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FAUNA OF WELLS NEAR THE SALINE WATER LINE OF THE EDWARDS AQUIFER, TEXAS

In line barrel sampler on an irrigation well in Bexar County, Texas.

Prepared for:

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Abstract

Zara Environmental LLC, funded by the Edwards Aquifer Authority, sampled over 21,000 ac-ft of water from 41 wells within 8 km of the saline water line between 2008 and 2014 in an attempt to gain insight to the current status and distribution of organisms in the deep portions of the Edwards Aquifer. This is the second major sampling effort in the deep portions of the Edwards Aquifer, the first one having been completed more than thirty years ago. We attempted to re-locate wells known to have historically produced stygobitic fauna; however, most could not be definitively located.

Twelve sites ranging from 111 to 652 m deep yielded invertebrate fauna, including 22 unique taxa in eight orders. Nearly every taxonomic record represents a new site locality for a rare species, and the collection efforts yielded a new species of copepod (*Diacyclops* sp.); an order of fauna (Bathynellacea) previously unknown from the Edwards Aquifer; and three new localities for the toothless blindcat, *Trogloglanis pattersoni,* one of which represents a 6 km range extension to the southwest. We collected representative material from 14 specimens of toothless blindcat from three wells, including entire intact specimens at one site and bones at the other two. Although collecting efforts for this study far exceeded historical collecting efforts, neither live blindcats nor material representing the widemouth blindcat, *Satan eurystomus*, was collected. The only currently active known locality, the Artesia Pump Station Well, was unavailable for sampling.

A community composition analysis of our sample set compared to historical samples collected by Henry Karnei in 1978 yielded no support for the hypothesis of an even distribution of species across the aquifer. The relative abundance of species in the two datasets was notably different, most likely attributable to the fact that only one of our sample sites overlapped. We used principal component analysis to test for correlations between stygobite diversity and factors that may influence species distribution, including distance to saline water line, well depth, temperature, and volume of water flow (an analog for conduit size). We hypothesized that sulfur-reducing bacteria near the saline water line may be a food source and therefore a limiting factor for distribution, but found no significant correlation between species richness and distance to saline water line. We also expected to see a positive correlation with flow, because greater flow represents a higher volume of water being sampled and also because high flow wells may represent larger caves which could have a greater number of different niches and available habitat than smaller caves. We found no significant correlation with flow, or any of the other factors tested. We recommend further research on the subterranean community to elucidate patterns of distribution, especially with respect to the microbial community and food sources, and to determine the status of the widemouth blindcat.

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Introduction

The San Antonio Pool of the Edwards Aquifer in central Texas is located directly below the city of San Antonio, where it provides the primary water source for over 2 million people. This aquifer contains an impressive diversity of subterranean aquatic fauna known from very deep wells, some more than 800 meters (m) below the surface. There is no way to see or directly sample this remote system; indirect sampling relies on a small sample of wells that are tiny windows into a geographically large, spatially, chemically, and thermally complex three-dimensional habitat. Obligate subterranean fauna are typically understudied due to the general inaccessibility of their habitats, leaving several information gaps about their distribution and habitat requirements. Nutrient flow, a limiting factor for subterranean life (Simon and Benfield 2002), is also complex, with energy arriving from both external (recharge-based allochthonous nutrients) and internal (microbial based autochthonous) sources. In the deep portions of the Edwards Aquifer, researchers describe a chemolithoautotrophic system with carbon sources coming from sulfur-reducing microorganisms, which in turn feed grazers including invertebrates and blind catfish (Summers-Engel 2007).

The Edwards Aquifer, renowned as one of the most diverse subterranean ecosystems in the world (Longley 1981), is contained within several porous limestone units within the Edwards Group. The length of the southern segment of the Edwards Aquifer in the San Antonio region extends approximately 290 kilometers (km), varying in width from 8 - 65 km from the northern limit of the recharge zone to the southern limit of fresh water. Two distinctive zones, fresh and saline, can be found in the Edwards Aquifer with a transition area between them. Locally, the point at which the TDS concentration reaches 1,000 ppm is referred to as the freshwater/saline-water interface, or bad-water line, and is the approximate southern extent of potable water (Pavilicek et al. 1987).

The Edwards Aquifer has four zones: the contributing zone, the recharge zone, the transition zone, and the confined zone (Figure 1). Most of the wells sampled for this project were contained within the confined zone. The confined zone, also known as the artesian zone, is the area where the Edwards Limestone is confined between two layers of low permeability rock which trap water inside the aquifer. Water flowing into the aquifer in the recharge zone pressurizes the water in the confined zone, forcing water upwards through artesian wells that penetrate the confining cap rock. In the confined zone, there is no water table and no vadose zone, as the entire aquifer is saturated.

The limestone units containing the aquifer are expressed at the surface in the recharge zone as caves and springs that lend themselves to the study of subterranean fauna in accessible zones (e.g., Holsinger and Longley 1980, Reddell 1993, Reddell 1994, Veni 2006, Krejca and Weckerly 2007, Gibson et al. 2008). Less well known are the fauna that occupy the deepest and most inaccessible regions of the aquifer within the confined zone, where caves and springs are less common. 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, over 40 species have been described from the aquifer (Longley 1986, Gibson et al. 2008).

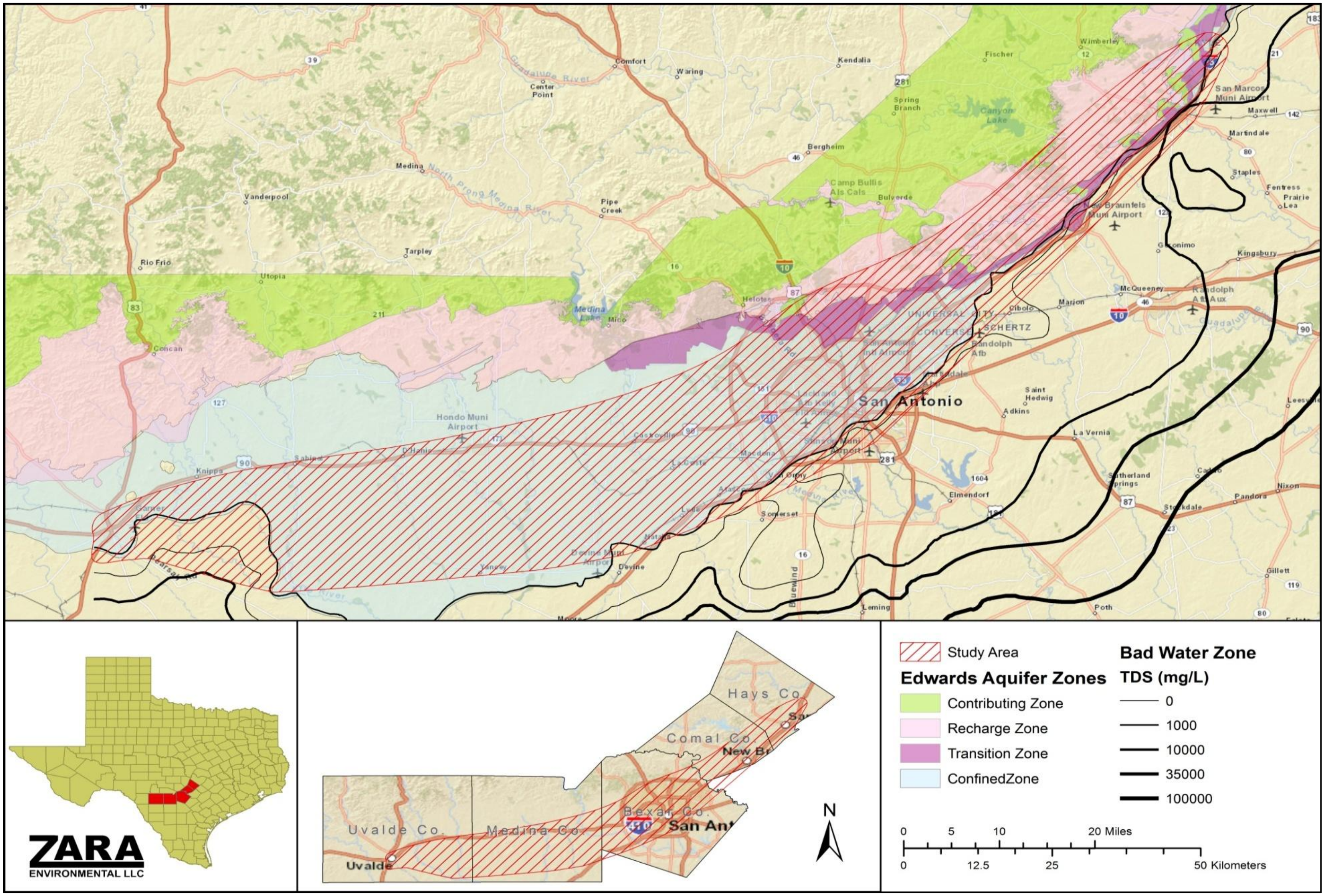

Figure 1. Edwards Aquifer regulatory zones, saline (bad water) zone, and study area. Geographic data for the Saline Zone was provided by the EAA (Steve Johnson, pers. comm. 2009).

The history of sampling the toothless blindcat, *Trogloglanis pattersoni*, and the widemouth blindcat, *Satan eurystomus*, from the Edwards Aquifer consists of sporadic collections in the early 1900s followed by a large sampling effort by Karnei (1978) and Longley and Karnei (1978a, b). Since these collections in the 1970s, new blindcat material has not been added to any museums, and blindcat observations have not been documented in the literature.

Prior to the current study, these blind catfish were the only two species of North American Ictalurid catfish for which no genetic data were on record. Eigenmann (1919) described the toothless blindcat from a single specimen retrieved from an artesian well in San Antonio by Mr. George W. Brackenridge. Two other specimens of the toothless blindcat, collected in 1934 and 1955, also came from artesian wells in San Antonio and were described in Hubbs and Bailey (1947) and Suttkus (1961) as likely being descended from the genus *Ictalurus*. Hubbs and Bailey (1947) described the widemouth blindcat from a single preserved specimen that Mr. William Kempin donated to the Witte Memorial Museum. Two additional specimens of the widemouth blindcat were retrieved in 1955 and 1960 and were evaluated by Suttkus (1961). These six specimens, three of each species, were the only collections made until Karnei (1978) and Longley and Karnei (1978a, b) embarked on a large collecting effort. As part of a survey of the subterranean fauna of the Edwards Aquifer in Bexar County, Karnei (1978) sampled 33 artesian wells and collected 26 toothless blindcats (Longley and Karnei 1978a) and 14 widemouth blindcats (Longley and Karnei 1978b) from three of those sites over two years of study, but preservation methods at the time precluded future DNA analyses.

