2016 Activities Report for the Lands Managed by the Williamson County Conservation Foundation in Williamson County, Texas

SWCA Project Number 31724.01

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Appendix C Population Trends, Annual Ecology, Habitat Evaluation, and Disease Prevalence for Federally Threatened Jollyville Plateau Salamanders (*Eurycea tonkawae*) in Williamson County, Texas

And

Effects of Temperature and Nitrogenous Wastes on Survival and Growth of the Barton Springs Salamander (*Eurycea sosorum*)

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EXECUTIVE SUMMARY

General preserve-wide and preserve-specific recommendations for Adaptive Management Committee consideration are summarized in Table ES1.

Table ES1. Recommendations for the Adaptive Management Committee; including general recommendations and preserve specific recommendations.

General Recommendations for All Preserves		
Increase invasive plant eradication efforts through strategic fire implementation across specific preserves		
Work with U.S. Fish and Wildlife Service (USFWS) to implement cave covers that would increase subterranean habitat value and develop system to quantify cave cover effects to karst habitat		
Implement game camera system across the preserves to monitor for unwanted human, hog, and cattle trespassers		
Contact Williamson County non-profit organizations for recurring vegetation surveys within Williamson County Conservation Fund (WCCF) administered preserves		
Final allocation for collected black-capped vireo habitat impact funds		
Preserve Specific Recommendations		
Preserve	**Cave**	**Recommendation**
	Whitney West	Flush cave interior to remove dust
	-	Excavate new feature washing open near parking lot
Beck	-	Excavate bore hole with *Ceuthophilus* cricket inhabitation
Cobbs Cavern	Cobbs Cavern	The WCCF may wish to consider legal action to remove pole mounted transformer or discontinue removal efforts altogether
Millennium	Millennium Cave	Excavate feature to prevent cave closure
Wilco	Venture Cave	Excavate cave to determine true cave extent
Big Oak Cave	Big Oak	Erect fence to keep out trash
Priscilla's Well	-	Strengthen perimeter fencing to exclude cattle
	Priscilla's Well	Excavate to allow greater access and examine nearby karst feature
	Yearwood Gold Mine	Excavate to determine if *Batrisodes* is present
Woodland Park	Duckworth Bat	Remove trash in dedicated effort
	-	Excavate potential new feature washing open
Karankawa	Quahadi	Excavate to allow access
	Pemmican	Rock wall needed to reduce sedimentation
Coffin	Coffin Cave	Reroute stream along natural contours
Shaded Fire Break Recommendations		
Seek additional funding to continue shaded fire break creation across other WCCF-administered preserves		
Give Twin Springs priority status for future shaded fire break creation		

Annual biota survey summary for listed karst invertebrates documented within Williamson County Conservation Foundation (WCCF) caves is shown in Table ES2. Coffin Cave was not monitored due to cave gate and rappelling bar installation, and Wild West surveys were not conducted due to a large number of western diamondback rattlesnakes in and around the cave that prevented safe entry.

Table ES2. Biota survey summary for all monitored caves shown alongside documented locations where federally listed karst invertebrates have been previously found.

Preserve	Monitored Cave Name	Known *Texella* Location?	Listed *Batrisodes* Location?	2016 *Texella?*	2016 *Batrisodes?*
Twin Springs	Sunless City	Yes	Yes	Yes	Yes
	Whitney West	Yes	No	No	-
Beck	Beck Bat	Yes	No	Yes	-
	Beck Pride	Yes	No	No	-
	Beck Horse	Yes	No	Yes	-
	Beck Tex-2	Yes	No	No	-
	Beck Salamander	No	No	-	-
Cobbs Cavern	Show Cave	Yes	Yes	Yes	No
	Wild Side	Yes	Yes	Yes	No
Millennium	Millennium	Yes	No	Yes	-
	Through Trip	No	No	-	-
	Little Demon	Yes	No	No	-
Wilco	Mongo	Yes	No	Yes	-
	Wilco	Yes	No	No	-
	Rock Ridge	Yes	No	No	-
	Wild West	Yes	No	*	-
	Prospector	No	No	-	-
	Venture	No	No	-	-
Chaos Cave	Chaos	Yes	No	Yes	-
	Poison Ivy	Yes	No	Yes	-
	Under The Fence	No	No	-	-
Priscilla's Well	Priscilla's	Yes	Yes	No	No
	Priscilla's Well	Yes	No	No	-
Woodland Park	Duckworth Bat	Yes	No	Yes	-
	Cat	Yes	No	No	-
Karankawa Cave	Angostura	No	No	Yes	-
	Armadon	No	No	No	-
	Karankawa	Yes	Yes	Yes	No
	Pemmican	Yes	No	Yes	-
	Polaris	Yes**	No	Yes	-
	Snake Dancer	No	No	No	-
	War Party	Yes	No	No	-
Coffin Cave	Coffin	Yes	Yes	Not monitored in 2016	
Beck Commons	Beck Sewer	Yes	No	Yes	-
Shaman Cave	Shaman	Yes	No	Yes	-
	Powwow	Yes	No	Yes	-
* Unable to enter cave due to multiple rattlesnakes blocking entrance					
** *Texella* is noted from the 2014 Annual Report, but report may have been from Pemmican Cave					

Table ES3 summarizes actual impacts to covered species habitat versus the Regional Habitat Conservation Plan (RHCP) anticipated impacts to habitat. The same table also portrays covered species

habitat mitigation available for use. The WCCF recorded three cave Zone A intrusions across 28.8 acres and recorded no intrusions to cave Zone B (see SWCA et al. 2008 for details regarding cave impact zones) during 2016. Shaman Cave karst fauna area (KFA) was incorporated as a *Batrisodes cryptotexanus* and Bone Cave harvestman (*Texella reyesi*) preserve during 2016. No black-capped vireo (*Vireo atricapilla*) habitat was impacted in 2016 and approximately 11.63 acres of golden-cheeked warbler (*Setophaga chrysoparia*) habitat were impacted during the same period. Table ES3 gives a brief overview of covered species impacts versus mitigation.

Table ES3. Covered species impacts versus RHCP anticipated impacts and available credits.

Covered Species	Actual Impacts		RHCP Anticipated Impacts		Mitigation			
KARST	2016 Impacted Caves	Impacted Caves Through 2016	Per Year Impacted Habitat (Caves)	Impacted Habitat Through 2016 (Caves)	2016 Karst Fauna Areas Added	Total Karst Fauna Areas	2016 Non-KFA Preserves Added	Total Non-KFA Preserves
Bone Cave Harvestman & Coffin Cave Mold Beetle	Impact Zone A: 3	Impact Zone A: 24	Impact Zone A: 5	Impact Zone A: 40	1	5	0	5
	Impact Zone B: 0	Impact Zone B: 0	Impact Zone B: 2	Impact Zone B: 16	1	5	0	1
BIRDS	2016 Impacted Habitat (Credits)	Impacted Habitat Through 2016 (Credits)	Per Year Impacted Habitat (Credits)	Impacted Habitat Through 2016 (Credits)	2016 Added Credits	WCCF Managed Credits-Total	Remaining Credits Available for Use	
Golden-cheeked Warbler	11.63	508.56	1,600	6,000	43.7	1,115.52	568.79	
Black-capped Vireo	0	22.5	1,138	4,267	0	0	-22.5	

1.0 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 endangered species 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 et al. 2008). The RHCP supports an incidental take permit that authorizes the *take* of the Bone Cave harvestman (*Texella reyesi*), the Coffin Cave mold beetle (*Batrisodes texanus*[1]), the golden-cheeked warbler (*Setophaga chrysoparia*; GCWA), and the black-capped vireo (BCVI; *Vireo atricapilla*); collectively defined as the Covered Species. Take is defined 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 [USC] 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 to preclude the need to list other species. These other species include, but are not limited to, 20 additional troglobitic cave invertebrates and four species of spring-adapted *Eurycea* salamanders; including the Georgetown salamander (GTS; *Eurycea naufragia*) and the Jollyville Plateau salamander (JPS; *Eurycea tonkawae*). See the RHCP for a complete list of permitted and additional species covered under this plan. This document describes the management activities conducted during fiscal year 2016 (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 relatively poor scientific understanding of habitat dynamics, monitoring is particularly important for terrestrial karst invertebrates. Monitoring data are the best and only available measure of preserve performance. Ecological monitoring of karst preserves is conducted 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 2017a).

Coordination with the local fire department regarding wildfire activity for the preserve system occurs annually.

1.1 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 *Williamson County Regional Habitat Conservation Plan*. Adaptive management issues are presented to the Adaptive Management Committee (AMC) within each *Yearly Activities Report* and actions are investigated by the group during the spring meeting (see SWCA et al. 2008 for AMC description). The AMC makes recommendations to the RHCP Administrator who then

[1] Chandler and Reddell (2001) split the listed *Batrisodes texanus* into two species, *B. texanus* and *B. cryptotexanus*, but the USFWS (2009) does not recognize the split. Species identified as *B. cryptotexanus* are known from 15 caves, all in Williamson County (Chandler and Reddell 2001; D.S. Chandler, personal communication to K. White, 2006). Both species are considered federally endangered and are protected under the Endangered Species Act. This report will reference each respective *Batrisodes* species by their taxonomically accepted Latin name.

presents recommendation to the WCCF Board. The WCCF Board votes on action funding and implementation.

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, there are several issues throughout most or all of the preserves that can be addressed with blanket comments. They include:

- Invasive plant species within preserves are an ongoing issue. 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. Privet (*Ligustrum* sp.), chinaberry (*Melia azedarach*), sacred bamboo (*Nandina domestica*), King Ranch bluestem (*Bothriochloa ischaemum* var. *songarica*), and bermudagrass (*Cynodon dactylon*) are common non-native plants in central Texas and require reduction management within the preserve. Identified invasive plants around cave entrances are generally removed. However, the areas adjacent to cave entrances constitute a fairly limited area as compared with the overall preserve. Invasive species are generally found throughout preserves and are only removed when they encroach on cave habitat. Removing individual plant species across the landscape would require large scale, expensive hand removal. However, many invasive plant species are not fire-adapted and the AMC may consider controlled burns at certain locations to reduce non-native plant density in a relatively cost- and time-efficient manner when compared with hand removal techniques. The AMC may wish to consider whether using fire as a tool is appropriate for WCCF-administered lands and if so, which preserves could potentially benefit. Coordination with local fire departments and USFWS is recommended.

- A cave entrance cover would likely decrease atmospheric exchange between the cave interior and the surface environment; which may increase cave habitat suitability to listed and non-listed troglobites. The WCCF is working with the USFWS to design an appropriate cover that allows trogloxene ingress and egress to caves and is not dangerous to other cave inhabitants. The AMC may wish to consider which caves to initiate cave cover trials prior to wider implementation across the preserve system. The AMC may also wish to design a data collection system for cave cover trials in order to gain better understanding regarding impact to indigenous fauna. Questions to consider include but are not limited to: How effective are the cave covers? How can potential effects be quantified? Did they improve habitat for listed and/or non-listed karst invertebrates? Can cave covers be improved?

- Trespassing, vandalism, and dumping are likely the most frequently encountered issues across the entire preserve system. Public education and outreach can be used to address these issues. However, some areas may be prone to undocumented trespassing that could negatively impact the preserve ecosystem. The AMC may wish to consider implementing a game camera system across sensitive areas or those areas where unauthorized access is suspected. These game cameras may also be a more accurate monitor for unwanted mammal presence such as feral hogs or cattle from neighboring properties.

- Continuing vegetation surveys are useful tools for assessing overall preserve health. The AMC may wish to consider having the Williamson County Master Gardeners, the Native Plant Society's Williamson County Chapter, or some other organization conduct 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.

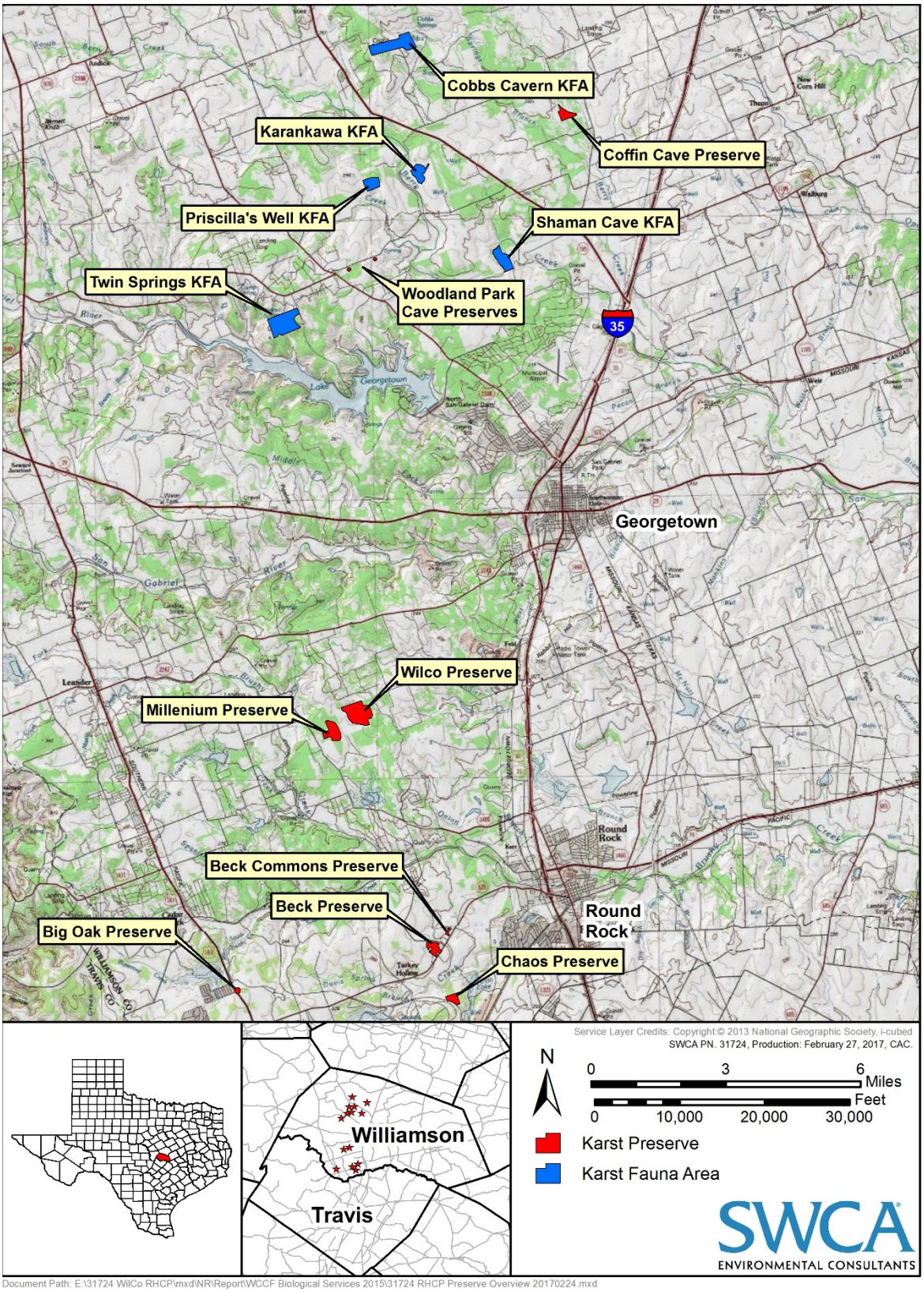


Figure 1. Location map of lands managed by the Williamson County Conservation Foundation.

2.0 METHODS

Yearly activities at each preserve are generally broken into two categories: monthly maintenance activities and biota surveys. Monthly maintenance activities are conducted around each cave's portal under the WCCF's care by the Preserve and Trails Steward (Mark Pettigrew). Monthly maintenance activities are restricted to the general vicinity around each cave's entrance and do not involve cave ingress. Monthly notes are included in this report under each preserve's designated section. Biota surveys involve annual physical entry within the caves during the cold season to document encountered organisms. Results from biota surveys are also included within each preserve's designated section. Other activities not related to monthly maintenance or annual biota surveys are discussed in separate subsections within each preserve's section.

2.1 Management Activities

Preserve inspections occur each month, conducted by the Preserve and Trails Steward, and general site conditions are examined for noticeable changes from the prior inspection. Preserve fences and signs are examined for damage and repaired if necessary. Vegetation damage from storms, fire, human, or mammal (i.e., pigs, cattle, deer) are documented and any trash is removed.

Monthly cave inspections generally involve examining a 200-foot area around the cave for noticeable changes to the immediate environment. Cave gates and locks are examined for damage from natural metal fatigue or from human attempts to gain access. Damage is generally fixed quickly and notes are taken for to inform this report. Locks are lubricated, if needed. Invasive plant encroachment is also noted around caves and removal efforts are carried out.

The RHCP indicates that red imported fire ant (*Solenopsis invicta*; RIFA) presence around cave entrances can have negative effects to the cave ecosystem and associated karst invertebrates. Monthly activities include documenting RIFA mounds within 10 meters (m) (33 feet) and 50 m (164 feet) from cave entrances with listed karst invertebrates. Biennial mound treatment with boiling water occurs regardless of mound density. Additional treatments occur if one RIFA mound is located within 10 m from the cave entrance or if mound density exceeds 80 mounds within 50 m from cave entrance. Additional details regarding RIFA control are included within the RHCP.

Monthly management activities are recorded at each cave and presented within this report's appropriate preserve section.

The RHCP Management Plan (Van Kampen-Lewis and White 2017b) describes in greater detail all activities to be completed within each preserve.

2.2 Annual Biota Surveys

All monitored caves are generally surveyed in the same manner. Climate (temperature, humidity) is recorded at the surface and is again recorded within the deepest accessible cave location. Cave size dictates the number of surveyors within each cave and the survey duration. Large caves require a large survey crew and may require many person-hours[2] of survey effort. Small caves may only allow for one surveyor and may not require much time to record species composition. Rock, wall, ceiling, and floor

[2] 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.

surfaces are investigated for invertebrate and vertebrate habitation. Rocks are also flipped (and then returned to original position) and void spaces examined to ensure thorough cave investigation. Species (if known) and estimated quantities of each species are recorded by the note taker. Notable cave characteristics are also documented. All collected specimens are preserved in 100 percent non-denatured alcohol and sent to James Reddell at the Texas Memorial Museum.

3.0 TWIN SPRINGS KARST FAUNA AREA

The Twin Springs Karst Fauna Area (KFA) is located adjacent to U.S. Army Corps of Engineers (USACE) 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 Management Activities

Maintenance activities included updating the locks on Sunless City and Whitney West Caves to reduce unauthorized access potential. The Whitney West gate was extended to fully cover the cave entrance in an effort to reduce unauthorized access potential. New warning signs were placed on both cave gates.

Table 1 describes notes from cave inspections occurring once per month.

Table 1. Preserve maintenance activities within the Twin Springs KFA.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Sunless City Cave	No	No	Yes/No	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Whitney West Cave	No	No	Yes/No	No	None	1.None. 2.Welded new lock on to gate. 3.Need to extend gate to cover hole.
* 80% Fence intact. Need new fence along S.E. corner.						
February						
Sunless City Cave	No	No	Yes/Yes	No	None	None.
Whitney West Cave	No	No	Yes/Yes	No	None	1.None. 2.None. 3.Need to extend gate to cover hole.
* 80% Fence intact. Need new fence along S.E. corner.						

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
March						
Sunless City Cave	No	No	Yes/No	No	1×1m W.	None.
Whitney West Cave	No	No	Yes/No	No	None	1.None. 2.None.3.Need to extend gate to cover hole.
* 80% Fence intact. Need new fence along S.E. corner.						
April						
Sunless City Cave	No	No	Yes/Yes	No	1×1m W. (Not active)	None.
Whitney West Cave	No	No	Yes/Yes	No	None	1.None. 2.None.3.Need to extend gate to cover hole.
* 80% Fence intact. Need new fence along S.E. corner.						
May						
Sunless City Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Whitney West Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.Need to extend gate to cover hole.
* 80% Fence intact. Need new fence along S.E. corner.						
June						
Sunless City Cave	No	No	Yes/No	No	1×2m W.	None.
Whitney West Cave	No	No	Yes/No	No	None	None.
* 80% Fence intact. Need new fence along S.E. corner.						
July						
Sunless City Cave	No	No	Yes/Yes	No	None	None.
Whitney West Cave	No	No	Yes/Yes	No	None	None.
* 80% Fence intact. Need new fence along S.E. corner.						
August						
Sunless City Cave	No	No	Yes/No	No	None	None.
Whitney West Cave	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* 80% Fence intact. Need new fence along S.E. corner.						
September						
Sunless City Cave	No	No	Yes/No	No	None	None.
Whitney West Cave	No	No	Yes/No	No	None	None.
* 80% Fence intact. Need new fence along S.E. corner.						
October						
Sunless City Cave	No	No	Yes/No	No	None	None.
Whitney West Cave	No	No	Yes/No	No	None	None.
* 80% Fence intact. Need new fence along S.E. corner.						
November						
Sunless City Cave	No	No	Yes/No	No	None	None.
Whitney West Cave	No	No	Yes/No	No	None	None.
* 80% Fence intact. Need new fence along S.E. corner.						
December						
Sunless City Cave	No	No	Yes/No	No	None	None.
Whitney West Cave	No	No	Yes/No	No	None	1.Removed dead tree from gate. 2.None. 3.None.
* 80% Fence intact. Need new fence along S.E. corner.						

3.2 Karst Biota Surveys

Karst biota surveys were conducted on October 27, 2016. Whitney West contained few troglobites with no listed karst invertebrates detected. The Sunless City survey was more productive due to detected presence of two Bone Cave harvestmen and two *Batrisodes texanus* during the approximately 2.25 person-hour search (Table 2). The cave was very wet with an actively dripping ceiling contributing to elevated humidity.

A potential karst feature appears to be washing open within approximately 50 feet of the designated parking lot on Twin Springs' northeast corner. This feature was noticeable during the previous karst biota survey 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 looked during the 2016 karst biota survey.

No specimens were collected.

Karst biota survey results are summarized in Table 2.

Table 2. Karst Biota Survey Results at Twin Springs Karst Fauna Area.

27-Oct-16		Inside Cave		Outside Cave		-			
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
Twin Springs	Whitney West	77.3	75.9	53.9	83.4	0.4	Stephen Van Kampen-Lewis	*Ceuthophilus secretus*	dozens
								Anapistula sp.	2
								Cicurina varians	2
								Leiobunum townsendi	dozens
								Collembola sp.	dozens
								Cambala speobia	1
								Procyon lotor	2
								Mosquito	dozens
								Unknown White Moth	1
Twin Springs	Sunless City	70.3	78.9	52.8	84.5	2.25	Stephen Van Kampen-Lewis, Craig Crawford, Kemble White	*Texella reyesi*	2
								Batrisodes texanus	2
								Ceuthophilus secretus	dozens
								Ceuthophilus cunicularis	3
								Collembola sp.	hundreds
								Cambala speobia	8
								Cicurina varians	5
								Cicurina vibora	dozens
								Rhadine noctivaga	1
								Helicodiscus eigenmanni	2
								Rana berlandieri	2
								Incilius valliceps	2
								Staphylinidae	5
								Annelid	1
								Mosquito	5

Photograph 1. Potential new karst feature as it existed during karst biota survey.

Sunless City Cave is the only cave on the preserve with documented sightings of both the Bone Cave harvestman and *Batrisodes texanus*. Bone Cave harvestman detection over time has been sporadic, with a notable detection spike in 2014, followed by no detected harvestmen in 2014, and two detected harvestmen in 2016 (Figure 2). Two *Batrisodes texanus* were detected within Sunless City and represents the first sighting since 2009 (Figure 2). Whitney West Cave has documented Bone Cave harvestman detection when it was first excavated, but none have been sighted since then. Increased exposure to surface environmental conditions occur within the cave because it is fairly shallow.

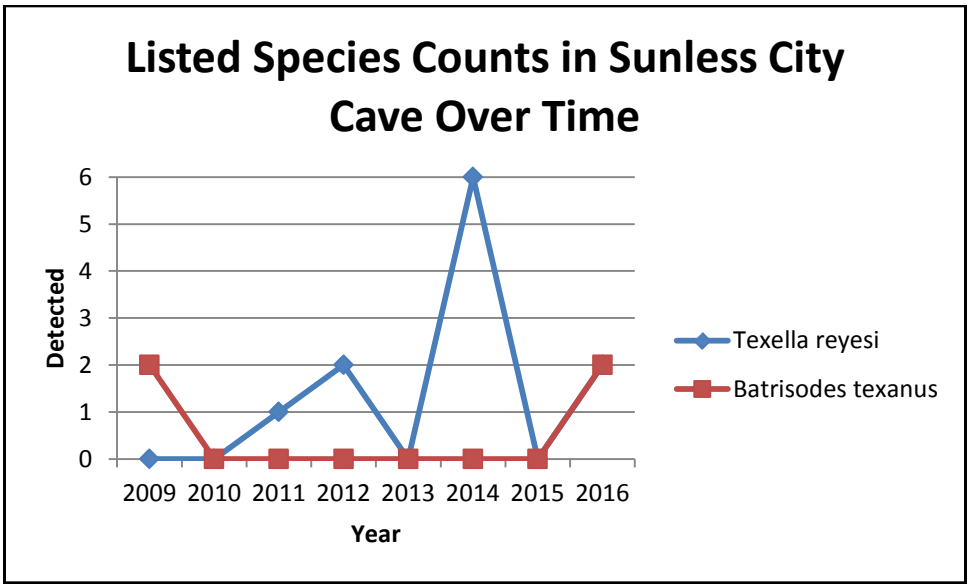


Figure 2. Endangered karst invertebrate species detection within the Twin Springs Preserve.

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 77.5% in 2016 (Figure 3). Temperature has fluctuated between 60 degrees Fahrenheit (°F) and 80°F over the eight survey periods, with 2009 and 2016 as the warmest documented survey years (Figure 3). The warmest years also coincide with *Batrisodes texanus* appearances. Limited data mean it is unclear if temperature plays a role in mold beetle detection; however, a trend may appear over time as more surveys are conducted.

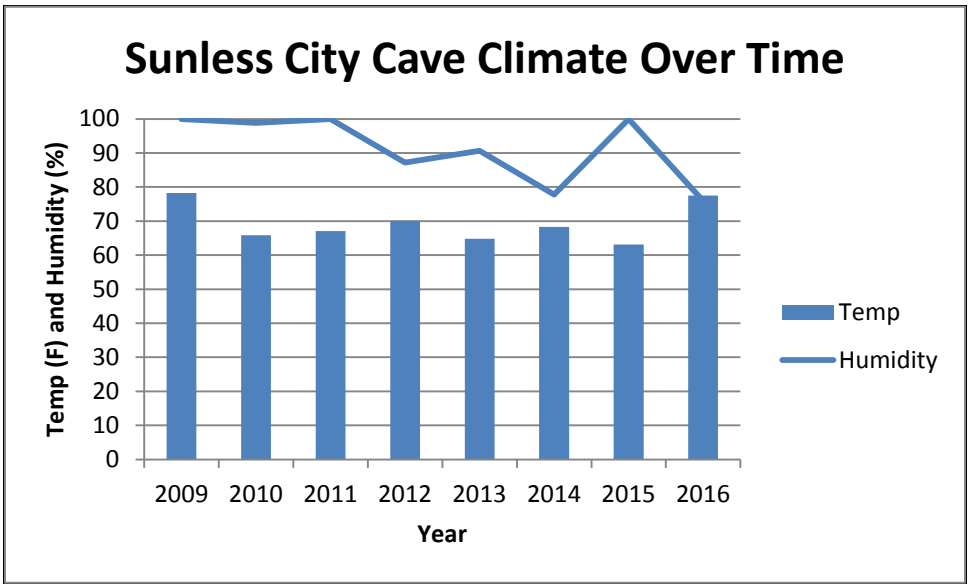


Figure 3. Climate readings within Sunless City Cave.

3.3 Golden-Cheeked Warbler Survey

SWCA documented 124 male GCWA, 24 female GCWA, eight GCWA fledglings, and one GCWA nest during five survey days between March 24 and May 31, 2016. Six to seven GCWA territories were estimated within the Twin Springs KFA during the 2016 breeding season. Most GCWA observations occurred within areas containing high deciduous tree densities, while few GCWA observations occurred within areas supporting mostly Ashe juniper (*Juniperus ashei*) stands. See Appendix A for full report.

3.4 Adaptive Management Issues

The following specific issues should be considered by the AMC.

- Whitney West was originally excavated using explosives, which left the cave interior covered in a fine limestone dust. Dust covering cave surfaces is not encountered within other caves containing *Batrisodes texanus*. Therefore, the dust presence may further inhibit the species from inhabiting the humanly accessible cave extent. A hose connected to water source could be used to quickly wash the cave interior to flush away the dust. A pressure washer used for the RIFA mound treatment is already employed by the WCCF and could be adapted to clean Whitney West.

- A new karst feature is washing open near the Twin Springs KFA parking lot (Photograph 1). This feature may contain cave passage inhabited by *Batrisodes texanus* and would be considered the second such cave within the KFA. Another consideration for the AMC may be Twin Springs KFA user safety. Sunless City has a similar cave entrance appearance and may be indicative of another vertical cave feature within the KFA. Its position close to both the trail and the parking lot may invite curious onlookers to get too close to an open or nearly open pit. Excavating the feature is recommended to reveal cave extent, biotic presence, and cave gate consideration to protect preserve users.

- 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 trespassing.

4.0 BECK PRESERVE

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

4.1 Management Activities

Maintenance activities included updating the locks on all caves to reduce unauthorized access potential. New warning signs were affixed to each cave. New gate hinges were welded onto Beck Creek Cave.

Scratches on the lock clasp were noted at Beck Bat Cave and it appears unauthorized access was attempted. No damage was done to the lock, cave, or gate and the security measures prevented cave access.

Table 3 describes notes from cave inspections occurring once per month.

Table 3. Preserve maintenance activities within the Beck Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Beck Bat Cave	No	No	Yes/Yes	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Beck Creek Cave	No	No	Yes/Yes	No	None	1.Gate hinges still snapped off after opening. 2.Welded new hinges and lock on to gate. 3.None.
Beck Crevice Cave	No	No	Yes/No	No	None	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/No	No	1×12m S.	1.Door stiff and lock needs replacing soon. 2.Welded new lock on gate and worked on gate to open freely. 3.None.
Beck Pride Cave	No	No	Yes/Yes	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Beck Salamander	No	No	Yes/Yes	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	Old RIFA mound not active.	1.None. 2.Welded new lock on to gate. 3.None.
* No fence.						
February						
Beck Bat Cave	Yes	No	Yes/Yes	No	None	1.Attempted break in of gate, lock clasp scratched, lock stayed secure. 2.Repainted lock clasp. 3.None.
Beck Creek Cave	No	No	Yes/Yes	No	None	None.
Beck Crevice Cave	No	No	Yes/No	No	None	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/Yes	No	No active mounds	None.
Beck Pride Cave	No	No	Yes/Yes	No	None	None.
Beck Salamander	No	No	Yes/Yes	No	None	None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	None	None.
* No fence.						
March						
Beck Bat Cave	No	No	Yes/Yes	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Beck Creek Cave	No	No	Yes/Yes	No	None	None.
Beck Crevice Cave	No	No	Yes/No	No	None	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/Yes	No	1×10m S.	None.
Beck Pride Cave	No	No	Yes/Yes	No	None	None.
Beck Salamander	No	No	Yes/Yes	No	None	None.
Beck Tex-2 Cave	No	No	Yes/Yes	No	1×2m W.	None.
* No fence.						
April						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	2×12m S.	1.None. 2.Treated RIFA with boiling water. 3.None.
Beck Crevice Cave	No	No	Yes/No	No	None	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/No	No	1×10m S.	1.None. 2.Treated RIFA with boiling water. 3.None.
Beck Pride Cave	No	No	Yes/No	No	None	None.
Beck Salamander	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	1×2m W.	1.None. 2.Treated RIFA with boiling water. 3.None.
* No fence.						
May						
Beck Bat Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Beck Creek Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Beck Crevice Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.Lock still corroded shut. 2.Put new "Warning" sign on gate. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Beck Pride Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Beck Salamander	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Beck Tex-2 Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.
* No fence.						
June						
Beck Bat Cave	No	No	Yes/No	No	None	None.
Beck Creek Cave	No	No	Yes/No	No	None	1.Gate covered in leaves and debris. 2.Cleared gate of debris. 3.None.
Beck Crevice Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/No	No	None	None.
Beck Pride Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Salamander	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Tex-2 Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
* No fence.						
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	Yes/No	No	Difficult to monitor due to thick vegetation.	1.Lock still corroded shut. 2.None. 3.Lock needs to be cut off.
Beck Horse Cave	No	No	Yes/Yes	No	None	None.
Beck Pride Cave	No	No	Yes/Yes	No	Difficult to monitor due to thick vegetation.	None.
Beck Salamander	No	No	Yes/Yes	No	Difficult to monitor due to thick vegetation.	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Beck Tex-2 Cave	No	No	Yes/Yes	No	None	None.
* No fence.						
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	Yes/No	No	None	1.Lock still corroded shut. Started covering entrance with rocks to close entrance. 2.None. 3.None.
Beck Horse Cave	No	No	Yes/No	No	None	None.
Beck Pride Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Salamander	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
* No fence.						
September						
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.
Beck Pride Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Salamander	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
* No fence.						
October						
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.Gate covered with rocks to produce a more natural environment. 2.None. 3.None.
Beck Horse Cave	No	No	Yes/No	No	1×4m S.	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Beck Pride Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Salamander	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	None	None.
* No fence.						
November						
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.Gate still covered with rocks. 2.None. 3.None.
Beck Horse Cave	No	No	Yes/No	No	1×4m S.	None.
Beck Pride Cave	No	No	Yes/No	No	None	None.
Beck Salamander	No	No	Yes/No	No	None	None.
Beck Tex-2 Cave	No	No	Yes/No	No	1×4m N.E.	None.
* No fence.						
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	No/No	No	None	1.Gate still covered with rocks. 2.None. 3.None.
Beck Horse Cave	No	No	Yes/No	No	1×4m S., 1×6m S.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Beck Pride Cave	No	No	Yes/No	No	None	None.
Beck Salamander	No	No	Yes/No	No	1×5m N.E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Beck Tex-2 Cave	No	No	Yes/No	No	1×4m N.E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
* No fence.						

4.2 Karst Biota Surveys

Karst biota surveys occurred on October 10, 2016. Beck Salamander Cave and Beck Tex II Cave are relatively small caves with high atmospheric exchange rates, creating a less hospitable environment for troglobitic invertebrates. Beck Horse Cave is a larger feature with less atmospheric exchange, which

resulted in four detected Bone Cave harvestmen. Eight Bone Cave harvestmen were detected within Beck Bat Cave and none were detected in Beck Pride Cave.

One *Ceuthophilus cunicularis* was collected from Beck Horse Cave and one potential *Anillinus affabilis* was collected from Beck Bat Cave. However, the eyeless *Anillinus* was unable to be identified by Dr. James Reddell (personal communication [pers. comm.] with Dr. Kemble White, 31 March 2017) and may represent an unknown taxon. A centipede was collected in Beck Pride Cave and may represent a troglobitic centipede from the Geophilomorphoda, which may represent the first such occurrence in the New World (pers. comm. with Dr. Kemble White, 31 March 2017).

Karst biota survey results are summarized in Table 4.

Table 4. Karst Biota Survey Results at Beck Preserve.

11-Oct-16		Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Beck	Beck Tex II	73.9	82.5	36.9	87.6	0.5	Stephen Van Kampen-Lewis, Ashley Wall	*Ceuthophilus secretus*	15
								Cicurina varians	9
								Collembola sp.	hundreds
								Solenopsis invicta	hundreds
								Lepisma saccharina	1
								Scutigeridae	1
Beck	Beck Salamander	67.8	86.4	36.1	91.2	0.25	Ashley Wall	*Cicurina varians*	2
								Isopod	3
								Collembola sp.	12
Beck	Beck Horse	83.5	75.3	54.1	81.3	2	Stephen Van Kampen-Lewis, Kemble White, Reed Lieble	Texella reyesi	4
								Ceuthophilus secretus	hundreds
								Ceuthophilus cunicularis	hundreds
								Batrisodes uncicornis	2
								Cambala bicornourus	4
								Speodesmus bicornourus	6
								Rhadine subterranea	1
								Eidmannella sp.	1
								Collembola sp.	hundreds
								Helicodiscus eigenmanni	2
								Gnat	1
								Mosquito (larvae)	hundreds
								Earwig	3
								Staphylinidae	6
Beck	Beck Bat	83.8	75.5	52.5	75.3	5	Stephen Van Kampen-Lewis, Kemble White, Craig Crawford, Ashley Wall, Matt Nordgren, Reed Lieble	*Texella reyesi*	8

11-Oct-16		Inside Cave		Outside Cave					
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
								Ceuthophilus cunicularis	1
								Ceuthophilus secretus	hundreds
								Speodesmus bicornourus	5
								Cambala speobia	dozens
								Cicurina vibora	3
								Cicurina varians	4
								Eidmannella sp.	1
								Anapistula sp.	3
								Collembola sp.	hundreds
								Batrisodes uncicornis	4
								Myotis velifer	1
								Perimyotis subflavus	2
								Anillinus affabilis	5
								Surface Millipede	3
								Fly	1
Beck	Beck Pride	77.1	74.7	42.1	81.0	2.25	Craig Crawford, Ashley Wall, Matt Nordgren	*Ceuthophilus secretus*	12
								Cambala speobia	5
								Speodesmus bicornourus	13
								White centipede	1

The Bone Cave harvestman is the only endangered karst invertebrate that is 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 (Figure 4). Beck Crevice Cave is known to contain the Bone Cave harvestman, but is no longer monitored due to its small size and connectivity with the larger, Beck Bat Cave. The Bone Cave harvestman was detected Beck Bat Cave and Beck Horse Cave during the 2016 surveys. Karst biota surveys were not conducted in 2013; therefore, 2013 is omitted from Figure 4.

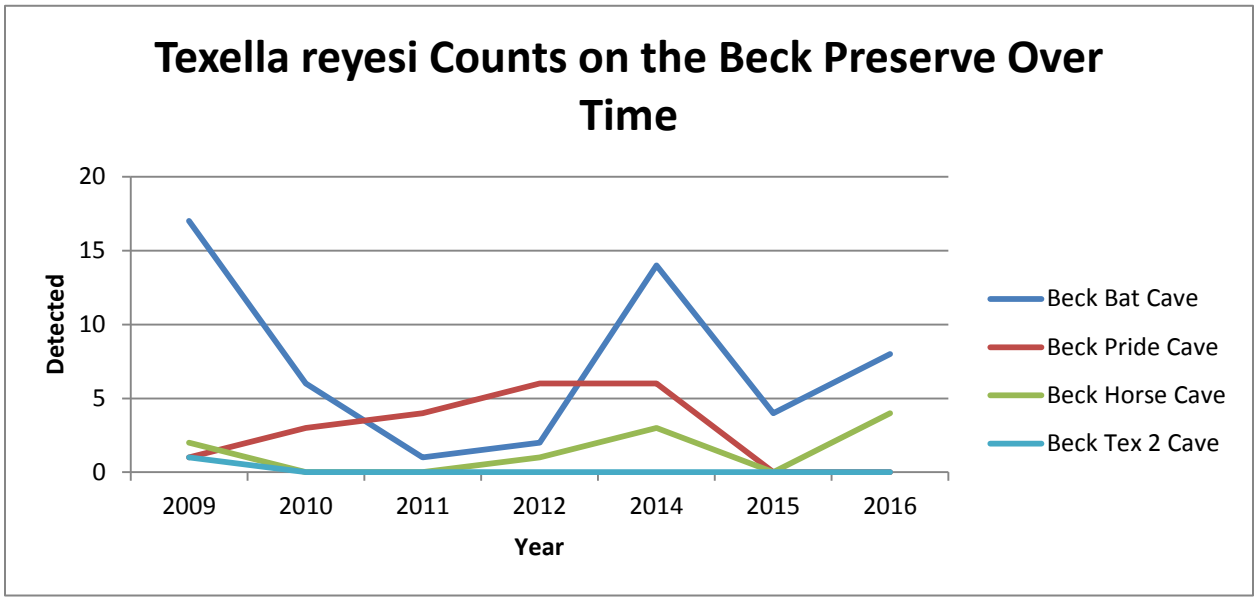


Figure 4. Endangered karst invertebrate species detection within the Beck Preserve.

The climate within the Beck Preserve caves known to contain the Bone Cave harvestman is variable within each cave (Figure 5). The humidity within these four caves dropped compared with the previous survey and may explain why fewer Bone Cave harvestmen were detected compared to the previous survey. Drier cave conditions can cause the troglobites to recede within interstitial spaces. Karst biota surveys were not conducted in 2013, therefore 2013 is omitted from Figure 5.

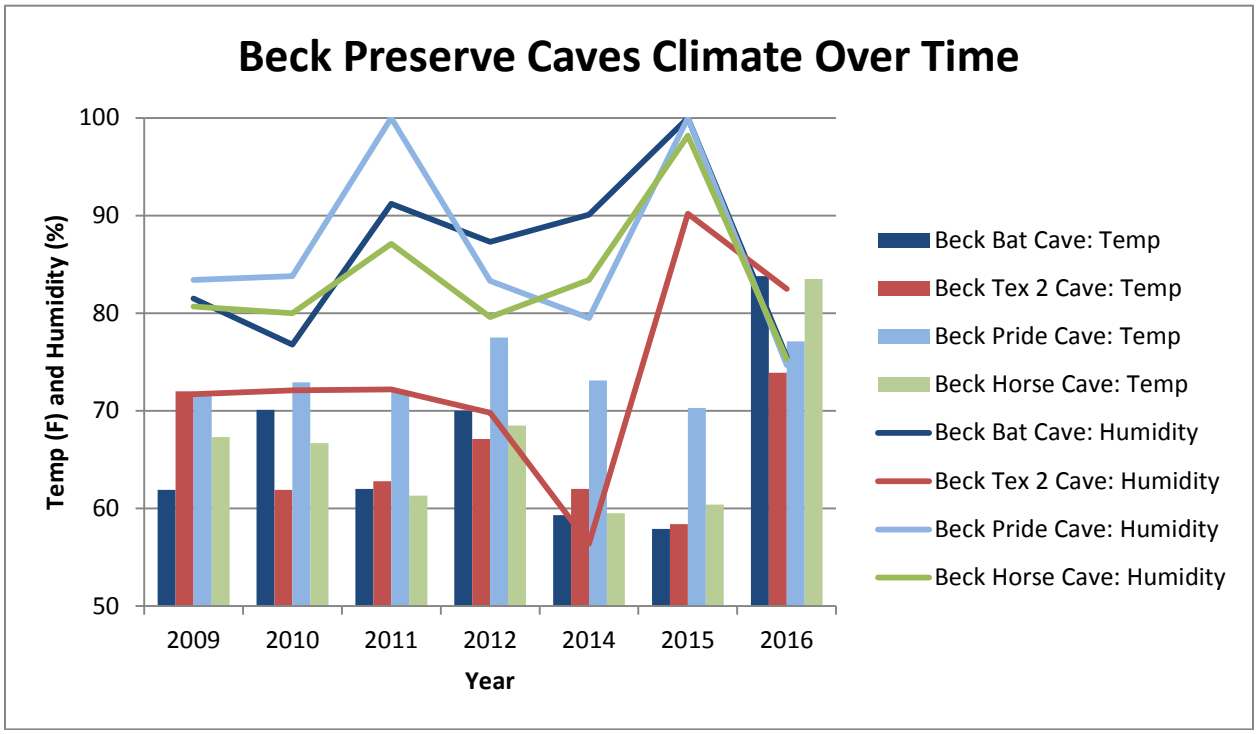


Figure 5. Climate readings within caves of the Beck Preserve.

4.3 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- The biota survey crew noticed what appeared to be soil piping into a small, unknown feature while walking between caves. Further investigation revealed a geotechnical bore-hole, likely from previous land survey efforts. The hole was funneling a significant amount of soil into a subterranean cavern of unknown size and the portal contained many cave crickets (*Ceuthophilus* spp.). The AMC may wish to consider full feature excavation to determine if cave extent exists within this feature.

- Several brush fires appeared to have burned several small grassland patches within the Beck Preserve directly adjacent to Farm-to-Market Road 620. No caves are located nearby and Photograph 2 depicts the burned grass in the largest affected area. The AMC may wish to consider prescribed burns within the Beck Preserve to reduce fuel load and limit wildfires.

Photograph 2. Small burned area within Beck Preserve showing fire extent.

5.0 COBBS CAVERN KARST FAUNA AREA

The 163-acre Cobbs Cavern KFA lies within the 1,670-acre Cobbs Ranch, located north of State Highway (SH) 195, approximately 5 miles northwest of the City of Georgetown in Williamson County, Texas.

Cobbs Cavern has two distinct sides. The eastern third was physically modified and used as a show cave to the general public in the late 1960s and early 1970s, while the cave remainder is relatively untouched by human activity and has been labeled the "Wild Side." Each area is surveyed separately due to Cobbs Cavern's large size.

5.1 Management Activities

RIFA treatment near the cave entrance occurred four times throughout 2016 due to mound density. It is unclear why RIFA are so prevalent in the area.

Table 5 describes notes from cave inspections occurring once per month.

Table 5. Preserve maintenance activities within the Cobbs Cavern Karst Fauna Area.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Cobbs Cavern	No	No	Yes/No	No	No active mounds	None.
* Fence around entire perimeter. Privately owned.						
February						
Cobbs Cavern	No	No	Yes/No	No	1×1m N., 1×6m N., 2×4m S.E.	None.
* Fence around entire perimeter. Privately owned.						
March						
Cobbs Cavern	No	No	Yes/No	No	3×5-6m E., 2×3m N., 1×10m S.	1.None. 2.Treated 6 RIFA mounds with boiling water. 3.None.
* Fence around entire perimeter. Privately owned.						
April						
Cobbs Cavern	No	No	Yes/No	No	1×5m S.E., 1×6m S., 2×5m N.	1.None. 2.Treated 4 RIFA mounds with boiling water. 3.None.
* Fence around entire perimeter. Privately owned.						
May						
Cobbs Cavern	No	No	Yes/No	No	1×8m S.E., 1×6m S., 1×5m N.	None.
* Fence around entire perimeter. Privately owned.						
June						
Cobbs Cavern	No	No	Yes/No	No	2×3m N.	1.None. 2.Treated 2 RIFA mounds with boiling water. 3.None.
* Fence around entire perimeter. Privately owned.						
July						
Cobbs Cavern	No	No	Yes/No	No	1×3m W.	None.
* Fence around entire perimeter. Privately owned.						
August						
Cobbs Cavern	No	No	Yes/No	No	1×3m E., 1×3m N.	1.None. 2.Treated RIFA boiling water. 3.None.
* Fence around entire perimeter. Privately owned.						
September						
Cobbs Cavern	No	No	Yes/No	No	None	1.None.
* Fence around entire perimeter. Privately owned.						
October						
Cobbs Cavern	No	No	No gate	No	None	None.
* Fence around entire perimeter. Privately owned.						

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
November						
Cobbs Cavern	No	No	No gate	No	1×2m S., 1×3m N.	None.
* Fence around entire perimeter. Privately owned.						
December						
Cobbs Cavern	No	No	No gate	No	1×2m S., 1×3m N.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
* Fence around entire perimeter. Privately owned.						

5.2 Karst Biota Surveys

The “Show Side” survey occurred on November 3, 2016. Surveyors detected nine Bone Cave harvestmen and one *Batrisodes texanus* during 7.3 person-hours. Two *Eidmanella* sp., one *Rhadine noctivaga*, and one *Tayshaneta anopica* were collected during this survey.

The “Wild Side” was not surveyed during the 2016 biota surveys due to time constraints.

Results from “Show Side” biota surveys are summarized in Table 6.

Table 6. Karst Biota Survey Results at Cobbs Cavern Karst Fauna Area.

4-Nov-16		Inside Cave		Outside Cave					
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Cobbs Cavern	Cobbs Cavern- Show Side	89.0	73.9	86.8	74.4	7.3	Stephen Van Kampen-Lewis, Craig Crawford, Reed Leible, Beth Banks	*Texella reyesi*	9
								Ceuthophilus secretus	hundreds
								Cicurina varians	4
								Cicurina vibora	7
								Eidmanella sp.	3
								Rhadine noctivaga	2
								Anillinus affabilis	2
								Cambala speobia	16
								Speodesmus bicornorus	23
								Tartarocreagris sp.	2
								Tayshaneta anopica	4
								Scolopendra sp.	1
								White Centipede	1
								Scutigeridae	1
								Eleutherodactylus marnockii	1
								Rana berlandieri	1
								Eptesicus fuscus	1
								Perimyotis subflavus	2
								Staphylinidae	3
								Batrisodes uncicornis	1
								White Moth	1
								Mite	10

Both the Bone Cave harvestman and *Batrisodes texanus* have been regularly detected within Cobbs Cavern since being established as a KFA in 2010. Typically, few mold beetles are observed during the annual biota survey, however the number of Bone Cave harvestman detected on these same surveys can fluctuate significantly (Figure 6). Bone Cave harvestman detection during recent surveys remained relatively high compared to previous years. Karst biota surveys were not conducted in 2013, therefore 2013 has been omitted from Figure 6.

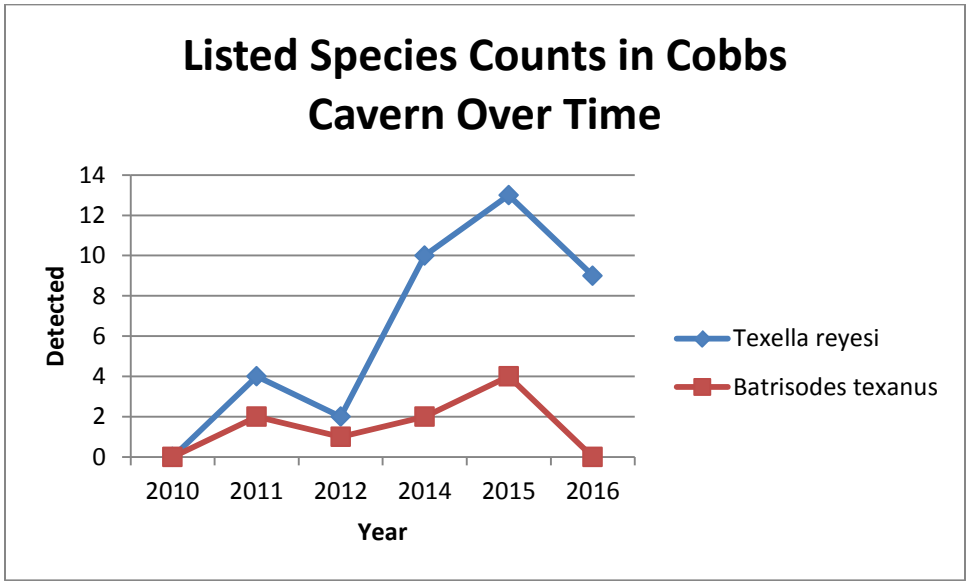


Figure 6. Endangered karst invertebrate species detection within the Cobbs Cavern KFA.

Cobbs Cavern is a very large feature and is able to maintain a relatively stable climate; as seen in Figure 7 where humidity fluctuates between 89% and 100%. The temperature was nearly 75°F during the most recent "Show Side" reading. Karst biota surveys were not conducted in 2013, therefore 2013 has been omitted from Figure 7.

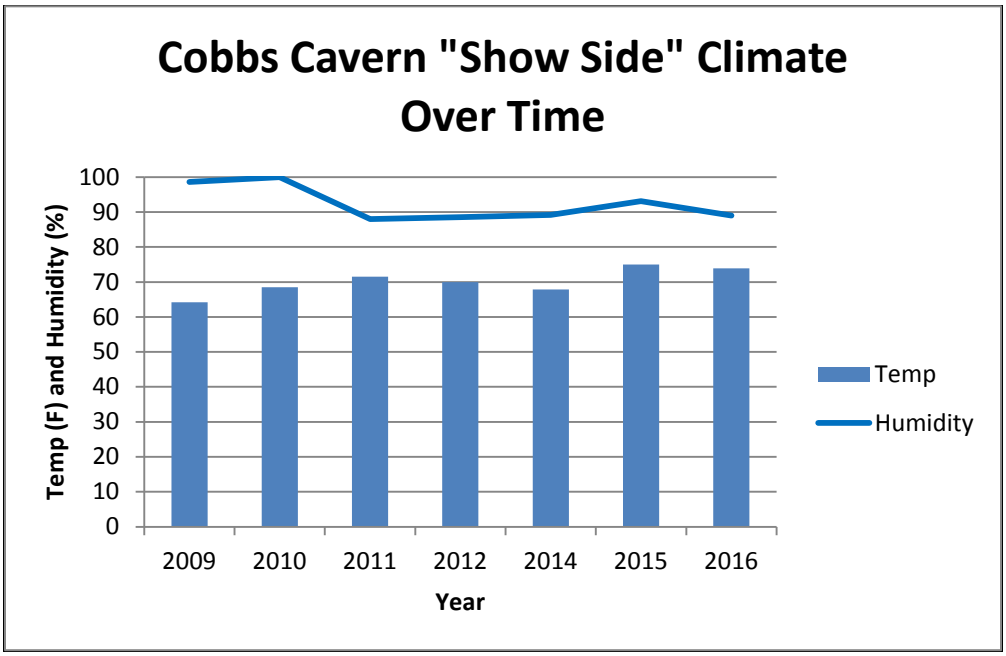


Figure 7. Climate readings from handheld device during biota surveys within Cobbs Cavern "Show Side."

Two Onset HOBO data loggers were placed at two separate locations within the Cobbs Cavern Show Side by Dr. Chris Maupin (Texas A&M). Logger A recorded temperature with relatively few fluctuations due to its placement at the back of the cave. The climate in this area is stable due to its long distance from any sources of surface climate input. Logger B recorded more erratic temperature fluctuations due to its placement near the skylight in the Show Side. The skylight is a drilled hole to allow artificial fill material placement within the cave prior to public attendance as a show cave. The skylight allows increased surface climate to infiltrate Cobbs Cavern as compared to Logger A's location, which is relatively insulated from surface climate. The skylight is currently left uncovered to encourage bat occupation within the Show Side. See Figure 8 for HOBO data logger results.

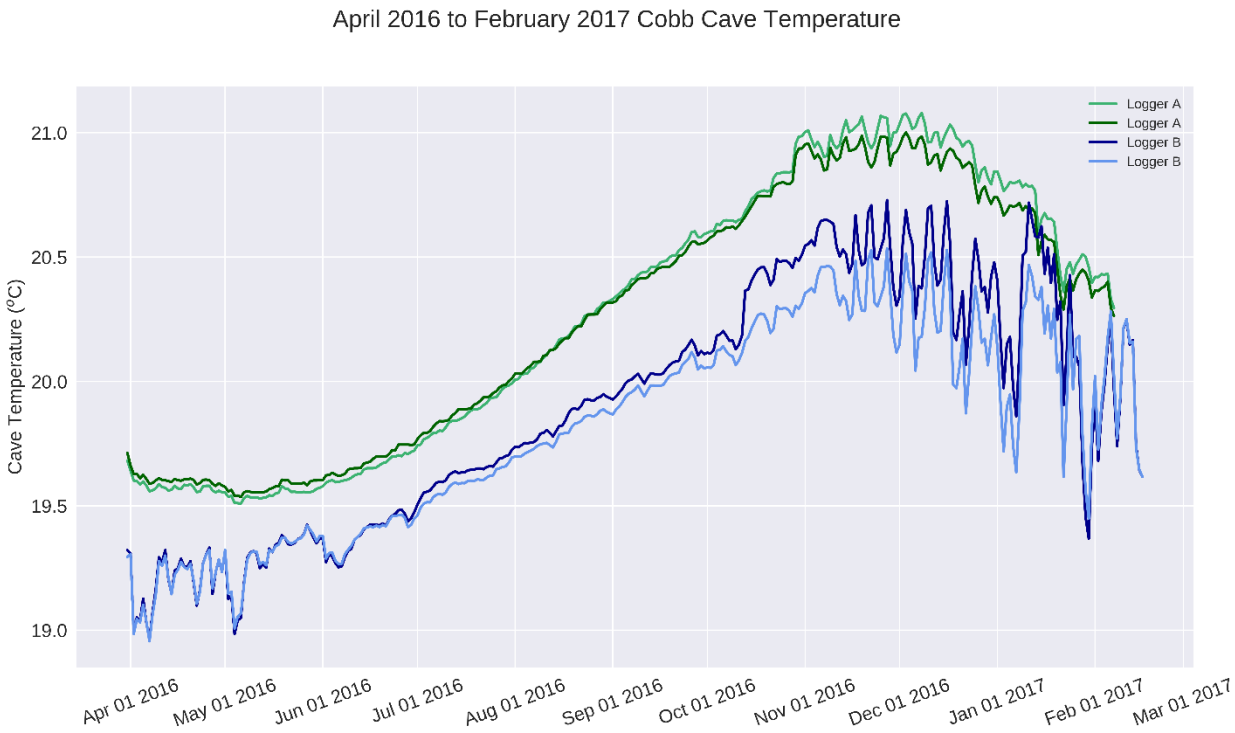


Figure 8. Cobbs Cavern temperature obtained with Onset HOBO data logger between April 2016 and February 2017. Graph courtesy of Ellen Bartow-Gilles and Dr. Chris Maupin at Texas A&M. Logger A was placed near the back of the Show Cave and Logger B was placed near the skylight in the Show Cave.

