INVERTEBRATE DIVERSITY FROM DEEP WELLS OF THE SOUTHERN BOUNDARY OF THE EDWARDS AQUIFER OF SOUTH-CENTRAL TEXAS

Kathryn E. Perez[1]*, Valentin Cantu[2], Vanessa Saenz[1], J. Randy Gibson[3], Patricia Duncan[4] and Benjamin Hutchins[5]
[1]School of Integrative Biological and Chemical Sciences, University of Texas Rio Grande Valley, Edinburg, TX, 78542
[2]Uvalde National Fish Hatchery, Uvalde, TX, 78801
[3]Aquatic Resources Center, United States Fish and Wildlife Service, San Marcos, TX 78666
[4]Ecological Aquatic Systems Research and Management, LLC, Norfolk, VA 23503
[5]Edwards Aquifer Research and Data Center, Texas State University, San Marcos, TX, 78666
**Corresponding author; Email: perezke@gmail.com*

Abstract.–This study examined the invertebrate fauna from three deep wells located at the Uvalde National Fish Hatchery in south-central Texas. These wells provide a water source from geological strata of the southern boundary of the Edwards Aquifer ranging in age from Lower Cretaceous to Recent. Forty-seven collections, sampled on a continuous basis from November 2020 to January 2022, produced a total of 738 specimens comprising 15 different species. These collections reveal a diverse crustacean assemblage along with dominant numbers of the stygobiotic snail *Balconorbis uvaldensis*. This study provides a preliminary survey for future investigations of unidentified crustacean species as well as revealing seasonal variations in the reproductive life cycle of *Balconorbis uvaldensis*.

Keywords: stygobiotic, stygosnails, Edwards-Trinity Aquifer System, reproductive seasonality

---

The Edwards, Trinity, and Edwards-Trinity aquifers of central Texas and northern Mexico are a biodiversity hotspot (Longley 1981) with a diverse stygobiotic (groundwater-obligate) fauna dominated by crustaceans, but also include gastropods, flatworms, insects, fishes and salamanders, among others (Nissen et al. 2018; Alvear et al. 2020a; Hutchins et al. 2021). Although biologic investigations of the Edwards Aquifer have occurred for more than 125 years (Stejneger 1896), the diversity and distribution of groundwater fauna are still poorly known. For example, a recent article examining patterns of diversity among

Published Online: 20 June 2024 | OPEN ACCESS DOI 10.32011/txjsci_76_1_Article2

groundwater sites in the Edwards Aquifer system (Hutchins et al. 2021) did not report any diverse sites in Uvalde County or adjacent counties because most sites remain unexplored or difficult to access due to private ownership. However, stygobiotic fauna have been documented there, including the gastropod *Balconorbis uvaldensis* Hershler & Longley, 1986 that was described from well sites at the Uvalde National Fish Hatchery (UNFH).

The UNFH was established in 1934 and operational by 1937, focusing primarily on culturing game fish for farm and ranch ponds (Wells 2000; Moore & Kalish 2022). During the mid-1970s, the hatchery began restoration and mitigation stocking efforts on Tribal, Federal and state lands (Wells 2000; Edwards Aquifer Authority 2019). Currently the UNFH holds captive populations of federally and state listed endangered and threatened species (invertebrates, amphibians, fish, and plants) in the event that propagation efforts become necessary to recover species in the wild (Edwards Aquifer Authority 2019). A continuous supply of near constant-temperature, high-quality water with chemistry similar to species' native habitat (i.e., $Ca\text{-}CO_3$ – type, low nutrients, with TDS < 1000 mg/L) is critical for the success of the refugia program. Our study relied on the hatchery wells to conduct sampling in otherwise inaccessible deep-aquifer habitats.

Due to difficulties with sampling subterranean habitats (habitat impediment) and obtaining sufficient samples for observation (biological impediment), the distribution, ecology, and life history of stygobiotic fauna are poorly known (Mammola et al. 2019; Mammola et al. 2021). This lack of basic biological information for groundwater fauna is problematic because subterranean species, as an ecological group, exhibit extremely high levels of imperilment owing to numerical rarity, limited range endemism, and potential sensitivity to contaminants and environmental variability (Culver & Pipan 2019). Consequently, biologic surveys for groundwater taxa are imperative for accurate characterization of species conservation status and basic life history information. Because of low detectability (Mammola et al. 2019), repeated surveys for stygobionts are important to provide accurate occurrence information. They can also shed light on reproductive biology (Carpenter 2021) such as whether a species reproduces continuously or with periodic episodes of reproduction.

Due to the scarcity of information on stygobiotic organisms, our predictions relied on previous work from the epigean snail *Lymnea stagnalis* (Lymnaeidae) which found five variables driving reproduction: photoperiod, food availability, water temperature, parasites, and water quality. As would be expected, long photoperiod and well oxygenated water enhanced reproduction and low temperature, low food availability, and high trematode parasite load decreased reproduction (Wayne 2001). Further studies have supported the key role of nutrient availability and demonstrated that there is plasticity in reproductive timing with clonal lineages of an epigean snail (*Potamopyrgus antipodarum* Tateidae) exhibiting either continuous or periodic reproduction depending on nutrient availability (Verhaegen et al. 2021). An epigean species in the family Cochliopidae (*Tryonia cheatumi*), displayed continuous reproduction throughout the year with periodic peaks in the appearance of juveniles (Perez et al. 2022).

