Yes	No	Yes/No	No	None.	None.
October	<u>-</u>	-	-		-	<u>-</u>
						1. Needs new larger gate installed.
Bat Well Cave	No	No	Yes/Yes	No	None.	 None. Needs new larger gate installed.
						1. None.
Berry Creek Cave	Yes	No	Yes/Yes	No	None.	2. Raised gates by 16 inches to keep lock out of water.
						3. None.
November						
						1. Needs new larger gate installed.
Bat Well Cave	No	No	Yes/No	No	None.	2. None.
						 Needs new larger gate installed.
Berry Creek Cave	Yes	No	Yes/No	No	None.	1. Signs of forced entry. No entry gained.
0010						2. None.
December						3. None.
December						1. Needs new larger
Bat Well Cave	No	No	Yes/No	No	Nono	gate installed. 2. None.
Dal VVEII GAVE	NU	No	t es/ino	No	None.	 None. Needs new larger gate installed.
Berry Creek Cave	No	No	Yes/No	No	None.	None.

*No fence.

16.2 Karst Biota Survey

The Bat Well Cave karst biota survey occurred on December 16, 2020. Poor terrestrial troglobitic habitat occurs within this cave because of its proclivity to flood during rain events. The entire cave is very muddy

and lacks dry areas that escape flooding. Neither the Bone Cave harvestman nor the Dragonfly Cave mold beetle were detected in Bat Well Cave in 2020. Due to poor oxygen levels, biologists spent little time at the stream passage and detected no aquatic stygobites that were present in previous years (Photograph 12).



Photograph 12. Collected *Caecidotea* sp. specimens from Bat Well Cave stream passage (2017).

Biologists collected no invertebrates at Bat Well Cave in 2020.

Karst biota survey results are summarized in Table 27.

In 2019, Mr. Reddell indicated the Berry Creek Cave may be located within the Bat Well Cave Preserve (see Van Kampen-Lewis and White 2020a for cave details) and that this cave may be another portal into the Edwards Aquifer. Berry Creek Cave is a mapped cave located within Berry Creek. Biologists confirmed that Berry Creek Cave occurs within the Bat Well Cave Preserve and that a subterranean stream passage flowing almost perpendicularly to Berry Creek is visible. Studies within Berry Creek Cave to determine species inhabitation are planned for 2021.

16.3 Adaptive Management Issues

There are currently no adaptive management considerations for the Bat Well Cave Preserve.

		In	side Cave				
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number
December 16, 2020	-	-	-	-	-	-	-
Bat Well	Bat Well	99.9	65.3	4.6	K. White, H. Beatty, I. Lord	Ceuthophilus cunicularis	Dozens
						Ceuthophilus secretus	Hundreds
						Cicurina varians	1
						Cambala speobia	9
						Lithobiomorpha	9
						Surface Millipede	1
						Carabidae	1
						Staphyllinidae	1
						Eleutherodactylus sp.	1
						Incilius nebulifer	2
						Thamnophis proximus	1
						Perimyotis subflavus	1
						Catfish	1
						Collembola	Dozens
						Surface Roach	1
						Unknown Spider	1

Table 27. Karst Biota Survey Results at Bat Well Cave Preserve

17 SNOWMELT CAVE PRESERVE

The Snowmelt Cave Preserve occupies 1.3 acres and is located near the intersection of County Road 176 and Parkside Parkway. Road construction bisected the cave, with half the cave being stabilized below County Road 176 and the other half (within Snowmelt Cave Preserve) remaining in it's natural state. Biota monitoring in such a cave is rare and might be a good opportunity to study the effects of significant impacts to a cave ecosystem. Road construction was completed in May 2020.

17.1 Maintenance Activities

There is one cave associated with this preserve: Snowmelt Cave. Regular maintenance activities at the Snowmelt Cave Preserve were limited to RIFA mound removal. Notes from the monthly cave inspections are provided in Table 28.

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
January	-	-	-	-	-	
Snowmelt	No	No	Yes/No	No	1×3 m W. (Not active).	 Road being built next to cave. None. None.
February						
Snowmelt	No	No	Yes/No	No	1×3 m W. (Not active).	 Road being built next to cave. None. None.
March						
Snowmelt	No	No	Yes/No	No	1×3 m W., 1×10 m N. (Not very active).	 Road being built next to cave. None. None.
April						
Snowmelt	No	No	Yes/Yes	No	1×3 m W., 1×10 m S.	 Road being built next to cave. None. None.
Мау						
Snowmelt	No	No	Yes/No	No	Old mounds no longer active.	 Road being built next to cave. None. None.
June						0.110110.

Table 28. Preserve Maintenance Triggers within the Snowmelt Cave Preserve

Preserve/Cave*	Vandalism, Trash Dumping and Unauthorized Entry	Damage to Vegetation, Pet Issues and Feral Animals	Gate Inspection / Lock Lubrication	Off-Trail Activity	RIFA Mounds Within 10 m of Cave Entrance	1. Comments 2. Tasks Completed 3. Tasks Outstanding
Snowmelt	No	No	Yes/No	No	1×2 m W.	1. None. 2. Treated RIFA with boiling water. 3. None.
July						
Snowmelt	No	No	Yes/Yes	No	None.	None.
August						
Snowmelt	No	No	Yes/No	No	None.	None.
September						
Snowmelt	No	No	Yes/No	No	None.	None.
October						
Snowmelt	No	No	Yes/Yes	No	None.	None.
November						
Snowmelt	No	No	Yes/No	No	None.	None.
December.						
Snowmelt	No	No	Yes/No	No	None.	None.

* No fence.

17.2 Karst Biota Survey

USFWS asked SWCA to perform presence/absence surveys within Snowmelt Cave in 2019 prior to the widening of County Road 176. The road widening required filling and stabilization of the portion of the Snowmelt Cave footprint underneath the road to provide strength for the increased traffic above. The goal of the presence/absence surveys was to locate the Bone Cave harvestman, which had not been documented within Snowmelt Cave in many years.

SWCA was unable to locate the Bone Cave harvestman in Snowmelt Cave during the 2019 biota survey and was unable to detect the species during the 2020 survey. Encountered species in 2020 include *Ceuthophilus secretus, Eidmannella* sp., *Cambala speobia*, and Collembola (Table 29).

Snowmelt Cave is a fairly small, shallow feature that experiences significant atmospheric exchange with the surface environment. Temperature was 77.9°F and relative humidity was 82.5°F within Snowmelt Cave during the 2020 biota survey.

		Inside Cave		Inside Cave					
Preserve	Cave	RH (%)	Temp (°F)	Person-Hours	Surveyors	Species	Number		
October 2, 2020	1	-	-			-			
Snowmelt	Snowmelt	82.5	77.9	1	S. Van Kampen-Lewis, M. Heimbuch, I. Lord	Ceuthophilus secretus	dozens		
						Cicurina varians	2		
						<i>Eidmanella</i> sp.	5		
						Cambala speobia	dozens		
						Collembola	thousands		

Table 29. Karst Biota Survey Results at Woodland Park Preserve

17.3 Adaptive Management Issues

There are currently no adaptive management considerations for the the Snowmelt Cave Preserve.

18 HIDDEN SPRINGS PRESERVE

The Hidden Springs Preserve is a new preserve acquired by the WCCF in 2020. The preserve occupies approximately 935.0 acres and is located near the northwestern corner of Williamson County. Photographs 13 through 15 show representative images of three ecotypes within the preserve.



Photograph 13. Scrubby juniper (*Juniperus* spp.) woodland on a hillside at Hidden Springs Preserve.



Photograph 14. Treeless portion of savannah mosaic at Hidden Springs Preserve.



Photograph 15. Forested lowland at Hidden Springs Preserve.

18.1 Maintenance Activities

There are no caves associated with this preserve. No monthly maintenance activities occurred at the Hidden Springs Preserve in 2020 because the purchase was finalized in the same year. However, the following activities occurred:

- Cattle grazing operations ceased.
- No hunting leases were distributed for fall 2020.
- A general cleanup around the ranch house occurred.
- Deer feeders were removed as they were identified.
- Maintenance on perimeter fences and replacement of four gates occurred.
- Ranch entrance signage was placed and gate maintenance occurred.
- The WCCF published a request for proposal on the ranch house for rehabilitation activities (e.g. leveling, painting, new roof).
- The WCCF is working with a university consortium to obtain funding for rangeland and native grassland improvement to the southern one-third of the ranch.
- The WCCF is working with the Williamson County Chapter of the Master Naturalists on game camera survey.

18.2 Biota Surveys

The Hidden Springs Preserve has no documented caves. However, this preserve was acquired as a GCWA preserve. The WCCF will send biologists to Hidden Springs during the 2021 GCWA breeding season to determine the number of breeding individuals that utilize this area. Afterwards, the WCCF intends to add approximately 240 acres of habitat within the Hidden Springs Preserve to its reserve of GCWA habitat credits for the species with an application to the USFWS that will be submitted in 2021. Initial surveys at Hidden Springs have shown the area to be occupied by breeding GCWA.

18.3 Adaptive Management Issues

There are currently no adaptive management considerations for the Hidden Springs Preserve.

19 ADDITIONAL ACTIVITIES

This section provides a summary of additional activities overseen or funded by the WCCF.

19.1 2020 Annual *Eurycea* Monitoring Activities Carried Out Under the Williamson County Regional Habitat Conservation Plan

This report by Cambrian details the 2020 results of Salado, Georgetown, and Jollyville Plateau salamander surveys funded by the WCCF at eight sites (Swinbank, Twin, Cobbs, Avery Springhouse, Avery Deer, Hill Marsh, PC, and Brushy Creek Springs) in Williamson County. Salamander habitat, observation, and capture data are compared to previous monitoring years, and population demographics are reported from capture-mark-recapture modeling for all sites. The full manuscript is included in Appendix A.

19.2 Salamander Manuscripts Published in 2020

Cambrian published three separate peer-reviewed notes in 2020 on the observation of eyeless *Eurycea* at Cobbs Spring, the documentation of crayfish as predators of Georgetown Salamanders, and the response of Jollyville Plateau Salamanders to spring drying. All three manuscripts in their final published form are included in Appendix B.

19.3 Salamander Manuscripts Accepted in 2020 and Currently In Press

Jollyville Plateau salamanders can be difficult to detect and capture in submerged leaf litter packs, woody debris, and vegetation. Therefore, performed studies within the WCCF managed preserves to determine more effective sampling methods than are currently utilized. Cambrian describes the modification of a water hyacinth sieve and introduces three designs of Hubbard rakes to effectively sample these cover objects. They captured the Jollyville Plateau salamanders using the sieve and all three rakes and additionally used these devices to capture other *Eurycea* species, including the Blanco River Springs salamander (*E. pterophila*), the Georgetown salamander, and the Salado salamander; and several co-occurring tadpoles, small fishes, and invertebrates. They also detail the application and success of these tools in various cover types, water depths, and substrates. The full manuscript is included in Appendix C.

19.4 Salamander Manuscripts Submitted in 2020 and Currently In Review

Cambrian submitted a manuscript regarding the macroscopic examination of 622 Salado salamanders collected between June 2018 and July 2020 from three springs (Cobbs, Cowan, Twin Springs) revealed the presence of encapsulated parasitic larvae of *Clinostomum* cf. *marginatum* (yellow grub) in three hosts. Two of these salamanders were examined and released unharmed but one was found dead. The dead specimen harbored six of these parasitic larvae, four on the head (including one behind the left eye), one near the left front leg, and one in the tail. Morphological identification of *C*. cf. *marginatum* was achieved by comparison to previous accounts. Molecular identification was accomplished by comparing sequence

homology and phylogenetic analysis using an 828 base pair partial sequence of the internal transcribed spacer region. This is the first report of any parasite from the Salado salamander, a federally listed threatened species. The full manuscript is included in Appendix D.

19.5 Investigation of Historic Georgetown Salamander Sites on the Avant Property in Georgetown, Texas

Cambrian performed population monitoring activities for Georgetown salamanders at three historically occupied sites in Williamson County, Texas, in 2020. The recent taxonomic reassignment of several salamander populations in Williamson County shrank the species' range, resulting in only one long-term monitoring site remaining as a locality for this species. As a response to gather natural history and population demographic data from other locations, the WCCF funded additional Georgetown salamander surveys at three historic locations on the Avant property in 2020. This report provides site descriptions and salamander habitat, observation, and capture data from 2020. Abundance estimates were generated for each site using capture-mark-recapture modeling and are compared to historic collection data. The full manuscript is included in Appendix E.

19.6 Preliminary Results of a Dye Trace Study for Krienke Spring

Cambrian performed a dye trace study designed to interpret perceived groundwater connections from nearby recharge features into Tonkawa Spring, also known as Krienke Spring, which is within a Jollyville Plateau salamander critical habitat unit (CHU 1) as mapped by the USFWS. Groundwater tracing using non-toxic dyes to characterize recharge areas, as well as groundwater flow paths and velocities, is a common diagnostic tool in karst aquifers worldwide, and has been used successfully within the Barton Springs segment of the Edwards Aquifer. Little dye trace work has been done in the northern segment of the Edwards Aquifer, and virtually none has been done in Williamson County prior to this work. Ultimately, dye was not detected near Krienke Spring (access to the spring was denied by landowner) nor at eight other sample sites. It is unclear why the dye was not detected at any site. The full results memorandum is included in Appendix F.

19.7 Southern Great Plains Climate Reconstruction

Texas A&M University paleoclimatologist Dr. Maupin continued to collaborate with SWCA and the WCCF to examine the history of the Southern Great Plains hydroclimate using absolutely dated oxygen isotope records from central Texas cave stalagmites collected from Cobbs Cavern in the summer of 2016.

The oxygen isotopic composition of stalagmite calcite is a unique fingerprint that identifies changes in the oxygen isotopic composition of the rainwater feeding the vadose zone (soil and rock above the Edwards Aquifer). The degree to which any initial radioactive uranium trapped in the calcite has decayed into the intermediate daughter isotope, thorium-230, allows determination of an absolute radiometric age for sampled growth layers in the stalagmites. These ages are precise, often within 99% certainty. Thus, speleothems serve as "rain gauges" throughout the time periods of their deposition in the cave.

Useful interpretations of speleothem oxygen isotope records require an understanding of region-specific controls on rainfall oxygen isotopic ratios. Dr. Maupin and Stephen Van Kampen-Lewis (SWCA) have continuously collected water from and measured the oxygen isotopic composition of every daily Austinarea rainfall event from 2015 and continuing into 2020. They have developed an interpretation linking rainfall (and therefore speleothem) oxygen isotope variability to the frequency and strength of mesoscale (>100 km) convective systems, which are often associated with strong thunderstorms.

These storms, ranking among the strongest on Earth, occur during late spring into early summer, and then again in fall. Recently, they have been responsible for climatologically frequent incidences of severe weather hazards, such as flash-flooding, hail, lightning, straight-line wind gusts, and tornados. However, in spite of the damage they portend, these systems supply central Texas and the Southern Great Plains with 50 to 90% of their annual rainfall totals. Years with a dearth of rainfall from such systems (e.g., 2011 and 2012) face catastrophic meteorological drought. Future trends in rainfall and severe weather over the Southern Great Plains remain highly uncertain, and understanding drivers of these storms has been highlighted as an indispensable scientific endeavor by the 2018 National Climate Assessment (U.S. Global Change Research Program 2018).

Collection and analyses of environmental parameters within Cobbs Cavern, germane to Dr. Maupin's paleoclimate work, remain ongoing. Two self-redundant HOBO (brand name) temperature loggers have recorded cave temperatures almost continuously for the past 5 years, with drip waters collected one to two times annually to ensure that oxygen isotope variations in the cave waters are transmitting the isotopic composition of the rainfall above. In May 2018, small watch glasses were suspended below active drips to "farm" cave calcite. Measurements of isotope partitioning between these watch glass speleothems and the drip waters supplying them were made in early 2019, confirming the stalagmites from Cobbs Cavern are best interpreted as recorders of central Texas precipitation changes. In 2020, they again collected the past year's calcite growth and replaced the plates for further calcite growth studies.

The first manuscript prepared from this work has now been peer-reviewed, accepted, and is now in press in *Nature Geoscience*. This paper presents a test case arguing their hypothesis and interpretation of the rainfall isotope data and its relationship to storms, while applying said interpretation to an interval of abrupt climate change ca. 30,000 to 50,000 years ago. Additional manuscripts in preparation examine changes and variability during the last interglacial-glacial-interglacial cycle, and the climate of central Texas through the past nearly 400,000 years, respectively. The goal of these additional manuscripts is to assess the combination of forcing and background conditions responsible for producing the mean climate state in the Southern Great Plains. These remaining manuscripts will be first submitted to the highestimpact peer-reviewed journals in the earth science fields and include all relevant personnel, Texas A&M University honors researchers, and organizations as coauthors and collaborators. The full manuscript in press is published copywrite rules of *Nature Geoscience* indicate that distributing a PDF is not permissible. However, the entire manuscript is accessible through this link: <u>https://rdcu.be/cj6Ao</u>.

20 WILLIAMSON COUNTY REGIONAL HABITAT CONSERVATION PLAN STATUS

This section provides a summary of the status of the Williamson County RHCP with respect to the participation rates, impacts, mitigation, and funding assumptions projected in the permit.

20.1 Leave No Trace Program

The WCCF offers preserve access to those who complete the Leave No Trace (LNT) program for Williamson County Preserves, which is based on the international Leave No Trace program for outdoor ethics. The program teaches outdoor enthusiasts to enjoy wild spaces while minimizing or eliminating potential impacts. Completing the LNT program allows access to Twin Springs KFA and Beck Preserve.

The WCCF issued 33 passes to LNT graduates after holding two classes (February 6, March 5) in 2020. The WCCF has three people trained to teach LNT classes and generate passes.

20.2 Endangered Songbird Credits

20.2.1 Golden-cheeked Warbler

The WCCF sold 100.3 GCWA credits during 2020. The WCCF held 181.6 GCWA credits through the end of 2020. All GCWA credits and debits from WCCF inception are shown in Table 30. The initially established GCWA conservation measures (Hickory Pass Ranch Conservation Bank credit purchases and the Twin Springs credits) still provide surplus credits, exceeding demand for songbird mitigation.

Date	Project Description	Credit s
Nov. 2007	Hickory Pass Ranch Conservation Bank – GCWA Credits	500.0
Jan. 2008	Twin Springs – GCWA Credits	115.5
Sept. 2010	Committed to Ronald Reagan Boulevard Phase III	-74.4
Dec. 2010	Hickory Pass Ranch – GCWA Credits	500.0
Oct. 2011	Committed to Vista Ridge Boulevard	-5.5
Feb. 2012	Committed to 1710 County Road 262 Georgetown	-1.0
Nov. 2013	Committed to Lakeline Boulevard Extension Phase 2 from Old Quarry Road to Old 2243 west of Leander	-24.3
Nov. 2013	Committed to a water transmission line Railroad Boulevard to County Road 175 (0.6 acres direct, plus 2.3 indirect)	-1.8
Aug. 2014	Committed to Reagan's Overlook subdivision (19.0 acres direct, plus 22.8 indirect)	-30.4
Nov. 2014	LAMY 2243 – Bluffview	-8.0
Dec. 2014	Wedemeyer	-346.0
Aug. 2015	City of Georgetown	-2.5
June. 2015	American Housing Ventures	-2.1
Sept. 2015	Sentinel Land / HWY 29 Ventures 2015 LP	-7.0
Jan. 2016	City of Georgetown – Southwest Bypass	-43.7
Jan. 2017	Enterprise Crude Pipeline	-151.4*
Jan. 2017	Enterprise Crude Pipeline	-38.4*
March. 2018	Parmer Cypress Development, LLC	-1.2
Dec. 2018	Lower Colorado River Authority (LCRA)	-24.5**
Feb. 2019	SFSG Investments, LP and R&M Global Group (also known as Patience Ranch)	-26.9
Dec. 2019	JCI Residential	-29.5
Jan. 2020	M2E3 Enterprise Pipeline	-100.3
	Total Available GCWA Credits Held by WCCF	196.6

Table 30. WCCF Managed Golden-cheeked Warbler Credits Through Time

* Note: Enterprise Crude from 2017 was incorrectly listed in the 2018 Activities Report as purchasing 341.2 credits, which did not account for most of those credits being calculated for indirect impacts at a 0.5:1 ratio. Therefore, the 2018 Activities Report overstated the number of purchased GCWA credits and underestimated the number of credits still maintained by the WCCF.

** Note: LCRA credits were incorrectly listed in the previous (2019) Activities Report as purchasing 39.5 credits, which did not account for indirect impacts at a 0.5:1 ratio. Therefore, the 2019 Activities Report overstated the number of purchased GCWA credits and underestimated the number of credits still maintained by the WCCF.

In 2020, 100.3 acres of GCWA habitat were impacted; therefore, 919.0 acres of GCWA habitat have been impacted directly or indirectly since RHCP inception. The RHCP anticipated that 200.0 acres of GCWA habitat would be impacted per year, totaling 6,000.0 impacted acres of habitat throughout the RHCP life (SWCA et al. 2008). Therefore, 2,400.0 acres of GCWA habitat were anticipated to have been impacted through 2020; however, less than half of this amount has actually been impacted.

20.2.2 Black-capped Vireo

No BCVI habitat participation was recorded during 2020; therefore, no additional coverage was added to the 22.5 acres already impacted. The RHCP anticipated 142.2 covered acres for BCVI habitat per year, totaling 4,267.0 impacted acres of habitat throughout the RHCP life; therefore, 1,706.7 BCVI habitat acres were expected to have been impacted through 2020 (SWCA et al. 2008).

The WCCF previously covered BCVI habitat impacts to 22.5 acres and all participation funds are expected to be put towards species habitat restoration. No additional BCVI habitat impacts were covered by the WCCF during 2020. The AMC did not make any recommendations for fund use. No BCVI habitat credits are currently held by the WCCF. USFWS (2018b) delisted the species due to recovery and the WCCF does not anticipate additional participations or credit disbursements for the BCVI.

20.3 Karst Participation

The WCCF is authorized to impact 210 occupied caves over the duration of the permit. This includes impacting 150 caves within Zone A (50–345 feet from cave footprint) and 60 caves within Zone B (within 50 feet of cave footprint).

Twenty-six new projects located in Williamson County participated for karst coverage within the RHCP across 1,464.1 acres during 2020, bringing the total enrolled karst zone participation through December 2020 to 10,771.3 acres during the RHCP's lifetime (9,307.2+1,464.1 acres).

Actual karst zone participation acreage is higher than assumptions discussed in the RHCP (SWCA et al. 2008). The permit projected 533.0 acres of karst zone participation per year, totaling 6,396.0 (5,863.0+533.0) participation acres after 12 years (2008 through 2022) of RHCP operation.

However, Zone A and Zone B impacts are significantly lower than assumptions discussed within the RHCP (see SWCA et al. 2008 for details regarding cave Zone A and Zone B). Sixty caves were projected to be partially impacted by Zone A intrusion over a 12-year period (five per year); however, the WCCF has recorded Zone A impacts to 31 caves through 2020. No Zone A participation impacts were recorded by the WCCF in 2020. Twenty-four caves were expected to be fully impacted by Zone B intrusion over 12 years (two per year); however, the WCCF has recorded Zone B impacts to five caves through 2020. The WCCF documented participation for one Zone B cave impact during 2020, which was assumed to be occupied by at least one covered species. Table 31 shows the 2020 karst participation in the RHCP.

Table 31. 2020 RHCP Enrolled Karst Zone Participation

Project	Enrolled Karst Zone (Acres)	Zone A intrusion (Acres)	Zone B intrusion (Caves)
Atmos Energy	0.2	None	None

Atmos Energy	0.2	None	None
Aura Avery Ranch – Trinsic	16.0	None	None
Georgetown ASLI IX	316.2	None	None
Canyons of HCH Ranch	364.2	None	None
Christensen Crest	20.0	None	None
City of Austin	1.0	None	None
City of Georgetown	3.5	None	None
City of Georgetown	1.0	None	None
City of Round Rock (University)	12.3	None	None
DH Holdings LLC	16.0	None	None
Hampton Park Estates	17.3	None	None
HM Parkside (Barton Tributary)	17.0	None	None
Lower Colorado River Authority	24.0	None	None
Lower Colorado River Authority	1.0	None	None
M2E3 (Enterprise)	67.5	None	1
Milestone Community Builders	7.2	None	None
Parkside (new water line)	15.2	None	None
Parkside on the River Phase 2	272.5	None	None
Parkside on the River Phase 1a	58.9	None	None
TxDOT (Williams Drive, etc.)	47.8	None	None
Williamson County Road Bond Program (WCRBP) – Hairy Man Road	10.6	None	None
WCRBP – Forest North III	5.5	None	None
WCRBP – Great Oaks Bridge, etc.	4.6	None	None
WCRBP – O'Connor Drive signals	0.9	None	None
Wolf Lakes Village	163.5	None	None
Total	1,464.1	0.0	1

The WCCF acquired the approximately 935.0-acre Hidden Springs Preserve in 2020 and maintained a total of four administered KFAs (Priscilla's Well, Cobbs Cavern, Twin Springs, Karankawa Cave). Three other existing preserves already administered by the WCCF are currently proposed for KFA status (Shaman, Wilco and Millennium Preserves).

20.4 Williamson County Conservation Foundation Statement of Activities

The unaudited Statement of Activities indicates the WCCF maintained a \$4.44 million balance at the end of 2019 and had changed that balance to approximately \$4.10 million by the end of 2020 (Table 32).

Williamson County Conservation Found Statement of Activities (Unaudited)					
Jan 1, 2020 to Dec 31, 202	0				
Balance as of 12/31/19	s	4,439,210.0			
Revenues:					
Donations	S	2,225.0			
Interest on Investments	S	41,356.9			
Miscellaneous Revenue	s	628.3			
Net Inc/Dec FMV Invest??	-	0400.0			
Participation Fees	S	2,574,889.0			
Tax Benefit Financing		2,511,487.2			
Tax benefit I mancing		2,511,407.2			
Total Revenues	\$	5,130,586.4			
Expenditures:					
Computer Equipment					
Contribution to Benefits (Insurance)					
Copier Rental & Supplies	S	3,131.3			
Educational Materials	\$	229.8			
Equipment	\$	20,632.1			
Facility Enhancements	s	426.4			
Gasoline	s	1,747.6			
Grounds Maintenance	S	23,562.6			
Land Acquisition	S	4,690,735.4			
Membership Dues	s	3,000.0			
Misc	S	1,162.6			
Office Furniture	S	111.9			
Office Supplies	S	392.7			
Postage	s	150.2			
Printed Materials & Binding	S	50.0			
Professional Services	S	301,963.6			
Publications/Books/Periodicals	20	N			
Research Activities	S	249,822.5			
Salaries and Fringes	S	158,641.2			
Small Equipment & Tools	S	353.3			
Training/Travel	s	3,296.9			
Transfer to Capital Projects	-				
Use of Donations	s	2,578.9			
Utilities	S	242.4			
Vehicle Insurance	S	335.3			
Vehicle Repairs & Maintenance	S	3,216.7			
Total Expenditures	\$	5,465,784.0			
Fund Equity as of 12/31/20	5	4,104,012.5			

Table 32. WCCF Statement of Activities for 2020

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APPENDIX A

2020 Annual *Eurycea* Monitoring Activities Carried Out Under the Williamson County Regional Habitat Conservation Plan This page intentionally left blank.



2020 Annual *Eurycea* Monitoring Activities Carried Out Under the Williamson County Regional Habitat Conservation Plan

Prepared for:

Williamson County Conservation Foundation 219 Perry Mayfield Leander, TX Attn: Gary Boyd

Prepared by:

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CAMBRIAN ENVIRONMENTAL

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April 2021

EXECUTIVE SUMMARY

We (Cambrian Environmental) conducted *Eurycea* salamander population monitoring activities at eight springs in Williamson County, Texas in 2020. These surveys were carried out on behalf of the Williamson County Conservation Foundation (WCCF) consistent with the biological goals and objectives of the Williamson County Regional Habitat Conservation Plan (Wilco RHCP). Monitoring activities at sites within the City of Georgetown and its extraterritorial jurisdiction are additionally intended to meet the water quality and salamander monitoring requirements of the special 4(d) rule (USFWS 2015).

Salamanders at three of the springs (i.e., Swinbank Spring, Twin Springs, and Cobbs Spring) were historically considered Georgetown Salamanders (E. *naufragia*; GTS; Chippindale et al. 2000), but a recent publication addressing the taxonomy of central Texas *Eurycea* suggests that two of these sites (i.e., Twin Springs and Cobbs Spring) are occupied by Salado Salamanders (*E. chisholmensis*; SS; Devitt et al. 2019). The other five springs (i.e., Avery Springhouse Spring, Avery Deer Spring, Hill Marsh Spring, PC Spring, and Brushy Creek Spring) are occupied by Jollyville Plateau Salamanders (*E. tonkawae*; JPS; Chippindale et al. 2000, Devitt et al. 2019).

This report details GTS and SS monthly monitoring results from Swinbank Spring (October 2015 – December 2020), Twin Springs (October 2015 – December 2020), and Cobbs Spring (July 2015 – February 2020), and JPS bimonthly monitoring results from Avery Springhouse Spring, Avery Deer Spring, Hill Marsh Spring, Brushy Creek Spring, and PC Spring (September/October 2016 – December 2020). Monitoring at Swinbank Spring and Twin Springs is a continuation of the monitoring program and capture-mark-recapture study established by Southwestern University (Pierce et al. 2010, Pierce et al. 2014, Gutierrez et al. 2018, Pierce and Gonzalez 2019). Monitoring at the five JPS sites is a continuation of the population and ecological studies established by Texas State University (Adcock et al. 2016).

We conducted 24 total monitoring events at Swinbank, Twin, and Cobbs Springs in 2020. Water conditions (i.e., temperature, pH, dissolved oxygen, conductivity) fell within a range reported as appropriate in the primary literature for *Eurycea* salamanders at all sites and during all surveys, with the exception of dissolved oxygen on few occasions. All three sites exhibited dissolved oxygens levels below the 4.5 mg/L threshold considered suboptimal for *E. nana* (Woods et al. 2010); however, we observed salamanders during each of these survey events.

We conducted 30 total monitoring events at Avery Deer, Avery Springhouse, Hill Marsh, PC, and Brushy Creek Springs in 2020. Water temperature and pH were suitable for *Eurycea* salamanders at all sites and during all survey events. Dissolved oxygen dropped below the 4.5 mg/L threshold considered suboptimal for *E. nana* (Woods et al. 2010) at each site on few occasions. Conductivity at Avery Springhouse and Brushy Creek Spring exceeded 900 μ S/cm which is associated with reduced JPS counts in a previous study (Bowles et al. 2006). It should be noted that despite apparent adverse dissolved oxygen and conductivity levels, Avery Springhouse Spring exhibits the one of the highest salamander relative abundances out of all monitored JPS springs. These results demonstrate that the tolerance of central Texas *Eurycea* to fluctuating values of dissolved oxygen and conductivity likely warrants reevaluation.

Relative abundance (i.e., the percentage of cover objects with salamanders) was highest in 2020 compared to previous years (2015-2019) at all sites except for Twin Springs, Avery Deer Spring, and Avery Springhouse Spring. Swinbank Spring had the highest number of observed salamanders in a single monitoring event (n = 110), as well as the largest number we were able to capture in a single monitoring event (n = 74). Additionally, Swinbank Spring had the greatest single-survey percentage of cover objects with salamanders (9.65%).

The percentage of *Eurycea* captures that were gravid in 2020 compared to previous monitoring years was greater or equal at Swinbank, Twin, Avery Springhouse, and Hill Marsh Springs. This percentage was reduced during 2020 relative to previous years at Cobbs, Avery Deer, PC, and Brushy Creek Springs. The variation in relative abundance metrics and reproduction are congruent with previous reports of large variations in surface counts of central Texas *Eurycea* (Bowles et al. 2006, Bendik et al. 2014, Pierce et al. 2014). We additionally provide data on clutch size for GTS, SS, and JPS which we acquired by counting the number of oocytes visible through the salamander's translucent venter. Clutch size ranged from 1 - 31 oocytes. Our 2019 and 2020 reports provide the first data reported on clutch size for any of these three species.

We analyzed all previous capture-mark-recapture data collected for all eight sites, using POPAN formulated Jolly-Seber models (Schwarz and Arnason 1996). This framework allows for the estimation of survival, probability of capture, probability of entry into these populations, and superpopulation size. Swinbank Spring harbors the largest superpopulation and demonstrates time varying probability of entry. Twin Springs was found to have the largest survival estimates for adult individuals and thus, a surprisingly large superpopulation size, despite seemingly few captures per event. Brushy Creek Spring was found to have the greatest estimated probability of capture (1.00), indicating that if a salamander is present at this site, it is near certain to be captured. Among more comparable sites Cobbs (10.5%) and Avery Deer Springs (9%) have the greatest probability of capture. Our 2019 and 2020 reports provide the first estimates of these demographic parameters for GTS and SS.

