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FINAL REPORT: BIOLOGICAL INVENTORY AND MONITORING AT JACOBS WELL

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Abstract

Jacobs Well is the second largest water-filled cave in Texas, and presents a rare opportunity to study the Trinity Aquifer from within. Between 2009 and 2011, we performed 20 SCUBA dives and several surface surveys in order to document the aquatic vertebrate and invertebrate fauna inhabiting the site, and to determine whether more than one species of salamander exists there. Data from fish and macroinvertebrate surveys were combined with past studies to yield a total of 72 benthic macroinvertebrate taxa, 15 fish species, one salamander species, and five subterranean crustaceans. A Lepidoptera in the genus *Oxyelophila* was collected, a congener of which exists at Comal Springs, but appears morphologically distinct from the Comal Springs species. Genetic analyses are underway to determine whether this is a unique species. At least one subterranean amphipod represents a new species, which is currently being described. Another amphipod displays morphological characters that are outside of the phenotypic variability currently described for known species, but requires further examination and genetic analysis before a determination can be made. Salamander specimens were genetically analyzed within a larger species framework, and it was determined that only one species, *Eurycea pterophila*, exists at Jacobs Well.

Introduction

River Systems Institute (RSI) at Texas State University initiated a study to document the aquatic species that occur at Jacobs Well and to characterize the genetics of a population of Fern Bank salamanders (*Eurycea pterophila*) known from there. Jacobs Well is a perennial spring that forms the headwaters of the lower third of Cypress Creek near Wimberley, Texas. The spring opening is enterable using SCUBA, providing access to the second longest underwater cave in Texas, and is renowned for consistent outflow from the Trinity aquifer. The cave is inhabited by spring and aquifer-adapted fauna that could be impacted by large-scale, imminent land use changes in the watershed. The vast majority of biological collections from this site prior to this study has been from the surface, or researchers working on larger regional projects (e.g. Levine 1999, Krejca 2005, Lucas et al. 2009).

Lucas et al. (2009) collected 22 *Eurycea pterophila* for genetic analysis from the surface at Jacobs Well, and a single historic specimen reportedly collected from within the cave exhibited a morphotype and haplotype that were unique from those specimens. This prompted research into the salamander population in order to determine whether the site hosts a single, recognized species of *Eurycea* or whether there is a spring associated morph as well as a cave adapted morph present. Such cases of sympatry have been noted in other species of central Texas *Eurycea*, such as *E. sosorum* and *E. waterlooensis* from Barton Springs in Travis County (Hillis et al. 2001).

Biological surveys of the subterranean chambers and the spring outflow area (referred to throughout this document as "surface") of Jacobs Well were performed from 2009 to 2011. The Texas Parks and Wildlife Department, including Dan Opdyke, Kenny Saunders, and Chad Norris[1], along with Krista McDermid from Zara, contributed to the study by sampling the fish and benthic macroinvertebrate community during a single, intense sampling event in October 2010, and compared those data with data obtained on previous TPWD surveys. Analysis of the TPWD data included the calculation of an Index of Biological Integrity (IBI), which is a synthesis of diverse biological information that numerically depicts associations between human influence and biological attributes. It is composed of several biological attributes, or metrics, that are sensitive to changes in biological integrity caused by human activities. The multi-metric approach is used to compare what is found at a monitoring site to what is expected using a regional baseline condition that reflects little or no human impact. Indices of Biological Integrity are developed for specific geographic areas and for specific sampling methodologies. Metrics are chosen based on their consistency in responding to several types of human disturbance and can generally be grouped into three broad categories: species composition, trophic composition, and species abundance and condition (Karr et al. 1986). IBI's have been identified by the USEPA as a suitable technique for conducting biological monitoring (Plafkin et al. 1989). In Texas, IBI's are analyzed in conjunction with water quality and habitat data to classify stream segments as having limited, intermediate, high, or exceptional aquatic life.

We combined the data that we gathered during these surveys with past survey data in order to develop the most complete species list from the site to date. This baseline community assemblage data will make it possible to detect and track species and population changes at the site.

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[1] Chad Norris led the team of Texas Parks and Wildlife researchers who surveyed the site on October 7, 2010, and wrote the corresponding portions of this report.

Methods

Surface surveys

The study area for benthic macroinvertebrate and fish surveys included all of the substrate of Cypress Creek that could be accessed without SCUBA gear within an area approximately 15 m upstream and 15 m downstream of the spring opening. Because different sampling methods may detect fauna at different levels of the water column, a variety of different sampling methods were used for collecting macroinvertebrates, including the use of kick-nets, drift nets, hand-picking, and the collection of woody debris. Ideally, kick net samples were taken in a "zig-zag" pattern from the downstream end to the upstream end within each mesohabitat. Kick-net samples were either sorted in the field with organisms picked from the net as they were encountered, or the entire contents of each kicknet sample were preserved in the field in and sorted later in the laboratory. Hand-collected sampling consisted of collecting invertebrates from stones, woody debris, and other substrates as they were encountered during the course of sampling. In general, hand-collecting was performed at each site, although the extent of the effort varied according to the effectiveness of other techniques. Snag habitats were sampled by collecting woody debris and hand-collecting organisms from the woody debris. All samples were preserved individually in the field in 90% ethanol. The preservative was replaced with fresh 70% ethanol within 24-72 hours to insure proper preservation.

Identifiable mesohabitats (e.g. riffle, run, pool, rapid, glide, backwater) were sampled for fish using the most appropriate gear (seine or backpack electrofisher). Fishes collected from each mesohabitat sampled using each type of gear were processed independently. Released fish were identified, measured, and examined for disease or other anomalies. Voucher specimens were preserved in 10% formalin for identification quality control checks and were deposited at the University of Texas Natural History Collection.

Figure 1. Researchers seined the creek at Jacobs Well as one method of collecting fish species for identification. Photo by Chad Norris.

Backpack shocking focused primarily on shallow areas containing cobble or debris where this technique is commonly effective. Seines were placed downstream of the backpack crew to assist in fish collection if deemed necessary. Output settings, shocking time, and species abundance were recorded for each habitat type sampled. Seining was conducted in various habitats using a 15 ft x 6 ft seine and a variety of seining techniques were used to complement shocking efforts. Seines were constructed of 3/16" delta weave mesh with double lead weights on the bottom line and double floats on the top line. The length of each haul, personnel, and site information was recorded for each seine haul.