The objective of this study was to evaluate the spatial distribution and abundance of fauna of the deep portions of the Edwards Aquifer by using two approaches: replicating and expanding on studies by Longley and Karnei (1978a, b), Eigenmann (1919), Hubbs and Bailey (1947), Suttkus (1961), and Karnei (1978) by including sampling from the sites identified in those studies; and investigating previously unsampled deep wells near the saline water line. In addition to an evaluation of stygobitic distribution and abundance over time, we hope to contribute to the understanding of this remote, inaccessible habitat, further evaluate the status, distribution, and taxonomy of these rarely observed species by providing material for genetic analysis to our collaborators, and explore some questions informed by our dataset.

In addition to evaluating the catch per unit effort (CPUE) and relative abundance of different taxa, we sought to compare our results to earlier sampling efforts and to address some hypotheses concerning the distribution and abundance of aquifer fauna. We examined a null hypothesis of no difference in faunal abundance in wells based on their distance to the saline water line; if hypotheses regarding chemolithoautotrophic nutrient sources are true, we expect more fauna closer to the food sources at the saline water line. Another null hypothesis we examined is that of no difference in species richness from wells that are deeper, warmer, or higher volume. Other authors have found that large cave systems contain most of the richness of fauna in an area (Schneider and Culver 2004); therefore, we expect higher volume wells to represent larger cave systems, and thus to have a higher species richness.

This study was funded by the Edwards Aquifer Authority (EAA) in an effort to better understand aquifer-dwelling species. The EAA is a regulatory agency established by the Texas Legislature in May 1993 to preserve and protect the unique groundwater resources in Atascosa, Bexar, Caldwell, Comal, Guadalupe, Hays, Medina, and Uvalde counties. The EAA manages groundwater withdrawals in its regulatory boundaries and collects significant quantities of groundwater and surface water data.

Methods

Sample Sites

Between 2007 and 2013, through extensive dialogue with various local water authorities, landowners, and scientists, we obtained landowner permission to sample at 41 sites, where we completed approximately 875 sampling events between 2008 and 2014. Our efforts were focused on emulating work done by Henry Karnei, who sampled 33 artesian wells in the late 1970s (Karnei 1978); thus, we compiled a list of historic blindcat sites and attempted to access each one, in addition to any new sites that were available. We prioritized sampling locations based on their proximity to the saline water line or their proximity to historical blindcat or invertebrate sites. Springs and other sites that have been heavily sampled by researchers in previous studies (e.g., Hueco Springs, Comal Springs, San Marcos Springs and Artesian Well, and Ezell's Cave) are reported elsewhere (Holsinger and Longley 1980, U.S. Fish and Wildlife Service 1996, Gibson et al. 2008, and others) and were excluded from our study.

The names of each site, method of sampling, and specific number of sample events per well are described in Table 1. Of those sites, only the San Antonio Zoo Well had been previously sampled by Karnei (1978), and none of them were previously known to have blind catfish. Most of these wells and sampling methods are previously described (Zara 2010). We assigned well names for ease of reference, often based on the owner's name. However, in many cases the owners and/or EAA did not have a name for the well, and since wells can change ownership over time, these names should be considered unofficial. Information we collected included the well name, meter ID, description of sampling method used, temperature, and a record of fauna or other observations made in the field. Temperature was measured using a standard mercury thermometer. We calculated the distance to the edge of the saline zone as mapped by EAA using the near function in ArcGIS 10.2 (ESRI, Redlands, CA). The EAA provided well depths. The average daily volume of water was obtained by averaging the difference in the well meter readings between the start and stop sample times. This reading was sometimes not recorded because the meter was inaccessible or unreadable. In these cases we extrapolated water volumes by estimating an average daily flow for that site multiplied by the number of days between sample events.

Table 1. Sampling methods and number of sampling events at wells included in this study between 2008 and 2014. Volume represents the volume of water flowed during our study.

Well	Method(s)	Sampling Events	Volume (ac-ft)
Aldridge 209	net, barrel	153	901.75
Aldridge Corporate	net	82	185216
Aquarena Springs*	bottle	2	N/A
Bexar Met #8	net	1	N/A
Bexar Met #91	net	1	N/A
Carroll Farms	net	12	429.26
City of Kyle	bottle	2	N/A
Constanzo	net	10	0.10

Well	Method(s)	Sampling Events	Volume (ac-ft)
Fort Sam Houston #2	net	6	0.15
Fort Sam Houston #5	net	15	21.39
Girl Scout	net	1	N/A
Homer Verstuyft	barrel	13	247.36
Hondo Index	bottle	1	N/A
Jeff Bailey	net, barrel	28	135.27
LCRA	net	2	0.33
Loop 353	net, barrel	25	447.18
Lyda Fallon (Partin)	net	10	379.43
Mark Verstuyft	barrel	50	192.81
Mission Bowling	bottle	6	N/A
Nelson Road	net, barrel	48	419.06
Panther Canyon	bottle	4	N/A
Paradise Alley	net	1	N/A
Parker	net	13	105.13
Persyn	barrel	36	912.55
Quail Crossing	net	16	476.45
Raymond Wauters	net, barrel	77	851.52
Roger Verstuyft	barrel	19	186.31
Ruiz	net	11	N/A
San Antonio Zoo	net	15	2005.87
San Marcos Flagpole	bottle	2	N/A
Schumann Artesian	bottle	6	N/A
Schumann House	net	10	0.56
Shavano Park #5	net	1	N/A
Steves house	net	1	N/A
Stull Farm	net	11	16.72
Tschirhart	net	65	426.03
Uvalde Index	bottle	4	N/A
Verstuyft Home	net	41	486.62
Verstufyft Farm	net; in-line	38	936.56
Woodley Farms #1	in-line	18	2154.04
Woodley Farms #2	in-line	19	2543.22

"Net" indicates that a net was cinched over the well outflow, "barrel" indicates that we sampled the irrigation line though a barrel sampler, "in-line" indicates that a portion of the flow was diverted to our sampling apparatus, and "bottle" indicates that we used a bottle trap to sample a non-flowing well. *Aquarena Springs monitoring well, not Texas State University's artesian well sampled by Glenn Longley.

Sample Methods

Methods for sampling varied depending on the accessibility of flow and the morphology of the well outlets. 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. Net types varied depending on flow and site morphology, with the largest being 4-m long, 1-m wide with a 500-μm mesh size, down to standard plankton nets with a 60-μm mesh size. Nets were cinched down over well outlets and covered with shade socks, large pieces of opaque vinyl sewn into a tube and placed over the net, used to slow or prevent algal growth on the nets. The stiff fabric of the shade sock directs water out the bottom of the net rather than the side of the net, so when used in conjunction with a water tub, it keeps specimens underwater, out of the sun, and reduces force on the specimens (Figure 2).

Figure 2. A shade sock secured over a net on the outlet at the Aldridge Corporate well. This net rests in a tub in order to reduce degradation of specimens that can result from battering.

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 several of these systems were appropriate for the installation of an in-line barrel sampler to filter water under high-pressure conditions by tapping into the system and excising a section of pipe (cover photo). In the latter part of the study, we modified some sites by adding a bypass pipe section and diverting the flow of water through a filter that could be accessed independently of the main flow of the pipe before water flowed into the main irrigation line.

Specimens large enough to see in the field were handpicked from the net and transferred directly into 95% ethanol. We transferred sediment samples that were 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, directly to ethanol. We examined these samples under microscopes and transferred apparent organismal material to a taxonomic expert for identification.

Catch per Unit Effort and Relative Abundance

Catch per unit effort (CPUE) and relative abundance were calculated separately for invertebrates and blindcats. For CPUE calculations, we considered any portion of an individual animal captured to represent one specimen (i.e., one "catch") when only a portion or portions (e.g. several legs, a bone or a few bones, fin spine portions) that could be reasonably considered to belong to the same animal were captured. When portions that obviously represented more than one animal were captured (e.g., two heads) then the appropriate number of specimens was assigned. When considering invertebrate CPUE at different taxonomic levels, the values are inclusive of all data from the lower levels. Catch per unit effort represents the volume of water it was necessary to sample from all of our wells combined in order to obtain one specimen of a given organism and was calculated by dividing the total volume of water sampled by the number of specimens per each taxon. We calculated relative abundance by dividing the number of organisms of particular taxa by the total number of organisms collected. The average daily acre-feet (ac-ft; 1 ac-ft equals 325,851 gallons) of water flowed during the study was calculated based on meter readings or estimated based on the gallons per minute of water flowed, as observed during site visits. Catch per unit effort from historical sites were calculated by converting flow measurements provided in Karnei (1978) and Longley and Karnei (1978a, b) to ac-ft and multiplying by the number of days sampled.

Comparison of Relative Abundance in Historical Datasets

Karnei (1978) performed the only other large-scale sampling effort of the San Antonio Pool of the Edwards Aquifer. We attempted to re-sample the exact locations he used, but only one well was available. Thus our taxonomic comparisons are not site-specific, but instead study-wide. Also since different sites demanded different sampling mechanisms due to their morphology (Zara 2010), in many cases the dimensions, orientation, and mesh size of the nets were different among these two studies.

Taxonomy

Randy Gibson, US Fish and Wildlife Service, identified most of the crustaceans. Janet Reid, Virginia Museum of Natural History, identified copepods, and Rosalie Maddocks, University of Houston, identified ostracods. The specificity of taxon determinations varied depending on their condition, lifestage, and sex. For the analyses, if the taxonomist could only verify the specimen to genus or even family, the specimen was left at that level. In the case where there is only one member of the genus known from Texas, we assumed that the specimen belonged to that species. Thus, any specimen receiving a designation *Palaemonetes* sp. was re-classified at *Palaemonetes antrorum*, *Cirolanides* sp. were re-classified as *Cirolanides texensis*, and *Speocirolana* were re-classified as *Speocirolana hardeni. Parabogidiella* sp. is considered *P. americana,* the only currently described species in that genus; however, the designation in this paper will remain *Parabogidiella* sp*.* due to taxonomic uncertainty presented in Holsinger and Longley (1980). Where the literature supports only one species of a given taxon occurring at a site, and gross morphological similarities suggest concurrence with our collections, we assume the taxon to belong to that species. For example, at the Brackenridge Zoo well we collected an eyeless, transparent, minute, flat snail. Hershler and Longley (1986) identified other specimens with this general morphology from this site as *Horatia nugax*, and also mentioned that Karnei (1978) illustrated this taxon as "Gastropod species no. 1." Consequently, we identified our collections as that species. We did not identify the specimens using precise techniques such as microscopic measurements, operculum shape, radula, or internal anatomy. Note that this species was incorrectly identified as *Phreatodrobia* sp. in Zara (2010).