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- 1. Location of Georgetown Salamander (*Eurycea naufragia*) and Salado Salamander (*Eurycea chisholmensis*) monitoring sites in Williamson County, Texas.
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1.0 INTRODUCTION

We (Cambrian Environmental) conducted Eurycea salamander population monitoring activities at eight springs in Williamson County, Texas in 2020. These surveys were carried out on behalf of the Williamson County Conservation Foundation (WCCF) consistent with the biological goals and objectives of the Williamson County Regional Habitat Conservation Plan (Wilco RHCP). We monitored salamander populations in accordance with the U.S. Fish and Wildlife (USFWS) protocol requirements (USFWS 2014b). Monitoring activities at sites within the City of Georgetown and its extraterritorial jurisdiction are additionally intended to meet the water quality and salamander monitoring requirements of the special 4(d) rule (USFWS 2015). Population monitoring improves our understanding of central Texas Eurycea salamander biology, including information on long-term population trends, status, relative abundance, reproductive ecology, and general natural history. Over time, these data may signal potential impacts of human activities on these taxa and will help inform and improve the ecological effectiveness of conservation policies for these species. One of the management goals of the WCCF is to ensure the continued ecological health of Eurycea salamander populations and their habitat. Therefore, overarching objectives of our monitoring activities are to document and evaluate short- and long-term Eurycea salamander population and habitat trends.

According to a recent taxonomic update by Devitt et al. (2019), three species of neotenic, permanently aquatic plethodontid salamanders within the genus Eurycea occur in Williamson County, Texas: Salado Salamanders (E. chisholmensis; SS), Georgetown Salamanders (E. naufragia; GTS), and Jollyville Plateau Salamanders (E. tonkawae; JPS). Salamanders at three of the springs included in this monitoring program (i.e., Swinbank Spring, Twin Springs, and Cobbs Spring) were historically considered GTS (Chippindale et al. 2000), but Twin Springs and Cobbs Springs are now considered occupied by SS (Devitt et al. 2019). The other five springs (i.e., Avery Springhouse Spring, Avery Deer Spring, Hill Marsh Spring, PC Spring, and Brushy Creek Spring) are occupied by JPS (Chippindale et al. 2000, Devitt et al. 2019). These three salamanders constitute the Septentriomolge clade of central Texas Eurycea salamanders and are all restricted to groundwater-fed aquatic habitats (i.e., springs, spring-fed creeks, and caves) associated with the northern segment of the Edwards Aquifer (Chippindale et al. 2000, Hillis et al. 2001). Despite their taxonomic status, Septentriomolge show little genetic divergence among one another (Chippindale et al. 2000, Devitt et al. 2019) and are biologically and ecologically similar. Therefore, when natural history data are incomplete for one taxon, congener data are typically an appropriate surrogate.

Septentriomolge require relatively shallow, cool, flowing water (Chippindale 2005) and typically do not occupy deep water (>60 cm) that can support predatory fish populations (Bowles et al. 2006, Bendik et al. 2016). Most individuals are encountered in close proximity, within 25 m, to springs or seeps (Sweet 1982, Bowles et al. 2006, Pierce et al. 2010, Bendik et al. 2014, Bendik et al. 2016), but JPS also occupy stream reaches between and downstream of springs (Bendik et al. 2016, Adcock et al. 2020a). *Septentriomolge* utilize various submerged cover objects (e.g., cobble, leaf litter, woody debris) as refugia from predators (Davis et al. 2001, Bowles et al. 2006, O'Donnell et al. 2008, Pierce et al. 2010), and areas with large cobble are considered preferred

habitat (Bowles et al. 2006; Pierce et al. 2010; USFWS 2013a, 2014a). However, Adcock et al. (2016) demonstrated no difference in JPS relative abundance among cover object types. Gravel substrates with interstitial spaces that provide habitat for prey items, refuge from predators, and access to sub-surface water are considered essential habitat components (Chippindale 2005; USFWS 2013a, 2014a). Little is known about *Septentriomolge* use of subsurface flooded karstic refugia, although it is believed that these areas are utilized to escape surface drying events and for reproduction (Davis et al. 2001, Chippindale 2005, O'Donnell et al. 2008, Bendik and Gluesenkamp 2013, Pierce et al. 2014, Bendik 2017, Adcock et al. 2020b). Only a few cave populations are known (e.g., Buttercup Creek area, Bat Well Cave, Water Tank Cave) and they are poorly understood (Chippindale et al. 2000). The vast majority of caves in Williamson County do not provide access to the water table where suitable habitat for *Eurycea* salamanders would occur (Reddell and Finch 1963).

Septentriomolge are suggested to require aquatic habitats with a narrow range of water chemistry associated with karst aquifers (USFWS 2013a, 2014a). Few studies have field tested occurrence or abundance of these taxa in relation to water chemistry (but see Davis et al. 2001, Bowles et al. 2006, Adcock et al. 2016), and most requirements are inferred from other central Texas congeners. San Marcos Salamanders (E. nana; SMS) and Barton Springs Salamanders (E. sosorum; BSS) lose their righting response in high water temperatures, at approximately 35 °C and 32 °C, respectively (Berkhouse and Fries 1995, Crow et al. 2016). Additionally, BSS demonstrate decreased growth rate when water temperatures are above 24 °C (Crow et al. 2016). JPS are documented in the field from a water temperature range of 10.6 °C to 32.0 °C and a pH range of 6.1 to 9.3 (Davis et al. 2001, Bowles et al. 2006). SMS and BSS are sensitive to dissolved oxygen (DO) concentrations below 4.5 mg/L (Woods et al. 2010), but JPS have been documented in springs with DO below this level (Davis et al. 2001, Bowles et al. 2006, O'Donnell et al. 2008, Adcock et al. 2016). DO is positively correlated with abundance of other Eurycea species (Willson and Dorcas 2003, Turner 2004, Turner 2009). Bowles et al. (2006) reported higher JPS counts from springs with conductivity approximately 600 µS/cm compared to springs with conductivity around 900 µS/cm and above. However, Adcock et al. (2016) documented increased JPS relative abundance and density at sites with conductivity greater than 900 µS/cm, and Woods and Poteet (2006) found no significant effects of conductivity up to 2,400 µS/cm for SMS.

JPS counts and density are negatively correlated with development and urbanization (Bowles et al. 2006, Bendik et al. 2014). The specific mechanism(s) resulting in reduced population sizes has not been identified, but degraded water quality, reduced groundwater quantity, exacerbated flash floods, and changes to the general faunal community are suggested (Bowles et al. 2006, Bendik et al. 2014). Several publications document a decrease in the abundance of stream dwelling salamanders due to changes in water quality (e.g., temperature, pH, DO) associated with urbanization (see Barrett and Price 2014). Willson and Dorcas (2003) and Bowles et al. (2006) reported that water conductivity increases in areas with increased impervious cover (a measure of urbanization) and is associated with a decrease in aquatic salamander density. Urbanization can also lead to increased siltation in water bodies from stormwater runoff, which can fill the interstitial spaces (and increase embeddedness) thought to be important refugia for *Eurycea* salamanders (Martin et al. 2012; USFWS 2013a, 2014a). JPS were listed as federally threatened in 2013 and

SS and GTS were listed as federally threatened in 2014 due to threats from urbanization, including loss of habitat, degraded water quality, and reduced water quantity (USFWS 2013a, 2014a).

Almost all of the published natural history information available for GTS and SS results from WCCF funded research, including refinement of survey techniques (Biagas et al. 2012, Pierce et al. 2010, McEntire and Pierce 2015), notes on morphology (Wall et al. 2020), description of general ecology (Pierce et al. 2010, Pierce and Gonzalez 2019, Jones et al. 2020), description of reproductive ecology (Pierce et al. 2014), population monitoring and estimates (Pierce et al. 2014), and investigation of movement patterns (Pierce et al. 2014, Gutierrez et al. 2018). JPS natural history information is largely attributed to the long-term monitoring program conducted by the City of Austin (see Bowles et al. 2006, Bendik et al. 2014, Bendik et al. 2016, Bendik 2017) and WCCF (Adcock et al. 2016). To date, WCCF funded research projects included the development of novel survey techniques, description of the reproductive ecology, evaluation and delineation of occupied surface habitat, evaluation of the USFWS critical habitat units, identification of small-scale environmental variables that influence salamander presence (microhabitat parameters), and evaluation of disease and parasite prevalence (Adcock et al. 2016, McAllister et al. 2018).

Herein, we summarize and report salamander observation, salamander observation rate, and water chemistry for all Cambrian collected data at all eight sites. We additionally include capture-markrecapture estimates of probability of capture, probability of entry, survival, and superpopulation size at all sites. We include the combination of Southwestern University, Texas State University, and Cambrian data (i.e., photographic recognition) for capture-mark-recapture analyses.

2.0 METHODS

2.1 Site Descriptions

2.1.1 Georgetown Salamander Sites

2.1.1.1 Swinbank Spring.— Swinbank Spring is a permanent spring located in the North Fork San Gabriel River watershed in Williamson County, Texas, N 30.662718°, W 97.710707° (Table 1, Figure 1). This spring occurs in an urban area within the City of Georgetown, and the spring emerges from the base of a spring house adjacent to a low-density residential area. From this point, the spring channel runs approximately 70 m downstream to the North Fork San Gabriel River. The spring run predominantly contains cobble, woody debris, and leaf litter refugia with gravel substrate.

Southwestern University initiated visual encounter surveys at Swinbank Spring in 2007 with photos for Wild-ID recapture analysis beginning in 2012. We acquired monitoring responsibilities in 2015 and continued visual encounter surveys and Wild-ID photographic mark-recapture through the current surveys. Following the protocol developed by Pierce et al. (2010), we survey the upper 24 m of the spring run which generally consists of a channelized run with water pooled by small natural dams. After 24 m, the spring run widens into a broad, cascading flow to the North Fork San Gabriel River. We measured water quality parameters at the spring orifice.

2.1.2 Salado Salamander Sites

2.1.2.1 Twin Springs.— Twin Springs is a permanent spring located in the North Fork San Gabriel River watershed in Williamson County, Texas, N 30.696906°, W 97.781845° (Table 1, Figure 1). This spring occurs within the Twin Springs Preserve adjacent to low-density residential neighborhoods and is one of the western-most known locations of SS. The spring run contains cobble, woody debris, and leaf litter refugia with bedrock and silt substrate.

Southwestern University initiated visual encounter surveys at Twin Springs in 2007 with photos for Wild-ID recapture analysis beginning in 2012. We acquired monitoring responsibilities in 2015 and continued visual encounter surveys and Wild-ID photographic mark-recapture through the current surveys. Following the protocol developed by Pierce et al. (2014), we survey the upper 38 m of the spring run. The spring run geomorphology has changed considerably over time due to scouring effects of flash flooding events. We measured water quality parameters at the spring orifice.

2.1.2.2 Cobbs Spring.— Cobbs Springs is an ephemeral spring located in the Berry Creek watershed in Williamson County, Texas, N 30.789586°, W 97.728700° (Table 1, Figure 1). This spring occurs on undeveloped ranchland. When groundwater level is high, water fills a nearby hand-dug well, and two additional springs discharge upstream of the main Cobbs Spring. The spring run contains cobble, vegetation, leaf litter, and woody debris refugia with bedrock, gravel, and silt substrate. The spring run regularly becomes choked with dense, rooted aquatic vegetation approximately 10 m downstream of the discharge location.

Sporadic surveys have occurred at Cobbs Spring since approximately 2002 when the site was first visited by Dr. Andrew Price of the Texas Parks and Wildlife Department. We began monthly visual encounter surveys at this site in July 2015 with photos for Wild-ID recapture analysis beginning in April 2016. The landowners denied access to this location in March of 2020. We measured water quality parameters at the spring orifice, as well as from the hand-dug well and other discharges, when possible.

2.1.3 Jollyville Plateau Salamander Sites

2.1.3.1 Avery Deer Spring.—Avery Deer Spring (CHU 6) occurs within the South Brushy Creek Watershed and is located within a residential neighborhood of Avery Ranch in Round Rock, Texas (Table 1, Figure 2). The site contains three discrete spring outlets, the first (N 30.507000°, W 97.749490°) discharging approximately 70 m upstream from the second and third outlets (N 30.507502°, W 97.749311°). The most upstream spring outlet is ephemeral, occasionally drying during summer months in the absence of rain, while the two downstream outlets permanently flow. The creek empties into a retention pond approximately 260 m downstream of the second and third spring outlets.

Texas State University initiated visual encounter surveys at Avery Deer Spring in 2013, and surveys from 2013 to 2016 focused primarily on habitat use, downstream distribution, reproductive ecology, and disease prevalence (Adcock et al. 2016). All captures (2013 to present) have photos

for Wild-ID analysis. We began surveys at Avery Deer Spring in September 2016. From September 2016 to December 2018, we conducted visual encounter surveys at each of the spring outlets and several downstream sections in general accordance with the methodology established by Adcock et al. (2016). No salamanders have ever been observed greater than 10 m from a spring outlet at this site. Consequently, beginning in January 2019 we ceased surveying beyond this point. In 2020, we conducted timed visual encounter surveys and measured water quality parameters within 10 m of all three spring outlets.

2.1.3.2 Avery Springhouse Spring.—Avery Springhouse Spring (CHU 6) is a permanent spring located in the South Brushy Creek Watershed and is located within a residential neighborhood of Avery Ranch in Round Rock, Texas, N 30.503698°, W 97.759829° (Table 1, Figure 2). This spring discharges near a spring house and runs for approximately 65 m before confluencing with a creek. The creek terminates in a golf course pond approximately 340 m downstream of the confluence point.

Texas State University initiated visual encounter surveys at Avery Springhouse Spring in 2013, and surveys from 2013 to 2016 focused primarily on habitat use, downstream distribution, reproductive ecology, and disease prevalence (Adcock et al. 2016). All captures (2013 to present) have photos for Wild-ID analysis. We began surveys at Avery Springhouse Spring in September 2016. From September 2016 to December 2018, we conducted visual encounter surveys at the spring outlet and several downstream sections in general accordance with the methodology established by Adcock et al. (2016). No salamanders have ever been observed greater than 125 m from the spring outlet at this site. Consequently, beginning in January 2019 we ceased surveying beyond this point. In 2020, we conducted timed visual encounter surveys in the 65 m spring run and first 60 m of the creek after its confluence with the spring run. We measured water quality parameters at the spring orifice, as well as the confluence of the spring run and creek.

2.1.3.3 Hill Marsh Spring.—Hill Marsh Spring (CHU 6) is a permanent spring located in the South Brushy Creek Watershed on the Avery Ranch Golf Course in Round Rock, Texas, N 30.507680°, W 97.755070° (Table 1, Figure 2). This spring has an approximate 45 m spring run before terminating in a golf course pond.

Texas State University initiated visual encounter surveys at Hill Marsh Spring in 2013, and surveys from 2013 to 2016 focused primarily on habitat use, downstream distribution, reproductive ecology, and disease prevalence (Adcock et al. 2016). All captures (2013 to present) have photos for Wild-ID analysis. We began surveys at Hill Marsh Spring in September 2016. From September 2016 to present, we conducted timed visual encounter surveys throughout the 45 m spring run. We measured water quality parameters at the spring orifice.

2.1.3.4 PC Spring.—PC Spring consists of two permanent spring discharges located in the Lake Creek watershed in Williamson County, Texas (Table 1, Figure 2). The most upstream discharge emerges from the bank of Davis Spring Branch under SH45 (N 30.481276°, W 97.742274°), and the second discharges from the base of a concrete culvert pad (N 30.481947°, W 97.742362°). At approximately 78 m from the culvert, water from the second discharge flows into Davis Spring Branch downstream of the first discharge.

Texas State University initiated visual encounter surveys at PC Spring in 2013, and surveys from 2013 to 2016 focused primarily on habitat use, downstream distribution, reproductive ecology, and disease prevalence (Adcock et al. 2016). All captures (2013 to present) have photos for Wild-ID analysis. We began surveys at PC Spring in September 2016. From September 2016 to December 2018, we conducted visual encounter surveys at each of the spring outlets and downstream sections in general accordance with the methodology established by Adcock et al. (2016). Few salamanders have ever been observed greater than 20 m from a spring outlet at this site. Consequently, beginning in January 2019 we ceased surveying beyond this point. In 2020, we conducted timed visual encounter surveys and measured water quality parameters in the upper 20 m of both spring runs.

2.1.3.5 Brushy Creek Spring.—Brushy Creek Spring is located within the Brushy Creek watershed in Round Rock, Williamson County, Texas, N 30.516834°, W 97.661271° (Table 1, Figure 2). Brushy Creek Spring discharges inside a storm water culvert that runs under US-79. Spring input is ephemeral, but when present, water emerges from three spring diversion pipes and several cracks in the concrete culvert. Water flows from the culvert over a large gabion and into a deep pool, which then constricts into a spring run that empties directly into Brushy Creek.

Texas State University initiated visual encounter surveys at PC Spring in 2013, and surveys from 2013 to 2016 focused primarily on habitat use, downstream distribution, reproductive ecology, and disease prevalence (Adcock et al. 2016). All captures (2013 to present) have photos for Wild-ID analysis. Surveys were focused on the shallow pool edges and the spring run outside of the culvert system from May 2014 to March 2015, as this appeared to be the most appropriate salamander habitat. From April 2015 to January 2018, surveys were conducted inside the culvert and in the spring run during every survey event. We began surveys at Brushy Creek Spring in September 2016. The landowner denied access to the spring run beginning in February 2018, but we maintained culvert surveys through current survey events. We measure water quality parameters at the eastern-most spring orifice inside the culvert.

2.2 Monitoring Methods

2.2.1 Abiotic Monitoring

2.2.1.1 Climate Conditions.—In accordance with USFWS (2014b) survey protocol, we report the air temperature during each survey event, the previous week's maximum and minimum air temperature, the total rainfall on the survey day, and the average daily rainfall for the previous week. We obtained temperature and rainfall data from the National Oceanic and Atmospheric Administration (https://www.noaa.gov) for Georgetown, Texas (Station USC00413507).

2.2.1.2 Habitat Conditions.—During each Eurycea survey we collected water quality and substrate data in accordance with USFWS (2014b) survey protocol. We recorded water temperature, pH, specific conductivity, and dissolved oxygen (DO) at each spring orifice per site. We measured water temperature and conductivity with a Com-100 from HM Digital (Culver City, California, USA), pH with a EcoTestr pH 2 from Oakton Instruments (Vernon Hills, Illinois, USA), and DO with a HI 9147 from Hanna Instruments. We additionally recorded water depth,

substrate type (e.g., silt, gravel), and estimated the average embeddedness of cover objects (see Sennatt et al. 2006, USFWS 2014b) during each survey.

2.2.2 Salamander Monitoring

2.2.2.1 Georgetown and Salado Salamander Survey Methods.—We conducted monthly salamander surveys at all Eurycea occupied sites following the survey protocol implemented by Pierce et al. (2014). Due to mandatory shutdowns associated with the 2020 COVID-19 pandemic, we did not survey in April, and therefore, we only conducted 11 surveys in 2020. During each survey, we methodically searched the spring run for salamanders by overturning or searching through potential cover objects (i.e., cobble, vegetation, leaf litter, woody debris), and attempting to capture all Eurycea observed. We began at the most downstream section and moved toward the spring orifice. We recorded the number cover objects searched to standardize salamander counts as the percentage of cover objects multiplied by 100; Pierce et al. 2010, 2014). We present counts and the percentage of cover objects with salamanders as metrics of relative abundance. We also recorded the number, size class, and location (i.e., distance from nearest spring discharge) of all observed Eurycea.

2.2.2.2 Jollyville Plateau Salamander Survey Methods.—We conducted bimonthly salamander surveys at all JPS occupied sites for a total of six surveys per site for 2020 We generally followed the methodology implemented by Adcock et al. (2016) and performed timed visual encounter surveys in each surveyed section. Unlike the methodology utilized for the above-referenced GTS and SS sites, methodology for these sites was originally intended to sample, rather than census, the salamander populations. During each survey, we methodically searched the spring run section for JPS by overturning or searching through potential cover objects (i.e., cobble, vegetation, leaf litter, woody debris). We began at the most downstream section and moved toward the spring orifice. We recorded the number cover objects searched to standardize salamander counts as the percentage of cover objects multiplied by 100; Pierce et al. 2010, 2014). We present counts and the percentage of cover objects with salamanders as metrics of relative abundance. We also recorded the number, size class, and location (i.e., distance from nearest spring discharge) of all observed JPS, and we attempted to capture all observed JPS.

2.2.2.3 Captures.—We captured salamanders using dip nets, sieves, or Hubbard rakes. Once captured, we measured each salamander using handheld calipers to the nearest 0.1 mm. We recorded the total length (TL; i.e., tip of snout to end of tail) and the snout vent length (SVL; i.e., tip of snout to posterior edge of the vent). We determined the gravidity of all captured *Eurycea* by visually checking for oocytes (eggs) through the salamander's translucent venter (Gillette and Peterson 2001, Pierce et al. 2014). If oocytes were present, we photographed the salamander's venter (Figure 3) and manipulated the body cavity to count oocytes. We took photos of the body and head of each salamander against a standardized grid background with the salamander in a water-filled dish. Pigmentation patterns on the head were used to identify recaptured salamanders using Wild-ID photographic recognition software (Figure 4; Bolger et al. 2012, Bendik et al.

2013). Photos were cropped and edited in ImageJ or Adobe Photoshop, to standardize the brightness, contrast, and orientation of each salamander within each photo. Cropped photos were uploaded into their respective site-specific Wild-ID database, then compared against all existing photos of previously captured salamanders. This allowed us to determine whether the new images represent recaptures of previously 'marked' individuals or individuals new to the study being 'marked' for the first time. Wild-ID reports photos from the top twenty potential matches from previous capture dates ranked by match probability. We review the first ten photos with the highest probability of being a potential match for every individual captured.

2.2.2.4 Capture-Mark-Recapture Modeling.—Capture-mark-recapture (CMR) studies are essential to acquiring demographic, life history, and behavioral data for listed species, and they allow the estimation of important population parameters when it is impractical to capture and count every individual. CMR studies require the identification and subsequent recognition of individuals in a study population. Results from Wild-ID enable us to create unique capture histories for each individual salamander encountered from each site and is much less invasive, easier, more time-and cost-effective than CMR studies using physical markers like visual implant elastomers (Bendik et al. 2013).

We survey once a month or once every other month under the current salamander monitoring program. The population is demographically "open" between survey occasions because births, deaths, and migration in and out of the population can occur in-between surveys. Therefore, we are required to use Open CMR models to analyze these data. Open CMR models were historically used to estimate survival, but several iterations were developed to allow estimation of other parameters. We chose to analyze the Eurycea data with a POPAN formulation of the classic Jolly-Seber model because it provides estimates for several parameters of interest (Schwarz and Arnason 1996) and has been suggested as the most appropriate for amphibians with prolonged breeding seasons (Wagner et al. 2011). This method allows us to estimate 1) the size of a superpopulation - the total number of salamanders that ever enter the sampled population between the first and last survey occasions, 2) survival (phi) - the probability that a salamander survives from one survey occasion to the next survey occasion, 3) capture probability (p) – the probability of capturing an individual salamander, and 4) probability of entrance (pent) – the probability that a salamander from the superpopulation enters the population between two survey occasions and survives to the next survey occasion (Schwarz and Arnason 1996, Williams et al. 2011). We compared models with constant and time-dependent survival, capture probability, and probability of entrance, i.e., we compared models where these parameters do not change over time to models where these parameters are different among survey occasions. We chose the best fit model as determined by Akaike's information criterion (Mazzarole et al. 2006). Jolly-Seber models have several assumptions that must be met for the model to yield reliable estimates, i.e., salamanders retain their marks (head patterns do not change), marks are properly read (Wild-ID properly identifies recaptures), sampling is instantaneous, survival probability is the same for all salamanders among sampling occasions (homogenous survival), catchability is the same for all salamanders at each sampling occasion (homogenous catchability), and the study area is constant (Schwarz and Arnason 1996, Williams et al. 2011). Sites were analyzed separately due to our belief that migration among them is unrealistic given the geographic distance separating them and previous genetic characterization of the animals from these sites (Devitt et al. 2019).

3.0 RESULTS

3.1 Georgetown Salamander Monitoring Results

3.1.1 Swinbank Spring

3.1.1.1 Habitat Monitoring.—Climate conditions for each 2020 Swinbank Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Swinbank Spring consistently had gravel and bedrock substrate throughout 2020 and estimated embeddedness ranged from 10 - 75%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.1.1.2 Salamander Monitoring.—We searched a total of 12,303 cover objects and observed 887 and captured 620 total GTS during the 11 monthly salamander surveys in 2020. Monthly GTS counts ranged from 50 - 110 and the percentage of cover objects with salamanders ranged from 5.05 - 9.65% (Table 5, Figures 9 and 10). We captured 98 gravid females which accounted for 15.8% of the total 2020 captures. Gravid females were observed in every month surveys were conducted, although none were observed during our July 8 survey, and no surveys were conducted during April. Most gravid females were observed in September through December, and December had the largest number of gravid females (n=18; Table 5).

From the onset of Cambrian monitoring of this site, we have observed 3,049 GTS, including the 887 observations in 2020 (Table 6). The percent of cover objects with salamanders was greater (7.21%) than the site average for this measure (5.20%), indicating increasing short term relative abundance at this site (Table 6, Figure 10).

A total of 1,919 unique GTS have been captured at Swinbank Spring since the onset of Wild-ID photographic documentation in October 2012. During 2020 monitoring, we captured 329 new GTS and recaptured 291 salamanders from previous monitoring events (47%). The greatest time between first capture and most recent capture for any single individual salamander was nearly five years (March 2014 – February 2019), and this salamander was captured four times within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included time varying probability of entry (*pent*) and capture probability (*p*), but constant survival (*phi*). Survival was estimated to be 0.89 ± 0.0037 (Table 7; Figure 11). In other words, an individual salamander has roughly an 89% chance of surviving from one sampling occasion to the next. Probability of capture was unique for each survey occasion, and estimates ranged from 0.012 to 0.19, meaning any observable salamander has between a 1% and 19% chance of being captured, contingent upon the conditions on a given survey date (Table 7; Figure 12). Probability of entry varied by time, ranging from 0 to 0.06, indicating that surveys are punctuated by pulses of new individuals after periods of high proportions of recaptures (i.e., low entrant probability; Table 7;

Figure 13). Finally, the superpopulation of salamanders at this site is estimated to be $4,307 \pm 128$ individual salamanders (Table 7; Figure 14).

3.2 Salado Salamander Monitoring Results

3.2.1 Twin Springs

3.2.1.1 Habitat Monitoring.—Climate conditions for each 2019 Twin Springs monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Twin Springs consistently had bedrock and silt substrate throughout 2019 and estimated embeddedness ranged from 10 - 50%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.2.1.2 Salamander Monitoring.—We searched a total of 10,225 cover objects and observed 79 and captured 68 total SS during the 11 monthly salamander surveys in 2020. Monthly SS counts ranged from 1 - 13 and the percentage of cover objects with salamanders ranged from 0.13 - 1.56% (Table 5, Figures 9 and 10). We captured 6 gravid females which accounted for 8.82% of the total 2020 captures (Table 6). Gravid females were observed in February, March, November, and December (Table 5).

From the onset of Cambrian monitoring of this site we have observed 432 SS, including the 79 observations in 2020 (Table 6). The percent of cover objects with salamanders was lower in 2020 (0.77%) than the site average for this measure (0.84%), indicating a decrease in salamander relative abundance at this site compared to previous years. We captured 6 gravid females during 2020 (8.8% of total captures), which is an average observation for this site among all previous years (Table 6).

A total of 396 unique SS have been captured at Twin Springs since the onset of Wild-ID photographic documentation in October 2012. During 2020 monitoring, we captured 33 new SS and recaptured 36 salamanders from previous monitoring events (53%). The greatest time between first capture and most recent capture for any single individual salamander was five years (December 2015 - December 2020), and this salamander was captured 12 times within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included constant survival (*phi*), capture probability (*p*), and probability of entry (*pent*). Survival was estimated to be 0.94 ± 0.004 (Table 7; Figure 11). In other words, an individual salamander has roughly a 94% chance of surviving from one sampling occasion to the next. Probability of capture was estimated to be 0.064 ± 0.004 , meaning any observable salamander has between a 6% and 7% chance of being captured within any given sampling event (Table 7; Figure 15). Probability of entry was estimated at 0.008, indicating that new individuals entering this site occurs rarely (Table 7; Figure 13). Finally, the superpopulation of salamanders at this site is estimated to be 791 ± 41 individual salamanders (Table 7; Figure 14).

3.2.2 Cobbs Spring

3.2.2.1 Habitat Monitoring.—Climate conditions for each 2020 Cobbs Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Cobbs Spring had gravel, silt, and bedrock substrate in the two 2020 surveys and estimated embeddedness ranged from 50 - 75%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.2.2.2 Salamander Monitoring.—We searched a total of 2,797 cover objects and observed 85 and captured 77 total SS during the 2 monthly salamander surveys in 2020. Salamander counts were 34 and 51 for the two surveys we were able to conduct, respectively. The percentage of cover objects with salamanders on these survey dates were 1.96% and 4.8%, respectively (Table 5, Figures 9 and 10). We did not capture any gravid females during either 2020 survey (Table 5).

From the onset of Cambrian monitoring of this site we have observed 886 SS, including the 85 observed in 2020 (Table 6). The percent of cover objects with salamanders in 2020 (3.04%) was higher than the site average for this measure (2.5%), indicating a slight increase in salamander relative abundance at this site.

A total of 523 unique SS have been captured at Cobbs Spring since the onset of Wild-ID photographic documentation in April 2016. During 2020 monitoring we captured 54 new individuals and recaptured 23 salamanders from previous monitoring events (30%). The greatest time between first capture and most recent capture for any single individual salamander was 14 months (December 2018 – February 2020), and this salamander was captured three times within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included time varying probability of entry (*pent*) and survival (*phi*), but constant capture probability (*p*). This is a departure from the model we observed last year, in which we observed constant survival. With the inclusion of data collected in 2020 survival was highly variable, ranging between 0 and 1, with a mean estimated to be 0.40 ± 0.13 (Table 7; Figure 11). In other words, an individual salamander has roughly a 40% chance of surviving from one sampling occasion to the next, contingent upon the conditions on a given survey date. Probability of capture was estimated to be 0.105 ± 0.014 , meaning any observable salamander has a 10.5% chance of being captured within any given sampling event (Table 7; Figure 15). Probability of entry varied by time, ranging from 0 to 0.14, indicating that surveys are punctuated by pulses of new individuals after periods of high proportions of recaptures (Table 7; Figure 13). Finally, the superpopulation of salamanders at this site is estimated to be $2,613 \pm 313$ individual salamanders (Table 7; Figure 14).

3.3 Jollyville Plateau Salamander Monitoring Results

3.3.1 Avery Deer Spring

3.3.1.1 Habitat Monitoring.—Climate conditions for each 2020 Avery Deer Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry

metrics are presented in Table 3. Avery Deer Spring consistently had bedrock, silt, and gravel substrate throughout 2020 and estimated embeddedness ranged from 10 - 30%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.3.1.2 Salamander Monitoring.—We searched a total of 5,701 cover objects and observed 45 and captured 39 total JPS during the six bimonthly salamander surveys in 2020. Monthly JPS counts ranged from 2 - 15 and the percentage of cover objects with salamanders ranged from 0.24 - 1.15% (Table 5, Figures 9 and 10). We captured two gravid females which accounted for 5.13% of all captures at Avery Deer Spring within 2020. Gravid females were only observed during March (Table 5).

From the onset of Cambrian monitoring of this site we have observed 279 JPS, including the 45 observed in 2020. The percent of cover objects with salamanders was lower (0.79%) than the site average for this measure (1.37%), indicating a decrease in overall salamander relative abundance at this site during 2020 (Table 6).