5.3 Adaptive Management Issues

The following specific issues should be considered by the AMC in consultation with the Lyda Family Trust:

- An electrical transformer on the Pedernales Electrical Cooperative (PEC) pole that is no longer active and is adjacent to the property's main entrance needs a spill containment structure installed or needs to be removed. Unfortunately, PEC has been unresponsive to requests for installation of such a structure or the transformer's removal; therefore, the issue continues to remain at large. The AMC may wish to coordinate with Williamson County leadership to secure their assistance in dealing with the PEC to get the pole removed. However, the electrical transformer is not energized and remains nearly two miles from the entrance to Cobbs Cavern. The AMC may also wish to consider a no action alternative; therefore, closing the issue completely.

6.0 MILLENNIUM PRESERVE

The approximately 74.4-acre Millennium Preserve is located within the Regional Park 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 Management Activities

An attempted break-in to Through Trip Cave's entrances was noticed in April. Rocks were broken on cave locks but no harm was done to either gates or locks.

A warning sign was noted missing from Fence and Trail Cave in April. New warning signs were posted to Little Demon, Millennium, and Through Trip Caves in May.

The entrances to Fence and Trail Cave and Knuckle Cave were covered with large rocks in an attempt to make these features less hospitable to unauthorized human access. Neither cave is gated, nor does cave biota monitoring occur due to these cave's relatively small size and exposure to the surface climate.

Table 7 describes notes from cave inspections occurring once per month.

Table 7. Preserve maintenance activities within the Millennium Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters 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/Yes	No	None	None.
Millennium Cave	No	No	Yes/Yes	No	None	None.
Through Trip North	No	No	Yes/Yes	No	None	None.
Through Trip South	No	No	Yes/Yes	No	None	None.
* No fence. Within park boundary.						
February						
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	Yes/Yes	No	1×2m S.	1.None. 2. Treated RIFA mounds with boiling water. 3.None.
Through Trip South	No	No	Yes/Yes	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1.Comments. 2.Tasks Completed. 3.Tasks Outstanding.
* No fence. Within park boundary.						
March						
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	Yes/Yes	No	None	None.
Through Trip South	No	No	Yes/Yes	No	None	None.
* No fence. Within park boundary.						
April						
Cap Cave	No	No	No Gate	No	None	None.
Fence Trail Cave	No	No	No Gate	No	None	1.Sign is missing. 2.None. 3.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	1.Attempted break in. 2.None. 3.None.
Through Trip South	No	No	Yes/No	No	None	1.Attempted break in. 2.None. 3.None.
* No fence. Within park boundary.						
May						
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	1.None. 2.Put new "Warning" sign on gate. 3.None.
Millennium Cave	No	No	Yes/No	No	1×5m S.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Through Trip North	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Through Trip South	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1.Comments. 2.Tasks Completed. 3.Tasks Outstanding.
* No fence. Within park boundary.						
June						
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	1×5m S.	None.
Through Trip North	No	No	Yes/No	No	1×1m S.	None.
Through Trip South	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
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	Yes/Yes	No	None	None.
Through Trip South	No	No	Yes/Yes	No	None	None.
* No fence. Within park boundary.						
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	Yes/No	No	1×3m W.	None.
Through Trip South	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1.Comments. 2.Tasks Completed. 3.Tasks Outstanding.
* No fence. Within park boundary.						
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.
Millennium Cave	No	No	Yes/No	No	None	None.
Through Trip North	No	No	Yes/No	No	None	None.
Through Trip South	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
October						
Cap Cave	No	No	No Gate	No	None	None.
Fence Trail Cave	No	No	No Gate	No	None	1.Entrance covered with large rocks to prevent unauthorized entry. 2.None. 3.None.
Knuckle Cave	No	No	No Gate	No	None	1.Entrance covered with large rocks to prevent unauthorized entry. 2.None. 3.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	None.
Through Trip South	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
November						
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	Yes/No	No	1×5m S.	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1.Comments. 2.Tasks Completed. 3.Tasks Outstanding.
Through Trip South	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
December						
Cap Cave	No	No	No Gate	No	2×10m E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Fence Trail Cave	No	No	No Gate	No	None	None.
Knuckle Cave	No	No	No Gate	No	1×3m S.	1.Too cold for fire ant mounds to be active. 2.None. 3.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	1×5m S.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Through Trip South	No	No	Yes/No	No	1×5 S.W.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
* No fence. Within park boundary.						

6.2 Karst Biota Surveys

Three caves were surveyed within the Millennium Preserve during two separate survey periods. The initial survey resulted in survey crew cave expulsion from Millennium Cave by a large western diamondback rattlesnake (*Crotalus atrox*) and from Little Demon Cave by a potentially dangerous raccoon (*Procyon lotor*) on November 28, 2016. Survey crews resurveyed these two caves on December 14, 2016. Two Bone Cave harvestman were located within Millennium Cave and no listed karst invertebrates were observed in other Millennium Preserve caves.

No specimens were collected.

Karst biota survey results are summarized in Table 8.

Table 8. Karst Biota Survey Results at Millennium Preserve. Note cave names with an asterisk were re-surveyed on December 14, 2016.

28-Nov-16	14-Dec-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Millennium	Millennium*	99.9	65.1	50.7	57.9	1.5	S. Van Kampen-Lewis, R. Jones, R. Leible	*Texella reyesi*	2
								Ceuthophilus cunicularis	hundreds
								Ceuthophilus secretus	hundreds
								Speodesmus bicornorus	1
								Cambala speobia	1
								Cicurina varians	7
								Anapistula sp.	1
								Eidmanella sp.	2
								Collembola sp.	hundreds
								Staphylinidae	1
								Batrisodes uncicornis	8
								Rhadine subterranea	1
								Labidus coecus	dozens
								Thamnophis proximus	1
								Isopod (pillbug)	10
Millennium	Little Demon*	99.9	70.7	52.4	59.7	0.8	S. Van Kampen-Lewis, A. Wall	*Ceuthophilus cunicularis*	dozens
								Ceuthophilus secretus	hundreds
								Cambala speobia	3
								Cicurina varians	4
								Cicurina browni	1
								Collembola sp.	dozens
								Heliodiscus eigenmanni	dozens
								Eleutherodactylus marnockii	2
								Incilius valliceps	2
								Rana berlandieri	2
								Mosquito	5

28-Nov-16	14-Dec-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Millennium	Through Trip	39.4	82.3	26.1	84.3	1.3	C. Crawford, R. Jones, R. Leible, A. Wall	*Ceuthophilus cunicularis*	dozens
								Ceuthophilus secretus	hundreds
								Collembola sp.	hundreds
								Cambala speobia	3
								Cicurina varians	dozens
								Gnat	

Few Bone Cave harvestman individuals are typically detected during biota surveys within Little Demon and Millennium Caves (Figure 9); with surveyors detecting no harvestmen in 2016.

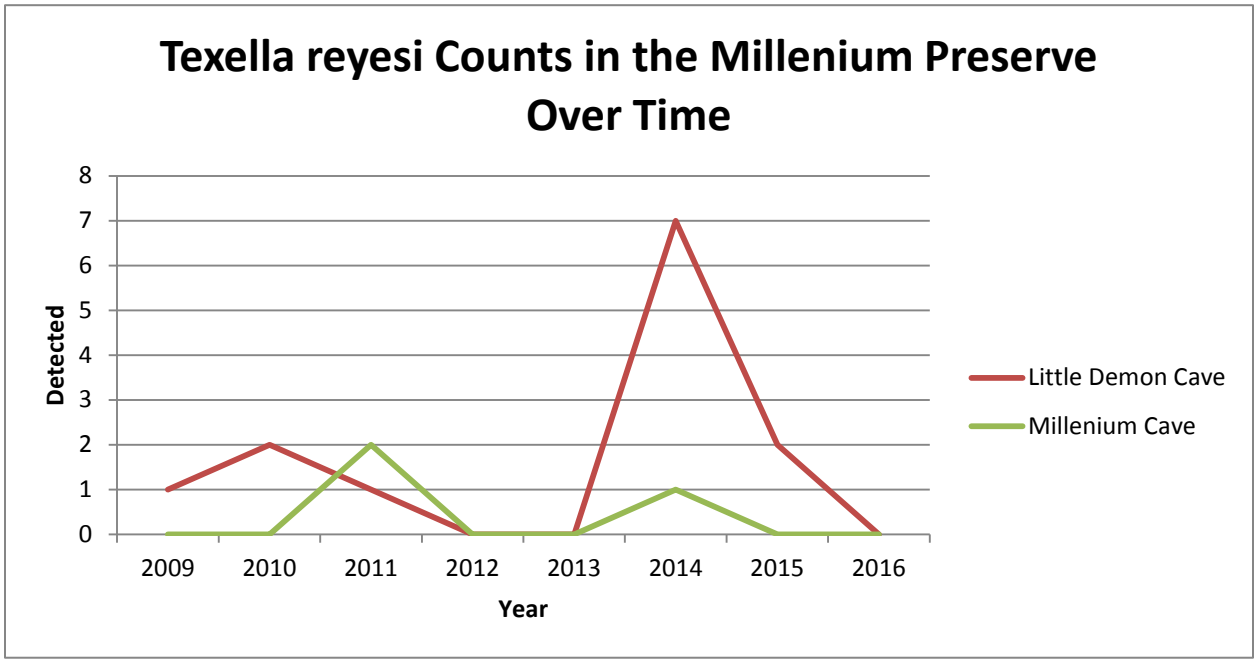


Figure 9. Endangered karst invertebrate species detection within the Millennium Preserve.

The humidity in Little Demon and Millennium Caves typically stays above 80% and the temperature regularly fluctuates between 60°F to 75°F (Figure 10).

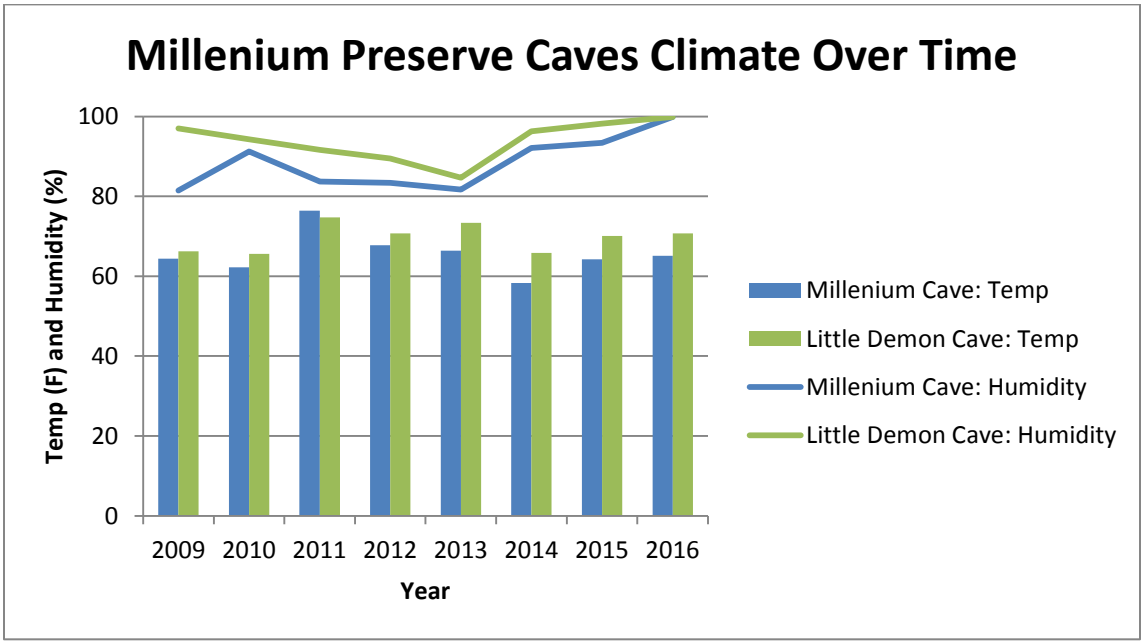


Figure 10. Climate readings from Millennium Preserve caves with documented Bone Cave harvestman sightings.

6.3 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- Millennium Cave entrance is partially filled in and the AMC may wish to consider cave excavation before it becomes sealed.

7.0 WILCO PRESERVE

The 152.5-acre Wilco Preserve is 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.

7.1 Management Activities

Cave gates and locks were upgraded at Mongo, Prospector, Rock Ridge, Venture, and Wild West Caves in January to further prevent unauthorized access.

Two small holes were noted in February approximately 3 m south from Venture Cave and their origins are unclear. No unauthorized access to Venture Cave was detected.

New warning signs were placed on Choya, Mongo, Prairie, Rock Ridge, and Venture Cave gates in May.

Large rocks were placed atop Poppin Rocks Cave in October to prevent unauthorized access to this ungated cave.

Table 9 describes notes from cave inspections occurring once per month.

Table 9. Preserve maintenance activities within the Wilco Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Choya	No	No	Yes/Yes	No	None	None.
Mongo	No	No	Yes/Yes	No	1×6m N.	1.None. 2.Welded new lock on to gate. 3.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	1.Need entrance gate. 2.Welded new lock on gate. 3.Need gate entrance to property.
Rock Ridge Cave	No	No	Yes/No	No	None	1.None. 2.Welded new lock on to gate. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Venture Cave	No	No	Yes/Yes	No	None	1.Need entrance gate. 2.Welded new lock on gate. 3.Need gate entrance to property.
Wilco Cave	No	No	Yes/Yes	No	None	1.Old RIFA mound not active. 2.Welded new lock on to gate. 3.None.
Wild West Cave	No	No	Yes/Yes	No	None	None.
* No fence. Within park boundary.						
February						
Choya	No	No	Yes/Yes	No	None	None.
Mongo	No	No	Yes/Yes	No	None active	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	1×3m W.	1.None. 2. Treated RIFA mounds with boiling water. 3.None.
Rock Ridge Cave	No	No	Yes/No	No	None	None.
Venture Cave	Yes	No	Yes/Yes	No	None	1.Two small holes dug 3 m South of cave entrance. 2.None. 3.None.
Wilco Cave	No	No	Yes/Yes	No	None	None.
Wild West Cave	No	No	Yes/Yes	No	None	None.
* No fence. Within park boundary.						
March						
Choya	No	No	Yes/Yes	No	None	None.
Mongo	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.
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.
* No fence. Within park boundary.						
April						
Choya	No	No	Yes/No	No	None	None.
Mongo	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
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	1×10m W.	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	1.Large rattlesnake beside gate. 2.None. 3.None.
* No fence. Within park boundary.						
May						
Choya	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Mongo	No	No	Yes/No	No	3×4m N.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Poppin Rock Cave	No	No	No gate	No	None	None.
Prairie Cave	No	No	Yes/No	No	1×8m N.E.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Prospector Cave	No	No	Yes/No	No	None	None.
Rock Ridge Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Venture Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Wilco Cave	No	No	Yes/No	No	1×5m S.	None.
Wild West Cave	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
June						
Choya	No	No	Yes/No	No	None	None.
Mongo	No	No	Yes/No	No	1×2m N.	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	1×6m W.	None.
Rock Ridge Cave	No	No	Yes/No	No	None	None.
Venture Cave	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Wilco Cave	No	No	Yes/No	No	None	None.
Wild West Cave	No	No	Yes/No	No	None	None.
* No fence. Within park boundary.						
July						
Choya	No	No	Yes/Yes	No	None	None.
Mongo	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.
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.
* No fence. Within park boundary.						
August						
Choya	No	No	Yes/No	No	None	None.
Mongo	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.						
September						
Choya	No	No	Yes/No	No	None	None.
Mongo	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.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Prospector Cave	No	No	Yes/No	No	1×4m S.W.	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.						
October						
Choya	No	No	Yes/No	No	None	None.
Mongo	No	No	Yes/No	No	None	None.
Poppin Rock Cave	No	No	No gate	No	None	1.Entrance covered with large rocks to prevent unauthorized entry. 2.None. 3.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	1.Rattlesnake beside cave. 2.None. 3.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.						
November						
Choya	No	No	Yes/No	No	1×5m E.	None.
Mongo	No	No	Yes/No	No	None	None.
Poppin Rock Cave	No	No	No gate	No	None	None.
Prairie Cave	No	No	Yes/No	No	4×4m E.	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.						
December						
Choya	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Mongo	No	No	Yes/No	No	None	None.
Poppin Rock Cave	No	No	No gate	No	None	None.
Prairie Cave	No	No	Yes/No	No	4×4m E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Prospector Cave	No	No	Yes/No	Yes	None	1.Tire tracks near cave. 2.None. 3.None.
Rock Ridge Cave	No	No	Yes/No	No	None	None.
Venture Cave	No	No	Yes/No	No	None	1.Too cold for fire ant mounds to be active. 2.None. 3.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

The Wilco Preserve had its share of troubling wildlife encounters on November 28 as well. Four very large (three were estimated as being longer than 4 feet) diamondback rattlesnakes were located at the entrance to Wild West Cave. A cold front had passed through the area only a few hours prior to biota survey initiation and this likely caused the snakes to seek subterranean shelter. The snakes were actively moving into the cave and conducting a survey under these conditions was deemed unsafe. The biota survey ended early in Rock Ridge Cave when a frightened skunk sprayed as it fled the area to avoid survey crews actively looking for cave inhabitants. The skunk avoided the survey crew but it was unknown if additional skunks remained in the cave. Neither cave was resurveyed due to their relatively small and shallow nature.

Two Bone Cave harvestmen were detected within Mongo Cave in the Wilco Preserve. No listed karst invertebrates were detected within other Wilco Preserve caves.

No specimens were collected.

Karst biota survey results are summarized in Table 10.

Table 10. Karst Biota Survey Results at Wilco Preserve.

28-Nov-16		Inside Cave		Outside Cave					
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
Wilco	Mongo	72.8	78.4	29.0	78.4	1.75	S. Van Kampen-Lewis, M. Nordgren, K. White	*Texella reyesi*	7
								Ceuthophilus cunicularis	dozens
								Ceuthophilus secretus	dozens
								Cicurina browni	6
								Eidmanella sp.	1
								Staphylinidae	1
								Crotalus atrox	1
								Gnat	1
								Flea	2
Wilco	Prospector	62.4	70.0	34.0	77.3	2	C. Crawford, R. Jones, R. Leible, A. Wall	*Ceuthophilus secretus*	dozens
								Ceuthophilus cunicularis	dozens
								Gnat	2
								Scutigeridae	1
								Leiobunum townsendi	dozens
								Assassin Bug	3
Wilco	Wild West					SURVEY NOT POSSIBLE DUE TO RATTLESNAKES			
Wilco	Venture	72.3	76.1	20.7	76.6	0.75	S. Van Kampen-Lewis, M. Nordgren, K. White	*Ceuthophilus secretus*	dozens
								Ceuthophilus cunicularis	5
								Cicurina varians	8
								Achaearanea sp.	1
								Collembola sp.	hundreds
								Leiobunum townsendi	5
								Scutigeridae	1
Wilco	Rock Ridge	58.0	75.2	18.8	78.2	0.5	S. Van Kampen-Lewis, R. Jones	*Ceuthophilus secretus*	dozens
								Ceuthophilus cunicularis	dozens

28-Nov-16									
		Inside Cave		Outside Cave					
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
								Speodesmus bicornorus	2
								Unknown white centipede	1
								Collembola sp.	hundreds
								Cicurina varians	3
								Helicodiscus eigenmanni	12
								Eleutherodactylus marnockii	1
								Mephitis mephitis	1
Wilco	Wilco	71.5	76.2	20.5	83.6	0.5	S. Van Kampen-Lewis, K. White	*Ceuthophilus secretus*	10
								Cicurina varians	3
								Staphylinidae	2
								Incilius valliceps	2
								Annelid	7
								Arenivaga investigata	6

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 2017a for Wilco Cave inhabitant descriptions). This species has been detected every year since biota surveys began in 2010 (Figure 11). Karst biota surveys were not conducted in 2013, therefore 2013 is omitted from Figure 11.

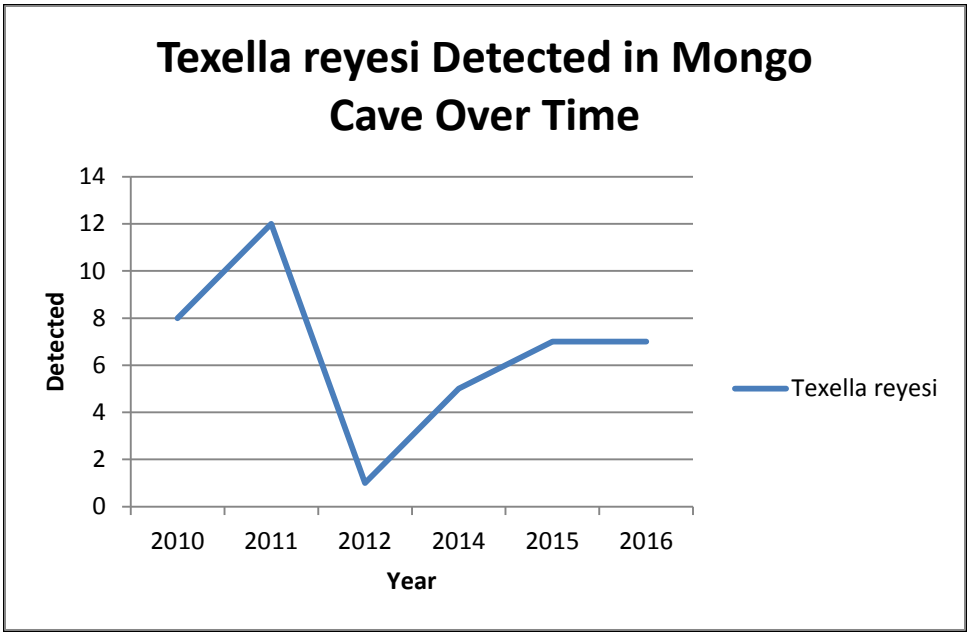


Figure 11. Endangered karst invertebrate species detection within the Wilco Preserve.

Mongo Cave has a relatively stable climate, with humidity typically reading at or above 90%; however, the 2016 survey documented significantly lower humidity (78.4%) than previous years. Temperature typically ranges between 65°F to 80°F (Figure 12). Karst biota surveys were not conducted in 2013, therefore 2013 has been omitted from Figure 12.

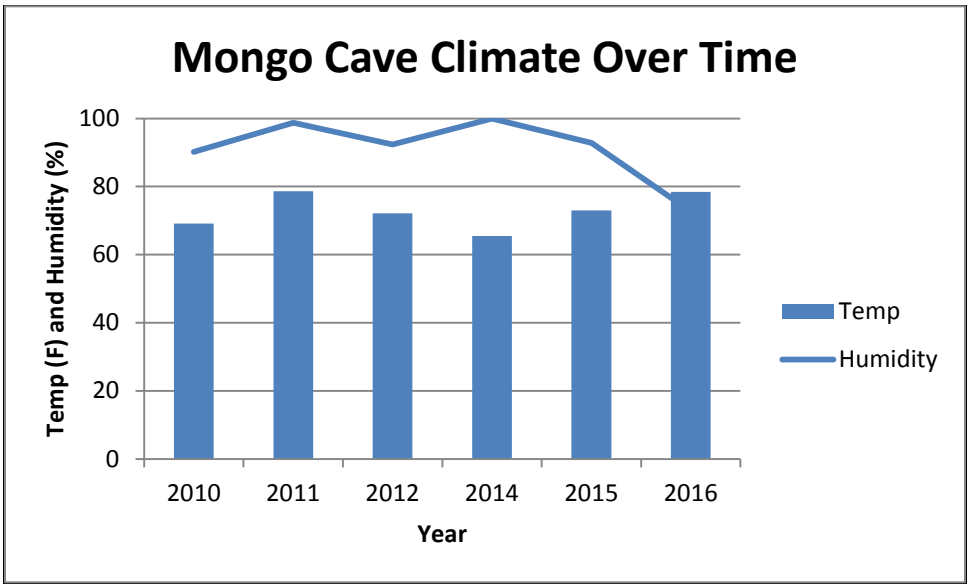


Figure 12. Climate readings within Mongo Cave.

7.3 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- Venture Cave has a noticeable breeze emanating from the deepest part of the cave. This feature may warrant further excavation and the AMC may wish to consider if determining the true cave extent is needed.

8.0 CHAOS CAVE PRESERVE

The 30-acre Chaos Cave Preserve is bounded by SH 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 Management Activities

The Chaos Cave preserve warranted no unusual or extraneous activities in 2016 beyond the basic monthly maintenance tasks.

Table 11 describes notes from cave inspections occurring once per month.

Table 11. Preserve maintenance activities within the Chaos Cave Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Chaos Cave	No	No	No gate	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
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						
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.
* Fence around entire perimeter						
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.
* Fence around entire perimeter						
April						
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						
May						
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						
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.
* Fence around entire perimeter						
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.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* Fence around entire perimeter						
August						
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						
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.
* Fence around entire perimeter						
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.
* Fence around entire perimeter						
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.
* Fence around entire perimeter						
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 20, 2016. Under the Fence Cave and Poison Ivy Cave are both relatively small features with high atmospheric exchange; therefore, no listed species were detected. However, Chaos Cave is much larger and was fairly damp during the 2016 biota survey. Three Bone Cave harvestmen were observed.

Two *Anillinus affabilis* were collected from Chaos Cave and one unknown white spider was collected from Poison Ivy Cave.

Karst biota survey results are summarized in Table 12.

Table 12. Karst Biota Survey Results at Chaos Cave Preserve.

20-Oct-16		Inside Cave		Outside Cave		-			
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
Chaos	Under the Fence	78.6	77.7	55.3	77.7	0.3	Stephen Van Kampen-Lewis, Ashley Wall	*Cicurina varians*	1
								Ceuthophilus secretus	12
								Leiobunum townsendi	dozens
								Mosquito	dozens
Chaos	Chaos Cave	85.9	75.7	55.3	78.6	2	Stephen Van Kampen-Lewis, Ashley Wall, Reed Leible, Craig Crawford	*Texella reyesi*	3
								Ceuthophilus cunicularis	dozens
								Ceuthophilus secretus	hundreds
								Batrisodes uncicornis	4
								Cambala speobia	6
								Cicurina varians	16
								Collembola sp.	hundreds
								Rhadine subterranea	1
								Anillinus affabilis	3
								Staphylinidae	1
								Scolopendra sp.	3
								Scutigera sp.	2
								Crotalus atrox	1
								Gastrophryne olivacea	1
								Plethodon albagula	1
								Arenivaga investigata	2
								Helicodiscus eigenmanni	4
								Red ant	1
								Coleoptera	3
								Surface Millipede	2
								Assassin Bug	1

20-Oct-16		Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
								Annelid	1
								Gnat	3
Chaos	Poison Ivy	70.5	79.3	77.3	47.5	1	Stephen Van Kampen-Lewis, Ashley Wall, Craig Crawford	*Texella reyesi*	1
								Ceuthophilus secretus	hundreds
								Collembola sp.	hundreds
								Cicurina varians	22
								Leiobunum townsendi	hundreds
								Batrisodes uncicornis	2
								Helicodiscus eigenmanni	
								Arenivaga investigata	8
								Scolopendra sp.	3
								Thamnophis cyrtopsis ocellatus	1
								Red Ant	1
								Unknown White Spider	1
								Coleoptera	1
								Isopod	12
								Mosquito	dozens

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 harvestmen, 2009 had the highest count of this species (Figure 13). Species detection is not consistent across all surveys and three Bone Cave harvestmen were detected during the 2016 survey.

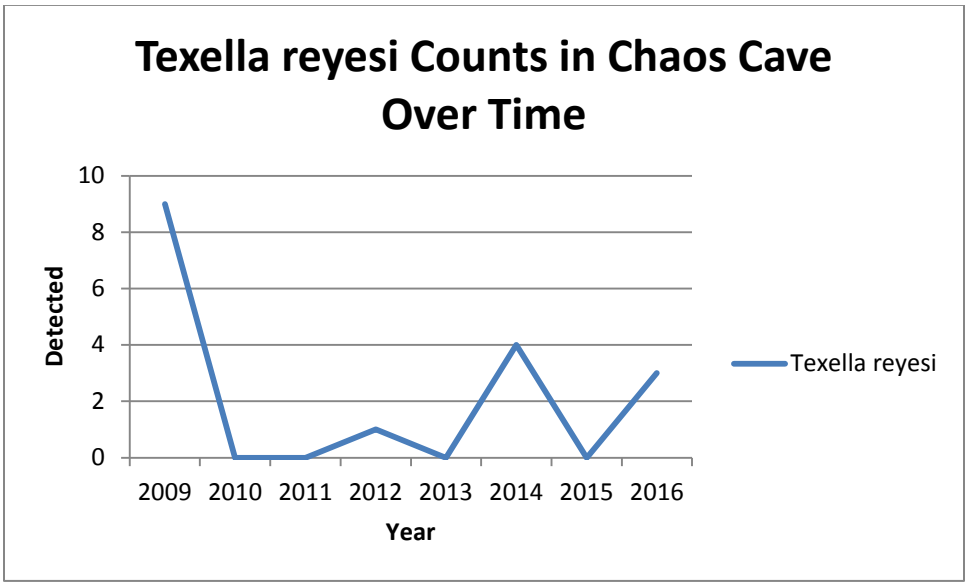


Figure 13. Endangered karst invertebrate species detection within the Chaos Cave Preserve.

The humidity within Chaos Cave is relatively stable and is usually over 80%. Temperature readings fluctuate from approximately 50°F to nearly 78°F (Figure 14).

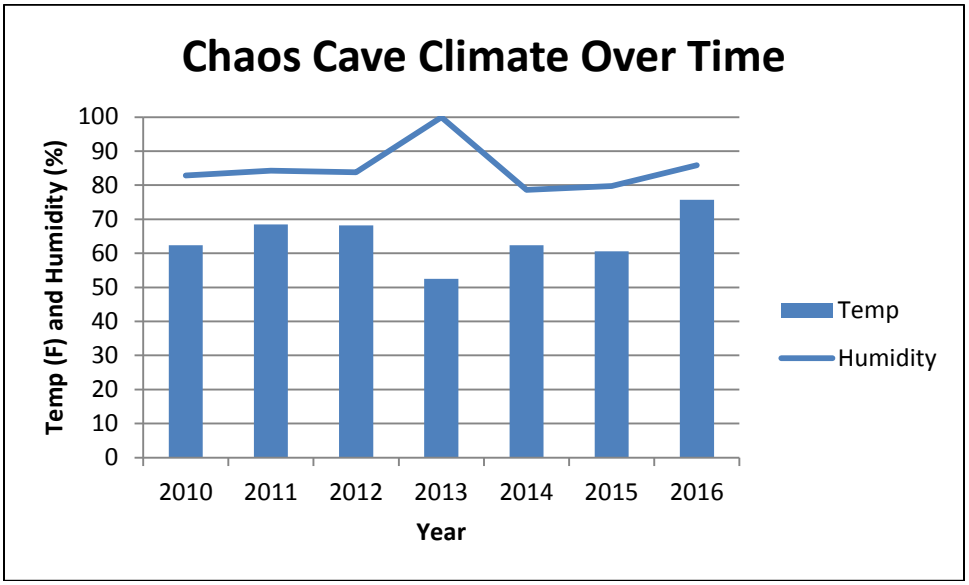


Figure 14. Climate readings within Chaos Cave.

8.3 Adaptive Management Issues

There are currently no noticeable issues that the AMC should consider for the Chaos Cave Preserve.

9.0 BIG OAK CAVE PRESERVE

Big Oak Cave occurs within a roughly 0.5-mile-long median area between U.S. Highway 183A (U.S. 183A) and the old U.S. 183 facilities across approximately 10 acres.

9.1 Management Activities

New warning sign was placed on the cave gate in May.

The Big Oak Cave preserve receives large quantities of trash due to its location between a major highway and adjacent access road. There is at least one homeless camp nearby and the trash problem appears unabated.

Table 13 describes notes from cave inspections occurring once per month.

Table 13. Preserve maintenance activities within the Big Oak Cave Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Big Oak Cave 1	No	No	Yes/No	No	No active mounds	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
Big Oak Cave 2	No	No	Yes/No	No	No active mounds	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
* No fence						
February						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. Welded new locks on gate. 3.More trash still needs to be removed. Ongoing problem.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. Welded new locks on gate. 3.More trash still needs to be removed. Ongoing problem.
* No fence						
March						
Big Oak Cave 1	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
Big Oak Cave 2	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
* No fence						
April						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
* No fence						
May						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. Put new "Warning" sign on gate. 3.More trash still needs to be removed. Ongoing problem.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. Baby buzzards in cave so couldn't check lock. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* No fence						
June						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. Baby buzzards in cave so couldn't check lock. 2.Picked up trash. 3.More trash still needs to be removed. Ongoing problem.
* No fence						
July						
Big Oak Cave 1	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed.
Big Oak Cave 2	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. Buzzard feces all over gate. 2.Picked up trash. Cleaned Buzzard feces off gate. 3.More trash still needs to be removed.
* No fence						
August						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash still needs to be removed.
* No fence						
September						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* No fence						
October						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* No fence						
November						
Big Oak Cave 1	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Big Oak Cave 2	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* No fence						
December						
Big Oak Cave 1	No	No	Yes/No	No	None	None.
Big Oak Cave 2	No	No	Yes/No	No	None	None.
* No fence						

9.2 Karst Biota Surveys

No endangered karst invertebrates have been observed within the Big Oak Cave Preserve since biota surveys commenced in 2009. Annual monitoring efforts will no longer occur at this location because surveys were only required for four years. Repeated monitoring events at this location also failed to yield additional *Rhadine persephone* sightings; likely due to limited accessible cave extent that is almost entirely exposed to surface atmospheric conditions, which proves inhospitable to karst-adapted invertebrates.

9.3 Adaptive Management Issues

Adaptive management for Big Oak Preserve is limited due to its small size and location within a roadway median. The following specific issues should be considered by the AMC:

- Roadway trash is a common issue and needs to be addressed further due to its location between the U.S. 183A main corridor and the adjacent access road. The AMC may wish to consider erecting a fence around the cave to restrict access and limit accumulation of trash.

10.0 PRISCILLA'S WELL KARST FAUNA AREA

The Priscilla's Well KFA is a 51.47-acre tract between Phase III of Ronald W. Reagan Boulevard and a residential portion of the Sun City Georgetown development.

10.1 Management Activities

Priscilla's Cave received an upgraded cave gate and lock in January to prevent unauthorized access. Priscilla's Well Cave received an upgraded cave gate and lock, plus new gate hinges, in February.

Table 14 describes notes from cave inspections occurring once per month.

Table 14. Preserve maintenance activities within the Priscilla's Well KFA.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Pricilla's Cave	No	No	Yes/No	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Pricilla's Well Cave	No	No	Yes/No	No	No active mounds	1.Hunting stand south. Hinges broken 2.None. 3.Hunting stand needs removal. Needs new hinges.
* Fenced area intact. Need to install fence on south side of property line.						
February						
Pricilla's Cave	No	No	Yes/Yes	No	None	None.
Pricilla's Well Cave	No	No	Yes/Yes	No	None	1.Hunting stand south. Hinges broken 2.Replaced hinges and welded on new locks. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
March						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.Removed trash from perimeter. 3.Hunting stand needs removal.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* Fenced area intact. Need to install fence on south side of property line.						
April						
Pricilla's Cave	No	No	Yes/Yes	No	None	None.
Pricilla's Well Cave	No	No	Yes/Yes	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
May						
Pricilla's Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.Put new "Warning" sign on gate.. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
June						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
July						
Pricilla's Cave	No	No	Yes/Yes	No	None	None.
Pricilla's Well Cave	No	No	Yes/Yes	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
August						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
September						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* Fenced area intact. Need to install fence on south side of property line.						
October						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
November						
Pricilla's Cave	No	No	Yes/No	No	None	None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						
December						
Pricilla's Cave	No	No	Yes/No	No	1×6m N.E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Pricilla's Well Cave	No	No	Yes/No	No	None	1.Hunting stand south. 2.None. 3.Hunting stand needs removal.
* Fenced area intact. Need to install fence on south side of property line.						

10.2 Karst Biota Surveys

Karst biota surveys were completed on November 30, 2016. Priscilla's Cave and Priscilla's Well Cave are relatively open to atmospheric exchange, and no listed species were located in either feature during the most current biota survey.

One *Batrisodes uncicornis* was collected from Priscilla's Cave.

Karst biota survey results are summarized in Table 15.

Table 15. Karst Biota Survey Results at Priscilla's Well Karst Fauna Area.

30-Nov-16		Inside Cave		Outside Cave		-			
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
Priscilla's	Priscilla's Cave	73.9	68.7	24.2	68.7	0.42	S. Van Kampen-Lewis, R. Leible, R. Jones	*Ceuthophilus secretus*	dozens
								Ceuthophilus cunicularis	5
								Scolopendra sp.	1
								Scutigeridae	1
								Cicurina vibora	2
								Cicurina varians	16
								Batrisodes uncicornis	1
								Anillinus affabilis	1
								Arenivaga investigata	12
								Rana berlandieri	2
								Perimyotis subflavus	12
								Unknown Surface beetle	1
								Assassin Bug	1
								Unknown Mite	2
								Gnat	3
Priscilla's	Priscilla's Well Cave	24.2	68.7	24.2	68.7	0.01	K. White	*Leiobunum townsendii*	1

Within the Priscilla's Well KFA, only Priscilla's Well Cave has had detection of the Bone Cave harvestman since biota surveys began in 2010. Even then, this species was only detected in 2011, and none have been located since that survey (Figure 15).

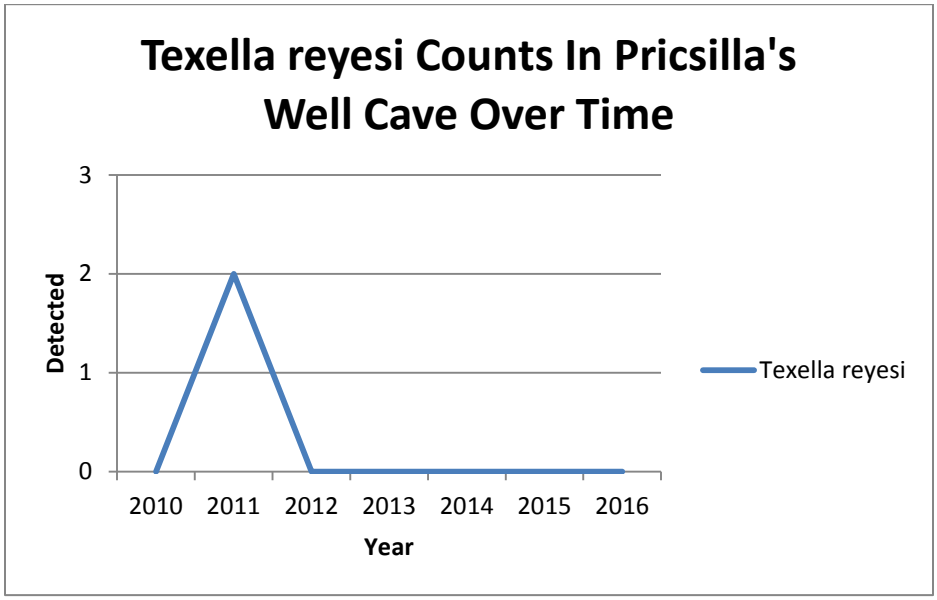


Figure 15. Endangered karst invertebrate species detection within the Priscilla's Well KFA.

The climate readings within Priscilla's Well Cave shows high variability. Humidity ranges from approximately 68% to 99%, and temperature ranges from approximately 45°F to 71°F (Figure 16).

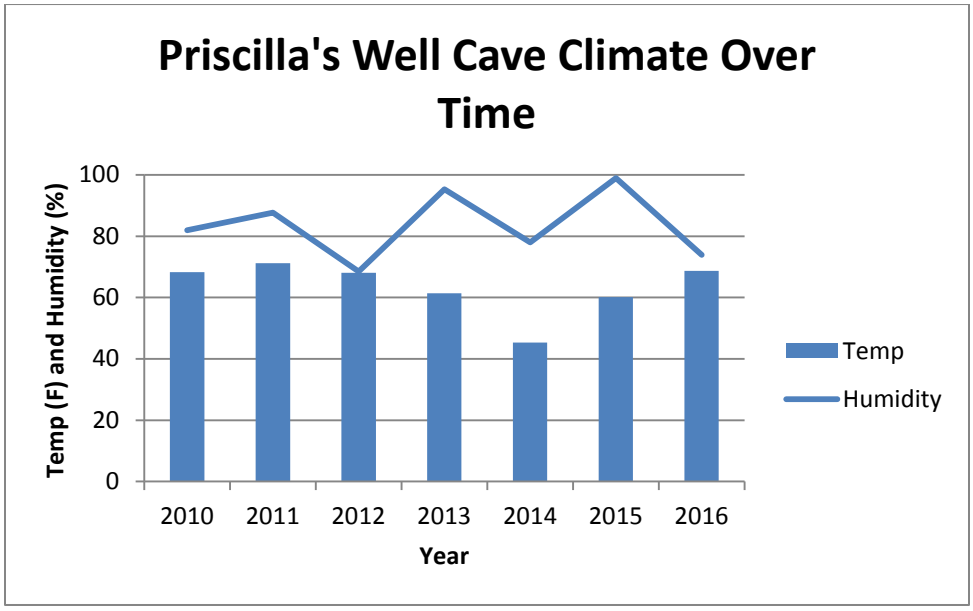


Figure 16. Climate readings within Priscilla's Well Cave.

10.3 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- Cows were seen within Priscilla's KFA during the karst biota survey and the AMC may wish to consider strengthening perimeter fencing to prevent further incidents.

- Priscilla's Well Cave was very difficult to get into during the most recent survey and it appears that a second entrance may be opening up. There is also a known lower room in the cave that has become plugged in recent years, making access too dangerous to attempt. Due to unsafe conditions within the cave, the AMC may wish to consider whether further excavation work at this feature is warranted.

- Yearwood's Gold Mine Cave is known within Priscilla's Well KFA and may be inhabited by the *Batrisodes cryptotexanus* due to placement within the species' range and proximity to Priscilla's Well Cave; a documented *B. cryptotexanus* location. Both monitored caves in this KFA are documented Bone Cave harvestman locations. The AMC may wish to consider excavating Yearwood's Gold Mine Cave to determine if either listed karst invertebrate can be documented within this feature.

- A hunting stand has been erected south from Priscilla's Well Cave and the AMC may wish to consider removing or destroying this feature.

11.0 WOODLAND PARK PRESERVE

Woodland Park Preserve is composed of two conservation areas located in the Woodland Park subdivision, approximately 2.3 miles northwest of the Heritage Oaks site.

11.1 Management Activities

Cat and Duckworth Bat Caves both received upgraded cave gates and locks in February to prevent unauthorized access.

Both cave gates also received new warning signs in May.

Table 16 describes notes from cave inspections occurring once per month.

Table 16. Preserve maintenance activities within the Woodland Park Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Cat Cave	No	No	Yes/No	No	None	1.None 2.None. 3.Gate needs to be extended.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
February						
Cat Cave	No	No	Yes/No	No	None	1.None 2.Welded new lock on gate and repaired hole in gate. 3.None.
Duckworth Bat Cave	No	No	Yes/No	No	None	1.None 2.Welded new lock on gate. 3.None.
* No fence						
March						
Cat Cave	No	No	Yes/No	No	None	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
April						
Cat Cave	No	No	Yes/Yes	No	None	None.
Duckworth Bat Cave	No	No	Yes/Yes	No	None	None.
* No fence						
May						
Cat Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Duckworth Bat Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
* No fence						
June						
Cat Cave	No	No	Yes/No	No	None	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
July						
Cat Cave	No	No	Yes/Yes	No	None	None.
Duckworth Bat Cave	No	No	Yes/Yes	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* No fence						
August						
Cat Cave	No	No	Yes/No	No	None	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
September						
Cat Cave	No	No	Yes/No	No	None	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
October						
Cat Cave	No	No	Yes/No	No	1×4m E.	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
November						
Cat Cave	No	No	Yes/No	No	None	None.
Duckworth Bat Cave	No	No	Yes/No	No	None	None.
* No fence						
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 survey with the Woodland Park Preserve occurred on October 25, 2016. Cat Cave is relatively open to atmospheric exchange and contained large quantities of old raccoon feces. No listed invertebrates were detected in Cat Cave. Duckworth Bat Cave is a larger feature and appears to have less atmospheric exchange than Cat Cave. Surveyors detected two Bone Cave harvestman during the most recent biota survey.

One juvenile *Leiobunum townsendii* was collected due to similar appearance with Bone Cave harvestman.

Karst biota survey results are summarized in Table 17.

Table 17. Karst Biota Survey Results at Woodland Park Preserve.

25-Oct-16		Inside Cave		Outside Cave					
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Woodland Park	Cat	84.2	76.1	53.3	79.5	1.3	Stephen Van Kampen-Lewis, Matt Nordgren, Craig Crawford, Kemble White	*Ceuthophilus cunicularis*	dozens
								Ceuthophilus secretus	thousands
								Cicurina varians	1
								Collembola sp.	hundreds
								Scolopendra sp.	1
								Eleutherodactylus marnockii	1
								Leiobunum townsendi	3
								Achaearanea	18
								Scutigeridae	2
								Arenivaga investigata	3
								Assassin Bug	dozens
								Isopod	hundreds
								Surface Millipede	1
Woodland Park	Duckworth Bat	81.3	73.0	63.6	82.9	2.7	Stephen Van Kampen-Lewis, Matt Nordgren, Craig Crawford, Kemble White	*Texella reyesi*	2
								Ceuthophilus secretus	hundreds
								Batrisodes uncicornis	1
								Cambala speobia	2
								Speodesmus bicornourus	14
								Cicurina varians	14
								Achaearanea sp.	2
								Eidmanella sp.	1
								Scolopendra sp.	3
								Perimyotis subflavus	1
								Incilius valliceps	1
								Rana berlandieri	1
								Eleutherodactylus marnockii	1
								Scolopendra sp.	3

Surveyors observed two Bone Cave harvestman during the most recent biota survey (Figure 17); which is the second detection since monitoring efforts began in 2012. Karst biota surveys were not conducted in 2013, therefore 2013 has been omitted from Figure 17.

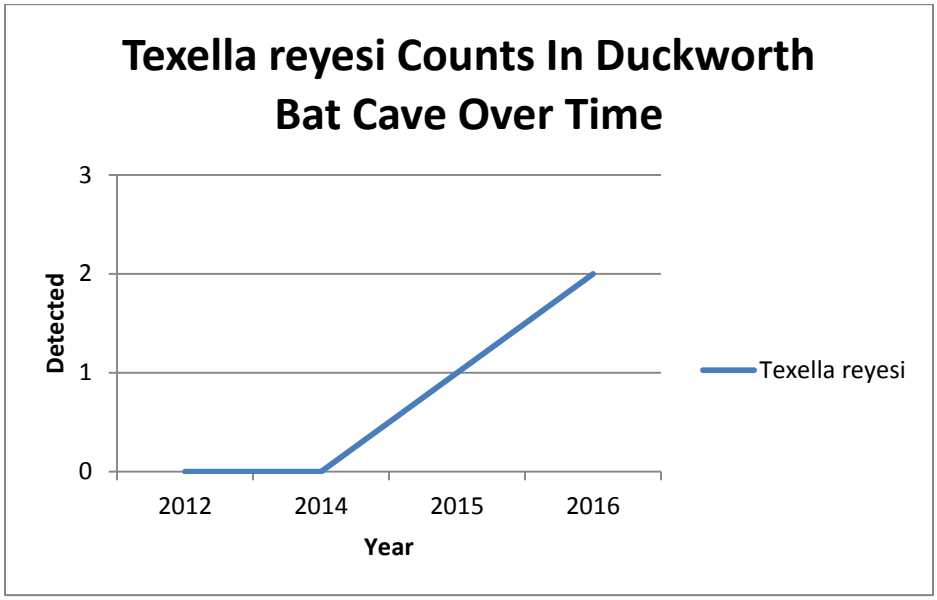


Figure 17. Endangered karst invertebrate species detection within the Woodland Park Preserve.

The Duckworth Bat Cave climate has been variable since monitoring began in 2012 (Figure 18). Temperature during biota surveys has ranged from 66°F to 73°F; while relative humidity has ranged from 70% to 90%. Karst biota surveys were not conducted in 2013, therefore 2013 has been omitted from Figure 18.

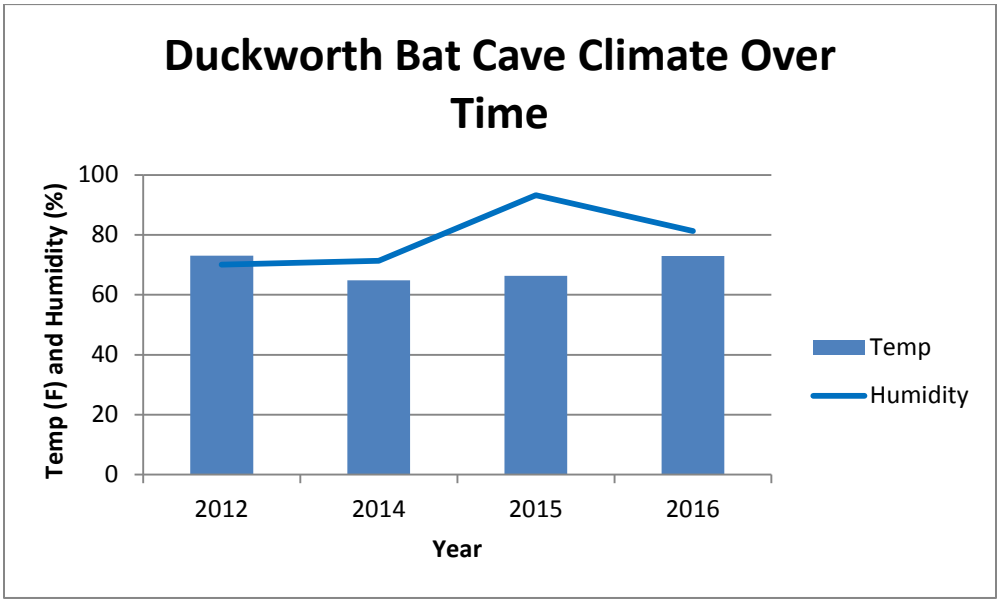


Figure 18. Climate readings within Duckworth Bat Cave.

11.3 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- Duckworth Bat Cave is a large feature that has accumulated a significant amount of trash. The AMC may wish to consider a dedicated trash removal effort be conducted to restore the habitat.
- A new potential cave appears to be opening near Duckworth Bat Cave, very near where biota survey teams park their cars. The AMC may wish to consider excavation to determine cave extent and potential for Bone Cave harvestman inhabitation.

12.0 KARANKAWA CAVE KARST FAUNA AREA

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

12.1 Management Activities

Angostura and Pemmican caves both received upgraded cave gates and locks in January to further prevent unauthorized access.

Table 18 describes notes from cave inspections occurring once per month.

Table 18. Preserve maintenance activities within the Karankawa Cave KFA.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Angostura Cave	No	No	Yes/Yes	No	None	1.None. 2.Welded new lock on to gate. 3.None.
Armadon Cave	No	No	No gate	No	None	None.
Karankawa Cave	No	No	No gate	No	None	None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/Yes	No	None	1.None. 2.Welded new lock on to gate. Gate repaired. 3.Rock wall needed to prevent erosion.
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.
* N.W. Fence intact, need rest of preserve fenced around property line.						

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
February						
Angostura Cave	No	No	Yes/Yes	No	None	None.
Armadon Cave	No	No	No gate	No	None	None.
Karankawa Cave	No	No	No gate	No	None	None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/Yes	No	None	1.None. 2.None. 3.Rock wall needed to prevent erosion.
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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
March						
Angostura Cave	No	No	Yes/No	No	None	None.
Armadon Cave	No	No	No gate	No	1×6m S., 1×8m S.	None.
Karankawa Cave	No	No	No gate	No	1×6m E., 1×5m N.E., 1×8m N.	None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/No	No	None	1.None. 2.None. 3.Rock wall needed to prevent erosion.
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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
April						
Angostura Cave	No	No	Yes/Yes	No	None	None.
Armadon Cave	No	No	No gate	No	1×6m S., 1×8m S.	1.None. 2.Treated RIFA with boiling water. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Karankawa Cave	No	No	No gate	No	1×6m E., 1×5m N.E., 1×8m N.	1.None. 2.Treated RIFA with boiling water. 3.None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/Yes	No	None	1.None. 2.None. 3.Rock wall needed to prevent erosion.
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	1×5m S.W.	None.
* N.W. Fence intact, need rest of preserve fenced around property line.						
May						
Angostura Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.None.
Armadon Cave	No	No	No gate	No	1×8m S.	None.
Karankawa Cave	No	No	No gate	No	None	None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/No	No	None	1.None. 2.Put new "Warning" sign on gate. 3.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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
June						
Angostura Cave	No	No	Yes/No	No	None	None.
Armadon Cave	No	No	No gate	No	1×8m S., 1×10m S.	None.
Karankawa Cave	No	No	No gate	No	None	None.
Kiowa 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.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
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	1×6m N.	None.
* N.W. Fence intact, need rest of preserve fenced around property line.						
July						
Angostura Cave	No	No	Yes/Yes	No	None	None.
Armadon Cave	No	No	No gate	No	1×10m S.	1.None. 2.Treated RIFA with boiling water. 3.None.
Karankawa Cave	No	No	No gate	No	1×3m S.	1.None. 2.Treated RIFA with boiling water. 3.None.
Kiowa Cave	No	No	No gate	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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
August						
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	1×5m E., 1×4m N.E.	None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/No	No	None	1.Rattlesnake in cave. 2.None. 3.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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
September						
Angostura Cave	No	No	Yes/No	No	None	None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Armadon Cave	No	No	No gate	No	None	None.
Karankawa Cave	No	No	No gate	No	None	None.
Kiowa 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	1.Rattlesnake in cave. 2.None. 3.None
War Party Cave	No	No	No gate	No	None	None.
* N.W. Fence intact, need rest of preserve fenced around property line.						
October						
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.
Kiowa 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.
* N.W. Fence intact, need rest of preserve fenced around property line.						
November						
Angostura Cave	No	No	Yes/No	No	1×10m E.	None.
Armadon Cave	No	No	No gate	No	1×4m S.	None.
Karankawa Cave	No	No	No gate	No	1×5m W.	None.
Kiowa 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.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
Snake Dancer Cave	No	No	No gate	No	None	None.
War Party Cave	No	No	No gate	No	None	None.
* N.W. Fence intact, need rest of preserve fenced around property line.						
December						
Angostura Cave	No	No	Yes/No	No	1×10m E. 1×3m S.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Armadon Cave	No	No	No gate	No	1×4m S.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Karankawa Cave	No	No	No gate	No	1×5m W. 1×9m E.	1.Too cold for fire ant mounds to be active. 2.None. 3.None.
Kiowa Cave	No	No	No gate	No	None	None.
Pemmican Cave	No	No	Yes/No	No	1×8m W.	1.Too cold for fire ant mounds to be active. 2.None. 3.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.
* N.W. Fence intact, need rest of preserve fenced around property line.						

12.2 Karst Biota Survey

Most karst biota surveys were completed on November 22, 2016, while Angostura Cave biota survey occurred November 30, 2016. Three Bone Cave harvestman were detected in Polaris Cave, six were detected in Karankawa Cave, and two were detected in Pemmican Cave. No Bone Cave harvestmen were detected in other caves. Dr. James Reddell (pers. comm. with Dr. Kemble White, 31 March 2017) confirmed *Batrisodes cryptotexanus* was detected in War Party Cave, which represents a new locality for the species.

Two large western diamondback rattlesnakes were within Armadon Cave and one was very agitated by the survey team's presence. The survey was cut short as a result.

Two *Achaearanea* sp. were collected in War Party Cave and one *Tartarocreagris* sp. was collected in Pemmican Cave.

Climate data were not collected in Polaris Cave.

Karst biota survey results are summarized in Table 19.

Table 19. Karst Biota Survey Results at Karankawa Cave Karst Fauna Area.