Analyses of samples obtained during a single sampling event in October 2010 included the calculation of diversity indices and biological metrics, incorporated into an Index of Biological Integrity (IBI), which provide a means of assessing the aquatic ecosystem health and provides a basis for comparison to past collections. For analysis of fish communities, the Regionalized IBI for Texas Streams (Linam et al 2002) was used. The regionalized IBI was used because the statewide IBI consistently underestimated the aquatic life use when compared to other methods. Additionally, the regionalized IBI contains biological criteria specific to regions of the state (i.e. ecoregions) rather than generic statewide criteria. Unfortunately, no such regionalized IBI exists for the analysis of qualitative benthic macroinvertenrate data. As such, the benthic macroinvertebrate community data was evaluated in accordance with the metric criteria used by the TCEQ.

Subterranean surveys

Dive surveys using SCUBA within the Jacobs Well took place on 20 occasions from May 2009 through June 2011 (Figure 2). These types of surveys can only be performed safely when the spring discharge is at or below 20 cubic feet per second (CFS), and heavy rains between

January and April 2010 increased the flow of the spring to levels that precluded SCUBA research during that time. In addition to SCUBA surveys, cotton cloth lures were placed within the shaft of the cave (Figure 3), following Gibson et al. (2008).

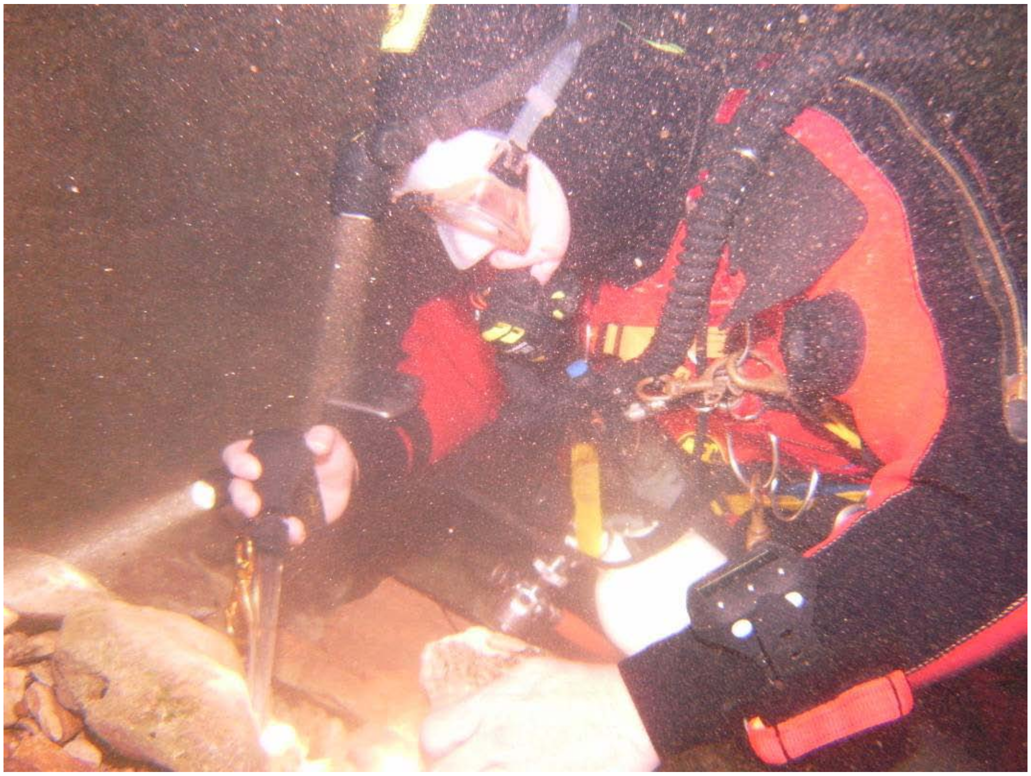

Figure 2. Diver performing a fauna survey in Jacobs Well, shown here using a baster to collect fauna.

Figure 3. Cloth lure secured to the side of the cave shaft in Jacobs Well.

The study area for subterranean surveys includes the shaft at the spring opening below about one meter depth, up to a maximum of 330 meters penetration. This includes the entrance shaft series, the vertical and horizontal constriction, through the "knife edge" to about 42 meters depth. Most dives went to about 215 meters penetration (through the deep section and back up to the 'shallow,' 30 m deep section). On several occasions this also included a portion of the "B tunnel." The majority of the survey area is shown in Figure 4.

During each sampling event, the cave was visually searched by highly trained, technically proficient divers collecting specimens using basters, dipnets and centrifuge vials. Passive trapping devices deployed inside the cave included a drift net placed at the first (vertical) constriction at about 25 meter depth and 42 meter penetration and eight salamander traps placed in the room at about 30 meter depth and 70 meter penetration (Figure 5). Traps were set in May 2009 and were checked periodically through June 2011; traps were baited with Wellness Brand Fish and Sweet Potato Formula Dog Food and weighted with two pound weights. The traps were retrieved and replaced with clean, freshly baited traps on each subsequent dive. The driftnet was installed in September 2009, removed in February 2010 for repairs necessitated by heavy rains that resulted in high discharge, and reinstalled in April 2010. The net remained in place consistently from April 2010 through the end of the sampling period (Figure 6).