A total of 266 unique JPS have been captured at Avery Deer Spring since the onset of Wild-ID photographic documentation in July 2013. During 2020 monitoring we captured 34 new individuals and recaptured 11 salamanders from previous monitoring events (28%). The greatest time between first capture and most recent capture for any single individual salamander was 3 years 7 months (September 2015 – May 2018), and this salamander was captured three times within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included time varying probability of entry (*pent*), and constant survival (*phi*) and capture probability (*p*). Survival was estimated to be 0.64 ± 0.037 (Table 7; Figure 11). In other words, an individual salamander has roughly a 64% chance of surviving from one sampling occasion to the next. Probability of capture was estimated to be 0.09 ± 0.018 , meaning any observable salamander has a 9% chance of being captured within any given sampling event (Table 7; Figure 15). Probability of entry varied by time, ranging from 0 to 0.12, indicating that surveys are punctuated by pulses of new individuals after periods of high proportions of recaptures (Table 7; Figure 13). Finally, the superpopulation of salamanders at this site is estimated to be 1,241 ± 189 individual salamanders (Table 7; Figure 14).

3.3.2 Avery Springhouse Spring

3.3.2.1 Habitat Monitoring.—Climate conditions for each 2019 Avery Springhouse Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Avery Springhouse Spring consistently had silt, bedrock, and gravel substrate throughout 2020 and estimated embeddedness ranged from 30 - 90%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.3.2.2 Salamander Monitoring.—We searched a total of 6,597 cover objects and observed 81 and captured 73 total JPS during the six bimonthly salamander surveys in 2020. Monthly JPS counts ranged from 4 - 20 and the percentage of cover objects with salamanders ranged from 0.53

-4.42% (Table 5, Figures 9 and 10). We captured eight gravid females which accounted for 10.95% of the total 2020 captures. Gravid females were observed in January and March (Table 6).

From the onset of Cambrian monitoring of this site we have observed 453 JPS, including the 81 observations in 2020. The percent of cover objects with salamanders was decreased in 2020 (1.23%) than the site average for this measure (1.71%), indicating a decrease in overall salamander relative abundance at this site over the previous years.

A total of 532 unique JPS have been captured at Avery Springhouse Spring since the onset of Wild-ID photographic documentation in May 2013. During 2020 monitoring we captured 63 new individuals and recaptured 10 salamanders from previous monitoring events (14%). The greatest time between first capture and most recent capture for any single individual salamander was 3 years 2 months (November 2015 – January 2019), and this salamander was captured twice within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included time varying probability of entry (*pent*), and constant survival (*phi*) and capture probability (*p*). Survival was estimated to be 0.78 ± 0.024 (Table 7, Figure 11). In other words, an individual salamander has roughly a 78% chance of surviving from one sampling occasion to the next. Probability of capture was estimated to be 0.035 ± 0.006 , meaning any observable salamander has a 3.5% chance of being captured within any given sampling event (Table 7, Figure 15). Probability of entry varied by time, ranging from 0 to 0.13, indicating that surveys are punctuated by pulses of new individuals after periods of high proportions of recaptures (Table 7, Figure 13). Finally, the superpopulation of salamanders at this site is estimated to be $3,843 \pm 485$ individual salamanders (Table 7, Figure 14).

3.3.3 Hill Marsh Spring

3.3.3.1 Habitat Monitoring.—Climate conditions for each 2020 Hill Marsh Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Hill Marsh Spring consistently had a gravel and silt substrate throughout 2020 and estimated embeddedness ranged from 10 - 40%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.3.3.2 Salamander Monitoring.—We searched a total of 1,819 cover objects and observed 62 and captured 50 total JPS during the six bimonthly salamander surveys in 2020. Monthly JPS counts ranged from 2 - 15 and the percentage of cover objects with salamanders ranged from 0.91 - 6.56% (Table 5, Figures 9 and 10). We captured three gravid females which accounted for 6.0% of the total 2020 captures. Gravid females were observed in January and March (Table 5).

From the onset of Cambrian monitoring of this site we have observed 212 JPS, including the 62 observations in 2020. The percent of cover objects with salamanders was greater in 2020 (3.41%) than the site average for this measure (2.55%), indicating increasing short term relative abundance.

A total of 319 unique JPS have been captured at Hill Marsh Spring since the onset of Wild-ID photographic documentation in May 2013. During 2020 monitoring we captured 45 new individuals and recaptured 5 salamanders from previous monitoring events (10%). The greatest time between first capture and most recent capture for any single individual salamander was 2 years 7 months (May 2015 – January 2018), and this salamander was captured twice within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included constant probability of entry (*pent*), survival (*phi*), and capture probability (*p*). Survival was estimated to be 0.85 ± 0.031 (Table 7, Figure 11). In other words, an individual salamander has roughly an 85% chance of surviving from one sampling occasion to the next. Probability of capture was estimated to be 0.017 ± 0.005 , meaning any observable salamander has a 1.7% chance of being captured within any given sampling event (Table 7, Figure 15). Probability of entry was estimated to be 0.0176 ± 0.0004 (Table 7, Figure 13) which indicates that on any given survey there's roughly a 1.8% chance of resulting in the observation of new individuals. Finally, the superpopulation of salamanders at this site is estimated to be $3,246 \pm 610$ individual salamanders (Table 7, Figure 14).

3.3.4 PC Spring

3.3.4.1 Habitat Monitoring.—Climate conditions for each 2020 PC Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. PC Spring consistently had bedrock, silt, and gravel substrate throughout 2020 and estimated embeddedness ranged from 20 - 60%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.3.4.2 Salamander Monitoring.—We searched a total of 4,018 cover objects and observed 102 and captured 62 total JPS during the six bimonthly salamander surveys in 2020. Monthly JPS counts ranged from 7-29 and the percentage of cover objects with salamanders ranged from 0.58 -5.35% (Table 5, Figures 9 and 10). We captured two gravid females which accounted for 3.23% of the total 2020 captures. One gravid female was observed in February and December (Table 5).

From the onset of Cambrian monitoring of this site we have observed 209 JPS, including the 102 observations in 2020. The percent of cover objects with salamanders was more than twice as high in 2020 (2.54%) than the site average for this measure (1.07%), indicating an increase in overall relative salamander abundance at this site for the second consecutive calendar year.

A third spring discharge, approximately 35 m west of the concrete culvert pad, increased in discharge in 2018 and now exhibits a typical gravel bottomed spring run with cobble cover objects. We monitored this third spring run during bimonthly monitoring at PC Spring, but observed no salamanders within this area during 2020. This spring run was dry during all surveys except those in February and June.

A total of 306 unique JPS have been captured at PC Spring since the onset of Wild-ID photographic documentation in May 2013. During 2020 monitoring we captured 58 new individuals and

recaptured 4 salamanders from previous monitoring events (6.5%). The greatest time between first capture and most recent capture for any individual salamander was 11 months, and this occurred for two individuals among recaptured JPS (March 2014 – February 2015; March 2016 – February 2017), and each salamander was captured twice within this period.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included constant probability of entry (*pent*), survival (*phi*), and capture probability (*p*). Survival was estimated to be 0.66 ± 0.07 (Table 7, Figure 11). In other words, an individual salamander has roughly a 66% chance of surviving from one sampling occasion to the next. Probability of capture was estimated to be 0.028 ± 0.01 , meaning any observable salamander has a 2.8% chance of being captured within any given sampling event (Table 7, Figure 15). Probability of entry was estimated to be 0.02 ± 0.0003 (Table 7, Figure 13) which indicates that on any given survey there's roughly a 2% chance of resulting in the observation of new individuals. Finally, the superpopulation of salamanders at this site is estimated to be $4,112 \pm 1,116$ individual salamanders (Table 7, Figure 14).

3.3.5 Brushy Creek Spring

3.3.5.1 Habitat Monitoring.—Climate conditions for each 2020 Brushy Creek Spring monitoring event and the previous week are presented in Table 2. Summary statistics for water chemistry metrics are presented in Table 3. Brushy Creek Spring consistently had silt substrate on top of the concrete culvert bottom throughout 2020 and estimated embeddedness ranged from 10 - 100%. Raw values for water chemistry and habitat data are presented in Table 4 and Figures 5 - 8.

3.3.5.2 Salamander Monitoring.—We searched a total of 1,123 cover objects and observed and captured four total JPS during the six bimonthly salamander surveys in 2020. Monthly JPS counts ranged from 0 - 2 and the percentage of cover objects with salamanders ranged from 0 - 1.31% (Table 5, Figures 9 and 10). We captured no gravid females at this site during 2020 (Table 5).

From the onset of Cambrian monitoring of this site we have observed 15 JPS, including the four observations in 2020. The percent of cover objects with salamanders was greater in 2020 (0.36%) than the site average for this measure (0.24%).

A total of 23 unique JPS have been captured at Brushy Creek Spring since the onset of Wild-ID photographic documentation in April 2015. During 2020 monitoring we captured four new individuals and recaptured zero salamanders from previous monitoring events. Among these 23 individuals, only two have ever been recaptured, and each recapture occurred during the survey immediately following first capture. No animals have been recaptured more than once at this site.

Among the POPAN formulated Jolly-Seber models we fit for this site, the best fit model included time varying probability of entry (*pent*), but constant survival (*phi*) and capture probability (*p*). Survival was estimated to be 0.08 ± 0.054 (Table 7, Figure 11). Probability of capture was estimated to be 1, meaning any observable salamander has a 100% chance of being captured during any given sampling event (Table 7, Figure 15). Probability of entry varied by time, ranging from 0 to 0.125 (Table 7, Figure 13). Finally, the superpopulation of salamanders at this site is estimated

to be 23 individual salamanders (Table 7, Figure 14). Sample size for models of this type should generally be larger than what we observe at this site, and thus these results should be seen as preliminary, at best.

3.4 Salamander Reproduction

We captured 119 gravid females among all monitored sites during 2020. These results are very close to what we observed during the 2019 monitoring season (n=120). We were able to count the number of oocytes through the translucent venter of each of these captured female salamanders, compared to only 68 measured during 2019. We pooled data from the previous two monitoring seasons and performed a linear regression to examine the relationship between snout-vent length and clutch size (the number of visible oocytes) counted in the field. There is a significant positive relationship ($\beta = 1.25$, p < 0.001, df = 185), indicating that larger females possess greater numbers of oocytes, as one might expect (Figure 16). Clutch size ranged from 1 – 31 oocytes.

4.0 **DISCUSSION**

4.1 Habitat Monitoring

Water temperature fluctuates seasonally at each of the eight monitored sites. Swinbank Spring experiences the smallest seasonal change in water temperature, whereas Hill Marsh Spring experiences the largest fluctuations. Among all sites, temperature rarely exceeds 24 °C which is associated with reduced growth rates in BSS (Crow et al. 2016). We did not record a temperature above the upper thermal tolerance of central Texas *Eurycea* salamanders at any site (Figure 5; Berkhouse and Fries 1995, Crow et al. 2016).

We found pH had a declining trend over time at all sites until 2020, when measurements began to increase relative to previous years. Regardless of these changes in pH over time, values among all sites are near neutral and fall within those known to be occupied by *Eurycea* salamanders (Figure 6; Davis et al. 2001, Bowles et al. 2006).

DO fluctuates with no discernable regularity at each site (Figure 7). In general, Swinbank Spring has the highest concentration of DO, whereas Cobbs Spring has the lowest, despite both sites supporting large populations of *Eurycea* salamanders. Cobbs, Avery Deer, PC, and Brushy Creek Springs all regularly experienced DO levels suggested to be detrimental to central Texas *Eurycea* salamanders (Woods et al. 2010). However, all of these sites maintain salamander populations. It is important to note that the Woods et al. (2010) manuscript had inconsistent results for salamander response to DO levels, and it is likely prudent to reexamine the importance of this water chemistry variable to central Texas *Eurycea*, especially in the field.

Among all water parameters, specific conductivity varied the most among sites (Figure 8). Brushy Creek Spring experiences sustained elevated levels of conductivity, potentially due to input from urban runoff. However, we see elevated levels at less anthropogenically modified sites as well, such as Avery Springhouse Spring and Hill Marsh Spring. Alternatively, Cobbs Spring consistently measures as the lowest conductivity among all sites. Conductivity at Avery Springhouse, Hill Marsh, and Brushy Creek Springs regularly exceeded 900 μ S/cm which is associated with reduced JPS counts in a previous study (Bowles et al. 2006). However, all of these sites exhibit stable relative abundance. Further, we note the contradicting information regarding the Bowles et al. (2006) publication and the Adcock et al. (2016) and Woods and Poteet (2006) reports. As with DO, the importance of this water chemistry parameter should be reevaluated.

4.2 Salamander Monitoring

Salamander observations follow the previously reported pattern of decreasing during winter months and spiking throughout the spring and summer (Figure 9; Bowles et al. 2006, Pierce et al. 2010, Bendik et al. 2014). The percentage of cover objects with salamanders is a measure of relative abundance and capture per unit effort (CPUE). At Avery Deer Spring CPUE increased from 2016 – 2019, with 2019 yielding the largest relative abundance by a large margin, and returned to more average values during 2020 (Figure 10). At Avery Springhouse Spring CPUE was reduced relative to 2019, but exhibited more variance among survey events than years previous. Brushy Creek Spring has also increased in CPUE each year, continuing in 2020. Cobbs Spring experienced a large increase in CPUE during 2019, and we suspect that this site experiences periods of boom and bust, however we do not have enough data at this time to understand the timing or nature of this cycle. Investigating these fluctuations became more challenging during 2020, when site access was revoked after two monitoring events. CPUE at PC Spring has increased during every year of Cambrian monitoring. Hill Marsh Spring, Swinbank Spring, and Twin Springs all increased in CPUE during 2020, exhibiting slightly above average captures, and exemplifying cyclical captures.

4.3 Population Modeling

During the 2020 monitoring season we were able to expand on our work on population modeling to include all JPS occupied sites (Avery Deer, Avery Springhouse, PC, Brushy Creek, and Hill Marsh Springs), in addition to Swinbank, Twin, and Cobbs Springs. Throughout 2020 we put extra effort into consolidating, organizing, and incorporating data beginning with those collected by Texas State University in 2013, up to present day. This means that all sites we are contracted to monitor by the WCCF are now analyzed using a framework that allows for the estimation of survival, probability of capture, probability of entry into these populations, and superpopulation size.

Among sites that are monitored monthly (Swinbank, Cobbs, and Twin Springs) Swinbank Spring harbors the largest superpopulation (Table 7; Figure 14). It is important to note that superpopulation in this context is a cumulative metric over the course of surveys, and that Cobbs has received half as many years of routine monitoring relative to Swinbank and Twin Springs. Probability of new entrants varied by time at Cobbs and Swinbank, but not at Twin Springs. This is likely caused by Twin Springs illustrating low counts that exhibit high survival, in other words

the same individuals are captured a large number of times, and new individuals are rare at this site. Swinbank Spring demonstrates a more predictable pattern (likely due to sustained high captures over time) that supports the hypothesis of subsurface reproduction, where reproducing animals becoming temporarily unobservable in winter, followed by a spike in observable entrants in the form of juveniles in spring (Figure 13). Likewise, Cobbs spring illustrates huge swings in entry due to its periodic drying. Despite Twin Springs having undergone reductions in CPUE over the last several seasons, this site shows extremely high survival estimates for adult individuals, and thus a surprisingly large superpopulation size (Table 7; Figures 11 and 14). Cobbs Spring has a large population size, but the lowest estimate of survival, likely due to observations of unprecedented reproductive events and boom in new captures after periodic drying of the springrun.

Among sites that are monitored bimonthly (Avery Deer, Avery Springhouse, PC, Brushy Creek, and Hill Marsh Springs) PC spring has the largest superpopulation (Table 7; Figure 14). However, the variance surrounding the estimate for this site is also the largest among all sites, indicating great uncertainty with respect to this metric. Probability of new entrants is time varying at all sites except Hill Marsh and PC Spring. It is difficult to determine why there is disagreement among these sites with respect to time varying versus constant probability of entry, given that these sites exhibit extremely reduced recapture rates when compared to the monthly monitored sites discussed above. Survival is greatest at Hill Marsh Spring among JPS sites. JPS monitored sites in general exhibited lower survival and more variance surrounding all estimates compared to monthly monitored sites. This is likely due to the fact that far fewer recaptures were observed at these sites. For reference, 11.7% of captures at bi-monthly sites were recaptures during 2020, on average. We believe this to be a consequence of the survey schedule being half as rigorous, but this likely requires further investigation.

4.4 Reproduction

Congruent with previous research, we observed a pulse of gravid animals among all sites beginning in September and continuing through March (Bowles et al. 2006, Pierce et al. 2014, Bendik 2017). These salamanders clearly reproduce seasonally although JPS demonstrate a single peak in gravid females per year (Bendik 2017) compared to a double peak in gravid GTS and SS females (Pierce et al. 2014). The cycle of reduced gravid females during the summer inversely corresponds to the patterns we observe among overall salamander observations being highest during the summer (Figure 9). This clearly illustrates how the salamander's reproductive cycle influences the outcomes of our monitoring efforts.

During 2019 and 2020, we began counting the number of oocytes within each gravid female in the field. This enabled us to examine clutch size and the relationship between oocyte counts and salamander body size for the first time for any of these taxa (Figure 16).

5.0 KEY PERSONNEL

Zachary C. Adcock—Senior Ecologist

Zach has over 15 years of experience in threatened and endangered wildlife ecology. He has conducted work on 27 federally listed species and many more state listed taxa in Texas and Florida with an emphasis on herpetofauna. His overarching specialties include threatened and endangered species research, surveys, habitat and population assessments, management plan development, and best management practices (BMPs). Zach is an expert on central Texas *Eurycea* salamanders with eight years of research and survey experience across seven species. His dissertation was designed to inform the ecology, conservation policy, and management of Jollyville Plateau Salamanders. He has over 15 peer-reviewed wildlife publications, including manuscripts on Jollyville Plateau, Georgetown, Salado, San Marcos, and Fern Bank Salamanders and Houston Toads.

Andrew R. MacLaren, Ph.D.—Senior Ecologist

Andrew has over seven years of experience in threatened and endangered wildlife ecology. His dissertation research focused on utilizing technological innovations to address issues pertinent to the conservation policy and management of the federally endangered Houston Toad (*Anaxyrus houstonensis*). Issues addressed within his research include evaluating the efficacy of the current federal protocol for conducting presence absence surveys for Houston Toads. His research on automated detection of Houston Toad vocalizations has received multiple awards, and was ultimately published in the *Journal of Fish and Wildlife Management*. Further research evaluating habitat induced bias in acoustic surveys for vocalizing birds and anurans has been published in *Ecology and Evolution*. Additional contributions to peer-reviewed research include best management practices and avoidance of impacts related to development within Houston Toad occupied habitat, as well as management of invasive aquatic vegetation occupied by the federally threatened San Marcos Salamander and new distribution records for Jollyville Plateau Salamanders.

Kemble White, Ph.D., P.G.—Owner, Senior Geoscientist

Kemble has served for over 20 years as senior geologist, karst specialist, and project manager in central Texas. Kemble specializes in the Endangered Species Act and water quality regulations as they pertain to caves, springs and the Edwards Aquifer. Kemble's doctorate was in biospeleology, the study of cave ecology, and his dissertation was one of the first involving central Texas endangered karst invertebrates. His research has been published in *Geology*, one of the world's flagship peer-reviewed scientific journals. He has discovered many new locations for rare and endangered species and two new species have been named in his honor. As a co-author of the Regional Habitat Conservation Plan/Environmental Impact Statement (RHCP/EIS), Kemble has had a direct hand in RHCP planning and implementation. Kemble is a licensed professional geoscientist and holds the applicable USFWS permits for working with threatened and endangered

karst species in the Austin-San Antonio growth corridor. He has been working with *Eurycea* in Williamson County since 1999.

Ryan Jones—Ecologist

Ryan Jones has over five years of experience working with *Eurycea* salamanders. He graduated with his B.S. in Biology from Texas State University in 2014. His specialties include population monitoring and conducting presence/absence surveys for *Eurycea* salamanders and karst invertebrates. He has contributed to peer-reviewed published manuscripts on Jollyville Plateau, Georgetown, and Salado Salamanders.

Craig Crawford, P.G.—Senior Geoscientist

Craig has over 15 years of experience with Edwards Aquifer compliance issues. His primary emphasis since 2005 has been the geomorphology and hydrogeology of the Edwards Aquifer as it relates to the status of several aquifer-related endangered species and 16 species of terrestrial karst invertebrates. His expertise is in conducting hydrogeologic evaluations of karst features, designing cave preserve management and monitoring plans, and conducting presence/ absence surveys and environmental assessment/habitat conservation plans for the USFWS. Craig is accomplished at the management and monitoring of springs and habitat for threatened and endangered *Eurycea* salamanders. This includes water quality and aqueous geochemistry studies. Craig is a licensed professional geoscientist and holds the applicable USFWS permits for working with threatened and endangered karst species in central Texas.

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2020 WCCF ANNUAL EURYCEA MONITORING REPORT

7.0 TABLES

Table 1. Location information for each spring monitored in 2020 as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

Eurycea Species	Site	Watershed	Latitude	Longitude
E. chisholmensis	Cobbs Spring	Berry Creek	N 30.789550	W 97.728550
E. chisholmensis	Twin Springs	San Gabriel River	N 30.698310	W 97.781810
E. naufragia	Swinbank Spring	San Gabriel River	N 30.662722	W 97.710709
E. tonkawae	Avery Deer Spring	Brushy Creek	N 30.507502	W 97.749311
E. tonkawae	Avery Springhouse Spring	Brushy Creek	N 30.503698	W 97.759829
E. tonkawae	Brushy Creek Spring	Brushy Creek	N 30.516834	W 97.661271
E. tonkawae	Hill Marsh Spring	Brushy Creek	N 30.507680	W 97.755070
E. tonkawae	PC Spring	Lake Creek	N 30.481276	W 97.742274

 Table 2. Climate data for each 2020 *Eurycea* salamander survey conducted as part of the Williamson County Regional Habitat

 Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site	Survey Date	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)	Previous Week Maximum Temperature (°C)	Previous Week Minimum Temperature (°C)	Daily Rainfall (cm)	Previous Week Cumulative Rainfall (cm)
E. chisholmensis	Cobbs	27-Jan-2020	22.8	7.2	25.6	1.1	0.0	2.9
E. chisholmensis	Cobbs	24-Feb-2020	17.2	10.6	26.1	-0.6	0.0	7.0
E. chisholmensis	Twin Springs	21-Jan-2020	13.9	1.1	25.6	1.1	0.0	3.0
E. chisholmensis	Twin Springs	21-Feb-2020	NA	NA	26.1	-0.6	NA	6.1
E. chisholmensis	Twin Springs	11-Mar-2020	25.0	13.3	23.3	6.1	0.0	3.2
E. chisholmensis	Twin Springs	28-May-2020	31.1	16.7	32.8	16.7	2.9	5.9
E. chisholmensis	Twin Springs	22-Jun-2020	33.3	23.3	33.9	17.8	0.0	2.5
E. chisholmensis	Twin Springs	16-Jul-2020	36.1	22.2	40.0	23.3	0.0	0.0
E. chisholmensis	Twin Springs	18-Aug-2020	39.4	22.2	40.0	23.3	0.0	0.0
E. chisholmensis	Twin Springs	23-Sep-2020	NA	NA	33.9	12.8	NA	5.4
E. chisholmensis	Twin Springs	9-Oct-2020	28.9	13.9	30.6	11.7	0.0	0.0
E. chisholmensis	Twin Springs	19-Nov-2020	23.9	4.4	27.2	2.8	0.0	0.0
E. chisholmensis	Twin Springs	16-Dec-2020	10.0	0.6	26.1	-1.7	0.0	0.2
E. naufragia	Swinbank	24-Jan-2020	15.6	3.3	25.6	1.1	0.0	3.0
E. naufragia	Swinbank	25-Feb-2020	21.1	7.2	26.1	-0.6	0.0	6.6
E. naufragia	Swinbank	17-Mar-2020	21.7	13.3	28.9	6.1	0.0	0.6
E. naufragia	Swinbank	28-May-2020	31.1	16.7	32.8	16.7	2.9	5.9
E. naufragia	Swinbank	8-Jul-2020	33.9	24.4	37.2	22.8	0.4	0.1
E. naufragia	Swinbank	27-Jul-2020	32.8	22.8	36.7	21.7	0.2	1.0
E. naufragia	Swinbank	26-Aug-2020	36.1	22.2	40.0	17.8	0.0	1.3

<i>Eurycea</i> Species	Site	Survey Date	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)	Previous Week Maximum Temperature (°C)	Previous Week Minimum Temperature (°C)	Daily Rainfall (cm)	Previous Week Cumulative Rainfall (cm)
E. naufragia	Swinbank	28-Sep-2020	30.6	11.7	30.6	12.8	0.0	10.0
E. naufragia	Swinbank	30-Oct-2020	15.0	5.6	30.0	2.8	0.0	0.7
E. naufragia	Swinbank	19-Nov-2020	23.9	4.4	27.2	2.8	0.0	0.0
E. naufragia	Swinbank	17-Dec-2020	10.0	-1.1	26.1	-1.7	0.0	0.2
E. tonkawae	Avery Deer	14-Jan-2020	14.4	9.4	23.3	0.0	0.1	1.9
E. tonkawae	Avery Deer	24-Mar-2020	25.6	16.7	28.3	11.7	0.0	5.5
E. tonkawae	Avery Deer	12-Jun-2020	33.3	16.1	36.7	16.7	0.0	0.0
E. tonkawae	Avery Deer	5-Aug-2020	37.2	22.2	37.8	21.7	0.0	1.2
E. tonkawae	Avery Deer	14-Sep-2020	NA	NA	33.9	12.8	NA	3.4
E. tonkawae	Avery Deer	18-Nov-2020	22.8	3.3	27.2	2.8	0.0	0.0
E. tonkawae	Avery Springhouse	23-Jan-2020	12.2	6.7	25.6	1.1	0.9	2.1
E. tonkawae	Avery Springhouse	23-Mar-2020	17.2	11.7	28.9	13.3	3.0	2.5
E. tonkawae	Avery Springhouse	15-Jun-2020	32.2	17.8	36.7	16.1	0.0	0.0
E. tonkawae	Avery Springhouse	12-Aug-2020	37.8	24.4	37.8	22.2	0.0	0.0
E. tonkawae	Avery Springhouse	18-Sep-2020	30.0	19.4	33.9	12.8	3.1	2.9
E. tonkawae	Avery Springhouse	18-Nov-2020	22.8	3.3	27.2	2.8	0.0	0.0
E. tonkawae	Brushy Creek Spring	5-Feb-2020	22.8	1.1	25.6	3.3	0.0	1.2
E. tonkawae	Brushy Creek Spring	11-Jun-2020	32.2	16.7	36.7	18.9	0.0	0.0
E. tonkawae	Brushy Creek Spring	15-Jul-2020	38.3	23.3	40.0	23.3	0.0	0.4
E. tonkawae	Brushy Creek Spring	13-Aug-2020	37.8	25.0	37.8	22.8	0.0	0.0
E. tonkawae	Brushy Creek Spring	7-Oct-2020	29.4	16.1	30.6	11.7	0.0	4.6

<i>Eurycea</i> Species	Site	Survey Date	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)	Previous Week Maximum Temperature (°C)	Previous Week Minimum Temperature (°C)	Daily Rainfall (cm)	Previous Week Cumulative Rainfall (cm)
E. tonkawae	Brushy Creek Spring	8-Dec-2020	18.9	3.3	23.3	-1.7	0.0	0.0
E. tonkawae	Hill Marsh	29-Jan-2020	19.4	5.0	22.8	1.1	0.0	4.0
E. tonkawae	Hill Marsh	24-Mar-2020	25.6	16.7	28.3	11.7	0.0	5.5
E. tonkawae	Hill Marsh	16-Jun-2020	33.3	20.6	36.7	16.1	0.0	0.0
E. tonkawae	Hill Marsh	6-Aug-2020	37.8	22.8	37.8	21.7	0.0	1.2
E. tonkawae	Hill Marsh	11-Sep-2020	20.6	12.8	33.9	12.8	0.0	4.0
E. tonkawae	Hill Marsh	16-Nov-2020	20.6	4.4	27.2	6.1	0.0	0.0
E. tonkawae	PC Spring	4-Feb-2020	NA	NA	22.8	3.3	NA	2.2
E. tonkawae	PC Spring	11-Jun-2020	32.2	16.7	36.7	18.9	0.0	0.0
E. tonkawae	PC Spring	15-Jul-2020	38.3	23.3	40.0	23.3	0.0	0.4
E. tonkawae	PC Spring	13-Aug-2020	37.8	25.0	37.8	22.8	0.0	0.0
E. tonkawae	PC Spring	7-Oct-2020	29.4	16.1	30.6	11.7	0.0	4.6
E. tonkawae	PC Spring	8-Dec-2020	18.9	3.3	23.3	-1.7	0.0	0.0

<i>Eurycea</i> Species	Site	Temperature (°C)		рН		Dissolved Oxygen (mg/L)		Specific Conductance (µS/cm)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
E. chisholmensis	Cobbs	13.2 ± 0.42	12.9 - 13.5	7.0 ± 0.00	7.0 - 7.0	3.0 ± 0.35	2.7 - 3.2	597.0 ± 7.07	592 - 602
E. chisholmensis	Twin Springs	17.9 ± 1.46	15.4 - 19.8	7.0 ± 0.19	6.8 - 7.4	6.3 ± 2.30	3.3 - 11.8	743.2 ± 17.15	715 - 766
E. naufragia	Swinbank	18.1 ± 0.50	17.0 - 18.6	7.1 ± 0.13	6.9 - 7.3	7.3 ± 2.65	4.3 - 12.7	729.8 ± 15.52	691 - 747
E. tonkawae	Avery Deer	18.0 ± 1.87	15.3 - 20.6	7.1 ± 0.17	6.9 - 7.4	4.8 ± 1.90	1.4 - 7.2	641.8 ± 28.54	586 - 698
E. tonkawae	Avery Springhouse	18.7 ± 1.68	16.5 - 20.7	7.3 ± 0.39	7.0 - 7.8	6.1 ± 1.61	4.1 - 8.0	748.7 ± 155.44	460 - 907
E. tonkawae	Brushy Creek Spring	19.3 ± 2.46	16.0 - 21.7	7.1 ± 0.27	6.9 - 7.6	7.0 ± 6.35	2.5 - 19.4	919.3 ± 29.85	896 - 974
E. tonkawae	Hill Marsh	18.0 ± 3.21	13.4 - 21.5	7.0 ± 0.11	6.9 - 7.2	4.9 ± 1.68	2.2 - 7.0	742.1 ± 33.87	702 - 776
E. tonkawae	PC Spring	19.6 ± 1.66	16.4 - 22.2	6.9 ± 0.13	6.8 - 7.2	5.2 ± 1.98	2.8 - 9.8	748.0 ± 62.84	590 - 821

Table 3. Summary statistics for habitat data (water conditions) for *Eurycea* salamander surveys conducted in 2020 as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

Table 4. Habitat data (water conditions) for each 2020 Eurycea salamander survey conducted as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site	Survey Date	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Specific Conductance (µS/cm)	Water Depth (cm)
E. chisholmensis	Cobbs	27-Jan-2020	13.5	7.0	2.7	602	10.0
E. chisholmensis	Cobbs	24-Feb-2020	12.9	7.0	3.2	592	8.0
E. chisholmensis	Twin Springs	21-Jan-2020	15.5	7.4	6.4	766	3.5
E. chisholmensis	Twin Springs	21-Feb-2020	15.4	7.3	5.5	764	5.0
E. chisholmensis	Twin Springs	11-Mar-2020	18.7	7.0	11.8	737	4.0
E. chisholmensis	Twin Springs	28-May-2020	17.6	6.8	7.9	755	4.0
E. chisholmensis	Twin Springs	22-Jun-2020	18.3	7.1	6.0	756	4.5
E. chisholmensis	Twin Springs	16-Jul-2020	18.6	7.0	3.3	758	4.5
E. chisholmensis	Twin Springs	18-Aug-2020	19.8	6.9	3.3	728	1.0
E. chisholmensis	Twin Springs	23-Sep-2020	18.7	6.8	5.9	731	3.0
E. chisholmensis	Twin Springs	9-Oct-2020	18.7	6.9	7.3	715	4.0
E. chisholmensis	Twin Springs	19-Nov-2020	19.0	6.9	6.0	729	4.0
E. chisholmensis	Twin Springs	16-Dec-2020	16.5	7.1	6.2	736	2.0
E. naufragia	Swinbank	24-Jan-2020	18.0	7.1	8.1	730	5.5
E. naufragia	Swinbank	25-Feb-2020	17.8	7.1	7.3	747	6.0
E. naufragia	Swinbank	17-Mar-2020	18.1	6.9	10.0	746	7.5
E. naufragia	Swinbank	28-May-2020	18.6	6.9	7.4	691	6.0
E. naufragia	Swinbank	8-Jul-2020	18.2	7.0	4.6	744	4.0
E. naufragia	Swinbank	27-Jul-2020	18.6	7.1	5.5	734	5.0
E. naufragia	Swinbank	26-Aug-2020	18.5	NA	4.3	727	4.0