22-Nov-16	30-Nov-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Karankawa	War Party	89.5	75.5	73.1	73.5	1.7	Stephen Van Kampen-Lewis, Harris Frampton, Ryan Jones, Craig Crawford, Reed Leible	*Batrisodes cryptotexanus*	1
								Ceuthophilus secretus	10
								Batrisodes uncicornis	1
								Cicurina varians	6
								Collembola sp.	3
								Achaearanea sp.	8
								Eidmanella sp.	5
								Rana berlandieri	3
								Pseudouroctonus reddelli	3
								Assassin Bug	1
								Mite	1
								White Moth	1
								Inchworm	1
Karankawa	Polaris			74.3	75.5	0.8	Stephen Van Kampen-Lewis, Craig Crawford, Reed Leible, Ryan Jones, Harris Frampton	*Texella reyesi*	3
								Ceuthophilus secretus	dozens
								Ceuthophilus cunicularis	2
								Speodesmus bicornourus	1
								Cicurina varians	2
								Cicurina vibora	1
								Achaearanea sp.	1
								Anapistula sp.	1
								Pseudouroctonus reddelli	7
								Arenivaga investigata	1
								Eleutherodactylus marnockii	1
								Rana berlandieri	1

22-Nov-16	30-Nov-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
								White Moth	1
								Isopod	1
								Assassin Bug	9
Karankawa	Snake Dancer	72.2	73.7	71.5	74.3	1.3	Stephen Van Kampen-Lewis, Harris Frampton, Reed Leible, Craig Crawford	*Ceuthophilus secretus*	hundreds
								Ceuthophilus cunicularis	7
								Batrisodes uncicornis	8
								Texoreddellia aquilonalis	1
								Achaearanea sp.	2
								Scutigera sp.	1
								Rana berlandieri	1
								Eleutherodactylus marnockii	2
								Assassin Bug	11
								Arenivaga investigata	2
								Mosquito	hundreds
								White Moth	1
Karankawa	Karankawa	88.1	73.7	78.6	71.7	2.3	Stephen Van Kampen-Lewis, Craig Crawford, Reed Leible, Ryan Jones	*Texella reyesi*	6
								Ceuthophilus secretus	dozens
								Ceuthophilus cunicularis	6
								Cambala speobia	7
								Speodesmus bicornourus	2
								Cicurina varians	11
								Cicurina vibora	6
								Anillinus affabilis	1
								Pseudouroctonus reddelli	3
								Staphylinidae	1
								Inchworm	1

22-Nov-16	30-Nov-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
								Assassin Bug	1
Karankawa	Armadon	83.2	72.5	74.0	73.0	2	Stephen Van Kampen-Lewis, Harris Frampton, Ryan Jones, Craig Crawford	*Ceuthophilus secretus*	3
								Ceuthophilus cunicularis	1
								Achaearanea sp.	1
								Crotalus atrox	2
								Gnat	1
Karankawa	Angostura	61.4	63.3	22.3	60.8	0.3	Stephen Van Kampen-Lewis, Kemble White	*Ceuthophilus secretus*	17
								Mosquito sp.	dozens
								Achaearanea sp.	5
Karankawa	Pemmican	93.2	75.9	73.0	74.4	2.1	Stephen Van Kampen-Lewis, Craig Crawford, Reed Leible, Ryan Jones, Harris Frampton	*Texella reyesi*	2
								Ceuthophilus secretus	hundreds
								Ceuthophilus cunicularis	dozens
								Cicurina vibora	4
								Cicurina varians	4
								Tartarocreagris sp.	1
								Anillinus affabilis	1
								Nerodia rhombifer	1
								Incilius valliceps	1
								Eleutherodactylus marnockii	1
								White Centipede	1

Bone Cave harvestman detection has been sporadic, with only Karankawa Cave consistently containing more than five detected individuals (Figure 19). Pemmican Cave was surveyed for the first time in 2015; therefore, data only exists for two years. Snake Dancer Cave is omitted from Figure 19 because the Bone Cave harvestman has not been located since regular biota surveys began in 2014.

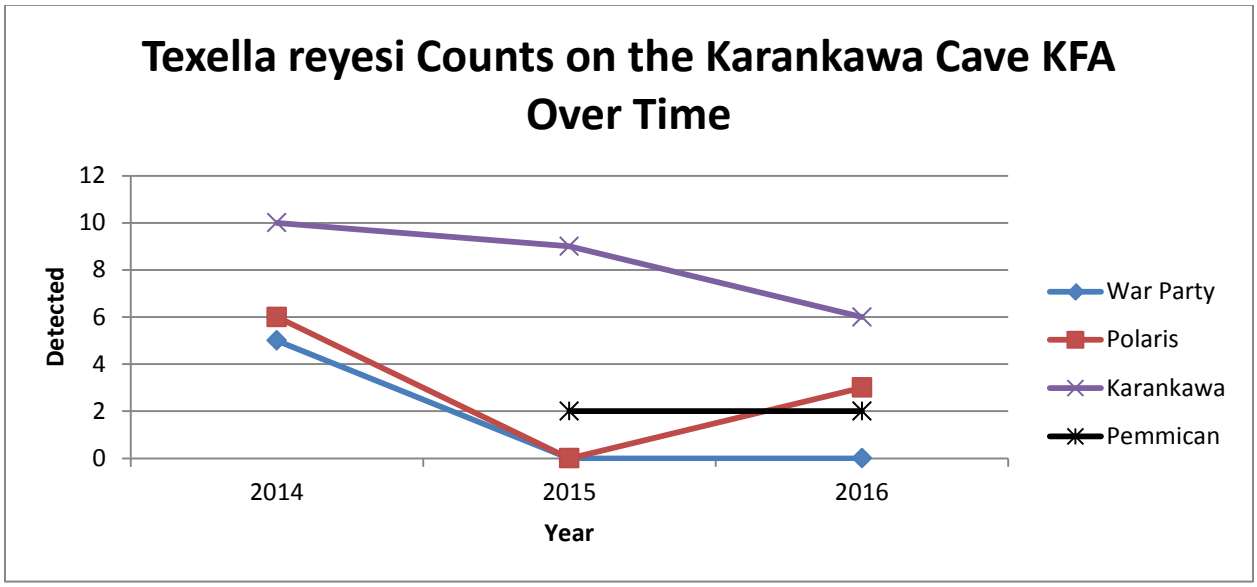


Figure 19. Endangered karst invertebrate species detection within the Karankawa Cave KFA.

Temperature and humidity readings in the Karankawa Cave KFA caves appear to fluctuate significantly (Figure 20). For example, relative humidity within War Party ranges from 63.0% to 89.5%, while temperature ranges from 52.0°F to 75.5°F. Pemmican Cave was surveyed for the first time in 2015; therefore, data only exists for two years.

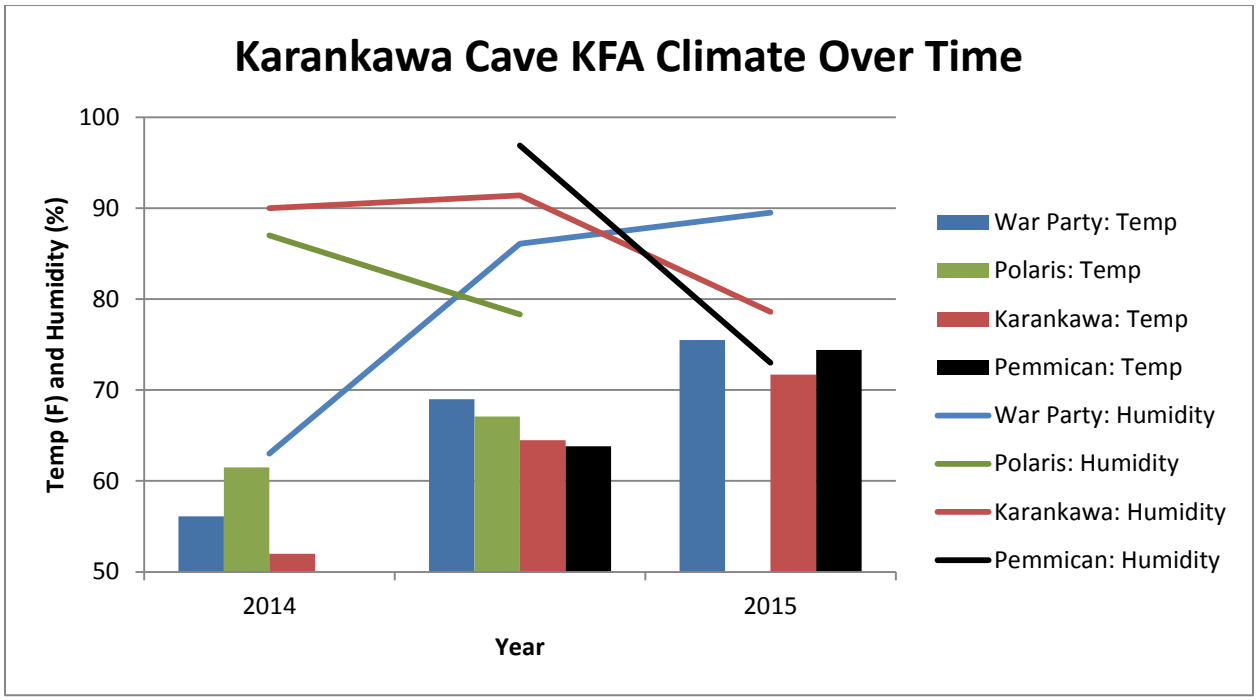


Figure 20. Climate readings within the Karankawa Cave KFA.

12.3 Adaptive Management Issues

The Karankawa Cave KFA is located in a currently largely undeveloped area, but plans for development in the area are in place. The following specific issues should be considered by the AMC:

- Quahadi Cave is not accessible for biota surveys and will require further excavation to enable future access. The AMC may wish to have this feature fully excavated.

- Pemmican Cave is directly adjacent to and downslope from the Ranch Road used to access the Karankawa Cave KFA interior. Its downslope location allows leaf litter and debris to wash into the cave during rain events. Current stormwater management includes an artificial silt fence to filter water moving towards the cave (Photograph 3). The AMC should consider installing a permanent rockwall or cobble filter dam to continue stormwater filtration indefinitely.

Photograph 3. Pemmican Cave silt fence filters stormwater entering the subsurface.

13.0 COFFIN CAVE PRESERVE

Coffin Cave Preserve is approximately 0.35 mile west from the Ronald Reagan Boulevard and County Road 234 intersection and occupies approximately 38 acres.

13.1 Management Activities

Two dumped tires were noted and subsequently removed by the Preserve and Trails Steward from the Coffin Cave Preserve entrance in May.

A large, heavy duty, steel A-frame was constructed atop the Coffin Cave entrance in November. This feature will allow 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. Cave gate construction across the Coffin Cave entrance was still underway in December. See Photograph 4 for the gate installation in progress.

Table 20 describes notes from cave inspections occurring once per month.

Photograph 4. Progress shot depicting cave gate and A-frame construction over Coffin Cave entrance.

Table 20. Preserve maintenance activities within the Coffin Cave Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Coffin Cave	No	No	No gate	No	1×6m W.	None.
* Fence intact around entire perimeter						
February						
Coffin Cave	No	No	No gate	No	No active mounds	1.None. 2.Cleared dirt and debris from around culvert over cave so it can be removed for monitoring. 3.None.
* Fence intact around entire perimeter						
March						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
April						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
May						
Coffin Cave	Yes	No	No gate	No	None	1.Two tires dumped at preserve entrance. 2.Removed tires. 3.None.
* Fence intact around entire perimeter						

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
June						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
July						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
August						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
September						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
October						
Coffin Cave	No	No	No gate	No	None	None.
* Fence intact around entire perimeter						
November						
Coffin Cave	No	No	No gate	No	None	1.Building repelling station over cave entrance. 2.None. 3.None.
* Fence intact around entire perimeter						
December						
Coffin Cave	No	No	No gate	No	None	1.Gate being built. 2.None. 3.None.
* Fence intact around entire perimeter						

13.2 Karst Biota Survey

Coffin Cave is unusually shaped for a Williamson County karst feature due to the approximately 40-foot vertical shaft that leads to the horizontal cave extent. As such, safety for biota surveyors is of paramount concern to the WCCF. Mark Pettigrew spent considerable effort designing and installing a heavy duty steel A-frame that allows biota survey crews to safely ingress and egress from the cave via rappelling ropes (Photograph 4). Previous expeditions utilized ropes tied to trees. A large cave gate was also installed in 2016. However, cave gate and A-frame completion around Coffin Cave was completed in late December and 2016 biota surveys did not occur as a result.

13.3 Adaptive Management Issues

The Coffin Cave Preserve is located in a mostly undeveloped area, but development in the region is ongoing. The following specific issues should be considered by the AMC:

- A small, ephemeral stream has been diverted around the entrance to the cave, but this modification represents an artificial condition for the cave. The AMC may consider rerouting the stream back to its previous course in order to restore more natural conditions within the cave.

14.0 BECK COMMONS PRESERVE

The Beck Commons Preserve occupies 5 acres and is approximately 0.25 mile north of the Beck Preserve, just off RM 620.

14.1 Management Activities

Upgraded gates and locks were installed on the Beck Sewer Cave and Beck Trash Cave entrances in January to further prevent unauthorized access. New warning signs were posted on both cave gates in May.

Trash along the preserve perimeter was noted as a recurring problem.

Table 21 describes notes from cave inspections occurring once per month.

Table 21. Preserve maintenance activities within the Beck Commons Preserve.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
January						
Beck Sewer Cave	No	No	Yes/Yes	No	1×6m S., 1×0.5m E.	1.Trash around perimeter. 2.Picked up trash. Welded new lock on to gate. 3.None.
Beck Trash Cave	No	No	Yes/Yes	No	None	1.Trash around perimeter. 2.Picked up trash. Welded new lock on to gate. 3.None.
* Fence intact around entire perimeter						
February						
Beck Sewer Cave	No	No	Yes/Yes	No	1×6m S., 1×0.5m E.	1.Trash around perimeter. 2.Picked up trash. Treated RIFA mounds boiling water 3.None.
Beck Trash Cave	No	No	Yes/Yes	No	None	1.Trash around perimeter. 2.Picked up trash. 3.None.
* Fence intact around entire perimeter						
March						
Beck Sewer Cave	No	No	Yes/Yes	No	None	None.
Beck Trash Cave	No	No	Yes/Yes	No	None	None.
* Fence intact around entire perimeter						

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
April						
Beck Sewer Cave	No	No	Yes/No	No	None	None.
Beck Trash Cave	No	No	Yes/No	No	None	None.
* Fence intact around entire perimeter						
May						
Beck Sewer Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.
Beck Trash Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.None. 2.Put new "Warning" sign on gate. 3.None.
* Fence intact around entire perimeter						
June						
Beck Sewer Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
Beck Trash Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	None.
* Fence intact around entire perimeter						
July						
Beck Sewer Cave	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash needs removal.
Beck Trash Cave	No	No	Yes/Yes	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.More trash needs removal.
* Fence intact around entire perimeter						
August						
Beck Sewer Cave	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Beck Trash Cave	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* Fence intact around entire perimeter						
September						
Beck Sewer Cave	No	No	Yes/No	No	Difficult to monitor due to thick vegetation.	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Beck Trash Cave	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.

Preserve/Cave	Vandalism, Trash Dumping & Unauthorized Entry	Damage To Vegetation, Pet Issues & Feral Animals	Gate Inspection & Lock Lubrication	Off Trail Activity	RIFA Mounds Within 10 Meters Of Cave Entrance	1. Comments. 2. Tasks Completed. 3. Tasks Outstanding.
* Fence intact around entire perimeter						
October						
Beck Sewer Cave	No	No	Yes/No	No	1×4m S.W.	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Beck Trash Cave	No	No	Yes/No	No	None	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* Fence intact around entire perimeter						
November						
Beck Sewer Cave	No	No	Yes/No	No	2×4m S.W.	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
Beck Trash Cave	No	No	Yes/No	No	Multiple mounds at 20m+	1.Trash around perimeter of preserve. 2.Picked up trash. 3.None.
* Fence intact around entire perimeter						
December						
Beck Sewer Cave	No	No	Yes/No	No	2×4m S.W.	1.Trash around perimeter of preserve. Too cold for fire ant mounds to be active. 2.Picked up trash. 3.None.
Beck Trash Cave	No	No	Yes/No	No	Multiple mounds at 20m+	1.Trash around perimeter of preserve. Too cold for fire ant mounds to be active. 2.Picked up trash. 3.None.
* Fence intact around entire perimeter						

14.2 Karst Biota Survey

There is surprisingly little life in the lower room in the deepest cave extent. However, surveyors detected 17 Bone Cave harvestmen during the 2016 biota survey.

No specimens were collected.

Karst biota survey results are summarized in Table 22.

Table 22. Karst Biota Survey Results at Beck Commons Preserve.

18-Oct-16		Inside Cave		Outside Cave					
Preserve	Cave	RH (%)	Temp (°F)	RH (%)	Temp (°F)	Person Hours	Surveyors	Species	Number
Beck Commons	Beck Sewer	95.5	75.4	80.3	80.0	12.8	Stephen Van Kampen-Lewis, Matt Nordgren, Kemble White, Craig Crawford, Reed Leible, Ashley Wall, Collin Haas	*Texella reyesi*	17
								Cambala speobia	16
								Speodesmus bicornourus	5
								Ceuthophilus cunicularis	dozens
								Ceuthophilus secretus	7
								Cicurina varians	20
								Cicurina vibora	1
								Anapistula sp.	1
								Collembola sp.	dozens
								Eidmanella sp.	2
								Lepisma saccharina	2
								Eleutherodactylus marnockii	2
								Eptesicus fuscus	1
								Isopod	1
								Surface Millipede	1

Surveyors have located the Bone Cave harvestman during every biota survey and observations have increased with each year of survey (Figure 21).

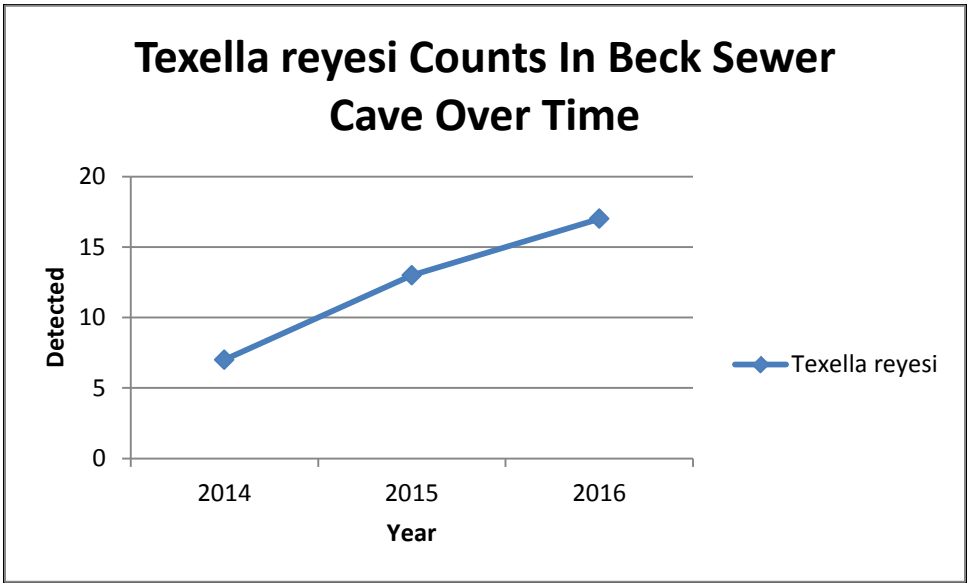


Figure 21. Endangered karst invertebrate species detection within the Beck Sewer Cave.

The climate readings within Beck Sewer Cave indicate relatively stable climate conditions. Humidity is generally close to 100% and temperatures range from 70°F to 75°F (Figure 22).

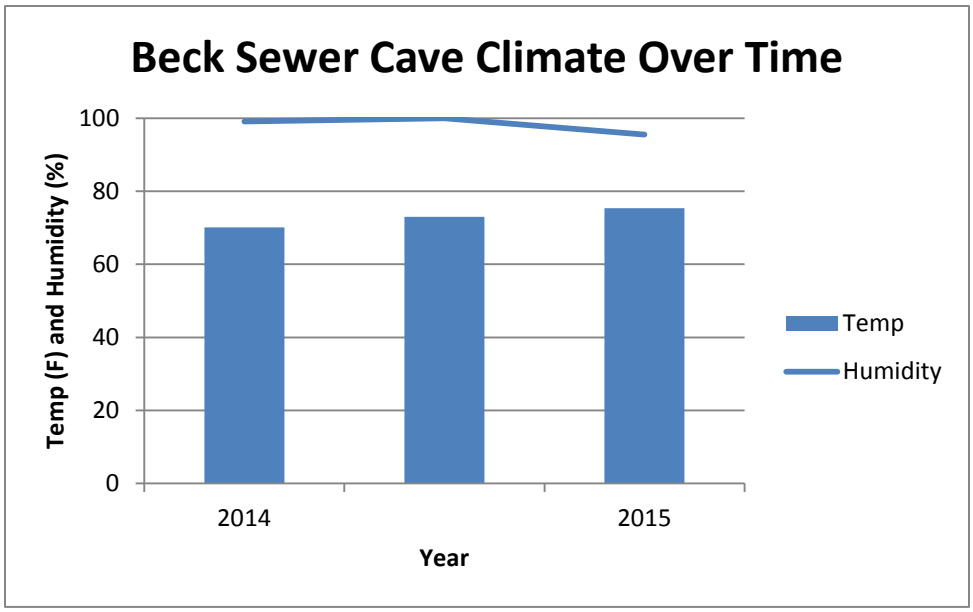


Figure 22. Climate readings within Beck Sewer Cave.

14.3 Adaptive Management Issues

There are currently no noticeable issues that the AMC should consider for the Beck Commons Preserve.

15.0 SHAMAN CAVE KARST FAUNA AREA

15.1 Management Activities

Regular maintenance activities did not occur at the Shaman Cave KFA due to its late 2016 acquisition by the WCCF. Monthly maintenance activities are expected to begin in early 2017.

15.2 Karst Biota Survey

An initial karst biota survey in Shaman Cave occurred on December 8, 2016. The cave is fairly extensive and water could be heard moving through the cave ceiling, down the wall, and into the cave floor. However, no moving water was visible to the survey crew. Eight Bone Cave harvestmen and two *Batrisodes cryptotexanus* were detected by the survey crew during the biota survey.

One *Rhadine noctivaga* was collected in Shaman Cave.

An attempt was made to enter Powwow Cave on the same day but sedimentation blocked the cave entrance. The debris was cleared and access allowed the Powwow biota survey on December 14, 2016. The biota survey crew detected 27 Bone Cave harvestmen, which is a record number of individuals observed in any cave during a single survey.

One *Batrisodes uncicornis* was collected from Powwow Cave on December 14, 2016. A voucher Bone Cave harvestman specimen will be collected during 2017.

Karst biota survey results and climate conditions are summarized in Table 23.

15.3 Adaptive Management Issues

There are currently no noticeable issues that the AMC should consider for the Shaman KFA.

Table 23. Karst Biota Survey Results at Shaman Cave Karst Fauna Area.

8-Dec-16	14-Dec-16	Inside Cave		Outside Cave		-			
Preserve	**Cave**	**RH (%)**	**Temp (°F)**	**RH (%)**	**Temp (°F)**	**Person Hours**	**Surveyors**	**Species**	**Number**
Shaman	Shaman	99.9	67.4	28.2	46.5	4	Stephen Van Kampen-Lewis, Kemble White, Ryan Jones, Harris Frampton	*Texella reyesi*	8
								Batrisodes cryptotexanus	2
								Ceuthophilus cunicularis	5
								Ceuthophilus secretus	3
								Speodesmus bicornorus	18
								Cambala speobia	2
								Cicurina vibora	13
								Anapistula sp.	1
								Achaearanea sp.	1
								Pseudouroctonus reddelli	4
								Anillinus affabilis	2
								Rhadine noctivaga	3
								Crotalus atrox	1
								Unknown caterpillar	1
								Unknown white moth	1
Shaman	Powwow	95.7	76.7	51.1	60.8	4	Stephen Van Kampen-Lewis, Craig Crawford, Reed Leible, Ryan Jones	*Texella reyesi*	27
								Speodesmus bicornorus	15
								Cicurina varians	6
								Cicurina vibora	2
								Rhadine noctivaga	2
								Batrisodes uncicornis	4
								Pseudouroctonus reddelli	1
								Gnat	1
								Unknown caterpillar	1
								Annelid	1

16.0 ADDITIONAL ACTIVITIES

16.1 Georgetown Salamander Survey

The GTS is included in the RHCP as a species of concern (SOC). Committed research for the species was mandated within the RHCP between years two through six (2009-2015), which is now complete. However, the RHCP indicates that additional funds cover research for SOC beginning with $25,000 in Year 2 and increasing by 2.5 percent through the 30-year RHCP plan. As such, the WCCF has continued funding the GTS research originally begun by Dr. Ben Pierce (Southwestern University) and continued by Dr. Kemble White (Cambrian Environmental). Survey results are attached to this report in Appendix B and summarized below.

Water quality monitoring and GTS population counts continued at Cobbs Spring, Twin Springs, and Swinbank Spring by Cambrian Environmental biologists, Dr. Kemble White, Craig Crawford, and Ashely Wall, between late 2015 through December 2016. Temperature, pH, dissolved oxygen, and specific conductivity were investigated at various locations throughout each spring run. Water quality results indicate parameters within each spring conform to conditions typically associated with the Edwards Aquifer water that is relatively unaffected by urbanization.

Salamander encounters remain sporadic, which is typical for the species. Wild.ID software is also being utilized for captured salamanders in order to determine recapture rates for encountered salamanders. Each GTS has a unique melanophore pattern that can be recorded with photographs. Wild.ID cross references photographs in order to determine if a photographed GTS has been previously documented within the database. This technique is less invasive and less time consuming to implement than previous mark-recapture techniques where captured salamanders were marked, released, and recorded when recaptured. Initial results indicate that relatively low recapture rates currently occur at these springs; however, the data are still being analyzed and recapture rates may increase as the database grows larger.

This report also discusses initial results pertaining to JPS surveys conducted at four spring sites by Dr. Michael Forstner (Texas State University), Zach Adcock (Texas State University), and biologists at Cambrian Environmental. More details are presented in Section 16.2.

16.2 Jollyville Plateau Salamander Studies

The JPS is also included within the RHCP as a SOC. The WCCF funded two, one-time *Eurycea* studies examining various environmental factors on the species' health as corollaries to potential urbanization effects. Authors contributing to one or both studies include Zachary Adcock, Andrea Villamizar Gomez, William K. Keitt, Dittmar Hahn, Justin C. Crow, Kenneth G. Ostrand, Joseph R. Tomasso, and Michael Forstner. Study results are attached to this report in Appendix C and summarized below. All results presented in this report are preliminary and may be altered through additional analysis for final publication.

The first study analyzed in-situ JPS population trends, habitat use, and disease prevalence in Williamson County. Water quality and population counts were conducted at five spring sites (Avery Deer, Avery Springhouse, Hill Marsh, PC, and Brushy Creek springs) from 2013-2016. Data indicate that water depth, pH, and conductivity were statistically significant predictors for JPS occurrence on a microhabitat scale and that flow velocity, water temperature, dissolved oxygen, and turbidity were not statistically significant; despite previous studies to the contrary.

Encountered JPS data indicate that abundance is significantly different between springs and that abundance decreases with distance from spring outlets. Data indicate that JPS may preferentially select woody debris over other cover structure types. Seasonal abundance was not detected for the species; however, a fall/winter reproductive season was observed.

The amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) was tested on all captured JPS throughout the study. Salamanders from each site tested positive for *Bd*; however, disease prevalence decreased from 2013 to 2015. Hill Marsh had significantly higher *Bd* rates than other springs. No observable effects were noticed on any infected JPS; which is notable due to the high mortality rates from this disease often seen in other amphibian taxa.

The second study examines laboratory effects of temperature and nitrogenous waste on captive-bred Barton Springs salamander (BSS; *Eurycea sosorum*) at the San Marcos Aquatic Resource Center as a surrogate for the JPS. Physiological responses (stress induced "loss-of-righting response and growth effects") to four stressors (temperature, ammonia, nitrite, nitrate) investigated. Data indicate the maximum mean temperature tolerance for BSS is 32.6±0.24 degrees Celsius (°C) and optimal temperature for BSS growth is 18.3°C. Nitrogenous waste studies indicate that the 2001 and 2013 U.S. Environmental Protection Agency water quality criteria recommendations for acute total ammonia-nitrogen exposure (17 mg/L un-ionized ammonia [UIA-N] and 0.066 mg/L UIA-N/L) are adequate to protect the BSS.

16.3 Shaded Fuel Break around the Regional Park

Wilco and Millennium Preserves are located within the Southwest Williamson County Regional Park (Regional Park). Land use around the Regional Park has become more urbanized as residential neighborhoods are built in the area. The AMC recommended seeking funding from the Federal Emergency Management (FEMA) for a shaded fuel break around the Regional Park's periphery in 2012. Agee et al. (2000) define shaded fuel break as such:

> "…a strategically located wide block, or strip, on which a cover of dense, heavy, or flammable vegetation has been permanently changed to one of lower fuel volume or reduced flammability."

Shaded fuel breaks alter surface fuels (i.e., remove or reduce flammable objects on the ground such as leaves or twigs), increase height to live crown base, and open the canopy with tree removal and are generally designed to assist fire fighters in the event a catastrophic blaze occurs within the Regional Park (Agee et al. 2000). Altering fire behavior to reduce the likelihood that forest fire can move easily across the landscape between the Regional Park and adjacent properties is the goal for creating this shaded fuel break. Coordination between USFWS, FEMA, and the Williamson County Director of Emergency Management generated an approach that all agencies agreed would create a fire barrier while maintaining the Regional Park's integrity as habitat for listed karst invertebrates.

The shaded fuel break project received FEMA Hazard Mitigation Grant Program funding, which was created after the devastating 2011 Texas Wildfires. A 50-foot-wide shaded fuel break was creating in 2016 that encircles the Regional Park in an approximately 4-mile-long corridor and covers 24.2 acres (Photograph 5). Jarred Thomas (Williamson County Director of Emergency Management) has indicated a desire for similar landscape style shaded fuel breaks to be implemented at other locations as well (J. Thomas, personal communication, 23 Jan 2017).

Photograph 5. Shaded fuel break near Fence & Trail Cave in the Millennium Preserve. Notice mature tree canopy is left in place while trees and shrubs near the ground have been mulched.

16.3.1 Adaptive Management Issues

The following specific issues should be considered by the AMC:

- Extending the shaded fuel break program from the Regional Park to other WCCF preserves may prove useful when combating future fire issues by reducing the ability for fire to easily move across the landscape. The Regional Park can act as template for implementing this type of program across other preserves and the AMC may wish to consider seeking additional funding for preserves with elevated fire risk.

- Twin Springs KFA has significant dead Ashe juniper stands due to recent drought and is located adjacent to low density housing developments; which elevates the need for shaded fire breaks at this location The AMC may wish to consider giving Twin Springs priority status for additional shaded fire breaks created within WCCF administered preserves.

16.4 Southern Great Plains Climate Reconstruction

Chris Maupin, Ph.D. is a paleoclimate researcher at Texas A&M University currently investigating southern Great Plains climate reconstruction using high-precision techniques for cave speleothem light stable isotope analysis. Rainwater infiltrates the Edwards limestone and slowly dissolves the calcium carbonate bedrock, where precipitate forms cave speleothems. The dissolved material picks up climate record proxies, such as oxygen and carbon isotopes and trace cations. Dr. Maupin uses uranium-thorium dating techniques to assign age to the examined speleothems and connect that age with past climate records trends and events.

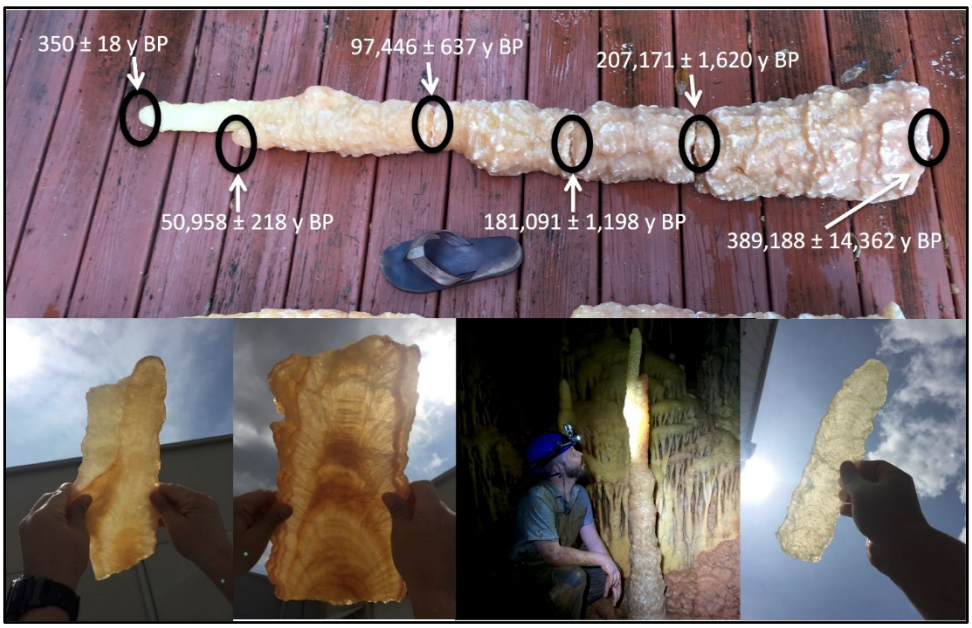
Dr. Maupin has also been collecting active drip waters inside the cavern (Photograph 6) and has separately collected precipitation in central Texas during every rainfall event since April, 2015; as a means to understand controls on precipitation and karst water isotopic composition which becomes recorded in speleothem calcite. Previous collaborative work between Dr. Maupin and SWCA has yielded

undergraduate presentations with SWCA staff coauthors at the 2016 American Geophysical Union Fall Meeting (the largest annual scientific conference in the world).

The WCCF has granted permission to Dr. Maupin to collect two carefully selected stalagmites in growth positions from within Cobbs Cavern's "Wild Side." Dr. Maupin identified these samples after thoroughly observing formations throughout the cave over multiple visits. One of the two standing samples collected has been reconnaissance dated with 12 uranium-thorium disequilibrium ages that indicate at least semi-continuous growth spanning 350 to 389,000 years before present (Photograph 7); a potentially unprecedented window into the Williamson County and the broader Southern Great Plains paleohydrology. Texas A&M University graduate student, Ellen Bartow-Gillies, is currently performing stable oxygen and carbon isotopic analyses on this particular sample. She is attempting to reconstruct the fundamental character of precipitation glacial to interglacial transitions for her Masters' thesis. Speleothems knocked over during the original, decades-old preparation of the "Show Side" were also collected for similar studies.

Dr. Maupin et al. became the first to identify the existence and precipitation response of abrupt climate change during the last glacial in Williamson County using samples from caves other than Cobbs Cavern. These results are currently in preparation for submission to a peer-reviewed journal.

Photograph 6. Chris Maupin (Ph.D.) examines speleothem purity and collects drip water in Cobbs Cavern.



Photograph 7. Montage showing nearly 400,000 year old speleothem collected from Cobbs Cavern and thin speleothem slices used for isotopic analysis.

17.0 WILLIAMSON COUNTY RHCP 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.

17.1 Endangered Songbird Credits

17.1.1 Golden-cheeked Warbler

The WCCF sold 43.7 GCWA credits during 2016 for the Southwest Bypass Project, which was the only participant requiring such credits for the entire year. The WCCF held 568.79 GCWA credits through the end of 2016. All GCWA credits and debits from WCCF inception are shown in Table 24. 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.

Table 24. WCCF managed golden-cheeked warbler credits through time.

WCCF Golden-cheeked Warbler Credits Through 2016		
Credits	**Project Description**	**Date**
500.00	Hickory Pass Ranch Conservation - Golden Cheeked Warbler Credits	Nov. 2007
115.52	Twin Springs - Golden Cheeked Warbler Credits	Jan. 2008
(74.40)	Committed to Ronald Reagan Phase III	Sept. 2010
500.00	Hickory Pass Ranch - Golden Cheeked Warbler Credits	Dec. 2010
(5.52)	Committed to Vista Ridge Boulevard	Oct. 2011

WCCF Golden-cheeked Warbler Credits Through 2016		
Credits	**Project Description**	**Date**
(1.00)	Committed to 1710 CR 262 Georgetown	Feb. 2012
(24.31)	Committed to Lakeline Blvd Extension Phase 2 from Old Quarry Rd to Old 2243 west of Leander	Nov. 2013
(1.77)	Committed to a water transmission line RR Blvd to CR 175 (0.62 acres direct, plus 2.29 indirect)	Nov. 2013
(30.41)	committed to Reagan's Overlook subdivision (19.03 acres direct, plus 22.76 indirect)	Aug. 2014
(8.00)	LAMY 2243 - Bluffview	Nov. 2014
(346.00)	Wedemeyer	Dec. 2014
(2.50)	City of Georgetown	Aug. 2015
(2.11)	American Housing Ventures	June 2015
(7.02)	Sentinel Land / HWY 29 Ventures 2015 LP	Sept. 2015
(43.70)	City of Georgetown - SW Bypass	Jan. 2016
568.79	**Total GCWA Credits Held By WCCF**	

In 2016, 43.70 acres of GCWA habitat were impacted; therefore 546.73 acres of GCWA habitat have been impacted directly or indirectly since RHCP inception. The RHCP anticipated 200 impacted GCWA habitat acres per year, totaling 6,000 impacted acres of habitat throughout the RHCP life. Therefore, 1,600 acres of GCWA habitat were anticipated for impact through 2016, while only 546.73 acres have actually been impacted.

17.1.2 Black-capped Vireo

No BCVI habitat participation was recorded during 2016; therefore, no additional coverage was added to the 22.5 acres already impacted. The RHCP anticipated 142 covered acres for BCVI habitat per year, totaling 4,267 impacted acres of habitat throughout the RHCP life; therefore 1,138 BCVI habitat acres were expected to have been impacted through 2016.

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 2016. The Adaptive Management Committee did not make any recommendations for fund use due to insufficient opportunities to do so. No BCVI habitat credits are currently held by the WCCF.

17.2 Karst Participation

Nineteen new projects participated for karst coverage within the RHCP across 492.8 acres during 2016; bringing the total enrolled karst zone participation through December 2016 to 7,309.8 acres during the RHCP's lifetime (6,817 + 492.8 acres).

Actual karst zone participation acreage is higher than assumptions discussed in the RHCP. The permit projected 533 acres of karst zone participation per year, totaling 4,264 participation acres after eight full years (2009 through 2016) of RHCP operation. Currently, the WCCF accounts for 7,309.8 karst zone participation acres after eight full permit operation years.

However, actual Zone A and Zone B impacts (see RHCP for details regarding cave Zone A and Zone B) are significantly lower than assumptions discussed within the RHCP. Fifty six caves were projected to be partially impacted by Zone A intrusion over an 8-year period (seven per year), while the WCCF has recorded Zone A impacts to 24 caves through 2016. Zone A participation included 28.8 acres for three caves in 2016.

Sixteen caves were expected to be fully impacted by Zone B intrusion over 8 years (two per year), while the WCCF has recorded Zone B impacts to three caves through 2016. The WCCF did not grant participation for Zone B cave impacts during 2016.

Table 25. Annual report tracking spreadsheet for 2016 RHCP participation.

Project	Enrolled Karst Zone (Acres)	Zone A intrusion (Acres)	Zone B intrusion (Caves)
Arterial H - Williamson County Road Bond	14.5	0	0
Brushy Creek MUD - Pepper Rock Creek	4.9	0	0
Cal Atlantic Homes - Ranch at Brushy Creek	2.7	0	0
City of Georgetown - SW Bypass	78.9	1.7	0
Continental 348 Fund LLC	22.6	0	0
Enterprise Crude Pipeline	5.2	0	0
Enterprise Crude Pipeline	4.9	0	0
Escalera Parkway - Williamson County Road	0.4	0	0
Forest North Drainage - Williamson County	6.6	0	0
Gatlin Creek (also Century Land Holdings)	96.1	0	0
Hwy 29 Ventures LP	74.7	24.4	0
Neenah Ave - Williamson County Road Bond	4.3	0	0
Pearson Ranch Road - Williamson County Road	18.7	0	0
RM 620 Phase 2 - Williamson County Road Bond	21.4	0	0
Round Rock ISD - McNeil HS (Williamson County Portion)	16.7	0	0
HWY 29 at Cedar Hollow - Williamson County	0.6	0	0
Pulte Homes of TX - SC 84	96.7	2.7	0
Pulte Homes of TX - SC 83 and 86	22.6	0	0
UBCWCID - Geotechnical Testing	0.5	0	0
Total 2016 Enrolled Units	**492.8**	**28.8**	**0**
Previous Enrolled Units	**6816.7**	**21**	**3**
Total Enrolled Units	**7309.5**	**49.8**	**0**

The WCCF acquired Shaman Cave KFA in 2016, bringing the total number of administered KFAs to five (Priscilla's Well, Cobbs Cavern, Twin Springs, Karankawa Cave) and two other existing preserves already administered by the WCCF are currently proposed for KFA status (Wilco and Millennium Preserves).

17.3 WCCF Statement of Activities

The unaudited Statement of Activities indicates the WCCF maintained nearly a $1.38 million balance at the end of 2015 and had increased that balance to nearly $1.41 million by the end of 2016 (Table 26).

Table 26. WCCF Statement of Activities for 2016.

Williamson County Conservation Foundation Statement of Activities (Unaudited) Jan 1, 2016 to Dec 31, 2016			
Balance as of 12/31/2015		$ 1,381,509.87	
Revenues:			
	Donations		$2,450.00
	Interest on Investments		$4,452.25
	Miscellaneous Revenue		$999.80
	Mitigation Credits		$2,900.00
	Participation Fees		$217,263.00
	Tax Benefit Financing	$ 109,609.41	
	Total Revenues		$337,674.46
Expenditures:			
	Contribution to Benefits (Insurance)		$13,488.00
	Copier Rental & Supplies		$3,263.34
	Equipment		$769.84
	Gasoline		$266.16
	Grounds Maintenance		$5,487.17
	Land Acquisition		
	Membership Dues		$100.00
	Misc		$1,251.87
	Office Furniture		$879.92
	Office Supplies		$258.03
	Postage		$106.63
	Printed Materials & Binding		$228.62
	Professional Services		$173,828.22
	Publications/Books/Periodicals		$293.74
	Research Activities		$6,355.50
	Salaries and related		$97,352.19
	Signs		$487.50
	Training/Travel		$4,038.51
	Use of Donations		$1,580.37
	Vehicle Repairs & Maintenance		$134.06
	Total Expenditures		$310,169.67
Fund Equity as of 12/31/2016		$ 1,409,014.66	

18.0 LITERATURE CITED

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APPENDIX A

2016 Golden-cheeked Warbler Monitoring Survey on the Twin Springs Preserve, Williamson County, Texas

2016 Golden-cheeked Warbler Monitoring Survey on the Twin Springs Preserve, Williamson County, Texas

SWCA Project Number 10717-139

July 2016

SUBMITTED TO:

Williamson County
350 Discovery Boulevard, Suite 207
Cedar Park, Texas 78613

SUBMITTED BY:

SWCA Environmental Consultants
4407 Monterey Oaks Boulevard
Building 1, Suite 110
Austin, Texas 78749

2016 GOLDEN-CHEEKED WARBLER MONITORING SURVEY ON THE TWIN SPRINGS PRESERVE, WILLIAMSON COUNTY, TEXAS

Prepared for

WILLIAMSON COUNTY
350 Discovery Boulevard, Suite 207
Cedar Park, Texas 78613

Submitted by

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SWCA Project Number 10717-139-AUS

21 July 2016

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1.0 INTRODUCTION

SWCA Environmental Consultants (SWCA) was retained by Williamson County to conduct a presence/absence survey for the federally listed endangered golden-cheeked warbler (*Dendroica chrysoparia*; GCWA) on the Twin Springs Preserve (Preserve) in the spring of 2016. The Preserve consists of approximately 158.3 acres located northwest of Lake Georgetown on the west side of County Road (CR) 262 approximately 0.25 mile south of its intersection with Farm-to-Market Road (F.M.) 3405, and roughly 6 miles northwest of the City of Georgetown, Williamson County, Texas (Figure 1).

The Preserve is the undeveloped portion of a formerly 193-acre tract known previously as the Russell Park Estates property. The U.S. Fish and Wildlife Service (USFWS) issued a section 10(a) permit on 25 July 2002 to Rockledge, Inc. that authorized the incidental take of the GCWA during development of approximately 53.5 acres of the Russell Park Estates property. As part of the Habitat Conservation Plan developed in support of the section 10(a) permit, Rockledge, Inc. agreed to preserve 127.3 acres of the property in perpetuity for the benefit of the GCWA, and to establish a 12.2-acre preserve around a cave (Sunless City Cave) known to support a federally listed endangered invertebrate species. After 22.5 acres of the property fronting CR 262 was developed, Williamson County purchased the 127.3-acre preserve area and the remaining 31-acre development area to create what is now the 158.3-acre Twin Springs Preserve.

As a condition of the Russell Park Estates section 10(a) permit, USFWS required that preserved portions of the property be surveyed for GCWAs on a bi-annual basis. The first GCWA monitoring survey was conducted in the spring of 2004. At that time, a road alignment had been cleared through the 22.5-acre development area on the Russell Park Estates property, but no home construction had commenced. The second GCWA monitoring survey was conducted in the spring of 2007, prior to Williamson County acquiring the Twin Springs Preserve in 2008. Williamson County was hereafter obligated to perform the GCWA monitoring surveys. The number of GCWA territories and female or fledgling GCWAs observed on the Preserve during all surveys is summarized in Table 1 below. During some years, female or fledgling warblers were not detected within the Preserve. Given the difficulties in detecting GCWA females, nest sites, and fledglings, and the limited time spent within the Preserve, failure to detect breeding success does not necessarily mean that GCWA did not breed successfully during that year.

Table 1. GCWA Survey Results within or Adjacent to the Twin Springs Preserve

Survey Year	Number of GCWA Territories	Number of Female GCWAs	Number of Fledgling Observations
2004	8	0	0
2007	9	4-5	5
2009	6-7	4	0
2012	5	3	4
2014	6*	4	0
2016	6-7	4	2

*One territory partially extended onto the Twin Springs Preserve from the south.

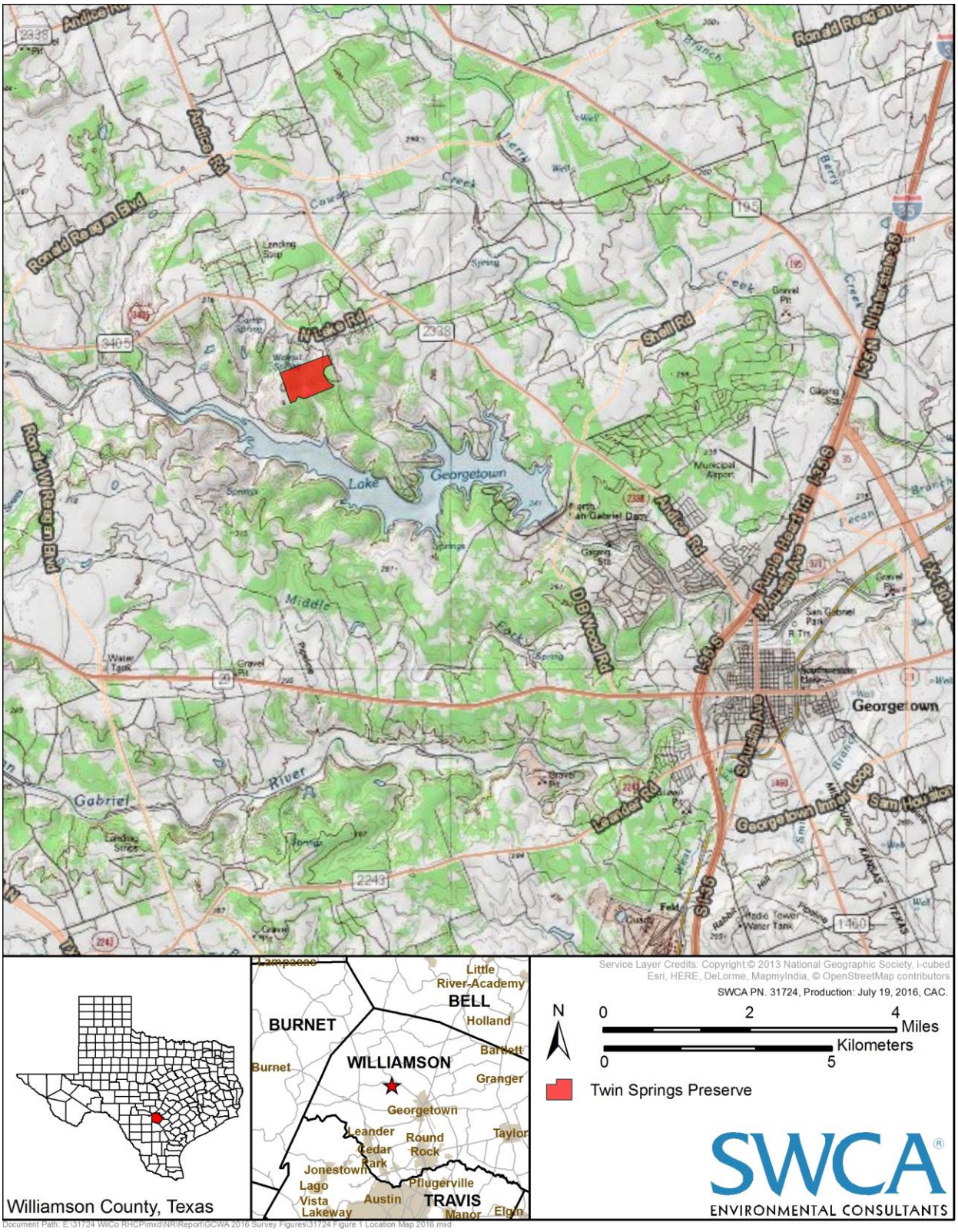

Figure 1. Twin Springs Preserve location map.

2.0 METHODS

The terms and conditions of the Russell Park Estates section 10(a) permit stipulated that the required GCWA monitoring surveys be performed in accordance with USFWS presence/absence survey protocols.[1] SWCA biologists experienced with GCWA vocalizations conducted surveys for the GCWA within the Preserve on five days between March 24 and May 31, 2016. All survey personnel were covered by SWCA's federal endangered species research and recovery permit (Permit No. TE800611) or were under direct supervision of a permitted biologist. Surveyors slowly walked throughout the entire Preserve looking and listening for the GCWA at a level of effort that exceeded the recommended minimum survey time of 20 hours per 100 acres of habitat. Locations of all GCWA seen or heard during surveys were mapped in the field on aerial photographs (1 inch = 250 feet) or recorded with a Garmin Etrex Global Positioning System device. A summary of survey dates and times is provided in Table 2.

Table 2. Summary of Survey Effort

Project Area	Date	Surveyor	Survey Time	Minutes
Twin Springs Preserve	24 March	Alyssa Martin	0704-1320	376
	31 March	Beth Banks	0735-1405	390
	28 April	Katie Snipes	0650-1150	300
	13 May	Beth Banks	0710-1338	388
	31 May	Beth Banks/ Katie Snipes	0720-1220	300
			Total	**1, 754 (29.23 hours)**

3.0 RESULTS

Over the course of five days, SWCA documented 124 observations of male GCWAs, 24 observations of female GCWAs, eight observations of GCWA fledglings, and one GCWA nest. Based on the distribution of warbler observations and patterns of contemporaneous vocalizations, it was estimated that all or portions of six to seven GCWA territories were present on the Preserve in 2016. Due to a lack of documented contemporaneous vocalizations, SWCA did not confirm if the seventh territory was actually part of the territory to the north or if it was a separate bird (Figure 2). Female GCWAs were observed in association with four of these territories. Fledglings were observed in association with two of these territories. The nest was documented within one of the territories that fledglings were observed.

Locations of all GCWA observations within the Preserve in 2016 are depicted on Figure 2. The distribution of GCWA observations correlated highly with the mixed deciduous/Ashe juniper (*Juniperus ashei*) woodland on the property. Most GCWA observations occurred in the north-central and western portions of the property, where a canyon supports higher densities of deciduous trees, especially Texas oak (*Quercus buckleyi*). Few GCWA observations were made on the northeast end of the property, which sits on a hilltop that supports woodlands dominated heavily by Ashe juniper trees.

[1] U.S. Fish and Wildlife Service (USFWS). 2010. USFWS section 10(a)(1)(A) scientific permit requirements for conducting presence/absence surveys and habitat assessments for endangered golden-cheeked warblers. USFWS Ecological Services Field Office, Austin, Texas.

On Figure 2, those observations believed to represent the same male GCWA are enclosed. Each enclosure generally contains a cluster of observations that developed over the course of the five survey visits. This type of distribution of observations is the typical result of surveys conducted over multiple days for birds that occupy discrete areas (territories). As can be seen on Figure 2, six to seven sets of observations are enclosed within the boundary of the Preserve. Several auditory observations were made in close proximity to one another and it is difficult to delineate these "territories;" therefore, these enclosures represent an approximate location.

The number of male GCWAs estimated on the Twin Springs Preserve in 2016 is the same or slightly higher than the number estimated in 2014, which is an increase from the number estimated in 2012 (see Table 1). In 2004, 2007, and 2009, it was estimated that three warbler territories were present on the east side of the conservation area, or the area east of the central canyon and below the area that is now developed. In 2012, 2014, and now in 2016, it is estimated that only two GCWA territories are present on this side of the conservation area. Consequently, the difference in number of GCWAs estimated present on the property through time arises from the number of birds believed present in, and west of, the central canyon. In 2014, this number was believed to be four, in 2012 this number was believed to be three, three to four in 2009, six in 2007, and five 2004.

GCWA numbers fluctuate naturally with time, so a slight increase or decrease in numbers from year to year is expected. Persistent drought conditions from 2008 through 2012 could have caused the temporary decrease in the local GCWA population, although that is simply speculation. Because no significant change in vegetation has been observed in the Preserve since 2004, and because the number of GCWA occurring near the developed area has not drastically decreased (meaning that it seems unlikely that the development has caused a decrease in number of warblers using the Preserve), we expect, barring some catastrophic event such as a wildfire, that future surveys could again result in the identification of up to eight or nine GCWA territories on the Preserve.

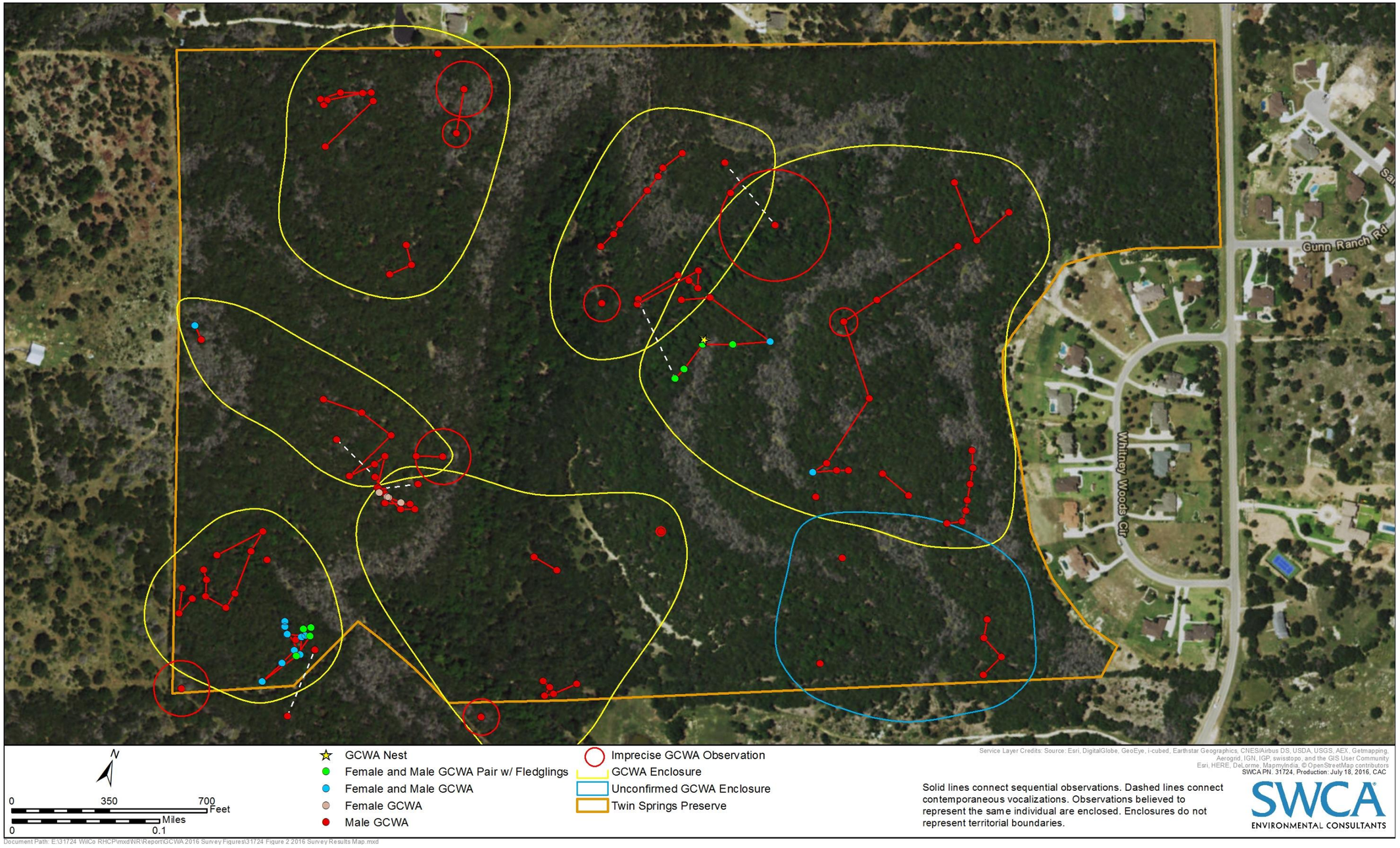

Figure 2. 2016 Twin Springs Preserve GCWA survey results.

APPENDIX B

Williamson County Conservation Foundation 2016 Annual Monitoring Report for the Threatened Georgetown Salamander (*Eurycea naufragia*) and Jollyville Plateau Salamander (*E. tonkawae*) at Eight Sites in Williamson County,

Williamson County Conservation Foundation 2016 Annual Monitoring Report for the Threatened Georgetown Salamander (*Eurycea naufragia*) and Jollyville Plateau Salamander (*E. tonkawae*) at Eight Sites in Williamson County, Texas

Cambrian Environmental
4422 Pack Saddle Pass, Suite 204
Austin, TX 78745
USFWS Permit # TE37416B-0

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

March 2017

As a licensed professional geoscientist I attest that the contents of this report are complete and accurate to the best of my knowledge.

