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Edwards Aquifer Habitat Conservation Plan 2016 Salvage Refugia Program Comal and Hays Counties, Texas

Prepared for

Edwards Aquifer Authority

Prepared by

SWCA Environmental Consultants in Partnership with the San Antonio Zoo and SeaWorld San Antonio

January 2017

EDWARDS AQUIFER
HABITAT CONSERVATION PLAN
2016 SALVAGE REFUGIA PROGRAM
COMAL AND HAYS COUNTIES, TEXAS

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EXECUTIVE SUMMARY

The purpose of the salvage refugia program is to collect and maintain captive stocks of listed Edwards Aquifer species so that individuals are available for reintroduction following a low-flow or other catastrophic event. The salvage refugia program has been established as part of compliance with the Edwards Aquifer Recovery Implementation Program Incidental Take Permit and the associated Edwards Aquifer Habitat Conservation Plan (EAHCP). The EAHCP requires the establishment of off-site refugia to maintain captive populations of federally listed Edwards Aquifer species when it is determined that a significant loss is imminent due to a catastrophic event, such as prolonged drought.

The Edwards Aquifer Authority (EAA) retained SWCA Environmental Consultants (SWCA), the San Antonio Zoo (SAZoo), and SeaWorld San Antonio (collectively referred to as the SWCA Team) in 2015 to assist with implementation of the salvage refugia program while the long-term refugia program was developed. The SWCA Team worked with the EAA to identify research and husbandry priorities for 2015 and 2016 that would advance the objectives of the refugia program and ensure preparedness for a salvage event. Thankfully, a salvage collection event did not trigger during 2015 or 2016 and biologists were able to focus their attention on advancing the scientific understanding of these species in a controlled setting.

Species Research

Research projects selected for 2016 focused on identifying the most efficient collection methods for use during a salvage collection event. Salvage collection is triggered when die-offs in the wild are imminent or ongoing as outlined in the EAHCP. During such an event, large numbers of individuals need to be collected in a short period of time. For this reason, research in 2016 focused on species for which bulk collection has not been successfully conducted in the past. The SWCA Team and the EAA selected the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and the Texas blind salamander (*Eurycea rathbuni*) for this initial research.

The SWCA Team developed a detailed research plan for EAA approval late in 2015. The research plan was presented to the EAHCP Science Committee on November 10, 2015. The SWCA Team incorporated comments received and updated the research plan accordingly.

The SWCA Team attempted to collect Comal Springs dryopid beetles for 12 weeks starting May 3, 2016, and ending on July 26, 2016. Ultimately, the methods did not result in the collection of any Comal Springs dryopid beetles, but other Edwards Aquifer-dependent species included in the EAHCP were collected and transferred to the SAZoo facility (Table ES-1).

Table ES-1. Edwards Aquifer Species Collected during the Comal Springs Dryopid Beetle Collection Methods Study

Species	Total Collected	Total Transferred to SAZoo
Comal Springs riffle beetle (*Heterelmis comalensis*)	451 (330 adults/121 larvae)	186 (152 adults/34 larvae)
Common riffle beetle (*Microcylloepus pusillus*)	2,161	0
Peck's Cave amphipod (*Stygobromus pecki*)	189	41
Water slater (*Lirceolus* sp.)	72	15

Note: These totals include specimens not included in the lure comparison study.

The SWCA Team collected Texas blind salamanders from three wells/fissures (Rattlesnake Well, Primer's Fissure, and Johnson's Well) in San Marcos, Hays County, Texas, from April to December 2016 (Table ES-2). In total, 22 Texas blind salamanders were collected during the research period using two different bait types. Unfortunately, this sample size is not large enough to conduct a statistical analysis on the preferred bait type.

Table ES-2. Collection Summary for the Texas Blind Salamander

Site	Total Collected	No. Collected and Transferred to SAZoo	No. Returned	Date Last Collected
Rattlesnake Well	**2**	1	1	7/5/2016
Johnson's Well	**14**	7	7	12/1/2016
Primer's Fissure	**6**	3	3	11/25/2016

Husbandry

Pursuant to the SWCA Team's agreement with the EAA and the terms and conditions of SWCA's scientific research permit from the U.S. Fish and Wildlife Service, a pre-determined quantity of species collected during research were transferred to the SAZoo. SAZoo biologists established protocols for species husbandry unique to the configuration of the research pods constructed for the salvage refugia program. Over the research period, the SAZoo housed 253 species of concern, including 11 Texas blind salamanders, 186 Comal Springs riffle beetles (*Heterelmis comalensis*), 41 Peck's Cave amphipods (*Stygobromus pecki*), and 15 water slaters (*Lirceolus* sp.).

The SAZoo was able to sustain individuals of each of these species throughout the term of the salvage refugia project. At least one reproductive event was observed in captivity for the Comal Springs riffle beetle, producing two larvae.

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1.0 INTRODUCTION

During the drought of record in Central Texas (1951–1956), the Comal Springs in Comal County, Texas, ceased flowing for 144 days. As a result, the fountain darter (*Etheostoma fonticola*) was extirpated from the Comal River. Many years later, a regional effort to prevent a similar catastrophic event from happening in the wild resulted in the creation and adoption of the Edwards Aquifer Habitat Conservation Plan (EAHCP). The EAHCP was developed in support of an Incidental Take Permit application submitted to the U.S. Fish and Wildlife Service (USFWS) in early 2012. The EAHCP includes a series of measures designed to reduce the likelihood and impact of a repeat drought of record event. These measures include aquatic habitat and riparian vegetation restoration, springflow protection measures, routine monitoring, and comprehensive modeling to predict how ecosystem changes will affect aquatic habitats. Modeling conducted during preparation of the EAHCP indicates that collectively these measures maintain spring flow in the Comal River during a repeat of the drought of record.

Recognizing that a future drought event could follow different patterns than the drought of record and the continued susceptibility to other catastrophic events, the EAHCP also includes a refugia program to procure a stock of captive held individuals. These captive individuals can be used to repopulate wild populations should the other measures anticipated in the EAHCP fail to sustain native populations. The refugia program relies on a collection schedule whereby species are collected early on to support research of captive breeding and increase the scientific understanding of these species to ensure a successful stock can be established for reintroduction, if necessary. Once pre-determined triggers are met in the Comal and/or San Marcos spring system, salvage refugia collection is initiated. Salvage refugia collection only occurs when conditions are trending towards a catastrophic event. During a salvage refugia collection event, the goal is to collect as many individuals as possible to protect them from declining conditions and build a salvage stock for resupply once the systems have restored to healthy conditions.

The Edwards Aquifer Authority (EAA) retained SWCA Environmental Consultants (SWCA), the San Antonio Zoo (SAZoo) and SeaWorld San Antonio (collectively referred to as the SWCA Team) in 2015 to assist with implementation of the salvage refugia program. Thankfully, a salvage collection event did not trigger during 2016 and biologists were able to spend the time advancing our scientific understanding of the Edwards Aquifer species in a controlled setting.

This report documents the SWCA Team's salvage refugia research and husbandry activities and results for the 2016 calendar year.

2.0 SALVAGE REFUGIA RESEARCH PROGRAM

The SWCA Team, together with the EAA, identified refining collection methods as the greatest research need for the salvage refugia program. Together, they designed the 2016 research program to evaluate collection methods for the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and the Texas blind salamander (*Eurycea rathbuni*). The 2016 research program focused on effective collection methods during a salvage collection event when a large number of individuals must be collected over a short period of time.

2.1 COMAL SPRINGS DRYOPID BEETLE

2.1.1 BACKGROUND INFORMATION

Comal Springs dryopid beetles are considered stygobiont (an obligate subterranean aquatic organism) and is the only known stygobiont within the beetle family dryopidae (Barr and Spangler 1992). The Comal Springs dryopid beetle occurs in the Edwards Aquifer and has only been collected in two general locations: Comal Springs in Comal County, Texas, and Fern Bank Springs in Hays County, Texas.

Adult Comal Springs dryopid beetles are aquatic, have vestigial eyes, and are flightless (Barr and Spangler 1992). Adults can absorb dissolved oxygen with the use of a plastron (a thin sheet of air) (Arsuffi 1993) and have been observed rising above the water surface in captivity (Barr and Spangler 1992). Comal Springs dryopid beetle larvae, and all other known dryopid larvae, do not possess gills and are considered terrestrial or semi-terrestrial (Brown 1987). Thus, it is presumed that the larvae inhabit air-filled void spaces within the aquifer. These void spaces may contain food sources such as organic detritus and tree roots (Barr and Spangler 1992) and possibly serve as oviposition and pupation sites.

To date, fewer than 300 Comal Springs dryopid beetles have been collected from the wild (Arsuffi 1993; Barr and Spangler 1992; Barr 1993; Gibson et al. 2008; personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015). Between 1987 and 1992, 58 adults and 27 larvae were collected, and from 2002 to 2013, 71 adults and 51 larvae were collected, for a total of 207 individuals (personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015). BIO-WEST has incidentally collected a small number (exact number unknown) of Comal Springs dryopid beetles during annual monitoring of Comal Springs riffle beetle (*Heterelmis comalensis*) populations (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015).

It is likely that the paucity of historic collections of Comal Springs dryopid beetle is attributable to methodologies that do not directly sample the Edwards Aquifer, which we understand to be the beetle’s habitat. Currently utilized methods only target the beetles that have been ejected from the aquifer and/or individuals that occur at the extreme fringe of their presumed habitat, such as near spring orifices and upwellings during major rainfall events. Previous collection methods have included kick nets, Hess samplers, hand sampling, bottle traps, drift nets, and cotton-cloth lures (Arsuffi 1993; Barr 1993; Barr and Spangler 1992; BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008; personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015). Kick netting and Hess sampling are likely destructive to Comal Springs dryopid beetles and their habitats which, coupled with the preexisting vulnerability present during a salvage collection event, make this a less-than-ideal collection method. Hand sampling has a historically low success rate, is labor intensive, and can also be potentially destructive to habitat. Bottle traps have been used to sample wells with limited success (personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015). Setting drift nets over spring orifices and upwellings is relatively non-invasive and has been successful in the collection of living specimens. However, as shown in Table 1, this method has a relatively low collection rate that appears to favor larvae over adults.

Cotton-cloth lures are composed of 60% cotton and 40% nylon and have been utilized to monitor Comal Springs riffle beetles in the Comal and San Marcos springs system (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008; Huston et al. 2015). Cotton-cloth lures have also occasionally attracted Comal Springs dryopid beetles (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008). The cotton-cloth lure method is the preferred non-lethal method for collecting Comal Springs riffle beetles, Comal Springs dryopid beetles, and other invertebrates (Gibson et al. 2008). Cotton-cloth lures are left out at least three to five weeks to foster biofilms (primarily bacteria and fungi) that are considered potential food sources that attract invertebrates. Cotton-cloth lures seem to catch low numbers of Comal Springs dryopid beetle but, unlike drift netting, tend to favor adults (Gibson et al. 2008; personal communication, R. Gibson, USFWS, to C. Collins, September 22, 2015).