Cochliopidae, the family that includes *Balconorbis uvaldensis*, includes several snail lineages that are adapted to subterranean environments (Alvear et al. 2020a; Alvear et al. 2020b). However, reproductive seasonality has not been investigated for any of the subterranean Cochliopidae. Other stygobiotic organisms, such as cave catfishes, have been observed to reproduce periodically in the rainy season (Secutti & Trajano 2021). However, stygobiotic shrimp (Strenth & Longley 1990) and three stygobiotic snails (Bichuette & Trajano 2003; Weck 2022): *Potamolithus troglobius, Potamolithus* sp. (Tataeidae), and *Fontigens antroecetes* (Fontigentidae) were observed to reproduce continually with reproductively mature females occurring throughout the year. Previous studies in subterranean animals have not determined how labile those characteristics are or the causative agents of these patterns.

Groundwater habitats are characterized by total darkness (Alqaragholi et al. 2021), and while reduced environmental variation and reduced energy availability relative to surface habitats are commonly-cited characteristics of subterranean environments, ecologists now acknowledge that they are more variable (Culver & Pipan 2019) than once thought, depending on depth, surface connectivity, recharge patterns, energy sources, and biogeochemistry. The subterranean aquatic environments sampled in this study, wells derived from relatively deep-water sources (Maclay 1995; Groschen 1996; Clark & Small 1997; Clark 2003) are constantly dark with relatively stable temperature and water quality, probably negating three of the primary cues for gastropod reproduction. The influence of parasites (e.g., trematode larvae) has not been investigated for subterranean snails. However, we speculate that if reproductive seasonality is observed in *B. uvaldensis,* it would most likely be correlated with seasonal increase in food availability associated with precipitation-driven allochthonous nutrient input. In this study, our objective was to continuously sample wells supplying aquifer water to the Uvalde National Fish Hatchery (and a static deactivated well) with two goals: 1) conduct a survey of the stygobiotic fauna, and 2) collect preliminary data on reproductive periodicity of *Balconorbis uvaldensis*.

MATERIALS & METHODS

Regional Groundwater Setting.—The UNFH is located in Uvalde County on the lower Gulf Coastal Plain on 0.40 km^2 of mesquite grasslands and shrubs (Moore & Kalish 2022). Major aquifers in Uvalde County include the Edwards Aquifer (Balcones Fault Zone), Edwards-Trinity Aquifer (Plateau), and Carrizo-Wilcox and Trinity Aquifers (Welder & Reeves 1962; Texas Water Development Board 2021). The Edwards is highly transmissive, porous, and cavernous, making it one of the most permeable and productive carbonate aquifers in the nation (Clark & Small 1997). Precipitation that occurs on the recharge zone infiltrates directly into the aquifer (Esquilin et al. 2001), while precipitation in the contributing zone (the Texas Hill Country) infiltrates the aquifer as rivers to the north (Nueces, Frio, and Sabinal rivers) and west (West Nueces River) (Welder & Reeves 1962) move south across the Edwards limestone outcrop (recharge zone). Groundwater flows south and east, supplying irrigation and municipal wells in Uvalde, Medina, and Bexar Counties before continuing northeast along the Balcones Fault Zone. Additional extraction occurs

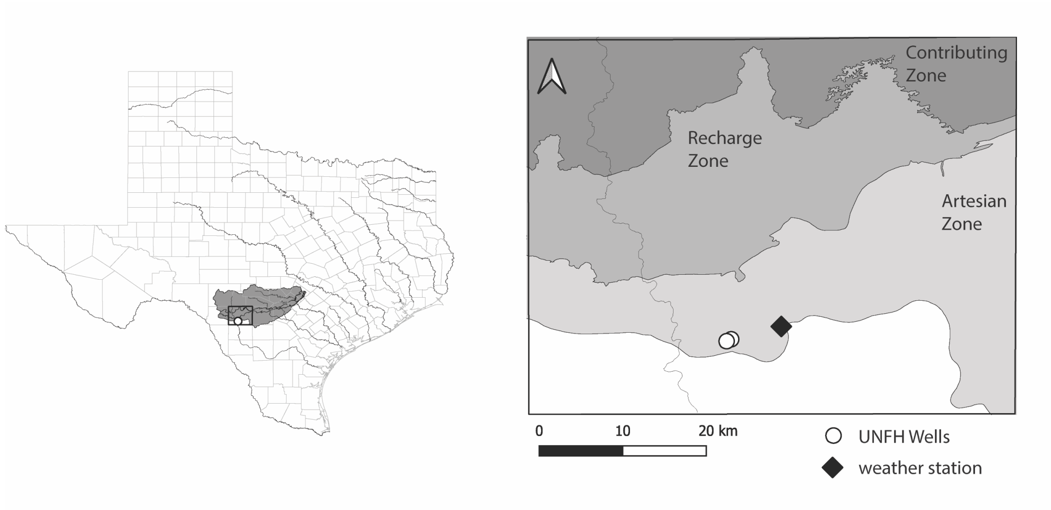

Figure 1. Map of Uvalde County, showing sites of UNFH wells and aquifer zones. The inset map shows the location of Uvalde County, major rivers, and the aquifer zones in Texas.

in Comal and Hays Counties before groundwater finally resurges at Comal and San Marcos Springs (Clark & Small 1997).