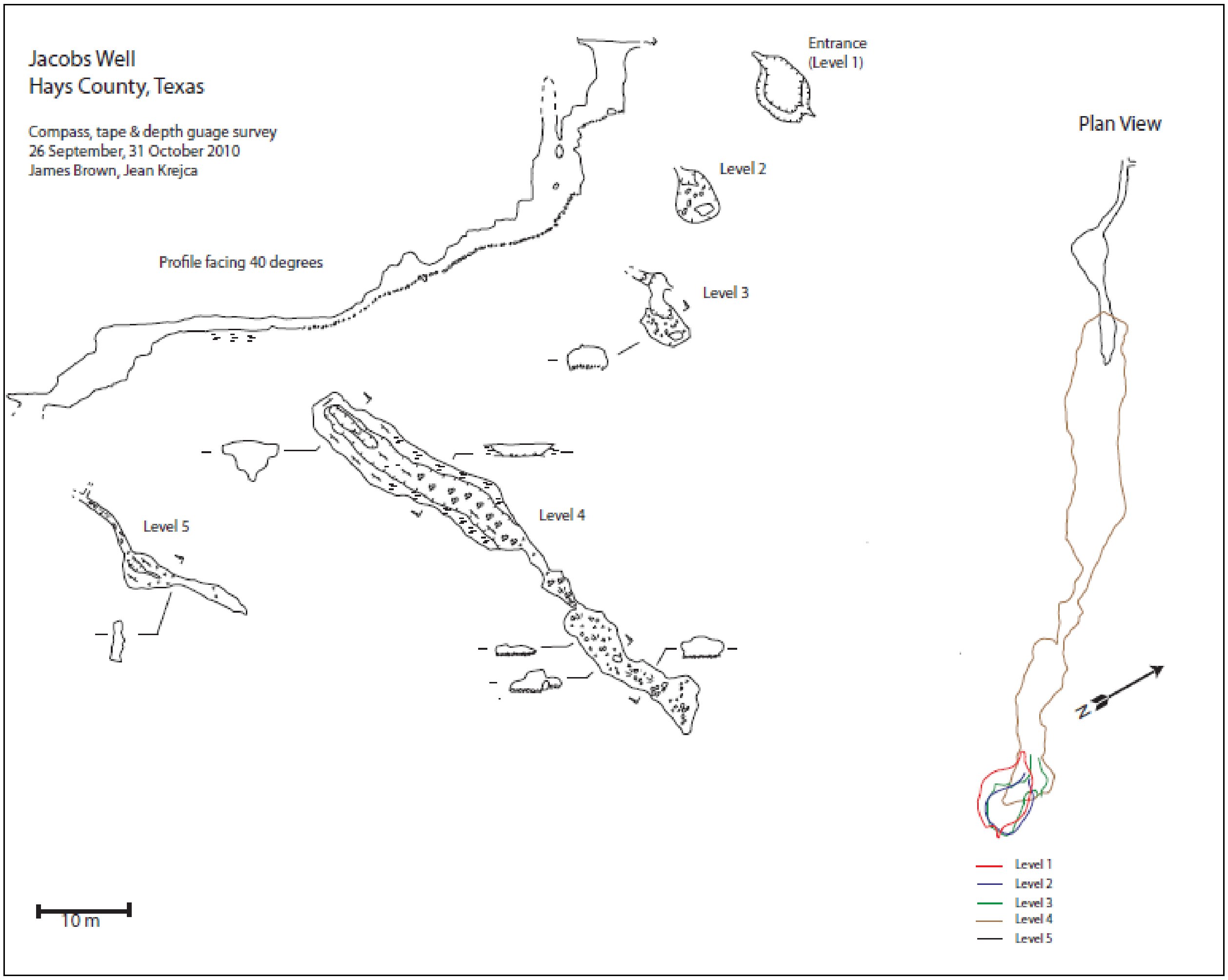

Figure 4. Cave map of Jacobs Well. Developed by Zara Environmental LLC.


Figure 5. Diver setting baited salamander traps in Jacobs Well.


Figure 6. Drift net set up at the constriction in Jacobs Well. During this low flow regime the drift net is flaccid.

Eurycea samples collected from Jacobs Well between May 2009 and May 2011 were combined with samples obtained from other central Texas springs for analysis. Samples were collected and stored in 70% ethanol at -80°C. Tissues were deposited in the Michael R. J. Forstner Frozen Tissue catalog at Texas State University–San Marcos. Invertebrate samples were preserved in ethanol immediately after collection and analyzed in the laboratory at Zara Environmental or at the USFWS Fish Hatchery in San Marcos.

To assess the phylogenetic relationships within central Texas *Eurycea*, maximum likelihood (ML, Felsenstein 1981), neighbor joining (NJ), and Bayesian analyses using mtDNA data were performed. In addition to sequences generated by us, 22 GenBank accessions were included in analyses. *Eurycea multiplicata* (GenBank AY014854) was used as an outgroup (Chippindale et al. 2000).

To assess populational relationships, a statistical parsimony network (Templeton et al. 1992) of mtDNA haplotypes was constructed. Differences in allele frequencies among sites were assessed by computing pairwise F_{ST} values with 10,000 permutations and a significance value of 0.05 and by performing Fisher's exact test of population differentiation. Isolation-by-distance was tested among individuals with a Mantel test (Mantel 1967) in ALLELES IN SPACE 1.0 (AIS, Miller 2005) with 1,000 permutations.

Results

Surface Survey Results

Benthic macroinvertebrate collections from Jacobs Well since 1999 have yielded a total of 72 unique taxa, which have been compiled in (Appendix A). Texas Parks and Wildlife Department (2003, 2005, 2010) data, including the tolerance and trophic position for each taxa, are in Appendix B. Table 1 describes the number of taxa recorded during each of six surveys at the site, however data collected by different sources are not directly comparable because collections methods varied. The only data that can be considered for year-to-year comparisons is the TPWD data, which was collected using comparable methods. Despite the apparent decrease in diversity as compared to past collections, the IBI score for Jacobs Well based on benthic macroinvertebrate collections remained exceptional in large part due to the balanced trophic structure (Table 2), which was calculated by TPWD, based on their survey data.

Table 1. Number of benthic macroinvertebrate taxa collected from Cypress Creek near Jacobs Well during six studies.

Date	Number of taxa	Source
1999	30	Levine (1999)
October 2003	34	TPWD[2]
March 2005	27	TPWD
2005	30	Dedden (2005)
2009	23	Zara (2009)
October 2010	25	TPWD

[2] Chad Norris, pers. comm.

Table 2. Index of Biological Integrity for the benthic macroinvertebrate community of Jacobs Well.