Table 1. Drift Rate Success Summary of Previous Comal Springs Dryopid Beetle Collections Utilizing Drift Nets in Comal Springs, Comal County, and Fern Bank Springs, Hays County, Texas

Location	Date	Drift Time (hours)	No. of Comal Springs Dryopid Beetles*	Drift Rate (No. per 24 hours)
Comal Springs	August 1992	397.5	5 (1A, 4L)	0.3
Comal Springs	May/August 2003	451.6	5 (2A, 3L)	0.3
Comal Springs	2003–2014	1,871.6	47 (4A, 41L, 1P)	0.6
Fern Bank Springs	August 1992	131.5	13 (1A, 12L)	2.4
Fern Bank Springs	May/August 2003	304.8	1L	0.1

Sources: BIO-WEST (2004–2008, 20014, 2015), Gibson et al. (2008)
* A = Adult, L = Larvae, P = Probable pupae

Using cotton-cloth lures, BIO-WEST has incidentally collected a few Comal Springs dryopid beetles in spring runs and upwellings in the Comal Springs system. BIO-WEST collected three Comal Springs dryopid beetles in 2007, six in 2013, and one in 2014 (BIO-WEST 2008, 2014, 2015). Gibson et al. (2008) also collected four Comal Springs dryopid beetles using cotton-cloth lures during invertebrate surveys of Comal Springs and Fern Bank Springs. Thus, if improved upon, a lure of some type and configuration may be suitable for collecting Comal Springs dryopid beetles at a higher rate.

2.1.2 Methods

2.1.2.1 RESEARCH QUESTIONS

Salvage triggers for Comal Springs dryopid beetle occur when spring flow at the Comal Springs falls below 30 cubic feet per second *and* when any standard or conventional water quality parameter within one of three Edwards Aquifer wells exceeds the historical range of water quality parameters by 10% or more (Recon Environmental et al. 2012). When triggers are met, it is expected that up to 500 individuals will be collected from the Comal Springs and placed in the salvage refugia facility. During a salvage collection event, it is likely that conditions will continue to decline and an efficient collection technique is essential to ensure that the maximum numbers of individuals are collected. Unfortunately, none of the current collection methods quickly yield adequate quantities of Comal Springs dryopid beetles. Thus, the SWCA Team aimed to identify alternative collection methods with a faster collection rate.

Specifically, the SWCA Team designed this study to answer the following questions:

- Which lure type performs the best overall?
- Do any perform better than the historical cotton-cloth method?
- Which site is best for collection?
- Does the use of a bell trap improve collection rates?
- What other potentially ecologically important species are collected with these methods?

2.1.2.2 STUDY AREAS

For the purposes of this study, the SWCA team selected four Study Areas, based on historic collection locations for Comal Springs dryopid beetles (Figure 1):

- Spring Run 2 Study Area
- Spring Run 3 Study Area
- Spring Island Study Area
- Pecan Island Study Area

From 2002 to 2014, biologists collected 23 adults and one larva from Spring Run 2; one adult and 20 larvae from Spring Run 3; and 29 adults from Spring Island upwellings (personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015). Biologists have not previously collected Comal Springs dryopid beetles in the upwellings of Pecan Island; however, Comal Springs dryopid beetles have been collected just north of Pecan Island along the western shoreline (Arsuffi 1993; Barr 1993; Barr and Spangler 1992; BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008; personal communication, R. Gibson, USFWS, to B. Hall, EAA, September 22, 2015).

2.1.2.3 LURE DESIGN

The SWCA Team developed several different lure types in an attempt to identify the most effective method for collected Comal Springs dryopid beetles. Due to previous successes with cotton-cloth lures, the SWCA Team used cotton-cloth lures consisting of 15 square centimeters (cm^2) (225 cm^2 of total surface area) cloth cut from 60% cotton and 40% nylon bed sheeting, placed into an approximately 5 × 1–cm hardware-cloth cage. The SWCA Team also used the invertebrate Hester-Dendy (HD) sampler in an attempt to identify alternative collection methods (Hester and Dendy 1962). The HD sampler is a multi-plate substrate sampler that is typically used for biological monitoring to sample macroinvertebrate communities. Like cotton-cloth lures, HDs likely foster the growth of biofilms that provide food sources for invertebrates. Comal Springs dryopid beetles have been found on pieces of wood lying on top of upwellings (Gibson et al. 2008). Thus, HDs made of native materials such as live oak (*Quercus fusiformis*) or bald cypress (*Taxodium distichum*), tree species found in and around Comal Springs (personal observation, C. Collins, SWCA, and others), may foster the appropriate type and amount of biofilms or habitat to attract Comal Springs dryopid beetle individuals.

The SWCA Team selected HDs made of three different materials, including live oak, bald cypress, and hardboard. Hardboard is a woody composite material that is commonly used for HD samplers. HDs are made of eight wooden plates, each a 3 × 3–inch square, 0.12-inch thick (7.6 × 7.6 cm × 0.3 cm thick, or 9 square inches [39.6 cm^2] of surface area per plate), totaling approximately 317 cm^2 of surface area per HD.

2.1.2.4 SCIENTIFIC DESIGN

Gas bubbles are readily observed arising from upwellings in Comal Springs (personal observation, C. Collins, SWCA, and LBG-Guyton Associates 2004) (Figure 2). The gas bubbles are an apparent indication of groundwater discharge and have been found to be largely atmospheric (LBG-Guyton Associates 2004). The gas likely goes into solution when atmospheric air is entrained and/or dissolved during surface-water recharge (LBG-Guyton Associates 2004). The gas comes out of solution with a reduction in pressure when aquifer water rises in elevation. The gas gets trapped within gravel and cobbles during the discharge of water into the spring system. This trapped gas may serve as potential habitat (air-filled void spaces) for the Comal Springs dryopid beetles. The larvae of Comal Springs dryopid beetle are thought to be terrestrial or semi-terrestrial, potentially relying on void spaces within the aquifer. It is also possible that adults favor void spaces, particularly dark spaces, as they are troglobitic

For this reason, the SWCA Team attempted to recreate these air-filled voids by utilizing bell traps (Figure 3). Bell traps consist of 1.5-liter black plastic containers with small holes drilled in the sides to create an air/water interface. Gas released from the spring orifice becomes trapped inside the bell trap and the water level within the bell trap is regulated at the appropriate water level height due to the holes drilled in the sides, so that the lures are partially underwater and partially above water. Inside the bell traps, the environment is dark and contains aquatic and terrestrial environments. Using this method, the lures will grow biofilms that may attract Comal Springs dryopid beetles, should they occur nearby within the upwelling.

The SWCA Team placed 16 lures over eight randomly selected upwellings (areas where gas bubbles were observed rising from floor) at each Study Area (64 lures in total). The SWCA Team placed two lures consisting of like material over each of the eight upwellings: one covered in rocks (unbelled) and one placed under a bell trap (belled). Each upwelling area had four lure pairs with each pair having a replicate. Lure pairs were set at least 2 meters apart. Sampling was conducted for 12 weeks. Lures were set on May 3, 2016, and checked once a week for 12 weeks, until July 26, 2016.

2.1.2.5 DATA REVIEW AND ANALYSIS

The SWCA Team tallied and recorded on data sheets the total number of collected invertebrates. Biologists used a binocular microscope to facilitate invertebrate identification in the field. Following inspection and collection, the lures were reassembled and placed back in their original sampling area. Pursuant to the terms and conditions of SWCA's scientific research permit, the SWCA Team collected living specimens of Comal Springs riffle beetles, *Stygobromus* amphipods, and water slater (*Lirceolus* sp.), and delivered them to the refugia facilities at the SAZoo.

The Spring Run 2 Study Area was vandalized repeatedly and experienced significant damage during a storm event during the early stages of the study period. For this reason, the SWCA Team determined that this site could not provide reliable results and discontinued collection at this Study Area for the remainder of the study period.

Following the field component of the study, SWCA statisticians reviewed the data and performed statistical analysis on the results. To the extent practicable, the SWCA Team conducted statistical analysis on the Comal Springs riffle beetle, the unlisted riffle beetle (*Microcylloepus pusillus*), the Peck's Cave amphipod (*Stygobromus pecki*), and an unconfirmed species of water slater (*Lirceolus* sp.). For this analysis, the SWCA Team combined the Study Areas to calculate the mean collection rates for the length of the project for each species by the eight lure types. This information was graphed with standard error used to create error bars. The four species were then combined and used to evaluate collection counts between the eight lure types and the three Study Areas over the length of the project. Additionally, each of the four species were compared individually over the length of the project.

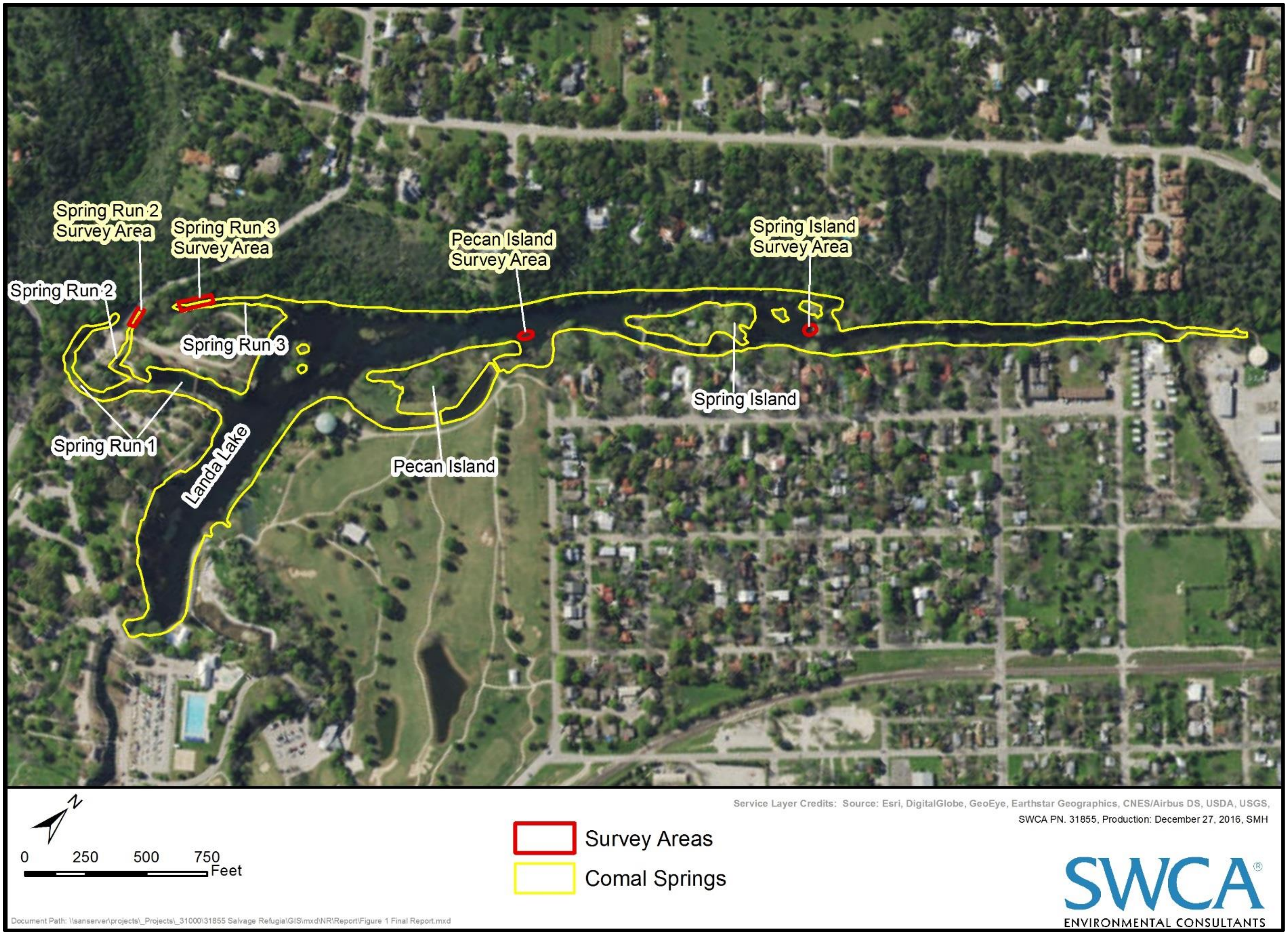

Figure 1. Aerial photographic map of the four Study Areas, Comal Springs, New Braunfels, Comal County, Texas.