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Texas Geoscience Firm Registration Number 50484
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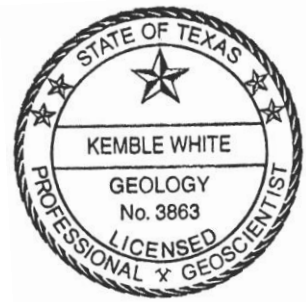

2016 Annual *Eurycea* Monitoring Activities Carried Out Under the Williamson County Regional Habitat Conservation Plan for Cambrian Environmental (Permit TE 37416B-0)

EXECUTIVE SUMMARY

Cambrian Environmental (under permit TE 37416B-0) conducted population monitoring activities at three springs occupied by the Georgetown salamander (*Eurycea naufragia*, GTS) and five sites occupied by the Jollyville Plateau salamander (*E. tonkawae*, JPS). 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). Population monitoring methods were in accordance with the U.S. Fish and Wildlife (USFWS) protocol requirements (USFWS 2014). Monitoring activities at these sites enhance understanding of *Eurycea* salamander biology, including information on long-term population trends, status, density, size, and reproductive ecology. Over time these data may signal potential impacts of human activities on the species, and may help improve the ecological effectiveness of conservation policies for the species in the future.

The GTS is endemic to Williamson County and is currently known to occur in at least seventeen springs, spring-fed creek segments, and caves within the San Gabriel River drainage basin. The JPS is endemic to Travis and Williamson counties and is currently known to occur in more than 100 springs, spring-fed creek segments, and caves within the Brushy Creek and Colorado River drainage basins. Habitat for the salamanders is closely tied to the northern segment of the Edwards aquifer whose host rock, the Edwards limestone (and associated hydrologic units), generally contains the caves, ground water and springs on which they depend. The salamanders have only been found in areas where discharge from the aquifer is sustained on a permanent or nearly-permanent basis. High quality spring habitat is characterized by clear water flowing over cobble substrate with an abundant macro-invertebrate community and aquatic vegetation. While these species are periodically known to retreat underground into the spring conduits (particularly following large rain events), how much of their life cycle is spent underground is unknown (Bendik 2011). Urbanization and declines in water quality and quantity are cited by the U.S. Fish and Wildlife Service (Service) as the primary threats to the species (USFWS 2012, 2013a, 2013b). The JPS was listed as federally threatened in August 2013 and critical habitat was designated for the species (USFWS 2013). The GTS was listed as federally threatened in February 2014 and critical habitat was proposed for the species (USFWS 2014).

This report details GTS monthly monitoring results that have been gathered at Cobbs Spring since July 2015 and Twin Springs and Swinbank Spring since October 2015, and raw data from JPS bimonthly monitoring results gathered at Avery Springhouse Spring, Avery Deer Spring, and Brushy Creek Spring since September 2016 and at Hill Marsh Spring and PC Spring since October 2016. Cambrian works in collaboration with Dr. Ben Pierce and his team from Southwestern University as part of an ongoing GTS capture-mark-recapture study using photo identification software (Wild.ID) at Twin Springs and Swinbank Spring. Wild.ID is a software designed to identify individual salamanders at a given site and serves as an information management platform to study population trends and dynamics. Wild.ID results from the GTS sites will be appended to the current report following completion. The five JPS sites were previously monitored by researchers at Texas State University (Dr. Mike Forstner and Zach Adcock) as part of a long-term project to evaluate population dynamics (including Wild.ID analysis) and habitat ecology. Cambrian commenced monitoring activities at these sites in the final months of 2016 and only raw data is presented herein. The overarching objective of monitoring activities is to determine short- and long-term population trends and to monitor water quality and quantity on a seasonal and annual basis. The management goal of the WCCF is to ensure the continued ecological health of salamander populations and habitat.

GEORGETOWN SALAMANDER

The Georgetown salamander (*Eurycea naufragia*, GTS) is endemic to spring outflows and wet caves of Williamson County. The species lacks a biphasic lifecycle and individuals never undergo metamorphoses into a terrestrial adult form. As an entirely aquatic species, the GTS is particularly vulnerable to water quality degradation (Pierce et al. 2014). The species requires relatively shallow, cool, moving water, an abundant macro-invertebrate community, aquatic vegetation, and substrates containing cover objects (including large rocks, cobble, and leaf litter) to provide sufficient refuge from predators (Pierce et al. 2010). Little is known about the salamanders' use of sub-surface flooded karstic refugia, although it is believed that these areas are utilized to escape surface drying events. The vast majority of caves within Williamson County do not provide access to the water table where suitable habitat for *Eurycea* salamanders would occur. The primary threats to the Georgetown salamander are urbanization and degradation of water quality and quantity; any activity that degrades the quality or quantity of water upstream of salamander habitat may result in take through habitat modification (Bowles et al. 2006; Chippendale and Price, 2005; USFWS 2012, 2013a, 2013b). The species is currently listed as threatened under the federal Endangered Species Act (USFWS 2014).

Cambrian conducted monitoring activities at three GTS-occupied sites, Twin Springs, Swinbank Spring, and Cobbs Spring, in Williamson County, Texas (Figure 1). Dr. Ben Pierce and his research team from Southwestern University have conducted monthly Georgetown salamander surveys for the WCCF at Twin Springs and Swinbank Spring since 2005 (https://sites.google.com/site/benapierce/). Dr. Pierce's regular teaching responsibilities have required him to reduce the amount of time spent in the field on this project. In October 2015, Cambrian personnel began supplementing Dr. Pierce's monitoring activities and by the end of the year had transitioned into the lead field role. Ashley Wall, M.S. is a former Pierce student who had been involved in the monitoring of these sites since 2010 (Pierce and Wall 2011; Pierce et al. 2014). Though a cooperative agreement between Cambrian, the WCCF, and Southwestern, Cambrian now supervises the Southwestern student research team in the field.

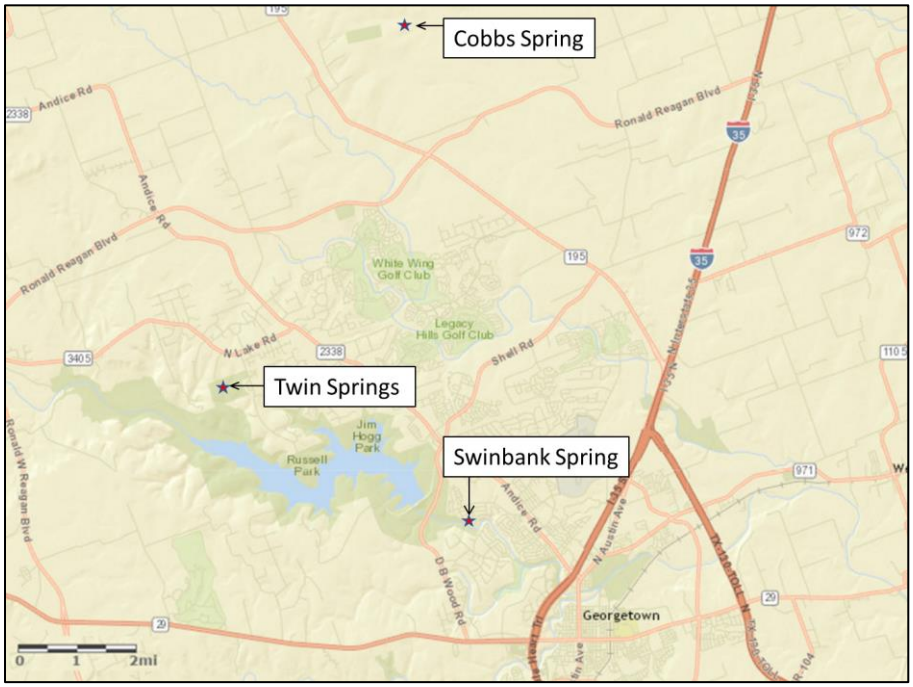

Figure 1. Location of GTS monitoring sites in Williamson County, Texas.

GTS Monitoring Methodology

The population monitoring survey area was delineated by running a transect from the spring origin to a specified distance downstream for each GTS site. The transect was divided into sections with a linear distance of approximately 5-m per section. Section size depends on stream characteristics at a given site. Utilizing the "Drive" method, we methodically searched the substrate for salamanders by overturning all potential cover objects (cobble, large rocks, leaf litter, vegetation) beginning at the most downstream point of the transect and moving upstream toward the spring head. Surveyors counted all cover objects overturned during each survey to standardize salamander counts as "number of salamanders per structure object" (Pierce et al. 2010; 2014).

All encountered salamanders were captured, when possible. Individuals were photographed on grid paper for future measurement and analysis using Wild.ID, a computer-assisted identification software that identifies individuals based on their unique melanophore patterns on the back of the head (Photo 1). This technique allows large numbers of individuals to be re-identified over time. Bendik et al. (2013) assessed the accuracy of using the Wild.ID identification technique in *Eurycea* salamanders compared with the use of physical markers (visual implant elastomers that are physically injected beneath the individual's skin) and found high matching success with the computer-assisted identification technique. Melanophore patterns shift and evolve over time and a negative correlation exists between match success and time between captures (Bendik et al. 2013). We therefore analyzed the first ten photos with the highest probability of being a potential match by eye for every individual captured.

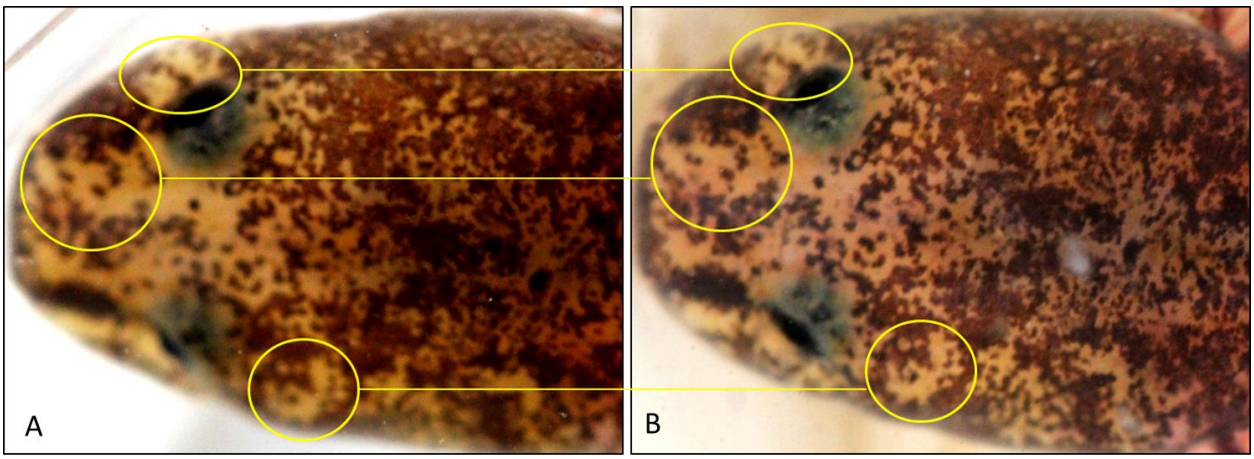

Photo 1. Melanophore recognition using the computer-assisted identification software Wild.ID. This individual from Cobbs Spring was captured first in April 2016 (A) and recaptured in May 2016 (B).

Salamanders were then checked for eggs and promptly released into the sections from which they were captured. In compliance with USFWS (2014) requirements to characterize salamander habitat, water quality parameters (water depth, water temperature, pH, dissolved oxygen, specific conductivity, and total dissolved solids) were taken with handheld meters following each survey and climate conditions (temperature and precipitation) for each survey date and the corresponding previous week were also recorded.

GTS Monitoring Results

Twin Springs. In collaboration with Dr. Ben Pierce and his research team from Southwestern University, Cambrian Environmental conducted monthly GTS monitoring at Twin Springs, a permanent spring located in the North San Gabriel River watershed in Williamson County, Texas (30°41′52.32″N, 97°46′53.15″W). The site is located within the Twin Springs Preserve, adjacent to low-density residential neighborhoods. The spring run contains silt, gravel, cobble, large rocks, and leaf litter with relatively high sedimentation, particularly in sections 4 and 5. We monitored salamanders by running a transect from the spring origin to 38 m downstream. The transect was divided into 6 sections, the first five sections being approximately 5 m long each and the last section (added to the originally 24-m transect by Dr. Ben Pierce in the last few years) being approximately 10 m long. Surveyors maintained a separate cover object count for Section 6.

Water quality parameters were measured at four distinct points (0 m, 14 m, 28 m, and 36 m from the spring outlet) along the transect and the average was recorded (Table 1, Figure 2). Total salamander encounters, number of salamanders captured for Wild.ID photographs, number of cover objects, and percentage of cover objects occupied by salamanders for Sections 1 – 5 and Section 6 at Twin Springs from November 2015-December 2016 are shown in Table 2. Percentage of cover objects occupied by salamanders on each survey date is shown in Figure 3. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 3).

Results from the Wild.ID analysis of salamanders from Twin Springs will be appended to the current report following completion and will include data from all monitoring events at the site (from October 2012 to December 2016).

Table 1. Water quality parameters and total salamanders encountered at Twin Springs during each survey event.

Date	Temp (°C)	pH	Dissolved Oxygen (mg/L)	Specific Conductivity (µS)	Total Dissolved Solids (ppm)	Embedded-ness (%)	Salamander Encounters
11/2/2015	21.4	7.4	6.9	640	329	60	7
11/20/2015	20.9	7.4	7.3	653.3	334.8	60	2
12/11/2015	20.4	7.5	7.5	652.5	332.3	50	5
1/22/2016	19	7.8	9	653.5	334	65	6
2/19/2016	20.6	7.8	5.9	638.5	325.3	65	13
3/22/2016	20.9	7.6	4.9	509.5	258.8	65	17
4/22/2016	21.3	7.6	8.95	747.3	381	65	4
5/16/2016	21.4	7.7	5.8	751.3	382.3	80	8
6/21/2016	21.8	7.6	8.0	762.8	388.5	70	3
7/20/2016	22.8	7.6	8.9	749.3	384.5	70	9
8/19/2016	23.0	7.6	5.6	721.0	366.8	70	10
9/23/2016	22.8	7.7	5.2	747.5	381.5	90	2
10/21/2016	22.4	7.8	X	751.0	382.3	90	13
11/23/2016	20.2	7.9	8.1	754.5	384.3	90	4
12/16/2016	20.98	7.8	7.4	750.75	381.75	90	11

*X indicates water parameter meter is under repair and measurements were unable to be taken.

Table 2. Total salamander encounters, number of salamanders captured for Wild.ID photographs, number of cover objects, and percentage of cover objects occupied by salamanders for Sections 1 – 5 and Section 6 at Twin Springs from November 2015-December 2016.

	Sections 1-5				Section 6			
Date	**Total Encounters**	**# Captured**	**CO**	**% CO with Salamanders**	**Total Encounters**	**# Captured**	**CO**	**% CO with Salamanders**
11/2/2015	7	6	729	0.96	0	0	349	0
11/20/2015	2	2	706	0.28	0	0	275	0
12/11/2015	5	5	572	0.87	0	0	219	0
1/22/2016	4	4	630	0.63	2	2	208	0.96
2/19/2016	6	6	656	0.91	7	6	281	2.49
3/22/2016	12	7	637	1.88	5	3	300	1.67
4/22/2016	4	2	549	0.73	0	0	483	0
5/16/2016	4	4	733	0.55	4	1	305	1.31
6/21/2016	2	2	551	0.36	1	0	294	0.34
7/20/2016	8	7	675	1.19	1	1	450	0.22
8/19/2016	8	8	790	1.01	2	1	261	0.77
9/23/2016	2	1	601	0.33	0	0	269	0
10/21/2016	11	11	636	1.73	2	1	336	0.60
11/23/2016	4	1	521	0.77	0	0	224	0
12/16/2016	5	3	658	0.76	3	2	263	1.14
1/27/2017	7	7	534	1.31	2	0	188	1.06

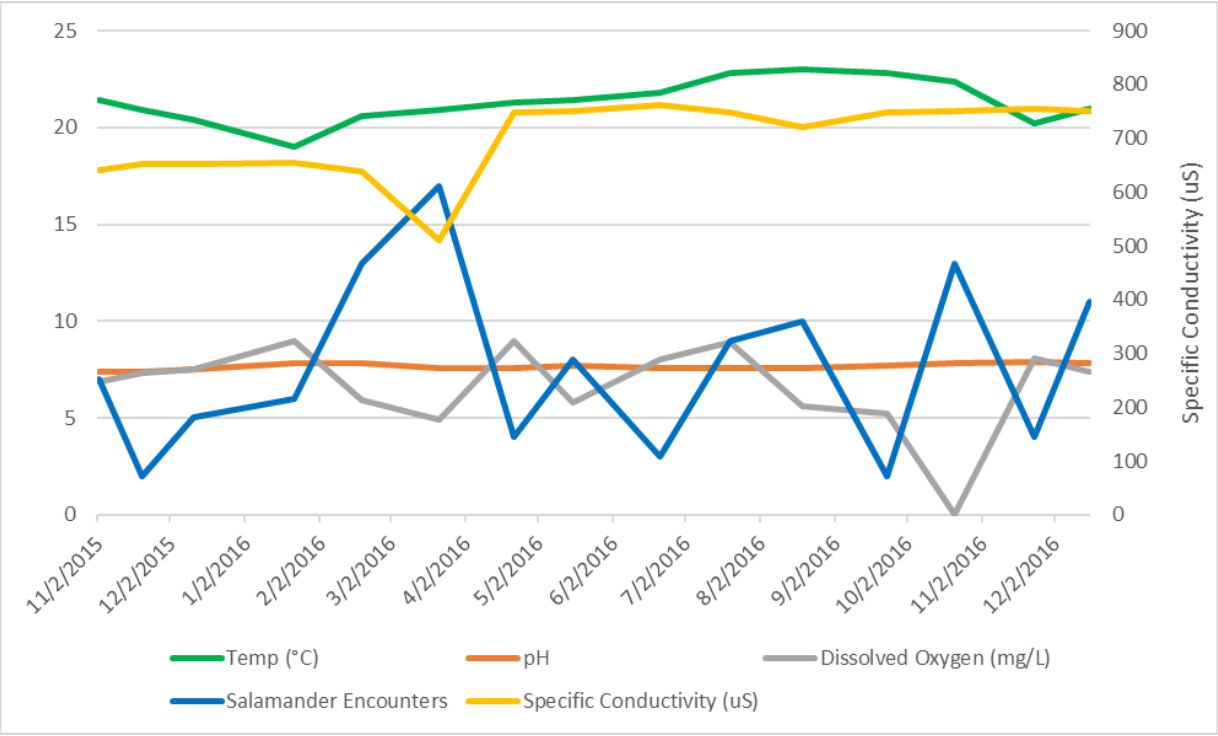

Figure 2. Water quality parameters (water temperature, pH, and dissolved oxygen) and total salamander encounters at Twin Springs from November 2015-December 2016.

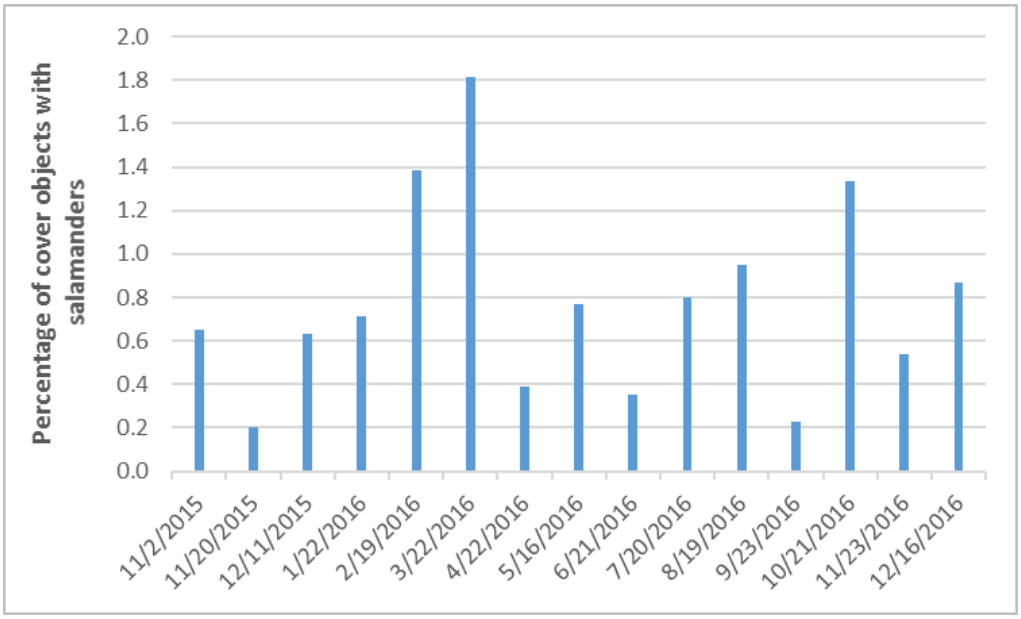

Figure 3. Percentage of cover objects occupied by salamanders at Twin Springs from November 2015-December 2016.

Table 3. Temperature and precipitation at Twin Springs on each survey event and its previous week.

Date	Current Temp. (°C)	Previous Week Min. Temp. (°C)	Previous Week Max. Temp. (°C)	Current Precipitation (in)	Prev. Week Precipitation (in)
11/2/2015	17.78	7.78	27.78	0	0.26
11/20/2015	15.56	8.89	30	0	0.06
12/11/2015	16.67	-1.11	18.89	0	0
1/22/2016	7.22	-3.33	21.67	0	0
2/19/2016	21.11	-2.22	27.78	0	0
3/22/2016	16.67	5.56	32.78	0	0
4/22/2016	20	10.56	31.67	0	0.13
5/16/2016	22.22	15.56	32.78	0.13	0.17
6/21/2016	28.89	23.89	35.56	0	0
7/20/2016	30	21.67	36.67	0	0.2
8/19/2016	28.33	23.89	38.89	0	0
9/23/2016	27.22	17.78	35.56	0	0
10/21/2016	16.67	8.89	31.67	0	0
11/23/2016	15.56	1.67	28.89	0.38	0
12/16/2016	15.56	-1.11	23.89	0	0

***Swinbank Spring*.** In collaboration with Dr. Ben Pierce and his research team from Southwestern University, Cambrian Environmental conducted monthly GTS monitoring at Swinbank Spring, a permanent spring located in the North San Gabriel River watershed in Williamson County, Texas (30°39′43.92″N, 97°42′37.80″W). This site 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-residential area. From this point, the spring channel runs approximately 50 m downstream to the North San Gabriel River. The spring run predominantly contains cobble, large rocks, and leaf litter. We monitored salamanders by running a transect from the spring origin at the base of the spring house to 24 m downstream. The transect was divided into 5 sections, each approximately 5 m long.

Water quality parameters were measured at three distinct points (0 m, 12 m, and 24 m from the spring outlet) along the transect and the average was recorded (Table 4, Figure 4). Total salamander encounters, number of salamanders captured for Wild.ID photographs, number of cover objects, and percentage of cover objects occupied by salamanders from November 2015-December 2016 are shown in Table 5. Percentage of cover objects occupied by salamanders on each survey date is shown in Figure 5. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 6).

Results from the Wild.ID analysis of salamanders from Swinbank Springs will be appended to the current report following completion and will include data from all monitoring events at the site (from October 2012 to December 2016).

Table 4. Water quality parameters and total salamanders encountered at Swinbank Spring during each survey event.

Date	Temp (°C)	pH	Dissolved Oxygen (mg/L)	Specific Conductivity (µS)	Total Dissolved Solids (ppm)	Embedded-ness (%)	Salamander Count
10/16/2015	21.6	7.5	7.7	626	317	5	49
11/13/2015	20.6	7.5	8.2	638	320	5	15
12/10/2015	20.8	7.5	7.6	650.3	329	7	13
1/15/2016	20.5	7.7	8	653.7	333.3	7	24
2/12/2016	20.9	7.7	6.9	636.3	326	7	31
3/24/2016	20.9	7.5	5	516	308.7	5	37
4/15/2016	21.4	7.6	6.9	722.7	362.7	5	23
5/19/2016	21.3	7.7	6.7	587.7	299	5	17
6/20/2016	21.6	7.6	2.2	724.3	371.3	5	19
7/17/2016	21.8	7.6	6.4	716	363.3	5	31
8/18/2016	22.4	7.9	5.2	559	284.3	7	15
9/9/2016	22.1	7.7	8.7	710	364	5	27
10/14/2016	21.6	7.7	3.1	728.7	372.3	5	27
11/18/2016	20.7	7.7	8.7	745.7	378.7	5	34
12/9/2016	20.5	7.7	10.1	749.3	380.7	5	22

Table 5. Total salamander encounters, number of salamanders captured for Wild.ID photographs, number of cover objects, and percentage of cover objects occupied by salamanders at Swinbank from November 2015-December 2016.

Date	Total Encounters	# Captured	CO	% CO with salamanders
10/16/2015	49	39	993	4.93
11/13/2015	15	5	849	1.77
12/10/2015	13	5	719	1.81
1/15/2016	24	17	887	2.71
2/12/2016	31	26	893	3.47
3/24/2016	37	23	708	5.23
4/15/2016	23	9	925	2.49
5/19/2016	17	8	834	2.04
6/20/2016	19	6	810	2.35
7/17/2016	31	18	1008	3.08
8/18/2016	15	4	724	2.07
9/9/2016	27	13	986	2.74
10/14/2016	27	16	890	3.03
11/18/2016	34	12	1111	3.06
12/9/2016	22	11	863	2.55

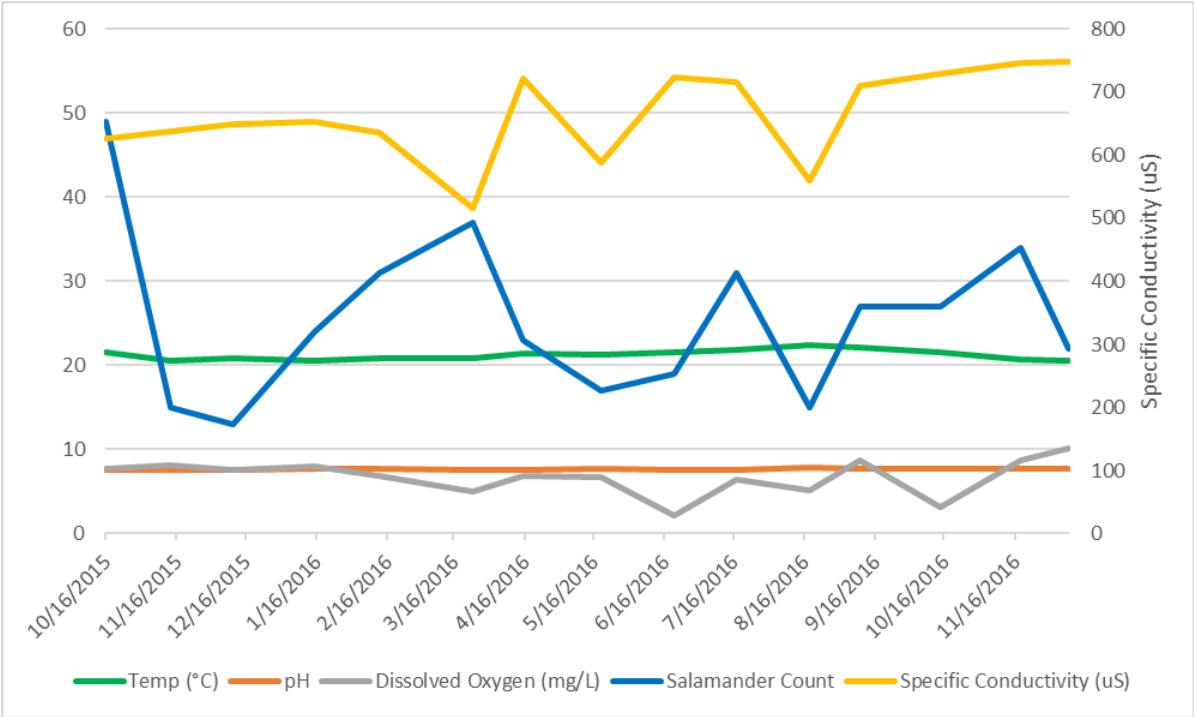

Figure 4. Water quality parameters (water temperature, pH, and dissolved oxygen) and total salamander encounters at Swinbank Spring from October 2015-December 2016.

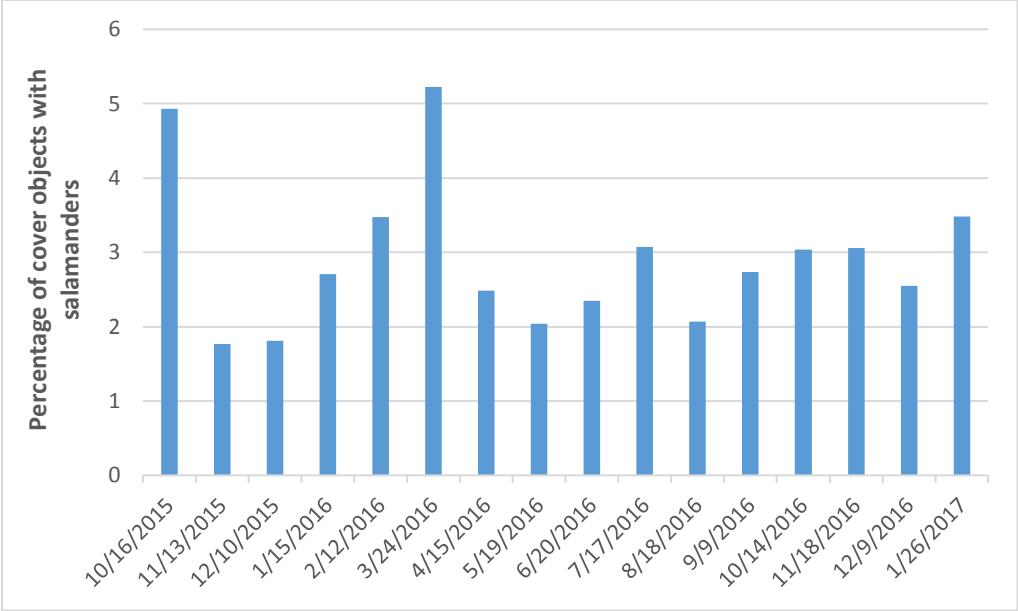

Figure 5. Percentage of cover objects occupied by salamanders at Swinbank Spring from November 2015-December 2016.

Table 6. Temperature and precipitation at Swinbank Spring on each survey event and its previous week.

Date	Current Temp. (°C)	Previous Week Min. Temp. (°C)	Previous Week Max. Temp. (°C)	Current Precipitation (in)	Prev. Week Precipitation (in)
10/16/2015	22.22	12.78	32.78	0	0
11/13/2015	14.44	11.67	28.89	0	0
12/10/2015	17.22	-1.1	18.89	0	0
1/15/2016	12.78	-1.1	21.67	0	0.03
2/12/2016	15	-3.33	27.78	0	0
3/24/2016	13.33	5.56	32.78	0.02	0
4/15/2016	17.22	6.67	28.89	0	0
5/19/2016	18.89	15.56	32.78	0.14	0.17
6/20/2016	28.89	23.89	35.56	0	0
7/17/2016	30	21.67	36.67	0	0.20
8/18/2016	25.56	23.89	38.89	0	0
9/9/2016	29.44	21.67	35	0	0.02
10/14/2016	23.33	11.67	32.78	0	0
11/18/2016	16.67	10.56	22.78	0	0.4
12/9/2016	12.22	-2.22	12.78	0	0.03

***Cobbs Spring*.** Cambrian Environmental conducted monthly GTS monitoring at Cobbs Spring, a nearly-permanent spring located in the Berry Creek watershed in Williamson County, Texas (30°47′27.27″N, 97°43′43.03″W). Sporadic monitoring has taken place at Cobbs Spring since around 2002 when the site was first visited by Andy Price of the Texas Parks and Wildlife Department. Following an interruption of previous monitoring efforts due to issues related to the listing, Cambrian Environmental began conducting monthly Georgetown salamander monitoring at Cobbs Spring in July 2015. The spring run predominantly contains cobble, large rocks, woody debris, and leaf litter with relatively high sedimentation. We monitored salamanders by running a transect from the spring origin to approximately 25 m downstream. The transect was divided into 5 sections, each approximately 5 m long. In April 2016, Cambrian began capturing all encountered salamanders when possible and individuals were photographed for future analysis using Wild.ID.

Water quality parameters were measured at the spring outlet (Table 7, Figure 6). Total salamander encounters, number of salamanders captured for Wild.ID photographs, number of cover objects, and percentage of cover objects occupied by salamanders from July 2015-December 2016 are shown in Table 8. Percentage of cover objects occupied by salamanders on each survey date is shown in Figure 7. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 9).

Preliminary results from the Wild.ID analysis of salamanders from Cobbs Spring show that out of a total of 81 captures from April to December 2016, only one individual was previously captured (see Photo 1). This individual was first captured in April 2016 and was recaptured one month later in May 2016. A summary of results and analysis from Cobbs Spring will be appended to the current report along with Wild.ID analysis from Twin Springs and Swinbank Spring.

Table 7. Water quality parameters, total salamanders encountered, and total cover objects overturned at Cobbs Spring during each survey event.

Date	Temp (°C)	pH	Dissolved Oxygen (mg/L)	Specific Conductivity (µS)	Total Dissolved Solids (ppm)	Embedded -ness (%)	Salamander Count	Cover Objects
7/9/2015	22.1	7.6	2.2	496	249	35	49	302
8/28/2015	23	7.2	2.5	551	281	90	6	294
9/25/2015	23.5	7.5	3.8	524	271	75	3	264
11/2/2015	20.9	7.9	6.5	455	233	10	8	321
11/24/2015	19.7	7.8	4.0	496	251	80	17	553
12/18/2015	17.8	7.9	5.6	495	254	75	20	713
1/22/2016	16.2	7.8	5.4	492	255	85	9	763
2/29/2016	17.8	8.0	3.0	475	236	95	6	338
3/31/2016	18.1	7.6	3.7	X	X	85	13	515
4/25/2016	20.9	7.7	8.9	560	271	90	32	646
5/25/2016	20.9	7.8	8.9	575	291	90	24	400
6/27/2016	22.5	7.6	5.3	568	292	85	50	893
7/26/2016	23	7.4	4.1	608	309	85	11	770
8/26/2016	23.5	7.7	1.5	588	304	90	6	1048
9/22/2016	23.4	7.5	3.3	623	422	90	7	858
10/31/2016	20.8	7.5	*X	651	332	95	1	586
11/18/2016	21	7.5	6.6	640	322	95	1	592
12/9/2016	18.1	7.8	7.5	642	330	95	6	550

*X indicates water parameter meter is under repair and measurements were unable to be taken.

Table 8. Total salamander encounters, number of salamanders captured for Wild.ID photographs (beginning in April 2016), number of cover objects, and percentage of cover objects occupied by salamanders at Cobbs Spring from July 2015-December 2016.

Date	Total Encounters	# Captured	CO	% CO with Salamanders
7/9/2015	49	X	448	10.94
8/28/2015	6	X	355	1.69
9/25/2015	3	X	264	1.14
11/2/2015	8	X	321	2.49
11/24/2015	17	X	553	3.07
12/18/2015	20	X	713	2.81
1/22/2016	9	X	763	1.18
2/29/2016	6	X	338	1.78
3/31/2016	13	X	515	2.52
4/25/2016	32	24	646	4.95
5/25/2016	24	11	400	6.00
6/27/2016	50	27	893	5.60
7/26/2016	11	7	770	1.43
8/26/2016	6	4	1048	0.57
9/22/2016	7	3	858	0.82
10/31/2016	1	1	586	0.17
11/18/2016	1	0	592	0.17
12/9/2016	6	4	550	1.09

*X indicates that no salamanders were captured during this time. Wild.ID photography began in April 2016.

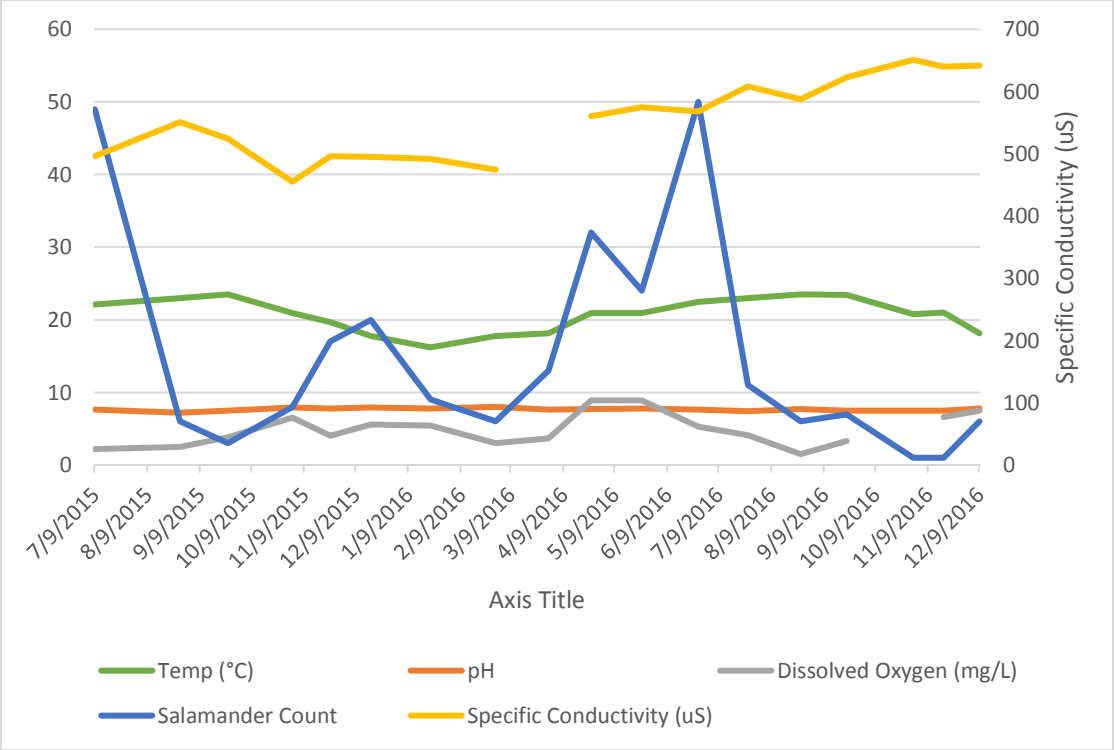

Figure 6. Water quality parameters (water temperature, pH, dissolved oxygen, specific conductivity) and total salamander encounters at Cobbs Spring from July 2015 through December 2016. *Gap in specific conductivity indicates water parameter meter is under repair and measurements were unable to be taken.

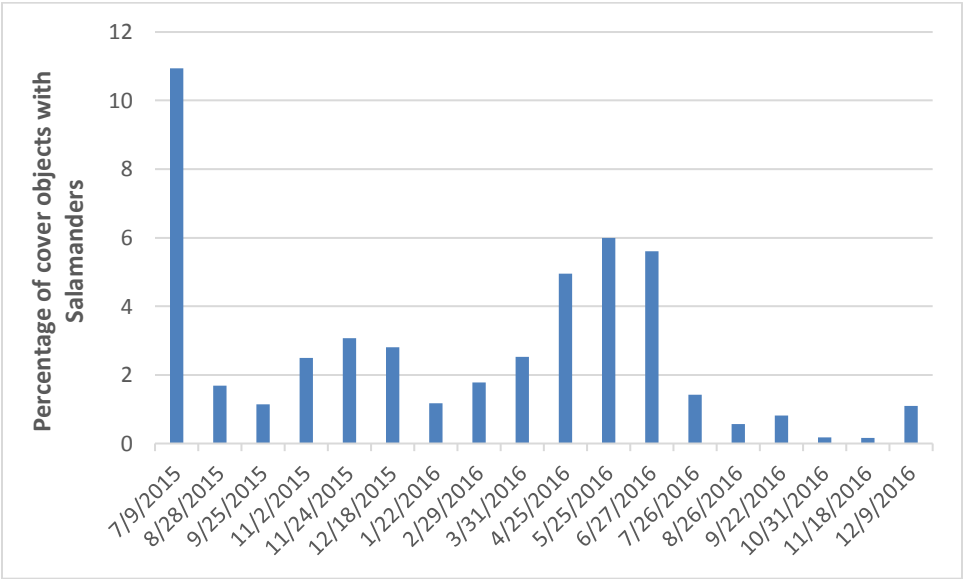

Figure 7. Percentage of cover objects occupied by salamanders at Cobbs Spring from July 2015-December 2016.

Table 9. Temperature and precipitation at Cobbs Springs on each survey event and its previous week.

Date	Current Temp. (°C)	Previous Week Min. Temp. (°C)	Previous Week Max. Temp. (°C)	Current Precipitation (in)	Prev. Week Precipitation (in)	Salamander Count
7/9/2015	33.89	20.56	33.89	0	0.01	49
8/28/2015	35.56	20.56	36.67	0	0	6
9/25/2015	33.89	13.89	35.56	0	0	3
11/2/2015	23.89	7.78	27.78	0	0	8
11/24/2015	11.67	2.78	25.56	0	0.07	17
12/18/2015	6.67	2.78	26.67	0	0	20
1/22/2016	6.11	-3.33	21.67	0	0	9
2/29/2016	20	1.67	21.67	0	0.22	6
3/31/2016	22.22	-1.11	26.67	0	0	13
4/25/2016	25.56	10.56	26.67	0	0.06	32
5/25/2016	26.67	15.56	27.78	0	0.1	24
6/27/2016	28.33	22.78	35.56	0	0	50
7/26/2016	28.89	23.89	36.67	0.09	0	11
8/26/2016	27.78	21.67	33.89	0	0.47	6
9/22/2016	26.67	17.78	35.56	0	0	7
10/31/2016	20	10.56	30.56	0	0	1
11/18/2016	16.67	10.56	22.78	0	0.4	1
12/9/2016	2.22	0.56	25	0	0.06	6

JOLLYVILLE PLATEAU SALAMANDER

The JPS is endemic to spring outflows, spring-fed creeks and cave streams of Travis and Williamson counties. The majority of known locations occur within the Bull, Lake, and Brushy creek drainage basins. The species lacks a biphasic lifecycle and individuals never undergo metamorphoses into a terrestrial adult form. As an entirely aquatic species, the JPS is particularly vulnerable to water quality degradation (USFWS 2012). The JPS requires relatively shallow, cool, moving water, an abundant macro-invertebrate community, aquatic vegetation, and substrates containing cover objects (including large rocks, cobble, and leaf litter) to provide sufficient refuge from predators (Bowles et al. 2006, Bonett and Chippindale 2006). Little is known about JPS use of sub-surface flooded karstic refugia, although it is believed that these areas are utilized to escape surface drying events. Only a few cave populations are known to occur (primarily in the Buttercup Creek area) and they are poorly understood. 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).

Since the JPS became federally listed as threatened (USFWS 2013), the Endangered Species Act's "take" prohibition has applied to all activities that would result in harm to the species. Protection under the Act goes well beyond prohibiting direct harm to salamanders at the site of their cave or spring run. The Service has indicated that any activity that degrades the quality or quantity of water upstream of salamander habitat may result in take through habitat modification (USFWS 2012, 2013a, 2013b). All known locations for the JPS rely on discharge from the Edwards aquifer. Since the aquifer is recharged by runoff originating in the contributing and recharge zones, those zones represent the geographic area from which impacts may arise.

In addition to the threatened listing, the Service also designated critical habitat for the JPS at every site where the species was historically known to occur. The designation of critical habitat is a separate federal action from the listing which is intended to highlight the geographic location and regulatory status of the JPS to other Federal agencies as well as project proponents using Federal funds, Federal permits, or Federal property. The critical habitat designation requires federalized projects to avoid adverse modification of critical habitat. In a critical habitat designation, the Service delineates areas that are deemed vital to the recovery of the species based on habitat components known as primary constituent elements (PCEs). In the case of the JPS, the Service defined two PCE boundary areas which formed the perimeter of multiple critical habitat units (CHUs) based on biological and hydrogeological assumptions. The first is the surface PCE boundary, which extends 262 feet (80 meters) in all directions from the spring outlet. This is the area within which it is presumed salamanders may travel on the surface within the spring run. The second is the sub-surface PCE boundary, which extends 984 feet (300 meters) in all directions from the spring outlet. This is the area within which it is presumed that salamanders may occur in sub-surface flooded karstic refugia (cave habitat) where they are able to survive periods of drought. It is important to note that neither of these PCE boundaries were developed with CHU-specific information. The surface PCEs were extrapolated for all JPS sites based on surveys conducted on a large spring containing JPS in the Bull Creek watershed. The sub-surface PCEs were extrapolated to all JPS locations based on the distance between locations for the Austin Blind salamander (*E. waterlooensis*) at Barton Springs in Austin. Being natural systems, the actual spatial distribution of these PCEs are likely different for each individual site and are not likely to conform to radial buffers extending the same distance in all directions. The five JPS sites reported on herein span three CHUs in Williamson County, Texas (Figure 8).

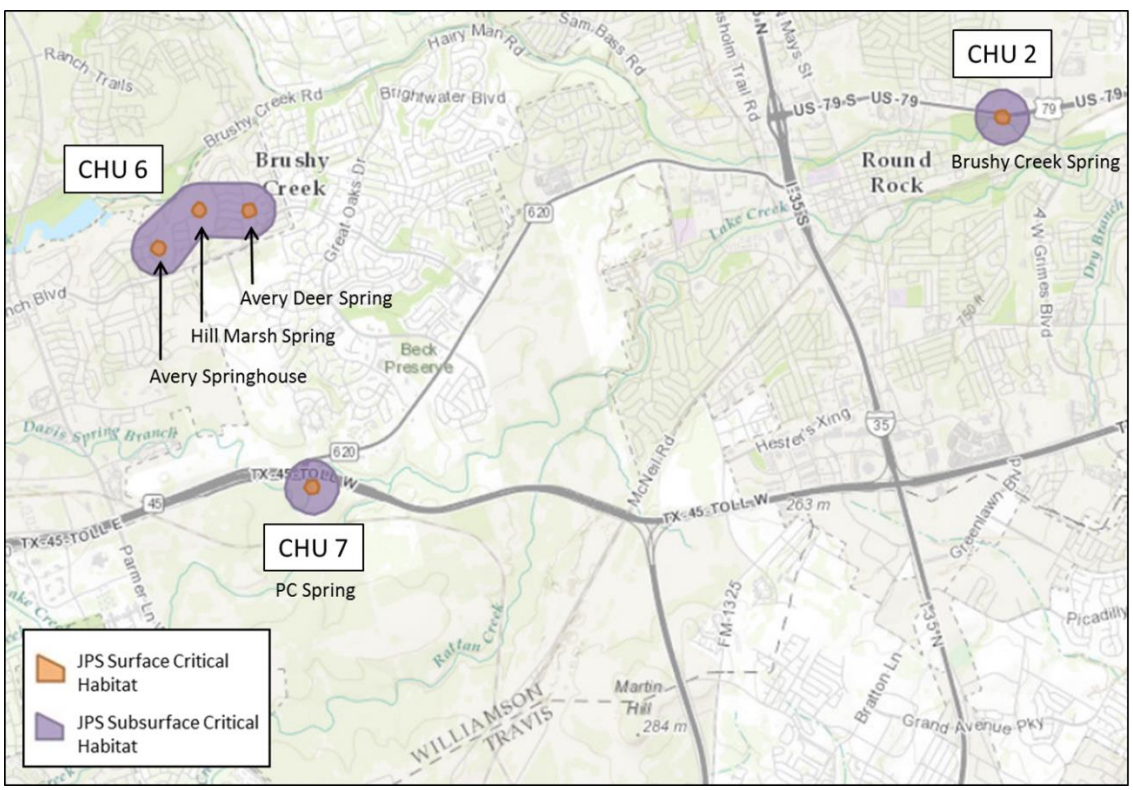

Figure 8. JPS monitoring sites and corresponding surface (orange) and subsurface (purple) critical habitat units (CHUs).

Researchers from Texas State University (under the direction of Mike Forstner and Zach Adcock) conducted monthly monitoring at these sites from May 2013 to September 2015 and commenced bimonthly surveys from October 2015 to August 2016 (see Dr. Mike Forstner and Zach Adcock's 2016 annual report to the WCCF). In August 2016, Cambrian personnel began supplementing monitoring activities at these sites and by September 2016 had appropriated JPS monitoring activities in Williamson County. Due to the collaborative and long-term nature of this mark-recapture monitoring study, this report details only raw data gathered since Cambrian's acquisition of monitoring activities at all JPS sites.

JPS Monitoring Methodology

Following the design implemented by researchers at Texas State University (Dr. Mike Forstner and Zach Adcock), Cambrian personnel conducted bimonthly, time-constrained surveys at each JPS site. We monitored salamanders by running a transect from the spring origin to a specified distance downstream for each JPS site. The transect was divided into sections with a linear distance of approximately 20-m per section. Section size was dependent on stream characteristics at a given site. Cambrian personnel also surveyed randomly selected downstream sections at each site (downstream of the immediate spring runs).

Unlike the methodology utilized for the above-referenced GTS sites, methodology for these sites was originally intended to sample rather than census the salamander populations. We therefore continued the approach used by Dr. Mike Forstner and Zach Adcock at Texas State University and conducted time-constrained surveys by searching the substrate within a given time frame (minimum of 20 person-minutes per 20 m section) for salamanders by overturning potential cover objects (cobble, large rocks, leaf litter, vegetation) beginning at the most downstream point of a given section and moving upstream toward the most upstream location of the section. Surveyors counted all cover objects overturned in every section that was surveyed on a given date to standardize salamander counts as "number of salamanders per structure object" (Pierce et al. 2010; 2014). All encountered salamanders were captured, when possible. Additionally, we utilized a specialized sieve (developed by Zach Adcock and Dr. Mike Forstner at Texas State University) designed to encourage salamander capture from within the substrate. Where the substrate permitted, we shoveled gravel, cobbles, sediment, vegetation, and organic debris into a 1 m x 1 m sieve and removed material gradually while searching the underlying mesh net for salamanders. Similar methods have been used effectively for closely-related *Eurycea* salamanders (Sweet 1978) and other aquatic vertebrates (Godley 1982).

Individuals were photographed on grid paper for future measurement and analysis using Wild.ID, a computer-assisted identification software that identifies individuals based on their unique melanophore patterns on the back of the head. Salamanders were then checked for eggs and promptly released into the sections from which they were captured. Water quality parameters (water depth, water temperature, pH, dissolved oxygen, specific conductivity, and total dissolved solids) were taken with handheld meters following each survey. Climate conditions (temperature and precipitation) for each survey date and the corresponding previous week were also recorded.

Cambrian acquired the following five JPS monitoring sites, Avery Springhouse Spring, Avery Deer Spring, Hill Marsh Spring, PC Spring, and Brushy Creek Spring, within the last months of the 2016 calendar year. This report details only raw data gathered at these sites since Cambrian's acquisition of monitoring activities. We intend to incorporate present and subsequent data and photographs for Wild.ID analysis with previous data gathered by Dr. Forstner's research lab when results become available for analysis (see Dr. Mike Forstner and Zach Adcock's 2016 annual report to the WCCF).

JPS Monitoring Results

Avery Springhouse Spring. Avery Springhouse Spring (designated CHU 6) occurs within 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°). The spring outlet is located adjacent to a springhouse. The spring run extends approximately 65 m before waterfalling into a larger creek system that empties into a pond approximately 400 m downstream. Cambrian began conducting surveys at Avery Springhouse Spring in September 2016. The spring run and subsequent channel was divided into twenty 20 m sections. On each survey date, we surveyed the first three sections from the spring outlet (0-60 m) and five randomly-selected downstream sections.

Water quality parameters (measured at the spring outlet), total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects (only within first three sections, 0-59 m from spring outlet), and percentage of cover objects occupied by salamanders at Avery Springhouse Spring in September and November 2016 are shown in Table 10. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 11).

Avery Deer Spring. Avery Deer Spring (designated CHU 6) also occurs within the South Brushy Creek Watershed and is located within a residential neighborhood of Avery Ranch in Round Rock, Texas. 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 are permanently wet (pers. comm. Zach Adcock, Texas State University). The creek empties into a retention pond approximately 260 m downstream of the second and third spring outlets. Cambrian began conducting surveys at Avery Deer Spring in September 2016. The spring run and subsequent channel was divided into seventeen approximately 20 m sections. On each survey date, we surveyed the first two sections from the first spring outlet (0-40 m), three sections that encompass the second and third spring outlets (60-100 m downstream from the first spring outlet), and five randomly-selected downstream sections.

Water quality parameters (measured at all three spring outlets and averaged together), total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects (only from Sections 1 and 2 which encompasses 0-39 m from the first spring outlet, and from Sections 4-6 which encompasses the second and third spring outlets and lies a linear distance of 60-107m from the first spring outlet), and percentage of cover objects occupied by salamanders at Avery Deer Spring in September and November 2016 are shown in Table 10. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 11).

Hill Marsh Spring. Hill Marsh Spring (designated CHU 6) occurs within the South Brushy Creek Watershed on the Avery Ranch Golf Course in Round Rock, Texas (N 30.507680°, W 97.755070°). The spring run extends approximately 45 m and empties into a large pond. Cambrian began conducting surveys at Hill Marsh Spring in October 2016. The spring run was divided into two approximately 25 m sections. Both sections were surveyed on each survey date.

Water quality parameters (measured at the spring outlet), total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects, and percentage of cover objects occupied by salamanders at Hill Marsh Spring in October and December 2016 are shown in Table 10. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 11).

PC Spring. PC Spring (designated CHU 7) is located within the Lake Creek Watershed at the intersection of SH45 and FM620. Two discrete discharge points occur at the site: the first (N 30.481276°, W 97.742274°) emerges from the bank of a creek under SH45 that originates offsite and the second (N 30.481947°, W 97.742362°) flows from the base of a concrete culvert pad. At approximately 78 m from the culvert, water from Discharge 2 flows into the larger creek system (downstream of the Discharge 1). Cambrian began conducting surveys at PC Spring in October 2016. The spring channel associated with Discharge 1 was divided into four approximately 20 m sections. The spring channel associated with Discharge 2 was divided into three 25 m sections. On each survey date, we surveyed the first section (0-20 m) from Discharge 1 and two randomly-selected sections downstream of Discharge 1, and the first section from Discharge 2 (0-25 m) and one randomly-selected section associated with the Discharge 2.

Water quality parameters (measured at both spring outlets and averaged), total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects, and percentage of cover objects occupied by salamanders at Hill Marsh Spring in October and December 2016 are shown in Table 10. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 11).

Brushy Creek Spring. Brushy Creek Spring (designated CHU 2) is located within the Lake Creek Watershed inside a storm water culvert that runs under highway 79 (N 30.516834°, W 97.661271°). 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 riprap structure and into a deep pool, which then constricts into a spring run that empties directly into Brushy Creek. Cambrian began conducting surveys at Brushy Creek Spring in November 2016. The run was divided into two 25 m sections and both sections were surveyed on each survey date.

Water quality parameters (measured at both outlets inside the culvert and at the base of the spring channel approximately 68 m from the outlets just before the spring channel waterfalls into Brushy Creek), total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects, and percentage of cover objects occupied by salamanders at Hill Marsh Spring in November 2016 are shown in Table 10. Climate conditions (temperature and precipitation) of each survey day and the corresponding previous week were also recorded (Table 11).

Table 10. Water quality parameters, total salamanders encountered, number of salamanders captured for Wild.ID, number of cover objects, and percentage of cover objects occupied by salamanders at Avery Springhouse Spring and Avery Deer Spring in September and November 2016, Hill Marsh Spring and PC Spring in October and December 2016, and Brushy Creek Spring in November 2016.

Location	Date	Total Encounters	# Captured	CO	% CO with salamanders	Temp (C°)	pH	Dissolved Oxygen (mg/L)	Spec. Conductivity (µS)	Total Dissolved Solids (ppm)
Avery Springhouse	9/27/2016	11	7	338	3.25	22.9	7.5	8.4	886	465
Avery Springhouse	11/29/2016	12	6	329	3.65	22.7	7.5	8.3	878	450
Avery Deer Spring	9/29/2016	7	4	815	0.86	23.7	7.5	3.8	660	336
Avery Deer Spring	11/21/2016	3	0	846	0.35	21.7	7.6	7.6	667	342
Hill Marsh Spring	10/21/2016	2	1	399	0.50	23.9	7.5	X	919	476
Hill Marsh Spring	12/5/2016	1	0	372	0.27	18.9	7.7	11.2	851	439
PC Spring	10/31/2016	0	0	543	0	23.7	7.5	X	817	425
PC Spring	12/5/2016	0	0	605	0	16.6	8.1	8.9	526	270
Brushy Creek Spring	11/29/2016	0	0	369	0	21.3	7.7	6.8	854	509

*X indicates water parameter meter is under repair and measurements were unable to be taken.

Table 11. Temperature and precipitation at Avery Springhouse Spring, Avery Deer Spring, Hill Marsh Spring, PC Spring, and Brushy Creek Spring on each survey event and its previous week.