	2003		2005		2010	
	Value	**Score**	**Value**	**Score**	**Value**	**Score**
Taxa richness	33	4	28	4	25	4
EPT Taxa	13	4	10	4	7	3
HBI	5.2	2	4.4	3	4.2	3
% Chironomidae	2.2%	4	7.5%	3	5.4%	3
% dominant taxa	8.2%	4	17.2%	4	19.5%	4
% dominant FFG	37.3%	3	36.9%	3	37.1%	3
% predators	37.3%	1	20.5%	3	23.5%	3
ratio I/T taxa	1.2	1	2.9	2	2.3	2
% Trich as Hydro	0.0%	4	0	4	0	4
# non-insect taxa	7	4	6	4	6	4
% CG	27.4%	3	28.9%	3	18.5%	4
% Elmidae	0.5%	1	0	1	0	1
	High	**35**	**Exceptional**	**38**	**Exceptional**	**38**

In 2009, we collected Lepidoptera in the genus *Oxyelophila*, which has an aquatic larval stage (Figure 7-Figure 8). This genus has also been collected from Comal Springs, but the specimens at Jacob's Well may be a different species (Pete Diaz, pers. comm. 2011, Appendix C). The specimen from Jacobs Well is currently being submitted for genetic analysis.

Figure 7. Full body ventral view of Oxyelophila from Jacobs Well. Photo by Pete Diaz.

Figure 8. Abdominal prolegs of Oxyelophila from Jacobs Well, showing crochets[3]. Photo by Pete Diaz.

[3] Crochets: small hardened hooklike structure on the end of the abdominal prolegs

Fish collections were made by TPWD in 2003, 2005 and 2010, resulting in the collection of 161 individuals representing seven unique species (Appendix D). The western mosquitofish (*Gambusia affinis*) was the most abundant species, accounting for 36% of the individuals collected.

The redspotted sunfish (*Lepomis miniatus*) was the most abundant member of the Family Centrarchidae and comprised 27% of the individuals captured. Along with the longear sunfish (*Lepomis megalotis*), the redspotted sunfish were collected primarily from undercut banks with the use of the backpack electrofisher. The Family Cyprinidae (i.e. minnows) was represented by only one species, the Guadalupe Roundnose minnow (*Dionda nigrotaeniata*). In comparison to past fish collections (Fall 2003 and Spring 2005), the collection made in October 2010 was much less diverse (Table 3 and Appendix D).

A total of 14 fish species were reported from Jacobs Well in past collections, including 12 species collected in September 2003 and 11 species collected in March 2005. This level of diversity represented the most fish species collected from any single spring system sampled by the Texas Parks and Wildlife Department during that period (2003-2005). The Texas shiner (*Notropis amabilis*) was the most abundant species, accounting for 33% and 20% of the individuals collected in September 2003 and March 2005, respectively. The Texas shiner was noticeably absent from the recent collection.

The family Centrarchidae contributed the most species in past collections as six species of sunfish (Lepomis sp.) and two species of black bass (*Micropterus* sp.) were collected in 2003 and 2005. The family Centrarchidae was represented by only three species (*L. megalotis, L. miniatus, and M. salmoides*) in the recent collection, less than half the number of species collected in 2003 and 2005. Centrarchid species previously collected at Jacobs Well not collected in 2010 include the redbreast sunfish (*L. auritus*), green sunfish (*L. cyanellus*), bluegill (*L. macrochirus*), redear sunfish (*L. microlophus*), and smallmouth bass (*M. dolomieu*). Most of these species were collected in small numbers (<4) and they may still persist in Cypress Creek despite being absent from our recent collection.

The longear sunfish (*L. megalotis*), which was the dominant sunfish species in past collections, persisted within the system but was replaced in dominance by the redspotted sunfish (*L. miniatus*) (Figure 9). The longear sunfish is typical of headwater springs in the Edwards Plateau, while the redspotted sunfish is found in most drainages in Texas, including streams, reservoirs, swamps, and estuaries.

Figure 9. The redspotted sunfish is newly dominant in the fish community at Jacobs Well. Photo by Chad Norris.

The Aquatic Life Use rating (ALU) for Jacobs Well based on metric scoring of the fish community sampling performed in October 2010 was intermediate (IBI score - 38). Despite the observed reduction in species diversity, the ALU rating for Jacobs Well at the headwaters of Cypress Creek are comparable to those from past fish collections. The ALU rating for Jacobs Well based on metric scoring of the fish community was also intermediate (IBI score - 40) for samples from 2003 and 2005.

Table 3. Index of Biological Integrity scores for the fish community of Jacobs Well.

	9/16/2003		3/29/2005		10/7/2010	
Metric Name	**Value**	**IBI Score**	**Value**	**IBI Score**	**Value**	**IBI Score**
Number of Fish Species	12	5	11	5	7	5
Number of Native Cyprinid Species	2	1	2	1	1	1
Number of Benthic Invertivore Species	2	5	1	3	1	3
Number of Sunfish Species	5	5	5	5	2	3
Number of Intolerant Species	2	5	2	5	1	3
% of Individuals as Tolerant Species	3.1	5	3.9	5	0	5
% of Individuals as Omnivores	16.5	1	15.7	3	19.2	1
% of Individuals as Invertivores	80.3	5	78.4	5	76.2	5
% of Individuals as Piscivores	3.1	1	5.9	3	4.6	3

	9/16/2003		3/29/2005		10/7/2010	
Number of Individuals in Sample	130	1	51	1	151	1
Number of Individuals/seine haul	20.4	1	5	1	13.7	1
Number of Individuals/min electrofishing	1.3	1	1.6	1	1.8	1
% of Individuals as Non-native Species	3.9	1	3.9	1	0	5
% of Individuals With Disease/Anomaly	0	5	0.8	3	0.8	3
IBI Numeric Score:		**40**		**40**		**38**
Aquatic Life Use:	**Intermediate**		**Intermediate**		**Intermediate**	

Subterranean survey results

Described subterranean aquatic invertebrates documented from Jacobs Well include two fairly common subterranean isopods: *Cirolanides texensis* and *Lirceolus hardeni,* and the amphipod *Stygobromus bifurcatus.* There are anecdotal accounts from divers of the stygobitic shrimp *Palaemonetes antrorum* occurring in the site, but no specimens have been observed or collected during our study.

