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FINAL REPORT FOR DEEP AQUIFER BIOTA STUDY OF THE EDWARDS AQUIFER

Prepared for:

Edwards Aquifer Authority
1615 N. St. Mary's Street
San Antonio, TX 78215

30 December 2010

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Executive Summary

From blind leeches to eyeless sucker-mouthed catfishes that live so far below San Antonio that they are actually below sea level, the biodiversity of the Edwards Aquifer is world-ranking and world renowned. These species are primarily known from the fresh water/saline water interface in this karst aquifer that provides water to millions of people, and they are essential components of that ecosystem. In an effort to improve our understanding of species' ranges and obtain new material from the deep portions of the Edwards Aquifer, biologists visited and contacted over 75 landowners in five counties, obtained permission to sample for biology, and then sampled 43 wells between 2008 and 2010 for aquifer organisms.

Outcomes of this project included:

- mapping, visiting and attempting to sample all known historic localities for the blind catfishes *Trogloglanis pattersoni* and *Satan eurystomus*
- collecting specimens and adding new locality records for both species of blind catfishes, which have not been documented since 1978
- obtaining samples and coordinating with laboratories to gather the first ever genetic analyses of blind catfish
- discovering 20 new localities for aquifer crustaceans
- documenting an entire order of fauna (Bathynellacea) previously unknown in the Edwards Aquifer
- discovering a species of copepod previously unknown to science, (*Diacyclops* sp.) from two sites
- obtaining samples used for organic carbon analysis
- obtaining samples used for stable isotope analysis
- developing an in-line sampler design for situations where water delivery from well casing to outlet is in an entirely closed system
- performing a sampling effort equal to or greater than any prior sampling effort in this system

The great successes itemized here are the result of careful landowner relations, close collaboration with the Edwards Aquifer Authority, and extensive collaboration with taxonomists, geneticists, and other scientific specialists. Based on our improving catch per unit effort during these three years, future work should include additional sampling at known localities and exploration and refined sampler placement at new localities. Because samples are sparse, we recommend continued evaluation of priorities for each specimen and collaboration with specialists in taxonomic and ecological studies.

INTRODUCTION

Project Logistics

The Edwards Aquifer contains a diverse array of stygobitic[1] fauna that, due to the general inaccessibility of the habitat, is rarely available for scientific research. The Edwards Aquifer Authority (Authority) awarded a contract to Zara Environmental LLC (Zara) in December 2007 to study the occurrence and distribution of aquatic animal life in the deep zones of the Edwards Aquifer (Contract No. 07-326-AQ). Subterranean aquatic habitats can sometimes be accessed through water filled caves within the recharge zone; however, these submerged passages are often too small and/or treacherous for human researchers to explore. Deeper portions of the aquifer are able to be accessed only through water wells, which have been likened to "windows to the aquifer." The objectives of this study were to compare the historic and present abundance and diversity of aquifer organisms by replicating and expanding on the work done by Longley and Karnei (1978a, 1978b), to make samples of aquifer fauna readily available to scientific institutions for study with modern techniques for genetic and isotopic analysis, and to sample the traditionally under-represented fauna of the deep aquifer. This report presents all of the findings and serves as fulfillment of the aforementioned contract.

A significant amount of effort has been invested in establishing and maintaining landowner relationships that provide not only access to wells on their land, but also offer valuable historical perspective on their sites (e.g. history of drilling, blindcat sightings, water levels), as well as providing information about other potential sampling localities. While requests to sample San Antonio Water System (SAWS) sites were denied, many private landowners in southern Bexar and Medina Counties have been receptive to the study and allowed access to sample their irrigation wells. Irrigation wells provide the best opportunity during this study to sample large volumes of water that are being drawn from the aquifer at a rate high enough to "vacuum" deep dwelling fauna.

The positive reaction of landowners by allowing access to their wells was greatly aided the by Authority granting an exemption to their permit limits for allowing sampling of their wells. This exception was granted using a "well letter," which constitute an agreement between the Authority and the landowner to exempt them from overuse penalties for water used in this study. The use of irrigation wells for sampling often restricts sampling to particular months of the year, however some landowners have agreed to flow their wells more consistently throughout the year as long as there is no permit limit penalty while sampling is occurring. The incentive of permit limit exemptions has spurred some landowners to invest in building custom samplers that divert flow from primary pipes for sampling, and then reintroduce the flow to the main pipe. An alternative to that custom sampler is the "in-line" sampling device that allows a net to be installed and accessed directly through a hatch in the irrigation line.

Project Development

[1] Underlined words are defined in the Glossary at the end of this document.

The kickoff meeting for this project was held on January 24, 2008, and sampling began on February 6. The bulk of the time dedicated to this project during the course of the first year was spent following "leads" to well sites, i.e. talking to landowners, pouring over well databases, talking to Authority personnel, SAWS and various other municipal water authorities to find out where wells are located, which wells might be appropriate, and what landowners might be amenable to sampling or to sharing information about local wells. Following leads and arranging site access, including the development and implementation of well letters, constituted much of the time spent working on this project during the period between January and October 2008.

About 20% of the effort during the first year of the project was spent arranging sampling infrastructure. The arrangement of sampling infrastructure involved site modification (debris removal, slight adjustment of well morphology to allow for sampling, creation of shade structures), the development of data sheets, personnel and vehicle arrangements for sampling, sampling equipment preparation and maintenance, and locating and hiring reliable taxonomists. Each of these steps was important for ensuring the success of the project, and for increasing sampling efficiency during the following years.

The actual amount of sampling that took place was lowest during the first year of the project compared to following years, as a result of increased sampling efficiency during subsequent years of the project (Table 1). During the second year of the project the majority of the sampling sites had been identified, but even then a portion of time spent sampling added to the knowledge base of what constituted a worthwhile site and what didn't. It was determined that to maximize efficiency and effectiveness sites should be easily accessible, productive (i.e. had potential for producing fauna based on available data about the water chemistry, history of fauna at the site, or amount of water available to sample), and the length of time a landowner or manager allowed a well to run continuously through the net.

Table 1. Approximate time-use break down (in percent) of project effort during sampling years.

Activity	**Percent Year 1 Effort**	**Percent Year 2 Effort**	**Percent Year 3 Effort**
Site access arrangements (and following "leads")	40	15	5
Infrastructure construction	20	25	10
Sampling, sorting and curation	30	50	70
Data analysis and reporting, meetings, administration	10	10	15
Total Effort Expended for Sampling Year	100	100	100

Sample Location Criteria

Sampling locations were prioritized based on their proximity to sites that have been historically reported to have blind catfish or invertebrate fauna, with an emphasis on sites that have artesian flow. Sites within 5 km of the saline water-fresh water interface, where the blindcat taxa are expected to occur, were also given priority. Sites that have been heavily sampled by researchers in previous studies (e.g. Hueco Springs, Comal Springs, San Marcos Springs, and Ezell's Cave) were excluded.

Well names were assigned for ease of reference, often based on the owner's name, but in most cases the owners and/or the Authority did not have a common name for the well, and wells can change ownership over time, so these names should not be considered official. The data sheets that were used during site visits include the location, site description,

description of sampling method used, temperature, checklist of photos, and also a place to record fauna seen in the field. Note that while very basic water chemistry data had a place on the datasheets, we did not always have access to water chemistry sampling equipment; water chemistry data should be obtained directly from the Authority. We explicitly recorded all data related to specimen captures and preservation to allow for maximal utilization of the specimens and repeatability of monitoring beyond the duration of this project.

Edwards Aquifer Fauna Sampling

Obligate subterranean fauna are typically understudied due to the general inaccessibility of their habitats, leaving a wealth of species undiscovered. As new ways to study these habitats are developed, the body of knowledge about these species is increasing. The number of known subterranean species in the United States and Canada more than quadrupled between 1960 and 1998, increasing from just 334 in 1960 to 1353 over the course of 38 years (Peck 1998). Stygobitic fauna is especially understudied, being represented by only 425 species at the time of Peck's (1998) research. Of these, Texas contains more than twice the number of stygobitic species recorded in any other state, with at least 42 known stygobites. The state with the second highest number of recorded species is Missouri, with only 13 (Hershler and Longley 1986, Peck 1998).

Researchers have conducted few investigations into the subterranean biota of the Edwards Aquifer, and most existing research addresses species associated with spring outflow (e.g. Gibson et al. 2008, Holsinger and Longley 1980). Less well known are the fauna that occupy the interior zone of the aquifer that is usually inaccessible. The typically small stygomorphic invertebrate fauna that inhabit these deep zones, like crustaceans (ostracods, copepods, amphipods, shrimp), and planaria have adapted to their unique habitat constraints such as a complete lack of light (and hence a lack of primary production) and a high pressure environment. The small size of many of these species allows them to easily inhabit the interstitial spaces of the aquifer that are not accessible to larger animals, such as subterranean fishes. The last intense study of the deep portion of the Edwards Aquifer was done by Karnei (1978) and yielded 16 stygobitic invertebrates, six of which were undescribed at the time. To date there are over 40 described species inhabiting the aquifer (Longley 1986, Gibson et al. 2008).

The focus of aquifer-related research on vertebrates has centered on salamanders of the genus *Eurycea*, which are typically collected from caves and springs along the Balcones Escarpment. The rarest of these, *Eurycea robusta*, has not been collected since the type material discovery in 1951 (Potter and Sweet 1981). This individual was collected from beneath the bed of the Blanco River between Kyle and San Marcos, Texas. Only one specimen exists, and none have been found since.

Two other aquifer-dwelling vertebrates are the widemouth blindcat, *Satan eurystomus*, and the toothless blindcat, *Trogloglanis pattersoni*. The populations of blind catfish that inhabit the San Antonio pool of the aquifer have seldom been sampled. These are the only two species of blind catfish in the United States, and the last published collection of either species was over 30 years ago, when Karnei (1978) collected them from wells in the San Antonio area. Prior to this study, these were the only catfish in the world for which a genetic analysis had not been conducted, so their relationship to other species had not yet been clearly defined. Obtaining access to the blind catfish collection sites in Karnei (1978) proved difficult and largely unproductive, as most of the sites no longer exist or are not accessible.

METHODS

Locating Sample Sites

Between the years 2007 and 2009, details regarding well locations were acquired for numerous sites, including those that were not suitable for this study at the time, but that might prove useful to future sampling efforts. These details were accumulated through many hours of dialog with various local water authorities, landowners, geologists, other scientists and university professors. The information that we have obtained from these sites has been compiled from field notes and is presented in **Error! Reference source not found.**.

Establishing Landowner Agreements (Well Letters)

The participation of landowners was essential to the success of this study. Well permit limits are set by the Authority, and penalties are imposed for overuse if a landowner draws more water than their permit allows. The use of permit limit exemptions has enabled Zara scientists to sample wells without subjecting the permit holding landowner to penalties for exceeding their permit limit when the study required more water than the permit allowed. An example of a well letter and associated meter reading form can be found on the following pages.

1.5-12.1-1a

April 28, 2009

Name of Landowner
1234 Mailing Address
San Antonio, TX 12345

Dear Mr. Landowner:

Thank you for participating in the Edwards Aquifer Authority's aquifer biota study being conducted by Zara Environmental. This study is being conducted to evaluate the distribution of aquifer-dwelling species, and the research could not be completed without the assistance of well owners like you.

Through the course of the study, Zara Environmental will withdraw groundwater from your well for sampling purposes. To ensure you will not be charged an aquifer management fee for the water used as part of this sampling process, Zara Environmental will confirm with you the readings on your meter at the time they begin their sample and when they conclude. You will be asked to sign two forms confirming these readings. You will keep one of the forms and we will keep the other form in our files. When you report your annual groundwater use for 2009, please indicate on the report how much water was used by Zara Environmental and reduce your use accordingly.

If you have any questions regarding the reporting process, please contact Mr. Name of Current Field Representative Supervisor at (210) 477-5104. For questions regarding the sampling process, please contact Mr. Current Chief Technical Officer at (210) 477-5128. Both of these people may be reached toll-free in Texas at (800) 292-1047.

Thank you again for participating in this study.

Sincerely,

Name of Current
Assistant General Manager

EDWARDS AQUIFER
AUTHORITY

VOLUNTARY AQUIFER SAMPLING
METER READING CONFIRMATION

Thank you for participating in the Edwards Aquifer Authority's aquifer biota study being conducted by Zara Environmental. This study is being conducted to evaluate the distribution of aquifer-dwelling species, and the research could not be completed without the assistance of well owners like you.

You will not be required to pay aquifer management fees for the groundwater used by Zara in the sampling process. Please initial and sign where indicated on this form to confirm the beginning and ending meter readings for the time Zara used your well. Please sign two forms and keep one for your records. When you report your annual groundwater use for 2009, please indicate on the report how much water was used by Zara Environmental and reduce your use accordingly. We will use our copy of this form to confirm your use.

________________________	____________	____________	____________
Beginning Meter Reading	Date	Owner Initials	Zara Initials
________________________	____________	____________	____________
Ending Meter Reading	Date	Owner Initials	Zara Initials

The meter readings above accurately reflect the amount of groundwater withdrawn from my well by Zara Environmental for the study of aquifer biota. I understand the Edwards Aquifer Authority will not assess aquifer management fees on this groundwater.

________________________________ ________________________________

Landowner Signature
Permit: XXXX-XXX

Zara Environmental Representative

Biological Sampling Procedures

Trapping Methods

Methods for sampling at wells were constantly refined over the 3 year course of this study. A variety of methods were used due to the diverse morphology of the sites that were sampled. Some locations allowed for continuous sampling of artesian flow, others allowed for periodic sampling of either artesian or pumped flow, and still others were accessible through a well opening where water did not flow out, but could be sampled by using a bottle trap (Figure 1). The bottle trap is ideal for catching salamanders, aquatic isopods and larger crustaceans, but the size and setup are inappropriate for capturing microfauna (too large) or catfish (too small).

Trap types that were used on a regular basis during the first year of the study included 60 μm plankton nets and larger drift nets with a mesh size up to 500 μm, with a capture bottle at the end (Figure 2). Methods used by Karnei (1978) were emulated for this study. In Karnei's (1978) work, "funnel nets" were attached to high flow sites, such as the well openings at the historic Verstraeten, Brackenridge Zoo, Artesia Pump Station and O.R. Mitchell wells. These large nets are useful in high flow locations. In areas with flow greater than approximately 0.63 liters per second, nets were arranged to rest in a tub or pool to minimize damage to specimens and ensure that the net remained submerged. The likelihood of finding microfauna such as ostracods, copepods, or bathynellaceans in such large nets is low because they are difficult to inspect and have larger mesh size, but they are effective for finding larger crustaceans and vertebrates. In some situations 60μm nets were placed inside of larger nets so that a small portion of the flow was filtered through them before entering the larger net.

Over the course of the first year of this study we improved our sampling methods by switching to the use of frameless nets, which could be cinched down over well outlets to help prevent surface contamination, and by the construction of shade socks (Figure 3). Shade socks are large pieces of opaque vinyl that are sewn into a tube and placed over the net. The benefit of shade socks is fourfold – they slow or prevent algal growth on the nets by providing shade, they direct the water out the bottom of the net underwater, thereby reducing force on the specimens, they help protect the net from wear and contamination, and they help shade the specimens so that a live capture is more likely.

Figure 1. Bottle traps were lowered down non-flowing wells to sample for small vertebrate and large invertebrate fauna.

Figure 2. Framed net on nursery tank outflow at the Aldridge 209 Well.

The use of framed nets was abandoned in favor of frameless nets cinched directly onto the well outlet (Figure 3). Some pipes have an outlet that is convenient for this type of sampling while others have to be modified to allow for net placement. Many landowners pump water directly to an irrigation system, and in some piping was tapped by excising a portion of it and installing an in-line "barrel sampler" to filter water under high pressure conditions (Figure 4). In the latter part of the study some sites were modified more drastically by the complete diversion of water to flow through a filter (which could be accessed and inspected for fauna) before returning to join the main irrigation line (Figure 5). Some locations allowed for continuous sampling of artesian flow, others allowed for periodic sampling of either artesian or pumped flow.

Figure 3. Zara biologist cinching a vinyl "shade sock" over the net on the nursery tank outflow of the Aldridge 209 Well.

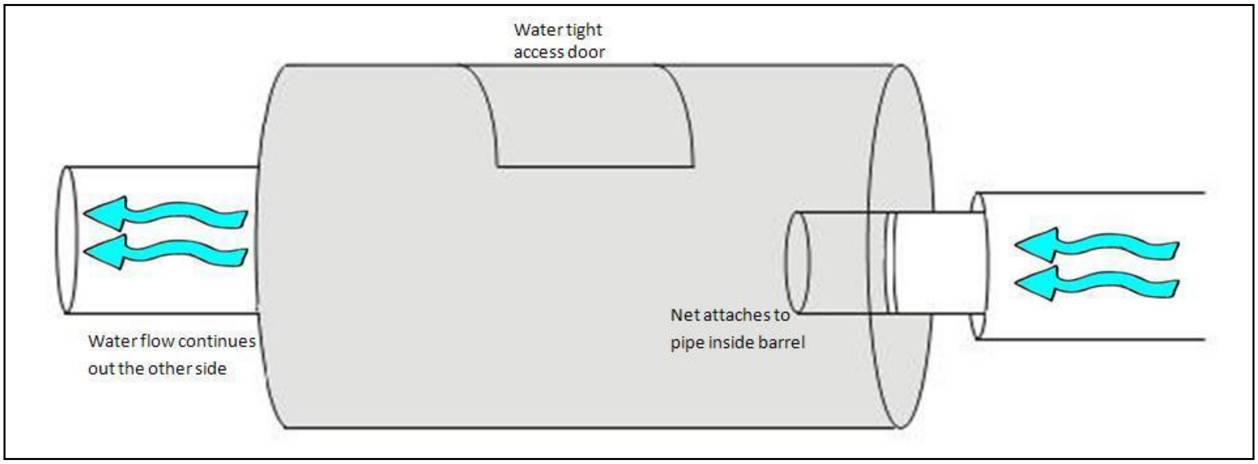

Figure 4. Schematic of in-line barrel sampler.

Figure 5. In-line filter sampler.

Specimen Collection and Identification

Specimens large enough to see in the field were handpicked from the net and transferred directly into 95% EtOH for preservation. These macrofauna included amphipods, decapods, isopods and catfish. Also transferred directly to ethanol were sediment samples that are gently scraped from the bottom of the capture bottles of 500 μm nets, which have a mesh size too large to reliably contain the smallest aquifer crustaceans. These samples were examined under microscopes in the laboratory at Zara, where any identifiable organisms were removed, labeled, and stored for later identification by a taxonomic expert.

Microfaunal samples from the capture bottles in the 60 μm plankton nets were returned to the lab in their native water. The ease of sorting out these live samples greatly exceeds the ease of sorting out preserved samples and allows scientists the opportunity for unique behavioral observations of live organisms. Living samples were sorted by taxon and preserved in 80% EtOH, unless another preservation method had been requested by the receiving taxonomist.

RESULTS

The site descriptions that follow include brief histories of some wells as well as descriptions of site-specific sampling methods. A summary of the wells sampled, including state well numbers, depths, average temperature and flow, the status of well letters and blindcat collections is presented in Table 2, and sample sites are mapped in Figure 6. A map showing our sampling sites in Bexar County along with historic blindcat localities as recorded by Suttkus (1961), Karnei (1978), and Longley and Karnei (1978a, 1978b) is also presented (Figure 7). Taxa lists are presented for sites containing aquifer fauna, otherwise taxa are listed in the results text.

Table 2. Sample sites, including historic blindcat localities that were not sampled during the current study, indicated in bold type.