Location	Date	Current Temp. (°C)	Previous Week Min. Temp. (°C)	Previous Week Max. Temp. (°C)	Current Precipitation (in)	Prev. Week Precipitation (in)	Salamander Count
Avery Springhouse	9/27/2016	22.2	20	35.6	0	0.01	11
Avery Springhouse	11/29/2016	16.7	2.8	23.9	0	0	12
Avery Deer Spring	9/29/2016	24.4	20	35.6	0	0.01	7
Avery Deer Spring	11/21/2016	14.4	5	28.9	0	0	3
Hill Marsh Spring	10/21/2016	34.4	11.7	35.6	0	0	2
Hill Marsh Spring	12/5/2016	8.9	3.9	25	0	0	1
PC Spring	10/31/2016	20	11.7	42.8	0	0	0
PC Spring	12/5/2016	8.9	3.9	25	0	0	0
Brushy Creek Spring	11/29/2016	16.7	2.8	23.9	0	0	0

DISCUSSION

Eurycea Surface Encounters

Surface counts ranged from 2 to 17 individuals at Twin Springs, 13 to 49 individuals at Swinbank Spring, and 1 to 50 individuals at Cobbs Spring. The results are consistent with long-term *E. naufragia* monitoring results which include high variability in numbers of salamanders observed between monitoring events and between sites and recognizable seasonal trends with abundance peaking in the spring at Twin Springs and Swinbank Spring and in the summer at Cobbs Spring. Similar temporal variation has been observed in counts of another species of *Eurycea* in central Texas with the highest counts observed during spring and summer (Bowles et al. 2006). Pierce et al. 2010 suggested that monthly differences in abundance of salamanders are influenced by variation in distribution of salamanders on the surface of the spring run and that during certain months, more salamanders may be near the surface of the flow. Contributing factors to variation in abundance on the surface among months may include differences in abundance of food, chemistry of the water, or discharge from the spring outlet. Continued monitoring will provide a better understanding of the GTS and JPS populations inhabiting the springs. Cambrian and Southwestern University researchers are currently scoring individual salamander photos from GTS spring sites for analysis in Wild.ID and analyzing reproductive patterns and population trends. Researchers from Texas State University are currently analyzing photos from JPS sites for Wild.ID analysis and data from subsequent surveys conducted by Cambrian will be contributed to this dataset.

Water Quality

Water quality parameters (water depth, water temperature, pH, dissolved oxygen, specific conductivity, and total dissolved solids) were measured at the conclusion of each survey to provide baseline water quality data and to monitor the long-term ecological health of each spring site. On average, water parameters such as pH, dissolved oxygen concentration (DO), and total dissolved solids (TDS) were all within the typical boundaries for a carbonate aquifer, such as the Edwards Aquifer. None of the analyses results indicated any type of anthropogenic contamination. Cambrian began monitoring JPS sites at the end of 2016 and no conclusions can be made about water quality parameters from these sites at this time (see Dr. Mike Forstner and Zach Adcock's 2016 annual report to the WCCF). Continuation of water chemistry analysis will provide a framework for analyzing the health of the salamander habitats, and will also serve to identify any contamination concerns that may arise.

pH Values. The acidity or alkalinity of water is based on its pH value, which is a measure of the activity of dissolved hydrogen ions (H^+). The value of pH ranges between 0 and 14, where 7 is considered neutral. Solutions in which the concentration of H^+ exceeds that of OH^- have pH values lower than 7.0 and are known as acids. Solutions in which OH^- exceeds H^+ have a pH value greater than 7.0 and are known as bases (or alkalis). Many acid-base reactions are occurring in the unsaturated and saturated zones of the subsurface. With groundwater, reactions involving carbon dioxide and carbonate minerals are particularly important. Water emerging from all GTS and JPS monitored sites had pH values ranging between 7.1 and 8.1, making these springs slightly alkaline, which is typical of carbonate aquifers.

Dissolved oxygen. Dissolved oxygen refers to the level of free, non-compound oxygen present in water or other liquids. A dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality. Dissolved oxygen is also produced as a waste product of photosynthesis from phytoplankton, algae, seaweed and other aquatic plants. Low DO is often an indicator of organic pollution. If organic material, such as untreated human or animal waste, DO levels diminish as microorganisms grow, using the organic material as an energy source and consuming oxygen in the process. DO concentrations fluctuate with amount of organic material, temperature, salinity, and pressure changes in the spring flow.

Dissolved oxygen has been found to be positively correlated with abundance of other *Eurycea* species (Willson and Dorcus 2003; Turner 2004). Turner (2004) suggested that salamander reproduction decreases as a result of low dissolved oxygen and that this effect can be seen in number of salamanders observed in the spring about six months later. Woods et al. (2010) observed a drop in metabolic rates of salamanders at lower oxygen concentrations and suggested that oxygen levels below 4.4 O_2/L would likely lead to a significant reduction in juvenile growth. According to USFWS (2005), it is believed that dissolved oxygen concentrations greater than 4 mg/L are suitable for *Eurycea* species.

The DO levels at Twin Springs (range 4.9-8.95 mg/L) and Cobbs Spring (range 4.5-8.9 mg/L) were indicative of well-oxygenated water and were higher than the minimal requirements for salamanders. At Swinbank, Spring, the DO concentrations were highly variable, ranging from 2.2 to 10.1 mg/L. However, salamander encounters remained stable from month to month and no negative effects in abundance were observed after the six-month lag time suggested by Turner (2004).

Conductivity. Conductivity is a measure of the spring water's capability to pass electrical flow and is directly related to the concentration of ions in the water. High conductivity is typically indicative of urbanization and is often found in sites within more developed watershed. Willson and Dorcas (2003) and Bowles et al. (2006) found that conductivity was negatively correlated with number of observed salamanders in a spring. However, researchers from Texas State University have found high abundances of salamanders at sites with high conductivity (pers. comm. Zach Adcock). Conductivity was relatively stable at GTS sites and ranged from 509 to 762 µS at Twin Springs, 516 to 749 µS at Swinbank, and 455 to 651 µS at Cobbs Spring.

Total Dissolved Solids. Total dissolved solids (TDS) refers to the total amount of solids that remain when a water sample is evaporated to dryness. In general, the total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) ions in the water, including inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulfates) and some small amounts of organic matter that are dissolved in water.

The TDS concentration in areas within the Edwards Aquifer Recharge Zone is typically between 200 and 400 mg/L (Duffin and Musick 1991). TDS values ranged from 258 to 388 mg/L at Twin Springs, 284 to 380 at Swinbank Spring, and 233 to 422 mg/L at Cobbs Spring. These levels indicate natural conditions, with no suggestion of contamination.

KEY PERSONNEL

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

Prior to starting Cambrian, Dr. Kemble White served for 15 years as senior geologist, karst specialist, and project manager for one of the largest ecological consulting firms in the country. 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.

Craig Crawford, P. G. *Senior Geoscientist*

Craig Crawford has ten 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.

Ashley Wall, M.S. *Karst Biologist*

Ashley Wall has six years of experience working with the threatened and endangered species of the Edwards Aquifer system. She provides biological assessments, ecological monitoring services, and support services for conservation entities and developers and ensures compliance with local and federal regulations. Her specialty is conducting habitat assessments and presence/absence surveys for *Eurycea* salamanders and endangered karst invertebrates within the northern segment of the Edwards Aquifer. In collaboration with Dr. Ben Pierce from Southwestern University, Ashley helped pioneer a long-term mark-recapture study involving the threatened Georgetown salamander, providing the first population estimates for the species as well as information on reproductive timing and movement (results published in *Herpetological Conservation and Biology*, research funded by the Williamson County Conservation Foundation). Though a cooperative agreement between Cambrian, the WCCF, and Southwestern, Cambrian now supervises the Southwestern student research team in the field. She provides cave and spring monitoring services, spring habitat management, and public outreach services.

Southwestern University Student Research

Cambrian works in collaboration with Dr. Ben Pierce and his student research team from Southwestern University as part of an ongoing capture-mark-recapture study using visual-encounter surveys and computer-assisted photo identification (Wild.ID) at Twin Springs and Swinbank Spring in Georgetown, Texas. Southwestern University students work under the direct supervision of Cambrian personnel at all times.

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APPENDIX C:

Population Trends, Annual Ecology, Habitat Evaluation, and Disease Prevalence for Federally Threatened Jollyville Plateau Salamanders (Eurycea tonkawae) in Williamson County, Texas

And

Effects of Temperature and Nitrogenous Wastes on Survival and Growth of the Barton Springs Salamander (Eurycea sosorum)

POPULATION TRENDS, ANNUAL ECOLOGY, HABITAT EVALUATION, AND DISEASE PREVALENCE FOR FEDERALLY THREATENED JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*) IN WILLIAMSON COUNTY, TEXAS

and

EFFECTS OF TEMPERATURE AND NITROGENOUS WASTES ON SURVIVAL AND GROWTH OF THE BARTON SPRINGS SALAMANDER (*EURYCEA SOSORUM*)

Prepared for:

Williamson County Conservation Foundation

Prepared by:

Department of Biology

Texas State University

San Marcos, Texas

31 August 2016

DISCLAIMER

Portions of this report will be submitted for publication in peer reviewed scientific journals. The results and/or conclusions reported herein may not be congruent with the final published product due to inclusion of additional data (data collection is not complete at the time of writing this report), inclusion of data from sites not covered in this report (i.e., sites outside of Williamson County), and the use of additional or alternative statistical analyses. The results and conclusions in this document should be viewed as preliminary findings until the final peer-reviewed articles are published.

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EXECUTIVE SUMMARY

This document summarizes two major bodies of work funded by the Williamson County Conservation Foundation. The first, Section 1, includes an analysis of *Eurycea tonkawae* population trends, habitat use, and disease prevalence from 2013-2016 in Williamson County, Texas. The second, Section 2, investigates the effects of temperature and nitrogenous wastes on the growth and survival of *Eurycea sosorum*. *Eurycea sosorum* was used as a surrogate to *Eurycea tonkawae* in Section 2 studies because *Eurycea tonkawae* was federally listed as a threatened species at the onset of this project, and studies that may result in take (as described by the federal guidelines) were not allowed. These sections represent complementary (field versus laboratory) but non-overlapping studies on two different species, and therefore, are presented herein as separate documents.

SECTION 1

Jollyville Plateau Salamanders (*Eurycea tonkawae*) occur north of the Colorado River in caves, springs, and creeks in Travis and Williamson Counties, Texas. This taxon is federally threatened, but natural history data are lacking to make sound conservation decisions. Our intention with this work was to provide data and results that not only add to the understanding of *Eurycea tonkawae* biology, but also inform conservation policy for this taxon. We conducted visual encounter surveys and quadrat surveys for *Eurycea tonkawae* at five spring sites (Avery Deer Spring, Avery Springhouse Spring, Hill Marsh Spring, PC Spring, and Brushy Creek Spring) and one creek (Brushy Creek) in Williamson County, Texas from 2013-2016. *Eurycea tonkawae* were observed at all five springs over the course of the study, but were never observed

in Brushy Creek. Data were reported and analyzed as the number of salamanders observed per structure object (i.e., cobble, vegetation, leaf litter, woody debris) searched. Three years is not sufficient to make conclusions on long-term population or reproductive trends, but our early observations can provide useful insight. We did not observe seasonal differences in salamander observation rate as previously reported in other central Texas *Eurycea* studies, but we did observe a fall/winter reproductive cycle (i.e., November to March) as previously reported. Independent analyses of visual encounter survey data and quadrat survey data determined that salamander observation rate was significantly different among the spring sites, suggesting that *Eurycea tonkawae* abundance differs among sites. Further, salamander observation rate was significantly different in downstream distance segments (i.e., distance from a primary spring discharge point) with most *Eurycea tonkawae* observations occurring within 25 m of a spring discharge point. Our visual encounter data yielded no difference in salamander observation rate among structure object types, but salamander observation rate in woody debris structure was significantly higher than observation rate in other structure object types in the quadrat surveys. Water depth, pH, and conductivity were statistically significant predictors of *Eurycea tonkawae* occurrence on a microhabitat scale, and flow velocity, water temperature, dissolved oxygen, and turbidity (all previously reported as critical components of *Eurycea tonkawae* habitat quality) were not statistically significant. We tested all captured *Eurycea tonkawae* for the amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*). Salamanders from each site tested positive for *Bd* over the course of the study. Overall, *Bd* prevalence decreased from 2013-2015, with 2014 having the lowest infection rate. *Bd* prevalence was significantly different among sites, and salamanders at Hill Marsh Spring had the highest infection rate. However, it is important to note that we never observed any *Eurycea tonkawae* demonstrating adverse signs of infection. These

data and results add to the available life history information for *Eurycea tonkawae*, and should inform future conservation decisions for this taxon.

SECTION 2

The Barton Springs Salamander (BSS), *Eurycea sosorum*, is a federally endangered obligate aquatic salamander found only in a few spring outflows located in a highly urbanized recreational area of Austin, Texas. The purpose of this study was to gain essential information regarding the physiological response of the BSS to thermal manipulations and three common aquatic nitrogenous toxins (ammonia, nitrite, and nitrate). All salamanders used in this study were produced at the San Marcos Aquatic Resource Center (U.S. Fish and Wildlife Service) as part of a captive breeding program. To examine thermal stressors, salamanders were subjected to a temperature increase of 0.5°C per day until a loss-of-righting response (LRR) was observed. Additionally, salamander growth was assessed following a 69 day trial in which young salamanders were reared at five different temperature treatments (nominal 15, 18, 21, 24 and 27°C). The cumulative ET50 of the LRR for the combined replicates observed in the BSS was 32.6 ± 0.24°C (mean ± SD). The optimal temperature for growth of the BSS for total length was estimated to be 18.3°C resulting in a 59.7 ± 21.09% increase in total length. To investigate the effects of nitrogenous wastes on the BSS, ninety-six hour median-lethal concentration (96-hour LC50) trials were conducted for un-ionized ammonia-N (UIA-N), nitrite-N, and nitrate-N. The 96-hour LC50 of UIA-N, nitrite-N, and nitrate-N to the BSS was 2.1 ± 0.19 mg/L, 27.7 ± 0.72 mg/L, and 851.1 ± 49.21 mg/L, respectively. These results will aid in the conservation, management, and ongoing efforts to culture the BSS in captivity.

POPULATION TRENDS, ANNUAL ECOLOGY, HABITAT EVALUATION, AND DISEASE PREVALENCE FOR FEDERALLY THREATENED JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*) IN WILLIAMSON COUNTY, TEXAS

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW OF THE CONSERVATION STATUS AND CURRENT LIFE HISTORY KNOWLEDGE OF JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*)

Eurycea is a genus of Plethodontid salamanders that includes several species complexes in eastern North America. Species boundaries within and among these species complexes are confusing, due in part to prevalent morphological variation and neoteny within the genus (Bendik et al., 2013a; Bonett et al., 2013). The phylogeny of the *Eurycea* genus and delineation of species boundaries and relationships have received considerable attention in the last two decades (Chippindale et al., 2000; Hillis et al., 2001; Bonett and Chippindale, 2004; Harlan and Zigler, 2009; Lucas et al., 2009b; Forstner and McHenry, 2010; Forstner, 2012; Bendik et al., 2013a; Steffen et al., 2014; Hillis, 2015). The cave and spring dwelling *Eurycea* salamanders of the Edwards Plateau in central Texas, clade Paedomolge (Hillis et al., 2001), are of particular interest, because this clade contains almost half of the *Eurycea* biodiversity in North America within an extremely limited geographic area (13 of the 28 currently described species; Chippindale et al., 2000; Hillis et al., 2001; AmphibiaWeb, 2015). Further, five more locations within this restricted range have *Eurycea* populations suggested to be elevated to species status: Comal Springs (Chippindale et al., 2000), Pedernales River (Chippindale, et al., 2000; Hillis, 2015), Travis County "off-plateau" (Chippindale, 2010), western Guadalupe River watershed (Hillis, 2015), and springs west of the Nueces River (Hillis, 2015).

The central Texas *Eurycea* are of high conservation concern because of their incredible level of endemism and virtually unprecedented range restrictions (Chippindale and Price, 2005). Seven of the 13 currently recognized species are listed as either threatened or endangered by the

U.S. Fish and Wildlife Service, USFWS (USFWS, 1967; 1980; 1997; 2013a; 2014a), and three additional species are listed as threatened or endangered by the Texas Parks and Wildlife Department, TPWD (2015). Four recent federal listings, *E. waterlooensis* (USFWS, 2013a), *E. tonkawae* (USFWS, 2013a), *E. chisholmensis* (USFWS, 2014a), and *E. naufragia* (USFWS, 2014a) were encouraged by the Center for Biological Diversity's lawsuit settlement with the USFWS that requires the USFWS to decide the listing status for 757 species before 2018 (Center for Biological Diversity, 2015). Because of the incredible backlog of species, the USFWS is forced to make listing determinations on several species without the adequate natural history data generally required to make informed and effective conservation decisions (i.e., listing status and designation of critical habitat). A total of four peer-reviewed, published articles that address natural history were available for the four recently listed central Texas *Eurycea* at the time of their respective listing decisions: *E. waterlooensis* (Hillis et al., 2001), *E. chisholmensis* (Chippindale et al., 2000), *E. naufragia* (Chippindale et al., 2000; Pierce et al., 2010), and *E. tonkawae* (Chippindale et al., 2000; Bowles et al., 2006). The USFWS relied heavily on data from unpublished reports, primarily from the City of Austin (COA) Watershed Protection Department, regarding natural history and population estimates for these taxa.

Eurycea tonkawae, along with *E. chisholmensis* and *E. naufragia*, constitute the Septentriomolge clade of central Texas *Eurycea* and are all found north of the Colorado River (Chippindale et al., 2000; Hillis et al., 2001). Its habitat consists of spring fed caves, pools, runs, and creeks fed by the northern segment of the Edwards Aquifer (Chippindale, 2005). Subsurface (aquifer) waters are utilized as refugia (Chippindale, 2005; Bendik and Glusenkamp, 2012) and as potential migration corridors (Chippindale, 2010) but to an unknown extent. The USFWS considers habitat modification, including degraded water quality and reduced water quantity, as

the primary threat to *E. tonkawae* because of its reliance on groundwater from the Edwards Aquifer system and the rapid urbanization of central Texas (USFWS, 2013a). The USFWS designated 32 critical habitat units, CHUs, (Figure 1) for the taxon in Travis and Williamson Counties, Texas (USFWS 2013b), which includes all known surface populations.

Despite increased attention from the federal listing process, published accounts are limited regarding important *E. tonkawae* natural history. This is apparent in the federal surface CHUs for *E. tonkawae*, which the USFWS established based on the maximum observed movement of *E. tonkawae* in a mark-recapture study (USFWS, 2013b), and not from basic data such as downstream surveys. Since the recognition of *E. tonkawae* as a species (Chippindale et al., 2000), only five peer-reviewed papers have been published regarding the natural history of this taxon. These address habitat (Bowles et al., 2006; Bendik et al., 2016), body shrinkage (Bendik and Glusenkamp, 2012), the use of photograph recognition software for mark-recapture studies (Bendik et al., 2013a), and population trends and the effects of urbanization on *E. tonkawae* density (Bowles et al., 2006; Bendik et al., 2014). Most data regarding natural history and population size estimates are from unpublished COA reports (e.g., Davis et al., 2001; O'Donnell et al., 2006; 2008; Bendik, 2010), and in fact, all peer-reviewed publications (except for Chippindale et al., 2000) originated from COA data and reports.

Effective design and implementation of conservation policy is not possible without adequate natural history knowledge of the target species. In our 2013-2016 monitoring efforts, we targeted several issues important to the conservation and management of *E. tonkawae* populations. Study objectives included monitoring population trends, describing annual ecology, delineating the full extent of occupied surface habitat (i.e., structure object use and occurrence in proximity to spring discharge locations), identifying small-scale environmental variables that

influence presence (microhabitat parameters), and evaluating disease prevalence within the studied populations. Our intention with this work is to provide data and results that not only add to the understanding of *E. tonkawae* biology, but also inform conservation policy for this taxon (e.g., refine CHUs, survey protocol, prioritize recovery sites).

CHAPTER 2

JOLLYVILLE PLATEAU SALAMANDER (*EURYCEA TONKAWAE*) SURVEY SITES IN WILLIAMSON COUNTY, TEXAS AND SURVEY METHODOLOGY

STUDY SITES

We conducted *Eurycea tonkawae* surveys at five spring sites spanning three CHUs and two watersheds in Williamson County, Texas (Figure 2). Sites included: 1) Avery Deer Spring, CHU6, South Brushy Creek Watershed; 2) Avery Springhouse Spring, CHU6, South Brushy Creek Watershed; 3) Hill Marsh Spring, CHU6, South Brushy Creek Watershed; 4) PC Spring, CHU7, Lake Creek Watershed; and 5) Brushy Creek Spring, CHU2, Lake Creek Watershed. We also surveyed a portion of the larger Brushy Creek system that is not part of a CHU. We conducted monthly salamander surveys at all sites from May 2013 to September 2015, and then surveyed every other month from October 2015 until August 2016 (exceptions are noted in *Site Descriptions*). No salamander surveys were conducted from October 2013 through February 2014 while we were waiting for federal approval to resume surveys after the USFWS decision to list *Eurycea tonkawae* as threatened.

Site Descriptions.—Avery Deer Spring is located in the Avery Ranch development and is surrounded by a residential neighborhood. The site consists of three spring discharges and receives considerable storm water input during heavy rain events. The most upstream spring discharge (N 30.507000°, W 97.749490°) is ephemeral, and during our monitoring period, it was common for the spring to stop flowing and the spring pool to dry during summer months without rain. Two additional discharges (N 30.507502°, W 97.749311°) occur on opposite banks of the creek approximately 69 m downstream from discharge one. The creek ends in a retention pond

approximately 235 m downstream of discharges two and three. However, from May 2015 until July 2016, the retention pond was flooded and restricted the creek to approximately 150 m downstream of discharges two and three. We conducted surveys at discharge one for the entire project, and added discharges two and three to the monitoring efforts beginning in July 2014.

Avery Springhouse Spring is also located in the Avery Ranch development and is surrounded by a residential neighborhood. The site has one primary spring discharge point (N 30.503698°, W 97.759829°) and the resulting spring run flows for approximately 65 m and waterfalls into a larger creek system. The creek terminates in a golf course pond approximately 340 m downstream of the confluence with the spring run. The creek portion receives storm water input during heavy rain events.

Hill Marsh Spring is located near the fourth tee box on the Avery Ranch Golf Course. The site has one primary spring discharge point (N 30.507680°, W 97.755070°) and a 45 m spring run. The spring run terminates in a golf course pond.

PC Spring is located at the intersection of SH45 and FM620 and consists of two primary spring discharge points. Discharge one (N 30.481276°, W 97.742274°) emerges from the bank of a creek that originates offsite. Surface water is often present in the creek upstream of the discharge point from either pond overflow, storm water input, or possibly another spring that may exist on private property. We had access to Texas Department of Transportation property during this study which included the creek 50 m upstream and 100 m downstream of discharge one. Discharge two (N 30.481947°, W 97.742362°) flows from the base of a concrete culvert pad and flows approximately perpendicular to the larger creek system with a confluence at 78 m. Both spring discharge points were surveyed for the entire project.

Brushy Creek Spring is located inside a storm water culvert that runs under highway 79 (N 30.516834°, W 97.661271°). Spring input is ephemeral, but when present, water emerges from three spring diversion pipes inside the culvert in addition to several cracks in the concrete culvert. Storm water flows over the top of spring discharge points during and after rain events. The spring discharges are located approximately 20 m inside the culvert. Water exits the culvert over a large riprap structure and into a pool. The pool constricts into a spring run which flows for approximately 20 m before it waterfalls into Brushy Creek. We began surveys in the Brushy Creek Spring run in July 2014 and regular surveys inside the storm water culvert in April 2015.

We also regularly surveyed a portion of the larger Brushy Creek system at Chisholm Trail Park (N 30.513070°, W 97.689822°) that is not near any known spring discharges, does not have any historical records of *Eurycea* salamanders, and is not part of a CHU. We monitored a section of Brushy Creek that spanned approximately 60 m upstream and 40 m downstream of the Chisholm Trail Road crossing.

SALAMANDER SURVEY DESIGN

We utilized two formal survey techniques in this study: visual encounter surveys (VES) and quadrat sampling. We also recorded incidental observations when salamanders were detected outside of either of the two formal surveys.

Visual Encounter Surveys.—VES consisted of researchers searching under structure objects (e.g., cobble, vegetation, leaf litter, woody debris) for *Eurycea* salamanders near primary spring discharge points (Bowles et al., 2006; Pierce et al., 2010; Bendik et al., 2014; Pierce et al., 2014). The number of structure objects overturned during VES was recorded to standardize salamander counts as “number of salamanders per structure object” (Pierce et al., 2010; 2014).

Surveys were conducted within 25m of a primary spring discharge point with most effort focused near the spring discharge. Unlike VES results reported by others (e.g., Pierce et al., 2010; Bendik et al., 2014; Pierce et al., 2014) we did not exhaustively search the upper stretches of the spring runs (i.e., overturn or remove all available cover objects). Our surveys were intended to sample rather than census the salamander population. Portions of the monitored sites are heavily covered by aquatic and emergent vegetation, and the only way to adequately search vegetation is destructive sampling (see *Salamander Sieve*). Vegetation patches destroyed during surveys quickly regrew, often by the next month's monitoring event. However, we avoided complete census in order leave most vegetation patches intact.

VES conducted within 25 m of a primary spring discharge point was our sole survey methodology from May 2013 to September 2013 and March 2014 to June 2014 (with the exception of Brushy Creek where there is not a spring discharge). Quadrat surveys were initiated in July 2014 (see *Quadrat Sampling*) and from July 2014 to March 2015, VES was a supplementary survey technique. From April 2015 through the present survey effort, VES were conducted for 20 minutes in each spring segment during each site visit. Segments are measured from the primary spring discharge point and are defined as 0-25 m, the historically reported distance for most *Eurycea* observations (Sweet, 1982; Pierce et al., 2010), 26-80 m, the remaining federal CHU (USFWS, 2013b), 81-125 m, and greater than 125 m. The 125 m boundary corresponds to the furthest downstream observation of *E. tonkawae* in these systems during our early survey effort. Structure object type, substrate, and distance from nearest primary spring discharge point were recorded for observed *E. tonkawae* during VES.

Quadrat Sampling.— We implemented quadrat surveys in July 2014 in which we exhaustively surveyed 30 cm x 30 cm samples, quadrats (Figure 3), of the spring run and

associated creek. Quadrat surveys were intended to 1) compare survey results from intensively sampling a small area in contrast to broadly surveying a large area (i.e., VES) and 2) to collect detailed microhabitat data associated with the presence or absence of *Eurycea* salamanders. False absences (i.e., incorrectly documenting no salamanders when a salamander(s) is present) are likely reduced during quadrat surveys because sampling is focused on a small area, rather than quickly surveying larger sections (Moilanan 2002). False absences can underestimate occupancy (MacKenzie and Royle, 2005).

At least two quadrats were surveyed in each distance segment of the spring run/creek. The sampled area spanned from the primary spring discharge point and terminated at a ponded water body (unless site conditions dictated otherwise). Quadrat locations were randomly chosen in each section. To prevent disturbing stream sections before sampling, we began at the most downstream quadrat and work upstream, concluding at the primary discharge. For each quadrat sample, we recorded distance from the primary spring discharge, the number and type of structure objects (e.g., cobble, leaf litter, aquatic vegetation, woody debris) searched, substrate (e.g., silt, sand, gravel, bedrock), substrate depth, water depth, estimated canopy cover, and duration of the survey. We removed all structure objects from the quadrat sample and documented the number of *E. tonkawae* observed and captured and the type of structure object that it occupied. Quadrat surveys were conducted in this manner monthly from July 2014 to March 2015.

Beginning in April 2015, we modified the quadrat sampling protocol to include additional habitat detail. Three quadrats were sampled per stream segment at each study site. Quadrat sample locations were randomly generated using R prior to field surveys. Randomly generated location included distance from primary discharge, position in spring run/creek (edge

or center), and spring run arm or channel (if the site has multiple primary discharge points or braided channels). Back-up quadrat sample locations were generated in case any of the three sample locations were not appropriate for surveys (e.g., off property, no structure objects).

Each quadrat was photographed before and after surveying. We collected the following data for each quadrat sample: survey duration, distance from primary discharge, position in spring run/creek (edge or center), stream habitat type (i.e., riffle, run, pool, bedrock glide), number of structure objects per type (i.e., cobble, vegetation, leaf litter, and woody debris) and size category (modified Wentworth scale as presented in USFWS, 2014b), substrate type (i.e., silt, sand, gravel, leaf litter, woody debris, bedrock) and percent cover, substrate depth at five locations, embeddedness (as suggested by Sennatt et al., 2006), water depth at five locations, water quality parameters (i.e., temperature, pH, dissolved oxygen, conductivity, and turbidity), water flow velocity, canopy species and percent cover, number of *Eurycea* observed, and number and type of potential *Eurycea* predators observed (e.g., dragon fly larvae, sunfish). Water quality parameters were measured with a Manta2® multiprobe from Eureka Water Probes, water flow velocity with a MF pro electromagnetic velocity sensor from Ott Hydromet, and canopy percent cover with convex, spherical densiometer. Water quality parameters and water flow velocity were measured after all structure objects were removed and searched for *E. tonkawae* to minimize flushing potential *E. tonkawae* from the quadrat sample prior to searching. Detailed quadrat surveys were conducted monthly from April 2015 to September 2015 and every other month from October 2015 to March 2016.

Salamander Sieve and Rake.— We modified an aquatic vegetation sieve (Goin, 1942; Godley, 1982) to a 30 cm x 30 cm size for sieve sampling aquatic vegetation, leaf litter, and woody debris structure objects. Vegetation, leaf litter, and woody debris material (and any *E.*

tonkawae present) are scooped into the sieve with a large dust pan and washed with water until all silt is removed. We then carefully sorted through the remaining material searching for *E. tonkawae* eggs, larvae, and adults. Once inside the sieve, salamanders could not escape, further reducing false absences associated with these difficult to sample structure types (Figure 4).

We also designed a salamander rake that combined the scoop and sieve into one sampling device (Figure 5). Six inch teeth were included on the front of the rake to comb through rooted emergent vegetation (e.g., *Ludwigia repens*). The rake was constructed by Hubbard Rakes in Jonesport, Maine, and is a custom design that combined aspects of their sea glass and cranberry rakes.

Quantifying Survey Effort.—The number of structure objects overturned was counted during all survey types, and we are therefore able to report results as the number of salamanders per structure object searched. Because of the known quadrat sample size and time surveyed, we are also able to report *E. tonkawae* observations from quadrat surveys as the number of salamanders observed per unit time searched, per number of structure objects (and by structure object type), and per m^2.

For objects that are not an obvious individual unit (e.g., leaf litter packs, vegetation), we treated each full sieve (0.09 m^2) as 12 objects. At this assumption, each "object" is approximately 87 mm x 87 mm, a size that falls within the small cobble category of the modified Wentworth scale (USFWS, 2014), and a size large enough to accommodate the average total length of an adult *E. tonkawae* (Chippindale, 2005).

Salamander Captures.—We attempted to capture all *E. tonkawae* observed during all survey events. Beginning in June 2014, the distance from primary discharge, substrate, and structure object were recorded for all salamander captures for both quadrat and TCS surveys. We

photographed each capture by placing the salamander in a petri dish filled with spring water, then placing the petri dish over a 6.35 mm grid paper. A photo of the dorsal view of the salamander with a grid background was then recorded. We measured the total length (TL), snout-vent length (SVL), inspected the body cavity for oocytes for all captures (oocytes are visible through the transparent venter), and externally inspected the salamander for abnormalities (e.g., parasites). Because sex is difficult to determine in the field, we only categorized captures as "not gravid" or "gravid female". Salamanders without oocytes could be males or non-gravid females. Salamanders were placed in small plastic bags to facilitate handling, measurements, and observations. A new, clean petri dish and plastic bag were used for each salamander.

In addition, we collected a tissue sample (i.e., small tail clip) from every capture, including potential recaptures. Recaptures were common at these spring sites and can be determined by either a truncated tail from a previous tail clip or a discolored tail indicating regrowth from a previous tail clip. Animals that appeared to have been previously tissue sampled were recorded as "potential recaptures" because missing tail tips may be from crayfish (*Procambarus* spp.) depredation (O'Donnell et al., 2008). We photographed and tissue sampled all captures and recaptures for future capture-mark-recapture analyses by photographic recognition software (Bendik et al., 2013b; Drechsler et al., 2015) and genetic fingerprinting (Lampa et al., 2013; McCall et al., 2013; Ringler et al., 2014; Drechsler et al., 2015), respectively.

WATER AND SITE CONDITIONS

Photographs of the spring discharge and spring run and/or creek were recorded at every site during every monitoring event for use as long term records of site conditions. From May

2013 to June 2014 water conditions (i.e., temperature, pH, dissolved oxygen, conductivity) were measured at spring discharges and in Brushy Creek, including the October 2013 to February 2014 when we could not survey for salamanders. We resumed these measures in April 2015 with the addition of turbidity, flow velocity, and spring discharge. Water discharge (volume of water per time) was measured at each primary spring discharge point either by building a temporary weir or by stream cross-section profile, depending on channel width and depth.

CHAPTER 3

POPULATION TRENDS AND ANNUAL ECOLOGY OF JOLLYVILLE PLATEAU SALAMANDER (*EURYCEA TONKAWAE*) POPULATIONS IN WILLIAMSON COUNTY, TEXAS

INTRODUCTION

Long term monitoring programs are useful for identifying trends in animal populations. The downside to these studies is the often substantial lag time necessary to accumulate enough data to be statistically informative. Further, effective design and implementation of conservation policy is not possible without adequate natural history knowledge of the target species. Despite increased attention from the federal listing process, there are few published reports of *Eurycea tonkawae* life history. Published data on *E. tonkawae* annual ecology (e.g., counts, size class, reproductive condition per month) are limited to Bowles et al. (2006), but additional data are available from unpublished reports (O'Donnell et al., 2008; Bendik, 2010) and for the closely related *E. naufragia* (Pierce et al., 2010; 2014).

Previous monitoring efforts indicate that surface counts for *E. tonkawae* (Bowles et al., 2006; Bendik, 2010) and *E. naufragia* (Pierce et al., 2014) juveniles and adults are highest in spring and summer months. Gravid female *E. tonkawae* are more abundant from November through March (Bowles et al., 2006), and gravid *E. naufragia* are most abundant in two seasonal peaks, November-December and February-March (Pierce et al., 2014). It is unknown if these peaks represent different females or females that double-clutch (Pierce et al., 2014). However, breeding may occur year round as juveniles of both species are reported throughout the year (Bowles et al., 2006; Bendik 2010; Pierce et al., 2014), and year round breeding is documented in *E. sosorum* in response to spring flow (Gillespie, 2011). *Eurycea tonkawae* eggs have only

been found on two occasions, one egg in September (O'Donnell et al., 2006) and one egg in March (O'Donnell et al., 2008). *Eurycea naufragia* eggs have never been observed during field studies (Pierce et al., 2014).

Our intention in this study was to 1) establish the foundation for long-term population monitoring of *E. tonkawae* at several sites in Williamson County, 2) establish long term monitoring of spring habitat conditions (e.g., photographic records, water conditions), and 3) investigate the annual ecology of this taxon with monthly captures over the course of several seasons.

MATERIALS AND METHODS

Study Sites and Survey Design.—All sites described in Section 2 were included in the study. VES surveys conducted within 25 m of a primary spring discharge point (as described in Chapter 2) were used to establish salamander observation trend data. This survey type and area constraint was selected so data may be comparable to other similar monitoring programs (Pierce et al., 2010; Bendik et al., 2014). The only exceptions are months where we did not perform VES surveys in the upper spring reach. In these instances, we used data from quadrats performed within 25 m from a primary discharge point as a surrogate for VES data. Quadrat data were used to express monthly counts and observation rate during the May 2014 survey event (Avery Deer, Avery Springhouse Spring, Hill Marsh Spring, and PC Spring), June 2014 event (Avery Deer Spring and Hill Marsh Spring), July 2014 event (Hill Marsh Spring), October 2014 (Avery Springhouse Spring), December 2014 (Avery Springhouse Spring and Hill Marsh Spring), and January 2015 (Avery Springhouse Spring).

Data from all captured salamanders, without regard to survey type, were used to evaluate reproductive trends. Captured salamanders were inspected for oocytes and measured as described in Chapter 2. Previous monitoring programs established size classes (i.e., < 25 mm, 25 – 50 mm, and > 50 mm total length, TL) to evaluate population structure (Bowles et al., 2006; O'Donnell et al., 2008; Pierce et al., 2014). Bruce (1976) examined 608 *Eurycea neotenes* and determined salamanders ≤ 25 mm snout-vent length (SVL) were invariably immature. We therefore present juveniles as all salamanders < 25 mm TL and also all salamanders ≤ 25 mm SVL. Rainfall data for the monitoring period was collated from a National Oceanic and Atmospheric Association (NOAA) gauge in Round Rock, Texas, and site condition data were collected as described in Chapter 2.

RESULTS

Count and Observation Rates.—Data from 113 VES totaling 23,848 structure objects searched and 764 *Eurycea tonkawae* observations were used to establish trend plots. Salamanders were observed at all spring sites but were not observed in the larger Brushy Creek system. VES observations and salamander observation rate are provided for each spring for the duration of the monitoring period (Figures 6 – 10). Salamander observation rate is the number of salamanders observed per structure object searched. Salamander observation rate for each spring was plotted with cumulative monthly rainfall for Round Rock, Texas (Figure 11). Salamander observation rate for each spring was also plotted with site condition data (i.e., spring discharge, temperature, pH, dissolved oxygen, conductivity, and turbidity) for each monitoring event (Figures 12 – 47).

Reproductive Cycle.—We captured 752 *Eurycea tonkawae* over the course of the study. Of those, 34 were gravid females, 49 were size class 1 juveniles (< 25 mm TL), and 255 were juveniles (≤ 25 mm SVL). We captured at least one gravid female and one juvenile at each site. The smallest gravid female was 23.6 mm SVL and 42.6 mm TL. All other gravid female were > 25 mm SVL. The smallest juvenile captured was 8.5 mm SVL and 13.3 mm TL. We caught 17 juveniles ≤ 10 mm SVL. We did not observe any oviposited eggs.

DISCUSSION

Count and Observation Rates.—Similar to previous studies, we observed high variability in monthly salamander counts and observation rate. Salamander observation rate does not appear to follow any pattern associated with rainfall or site conditions. We did not observe a pattern of higher salamander abundance in the summer and low numbers in the winter as reported in other studies (Bowles et al, 2006; Pierce et al., 2014). This may be due to the relatively short monitoring period (just over three years), and the five month data gap after the listing decision (see Chapter 2 for explanation). There appears to be a difference in observation rate among the sites, and this is addressed in detail in Chapter 4.

Reproductive Cycle.—We do not consider 34 gravid female captures over three years enough data to determine reproductive cycle, but our limited results agree with the results from Bowles et al. (2006). The percentage of gravid female salamanders peaked from November to February in the two years with adequate data (Figure 48). We did not observe a double peak in gravid females as in *E. naufragia* (Pierce et al., 2014).

Percentage of size class 1 juveniles and all juveniles was highest from approximately March to September, with the peak approximately two to three months after the peak in gravid

females (Figure 48). Eighty percent of size class 1 *E. tonkawae* were captured in aquatic vegetation. The dense root systems likely provide the necessary structure complexity to keep tiny juveniles from flushing downstream. Bendik et al. (2016) found the highest numbers of size class 1 juveniles at approximately 80 m from spring discharge points in the Bull Creek system. Approximately half of our size class 1 captures occurred around 24-27 m from the primary discharge point, but none occurred past 36 m.

These data provide a solid foundation for future monitoring work to build upon. However, more data are needed before definitive statements regarding *E. tonkawae* life history can be supported.

CHAPTER 4

DISTRIBUTION OF JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*) AMONG AND WITHIN SPRING RUNS IN WILLIAMSON COUNTY, TEXAS

INTRODUCTION

Defining habitat for endangered and threatened species is critical for designing effective conservation plans and survey protocols. For example, the Desert Night Lizard (*Xantusia vigilis*) was considered a rare species for three decades following its formal description, until researchers determined that its preferred habitat is downed yucca (*Yucca brevifolia*). With the proper habitat documented, subsequent surveys have identified that this lizard constitutes the highest single species biomass in some desert areas (Bezy, 1988 as described in Gray-Lovich and Lovich, 2012).

Eurycea tonkawae habitat consists of spring fed caves, pools, runs, and creeks fed by the northern segment of the Edwards Aquifer (Chippindale, 2005). Subsurface (aquifer) waters are utilized as refugia (Chippindale, 2005; Bendik and Glusenkamp, 2012) and as potential migration corridors (Chippindale, 2010) but to an unknown extent. Determining which portions of these surface habitats are occupied is important for designating CHUs and developing survey protocols to determine presence and estimate abundance.

The habitat parameters for *E. tonkawae* and other central Texas *Eurycea* have persisted with few updates since their original descriptions (Chippindale et al., 2000; Chippindale, 2005; Bowles et al., 2006). *Eurycea tonkawae* habitat is described as submerged cobble and leaf litter cover on gravel and bedrock substrate near spring discharge points (Sweet, 1982; Chippindale,

2005; Bowles et al., 2006). Most habitat descriptions for other surface-dwelling central Texas *Eurycea* are identical (Petranka, 1998).

Previous studies report that central Texas *Eurycea* are rarely found greater than 25 m from spring discharge points (Sweet, 1982; Pierce et al., 2010). Sweet (1982) suggested that maintaining a close proximity to the spring discharge provided necessary thermal stability, especially in the hot, semi-arid climate of central Texas. However, Sweet (1982) only surveyed during summer months when thermal stability may be most important. The USFWS designated the surface CHUs for *E. tonkawae* as an 80 m radius around the primary spring discharge (USFWS, 2013b). This distance is based on the maximum observed movement of *E. tonkawae* in a mark-recapture study (USFWS, 2013b) and not from surveys that were conducted further downstream from discharge points. In a recent study, Bendik et al. (2016) surveyed creek sections in between known spring discharge points in the Bull Creek system in Travis County and frequently found *Eurycea tonkawae* outside of the vicinity of spring discharges. Bendik et al.'s (2016) work is an important first step in understanding the distribution of *Eurycea tonkawae* in spring runs and spring fed creeks.

All aquatic *Eurycea* species, including central Texas *Eurycea*, utilize submerged structure objects (e.g., cobble, vegetation, leaf litter, woody debris) as refugia (Petranka, 1998; Chippindale, 2005). Cobble are considered the preferred structure object for *E. tonkawae* (Bowles et al., 2006) and closely related congeners, *E. naufragia* (Pierce et al., 2010) and *E. sosorum* (Dries, 2012). All three studies report that greater than 90% of observed *Eurycea* are documented under cobble (Bowles et al., 2006; Pierce et al., 2010; Dries, 2012). Vegetation, leaf litter, and woody debris are considered minor habitat components (USFWS, 2013a), and collectively account for less than 2% of observed *Eurycea* (Bowles et al., 2006; Pierce et al.,

2010; Dries, 2012). However, *E. nana* is most abundant in algae, moss, and aquatic vegetation structure objects (Chippindale and Fries, 2005), and there are several contradicting reports regarding *E. tonkawae* structure object use. O'Donnell et al. (2008) states that *E. tonkawae* are found in roots and vegetation along creek banks and are "commonly found in leaf litter", but the authors fail to quantify these observations. Davis et al. (2001) documented *E. tonkawae* observations under aquatic plants, algae, and organic debris, but also did not survey heavily vegetated spring sections due to difficulty of searching these areas. Bowles et al. (2006) and Pierce et al. (2010) also acknowledge that *Eurycea* use of vegetation, leaf litter, and woody debris may be underestimated due to the difficulty in searching these structure objects.

Most studies and the current federal survey protocol focus on searching cobble near primary spring discharge points (Bowles, 2006; Pierce et al., 2010; Bendik et al., 2014; Pierce et al., 2014; USFWS, 2014b). Our intent in this study was to 1) determine the maximum distance from primary discharge points commonly utilized by *E. tonkawae*, 2) determine the type and frequency of structure object use, and 3) simultaneously collect abundance data comparable to other long-term population studies. Our ultimate goal is a less biased approach to sampling that can be applied across central Texas *Eurycea* sites and taxa that yields a sample (*Eurycea* observations) representative of the entire surface population (Hayek, 2012).

MATERIALS AND METHODS

Study Sites and Survey Design.—All sites described in Chapter 2 were included in the study. However, survey data from Brushy Creek were excluded from analyses because salamanders were never observed at this location. Data from VES and both quadrat survey types were utilized (see Chapter 2 for survey descriptions). Analyses for VES data include 369 surveys

totaling 46,708 structure objects searched and 871 *E. tonkawae* observations. Analyses for quadrats include 578 quadrat samples totaling 31,156 structure objects searched and 95 quadrats with salamanders observed.

Data Analyses.—Results from VES surveys were represented as a salamander observation rate (number of salamanders observed per structure object searched) per structure object type, per distance segment, per site. An unbalanced three-factor analysis of variance (ANOVA) was used to analyze these data with structure object type, distance segment, and site as the three categorical predictors and salamander observation rate as the response variable. A fully saturated model was analyzed to evaluate the influence of each predictor and the interactions among predictors.

Results from quadrat surveys were represented as salamander "observed" or "not observed". Data were modeled using logistic regression coupled with a chi-square goodness of fit test. The response variable was binary, and predictors were again structure object type, distance, and site. Distance was treated as a continuous variable in this analysis as the exact distance was known for every quadrat surveyed.

RESULTS

Salamanders were observed at all sites and in/under all structure object types. The furthest downstream observation at each site was: 9 m at Avery Deer Spring, 121.5 m at Avery Springhouse Spring, 45 m at Hill Marsh Spring, 93 m at PC Spring, and 45 m at Brushy Creek Spring.

Site ($p < 0.001$) and distance segment ($p < 0.001$) were significant predictors of salamander observation rate from the VES data. Structure type ($p = 0.1461$) and interactions between predictor variables were not significant (Table 1).

Site ($p < 0.001$), distance ($p < 0.001$), and structure object type ($p = 0.005$) were all significant predictors of salamander observation from the quadrat data (Table 2). In the logistic regression model, the site "Avery Deer Spring" and the structure object "cobble" were treated as the model intercepts (i.e., all other sites were compared to Avery Deer Spring and all other structure object types were compared to cobble). Salamander observations at Avery Springhouse Spring ($p < 0.001$) and Hill Marsh Spring ($p = 0.003$) were significantly different than Avery Deer Spring, but salamander observations at Brushy Creek Spring ($p = 0.983$) and PC Spring (0.259) were not. Salamander observations in woody debris structure were significantly different than cobble ($p = 0.013$), but salamander observations in vegetation ($p = 0.990$) and leaf litter ($p = 0.867$) were not significant (Table 3).

DISCUSSION

Site and distance were significant predictors in both analyses, strongly indicating that *Eurycea tonkawae* populations, whether modeled as an observation rate or simply as observed, are different among the five sites and salamanders are not uniformly distributed throughout the spring runs and creeks. These results are not surprising, as it is not expected for salamander populations to be similar among sites that have variable habitat characteristics and configuration. In addition, it is well documented in central Texas *Eurycea* that the majority of the surface population occurs in close vicinity to a spring discharge point (Sweet, 1982; Bowles et al., 2006;

Pierce et al., 2010; Bendik et al., 2014; Pierce et al., 2014; Bendik et al., 2016). However, it is interesting that the sites vary in salamander downstream occurrence.

Eurycea tonkawae were commonly observed throughout the 45 m spring run at Hill Marsh Spring and up to approximately 120 m downstream at Avery Springhouse Spring (Table 4). In contrast, only four total salamanders were observed beyond 25 m from a primary spring discharge at the other three sites during: three at PC Spring (at 32 m, 36 m, and 93 m), one at Brushy Creek Spring (at 45 m), and none at Avery Deer Spring. All three of these sites have downstream creek sections that appear suitable for *Eurycea* salamanders, but observations appear to be an anomaly. Three of these four observations (36 m and 93 m at PC Spring and 45 m at Brushy Creek Spring) occurred after the heavy rainfall events in April 2015 and March 2016. It is possible that these salamanders were washed downstream, but surveys after other strong storm events (e.g., October 2013, May 2015, October 2015) did not yield downstream observations.

Avery Deer Spring, PC Spring, and Brushy Creek Spring all have pooled sections where flow velocity slows, water deepens, and centrarchid fish may be observed. Pools with predatory fish have been suggested as potential dispersal barriers for *Eurycea tonkawae* (Bendik et al., 2014). Notably, Avery Springhouse Spring and Hill Marsh Spring both have relatively uniform habitat consisting of shallow riffles, runs, and glides without pooled sections. It is unknown if any of the five spring sites have downstream portions of the spring run that gain groundwater, and therefore, may offer similar habitat as a primary discharge point. Additional work is needed to determine the mechanism that is limiting downstream salamander distribution in these sites.

Salamander observation rate did not differ among the structure object types in our VES data, but woody debris was significantly different in the quadrat data. These conflicting results may be due to a small sample size of quadrats with woody debris structure. The raw salamander

observation rates from VES by structure type and distance segment are shown in Figure 49. Most other North American *Eurycea* salamanders indiscriminately use submerged structure objects (Petranka, 1998), and it is not surprising that our results are congruent with the published studies of *Eurycea* from outside of Texas. Our use of a sieve and salamander rake to sample vegetation, leaf litter, and small woody debris structure undoubtedly increased detection in these difficult to sample habitats, and it is likely that our results contrast with other central Texas *Eurycea* studies because we specifically targeted the breadth of available habitats (Bowles et al., 2006; Pierce et al., 2010; Dries , 2012). When performing surveys for central Texas *Eurycea*, we strongly recommend searching all potential structure present at a site to yield an appropriate sample of the surface population.

CHAPTER 5

MICROHABITAT ASSOCIATIONS FOR JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*) IN WILLIAMSON COUNTY, TEXAS

INTRODUCTION

Amphibians are often irregularly distributed within their habitat into smaller microhabitat units, and management and conservation decisions are enhanced if detailed microhabitat data for individual observations are available (Inger, 1994). Several microhabitat factors are suggested to be important for *Eurycea tonkawae* occurrence and conservation (e.g., water temperature, water flow velocity, embeddedness of structure objects), but few have been field tested (Bowles et al., 2006; USFWS, 2013a). Many of the microhabitat parameters thought to be important to this taxon are negatively affected by urbanization within the watershed (Bowles et al., 2006; USFWS, 2013a).

The USFWS considers habitat modification, including degraded water quality and decreased water quantity, as the primary conservation threat to *Eurycea tonkawae* (USFWS, 2013a). Several publications document a decrease in abundance of stream dwelling salamanders due to changes in water quality (e.g., dissolved oxygen, temperature, pH) associated with urbanization (see Barrett and Price, 2014). Laboratory studies demonstrated that central Texas *Eurycea* are sensitive to low dissolved oxygen concentrations (Woods et al., 2010) and high temperatures (Berkhouse and Fries, 1995), and Chapter 2 of this final report investigates the effects of temperature and nitrogenous wastes on *Eurycea sosorum*. 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 *E. tonkawae* density. Urbanization can also

lead to increased siltation in waterbodies 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).

Only one published study has field tested microhabitat parameters for *E. tonkawae* and this study collected data at the spring discharge, and not necessarily at the location where salamanders were observed (Bowles et al., 2006). In accordance with the perceived importance of historically suggested microhabitat parameters, the current federal survey protocol for *E. tonkawae* requires a description of substrate, an estimation of embeddedness (see Sennatt et al., 2006), and measures of water temperature, pH, conductivity, dissolved oxygen, and water quantity (USFWS, 2014b). In this study, we sought to determine detailed microhabitat parameters that are associated with *Eurycea tonkawae* presence within spring runs and creeks.

MATERIALS AND METHODS

Study Sites and Survey Design.—All sites described in Chapter 2 were included in the study. However, survey data from Brushy Creek were excluded from analyses because salamanders were never observed at this location. We used the quadrat sampling methodology implemented from April 2015 to March 2016 (as described in Chapter 2) to evaluate microhabitat. A total of 247 quadrats, 38 of which had detected salamanders, were included for analyses.

Data Analyses.—We performed principal component analyses (PCA) to visualize data in two groups: microhabitat structure and water conditions. An additional logistic regression model was constructed with water depth and the water condition data. Results from quadrat surveys were represented as salamander “observed” or “not observed”. The response variable was binary,

and predictors were water depth, flow velocity, temperature, pH, dissolved oxygen, conductivity, and turbidity.

RESULTS

The range of sampled microhabitat conditions and the range for quadrats with *Eurycea tonkawae* present are provided in Table 5. The microhabitat structure PCA demonstrates that salamanders were present in almost the full range of measured conditions, but downstream quadrats with deeper water and substrate had fewer salamander observations (Figure 50). The water condition PCA shows that quadrats with salamanders typically had higher conductivity, lower dissolved oxygen, and lower pH. Flow velocity, temperature, and turbidity had little influence (Figure 51).

Water depth ($p = 0.007$), pH ($p = 0.004$), and conductivity ($p = 0.007$) were significant predictors in the logistic regression model. Flow velocity ($p = 0.392$), temperature ($p = 0.282$), dissolved oxygen ($p = 0.695$), and turbidity ($p = 0.110$) were not significant (Table 6).

DISCUSSION

Results from the PCAs were generally congruent with results from our study of distance segment and structure object use in Chapter 4 and with results of the logistic regression model of water condition data. The microhabitat structure PCA (Figure 50) shows that substrate type and canopy percent cover have little to no influence on salamander presence, with exception of silt and substrate depth. Fewer salamanders occurred in quadrats with high silt percent cover and deep substrate. Similar to our results in Chapter 4, fewer salamanders occurred further from the primary discharge points, but structure object type was not influential.

The water condition PCA and the logistic regression model both demonstrate that water depth, pH, and conductivity are important predictors of salamander occurrence. Specifically, as water depth increases, pH increases (moves away neutral), and conductivity decreases, fewer quadrats had salamanders present. Dissolved oxygen appears to be an important variable in the PCA, but the logistic regression results clearly show the dissolved oxygen is not a significant predictor. Interestingly, the four water condition variables that were not significant (i.e., flow velocity, temperature, dissolved oxygen, and turbidity) are all historically considered to be critical components of *Eurycea tonkawae* habitat quality (Sweet, 1982; Bowles et al., 2006; USFWS, 2013a). Further, the one study that investigated similar microhabitat parameters concluded that increasing water conductivity was correlated with decreasing salamander population sizes (Bowles et al., 2006). Our data are in stark contrast and demonstrate that salamanders are present more frequently in water with higher conductivity.

CHAPTER 6

PREVALENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN POPULATIONS OF JOLLYVILLE PLATEAU SALAMANDERS (*EURYCEA TONKAWAE*) IN WILLIAMSON COUNTY, TEXAS

INTRODUCTION

Chytridiomycosis is a disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), and is one of the leading causes of global amphibian decline (Skerratt et al., 2007). Due to the large number of affected species and elevated extinction rates, it is considered to be the worst infectious disease present in vertebrates (Briggs et al., 2005; Stuart et al, 2004; La Marca et al, 2005). *Bd* is likely a leading cause of salamander population declines in Central America (Rovito et al., 2009; Cheng et al, 2011), and while similar population declines have not been observed, the fungus has also been detected in a variety of salamander species across North America (Davidson et al., 2003; Byrne et al., 2008; Rothermel et al., 2008; Gaertner et al., 2009; Hossack et al., 2010; Rollins et al., 2013).

The disease negatively affects electrolyte transport by disrupting the cutaneous function, causing mortality in susceptible amphibian populations (Voyles et al., 2012). Even though *Bd* is able to infect a wide variety of amphibians, some species do not exhibit any clinical signs of the disease (Gahl et al., 2012). These species can serve as important vectors of disease, especially if the species is wide spread, invasive, farmed, or commonly traded in pet markets (Garner et al., 2006; Fisher and Garner, 2007). The most prominent risk of dealing with unaffected carriers of *Bd* is their ability to spread the disease to susceptible amphibians in areas of cohabitation.

Bd presence and its response to environmental variables is understudied in Texas amphibian populations. Gaertner et al. (2009) and Saenz et al. (2010) evaluated the spread of *Bd*

across Texas, but neither study addressed the correlation of *Bd* prevalence with environmental variables. In addition, and importantly, there are no reports of amphibian declines or mass die offs associated with the presence of the pathogen in Texas, as there are in other parts of the country.

METHODS

Study Sites and Survey Design.—All sites described in Chapter 2 were included in the study. In addition, salamanders captured from all survey techniques were evaluated for *Bd.* We were cautious to not spread *Bd* from one site to another. In between sites, all equipment that touched the banks or water (e.g., salamander sampling equipment, boots) was sprayed with a solution of at least 15-20% bleach and allowed to air dry before use at another site.

All captured *Eurycea tonkawae* (as described in Chapter 2) were swabbed using sterile cotton tips with a plastic handle, according to the protocol of Kriger et al. (2007). The cotton tips were placed in PrepMan tubes, and DNA was extracted from the swabs using a PrepMan assay (Gaertner et al., 2009; Kilburn et al., 2010). *Bd* presence was assessed using a real time Taqman qPCR assay (Boyle et al., 2004; Kilburn et al., 2010). We used a ChytrMGB2 probe with two species-specific primers, ITS1-3 Chytr and 5.8S Chytr, as summarized in Boyle et al. (2004) and Garland et al. (2010). Each sample was run in triplicate and the results were compared to a regression line based on a consecutive 10-fold dilution of five standards. Using the JEL423 strain as a genome reference and following the suggestions of Rebollar et al. (2014), we ensured that quantifications had an efficiency between 95 and 100%, with an average of 98.15%, and a R^2 value greater than 0.997. *Bd* presence was evaluated for all salamander swab samples collected

from 2013 through 2015. We evaluated potential differences in *Bd* prevalence among sites and potential temporal differences (i.e., among years) with a logistic regression model.