Dr. Dean Hendrickson, the Curator of Icthyology at the Texas Memorial Museum (TMM) managed 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 Ichthyology Department of the Academy of Natural Sciences in Philadelphia, Pennsylvania, and his staff identified 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-principal 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.

Habitat Criteria

We used a principal components analysis (PCA) to help identify patterns in the data in terms of the differences and similarities among several factors. The PCA was performed using the XLSTAT plugin for Microsoft Excel with the following input factors for each well: average temperature, distance to the saline zone, depth, number of stygobitic taxa, and average ac-ft per day flowed during study. Other researchers have proposed that the deepest parts of the aquifer represent a chemolithoautotrophic system where microbes flourish near the saline zone and provide the base of the food web in that area (Karnei 1978, Longley and Karnei 1978a, Longley and Karnei 1978b, Randall 2006, Birdwell and Engel 2009, RECON et al. 2011), thus we expect the diversity and abundance of deep aquifer organisms to increase proximal to the saline zone. Species abundance may also be related to volume of flow (Karnei 1978, Longley and Karnei 1978a and b), presumably because wells with higher flow may indicate intersections with conduits and caves, resulting in more local habitat and resource availability. Only wells with complete information for each factor can be considered for PCA, and the software automatically excluded sites with incomplete or missing data.

Results

Twelve of the 41 wells we sampled yielded stygobitic fauna, representing 22 unique taxa in nine orders. Aldridge 209 was the most taxonomically diverse with eight different taxa, followed by Tschirhart with seven taxa, and San Antonio Zoo with six taxa. The isopod *Speocirolana hardeni* was the most widespread invertebrate recorded, with six site records from this study, followed by the isopod *Cirolanides texensis* and amphipods in the genus *Parabogidiella* from five sites each, and amphipods in the genus *Holsingerius* and *Stygobromus* and the shrimp *Palaemonetes antrorum* from three sites each (Table 2).

Table 2. Number of individuals of each taxa encountered in 12 wells sampled during this study, including the number of wells each taxon was recorded from (right column) and the number of taxa recorded from each well (bottom row). The taxonomic designation represents the lowest taxonomic level of identification that was made for the specimen(s), sometimes represented by family or genus.

Taxon	Aldridge 209	Aldridge Corporate	FSH #2	Homer Verstuyft	Jeff Bailey	Loop 353	Mark Verstuyft	Nelson Rd.	Ruiz	SA Zoo	Schumann	Tschirhart	**Wells**
Allotexiweckelia hirsuta (amphipod)					1								**1**
Hadziidae (amphipod)	8	1											**2**
Holsingerius sp. (amphipod)	15	8										4	**3**
Parabogidiella sp. (amphipod)	15	5			4			1				30	**5**
Stygobromus sp. (amphipod)									3	1		6	**3**
Acanthocyclops sp. (copepod)											74		**1**
Diacyclops new sp. (copepod)			20										**1**
Epactophanes richardi (copepod)										1			**1**
Nitocra spinipes (copepod)										3			**1**
Paracyclops chiltoni (copepod)											61		**1**
Paracyclops poppei (copepod)										1			**1**
Horatia nugax (gastropod)										12			**1**
Cirolanidae (isopod)	15												**1**
Cirolanides texensis (isopod)	8				1		2		4			3	**5**
Mexistenasellus coahuila (isopod)										2		1	**2**
Speocirolana hardeni (isopod)	76	3		7		1	1	1					**6**
Chlamydotheca arcuata (ostrocod)						1						221	**2**
Cypridopsis vidua (ostracod)			51										**1**
Palaemonetes antrorum (shrimp)	39	1			1								**3**
Tethysbaena texana (thermosbaenacean)			1										**1**
Texanobathynella bowmani (bathynellacean)											7		**1**
Trogloglanis pattersoni (blindcat)	11				2							1	**3**
Number of taxa per well	**8**	**5**	**3**	**1**	**5**	**2**	**2**	**2**	**2**	**6**	**3**	**7**	**--**

Material representing 14 confirmed specimens of the toothless blindcat was obtained from three wells within our study area (Table 2); confirmation on four additional specimens, one of which may add a new site record for the species (Table 3, Figure 3), is pending. Only our confirmed sites are discussed in the following descriptions. Karnei collected 14 individuals of the widemouth blindcat (Longley and Karnei 1978b) and 26 individuals of the toothless blindcat (Longley and Karnei 1978a) from three wells and gathered reports of other purported blindcat localities in the deep artesian portion of the Edwards Aquifer in Bexar County. We attempted to locate and access these sites, as discussed below (Table 4).

Table 3. Toothless blindcat material collected during current study.

Zara ID Number	Collection Date	Locality	TNHC Accession Number	Notes
Zara 3889	8/28/2008	Aldridge 209	N/A	opercular bone, partial hyomandibular, bits of hyoid bar with a few branchiostegal rays
N/A	3/23/2009	Aldridge 209	TNHC 42586	partial fish
Zara 5335	3/24/2010	Aldridge 209	TNHC 45369	partial fish
Zara 5343	3/24/2010	Tschirhart	TNHC 45367	bones
Zara 5344	3/24/2010	Aldridge 209	TNHC 45368	fish parts
Zara 5347	3/29/2010	Aldridge 209	TNHC 45366	fish parts
Zara 5921	7/22/2010	Aldridge 209	TNHC 45860	whole fish
Zara 6149	9/29/2010	Aldridge 209	TNHC 46823	partial fish
Zara 6730	9/30/2011	Jeff Bailey	N/A	dorsal fin pterygiophore
Zara 6731	10/6/2011	Jeff Bailey	N/A	complete pectoral-fin spine and cleithrum fragment
Zara 6736	10/14/2011	Aldridge 209	N/A	no ID received yet
Zara 6739	10/21/2011	Aldridge 209	N/A	no ID received yet
Zara 7036	4/21/2012	Aldridge 209	N/A	paired set of pectoral fin spines and likely associated dorsal fin spine articulated to its pterygiophore
Zara 7040	4/27/2012	Aldridge 209	N/A	at Cornell
Zara 7969	6/22/2012	Mark Verstuyft	N/A	no ID received yet
Zara 7073	6/22/2012	Tschirhart	N/A	no ID received yet
Zara 7977	11/6/2012	Aldridge 209	N/A	at Cornell
Zara 8037	4/19/2013	Aldridge 209	N/A	at Cornell

Table 4. Summary of all blindcat sites, organized chronologically by year specimen was collected.

Site	Current Status	Species and number Collected	Year(s)	Notes
George W. Brackenridge	capped	Trogloglanis (1)	prior to October 1919	type locality; given to J.T. Patterson at the University of Texas (Eigenmann 1919)
R.P. Persyn	well could not be located	unknown (anecdotal, no collections made)	1929	blindcats referred to in newspaper article, supposedly San Antonio Light (9/7/29) (Hubbs and Bailey 1947); probably incorrect reference since Longley and Karnei (1978a) couldn't find it
Josef Boeke	capped (covered by highway construction; I-35)	Trogloglanis (1)	1934	Anecdotally 20 fish were observed in irrigation ditches (Longley and Karnei 1978b) (Hubbs and Bailey 1947)
William Kempin	well could not be located	Satan (1)	prior to 1938	type locality; Hubbs and Bailey (1947)
Alamo Dressed Beef Company	well could not be located	unknown (anecdotal, no collections made)	prior to 1947	Hubbs and Bailey (1947)
Bexar Metropolitan Water District Well	capped	Satan (1)	1953	Three fish came out, only one was preserved (Longley and Karnei 1978b)
O.R. Mitchell	inactive; part of Union Pacific Rail Yard	Trogloglanis (4), Satan (4)	1955	1955 specimens (1 of each species) collected by John Werler (Suttkus 1961); 1977 specimens (3 of each) by Longley and Karnei (1978a); recent sample efforts declined (Zara 2010)
El Patio Foods	capped	Satan (1)	1960	San Antonio News reported approx. 12 blindcats came out with initial flow when well was first drilled (Suttkus 1961); 50 additional blindcats (both species) observed by employees when water tower was drained in 1964 (Longley and Karnei 1878a); none collected
Verstraeten	capped	Trogloglanis (1)	1977-1978	Karnei (1978a); capped in 2008 (Zara 2010)
Artesia	active	Trogloglanis (22), Satan (11)	1978	Karnei (1978a); recent sample efforts declined by SAWS (Zara 2010)
Aldridge 209	active	Trogloglanis (11)	2008 - 2010	Zara (2010)
Tschirhart	active	Trogloglanis (1)	2010	Zara (2010)
Jeff Bailey	probably capped after our sampling was terminated by landowner	Trogloglanis (2)	2011	Reported here for the first time