The *Cirolanides* was a new genus that Benedict described as a blind isopod with distinct maxillipeds and mouth-parts. The *C. texensis* is common in Texas; it has been found in 38 different sites in 14 counties. It's type locality is its original site described by Benedict, well No. 19327, U.S.N.M in Hays County. The other locations include: Bexar County – Artesia Pump Station Well; Isopit; Leon Creek Powerplant Well No. 1; O.R. Mitchell Well; Twin Pits; Verstraeten Well No. 1; Verstraeten Well No. 2. Burnet County – Longhorn Caverns. Comal County – Honey Creek Cave; Klar Well; LCRA Well; Python Pit. Crockett County – 0-9 Well. Edwards County – Devil's Sinkhole. Hays County – Artesian well at San Marcos; Ezell's Cave; Frank Johnson's Well; Marcia's Well; Wonder Cave. Kendall County – Bufo Cave. Kerr County – Boxed Spring; Stowers Cave. Medina County – Valdina Farms Sinkhole. Real County – Bonner Fallout Shelter Cave. Schleicher County – Cave Y. Terrell County – Sorcerer's Cave. Uvalde County – Carson Cave; Indian Creek Cave; McNair Cave; Rambie's Cave. Val Verde County – Unnamed spring on east side of Devils River, ca. 32 km N of Del Rio; Diablo Cave; Four-Mile Cave; H.T. Miers Cave; Little Diablo Cave; Slaughter Bend Springs.

Lirceolus hardeni has been recorded at spring outflows in five central Texas counties, including Blanco, Comal, Hays, Kendall and Travis.

Stygobromus bifurcatus has been found in eight Texas counties including Bell County at Anderson Springs and Critchfield Springs; Coryell County at Tippit Cave; Hays County at San Marcos Springs; Kendall County at Cave-Without-A-Name and Schneider Ranch Cave; Lampasas County at Sullivan Knob Cave; San Saba County at Gorman Cave, Harrell's Cave, and an Unknown Cave; Travis County at Adobe Springs Cave, Armadillo Ranch Sink, Barton Springs (Concession Spring), Jack's Joint, and Talus Spring Cave; and Williamson County at Great Mud Cave.

Two undetermined amphipod species have been collected from the site, one is *Stygobromus* sp. (near *longipes*), which is not considered a new species until genetics or more specimens with consistent morphological differences are collected (Figure 10). The cleft telson is unique and does not currently fit in any known species group (Figure 11).

Figure 10. *Stygobromus* (near *longipes*) collected from Jacobs Well. Photo by Randy Gibson.

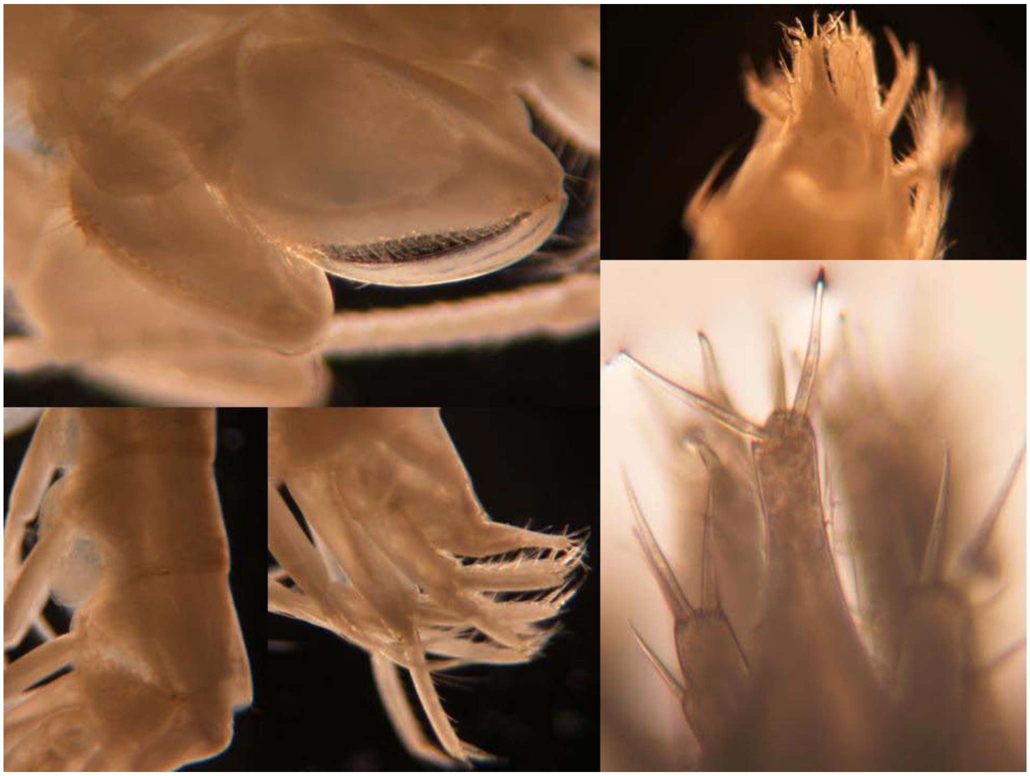

Figure 11. The cleft telson of these *Stygobromus* collected from Jacobs Well is one unique character exhibited by this population. Photos by Randy Gibson.

The other unique amphipod collected during this study is definitively a new species also known from John Knox Spring. It is extremely small as an adult, therefore difficult to see and collect (Figure 12). Images of this species preserved and in life next to a *Lirceolus* isopod are shown in (Figure 13 and Figure 14).

Figure 12 New species of amphipod showing small body size.

Figure 13. Live *Lirceolus hardeni* isopod (upper right) and *Stygobromus* new species amphipod on a decaying leaf.

Figure 14. Preserved *Stygobromus* new species.

In addition to invertebrates, *Eurycea pterophila* were observed or captured by hand during SCUBA surveys at an average rate of 0.38 per person-hour of search effort, and in the drift net at an average rate of 0.05 per million gallons of water (Table 4).