Three UNFH wells that penetrate the Edwards Aquifer were selected for repeated biologic sampling (Figure 1). Spurgeon Well (YP-69-50-501) supplies most of the water for hatchery operations, while Wilson Well (YP-69-50-5xx) serves as a backup. Burkett Well (YP-69-50-402) has not been used since November 2008 because of high iron and sulfur content. Spurgeon Well was drilled in 1950 to a depth of 183 m (Texas Water Development Board 1966–2004). Water is derived primarily from the Austin Chalk, confined above Edwards limestones (Groschen 1996), with additional contribution from the Leona Formation (sand and gravel above the Austin Chalk) (Welder & Reeves 1962; Snyder 2004) (Table 1). The well is constructed open to lower units, including Edwards limestones, but the bore is partially obstructed in the Del Rio Formation, possibly due to swelling and sloughing (Snyder 2004). Wilson Well was drilled in 2000 to a depth of 251 m (Texas Department of License and Regulation 2000). Water was derived from the Edwards Aquifer (Salmon Peak Formation, Table 1)

Table 1. Table showing a summary of the lithologic and hydrologic properties of the hydrogeologic subdivisions of the Edwards Aquifer and upper/ lower confining units in the vicinity of the Uvalde National Fish Hatchery, central Uvalde County, Texas. Hydrogeologic subdivisions, groups, formations, members, thickness, lithology, and water-bearing properties modified from Clark and Small (1997) and Welder and Reeves (1962). * not present at UNFH

System (Hydrogeologic subdivision)	Group, formation, or member	Hydrologic function	Thickness (m)	Lithology	Water-bearing properties
Quarterly (Local aquifers)	Leona Formation	AQ	25-40	Fine calcareous silt grading down into coarse chert gravel.	Yields small to large quantities of fresh to slightly saline water, locally
Upper Cretaceous (Upper confining unit)	Anacacho Limestone*	CU	470-490	Massive mudstone to packstone, with interbedded bentonitic clay	Yields small quantities of slightly saline to moderately saline water. Relatively impermeable.
	Austin Group	AQ	400	Massive, chalky to marly, fossiliferous mudstone grading downward into interbedded limestone and shale	Yields small to moderate quantities of fresh to slightly saline water in upper part.
	Eagle Ford Group	CU	130-150	Dark grey to brown, flaggy, sandy shale and argillaceous limestone	Yields very small to small quantities of slightly to moderately saline water
	Buda Limestone	AQ	70-90	Buff to light grey, dense mudstone, hard, massive.	Yields small to moderate quantities of fresh to slightly-saline water

	Del Rio Clay	CU	90-110	Blue-green to yellow-brown fossiliferous clay	Not known to yield water
Lower Cretaceous (Edwards Aquifer)	Salmon Peak Formation	AQ	385	Thick, massive grainstone grading down into cherty mudstone	Yields moderate to large quantities of fresh water especially in upper part, principal aquifer in area
	McKnight Formation	CU	160-170	Thinly bedded wackestone and grainstone grading downward into dark fissile, carbonaceous mudstone with evaporite collapse	Yields small quantities of water
	West Nueces Formation	CU	140-160	Fossiliferous grainstone to wackestone, mudstone, and packstone	Yields small quantities of water
Lower Cretaceous (Lower confining unit)	Upper member of the Glen Rose Formation	CU	350-500	Yellowish tan, thinly bedded limestone and marl	Not known to yield water to wells in central Uvalde County

(Snyder 2004). Well testing demonstrated hydraulic communication between Spurgeon and Wilson Wells (Snyder 2004). Burkett Well was drilled in 1958 to a depth of 270 m (Texas Water Development Board 1958). Water was derived from the upper portion of the Salmon Peak Formation (Edwards Aquifer), but along the Edwards Aquifer freshwater/ saline-water interface Snyder (2004) where total dissolved solids exceeded 1000 mg/L and oxygen rapidly decreased to near 0 mg/L.

Sampling methods.—Samples were taken from UNFH wells using two different methods. From the active Wilson and Spurgeon Wells, samples were collected in a 100 µm mesh net placed over the 15.24 cm PVC outflow pipe that supplies water to hatchery ponds (Pond 17). A capped PVC pipe at the end of the net serves as a refuge for invertebrates that were caught in the net (Figures 4 and 28 in Barr et al. 2015). After the first sampling periods, algal growth clogged the mesh, so a piece of PVC fabric was cut to fit over the drift net and discharge pipe and used to cover and shade the net.

The sampling of Burkett Well from a discharge pipe was not conducted as the pump was no longer operable. The above ground pump obstructs most of the well opening, however, a small opening at the base allowed limited access to the aquifer. Stygobionts were initially collected using a small PVC bottle (13 cm length by 4 cm width) with holes drilled in the top to allow invertebrate access. The trap was baited with cotton strings (for substrate) and pistachios, weighted with fishing weights, and lowered to the water table (~100 m from the top of the bore). Unfortunately, the PVC trap became stuck deep in Burkett Well on the 22 December 2020 sampling effort, therefore only polyethylene terephthalate (PETE) bottle traps were used afterwards to sample the well. PETE plastic water bottle traps (354 mL) were tied with twine, baited and weighted, then lowered down to the same depth. The top portion of the bottle was cut to make a small funnel and zip-tied upside down back onto the bottle to make the trap. When lowering the bottle traps, an obstruction was reached at ~18 m from the ground surface. One measurement indicated that traps were lowered to ~1 m of aquifer water before reaching this obstruction with a depth that varied depending on the amount of rain that infiltrated the recharge zone. During low rain or drought events no water was present at this sampling depth of the water column.