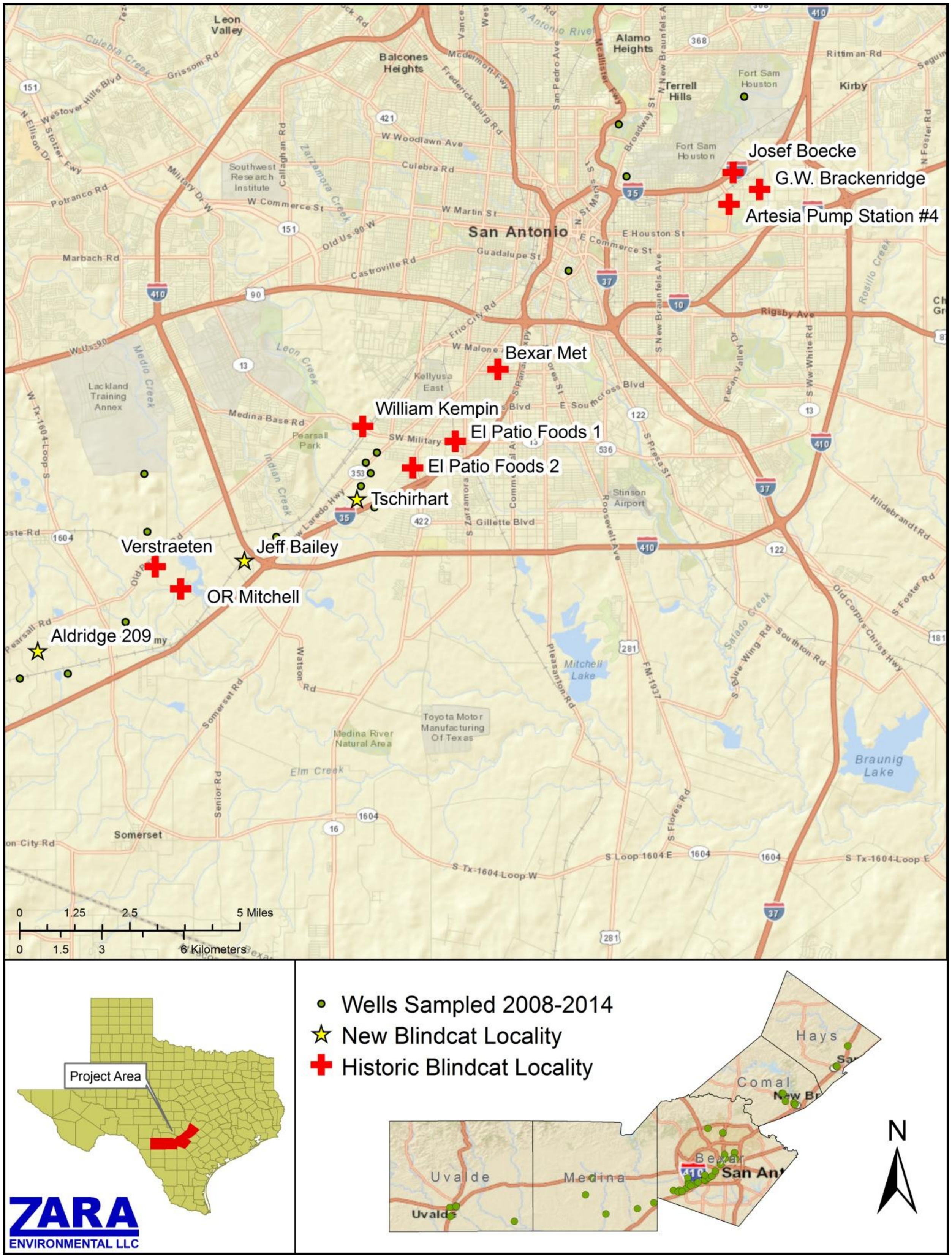

Figure 3. Distribution of known historic and confirmed new blindcat localities in Bexar County, Texas.

Blindcat Localities

George W. Brackenridge Well (29.439394, -98.422865)
This is the type locality for the toothless blindcat. Longley and Karnei (1978a) reported that the original description did not specify which of George W. Brackenridge's wells produced the catfish. Longley and Karnei (1978a) deduced that the probable type locality is a 308-m deep well at the intersection of Belgium Lane and Kono Road, noting that it was likely to be capped soon. This location currently lies at the edge of a suburban development consisting of single family homes immediately to the north, apartment complexes to the southeast, and a ploughed field to the southwest, near the Salado Creek Greenway. Its coordinates are based on the intersection of these two roads; there are no visible irrigation wells in this area.

R.P. Persyn (no coordinates available)
Hubbs and Bailey (1947) indicated that a Mrs. R.P. Persyn referenced a blind catfish in a San Antonio Light newspaper article from 7 September 1929. Karnei (1978a, b) could locate neither the original article nor living relatives recognizing the name R.P. Persyn. This well is not represented in Figure 3.

Josef Boecke Well (29.444867, -98.432673)
The second known specimen of toothless blindcat was collected by Josef Boecke from the outflow of his artesian well (Hubbs and Bailey 1947). At the time of Longley and Karnei (1978a), the location was in the right of way of I35, and the 308-m deep well was covered by highway-related construction. We did not attempt to revisit this site. Its coordinates are based on the map from Figure 6 in Longley and Karnei (1978a) and the description of the collection locality as being 4.4 km east and 3.0 km north of the Alamo.

William Kempin Well (29.361798, -98.572501)
This is the type locality for the widemouth blindcat. 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 m deep and located in southwest San Antonio near the East Kelly Air Force Base. Longley and Karnei (1978a) reported the area was under development, and no well site could be found. Peter Sprouse tried to locate this site in August 2008 but was unsuccessful. Best estimates of the historical 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 4).

Figure 4. A utility building west of the railroad may house the William Kempin Well, a historic blindcat locality.

Alamo Dressed Beef (no coordinates available)

Hubbs and Bailey (1947) indicated that this was a blindcat locality; however, Karnei (1978a, b) was unsuccessful at locating the business. The business was not listed in city or county records. Aside from attempting to locate this business through the internet, we did not attempt to locate this site.

Bexar Metropolitan Water District Well (29.380457, -98.521515)

Also called the "Bexar Metropolitan Well," this is a historical locality for the widemouth blindcat. Longley and Karnei (1978b) reported that the manager of the Bexar Metropolitan Water District collected one of three specimens observed of widemouth blindcat in 1953. The well from which it was collected was 15 cm in diameter, of an unknown depth, and located on approximately the 500 block of Carlisle in southwest Bexar County. Longley and Karnei (1978b) found this well to be capped at the time of their study. Our coordinates are based on aerial imagery and centered on the 500 block of Carlisle; thus, we only consider them as accurate as the address in the literature.

O.R. Mitchell Well (29.308458, -98.641140)

This is a historical locality for widemouth and toothless blindcats. Suttkus (1961) reported 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 toothless blindcats in 1955. He reported the depth of the artesian well as 582 m, and the ranch location as 22.5 km southwest of San Antonio in the Von Ormy area (USGS No AY-68-43-601). According to Longley and Karnei (1978a), three specimens of each of these two species were collected at this location between 30 March and 30 June 30 1977. One of the widemouth blindcats was kept alive for 164 days. The well had a reported flow of 315 L/s. Their request to re-sample this site in 1978 was denied by the ranch foreman. In August 2008, this property was being developed as the Union Pacific San Antonio Intermodal Yard, and our requests to access the property were denied.

El Patio Foods Well no. 1 (29.356731, -98.537691)

This is a historical locality for the widemouth and possibly toothless blindcat. Suttkus (1961) reported on the collection of widemouth blindcats from this site on 1 June 1 1960. Suttkus gave the address of the artesian well (427 m deep), and Longley and Karnei (1978a) reported the well was capped or plugged and closed down in 1964 due to infiltration of oil and sulfur from the bad water zone. Peter Sprouse attempted to relocate this well on 14 August 2008 at 2600 Military Drive. At this location was a large slab left over from a demolished industrial facility that could have been El Patio Foods (Figure 5). Also at this location was a terminal power pole (typical of well sites) that is overgrown with vegetation and could be a sealed well site. Its coordinates are based on the location of that terminal power pole, and may or may not be accurate based on our interpretation of the historical literature.

Figure 5. At 2600 Military Drive, a historical blind catfish locality, lies a demolished industrial facility at that location that could have been El Patio Foods.

Verstraeten Well (29.315792, -98.650708)

This is a historical locality for the toothless blindcat. Longley and Karnei (1978a) netted this well starting on 16 March 1977 and were still netting there when they wrote their report. One toothless blindcat was collected, and many invertebrates were collected from the 513-m deep well, which had a reported flow of 315.4 L/s. This site was capped in February 2008, and another well on the property was inaccessible for sampling during our study.

Figure 6. View of property where Verstraeten Well is located.

Artesia Pump Station Well (29.433056, -98.440278)

Also sometimes called the "Artesia Well #4," this is a historical locality for the widemouth blindcat, although there is some confusion because records in the Texas Memorial Museum show the toothless blindcat here, not the widemouth. According to Longley and Karnei (1978b), sampling of this well began on 22 February 1978, and was continuing when their report was written. Those authors locate Artesia Well Number 4 of five artesian wells at the pump station as 3.2 km southwest of the historical location of toothless blindcat near the Joe Freeman Coliseum on Coliseum Road (which has since been re-named to AT&T Center Parkway) and Aniol Roads. They described the well as 402 m deep and flowing at 244 L/s. Those authors found 11 widemouth and 22 toothless blindcats at this locality during their study. This well is probably in use, and SAWS declined our request to sample this well during our study. Its coordinates are based on SAWS address for the Artesia Pump Station at 703 Coliseum Road combined with aerial imagery, which places the pump station approximately 610 m west of the San Antonio Stock Show and Rodeo Grounds.

Aldridge 209 Well (29.288220, -98.695016)

David Aldridge sighted a blind catfish coming out of the "Aldridge 209" irrigation well ca. 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 m 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, but the sampling technique was refined in 2009, when a 500-μm net covered with a vinyl shade sock was cinched over the outlet. In 2010 an in-line sampler was installed to allow sampling at the pump site, before the water flows to the various outlets, 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 7). This well flows under artesian pressure and is also pumped at times, depending on water needs and aquifer level.

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 EAA 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 m. Under artesian flow, this site was noted to discharge at a rate of approximately 40 L/s in October 2009.

Figure 7. Wooden shade tent over in-line sampler at Aldridge 209 pump site.

A sample collected on 28 August 2008, which contained just a bit of white bone material, was putatively identified as belonging to the widemouth blindcat, based on a well-preserved opercular bone, partial hyomandibular, and bits of hyoid bar with a few branchiostegal rays (Zara 2010); however, cytochrome *b* sequencing later resulted in a match with the toothless blindcat (J. Lundberg, pers. comm., 13 May 2014). The first sample readily identifiable as fish parts were retrieved from this well outlet on 23 March 2009, after 26 sampling events spanning almost exactly one year (Figure 8), and an intact specimen was retrieved during the summer of 2010

(Figure 9). 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 10 and Figure 11. Six blindcats or blindcat parts were collected from this site between April 2008 and January 2014.

Figure 8. Partial toothless blindcat collected from Aldridge 209 Well outlet, March 2009.

Figure 9. Toothless blindcat collected from the Aldridge 209 well outlet, July 2010.