Figure 2. Gas escaping from an upwelling. The released gas is largely atmospheric LBG-Guyton Associates 2004).

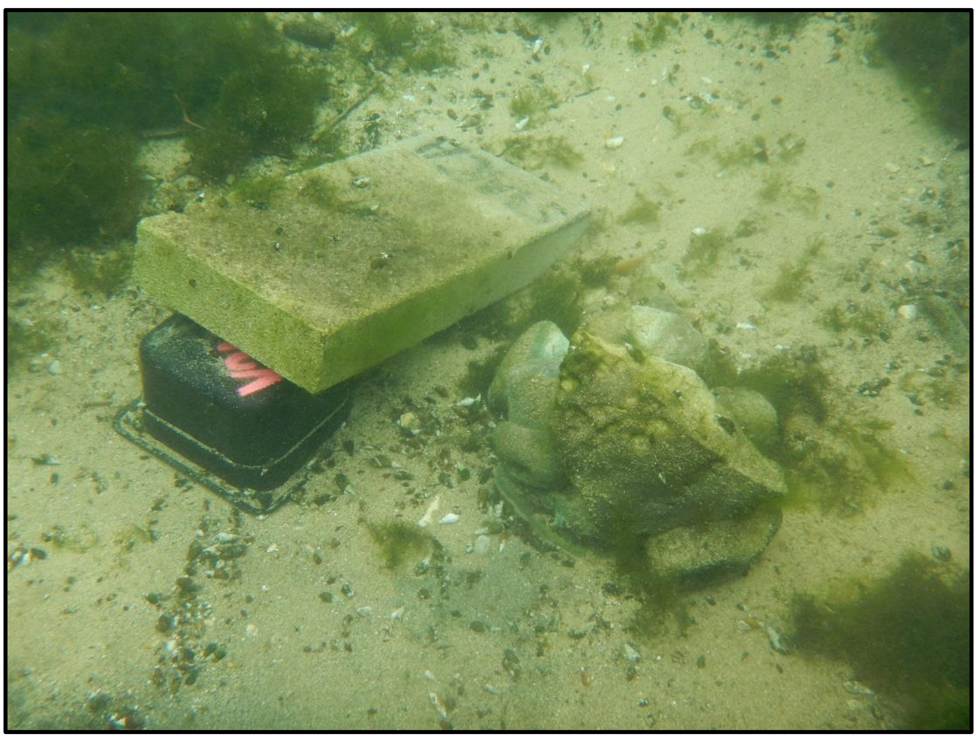

Figure 3. Bell trap (1.5-liter black plastic container) covering a lure. Bell traps are held down with cement brick. Gas released from the upwellings will be trapped within the bell trap. The water level is regulated with holes punched at the appropriate level. Portions of the lure will be both underwater and above water.

2.1.3 Results and Discussion

After 12 weeks of sampling, the SWCA Team did not collect any Comal Springs dryopid beetles from the Comal Spring system. However, the SWCA Team did incidentally collect several other species of interest to the Edwards Aquifer, including the Comal Springs riffle beetle, an unlisted riffle beetle (*Microcylloepus pusillus*), Peck's Cave amphipod, and an unconfirmed species of water slater (*Lirceolus* sp.). Though not the direct objective of the research design, collection of these species contributes to the general scientific understanding of the Edwards Aquifer ecosystem and provides insight into other facets of the refugia program and is therefore analyzed herein. Collection results for these species over the 12-week study period are presented in Figure 4.

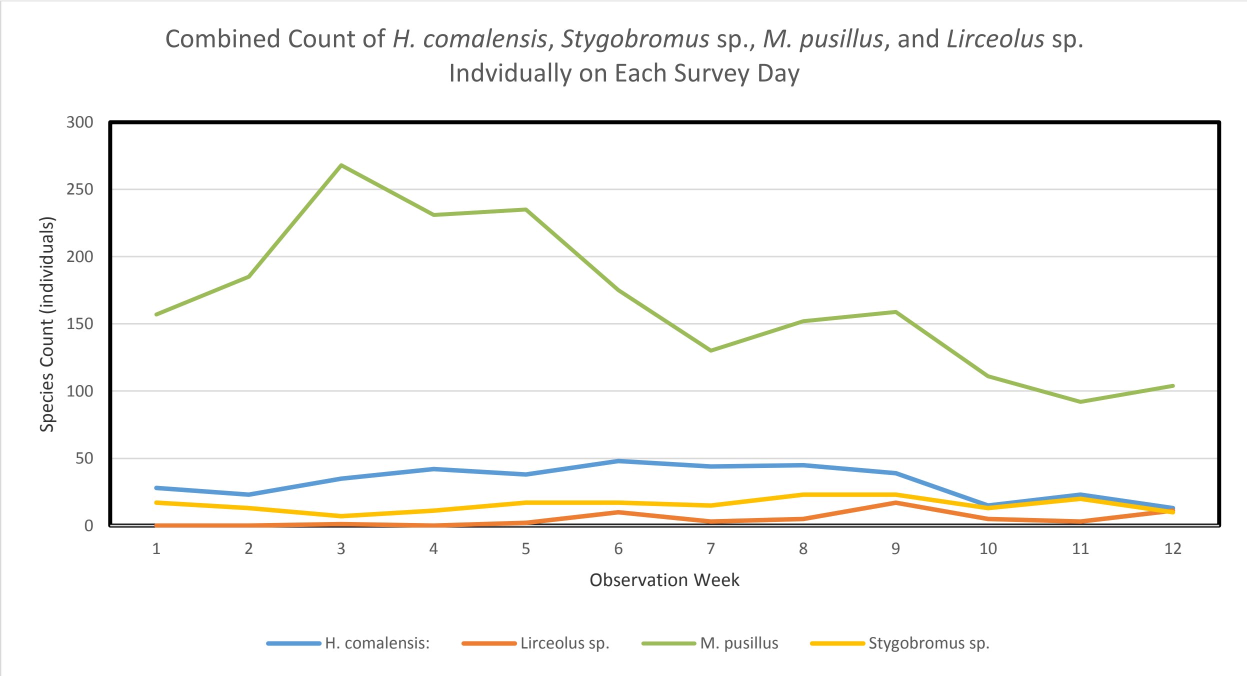

Figure 4. Count of *H. comalensis, Stygobromus* spp., *M. pusillus,* and *Lirceolus* sp. individuals collected on each survey day across the 12-week study.

While the results of this research left several unanswered questions, they did identify some preliminary trends for the handful of species that were most frequently collected. For example, the research yielded some interesting data on how abiotic factors like weather conditions affect collection success. Observations like this are directly applicable to the goals of this study (Figure 5). Predictably, the total number of species collected increased following rain events commensurate with increased springflow. The SWCA Team observed a spike in stream flow in week 3 that correlates to a spike in species collected at the same time. After June 7, 2016 (which corresponds with observation week 7), the average stream flow begins to experience a gradual decline. The total count of collected species initially rises, then experiences a rapid decline as stream flow decreases (see Figure 5).

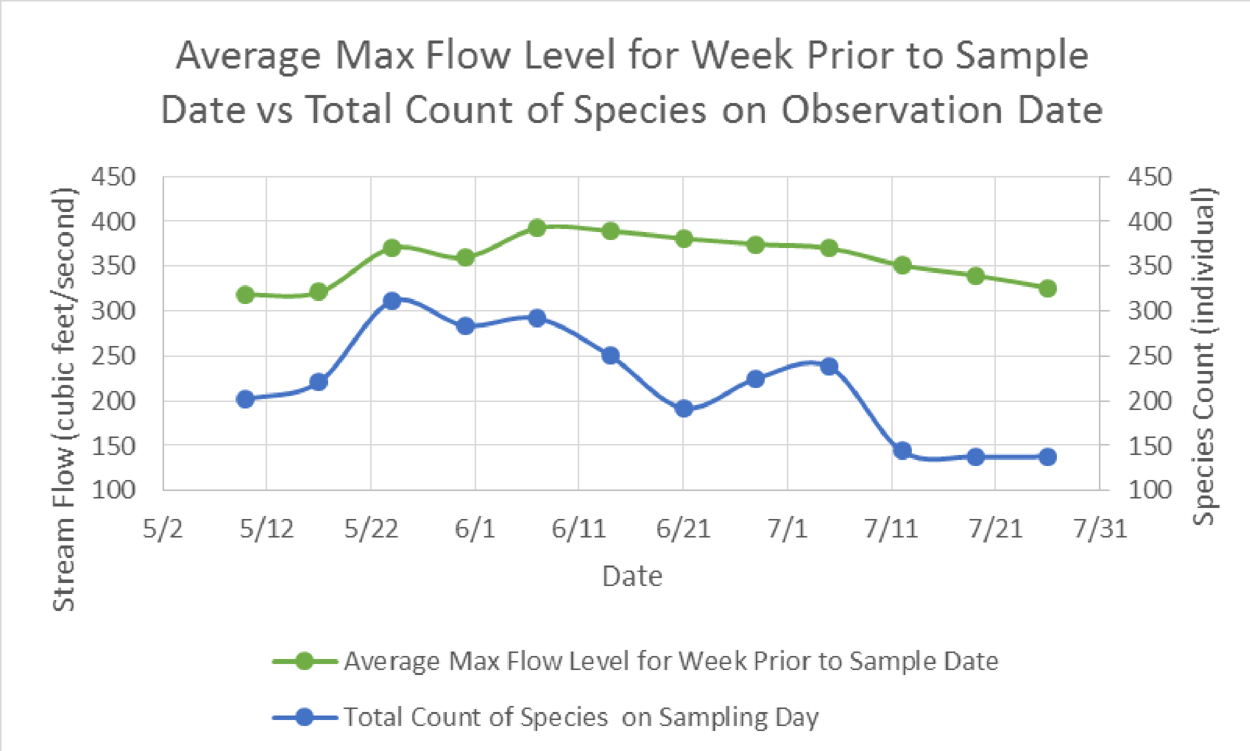

Figure 5. Average max flow at Comal Springs for the week prior to the sample date vs. the total count of H. comalensis, Stygobromus spp., M. pusillus, and Lirceolus sp. individuals collected per sample date.