RESULTS

Batrachochytrium dendrobatidis was detected in all sampled sites and during every surveyed month from 2013 through 2015. In 2013, 45.8% of the 96 *Eurycea tonkawae* sampled had *Bd*. In 2014, the prevalence of *Bd* dropped to 14.1% out of the 192 sampled salamanders. In 2015, the overall prevalence increased to 31.4% of the 258 sampled *E. tonkawae* (Figure 52). *Bd* prevalence in 2013 was significantly higher than in both 2014 ($p < 0.001$) and 2015 ($p = 0.001$) (Table 7).

Bd was detected at all sampling sites, but prevalence fluctuated among years (Figure 53). Hill Marsh Spring had significantly higher prevalence ($p = 0.011$) compared to the other spring sites. Prevalence ranged from less than 10% at Avery Springhouse Spring in 2014 to greater than 60% at Hill Marsh Spring in 2013.

DISCUSSION

Bd prevalence has not shown a directional pattern over the three studied years, but has decreased overall since the beginning of the study. It is unclear why there are large differences in prevalence among the years. We received progressively more rain and associated flood events from 2013 to 2015, but no obvious environmental correlate explains the large drop in prevalence in 2014 and subsequent upswing in 2015. Several more years of data are likely necessary to illuminate a pattern.

All monitored sites are urban with either large potential for human traffic (i.e., Avery Deer Spring, Avery Springhouse Spring, Hill Marsh Spring) or runoff from major roads (i.e., PC Spring, Brushy Creek Spring). *Bd* prevalence, however, is fairly uniform with the exception of Hill Marsh Spring being significantly different. Surprisingly, of all the residential neighborhood sites, Hill Marsh likely gets the least amount of foot traffic because it is deep in the rough of the golf course and portions are fenced, whereas hiking trails go through Avery Springhouse Spring and Avery Deer Spring.

Amphibian communities with low levels of species richness and a community dominated by known reservoir species (i.e., species that are known to be carriers of *Bd*) present a higher prevalence of the pathogen compared to communities that show greater amphibian diversity, or no presence of known reservoirs. In 2015, we collected anurans opportunistically while doing our regular sampling. A total of 9 cricket frogs (*Acris crepitans*) were collected for *Bd* sampling, as they are a known reservoir species for *Bd*. Of the nine *A. crepitans* sampled, six tested positive for *Bd*. This evaluation demonstrates the increased risk of continued spread of the pathogen by reservoir species across Williamson County.

While our sample size is considerably larger, our results are similar to Gaertner et al. (2009) in which *Bd* was present in six of 14 sampled *E. tonkawae.* Like in Gaertner et al. (2009), no *E. tonkawae* evaluated in this study showed physical symptoms of infection, and we never observed a salamander that appeared to die from disease. However, it is important to note that a high prevalence of the pathogen was detected. Furthermore, while this preliminary analysis shows a decrease in the overall prevalence throughout the years, it should be noted that there are confounds which require intensive evaluation to determine their effect on the statistical analyses. While more intensive analyses, and sampling, are still on-going, this represents an extensive

evaluation of the pathogen in the county, and one of the few in the state. However, future research is required, and ongoing, to evaluate the interaction between *Bd* and the environment.

CHAPTER 7

CONTINUING RESEARCH

All portions of this report are considered in progress and results and conclusions should be considered as the baseline data necessary for characterizing the habitat, surface occupancy, and occurrence for *Eurycea tonkawae* at these sites. All chapters will be updated prior to publication with additional data when field work is completed this year. In many chapters, data will be added from other sites that were not included as part of this report. All statistical analyses will be rerun and may be refined, supplemented, or replaced as the final data are completed in 2016. In particular, additional modeling techniques (e.g., occupancy modeling) are intended to be added to the current analyses.

In addition to work to be completed for the chapters in this report, we have accumulated a substantial database of *Eurycea tonkawae* photographs and tissue samples. We intend to utilize Wild ID and genetic fingerprinting for capture-mark-recapture analyses to estimate demographic parameter estimates (e.g., survival, recruitment, abundance) at the surveyed sites. We also intend to explore potential influences of microhabitat on *Bd* prevalence in *E. tonkawae* and further analyze all swab samples for *Batrachochytrium salamandrivorans* (Bsal), another chytrid pathogen causing declines in salamander populations in Europe (Martel et al., 2013).

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TABLES

Table 1. Fully saturated model results for an unbalanced three-factor analysis of variance on visual encounter survey data with site, distance segment, and structure object type as predictors and *Eurycea* salamander observation rate as the response variable.

	Degrees of Freedom	Sum of Squares	Mean Square	F Value	p Value
Site	4	0.241	0.06020	5.974	**<0.001**
Distance	3	0.548	0.18250	18.110	**<0.001**
Structure Object Type	3	0.055	0.01826	1.812	0.144
Site*Distance	7	0.115	0.01639	1.626	0.126
Site*Structure	12	0.155	0.01288	1.278	0.228
Distance*Structure	9	0.043	0.00478	0.475	0.892
Site*Distance*Structure	20	0.203	0.01013	1.006	0.454
Residuals	443	4.464	0.01008		

Table 2. Analysis of deviance table of a chi-square goodness of fit test on a logistic regression model of quadrat sample data with site, distance, and structure object type as predictors and *Eurycea* salamander observation (observed or not observed) as the binary response variable.

	Degrees of Freedom	Deviance	Residual Degrees of Freedom	Residual Deviance	p Value
Null			935	661.29	
Site	4	42.462	931	618.83	**<0.001**
Distance	1	118.024	930	500.80	**<0.001**
Structure Object Type	3	12.642	927	488.16	**0.005**

Table 3. Logistic regression model results of quadrat sample data with site, distance, and structure object type as predictors and *Eurycea* salamander observation (observed or not observed) as the binary response variable.

	Estimate	Standard Error	z Value	p Value
Intercept	-1.368291	0.334350	-4.092	**<0.001**
Avery Springhouse	1.592343	0.378750	4.204	**<0.001**
Brushy Creek Spring	-14.970735	705.273387	-0.021	0.983
Hill Marsh Spring	1.080495	0.362001	2.985	**0.003**
PC Spring	0.449211	0.398162	1.128	0.259
Distance	-0.043755	0.006338	-6.904	**<0.001**
Leaf Litter	-0.081195	0.483188	-0.168	0.867
Vegetation	0.003094	0.252890	0.012	0.990
Woody Debris	-1.368544	0.448496	-3.051	**0.002**

Table 4. *Eurycea* salamander observation rate (salamanders observed per structure object searched) for each distance segment (distance from primary spring discharge point) at each spring site for visual encounter survey data.

	0 – 25 m	26 – 80 m	81 – 125 m	> 125 m
Avery Deer	0.0191	0	0	0
Avery Springhouse	0.0742	0.0480	0.0072	0
Hill Marsh	0.0431	0.0284	NA	NA
PC Spring	0.0319	0.0004	0.0004	NA
Brushy Creek Spring	0.0060	0.0004	NA	NA

Table 5. Data range for microhabitat variables measured during quadrat samples and range of variables in quadrats with *Eurycea tonkawae* detection.

	Range of All Measured Microhabitat Variables		Range of Microhabitat Variables in Quadrats with *Eurycea* detected	
	Minimum	Maximum	Minimum	Maximum
Substrate Depth (mm)	0	159	0	110
Embeddedness (%)	0	100	0	100
Water Depth (mm)	10	636	10	269
Canopy Cover (%)	0	100	0	96.4
Flow Velocity (m/s)	0.00	0.765	0.00	0.581
Temperature (°C)	18.18	30.98	18.74	26.01
pH	6.82	8.09	6.95	8.06
Dissolved Oxygen (mg/L)	0.90	11.61	5.70	8.84
Conductivity (µS/cm)	318.6	1122.0	674.2	1122.0
Turbidity (NTU)	0.0	70.1	0.0	14.7

Table 6. Logistic regression model results of quadrat sample data with as water condition variables as predictors and *Eurycea* salamander observation (observed or not observed) as the binary response variable.

	Estimate	Standard Error	z Value	p Value
Intercept	24.778185	8.071194	3.070	0.002
Water Depth	-0.019356	0.007213	-2.683	**0.007**
Flow Velocity	-2.195166	2.563702	-0.856	0.392
Temperature	-0.136925	0.127254	-1.076	0.282
pH	-3.499828	1.215531	-2.879	**0.004**
Dissolved Oxygen	0.122428	0.312311	0.392	0.695
Conductivity	0.004427	0.001632	2.712	**0.007**
Turbidity	-0.232757	0.145598	-1.599	0.110

Table 7. Logistic regression model results of disease data with site and year as predictors and *Batrachochytrium dendrobatidis* presence (present or absent) as the binary response variable.

	Estimate	Standard Error	z Value	p Value
Intercept	-0.5528	0.3707	-1.491	0.136
Avery Deer	0.2145	0.35	0.616	0.538
Avery Springhouse	0.2677	0.8674	0.309	0.758
Hill Marsh	0.8533	0.3347	2.549	**0.011**
PC Spring	0.1231	0.3486	0.353	0.724
2014	-1.5333	0.2928	-5.236	**<0.001**
2015	-0.8135	0.2532	-3.212	**0.001**

FIGURES

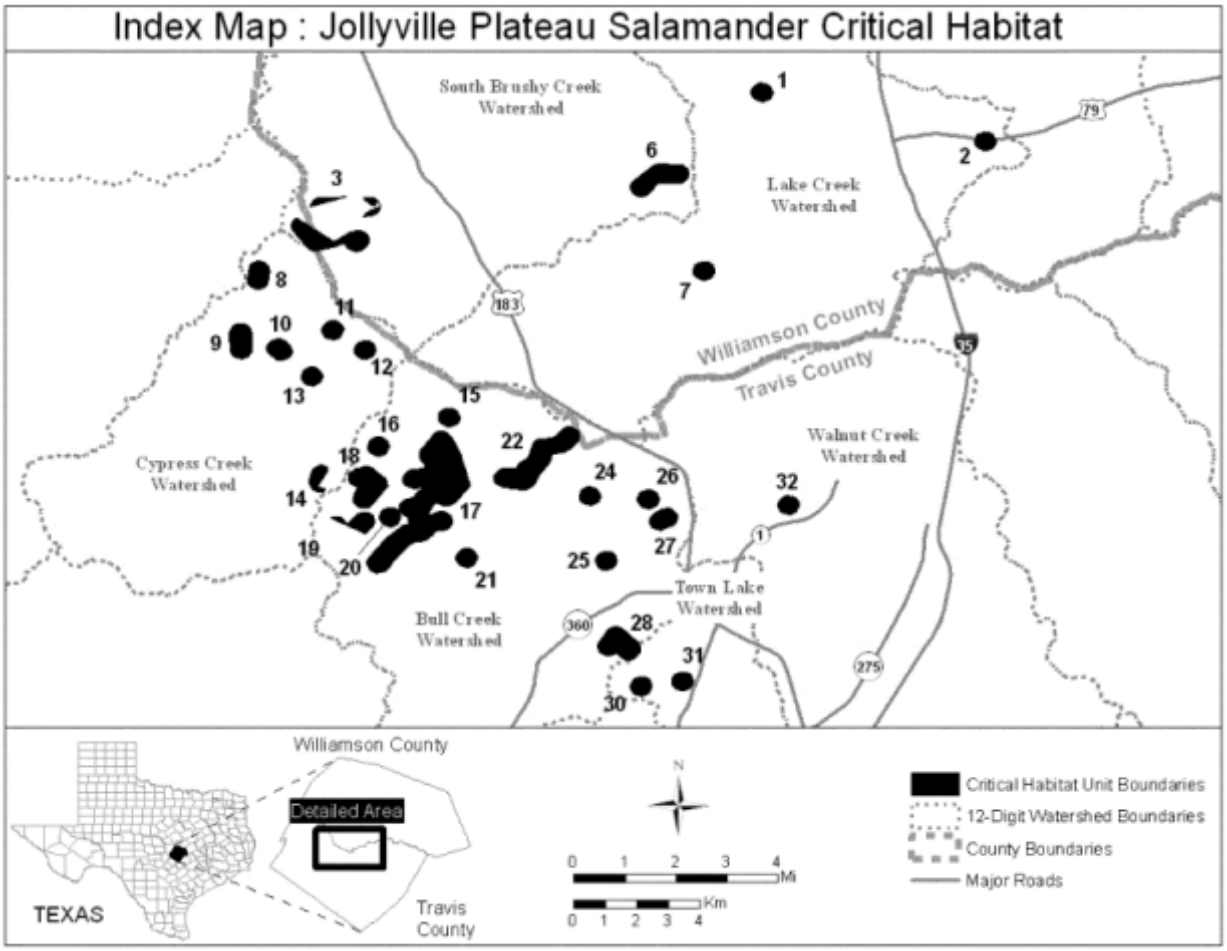


Figure 1. U.S. Fish and Wildlife Service designated critical habitat units for the Jollyville Plateau Salamander (*Eurycea tonkawae*). Figure is reprinted from USFWS (2013b).

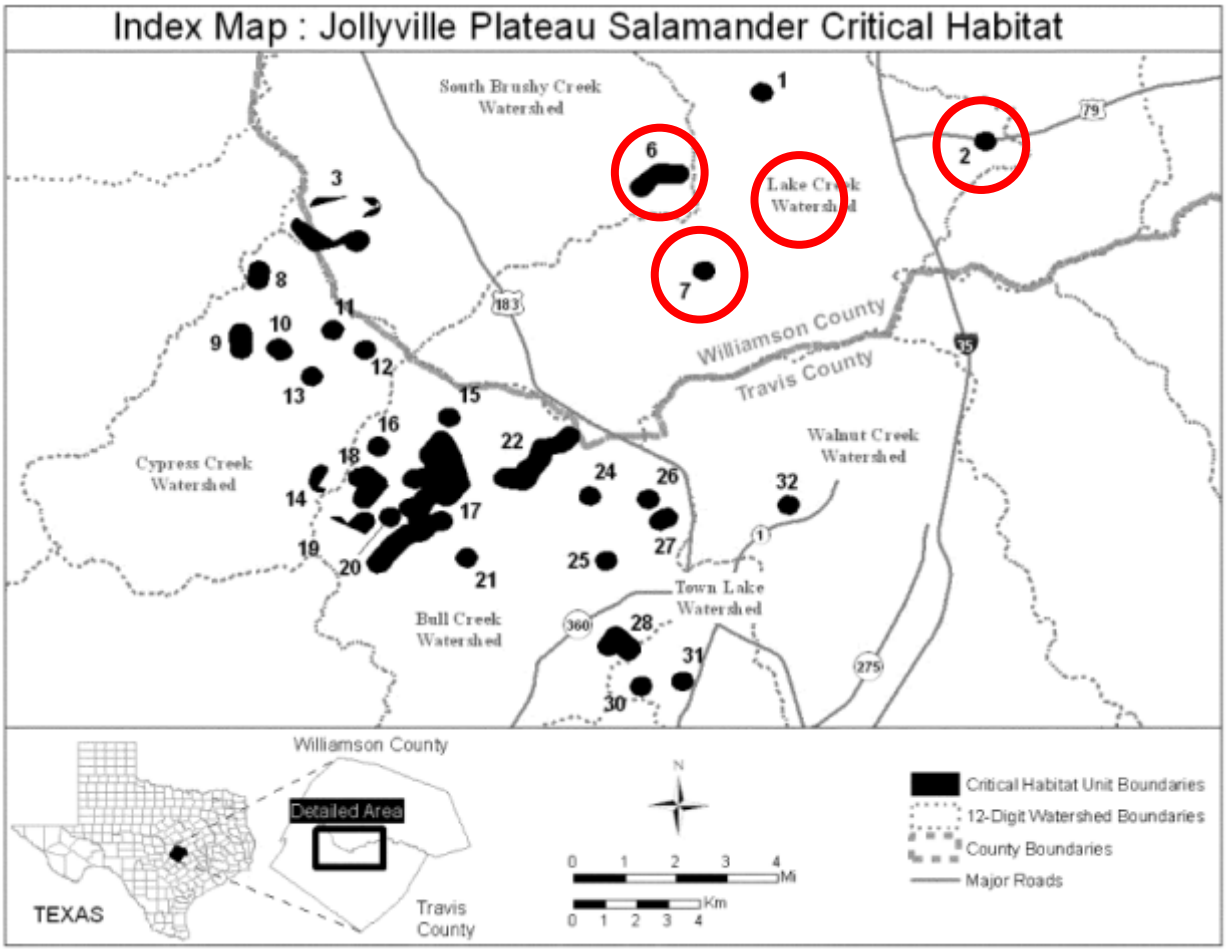


Figure 2. U.S. Fish and Wildlife Service designated critical habitat units for the Jollyville Plateau Salamander (*Eurycea tonkawae*) and five spring survey sites included in this study (red circles). Critical habitat unit map is reprinted from USFWS (2013b).

Figure 3. Example quadrats demonstrating common structure objects (i.e., cobble, vegetation, leaf litter, woody debris) used by *Eurycea tonkawae* as refugia.

Figure 4. Spring quadrat sample completely covered by aquatic vegetation, which is scooped into the salamander sieve, washed until the silt is removed, and then the remaining vegetative material is carefully removed to reveal a *Eurycea* salamander.

Figure 5. Salamander rake which is used by combing through and scooping vegetation and any inhabitants into the catchment, washed until the silt is removed, and then the remaining vegetative material is carefully removed to reveal a *Eurycea* salamander(s).

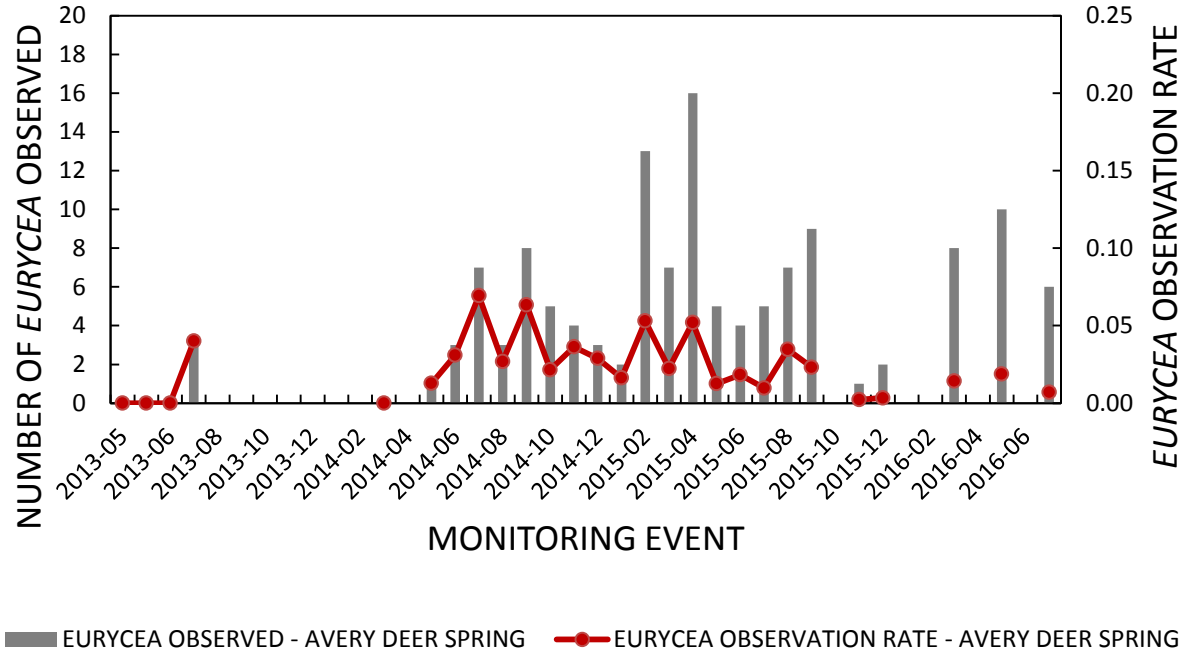


Figure 6. Total number of *Eurycea tonkawae* observed and the observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at Avery Deer Spring.

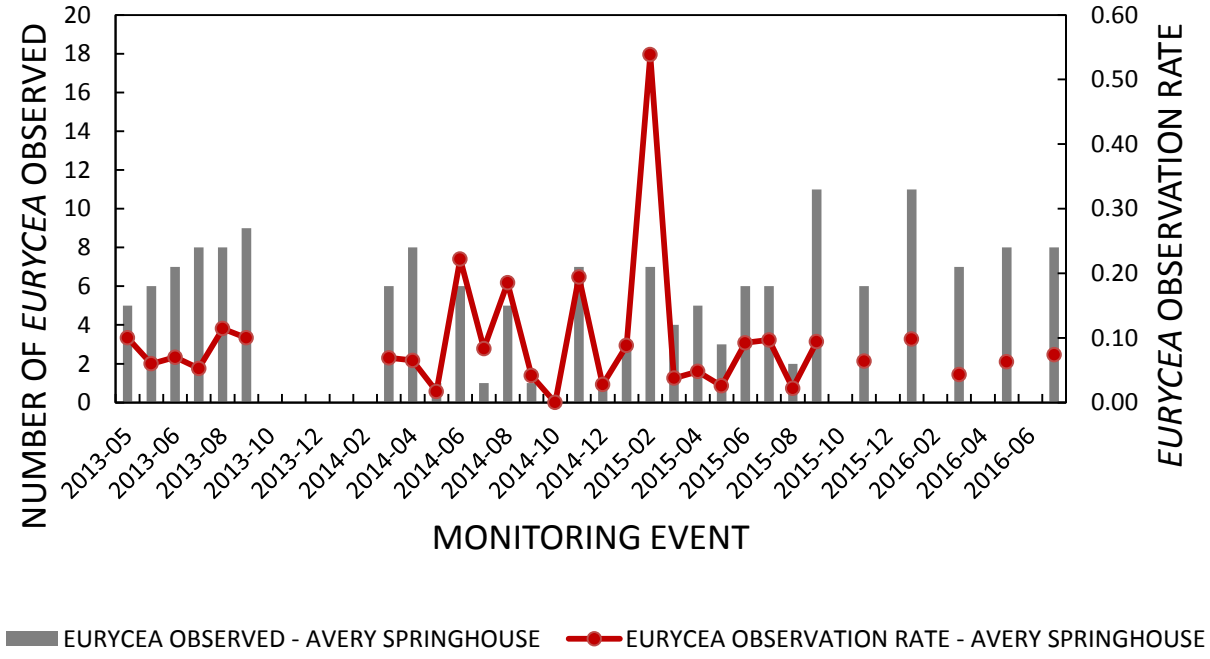


Figure 7. Total number of *Eurycea tonkawae* observed and the observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at Avery Springhouse Spring.

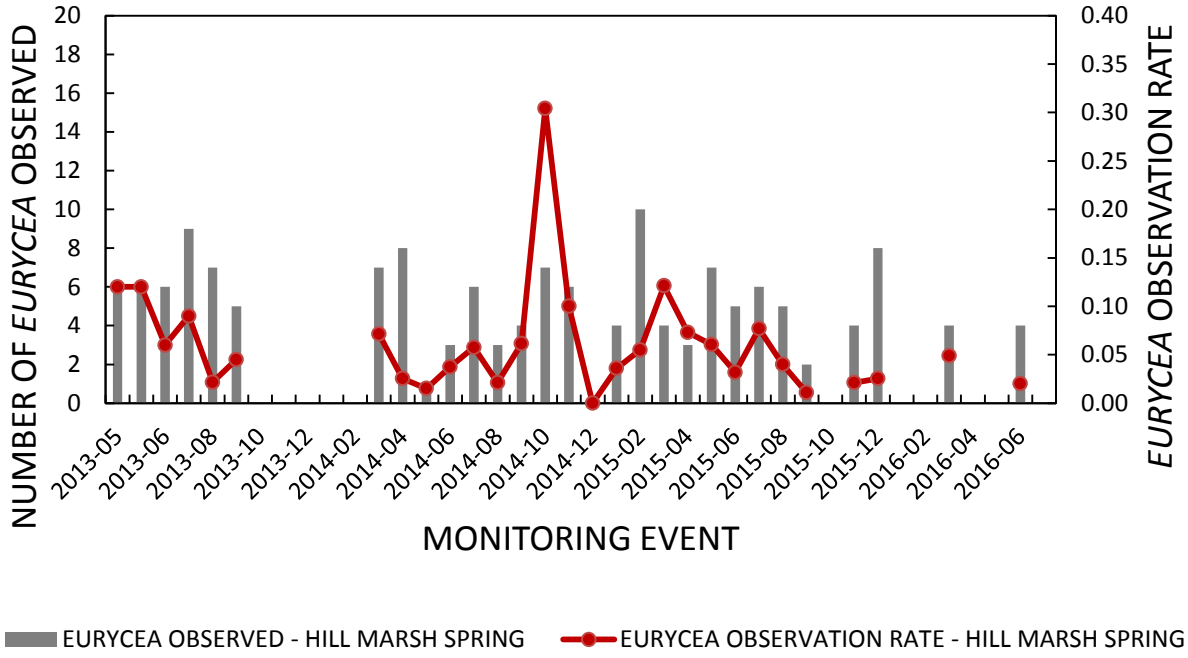


Figure 8. Total number of *Eurycea tonkawae* observed and the observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at Hill Marsh Spring.

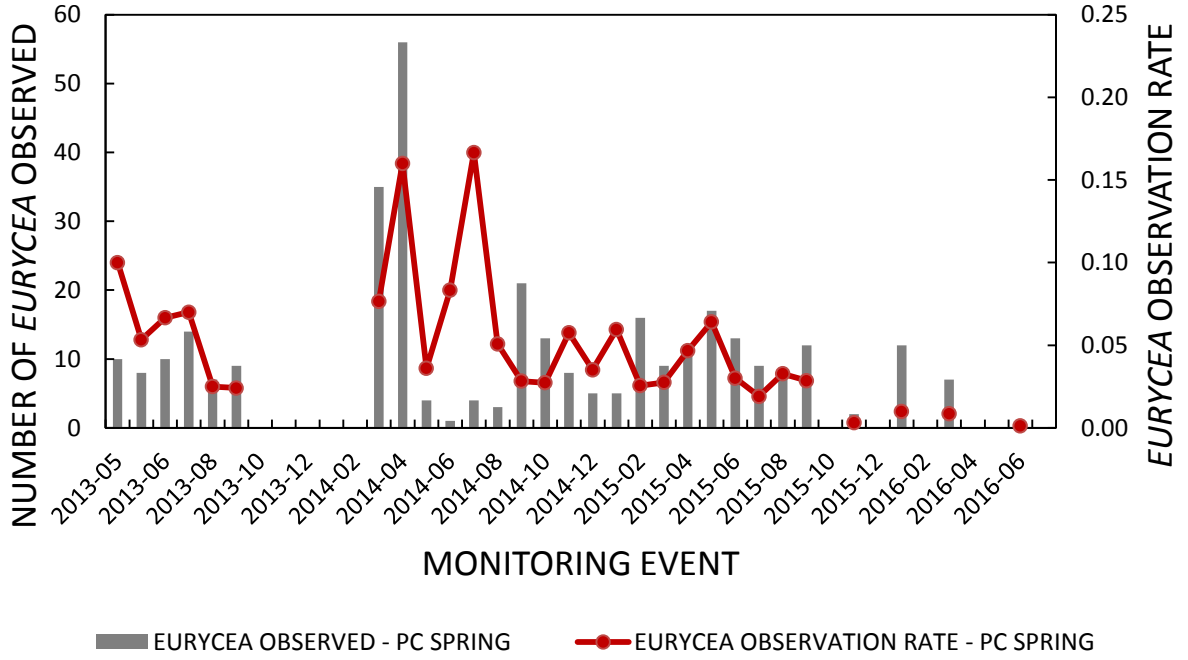


Figure 9. Total number of *Eurycea tonkawae* observed and the observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at PC Spring.

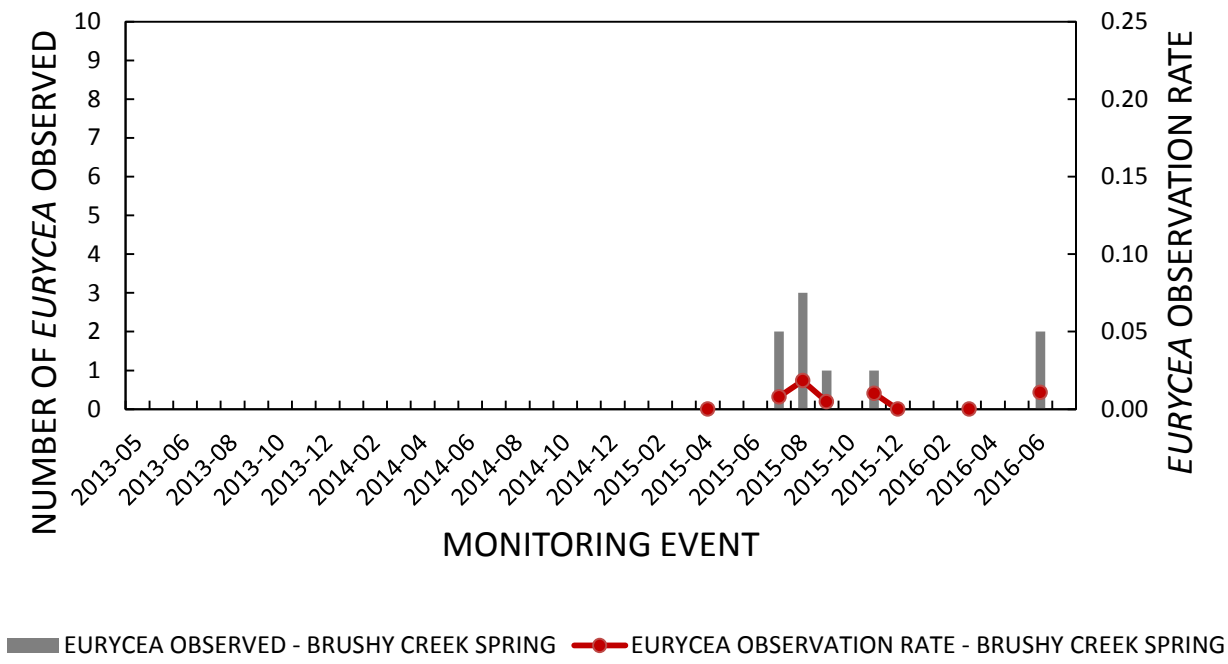


Figure 10. Total number of *Eurycea tonkawae* observed and the observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at Brushy Creek Spring.

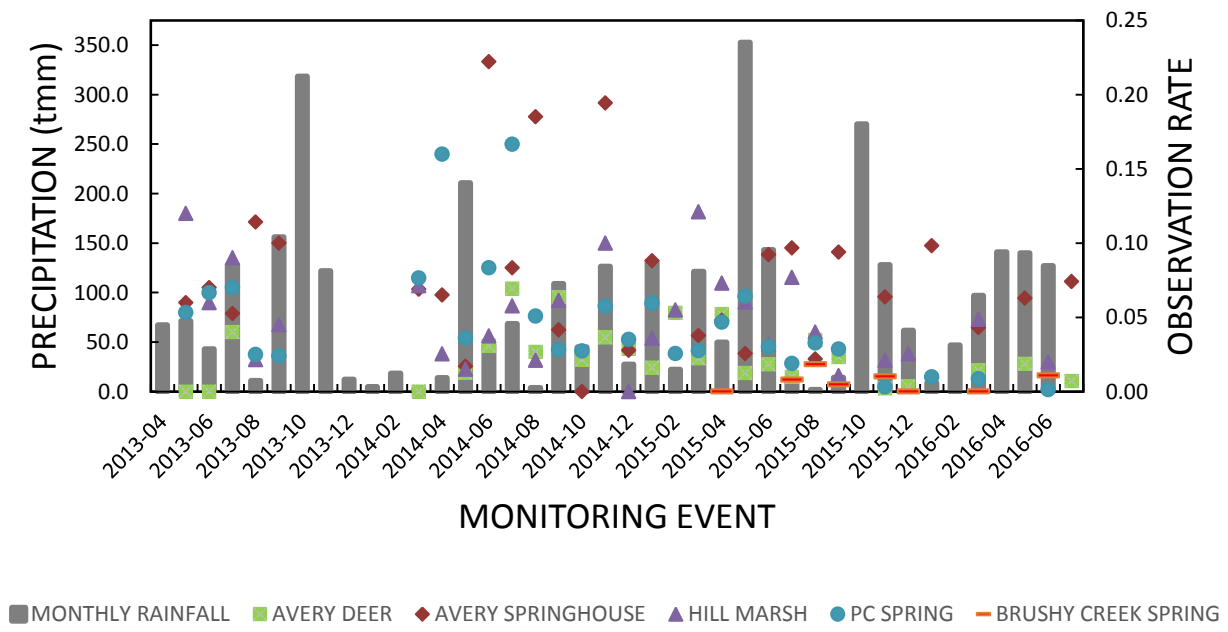


Figure 11. Monthly precipitation and *Eurycea tonkawae* e observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge point at all monitored springs. Not depicted are observations rate of 0.54 at Avery Springhouse on 2015-02 and 0.30 at PC Spring on 2014-10.

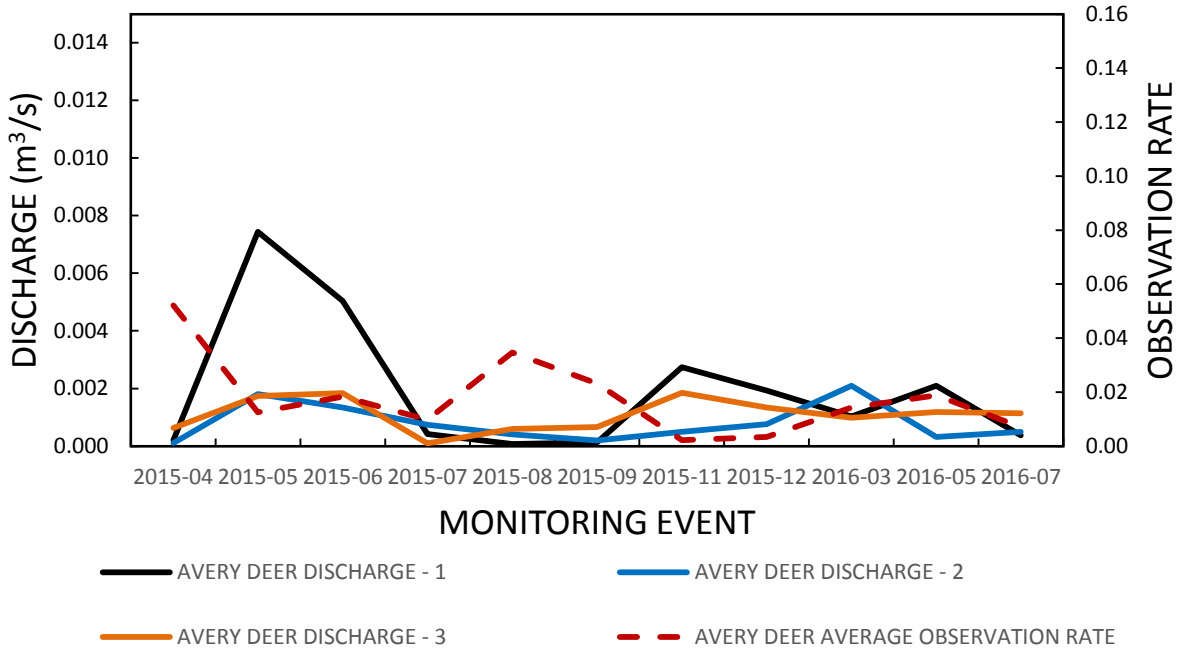


Figure 12. Spring discharge measurements at each primary discharge point and *Eurycea tonkawae* observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge points at Avery Deer Spring.

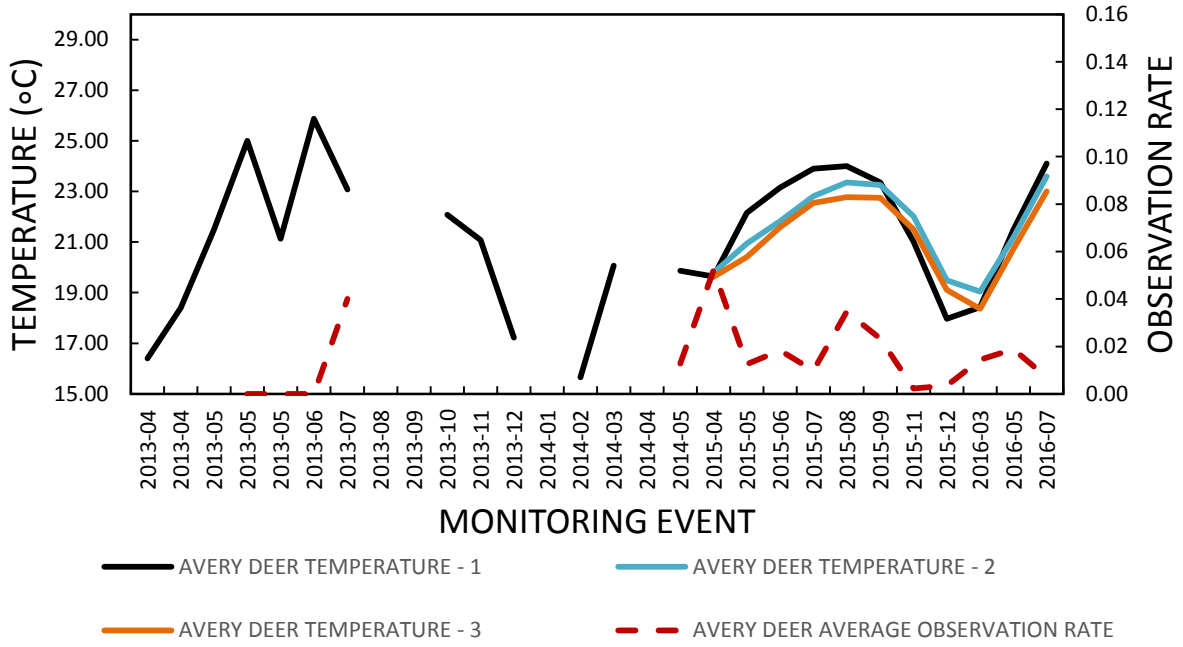


Figure 13. Spring discharge temperature at each primary discharge point and *Eurycea tonkawae* observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge points at Avery Deer Spring.

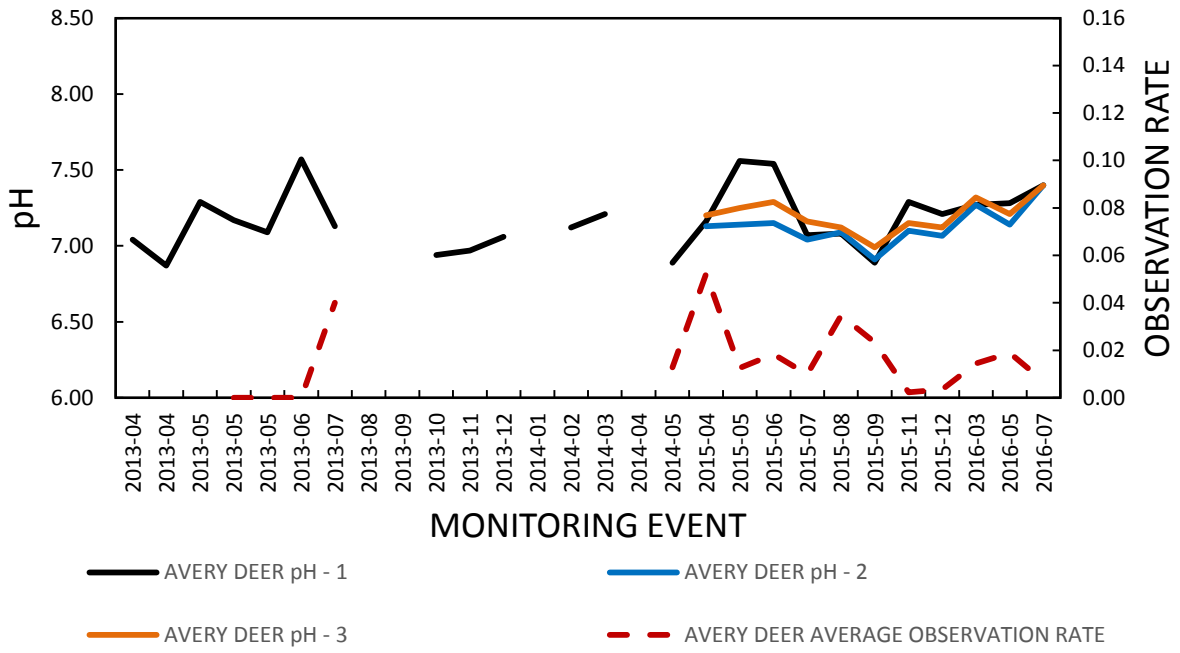


Figure 14. Spring discharge pH at each primary discharge point and *Eurycea tonkawae* observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge points at Avery Deer Spring.

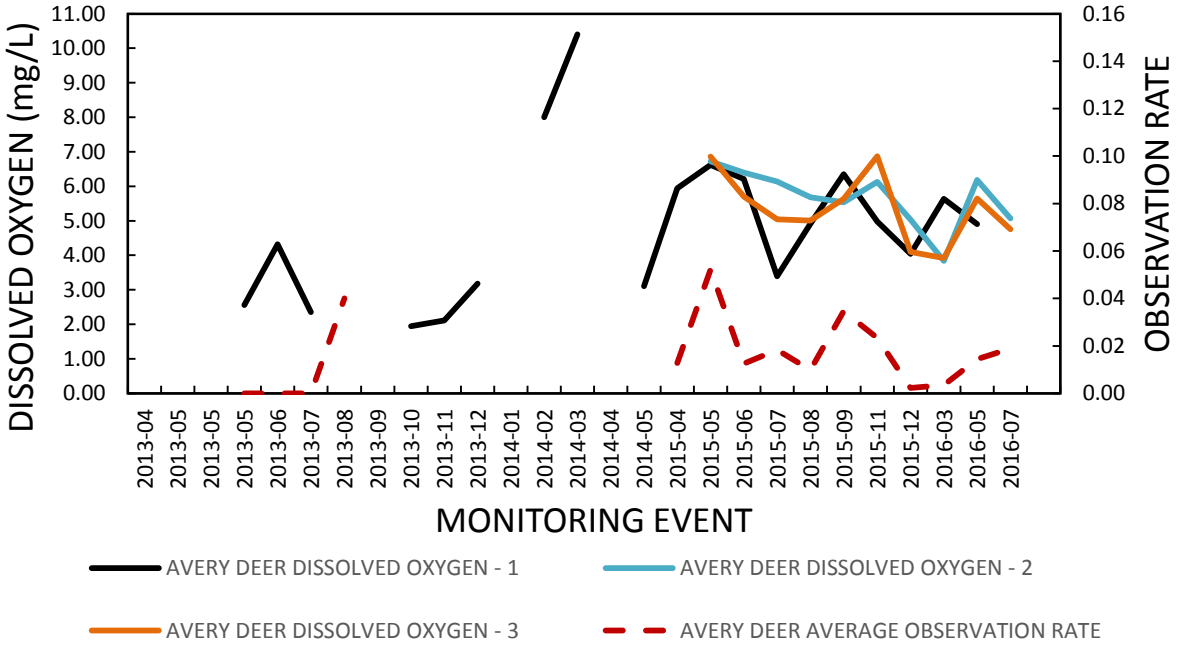
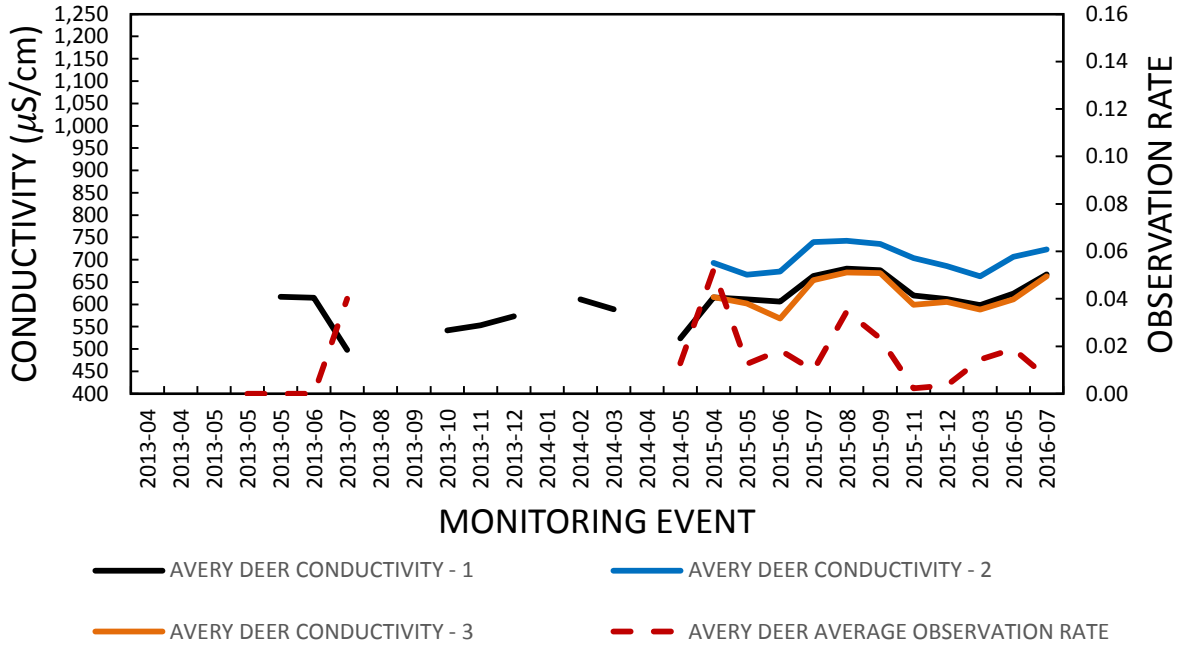


Figure 15. Spring discharge dissolved oxygen at each primary discharge point and *Eurycea tonkawae* observation rate (number of salamanders per structure object searched) during visual encounter surveys within 25m of the primary spring discharge points at Avery Deer Spring.



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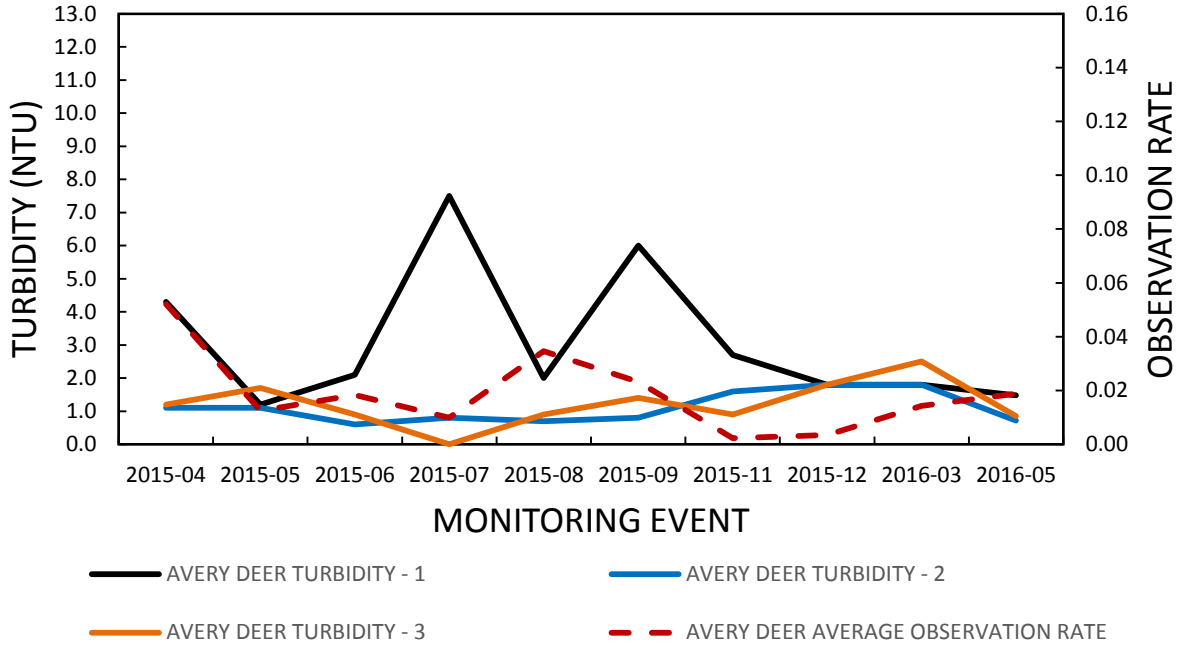

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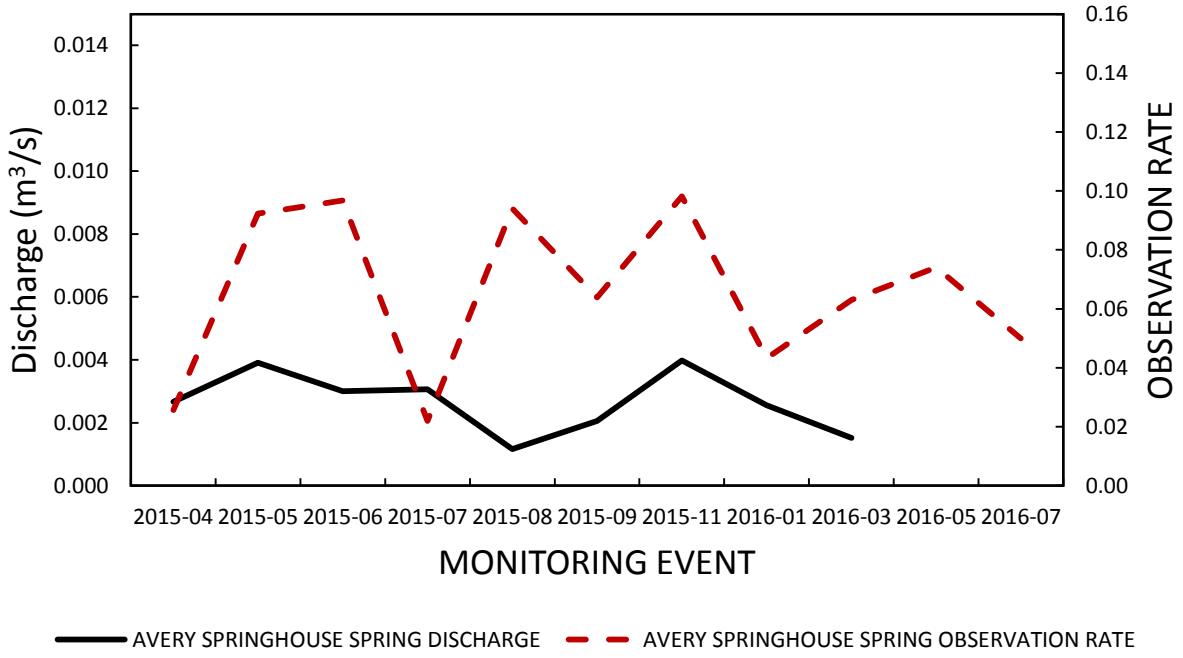

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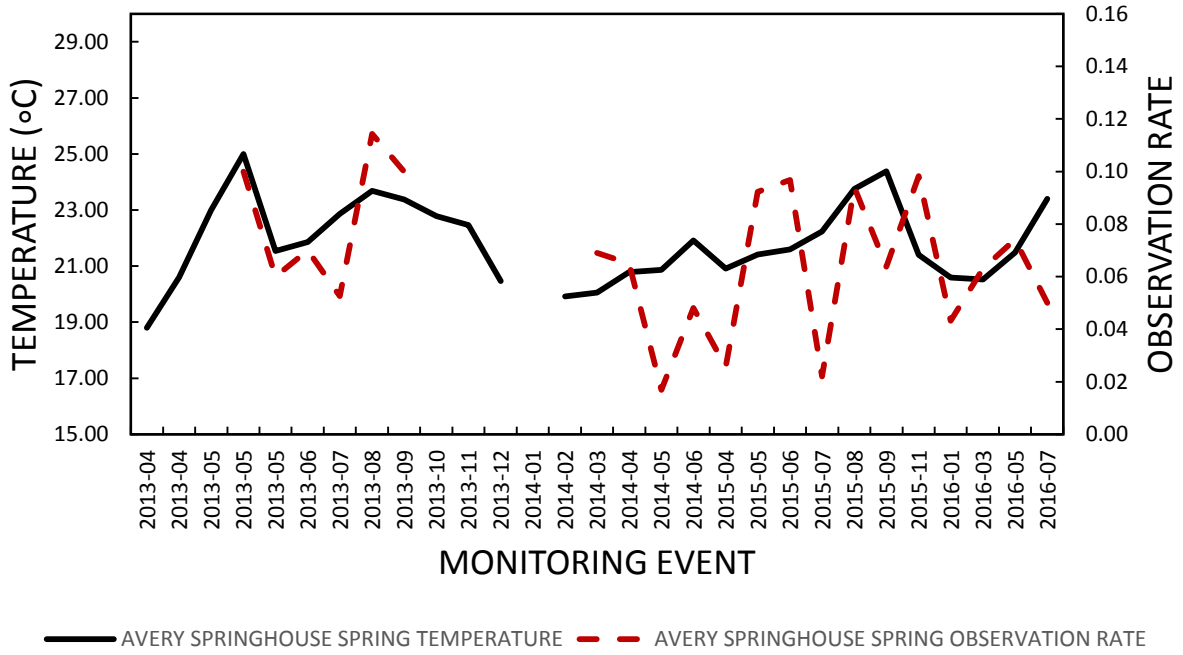

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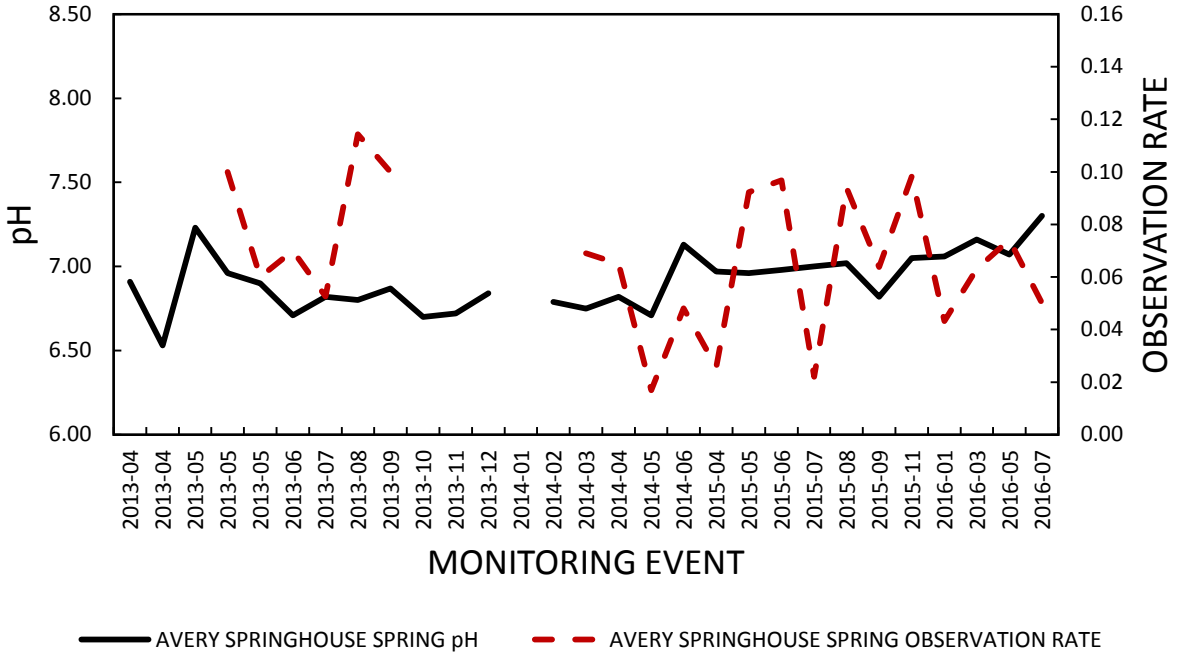

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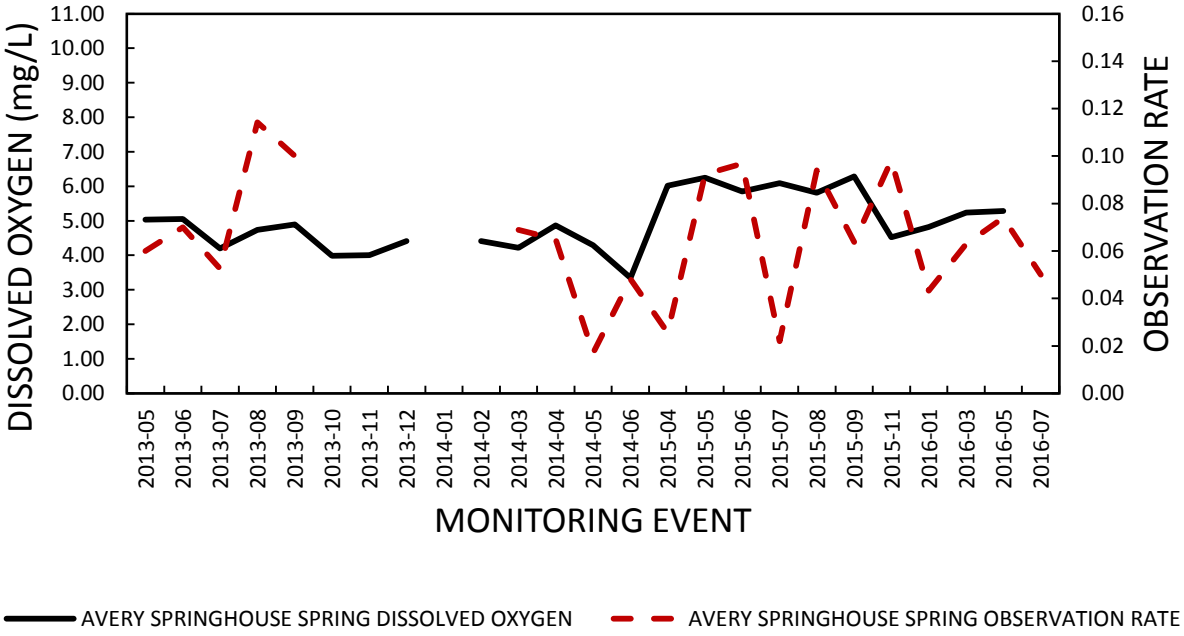