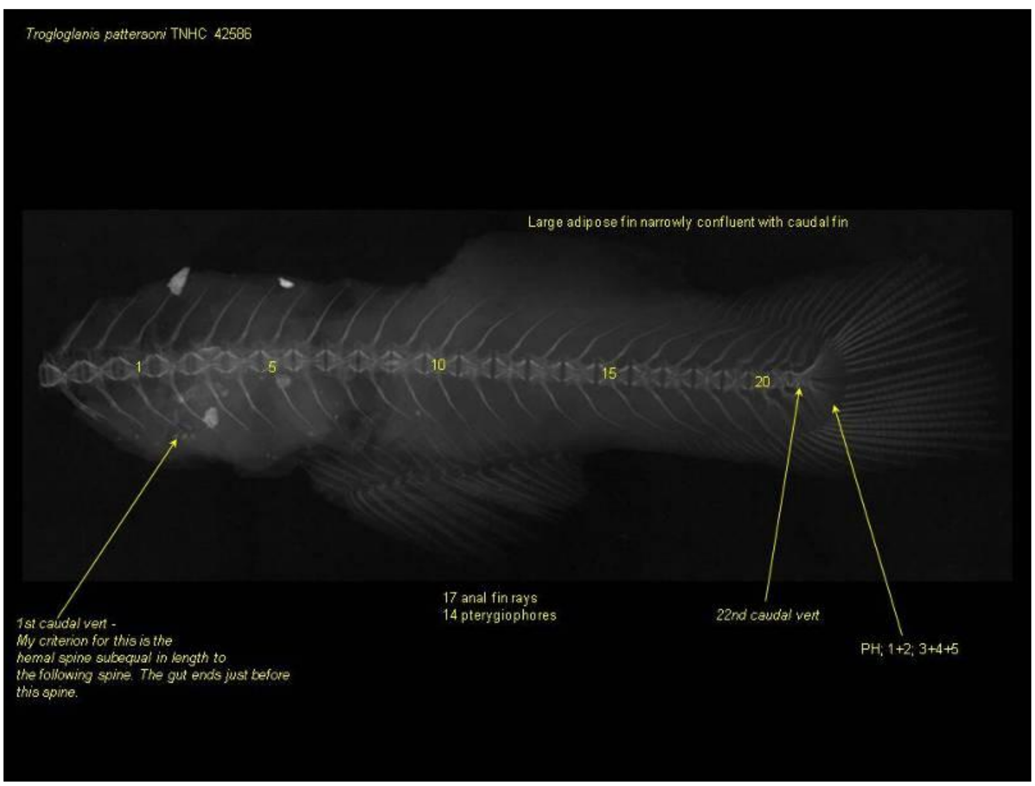

Figure 10. X-ray image showing diagnostic characteristics of partial toothless blindcat collected from Aldridge 209 Well outlet, March 2009.

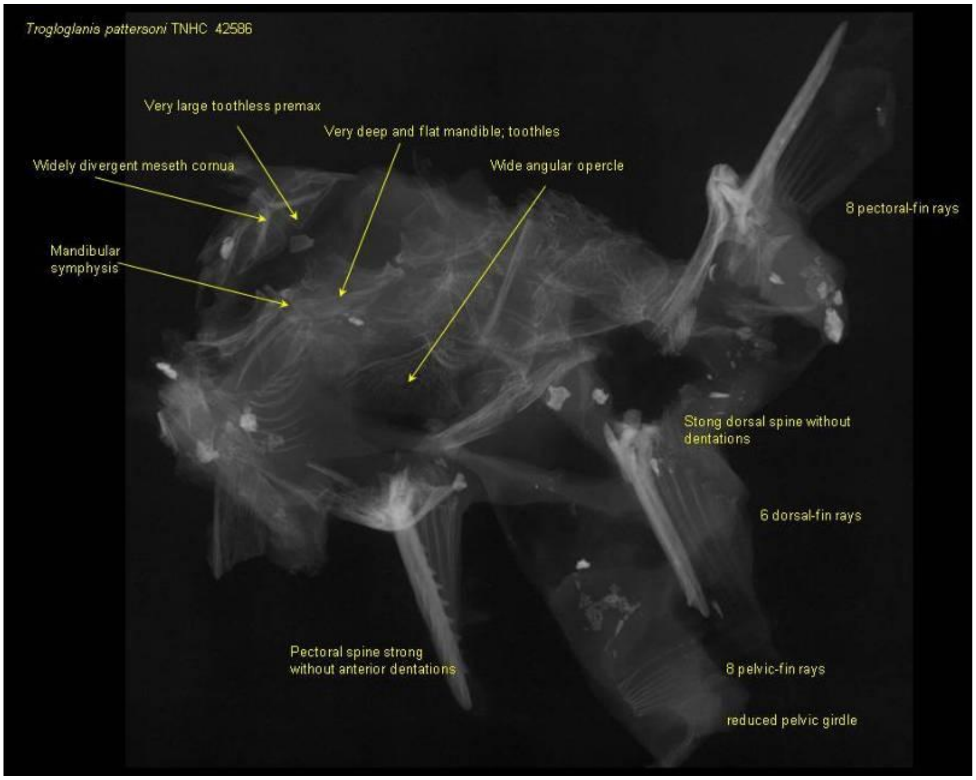

Figure 11. X-ray image of toothless blindcat showing diagnostic features, March 2009.

Tschirhart Well (29.337813, -98.574808)

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, 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 12). This net often contained some amount of surface contamination, including millipedes and terrestrial isopods, but also yielded aquifer-associated material including unidentified (bacterial?) mats, stygobitic invertebrates, and a single identifiable catfish bone belonging to the toothless blindcat (Figure 13).


Figure 12. Net cinched inside of an in-line barrel sampler at the Tschirhart well.

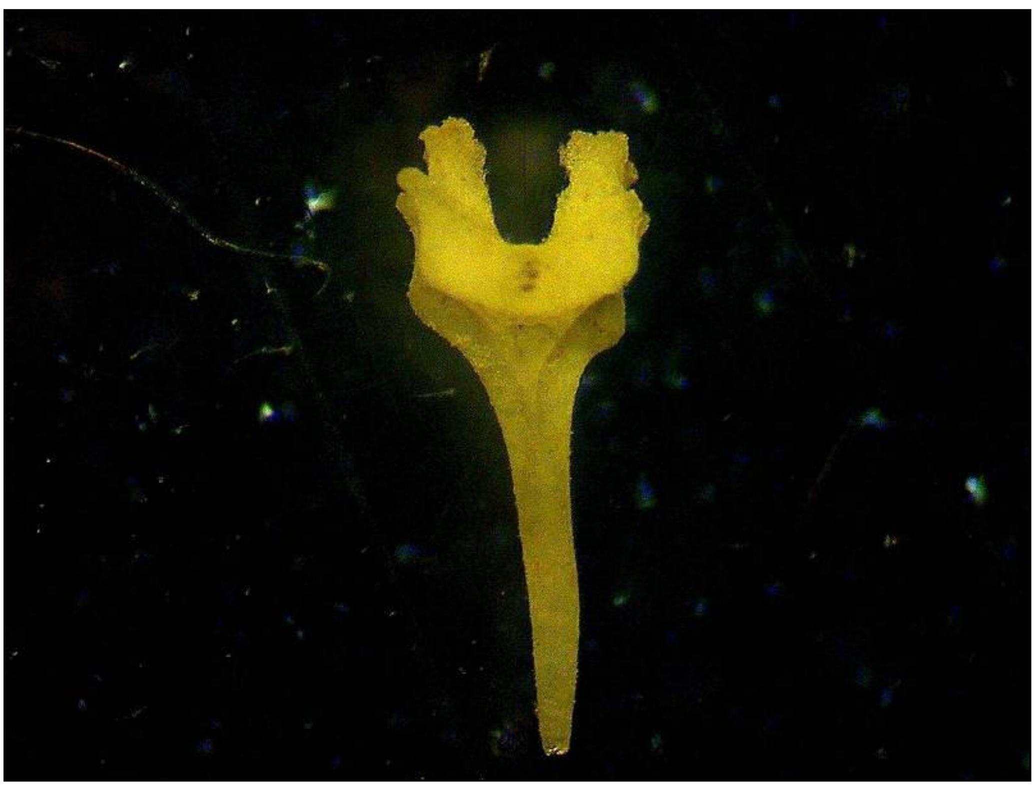
Figure 13. Vertebra collected from the Tschirhart Well.

Jeff Bailey Well (29.317793, -98.617179)
This well is located in Bexar County, and flows sporadically based on irrigation needs. As of Zara (2010), no fauna had been retrieved from the site; however, during a site visit on 30 September 2011, a single bone was retrieved and subsequently identified as belonging to the toothless blindcat. Another sample containing a complete pectoral-fin spine and a small fragment of cleithrum representing the toothless blindcat were collected on 6 October 2011. The specimens were retrieved after the sampling infrastructure had been improved from open barrels and nets cinched over outflow (see Zara 2010 for details) to a fully contained in-line barrel sampler (Figure 14). Sampling ended when the landowner sold the well, and this site was due to be capped by the new landowner.

Figure 14. In-line barrel sampler at the Jeff Bailey Well.

Catch per Unit Effort and Relative Abundance

Catch per unit effort represents the volume of water (in ac-ft) necessary to sample in order to obtain one specimen of a specific organism. The volume of water sampled necessary to obtain a specimen of any organism listed in Table 5 ranges from 96.1 ac-ft (*Chlamydotheca arcuata*) to 21,343 ac-ft (*Epactophanes richardi*, *Paracyclops chiltoni*, *Mexistenasellus coahuila, Tethysbaena texana*); on average it is necessary to sample 6,425 ± 8,558 ac-ft of water in order to obtain one specimen of any of the stygobitic invertebrates listed. The volume of water sampled necessary to obtain a sample of toothless blindcat varies by well, and in this study ranged from 67.6 ac-ft at the Jeff Bailey Well to 426 ac-ft at the Tschirhart Well (Table 6). The high standard deviation is due to the large variance in flow volumes, with many species needing over 21,000 ac-ft of water flowed before detecting one individual. We present CPUE for invertebrates at two distinct levels: the ordinal and specific. In the case of some identifications, the most specificity was only to the genus or family level based on poor specimens or partial specimens obtained during sampling. The ordinal level represents the volume of water that is necessary to sample in order to catch a single specimen belonging to that order. Ostracods were the most abundant organisms in our samples, and thermosbaenaceans were the least abundant (Table 5). The higher an organism's relative abundance found in our samples, the lower the volume of water was necessary to capture a single specimen of that organism (Table 5).

Table 5. Catch per unit effort (CPUE; in ac-ft) and relative abundance of fauna captured during this project. The species column represents the lowest taxonomic level of identification that was made for the specimen(s), sometimes represented by family or genus. An asterisk (*) indicates species that are not obligate subterranean fauna, though they are known to be associated with groundwater. Relative abundance and CPUE at the ordinal level are presented in bold.