Common Name	Depth (m)	Temp(°C) (Avg. ± SD)	Well Letter	Year(s) Sampled	Aquifer Fauna	State Well Number	EAA Well Number	lat	long
Aldridge 209 Well	>481.8	26.9±0.1	Y	2008-10	Y	AY6843802	W100-400	29.28822	-98.695
Aldridge Corporate	605.6	31.5±0.2	Y	2008-10	Y	AY6843700	W100-223	29.28642	-98.7255
Artesian Pump Station	122.5	--		1978-79	Y	--	--	--	--
Bexar Metropolitan Well	--	--		1977-78	Y	--	--	--	--
Bexar Met #8	328.6	--		2008		AY6829415	W100-277	29.56638	-98.4836
Bexar Met #91	328.6	--		2008		AY6829419	W100-459	29.5675	-98.4847
Boecke Artesian Well	93.9	--		1977-78	Y	--	--	--	--
Constanzo Well (Vogal Well) #2	--	32.5±6		2008		--	W100-303	29.27904	-98.7018
El Patio Foods Well	131.1	--		1977-78	Y	--	--	--	--
Fort Sam Houston #2	--	17.1		2008		--	--	29.44368	-98.4729
Fort Sam Houston #5	--	23.6±.8		2008		--	--	29.46978	-98.4284
George W. Brackenridge	93.9	--		1919	Y	--	--	--	--
Homer Verstuyft Well	--	26.5	Y	2009-10		--	W100-211	29.34978	-98.5714
Jeff Bailey Well	--	27.2±1	Y	2009-10		--	W100-209	29.31779	-98.6172
Kempin Well	116.1	--		1977-78	Y	--	--	--	--
Loop 353 Well	--	25.5	Y	2008-10		--	W100-508	29.35307	-98.5673
Lyda Fallon (Partin) Well (Bryce Britsch and David Jones dba Uno Mas)	--	28±1.5	Y	2010		--	W101-109	29.1717	-99.0549
Mark Verstuyft (Otto A. Reeh)	--	25.8±0.4	Y	2010		--	W100-212	29.32541	-98.6052
Nelson Road Well (Marvin G. Verstuyft)	548.6	24.5	Y	2009-10		AY843605	W100-058	29.32728	-98.6537
O. R. Mitchell Ranch	177.4	--		1977-78	Y	AY6843601	--	--	--

Common Name	Depth (m)	Temp(°C) (Avg. ± SD)	Well Letter	Year(s) Sampled	Aquifer Fauna	State Well Number	EAA Well Number	lat	long
Persyn Well	--	31.4±0.5	Y	2010		7E+06	W100-299	29.33507	-98.5683
Raymond Wauters Well	--	25.5±0.5	Y	2009-10		--	--	29.34207	-98.5733
Roger Verstuyft	--	26.1±.4	Y			--	--	29.34628	-98.5696
Roosevelt Bridge Springs	--	--		2008		--	--	29.37462	-98.4813
San Antonio Zoo Well	124.1	24.1±0.2		2008-09	Y	AY6837127	W100-052	29.46089	-98.4757
Shavano Park Well #5	132.5	--		2008		AY6828203	W100-650	29.58861	-98.5575
Steve's Homestead (509 King William / SA Conservation Society)	--	--		2008		--	W105-307	29.41274	-98.4949
Tschirhart Well	--	27±.1	Y	2009-10	Y	--	--	29.3379	-98.5748
Verstraeten Well	156.4	--		1977-78	Y	--	--	--	--
Verstuyft Farms Well	--	32	Y	2008-10		--	W100-505	29.29756	-98.662
Verstuyft Home Farm Well	--	31.8±0.4	Y	2008-10		68-3-10	W100-506	29.28061	-98.6838
Von Ormy Growers Well	607.5	--		2008		AY6843816	W100-222	29.2762	-98.6901
W. H. Guenther Well	--	25.8		2009		--	W100-066	29.4111	-98.4969
Girl Scout Well	--	--		2008		DX6823619	W104-975	29.70666	-98.1358
LCRA Well	--	NR		2008-10	Y	DX6823304	--	29.71111	-98.1375
Mission Bowling Well	--	--		2008	Y	DX6823209	--	29.72107	-98.1792
EAA New Braunfels Transect Well	--	--		2008		6823617/8	W104-973	29.70583	-98.1344
Schumann Artesian Well	--	--		2008		AY6815801	--	29.75961	-98.1968
Schumann House Well	--	--		2008	Y	AY6815801	--	29.75891	-98.1919
Panther Canyon Well	--	--		2008	Y	7E+06	W104-970	29.71361	-98.1383
EAA Paradise Alley Well	--	--		2008		6823616A/B	W104-972	29.70444	-98.1331
City of Kyle Well	181.4	--		2008		LR6702303	W104-869	29.99	-97.8748
EAA Aquarena Springs	226.2	--		2008		LR6701814	W104-983	29.89194	-97.9315

Common Name	Depth (m)	Temp(°C) (Avg. ± SD)	Well Letter	Year(s) Sampled	Aquifer Fauna	State Well Number	EAA Well Number	lat	long
EAA San Marcos Flagpole Well	213.1	--		2008		LR6701813	W104-982	29.89117	-97.9293
Carroll Farms Well	--	34.7±1.2		2009-10		--	--	29.22613	-98.8214
Hondo Index Well	457.2	27.2±1		2008		TD6947306	W104-875	29.34819	-99.1215
Stull Farms	--	34		2010		--	W101-200	29.19722	-98.9006
Yanta Well	--	--		2010		--	W101-004	29.17413	-99.051
Uvalde Index Well	87.5	--		2008		YP6950302	W104-880	29.20874	-99.7844
Woodley Farms Well #1 (Blackstone Dilworth)	--	36±1.2	Y	2010		--	W101-504	29.14822	-99.5071
Woodley Farms Well #2 (Blackstone Dilworth)	--	37.1±0.3	Y	2010		--	W101-505	29.13547	-99.5014

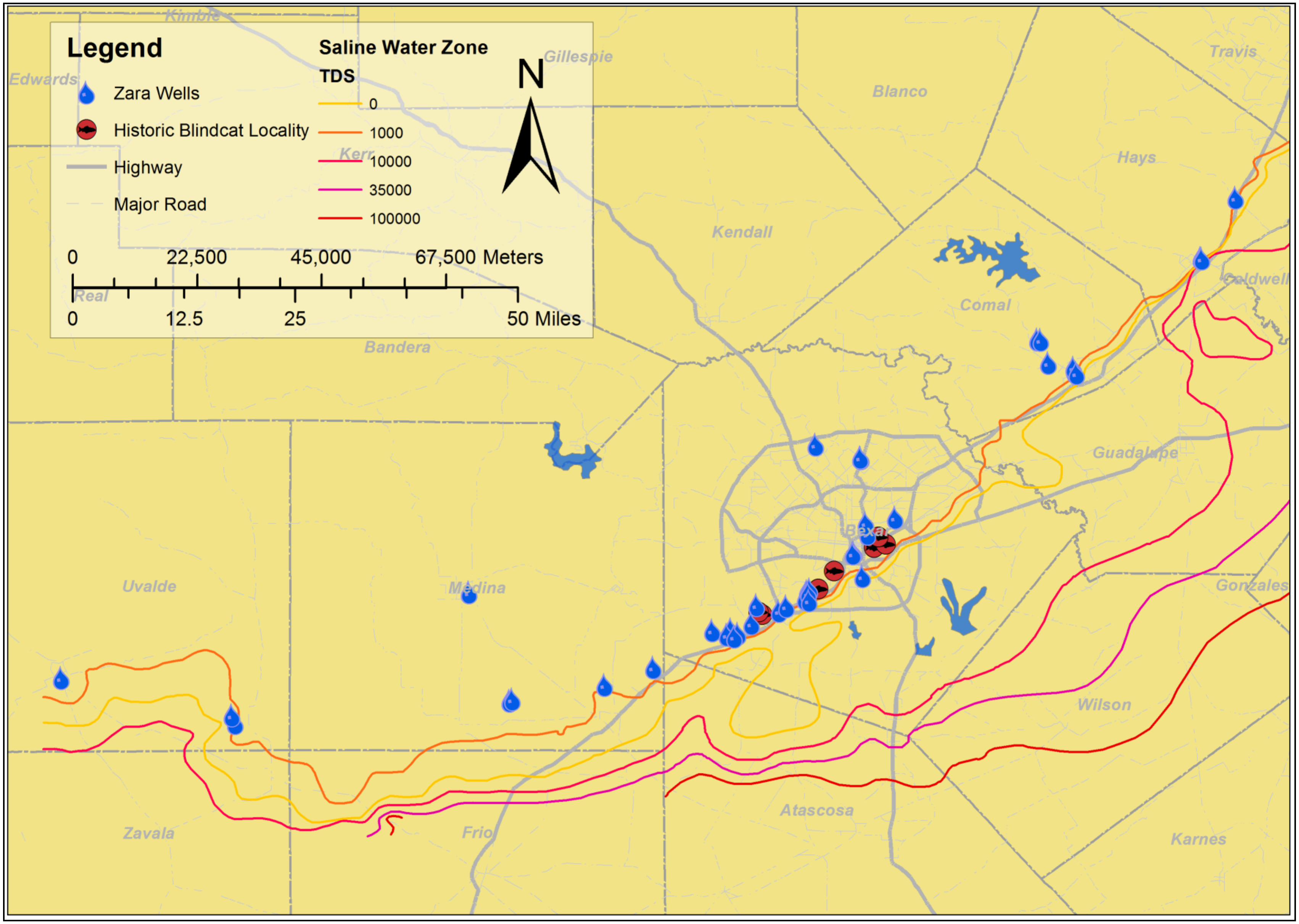

Figure 6. Well locations sampled by Zara during this study.

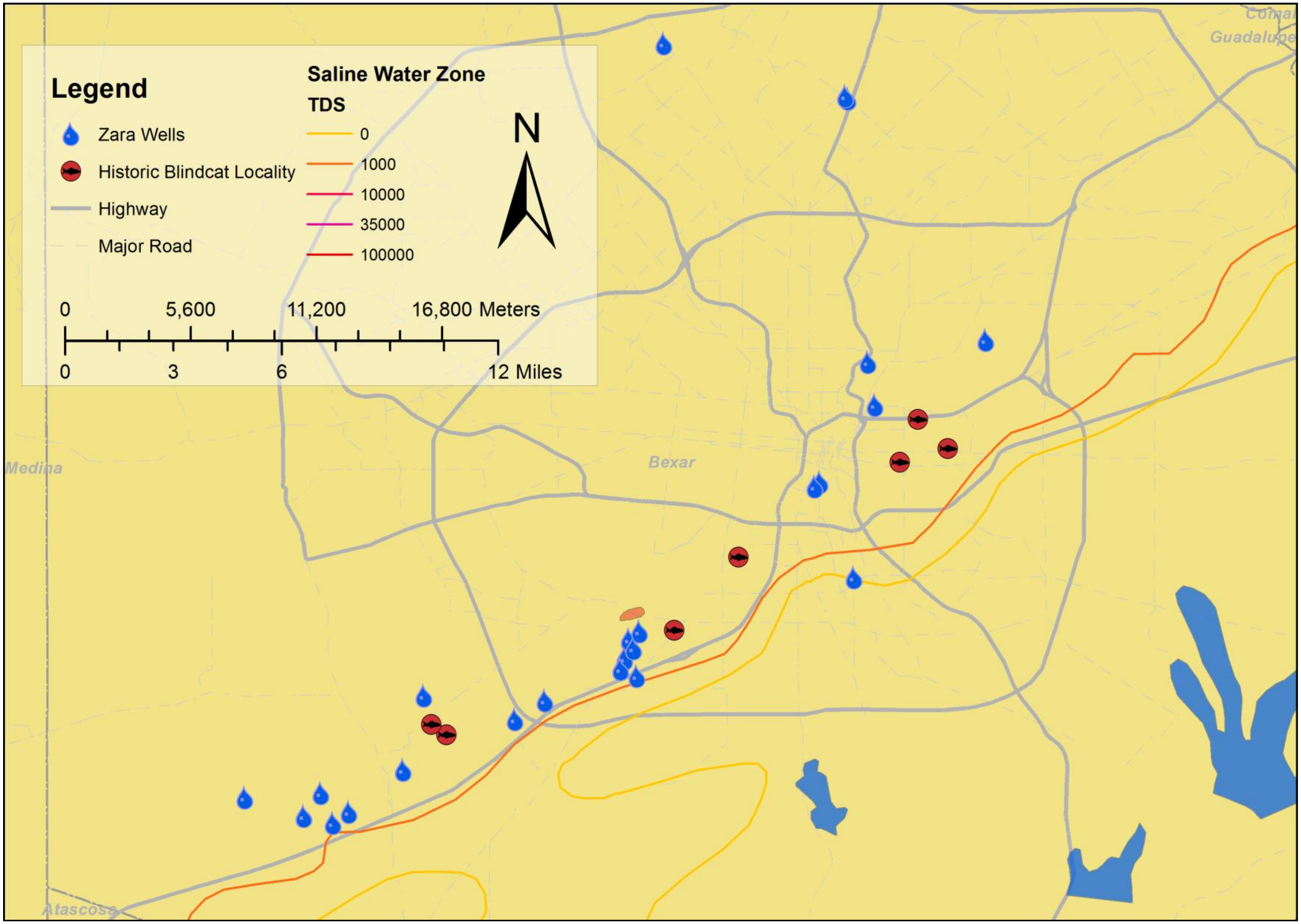

Figure 7. Distribution of selected sampling sites in relation to historic collection localities for blind catfish.

Historic Blind Catfish sites

Karnei (1978) collected 13 individuals of *S. eurystomus* and 26 individuals of *T. pattersoni* from the deep artesian portion of the Edwards Aquifer in Bexar County, and was able to gather reports of approximately 40 other individual blindcats that had been flushed out of wells prior to the inception of his study. Unfortunately the preservation methods of the time dictated that the specimens be preserved in formalin, so their DNA was not available for analysis. The following pages summarize our efforts to emulate Karnei's research. While our goal was to gain a more complete understanding of all of the fauna that live in the Edwards Aquifer, our efforts were focused on emulating work done by Longley and Karnei (1978a, 1978b) in locating the blind catfishes of the aquifer. In our efforts to follow up on those studies we compiled a list of historic blindcat sites and attempted to access each one.

Artesia Pump Station Well

Also sometimes called the "Artesia Well #4," this is a historic locality for *Satan eurystomus,* although there is some confusion because records in the Texas Memorial Museum show *Trogloglanis pattersoni* here, not *S. eurystomus*. According to Longley and Karnei (1978a), sampling of this well began on February 22, 1978, and was continuing when their report was written. Those authors locate the Well Number 4 of five artesian wells at the pump station as 3.2 km southwest of the historical location of *T. pattersoni* near the Joe Freeman Coliseum on Coliseum Road and Aniol Roads (Figure 8 and Figure 9). They describe the well as 402 m deep and having a flow of 244 liters per second. Those authors found 11 *S. eurystomus* and 22 *T. pattersoni* at this locality during their study. SAWS declined our request to sample this well during our 2007-2010 study.

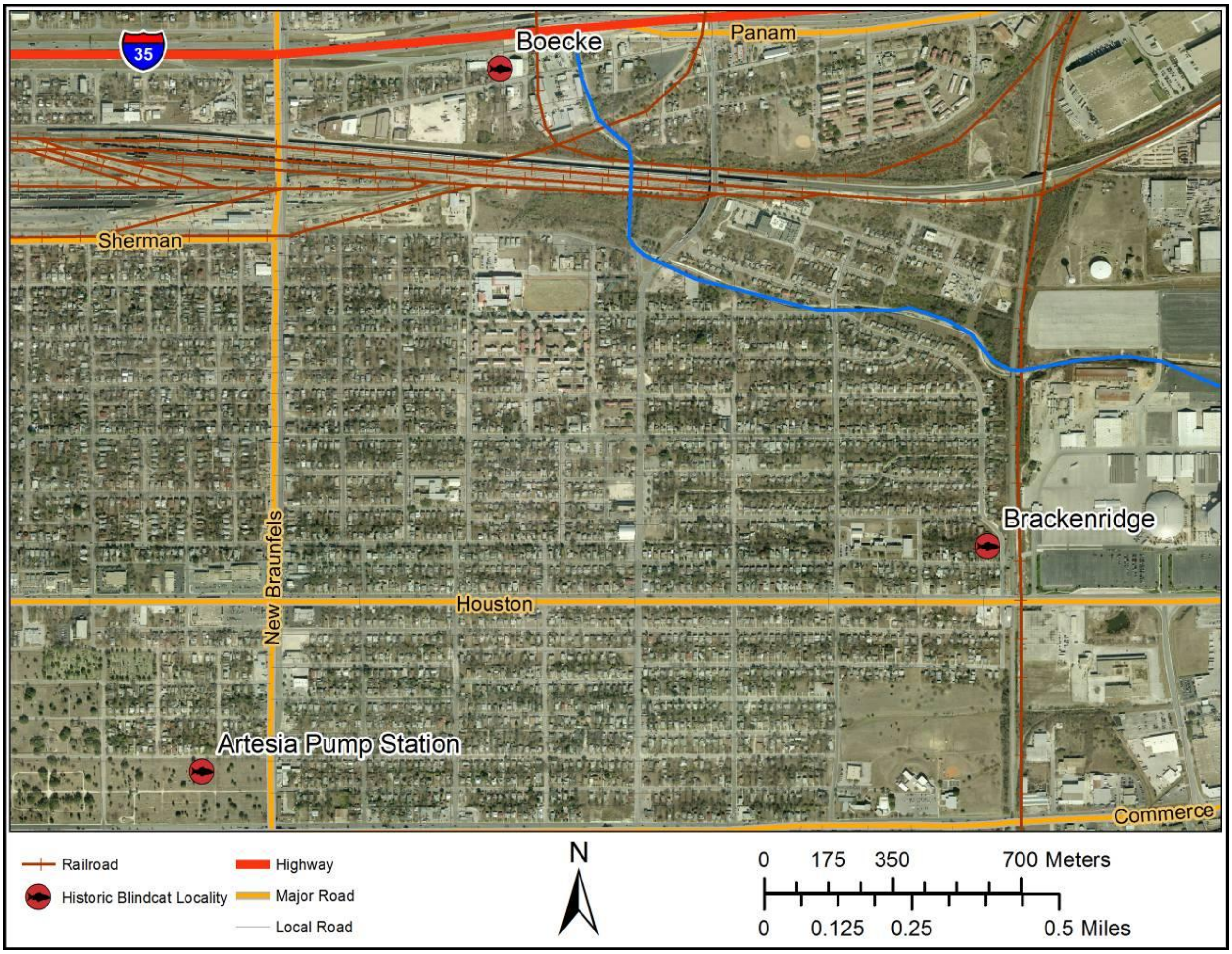

Figure 8. Location of Artesia Pump Station Well in relation to the Josef Boecke Well and the George W. Brackenridge Well.

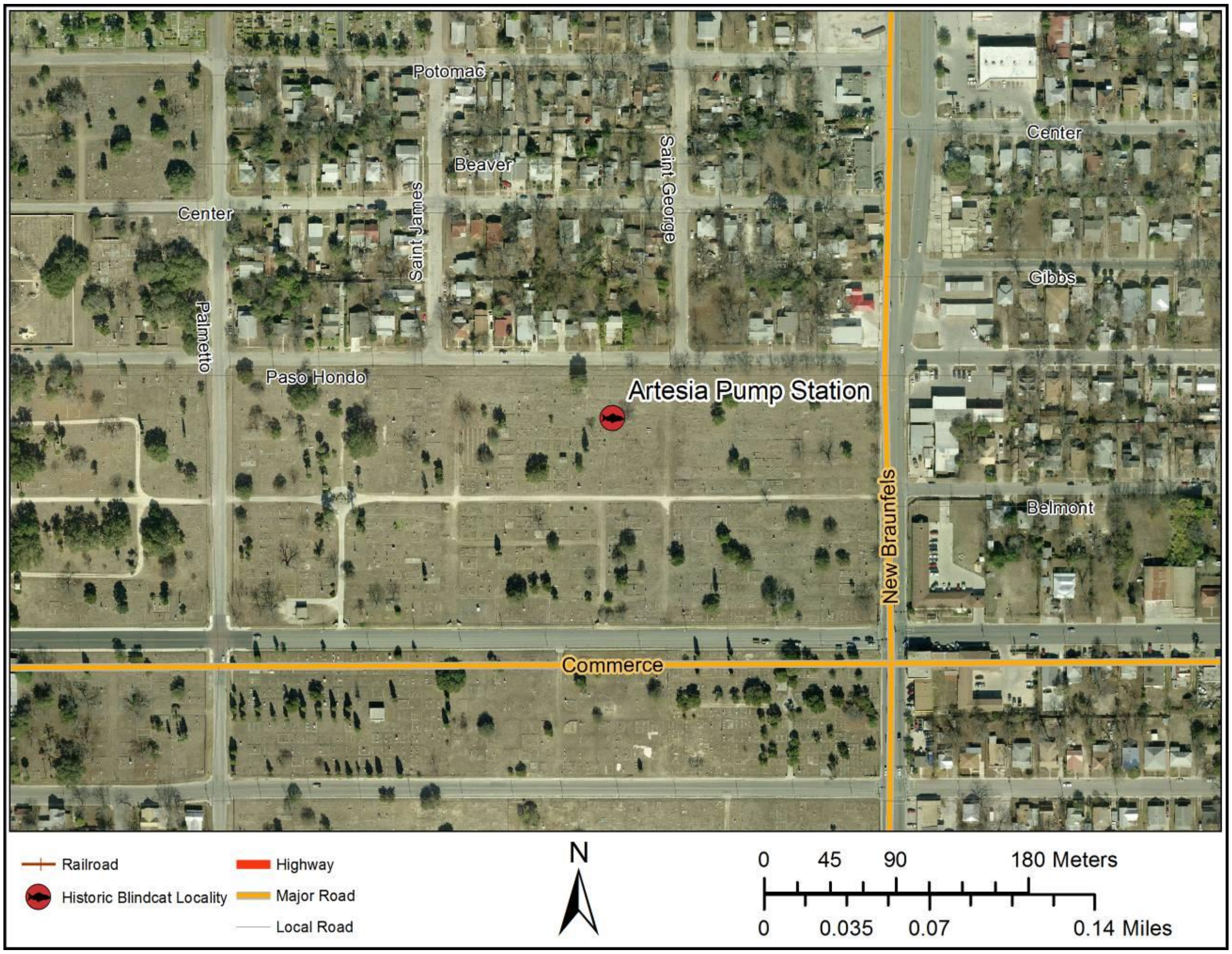

Figure 9. Approximate location of the Artesia Pump Station Well.