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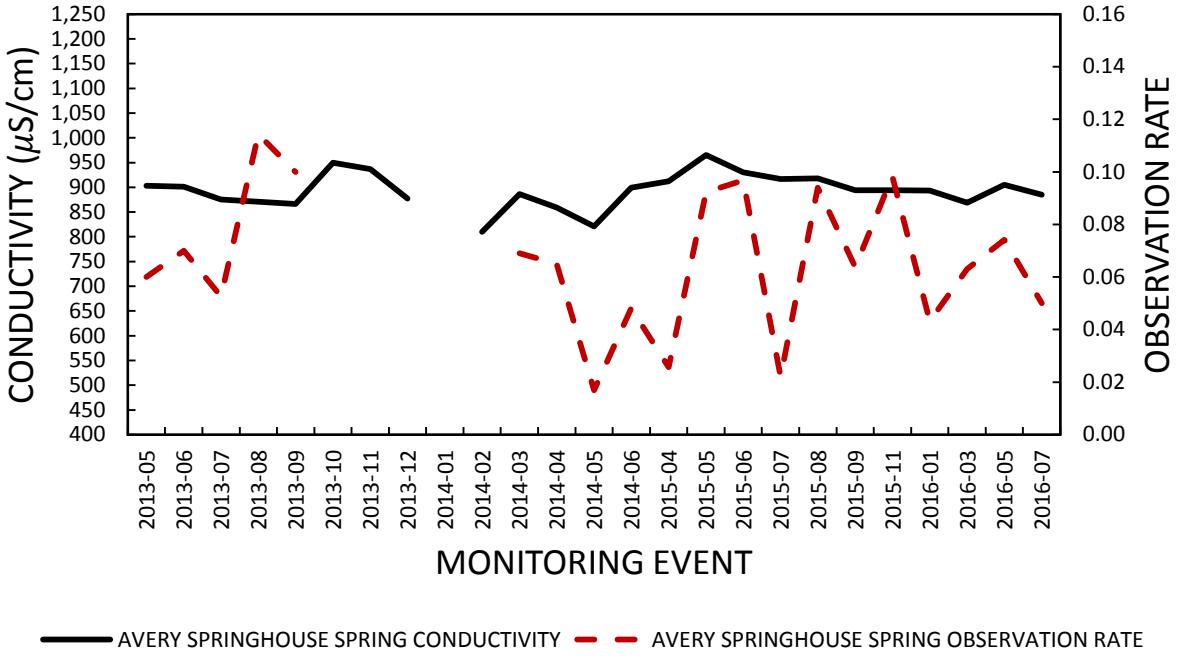

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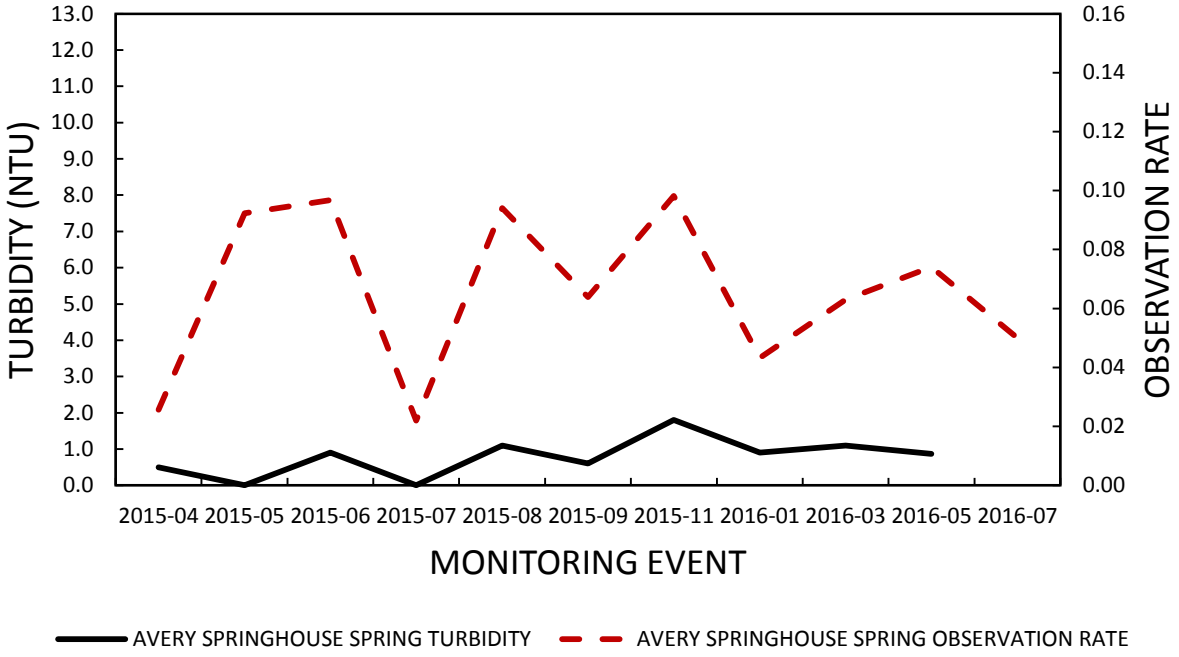

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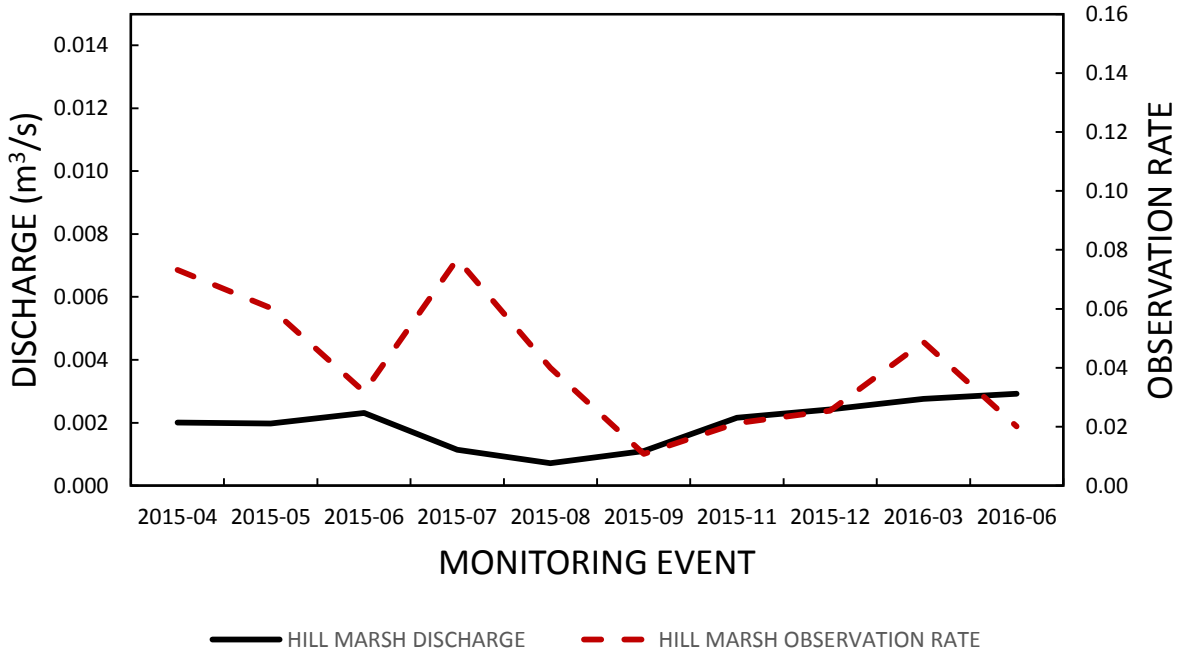

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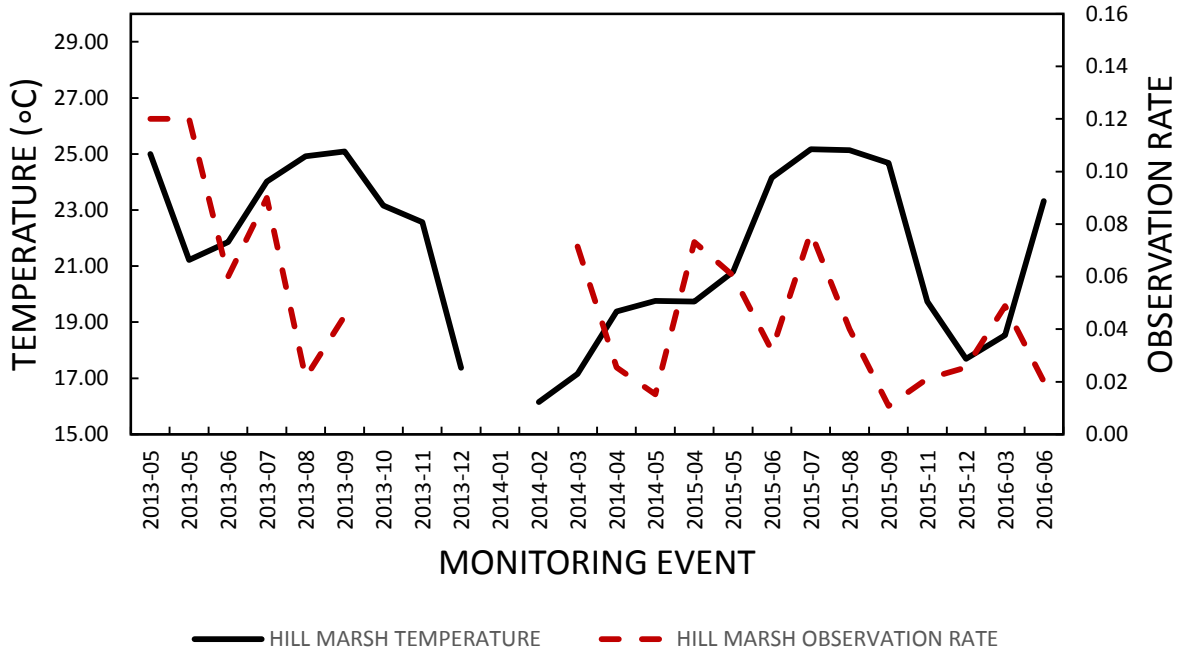

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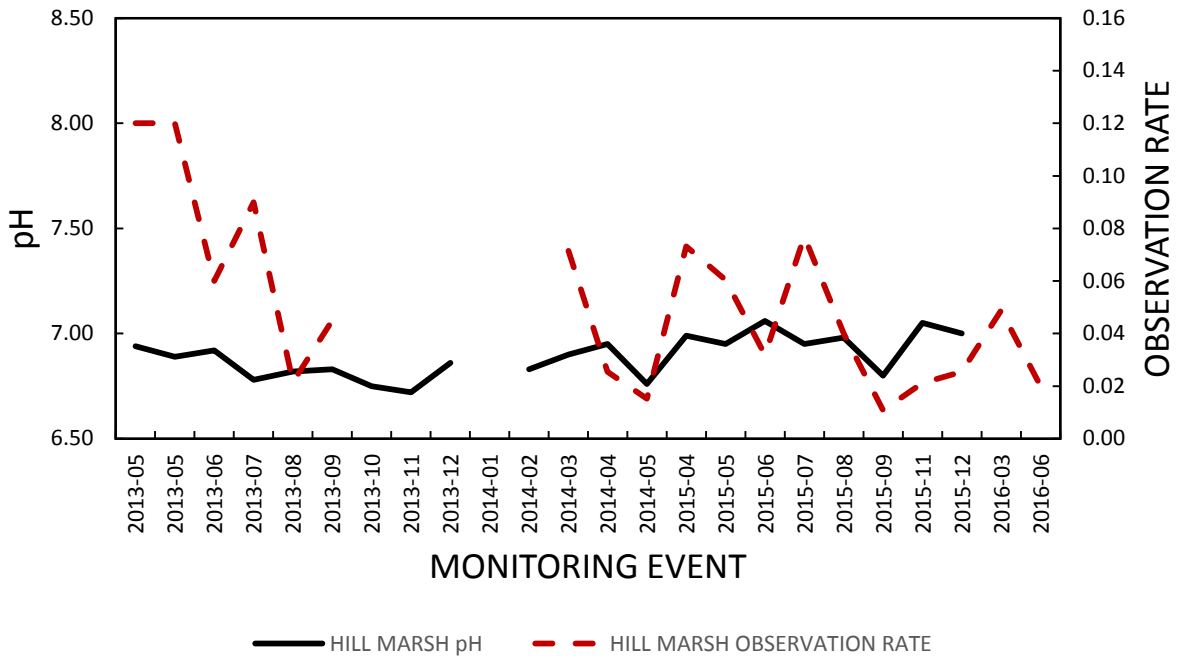

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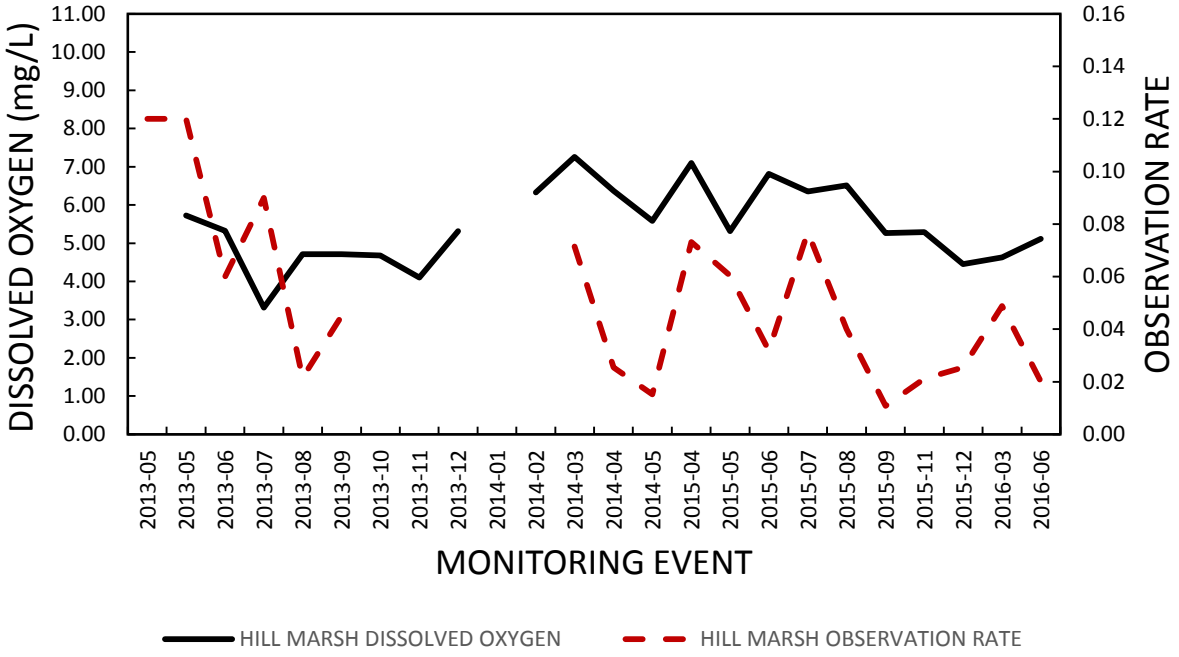

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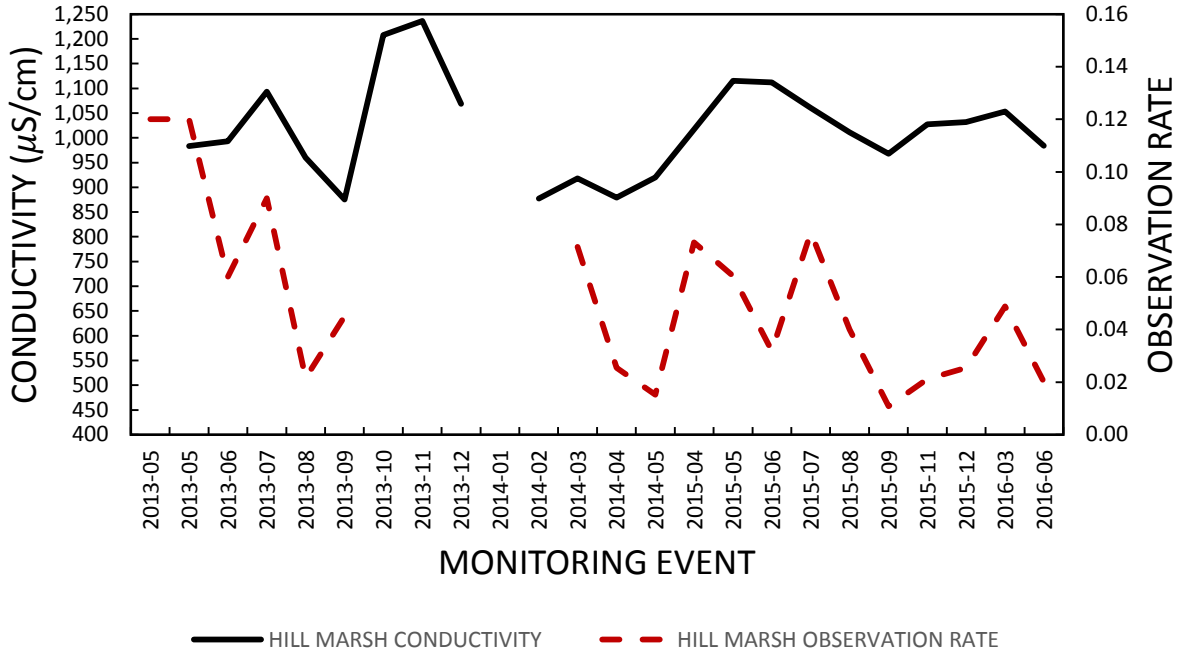

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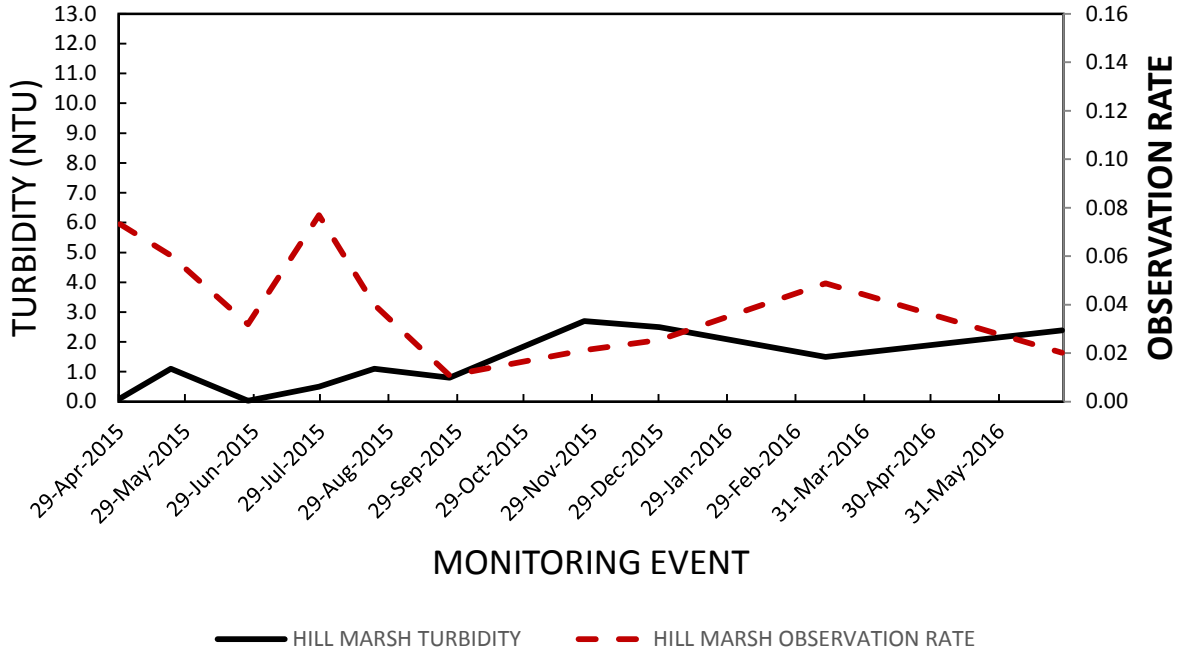

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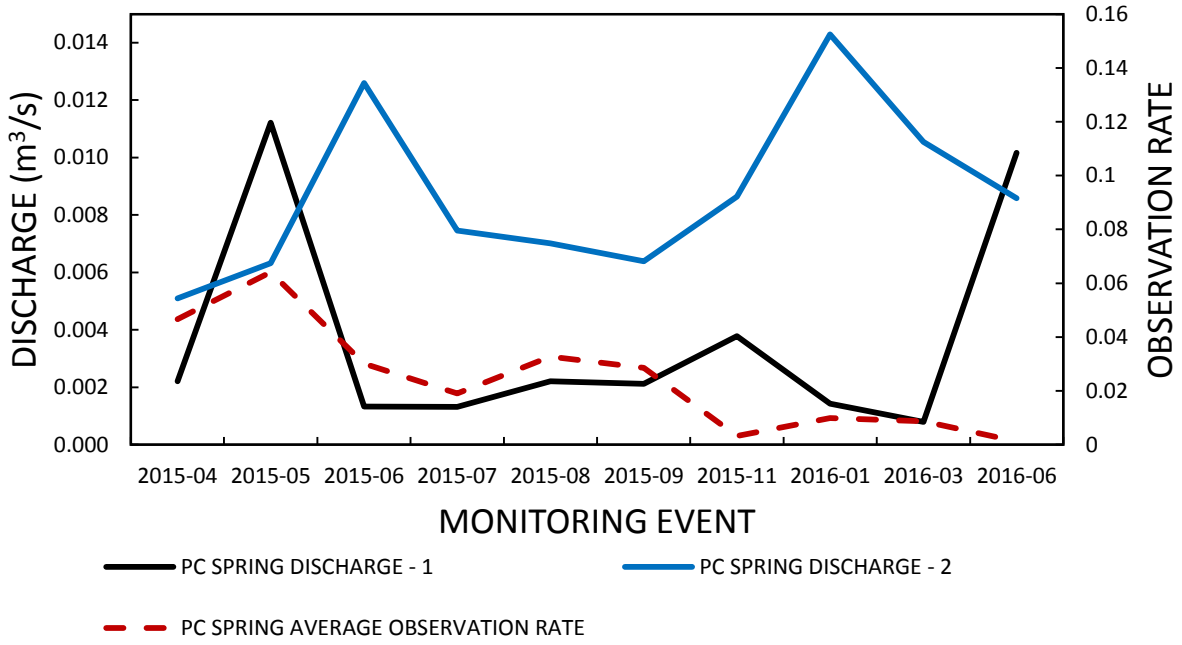

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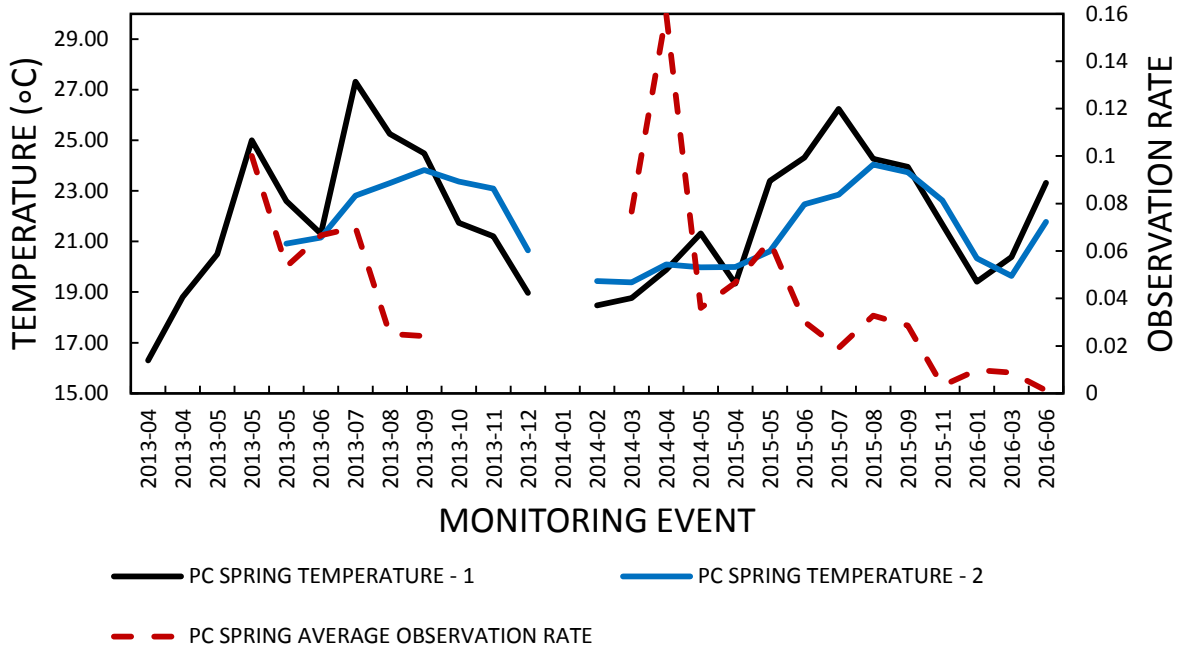

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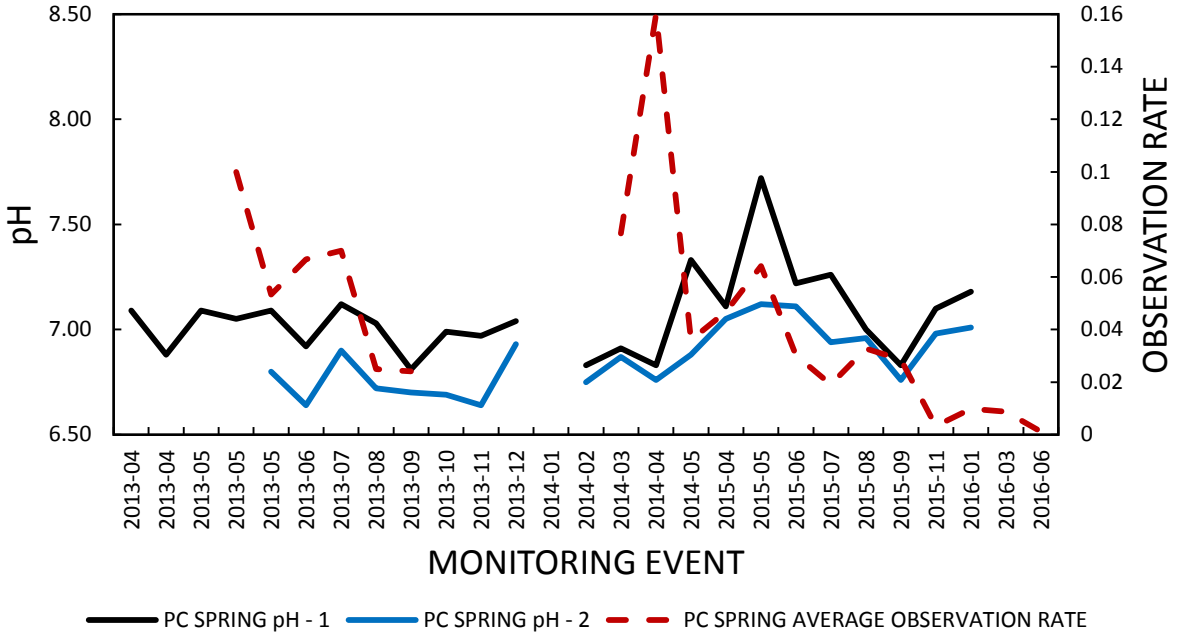

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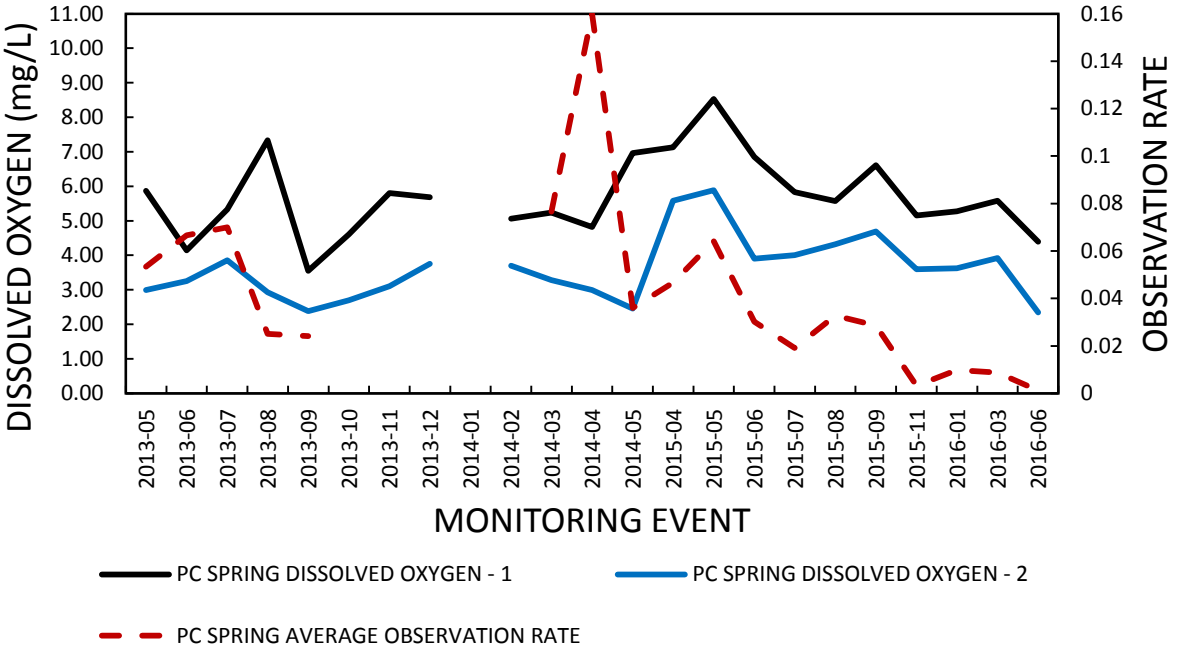

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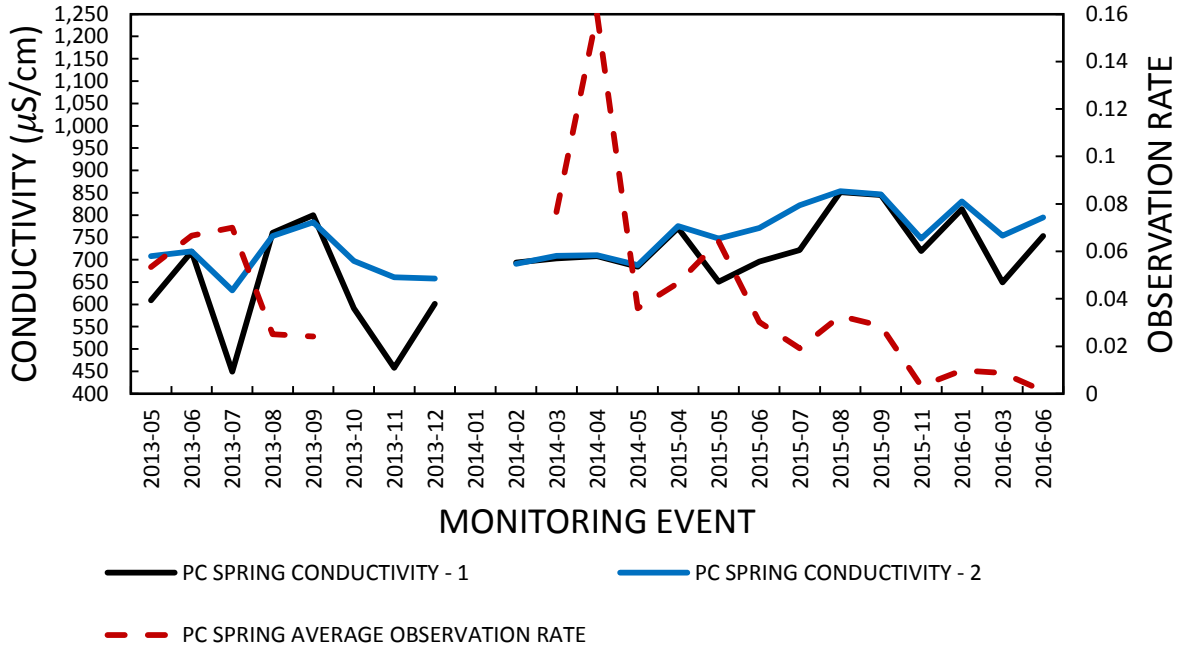

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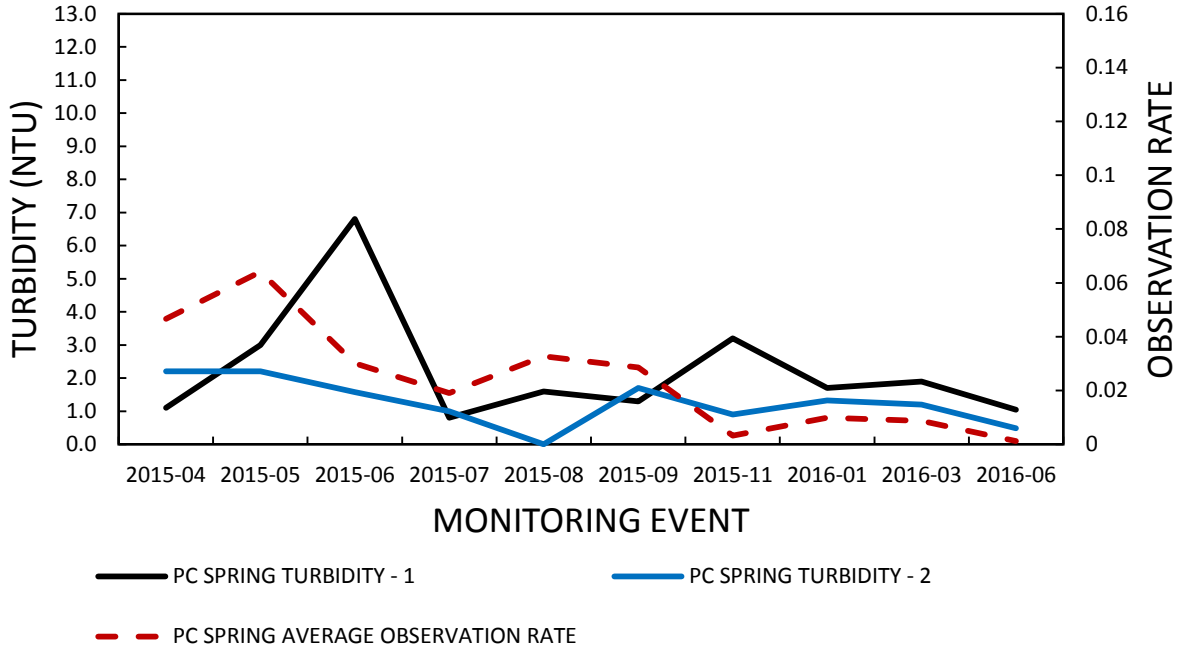

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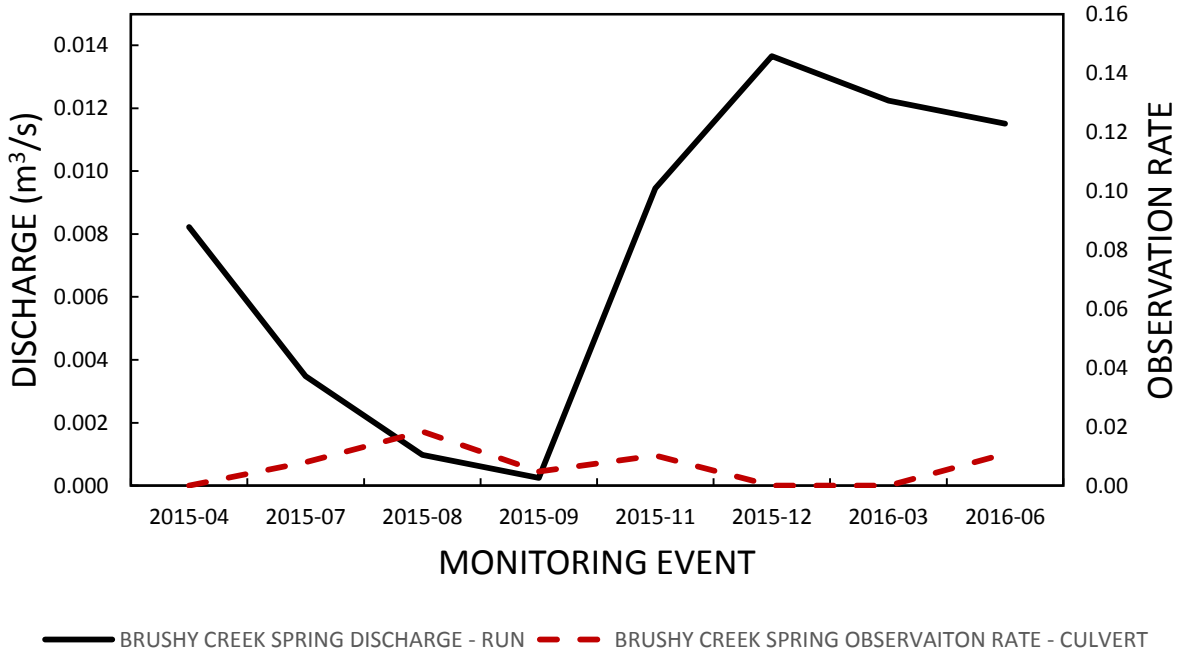

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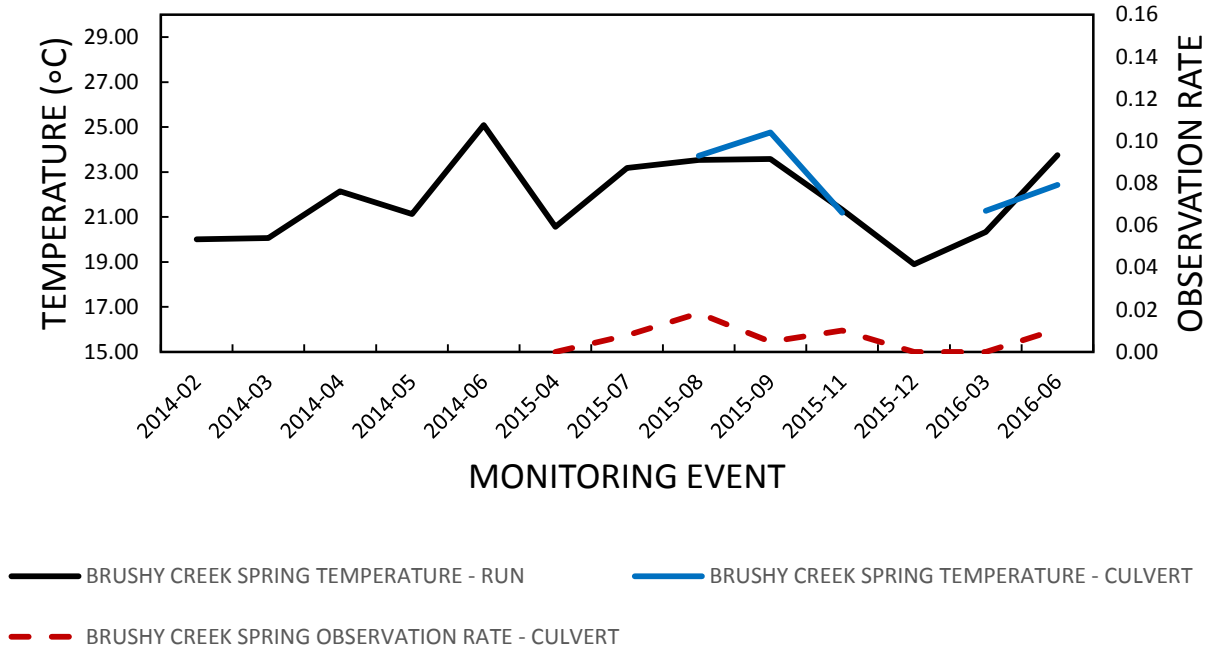

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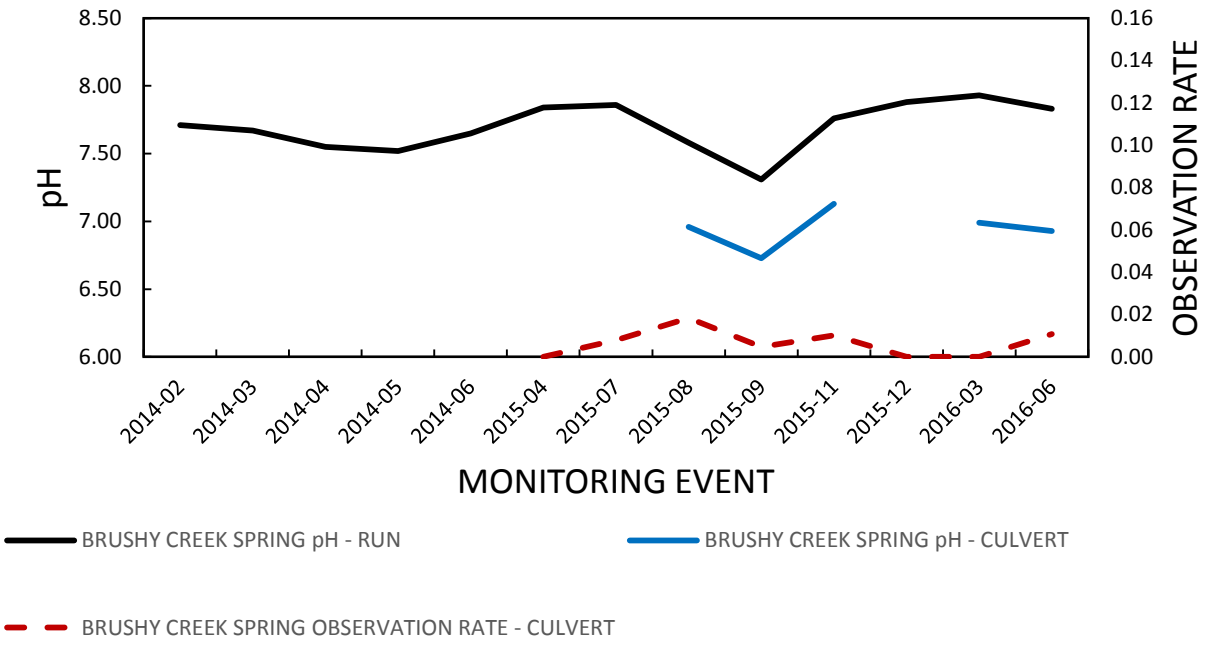

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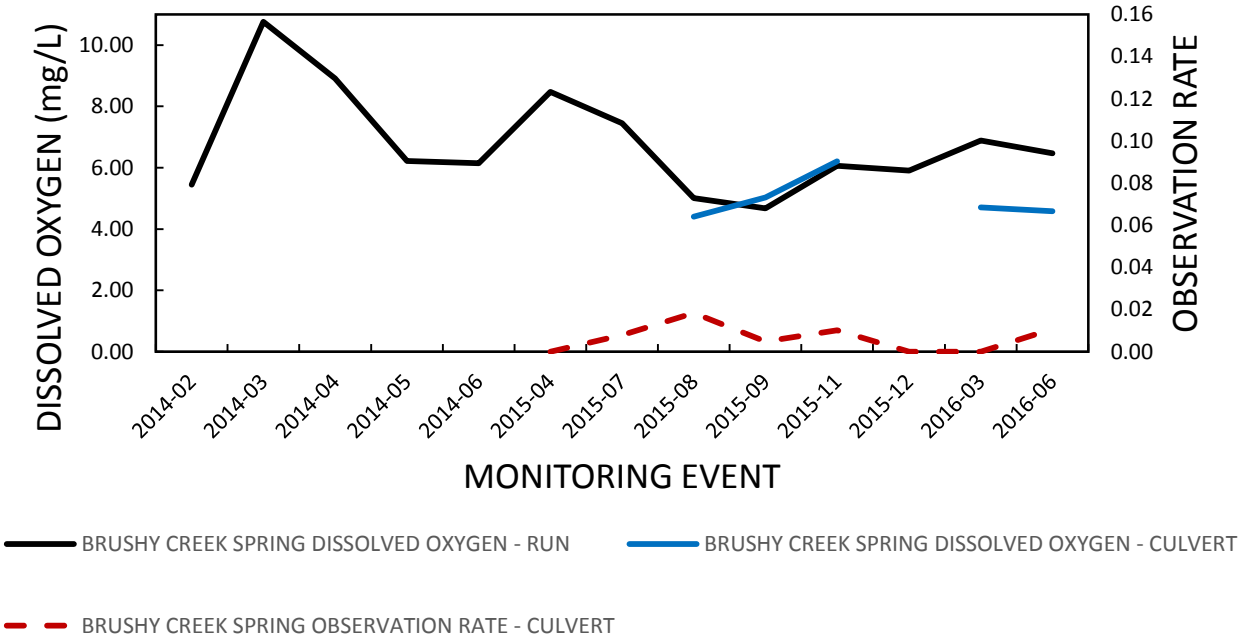

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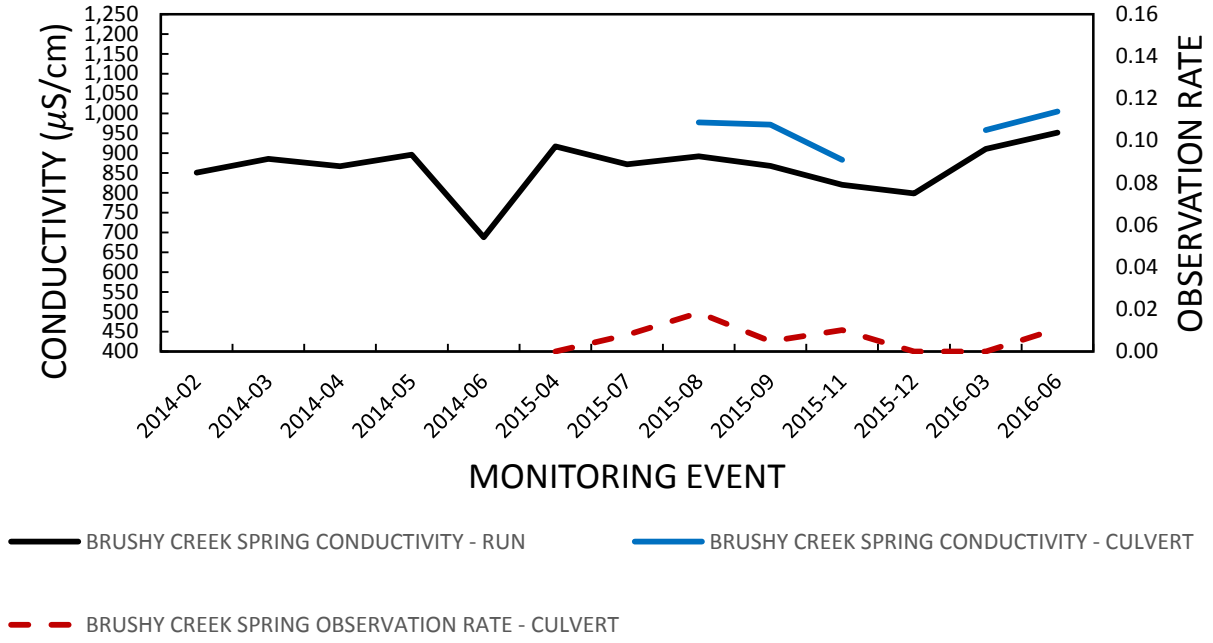

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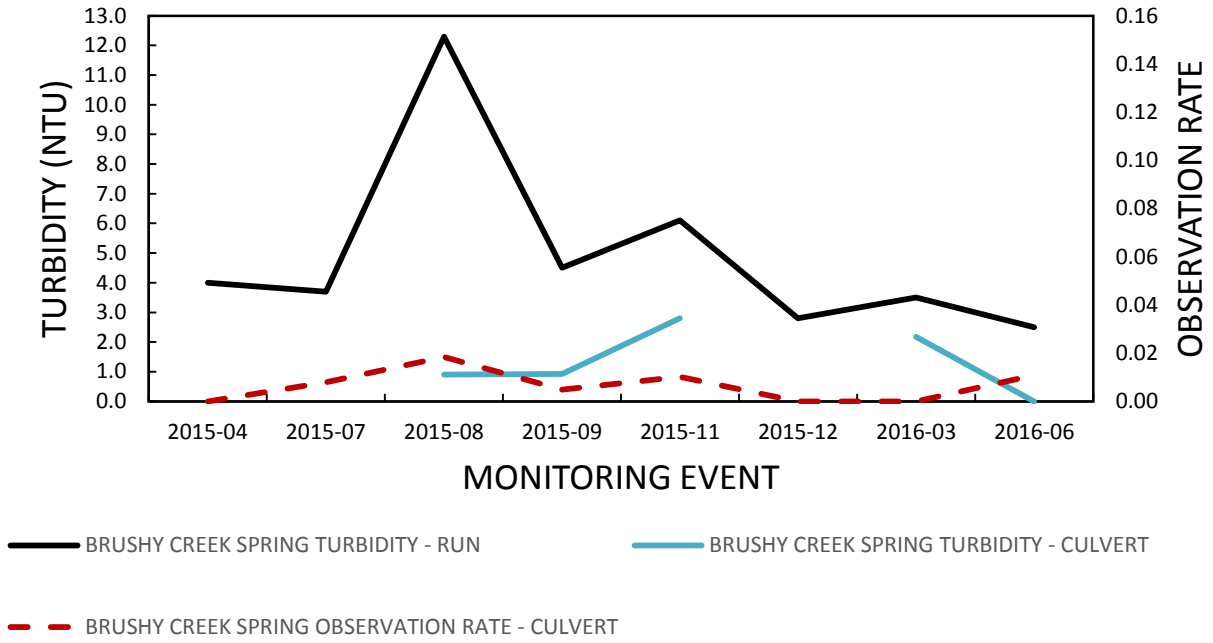

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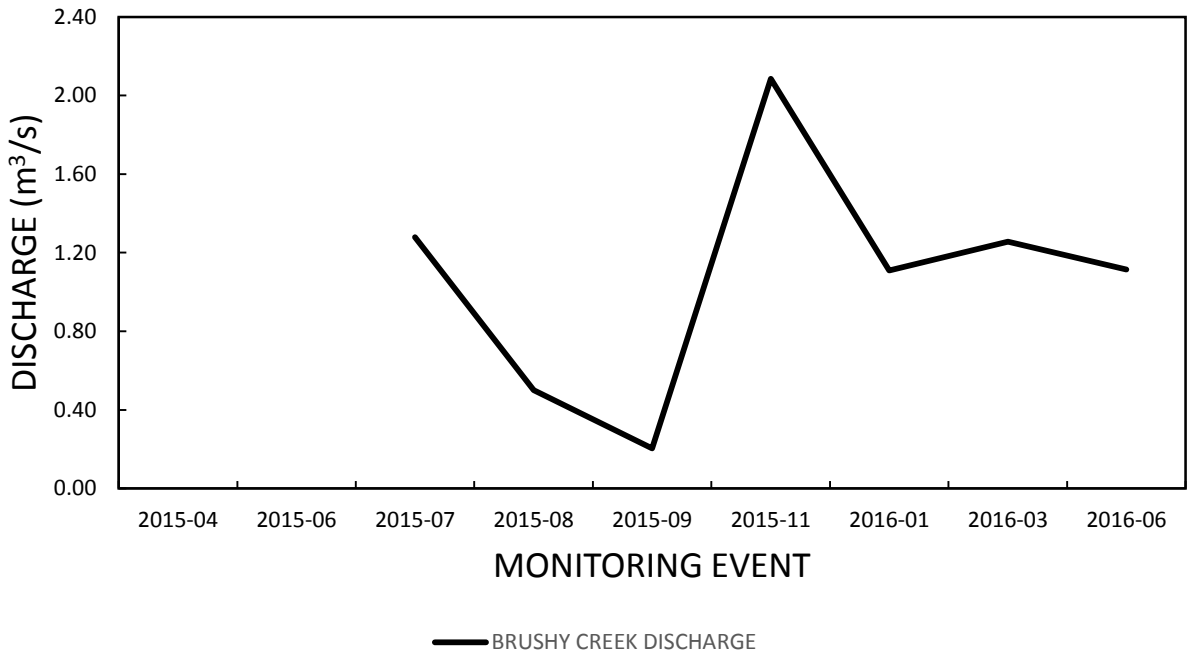

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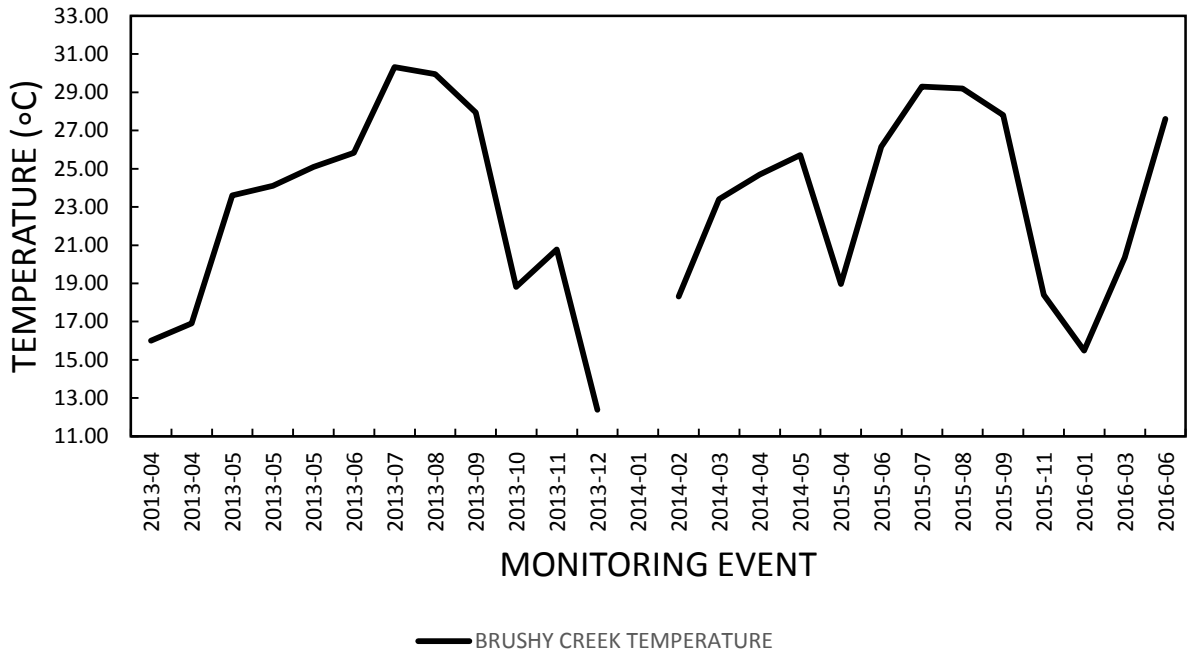

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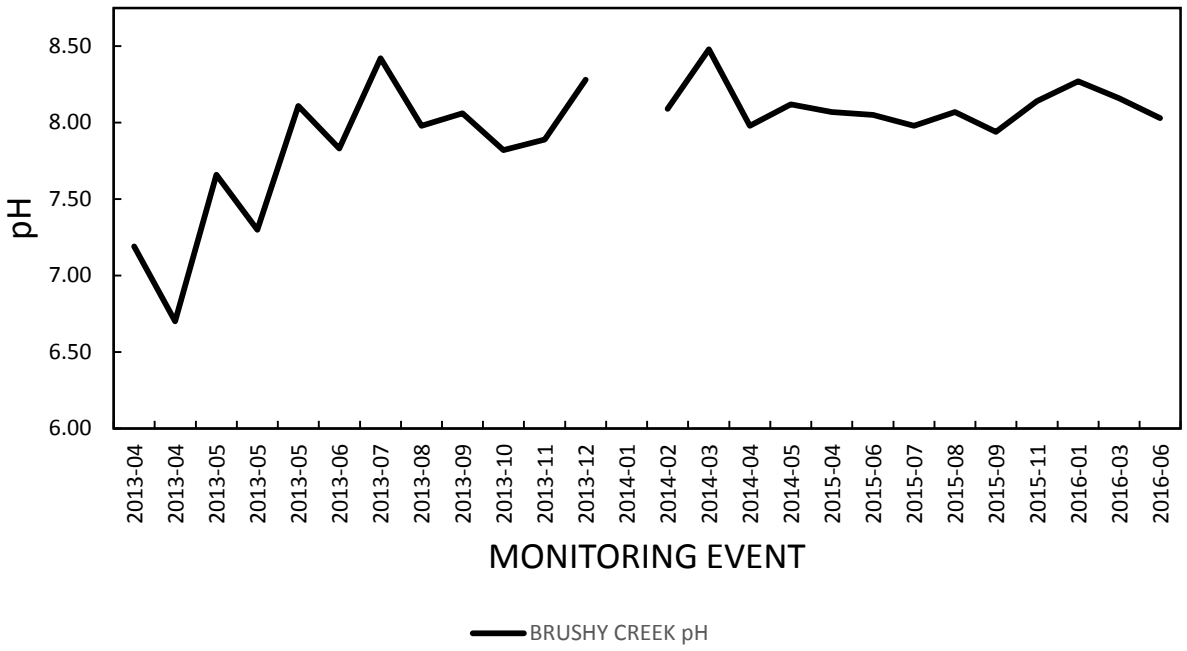