Order	Species	CPUE (ac-ft)	Relative Abundance
Ostracoda	**Overall (cumulative)**	**78**	**33.7**
Ostracoda	*Chlamydotheca arcuata**	96	27.31
Copepoda	**Overall (cumulative)**	**98**	**26.8**
Isopoda	**Overall (cumulative)**	**158**	**16.6**
Amphipoda	**Overall (cumulative)**	**177**	**14.9**
Isopoda	*Speocirolana hardeni*	240	10.95
Copepoda	*Acanthocyclops* sp.*	288	9.1
Copepoda	*Paracyclops poppei**	350	7.5
Copepoda	Copepoda (undetermined) *	368	7.13
Amphipoda	*Parabogidiella* sp.	395	6.64
Ostracoda	*Cypridopsis vidua**	418	6.27
Decapod	***Palaemonetes antrorum***	**521**	**5.04**
Amphipoda	*Holsingerius* sp.	790	3.32
Copepoda	*Diacyclops* sp.	1,067	2.46
Amphipoda	Amphipoda (undetermined)	1,123	2.33
Gastropod	***Horatia nugax***	**1,779**	**1.48**
Amphipoda	*Stygobromus* sp.	2,134	1.23
Amphipoda	Hadziidae	2,668	0.98
Bathynellacea	***Texanobathynella bowmani***	**3,049**	**0.86**
Isopoda	Isopoda (undetermined)	3,557	0.74
Crustacea	Undetermined	5,336	0.49
Copepoda	*Nitocra spinipes* *	7,114	0.37
Isopoda	*Mexistenasellus coahuila*	7,114	0.37
Amphipoda	*Allotexiweckelia hirsuta*	21,343	0.12
Copepoda	*Epactophanes richardi* *	21,343	0.12
Copepoda	*Paracyclops chiltoni* *	21,343	0.12
Ostracoda	Ostracoda (undetermined)	21,343	0.12
Thermosbaenacea	***Tethysbaena texana***	**21,343**	**0.12**
Isopoda	*Cirolanides texensis*	970	0.03
Isopoda	Cirolanidae	1423	0.02

The two most abundant taxa in our samples, ostracods and copepods, contain some species that are not restricted to groundwater habitats. For example, *Acanthocyclops vernalis* (Fischer, 1853) is widespread in the Northern (and possibly Southern) Hemisphere temperate zones. It usually occurs in ephemeral (vernal) pools and other small waterbodies but occasionally is found in wells and caves. Although the ostracods and copepods we collected were from nets attached directly to artesian outflow, and we made every effort to avoid surface contamination, in some cases the sample container did rest in water that overflowed to a stock tank or irrigation ditch. We acknowledge the possibility that some of the samples originated surface sources; those samples are identified with asterisks in Table 3.

Comparison to Historical Datasets

Over half of the invertebrates reported by Karnei (1978) were the blind shrimp *Palaemonetes antrorum*, whereas this species accounted for only 5% of the invertebrates in our samples. Conversely, the relative abundance of copepods, which made up more than a quarter of the total number of invertebrates that were collected, represented only about 2% of Karnei's samples (Figure 15). Considering the two sample set overlapped with only one site, and there were also differences in net morphology, the observed differences cannot be confidently attributed to any one thing.

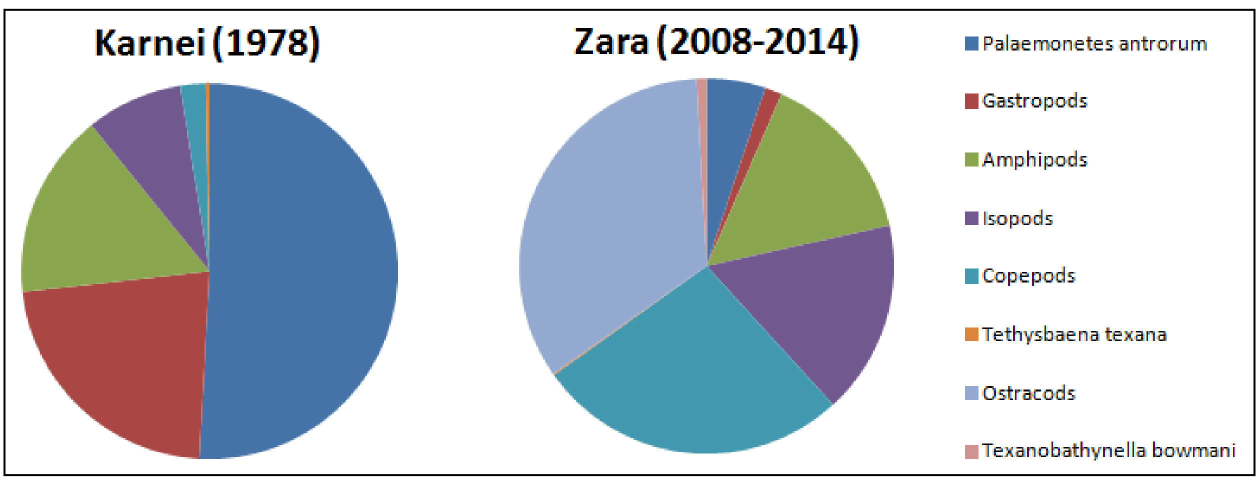

Figure 15. Relative abundance of invertebrates reported in Karnei (1978) compared to invertebrates collected for this study. Results are reported at the ordinal level, except for when specimens were represented by only a single species.

Table 6. Catch per unit effort (in ac-ft) of blind catfish captured during our study and compared to historical data.

Well	Toothless	Widemouth
Artesia Pump Station*	52.6964	105.3927
Aldridge 209	150.2919	N/A
Jeff Bailey	67.6329	N/A
Tschirhart	426.0275	N/A
O.R. Mitchell*	728.1124	728.1124
Verstraeten*	4,816.0623	N/A
* Longley and Karnei (1978a, 1978b)		

Habitat Characterization

Characteristics for each sampled well are given in Table 7. Temperatures of wells yielding stygobitic fauna ranged between 22.13 and 31.2°C, with a mean temperature of 26.1 ± 2.5°C. The distance of those wells to the nearest line feature of the saline zone ranged from 0.77 to 7.50 km, with a mean distance of 3.33 ± 2.18 km (Figure 16). Depths of wells with fauna ranged from 111 to 652 m, with a mean depth of 390.3 ± 203.0 m.

Figure 17 displays the direction and magnitude of principal components considered for this analysis, and Figure 18 displays the PCA plot. For example, deeper wells with higher temperatures, such as Woodley Farms and Carroll Farms, display toward the right hand side of the graph, wells with more taxa, such as the Aldridge Wells, Jeff Bailey and Tscirhart, display closer to the top of the graph, and wells farthest from the saline zone, such as Shavano Park, Quail Crossing, and Bexar Met #8, display to the lower left. Only wells containing data for each component considered (temperature, distance to saline zone, depth, species richness, and average flow) are included in the analysis. Depth and average volume of water flowed increased as temperature increased; well depth decreased with increasing distance from the saline zone; and wells with similar characteristics, such as high temperatures (e.g. Woodley and Carroll Farms wells), clustered closely together (Table 8, Figure 18). We expected warmer temperatures in deeper wells near the saline zone because the faulting of the Balcones Fault Zone may provide more effective convection pathways for upwelling of warmer saline fluids or may slow fluids down, resulting in a stagnated hydrologic system with an increase in temperatures over the long-term. The geothermal gradient typically results in temperature increases of 1.8 to 2.7° C for 1 km in depth (Woodruff and McBride 1979), and our wells ranged beyond 0.5 km deep. We expected deeper wells closer to the saline water line because of the dominant dip of the Edwards Formation to the east and south toward the saline zone. The relevant finding for exploring hypotheses of habitat characteristics is that none of these factors varied significantly with respect to the diversity of stygobitic taxa, which is distributed rather evenly across the plot (Table 8, Figure 18).

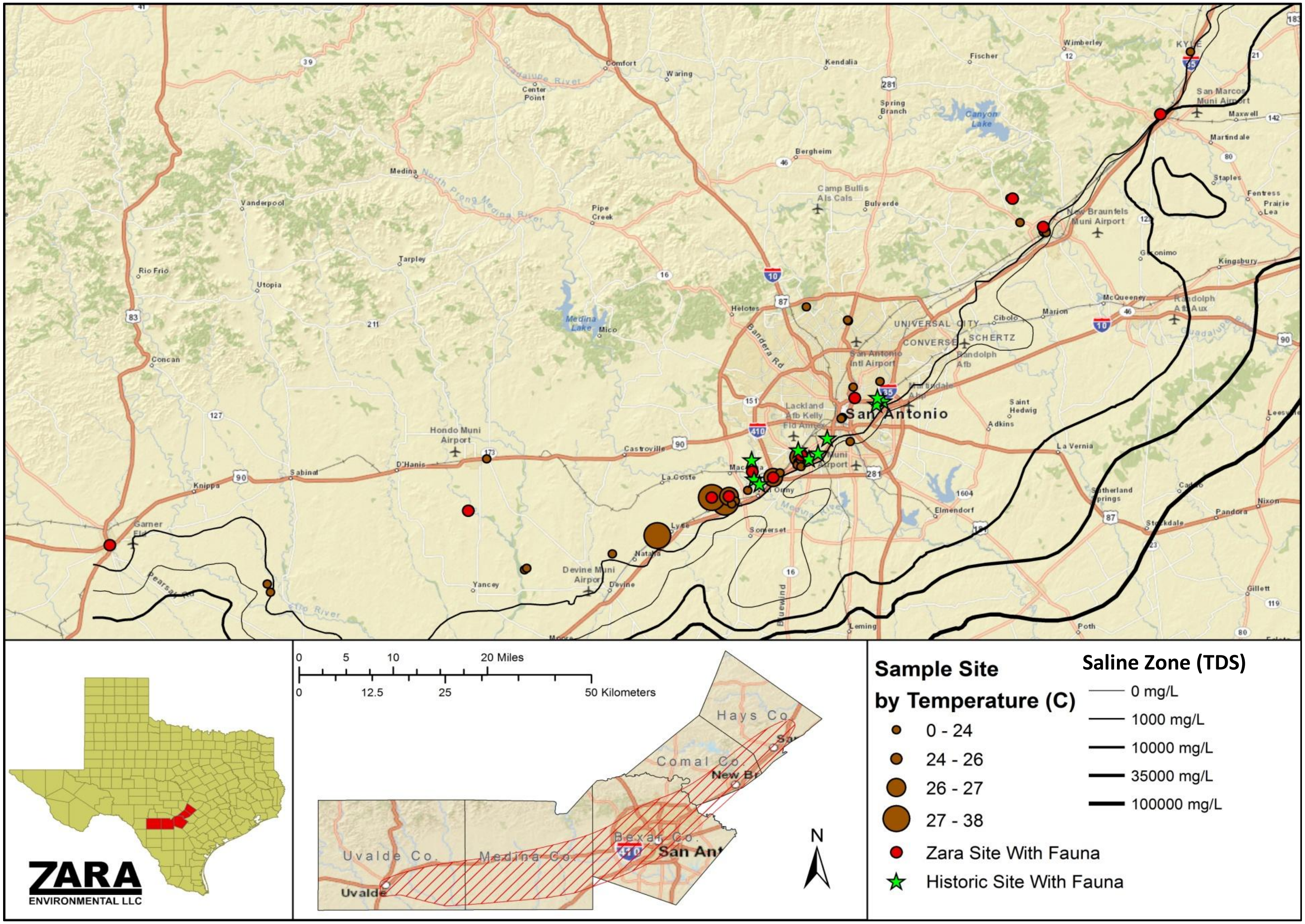

Figure 16. Distribution of wells included in this study, including temperature gradients, fauna, and historical fauna. Geographic data for the saline zone was provided by the EAA (Steve Johnson, pers. comm. 2009)