Artesian well in San Antonio

This is the type locality for *Trogloglanis pattersoni*.

Bexar Metropolitan Water District Well

Also called the "Bexar Metropolitan Well," this is a historic locality for *Satan eurystomus*. Longley and Karnei (1978a) report that the manager of the Bexar Metropolitan Water District collected one of three specimens observed of *S. eurystomus* in 1953. The well it was collected from was 15 cm in diameter, of an unknown depth, and located on approximately the 500 block of Carlisle in southwest Bexar County. The well has since been capped or plugged (Longley and Karnei 1978a).

Figure 10. Approximate location of the Bexar Met Well.

El Patio Foods Well

This is a historic locality for *Satan eurystomus* and possibly for *Trogloglanis pattersoni*. Suttkus (1961) reported on the collection of *S. eurystomus* from this site on June 1, 1960. Suttkus gives the address of the artesian well (427 m deep), and Longley and Karnei (1978a) report the well was capped or plugged and closed down due to infiltration of oil and sulfur from the bad water zone in 1964. Peter Sprouse attempted to relocate this well on 14 August 2008 (Figure 11). At 2600 Military Drive there is a large slab left over from a demolished industrial facility at that location that could have been El Patio Foods (Figure 12). There is a terminal power pole (typical of well sites) that is overgrown with vegetation that could be a sealed well site.

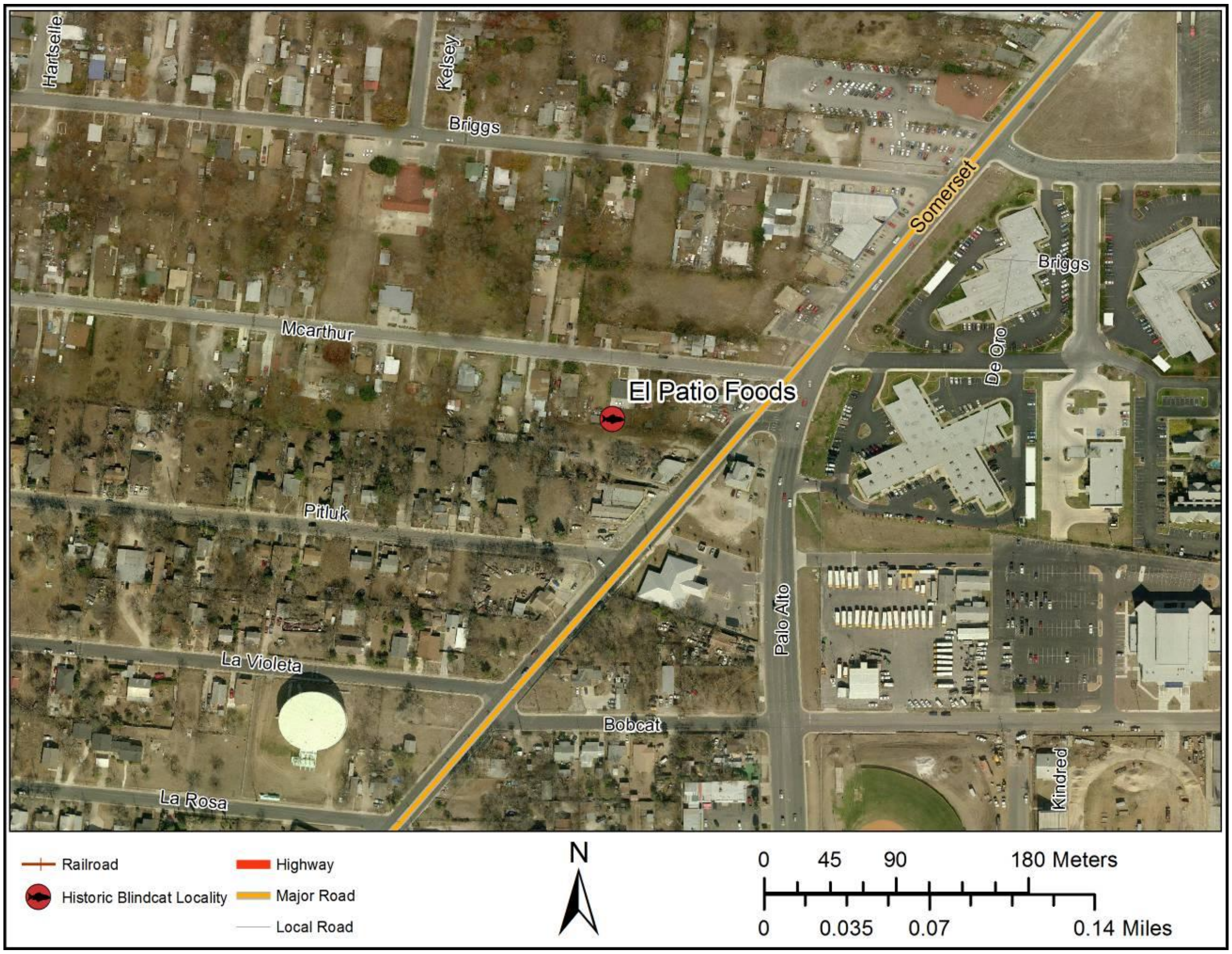

Figure 11. Approximate location of the El Patio Foods Well.


Figure 12. At 2600 Military Drive, a historic blind catfish locality, lies a demolished industrial facility at that location that could have been El Patio Foods.

El Patio Foods Well no. 2

Estimates of the historic location for this well place it in the middle of a long-established residential neighborhood, across from a school district facility. Efforts to locate this site on 14 August 2008 were unsuccessful. Peter Sprouse spoke with a resident a few houses away from the coordinate location who had moved there in 1961, and he said the houses had already been there a long time then. He said the school was also in place, suggesting that the well may have not been there.

George W. Brackenridge Well

This is the type locality for *Trogloglanis pattersoni*. Longley and Karnei (1978b) report that the original description did not specify which of George W. Brackenridge's wells produced the catfish. Longley and Karnei (1978b) deduced through unsuccessful attempts at the Brackenridge Zoo well and via the history of the land ownership that the probable type locality is a 308 m deep well at the intersection of Belgium Lane and KONO Road (Figure 13), noting that it was likely to be capped soon.

Figure 13. Approximate location of the George W. Brackenridge Well.

Josef Boecke Well

The second known specimen of *Trogloglanis pattersoni* was collected at this site (Hubbs and Bailey 1947). According to Longley and Karnei (1978b), the location is now in the right of way of I35 (Figure 14), and the 308 m deep well was covered by highway-related construction.

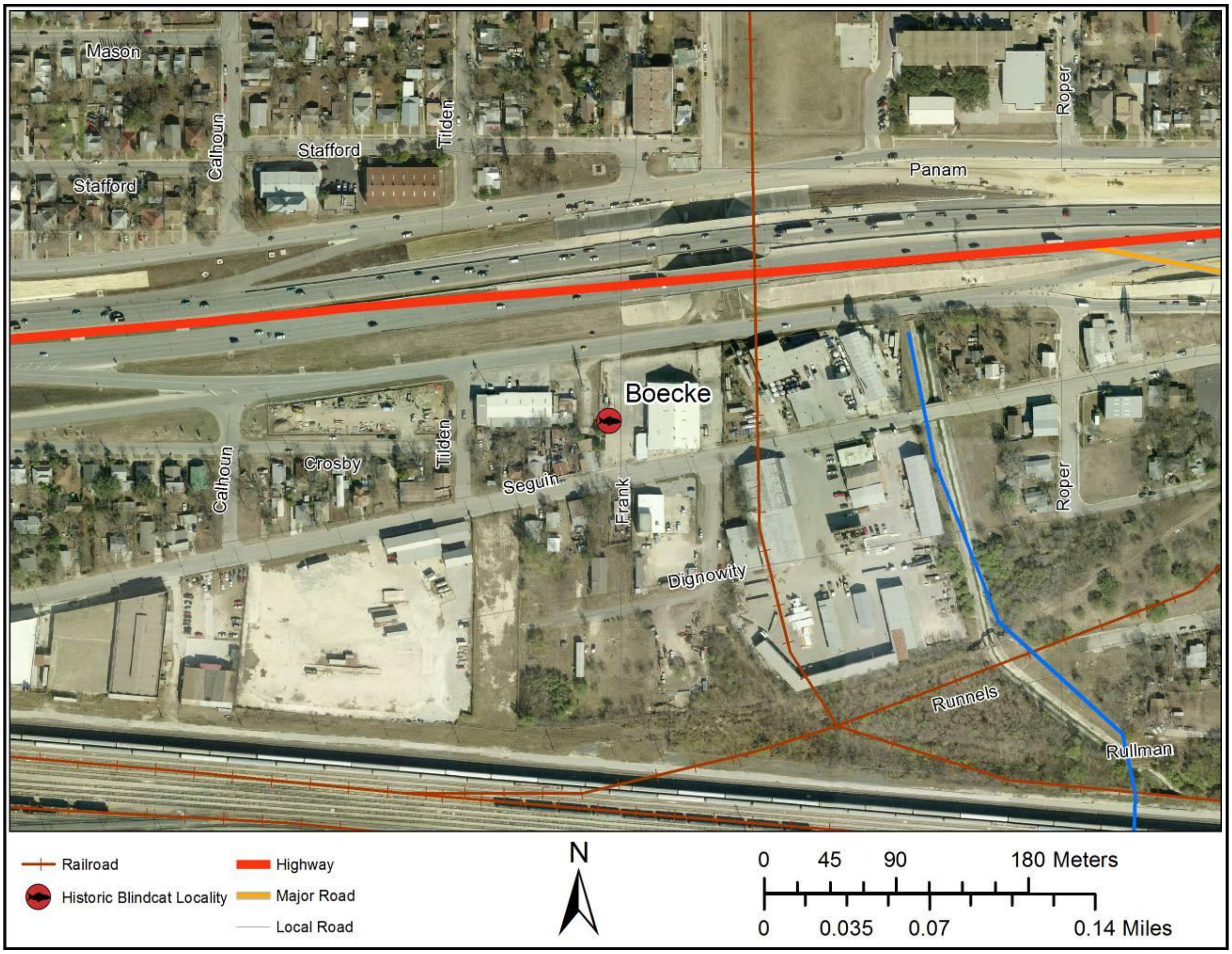

Figure 14. Approximate location of the Josef Boecke Well.

O.R. Mitchell Well

This is a historic locality for *Satan eurystomus* and *Trogloglanis pattersoni*. Suttkus (1961) reports on the first collection at this site of a single specimen of *S. eurystomus* from the O.R. Mitchell Ranch, and an additional specimen of *T. pattersoni* in 1955. He reports the depth of the artesian well as 582 meters, and the ranch location as 22.5 km southwest of San Antonio in the Von Ormy area (USGS No AY-68-43-601) (Figure 15). According to Longley and Karnei (1978a), three specimens of each of these two species were collected at this location between March 23 and June 30, 1977. One of the *S. eurystomus* was kept alive for 164 days. The well had a reported flow of 315 liters per second. Their request to re-sample this site in 1978 was denied by the ranch foreman.

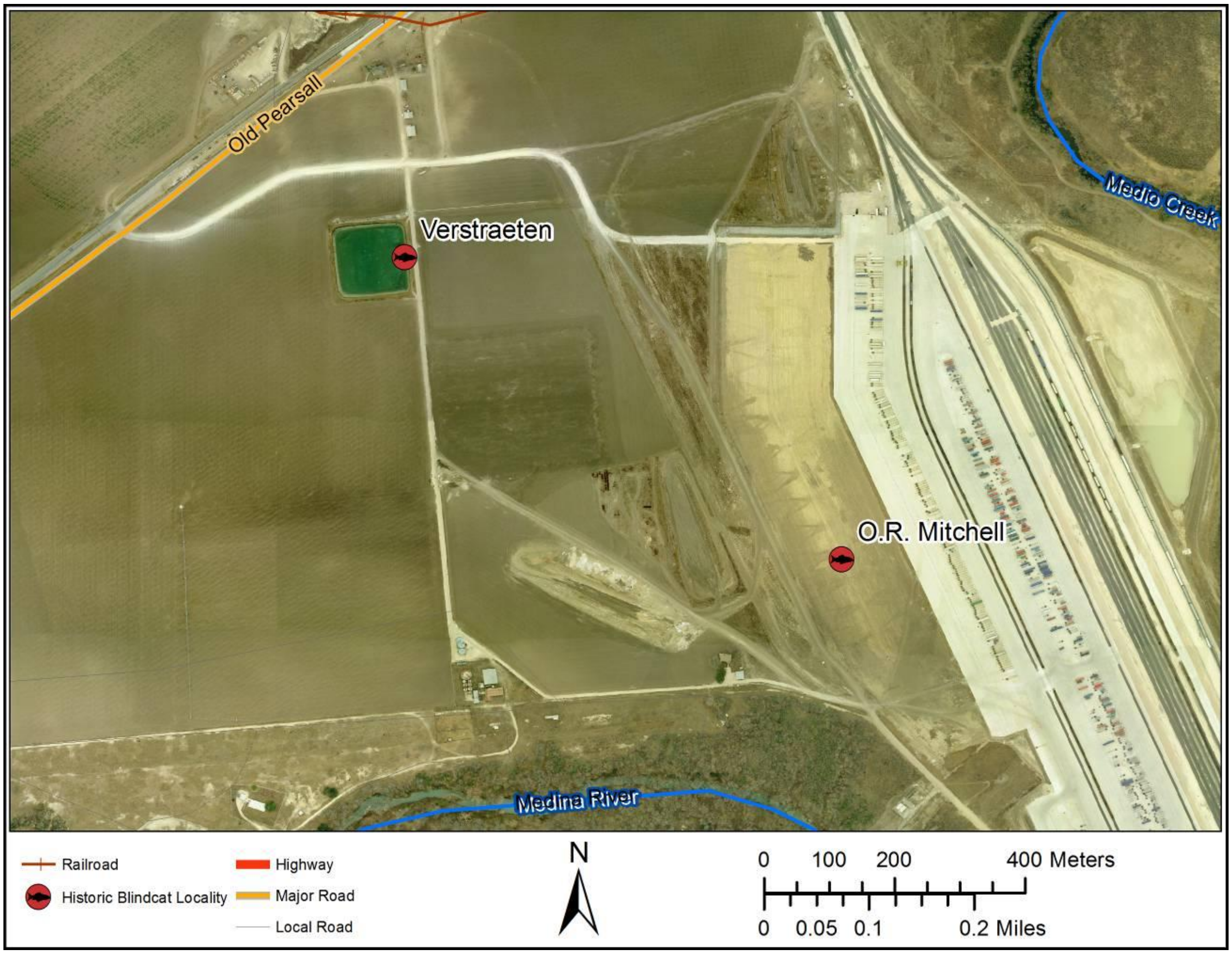

Figure 15. Approximate sites of the O.R. Mitchell and Verstraeten Wells.

Verstraeten Well No. 1

This is a historic locality for *Trogloglanis pattersoni*. Longley and Karnei (1978b) netted this well starting on March 16, 1977 and were still netting there when they wrote their report. One *T. pattersoni* was collected and many invertebrates were collected from the 513 m deep well, which had a reported flow of 315.4 liters per second. They describe the location and morphology of the well pipe. This site (Figure 15**Error! Reference source not found.**) has recently been capped and is not accessible for sampling.

Figure 16. View of property where Verstraeten Well is located.

William Kempin Well

This is the type locality for *Satan eurystomus*. William Kempin gave the type specimen to the Witte Memorial Museum in San Antonio in 1938, and Hubbs and Bailey (1947) described the species from this specimen. According to contacts reported in Longley and Karnei (1978a), the Kempin artesian well was 381 meters deep and located in southwest San Antonio near the East Kelly Air Force Base (Figure 17). Longley and Karnei (1978a) reported the area was under development and no well site could be found. Peter Sprouse tried to locate this site on 14 August 2008. Best estimates of the historic location placed it at the southeast corner of the air base, northwest of where Military Drive crosses the railroad. No signs of a well could be seen there by looking through the fence. There is a utility building on the south side of the road, west of the railroad, but its purpose is unknown. It could house a well, or it could be related to other water or gas utilities (Figure 18).

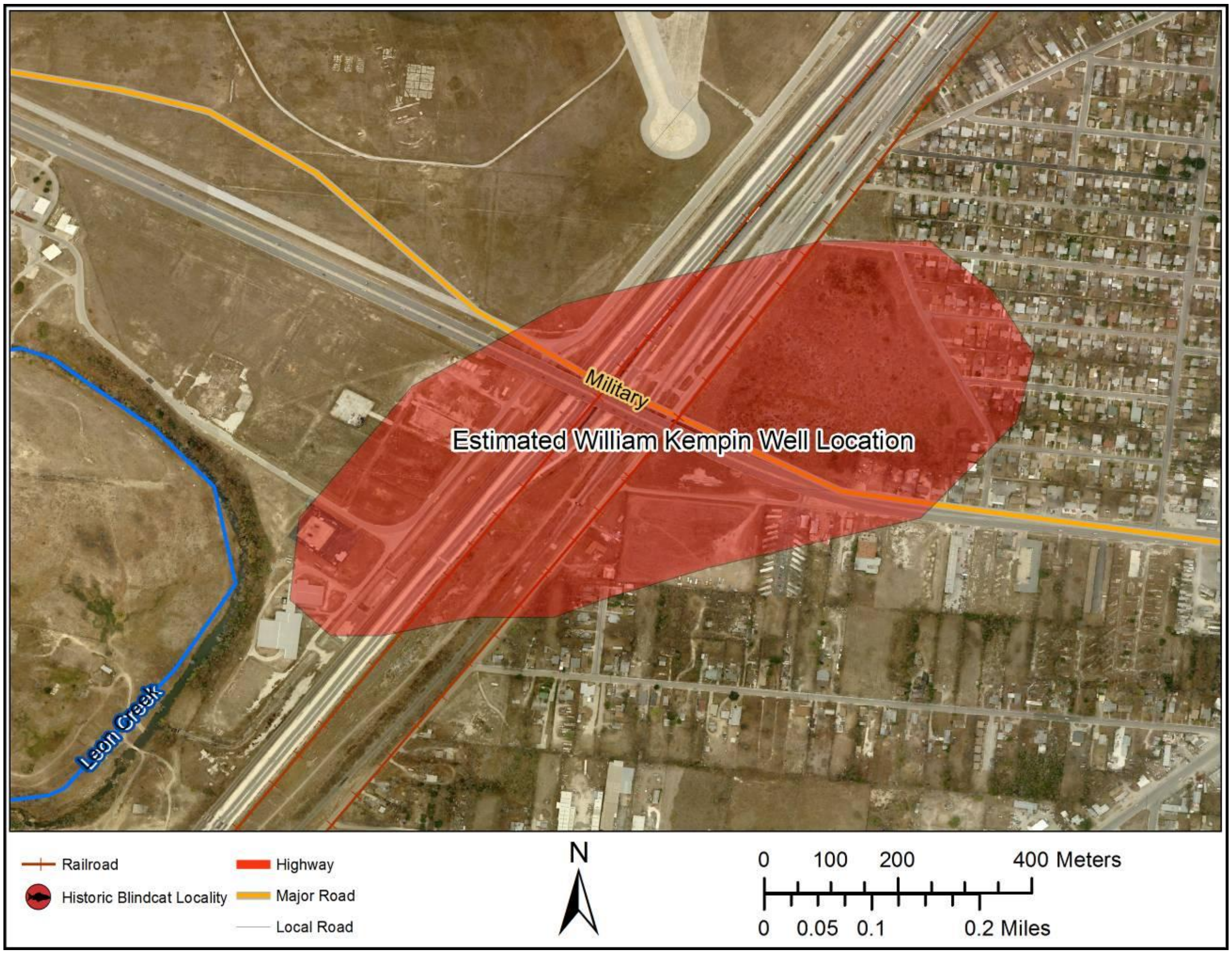

Figure 17. Approximate location of the William Kempin Well.