At the end of the two-week sampling period, drift net and bottle trap contents were washed into a sorting tray and preserved in 95% EtOH. Bottle traps and drift nets were left in place for 14-day sampling periods from 6 Nov 2020 through 5 Jan 2022, but sampling was not continuous nor consistent across sites because of sampling logistics (e.g., routine hatchery maintenance operations or well failures did not allow for continuous flow from wells).

Material examined.—Vouchers for each species and site are deposited at the Aquifer Biodiversity Collection (Texas State University, Edwards Aquifer Research & Data Center) under the following ABC numbers: 000335, 000347, 000356, 002902-07, 002913-14, 002916, 002919, 002935-40, 003194, 003197; 003302-4; 003377; 003379; 003390; 003450; 004472; 004497-8; 004744; 005623-24; 005655; 007761-007778; 008201-008244.

Laboratory methods.—In the lab, samples were washed through a 100 µm sieve and fully sorted under 10X magnification. Individual specimens were identified by the authors. Terrestrial and epigean taxa were removed from analyses.

All *Balconorbis uvaldensis* shells that were intact with tissues present (live when collected) and with complete, unbroken shells were measured using a Leica S9i stereo microscope and the LASX software to the nearest 0.01 mm (n=500). To estimate the size of snails at reproductive maturity, individuals across a range of sizes (n=7) were examined. The shell was dissolved with 50% hydrochloric acid and the tissues were fixed in Bouin's solution before being dissected and examined for the presence of reproductive organs.

Analysis.—Species lists were based on material collected from this study, along with historic material in the authors' institutional collections (Aquifer Biodiversity Collection, San Marcos Aquatic Resources Center). Precipitation data were taken from the Garner Field Airport, Station ID WBAN 12985 (https://www.ncdc.noaa.gov/cdo-web/datasets#LCD), 7 km from the UNFH. If precipitation fell within a sampling period, it was summed across days. JMP®, Version Pro 16 software (SAS Institute Inc., Cary, NC) was used to calculate summary statistics. To visualize how community composition varied among samples, abundance data from Wilson and Spurgeon Wells was Hellinger transformed (Borcard et al. 2011) and plotted using PCA. Data from Burkett was excluded because it was collected using a different method (baited bottle-trap versus drift net) and therefore not directly comparable. Analysis was conducted using R 4.3.2 using the vegan package. Total species richness was estimated using two methods: Chao1 (iChao1) (Chiu et al. 2014) and abundance-based coverage (ACE) with k = 10 (Chao & Chiu 2016). The number of shared species among sample sites was not estimated because of different sampling methods employed, and because samples were collected at non-overlapping times. Estimates were calculated in R 4.3.2 using the SpadeR package.

RESULTS

In total, 738 stygobionts were collected, with a range from 0–7 taxa per sample (Figure 2, Tables 2 & 3). Also observed were calanoid copepods and *Hyalella* (amphipoda), both of which are epigean species. The other copepod groups may also include epigean and stygobitic species. Samples with no organisms collected were retained to calculate summary statistics for each well sample. Spurgeon and Wilson Wells had greater species richness and abundance than Burkett Well, though this might be an artifact of different sampling methods.

Significant precipitation (>2.54 cm) was observed at the regional weather station in April, May, August, and October of 2021 (Figure 3). We observed snails in all months, but more juvenile individuals were observed March–June and then again in October. Among the seven dissected *B. uvaldensis* individuals, reproductive organs were observed in four individuals (shell widths 0.81–1.22 mm) but not in the remaining three individuals (shell widths 0.54–0.65 mm).