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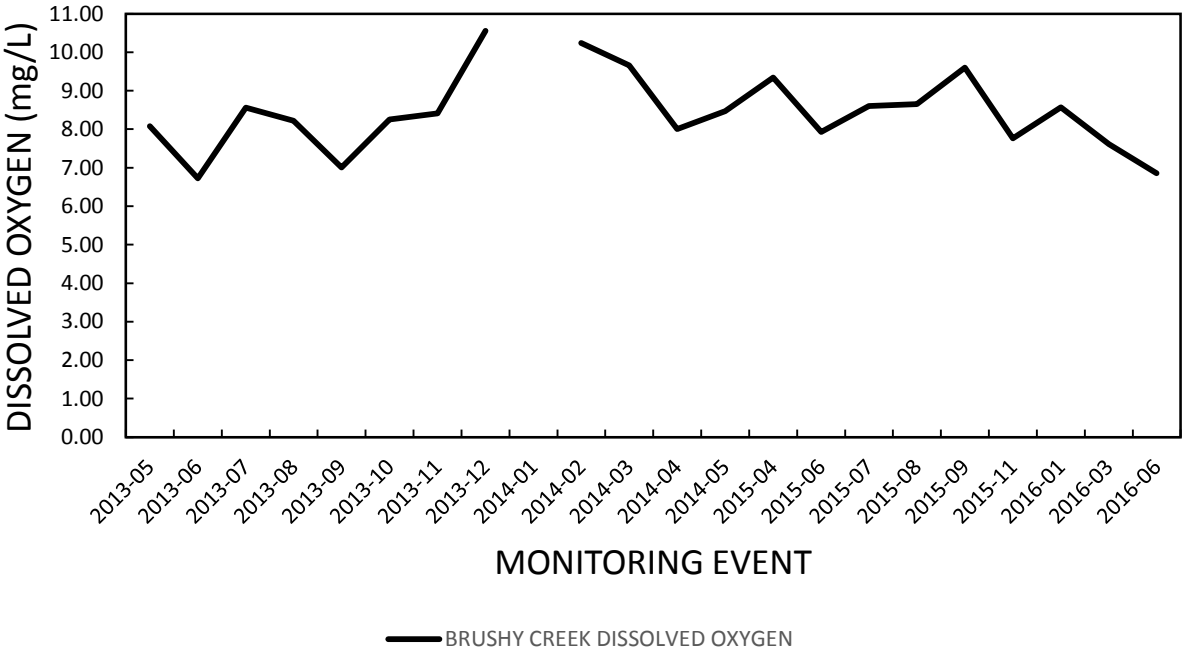

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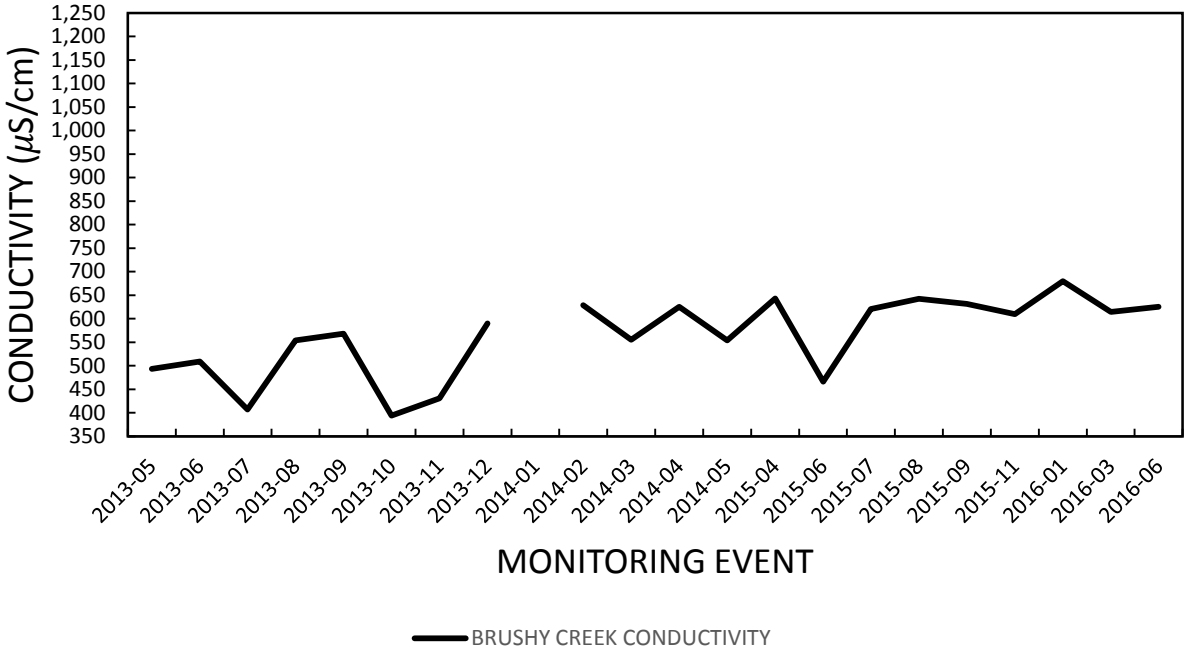

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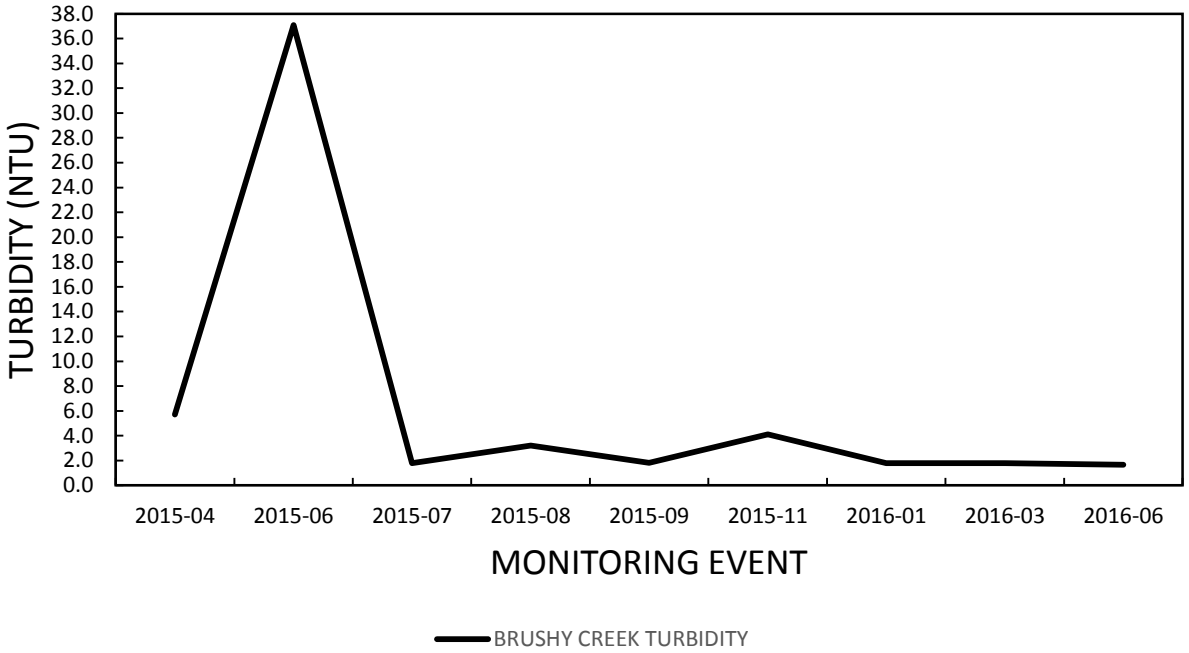

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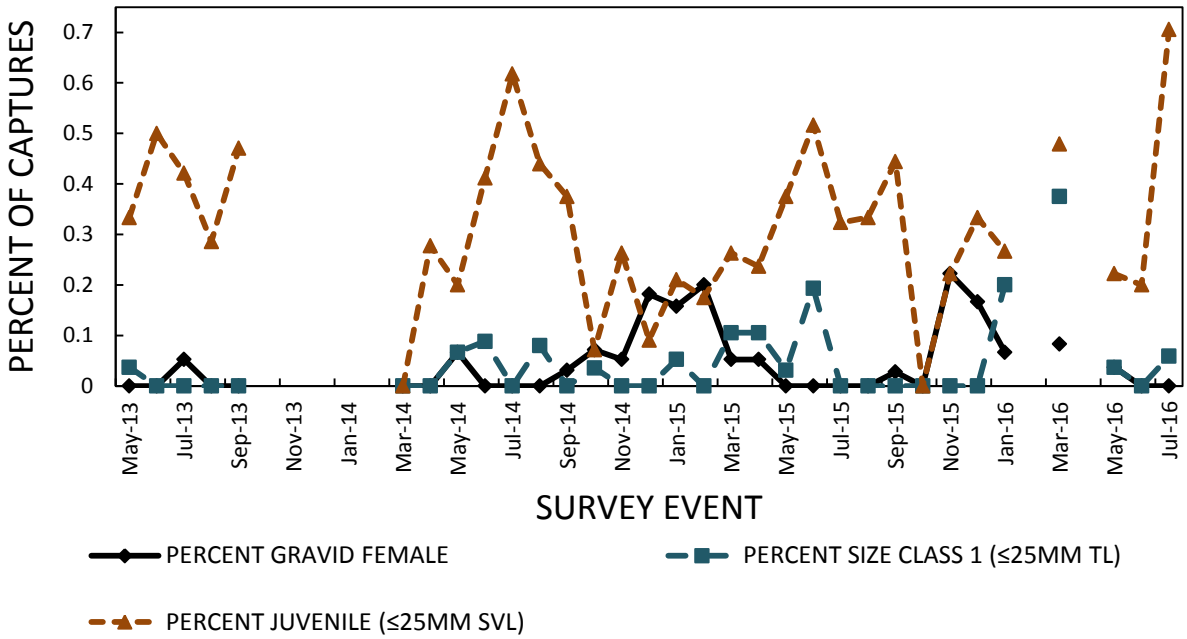

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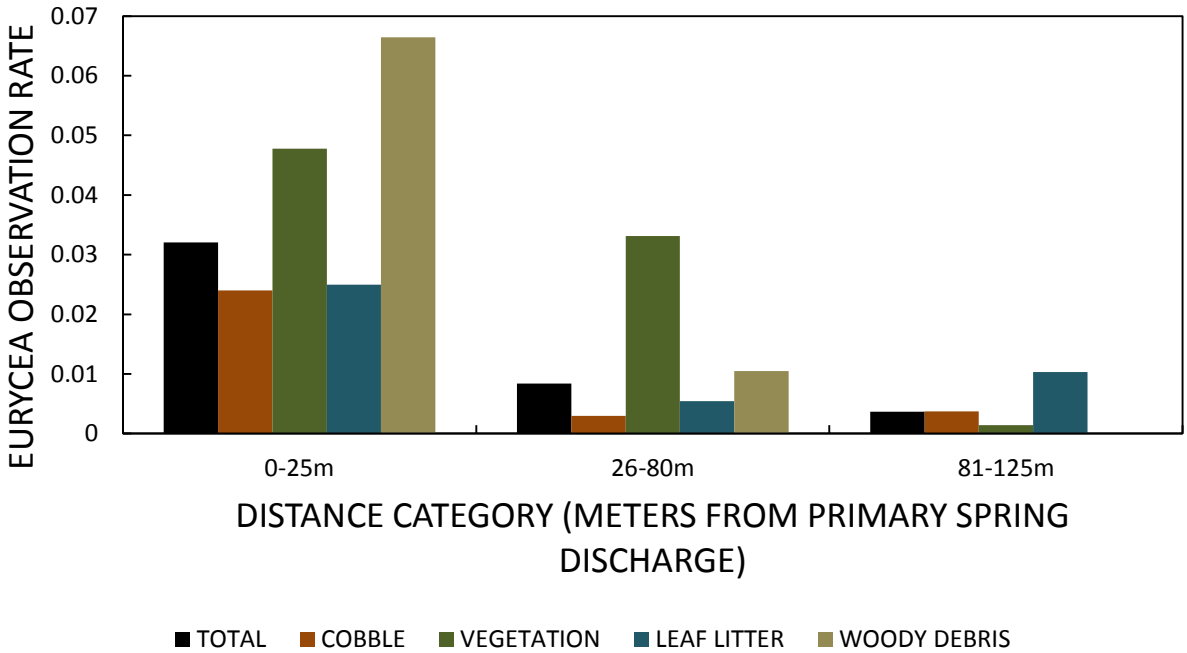

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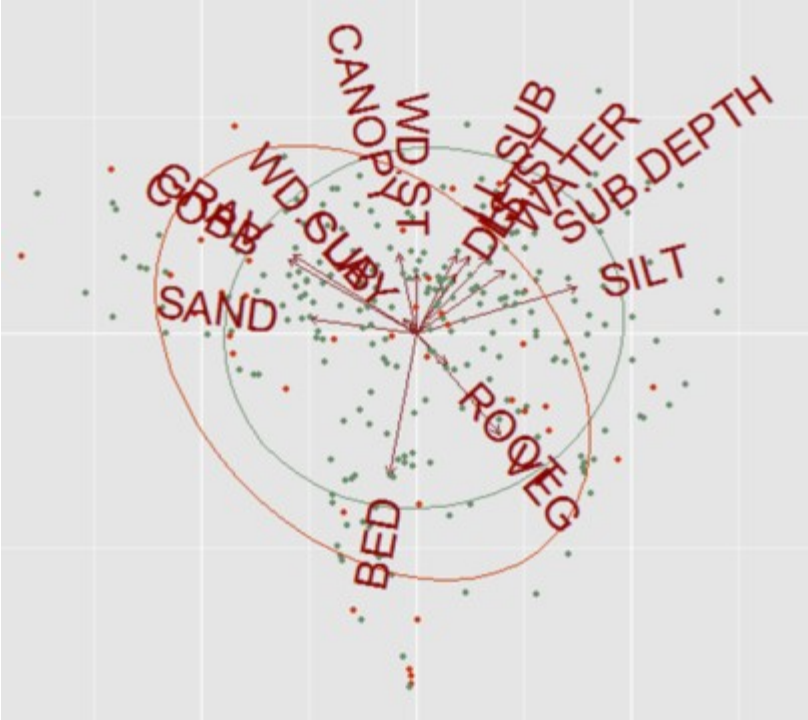

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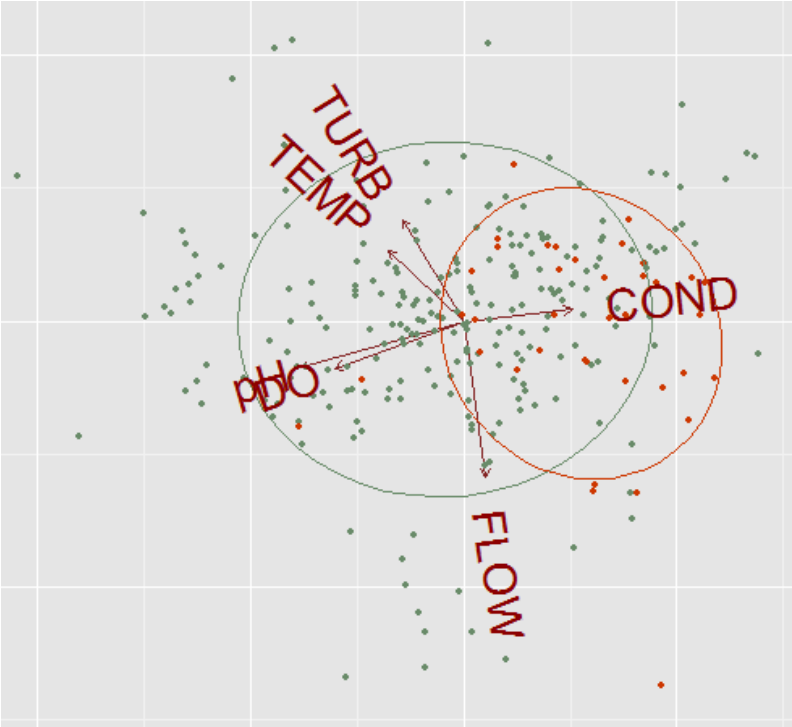

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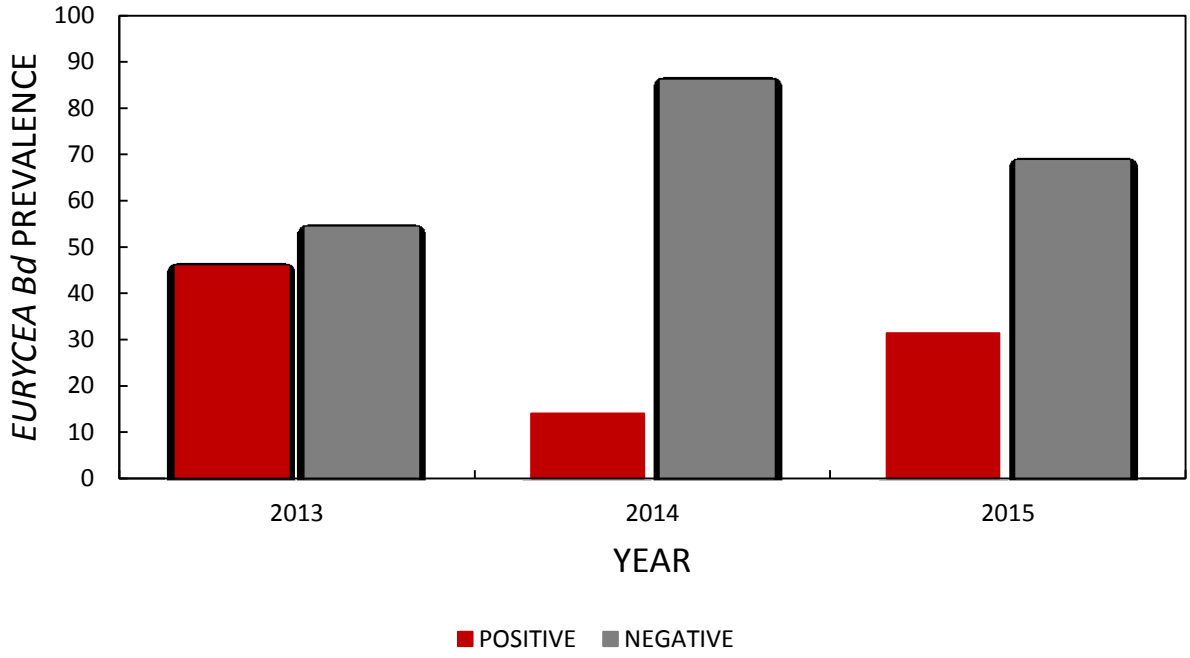

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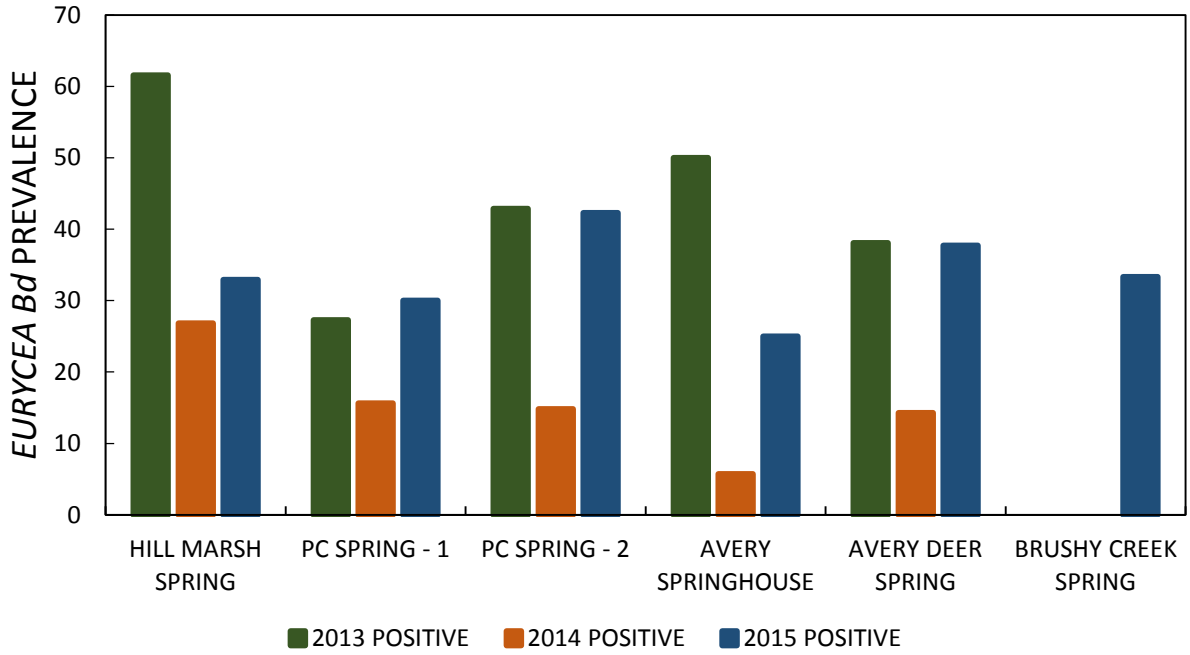

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EFFECTS OF TEMPERATURE AND NITROGENOUS WASTES ON SURVIVAL AND GROWTH OF THE BARTON SPRINGS SALAMANDER *EURYCEA SOSORUM*

by

Justin C. Crow, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Biology
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Committee Members:

Joseph R. Tomasso, Chair

Michael R. J. Forstner

Kenneth G. Ostrand

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2. Relationship between mean temperature (°C) and growth by percent total length (A) and percent weight (B) of the BSS after a 69 day exposure to nominal temperatures of 15, 18, 21, 24 and 27°C. Growth by TL (A) was equivalent at all tested temperatures, except 27°C, where a significant decline ($P = 0.005$) in growth occurred 12

ABSTRACT

The Barton Springs Salamander (BSS), *Eurycea sosorum*, is a federally endangered obligate aquatic salamander found only in a few spring outflows located in a highly urbanized recreational area of Austin, Texas. The purpose of this study was to gain essential information regarding the physiological response of the BSS to thermal manipulations and three common aquatic nitrogenous toxins (ammonia, nitrite, and nitrate). All salamanders used in this study were produced at the San Marcos Aquatic Resource Center (U.S. Fish and Wildlife Service) as part of a captive breeding program. To examine thermal stressors, salamanders were subjected to a temperature increase of 0.5°C per day until a loss-of-righting response (LRR) was observed. Additionally, salamander growth was assessed following a 69 day trial in which young salamanders were reared at five different temperature treatments (nominal 15, 18, 21, 24 and 27°C). The cumulative ET50 of the LRR for the combined replicates observed in the BSS was 32.6 ± 0.24°C (mean ± SD). The optimal temperature for growth of the BSS for total length was estimated to be 18.3°C resulting in a 59.7 ± 21.09% increase in total length. To investigate the effects of nitrogenous wastes on the BSS, ninety-six hour median-lethal concentration (96-hour LC50) trials were conducted for un-ionized ammonia-N (UIA-N), nitrite-N, and nitrate-N. The 96-hour LC50 of UIA-N, nitrite-N, and nitrate-N to the BSS was 2.1 ± 0.19 mg/L, 27.7 ± 0.72 mg/L, and 851.1 ± 49.21 mg/L, respectively. These results will aid in the conservation, management, and ongoing efforts to culture the BSS in captivity.

PREFACE

Public awareness of the recent decline in amphibians has increased considerably since this phenomenon was first acknowledged at a National Research Council workshop in the early 1990's (Wake 1991). Around the same time, public attention in Central Texas was focused on the Barton Springs Salamander (BSS), *Eurycea sosorum*, with habitat located in the rapidly developing area of Austin, Texas. Politicians, citizens, and developers fervently deliberated proposed conservation measures for the BSS, and ultimately the BSS was granted endangered status by the U. S. Fish and Wildlife Service in 1997 (USFWS 1997). In order to develop and implement effective conservation measures, an understanding of the environmental requirements of the organism of concern is necessary. One of the most influential environmental factors affecting the BSS is temperature, which remains almost constant in its stenothermal habitat. However, information concerning the physiological response of the BSS to thermal changes is lacking. Further, studies have been conducted on the physiological and developmental effects of several insecticides on the BSS (USEPA 2007a and b), yet information concerning the physiological response of the BSS to common aquatic nitrogenous toxins present in its environment remain unknown. Thus, the following study sought to examine the physiological tolerances of the BSS to thermal manipulations (Chapter 1) and three common aquatic nitrogenous toxins (Chapter 2).

CHAPTER I

EFFECTS OF MULTIPLE THERMAL STRESSORS ON THE BARTON SPRINGS SALAMANDER (*EURYCEA SOSORUM*)

Introduction

The Barton Springs Salamander (BSS), *Eurycea sosorum,* (Chippendale *et al*. 1993) is a state and federally listed endangered plethodontid salamander found in the Barton Springs segment of the Edwards Aquifer. The known geographic range of this species is restricted to four spring openings, collectively known as Barton Springs, in the Barton Creek drainage located near downtown Austin, Texas (Petranka 1998). This species occupies a stenothermal habitat with perennial spring flow and substrates consisting of rock, cobble, gravel, and various macrophytes. Given the adaptation of the BSS to a mostly stable thermal environment, it has been reasonably concluded that the BSS is at minimum partially dependent on consistent water temperatures (USFWS 2005).

Temperature is one of the most important abiotic factors affecting amphibians and influences physiological functions, such as metabolic rate, gas exchange, reproduction, development and growth (Hillman *et al*. 2009). For stenotherms, maintenance of a narrow range of environmental temperatures is necessary for optimal physiological function. Numerous studies have demonstrated the detrimental effects to salamanders caused by temperature extremes (Zweifel 1957; Hutchison 1961; Rohr and Palmer 2012). Annual mean water temperatures in Barton Springs normally range from 21 to 22°C (USFWS 2005). However, intermittent spring flow is reported to occur at Upper Barton Springs (Dries *et al*. 2013). While no thermal information is available, it is reasonable to

think that water temperatures vary more widely during the interruption of flow than during normal flow. The objectives of this study were to determine the relationship between a slow increase in temperature (mean increase of 0.5°C per day) on the loss-of-righting response reflex (LRR), and determine the effect of temperature on growth of the BSS. This information can be incorporated into the recovery plan (USFWS 2005) for this federally listed species.

Materials and Methods

Thermal manipulation experiments were conducted at the San Marcos Aquatic Resource Center (SMARC, U.S. Fish and Wildlife Service), in San Marcos, Texas. All salamanders used in this study were produced at the SMARC as part of a captive breeding program (USFWS 2005). Multiple, insulated, temperature-controlled, 1,135-L fiberglass tank systems were filled approximately half full with well water from the Edwards Aquifer to serve as thermally stable water bath reservoirs. Three 75-L experimental tanks were placed in each water bath reservoir approximately two-thirds submerged and used to hold salamanders during trials. Polyvinyl chloride (PVC) pipe halves (5 cm diameter; thermal maxima experiment) or rocks and macrophytes (optimal growth experiment) were placed in each experimental tank in order to provide shelter for the salamanders. A submersible pump was also placed in each experimental tank to provide circulation.

Thermal maxima experiment. Fifteen BSS were placed into each of three experimental tanks. The salamanders were then allowed to acclimate for 24 hours prior to treatment. Initial water temperature was 21 ± 1°C, which is similar to their natural spring

habitat and captive assurance colony holding systems. The temperature was then raised a nominal 0.5°C per day (actual mean temperature increase = 0.46°C per day, $r^2 = 0.997$) until all salamanders had exhibited a LRR. The LRR is characterized by a loss of equilibrium so that upon sinking to the bottom after a period of swimming, the salamander may rest upon its back for a time before righting itself (Zweifel 1957). The gradual increase in temperature should give the animals an opportunity to physiologically acclimate to the increasing temperature (Lowe and Vance 1955; Spotila 1972), and serve as an indicator of maximum tolerable temperature. Once the temperatures approached the salamanders suspected upper thermal limits, based on decreased feeding, hyperactivity, and thermal maxima values for salamanders obtained from literature (Zweifel 1957; Sealander and West 1969; Spotila 1972; Berkhouse and Fries 1995; Lutterschmidt and Hutchison 1997), we began monitoring the salamanders for several minutes every hour until the temperature stopped increasing, and then every three hours after that. At the first indication an individual was experiencing a LRR, that individual was removed, weighed (grams), and measured (SVL, snout to vent length; the distance from the tip of the snout to the posterior margin of the cloaca; and TL, total length; the distance from the tip of the snout to the tip of the tail). Immediately following measurements, salamanders were placed in a separate experimental tank maintained at the same experimental temperature, and allowed to cool gradually (approximately 1°C per hour) in an attempt to facilitate recovery of the salamander. Mean salamander weight in the thermal maxima experiment was 1.2 ± 0.40 g (mean ± SD), and mean SVL and TL were 37 ± 3.5 mm and 73 ± 9.4 mm, respectively.

Temperature, pH (8.0 ± 0.31), and dissolved oxygen saturation (86 ± 10.0%) were measured daily during the trial using a Hydro-tech MS5 (Hach Co., Loveland, CO). Total ammonia-nitrogen (0.3 ± 0.31 mg/L) was measured up to three times per week throughout the trial by direct Nesslerization (APHA 1989). The salamanders were fed to excess a diet of commercially produced live black worms, *Lumbriculus variegatus*, (California Blackworm Co., Fresno, CA) and brine shrimp, *Artemia salina*, (Mariculture Technologies International, Inc., Oak Hill, FL). Light cycle was maintained under natural photoperiod conditions via overhead skylights and uncovered windows.

Optimal growth experiment. Six to seven salamanders (TL range from 15 to 40 mm) were stocked into each of fifteen 75-L experimental tanks. Each experimental tank was partially submerged in one of five temperature-controlled fiberglass tank systems which served as a thermally stable water bath reservoir (three experimental tanks per water bath reservoir). Salamanders were then given 24 hours to acclimate at a water temperature of 21 ± 1°C. The temperature in each of the five water bath reservoirs was then adjusted to a nominal 15, 18, 21, 24 and 27°C at a rate of ± 1°C per day (Sadler 1979) to allow the salamanders to acclimate to treatment temperatures. Once each water bath reservoir reached the assigned temperature, salamanders were removed, weighed (grams), measured (TL in mm), and returned to their respective experimental tanks. Mean weight and TL of salamanders at the start of the trial were 0.1 ± 0.06 g and 31 ± 4.6 mm, respectively.

While in the experimental tanks, salamanders were observed at least two times per day and fed an alternating diet of *Artemia* nauplii, *Artemia salina*, (INVE Aquaculture, Inc., Salt Lake City, UT), zooplankton (pond raised at the SMARC), and

commercially produced live black worms, *Lumbriculus variegatus*, (California Blackworm Co., Fresno, CA) to excess. Temperature was monitored daily using a Hach HQ40d meter (Hach Co., Loveland, CO) and remained within a mean of 0.2 ± 0.21°C of nominal settings across temperature treatments. Dissolved oxygen saturation (92 ± 7.3%, across all treatments), and pH (8.3 ± 0.11, across all treatments) was monitored in each experimental tank daily using a Hach HQ40d meter. Total ammonia-nitrogen (0.8 ± 0.59 mg/L, across all treatments) was monitored in each experimental tank twice per week by direct Nesslerization. If total ammonia-nitrogen levels exceeded 0.5 mg/L in experimental tanks, a 40% water change was performed using fresh Edwards Aquifer well water that had been maintained at the same experimental temperature as the respective experimental tank.

Once salamanders had spent 69 days at their respective temperature treatments they were removed from their experimental tanks and individually weighed and measured. The salamanders were then returned to their respective experimental tanks and acclimated back to their natural habitat and holding temperature of 21°C, using the method of 1°C per day (Sadler 1979) as previously described.

Regression analysis was used to analyze data from both experiments, with a significance level set at a $p \leq 0.05$. The statistical software package R Studio (R Core Team 2014) was used to determine probability values. In the thermal maxima experiment linear regression was applied to each replicate ($N = 3$) and to all replicates combined. The regression of the combined replicates is shown in Figure 1. The effective temperature at which half of the salamanders displayed a LRR (ET50) was calculated for each replicate, as well as the combined replicates along with the 95% confidence interval. Linear

equations generated by each regression were used to calculate ET50 values by assuming a 50% value for the dependent variable (% LRR) and solving for the independent variable value (Temperature). For the optimal growth experiment, the mean of all logged temperature readings (N=70 temperature readings per experimental tank) obtained during the trial and mean salamander growth (% increase in weight and TL of salamanders in experimental tank) in each experimental tank were used in the polynomial regression analyses. This resulted in an *N* of three for each temperature treatment. Optimal growth temperatures and associated 95% confidence intervals were calculated for regressions. Optimal growth temperatures were estimated from the quadratic equation generated by regression curves by calculating the estimated vertex of the curve using the following equation; $\hat{x} = -\frac{b}{2a}$

Results and Discussion

Thermal maxima experiment. The first observed instance of LRR in the BSS occurred at 31.7°C and all BSS had exhibited a LRR or died by 33.5°C. Episodes of LRR were distinguished by the preliminary loss of posterior limb function, consistent with Hutchison (1961), followed by moments of disoriented swimming. Linear regression was applied to each replicate, as well as all replicates combined. However, only the regression of combined replicates ($r^2 = 0.98$, $P < 0.001$) is shown in Figure 1. The ET50 for the LRR in the BSS ranged from 32.4 ± 0.45°C (95% confidence interval of 32.17 to 32.63°C) to 32.9 ± 0.44°C (95% confidence interval of 32.67 to 33.12°C) for all three replicates. For all replicates combined the ET50 for the LRR in the BSS was of 32.6 ± 0.24°C (95% confidence interval of 32.45 to 32.75°C, Figure 1).

Our results were consistent with numerous thermal tolerance studies of plethodontids (Sealander and West 1969; Spotila 1972; Berkhouse and Fries 1995; Lutterschmidt and Hutchinson 1997), which indicate episodes of mean LRR at similar temperatures for *Plethodon ouachitae* (30.6 ± 0.25°C), *Plethodon richmondi* (31.3 ± 0.48°C) and *Desmognathus ochrophaeu* (29.2 ± 0.49°C). However, many past studies utilized heating rates of 0.5 to 1.0°C per minute. These rapid heating rates were justified as necessary to account for the rapid rate at which most reptiles absorb or lose heat (Cowles and Bogert 1944). A reduced heating rate of 0.5°C per day was chosen for the current study to allow the animals to physiologically acclimate to changing temperatures, perhaps giving a better estimate of their upper thermal limits. Moreover, these are more likely to approximate conditions in the field in the absence of spring flow or during reduced spring flows.

Optimal growth experiment. A total of 3 BSS died during the temperature treatment exposures (97% survival). Initial weights and initial TL were not significantly different among treatments at the beginning of the experiment ($P = 0.265$ and $P = 0.464$, respectively). Temperature had a significant effect on the percent increase in TL of the BSS ($P = 0.018$, Fig 2 A). However, when measured as percent increase in weight of the BSS, temperature did not have a significant effect ($P = 0.175$, Fig 2 B). The optimal temperature for growth of the BSS for increase in TL was estimated to be 18.3°C resulting in a 59.7% increase in TL (95% confidence interval of 27.96 to 91.49%). Further, the percent increase in TL at all tested temperatures, except 27°C, fall within the 95% confidence interval for the percent increase in TL among optimal temperature estimates. The optimal temperature for growth of the BSS for increase in weight was not

estimated due to the lack of a significant treatment effect. Qualitatively, a trend toward reduced increases in TL began to occur just beyond 21°C, but as only the 27°C treatment was significantly different, a range of the tested temperatures (15-24°C) accommodates similar growth. The least overall increases in both weight and TL was observed in the 27°C treatment (actual mean temperature = 27.7°C), which is well outside the normal thermal range currently encountered in the BSS habitat.

The optimal growth temperature estimate derived from the intercept of our model is slightly below the lower end of the temperature range typically occurring in BSS surface habitat (21 to 22°C). However, as none of the treatments in the range of 15-24°C were significantly different, we can state that the surface habitat temperatures known for the BSS are within the range of temperatures bound by our confidence interval estimates for growth by TL. Our results were consistent with other studies, which indicate that within the temperature range normally encountered by an organism, a decrease in rearing temperatures causes a majority of ectotherms to attain larger sizes at a given developmental stage (Atkinson 1994). Additionally, it has been shown that reduced growth occurs with increased rearing temperatures in ectotherms (Atkinson 1995). However, this reduced growth generally results when an ectotherm is outside of its optimal temperature range for performance (Huey and Kingsolver 1989). Temperature essentially controls the metabolic rate and metabolic scope of such organisms. Metabolic scope is described as the ratio of the maximum sustained metabolic rate to the standard metabolic rate (SMR) for poikilotherms (Randall *et al*. 1997). At SMR for poikilotherms, metabolic oxygen demand generally increases exponentially with temperature. However, the physiological capacity to supply this oxygen increases sigmoidally with increasing

temperatures (Neill and Bryan 1991). Thus, the temperature of maximum metabolic scope is the optimal temperature for an organism. As temperatures increase beyond an organism's optimal temperature, metabolic scope begins to decrease, subsequently affecting the physiology and growth of an organism. The results from this study would support an optimal temperature range for optimal growth of *Eurycea sosorum* that is much broader than is often speculated (USFWS 2005; Pierce and Wall 2011).

Given the ongoing efforts to culture the BSS in captivity (Chamberlain and O'Donnell 2003; USFWS 2005), these results will provide basic thermal information for the culture of the BSS for captive assurance colonies. Further, our results provide vital thermal tolerance information for the BSS, which may be of value to habitat managers as they try to estimate the impacts of changing habitat temperatures and conditions on the BSS (Turner 2004). Temperatures exceeding 26°C in Old Mill Spring (Dries *et al*. 2013), a known spring habitat for the BSS, have been documented. In addition, given the previously speculated thermal sensitivity of other central Texas *Eurycea* (Sweet 1982; Pierce and Wall 2011), our results will aid in the assessment of the potential impacts of climate change in the Edwards Plateau region (Loaiciga *et al*. 2000) on habitat temperatures of these species. For example, temperatures up to and exceeding 30°C have been documented (Bowles *et al*. 2006) in a known habitat of the Jollyville Plateau Salamander, *Eurycea tonkawae,* a federally listed threatened (USFWS 2013) species found along the Jollyville segment of the Edwards Plateau. Our data would support this temperature nearing the limits of the salamander's thermal maxima, but clearly beyond the temperatures supporting optimal growth. With the potential for continued increases in habitat temperatures for the BSS, as well as other central Texas *Eurycea*, an

understanding of their thermal requirements will be necessary for their continued conservation.

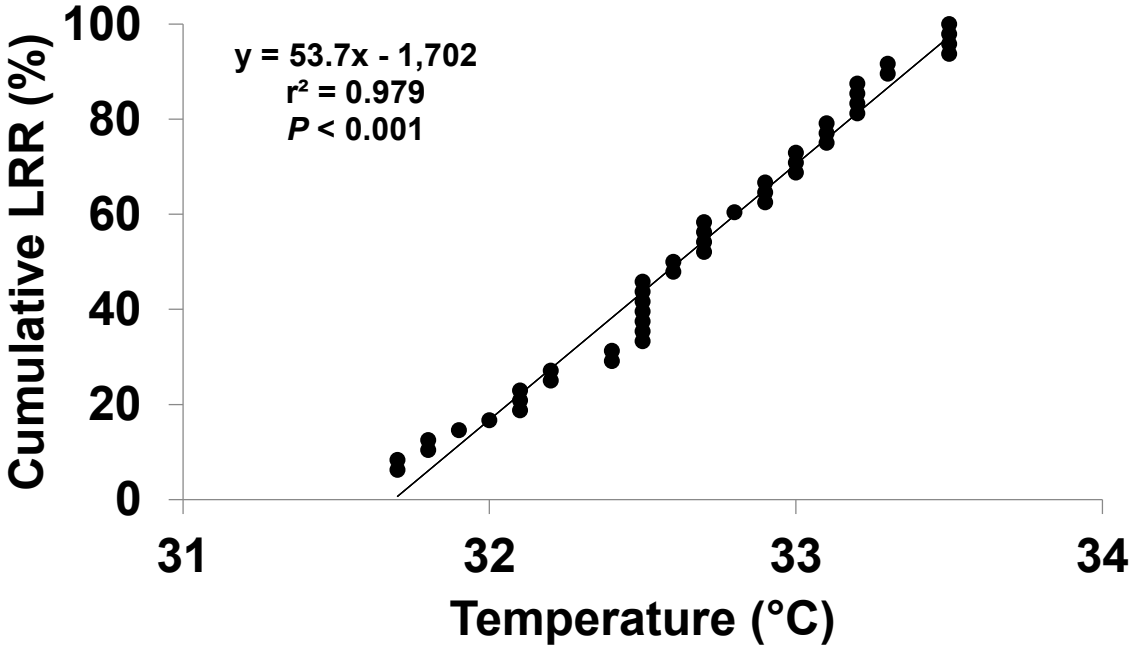

Figure 1. Cumulative Loss of Righting Response (%), for combined replicates, of the BSS in the course of daily temperature increases of 0.45°C (initial temperature = 20.8°C).

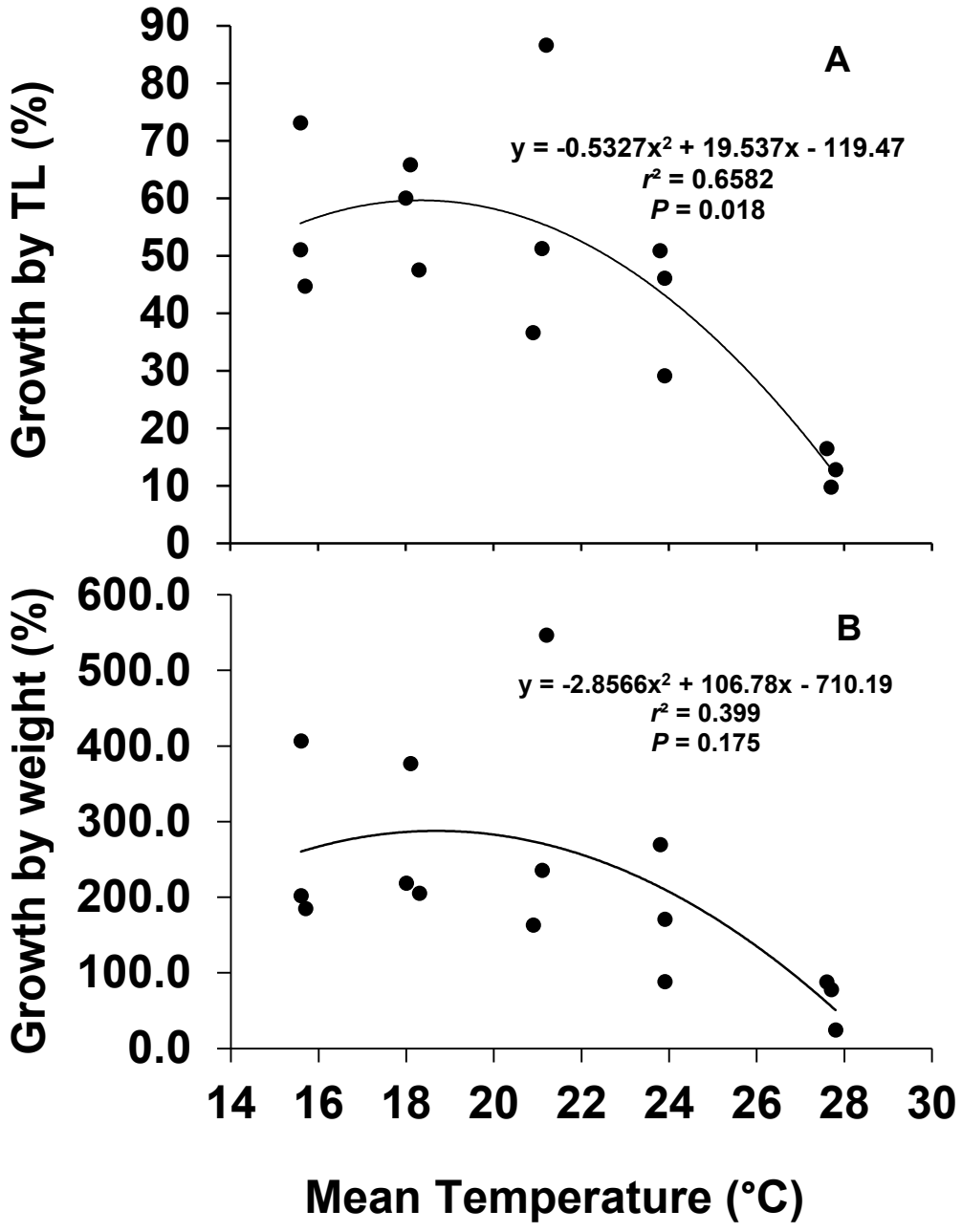

Figure 2. Relationship between mean temperature (°C) and growth by percent total length (A) and percent weight (B) of the BSS after a 69 day exposure to nominal temperatures of 15, 18, 21, 24 and 27°C. Growth by TL (A) was equivalent at all tested temperatures, except 27°C, where a significant decline ($P = 0.005$) in growth occurred**.**

CHAPTER II

ACUTE TOXICITY OF AMMONIA, NITRITE, AND NITRATE IN THE BARTON SPRINGS SALAMANDER (*EURYCEA SOSORUM*)

Introduction

The federally endangered Barton Springs Salamander (BSS), *Eurycea sosorum,* occurs in a few spring outflows, collectively known as Barton Springs, emanating from the Barton Springs Segment of the Edwards Aquifer (Green *et al*. 2014). This spring system is located in a rapidly growing, highly urbanized area near downtown Austin, Texas. Barton Springs also serves as an important recreational resource that receives around 350,000 paid visits per year (Smith *et al*. 2001). Highly urbanized environments, such as this, often produce storm water runoff with elevated levels of pollutants (Booth and Reinelt 1993). Analysis of long-term water quality data (1975 to 1999) from Barton Springs indicates increases in sulfates, total organic carbon, and specific conductance, which are presumed to be related to increased urbanization (Turner 2005). A recent study indicates that nitrate plus nitrite concentrations at Barton Springs were consistently greater than the national background concentration for groundwater and were positively correlated with spring discharge (Mahler *et al*. 2011). Given current pressures and projected increases in urbanization, it is reasonable to anticipate an increase in contaminant loads occurring in BSS habitat.

The objectives of this study were to determine the acute toxicity of three common aquatic nitrogenous toxins, (ammonia, nitrite, and nitrate) to the BSS. The results of this study were compared with other aquatic species to determine if current water quality

criteria are adequate to protect the BSS. Additionally, the following study establishes a benchmark for the tolerance of the BSS to these three toxins.

Materials and Methods

A total of 96 adult BSS were obtained from the San Marcos Aquatic Resource Center (SMARC, U.S. Fish and Wildlife Service) in San Marcos, Texas and transferred to Texas State University in San Marcos, Texas. Salamanders used in this study ranged in size from 45 to 80 mm (mean ± SD = 65 ± 12.8 mm) and were produced at the SMARC as part of a captive breeding program (USFWS 2005). All salamanders were held in the BSS captive breeding refugium at the SMARC until 24 hours prior to exposure. At which time eight individuals per toxicity trial were transported to Texas State University.

Toxicity tests were conducted at Texas State University. A single 720-L fiberglass tank served as a thermally stable water bath reservoir by receiving a constant flow of Edwards Aquifer water adequate to maintain a temperature of approximately 22°C. Ninety-six hour median-lethal concentration (96-hour LC50) trials were conducted in eight 30-L experimental tanks that were partially submerged in the reservoir to serve as water baths. One salamander was placed into each of the eight experimental tanks so that there were a total of eight independent observations for each trial. Several sterile rocks were placed into each experimental tank to provide habitat. A submersible pump was placed into each experimental tank to provide water flow. Each experimental tank contained 10-L of Edwards Aquifer water (14 mg sodium/L, 1 mg potassium/L, 60 mg calcium/L, 20 mg magnesium/L, 23 mg chloride/L, 30 mg sulfate/L) in order to reduce chemical usage and standardize dosage calculations.

Salamanders were stocked into experimental tanks 24 hours before toxin exposure. A modified version of the USEPA toxicity testing protocol (USEPA 2002) was used. The modification was a reduction of the number of animals from 15 to 1 per replicate, reducing by approximately 93% the number of animals needed for the study.

Initial exposure concentrations were estimated from literature (Camargo *et al*. 2005; Griffis-Kyle 2007; USEPA 2010; USEPA 2013) and concentrations were increased by a geometric progression factor of two. For each trial eight animals were exposed at a time (including a control which received no toxin). Based on the results of each trial, more animals were exposed until we had four concentrations, near the anticipated LC50, to each of which five animals had been exposed. Thus, the ammonia, nitrite, and nitrate toxicity trials were replicated five times each. Dead salamanders (total N = 85) were removed from aquaria, measured, and preserved (95% ethanol). A summary of toxin concentrations can be found in Table 1.

Salamanders were fed during the exposure an alternating diet of live brine shrimp, *Artemia salina*, (Mariculture Technologies International, Inc., Oak Hill, FL) and commercially produced live black worms, *Lumbriculus variegatus*, (California Blackworm Co., Fresno, CA). Temperature (22 ± 1.0°C for all trials) and dissolved oxygen saturation (90 ± 9.0% for all trials) were measured daily during exposure using a YSI 2030 meter (YSI, Inc., Yellow Springs, Ohio). At the end of each exposure, pH (8.3 ± 0.31 for all trials) was measured (Accumet AB15 plus pH meter; Thermo Fisher Scientific Inc., Walthma, MA). At the end of each exposure, total ammonium-nitrogen, nitrite-nitrogen, or nitrate-nitrogen levels were measured, depending on exposure treatment. Total ammonia-nitrogen was determined by direct Nesselarization (APHA

1989). Nitrite-nitrogen and nitrate-nitrogen were determined spectrophotometrically using a GENESYS 20 spectrophotometer (Thermo Fisher Scientific Inc., Walthma, MA) with the reagents NitriVer® 2 nitrite reagent and NitraVer® 5 nitrate reagent (Hach Company, Loveland, CO), respectively. Nominal concentrations of ammonia, nitrite, and nitrate were developed by the addition of ammonium chloride, sodium nitrite, and sodium nitrate (Thermo Fisher Scientific Inc.), respectively. Un-ionized ammonia-nitrogen (UIA-N) concentrations were calculated from tables in standard methods for the examination of water and wastewater (APHA 1989). At the end of the toxicity tests, the mean percent nominal concentrations of UIA-N, nitrite-N, and nitrate-N were 90 ± 5.8%, 92 ± 4.6% and 101 ± 13.6%, respectively. Following each toxicity trial, each experimental tank was drained, rinsed, dried, and refilled with fresh aquifer water. The laboratory light cycle was maintained on a 12:12 light dark photoperiod.

Data were analyzed using the Spearman-Karber Method (Finney 1978), which is a non-parametric procedure used to estimate median-lethal dose/concentration values and associated 95% confidence intervals (USEPA 2002). This method estimates the mean of the distribution of the logarithm of the tolerance. This non-parametric procedure was chosen due to the violation of the assumptions of normality and homoscedasticity caused by a binary response variable. The 96-hour time course was retained for this study because the toxicity-testing database consists of mostly 96-hour studies, allowing better integration of our results with the literature.

Results and Discussion

The 96-hour LC50 of UIA-N to the BSS was 2.1 ± 0.19 mg UIA-N/L (95% confidence interval of 1.6 to 2.9 mg UIA-N/L). The first mortality was observed at 2 mg UIA-N/L (Table 1). All mortalities occurred in the first 24 hours. The 96-hour LC50 of UIA-N to the BSS is similar to that of the Leopard frog, *Rana pipiens*, when exposed as embryos (1.2 mg UIA-N/L, Diamond *et al*. 1993) and eggs (2.2 mg UIA-N/L, Jofre and Karasov 1999). The acute toxicity of the BSS to UIA-N is generally higher than that of many freshwater fishes (Haywood 1983; Tomasso 1994; USEPA 2013).

The 96-hour LC50 for nitrite-N was 27.7 ± 0.72 mg NO_2-N/L (95% confidence interval of 21.8 to 35.1 mg NO_2-N/L). The first mortality was observed at 32 mg NO_2-N/L. The BSS shows greater resistance to nitrite-N than small-mouth salamander *Ambystoma texanum* larvae, which have a 96-hour LC50 of 1.09 mg NO_2-N/L (Huey and Beitinger 1980). However, the tolerance of the BSS to nitrite-N appears average when compared to freshwater fish species, with the LC50 of nitrite-N to the BSS falling midway between most LC50 values of many freshwater fishes (Russo and Thurston 1977; Tomasso 1986; Lewis and Morris 1986).

The 96-hour LC50 for nitrate-N was 851.1 ± 49.21 mg NO_3-N/L (95% confidence interval of 562.7 to 1,287.4 mg NO_3-N/L). The first mortality was observed at 500 mg NO_3-N/L. The 96-hour LC50 of nitrate-N to the BSS is similar to that of the washboard mussel *Megalonaias nervosa* (937 mg NO_3-N/L, USEPA 2010). The BSS appears to have a higher tolerance for nitrate-N than Pacific tree frog *Pseudacris regilla* embryos (643 mg NO_3-N/L, Schuytema and Nebeker 1999a). However, when the Pacific tree frog is exposed to nitrate-N at the larval stage, the BSS has a considerably lower threshold for

nitrate-N than the Pacific tree frog (1,749.8 mg NO_3-N/L, Schuytema and Nebeker 1999b). A considerable amount of literature has tested the acute effects of nitrate-N on amphibian eggs and/or larvae (Laposata and Dunson 1998; Camargo *et al*. 2005; Meredith and Whiteman 2008). However, it is difficult to draw a direct comparison to our results, which are based on adult organisms.

The toxicity of nitrogenous wastes can vary based on water quality characteristics, such as temperature, pH, and chloride (Tomasso *et al*. 1980; Williams and Eddy 1986; Russo and Thurston 1991; Tomasso 1994). The effects of chemical constituents in water must be considered when conducting and interpreting toxicity tests. Lack of consideration of the variation in water chemistry between test locations may result in disparities in toxicity estimates across species and within species, resulting in wide-ranging and confusing LC50 values (Lewis and Morris 1986).

The United States Environmental Protection Agency (USEPA) establishes National Ambient Water Quality Criteria (AWQC) under the Clean Water Act (Clean Water Act 1972). These criteria serve as recommendations for defining ambient water concentrations that will protect against adverse ecological effects to aquatic life as a result of pollutants (USEPA 2013). Documents are available evaluating the toxicity of specific chemicals, including ammonia (USEPA 2013), while nutrient criteria documents are available for nitrite and nitrate (USEPA 2001). As data become available, AWQC are refined and adjusted. In 1999, the USEPA updated the AWQC to include and account for the effects of temperature and pH on the toxicity of ammonia (USEPA 1999), adjusting all subsequent AWQC documents. Additionally, as data become available, more species will be added and subsequently used to estimate water quality criteria. Currently, out of

the 100 species that were included in the 2013 AWQC document for ammonia, only four were amphibians, all of which were anurans. This lack of toxicity data available for amphibian species is concerning. Without sufficient knowledge of an organism's environmental requirements and tolerances, governmental agencies may fail to provide adequate protections for sensitive organisms.

Our results demonstrate that current USEPA water quality criteria recommendations for acute exposures to total ammonia-nitrogen, 17 mg/L, or UIA-N, 0.066 mg UIA-N/L (USEPA 2013), are adequate to protect the BSS. Further, the USEPA ranks the sensitivity of the organisms to total ammonia-nitrogen based on a ranked genus mean acute value (GMAV). These values are then ordered by sensitivity, with the most sensitive organisms given the lowest rankings. Based on our results, the BSS would rank 44 out of 69 genera with a ranked GMAV of 156.9 mg total ammonia-nitrogen/L (adjusted for a pH = 7.0) in the USEPA AWQC for ammonia (USEPA 2013). Our results also demonstrate that current water quality criteria recommendations for both nitrite and nitrate measured as the sum of nitrite and nitrate, 0.05 mg/L for Ecoregion 32 (USEPA 2001), are adequate to protect the BSS.

Table 1. Results of 96-hour LC-50 toxicity trials on the BSS

N	Dosage (mg/L)	% Survival
	UIA-N	
1	0.25	100
1	0.5	100
5	1	100
5	2	60
5	4	0
5	8	0
1	16	0
	Nitrite-N	
1	0.5	100
1	1	100
1	2	100
1	4	100
6	8	100
6	16	100
7	32	29
6	64	0
	Nitrate-N	
2	1	100
2	10	100
6	100	100
7	500	86
6	1000	50
5	2000	0
4	4000	0

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