<i>Eurycea</i> Species	Site	Survey Date	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Specific Conductance (µS/cm)	Water Depth (cm)
E. naufragia	Swinbank	28-Sep-2020	18.5	7.0	12.7	722	5.0
E. naufragia	Swinbank	30-Oct-2020	17.6	7.3	NA	735	5.0
E. naufragia	Swinbank	19-Nov-2020	18.3	7.0	8.5	725	3.0
E. naufragia	Swinbank	17-Dec-2020	17.8	7.2	5.0	727	3.0
E. tonkawae	Avery Deer - 1	14-Jan-2020	15.8	7.0	5.1	638	4.0
E. tonkawae	Avery Deer - 1	24-Mar-2020	17.0	7.2	7.0	651	7.5
E. tonkawae	Avery Deer - 1	12-Jun-2020	18.6	7.4	7.2	624	19.0
E. tonkawae	Avery Deer - 1	5-Aug-2020	20.2	7.4	2.4	619	15.0
E. tonkawae	Avery Deer - 1	14-Sep-2020	NA	NA	NA	NA	NA
E. tonkawae	Avery Deer - 1	18-Nov-2020	16.3	7.3	4.5	678	11.0
E. tonkawae	Avery Deer - 2	14-Jan-2020	15.6	7.0	5.5	635	3.0
E. tonkawae	Avery Deer - 2	24-Mar-2020	16.6	6.9	6.6	677	3.0
E. tonkawae	Avery Deer - 2	12-Jun-2020	18.7	7.3	6.6	635	2.0
E. tonkawae	Avery Deer - 2	5-Aug-2020	20.1	7.0	3.0	670	3.5
E. tonkawae	Avery Deer - 2	14-Sep-2020	20.6	NA	2.5	662	2.5
E. tonkawae	Avery Deer - 2	18-Nov-2020	17.9	7.0	5.2	698	3.0
E. tonkawae	Avery Deer - 3	14-Jan-2020	15.3	7.0	5.0	622	8.5
E. tonkawae	Avery Deer - 3	24-Mar-2020	15.7	7.3	5.9	640	5.0
E. tonkawae	Avery Deer - 3	12-Jun-2020	18.2	7.2	7.2	614	5.0
E. tonkawae	Avery Deer - 3	5-Aug-2020	20.0	7.1	2.2	614	5.0
E. tonkawae	Avery Deer - 3	14-Sep-2020	20.5	NA	1.4	586	3.0

<i>Eurycea</i> Species	Site	Survey Date	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Specific Conductance (µS/cm)	Water Depth (cm)
E. tonkawae	Avery Deer - 3	18-Nov-2020	18.1	7.0	4.3	648	7.0
E. tonkawae	Avery Springhouse	23-Jan-2020	16.5	7.1	6.4	907	6.5
E. tonkawae	Avery Springhouse	23-Mar-2020	14.7	6.9	6.3	880	5.0
E. tonkawae	Avery Springhouse	15-Jun-2020	18.8	7.1	6.9	781	6.0
E. tonkawae	Avery Springhouse	12-Aug-2020	20.5	7.0	4.1	773	4.0
E. tonkawae	Avery Springhouse	18-Sep-2020	20.7	7.8	4.1	722	4.0
E. tonkawae	Avery Springhouse	18-Nov-2020	18.4	7.0	6.9	849	6.0
E. tonkawae	Brushy Creek Spring	5-Feb-2020	16.8	6.9	19.4	933	1.0
E. tonkawae	Brushy Creek Spring	11-Jun-2020	18.9	7.6	5.8	896	1.0
E. tonkawae	Brushy Creek Spring	15-Jul-2020	21.0	7.0	2.9	907	1.0
E. tonkawae	Brushy Creek Spring	13-Aug-2020	21.7	7.0	2.5	908	1.0
E. tonkawae	Brushy Creek Spring	7-Oct-2020	21.4	7.0	4.0	898	1.0
E. tonkawae	Brushy Creek Spring	8-Dec-2020	16.0	6.9	7.4	974	1.0
E. tonkawae	Hill Marsh	29-Jan-2020	13.4	7.1	6.3	762	5.0
E. tonkawae	Hill Marsh	24-Mar-2020	15.1	7.0	4.3	703	6.0
E. tonkawae	Hill Marsh	16-Jun-2020	19.5	7.2	5.0	736	5.0
E. tonkawae	Hill Marsh	6-Aug-2020	21.5	6.9	4.7	774	6.0
E. tonkawae	Hill Marsh	11-Sep-2020	20.7	NA	2.2	702	4.0
E. tonkawae	Hill Marsh	16-Nov-2020	17.9	7.0	7.0	776	5.0
E. tonkawae	PC Spring - 1	4-Feb-2020	16.4	6.8	4.9	786	9.0
E. tonkawae	PC Spring - 1	11-Jun-2020	19.3	7.2	5.7	720	5.0

<i>Eurycea</i> Species	Site	Survey Date	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Specific Conductance (µS/cm)	Water Depth (cm)
E. tonkawae	PC Spring - 1	15-Jul-2020	21.0	7.0	3.8	678	3.0
E. tonkawae	PC Spring - 1	13-Aug-2020	22.2	6.8	4.2	590	4.0
E. tonkawae	PC Spring - 1	7-Oct-2020	20.6	6.9	9.8	767	5.0
E. tonkawae	PC Spring - 1	8-Dec-2020	18.0	6.9	7.5	809	5.0
E. tonkawae	PC Spring - 2	4-Feb-2020	17.6	6.9	5.0	781	23.0
E. tonkawae	PC Spring - 2	11-Jun-2020	19.0	7.1	3.6	736	23.0
E. tonkawae	PC Spring - 2	15-Jul-2020	20.0	6.9	2.8	760	16.0
E. tonkawae	PC Spring - 2	13-Aug-2020	21.0	6.8	3.2	769	17.0
E. tonkawae	PC Spring - 2	7-Oct-2020	20.5	6.9	6.0	759	19.0
E. tonkawae	PC Spring - 2	8-Dec-2020	19.0	6.8	5.7	821	17.0

Table 5. Results for each 2020 Eurycea salamander survey conducted as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site	Survey Date	Number of Cover Objects Searched	Number of <i>Eurycea</i> Observed	Number of <i>Eurycea</i> Captured	Percentage of Cover Objects with Salamanders	Number of Gravid <i>Eurycea</i>	Percentage of Captures that were Gravid
E. chisholmensis	Cobbs	27-Jan-2020	1734	34	31	1.96	0	0.0
E. chisholmensis	Cobbs	24-Feb-2020	1063	51	46	4.80	0	0.0
E. chisholmensis	Twin Springs	21-Jan-2020	966	1	1	0.10	0	0.0
E. chisholmensis	Twin Springs	21-Feb-2020	992	6	5	0.60	1	20.0
E. chisholmensis	Twin Springs	11-Mar-2020	1060	7	7	0.66	3	42.9
E. chisholmensis	Twin Springs	28-May-2020	1013	9	9	0.89	0	0.0
E. chisholmensis	Twin Springs	22-Jun-2020	1115	13	12	1.17	0	0.0
E. chisholmensis	Twin Springs	16-Jul-2020	973	11	9	1.13	0	0.0
E. chisholmensis	Twin Springs	18-Aug-2020	716	11	8	1.54	0	0.0
E. chisholmensis	Twin Springs	23-Sep-2020	891	5	3	0.56	0	0.0
E. chisholmensis	Twin Springs	9-Oct-2020	891	5	5	0.56	0	0.0
E. chisholmensis	Twin Springs	19-Nov-2020	909	7	6	0.77	1	16.7
E. chisholmensis	Twin Springs	16-Dec-2020	699	4	3	0.57	1	33.3
E. naufragia	Swinbank	24-Jan-2020	1277	75	42	5.87	4	9.5
E. naufragia	Swinbank	25-Feb-2020	1393	94	64	6.75	13	20.3
E. naufragia	Swinbank	17-Mar-2020	1120	90	59	8.04	13	22.0
E. naufragia	Swinbank	28-May-2020	933	90	53	9.65	4	7.5
E. naufragia	Swinbank	8-Jul-2020	1181	110	74	9.31	0	0.0
E. naufragia	Swinbank	27-Jul-2020	1019	81	59	7.95	2	3.4
E. naufragia	Swinbank	26-Aug-2020	781	75	57	9.60	5	8.8

<i>Eurycea</i> Species	Site	Survey Date	Number of Cover Objects Searched	Number of <i>Eurycea</i> Observed	Number of <i>Eurycea</i> Captured	Percentage of Cover Objects with Salamanders	Number of Gravid <i>Eurycea</i>	Percentage of Captures that were Gravid
E. naufragia	Swinbank	28-Sep-2020	1107	77	61	6.96	15	24.6
E. naufragia	Swinbank	30-Oct-2020	975	50	34	5.13	11	32.4
E. naufragia	Swinbank	19-Nov-2020	1546	78	62	5.05	13	21.0
E. naufragia	Swinbank	17-Dec-2020	971	67	55	6.90	18	32.7
E. tonkawae	Avery Deer	14-Jan-2020	1469	12	9	0.82	0	0.0
E. tonkawae	Avery Deer	24-Mar-2020	1327	15	13	1.13	2	15.4
E. tonkawae	Avery Deer	12-Jun-2020	822	7	7	0.85	0	0.0
E. tonkawae	Avery Deer	5-Aug-2020	623	2	2	0.32	0	0.0
E. tonkawae	Avery Deer	14-Sep-2020	609	7	6	1.15	0	0.0
E. tonkawae	Avery Deer	18-Nov-2020	851	2	2	0.24	0	0.0
E. tonkawae	Avery Springhouse	23-Jan-2020	1784	19	16	1.07	4	25.0
E. tonkawae	Avery Springhouse	23-Mar-2020	1400	18	17	1.29	4	23.5
E. tonkawae	Avery Springhouse	15-Jun-2020	896	20	19	2.23	0	0.0
E. tonkawae	Avery Springhouse	12-Aug-2020	937	12	12	1.28	0	0.0
E. tonkawae	Avery Springhouse	18-Sep-2020	834	8	6	0.96	0	0.0
E. tonkawae	Avery Springhouse	18-Nov-2020	746	4	3	0.54	0	0.0
E. tonkawae	Brushy Creek Spring	5-Feb-2020	341	0	0	0.00	0	0.0
E. tonkawae	Brushy Creek Spring	11-Jun-2020	158	2	2	1.27	0	0.0
E. tonkawae	Brushy Creek Spring	15-Jul-2020	153	2	2	1.31	0	0.0
E. tonkawae	Brushy Creek Spring	13-Aug-2020	107	0	0	0.00	0	0.0
E. tonkawae	Brushy Creek Spring	7-Oct-2020	125	0	0	0.00	0	0.0

<i>Eurycea</i> Species	Site	Survey Date	Number of Cover Objects Searched	Number of <i>Eurycea</i> Observed	Number of <i>Eurycea</i> Captured	Percentage of Cover Objects with Salamanders	Number of Gravid <i>Eurycea</i>	Percentage of Captures that were Gravid
E. tonkawae	Brushy Creek Spring	8-Dec-2020	239	0	0	0	0	0.0
E. tonkawae	Hill Marsh	29-Jan-2020	331	12	11	3.63	2	18.2
E. tonkawae	Hill Marsh	24-Mar-2020	560	14	8	2.50	1	12.5
E. tonkawae	Hill Marsh	16-Jun-2020	183	12	10	6.56	0	0.0
E. tonkawae	Hill Marsh	6-Aug-2020	339	15	14	4.42	0	0.0
E. tonkawae	Hill Marsh	11-Sep-2020	186	7	6	3.76	0	0.0
E. tonkawae	Hill Marsh	16-Nov-2020	220	2	1	0.91	0	0.0
E. tonkawae	PC Spring	4-Feb-2020	1214	7	5	0.58	1	20.0
E. tonkawae	PC Spring	11-Jun-2020	458	22	10	4.80	0	0.0
E. tonkawae	PC Spring	15-Jul-2020	710	29	18	4.08	0	0.0
E. tonkawae	PC Spring	13-Aug-2020	430	23	17	5.35	0	0.0
E. tonkawae	PC Spring	7-Oct-2020	389	7	5	1.80	0	0.0
E. tonkawae	PC Spring	8-Dec-2020	817	14	7	1.71	1	14.3

Table 6. Summary of *Eurycea* observation, capture, and gravid female data for each spring monitored as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services. "Total" refers to the data history for each site, i.e., onset of Cambrian monitoring to end of 2020.

<i>Eurycea</i> Species	Site	Number of Surveys		Number of Salamander Observations		Number of Salamander Captures		Percentage of Cover Objects with Salamanders		Number of Gravid Females		Percentage of Captures that were Gravid	
		2020	Total	2020	Total	2020	Total	2020	Total	2020	Total	2020	Total
E. chisholmensis	Cobbs	2	47	85	886	77	673	3.04	2.51	0	18	0.0	2.7
E. chisholmensis	Twin Springs	11	62	79	432	68	342	0.77	0.84	6	30	8.8	8.8
E. naufragia	Swinbank	11	62	887	3049	620	1957	7.21	5.20	98	254	15.8	13.0
E. tonkawae	Avery Deer	6	26	45	279	39	187	0.79	1.37	2	10	5.1	5.3
E. tonkawae	Avery Springhouse	6	25	81	453	73	376	1.23	1.71	8	32	11.0	8.5
E. tonkawae	Brushy Creek Spring	6	25	4	15	4	14	0.36	0.24	0	3	0.0	21.4
E. tonkawae	Hill Marsh	6	26	62	212	50	156	3.41	2.55	3	9	6.0	5.8
E. tonkawae	PC Spring	6	26	102	209	62	125	2.54	1.07	2	6	3.2	4.8

Table 7. Results from POPAN formulated Jolly-Seber capture-mark-recapture models for each site surveyed as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services. The best fit model parameters either varied by time (~time) or were constant over all surveys (~1). The range of estimated values are provided for model parameters that were time dependent, and estimates with confidence intervals are provided for constant model parameters.

<i>Eurycea</i> Species	Site	Sur	vival (<i>phi</i>)	Capture	e Probability (p)	Probabilit	y of Entry (<i>pent</i>)	Superpopulation	
		Model	Estimate	Model	Estimate	Model	Estimate		
E. chisholmensis	Cobbs	~time	0.00 - 1.000	~1	0.105 ± 0.014	~time	0.000 - 0.1400	2613 ± 313	
E. chisholmensis	Twin Springs	~1	0.94 ± 0.004	~1	0.064 ± 0.004	~1	0.008 ± 0.0003	791 ± 41	
E. naufragia	Swinbank	~1	0.89 ± 0.004	~time	0.012 - 0.190	~time	0.000 - 0.0600	4307 ± 128	
E. tonkawae	Avery Deer	~1	0.64 ± 0.037	~1	0.090 ± 0.018	~time	0.000 - 0.1200	1241 ± 189	
E. tonkawae	Avery Springhouse	~1	0.78 ± 0.024	~1	0.035 ± 0.006	~time	0.000 - 0.1300	3843 ± 485	
E. tonkawae	Brushy Creek Spring	~1	0.08 ± 0.054	~1	1.000 ± 0.000	~time	0.000 - 0.1250	23 ± 00	
E. tonkawae	Hill Marsh	~1	0.85 ± 0.031	~1	0.017 ± 0.005	~1	0.018 ± 0.0004	3246 ± 610	
E. tonkawae	PC Spring	~1	0.66 ± 0.070	~1	0.028 ± 0.010	~1	0.020 ± 0.0003	4112 ± 1116	

8.0 FIGURES



Figure 1. Location of Georgetown Salamander (*Eurycea naufragia*) and Salado Salamander (*Eurycea chisholmensis*) monitoring sites in Williamson County, Texas.

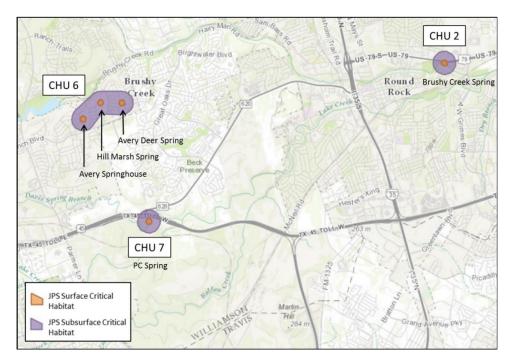


Figure 2. Location of Jollyville Plateau Salamander (*Eurycea tonkawae*) monitoring sites in Williamson County, Texas. U.S. Fish and Wildlife Service critical habitat units are displayed.



Figure 3. Oocytes visible through the venter of a gravid female *Eurycea naufragia* from Swinbank Spring.

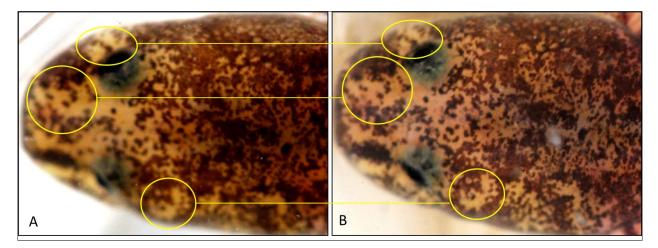


Figure 4. Melanophore recognition using the computer-assisted identification software Wild-ID. This individual from Cobbs Spring was captured first in April 2016 (A) and recaptured in May 2016 (B).

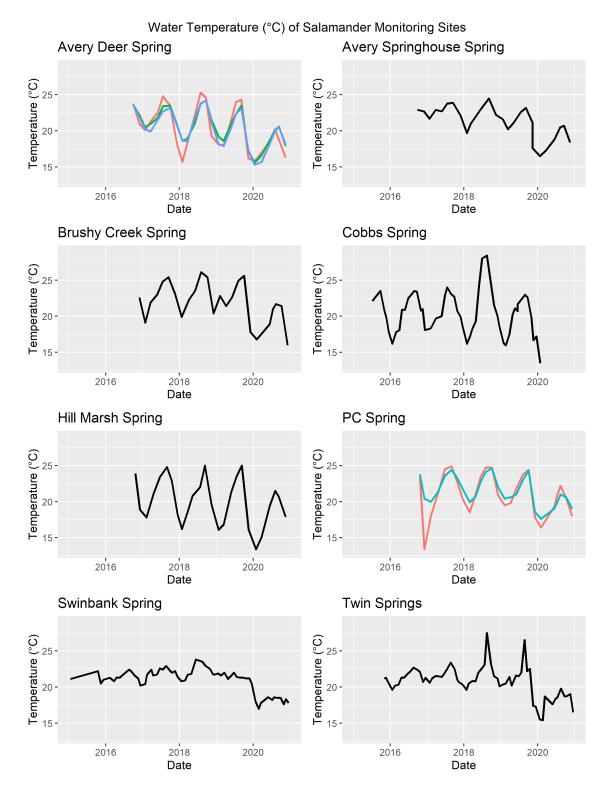


Figure 5. Water temperature at each permanent spring outlet at each salamander monitoring site through time. Avery Deer Spring has three nearly permanent spring outlets and PC Spring has two permanent spring outlets.



Figure 6. pH at each permanent spring outlet at each salamander monitoring site through time. Avery Deer Spring has three nearly permanent spring outlets and PC Spring has two permanent spring outlets.



Figure 7. Dissolved oxygen at each permanent spring outlet at each salamander monitoring site through time. Avery Deer Spring has three nearly permanent spring outlets and PC Spring has two permanent spring outlets.

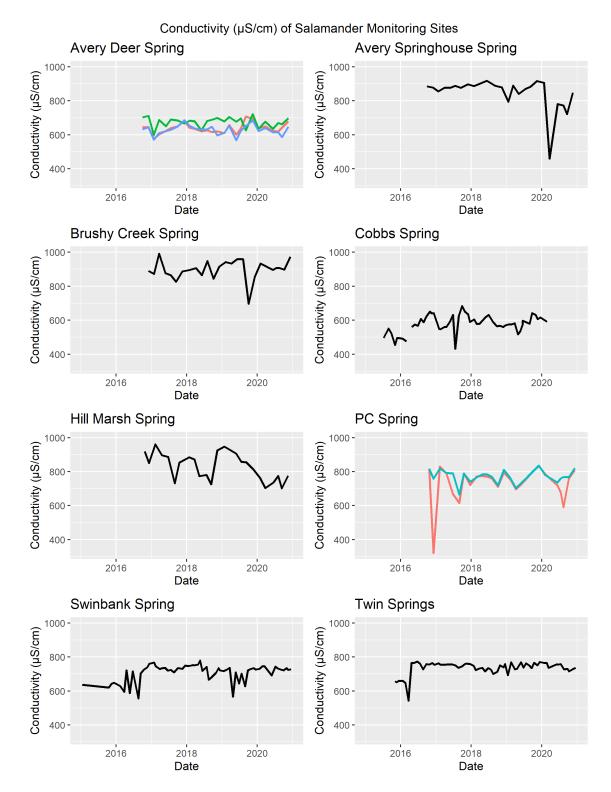


Figure 8. Conductivity at each permanent spring outlet at each salamander monitoring site through time. Avery Deer Spring has three nearly permanent spring outlets and PC Spring has two permanent spring outlets.

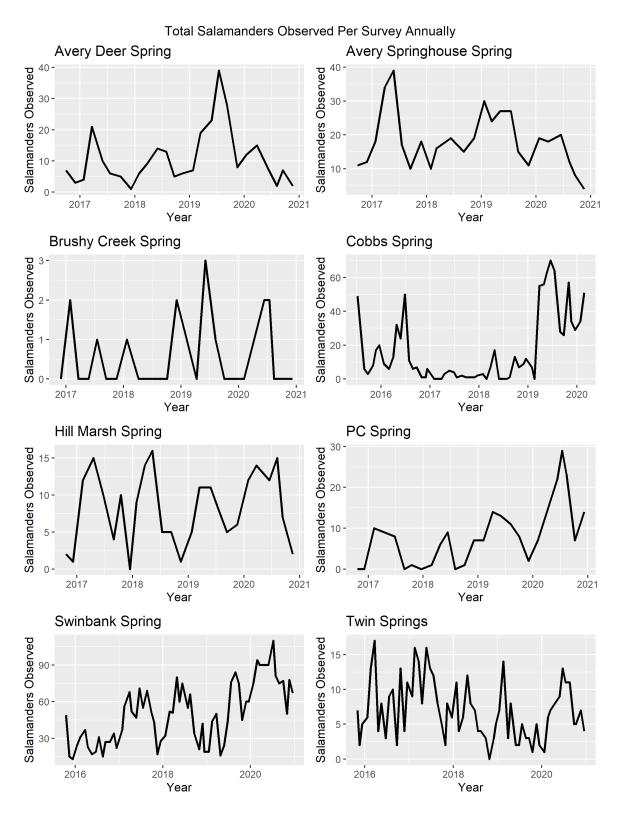
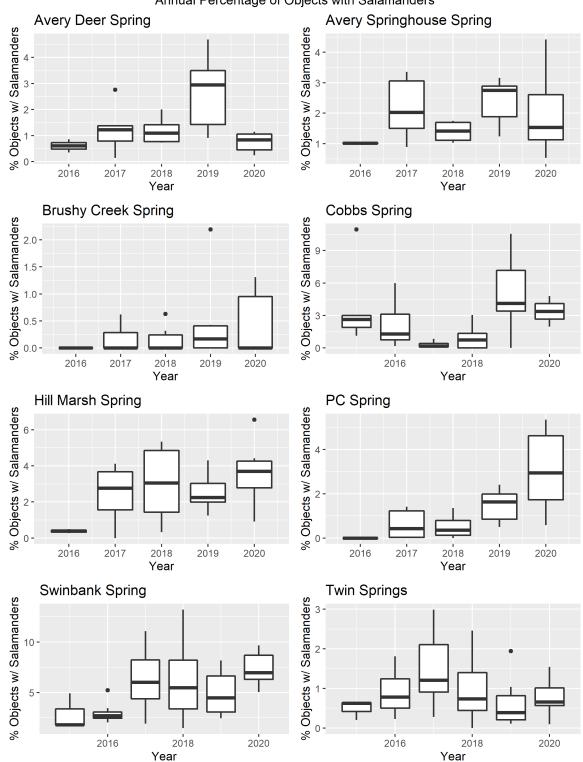


Figure 9. Number of *Eurycea* salamanders observed during every survey event, at each site, through time.



Annual Percentage of Objects with Salamanders

Figure 10. Boxplots comparing the variation in the percentage of cover objects with *Eurycea* salamanders (catch per unit effort), both within and among years, for each site.

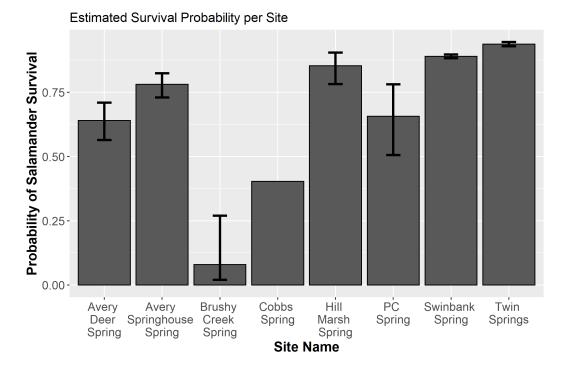


Figure 11. Estimated salamander survival probability at all eight monitored sites.

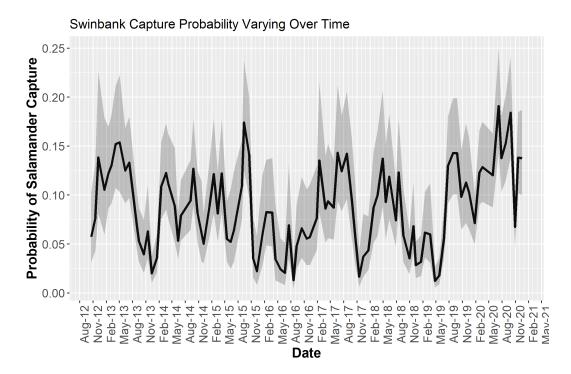


Figure 12. Time-varying salamander capture probability at Swinbank Spring.

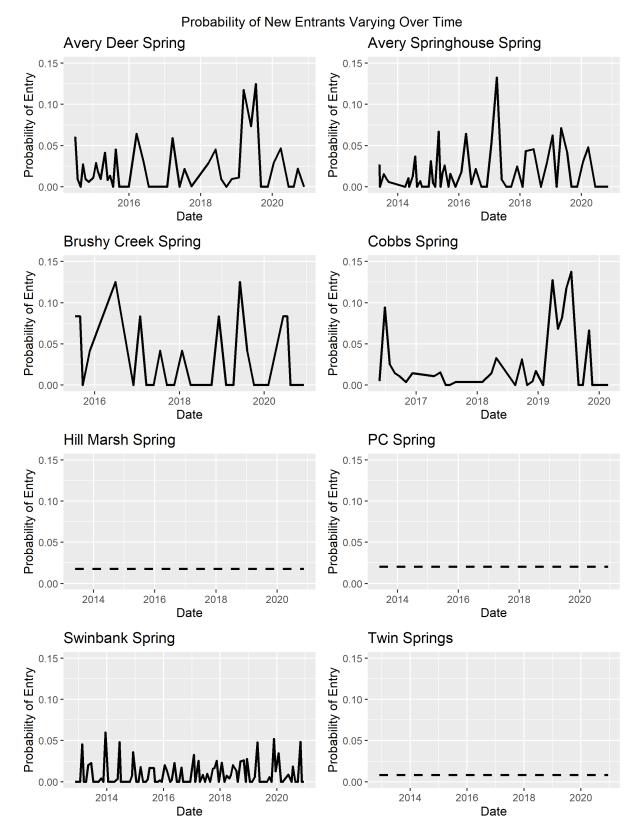


Figure 13. Probability of new entrants into each salamander population at eight monitored sites.

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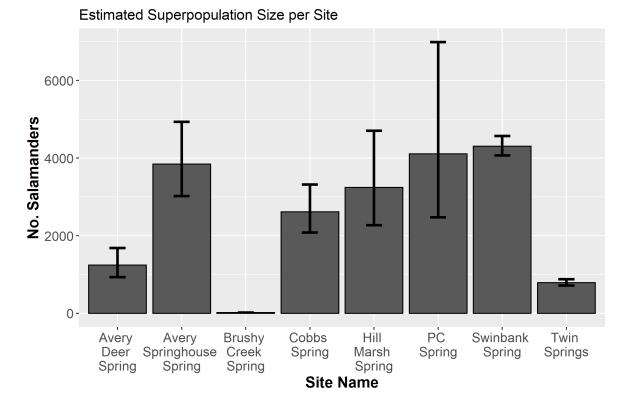
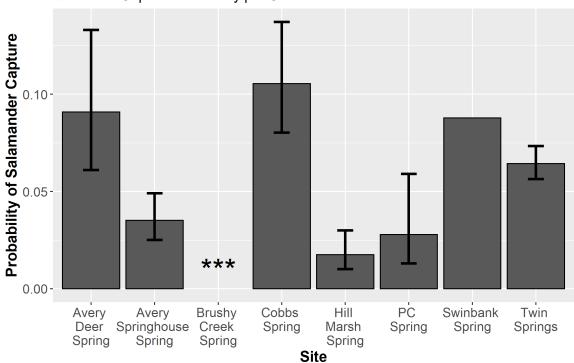


Figure 14. Estimated superpopulation size at eight monitored sites.



Estimated Capture Probability per Site

Figure 15. Estimated salamander capture probability at eight monitored sites.



Figure 16. Scatterplot of the number of oocytes visible within each gravid female versus snout-vent length.

APPENDIX B

Salamander Manuscripts Published in 2020

- Wall, A.E., Z.C. Adcock, R. Jones, and K. White. 2020. Eurycea naufragia (Georgetown Salamander) morphology. Herpetological Review 51:93.
- Jones, R. Z.C. Adcock, and K. White. 2020. *Eurycea naufragia* (Georgetown Salamander) predation. *Herpetological Review* 51:291–292.
- Adcock, Z.C., A. Parandhaman, W.W. Keitt, and M.R.J. Forstner. 2020. *Eurycea tonkawae* (Jollyville Plateau Salamander) response to spring drying. *Herpetological Review* 51:808–809.

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and flip free (Brodie et al. 1989. Herpetologica 45:167–171). Moreover, it may be beneficial for snakes to periodically attack large or difficult prey even if these attacks are unsuccessful given the low cost to the snake of expending energy for a large potential energetic reward (Feder and Arnold 1982. Oecologica 53:93–97). Given their overlap in habitat and geographic range, it is likely *N. sipedon* consumes variously sized *C. alleganiensis*, possibly during summer months when adult Hellbenders are more active.

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EURYCEA NAUFRAGIA (Georgetown Salamander). MORPHOL-OGY. Eurycea naufragia is a neotenic, federally threatened salamander restricted to 17 springs and caves in the San Gabriel River Basin in Georgetown, Williamson County, Texas, USA (U.S. Fish and Wildlife Service 2014. Fed. Reg. 79:10236-10293). However, recent phylogenetic and population genetic analyses suggest E. naufragia populations in the Berry Creek drainage and some within the North Fork San Gabriel drainage may be E. chisholmensis (Salado Salamander; Devitt et al. 2019. Proc. Natl. Acad. Sci. USA 116:2624–2633). Eurycea naufragia, along with E. tonkawae (Jollyville Plateau Salamander) and E. chisholmensis constitute the Septentriomolge clade of central Texas Eurycea that occupies the Northern segment of the Edwards Aquifer (Chippindale et al. 2000. Herpetol. Monogr. 14:1-80; Hillis et al. 2001. Herpetologica 57:266-280). In members of the Blepsimolge clade of central Texas Eurycea, there is a well-documented morphological continuum between epigean forms, that have pigmented skin and well-developed eyes, to subterranean (troglomorphic) forms that lack pigmentation and have reduced or vestigial eves (Sweet 1984. Copeia 1984:428-441; Bendik et al. 2013. BMC. Evol. Bio. 13:201). Eurycea naufragia are typically pigmented, with a broad, short head and well-developed eyes (Chippindale et al. 2000, op. cit.). A previous report of troglomorphic individuals of this taxon is based on unpublished, anecdotal observations (U.S. Fish and Wildlife Service 2014, op. cit.), but some cave populations of closely related E. tonkawae exhibit subterranean morphology (Chippindale et al. 2000, op. cit.). However, neither the E. naufragia nor E. tonkawae reports include information regarding the extent of troglomorphism or a description of the character modification (e.g., eyes, pigmentation, head shape). In comparison to the other Septentriomolge species, E. chisholmensis has reduced eyes and a more flattened head, but we are unaware of any observations of subterranean morphology in this taxon (Chippindale et al. 2000, op. cit.).