Table 4. Eurycea salamander capture rate per unit search effort in Jacobs Well. (n=number of *Eurycea*, --=no data exists)

Dive Date	Person-hours dive time	Hand captured or seen (n)	Driftnet capture (n)	Catch per hour dive time (n)	Catch per day drift time (n)	Average Discharge (CFS) during period	Driftnet Catch per million gallons of water
28-May-09	1.033333	1	0	0.967742	--	--	--
4-Jun-09	1.833333	0	0	0	--	4.25	--
13-Aug-09	1.016667	1	0	0.983607	--	2.97	--
12-Sep-09	0.916667	0	Driftnet installed			3.64	--
21-Oct-09	3.533333	0	0	0	0	4.84	0.00
5-Nov-09	2.066667	0	0	0	0	4	0.00
21-Feb-10	2	0	Driftnet removed for repair			23.75	--
29-Apr-10			Driftnet re-installed				
14-May-10	3.666667	2	5	0.545455	0.3333333	10.14	0.05
21-May-10	4	1	11	0.25	1.5714286	13.29	0.18
5-Jun-10	1.333333	1	0	0.75	0	9.31	0.00
10-Jun-10	5	0	12	0	2.4	7.32	0.51
3-Jul-10	5.933333	9	0	1.516854	0	5.55	0.00
4-Aug-10	3.166667	0	2	0	0.0625	6.14	0.02
31-Aug-10	3.666667	0	0	0	0	2.90	0.00
26-Sep-10	--	0	0	--	0	15.65	0.00
31-Oct-10	--	0	0	--	0	7.63	0.00
12-Feb-11	4.5	5	0	1.111111	0	2.64	0.00
31-May-11	2.066667	0	0	0	0	2.08	0.00
14-Jun-11	2.833333	1	0	0.352941	0	0.63	0.00
			Average:	**0.381042**	**0.2911508**		**0.05**

The samples from Jacobs Well were combined with *Eurycea* collected from seven counties, and added to already published GenBank accessions, for a total of 149 *Eurycea pterophila* individuals analyzed. The 1026-bp cytochrome b alignment resulted in 3 haplotypes being detected at Jacobs Well; all were unique to that site. Jacobs Well was different from the other locations within the clade that contains *E. pterophila* sites (Fern Bank Springs and Ott's Spring), but there was no evidence for more than a single, well supported species at Jacobs Well.

Discussion

The continued persistence of flow at Jacobs Well is threatened by increasing urbanization and the resulting demand on local resources. The factors favoring the maintenance of the area as a natural preserve are many, including the detection of one federally listed terrestrial species, a Golden-cheeked Warbler (*Dendroica chrysoparia*), that was identified by song approximately 10 meters from the spring on 30 March 2009.

Overall, analysis of the fish and benthic macroinvertebrate collections made by TPWD in 2010 display a reduction in diversity as compared to past TPWD collections. Given the historical persistence of discharge from Jacobs Well, the likely reason for the displayed declines in diversity is the cessation of flow that occurred during the drought of 2008-2009. The persistence of particular species and apparent loss of others is likely the result of differing life history requirements and adaptations. For instance, the Texas shiner was the most abundant species collected in 2003 and was absent from collections in 2010. This species is generally restricted to medium sized spring-fed streams, while the Guadalupe roundnose minnow is more common in small, headwater spring habitats. As habitat quantity and quality was reduced during the drought, it is likely conditions were unsuitable for the Texas shiner and favored the roundnose minnow. The Guadalupe roundnose minnow was the only species collected that is recognized as intolerant to water quality and habitat degradation. This minnow is endemic to the Guadalupe River Basin in Central Texas and requires clean, clear flowing water with a silt-free gravel and cobble substrate.

The overall reduction in fish species diversity suggests the headwaters of Cypress Creek have degraded as compared to the recent past. Possible causes of the apparent reduced diversity include the cessation of flow during the drought of 2008-2009 and/or flash floods that occurred in late 2007, although Levine (1999) found that surface macroinvertebrate populations had taken only two months to completely recovered from a flood in 1997. Despite the apparent reduced diversity, ALU ratings for Jacobs Well remained similar to those calculated in the past suggesting Jacobs Well has an overall Aquatic Life Use rating of High.

It is important to note that the IBI's are intended for use in streams and rivers and their applicability to use in spring and springrun habitats has not been tested. Because the overall diversity of river and stream systems generally increases with increasing stream order, IBI scores for first-order, spring fed systems would likely underestimate the aquatic life use. However, because the IBI's incorporate structural and functional attributes of the benthic macroinvertebrate and fish communities, they will still provide us with a means of comparing the biological integrity of the springs studied as well as a basis from which to compare future collections and detect changes in the biotic integrity.

Salamander samples analyzed from Jacobs Well were collected at a variety of depths, and salamanders were observed as deep as41 meters below the surface within the cave. This is the deepest recorded observation of spring associated *Eurycea* in the world.

Genetic analyses revealed no substantial genetic differences among collected specimens, regardless of depth, indicating there is only a single species of salamander at the site. The results of comparative analyses between Jacobs Well *Eurycea* and those from other sites indicate that the Jacobs Well salamanders are part of a geographically broad group of E. *pterophila* populations. When these sequences were compared against material from across Texas, it became clear that some of the species boundaries do not follow monophyletic groups, and that significantly more work will be required before the statewide taxonomy can be shown to accurately portray the underlying evolutionary relationships among the salamanders from these localities.

Jacobs Well is one of a handful of moderately large to large springs that still flow in central Texas (Brune 1975). This site was known to flow continuously through the drought of record (1956), and recorded to stop for the first time in 2000. The reduction of spring flow is a state-wide phenomenon and one that is unlikely to reverse trend. While *E. pterophila* from Jacobs Well may not represent a distinct evolutionary lineage from others in the area based on our analyses of mtDNA sequence data, they do contain genetic variation unique to the species, and as such, the site should be preserved as a discrete management unit, as suggested in Lucas (2009). This spring is unique in its large size compared to adjacent localities, and it is one of the only central Texas springs with an associated cave that is accessible enough to study the subterranean aquatic fauna in situ at depths inaccessible at other spring sites. Most central Texas spring orifices are smaller and inaccessible at depth; this humanly accessible connection to the deeper parts of the Trinity Aquifer make Jacobs Well an important and unique natural laboratory. The conjunction of all of those benefits increase the overall value of this site as a stable locality of *Eurycea* with all the attendant benefits to future research and conservation goals.