To assess how flow affected collection counts on a certain date, SWCA statisticians calculated the average maximum flow for the week prior to the observation day. The average maximum flow rate was compared to the full maximum flow data set for the length of the project to ensure that averages provided an appropriate estimation of flow rates for the study period. SWCA statisticians calculated the total count of Comal Springs riffle beetles, *M. pusillus*, Peck's Cave amphipods, and *Lirceolus* sp. across all lure types and sites to identify the total number of individuals collected per observation date. SWCA statisticians then graphed the average maximum flow and the total individual collection counts for comparison and performed a regression analysis to assess if the average maximum flow accounted for trends in the total count observed.

About 24% of the variation in the total count can be explained by the independent variable of the average max flow for the week prior to the sample date as seen by the adjusted R square value. The Significance F and p-value associated with average max flow show a trend in the data toward significance (Table 2).

Table 2. Output for Regression Analysis of the Average Max Flow at Comal Springs for the Week prior to the Sample Date and the Total Count of *H. comalensis, Stygobromus* spp., *M. pusillus, and Lirceolus* sp. Individuals Collected per Sample Date

Regression Statistics	
Multiple R	0.557933875
R Square	0.311290209
Adjusted R Square	0.24241923

Table 2. Output for Regression Analysis of the Average Max Flow at Comal Springs for the Week prior to the Sample Date and the Total Count of *H. comalensis, Stygobromus* spp., *M. pusillus, and Lirceolus* sp. Individuals Collected per Sample Date (Continued)

Regression Statistics					
Standard Error	51.9198734				
Observations	12				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	12184.18412	12184.18412	4.519903926	0.059419604
Residual	10	26956.73254	2695.673254		
Total	11	39140.91667			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	
Intercept	-232.7775372	213.3021441	-1.091304254	0.300734361	
Average Max	1.264250503	0.594659736	2.126006568	0.059419604	

Note: Highlighting indicates instances where the results are statistically significant.

After the field component of the study was complete, SWCA statisticians performed multivariate regressions for each Study Area to compare abiotic factors (dissolved oxygen [DO], temperature, specific conductance, turbidity, and pH) with the total species collection count for that site. Total count was calculated as explained above. This analysis was performed to assess if any of the abiotic factors could explain the variation in the total count at that site (Table 3).

Table 3. Output for Regression Analysis of the Abiotic Factors Collected at Pecan Island on Each Sampling Day and the Total Count of *H. comalensis*, *Stygobromus* spp., *M. pusillus*, and *Lirceolus* sp. Individuals Collected at the Pecan Island Study Area

Regression Statistics	
Multiple R	0.448830232
R Square	0.201448577
Adjusted R Square	-0.464010942
Standard Error	69.99073233
Observations	12
ANOVA	

Table 3. Output for Regression Analysis of the Abiotic Factors Collected at Pecan Island on Each Sampling Day and the Total Count of *H. comalensis*, *Stygobromus* spp., *M. pusillus*, and *Lirceolus* sp. Individuals Collected at the Pecan Island Study Area (Continued)

	df	***SS***	***MS***	***F***	***Significance F***
Regression	5	7414.700995	1482.940199	0.302721009	0.894608028
Residual	6	29392.21567	4898.702612		
Total	11	36806.91667			
	Coefficients	***Standard Error***	***t Stat***	***P-value***	
Intercept	-1168.228648	1563.692164	-0.747096311	0.483243986	
DO (mg/L)	33.48065577	29.65120264	1.12915001	0.301947299	
Temperature (°C)	30.03843353	65.41861706	0.459172555	0.662275277	
Specific conductance (mS/cm)	-0.105900762	0.176854098	-0.598802987	0.571203796	
Turbidity (NTU)	-7.85037469	8.564373003	-0.916631572	0.394685229	
pH	61.88421958	307.8823449	0.200999572	0.847339529	

For the Spring Island Study Area there is a trend (though not significant) to explain up to 54% of the independent variables. The only independent variable that is significant to drive this overall trend is Turbidity (nephelometric turbidity unit [NTU]) (Table 4).

Table 4. Output for Regression Analysis of the Abiotic Factors Collected at Spring Island on Each Sampling Day and the Total Count of H. comalensis, Stygobromus spp., M. pusillus, and Lirceolus sp. Individuals Collected at the Spring Island Study Area

Regression Statistics					
Multiple R	0.866190793				
R Square	0.75028649				
Adjusted R Square	0.542191899				
Standard Error	3.479602993				
Observations	12				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	218.2708447	43.65416895	3.605506921	0.074967735
Residual	6	72.64582192	12.10763699		
Total	11	290.9166667			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	
Intercept	40.0281134	85.69197612	0.467116237	0.656898109	

Table 4. Output for Regression Analysis of the Abiotic Factors Collected at Spring Island on Each Sampling Day and the Total Count of H. comalensis, Stygobromus spp., M. pusillus, and Lirceolus sp. Individuals Collected at the Spring Island Study Area (Continued)

Regression Statistics				
DO (mg/L)	-2.14944668	1.001283259	-2.146691918	0.075450036
Temperature (°C)	1.938608106	2.487858096	0.779227766	0.465459855
Specific conductance (mS/cm)	-0.002834058	0.009560268	-0.296441232	0.776886597
Turbidity (NTU)	-1.079584471	0.332028473	-3.251481597	0.017433923
pH	-7.32704693	9.871360884	-0.74225297	0.485964821

Note: Highlighting indicates instances where the results are statistically significant.

None of the abiotic factors are a good fit for explaining any of the variation in the total counts collected at the Spring Run 3 Study Area (Table 5).

Table 5. Output for Regression Analysis of the Abiotic Factors Collected at Spring Run 3 on Each Sampling Day and the Total Count of *H. comalensis, Stygobromus* spp.*, M. pusillus, and Lirceolus* sp. Individuals Collected at the Spring Run 3 Study Area

Regression Statistics					
Multiple R	0.736966431				
R Square	0.54311952				
Adjusted R Square	0.162385788				
Standard Error	10.43837386				
Observations	12				
ANOVA					
	df	***SS***	***MS***	***F***	***Significance F***
Regression	5	777.1587739	155.4317548	1.426507487	0.335779976
Residual	6	653.7578928	108.9596488		
Total	11	1430.916667			
	Coefficients	***Standard Error***	***t Stat***	***P-value***	
Intercept	51.33880394	169.1696395	0.303475281	0.771775605	
DO (mg/L)	1.309062542	1.99003898	0.657807488	0.535069228	
Temperature (°C)	4.80127516	4.533820628	1.058990982	0.330362675	
Specific conductance (mS/cm)	-0.008600589	0.026585924	-0.323501619	0.757293726	
Turbidity (NTU)	1.009057486	0.971414356	1.038750847	0.338957543	
pH	-19.03850976	14.45470984	-1.317114627	0.235864169	

Since the study design for this research depended on historical sites for Comal Springs dryopid beetle collections, the most interesting and innovative results from this research are the differences in productivity of the various lure types. Interestingly, this initial research showed that the preferred lure type varies by species but that the bald cypress lures consistently had the lowest collection counts throughout the study. Bald cypress contains a chemical compound that prevents the growth of biofilm, which may explain the unpopularity of this lure type. The other lures appeared to have noticeable biofilm growing on them and followed a similar trend of increasing species counts over time, possibly as more biofilms formed on the lure, until peaking about halfway through the study (weeks 3, 5, and 6). After this point, collection counts generally began to decline for all lure types (Table 6; Figure 6).

The SWCA Team included cotton-cloth lures in the scientific design because this is the current standard method for capturing aquatic troglobitic beetles such as Comal Springs riffle beetles and Comal Springs dryopid beetles (Huston et al. 2015). Because neither the cotton-cloth lures nor the experimental HD lures collected any of the dryopid beetles, the SWCA Team cannot assess whether the additional lure types included in this study were significantly better or worse at capturing these species.

In conducting this study, the SWCA Team identified some confounding factors and disadvantages with using eight-plate HDs. HDs have 31% more surface area than cotton-cloth lures, which could potentially confound collection comparisons between the cotton-cloth and HD lure types. HDs also take longer to inspect for specimens. Every time an HD is inspected it must be disassembled and reassembled. The cotton-cloth lure appears to be a more efficient method for general monitoring (not necessarily for collection) of spring invertebrates such as the Comal Spring riffle beetle. Furthermore, dark-colored invertebrates are harder to see on dark-colored HD plates such as live oak and hardboard materials. However, light-colored specimens such as *Lirceolus* are very easy to see. While the SWCA Team did not collect any *Lirceolus* on cloth lures, a *Lirceolus* specimen on the light-colored cloth could have been easily overlooked, especially since these individual are relatively small.

Due to the use of bell traps in the lure design, the SWCA Team could only sample on vertical upwellings. Future studies may consider identifying a technique to similarly sample horizontal upwellings to determine if these are more productive.

Table 6. Combined Count of Species Collected throughout the 12-week Dryopid Beetle Study, by Lure Type (Regardless of Presence of Bell Trap)

Species Collected	Live Oak	Bald Cypress	Hardboard	Cotton Cloth	Total
H. comalensis	94	31	208	60	393
Lirceolus sp.	43	3	11	0	57
M. pusillus	612	99	594	694	1,999
Psephenus sp.	90	21	78	5	194
Stenelmis sp.	1	1	2	0	4
Stygobromus spp.	54	39	66	27	186
Stygoparnus comalensis	0	0	0	0	0
Total	**894**	**194**	**959**	**786**	**2,833**

Note: Labels in red are species of potential importance in the EAHCP. Highlighting indicates instances where the collection total was markedly different from the other lure types.

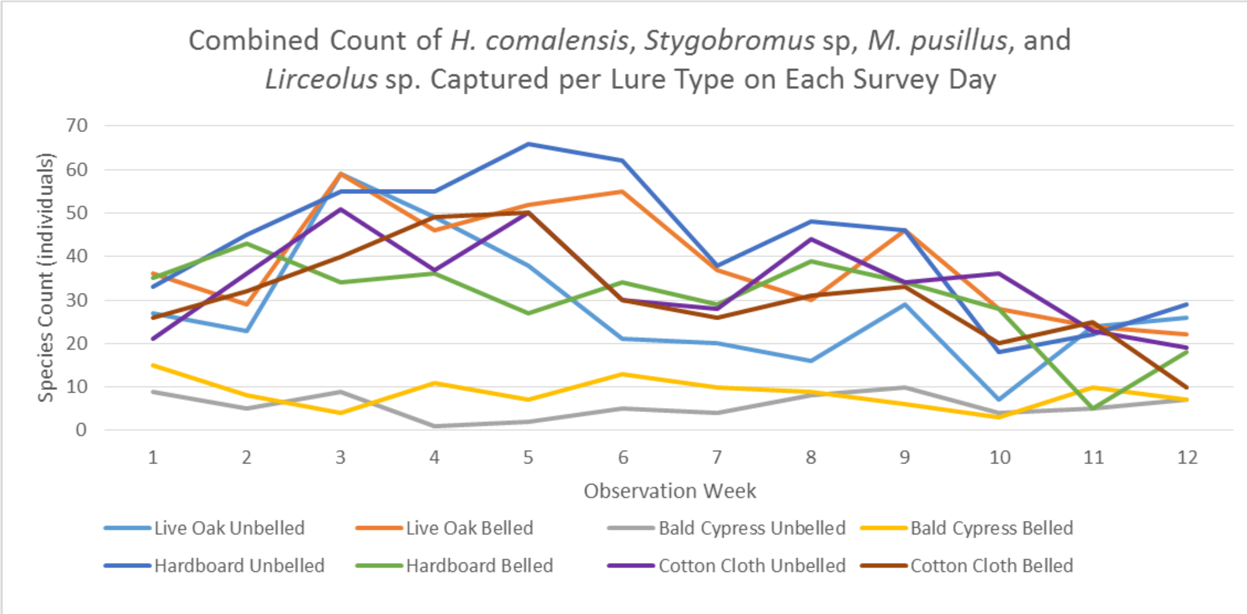

Figure 6. Count of all individuals collected at all Study Areas on each survey day across the 12-week study period, by lure type.