Here, we report the capture of three *E. naufragia* salamanders with the reduction or absence of at least one eye. On 24 May 2017, we captured an eyeless *Eurycea* salamander (41.8 mm total length) with a slightly flattened head and typical epigean pigmentation at a small spring in Williamson County, Texas (precise locality withheld due to conservation concerns). This spring discharges in the Berry Creek watershed, and therefore, salamanders at this location are historically considered *E. naufragia*, but may be *E. chisholmensis* (Chippindale et al. 2000, *op. cit.*; U.S. Fish and Wildlife Service 2014, *op. cit.*; Devitt et al. 2019, *op. cit.*). We recaptured this individual on 26 June 2017, but it has not been detected in subsequent surveys (n = 24). At

this same spring, we captured a one-eyed salamander (37.6 mm total length) on 22 May 2019, and one with a reduced eye (64.3 mm total length) on 29 January 2019. Both of these salamanders demonstrated typical epigean pigmentation. We are unable to determine if the reduced eye was a consequence of development or trauma.

This combination of pigmented but eyeless morphology is previously undocumented for this taxon despite long-term population monitoring (AEW, unpubl. data; Pierce et al. 2010. Southwest Nat. 55:291–297; Pierce et al. 2014. Herpetol. Conserv. Biol. 9:137–145; Pierce et al. 2018. Herpetol. Conserv. Biol. 13:383–390). Further, we are unaware of any previous records of eyeless *Septentriomolge* taxa, or any central Texas *Eurycea* taxa demonstrating one developed and one reduced or absent eye. The epigean to subterranean continuum of morphological characters exhibited by other central Texas *Eurycea* likely occurs in *E. naufragia*, but these individuals may be rare, difficult to detect (occur subsurface), or inhabit springs and caves on private property with restricted access.

We conducted surveys in accordance with Federal Fish and Wildlife Permit TE37416B-0. We thank the Williamson County Conservation Foundation for funding and the property owner for site access.

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PLETHODON CINEREUS (Eastern Red-backed Salamander). **PREDATION.** Plethodon cinereus is perhaps the most abundant vertebrate in temperate forests of eastern North America (Burton and Likens 1975. Copeia 1975:541-546), where it is an important predator on invertebrate decomposers within the detritus-based food web of the forest-floor (Walton 2013. Herpetologica 69:127-146). Plethodon cinereus shares this role with several abundant arthropod predators (e.g., spiders, centipedes, and carabid beetles) that are large enough to consume juvenile and perhaps adult salamanders. However, actual observations of arthropod predation on P. cinereus are few. Reports of arthropods preving on *P. cinereus* are limited to observations of predation by a spider and ants (species not specified; Lotter 1978. J. Herpetol. 12:231–236), a praying mantis (Mantis religiosa; Stein 1989. Bull. Maryland Herpetol. Soc. 25:60-61), and a rove beetle (Platydracus viduatus; Jung et al. 2000. Herpetol. Rev. 31:98-99).

We observed predation on a juvenile P. cinereus by Callobius bennetti (Bennett's Hacklemesh Weaver; Amaurobiidae). The predation event was observed at 1123 h on 11 September 2015 during a survey of an array of artificial cover objects. The array consisted of 108 ceramic floor tiles (296 × 296 mm) in a beechmaple woodlot on the campus of University School in Hunting Valley, Cuyahoga County, Ohio, USA (41.87860°N, 81.42630°W; WGS 84; 327 m elev.). A female C. bennetti and the salamander were on the underside of a tile. The spider straddled the salamander, which was ensnared in webbing. The salamander was a juvenile in its first season (18.1 mm SVL), and the spider was 8.5 mm (total length), based on the analysis of digital photographs using image analysis software (ImageJ, Schneider et al. 2012. Nat. Methods 9:671-675). Temperature under the plate was 19.4°C (measured with a Raytek infrared thermometer). Temperature at the leaf litter surface was 20.9°C and relative humidity was 78.2% (temperature/RH logger, Onset Computers, Inc.). The spider did not move from the salamander when the tile was turned. The salamander was immobile, apparently dead This page intentionally left blank.

We thank the Forests and Fish Adaptive Management Program facilitated via the Washington Department of Natural Resources for funding support. This is contribution number 36 of the Aquatic Research Section of the Washington Department of Fish and Wildlife's Habitat Program.

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EURYCEA LUCIFUGA (Cave Salamander). DIET. Eurycea lucifuga is a common and widespread facultative inhabitant of caves and springs in the southeastern and central United States (Powell et al. 2016. Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America. Houghton Mifflin Harcourt, Boston, Massachusetts. 512 pp.). This species is known to feed on a variety of invertebrates, such as snails, crustaceans, beetles, and flies (Peck 1974. Nat. Speleol. Soc. Bull. 36:7-10). Although several millipede species also can serve as prey for E. lucifuga, some are presumably avoided due to the presence of defensive secretions (e.g., Abacionidae, Paradoxosomatidae). Captive feeding trials and field study show that E. lucifuga do not actively prey upon cave-obligate Tetracion millipedes, which produce defensive secretions comprised primarily of the compound p-cresol (Peck and Richardson 1976. Annales de Speleologie 31:175-182). On the other hand, Pseudotremia millipedes, which do not possess chemical defenses, are a significant component of the diet of cave-dwelling E. lucifuga (Peck and Richardson 1976, op. cit.).



FIG. 1. *Eurycea lucifuga* predating *Oxidus gracilis* in the dark zone of Eblen Cave (TCS #TRN6), Roane County, Tennessee.

The Greenhouse Millipede (Oxidus gracilis) has been introduced from Asia to temperate and tropical regions worldwide. This species produces a mixture of defensive secretions, with *p*-cresol serving as a minor component (Taira et al. 2003. Appl. Entomol. Zool. 38:401-404). Although it has been reported from numerous caves from over seven states (Reeves 1999. Proceedings of the 1999 National Cave and Karst Management Symposium. Southeastern Cave Conservancy, pp. 164-166; Niemiller et al. 2019. In W. White, D. Culver, and T. Pipan [eds.], Encyclopedia of Caves 3rd edition, pp. 163–176. Academic Press, Cambridge, Massachusetts), we know little about its impact on, or interactions with, native cave inhabitants. In surface habitats, O. gracilis has been shown to invade and prey upon Ambystoma opacum eggs, but it is unlikely that nestguarding females attempted to feed on invading millipedes (Croshaw and Scott 2005. Am. Midl. Nat. 154:398-412).

At ca. 1100 h on 6 July 2019, at Eblen Cave (Tennessee Cave Survey no. TRN6) in Roane County, Tennessee, USA, we encountered an *E. lucifuga* preying upon an *O. gracilis*. The salamander captured the millipede by the posterior half of its body (Fig. 1) and proceeded to move over 10 m away from the point of capture over several minutes before ingesting the millipede. After ingestion, the salamander did not seem perturbed by the defensive secretions. This is the first known observation of a vertebrate cave-inhabiting species preying upon *O. gracilis*, and the first observation of *E. lucifuga* consuming a millipede species in the dark zone of a cave system that produces defensive secretions.

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EURYCEA NAUFRAGIA (Georgetown Salamander). PREDA-**TION.** Eurycea naufragia is a permanently aquatic, Federally Threatened salamander endemic to springs and groundwater in the San Gabriel River watershed in the vicinity of Georgetown, Williamson County, Texas, USA (U.S. Fish and Wildlife Service 2014. Fed. Reg. 79[36]:10236-10293; Devitt et al. 2019. Proc. Natl. Acad. Sci. USA 116:2624-2633). Eurycea naufragia use cobbles, woody debris, leaf litter, and detritus as habitat for foraging and refuge from predators (Pierce et al. 2010. Southwest. Nat. 55:291-297). Procambarus clarkii (Louisiana Crayfish) is a highly invasive species on several continents due to its regional culinary importance, adaptability, and high fecundity (Gheradi 2006. Mar. Freshw. Behav. Phy. 39:175-191). Although introduced around the world, our study site is within P. clarkii native range from northern Mexico to the Florida panhandle, and the type locality is in central Texas (Faxon 1885. Mem. Mus. Comp. Zool. Harvard 10:26-27).

On 28 July 2018 during a population monitoring survey at Swinbank Spring, Georgetown, Williamson County, Texas, we observed a *P. clarkii* consuming a *E. naufragia*. We do not know if the interaction was predation or scavenging by *P. clarkii*, as we only observed a dead *E. naufragia* in the chelipeds and mouth of the crayfish. We took photographs and video of the interaction, but we did not collect the crayfish or the salamander. However,



FIG. 1. *Procambarus clarkii* with *Eurycea naufragia* in chelipeds and mouth at Swinbank Springs, Georgetown, Texas, USA.

we note that tissue degradation on the salamander is not noticeable in any of the photos, which would help confirm the animal was dead before capture. *Procambrus clarkii* predation on *E. sosorum* has been documented (Owen et al. 2016. Herpetol. Rev. 47:275), but to our knowledge, this report documents the first observation of *P. clarkii* consuming *E. naufragia* (Pierce and Gonzalez 2019. J. Herptol. 53:81–86; Pierce et al. 2010, *op. cit.*). Pierce et al. (2010, *op. cit.*) found high probability of co-occurance of *E. naufragia* and *P. clarkii* under the same cover object. This high probability of co-occurance and sharing microhabitat likely leads to increased frequencies of interactions between *E. naufragia* and *P. clarkii*.

This work was conducted under U.S. Fish and Wildlife Permit No. TE37416B-0 and Texas Parks and Wildlife Scientific Research Permit No. SPR-0319-056. We thank Dan Johnson for visual identification of *P. clarkii* from photos, and Pete Diaz for identifying several large male crayfish collected from the spring as *Procambarus* spp. We thank the Williamson County Conservation Foundation for funding and the property owner for site access.

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NOTOPHTHALMUS VIRIDESCENS VIRIDESCENS (Red-spotted Newt). HABITAT. Saline habitats are considered hazardous environments for amphibians because of their permeable and hypoosmotic skin. However, recent research suggests that several amphibians, especially salamanders, are more tolerant to saline environments than currently recognized (Hopkins and Brodie 2015. Herpetol. Monogr. 29:1–27). *Notophthalmus viridescens viridescens* is a semi-aquatic salamander associated with lentic freshwater habitats including ephemeral wetlands, ponds, and oxbow lakes (Petranka 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. 587 pp). While this species has been reported in brackish water in east-central Pennsylvania (Pawling 1939. Herpetologica

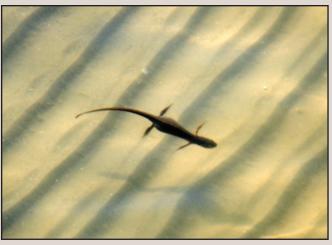


FIG. 1. *Notophthalmus viridescens viridescens* photographed swimming in the Chesapeake Bay at Brownies Beach, Calvert County, Maryland, USA.

1:165–169), no records of *N. viridescens viridescens* using saline habitats are known from estuarine or coastal regions.

On 12 March 2019, one adult N. v. viridescens was observed swimming in the surf zone of the Chesapeake Bay along Brownies Beach, Bayfront Park, Calvert County, Maryland, USA (38.67753°N, 76.53215°W; WGS 84). The salamander did not show signs of lethargy when discovered and was photographed quickly and released on forested land well beyond the backshore of the beach. Notophthalmus v. viridescens is a locally abundant salamander in Calvert County, Maryland and several individuals have been recorded near the vicinity of Brownies Beach (Cunningham and Nazdrowicz 2018. The Maryland Amphibian and Reptile Atlas. John Hopkins University Press. Baltimore, Maryland. 312 pp). The surrounding habitat west of the beach consists of mesic temperate deciduous forests and shallow ephemeral wetlands typical of the Atlantic Coastal Plain ecoregion. The shores are abutted in several areas by steep eroding cliffs containing Miocene fossil deposits from the Calvert Formation (Godfrey 2018. Smithson. Contrib. Paleobiol. 100:1-274). These cliffs are steep and unstable with large vertical drop-offs that can cause terrestrial animals near the cliff to fall down to the beach from the forest above. Early spring is considered peak activity season for amphibians in the Mid-Atlantic Coastal Plain and N. viridescens are known to move long distances during the breeding season (Roe and Grayson 2008. J. Herpetol. 42:22-30). Because of this, we hypothesize that the salamander fell down from the cliffs to the Chesapeake Bay during a breeding-based dispersal event which may have been facilitated by heavy rains the previous night. It is also possible the salamander was swept down from the floodwaters of nearby Brownies Creek, which feeds into Brownies Beach ca. 250 m north of our observation. However, given that this creek largely contains lotic wetland habitats, we believe this scenario is more improbable than the former.

This record represents the first live observation of a salamander from the genus *Notophthalmus* in an estuarine environment and the first observation of any salamander species in Chesapeake Bay. Our findings support a laboratory study that noted high sodium tolerance in individual *N. viridescens* (Wittig and Brown 1977. Comp. Biochem. Physiol. 58:49–52), which implies that *N. viridescens*, like some other salamanders, may be able to tolerate exposure to saltwater habitats longer than previously anticipated.

OTO BY BRETTON W.

EURYCEA TONKAWAE (Jollyville Plateau Salamander). RE-SPONSE TO SPRING DRYING. Eurycea tonkawae is a neotenic, federally threatened salamander restricted to springs, spring-fed creeks, and caves in Travis and Williamson counties, Texas, USA (U.S. Fish and Wildlife Service 2013. Fed. Reg. 78:51278-51326). Some of these occupied springs and creeks are ephemeral, and as surface water recedes, E. tonkawae will retreat subsurface into the aquifer to avoid desiccation (Sweet 1977. Herpetologica 33:364-375; Bendik and Gluesenkamp 2013. J. Zool. 290:35-41). Sweet (1977, op. cit.) and Bendik (2017. Ecol. Evol. 7:5002-5015) reported regularly finding stranded juvenile Eurycea salamanders as spring flow ceased and habitats dried. Here, we report our survey results for E. tonkawae at two springs monitored during both dry and flowing conditions. At both sites, we attempted to capture and photograph all observed E. tonkawae. We used Wild-ID photographic recognition software to evaluate pigmentation patterns on the salamander's head to identify recaptured individuals (Bolger et al. 2012. Methods Ecol. Evol. 3:813-822; Bendik et al. 2013. PLoS ONE 8:e59424).

We report observations from Avery Deer Spring in Williamson County, Texas, USA (30.50700°N, 97.74949°W; WGS 84; 254 m elev.) from May 2013 to July 2014. During this timeframe, we conducted 11 surveys and observed the following conditions: no surface water and no spring flow (dry; N = 3), surface water present but no spring flow (N = 3), and surface water present with spring flow (N = 5). We never observed *E. tonkawae* when the spring was not flowing, and therefore, never observed stranded salamanders. We observed E. tonkawae in four of the five surveys conducted with spring flow. The single survey with spring flow but without E. tonkawae observations occurred during very low flow conditions and between surveys without spring flow (Table 1). The area received ca. 12 cm of rainfall between our 7 May and 19 May 2014 surveys resulting in the return of spring flow, surface water, and E. tonkawae (Table 1). We regularly observed E. tonkawae retreating into the spring orifice to avoid capture, but we did not recapture any individuals after dry events. Therefore, we confirmed salamander access to subsurface water, but did not document an individual's successful recolonization of surface habitat.

We additionally surveyed MacDonald Well in Travis County, Texas, USA (30.45026°N, 97.85434°W; WGS 84; 233 m elev.). We

TABLE 1. *Eurycea tonkawae* survey data for Avery Deer Spring in Williamson County, Texas during and immediately following dry conditions. Observation rate is the percentage of searched refugia harboring salamanders.

Survey date	Spring discharging?	Refugia searched	Salamanders observed	Observation rate (%)
20 May 2013	Ν	25	0	_
27 May 2013	Y (low flow)	25	0	_
05 Jun 2013	Ν	25	0	
19 Jul 2013	Y	75	3	4.00
23 Aug 2013	N (dry)	0	_	
16 Sep 2013	N (dry)	0	_	
31 Mar 2014	Ν	94	0	
07 May 2014	N (dry)	0	_	
19 May 2014	Y	80	1	1.25
02 Jul 2014	Υ	42	2	4.76
29 Jul 2014	Y	89	4	4.49

had access to a 32.5-m portion of the spring run approximately 60 m downstream of the spring. As we did not have access to the spring orifice, we only report on conditions of the creek channel. We surveyed for E. tonkawae six times from September 2014 to July 2015. Surveys included six visual encounter surveys (VES; totaling 1035 searched refugia objects) and three quadrat surveys (totaling 10 randomly placed 30 × 30 cm quadrats). We observed 54 E. tonkawae during this timeframe, including 11 observations on 28 July 2015. No surface water was present during our next site visit (17 August 2015), but soil in the deeper portions of the creek was still wet, indicating recent drying (Fig. 1). We exhaustively searched all available refugia in the dry creek bed (N = 358) finding five small Lithobates berlandieri, many Incilius nebulifer metamorphs, and three crayfish (one alive and two dead). We did not observe any stranded E. tonkawae. It is possible that salamanders were scavenged prior to our survey, but we consider it unlikely that all E. tonkawae would be selectively consumed while other amphibians remained in the desiccating streambed. This portion of the MacDonald Well spring run does not contain any obvious aquifer access, as it has soil and concrete substrate rather than gravel or porous bedrock (Fig. 1). Salamanders either utilized conduits in the soil and roots not visible to us or had to travel upstream to areas with subsurface



FIG. 1. MacDonald Well spring run in Travis County, Texas, USA inundated and flowing on 28 July 2015 (A) and dry on 17 August 2015 (B). Moist soil in deepest areas indicates recent drying. The soil and concrete streambed lack obvious subsurface (aquifer) access.

water access. This is especially notable because seven of our 11 observations on 28 July 2015 occurred in the most downstream 10 m, indicating that these salamanders may have traveled at least 20-30 m upstream in about two weeks. MacDonald Well remained dry until the area received 10 cm of rain on 24 October 2015. Salamanders immediately returned to the flooded creek channel and were observed the following day. We conducted two additional VES (totaling 593 searched refugia objects) and quadrat surveys (totaling eight randomly placed 30 × 30 cm quadrats) in November 2015 and January 2016. We observed 15 E. tonkawae during this timeframe. We recaptured two E. tonkawae on 29 January 2016 that were initially captured on 26 June 2015, prior to the stream channel drying. Both of these animals were originally caught near the downstream limit of surveys and were recaptured 3.5 m and 5 m upstream of their original capture location. The E. tonkawae observation rate (the percentage of searched refugia harboring salamanders) and density both decreased when comparing surveys prior to and after the dry period (4.73% to 2.36% and 5.56/m² to 1.39/m², respectively). Conversely, Bendik (2017, op. cit.) did not find differences in E. tonkawae abundance after drought. However, we are cautious not to over interpret these results, as this decrease may reflect the normal pattern of decreased E. tonkawae observations in winter months (Bowles et al. 2006. Hydrobiologia 553:111-120; Pierce et al. 2010. Southwest. Nat. 55:291-297).

In contrast to previous studies, we never observed a stranded salamander, and we never observed a salamander after spring flow ceased, even if surface water was still present. These previous studies reported that stranded juveniles are more frequently observed, and adults are more capable of migrating into subsurface habitat (Sweet 1977, op. cit.; Bendik 2017, op. cit.). We acknowledge that Avery Deer Spring and MacDonald Well went dry during a timeframe when fewer E. tonkawae juveniles are on the landscape (Bowles et al. 2006, op. cit.; Bendik 2017, op. cit.). However, we observed juvenile and subadult size salamanders (see Sweet 1977, op. cit.; Bendik 2017, op. cit.) immediately before and after the dry period at MacDonald Well. Our limited data suggest that E. tonkawae responds to environmental cues (likely flow velocity) to seek subsurface refuge from drying conditions. At MacDonald Well, this may have required more than 20-30 m of travel over a short period of time (ca. two weeks). At both sites, E. tonkawae returned to surface habitat immediately with the return of spring flow, and at MacDonald Well, two salamanders were recaptured within 5 m of their original capture locations.

We conducted surveys in accordance with U.S. Fish and Wildlife Permit TE039544-1. We thank the Williamson County Conservation Foundation and Texas Department of Transportation for funding and site access.

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NOTOPHTHALMUS VIRIDISCENS (Eastern Newt). HABITAT USE. *Notophthalmus viridiscens* is a common salamander species in Pennsylvania, USA, and possesses a terrestrial form, commonly called a red eft, which is frequently observed foraging on rainy and humid days (Petranka 2010. Salamanders of the United States and Canada, Smithsonian Institution Press, Washington, D.C. 592 pp.). The following observations occurred during a larger camera-trap study on the association of snakes and active ant mounds in northeastern Pennsylvania. Due to the presence of



FIG. 1. Surface activity of *Notophthalmus viridiscens* outside of an ant mound constructed by *Formica exsectoides* (Allegheny Mound Ants): A) initial capture of a *Notophthalmus viridiscens* walking up the mound; B) a second capture, at the same mound a day after the initial encounter.

sensitive species at this site, we cannot disclose coordinates of the observation. On 8 October 2019 at 1631 h, we recorded surface activity of a N. viridiscens outside of an active ant mound built by Formica exsectoides (Allegheny Mound Ant). This initial encounter consisted of two photo captures spanning 1 min. The individual was observed at the base of the mound making its way up (Fig. 1A). On 9 October 2019 at 1705 h, at the same mound, we captured another N. viridiscens, or perhaps the same individual, moving across the base of the mound (Fig. 1B). This second encounter spanned roughly 4.5 min. It is unclear what the nature of this association was. The diet of N. viridiscens consists primarily of leaf litter invertebrates, with collembolans, spiders, mites, and fly larvae making up the majority of prey items (MacNamara 1977. Herpetologica 33:127-132). Whether the salamanders were foraging is uncertain. To our knowledge, this observation is the first to document Notophthalmus viridiscens utilizing habitat engineered by F. exsectoides.

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PLETHODON CINEREUS (Eastern Red-backed Salamander) and PLETHODON ELECTROMORPHUS (Northern Ravine Salamander). HYBRIDIZATION. Plethodon cinereus and P.

APPENDIX C

Salamander Manuscripts Accepted in 2020 and Currently In Press

Adcock, Z.C., M.E. Adcock, B.E. Hall, and M.R.J. Forstner. Modification of a water hyacinth sieve and description of Hubbard rakes for sampling small, aquatic salamanders. *Amphibian & Reptile Conservation*.

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1	Modification of a water hyacinth sieve and description of Hubbard rakes for sampling
2	small, aquatic salamanders
3	
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11	Abstract.—Jollyville Plateau salamanders (Eurycea tonkawae) can be difficult to detect and
12	capture in submerged leaf litter packs, woody debris, and vegetation. We describe the
13	modification of a water hyacinth sieve and introduce three designs of Hubbard rakes to
14	effectively sample these cover objects. We captured E. tonkawae using the sieve and all three
15	rakes and additionally used these devices to capture E. pterophila, E. naufragia, E.
16	chisholmensis, and several co-occurring tadpoles, small fishes, and invertebrates. We detail the
17	application and success of these tools in various cover types, water depths, and substrates.
18	
19	Keywords. Amphibia, Caudata, central Texas Eurycea, cover objects, monitoring, sampling
20	
21	Introduction
22	Jollyville Plateau salamanders (Eurycea tonkawae) are small, fully-aquatic salamanders
23	endemic to central Texas, USA (Chippindale et al. 2000) and listed as threatened by the U.S.

24	Fish and Wildlife Service (USFWS 2013). Bowles et al. (2006) and USFWS (2013) consider
25	submerged cobble and gravel to be preferred habitat for this taxon, but they are also documented
26	from leaf litter, woody debris, and aquatic vegetation (Bowles et al. 2006; Chippindale 2005;
27	Davis et al. 2001; O'Donnell et al. 2008). Submerged cobble is easily surveyed by overturning
28	and visually searching for salamanders underneath (Bowles et al. 2006; Pierce et al. 2010; Sweet
29	1977). In contrast, E. tonkawae are difficult to detect in leaf litter packs, woody debris, and
30	vegetation because these cover objects can be dense and often occur on silty substrate (Bowles et
31	al. 2006; Davis et al. 2001). Bowles et al. (2006) recognized that this difficulty may have caused
32	underestimates of <i>E. tonkawae</i> relative abundance in large leaf packs.
33	Previous researchers have surveyed for salamanders in submerged, dense leaf litter and
34	vegetation with pipe and box samplers, dip nets, and seines (Shaffer et al. 1994; Skelly and
35	Richardson 2010), but these techniques are difficult to apply in shallow water (< 15 cm) and in
36	areas with gravel or bedrock substrate that characterize E. tonkawae habitat (Z.C. Adcock, pers.
37	obs.). Sweet (1977) collected central Texas Eurycea in gravel substrates by shoveling the
38	material onto a wire-mesh screen suspended over a large tray. Sweet's (1977) method could be
39	applied to leaf litter, woody debris, and vegetation, but it requires bulky gear that may be
40	difficult for one surveyor to use or to transport in the field. O'Donnell et al. (2008) reported
41	catching <i>E. tonkawae</i> by sweeping leaf litter into a large net, but this method does not allow for
42	easy quantification of the surveyed area and has limited applicability to other cover objects.
43	Passive and active traps, such as funnel traps, drift nets, leaf litter bags, and mopheads, can
44	capture Eurycea salamanders in various aquatic cover (Devitt and Nissen 2018; Pauley and Little
45	1998; USFWS 2014; Waldron et al. 2003; Willson and Dorcas 2003; Willson and Gibbons
46	2010). However, captured animals can die if passive traps are not checked frequently (Willson

and Gibbons 2010), and all trapping methods require several subsequent site visits which may 47 not always be practical. Traps can also result in a size-biased sample (Luhring et al. 2016). 48 Here, we detail a modification of the Godley water hyacinth (Eichhornia crassipes) sieve 49 (Godley 1982) and describe Hubbard rakes to sample E. tonkawae in a variety of cover types, 50 water depths, and substrates. We designed these tools to be small so they would work efficiently 51 52 within the often-narrow spring runs occupied by these salamanders and to allow a single researcher to easily carry and operate the equipment. Like the Godley sieve, and its predecessor, 53 the Goin dredge (Goin 1942), our modified sieve and rakes sample a known area, thus enabling 54 55 estimates of salamander densities. Although we designed these sampling devices to capture E. tonkawae, we demonstrate they are also effective for other central Texas Eurycea salamanders 56 and several co-occurring vertebrates and invertebrates. 57

58

59 Materials and Methods

Sieve.—Our modified sieve design required 1.25 m of 1.9-cm x 8.9-cm (standard 1-inch x 4-60 inch) untreated pine lumber, eight 3.8-cm (1.5-inch) galvanized corner braces, two galvanized 61 gate handles, a 91-cm x 2.1-m (3-foot x 7-foot) roll of fiberglass window screening, a 1.27-cm x 62 63 61-cm x 152-cm (0.5-inch x 2-foot x 5-foot) roll of 19-gauge galvanized steel hardware cloth, a box of 1.27-cm (0.5-inch) stainless steel staples, and a can of spar urethane (Table 1). We cut the 64 lumber into two 30-cm and two 32-cm lengths to form a box frame with a 30 cm x 30 cm interior 65 66 dimension (Fig. 1A), but we note that the interior dimension should be adjusted to meet the researcher's needs. We attached the corner braces on the outside of the frame to eliminate any 67 sharp corners inside the sieve that may harm captured animals (Fig. 1B). We then sealed the 68 69 frame with spar urethane and attached the gate handles after the frame dried. The bottom was

constructed by attaching window screening supported by hardware cloth to the bottom of the
frame with staples (Fig. 1C). Because of the small sieve size, staples adequately supported the
bottom, and a bottom brace as described by Godley (1982) was not required.

Materials to construct one sieve cost ≈ \$56.00 USA. However, much of this cost was
associated with excess materials because the smallest amount available for purchase exceeded
the amount needed for construction (see Table 1). Constructing additional sieves from the excess
material (up to 10 total) would only require purchasing more lumber, corner braces, and gate
handles for ≈ \$20.50 USA per sieve.

If the water was deep enough and floating cover objects (e.g., woody debris, unrooted 78 vegetation) were present, the sieve could be positioned underneath the material and lifted straight 79 80 up, as described by Goin (1942, 1943) and Godley (1980, 1982). In shallow water and in rooted vegetation, we used a large dustpan to scoop gravel, leaf litter, woody debris, vegetation, and the 81 inhabitants into the sieve (Fig. 2A). Dustpans in similar dimensions to our modified sieve design 82 83 are available at most hardware stores for \approx \$7.00 USA. Nets, strainers, or other scooping devices can also be used to fill the sieve, but we chose a dustpan because we wanted to collect the sample 84 in a single scoop, rather than multiple small scoops which may cause animals to flee before 85 capture. We washed the collected cover material with water to rinse away silt, then carefully 86 sorted through it searching for salamanders and co-occurring fauna (Fig. 2 B,C). Once inside the 87 88 sieve, salamanders are unlikely to escape (Fig. 2 D), reducing false absences associated with 89 these difficult-to-sample cover types (Bowles et al. 2006; Davis et al. 2001). Hubbard Rakes.—Aluminum rakes were constructed by Hubbard Rakes in Jonesport, Maine, 90 91 USA (www.hubbardrakes.com) and are custom designs that combine aspects of their lowbush

blueberry, cranberry, and sea glass rakes. Each rake cost \approx \$50.00 plus shipping and handling.

93 The interior (30 cm x 30 cm x 11.5 cm) matches our modified sieve dimensions for comparable 94 density estimates. All rakes have a (30 cm x 14 cm x 11.5 cm) backend enclosed on all sides that 95 serves as a receptacle for scooped material. We drilled large drain holes and small holes for 96 window screen attachment into the receptacle and lined the rakes with window screening to 97 prevent fauna from escaping through the teeth and drain holes (Fig. 3).

We designed three rakes that differ in the sampling edge (i.e., flat edge, short teeth, and long teeth) to accommodate different cover objects (Fig. 4). The flat-edged rake is scooped through the cover objects, like the dustpan, but as it is drawn through the water column, all material and inhabitants are entrapped in the receptacle. The short-toothed rake has ≈ 6.5 cm-long teeth, and the long-toothed rake has ≈ 15 cm-long teeth. Both are designed to rake through dense, rooted vegetation and comb any resident fauna out of the vegetation and into the rake receptacle. As with the sieve, salamanders are unlikely to escape once inside the rakes (Fig. 5).