Table 7. Characteristics of wells sampled during this study; wells with stygobitic fauna in boldface type. Average flow represents the daily average volume of water flowed during our study.

Well	Mean Temp. (°C)	Distance to Saline Zone (m)	Well Depth (m)	Average flow (ac-ft)
Aldridge 209	**26.7**	**1801**	**606**	**0.3955**
Aldridge Corporate	**31.2**	**3706**	**652**	**2.1201**
Aquarena Springs	N/A	335	N/A	0
Bexar Met #8	22.7	15830	329	0
Bexar Met #91	N/A	15994	279	0
Carroll Farms	34.7	2518	741	4.9689
City of Kyle	N/A	462	181	0
Constanzo	32.5	1334	575	0.0008
Fort Sam Houston #2	**N/A**	**4802**	**230**	**0.0011**
Fort Sam Houston #5	23.7	4204	194	0.1768
Girl Scout	24.05	350	199	0
Homer Verstuyft	**26.5**	**1905**	**610**	**0.7092**
Hondo Index	N/A	26206	457	0
Jeff Bailey	**26.5**	**819**	**467**	**0.3821**
LCRA	23.6	631	294	0.0005
Loop 353	**25.6**	**2116**	**335**	**0.4529**
Lyda Fallon (Partin)	27.8	6000	808	1.2208
Mark Verstuyft	**26.5**	**784**	**477**	**0.2847**
Mission Bowling	N/A	4478	74	0
Nelson Road	**25.1**	**4083**	**549**	**0.2133**
Panther Canyon	N/A	774	98	not flowing
Paradise Alley	N/A	10	286	not flowing
Parker	31.09*	1985	293	0.6748
Persyn	29.1	266	510	0.6700
Quail Crossing	27.3	17688	105	1.5603
Raymond Wauters	26	1160	335	0.5253
Roger Verstuyft	26.5	1481	427	0.6133
Ruiz	**N/A**	**2868**	**191.1****	**0**
San Antonio Zoo	**24**	**6214**	**124**	**1.3258**
San Marcos Flagpole	N/A	119	N/A	0
Schumann Artesian	N/A	7940	610	0
Schumann House	**22.13***	**7501**	**111**	**0.0044**
Shavano Park #5	22.4*	21922	133	0
Steves house	N/A	3162	N/A	0
Stull Farm	34*	1084	841	0.0448
Tschirhart	**27**	**772**	**419**	**0.3086**
Uvalde Index	N/A	3966	107	0
Verstuyft Home	30.3	879	670	0.4445
Verstufyft Farm	31.2	2140	566	0.5964
Woodley Farms #1	36.9	1031	708	3.6637
Woodley Farms #2	38.1	384	683	3.9616

*Only one temperature data point available **well depth estimated based on depths of nearby wells

Table 8. Correlation matrix resulting from PCA. In bold, significant values (except diagonal) at the level of significance alpha=0.050 (two-tailed test). Note that diversity of stygobitic taxa was not significantly associated with any parameters.

	Average Temperature	**Distance to saline water line (m)**	**Depth (m)**	**Species Richness**	**Average ac-ft/day**
Average Temperature (°C)	1	-0.357	**0.737**	-0.119	**0.689**
Distance to saline water line (m)		1	-0.448	-0.116	-0.094
Depth (m)			1	**0.087**	**0.444**
Species Richness				1	-0.049
Average acre/feet per day					1

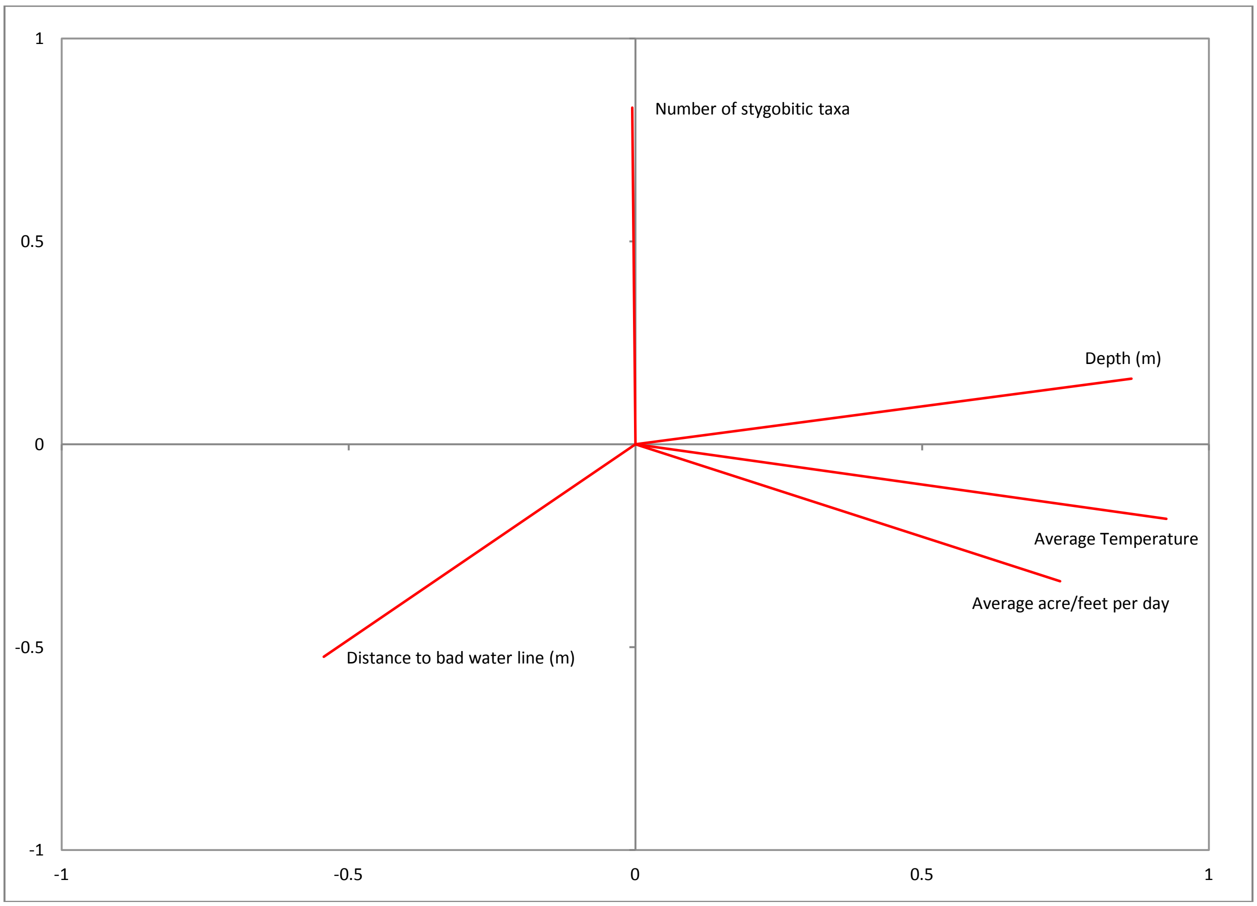

Figure 17. The direction and magnitude of principal components considered for this analysis help to explain the graph in Figure 8.