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Personnel

Jean K. Krejca, Ph.D. performed field work and edited this report. She is a biologist specializing in karst fauna. Jean has a Bachelor's degree in Zoology, and a Ph.D. in Evolution, Ecology and Behavior from the University of Texas. Her dissertation work focused on cave-adapted aquatic fauna, biogeography and hydrology of Texas and North Mexico. Since 1991 she has worked as a cave biologist and her experience in that area spans across the United States (California, Texas, Nevada, Illinois, Missouri, Indiana, Tennessee, North and South Carolina) as well as Mexico, Belize, Thailand, and Malaysia. She has published extensively on these studies. Her Texas cave biology experience started in 1997 and includes detailed collections of aquatic cave fauna for research, monitoring for endangered species, and working as a Karst Invertebrate Specialist for the U.S. Fish and Wildlife Service. In 2003 she co-founded Zara Environmental LLC, which offers land management and endangered species permit consultation in addition to conducting various research projects. She has been involved with a variety of public outreach efforts including presentations, leading field trips, and cave biology photography. She holds USFWS Endangered Species Permit TE028652-0.

Krista McDermid performed field work and wrote this report (with Chad Norris). Krista holds a Master's degree in Wildlife Ecology from Texas State University in San Marcos, where she studied the Common Musk Turtle, *Sternotherus odoratus*. She also holds a bachelor's degree in Evolution, Ecology and Behavior from The University of Texas at Austin, where she worked on behavioral and genetic development of the zebra fish, *Danio rerio.* Krista has worked as a biologist for Texas Parks & Wildlife Department monitoring white-winged dove migration and population, and the City of Austin assisting with a mark-recapture study on the Jollyville Plateau Salamander, *Eurycea tonkawae*. Krista is a GIS technician; she received her certification in ArcView 3.x in 2005, and completed the postbaccalaureate certification program in geographic information systems through Penn State University in 2010. She has worked with Zara since 2007 and in that time has participated in numerous habitat surveys for listed karst invertebrates, cave fauna surveys, karst feature surveys, presence/absence surveys and biological monitoring for listed karst invertebrates. She has also conducted aquatic macro-invertebrate habitat and presence/absence surveys for aquifer species in Hays, Bexar, Uvalde and Medina Counties. She holds Texas Parks and Wildlife Scientific Research Permit SPR-0608-082 to collect and study aquifer fauna and U.S. Fish and Wildlife Service Permit TE192229-0 to collect and study federally listed endangered Texas karst invertebrate species.

Appendix A

Benthic Macroinvertebrate taxa collected from Jacobs Well over multiple studies from 1999-2010

Order	Family	Genus	Species
Annelida			
Ephemeroptera	Baetidae	*Baetis*	*inermis*
		Callibaetis	*floridanus*
		Camelobaetidius	
		Centroptilum	
		Fallceon	*quilleri*
		Procleon	
	Caenidae	*Caenis*	
		Brachycercus	
	Ephemeridae	*Hexagenia*	
	Heptageniidae	*Stenacron*	
		Stenonema	*femoratum*
	Isonychiidae	*Isonychia*	
	Leptophlebiidae	*Neocoroterpes*	
		Farrodes	
	Tricorythidae	*Tricorythodes*	
Trichoptera	Helicopsychidae	*Helicopsyche*	*borealis*
	Hydropsychidae	*Cheumatopsyche*	
		Hydropsyche	
	Hydroptilidae	*Leucotrichia*	
		Oxytheria	
	Leptoceridae	*Nectopsyche*	
	Odonioceridae	*Marilia*	
	Philopotamidae	*Chimarra*	
	Polycentropidae	*Polycentropus*	
Odonata	Calopterygidae	*Hetaerina*	
	Coenagrionidae	*Argia*	*sp.*
		Argia	*translata*
		Enallagma	
		Telebasis	
	Gomphidae	*Erpetogomphus*	
	Lestidae	*Lestes*	
	Libellulidae	*Brechmorhoga*	
		Dythemis	
		Libellula	
		Sympetrum	*vicinum*
	Macromiidae	*Macromia*	
Hemiptera	Gerridae	*Aquarius*	
		Metrobates	
	Naucoridae	*Ambrysus*	*lunatus*
	Nepidae	*Ranatra*	
	Velildae	*Microvelia*	
		Rhagovelia	

Order	Family	Genus	Species
Coleoptera	Cirulionidae		
	Dytiscidae	*Agabus*	
		Laccophilus	
	Elmidae	*Stenelmis*	
	Gyrinidae	*Dineutus*	
	Haliplidae	*Peltodytes*	
	Hydrophilidae	*Tropistemus*	
	Psephenidae	*Psephenus*	*texanus*
Lepidoptera	Crambidae	*Oxyelophila*	
Megaloptera	Corydalidae	*Corydalus*	
Diptera	Athericidae	*Atherix*	
	Ceratopogonidae		
	Chironomidae	(Tribe: Chironomini)	
		(Subfamily: Tanypodinae)	
	Simulidae	*Simulium*	
	Tabanidae	*Tabanus*	
Decapoda	Cambaridae	*Procambarus*	*clarkii*
Amphipoda	Hyalellidae	*Hyalella*	*azteca* complex
Hydracarina			
Limnophila	Planorbidae	*Helisoma*	*anceps*
Mesogastropoda	Pleuroceridae	*Elmia*	*comalensis*
	Physidae	*Physella*	
Pelecypoda	Corbiculidae	*Corbicula*	
Hirudinea			
Tricladida			
Ostracoda			
Branchiopoda	Chydoridae		
	Daphniidae		
Copepoda			

Appendix B

Benthic Macroinvertebrates collected from Jacobs Well by TPWD in 2003, 2005 and 2010, including species' tolerance and trophic niche