105 Sampling.—We surveyed for E. tonkawae at springs in the vicinity of Round Rock and Cedar

106 Park, Texas, USA from 2014 through 2019. From July 2014 to August 2016, we quantified *E*.

107 *tonkawae* captures and survey effort using the sieve and Hubbard rakes as well as standard visual

108 encounter surveys by searching under cobble (see Bendik et al. 2014; Pierce et al. 2010). In

subsequent years, we used the sieve and Hubbard rakes to survey for other species of central

110 Texas Eurycea salamanders (i.e., E. pterophila, E. naufragia, and E. chisholmensis) at springs in

111 Hays and Williamson counties, Texas, USA.

112

113 **Results**

From July 2014 to August 2016, we captured 325 *E. tonkawae* using the sieve and Hubbard rakes, compared to 342 *E. tonkawae* in cobble surveys. We captured 0.53 salamanders per

sieve/rake sample and 0.02 salamanders per searched cobble. We used sieve and Hubbard rakes 116 to capture E. tonkawae in submerged gravel, leaf litter packs, small woody debris, silt, and 117 several types of vegetation (e.g., floating, aquatic, emergent). We also caught *E. pterophila*, *E.* 118 naufragia, and E. chisholmensis in these same cover types at their respective springs. 119 In addition to the targeted *Eurycea*, these tools captured a number of co-occurring tadpoles, 120 121 fishes, and invertebrates. Bycatch included Blanchard's cricket frog (Acris blanchardi) tadpoles, Rio Grande leopard frog (Lithobates [Rana] berlandieri) tadpoles, juvenile sunfish (Lepomis 122 spp.), juvenile bass (Micropterus spp.), western mosquitofish (Gambusia affinis), slough darters 123 (Etheostoma gracile), crayfish (Family Cambaridae), dragonfly and damselfly larvae (Order 124 Odonata), mayfly larvae (Order Ephemeroptera), giant water bugs (Family Belostomatidae), 125 beetles (Order Coleoptera), snails (Order Gastropoda), hellgrammites (Family Corydalidae), 126 annelid worms (subclasses Hirudinea and Oligochaeta), and amphipods (Order Amphipoda). 127

128

129 **Discussion**

We captured approximately 49% of E. tonkawae using the sieve and Hubbard rakes, and we 130 caught the remaining 51% in traditional cobble searching surveys. The frequency of salamander 131 132 observations per cobble (= 0.02) was comparable to those reported by Pierce et al. (2010) for E. *naufragia* but was substantially lower than the salamander observations per sieve/rake sample (= 133 134 (0.53). However, we acknowledge that these tools sample a larger area than the average cobble 135 size. Our goal was not to evaluate the best survey methodology or overall tool, but to demonstrate that most cover objects can be efficiently sampled with proper tool design and 136 137 selection. Any potential differences in salamander or faunal captures among sampling tools 138 would be more indicative of differences in cover object availability and use (Z.C. Adcock,

unpublished data). Importantly, our efforts demonstrate that the sieve and Hubbard rakes 139 effectively capture central Texas Eurycea salamanders in cover objects previously described as 140 difficult to sample (Bowles et al. 2006; Davis et al. 2001) and in cover objects the USFWS 141 considers suboptimal habitat (USFWS 2013). 142 Our modified sieve and dustpan combination worked particularly well in shallow water when 143 144 cover objects could be scooped without losing water and material over the edges of the dustpan. The dustpan was effective at scooping gravel, leaf litter, small woody debris, silt, and unrooted 145 or weakly rooted vegetation into the sieve (Fig. 6). The sieve was also effective when floating 146 cover objects were present in deep water, as previously described (Goin 1942, 1943; Godley 147 1980, 1982). We found the sieve and dustpan combination to be ineffective for submerged cover 148 objects in deep water (> 30 cm deep) and in vegetation with durable stems and roots. When 149 scooping material in deeper water, cover objects (and likely fauna) spilled over the sides of the 150 dustpan as we brought it up through the water column. Likewise, the dustpan was inadequate at 151 pushing through durable roots or emergent vegetation, undoubtedly causing salamanders to 152 retreat undetected. These deficiencies prompted our combination of the sieve and dustpan into a 153 single tool, the Hubbard rakes. 154

The Hubbard rakes were capable in all water depths due to the enclosed backend receptacle. The flat-edged rake was effective in scooping all cover types except for vegetation with durable stems and roots; we used the toothed rakes in these situations. The short-toothed rake performed well in aquatic vegetation and in emergent vegetation along creek edges. In emergent vegetation zones (Fig. 6C), the long-toothed rake often hit hard substrate (e.g., soil along the bank) before the vegetation encountered the receptacle edge, which allowed fauna to escape through the teeth. However, the long-toothed rake worked particularly well in large patches of aquatic vegetation in water > 15 cm deep (Fig. 6D). The toothed rakes did not perform well in gravel, leaf litter, and
woody debris because these smaller items fell through the teeth while scooping. We note that we
never impaled salamanders with the rake teeth, and researchers are unlikely to do so if the rakes
are used in a combing motion.

These tools also allowed us to sample exposed vegetation roots in addition to stems and leaves. We frequently captured *Eurycea* salamanders by placing submerged, exposed root clumps in the sieve or rakes and washing with water, by combing through roots with the toothed rakes, and by scraping the bottom of dense root mats and undercut stream banks with the flatedged rake or the wood edge of the sieve.

Central Texas *Eurycea* typically escape predators (and researchers) by diving into the 171 interstitial spaces of substrate. Sweet (1977) exploited this behavior with his wire-screen method, 172 allowing salamanders to "escape" through the screen and into the collection tray. Our modified 173 sieve and Hubbard rakes exploit this same behavior. Washing the sampled material created 174 175 enough disturbance to cause most entrapped salamanders to retreat out of the cover objects and to the bottom of the sieve or rakes. We note that adult central Texas Eurycea were often easily 176 noticed in the sieve and rake as they actively sought shelter. Small juvenile salamanders (< 15 \pm 177 178 mm total length) typically were less active and frequently laid motionless. Therefore, we caution researchers to carefully search the sampled material and the sampling devices for juveniles. 179 180 We replaced sampled gravel, leaf litter, woody debris, silt, and similar cover objects back 181 into the streams to minimize habitat impacts. The toothed rakes caused minor damage to aquatic and emergent vegetation when combing through roots, stems, and leaves. Using the sieve and 182 183 rakes results in destructive sampling for weakly rooted vegetation, but we rarely noticed the 184 sampling impacts in subsequent survey events. The fast-growing watercress (Nasturtium

officinale) constituted much of our sampled vegetation, and a monthly survey timeframe allowed
 ample time for regrowth. We suggest researchers be cognizant of potential oversampling by
 considering vegetation growth rates and their planned survey timing.

We modified the water hyacinth sieve and designed the Hubbard rakes to capture *E*. *tonkawae*, but they proved effective for other central Texas *Eurycea* salamanders and several cooccurring vertebrates and invertebrates. The density of salamanders and co-occurring fauna can be easily calculated by dividing the number of captures by the number of samples multiplied by the size of the sampling device. These tools are undoubtedly applicable to a wide variety of small, aquatic salamanders, tadpoles, fishes, and invertebrates if the appropriate device is matched to the cover objects to be sampled.

195

Acknowledgements.—We thank Steve Godley for recommending that we explore designs for a 196 hybrid sieve and scoop, Ike Hubbard for design input and manufacturing the rakes, and field help 197 from Texas State University and Cambrian Environmental. Andrew MacLaren, Steve Godley, 198 and the reviewers provided helpful comments on earlier drafts of the manuscript. The 199 Williamson County Conservation Foundation, Texas Department of Transportation, and 200 201 PulteGroup, Inc. provided funding and site access. We conducted this work in compliance with Texas State University Institutional Animal Care and Use Committee (0417 0513 07), Texas 202 Parks and Wildlife Department (SPR-0102-191 and SPR-0319-056), and the U.S. Fish and 203 204 Wildlife Service (TE039544-1 and TE37416B-0).

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288

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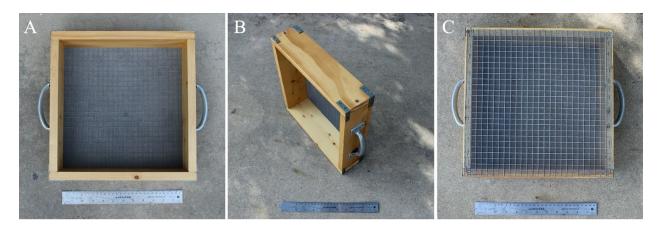


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working with him seek to provide genetic and ecological data relevant to those conservation
efforts.

M - 4	0	Total Approximate Cost (US dollars)	
Material	Quantity		
1.9-cm x 8.9-cm x 1.8-m (standard 1-inch x 4-inch x	1	\$3.50	
6-foot) untreated pine lumber	1	\$3.3U	
3.8-cm (1.5-inch) galvanized corner brace	8	\$7.00	
Spar urethane*	1 can	\$10.00	
Galvanized gate handle	2	\$10.00	
91-cm x 2.1m (3-foot x 7-foot) roll of fiberglass	1 11	¢7.00	
window screening*	1 roll	\$7.00	
1.27-cm x 61-cm x 152-cm (0.5-inch x 2-foot x 5-			
foot) roll of 19-gauge galvanized steel hardware	1	\$6.50	
cloth*			
	Box of	¢1 2 00	
1.27-cm (0.5-inch) stainless steel staples*	1,000	\$12.00	
Total Cost		\$56.00	

Table 1. Cost of materials to construct one salamander sieve.

*The quantity of these materials indicates the smallest amount available for purchase, not the
quantity needed for construction.



- Fig. 1. (A) Top, (B) side, and (C) bottom of a salamander sieve. Scale is 30 cm. *Photos by*
- 283 Michelle Adcock.





Fig. 2. Salamander sieve demonstration. (A) Cover objects are scooped into the sieve using a
dustpan and (B) carefully searched for fauna to (C, D) reveal a salamander. Red arrows identify a
Jollyville Plateau salamander (*Eurycea tonkawae*) trapped in the sieve. *Photos by Madison Torres (A) and Zach Adcock (B-D)*.

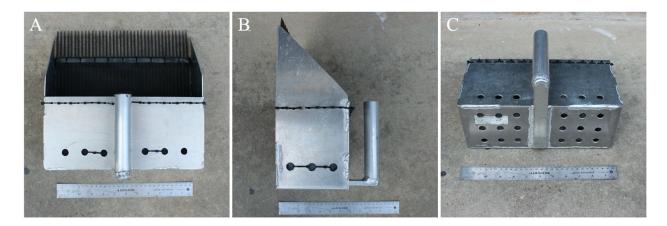
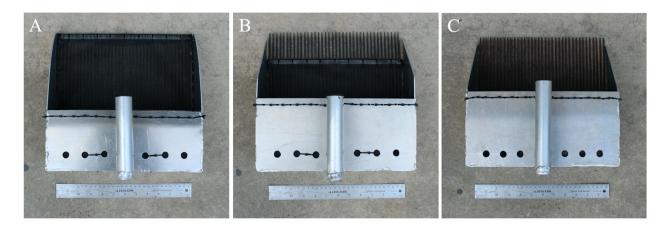
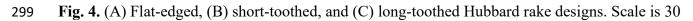


Fig. 3. (A) Top, (B) side, and (C) back of a Hubbard rake showing receptacle backend with drain

holes and holes for window screen attachment using zip ties. Scale is 30 cm. *Photos by Michelle*

- *Adcock*.





300 cm. *Photos by Michelle Adcock*.



- 302
- **Fig. 5.** Hubbard rake demonstration. (A) Cover objects are scooped into the rake receptacle and
- 304 (B, C) carefully searched for fauna to reveal a salamander. Red arrow identifies a Jollyville
- 305 Plateau salamander (*Eurycea tonkawae*) trapped in the rake. *Photos by Zach Adcock*.



307

Fig. 6. Examples of Jollyville Plateau salamander (*Eurycea tonkawae*) cover objects that are

- 309 effectively sampled using the salamander sieve and Hubbard rakes. (A) Submerged leaf litter and
- exposed roots, (B) submerged woody debris, (C middle of springrun) aquatic vegetation with
- 311 weak roots, (C springrun edges) shallow, emergent vegetation, and (D) deep, aquatic
- 312 vegetation with durable roots and stems. *Photos by Zach Adcock*.

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APPENDIX D

Salamander Manuscripts Submitted in 2020 and Currently In Review

McAllister, C.T., Z.C. Adcock, A. Villamizar-Gomez, R.M. Jones, and M.R.J. Forstner. A new host record for *Clinostomum* cf. *marginatum* (Trematoda: Digenea: Clinostomidae) from the endemic Salado Salamander, *Eurycea chisholmensis* (Caudata: Plethodontidae), from the Edwards Plateau, Texas, U.S.A. *Comparative Parasitology*.

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Research Note

A New Host Record for *Clinostomum* cf. *marginatum* (Trematoda: Digenea: Clinostomidae) from the Endemic Salado Salamander, *Eurycea chisholmensis* (Caudata: Plethodontidae), from the Edwards Plateau, Texas, U.S.A.

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ABSTRACT: Macroscopic examination of 622 Salado salamanders, *Eurycea chisholmensis* Chippindale, Price, Wiens and Hillis, 2000 collected between June 2018 and July 2020 from 3 springs in Williamson County, Texas, revealed the presence of encapsulated metacercariae of *Clinostomum* cf. *marginatum* ("yellow grub") in 3 (0.5%) hosts. Two of these 3 salamanders were examined and released unharmed per permit requirements but 1 was found dead and it harbored 6 total metacercariae, 4 on the head region (including 1 behind the left eye), 1 near the

⁴ Corresponding author.

left front leg, and 1 in the tail. Morphological identification of *C*. cf. *marginatum* was achieved by comparison to previous accounts. Molecular identification was accomplished by comparing sequence homology and phylogenetic analysis using an 828 base pair partial sequence of the internal transcribed spacer region. This is the first report of any parasite from *E. chisholmensis*, a federally threatened species.

KEY WORDS: *Eurycea chisholmensis*, *Clinostomum* cf. *marginatum*, Trematoda, Digenea, Clinostomidae, Salado Salamander, Endemism, Edwards Plateau, Texas.

The Salado salamander, Eurycea chisholmensis Chippindale, Price, Wiens and Hillis is a paedomorphic salamander restricted to groundwater-fed habitats and known from less than 20 locations in 2 Texas counties (Bell and Williamson) (Chippindale, 2005; Tipton et al., 2012; Dixon, 2013; LaDuc, 2017; Devitt et al., 2019). The Salado salamander is part of the Septentriomolge group (clade) of central Texas Eurycea salamanders that are located in the northern part of the Edwards Aquifer (Hillis et al., 2001). This species was originally included in E. neotenes Bishop and Wright (Texas Salamander) by Sweet (1982) and in publications until 2000 (see Dixon, 2000, 2013). Populations in a portion of its range (north of Lake Georgetown in Williamson County, Texas) were considered E. naufragia Chippindale, Price, Wiens and Hillis (Georgetown salamander) from 2000 until the taxonomic revisions of Devitt et al. (2019). The Salado salamander is listed as vulnerable on the IUCN Red List of Threatened Species (Hammerson and Chippindale, 2008), as critically imperiled (G1) by NatureServe (2020), and as a federally threatened species afforded protection by the U.S. Fish and Wildlife Service (USFWS) (USFWS, 2014). The primary conservation concerns for this taxon include its restricted geographic distribution, urban growth within this limited distribution, and potential

impacts to the aquifer including water quantity reduction and water quality degradation (Chippindale et al., 2000; USFWS, 2014; Devitt et al., 2019). Some known spring sites are heavily modified and groundwater contamination events have occurred within its proposed critical habitat (Chippindale et al., 2000; Tipton et al., 2012; USFWS, 2014).

Conservation concerns for *E. chisholmensis* have prompted ecological studies on abundance, reproduction, movement, predation, morphology, and water contaminants (see Pierce et al., 2014; Diaz and Bronson-Warren, 2018; Gutierrez et al., 2018; Pierce and Gonzalez, 2019; Diaz et al., 2020; Wall et al., 2020). However, to the best of the authors' knowledge, there are no records of parasites reported from this species. Herein, the first trematode is reported from *E. chisholmensis*.

Between June 2018 and July 2020, 810 *E. chisholmensis* were collected with dipnet from 3 springs (Cobbs [n = 480], Cowan [n = 220] and Twin [n = 110]) in Williamson County. We used dial calipers to measure snout-vent length (SVL) to the nearest 0.1 mm for all captured salamanders. All salamanders were photographed and Wild-ID photographic recognition software was used to identify recaptured specimens (Bolger et al., 2012; Bendik et al., 2013). Because this salamander is a protected species (no removal per federal listing), the epidermis of specimens was examined in the field for encapsulated helminths via external visual macroexamination. Those same restrictions precluded examination of the coelomic cavity or organs of live salamanders. When parasites were observed, the infection site was photographed, and all salamanders were released unharmed at their collection site. Salamanders found dead were salvaged, placed in 95% (v/v) DNA grade ethanol in the field, and completely examined for parasites in the lab. Parasites were stained with Semichon's acetocarmine, dehydrated to absolute ethanol, cleared in methyl salicylate, and mounted in Kleermount®. Two voucher specimens

were deposited in the Harold W. Manter Laboratory of Parasitology (HWML 216372), University of Nebraska, Lincoln, Nebraska, U.S.A. Tissue samples of the parasites were deposited in the M. R. J. Forstner Frozen Tissue Catalog currently held at Texas State University, San Marcos, Texas, U.S.A. (MF41139). We follow the common and scientific names of North American herpetofauna of Crother (2017).

We assessed the molecular identity of a single metacercaria of *Clinostomum* sp. recovered from an *E. chisholmensis* found dead and salvaged from Cobbs Spring. DNA was isolated from tissue with a DNeasy® Tissue Kit (Qiagen Inc., Venlo, Netherlands) using standard procedures. Two sets of primers were used to target the ITS1+5.8S+ITS2+28S region (Moszczynska et al., 2009; Haarder et al., 2013). Sequences and annealing temperatures for each primer are provided (Table 1). All PCR reactions had a total volume of 25.0 μ l. A dilution of 1-5× B\buffer (10× GenScript Buffer), 2-2.5 mM MgCl₂, 10 μ M concentration of primers and dNTPs, and 0.6 U of Taq Polymerase (GenScript) were used. The PCR product was cleaned using the ExoSAP-IT enzyme (Affymetrix) and sequencing was performed using the ABI 3500 Genetic Analyzer, using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems®). The 4 sequences were assembled in Geneious Prime 2020.2.3 (www.geneious.com) and a consensus sequence was generated at a 65% threshold. The final gene sequence used in the phylogenetic analyses was 828 base pairs (bp) and was accessioned into GenBank (MW261284).

Seventy-six *Clinostomum* sp. ITS1+5.8S+ITS2 (ITS) sequences were assembled from GenBank (Table 2) to compare to our generated sequence. ITS sequences from 18 described taxa were included as well as documented but unnamed species (Locke et al., 2015), lineages (Pérez-Ponce de León et al., 2016), and morphotypes (Caffara et al., 2017). When possible, at least 3 sequences per taxon, unnamed taxon, lineage, and morphotype were included. *Ithyoclinostomum* sp. (*n* = 2) was used as a non-*Clinostomum* outgroup (Briosio-Aguilar et al., 2018b; Table 2). An alignment of the 79 total sequences was made using MUSCLE (Edgar, 2004) implemented in Geneious Prime 2020.2.3. Nucleotide substitution models were evaluated with Akaike Information Criterion (AIC) using Modeltest (Posada and Crandall, 1998) in PAUP* (Swofford, 2002) implemented in Geneious Prime 2020.2.3. Maximum likelihood (RAxML; Stamatakis, 2006, 2014) and Bayesian (MrBayes; Huelsenbeck and Ronquist, 2001) phylogenetic analyses were conducted, with both analyses implemented through Geneious Prime 2020.2.3. The Modeltest results determined input parameters, and the general time reversible (GTR) substitution model with gamma rate variation (Tavaré, 1986) was used for both analyses. For the RAxML analysis, a rapid bootstrapping algorithm for 10,000 bootstrap replicates was used. The Bayesian analysis parameters included 4 heated chains and 1,100,000 Markov chain Monte Carlo (MCMC) generations, saving every 100th tree, with a burn-in of 100,000 generations.

The model of evolution that best fit the sequence data was GTR+I+G (chosen by AIC) as determined by Modeltest. Maximum likelihood and Bayesian phylogenetic analyses resulted in similar topologies that do not differ in taxonomic assignments. Maximum likelihood results are presented on the Bayesian phylogram (Fig. 1). The *Clinostomum* sp. found in the *E. chisholmensis* from Cobbs Spring clearly groups in the *C. marginatum/C. attenuatum* clade (Fig. 1). This clade has a posterior probability of 0.98 and 89% bootstrap support in our analyses (Fig. 1) and includes the *C. marginatum* identified from Jollyville Plateau salamanders, *E. tonkawae* Chippindale, Price, Wiens, and Hillis in southern Williamson County, Texas (McAllister et al., 2018). Our topologies are generally consistent with other recent *Clinostomum* phylogenetic studies, especially in the deep phylogenetic split between old and new world taxa (e.g., Locke et al., 2015; Pérez-Ponce de León et al., 2016; Caffara et al., 2017; Rosser et al., 2018; Briosio-

Aguilar et al., 2018a, b). The ITS genetic sequence from the *Clinostomum* sp. found in the Cobbs Spring *E. chisholmensis* was 99.75–100% similar to *C. marginatum*, 100% similar to *C. attenuatum*, 99.64% similar to *C. poteae*, and 99.13% similar to *C. album* sequences included in the phylogenetic analyses. The present analyses show some instances of paraphyly among closely related species, lineages, and morphotypes considered distinct in previous publications, but these other studies also used additional genes and/or morphometrics to support their conclusions. Of importance, the *C. marginatum/C.attenuatum* clade resolved without ambiguity and with strong support in both of our analyses. However, because of the close similarity of the ITS genetic sequence among some species, we conservatively identify the unknown as *C. cf. marginatum*.

As far as distinguishing between metacercariae of North American *Clinostomum* spp. based solely on morphological characters, there are not many reliable features to help tell them apart, despite information from multiple hosts (primarily fish). In addition, this is further complicated by the fact that metacercaria of *Clinostomum poteae* Rosser, Baumgartner, Alberson, Noto, Woodyard, King, Wise, and Griffin, 2018 has not yet, to date, been described from any intermediate host (see Rosser et al., 2018). However, we identified the parasite as *C. marginatum* Rudolphi, 1819 based on morphological characteristics that help distinguish its metacercariae from metacercariae of *Clinostomum attenuatum* Cort, 1913. For example, the placement of the primordial genitalia within the posterior half of the body and the uniformity of the body widths in *C. attenuatum* (Miller et al., 2014) differs from *C. marginatum*. Caffara et al. (2011) reported that metacercariae of *Clinostomum complanatum* Rudolphi, 1814 can be distinguished from metacercariae of *C. marginatum* by the distance between the ventral and oral sucker and by the width of the body; however, they did not investigate different stages of metacercarial development. Further, current literature (Caffara et al., 2011; Rosser et al., 2018) suggests that *C. complanatum* is a European species and is not presently found in hosts from the Americas. Unfortunately, metacercariae of *Clinostomum album* Rosser, Alberson, Woodyard, Cunningham, Pote and Griffin, 2017, are morphologically ambiguous and ranges for most morphological characters used to describe metacercaria overlap with *C. marginatum* (see Rosser et al., 2017, 2018).

Our survey results identified 622 individual *E. chisholmensis* from the 3 springs (Cobbs [n = 384], Cowan [n = 165] and Twin [n = 73]). Three of 622 (0.5%) *E. chisholmensis* were found to be infected with metacercariae. This included, 2 (0.5%) salamanders from Cobbs Spring and 1 (0.6%) from Cowan Spring; none of the 73 specimens from Twin Springs were infected. One salamander from Cobbs Spring (32.3 mm SVL) had 5 metacercariae in its trunk and tail and the Cowan Spring salamander (25.7 mm SVL) had a single metacercaria in its tail. Both of these specimens were alive and released unharmed but were never recaptured. The other Cobbs Spring salamander (33.0 mm SVL) was found dead, and it harbored 6 total metacercariae, 4 on the head region (including 1 behind the left eye), 1 near the left front leg, and 1 in the tail (Fig. 2).

In conclusion, we document the first parasite (*C*. cf. *marginatum*) from *E*. *chisholmensis* and accession both voucher specimens and a molecular sequence. The low observed infection prevalence in *E*. *chisholmensis* (0.5%) is similar to previously reported values from some other Eurycea including Valdina Farms salamander, *E*. *troglodytes* Baker (reported as *E*. *neotenes*) in Real County, Texas (1.4%; McAllister 1990), cave salamander, *E*. *lucifuga* Rafinesque, in Tennessee (0.2%; McAllister et al., 2007), *E*. *tonkawae* in Williamson County, Texas (0.4%; McAllister et al., 2007), E. tonkawae in Williamson County, Texas (0.4%; McAllister et al., 2018). However, higher prevalences have been reported for *C*. *marginatum* in 2 other *Eurycea* spp., including Oklahoma salamander, *E*. *tynerensis* Moore and Hughes, and

grotto salamander, *E. spelaea* Stejneger in Cherokee County, Oklahoma (56% and 8.3%, respectively; Bonett et al., 2011). Examination of additional endemic spring-inhabiting *Eurycea* spp. of the Edwards Plateau in Texas (Tipton et al., 2012) for *C. marginatum* and other helminths is certainly warranted.

We thank the Williamson County Conservation Foundation and PulteGroup, Inc., for funding, the landowners for site access, Andrew MacLaren for assistance with recapture identifications, and Drs. Scott L. Gardner and Gabor Racz (HWML) for expert curatorial assistance. Salamanders were collected under IACUCs 1202_0123_02 and 0417_0513_07, Texas Parks and Wildlife Department Scientific Collecting Permits SPR-0102-191 and SPR-0319-056, and U.S. Fish and Wildlife Federal Fish and Wildlife Permits TE039544-1 and TE37416B-0.

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Figure Legends

Figure 1. Bayesian consensus phylogram of 77 *Clinostomum* ITS sequences rooted with *Ithyoclinostomum*. All Bayesian posterior probabilities and maximum likelihood (RAxML) bootstrap support values >70 are provided. Bayesian posterior probabilities are labeled above branches and RAxML bootstrap support values are labeled below branches. Some labels occur at nodes for figure clarity. *Clinostomum* cf. *marginatum* MW261284 sequence from *Eurycea chisholmensis* is bolded.

Figure 2. *Clinostomum* cf. *marginatum* from a salvaged *Eurycea chisholmensis* from Cobbs Spring, Williamson County, Texas. **A.** View showing host with encapsulated metacercaria in head, trunk, and tail (arrows). **B.** View of head showing deformation due to encapsulated metacercaria. **C.** *Clinostomum* cf. *marginatum* (arrow) dissected out of the salamander's body near the left, front leg. **D.** Encapsulated metacercaria behind the salamander's left eye (arrow). Scale bars = 5 mm.

Table 1. PCR primers used to amplify ITS1+5.8S+ITS2+28S gene fragments, fragment size

(number of base)	pairs), and anne	aling temperatures	for	Clinostomum sp.
(number of buse	pull by, und unne	uning temperatures	101	cunoscontant sp.

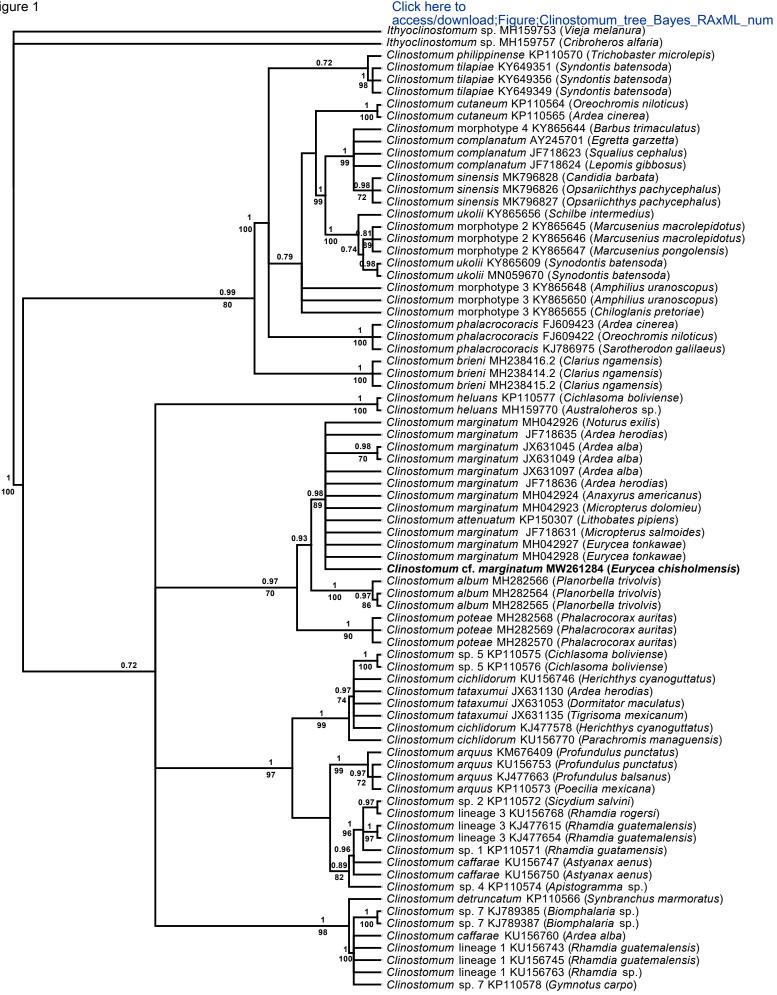
Primer Name	Gene Fragment	Sequence	Size	Annealing Temperature (C)
NC5	ITS1+5.8S+ITS2+28S	TTAGTTTCTTTTCCTCCGCT	1300	52
NC2	ITS1+5.8S+ITS2+28S	GTAGGTGAACCTGCGGAAGGATCATT	1300	52
D1	ITS1+5.8S+ITS2rDNA	AGGAATTCCTGGTAAGTGCAAG	1100	55
D2	ITS1+5.8S+ITS2rDNA	CGTTACTGAGGGAATCCTGG	1100	55

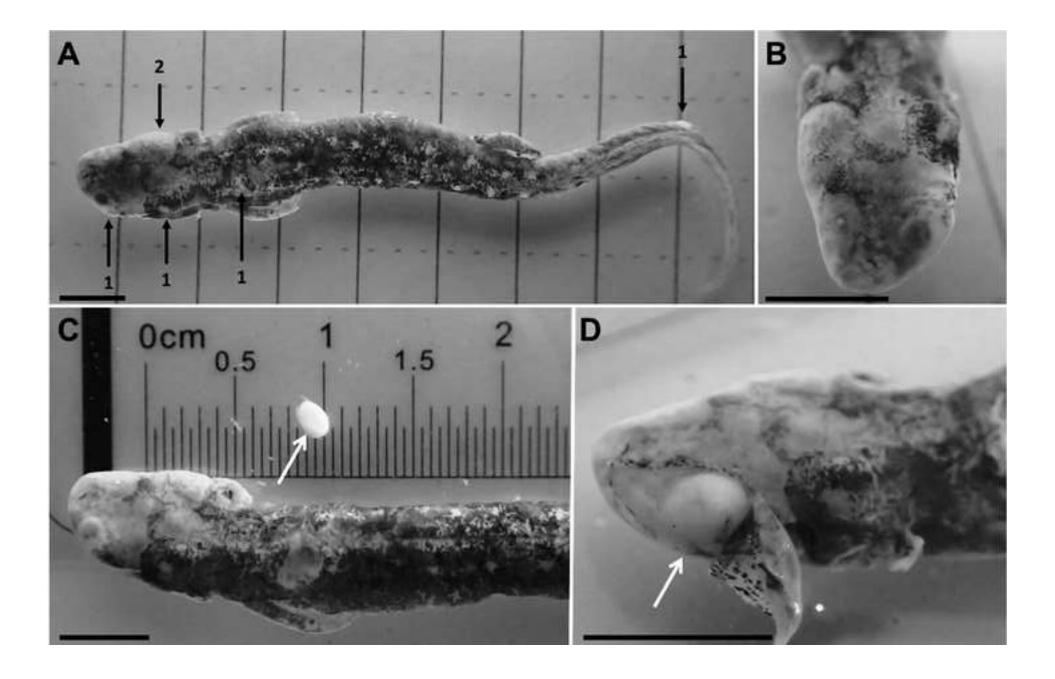
Species, lineage, or		
morphotype	GenBank accession number and citation source	
Clinostomum album	MH282564–MH282566 (Rosser et al., 2018)	
Clinostomum arquus	KJ477663, KM676409, KU156753 (Pérez-Ponce de León et al., 2016); KP110573 (Locke et al., 2015)	
Clinostomum attenuatum	KP150307 (Locke et al., 2015)	
Clinostomum brieni	MH238414.2–MH238416.2 (Caffara et al., 2019)	
Clinostomum caffarae	KU156747, KU156750, KU156760 (Pérez-Ponce de León et al., 2016)	
Clinostomum cichlidorum	KJ477578, KU156746, KU156770 (Pérez-Ponce de León et al., 2016)	
Clinostomum	AY245701 (Dzikowski et al., 2004); JF718623–JF718624 (Caffara et	
complanatum	al., 2011)	
Clinostomum	KP110564–KP110565 (Locke et al., 2015 [from Gustinelli et al.,	
cutaneum	2010])	
Clinostomum detruncatum	KP110566 (Acosta et al., 2016)	
Clinostomum heluans	MH159770 (Briosio-Aguilar et al., 2018a, b); KP110577 (Locke et al. 2015)	
Clinostomum marginatum	JF718631, JF718635, JF718636 (Caffara et al., 2011); JX631045, JX631049, JX631097 (Sereno-Uribe et al., 2013); MH042923-MH042924, MH042926–MH042928 (McAllister et al., 2018)	
Clinostomum	FJ609422–FJ609423 (Gustinelli et al., 2010); KJ786975 (Caffara et al.	
phalacrocoracis	2014)	
Clinostomum philippinensis	KP110570 (Locke et al., 2015)	
Clinostomum poteae	MH282568–MH282570 (Rosser et al., 2018)	
Clinostomum sinensis	MK796826–MK796828 (Locke et al., 2019)	
Clinostomum tataxumui	JX631053, JX631130, JX631135 (Sereno-Uribe et al., 2013)	
Clinostomum tilapiae	KY649349, KY649351, KY649356 (Caffara et al., 2017)	
Clinostomum ukolii	KY865609, KY865656 (Caffara et al., 2017); MN059670 (Caffara et al., 2020)	
<i>C</i> . sp. 1*	KP110571 (Locke et al., 2015)	
<i>C</i> . sp. 2*	KP110572 (Locke et al., 2015)	
<i>C</i> . sp. 3*	= <i>C. arguus</i> (Sereno-Uribe et al., 2018)	
<i>C</i> . sp. 4*	KP110574 (Locke et al., 2015)	
<i>C</i> . sp. 5*	KP110575–KP110576 (Locke et al., 2015)	
<i>C</i> . sp. 6*	= <i>C. heluans</i> (Briosio-Aguilar et al., 2018a,b)	
<i>C</i> . sp. 7*	KJ789385, KJ789387 (Locke et al., 2015 [from Pinto et al., 2015]); KP110578 (Locke et al., 2015)	
<i>C</i> . sp. 8*	= <i>C. sinensis</i> (Locke et al., 2019)	
<i>C</i> . sp. 9*	no sequence available (Locke et al., 2019)	

Table 2. Species, accession number(s), and citation source for all GenBank ITS+5.8S+ITS2+28S sequences used in the phylogenetic analyses.