Unlike the lure type results, the productivity of the different Study Areas remained consistent for multiple species (Table 7; Figure 7). Specifically, the Pecan Island Study Area consistently yielded the highest collection results overall. This Study Area experienced a peak of individuals caught in week 3 that began to plateau through week 5; after sharp decline, a secondary smaller peak in collection count occurred between weeks 8 and 9, with a final decline through the terminus of the experiment. Interestingly, even though golfers use this Study Area, it still appears to maintain a high quality of habitat that is mostly undisturbed. Pecan Island has high walls and restricted public access. It is not altogether surprising that this area would have a high invertebrate diversity due to this relative isolation in comparison to the other Study Areas.

The SWCA Team limited the number of Study Areas to avoid interfering with ongoing monitoring activities that may be prime collection sites (such as along the western shoreline). For future research, sampling additional upwellings across the Comal Springs system may result in identifying more sites that consistently produce high collection rates. While it is impossible to know with any certainty if sampling at these additional upwellings would have resulted in higher collection rates or collection of Comal Springs dryopid beetle, future studies may benefit from an expanded research area.

Table 7. Combined Count of the Species Collected throughout the 12-week Dryopid Beetle Study on Each Individual Study Area

Species Collected	Pecan Island	Spring Island	Spring Run 3	Total
H. comalensis	203	29	161	393
Lirceolus sp.	53	1	3	57
M. pusillus	1629	192	178	1,999
Psephenus sp.	69	70	55	194
Stenelmis sp.	0	4	0	4

Table 7. Combined Count of the Species Collected throughout the 12-week Dryopid Beetle Study on Each Individual Study Area (Continued)

Species Collected	Pecan Island	Spring Island	Spring Run 3	Total
Stygobromus spp.	18	23	145	186
Stygoparnus comalensis	0	0	0	0
Total	**1,972**	**319**	**542**	**2,833**

Note: Labels in red are species of potential importance in the EAHCP. Highlighting indicates instances where the collection total was markedly different from the other lure types.

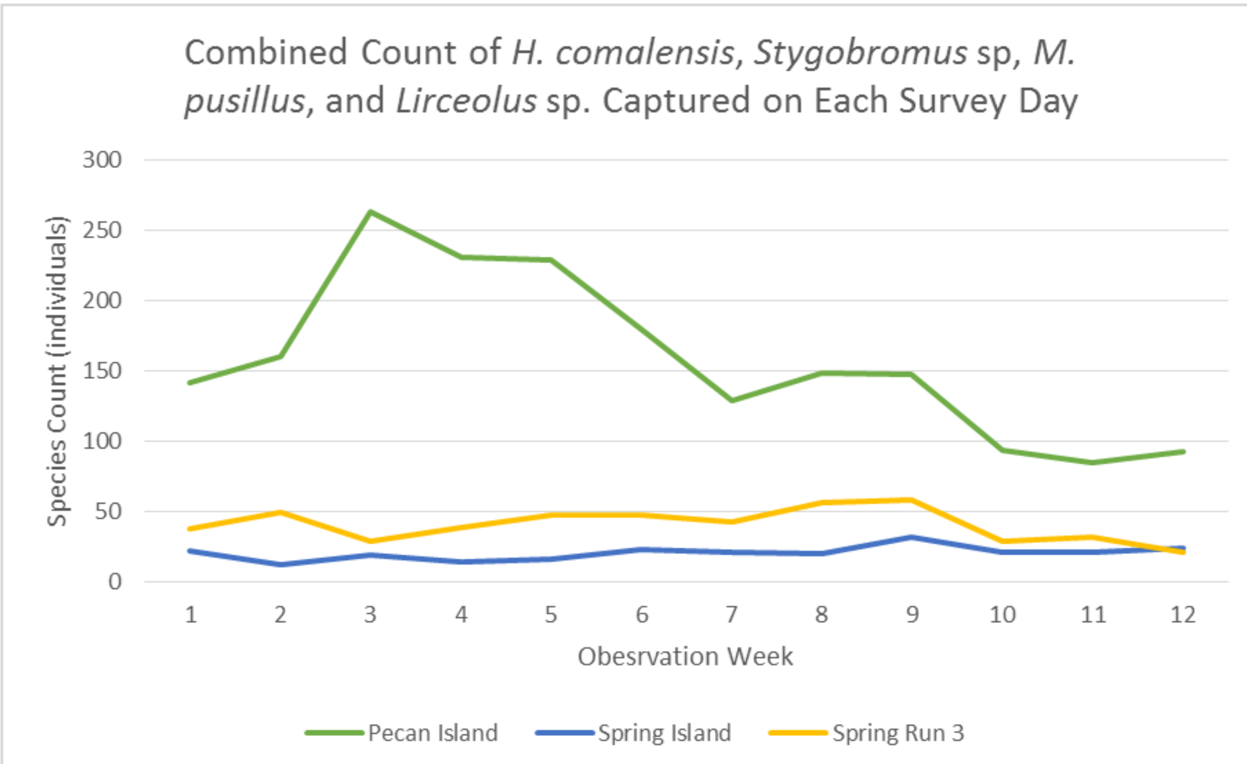

Figure 7. Count of all individuals collected on each survey day across the 12-week study period, by Study Area.

2.1.3.1 COMAL SPRINGS RIFFLE BEETLE

The Comal Springs riffle beetle is a rare species of beetle also endemic to Comal and San Marcos Springs. This species, as with the target Comal Springs dryopid beetle, is currently federally listed as endangered. This species is often associated with spring upwellings, tending to have higher abundance within a very small, approximately 20-cm radius around the upwellings (Cooke et al. 2015). The beetle is small, only obtaining a maximum length of approximately 0.2 cm (1/16 inch). Adults are flightless and lack gills. Most natural history information gathered from this species has come from in-situ observations.

Collection of the Comal Springs riffle beetle steadily increases from about 25 in the first two weeks until a maximum in week 6 of 48 individuals, after which there is a steep decline between weeks 9 and 10. Hardboard lures, regardless of presence of bell trap, had the highest average collection rate (2.89 ± 0.43 individuals/week) and the belled live oak lures captured the next highest number (2 ± 0.31 individuals/week). All of the other lure types collected *H. comalensis* at approximately the same rate, with the exception of the bald cypress unbelled-type lures, which had the lowest overall collection rates (0.2 ± 0.05 individuals/week) (Figure 8).

Notably, the hardboard lures outperformed the standard cotton-cloth lure for collecting Comal Springs riffle beetles (see Figure 8). Previous studies using cotton-cloth lures observed a decrease in collection success after the tenth week of study due to decomposition of the lure (Huston et al. 2015). Collection success with our cotton-cloth lures was fairly constant throughout our study period with a slight decrease near the study's end. HDs, while collecting more Comal Springs riffle beetles than cotton-cloth lures, also saw a decrease in collection success after the sixth week.

Based on the information collected during this 12-week study, the best way to optimize collection of the Comal Springs riffle beetle is to use hardboard lures placed at the Pecan Island Study Area.

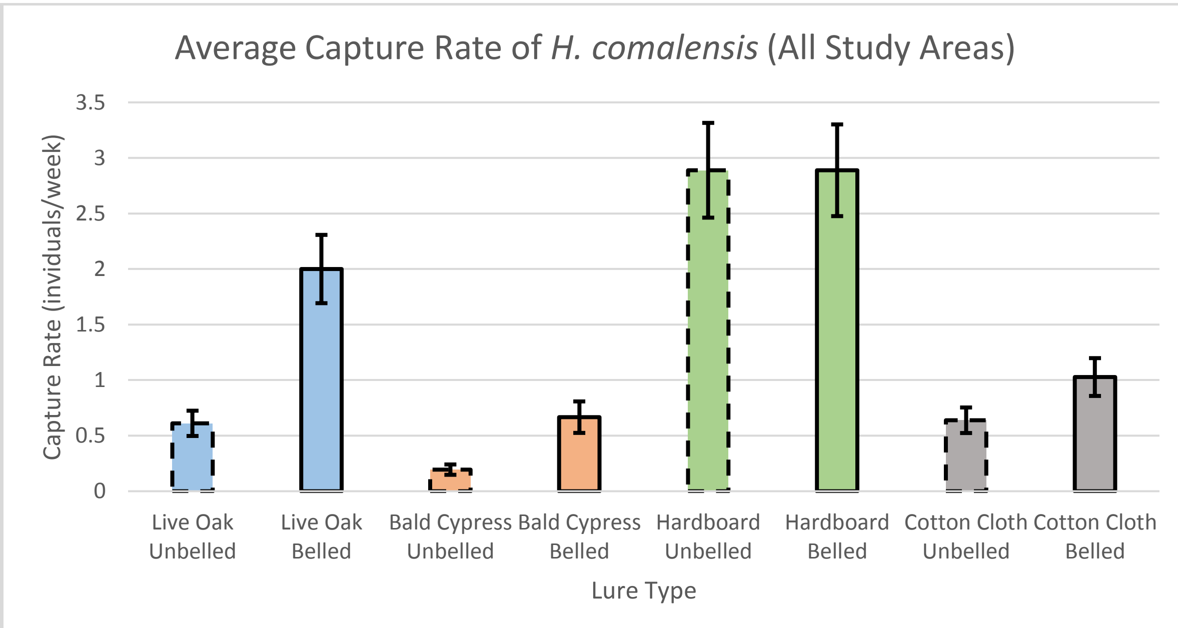

Figure 8. Average collection rate of *H. comalensis* across the 12-week study period all Study Areas combined (standard error used for error bars).

2.1.3.2 PECK'S CAVE AMPHIPOD

The Peck's Cave amphipod (*Stygobromus pecki*) is a subterranean, aquatic crustacean that is endemic to Comal and Hueco Springs (Holsinger 1967). This species is eyeless, unpigmented, with elongated appendages (Ethridge et al. 2012). However, individuals found within the Comal Springs system are orange is color (personal observation, C. Collins, SWCA). Current knowledge lends towards this species occurring in and around spring upwellings and not necessarily dwelling throughout the aquifer as previously thought. As juvenile *Stygobromus* are hard to identify to species, all blind amphipods collected during this study are referred to as *Stygobromus* spp.