Order	Family	Genus	Species	# individuals				
				2003	2005	2010	Tolerance	Trophic
Ephemer-optera	Baetidae	*Baetodes*	*inermis*		7		4	scr
		Callibaetis	*floridanus*	4	8		4	scr/cg
		Camelobaetidius	sp.	1	1		4	scr/cg
		Fallceon	*quilleri*	18	24	21	4	scr/cg
		Plauditus	sp.		2			scr/cg
	Caenidae	*Caenis*	sp.	13		7	7	scr/cg
		Brachycerus	sp.	6			3	cg
	Ephemeridae	*Hexagenia*	sp.	1			6	cg
	Heptageniidae	*Stenonema*	*femoratum*	5	7	6	4	scr/cg
	Tricorythidae	*Tricorythodes*	sp.	8	15	4	5	cg
Trichoptera	Helicopsychidae	*Helicopsyche*	*borealis*	12	7	16	2	scr
	Hydroptilidae	*Leucotrichia*	sp.	4		2	3	cg/scr
	Leptoceridae	*Nectopsyche*	sp.	3	28		3	shr/cg/p
	Odontoceridae	*Marilia*	sp.	1	5	6	0	shr
Odonata	Coenagrionidae	*Argia*	sp.	7	1	8	6	p
		Argia	*translata*		2		6	p
		Enallagma	sp.	3	8		6	p
	Lestidae	*Lestes*	sp.		1	3		p
	Libellulidae	*Libellula*	sp.	2	1	2	8	p
		Sympetrum	*vicinum*		2			p
Hemiptera	Gerridae	*Aquarius*	sp.	10	2	6		p
		Metrobates	sp.	4				p
	Naucoridae	*Ambrysus*	*lunatus*	3		3	5	p
	Veliidae	*Microvelia*	sp.	5		2		p
		Rhagovelia	sp.	8	16	9		p
Coleoptera	Dystiscidae	*Agabus*	sp.		1			p

Order	Family	Genus	Species	# individuals				
				2003	2005	2010	Tolerance	Trophic
		Laccophilus	sp.	3		2	8	p
	Elmidae	*Stenelmis*	sp.	1			7	cg/scr
	Haliplidae	*Peltodytes*	sp.	4		3	8	shr/p
	Psephenidae	*Psephenus*	*texanus*	7	17	19	4	scr
Diptera	Athericidae	*Atherix*	sp.		2	1	4	p
	Ceratopogonidae			1			5	p/cg
	Chironomidae	Chironomini		4	18	12	6	p/cg/fc
		Tanypodinae			2		6	p/cg/fc
Decapoda	Cambaridae			3	13	3	5	cg
Amphipoda	Taltridae	*Hyallela*	*azteca* complex	12	27	43	8	cg/shr
Hydracarina				8		6	6	p
Limnophila	Planorbidae	*Helisoma*	*anceps*	2		2	7	scr
Mesogastropoda	Pleuroceridae	*Elimia*	*comalensis*	9	46	29	2.5	scr
	Ampullaridae				1			scr
	Physidae	Physella sp.			1		9	scr
Hirudinoidea				7			8	p
Tricladida	Planariidae	*Dugesia*	sp.	3	3	6	7.5	p
			# ind	182	268	221		
			# taxa	34	28	25		

Appendix C

Personal Communication from Pete Diaz, re: Oxyelophila

From:	Pete_Diaz@fws.gov
To:	krista@zaraenvironmental.com
Subject:	Oxyelophila
Date:	Thursday, June 16, 2011 8:48:49 AM
Attachments:	TWAP_Invertebrates_v4.0.xls

Hey Krista,

The Lep from Jacobs Well keys out to Oxyelophila. The species from Comal Springs is on the state list of species of concern (I attached it). To my knowledge, this species is only recorded from Comal Springs. The specimen from Jacobs Well looks like it is in the same genus, however it may be a different species. The gills on this specimen go to at least segment 8 dorsally. Other specimens that I have collected don't seem to have that many segments covered with gills like that. This genus differs from Petrophila in the fact that the body shape is more rounded, there are dorsal gills, and the head is hypognathus (directed downwards).

I am in the process of sending the specimen off for DNA analysis. I have others from other springs. I believe this to be a spring associated genus. I don't have any empirical data, I have just caught specimens only from spring runs. I would like to get back to Jacobs Well and search for some more. It would be good to go sooner than later (this month or next). If you can't go can I get the contact for the area so that I can get in there. I would also like to run our toxicity study by them to see if I can get permission to collect 3 or 4 sallies from the springs for analysis.

Hopefully the DNA analysis will help with the species part of the identification. There aren't any keys available, and the larval stages of the species from Comal, Oxyelophila callista haven't even been described. I have been making collections from the area, but the species is rare and tough to find even in its type locality. The key in Merritt and Cummins was based off of South American Oxyelophila which are more common in that region. I am working with Alma Solis who helped write the chapter in the newest M&C.

Very Cool,

Pete

Pete Diaz
Texas Fish and Wildlife Conservation Office
U. S. Fish and Wildlife Service
500 East McCarty Lane
San Marcos, Texas 78666
(512) 353-0011 ext 235
www.visualtaxonomy.com

Appendix D

Fish species collected from Jacobs Well by TPWD in 2003, 2005 and 2010, including species' tolerance and trophic niche

Tolerance	Trophic	Genus species	Common Name	2003	2005	2010
I	O	*Dionda nigrotaeniata*	Guadalupe roundnose minnow	21	8	29
	IF	*Notropis amabilis*	Texas shiner	42	10	
	IF	*Astyanax mexicanus*	Mexican tetra	1		2
T	IF	*Gambusia affinis*	Western mosquitofish	31	9	58
	IF	*Lepomis auritus*	Redbreast sunfish	5	1	
T	P	*Lepomis cyanellus*	Green sunfish	2	1	
T	IF	*Lepomis macrochirus*	Bluegill	2	1	
	IF	*Lepomis megalotis*	Longear sunfish	6	9	14
	IF	*Lepomis microlophus*	Redear sunfish	1		
	IF	*Lepomis miniatus*	Redspotted sunfish		4	43
	IF	*Lepomis sp.*	Sunfish sp.	4		
	P	*Micropterus dolomieu*	Smallmouth bass		1	
	P	*Micropterus salmoides*	Largemouth bass	2	1	7
I	IF	*Etheostoma lepidum*	Greenthroat darter	2		
	IF	*Etheostoma spectabile*	Orangethroat darter	11	6	8