Figure 18. A utility building west of the railroad may house the William Kempin Well, a historic blindcat locality.

Collaborative Results

Fish Collections

Dr. Dean Hendrickson, the Curator of Icthyology at the Texas Memorial Museum (TMM) and his assistant Jessica Rosales have been managing the fish material that has been collected during the course of this study. Doctor Hendrickson's lab accessions and catalogues the specimen at the Texas Natural History Center (TNHC) prior to sending them to Mr. Lundberg's lab in Philadelphia. The TMM will be the ultimate repository for the majority of the specimens collected during this study.

John Lundberg, Curator and Chaplain Chair of the Icthyology Department of the Academy of Natural Sciences in Philadelphia, Pennsylvania, and his staff have been identifying many of the samples that were collected during the course of this study. Mr. Lundberg's taxonomic expertise includes catfish and electric fishes, and he is co-principle investigator in the All Catfish Species Inventory. The computed tomography (CT) (x-ray) images were done by Kyle Luckenbill and the genetic sequencing work has been done by John Sullivan, both of whom are currently working in the Lundberg Lab.

Of the sites sampled so far, the Aldridge 209 Well has produced the greatest number of blind catfish (nine samples, see Table 3). The re-discovery of both of the blind catfishes from the Edwards Aquifer was very encouraging, since these are the first recorded collections since Karnei's 1978 study. We sampled Aldridge 209 for more than a year before the fish was collected; improved sampling equipment since that time greatly increased the success rate (also see full discussion of this site in "Current Study Results" section below). John Lundberg's lab successfully extracted DNA from the Toothless Blindcat, *Trogloglanis pattersoni,* which is one of the few known catfish species in the world that had not yet been sequenced. The other species is *Satan eurystomus*, the widemouth blindcat, which also lives in the deep portion of the Edwards Aquifer. Bones and fleshy material revealed a well-preserved opercle bone, partial hyomandibular, bits of hyoid bar with a few branchiostegal rays inside the largest chunk of material. The shape of the opercle differs from the distinctive opercle of *Trogloglanis,* and instead closely resembles the epigean flathead catfish, *Pylodictis olivaris*. Based on several uniquely diagnostic features, *Satan eurystomus* is most closely related to *Pylodictis* (John Lundberg, pers. comm.).

A single fish collection from the Aldridge Corporate Well (Zara 5954) is especially interesting: it represents well preserved and just disarticulated parts of one fish skeleton, indicating that the fish was whole until soon before or soon after it arrived in the net. The identity of the material remains in question, but it has been ruled out as a catfish, and further ruled out as belonging to any of the following: *Morone chrysops*, *Perca*, bass, rock bass, or Crappie, *Lepomis cyanellus* or *humilis* (J. Lundberg pers. comm. from Jerry Smith, 25 October 2010). We have explored several different possible routes that surface contamination could have arrived in the net, including the possibility that a surface fish washed into a distant karst feature and somehow made its way through the aquifer to the well. All of the possibilities thus far seem highly unlikely; the probability of this material being the result of surface contamination is quite low at this particular site (see site description in "Current Study Results" section below). The material is currently in the possession of John Lundberg, who is attempting to further image it and will attempt to extract DNA and match it with a known sequence over the next several months.

Table 3. Vertebrate material collections. TNHC=Texas Natural History Center (Texas Memorial Museum); JL=Positive identification by John Lundberg.

Collection Site	Catalog #s Zara	Catalog #s TNHC	Accession Number	Date Collected	Identification
Aldridge 209	3889			28 Aug 2008	*Satan eurystomus* (JL)
Aldridge 209	No data	42586	2009-10	23 Mar 2009	*Trogloglanis pattersoni* (JL)
Aldridge 209	5344	45368	2010-04	24 Mar 2010	possible fish parts
Aldridge 209	5335	45369	2010-04	24 Mar 2010	possible fish parts/bones
Aldridge 209	5347	45366	2010-04	29 Mar 2010	*Trogloglanis pattersoni*
Aldridge 209	5424			14 Apr 2010	possible bone
Aldridge 209	5430			14 Apr 2010	possible fish flesh
Aldridge 209	5921	45860	2010-17	22 Jul 2010	*Trogloglanis pattersoni*
Aldridge 209	6149	46823	2010-21	29 Sep 2010	*Trogloglanis*? (JL-prelim. ID)
Aldridge Corporate	5954	46824	2010-04	14 July 2010	acanthopterygian fish
Loop 353	5920	45861	2010-17	5 June 2010	preliminarily identified as fish, later identified as *Eurycea* sp.
Tschirhart	5343	45367	2010-04	24 Mar 2010	Ictaluridae (ID by TMM)

Crustacean Collections

Pete Diaz and Randy Gibson volunteered several hours of their time to identify, curate and photograph several specimens and partial specimens of amphipods and isopods that were collected during this study. Janet Reid identified copepods, including one species that is new to science and not yet described. Rosalie Maddocks identified ostracod specimens and shared her wealth of knowledge about the variation and life history of the species that we collected. These species are discussed in more detail in the site-by-site descriptions that follow.

Microbial analysis

Several raw samples (unsorted for fauna) were shared with Annette Summers Engel for microbial analysis. Doctor Engel is trained in geology, geochemistry, and microbiology. She currently has several samples that were obtained through this study, and will process and analyze them at some time in the future. Below is an excerpt from Dr. Engel's proposal:

> The eastern edge of the Edwards Aquifer is marked by a steep, freshwater to saline water interface, known locally as the bad-water line. The juxtaposition of the two geochemically distinct waters creates an energy (redox) gradient that can support diverse ecological systems, and especially microbial communities. Engel is currently funded (2006-2009) by the Louisiana Board of Regents (BOR) to examine the ecological and geological significance of saline water microbes. As a part of this ongoing work, it has become clear that there are changes in the microbial community diversity and structure across the transition zone from the freshwater to saline water. These changes need to be further investigated by examining the diversity of aquifer microbes where the transition zone can be accessed.
>
> Virtually nothing is known about saline water microbiology or about the possible effects of microbes to aquifer processes. Reduced sulfur compounds, like H_2S, serve as rich energy sources for sulfur-oxidizing bacteria, which produce sulfuric acid as a metabolic byproduct. Colonization of aquifer rocks by these microbes causes significantly more carbonate dissolution compared to possible dissolution from bulk aquifer fluids. Dissolved organic matter (DOM) types in the saline water have also been studied, finding that there is significantly less quinone-like fluorophores associated with terrestrial or sediment (allochthonous) DOM. Most saline waters have a more heterogeneous collection of fluorophores suggestive of a lack of humified DOM. Instead, the DOM has higher relative amounts of protein-like and microbial DOM, indicating a microbial source for DOM in the saline-waters. This has important implications for carbon sources and cycling in the Edwards Aquifer ecosystem. Thus far, microbial communities identified from molecular methods in several saline water wells (at Sonterra and Kyle) include groups known for sulfur cycling (e.g., *Gammaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria* and OP11 candidate division groups), as well as others with poorly to unknown metabolism.

In future years of this project we hope to provide Dr. Engel with samples that will allow her to study the microbial diversity from aquifer transect wells in order to understand community shifts across the transition zone, and specifically from freshwater wells. Molecular methods allow for the characterization of the microbial communities that may be difficult, if not impossible, to cultivate.

Stable isotope analysis

Ben Hutchins, a student in Dr. Benjamin Swartz's lab at Texas State University, is studying stable isotopic signatures from samples drawn from the aquifer in an attempt to describe the trophic ecology of the aquifer. Ben currently possesses only a few samples from our study sites, however in future years of this project we hope to provide Ben with samples that will allow him to further these investigations. An abstract of Ben Hutchin's (2010) research proposal follows:

> The study presented here aims to elucidate carbon and energy sources utilized by the diverse biota of the Edwards Aquifer. A variety of potential nutrient sources exist including microbial biomass produced chemolithoautotrophically near the freshwater saline water interface, photosynthetically derived material washed in at recharge features, and hydrocarbons migrating updip from deep-seated reservoirs. An analysis of the stable isotope ratios for multiple isotopes including carbon, nitrogen, and sulfur in these nutrient sources and in animal tissues will elucidate which of these sources are important, and how nutrients pass through food chains from producers to consumers to predators in the Edwards Aquifer. A major component of this study is an analysis of how hydrology and geochemistry affect the availability and movement of nutrients through the aquifer. Specifically, I aim to compare patterns between biotic isotopes and inorganic geochemistry, including stable isotopes of water and strontium along known and inferred flowpaths to evaluate the utility of isotopes as natural tracers. Furthermore, I aim to investigate how faults, fault blocks, recharge features, and the saline water zone affect the availability, utilization, and movement of nutrients important to the Edwards Aquifer ecosystem. Approximately 7 surface rivers and 40 wells and caves will be sampled for biota, carbon sources, and aqueous geochemistry. Geochemical parameters analyzed include stable isotopes of oxygen, hydrogen, and strontium, major anions and cations, and basic physical parameters such as temperature, pH, and dissolved oxygen. Importantly, sampling sites will be chosen to transect the freshwater-saline water (FW-SW) interface and major faults, and to sample along flowpaths between recharge features and resurgence features. This sampling regime will allow for investigation of three distinct research questions. 1) How does proximity from recharge features and the FW-SW interface affect the abundance and utilization of allochthonous and chemoautotrophic resources, respectively? 2) Can stable isotopes in organic matter and animal tissue be used to trace flowpaths? 3) What role do faults play in facilitating or restricting the movement of organic matter and animal migration within the Edwards Aquifer?

Current Study Results

New locality records

This study has resulted in the collection of thirteen stygobitic invertebrates from wells penetrating the Edwards Aquifer (Table 4). These are all new locality records for these species, which are discussed in more detail in the site-by-site descriptions that follow.

Table 4. Stygobitic invertebrates collected during this study. Most of these represent new locality records for the species that were collected.

Species	Collection Site
Palaemonetes antrorum	Aldridge 209
Holsingerius samacos	Aldridge 209, Tschirhart
Paraholsingerius sp.	Aldridge 209
Speocirolana hardeni	Aldridge 209, Aldridge Corporate, Loop 353
Cirolanides texensis	Aldridge 209, San Antonio Zoo, Tschirhart
Parabogidiella americana	Aldridge 209, Tschirhart
Parabogidiella sp.	Nelson Road
Allotexiweckelia hirsuita	Aldridge Corporate

Species	Collection Site
Diacyclops new sp.	Fort Sam Houston #2, Schumann House Well
Tethysbaena texana	Fort Sam Houston #2
Mexistenasellus coahuila	Tschirhart
Phreatodrobia sp.	San Antonio Zoo
Texanobathynella bowmani	Schumann House Well

The Appendix contains meter readings and temperature data from sites where that information was recorded, and Table 2 presents the geographic coordinates of the sites sampled during this study.

Aldridge 209 Well

David Aldridge sighted a blind catfish coming out of the "Aldridge 209" irrigation well circa 1988, when his family owned it. Partial outflow from this well is piped approximately 1 km to the Aldridge Nursery office grounds, where the pipe rises 1.5 meters off the ground before bending 90 degrees to flow into a round open concrete tank. In 2008 this well was sampled through a framed net attached to the pipe outlet (Figure 2), but the sampling technique was refined in 2009, when a 500 μm net covered with a vinyl shade sock was cinched over the outlet (Figure 3). In 2010 an in-line sampler was installed to allow sampling at the pump site, before the water flows to the various outlets (Figure 19), and a wooden shade tent was constructed to protect samples from getting too hot and to keep the sampler from getting too hot to touch (Figure 20). This well flows under artesian pressure and is also pumped at times, depending on water needs and aquifer level.

In 2008 the Aldridge 209 Well was sampled from March 28 to September 30, 2008. A total of 18 samples were taken from this site at irregular intervals, averaging one sample every 10 days. The president of Aldridge Nursery, Thomas Trautner, agreed to allow continuous access to this well without requiring that the net be removed until the termination of this study.

The net was left on the well continuously in 2009, and samples were collected from the site on 22 occasions. The well pump and meter were removed for maintenance in October 2009, which provided a valuable opportunity for sampling of unpumped artesian flow from the site and for Authority personnel to log the well. During the well logging, it was noted that an oily sheen could be observed at the water surface and that small "globs" of oil were ejected from the well. The well casing was covered with calcite precipitates, which built up to such a thickness that the logging equipment could not proceed past a depth of 481.9 meters. Under artesian flow, this site was noted to discharge at a rate of approximately 40 liters per second in October 2009.

In order to sample the artesian flow at the open wellhead, a paint strainer was secured over the top the well outlet for a period of 7 hours (Figure 21). During that time one live stygobitic isopod, *Speocirolana hardeni* was collected (Figure 22), then transferred into an aquarium at Texas Memorial Museum, where it survived for over a month. During that time, the animal was observed to swim almost continuously (Dean Hendrickson, pers. comm.).

Aldridge 209 Well was visited 30 times between 20 January 2010 and 13 October 2010. This increased sampling frequency combined with the use of the in-line sampling system facilitated the collection of several specimens of whole or partial blindcats and crustaceans that may otherwise have been degraded beyond usefulness.


Figure 19. In-line sampler at the Aldridge 209 Well.


Figure 20. Wooden shade tent over in-line sampler at Aldridge 209 pump site.

Figure 21. Paint strainer attached to artesian flow while the well was uncapped*.*

Figure 22. The stygobitic isopod *Speocirolana hardeni* from the Edwards Aquifer.

Partial fish parts belonging to *Trogloglanis pattersoni* were first retrieved from this well outlet on 23 March 2009 (Figure 23), and an intact specimen was retrieved during the summer of 2010 (Figure 24). They were examined and genetically sequenced by John Lundberg, who is the co-principal investigator on the National Science Foundation project "All Catfish Species Inventory." X-ray images detailing the diagnostic features from the specimen are shown in Figure 25 and Figure 26.

A sample collected on 28 August 2008, which contained just a bit of white bone material, was examined and determined to belong to *Satan eurystomus*, based on a well preserved opercle bone, partial hyomandibular, and bits of hyoid bar with a few branchiostegal rays. The opercle differs from the distinctive opercle of *Trogloglanis* and closely resembles the epigean flathead catfish, *Pylodictis olivaris*. Based on several uniquely diagnostic features *Satan eurystomus* is most closely related to *Pylodictis* (John Lundberg, pers. comm.). *Satan* material collected from the Aldridge 209 well has been genetically sequenced by John

Lundberg. Records of putative fish material collected from the well are presented in Table 3 (and also see discussion in "Collaborative Results" section).

The crustaceans collected from the Aldridge 209 Well, represent members of a diverse subterranean ecosystem. Stygobitic crustacean fauna from this site represents new locality records for several species including *Palaemonetes antrorum* (Figure 27), which was not collected at any other sites during this study. The amphipod *Holsingerius samacos* was collected from this site and from the Tschirhart well. These site records might represent the first confirmed Bexar County records of the species, although there is an unconfirmed record of *H. samacos* from the San Antonio Well D-1 of the bad water transect (J. Reddell and R. Gibson, pers. comm.).

Aldridge 209 is also a new locality for the Bogidiellid amphipod *Parabogidiella americana* (Figure 28), which was previously identified from an artesian well in San Marcos (Hays County) and from two historic blindcat localities, the O.R. Mitchell Well and the Verstraeten Well. This site also yielded a third amphipod species that is not *Holsingerius* sp., which may be *Paraholsingerius* (Randy Gibson, pers. comm.). and the widespread isopod species *Cirolanides texensis.*

None of the worms or snails from the Aldridge 209 Well represent groundwater fauna; the leech (*Helobdella* sp.) is not troglobitic and in fact has quite prominent eyes, and the worms (*Dero* (*Aulophorus*) *furcata*) do not have any published affinities with groundwater, and the snail *Physa* sp. is a common inhabitant of freshwater ponds. All of these are likely to be surface contamination resulting from early sampling efforts at this site, when the net rested in a perennial stock tank inhabited by typical pond fauna.

List of taxa collected from Aldridge 209 Well

- Worms
  - Annelida
    - Hirudinea
      - Glossiphoniidae
        - *Helobdella* sp. (ID by Boris Sket)
      - Enchytraeidae (immature) (ID by M. Wetzel)
      - Naididae
        - *Dero (Aulophorus) furcata* (Müller, 1773) (ID by M. Wetzel)
- Mollusks
  - Gastropoda
    - Physidae
      - *Physa* (ID by J. Krejca)
- Crustaceans
  - Ostracoda
  - Decapoda
    - Palaeomonidae
      - *Palaemonetes antrorum* (Benedict, 1896) (ID by R. Gibson)
  - Amphipoda
    - Haziidae
      - *Holsingerius samacos* (ID by Randy Gibson)
      - *?Paraholsingerius* sp. (ID by Randy Gibson)
    - Bogidiellidae
      - *Parabogidiella americana* (Holsinger, 1980) (ID by J. Krejca and R. Gibson)
  - Isopoda
    - Cirolanidae
      - *Cirolanides texensis* (sight ID by JEAN KREJCA, needs microscope verification)
      - *Speocirolana hardeni* (ID by R. Gibson)
- Fish
  - Siluriformes
    - Ictaluridae
      - *Trogloglanis pattersoni* (ID by J. Lundberg)
      - *Satan eurystomus* (ID by J. Lundberg)

Figure 23. Partial *Trogloglanis pattersoni* collected from Aldridge 209 Well outlet, March 2009.

Figure 24. Fish, believed to be *T. pattersoni*, collected from the Aldridge 209 well outlet, July 2010.

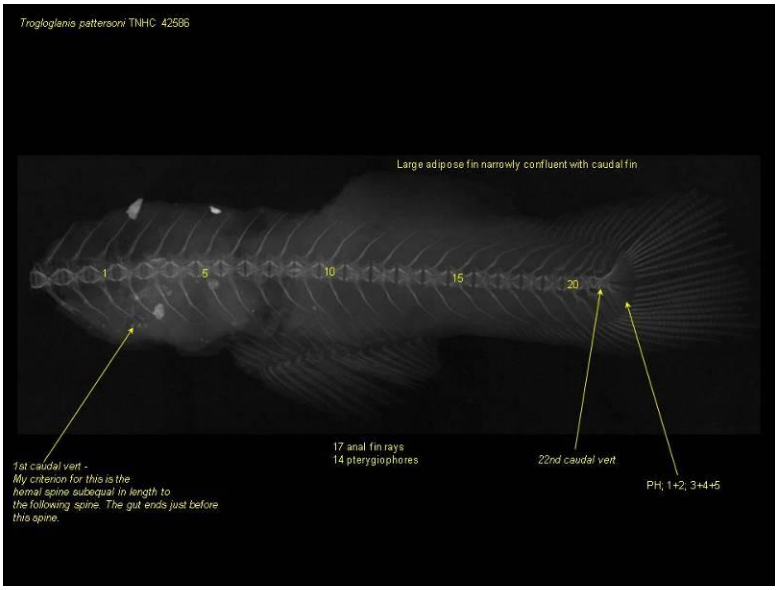

Figure 25. X-ray image showing diagnostic characteristics of partial *Trogloglanis pattersoni* collected from Aldridge 209 Well outlet, March 2009.