The SWCA Team collected Peck's Cave amphipods at all three Study Areas. Collection counts of Peck's Cave amphipods remained relatively constant throughout the 12-week study period, ranging between 10 and 20 individuals each week. The highest number of amphipods were collected from Spring Run 3, with six times as many amphipods collected at this location than the next highest location, or 78% of the total individuals collected there (see Table 7). Of all of the species collected, the Peck's Cave amphipod is the only one to have had a higher total at the Spring Run 3 Study Area. Slight habitat differences between this Study Area and the other two may shift preference for this species to have higher densities there than the other sites that were sampled. The SWCA Team does not know what those habitat difference may be at this time. However, of the Study Areas, the Spring Run 3 Study Area has the highest spring flow rate (Norris and Gibson 2013). This may explain the larger collection rate of Peck's Cave amphipods at this Study Area.

Both belled and unbelled hardboard lures, and the belled live oak and bald cypress lures, had similar average collection rates for Peck's Cave amphipod (Figure 9). The cotton-cloth lures had a mean collection rate much closer to the unbelled live oak and bald cypress, which averaged about half that of the more productive lure types. This indicates that for Peck's Cave amphipod, HD lures, without regard to composition, and bell traps are more productive compared to the traditional cotton-cloth lure. Lures utilizing bells trap may facilitate the collection success of Peck's Cave amphipods. As this species appears to be relatively light sensitive, the dark environment provided by the bell traps may improve the collection success of this species. The average collection rates for *Stygobromus* spp. fell basically into two groups. Live oak belled, bald cypress belled, and both hardboard belled and unbelled lures averaging between 0.72 to 1.0 individuals/week. The live oak unbelled, bald cypress unbelled, and both types of cotton-cloth lures had a lower average of between 0.36 to 0.5 individuals/week (see Figure 9).

Based on the information collected during this 12-week study, the best way to potentially optimize collection of Peck's Cave amphipod is to use HD lures with bell traps placed at the Spring Run 3 Study Area.

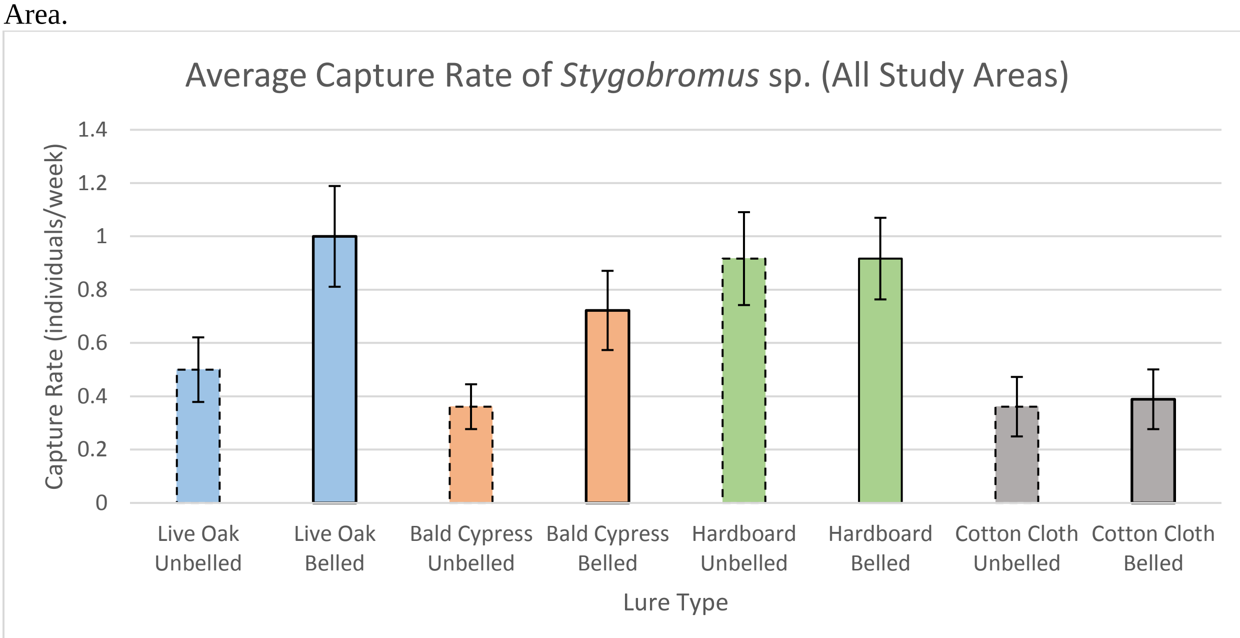

Figure 9. Average collection rate of *Stygobromus* spp. across the 12-week study period all Study Areas combined (standard error used for error bars).

2.1.3.3 WATER SLATER

Isopods in the genus *Lirceolus* are typically stygobitic. These isopods occasionally get expelled or emerge from the aquifer through upwellings and springs. Capturing these isopods could provide information on methods that are successful in capturing these and other important aquifer-dwelling species, such as the Texas troglobitic water slater (*Lirceolus smithii*).

The SWCA Team collected a negligible amount of *Lirceolus* until the sixth week of the study when collection increased to approximately five to 20 individuals per week. The SWCA Team collected *Lirceolus* isopods primarily at the Pecan Island Study Area, with a few individuals collected at the other Study Areas (see Table 7). Live oak lures collected a significant number of individual isopods, though the belled and unbelled lures had a nearly identical average collection rate (Figure 10). The cotton-cloth lures did not collect any individual *Lirceolus*; the bald cypress and hardboard lures each collected few individuals (see Figure 10).

Live oak lures had the highest average collection rates (0.58 ± 0.20 and 0.61 ±0.18 individuals/week) with a steep decline to the next highest lure type hardboard (0.22 ± 0.10 and 0.08 ±0.03 individuals/week). The bald cypress lure types collected minimal numbers of *Lirceolus* sp. and the cotton-cloth lure types caught no individuals (see Figure 10).

These findings suggest that the most efficient way to collect isopods is using live oak HD lures placed at the Pecan Island Study Area.

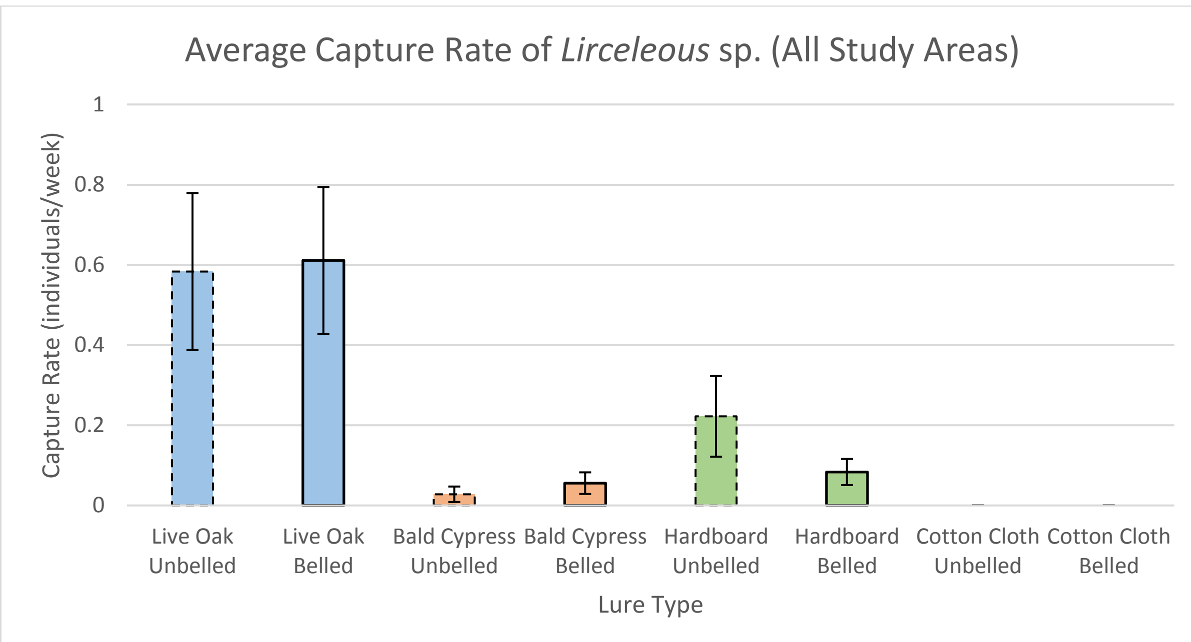

Figure 10. Average collection rate of *Lirceolus* sp. across the 12-week study period all Study Areas combined (standard error used for error bars).

2.1.3.4 RIFFLE BEETLE

A relatively common species of riffle beetle (*Microcylloepus pusillus*) is found within the Comal Springs ecosystem. This species is often collected while monitoring for the Comal Springs riffle beetles. Capturing this species will also aid in identifying the collection success of aquatic invertebrates within Comal Springs.

The SWCA Team collected approximately 1,999 individual riffle beetles (*M. pusillus*) during the study period. Collection counts for this species peaked in week 3 and declined after that, with a smaller secondary peak in the ninth week. The majority of these beetles (81.5%) were collected at the Pecan Island Study Area. The other two Study Areas had approximately the same number of total collections with approximately 200 individuals collected at each Study Area (see Table 7). There was no significant difference in collection rates among HD lure compositions with the exception of the bald cypress material, which collected virtually no *M. pusillus* regardless of presence of the bell trap (Figure 11).

The live oak, hardboard, and cotton-cloth lure types—regardless of bell trap—had similar average collection rates (6.17 ± 0.93 to 10.36 ± 1.21 individuals/week). The Bald Cypress type lures had much lower average collection rates, comparatively averaging only 1.33 ± 0.23 individuals per week without a bell trap, and 1.42 ± 0.18 individuals per week with a bell trap (see Figure 11).

These findings suggest that the most efficient way to collect *M. pusillus* is using HDs composed of live oak or hardboard or cotton-cloth lures placed at the Pecan Island Study Area.

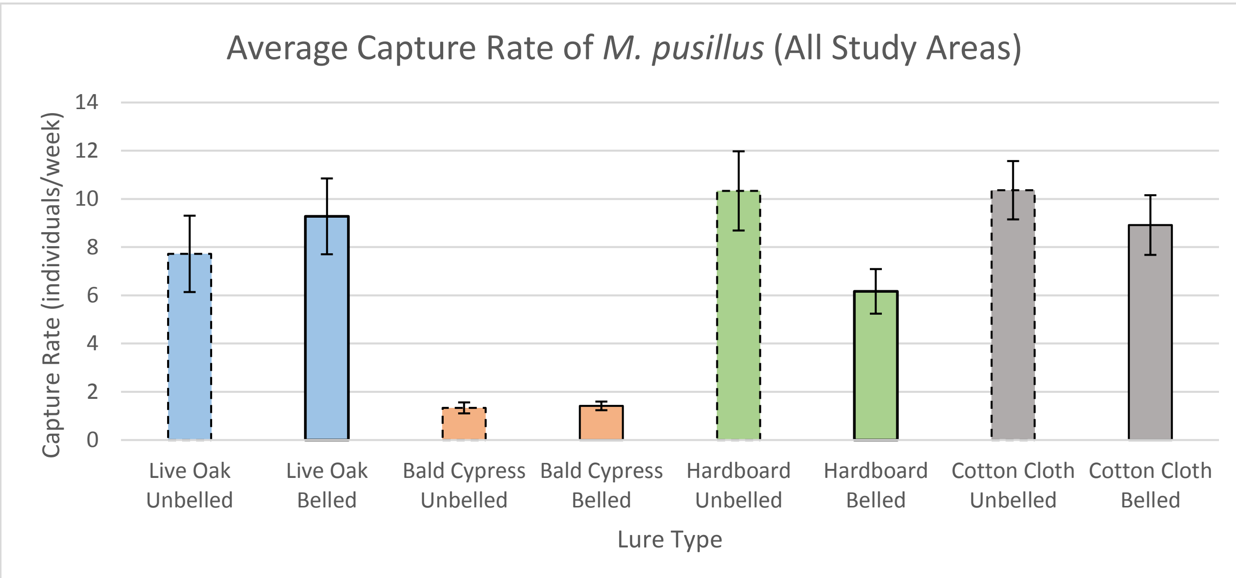

Figure 11. Average collection rate *M. pusillus* across the 12-week study period all Study Areas combined (standard error used for error bars).