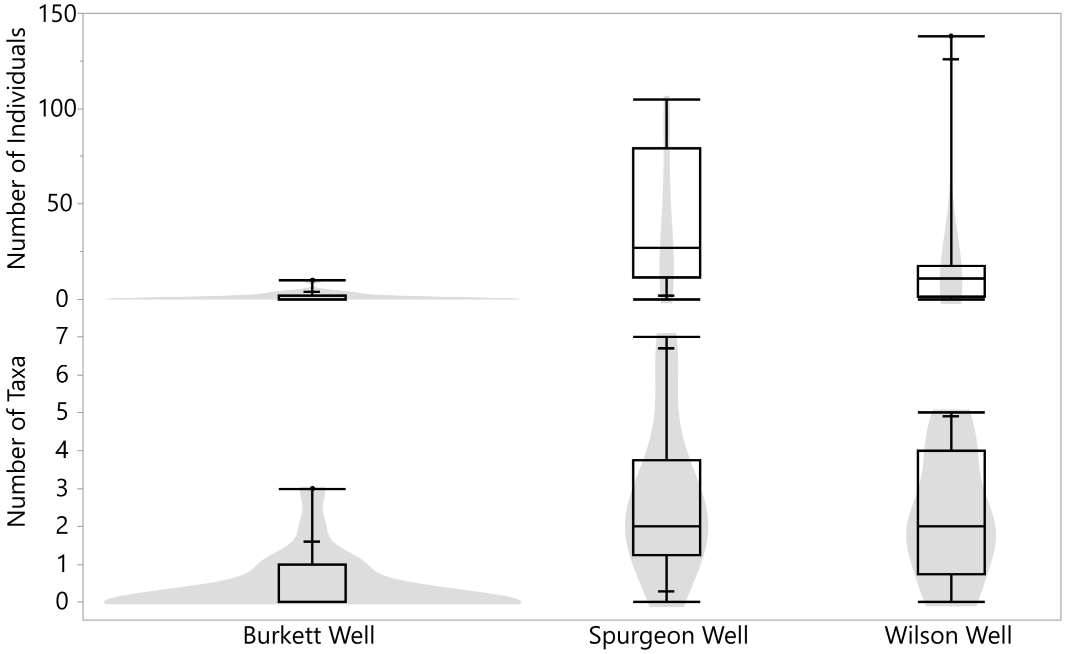

Figure 2. Number of individuals and species from all sampling sites collected during this study. Burkett Well, n=23, Spurgeon Well, n=15, Wilson Well, n=9.

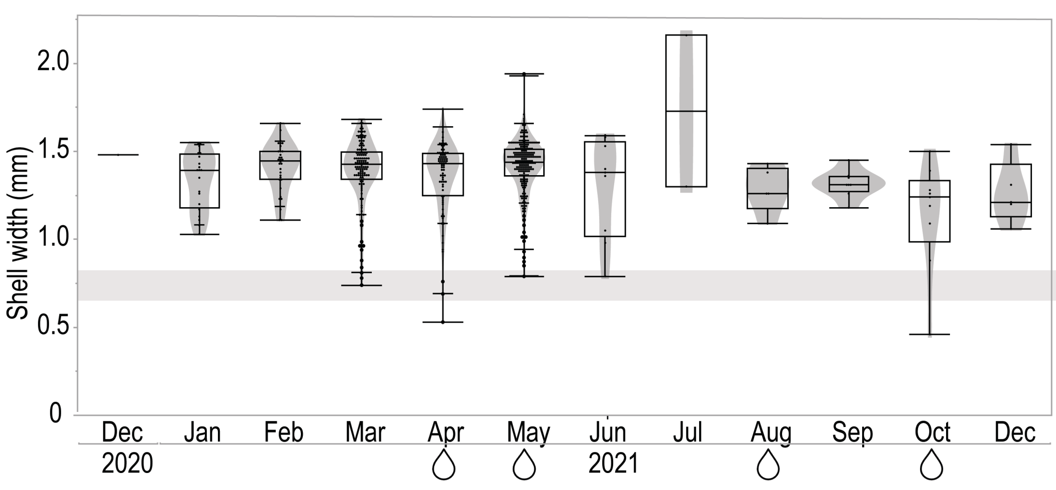

Figure 3. Width (mm) of *Balconorbis uvaldensis* individuals (n=500) with samples combined into monthly intervals. The outlines are quantiles. Significant precipitation (>2.54 cm) was observed at the nearby weather station in April, May, August, and October of 2021, indicated by the droplet outlines on the figure. A bar is drawn indicating the approximate size when reproductive organs can be observed.

Table 2. Number of samples per site and mean number of species and individuals (standard deviation in parentheses) per sample collected during the study.

Well Site	Number of samples	Mean Number of Species	Mean Number of Individuals
Burkett	23	0.39 (0.78)	1.09 (2.35)
Spurgeon	15	2.75 (2.05)	41.25 (38.40)
Wilson	9	2.2 (1.69)	21.8 (41.35)