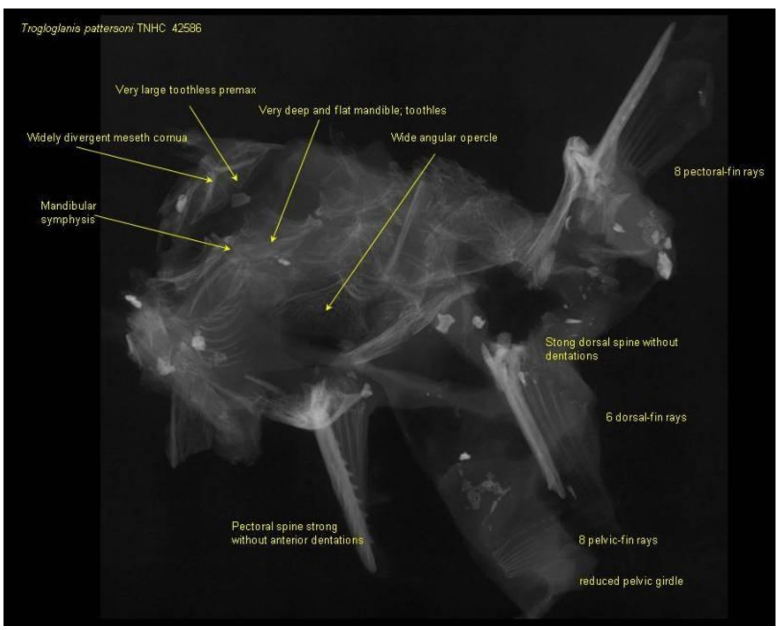

Figure 26. X-ray image of *Trogloglanis pattersoni* showing diagnostic features, March 2009.

Figure 27. The stygobitic shrimp *Palaemonetes antrorum* from the Aldridge 209 Well.

Figure 28. The stygobitic amphipod *Parabogidiella americana* from the Aldridge 209 Well.

Aldridge Corporate Well

The Aldridge Nursery owns property on Barker Road where the Aldridge Corporate Well is located; this well is used for irrigation. The well outlet used for sampling is angled slightly upwards, and when turned on flows freely down drainage into a creekbed at an average rate of about 2,271 liters per minute. To sample this site, a 500 μm net covered with a vinyl shade sock was cinched over the outlet, and a metal tub was placed just below the outlet so that the end of the net would remain submerged (Figure 29). Samples were retrieved by removing the net from the outlet and examining the inside of the net, usually by turning it inside out.

During the first year of the study, the Aldridge Corporate Well was sampled from April 10 to July 22, 2008, and again from August 4 to September 30, 2008. A total of 11 samples were taken from this site at irregular intervals in 2008, averaging one sample every 14 days. The president of Aldridge Nursery, Thomas Trautner, agreed to allow continuous access to this well without requiring that the net ne removed until the termination of this study, however on several visits the water had been re-directed for irrigation and was not flowing through our nets. Therefore, flow has not always been continuous.

The well was sampled 11 times from February 20 to September 1, 2009, with visits occurring approximately every three weeks. The well was visited 22 times between January 20, 2010 and October 13, 2010, with site visits occurring approximately weekly.

This well has yielded amphipods and isopods (*Speocirolana hardeni*), and occasionally biological sludge of unknown composition (Figure 30) that resembles skin. A sample of this material is currently awaiting genetic analysis[2]. Bones of an unknown acanthopterygian fish species were also retrieved from the net. Fish experts examining the material have determined that the bones do not belong to either species of blindcat, and are currently attempting to identify the material to family (Figure 31, and see discussion in "Collaborative Results" section above).


Figure 29. Aldridge Corporate Well flows over the ground down to a creek bed.

[2] The sample in Figure 30 is currently in the possession of John Lundberg. X-ray imaging of the material revealed no skeletal parts. Without bones, positive results for these samples would have to be based on a sequence match with a known organism (John Lundberg, pers. comm., 10 May 2010).

Figure 30. Unidentified biological material collected from the Aldridge Corporate Well on April 18, 2010 is currently awaiting genetic sequencing at John Lundberg's lab in Philadelphia.

Figure 31. Unidentified bones collected from the Aldridge Corporate Well on July 14, 2010. These bones do not belong to either species of blind catfish.

In addition to being a locality for *S. hardeni* and an unidentified fish, the Aldridge Corporate Well represents a new locality record for the amphipod *Allotexiweckelia hirsuita,* which was previously documented from Bexar County, but not from that site.

List of taxa collected from Aldridge Corporate Well
Crustaceans
  Amphipoda
    Haziidae
      Allotexiweckelia hirsuita
    Bogidiellidae
  Isopoda
    Cirolanidae
      Speocirolana hardeni (ID by Randy Gibson)
Fish (undetermined material)

Aquarena Springs Well

The Aquarena Springs Well is a monitoring well located in the parking lot of Texas State University's Aquarena Center in San Marcos, Hays County (Figure 32). This 226 meter deep well, located inside of a locked fence for protection, does not flow. A bottle trap was placed in this well on April 9, 2008. No specimens were recovered from the trap and sampling at this location was not repeated in 2009 or 2010.

Figure 32. Monitoring Well at Aquarena Center.

Bexar Met Well #8

On February 27, 2008 this monitoring well was sampled while the Authority purged it. A 60 µm plankton was attached to the outflow and one sample was collected after a cumulative sample time of approximately 1 hour, after which the net was removed. No fauna were present in the sample, and sampling at this location was not repeated in 2009 and 2010.

Bexar Met Well #91

On February 27, 2008 this monitoring well was sampled while the Authority purged it. A 60 µm plankton was attached to the outflow and one sample was collected after a cumulative sample time of approximately 1 hour, after which the net was removed. No fauna were present in the sample, and sampling at this location was not repeated in 2009 and 2010.

Carroll Farms Well

This warm water Medina County irrigation well could only be sampled seasonally, when the farmer irrigated, and due to the high discharge rate the net could only be attached when the well was not flowing. The net blew off of this outlet several times and had to be replaced. This well was sampled seven times in 2009, and seven times in 2010, but no fauna have been collected. The outflow from this well flows into a large reservoir (Figure 33).

Figure 33. Carroll Farms Well outflow into stock tank.

City of Kyle Well

A bottle trap was placed into this 181 meter deep monitoring well on March 19th, 2008 (Figure 34). This trap was checked when the well data was being downloaded by the Authority, approximately once per month. No specimens were retrieved from the trap. Sampling at this location was not repeated in 2009 or 2010.


Figure 34. A bottle trap was used to sample the City of Kyle well, located underneath the Kyle water tower.

Constanzo Well #2

This well is located along West Skaggs Road, near the Aldridge 209 Well. Two pipes lead from the well underneath the road and discharge into the BMA Canal. This water consistently left thick, oily residue on the nets. This outlet was first sampled with a 60 µm plankton net secured over the outlet and resting in a bucket (Figure 35), but the nature of the discharge destroyed the net, so sampling methods were altered by securing a disposable paint strainer to the outlet and resting the end in a bucket under the outlet. The strainers were replaced during each visit and discarded after being checked for fauna. Flow from this site was typically quite low, approximately 3.8 liters per minute.

This site was sampled from May 12, 2008 until August 11, 2008, at which time all sampling equipment was removed from the site. No fauna were detected, and due to the thickness of the oily residue left on the sampling equipment it was not likely that extending the sampling period would yield positive results. In addition, the net location was often inundated in the canal, making sampling more difficult. Sampling at this location was not repeated in 2009 or 2010.


Figure 35. Constanzo Well #2 discharged into the BMA canal.

Fort Sam Houston Well #2

This well has a 5 centimeter diameter overflow drain pipe that flows into a street drain covered by a metal sheet. A 60 μm plankton net was attached to the drainpipe so that the bottle rested in a small pool of accumulated water in the bottom of the drain (Figure 36). This site must be accessed through a locked gate that can be opened only by Fort Sam Houston personnel with a code, and it has proven logistically difficult to access on occasion. A larger ouflow exists that would have been far superior, but the valve that opens it was seized.

This outlet was sampled nine times from February 19 to June 19, 2008. On the last visit the net was completely dry and there was no water coming from the drainpipe. Sampling at this location was not continued in 2009 or 2010.

Although the Fort Sam Houston (FSH) #2 sample site did not have high flow (less than 7.5 liters per minute) and did not provide much water to sample, interesting collections resulted from the site. The ostracod *Cypridopsis vidua* (Figure 37), also recorded from the Schumann House Well, is cosmopolitan, occurring on most continents and in most freshwater habitats, including ponds, ditches, springs, and ephemeral wetlands. Although morphologically variable in size and color, collections from FSH #2 were atypical in several respects, and Rosalie Maddocks had to perform dissections in order to verify that the specimens fell within the report range of variation for the species. This variation probably has both environmental and genetic sources.

The FSH #2 site also produced a copepod species that is new to science and not yet described, another species that the site shares with the Schumann House Well. *Diacyclops* is a very speciose genus and it is especially successful in subterranean environments (Janet Reid, pers. comm., 11 April 2008). This species is now known only from FSH #2 and the Schumann House Well in Comal County.

The insects from this site are the result of surface contamination, due to the morphology of the site the net rests in standing water. The thermosbaenacean is probably *Tethysbaena* (=*Monodella*) *texana*, which represents a new locality for the species (Figure 38). The order is interesting due to its relict Tethys Sea distribution, and that it is the only known species in this order from the continental United States.

List of taxa from FSH Well #2

Gastropoda
- Ostracoda
  - Cyprididae
    - *Cypridopsis vidua* (Müller, 1776) (ID by Rosalie Maddocks)
- Maxillopoda (Copepods)
  - Cyclopidae
    - *Diacyclops* new sp. (ID by Janet Reid)
    - undetermined juvenile copepod (nauplii) (ID by Janet Reid)

Insecta (larvae) (accidental surface contamination) (ID by Jean Krejca)
- Chironomidae
- Diptera
- Coleoptera

Thermosbaenacea
- Thermosbaenidae
  - probably *Tethysbaena texana* (ID by Jean Krejca)

Figure 36. A plankton net was attached to the outflow at FSH #2. The collection bottle remains submerged when there is water pooled in the drain.

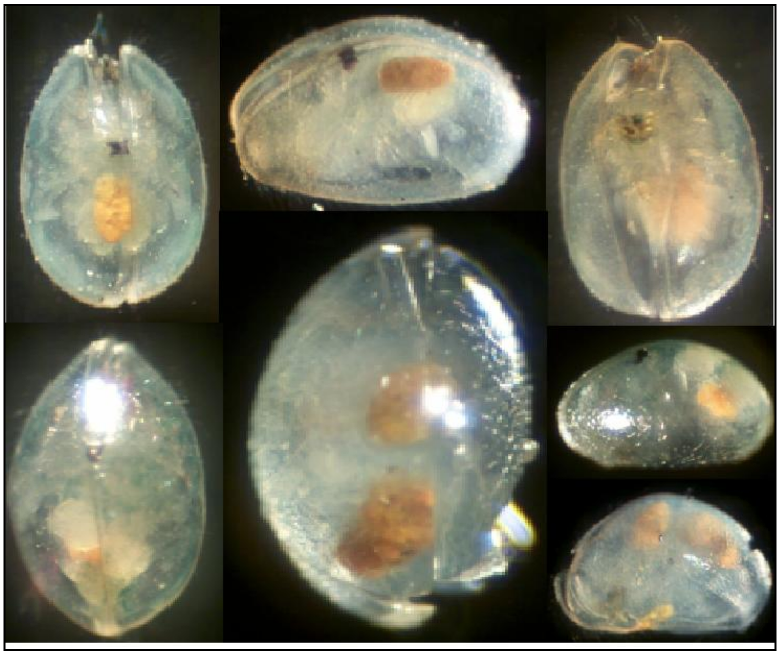

Figure 37. *Cypridopsis vidua* collectedfrom FSH #2; photographed by Rosalie Maddocks.

Figure 38. *Tethysbaena texana;* photograph by Jean Krejca from Ezell's Cave in Hays County.

Fort Sam Houston Well #5

This well flows from a 25 centimeter diameter cast iron pipe down a bank and into Salado Creek. Concrete chunks were excavated from the area beneath the opening in order to fit a large plastic tub under the outflow. A 500 μm plankton net was cinched over the opening. The net rested in the plastic tub, where the bottom of the net remained submerged when water was flowing (Figure 39).

This location was visited18 times from 19 February to 19 June 2008. This well was sampled by visually inspecting the net for macrofauna and scraping sediment from the collection container at the end of the net to examine in the lab at Zara. No subterranean fauna were retrieved from this site. Fort Sam Houston personnel decided to turn off the flow in summer 2008. Sampling at this location was not repeated in 2009 or 2010.

Figure 39. We set up a tub at Fort Sam Houston Well #5 to ensure that samples would remain submerged.

Girl Scout Well

On April 2, 2008 this monitoring well was sampled while the Authority purged it. A 60 µm plankton net was attached to the end of a garden hose and a sample was collected after approximately 1 hour, after which time the net was removed. Sampling at this location was not repeated in 2009 and 2010.

No notable taxa were recorded from this location. The water sample that was retrieved was thought to contain ostracods, however upon further examination it was determined that these were actually small rotifers or flagellates (Figure 40) and a thecamoebian test (Figure 41). The sample also contained one mite that was probably the result of surface contamination.

Figure 40. Rotifer or flagellate collected from Girl Scout Well; photographed by Rosalie Maddocks.

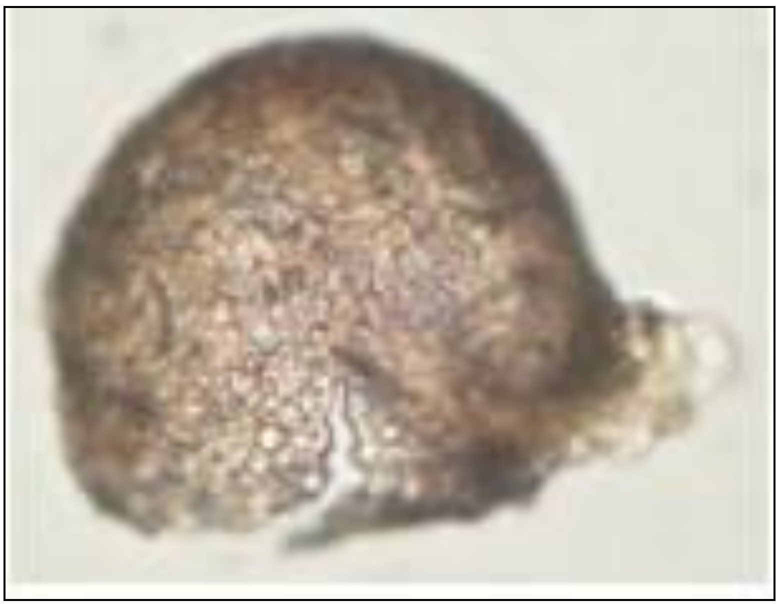

Figure 41. Thecamoebian test collected from Girl Scout Well; photographed by Rosalie Maddocks.

Homer Verstuyft Well

This well flows through an irrigation line where an in-line sampler was installed (Figure 42), and also has a smaller outflow into a household water supply tank (Figure 43) where a plankton net was attached to an overflow pipe. There was a third outflow into an irrigation ditch, and initially that outlet was sampled by situating a tub under the flow and cinching a net to the end of the irrigation line, but the installation of the in-line sampler in the ditch made this unnecessary.

This site was sampled five times between May and December 2009, and then ten times between January 2010 and June 2010. In June 2009, the net in the sampler in Figure 42 was dry and full of ants. In July 2009, a common species of surface-dwelling amphipod was recorded from the ditch outlet (Figure 44). Other taxa recorded at this site can be defined as surface contamination, including mites (Figure 45) and fly larvae (Figure 46), but no aquifer fauna have been collected.

Figure 42. In-line barrel sampler at the Homer Verstuyft Well.

Figure 43. Homer Verstuyft Well outflow into irrigation ditch.


Figure 44. *Hyalella* amphipod collected from the Homer Verstuyft Well, a common non-subterranean genus.


Figure 45. Acari (mites) collected from the Homer Verstuyft Well.


Figure 46. *Pericoma* fly larvae collected from the Homer Verstuyft Well.

Hondo Index Well

The Hondo Index Well is a monitoring well located in Hondo, Medina County. This 457.2 m deep well, located inside of a locked fence for protection, does not flow. A bottle trap was placed in this well on June 12, 2008. No fauna were recovered from this location, and sampling was not repeated in 2009 or 2010.

Jeff Bailey Well

This well is located in Bexar County, and was visited seven times in 2010, although water had only flowed through the net prior to four of those sampling events. An in-line sampler was initially installed at an irrigation pipe outflow to the north (Figure 47), which was used for about four months out of the year, and a shade structure similar to that in Figure 20 was placed over the sampler. The design was changed to sample from the end of the pipe shown to the right of the valves that are visible in Figure 47; the modified sampling setup involved cinching a net with a shade sock over the end of the pipe, which flows into a stock tank (Figure 48). The stock tank is used for irrigation year-round, allowing more continuous sampling opportunities than the previous set up would have. No fauna have been retrieved from this site.

Figure 47. In-line sampler at the Jeff Bailey Well.

Figure 48. The Jeff Bailey well flows through our net to fill a stock tank that is used for irrigation.

LCRA Well

This 294 m deep artesian well flows up a vertical pipe approximately 4.9 m into the air before surfacing with an additional 0.3 to 1 m head, depending on the level of the aquifer (Figure 49). The well is located in Comal County. Personnel from the Authority attached a hose approximately 15 cm in diameter to the well at about 2.1 m above the ground and strung the hose down Fredericksburg Road approximately 365 m to a drain where the hose discharged to. A 60 μm plankton net was attached to the end of the hose, a sample was collected after 3 hours (Figure 50). This method of sampling was performed on 12 March 2008, 4 March 2009, and 3 March 2010, however no fauna have been identified in samples from this site during this study.

Figure 49. Artesian flow at the LCRA well.

Figure 50. A plankton net secured to the end of the net captures outflow from the LCRA well.

Loop 353 Well

Loop 353 Well is an irrigation well with east and west outlets. The west outlet flows into a concrete basin before irrigating a field. Owner Tom Verstuyft says that this well was drilled about 100 years ago by his grandfather and that blind catfish came out at that time. Mr. Verstuyft has been irrigating from this well for 50 years, and has not seen any of these fish since he started irrigating. A 500 µm net was attached to the outlet and situated in a plastic pool inside of the concrete basin to keep the end of the net submerged (Figure 51), but the design was improved with an in-line barrel sampler in 2010 (Figure 52). This well is run occasionally by the owner, Tom Verstuyft, for irrigation, but cannot be left on continuously because it will flood his field. Nets were removed in May 2008 and reattached in February 2009. The outlet to the east was fitted with an in-line sampling device and sampling of that outlet started on 13 May 2009 (Figure 53).

The Loop 353 well was sampled on 11 occasions between January and June 2010. On June 5, a sample was retrieved that appeared to contain skeletal material, and was sent to John Lundberg's laboratory for imaging and genetic analysis. The images revealed a pair of gecko skeletons embedded in ground, white flesh, with clear limbs, backbones and two sets of mandibles, that unfortunately represent surface contamination into the net (Figure 54**Error! Reference source not found.**).

Figure 51. A plastic tub was put into place at the Loop 353 Well to ensure that any samples collected remained submerged until they could be retrieved.

Figure 52. A closed barrel sampler was installed on the west outlet of the Loop 353 Well in 2010.

Figure 53. The east outlet of the Loop 353 Well is fitted with an in-line sampler.

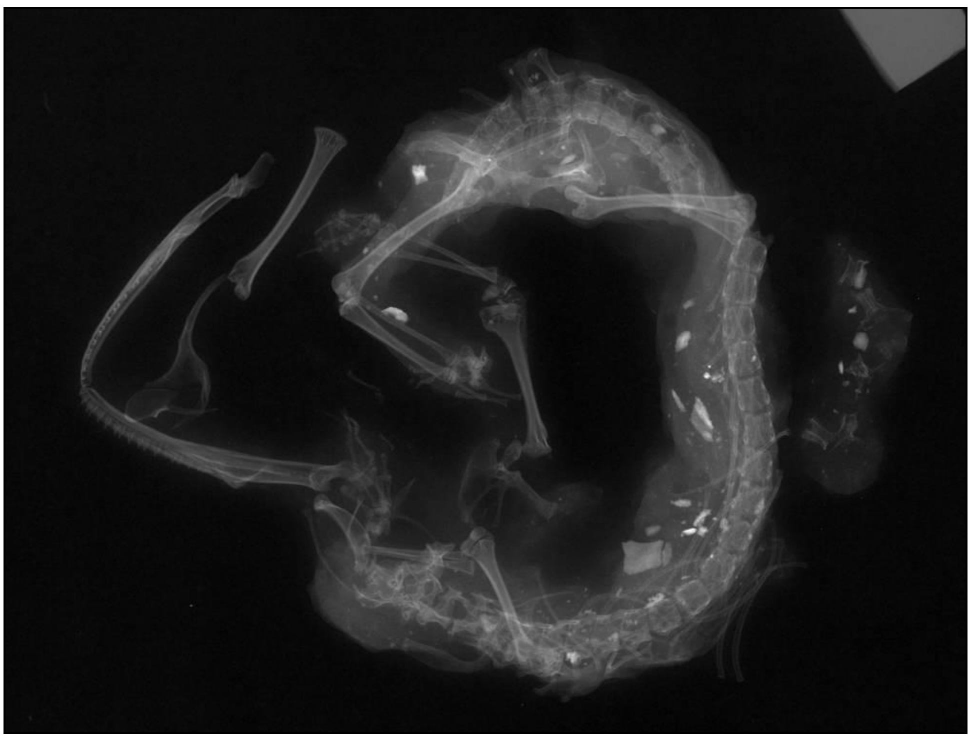

Figure 54. Digital scan of gecko material from Loop 353 Well, showing almost an entire intact skeleton.