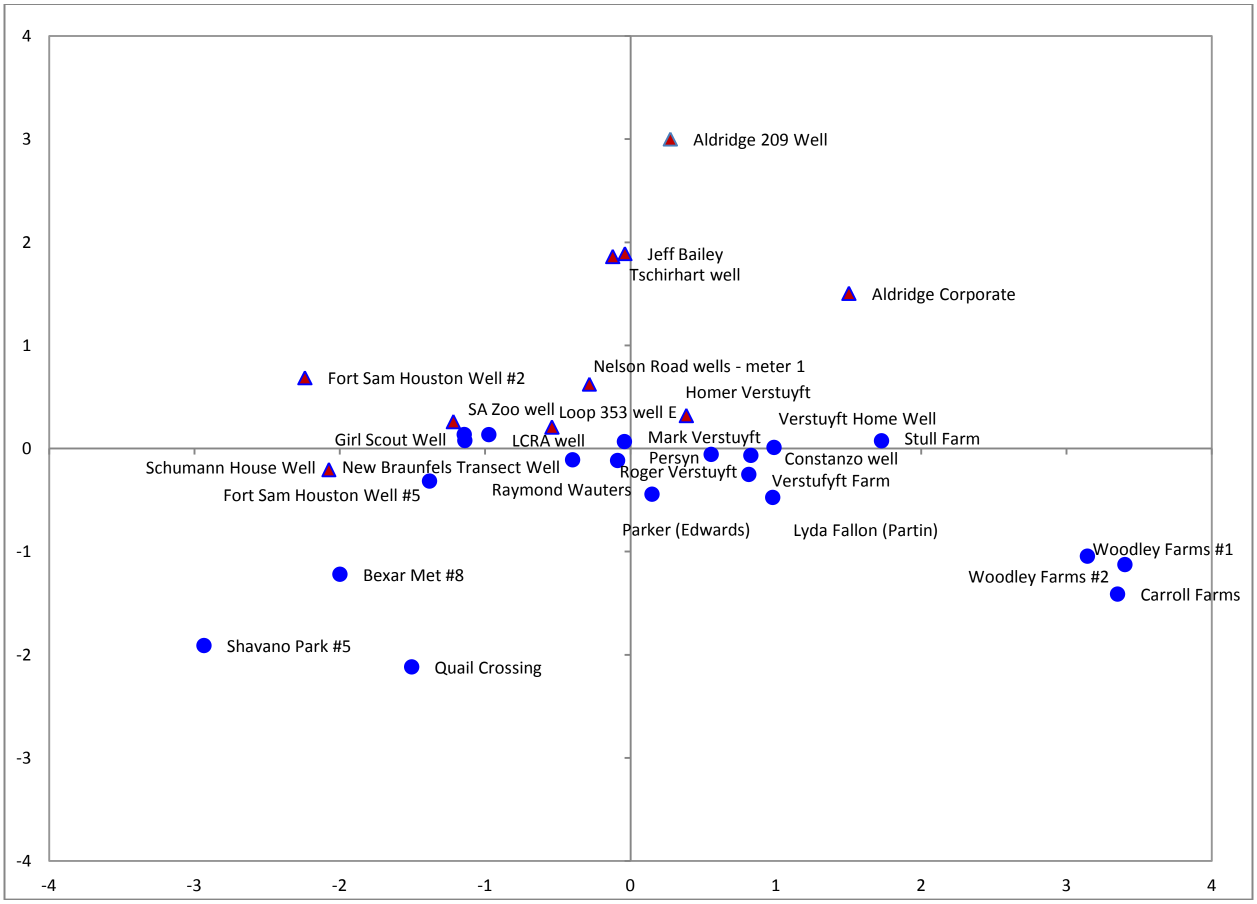

Figure 18. PCA plot showing wells with fauna records in red. The number of taxa was included as a principal component in order to help separate these records for visual display.

Discussion

The sample effort in this study is the largest ever for deep portions of the Edwards Aquifer. After sampling over 21,000 ac-ft of water across 41 sites over the span of six years, we can only confirm three sites for the toothless blindcat. Has the widemouth blindcat gone extinct since its last collection 36 years ago in 1978?

Our dataset finds material from 14 confirmed specimens of the toothless blindcat at three wells, and if the historical 2:1 ratio estimated by Longley and Karnei (1978a, b) at Artesia Pump Station, or the 1:1 ratio at O.R. Mitchell Well holds across the aquifer, then 7 to 14 samples of the widemouth blindcat could be expected. Our dataset contains three wells with toothless blindcat, with an average CPUE that is more abundant than the findings of Longley and Karnei (1978a, b; Table 5). This finding combined with a sampling effort that exceeded Karnei (1978) suggests that the lack of widemouth specimens in our collections was not due to an ineffective sampling scheme (e.g., different net design, too long between checking nets, too little volume of water, too far from appropriate blindcat habitat).

While the dataset is not comprehensive enough to conclude that the widemouth blindcat is extant, we think the lack of collections is better explained by the hypothesis that the species is unevenly distributed through the aquifer and also very rare. There appear to be differences in the species composition of wells near the saline zone of the Edwards Aquifer; however, our site selection was biased based, with preference given to wells in close proximity to the saline zone. It is easy to think of the aquifer as a giant sponge of relatively homogenous passages with nutrients and animals circulating throughout, however species distributions are more likely governed by a complex set of parameters. Carbon sources are cited as a limiting factor for subterranean life (Simon and Benfield 2002), thus the location of nutrient sources is likely a major factor in the Edwards Aquifer. These sources could be both autochthonous microbial primary production near the saline zone (Summers-Engel 2007), and allochthonous sources from losing streams, sinkholes, and caves in the recharge zone (Koehn, Keuhn and O'Neil 1991). Water chemistry varies across the aquifer, and parameters such as dissolved oxygen and temperature are well known to impact survivorship of aquatic species. Flowpaths may connect areas with good food or water quality, or may be so narrow as to exclude larger species. High flow could make travel difficult, and areas with low flow are likely to settle out organic matter that is a food source or sheltering opportunity. The characteristics of the passageways in deep wells as seen in downhole cameras are variable with enlarged bedding planes, joints, and breakdown that could serve as shelter for invertebrates or blindcats. The myriad characteristics are a likely explanation for the uneven distribution of species within the aquifer.

In our evaluation of the relationship of the parameters temperature, distance to saline water line, depth, flow (ac-ft/day), and number of taxa, we were surprised to learn that species richness was not significantly correlated with any of the factors (Table 8). The positive associations between temperature, depth and flow are easily explainable by the mechanics of the system, with wells getting warmer, deeper, and higher in flow as they are closer to the saline water line. The lack of association of any of these factors with stygobite richness is explainable by several hypotheses. First, it may be that the geographic spread of our sample sites was too small to detect the effects of distance from the saline zone. Our dataset included sites ranging from 0.72 - 7.50 km from the saline zone, but possibly the drop-off point for diversity changes was farther out than our sample, or so close to the edge of our sample that we did not detect the change. Secondly, and possibly overlapping with the first explanation, is that the lack of correlation is a real pattern, showing that the communities are not restricted to nutrient sources at the saline water line. In this scenario, nutrients from surface sources at recharge sites to the northwest of sample locations (Figure 1) may provide some or all of the food sources for those communities, and thus the invertebrate communities are truly contiguous between the saline water line to the recharge zone. Thirdly, it may be that the invertebrate communities are partially or totally reliant on nutrient sources at the saline zone; however, their biology allows them to range far from food sources, thus they are not preferentially distributed near the saline zone. Since deeper, warmer, and higher volume wells are also associated with distance to the saline zone, similar explanations can be given for the lack of correlation. The patterns could be real or simply not detected due to a small sample size considering the variation in that parameter.

Catch per unit effort was low within the system, as expected for a deep subterranean system and similar to Karnei's (1978) results. The most common invertebrate species, *Speocirolana hardeni*, was only present in units of one per 240 ac-ft. of water, while rare species were found in units of one per 21,000 ac-ft. Each of the larger groups contained some species that were more abundant, and some species that were more rare, also typical of an invertebrate community. Catch per unit effort varies at each site, and we chose to evaluate invertebrate CPUE of all of our sites together in order to help estimate the amount of sampling that needs to be performed system-wide in order to capture and study any significant numbers of these species. This is valuable because any given site may or may not yield fauna.

In order to document community trends over time, we spent a large amount of effort attempting to locate and sample sites that Karnei (1978) documented to have produced fauna. All but one of those sites could not be located, were altered in such a way as to preclude sampling efforts, or were unavailable for sampling (Zara 2010). The San Antonio Zoo (Brackenridge Zoo in Karnei [1978]) was the only historical site that allowed access. The collection of a significantly greater number of very small fauna (e.g., copepods, ostracods) in our study compared to Karnei (1978) is explainable because some troglobitic and interstitial species may only approach 200 µm, and he utilized 500-µm nets while we used a combination of nets ranging down to only 60 µm. Karnei may have not detected these taxa or not included them because they are not known to be fully aquifer limited species.

Given a null hypothesis of an even distribution of taxa across the aquifer, the difference in the relative abundance of fairly common organisms, such as shrimp and gastropods, between our study and Karnei's (1978) is unexpected. What represents a real locality, and what is the actual range for each of these species? Many of the historical localities for these species are ill-defined in the literature, have been capped or plugged, or are unavailable for sampling. While our relative abundance numbers are somewhat distorted by the inclusion of ostracods, we still collected notably fewer shrimp and snails. We also collected the bathynellacean *Texanobathynella bowmani* from Comal County, while Karnei (1978) sampled only wells in Bexar County. These differences are most likely a result of sampling at different localities and demonstrate that taxa are not evenly distributed across the aquifer. *Trogloglanis* bones at Tschirhart and Jeff Bailey may have travelled some distance before getting pulled into our nets. There are few modern blindcat sites, and we can no longer demonstrate whether the species exist at historical sites. An alternate hypothesis that we cannot test given our non-overlapping sites is that there has been a shift in community composition, and these organisms have become less common over time. Thus, we recommend a conservative approach to answering questions regarding habitat effects. Given the apparent patchy distribution of stygobitic species, it is possible that appropriate habitat is rather rare, and wells that produce fauna, particularly blindcat material, may warrant conservation.

Recommendations

The distribution and abundance of aquifer dwelling organisms remains poorly understood, with this study representing only the second major effort at widespread macrofauna sampling of one of the most diverse aquifers in the world. Since abundances of stygobitic species in general are very low, additional data are needed to find trends in populations or habitat; additional physical sampling of many ac-ft of water over many years may be the key to identifying those trends.

We envision future datasets using a combination of new and more accessible technologies, including e-DNA, continuous water chemistry and contaminant sampling, or even robotic sampling of deep wells. A major gap in our understanding is the lack of microbial sampling for diversity, abundance, and ecosystem function. Given that microbes may be the primary carbon source for this community, there is much to learn about them.

It is important to verify whether the widemouth blindcat still exists within the Edwards Aquifer; this is the only North American Ictalurid for which no genetic data is on file, thus material collected (e.g., eDNA) may never be confirmed until comparative tissue is obtained that can be first matched morphologically to museum specimens. The most obvious mechanism to obtain this match is to sample the wells at the only known available locality for the species: Artesia Pump Station. It may be possible to access chlorination tanks at this site using confined space SCUBA techniques. Even if access to this site is denied in the future, efforts should be made to continue and expand upon sampling within the known range of this species.

Drawdown, contamination, and altered flow regimes are continual threats to aquifer species in this urban area (Bowles and Arsuffi 1993). Since the aquifer communities likely receive some of their carbon source from recharge, it is logically important that recharge features and their contributing zones are in a natural state so that native organic material can be washed in. It may be just as important for the health of aquifer fauna that springflow is maintained at the downstream end of that system in order to push out waste materials and refresh the food sources. Springflow is under threat from overpumping by an increasing urban population (Bowles and Arsuffi 1993) and a long-term severe drought. Water levels may influence the connectivity of conduits and the location of the saline zone, which is likely to contribute to the base of the food chain. Although our results do not strongly imply that the richness or abundance of invertebrate aquifer fauna co-varies with distance to the saline zone, we demonstrate that fauna are not evenly distributed across the aquifer. We hypothesize that stygobitic richness and diversity may co-vary with an assortment of other factors, including water chemistry, flow volumes and specific flowpaths, habitats within the substrate, and an uneven distribution of nutrient sources yet to be identified.

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