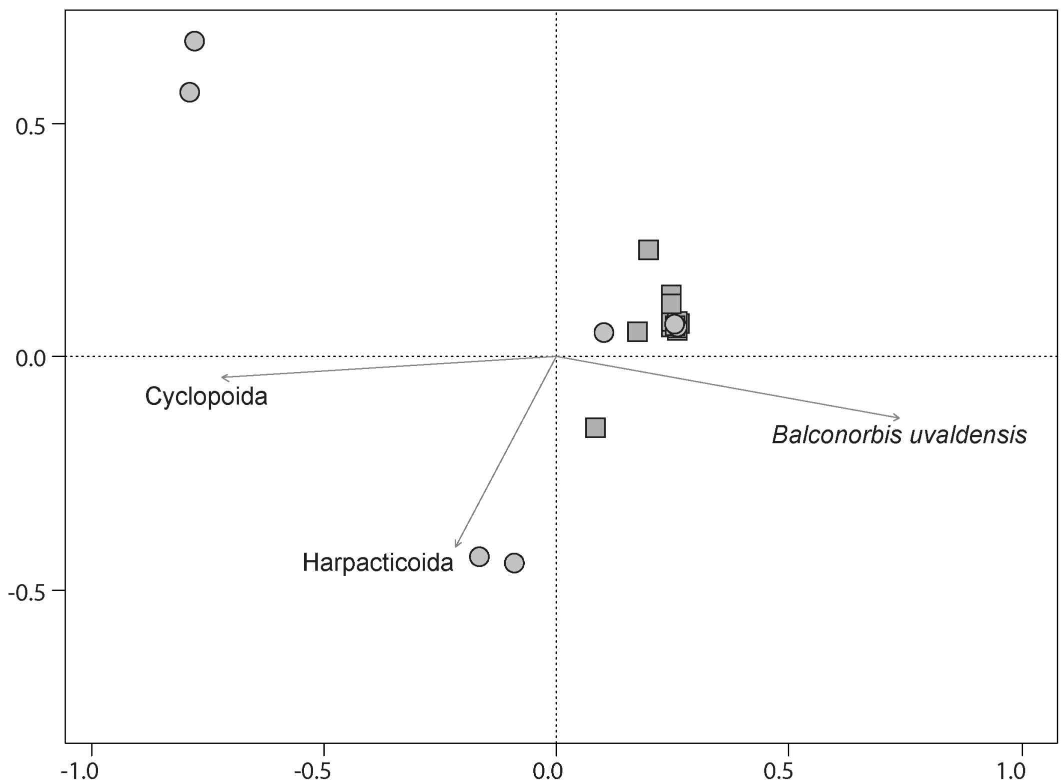

Figure 4. PCA comparing community composition among Wilson (circles) and Spurgeon Wells (squares). PC axis 1 comprised 68% of the variance in community composition and PC axis 2 comprised 12%.

The first two axes of a PCA of Hellinger-transformed species data from Wilson and Spurgeon Wells explained 68% and 12% of variance in community composition, respectively (Figure 4). Axis 1 primarily described gradients in the numbers of cyclopoid copepods and the snail

Table 3. Number of individuals of each species collected from the three well sampling sites (SP = Spurgeon, WI = Wilson, BU = Burkett). [1] = Texas species of greatest conservation need (identified to species level) or potential species of greatest conservation need (identified to genus level). P = taxa represented in historic samples but not collected during this study.

Phylum	Class	Family	Identification	SP	WI	BU
Mollusca	Gastropoda	Cochliopidae	*Balconorbis uvaldensis*	451	172	19
Crustacea	Malacostraca	Artesiidae	*Artesia subterranea*[1]	P	2	1
		Bathynellacea	*Montanabathynella* sp.	0	1	0
		Cirolanidae	*Cirolanides* sp.	0	2	0
		Crangonyctidae	*Stygobromus hadenoecus*[1]	5	0	1
			Stygobromus sp.[1]	1	1	0
		Hadziidae	*Mexiweckelia hardeni*[1]	P	0	0
			Texiweckelia sp.[1]	0	1	0
		Ingolfiellidae	*Ingolfiella* sp.[1]	2	0	0
		Microcerberidae	*Texicerberus* sp.	1	0	0
		Monodellidae	*Tethysbaena texana*	1	2	0
		Parabogidiellidae	*Parabogidiella cf. americana*	1	0	0
			Parabogidiella sp.	1	1	0
		Seborgiidae	*Seborgia* cf *relicta*[1]	4	0	0
		Stenasellidae	*Mexistenasellus coahuila*	P	0	0
	Hexanauplia	Cyclopoida	Undetermined	8	27	0
		Harpaticoida	Undetermined	2	3	1
			# of individuals	495	218	25
			# of species	14	10	4

Table 4. Observed and estimated species richness (Chao1 and ACE with 95% confidence intervals in parentheses) for the three sampled wells. Only samples with one or more species present were used in analysis. Singletons are a species detected in only one sample.

Well	Samples	# Observed Taxa	# Singletons	Chao1	ACE
Burkett Well	6	5	4	10 (6-38)	20 (7 - 124)
Spurgeon Well	11	12	6	33 (16-132)	21 (14-52)
Wilson Well	8	10	5	17 (11-47)	16 (11-42)

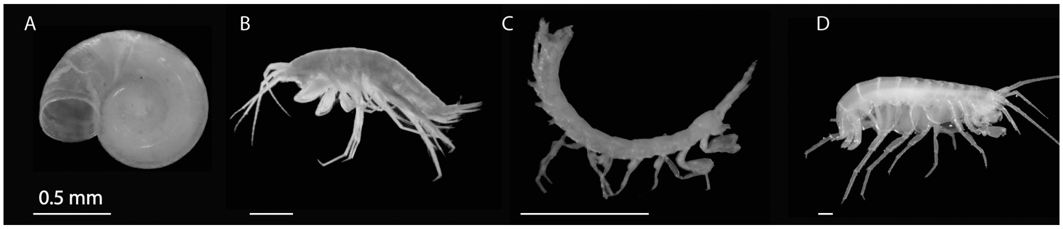

Figure 5. Selected invertebrates observed in samples from Uvalde National Fish Hatchery, Uvalde County, TX. A. *Balconorbis uvaldensis*; B. *Artesia subterranea*; C. *Ingolfiella* sp.; D. *Stygobromus* cf. *hadenoecus*. Scale bars = 0.5 mm. The scale bar is approximate for the crustaceans based on the sizes reported in the species descriptions or other members of the genus. Photo credits: A: Kathryn Perez, B–D: J. Randy Gibson.