List of taxa collected from Loop 353 Well

Crustaceans
- Ostracoda
  - *Chlamydotheca arcuata* (ID by Rosalie Maddocks)
- Isopoda
  - Cirolanidae
    - *Speocirolana hardeni* (ID by R. Gibson)

Gecko
- Squamata
  - Gekkonidae

Lyda (Fallon) Partin Well

The Lyda (Fallon) Partin well was first sampled on 10 March 2010, and visited on eight occasions between March and the end of September 2010. Samples collected in May and June contained black and brown sludge of unknown origin and composition, however no fauna were observed in the samples.

Mark Verstuyft Well

Thirteen visits were made to the Mark Verstuyft Well during the 2010 sampling year. This well was sampled using a standing sampler very similar to what was used at the Roger Verstuyft Well site (Figure 55). This well did not yield any stygobitic fauna.

Figure 55. Riser sampler on irrigation pipe at the Mark Verstuyft Well. This structure was built for the purposes of this study.

Mission Bowling Well

There were two bottle traps placed in Mission Bowling Well, a known *Eurycea sp.* site. These traps were checked approximately monthly from June through November 2008, but no fauna were retrieved. This well is under study by other researchers, namely Dr. Andrew Gluesenkamp of Texas Parks and Wildlife Department, and any significant findings remain in his possession. The trap was removed at the end of 2008, sampling as part of this study was not repeated in 2009 or 2010.

Nelson Road Well

A 500 micron net was set in an outflow to a reservoir on 4 May 2009. Temperature was not recorded at this site during the 2009 sampling period because the outflow was typically below the water level of the pond (Figure 56). On June 2, 2009 the net was removed because it had been sucked back through the pipe and torn. Fauna collected from this well includes unidentified gastropods, ostracods, copepods, mites, and amphipods. There is a high occurrence of surface contamination at this site, including snakes (*Nerodia sp.*), sunfish (*Lepomis* sp.), non-subterranean aquatic mites, pond snails (*Physella* sp.) and non-subterranean aquatic insects. An in-line sampling device was developed for this well and installed in 2010 (Figure 57) to assist with accessibility of the net and reduce the likelihood of contamination from surface waters.

An individual of the *Parabogidiella* amphipod was documented at the Nelson Road Well, another new site locality for the genus. If the Nelson Road Well specimen represents *P. americana* then the Nelson Road Well will represent the only Bexar County site for the species where no catfish material has yet been retrieved. That could change, since sampling equipment has recently been upgraded.

<u>List of taxa collected from Nelson Road Well</u>

- Mollusks
  - Gastropoda
    - Physidae
      - *Physella* (ID by R. Hershler)
    - Planorbidae
      - *Helisoma* (ID by R. Hershler)
- Crustaceans
  - Branchiopoda
    - Cladocera (ID by Rosalie Maddocks)
  - Copepoda
  - Amphipoda
    - Bogidiellidae
      - *Parabogidiella* sp. (ID by Randy Gibson)
- Insects
  - Acarina

Figure 56. Prior to the installation of an in-line sampler, the net at the Nelson Road Well was typically underwater, so we did not record outflow temperature at this site.

Figure 57. An in-line sampler was installed at the Nelson Road Well in 2010.

New Braunfels Transect Wells

On March 31, 2008 The New Braunfels Transect wells were purged by the Authority at a rate of 11 liters per minute. A 60 µm plankton net to the end of a 4.6 m hose attached to the outflow at this site and collected one sample after a cumulative sample time of approximately 1 hour, after which Krista removed the net. No fauna were present in the sample, and sampling at this site was not repeated in 2009 or 2010.

Panther Canyon Well

On April 1, 2008 a bottle trap was placed in Panther Canyon Well. The opening to this well is located inside a locked well house (Figure 58) and the well is only suitable for sampling with a bottle trap (Figure 59). This trap was checked on April 16 after approximately 360 hours of sampling and again on May 15 after approximately 1080 hours of sampling. The trap was removed on May 15 and replaced on June 10, 2008. The trap was removed at the end of 2008, and was not replaced. Sampling at this location was not repeated in 2009 or 2010.

The aquifer adapted isopod *Cirolanides texensis* was collected from this site, which is a known locality for this species and several other stygobitic crustaceans, including the federally listed amphipod, *Stygobromus pecki* (Krejca 2005) (Figure 60).

Figure 58. Panther Canyon well house.

Figure 59. The opening of Panther Canyon Well.

Figure 60. *Cirolanides texensis* and *Stygobromus pecki* from Panther Canyon Well.

Paradise Alley Well

On April 2, 2008 Authority personnel purged this site at a rate of 11 liters per minute. A 60 µm plankton net was attached to the end of a 4.6 m hose attached to the outflow at this site and one sample was collected one sample after approximately 1 hour. No fauna were retrieved from this site, and sampling was not repeated in 2009 or 2010.

Persyn Well

Mark Verstuyft arranged access to the Persyn Well, which is located east of I-35 near Somerset Road. This well was sampled six times in 2010, through the use of an in-line sampler (Figure 61). Inorganic materials, including rocks, rust and clumps of clay and tar have been retrieved from this net. Samples from this site often smelled sulfurous, although no water chemistry analysis has been performed. No fauna has been recorded from this site, however some samples have been shared with Ben Hutchins and Annette Summers-Engel.

Figure 61. A barrel sampler was installed on the Persyn Well.

Raymond Wauters Well

This well has two outlet pipes; an in-line sampler was installed at the north outlet in 2009 (Figure 62), and replaced with an improved design in 2010 (Figure 63). A net was also attached to the south outlet, which was utilized less frequently. Water from this well contained rust-colored sediments that tended to clog nets, however examination of the net and sediment have not resulted in any fauna collections.

Figure 62. An open barrel style sampler was used to samplethe Raymond Wauters Well in 2009.

Figure 63. A closed riser sampler was installed at the Raymond Wauters Well in 2010.

Roger Verstuyft Well

According to Roger Verstuyft, this well was sampled during the Karnei (1978) study, though it was not documented. Discussions with Glenn Longley at Texas State University-San Marcos, who was also heavily involved in the study, indicated that unproductive wells may not have been included in their reports. With the assistance of manager Doug Verstuyft, a steel riser structure was modified to allow installation of a net (Figure 64).

This well was sampled five times from May 22, 2009 through November 10, 2009, and nine more times between January 20, 2010 and June 30, 2010. Although unidentified sludgy material has been collected from this site, no fauna were observed in the samples.

Figure 64. The top of this riser structure was modified with a lid that flips open, allowing access to a net attached to the pipe outlet.

Roosevelt Bridge Springs

This site is a fist sized spring outlet located about 30 m upstream of Roosevelt Bridge on the northeast bank of the San Antonio River. A mop head covered with an aquarium net was installed in the spring orifice on February 19, 2008 (Figure 65). This site is documented to have yielded stygobitic isopods in the genus *Mexistenasellus (*Stenasellidae), however none were encountered during this study. Two samples were collected from this site in 2008, but the only fauna that was collected was an unidentified (eyed) planarian. Sampling at this site was not repeated in 2009 or 2010.

Figure 65. Roosevelt Bridge Spring outlet with aquarium net. The mop head is pushed back into the spring orifice, out of sight.

San Antonio Zoo Well

This high flow site was first sampled with a 500 µm net with a rectangular metal frame that was tied over the well opening. The net that was installed on April 17, 2008 was found to have blown off when checked on April 22. The net was reinstalled on April 22, 2008 and then removed again on May 9, 2008 for repairs. A replacement net without a frame was cinched over the well opening on June 3, 2008 (Figure 66). This net tore and was replaced on June 20, 2008. The replacement net was protected from the sharp edges of the outlet by securing rubber inner tubing directly over the pipe. When this well was checked on July 14, 2008 it had torn again, and was removed for the season.

On March 12, 2009 a 500 µm net was installed over the rubber tubing that was still in place from 2008. The net was maintained until June 12, 2009, when it was found torn and permanently removed. Sampling at this site was not repeated in 2010.

The meter for this site is located inside of a locked well house that must be opened by a zoo employee. Groundspersons were typically unavailable to unlock the well house, so meter readings were not recorded at this site.

While the San Antonio Zoo Well was a logistical challenge to sample due to the repeated destruction of sampling equipment resulting from the high flow at the site, three groundwater species were collected from there, including the regionally common stygobitic isopod *Cirolandies texensis.* A slightly less well-documented isopod that was collected from this site as well as from the Tschirhart Well is *Mexistenasellus coahuila*, which is classified as "endangered" on the IUCN list of threatened species (Inland Water Crustacean Specialist Group 1996) (e.g.-Figure 67). Also from the Zoo well we collected a minute snail in the genus *Phreatodrobia* (Figure 68). This genus occurs primarily in the Balcones Fault Zone region of Texas, and the only records from Bexar County prior to this come from two other wells (Hershler and Longley, 1986), both of the species *P. imitata*, which is likely what the specimen from the Zoo well represents. While there are no current state or federal mandates in place to protect this species, it was proposed for listing and received a positive 90 day finding from USFWS in 2009, indicating that listing for the species may be warranted. The discovery of this probable new site locality for the species will be important information if USFWS moves forward with the listing of this species.

<u>List of taxa collected from San Antonio Zoo Well</u>

Worms
  Annelida

Gastropoda
  Hydrobiidae
    Phreatodrobia sp. (ID by J. Krejca, R. Gibson)

Crustaceans
- Amphipoda
  - Haziidae (ID by R. Gibson)
- Copepoda
  - Cyclopidae
    - *Paracyclops chiltoni* (ID by J. Reid)
  - Ameiridae
    - *Nitokra spinipes* (ID by J. Reid)
  - Canthocamptidae
    - *Epactophanes richardi* (ID by J. Reid)

Isopods
- Cirolanidae
  - *Cirolanides texensis* (ID by R. Gibson)
- Stenasellidae
  - *Mexistenasellus* (*coahuila*?) (ID by R. Gibson)

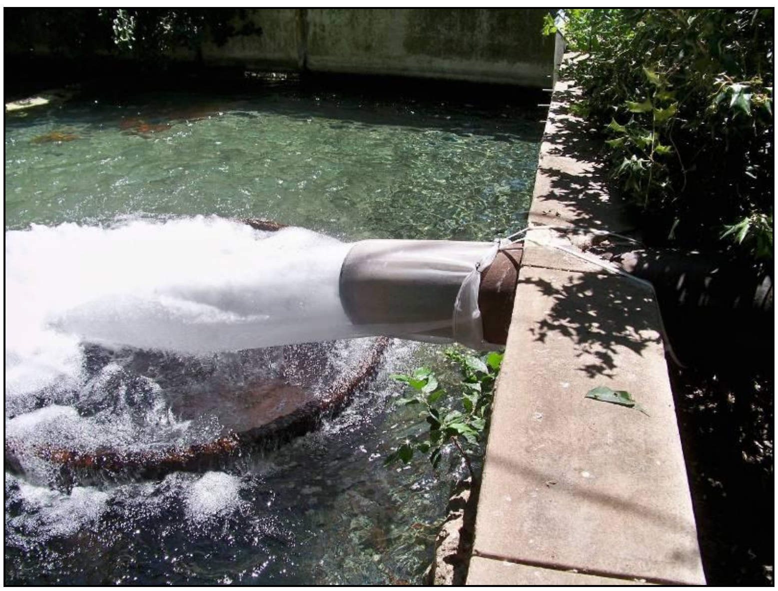
Figure 66. High flow at the San Antonio Zoo Well often damaged the nets and jeopardized samples.

Figure 67. The isopod *Mexistenasellus coahuila*. The individual pictured was collected in Mexico.

Figure 68. The hydrobiid snail *Phreatodrobia* sp. collected from the San Antonio Zoo well.

San Marcos Flagpole Well

This well is located near the Texas State University informational map, by the flagpole on Aquarena Springs Drive in San Marcos, Hays County. This artesian well does not flow, but was sampled using a bottle trap (Figure 69) beginning in the summer of 2008. The trap was removed at the end of 2008, and was not replaced. No fauna were recovered from this site, and sampling was not repeated in 2009 or 2010.

Figure 69. The San Marcos Flagpole Well was only large enough to drop a bottle trap into. There was no flow at this site.

Schumann Artesian Well

This non-flowing artesian well was accessed through a large diameter PVC pipe (Figure 70). This site was sampled with bottle traps beginning in February 2008. The traps were removed at the end of 2008, and were not replaced. No fauna were retrieved from this site, and sampling at this location was not repeated in 2009 or 2010.

Figure 70. The Schumann Artesian Well is accessed through a PVC pipe.

Schumann House Well

Water from the Schumann House Well flowed out of a small pipe and into a concrete stock tank (Figure 71). This site was sampled by placing a plankton net over the outflow and collecting samples from the collection bottle at the base of the net. Samples were collected from this site approximately monthly until the end of 2008, when the net was removed. Sampling at this location was not continued in 2009 or 2010.

The widespread freshwater copepod species *Acanthocyclops vernalis* and widespread surface freshwater ostracod *Cypridopsis vidua* (Figure 37) were collected from this site. Additionally, eight individuals of the obscure order of aquifer crustaceans Bathynellacea have been identified from this site (Figure 72). The bathynellacean species is the first record in this order for the Edwards Aquifer. Previously the species was only known from Dickens and San Saba counties, Texas.

<u>List of taxa from Schumann House Well</u>
Annelida (worms)
Crustaceans
- Ostracoda
  - Cyprididae
    - *Cypridopsis vidua* (Müller, 1776) (ID by Rosalie Maddocks)
- Maxillopoda (Copepods)
- Cyclopidae
    - *Diacyclops* new sp.
    - *Acanthocyclops vernalis* (Fischer, 1853) (ID by Janet Reid)
    - undetermined juvenile copepod (nauplii) (ID by Janet Reid)
- Bathynellacea
  - Parabathynellidae
    - *Texanobathynella bowmani* (ID by Janet Reid)

Insecta (larvae) (accidental surface contamination) (ID by Jean Krejca)
Acarina

Figure 71. Sampling setup at Schumann House Well, where the water flows into a large concrete tank.

Figure 72. *Texanobathynella bowmani* belongs to an order of crustaceans not previously recorded in the Edwards Aquifer.

Shavano Park #5

On February 25, 2008 Authority personnel discharged water from the Shavano Park well at a rate of 11 liters per minute. A 60 µm plankton net was attached to the end of a 4.6 m hose attached to the outflow at this site, and one sample was collected after a approximately one hour, after which time the net was removed. No fauna were retrieved at this site, and sampling was not repeated in 2009 or 2010.

Steve's Homestead (San Antonio Conservation Society)

The historic Edward Steve's Homestead has an artesian well that had previously been used to fill a swimming pool on the property (Figure 73). The well has since been capped and can now be turned on with a valve that allows water to flow through a garden hose attachment (Figure 74). This site was sampled for one hour on March 4, 2008 using a60 µm plankton net. No fauna were detected in the sample, and sampling at this site was not repeated in 2009 and 2010.

Figure 73. This swimming pool on the Steve's Homestead used to be filled solely from artesian flow from the well on the property.

Figure 74. The artesian well at Steve's Homestead has been capped and was accessible for sampling only through a garden hose.

Stull Farms Well

A net was initially installed on the Stull Farms Well outlet on October 23, 2009 (Figure 75), and checked on November 3, 2009, but no fauna were present. This site was visited ten times between January 20, 2010 and September 15, 2010. Flow through the net was intermittent during that time, and surface contamination was sometimes observed in the net, but no aquifer fauna have been retrieved from the site.

Figure 75. A net covered with a vinyl shade sock rests in a tub to keep it submerged when the reservoir water level drops at Stull Farms.

Tschirhart Well

This well outlet occasionally flows into a reservoir containing thick emergent vegetation, however the flow is also diverted into irrigation lines. A net was attached to the outlet in the reservoir (Figure 77), but this valve could only be turned on for limited periods of time or the reservoir would overflow. An in-line (closed barrel) sampler was used where the flow was directed into irrigation pipes (Figure 78). This well often contains some amount of surface contamination, including millipedes (Figure 79) and surface snails (Figure 80), but also yields high amounts of aquifer-associated material, including unidentified (bacterial?) mats and unidentified amphipods (Figure 81) with stygobitic morphology.

This well has the second highest diversity of groundwater fauna found at any of the sample sites and represents new locality records for four stygobites. Two species from two different genera of amphipods were collected(e.g.-Figure 83): *Holsingerius samacos* (Figure 83), which together with collections from Aldridge 209 represent the first confirmed record of this species in Bexar County, and *Parabogidiella americana.* The isopods *Cirolanides texensis* (Figure 84) and *Mexistenaselus coahuila* were also collected from this site.

The ostracod *Chlamydotheca arcuata* was collected from Loop 353 as well as Tschirhart. This large (up to 2.7 mm) North American species varies in color and size, some with dark green irregular bands radiating from the central region, as in Figure 86. Although burrowing, interstitial, aquifer and cave-dwelling Ostracoda are typically much smaller, unpigmented and blind, Rosalie Maddocks (pers. comm., 14 June 2010) cautioned against discounting this species as surface contamination, stating that "clearly these populations are living and reproducing in the Tschirhart well consistently (November-April) in large numbers."

Another significant collection at this site includes unidentified fish bones (Figure 87), which may represent blind catfish. These bones are currently awaiting analysis at the laboratory of John Lundberg, but the sample is very small and diagnostic features have not yet been identified.

Figure 76. Water flows through two large tank reservoirs before outflowing through our net.

<u>List of taxa collected from Tschirhart Well</u>

Worms
- Annelida

Gastropoda
- Physidae
  - *Physa* sp.

Crustaceans
- Ostracoda
  - *Chlamydotheca arcuata* (ID by Rosalie Maddocks)
- Amphipoda
  - Haziidae
    - *Holsingerius samacos* (ID by R. Gibson)
  - Bogidiellidae
    - *Parabogidiella americana* (ID by Randy Gibson)

Isopods
- Cirolanidae
  - *Cirolanides texensis* (ID by R. Gibson)
- Stenasellidae
  - *Mexistenasellus coahuila* (probable ID; R. Gibson)

Fish
- Ictaluridae

Figure 77. The Tschirhart well flows into an overgrown reservoir.

Figure 78. Inline barrel sampler in the irrigation line at the Tschirhart Well.


Figure 79. Millipedes were often encountered on or in the net at the Tschirhart Well.


Figure 80. Common pond snail, *Physa*, collected from the Tschirhart Well.


Figure 81. Amphiopod collected from the Tschirhart Well.

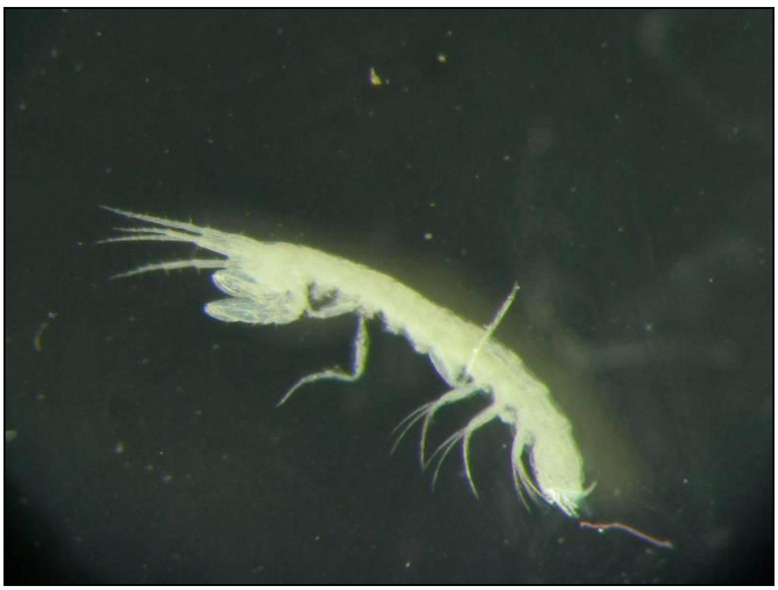
Figure 82. Amphipod colelcted from the Tschirhart Well.