C. lineage 1†	KU156743, KU156745, KU156763 (Pérez-Ponce de León et al., 2016)
C. lineage 2†	= <i>C. caffarae</i> (Sereno-Uribe et al., 2018)
C. lineage 3†	KJ477615, KJ477654, KU156768 (Pérez-Ponce de León et al., 2016)
C. lineage 4†	= <i>C. arquus</i> (Sereno-Uribe et al., 2018)
C. lineage 5†	= <i>C. cichlidorum</i> (Sereno-Uribe et al., 2018)
C. morphotype 1‡	<i>=C. ukolii</i> (Caffara et al., 2020)
C. morphotype 2 [‡]	KY865645–KY865647 (Caffara et al., 2017)
<i>C</i> . morphotype 3‡	KY865648, KY865650, KY865655 (Caffara et al., 2017)
C. morphotype 4‡	KY865644 (Caffara et al., 2017)
Ithyoclinostomum sp.	MH159753, MH159757 (Briosio-Aguilar et al., 2018b)

**Clinostomum* sp. 1–9 identified by Locke et al. (2015). †*Clinostomum* lineages 1–5 identified by Pérez-Ponce de León et al. (2016). ‡ *Clinostomum* morphotypes 1–4 identified by Caffara et al. (2017).





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APPENDIX E

Investigation of Historic Georgetown Salamander (*Eurycea naufragia*) Sites on the Avant Property in Georgetown, Texas

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Investigation of Historic Georgetown Salamander (*Eurycea naufragia*) Sites on the Avant Property in Georgetown, Texas

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CAMBRIAN ENVIRONMENTAL

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April 2021

EXECUTIVE SUMMARY

We (Cambrian Environmental) conducted population monitoring activities for Georgetown Salamanders (*Eurycea naufragia*) at three historically occupied sites in Williamson County, Texas in 2020. These surveys were carried out on behalf of the Williamson County Conservation Foundation (WCCF) consistent with the biological goals and objectives of the Williamson County Regional Habitat Conservation Plan (Wilco RHCP) and in response to the results of a recent taxonomic revision of central Texas *Eurycea*.

This taxonomic reassignment of salamander populations in Williamson County caused a reduction in the number of Georgetown Salamander sites that are routinely monitored or academically studied. Additionally, the U.S. Fish and Wildlife Service recently proposed critical habitat for the Georgetown Salamander. These events highlighted the need for further research for this taxon.

This report details Georgetown Salamander surveys performed at Buford Hollow Spring, Capitol Aggregates Spring, and Cedar Breaks Hiking Trail Spring. Each of these springs are proposed critical habitat units. We conducted four monitoring events at each spring within an eight-day period. This allows us to apply capture-mark-recapture models for demographically closed populations to estimate the abundance and the probability of capturing and recapturing salamanders.

We observed salamanders at each site but not on every survey occasion. Models indicate that, during our surveys, Buford Hollow Spring harbored a small population of at most 10 salamanders, Capitol Aggregates Spring harbored at most 34 salamanders, and Cedar Breaks Hiking Trail Spring harbored at most 116 salamanders. We observed gravid females at every site.

We intend to repeat this approach at regular intervals, allowing for demographic parameter estimates that have not been calculated for Georgetown Salamanders to date. The results presented in this report represent the first of many steps intended to provide information on the ecology and natural history that is currently absent from the primary literature for this species.

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1.0 INTRODUCTION

We (Cambrian Environmental) expanded Georgetown Salamander (*Eurycea naufragia*) population monitoring activities to include three additional springs in Williamson County, Texas in 2020. These surveys were carried out on behalf of the Williamson County Conservation Foundation (WCCF) consistent with the biological goals and objectives of the Williamson County Regional Habitat Conservation Plan (Wilco RHCP) and in response to taxonomic revisions proposed by Devitt et al. (2019). We monitored salamander populations in accordance with the U.S. Fish and Wildlife (USFWS) protocol requirements (USFWS 2014b).

Prior to 2000, all Eurycea salamanders in the northern segment of the Edwards Aquifer were classified as the wide-ranging Texas Salamander (E. neotenes; Baker 1961, Sweet 1982). Chippindale et al. (2000) formally described Salado Salamanders (E. chisholmensis; SS), Georgetown Salamanders (E. naufragia; GTS), and Jollyville Plateau Salamanders (E. tonkawae; JPS) as distinct species from Texas Salamanders and other central Texas Eurycea based on comparisons of allozymes, mitochondrial DNA, and morphology. These three taxa constitute the Septentriomolge clade of central Texas Eurycea salamanders, and all are restricted to groundwaterfed aquatic habitats (i.e., springs, spring-fed creeks, and caves) associated with the northern segment of the Edwards Aquifer (Chippindale et al. 2000, Hillis et al. 2001). Chippindale et al. (2000) described JPS as occurring in Travis County north of the Colorado River and in southern Williamson County in the Brushy Creek drainage basin. GTS were restricted to springs in the San Gabriel River watershed in the vicinity of the City of Georgetown, and SS were restricted to the Salado Creek drainage in Bell County (Chippindale et al. 2000). However, Chippindale et al. (2000) acknowledged that some GTS populations in the Berry Creek drainage were not included in the genetic analyses and also had morphological features similar to SS, and that these populations were tentatively assigned to GTS based on proximity to other confirmed GTS locations. The genetic analyses of Devitt et al. (2019) taxonomically reassigned five GTS locations in Williamson County to SS (i.e., Bat Well Cave and Cobbs, Cowan, Walnut, and Twin Springs [Taylor Ray Hollow]). These locations included two of the three GTS long-term monitoring locations funded by the WCCF (Cobbs Spring and Twin Springs) and another site funded by Pulte Group, Inc. (Cowan Spring). Prior to the findings of Devitt et al. (2019), GTS were known from 16 sites. Currently, Garey Ranch, Shadow Canyon, Capitol Aggregates (Avant Spring), Swinbank, Buford Hollow, Cedar Breaks Hiking Trail, Crocket Garden Falls (Knight Spring), Cedar Hollow, Hogg Hollow (I & II) Springs, and Water Tank Cave are considered to be occupied by GTS. Therefore, Swinbank Spring is the only long-term monitoring location for GTS, and almost all ecological and population data for the taxon originate from this site alone.

The WCCF recognized the need to collect GTS data from other sites, and in satisfaction of the "adaptive management" guidelines of the 4(d) rule, negotiated access and is now funding surveys at three additional GTS springs. With the addition of these extra sites, the WCCF is currently funding research and monitoring of GTS at four of the nine proposed critical habitat units (CHUs; USFWS 2020). Herein, the results of the first set of surveys at the newly added sites are presented and compared to historical data.

2.0 METHODS

2.1 Site Descriptions

2.1.1 Buford Hollow Spring

Buford Hollow Spring (N 30.659063°, W 97.728310°) is a permanent spring located on the western bank of Buford Hollow, a tributary of the North Fork San Gabriel River. The spring occurs within proposed CHU #5 (Figures 1; USFWS 2020). The spring has a low rate of discharge, and the spring run consists of approximately 30 m of woody debris with sparse cobble embedded into a silt and mud substrate (Figure 2). The site is impacted by cattle and appears to flash flood. Dominant canopy surrounding the spring consists of Privet (*Ligustrum sp.*), Chinese Tallow (*Triadeca sebifera*), Carolina Buckthorn (*Rhamnus caroliniana*), Yaupon Holly (*Ilex vomitoria*), Pecan (*Carya illinoinensis*), Green Ash (*Fraxinus pennsylvanica*), and Cedar Elm (*Ulmus crassifolia*). This location is the type locality for GTS.

2.1.2 Capitol Aggregates (Avant) Spring

Capitol Aggregates Spring (N 30.646195°, W 97.737744°) is a permanent spring located on the northern bank of the Middle Fork San Gabriel River. The spring occurs within proposed CHU #7 (Figure 1; USFWS 2020). The spring is comprised of a collection of groundwater outlets that begin just above the resting level of the Middle Fork San Gabriel River and continue up the steep canyon wall approximately 3 m. The spring run is formed of a series of small ledges, fringed with Maidenhair Fern (*Adiantum* sp.) and waterfalls descending the steep canyon wall. It forms a silt bottomed run 1 - 2 m long containing rooted aquatic vegetation and leaf litter, then continues into a small but deep silt bottomed pool of lilies, ultimately forming a narrow 15 m long run containing cobble and woody debris that continues until its confluence with the Middle Fork San Gabriel River (Figure 3).

A second spring (N 30.646264°, W 97.738791°) occurs approximately 100 m upstream of the spring referenced by the USFWS (USFWS 2020). This additional spring falls outside of the 80 m boundary delineating the extent of the proposed surface CHU, but within the 300 m buffer suggested to include the extent of the underground area occupied by the salamander population observed downstream. This spring also emerges from the northern bank of the Middle Fork San Gabriel River and forms a spring run approximately 75 m long before its confluence with the river. This spring has a large discharge and flows rapidly, forming a series of pools and waterfalls before widening into a springrun with rapid but shallow flow. It is predominantly formed of cobble structure loosely embedded in silt substrate, but also contains portions bordered with aquatic vegetation where cobble does not occur (Figure 4). Capitol Aggregates Spring is also referred to as Capital Aggregates and Avant Spring throughout historical records and literature.

2.1.3 Cedar Breaks Hiking Trail Spring

Cedar Breaks Hiking Trail Spring (N 30.659962°, W 97.750637°) is a permanent spring located just south of Lake Georgetown and serves as the headwaters of an unnamed tributary that flows north into the lake. This spring, along with Knight (Crocket Garden) Spring, form the Lake Georgetown proposed CHU #4 (Figures 1; USFWS 2020). Cedar Breaks Hiking Trail Spring

originates at the base of a large sycamore tree (*Platanus occidentalis*) in the center of a deep limestone canyon. The spring run begins as 4 m of short networked channels that confluence into a single narrow, deep channel that continues for an additional 20 m. From 20 m to 46 m downstream the run widens into a bedrock glide with slow, shallow flow and few cover objects with the exception of seasonally occurring leaf litter (Figure 5). The spring run ends in a large deep pool containing aquatic vegetation and predatory fish. At approximately 55 m downstream there are several other small groundwater outlets (orifices) that weep from the western bank of the channel into the deep pool. The springrun is frequented by cattle.

2.2 Monitoring Methods

2.2.1 Abiotic Monitoring

In accordance with USFWS (2014b) survey protocol, we report the air temperature during each survey event, the previous week's maximum and minimum air temperature, the total rainfall on the survey day, and the average daily rainfall for the previous week. We obtained temperature and rainfall data from the National Oceanic and Atmospheric Administration (https://www.noaa.gov) for Georgetown, Texas (Station USC00413507).

During each *Eurycea* survey we collected water quality and substrate data in accordance with USFWS (2014b) survey protocol. We recorded water temperature, pH, specific conductivity, and dissolved oxygen (DO) at each spring orifice per site. We measured water temperature and conductivity with a Com-100 from HM Digital (Culver City, California, USA), pH with a EcoTestr pH 2 from Oakton Instruments (Vernon Hills, Illinois, USA), and DO with a HI 9147 from Hanna Instruments. We additionally recorded water depth, substrate type (e.g., silt, gravel), and estimated the average embeddedness of cover objects (see Sennatt et al. 2006, USFWS 2014b) during each survey.

2.2.2 Salamander Monitoring

During each survey, we methodically searched the spring run for salamanders by overturning or searching through potential cover objects (i.e., cobble, vegetation, leaf litter, woody debris), and attempting to capture all *Eurycea* observed. On the first day of surveys, we deployed leaf litter traps to bolster cover objects when loose leaf litter was likely to be removed via our survey efforts. We began at the most downstream section and moved toward the spring orifice. We recorded the number of cover objects searched to quantify survey effort among survey dates. We also recorded the number, size class, and location (i.e., distance from nearest spring discharge) of all observed *Eurycea*. We standardize salamander counts as the percentage of cover objects with salamanders (i.e., number of salamanders observed divided by number of searched cover objects multiplied by 100; Pierce et al. 2010, 2014). We refer to this throughout as capture per unit effort (CPUE).

We captured salamanders using dip nets, sieves, or Hubbard rakes. Once captured, we measured each salamander using handheld calipers to the nearest 0.1 mm. We recorded the total length (TL; i.e., tip of snout to end of tail) and the snout vent length (SVL; i.e., tip of snout to posterior edge of the vent). We determined the gravidity of all captured *Eurycea* by visually checking for oocytes (eggs) through the salamander's translucent venter (Gillette and Peterson 2001, Pierce et al. 2014).

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If oocytes were present, we photographed the salamander's venter (Figure 6) and manipulated the body cavity to count oocytes. We took photos of the body and head of each salamander against a standardized grid background with the salamander in a water-filled dish. Pigmentation patterns on the head were used to identify recaptured salamanders using Wild-ID photographic recognition software (Figure 7; Bolger et al. 2012, Bendik et al. 2013). Photos were cropped and edited in ImageJ or Adobe Photoshop, to standardize the brightness, contrast, and orientation of each salamander within each photo. Cropped photos were uploaded into their respective site-specific Wild-ID database, then compared against all existing photos of previously captured salamanders. This allowed us to determine whether the new images represent recaptures of previously 'marked' individuals or individuals new to the study being 'marked' for the first time. Wild-ID reports photos from the top twenty potential matches from previous capture dates ranked by match probability. We review the first ten photos with the highest probability of being a potential match for every individual captured.

Capture-mark-recapture (CMR) studies are essential to acquiring demographic, life history, and behavioral data for listed species, and they allow the estimation of important population parameters when it is impractical to capture and count every individual. CMR studies require the identification and subsequent recognition of individuals in a study population. Results from Wild-ID enable us to create unique capture histories for each individual salamander encountered from each site and is much less invasive, easier, more time- and cost-effective than CMR studies using physical markers like visual implant elastomers (Bendik et al. 2013).

We are implementing a robust-design CMR study at these three sites (Kendall et al. 1997). Robustdesign CMR requires repeated "primary" survey periods per year that each have 3-4 surveys ("secondary" survey events) within a short period of time (e.g., two weeks). This survey cadence allows abundance to be estimated from each "primary" period and survival to be estimated in the timeframe between "primary" periods in addition to several other demographic parameters that may be of interest. We conducted four surveys within an 8-day window at each site, allowing for at least 1 day between each survey, serving as the first "primary" period. This survey frequency allows us to assume the population is demographically "closed" because births, deaths, and migration in and out of the population are not likely to occur between these secondary surveys. We recognize that it is possible for salamanders to move in and out of the subsurface environment even during this 8-day period. So, we consider the modeled population to include salamanders in the surface and near-surface environment. These four surveys serve as the first "primary" period towards robust-designed CMR; however, this approach requires a reasonably large sample of repeated "primary" periods to be useful. Thus, for this first primary period we used closepopulation models to generate estimates of abundance (N), capture probability (p), and recapture probability (c), for each site. We tested models with time-varying capture probability, a behavioral effect (trap-shy or trap-happy effect), a model with no behavior or time effect (null model), a model that allows for individual heterogeneity in capture probability, as well as a null model and behavioral model with the influence of salamander size (six total models). Models were ranked using Akaike's information criterion corrected for small sample size (AICc; Burnham & Anderson 2002). We report model averaged estimates of abundance and capture probability, when applicable.

3.0 RESULTS

3.1 Habitat Monitoring.—Climate conditions for each monitoring event and the previous week are presented in Table 1. Average water metrics at Buford Hollow Spring were 19.06 °C (temperature), 7.34 (pH), 6.24 mg/L (DO), and 472 μ S/cm (conductivity). Average water metrics for the downstream portion of Capitol Aggregates Spring were 18.88 °C (temperature), 7.14 (pH), 7.6 mg/L (DO), and 566.8 μ S/cm (conductivity). Average water metrics for the upstream portion of Capitol Aggregates Spring were 18.23 °C (temperature), 7.0 (pH), 8.88 mg/L (DO), and 845.3 μ S/cm (conductivity). Average water metrics at Cedar Breaks Hiking Trail Spring were 17.4 °C (temperature), 6.98 (pH), 5.32 mg/L (DO), and 690.6 μ S/cm (conductivity). Water chemistry metrics from each site, and individual spring outlets, are presented in Table 2.

3.2 Salamander Monitoring.—At Buford Hollow Spring, we searched a total of 1,713 cover objects and observed seven and captured seven total GTS during the four salamander surveys in 2020. Counts ranged from 1-3 salamanders during each survey, CPUE was 0.41 salamanders per 100 objects searched. We captured a single gravid female that contained 15 large oocytes. At Capitol Aggregates Spring we searched a total of 2,721 cover objects and observed 22 and captured 22 GTS during the four salamander surveys in 2020. Counts ranged from 5-6 salamanders during each survey, CPUE was 2.03 salamanders per 100 objects searched (within the downstream portion alone). We never observed a salamander within the upstream most springrun at this site. We captured four gravid females, and oocyte counts ranged from 9-21. At Cedar Breaks Hiking Trail Spring we searched 3,830 cover objects and observed 92 and captured 85 total GTS during the four surveys within 2020. Counts ranged from 11-30, and CPUE was 2.4 salamanders per 100 objects searched. We observed three gravid females, and oocyte counts ranged from 15-18. Daily data for counts, observations, CPUE, and gravidity are presented in Table 3. We collected genetic samples (i.e., tail clips) from a number of animals at each site, including a whole specimen that was found deceased (Table 4).

Results from Wild-ID photographic recognition reveal we captured four individuals seven times at Buford Hollow Spring, and the most any individual was captured was three times. We 'marked' 1, 1, 2, and 0, new individuals on each survey respectively. At Capitol Aggregates we captured 16 individuals 22 times, and captured numerous individuals up to twice each. We 'marked' 5, 6, 3, and 2, new individuals on each survey respectively. At Cedar Breaks Hiking Trail Spring we captured 61 animals 85 times, two individuals were captured during all surveys and numerous others were captured on three occasions. We 'marked' 20, 19, 17, and 5, new individuals on each survey respectively.

With so few captures, no clear top model was recovered for Buford Hollow Spring. The null model (no variation in p, p=c) both without and with the influence of salamander body size, as well as the behavior model (p differs from c, but both are constant among occasions) contributed to model averaged estimates. The model with individual heterogeneity did not converge, and was removed from our analysis for this site. Model averaged abundance was estimated to be 5.43 ± 5.4

salamanders, indicating very little confidence in our immediate results. Probability of capture was estimated (via the null model) to be 0.4375 and did not differ from recapture probability.

We observed similar results at Capitol Aggregates Spring. No model was found to clearly outperform the others, and the same three models presented for the Buford Hollow results contribute to model averaged estimates at this site. The model with individual heterogeneity did not converge, and was removed from our analysis for this site. Model averaged abundance was estimated to be 25.83 ± 8.79 salamanders. Probability of capture was estimated (via the null model) to be 0.2228 and did not differ from recapture probability.

Cedar Breaks Hiking Trail is the only site for which we recovered all models tested, likely due to large number of captures. Time varying capture probability (while p=c) was found to be the best fit model. The model averaged abundance estimate was 97.29 ± 17.78 salamanders. Probability of capture (and thus, recapture probability) varied by survey occasion, and was estimated to be 0.212, 0.286, 0.297, and 0.106, for each survey, respectively.

4.0 DISCUSSION

4.1 Habitat Monitoring

Water temperature was comparable at all three sites we monitored on the Avant family tract. Buford Hollow had the highest water temperature, likely due to the shallow depth and extremely low flow at this site. No site demonstrated temperatures exceeding 25 °C, and never exceeded the upper thermal tolerance of central Texas *Eurycea* salamanders (Crow et al. 2016). Among sites, pH followed the same trend as temperature, but did not demonstrate much variability (Table 2). Although we did not directly measure flow velocity, it is readily apparent that both dissolved oxygen and conductivity are elevated at springs with the greatest flow and decrease along this trend in the following order: Capitol Aggregates Spring (upstream), Capitol Aggregates Spring (downstream), Cedar Breaks Hiking Trail Spring, and Buford Hollow Spring (Table 2).

4.2 Salamander Monitoring

Previous work has demonstrated that captures of new GTS individuals diminish as surveys are repeated during periods of "closure" (Pierce et al. 2014). We observed this pattern at two of the three sites on the Avant family tract. Buford Hollow did not follow this pattern, but resulted in only four individual animals being captured, likely too few to allow any pattern to be observed. CPUE was similar at Capitol Aggregates and Cedar Breaks Hiking Trail Springs, indicating that given the disparity regarding the length and area of these springruns they have similar density of salamanders.

Among the three occupied springs we monitored on the Avant Family tract, Cedar Breaks Hiking Trail Spring harbors the largest population. CMR models indicate 15-51 unobserved animals may occur at this site. Thus, population size could range from 80-116 salamanders. Despite the large number of unobserved individuals, probability of detection was generally higher at this site than other *Eurycea* sites monitored on behalf of the WCCF (Cambrian 2021). We observed survey

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(time) dependent probability of capture (and recapture), which can be interpreted in a number of ways. Perhaps as surveyors become more familiar with this site, they become more effective at finding salamanders. Another possibility is that the use of leaf litter traps allowed for salamanders to be better observed on surveys 2-4 at a different rate than initial surveys.

At Buford Hollow Spring and Capitol Aggregates Spring (downstream) CMR models indicate that its possible that between 0-6 and 1-18 unobserved salamanders, respectively, may occur at these sites. Buford Hollow Spring harbors very few salamanders, and among all sites monitored they are the easiest to find (Cambrian 2021). Probability of capture at this site is more than twice what we observe for the remaining sites. This is a reflection of the small area the site occurs in, and the extremely low density of salamanders that occur here. That is to say, if observable animals occur on any given survey date, we are very likely to find them. Sample sizes at this site are truly too low to support the type of models we attempted to apply, so results should be accepted with caution. Salamanders are less likely to be found at Capitol Aggregate Spring, but the habitat they occupy at this site is highly variable and presents challenges to surveyors that are unlike many other *Eurycea* occupied sites.

Records indicate GTS were collected from these sites between 1991 and 1998. The current estimated population size from Buford Hollow Spring and Capitol Aggregates Spring compare favorably to these past collection numbers, while the results at Cedar Breaks Hiking Trail demonstrate a large population increase compared to past voucher numbers (Table 5). We do note that the voucher records may not be equivalent to all observations, but observation data are not included in these records. Further, at the time of listing, salamanders had not been observed at Buford Hollow Spring in over a decade, and only a few recent observations occurred at Capitol Aggregates Spring (USFWS 2014a). Chippindale et al. (2000) stated that A.H. Price determined that quarrying activities conducted by Capitol Aggregates were unlikely to jeopardize recharge and spring flow in the area. We do not have historic discharge data for comparison, but all springs were discharging at a rate comparable to other small- to medium-sized springs in Williamson County. Further, Price et al. (1999) predicted long-term viability for salamander populations on the south shore of Lake Georgetown unless unrestricted development occurred. Comparable and increased observations over these 22-29 years appear to support this prediction. We believe capture success and overall surface population size could be improved at these sites with some restoration effort.

These sites are all proposed to be critical habitat for the GTS, and these data represent the first proper estimate of population size, albeit at a minimal timescale, for any proposed CHU with the exception of Swinbank Spring (Cambrian 2020, 2021). The continuation of monitoring and eventual robust CMR modeling will provide population estimates that are currently absent from the GTS literature and critical for proper conservation planning.

5.0 KEY PERSONNEL

Andrew R. MacLaren, Ph.D.—Senior Ecologist

Andrew has over seven years of experience in threatened and endangered wildlife ecology. His dissertation research focused on utilizing technological innovations to address issues pertinent to the conservation policy and management of the federally endangered Houston Toad (*Anaxyrus houstonensis*). Issues addressed within his research include evaluating the efficacy of the current federal protocol for conducting presence absence surveys for Houston Toads. His research on automated detection of Houston Toad vocalizations has received multiple awards, and was ultimately published in the *Journal of Fish and Wildlife Management*. Further research evaluating habitat induced bias in acoustic surveys for vocalizing birds and anurans has been published in *Ecology and Evolution*. Additional contributions to peer-reviewed research include best management practices and avoidance of impacts related to development within Houston Toad occupied habitat, as well as management of invasive aquatic vegetation occupied by the federally threatened San Marcos Salamander and new distribution records for Jollyville Plateau Salamanders.

Zachary C. Adcock—Senior Ecologist

Zach has over 15 years of experience in threatened and endangered wildlife ecology. He has conducted work on 27 federally listed species and many more state listed taxa in Texas and Florida with an emphasis on herpetofauna. His overarching specialties include threatened and endangered species research, surveys, habitat and population assessments, management plan development, and best management practices (BMPs). Zach is an expert on central Texas *Eurycea* salamanders with eight years of research and survey experience across seven species. His dissertation was designed to inform the ecology, conservation policy, and management of Jollyville Plateau Salamanders. He has over 15 peer-reviewed wildlife publications, including manuscripts on Jollyville Plateau, Georgetown, Salado, San Marcos, and Fern Bank Salamanders and Houston Toads.

Kemble White, Ph.D., P.G.—Owner, Senior Geoscientist

Kemble has served for over 20 years as senior geologist, karst specialist, and project manager in central Texas. Kemble specializes in the Endangered Species Act and water quality regulations as they pertain to caves, springs and the Edwards Aquifer. Kemble's doctorate was in biospeleology, the study of cave ecology, and his dissertation was one of the first involving central Texas endangered karst invertebrates. His research has been published in *Geology*, one of the world's flagship peer-reviewed scientific journals. He has discovered many new locations for rare and endangered species and two new species have been named in his honor. As a co-author of the Regional Habitat Conservation Plan/Environmental Impact Statement (RHCP/EIS), Kemble has had a direct hand in RHCP planning and implementation. Kemble is a licensed professional geoscientist and holds the applicable USFWS permits for working with threatened and endangered karst species in the Austin-San Antonio growth corridor. He has been working with *Eurycea* in Williamson County since 1999.

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7.0 TABLES

Table 1. Climate data for each 2020 <i>Eurycea</i> salamander survey on the Avant Property conducted as part of the Williamson
County Regional Habitat Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site	Survey Date	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)	Previous Week Maximum Temperature (°C)	Previous Week Minimum Temperature (°C)	Daily Rainfall (cm)	Previous Week Cumulative Rainfall (cm)
E. naufragia	Buford Hollow	30-Sep-2020	NA	NA	30.6	12.8	NA	10.0
E. naufragia	Buford Hollow	16-Oct-2020	29.4	16.7	32.8	13.3	0.0	0.0
E. naufragia	Buford Hollow	19-Oct-2020	31.1	20.0	32.8	13.3	0.0	0.0
E. naufragia	Buford Hollow	21-Oct-2020	30.6	20.6	32.8	13.3	0.0	0.0
E. naufragia	Buford Hollow	23-Oct-2020	30.0	21.1	32.8	13.3	0.0	0.0
E. naufragia	Capitol Aggregates	30-Sep-2020	NA	NA	30.6	12.8	NA	10.0
E. naufragia	Capitol Aggregates	16-Oct-2020	29.4	16.7	32.8	13.3	0.0	0.0
E. naufragia	Capitol Aggregates	19-Oct-2020	31.1	20.0	32.8	13.3	0.0	0.0
E. naufragia	Capitol Aggregates	21-Oct-2020	30.6	20.6	32.8	13.3	0.0	0.0
E. naufragia	Capitol Aggregates	23-Oct-2020	30.0	21.1	32.8	13.3	0.0	0.0
E. naufragia	Cedar Breaks Hiking Trail	30-Sep-2020	NA	NA	30.6	12.8	NA	10.0
E. naufragia	Cedar Breaks Hiking Trail	2-Nov-2020	23.3	8.9	26.1	2.8	0.0	0.7
E. naufragia	Cedar Breaks Hiking Trail	4-Nov-2020	25.6	6.1	26.1	2.8	0.0	0.7
E. naufragia	Cedar Breaks Hiking Trail	6-Nov-2020	26.7	13.3	25.6	2.8	0.0	0.4
E. naufragia	Cedar Breaks Hiking Trail	9-Nov-2020	25.6	13.9	26.7	6.1	0.0	0.0

Table 2. Habitat data (water conditions) for each 2020 Eurycea salamander survey on the Avant Property conducted as part of
the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site/Spring	Survey Date	Temperature (°C)	рН	Dissolved Oxygen (mg/L)	Specific Conductance (µS/cm)	Water Depth (cm)
E. naufragia	Buford Hollow	30-Sep-2020	18.4	7.4	5.4	479	5.0
E. naufragia	Buford Hollow	16-Oct-2020	19.0	7.3	5.6	473	4.0
E. naufragia	Buford Hollow	19-Oct-2020	19.1	7.3	9.1	472	6.5
E. naufragia	Buford Hollow	21-Oct-2020	19.3	7.3	5.7	467	3.0
E. naufragia	Buford Hollow	23-Oct-2020	19.5	7.4	5.4	469	6.0
E. naufragia	Capitol Aggregates - up	30-Sep-2020	18.3	6.9	9.6	835	8.0
E. naufragia	Capitol Aggregates - up	16-Oct-2020	18.1	7.0	9.2	855	7.0
E. naufragia	Capitol Aggregates - up	19-Oct-2020	NA	NA	NA	NA	NA
E. naufragia	Capitol Aggregates - up	21-Oct-2020	18.2	7.0	8.7	853	5.0
E. naufragia	Capitol Aggregates - up	23-Oct-2020	18.3	7.1	8.0	838	6.0
E. naufragia	Capitol Aggregates - down	30-Sep-2020	18.7	7.2	9.8	569	5.0
E. naufragia	Capitol Aggregates - down	16-Oct-2020	21.1	7.3	7.9	570	3.0
E. naufragia	Capitol Aggregates - down	19-Oct-2020	18.0	6.9	6.8	569	0.5
E. naufragia	Capitol Aggregates - down	21-Oct-2020	18.8	7.0	6.0	567	4.0
E. naufragia	Capitol Aggregates - down	23-Oct-2020	19.3	7.3	7.5	559	3.0
E. naufragia	Cedar Breaks Hiking Trail	30-Sep-2020	18.5	7.1	4.7	689	8.0
E. naufragia	Cedar Breaks Hiking Trail	2-Nov-2020	16.1	6.9	5.2	692	8.0
E. naufragia	Cedar Breaks Hiking Trail	4-Nov-2020	16.3	7.0	4.6	691	5.0
E. naufragia	Cedar Breaks Hiking Trail	6-Nov-2020	18.0	6.9	6.2	691	6.0
E. naufragia	Cedar Breaks Hiking Trail	9-Nov-2020	18.1	7.0	5.9	690	6.5

<i>Eurycea</i> Species	Site/Spring	Survey Date	Number of Cover Objects Searched	Number of <i>Eurycea</i> Observed	Number of <i>Eurycea</i> Captured	Percentage of Cover Objects with Salamanders	Number of Gravid <i>Eurycea</i>	Percentage of Captures that were Gravid
E. naufragia	Buford Hollow	30-Sep-2020	NA	NA	NA	NA	NA	NA
E. naufragia	Buford Hollow	16-Oct-2020	558	1	1	0.18	0	0.0
E. naufragia	Buford Hollow	19-Oct-2020	439	2	2	0.46	1	50.0
E. naufragia	Buford Hollow	21-Oct-2020	430	3	3	0.70	0	0.0
E. naufragia	Buford Hollow	23-Oct-2020	286	1	1	0.35	1	100.0
E. naufragia	Capitol Aggregates - up	30-Sep-2020	NA	NA	NA	NA	NA	NA
E. naufragia	Capitol Aggregates - up	16-Oct-2020	933	0	0	0.00	0	0.0
E. naufragia	Capitol Aggregates - up	19-Oct-2020	215	0	0	0.00	0	0.0
E. naufragia	Capitol Aggregates - up	21-Oct-2020	314	0	0	0.00	0	0.0
E. naufragia	Capitol Aggregates - up	23-Oct-2020	174	0	0	0.00	0	0.0
E. naufragia	Capitol Aggregates - down	30-Sep-2020	NA	NA	NA	NA	NA	NA
E. naufragia	Capitol Aggregates - down	16-Oct-2020	238	5	5	2.10	1	20.0
E. naufragia	Capitol Aggregates - down	19-Oct-2020	342	6	6	1.75	1	16.7
E. naufragia	Capitol Aggregates - down	21-Oct-2020	276	6	6	2.17	0	0.0
E. naufragia	Capitol Aggregates - down	23-Oct-2020	229	5	5	2.18	2	40.0
E. naufragia	Cedar Breaks Hiking Trail	30-Sep-2020	NA	NA	NA	NA	NA	NA
E. naufragia	Cedar Breaks Hiking Trail	2-Nov-2020	964	23	20	2.39	0	0.0
E. naufragia	Cedar Breaks Hiking Trail	4-Nov-2020	924	30	27	3.25	2	7.4
E. naufragia	Cedar Breaks Hiking Trail	6-Nov-2020	1054	28	28	2.66	1	3.6
E. naufragia	Cedar Breaks Hiking Trail	9-Nov-2020	888	11	10	1.24	0	0.0

 Table 3. Results for each 2020 Eurycea salamander survey on the Avant Property conducted as part of the Williamson County

 Regional Habitat Conservation Plan (RHCP) support services.