Balconorbis uvaldensis. Collectively, these two species made up 83% of all individuals collected across both sites. The second axis primarily described a gradient in the numbers of harpacticoid copepods. PCA did not reveal an obvious separation between the two wells, in terms of community composition, though Wilson Well samples exhibited more compositional variability and generally had more copepods and fewer *B. uvaldensis*, with the exception of one sample, collected on 25 May 2021. This was the first sample collected from the Wilson Well, and it had the most *B. uvaldensis* of any samples collected during the project.

Species richness estimators suggest that additional, undiscovered taxa are present at all three wells sampled (Table 4). Uncertainty around the species richness estimates is large, in part due to the large numbers of singletons (species observed in only one sample), but also because of limited sampling. Species richness estimates ranged from 10 (Chao1) at Burkett Well to 33 (Chao1) at Spurgeon Well.

Discussion

We attempted to sample three well sites of different hydrogeologic subdivisions, groups, and formations of the Edwards Aquifer continuously for a year. Stygobiont sampling was most extensive from

Burkett Well, followed by Spurgeon Well, then Wilson Well. Except for *Balconorbis uvaldensis* at Spurgeon Well, all of the occurrences in Table 2 represent new site records. Documentation of additional occurrence records for these taxa is important, as several have been identified by Texas Parks and Wildlife Department as species of greatest conservation need (Table 2). These wells varied in diversity and abundance of invertebrates sampled, with Spurgeon Well having the highest species richness and abundance, followed by Wilson Well, and with Burkett Well having the fewest species and individuals. However, the limitations on sampling Burkett Well due to the small opening size, the obstruction within the well, the variable water table depths that varied, and the limited volume of water moving through the PVC or bottle trap when compared to the drift net method, precludes diversity and abundance comparisons between the sites. It is possible that the region of the aquifer that Burkett Well draws from may have yielded a greater diversity and abundance of invertebrates than observed in this study if the well had been pumped and sampled via the drift net method used for the other wells. Nonetheless, invertebrates found in Burkett Well still allow us to examine rare and unique species found at this site (Table 3; Figure 4). Although our original goal was to sample continuously from Spurgeon Well, the well failure provided an opportunity to sample from Wilson Well resulting in different sample sizes taken from the active wells. These limitations on sampling precludes diversity and abundance comparisons between the sites. We can, however, collate data from across sites to examine diversity in the region (Table 3; Figure 5).

The most abundant species encountered from all wells was *Balconorbis uvaldensis*, the Balcones ghostsnail. *Cirolanides texensis* was also abundant and present at all three wells. *Hyalella* sp. was also found in each well sample but is epigean and most likely entering samplers from the surface. A faunal listing from these samples provides an initial database for understanding species diversity in this section of the Edwards Aquifer, but it is no doubt incomplete. For example, new species are still being encountered even with >100 years of continuous sampling (Hutchins et al. 2021) at an Edwards well in San Marcos, TX (San Marcos Artesian Well). Multiple taxa were rarely encountered at UNFH, including *Artesia subterranea*, *Stygobromus* cf. *hadenoecus*, *Texiweckelia* sp., *Ingolfiella* sp., *Texicerberus* sp., *Seborgia* cf. *relicta*, and *Parabogidiella* sp. The presence of singletons and doubletons (taxa only recovered from one or two samples, respectively), strongly suggests the presence of additional, undetected taxa at the well sites. Several of the crustaceans encountered (*Ingolfiella* and *Texicerberus*) were recognizably members of a known genus but had features distinctive from currently described species and could not be assigned to a nominal species. While some species are found in Spurgeon/Wilson Wells but not Burkett Well and vice versa, this sampling is too limited to draw conclusions about species absence from a particular site. The hydrologic connectivity between Spurgeon and Wilson Wells (Snyder 2004) may explain why both share similar species, however, it is possible that species diversity may differ between them due barriers to dispersal from differences in lithologic and hydrologic properties found in the different groups, formations, or members of the wells. Spurgeon Well derives water primarily from the Austin Chalk formation (Groschen 1996) while Wilson Well derives water from the from the Edwards Aquifer (Salmon Peak Formation) (Snyder 2004).

Previous studies on stygobiotic snail reproductive periodicity have observed continuous reproductive effort inferred from the presence of reproductively mature females (Bichuette & Trajano 2003). In this study we determined the approximate size of reproductively mature individuals (male or female) and used that to delimit juvenile from adult individuals. We consider this a rough estimate, as relatively few individuals from a single region were examined and there could be variability in size at reproductive maturity among individuals and/or among populations. We found two time periods when snails in the juvenile size range were observed, March–June and October. In samples from July–September, we encountered relatively few individuals, perhaps not sampling juveniles due to low overall sample sizes.