Figure 83. Stygobitic amphipods (*Holsingerius samacos*) collected from the Tschirhart Well.

Figure 84. Stygobitic isopod *Cirolanides texensis* collected from the Tschirhart Well.


Figure 85. Ostracods (Chlamydotheca arcuata) collected from the Tschirhart Well.

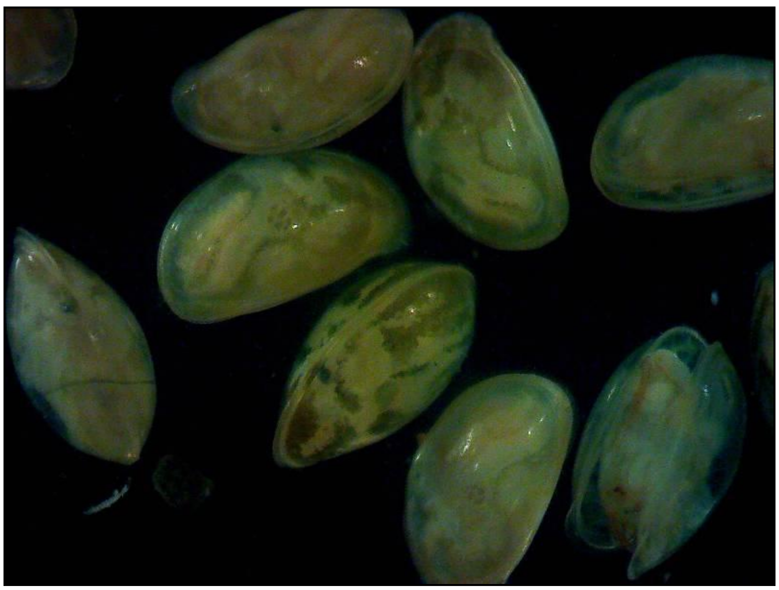
Figure 86. The ostracods Chlamydotheca arcuata can have varying color patterns. These were collected from the Tschirhart Well.

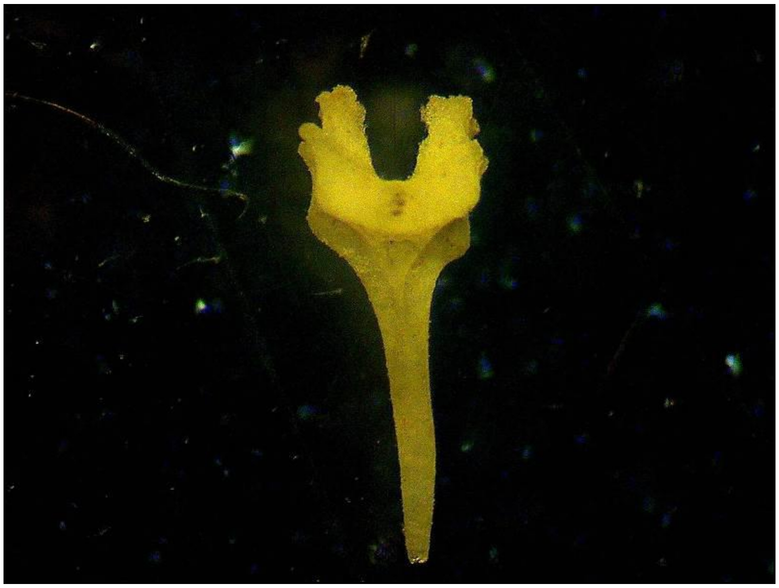
Figure 87. Vertebra collected from the Tschirhart Well on June 5, 2010.

Uvalde Index Well

The Uvalde Index Well is located inside of a chain link fence and locked well house. A bottle trap was installed in this monitoring well on June 12, 2008. This trap was checked sporadically during the 2008 sampling season. The trap was removed at the end of 2008, and was not replaced. No fauna were retrieved from this site, and sampling was not repeated in 2009 or 2010.

Verstuyft Farms Well

This site was visited 14 times from February 20, 2008 and August 14, 2008. Sampling this site initially proved challenging because the water flows out from the bottom of a pool in a concrete aqueduct, and while a net was attached to the ouflow that could be accessed and reattached by using a snorkel, it was logistically difficult to do. This site was also sampled by securing a net in the water flowpath downstream of the outlet, however the net was subjected to surface contamination and it was difficult to ensure that all of the water flowed through the net (Figure 88).

This site was visited 15 times between January 20, 2010 and October 13, 2010. In late 2009, a filter sampler was designed and installed at this site to allow more accessible and efficient sampling (Figure 89). Unidentified ostracods and gastropods have been observed at this site, however no official taxonomic determinations have been made.

Figure 88. The Verstuyft Farms Well outlet is located at the bottom of this aqueduct head, and one method of sampling involved securing a net in the path of the water downstream of the outlet (shown).

Figure 89. A filter style sampler was installed on the aqueduct outflow at Verstuyft Farms to facilitate easier sampling. The filter is shown here in its disassembled state.

Verstuyft Home Farm Well

The sampled outlet from this well discharges into an unlined ditch; this site was initially sampled by attaching a net to the outlet pipe and situating a metal tub underneath (Figure 90), but modified in 2010 so that samples could be obtained directly from the irrigation line

(Figure 91). A net was cinched over the outlet pipe in July 2008. This net tended to get clogged with white sulfur biomat sludge, although no other fauna has been recorded.

Figure 90. The initial sampling setup at the Verstuyft Home well involved flowing water through a net into a metal tub to keep the net submerged.

Figure 91. The modified sampling setup at the Verstuyft Home well involved detouring the water to run through a filter that could be sampled before the water returned to the main irrigation line.

Von Ormy Growers Well

This site was sampled for approximately 36 hours between May 9 and May 12, 2008. This well outlet discharged to an irrigation canal, however a plastic pool was placed beneath the discharge to ensure that the net remained underwater and that samples were battered as little as possible (Figure 92). Although no fauna was collected, the discharge from this site consisted of large amounts of white, filamentous material (Figure 93) that may have been bacteria, and smelled sulfurous. The owner gave limited access to sample the well, however this site was not ideal for the study, so sampling was discontinued.

Figure 92. A plastic pool was placed underneath the outlet from the Von Ormy Growers Well to keep the net submerged when the discharge was not flowing into the irrigation canal.

Figure 93. White filamentous material on net at Von Ormy Growers Well.

W.H. Guenther Well

This site consists of a water storage tank from which a 2” diameter pipe can be opened. On 23 March 2009 this site was sampled by running water from the storage tank through a 500 µm net for 15 minutes. No fauna were retrieved and sampling was not repeated due to difficult access and low available flow.

Woodley Farms Well No. 1

At Woodley Farms Well No. 1 (Figure 94) the landowner installed a high-pressure filter unit that allows water to flow through, but can be turned off by valves in order to check the filter. Sampling at this site began in 2010, however no fauna were retrieved.

Figure 94. Woodley Farms Well No. 1 prior to installation of a filter for sampling.

Woodley Farms Well No. 2

At Woodley Farms Well No. 2 the sampling infrastructure is such that water is diverted from the regular irrigation pipe to flow through an alternate pipe (Figure 95). There are valves on both sides that allow the alternate pipe to be sealed off during sample collection. After flowing through the filter, the water continues back into the regular irrigation pipe.

Figure 95. Sampling occurred at Woodley Farms Well No. 2 by examining a removable filter that the landowner had installed in the irrigation line for that purpose.

Yanta Well

This well is 0.3 m in diameter. The owner indicated that the well is 365 m deep and intersects a cave. A net was installed on the outflow pipe into the reservoir at this site on 23 October 2009, however on 28 October 2009 from the well owner's legal representative indicated that they did not wish to continue participating in the study. The net was removed on 3 November 2009; no samples were collected.

DISCUSSION AND FUTURE WORK

The subterranean diversity of the Edwards Aquifer is documented from limited areas where researchers have sampled intensively (e.g. Holsinger and Longley 1980; Culver and Sket 2000; Gibson et al. 2008), however the large number of new locality records discovered in this relatively short study (Table 4) demonstrate how much more remains to be found. Throughout these three years we have observed a gradual "ramping up" of sample numbers and species diversity, indicating that at most sites we have not reached an "asymptote" of productivity. In fact we have observed that the catch per unit effort has increased each year, and the most efficient sampling efforts may still be in front of us.

Springs, caves and artesian wells serve as sporadic, sometimes distantly spaced "windows" into a large aquifer. In central Texas, and particularly in the deep portion of the Edwards Aquifer, exceedingly few of these windows have ever been monitored by biologists, and typically only for very short periods of time. For many aquifer taxa, our current state of knowledge may include 10% or less of the total localities where the species actually occur. However, the fact that we sampled for a year or more at a single locality before a new record for that site was found indicates that the abundance and detectability of aquifer species is very low. Given that aquifer environments are probably similar to water-filled caves, our qualitative observations indicate sparse and patchy distributions of species. For a full understanding of the distribution of deep aquifer species, we are currently limited to making inferences about the environment 'in-between' the known well sites.

Future work most valuable to improving the inferences we make about the environment between known localities of rare aquifer species includes at least six immediately available technologies. (1) Sampling at more localities is the "low-hanging fruit" to help increase our understanding of these species. (2) Sampling further at existing localities is likely to lead to more discoveries, as is evidenced in this study by sites like the Aldridge 209 Well, where only after a year of continuous sampling did a single specimen of the toothless blindcat (a new locality) appear in our nets. Since then that site produced eight other fish collections, including the widemouth blindcat. (3) Population genetic analysis of abundant species that co-occur at multiple localities could provide estimates of migrants per generation between sites, and therefore give an indication of connectedness between known localities. For spring-dwelling *Eurycea* salamanders in central Texas, for example, analyses showed that each of the known localities were essentially unconnected, and that there was no evidence for dispersal via aquifers or surface waterways (Lucas et al. 2009). Aquifer species may show quite a different trend, since we are not aware of discontinuities in the deep aquifer habitat that would lead to population differentiation (beyond simply the large size of the aquifer). Conversely, differences in observed organic material emerging from nearby wells do suggest habitat differentiation that should be examined. Some of these differences may have as much to do with the varying depths of wells as much as their proximity to the bad water line. The difficulty with performing these analyses is that large sample sizes are needed from a variety of sites, thus the analysis may be limited to few localities and a few, more abundant taxa. (4) We can develop our understanding of microbial species. Studying microbes is beneficial because they are more abundant than macroinvertebrates, and with larger sample sizes statistical inferences can be made about the similarity and potential 'connectedness' of well sites at varying distances from one another. Birdwell and Engel (2009) demonstrated that a significant portion of dissolved organic matter in Edwards Aquifer samples may originate from chemosynthesis within the aquifer, rather than from organic matter that is flushed in from recharge features. In addition to comparing these dissolved organic matter signatures, microbial diversity patterns as measured through gene sequencing may provide insight into the habitat continuity between known rare species (e.g. blindcat) localities. In addition to documenting the micro and macro fauna species composition, a fifth available route of study is to examine food chains. Taylor et al. (2007) showed significant differences of isotope signatures between urban versus rural cave sites in central Texas, and similar work may yield insight into differences between sites that are affected by droughts and floods or contamination. During this study we provided some material for examination using this method, however in order to perform meaningful comparisons among sites, many samples are needed over a longer period. Other ways to examine food chains are through simple dissection of gut contents. Some work has been done on the blindcats (Longley and Karnei 1978a and 1978b, Langecker and Longley 1993), but higher sample sizes combined with genetic analysis are bound to be more informative. (5), it is worth opening our imaginations to developing technologies and non-traditional uses of existing ones. For example, it is possible to utilize deep aquifer wells for the insertion of advanced data collection devices, cameras, fauna traps, and remotely operated vehicles (ROVs). The potential of what could be learned via direct observation is huge, and includes but is not limited to behavior, breeding, species interaction, feeding, and abundance. An enterable shaft designed for ROV travel even into a shallow section of the aquifer may provide enormous benefits in terms of species understanding, conservation, and education. (6) Perform limited water quality analysis on all wells to determine whether water quality can be an indicator for the suitability of wells for sampling. This would include the collection of basic data, such as cation/anion, pH, temperature, conductivity and alkalinity.

Regarding the first and second options introduced above, there are two basic methods for increasing sampling productivity in the deep portion of the aquifer. The first is to increase the amount of water that can be flowed through nets. There are a number of sites identified during this study that could be developed with further work. Typically these are sites that were visited early in the project where we were told that "there is no way to sample this water" due to a well being sealed or there being no where to flow water to. With three years of sampling experience behind us, we may now be able to modify infrastructure at some of these sites to enable us to sample water in crucial areas. Some wells that are no longer used are under threat of being permanently sealed under regulatory mandate. Some of these could be very important research sites if they could be rescued from closure and modified to allow for flow sampling or down-hole device insertion. Secondly, we can increase productivity by continuing to improve upon the sampling devices that have proven effective in this study. The installation of in-line sampling devices in farm irrigation systems is dependent upon not interrupting the effectiveness of those systems. Our experience to date with building samplers that will not leak in a pressurized system will now allow us to move forward with installing these at more sites, as well as improve the infrastructure at existing sites. In particular, building larger enclosed samplers would allow us to utilize larger nets, which would reduce the flow force on samples, thereby allowing us to collect them in a more intact condition for study, or even to obtain live samples which is very difficult to do from wells.

Another key component to the successful sampling of new and existing sites is the maintenance of landowner relations. Typically this is part of the background of any such project and is generally understood, however the setting for this project has a number of complications. There are a finite number of active wells, and they are crucial to agricultural output. These wells have come under increased regulation in recent decades, which can create tension between users and regulators. To some, the idea of allowing biological sampling in their wells may be perceived as risking additional regulations regarding endangered species. One critical component of the success of this project included the assignment of staff with decades of experience in sensitive landowner relations in Texas. During the sampling seasons, we made every effort to send the exact same field crew out so that the landowners were comfortable with those individuals. A key component was the use of "well letters" (described in methods section), something that provided significant incentive for landowner involvement.

All future specimens need to be examined and prioritized based on all of the available collaborators and their research specialties. The material is so rare, and represents such a large sampling effort, that we often assessed specimens on a case-by-case basis regarding the preservation method and the destination of the sample. Most blindcat material was not intact, therefore x-rays and gene sequencing were most appropriate. Some material was used for stable isotopes, others preserved for morphology. Most of this collaboration was done as "matching" efforts to this study, greatly increasing the benefit to the client. Future workers should consider these possibilities as well.

STAFF AND CONTRIBUTORS

Zara Staff

A number of Zara personnel contributed to this project, those with the largest contributions are listed here.

Peter Sprouse performed field work, sorted specimens, coordinated with the Authority, contacted landowners, built and installed infrastructure, helped manage this project, and edited this report. Peter has been exploring and studying caves since 1970, having led the exploration of Sistema Purificación in Mexico, one of the longest and deepest caves in the world. He began collecting cave fauna for study by taxonomists in 1977, and has nine species named in his honor. He attended the University of Texas at Austin as a geology major, and since 1991 he has worked professionally in the fields of cave biology, land management, and cartography. He holds US Fish and Wildlife Service endangered species permit number TE014168-0 (covering endangered karst invertebrates in Texas) and Texas Dept. of Agriculture Pest Control License number 0362274. He has extensive experience in conducting karst surveys for invertebrate cave fauna habitat and biological inventories in caves and wells. He is a director of the Texas Speleological Survey and serves on the Balcones Canyonlands Preserve Scientific Advisory Committee Karst Subcommittee. The National Speleological Society has given him the prestigious Lew Bicking Award, named him an NSS Fellow, and he was the medal winner in the 1980 and 1986 NSS Cartographic Salons.

Krista McDermid performed field work, sorted specimens, helped manage this project, and wrote this report. 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 (expires 5 June 2011) to collect and study aquifer fauna and U.S. Fish and Wildlife Service Permit TE192229-0 (expires 30 October 2011) to collect and study federally listed endangered Texas karst invertebrate species.

Dr. Jean Krejca performed field work, identified specimens and reviewed and edited this report. Jean has a Bachelor's degree in Zoology, and a Ph.D. in Evolution, Ecology and Behavior from the University of Texas at Austin. 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 (Arkansas, California, Texas, Nevada, Illinois, Missouri, Indiana, Tennessee, North and South Carolina) as well as Mexico, Belize, Thailand and Malaysia. Her publication list on these areas is extensive. 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 where she continued her work from independent consulting and expanded to perform land management for landowners with endangered species, consult on endangered species permits, and perform custom research projects. In addition she has been involved with a variety of public outreach efforts such as public talks, field trips, and cave biology photography. She holds a USFWS endangered species permit (TE028652-0) and several state permits covering karst invertebrates and salamanders in Texas.

Bill Larsen performed countless hours of fieldwork and installed infrastructure at several sampling sites. Bill began exploring and studying caves in 1986 and has since discovered over 300 cave in central Texas. He began working as a karst technician in 1990, subcontracting to George Veni and Associates. In 2009 he became an employee of Zara Environmental. Much of his work has been in the San Antonio to Austin area and has included grid searches for karst features, excavation of caves and karst features, collection of invertebrate karst species for study, biomonitoring caves, and well sampling for fauna. Bill holds a USFWS Endangered Species Permit.

Taxonomic Experts

We thank Randy Gibson for his assistance with sampling and for identification of amphipods and bathnellaceans; Janet Reid for her identification of copepods; Mark Wetzel for his curation and identification of worms; Rosalie Maddocks for her identification of ostracods; Andrea Radwell for her assistance with the disposition of the mites; and Boris Sket for his contribution with the leech. John Lundberg and Dean Hendrickson have been indispensible for their contributions to the existing knowledge base/data availability of images and genome sequencing pertaining to the blind catfishes of the aquifer. Additionally, Ben Hutchins of Benjamin Schwartz's laboratory in San Marcos is working with Annette Summers-Engel to complete stable isotope analyses and geomicrobiology.

Expert	Taxa	Requested Curation Method	Mailing Address	Contact
Randy Gibson	Amphipods Isopods	80% EtOH	National Fish Hatchery 500 East McCarty Lane San Marcos, Texas 78666	512-353-0011 ext.226 randy_gibson@fws.gov
Janet Reid	Copepods	80% EtOH	1100 Cherokee Court Martinsville, VA 24112-5318	(276) 656-6719 jwrassociates@sitestar.net
Andrea Radwell (with Ian Smith)	Mites	40% glycerin 10% acetic acid 50% water	Department of Biological Sciences University of Arkansas Fayetteville, AR 72701	(479) 575-3534 aradwell@uark.edu
Rosalie Maddocks	Ostracods	80% EtOH	Geosciences Department University of Houston Houston, Texas 77204	(713) 743-3429 rmaddocks@uh.edu
Mark Wetzel	Worms	10% Formalin	1816 S. Oak Street 1021 I-Bldg., MC-652 Champaign, IL 61820	(217) 714-4177 vermes1@gmail.com mjwetzel@uiuc.edu

Expert	Taxa	Requested Curation Method	Mailing Address	Contact
Boris Sket	Leeches	Fixed; 80% EtOH	Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, p.p. 2995, 1001 Ljubljana, Slovenia	tel. +386 1 42 333 88 boris.sket@bf.uni-lj.si
Benjamin Schwartz	Stable isotopes	Water Sample	Texas State University 601 University Drive San Marcos, TX 78666	(512) 245-8713 bs37@txstate.edu
Annette Summers Engel	Geomicro biology	Water Sample	Louisiana State University Dept. of Geophysics E235 Howe-Russell Geoscience Complex Baton Rouge, LA 70803 USA	(225) 578-2469 aengel@lsu.edu
Dean Hendrickson	Fish (and live isopods)	95% EtOH (or water if live)	Texas Natural History Collections, PRC 176 / R4000, 10100 Burnet Road, Austin, TX 78758	512 471-9774 deanhend@mail.utexas.edu
John Lundberg	Blindcat material	95% EtOH	Academy of Nat. Sci. 1900 Ben Franklin Pkwy Philadelphia, PA 19103	215 405-5069 lundberg@ansp.org

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APPENDIX

Particular sites were appropriate for recording meter readings and water temperatures. These data are presented in the following tables, and are preceded by the common name indicated in Table 2.