Table 4. Data for each 2020 Eurycea salamander voucher from the Avant Property collected as part of the Williamson County Regional Habitat Conservation Plan (RHCP) support services.

<i>Eurycea</i> Species	Site	Date	Number	Voucher Type	Notes
E. naufragia	Buford Hollow	16-Oct-2020	1	tissue sample	tail clip
E. naufragia	Buford Hollow	10-Oct-2020	1	tissue sample	tail clip
E. naufragia	Buford Hollow	21-Oct-2020	2	tissue sample	tail clip
E. naufragia	Capitol Aggregates	16-Oct-2020	5	tissue sample	tail clip
E. naufragia	Capitol Aggregates	19-Oct-2020	6	tissue sample	tail clip
E. naufragia	Capitol Aggregates	21-Oct-2020	2	tissue sample	tail clip
E. naufragia	Cedar Breaks Hiking Trail	2-Nov-2020	19	tissue sample	tail clip
E. naufragia	Cedar Breaks Hiking Trail	4-Nov-2020	1	whole specimen voucher	found dead and whole specimen salvaged

Table 5. Historical voucher records for springs on the Avant Property in Georgetown, Williamson County, Texas.

<i>Eurycea</i> Species	Site	Survey Date	Researcher(s) ¹	Number of Vouchers	Museum and Identification ²
E. naufragia	Buford Hollow Spring	20 Jan 1991	D.M. Hillis	1	TNHC 51007
E. naufragia	Buford Hollow Spring	12 Dec 1991	P.T. Chippindale, A.H. Price	8	TNHC 51008 - 51015
E. naufragia	Buford Hollow Spring	14 Aug 1998	D.M. Hillis, L. Dries	4	TNHC 58860, 58861, 59691, 59692
E. naufragia	Capitol Aggregates Spring	17 Mar 1992	P.T. Chippindale, A.H. Price	10	TNHC 51023 – 51031, 55386
E. naufragia	Capitol Aggregates Spring	11 Mar 1995	J.R. Dixon	22	TCWC 71491 - 71512
E. naufragia	Cedar Breaks Hiking Trail Spring	13 Sep 1991	P.T. Chippindale, A.H. Price	9	TNHC 50999 – 51006, 57752

¹Researcher Affiliation

University of Texas (DMH, PTC, LD) Texas Parks and Wildlife Department (AHP) Texas A&M University (JRD)

² Museum Information

Texas Natural History Collections (TNHC) Herpetology is now known as the Biodiversity Collections at the University of Texas at Austin Texas Cooperative Wildlife Collection (TCWC) is now known as the Texas A&M Biodiversity Research and Teaching Collections

8.0 FIGURES

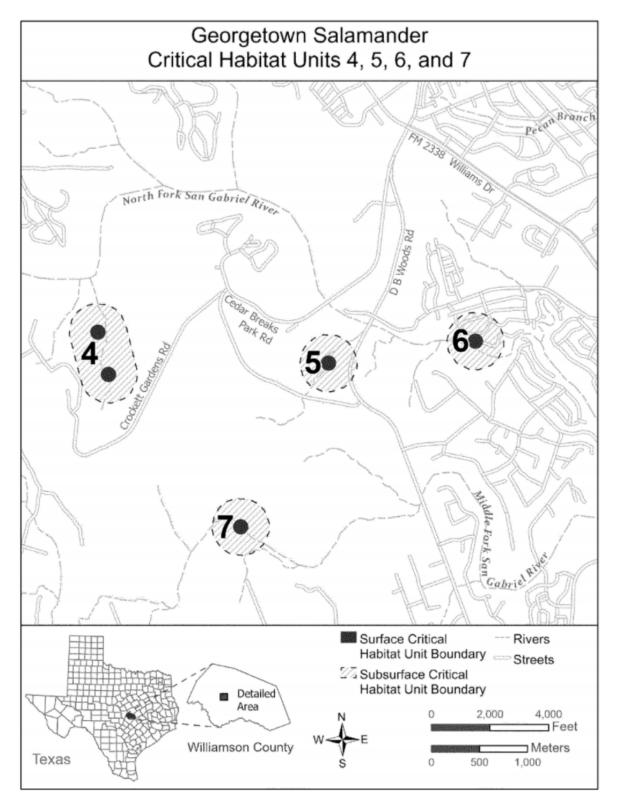


Figure 1. Location of Georgetown Salamander (*Eurycea naufragia*) proposed critical habitat units 4-7 within Williamson County, Texas (USFWS 2020). Units 4, 5, and 7 occur on the Avant family tract.



Figure 2. Buford Hollow Spring, September 2020, in context (left), and at the spring orifice (right).



Figure 3. Capitol Aggregates (Avant) Spring, downstream portion, September 2020. Cliffside pools (left), and silt bottomed pool below cliffside portions (right).



Figure 4. Capitol Aggregates (Avant) Spring, upstream portion, September 2020. Pool containing the spring orifice (left), and the springrun with rapid streamflow immediately following the orifice (right).



Figure 5. Cedar Breaks Hiking Trail Spring, September 2020. Upper four meters of the spring run, including multiple orifices (left), and the wide shallow bedrock glide following the first 20 meters (right).



Figure 6. Oocytes visible through the venter of a gravid female *Eurycea naufragia* from Swinbank Spring.

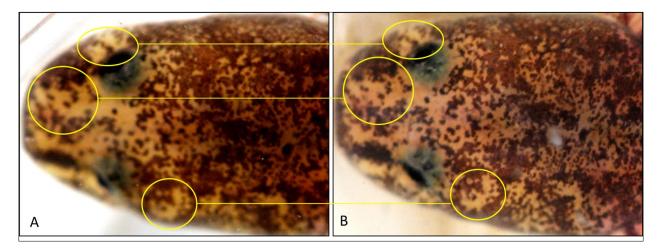


Figure 7. Melanophore recognition using the computer-assisted identification software Wild-ID. This individual from Cobbs Spring was captured first in April 2016 (A) and recaptured in May 2016 (B).

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APPENDIX F

Preliminary Results of a Dye Tract Study for Krienke Spring

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9 March 2021

Gary Boyd Environmental Program Coordinator Williamson County Conservation Foundation 219 Perry Mayfield Leander, Texas 78641

Re: Preliminary Results of a Dye Trace Study for Krienke Spring, Round Rock, Williamson County, Texas

Dear Gary,

This letter report summarizes our preliminary results for a dye trace study conducted between August 2019 and September 2020, in support of the Williamson County road bond program. The dye trace study was designed to establish groundwater connections between nearby recharge features and Tonkawa Spring (also known as Krienke Spring) which is within Jollyville Plateau Salamander (JPS) critical habitat unit (CHU 1) as mapped by the U.S. Fish and Wildlife Service (the Service). Groundwater tracing using non-toxic dyes to characterize recharge areas, as well as groundwater flow paths and velocities, is a common diagnostic tool in karst aquifers worldwide, and has been used successfully within the Barton Springs segment of the Edwards Aquifer¹. Little dye trace work has been done in the northern segment of the Edwards Aquifer, and virtually none has been done in Williamson County prior to this work.

Krienke Spring is located in the Tonkawa Springs neighborhood north of Brushy Creek and south of Sam Bass Road approximately two miles west of IH 35 in Round Rock. The alternate name for the Tonkawa Spring (Krienke Spring) is after the Krienke family, some of whom settled in the Round Rock area. This historic site is described in The Springs of Texas² as being 5 km (3.11 miles) west-northwest of the town of Round Rock with a discharge rate of 1.9 liters per second (30 gallons per minute) in 1940 and 1978. Presence of the JPS at the spring was cited by the Service when they listed the salamander as threatened and designated critical habitat in 2013. The Service stated with their listing that they seek to understand more about this spring site and other JPS springs sites because "*hydrology in Central Texas is very complex and information on hydrology of specific spring sites is largely unknown*". Because of this uncertainty, the Service designated a 300 m (984 ft) circular subsurface critical habitat unit around the spring. This preliminary dye trace study was intended to provide some clarity to the hydrogeology of Krienke Spring. Local hydrogeology and the reported constant flow dynamic of the spring indicate that the spring-shed must be large, extending well beyond the boundaries of CHU 1.

Cambrian Environmental began the study in August 2019 by developing a hydrogeological model of the subsurface drainage basin taking into account structural geology, surface topography and the prevailing groundwater flow direction (Figure 1). Krienke Spring issues from the water table portion of the Edwards

¹ Summary of Groundwater Tracing in the Barton Springs Edwards Aquifer from 1996 to 2017. <u>https://bseacd.org/uploads/Zappitello-et-al.-2019-Dye-Tracing-Summary.pdf</u>

² Brune, G. M. (2002). Springs of Texas (Vol. 1). Texas A&M University Press.



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Aquifer where the Edwards and Georgetown Limestones are exposed at the surface. Rainfall can infiltrate on these outcropping units and recharge the Edwards Aquifer. Krienke Spring is one of many low-flow springs in this setting that discharges from the base of Edwards Limestone above the Comanche Peak Formation which is stratigraphically below the Edwards Limestone and serves as the lower confining unit of the Edwards Aquifer. Flow at the spring is thought to also be bounded by the intersection with the Onion Fault, although we established sampling locations on the opposite side of the fault. Topographically our model confines recharge to the north side of the Dry Fork Creek since the spring issues from the north bank and because the Edwards Limestone has been removed by erosion along the bed of the creek. Honey Bear and Vista Oaks (HB/VO) springs to the north provide a logical place to draw a divide between spring sites since surface drainage flows south towards these springs in the upper drainages of Dry Fork Creek. Downstream of HB/VO springs, surface water flows to a reservoir (SCS Site 13a) where an outlet re-joins the Dry Fork Branch. The western boundary of our presumed Krienke springshed coincides with the westmost tributary of Dry Fork Branch above the base of the Edwards Aquifer outcropping units. We make the eastern boundary for both the Krienke and the HB/VO springsheds coincide with a topographic divide between the Dry Fork and Onion branches of Brushy Creek. Based on regional studies of the northern Edwards Aquifer and on potentiometric surface mapping conducted for a nearby hydrocarbon plume from an underground storage tank located at 1901 Hermitage Dr.³, we accept that the prevailing groundwater flow direction in the unconfined aquifer of Williamson County is northeasterly north of Brushy Creek. Our small-scale hydrogeologic model has a southern groundwater flow direction proximal to selected recharge features, and southeastern flow direction along the dip of Edwards Aquifer strata to the Krienke Spring outlet.

We discussed our model with Jason Krothe of Hydrogeology, Inc. who then developed protocols for a dye trace study. Sampling protocols, injection logistics and laboratory analyses were all provided by Hydrogeology, Inc. The study was designed to include injection into two recharge features, four sampling locations, three surface water locations and one well location. We selected two caves in the Walsh Ranch neighborhood that were 0.28 and 0.65 miles north of Krienke Spring. Walsh Ranch (WR) Cave 1 and Cave 2 are informally assigned names and not registered cave names in Williamson County (Figure 2). We were able to access these recharge features with permission from the Walsh Ranch municipal utility district (MUD). These two caves are the most proximal known potential recharge features to the spring.

The Williamson County Public Information Office assisted with communications with the local community and request for access to private land for water sampling. While permission for direct access to the spring was denied, receptors were deployed within the impounded section of Dry Berry Creek just downstream of the pond.

Sampling Methods

The primary sampling method for the dye traces was activated charcoal samples (ACS). An ACS consists of 10 grams of coconut shell carbon in a fiberglass mesh pouch. To establish a sampling station, an ACS packet was suspended by wire within the portion of the spring or stream with the highest visible flow. The

³ Hall Southwest, 1994. Potentiometric surface map for 7-Eleven store 25945 on 7/26/94. Available from the Texas Commission on Environmental Quality, LPST File 106895.



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ACS were typically left in place for one week. Grab water samples were the secondary sample method. Water samples were typically collected when an ACS packet was missing. Figures 3 and 4 are representative photos of Krienke Spring and of selected sampling locations.

Results

Pre-injection field reconnaissance site visits and initial background sampling were conducted in late 2019. The first round of dye trace work (dye trace 1) was conducted between January and March of 2020 (Tables 1 and 2). In accordance with the project plan, two rounds of background sampling were conducted before dye injections. Based on the results of the background sampling, the dyes Fluorescein and Rhodamine WT were selected for injection. Since the likely resurgence point (Krienke Spring) for these dye traces is in a neighborhood and flows into a recreational water body, the volume of dye used for injection was selected to prevent a strong dye visual at Krienke Spring. On the dates of the injections, the Williamson County road and bridge department provided valuable assistance by delivering a water truck that was used to flush the dye into the karst system during the injection. The dye injections were conducted in coordination with the Texas Commission on Environmental Quality (TCEQ) Edwards Aquifer Protection Program, the U.S. Fish and Wildlife Service, and coordination with the Texas Parks and Wildlife Department. TCEQ sent junior investigators to observe the dye injection on the 19th February. That day one pound of Rhodamine WT liquid dye was injected into Walsh Ranch Cave 2. Prior to injecting the dye, 1000 gallons of potable water was flushed into the cave. The dye was then mixed with 4000 gallons of potable water and injected into the cave. On the 20th of February, 0.6 pounds of Fluorescein liquid dye was injected into Walsh Ranch Cave 1. Prior to injecting the dye, 1000 gallons of potable water was flushed into the cave. The dye was then mixed with 2000 gallons of potable water and injected into the cave. Post-injection visual observations did not reveal colored water at any of the nearby surface water bodies.

Injection Date	Recharge Feature	Non-Toxic Dye	Overall Result
2/19/2020	Walsh Ranch Cave 2	Rhodamine WT	No positive detection
2/20/2020	Walsh Ranch Cave 1	Fluorescein	No positive detection
7/28/2020	Walsh Ranch Cave 2	Rhodamine WT	No positive detection

Table 1. Dye injection summary. Dye trace 1No positive dye detections were made from these injections.

Study modifications were implemented in the summer of 2020 based on initial negative results. Additional sites were added to the sampling network in conjunction with a second dye trace (dye trace 2). A new set of background samples were analyzed in preparation for a re-injection into Walsh Ranch Cave 2. The goal was to increase the mass of the dye used in the closest feature to Krienke Spring. In conjunction with adding more dye into the closest feature, another slug of water was flushed into Walsh Ranch Cave 1. Sampling found no positive laboratory detections from these dye injections (Table 3).



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Monitoring Site ID	Description	Status
#1 Krienke Spring PO	Pond outlet below spring	Monitored since Oct. 2019
#2 Brushy Creek WS	South bank at Wyoming Springs	Monitored since Oct. 2019
#3 Brushy Creek CB	Close to north bank below Creek Bend	Monitored since Oct. 2019
#4 Brushy Creek LO	South bank near Ledbetter Oaks subdivision	Monitored since Oct. 2019
#5 Pond on Fault	Hidden Glen greenbelt semi-perennial pond	Monitored since Oct. 2019
#6 Krienke Pond	Weighted float within pond	Abandoned location
#7 West Seep	Weighted rock at seep	Added summer 2020
#8 Tree Stump	Near south bank on pond	Added summer 2020
#9 South Pond FS	Pond south of Fire Station	Added summer 2020
#10 Pond BR	Stormwater pond in Behrens Ranch subdiv.	Abandoned location
#11 Aqua TX	Near a Tonkawa Springs neighborhood pond	Not used
#12 Brushy Creek at AW	Beneath AW Grimes Blvd overpass	Added summer 2020
#13 Kinney Fort	Downstream of Kinney Fort Spring	Possible future location
#14 Westinghouse Seep	Issues from concrete culvert at IH 35	Possible future location

Table 2. Monitoring network summary. Direct access to Krienke Spring has not been granted. ID #8 was used once for a north bank seep and re-located to the tree stump within the main body of the Krienke pond. The seep at IH 35 and Westinghouse Road could be added to monitor possible northeastern groundwater flow.

Limitations and Recommended Next Steps

A major limitation to the dye trace study was the lack of access to Krienke Spring. Standard water quality and flow measurements of spring water would inform our hydrogeological model, although the amount of dye used in this study was sufficient to detect a color change at the outlet and at the downstream pond outlet as well as at locations further downgradient.

Sampling one or more water wells would enable detection of groundwater flow directions that might be outside the hypothesized springshed. It is possible the recharging groundwater upgradient of Krienke Spring is transmitted along mapped or unmapped faults and that groundwater might remain in the body of the aquifer for a long distance. Having sampling sites such as at the Westinghouse seep or wells/springs located northeast of the area may increase the dye detection probability. Coordination with Brushy Creek MUD for access to their Edwards Aquifer wells was limited. Access to sample at one or more of the groundwater-sustained ponds in the Tonkawa Springs neighborhood would be helpful.

Given the close proximity between the spring and the injection sites, the lack of a successful dye detection was unexpected. We believe that the most likely explanation for this result is related to the history of construction in the area (Figure 6). A large water quality pond occurs between the injection sites and the spring which would have required significant excavation to construct. We also know from personal experience that karst voids have been encountered and mitigated beneath the pond adjacent to Alexandrite Way and south of Walsh Ranch Cave 2. As a result of construction, it is possible that a blockage in the Walsh Ranch cave network prevents a groundwater connection to Krienke Spring even though there is sufficient recharge capability at the entrances. Some degree of excavation of one or more of these caves may be necessary. The idea of a subsurface blockage is supported given the short distance between the



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recharge features and spring. We hypothesize that nearby stormwater ponds could be acting as a barrier to flow. Conversely, different injection sites may be considered to test other recharge features.

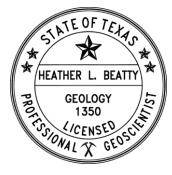
Another compounding issue relates to the history of the Tonkawa Springs neighborhood, which sits on the location of a former limestone quarry. Homes on the north side of Dry Berry Creek are constructed around as series of ponds which are old quarry pits filled with groundwater. The hydrological relationship between the ponds and the spring is unknown, but presumable they are both fed by the same source of groundwater. Permission to sample the ponds for dye was not granted. Clearly the hydrogeology of Tonkawa Springs is more complicated than represented by the boundaries of JPS CHU 1.

Please let us know if you have any questions regarding the dye trace study or associated next step recommendations.

Sincerely,

tom Ben

Heather L. Beatty, P.G. Senior Karst Geoscientist <u>hbeatty@cambrianenvironmental.com</u>



Tel

Kemble White Ph.D., P.G. Senior Karst Geoscientist, Owner kwhite@cambrianenvironmental.com

Attachments:

Figure 1. Geologic map with hypothesized Krienke and Honey Bear springsheds.

Figure 2. Location map showing Krienke Spring, dye injection sites (recharge features) and sampling network.

Figure 3. Representative photos of Krienke Spring.

- Figure 4. Photos of selected sampling locations.
- Figure 5. Injection site photos.
- Figure 6. Injection site maps.
- Table 3. Dye Trace Analytical Results.



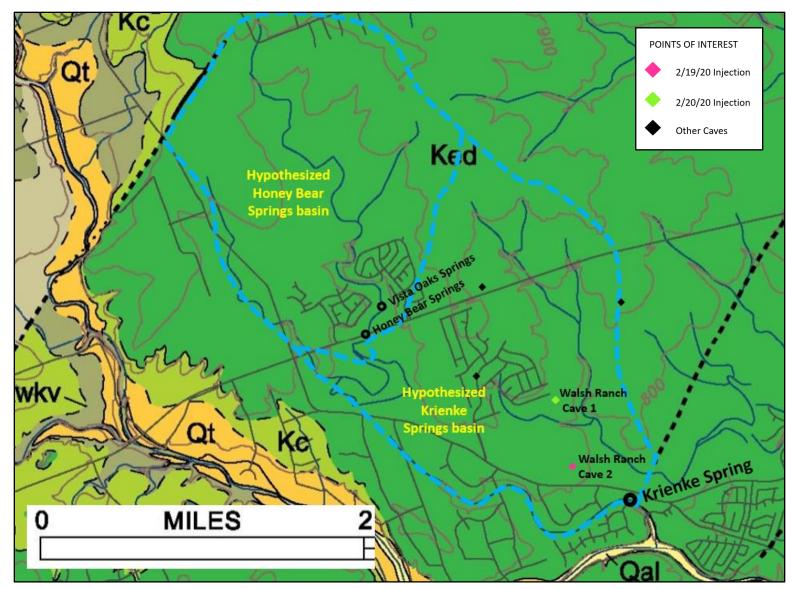


Figure 1. Presumed catchment basin for Krienke Spring south of Honey Bear and Vista Oaks Springs. Diamond shapes represent nearby caves or potential recharge features. The two closest known caves were selected for dye injection.

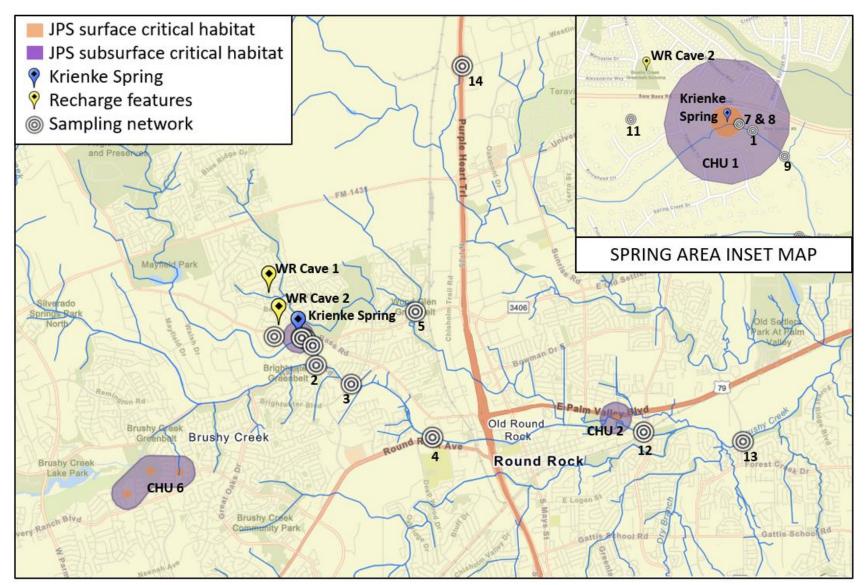


Figure 2. Krienke Spring, dye injection sites (caves/recharge features) and sampling network. JPS critical habitat units in the Round Rock area of Williamson County.

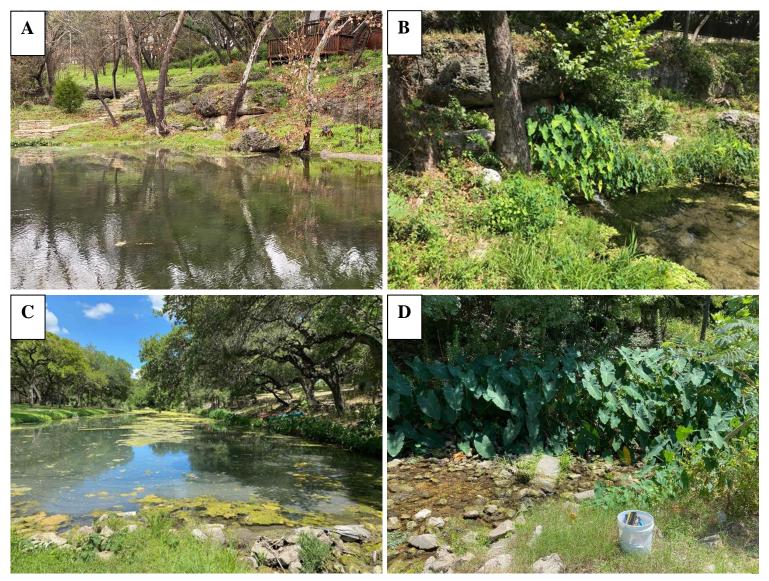


Figure 3. Representative photos of Krienke Spring. **A.** Spring issues from the north bank. **B.** Flowing Krienke Spring below elephant ear plants. **C.** Pond downstream of the spring (facing west). **D.** Rifles that form the Krienke pond outlet.

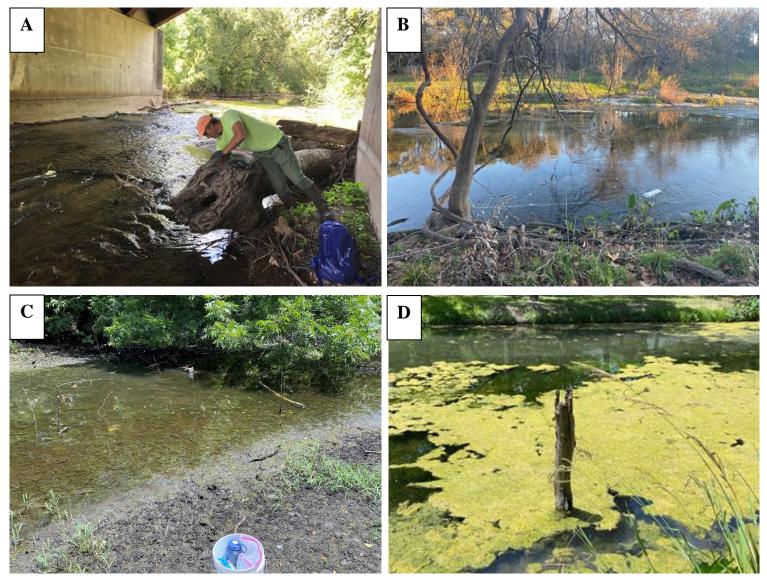


Figure 4. Photos of selected sampling locations. **A.** #3 Brushy Creek **B.** #4 Ledbetter Oaks (LO) protected in a white perforated canister. **C.** #5 Pond on Fault nearly dry in summer 2020. **D.** #8 packet was attached to the tree stump in the Krienke pond, the closest sampling point with access to the spring during the study.

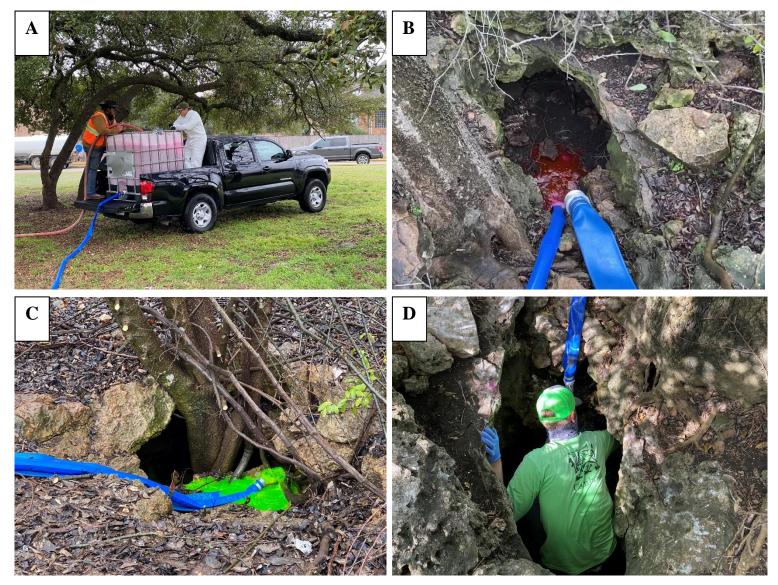


Figure 5. Injection site photos. **A.** Dye mixing operation. **B.** Walsh Ranch cave 2 injection 19 February 2020. **C.** Walsh Ranch cave 1 injection 20 February 2020. **D.** Re-injection of Walsh Ranch cave 2 on 28 July 2020.



Figure 6. Injection site maps. The image on the left (April 2006) illustrates construction progress in the Walsh Ranch Subdivision (left side). The bottom of a water quality pond to the south of Walsh Ranch Cave 1 is approximately 12 feet below the cave entrance. The image on the right (November 2009) shows exposed karst void on the north end of the gabion wall. The bottom of the water quality pond is approximately 15 feet below the cave entrance. These caves were selected as injection points because they are the closest caves to Krienke Spring. Negative dye detections may indicate a blockage in the karst network such as from pond structures.

TABLE 3: DYE TRACE ANALYTICAL RESULTS

			DYE TRACE 1		
Location	Date	Fluorescein (ppb)	Rhodamine WT (ppb)	Sample Type	Comments
1	1/31/2020	ND	ND	ACS	
2	1/31/2020	ND	ND	ACS	
3	1/31/2020	ND	ND	ACS	
4	1/31/2020	ND	ND	ACS	
1	2/7/2020	ND	ND	ACS	
2	2/7/2020	ND	ND	ACS	
3	2/7/2020	ND	ND	ACS	
4	2/7/2020	ND	ND	ACS	
5	2/7/2020	ND	ND	ACS	
1	2/28/2020	ND	ND	ACS	
2	2/28/2020	ND	Ν	ACS	
3	2/28/2020	ND	ND	ACS	
4	2/28/2020	ND	ND	ACS	
5	2/28/2020	ND	ND	ACS	
1	3/6/2020	No sample	No sample	No sample	ACS missing
2	3/6/2020	No sample	No sample	No sample	ACS missing
3	3/6/2020	ND	ND	ACS	
4	3/6/2020	ND	ND	ACS	
5	3/6/2020	ND	ND	ACS	
1	3/12/2020	ND	ND	ACS	
2	3/12/2020	ND	ND	ACS	
3	3/12/2020	ND	ND	ACS	
4	3/12/2020	No sample	No sample	No sample	ACS missing
5	3/12/2020	ND	ND	ACS	
6	3/12/2020	ND	ND	ACS	
1	3/26/2020	ND	ND	ACS	
2	3/26/2020	ND	ND	ACS	
3	3/26/2020	ND	ND	ACS	
4	3/26/2020	ND	ND	ACS	
5	3/26/2020	ND	ND	ACS	
6	3/26/2020	ND	ND	ACS	
7	3/26/2020	ND	ND	ACS	
8	3/26/2020	ND	ND	ACS	
9	3/26/2020	ND	ND	ACS	
10	3/26/2020	ND	ND	ACS	

Rhodamine WT (ppb) Loaction Date Sam 7/28/2020 ND 1 ND 2 7/28/2020 ND 3 7/28/2020 7/28/2020 ND 4 ND 5 7/28/2020 7/28/2020 ND 7 ND 8 7/28/2020 ND 7/28/2020 9 ND 8/2/2020 1 8/2/2020 ND 2 ND 3 8/2/2020 ND 4 8/2/2020 ND 5 8/2/2020 ND 7 8/2/2020 ND 8/2/2020 8 ND 8/2/2020 9 8/9/2020 ND 1 ND 2 8/9/2020 3 8/9/2020 ND 8/9/2020 ND 4 ND 5 8/9/2020 ND 8/9/2020 7 ND 8/9/2020 8 9 8/9/2020 ND . ND 1 8/20/2020 2 8/20/2020 No No sample 8/20/2020 ND 3 ND 4 8/20/2020 5 8/20/2020 ND ND 7 8/20/2020 ND 8/20/2020 8 ND 8/20/2020 9 12 8/20/2020 ND 13 8/20/2020 ND ND 9/10/2020 1 9/10/2020 ND 7 ND 8 9/10/2020 ND 9 9/10/2020 F ND 9/10/2020 13 V

BACKGROUND SAMPLE

POST-INJECTION SAMPLE

ACS = ACTIVATED CHARCOAL SAMPLE ND = NON-DETECT

DYE	TRACE 2	
(ppb)	Sample Type	Comments
	ACS	
	No sample	ACS missing
	ACS	
	Water	
	Water	
	ACS	
	Water	