2.2 Texas Blind Salamander

2.2.1 Background Information

The Texas blind salamander is endemic to underground caves within the Edwards Aquifer and has been found in Hays County, Texas (Chippindale et al. 2000; Epp et al. 2010). The natural habitat of this species is underground within the aquifer and as such is largely inaccessible for study. Known collection sites for Texas blind salamanders include features with small openings that allow limited access to the Edwards Aquifer. These conditions inherently present significant collection limitations. Features where the collection of Texas blind salamander have occurred include wells (under artesian pressure and at water table), springs, and caves of various sizes and configurations. Depending on the configuration of these features (e.g., the size of well casing or cave entrance and interior dimensions), collection methods need to be designed to specifically fit each collection site. For example, during a mark and recapture study, Krejca and Gluesenkamp (2007) collected Texas blind salamanders from Rattlesnake Cave using a telescoping net and at Ezell's Cave using SCUBA gear. The Texas blind salamander has been collected from Rattlesnake Well, Texas State Artesian Well, Federal Fish Hatchery Well, Well at Aquarena Springs, Johnson's Well, Diversion Spring, Sessom Spring, Rattlesnake Cave, Ezell's Cave, Wonder Cave, and Primer's Fissure (Krejca and Gluesenkamp 2007; Longley 1978).

Most of the available scientific knowledge of Texas blind salamanders comes from studies examining captive populations (Epp et al. 2010). Texas blind salamanders are smooth and unpigmented (a chalky, almost translucent white), troglobitic (underground/cave adapted) species (USFWS 1997). Adults tend to reach 10 to 13 cm (4–5 inches) in size. Their heads are large with wide jaws, which aid in their diet, which has been documented to include amphipods, blind shrimp, snails, and even other blind salamanders (USFWS 1996). Texas blind salamanders have vestigial eyes and long, slender fore and hind limbs. They do not exhibit external secondary sexual characteristics that allow for reliable sex determination (USFWS 1997).

Previous researchers have attempted to collect Texas blind salamanders and other aquifer-residing salamanders using dip netting, hand collection, drift netting, and various other trapping methods (Krejca and Gluesenkamp 2007; Mitchell and Reddell 1965; Uhlenhuth 1921; USFWS 2014). Researchers have used bottle traps and funnel traps to successfully collect groundwater organisms (Hutchins et al. 2010; Hutchins and Orndorff 2009; Malard et al. 2002; Purvisa and Opsahl 2005) including blind salamanders (personal observation, D. Fenolio, SAZoo, 2015).

2.2.2 Methods

Salvage triggers for the Texas blind salamander occur when springflow at San Marcos Springs falls below 50 cubic feet per second *and* when any standard or conventional water quality parameter within one of three Edwards Aquifer wells exceeds the historical range of water quality parameters by 10% or more (Recon et al. 2012). When triggers are met, it is anticipated that biologists will collect up to 500 individuals from collection sites around the San Marcos area for placement in the salvage refugia facility. In a salvage situation, it is likely that conditions will continue to decline and efficient collection methods are essential to ensure that the maximum numbers of individuals are collected. Unfortunately, none of the current collection methods effectively collect large quantities of Texas blind salamanders in a short period of time. Thus, this research attempts to evaluate bait preference by Texas blind salamanders in an attempt to improve collection success should a salvage event trigger.

The SWCA Team used standard minnow traps (40 × 22 cm with 2.5-cm openings, mesh size 0.5 × 0.5 cm) baited with either store-bought shrimp (penaeid shrimp) or pistachio (*Pistacia vera*) nuts for this study. Three sites were trapped, with one trap placed within each well/fissure site. The SWCA Team trapped at three sites: Rattlesnake Well, Johnson’s Well, and Primer’s Fissure (Figure 12). Biologists baited the traps with six pistachio nuts for six weeks, then changed the bait to a small portion of shrimp. The SWCA Team rotated bait types between pistachio nuts and shrimp every six weeks for the duration of the study. To hold the bait in the center of the trap, biologists placed the bait in pantyhose suspended in the middle of the trap with a bungee cord. Authorized biologists from SWCA and the SAZoo checked the traps for salamanders and replaced old bait with fresh bait twice a week between April 1, 2016, and December 1, 2016.

Biologists collected morphometrics on 14 of the 22 salamanders collected, including total length, snout to vent length, and head width, and swabbed a few salamanders for diseases. Thus far, no obvious diseases have been found. Pursuant to the USFWS scientific research permit, every other salamander collected was kept and transferred to the San Antonio Zoo refugia facility. The SWCA Team originally anticipated conducted a statistical analysis assessing the productivity of the bait types used, but the research did not yield sufficient results for a meaningful statistical analysis. SWCA statisticians did calculate total captures and determined the collection rates for each bait type over the study period, as shown in Section 2.2.3.

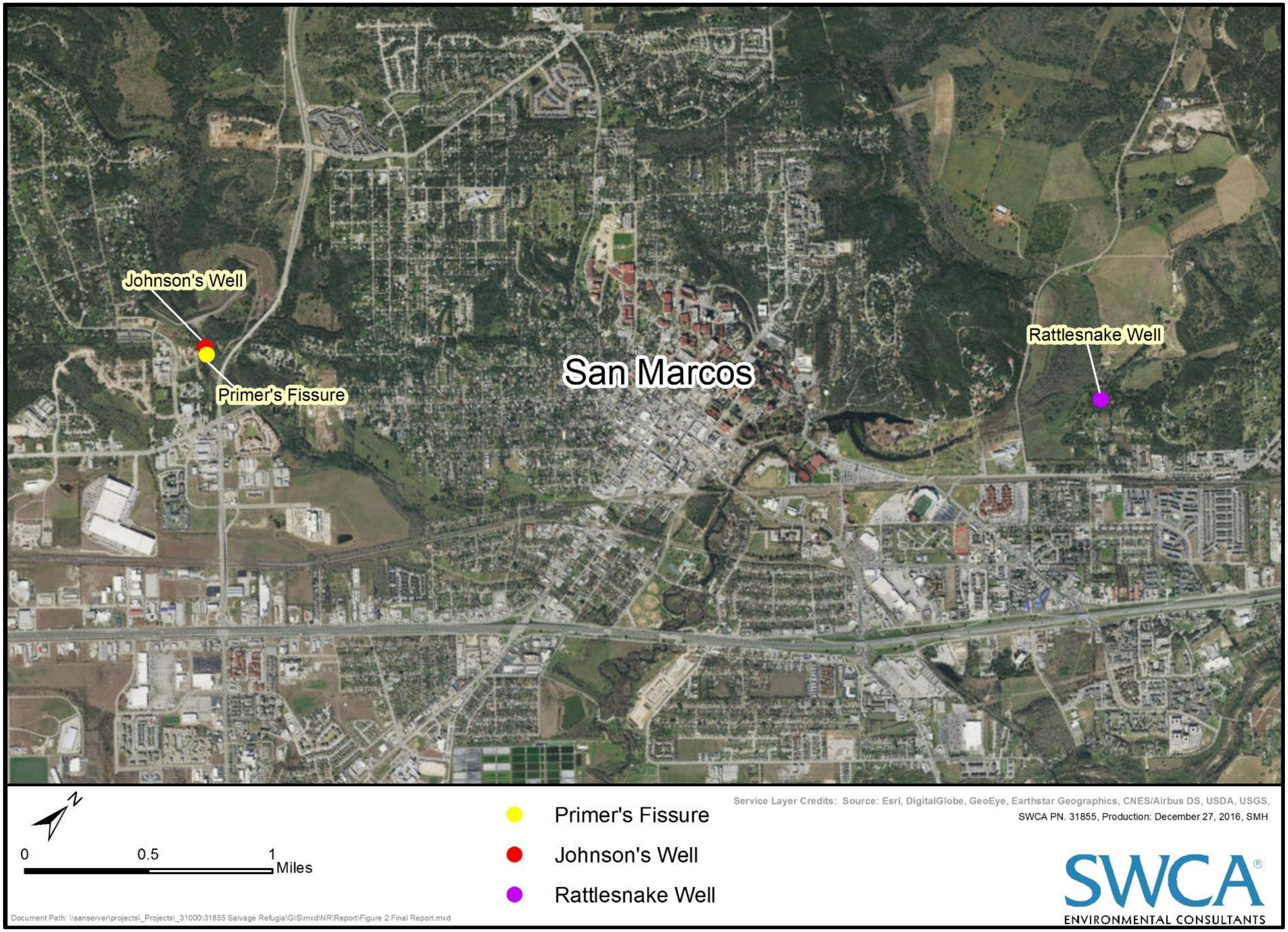

Figure 12. Aerial photographic map of the three Texas blind salamander collection sites, San Marcos, Hays County, Texas.

2.2.3 Results and Discussion

The SWCA Team collected 22 salamanders over 244 study days. Eleven of the 22 salamanders were released at the point of collection and 11 were taken to the San Antonio Zoo. Of the 22 collected Texas blind salamanders, the SWCA Team collected 14 from Johnson's Well, six at Primer's Fissure, and two at Rattlesnake Well over eight months of sampling (Table 8). Morphometrics of 14 of the 22 collected salamanders are found in Table 9.

The SWCA Team successfully collected salamanders using both shrimp and pistachios during this study. The bait types differed in collection rates at all three sample sites with the Texas blind salamanders appearing favor shrimp over pistachios. The SWCA Team stresses, however, that the number of salamanders collected (n=22) and the number of sample locations (n=3) were too small to statistically determine bait preference at this time. The collection of Texas blind salamanders is too uncommon an occurrence to determine bait preference in eight months. Adding additional sample locations and extending the sampling during will be needed to more definitively determine bait preference of Texas blind salamanders. The SWCA Team also suggests adding additional bait types including a "no bait" choice. With the current scientific design, the SWCA Team cannot know if the salamanders collected entered the minnow traps by chance or in response to the presence of the bait. Further, ex-situ bait preference studies would be helpful with whittling down bait types that would be useful in field trials. There are likely many factors other than just bait preference that affect the collection rate success of Texas blind salamanders. Aquifer flow rate, aquifer depth, and dissolved oxygen are just some factors which would fluctuate during salvage conditions. It would also be interesting to study collection rates during varied flow conditions since this study was conducting during a period of relatively high aquifer levels. It is possible that Texas blind salamanders under stress (as they would be during a drought situation) would have a different bait preference or would be easier or more difficult to collect using this method. Ex-situ experiments would be helpful in determining bait preference under a variety of controlled conditions.