Aldridge 209

Sample Date	Meter 00-6-1558N	Temperature (°C)
28 Mar 2008	NR	26.9
2 Apr 2008	NR	NR
10 Apr 2008	NR	26
16 Apr 2008	NR	26.99
30 Apr 2008*	NR	NR
12 May 2008	NR	26.5
28 May 2008	NR	27.23
11 Jun 2008	NR	NR
13 Jun 2008	NR	NR
19 Jun 2008	NR	NR
24 Jun 2008	NR	NR
3 Jul 2008	NR	NR
9 Jul 2008	NR	NR
10 Jul 2008	NR	NR
22 Jul 2008	NR	27.5
4 Aug 2008	193	NR
7 Aug 2008	194.61	NR
11 Aug 2008	NR	NR
14 Aug 2008	197.2	27
29 Aug 2008*	NR	NR
20 Feb 2009*	257.715	NR
26 Feb 2009	NR	NR
12 Mar 2009	NR	NR
23 Mar 2009*	26 6.035	25.1
31 Mar 2009	267.515	26.9
10 Apr 2009	270.458	NR
20 Apr 2009	273.048	24.4
4 Apr 2009	277.041	NR
13 May 2009	284.545	27
22 May 2009	NR	NR
2 June 2009	NR	27
12 June 2009	NR	NR
19 June 2009*	311.89	27.5
3 July 2009	322.13	27
1 Sept 2009	373	NR

20 Sept 2009	NR	NR
28 Sept 2009	382	NR
12 Oct 2009	386.25	NR
23 Oct 2009	NR	27
3 Nov 2009	NR	24.9
Meter 09-6-1356		
10 Nov 2009*	0.0305	NR
17 Nov 2009	1.74	NR
17 Dec 2009	6.087	26.5
20 Jan 2010	11.366	NR
2 Feb 2010	14.818	NR
25 Feb 2010	19.213	25.8
10 Mar 2010	22.974	26.5
24 Mar 2010*	27.772	27
29 Mar 2010*	29.166	27
5 Apr 2010	31.877	27
14 Apr 2010*	34.064	27
21 Apr 2010	36.095	27.5
28 Apr 2010	38.213	26.25
5 May 2010	41.229	27
12 May 2010	45.213	27
19 May 2010	46.358	28.5
20 May 2010	46.886	27
22 Jun 2010	61.669	27.5
23 Jun 2010	62.179	27.5
30 Jun 2010	65.563	27.5
6 Jul 2010	67.131	27.5
22 Jul 2010*	75.143	27
23 Jul 2010	76.209	27
28 Jul 2010	76.206	27
4 Aug 2010	81.904	27
25 Aug 2010	108.3	27.5
1 Sep 2010	114.04	27.5
8 Sep 2010	118.95	27.5
15 Sep 2010	121.28	27.5
22 Sep 2010	123.01	26.5
29 Sep 2010*	124.36	27
6 Oct 2010	126.96	27
13 Oct 2010	130.84	27.5

Aldridge Corporate

Sample Date	Meter 00-10-1316N	Temperature (^oC)
28-Mar-08	NR	30.7
10-Apr-08	689.858	NR
11-Jun-08	690.156	NR
13-Jun-08	NR	NR
19-Jun-08	NR	NR
20-Jun-08	702.139	NR
24-Jun-08	706.843	NR
10-Jul-08	NR	NR

Sample Date	Meter 00-10-1316N	Temperature (^oC)
22-Jul-08	NR	NR
4-Aug-08	720.194	NR
7-Aug-08	725.872	NR
10-Aug-08	719.342	NR
11-Aug-08	NR	NR
14-Aug-08	732.248	NR
29-Aug-08	753551	NR
20-Feb-09	866.662	NR
12-Mar-09	NR	NR
23-Mar-09	885.963	NR
31-Mar-09	892.313	NR
10-Apr-09	899.905	NR
20-Apr-09	905.326	NR
4-May-09	912.252	NR
22-May-09	920.765	NR
2-Jun-09	924.184	NR
12-Jun-09	928.089	NR
19-Jun-09	932.272	NR
1-Sep-09	950.491	NR
20-Jan-10	NR	NR
24-Mar-10	951.596	32.5
29-Mar-10	963.151	31.5
5-Apr-10	980.363	32
14-Apr-10	985.901	31.5
21-Apr-10	998.402	32.5
28-Apr-10	16.118	NR
5-May-10	33.868	NR
12-May-10	19.524	32
19-May-10	64.248	32
20-May-10	66.269	32
22-Jun-10	102.532	29
30-Jun-10	117.799	32
14 Jul 2010*	NR	NR
23-Jul-10	163.955	31
28-Jul-10	163.973	30
25-Aug-10	216.746	30
1-Sep-10	229.908	31.5
8-Sep-10	243.966	31.5
15-Sep-10	259.697	32
22-Sep-10	275.459	32
29-Sep-10	292.383	32

Sample Date	Meter 00-10-1316N	Temperature (^oC)
6-Oct-10	309.526	31.5
13-Oct-10	325.957	32

Carroll farms

Sample Date	Meter 97-8-2386N	Temperature (^oC)
4 May 2009	697.112	32.9
13 May 2009	739.565	NR
22 May 2009	763.203	NR
2 June 2009	812.667	31
12 June 2009	861.801	32.9
19 June 2009	895.297	NR
3 July 2009	922.221	NR
12 May 2010	60.687	35
20 May 2010	68.536	NR
23 Jun 2010	171.940	38
6 Jul 2010	180.973	NR
28 Jul 2010	204.152	NR
1 Sep 2010	254.496	38.5
15 Sep 2010	254.996	NR

Constanzo #2

Sample Date	Meter	Temperature (^oC)
10 Apr 2008	(meter broken)	33.5
9 May 2008	001.475	32.5
12 May 2008	NR	33
28 May 2008	NR	30.91
19 Jun 2008	NR	NR
20 Jun 2008	1.558	NR
24 Jun 2008	1.558	NR
10 Jul 2008	1.57	NR
22 Jul 2008	1.577	NR
4 Aug 2008	1.557	NR
10 Aug 2008	1.558	NR
11 Aug 2008	1.577	NR

Homer

Sample Date	Meter 01-8-2232N	Temperature (^oC)
13 May 2009	589.836	NR
2 June 2009	Overflow pond: 631.367 ac/ft Irrigation Line: 605.905 ac/ft	Overflow pond: 26.2
3 Jul 2009	679.554	
17 Dec 2009	680.721	
20 Jan 2010	680.721	NR
2 Feb 2010	680.721	NR
25 Feb 2010	680.721	NR
24 Mar 2010	680.728	no flow
28 Apr 2010	680.721	no flow

13 May 2010	690.496	26.5
19 May 2010	693.535	26.5
5 June 2010	728.934	26.5
22 June 2010	758.370	26.5
30 Jun 2010	773.691	26.5

Jeff Bailey

Sample Date	Meter 06-70-567	Temperature (°C)
20 Jan 2010	0.009	no flow
2 Feb 2010	0.009	no flow
10 Mar 2010	0.009	no flow
13 May 2010	0.010	27
19 May 2010	0.011	27
22 Jun 2010	11.196	27.2
6 Jul 2010	15.464	27.5

Loop 353

Sample Date	Meter 97-6-1767N	Meter 97-6-1773N	Temperature (°C)
12 May 2008	184.519[1]		25.5
20 Feb 2009	2198.842[1]		no flow
26 Feb 2009	248.261[1]		no flow
23 Mar 2009	228.3665[1]		no flow
31 Mar 2009	228.3665[1]		no flow
10 Apr 2009	230.849[1]		no flow
13 May 2009	238.094	298.582	NR (flowing)
2 June 2009	246.676	NR	NR (flowing)
12 June 2009	255.270	NR	no flow
3 July 2009	NR	370.171	no flow
17 Dec 2009	260.138	370.171	no flow
20 Jan 2010	260.138	370.171	no flow
2 Feb 2010	260.138	370.171	no flow
25 Feb 2010	260.138	370.171	no flow
24 Mar 2010	260.138	370.171	no flow
14 Apr 2010	260.138	380.716	24.5
28 Apr 2010	260.130	380.716	23
13 May 2010	260.138	394.656	26.5
19 May 2010	NR	397.317	25
5 Jun 2010*	272.559	NR	26.6
22 Jun 2010	280.640	437.240	26.5
30 Jun 2010	283.598	447.976	25.5

Lyda Fallon

Sample Date	Meter 97-10-1454N	Temperature (^oC)
10 Mar 2010	167.454	NR
12 May 2010	212.539	25.5
20 May 2010	215.082	NR
23 Jun 2010	276.314	31
6 Jul 2010	300.167	30
28 Jul 2010	330.859	NR
15 Sep 2010	452.447	NR
29 Sep 2010	452.447	25.5

Mark Verstuyft Well

Sample Date	Meter 00-8-1181N	Temperature (^oC)
2 Feb 2010	568.879	no flow
10 Mar 2010	568.879	no flow
5 May 2010	570.001	no flow
13 May 2010	573.891	25.5
19 May 2010	573.892	24.5
5 Jun 2010	582.500	26.5
6 Jul 2010	609.642	no flow
1 Sep 2010	623.857	27
8 Sep 2010	624.570	no flow
15 Sep 2010	624.570	no flow
22 Sep 2010	625.955	25.5
29 Sep 2010	626.410	26
13 Oct 2010	269.235	no flow

Nelson Road

Sample Date	Meter 97-8-2627N	Temperature (^oC)
4 May 2009	450.210 ac/ft	NR
13 May 2009	459.284 ac/ft	NR
22 May 2009	NR	NR
2 June 2009	NR	NR
17 Dec 2009	581.257	no flow
20 Jan 2010	581.638	no flow
25 Feb 2010	581.682	no flow
10 Mar 2010	582.253	NR
24 Mar 2010	582.255	NR
5 Apr 2010	582.706	NR
13 May 2010	609.740	NR
19 May 2010	609.740	no flow
5 Jun 2010	618.490	24.5
22 Jun 2010	631.016	24.5
6 Jul 2010	645.425	NR
25 Aug 2010	656.321	NR
15 Sep 2010	658.826	NR
6 Oct 2010	658.826	NR

Persyn

Sample Date	Meter 087-1508	Temperature (^oC)
13 May 2010	5.992	31.5
19 May 2010	12.179	32

20 May 2010	13.401	32
5 Jun 2010	31.957	32
22 Jun 2010	45.529	31.8
30 Jun 2010	54.130	29

Raymond Wauters

Sample Date	**Meter Number**		**Temperature (°C)**
	00-6-1560N	**01-6-1083N**	
20 Apr 2009	28.001		NR
4 May 2009	30.361		NR
13 May 2009	33.092	327.132	NR
22 May 2009	35.961	331.974	NR
2 June 2009	37.790	341.072	NR
12 June 2009	42.449	357.403	NR
3 Jul 2009	44.320		
10 Nov 2009	44.3205		NR
17 Nov 2009	44.320		NR
20 Jan 2010	44.323	382.657	no flow
25 Feb 2010	44.323	382.657	NR (flowing)
24 Mar 2010	44.323	382.661	NR
5 Apr 2010	223.323	382.662	no flow
28 Apr 2010	44.323	382.662	23.5
13 May 2010	44.323	405.323	27
19 May 2010		408.998	24.5
5 Jun 2010		434.788	25.8
22 Jun 2010		458.124	26.7
30 Jun 2010		476.224	25.5

Roger Verstuyft

Sample Date	Meter	Temperature (°C)
13 May 2009	347.648 ac/ft	NR
22 May 2009	359.478 ac/ft	NR
2 June 2009	355.015 ac/ft	NR
12 June 2009	378.523 ac/ft	NR
20 Jan 2010	418.036	nR
25 Feb 2010	69: 418.037 70: 202.107 65: 393.422	
24 Mar 2010	97-6-1769N: 418.037 97-6-1765N: 393.454 97-6-1770N: 202.107	NR
14 Apr 2010	69: 436.188 65: 393.475 70: 203.832	26.8
13 May 2010	69: 451.313 65: 393.523 70: 207.671	26.5
19 May 2010	70: 207.953 65: 393.556 69: 454.154	24.5
5 Jun 2010	69: 483.923 70: 213.452 65: 393.558	26.5
22 Jun 2010	69: 518.605	26.8
30 Jun 2010	69: 527.504 70: 220.782 65: 393.597	25.5

SA Zoo

Sample Date	Temperature (°C)
14 Aug 2008	24
12 Mar 2009	NR
23 Mar 2009	NR
31 Mar 2009	23.8
10 Apr 2009	24
20 Apr 2009	24.7
22 May 2009	NR
12 June 2009	23.9

Stull Farms

Sample Date	Meter 06-70-0615	Temperature (°C)
23 Oct 2009	253.247	NR
3 Nov 2009	253.899	NR
20 Jan 2010	001.206	NR
10 Mar 2010	001.339	NR
29 Mar 2010	001.339	NR
14 Apr 2010	001.739	NR

Sample Date	Meter 06-70-0615	Temperature (^oC)
12 May 2010	003.528	34
20 May 2010	003.675	NR
23 Jun 2010	006.756	NR
6 July 2010	007.428	NR
1 Sep 2010	015.810	NR
15 Sept 2010	016.066	NR

Tschirhart

Sample Date	Meter 00-6-1556N	Temperature (^oC)
31 Mar 2009	117.894 ac/ft	NR
10 Apr 2009	117.894 ac/ft	27
20 Apr 2009	117.895 ac/ft	26.5
4 May 2009	128.736 ac/ft	no flow
13 May 2009	139.232 ac/ft	NR
22 May 2009	141.672 ac/ft	NR
2 June 2009	134.677	NR
12 June 2009	144.713 ac/ft	27
3 Jul 2009	154.599	no flow
8 Jul 2009	NR	26.8
1 Sep 2010	NR	NR
28 Sep 2009	154.599	27.4
23 Oct 2009	154.599	NR
3 Nov 2009	154.599	25
10 Nov 2009	154.599	27.2
17 Nov 2009	154.599	25.5
17 Dec 2009	154.599	NR
20 Jan 2010	154.610	26.9
2 Feb 2010	154.610	26.5
25 Feb 2010	154.610	25.5
10 Mar 2010	154.610	27.2
24 Mar 2010	154.610	27
5 Apr 2010	154.610	27.5
21 Apr 2010	154.610	27.5
28 Apr 2010	154.610	27
6 May 2010		27.5
13 May 2010	165.035	27.5
19 May 2010	165.035	27
5 Jun 2010	174.479	27.5
22 Jun 2010	186.389	27
30 Jun 2010	196.540	27.5
22 Jul 2010	196.540	27
23 Jul 2010	196.540	27
28 Jul 2010	196.540	27.5
25 Aug 2010	196.540	27.5
1 Sep 2010	196.540	27.5
22 Sep 2010	196.540	27
29 Sep 2010	196.540	27
6 Oct 2010	196.540	27.5
13 Oct 2010	196.540	27.5

Verstuyft Farms

Sample Date	Meter	Temperature (^oC)
14 Aug 2008	898.228	NR
20 Feb 2009	106.2245	NR
10 Nov 2009	37:552.353 75:264.366	no flow
17 Nov 2009	264.366	NR
20 Jan 2010	264.366	no flow
2 Feb 2010	37:555.440 75:264.366	no flow
25 Feb 2010	75: 264.366 37:555.440	no flow
10 Mar 2009	37: 555.702 75: 264.366	NR
24 Mar 2010	02-12-1037: 556.593 01-12-1075N: 264.366	NR
5 Apr 2010	75: 264.366 37: 558.996	NR
20 May 2010	30: 565.444	NR sampler not on yet
22 Jun 2010	37: 565.903	32
22 Jul 2010	37: 565.907	NR
25 Aug 2010	565.920	NR
15 Sep 2010	567.1037	NR
22 Sep 2010	568.470	NR
6 Oct 2010	569.676	32
13 Oct 2010	571.888	32

Verstuyft Home

Sample Date	Meter 99-8-2219N	Temperature (^oC)
10 Nov 2009	080.575	no flow
17 Nov 2009		32
20 Jan 2010	NR	no flow
2 Feb 2010	090.058	no flow installed new net and tub
24 Mar 2010	104.698	no flow
5 Apr 2010	117.407	32.5
13 May 2010	152.298	32.5 (no sampler)
25 Aug 2010	250.379	NR
15 Sep 2010	272.074	30
6 Oct 2010	284.644	32
13 Oct 2010	292.636	32

WH Guenther

Sample Date	Meter	Temperature (^oC)
23 Mar 2009	NR	25.8

Woodley Farms #1

Sample Date	Meter 97-12-1104	Temperature (°C)
23 Mar 2009	NR	25.8
2 Feb 2010	636.807	no flow
30 Mar 2010	746.458	36.5
22 Apr 2010	834.132	37
28 Apr 2010	834.159	37
12 May 2010	834.418	37
23 Jun 2010	(meter broken)	39
6 Jul 2010	(meter broken)	no flow
4 Aug 2010	(meter broken)	37
25 Aug 2010	(meter broken)	37
15 Sep 2010	(meter broken)	37
29 Sep 2010	(meter broken)	37

Woodley Farms #2

Sample Date	Meter 97-12-1094N	Temperature (°C)
2 Feb 2010	994.892	no flow
10 Mar 2010	995.958	no flow
30 Mar 2010	(meter broken)	37.5
22 Apr 2010	(meter broken)	37.5
28 Apr 2010	(meter broken)	NR
12 May 2010	(meter broken)	35.5
23 Jun 2010	(meter broken)	38
6 Jul 2010	(meter broken)	NR
4 Aug 2010	(meter broken)	37
25 Aug 2010	(meter broken)	37
15 Sep 2010	(meter broken)	37
29 Sep 2010	(meter broken)	37

GLOSSARY

Acanthopterygian: spiny-finned (ray-finned) fishes, so called for the characteristic bony, sharp rays in their fins

Amphipod: an order of small, shrimp-like crustaceans; sometimes referred to as scud

Atypical: not the normal, representative form of a group, class or type

Bathynellacean: an order of crustaceans which live interstitially in groundwater

Branchiostegal rays: small, slender rays that support the gill membranes

Copepods: group of small (usually 1-2 mm) crustaceans, usually having six pairs of limbs on the thorax

Cosmopolitan: occurring or growing in many parts of the world

Crustacean: large group of mainly aquatic arthropods having a segmented body and chitinous exoskeletons; includes crabs, lobsters, crayfish, shrimp, and many other groups

Decapod: a crustacean order having five pairs of legs, one on each segment of the thorax

Ephemeral: being present only briefly; flowing seasonally in response to inundation

Epigean: pertaining to the biological domain or to an organism's activity at or above the soil surface

Flagellates: organisms with one or more whip-like organelles called flagella

Hydrobiid: belonging to the taxonomic order Hydrobiidae

Hyoid bar: cartilaginous plates forming the second visceral arch, from which a part of the hyoid bone is developed

Hyomandibular: a set of bones that is found in the hyoid region in most fishes

Isopod: any of various small terrestrial or aquatic crustaceans with seven pairs of legs adapted for crawling

Macrofauna: animals that are 0.5mm or larger, living within sediments

Microfauna: small, mostly microscopic animals such as protozoa, nematodes, etc

Morphology (in text: morphologically): of or having to do with an organism's form and structure (size, shape, color pattern, skeletal structure, etc)

Obligate: restricted to a particular condition of life

Opercle: a hard bony flap covering the gill of a fish

Ostracods: seed shrimp; tiny crustaceans enclosed in a bivalve shell

Rotifers: microscopic, planktonic aquatic (usually freshwater) organisms with a ciliate wheel-like organ that is used for feeding and for moving from place to place

Speciose: refers to a taxa with a large number of species

Stygobitic: an aquatic troglobite

Stygomorphic: having the form of an aquatic troglobite

Thecamoebian test: the exoskeleton of a particular type of microscopic aquatic organism

Thermosbaenacean: a small crustacean whose distributional pattern lies within the limits of the ancient Tethys Sea

Troglobite: organisms that carry out their entire life cycle in a cave and cannot survive on the surface; typically exhibiting cave-adapted morphologies including the loss of pigment and the loss (or reduction) of eyes, and elongated appendages

Vertebrates: a group of animals with a backbone