Table 8. Collection Summary for the Texas Blind Salamander

Site	Total Collected	Number Transferred to SAZoo	Number Released	Date Last Collected
Rattlesnake Well	2	1	1	7/5/2016
Johnson's Well	14	7	7	12/1/2016
Primer's Fissure	6	3	3	11/25/2016
Total	**22**	**11**	**11**	

Table 9. Morphometrics of 14 of 22 Texas Blind Salamanders Collected during the Study

Texas Blind Salamander	Location	Total Length (mm)	Snout/Vent Length (mm)	Head Width (mm)
1	Rattlesnake Well	66.00	33.00	No Data
2	Johnson's Well	78.30	38.44	8.93
3	Johnson's Well	86.14	43.67	11.59
4	Johnson's Well	100.00	55.00	14.00
5	Johnson's Well	101.66	50.61	11.92

Table 9. Morphometrics of 14 of 22 Texas Blind Salamanders Collected during the Study (Continued)

Texas Blind Salamander	Location	Total Length (mm)	Snout/Vent Length (mm)	Head Width (mm)
6	Johnson's Well	86.22	46.68	11.21
7	Johnson's Well	88.00	43.00	11.00
8	Johnson's Well	80.92	43.69	10.55
9	Johnson's Well	87.50	55.00	10.55
10	Johnson's Well	87.12	44.74	10.25
11	Primer's Fissure	65.66	36.30	8.74
12	Primer's Fissure	104.00	49.00	13.00
13	Primer's Fissure	76.09	42.63	12.16
14	Primer's Fissure	57.87	31.17	8.39

Unfortunately, due to low collection numbers, a meaningful statistical analysis comparing the bait types could not be conducted. Regardless, a brief summary of the results is captured in Table 10. The two salamanders collected at Rattlesnake Well were collected with shrimp while none were collected with pistachios. Salamanders collected at Johnson's Well appeared to equally favor shrimp and pistachios; while salamanders at Primer's Fissure appeared to favor shrimp over pistachios.

Table 10. Collection Summary by Bait Type for the Texas Blind Salamander

Site	Number Collected with Pistachio Bait	Number Collected with Shrimp Bait	Days per Collection: Pistachio	Days per Collection: Shrimp
Rattlesnake Well	0	2	-	55.50
Johnson's Well	7	7	16.43	15.86
Primer's Fissure	2	4	57.50	27.75

3.0 HUSBANDRY

3.1 Lab Design

Animals collected by the SWCA Team and kept for the salvage refugia facility were transferred to the SAZoo. In 2015, the SAZoo completed construction on the salvage refugia laboratory (lab) facility. The lab units are modified temperature-controlled shipping containers with insulated, fiber-reinforced plastic–covered walls and nose-mounted Bard© Heating/cooling units. The lab facility has a back-up generator installed to supply continuous power in the event of a power failure. The SAZoo facility was designed with biological security (bio-security) as a primary objective. The entrance to each lab unit has a footbath with Peroxy 4D disinfectant, and rubber Crocs© footwear are available for biologist use inside each lab facility. The rubber Crocs, as well as lab coats, are assigned to lab units and are available for biologists working within the labs. These articles of clothing ensure that contaminants from people's clothing is not brought into the lab unit, and also prevents the transfer of any contaminants from one laboratory to another. Hand sanitizers are present in each lab. Biologists working in the SAZoo facility are required to use disposable, powder-free, nitrile gloves at all times during maintenance, and gloves are changed between each and every enclosure. Each enclosure is its own closed system to prevent potential cross contamination. Tools are used once per enclosure, then disinfected with a 20% sodium hypochlorite solution before being rinsed and dried. Some items are single use only (pipettes, airstones, etc.).

Well water from the Edwards Aquifer is pumped directly to the Conservation and Research Center at the SAZoo. To reduce the risk of contamination, the well water is stored temporarily in 100-gallon stock tanks with recirculating carbon filtration for a minimum of one week prior to use in animal enclosures. Water testing (ammonia, nitrites, nitrates, pH, hardness) are performed bi-weekly, and water changes (10%–20%) are done weekly to bi-weekly. Wastewater is collected in rain barrels, treated, and used for irrigation. Each enclosure is marked with the scientific name, sex (if known), date animal was collected, and collection location. All data, along with notable behavior, are recorded in a Samsung Note tablet provided by SWCA. Mortalities are recorded and preserved in 100% ethanol (ETOH) for transfer to the University of Texas in Austin. All mortalities are investigated immediately and water-quality tests performed.

3.2 Comal Springs Riffle Beetles

Comal Springs riffle beetles (*Heterelmis comalensis*), both adults and larvae, are kept in two ways at the San Antonio Zoo:

- 10-gallon glass aquaria filled two-thirds with well water and overflow filters (filter intakes are covered with pantyhose to prevent beetles from being drawn into filter). The return water from the filter falls over limestone rocks. Two airstones are present in the aquarium to increase oxygen levels.

- 3-gallon food-safe (Carlisle©) tubs are plumbed with sponge filters and airstones. Limestone rocks are added to the enclosures.

Both Comal Springs riffle beetles set-ups contain multiple small pieces of fabric that were cultured in Spring Run 3 for biofilms that the beetles are believed to ingest. The beetles appear to prefer the cloth pieces to the rocks in their enclosures.

At the beginning of keeping the Comal Springs riffle beetles at SAZoo, the beetles were fed three times per week in addition to having the cultured cloth present at all times. They were fed Hikari™ microwafers, spirulina flakes, and algae flakes. Feeding was reduced to once per month to avoid water contamination and waste build-up from uneaten food items.

Comal Springs riffle beetles are very small (approximately 0.3 cm [1/8 inch] in length), and often difficult to see during daily counts. On November 23, 2016, two Comal Springs riffle beetle larvae were seen on the cloth and airline tubing in one of the glass enclosures. This enclosure had only adult beetles previously.

The total mortality count for the captive population of Comal Springs riffle beetles is 38 out of 66 adults and 18 out of 19 larvae from the Pecan Island Study Area; 14 out of 15 adults and 7 out of 7 larvae from the Spring Island Study Area; 40 out of 48 adult and 7 out of 7 larvae from the Spring Run 3 Study Area, and two adults and no larvae from the Spring Run 2 Study Area (Table 11). As of December 21, 2016, there are 94 adult and 32 larvae Comal Springs riffle beetles held at the SAZoo.

Table 11. Total Mortality Count for Comal Springs Riffle Beetles, by Study Area

Study Area	Total Species Count Adults/Larvae	Total Mortality Count Adults/Larvae
Pecan Island	60/19	22/1
Spring Island	15/7	1/0
Spring Run 3	48/7	8/0
Spring Run 2	2/0	0/0

3.3 Peck's Cave Amphipods

Peck's Cave amphipods (*Stygobromus pecki*) are kept in two different set-ups at SAZoo:

- 5.5-gallon glass aquaria with overflow filters (pantyhose covering filter intake to prevent the amphipods from being drawn into the filter) contain airstones, and limestone rocks are provided as cover.

- 3-gallon food-safe (Carlisle©) tubs are plumbed with sponge filters and/or airstones, and have limestone rocks to provide cover.

Each Peck's Cave amphipod is kept singly as they are known to have cannibalistic tendencies. Peck's Cave amphipods were initially fed three times per week, which was reduced to once per week to avoid fouling the water. The Peck's Cave amphipods are fed a variety of foods: live amphipods (*Gammarus* and *Hyalella* sp.), frozen/thawed brine shrimp (*Artemia* sp.), frozen/thawed Gammarus, and Hikari™ microwafers. All food items appear to be accepted by the Peck's Cave amphipods.

The total mortality count for the captive population of Peck's Cave amphipods is three out of five from the Pecan Island Study Area, four out of 11 from the Spring Island Study Area, and 13 out of 25 from the Spring Run 3 Study Area (Table 12). As of December 21, 2016, there are 21 adult Peck's Cave amphipods held at the SAZoo.

Table 12. Total Mortality Count for Peck's Cave Amphipods, by Study Area

Study Area	Total Species Count	Total Mortality Count
Pecan Island	5	3
Spring Island	11	4
Spring Run 3	25	13

3.4 Water Slaters

Water slaters (*Lirceolus* sp.) are kept in 3-gallon food-safe (Carlisle©) tubs with sponge filters, limestone rocks, and pieces of cultured cloth. The *Lirceolus* sp. are kept in groups based on collection location. *Lirceolus* are fed once per week from the following items: live amphipods (*Gammarus* sp., *Hyalella* sp.), frozen/thawed brine shrimp (*Artemia* sp.), and frozen/thawed mysis shrimp (*Mysida* sp.).

These animals are very difficult to see in their enclosures, as they are very small, and their white coloration blends in with the white of the tub.

The total mortality count for the captive population of water slaters is 1 out 12 from the Pecan Island Study Area. None of the specimens collected from the Spring Run 3 Study Area have passed. As of December 21, 2016, there are 14 water slaters held at the SAZoo.

Table 13. Total Mortality Count for Water Slaters, by Study Area

Study Area	Total Species Count	Total Mortality Count
Pecan Island	12	1
Spring Run 3	3	0

3.5 Texas Blind Salamanders

Texas blind salamanders (*Eurycea rathbuni*) are housed in two styles of enclosures:

- 20-gallon "high" aquaria (30 × 12 × 16 inches) with overflow and sponge filters, limestone rocks, and floating plastic aquarium-safe plants. The sponge filters were disconnected, as the water was being over-oxygenated, and the salamanders started rapidly losing their gills.

- 13-gallon food-safe (Carlisle©) tubs plumbed with a sponge filter, limestone rocks, and plastic aquarium-safe plants.

Each salamander is kept singly (at the moment). Salamanders were being fed three times per week, following the San Marcos Aquatic Resources Center schedule. This has recently been modified to one feeding per week. The salamanders have the greatest variety in their diet: live white worms (*Enchytraeus albidus*), flatworms (*Planaria* sp.), amphipods (*Gammarus* sp. and *Hyalella* sp.), frozen/thawed bloodworms (Chironomidae), frozen/thawed brine shrimp (*Artemia* sp.), frozen/thawed mysis shrimp (*Mysida* sp.), and frozen/thawed gammarus (*Gammarus* sp.). Commercially available "Newt and Salamander minis," a pelleted food, is also fed.

The total mortality count for the captive population of Texas blind salamanders is two out of the seven salamanders collected from Johnson's Well (Table 14). As of December 21, 2016, there are eight Texas blind salamanders held at the SAZoo.

Table 14. Total Mortality Count for Texas Blind Salamanders, by Study Area

Study Area	Total Species Count	Total Mortality Count
Rattlesnake Well	1	0
Johnson's Well	7	2
Primer's Fissure	3	1

4.0 LITERATURE CITED

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