One of our hypothesized cues for reproduction in a subterranean environment was precipitation-driven nutrient availability (Hawes 1939). During our sampling year, precipitation patterns followed the usual pattern for the region with a peak of precipitation in late spring and again in early Fall (Welder & Reeves 1962). We found juveniles in months corresponding to nearby rainfall events, though we also collected juveniles in the month before a significant precipitation event. Local rainfall may not correspond to rapid changes in nutrient resources at our sample sites. Hutchins et al. (2016) showed that portions of the confined zone of the Edwards Aquifer were supported more by *in-situ* chemolithoautotrophic production by microbes than by allochthonous organic matter from the surface, though organic matter sources have not been assessed in the UNFH area. Local rainfall may also not correspond to rapid changes in hydraulic conditions in the aquifer, as it was gauged at a weather station seven kilometers from our site and about ten kilometers from the aquifer recharge zone. We observed signs of reproduction in *Balconorbis uvaldensis* in late spring and early fall, coincident with regional rainfall patterns, however, we do not feel this is conclusive. There are number of potential mechanisms by which changes in hydraulic conditions, driven by rainfall, might influence community structure and age-class distribution (Hutchins et al. 2016; Hutchins et al. 2021), but additional sampling and better knowledge of resource availability would be needed to confirm whether the two periods of juvenile presence are a consistent annual pattern or determined by resource availability or other cues.

Given the current, limited sampling, two wells, Spurgeon and Wilson, both have more than ten documented species—an important milestone considering that only 13 groundwater sites in Texas have ten or more documented species (Hutchins et al., 2021). But species richness estimators (Chao1 & ACE; Table 4) suggest that fewer than half of the species occurring at sampled wells were collected in the current sampling effort. Although uncertainty around estimates is high because of small sample size and a high number of singletons, they suggest that at least Spurgeon Well may be a globally notable site of groundwater diversity, with 20 or more groundwater-obligate species (Culver et al. 2021). Obviously, more sampling is needed, across variable hydrologic conditions (Hutchins et al. 2021), to better characterize the UNFH groundwater community.

Acknowledgments

We thank the Uvalde National Fish Hatchery staff for site access and assistance with sampling. We also thank Benjamin Schwartz and the Edwards Aquifer Research & Data Center at Texas State University for material support. We thank UTRGV School of Integrative Biological and Chemical Sciences and the College of Sciences. Initial sampling was supported by Texas Parks and Wildlife Department (U.S. Fish and Wildlife Service, TPWD Interagency Cooperation Contract #532109). We thank UTRGV Biology students for their assistance with sample processing: Jonathan Dominguez, Brittany Lazo, Aaron Olivarez, Elisa Saenz, Natalia Salazar-Lozano, Mariana Gonzalez-Tiscareño, Ulyses Yakovlevich. The views presented herein are those of the authors and do not necessarily reflect those of the U.S. Fish and Wildlife Service.

LITERATURE CITED

Alqaragholi, S. A., W. Kanoua & P. Göbel. 2021. Comparative investigation of aquatic invertebrates in springs in Münsterland area (Western Germany). Water 13:359.

Alvear, D., P. H. Diaz, J. R. Gibson, B. Hutchins, B. F. Schwartz & K. E. Perez. 2020a. Expanding the known ranges of the endemic, phreatic snails (Mollusca, Gastropoda) of Texas, USA. Freshw. Moll. Biol. Conserv. 23:1–17.

Alvear, D. A., P. H. Diaz, R. Gibson & K. E. Perez. 2020b. An unusually sculptured new species of *Phreatodrobia* Hershler & Longley (Mollusca; Caenogastropoda; Cochliopidae) from central Texas. Zootaxa 4810:143–152.

Barr, C. B., J. R. Gibson & P. H. Diaz. 2015. *Typhloelmis* Barr (Coleoptera: Elmidae: Elminae), a new stygobiontic riffle beetle genus with three new species from Texas, USA. Coleopterist Bull. 69:531–558.

Bichuette, M. E. & E. Trajano. 2003. A population study of epigean and subterranean *Potamolithus* snails from southeast Brazil (Mollusca: Gastropoda: Hydrobiidae). Hydrobiologia 505:107–117.

Borcard, D., F. Gillet & P. Legendre. 2011. Numerical Ecology with R. Vol. 2, Springer, New York, 435 pp.

Carpenter, J. H. 2021. Forty-year natural history study of *Bahalana geracei* Carpenter, 1981, an anchialine cave-dwelling isopod (Crustacea, Isopoda, Cirolanidae) from San Salvador Island, Bahamas: reproduction, growth, longevity, and population structure. Subterr. Biol. 37:105–156.

Chao, A. & C.-H. Chiu. 2016. Species Richness: Estimation and Comparison. In: Wiley StatsRef: Statistics Reference Online. https://doi.org/10.1002/9781118445112.stat03432.pub2

Chiu, C., Y. Wang, B. Walther & A. Chao. 2014. An improved nonparametric lower bound of species richness via a modified good-turing frequency formula. Biometrics 70:671–682.

Clark, A. K. 2003. Geologic framework and hydrogeologic characterisitics of the Edwards Aquifer, Uvalde County, Texas. U.S. Geological Survey, Water-Resources Investigations Report 03-4010, Austin, TX, iii+17 pp.

Clark, A. K. & T. A. Small. 1997. Geologic framework of the Edwards Aquifer and upper confining unit, and hydrogeologic characterisitics of the Edwards Aquifer, south-central Uvalde County, Texas. U.S. Geological Survey, Water-Resources Investigations Report 97-4094, Austin, TX, iii+11 pp.

Culver, D. C., L. Deharveng, T. Pipan & A. Bedos. 2021. An overview of subterranean biodiversity hotspots. Diversity 13:487. https://doi.org/10.3390/d13100487

Culver, D. C. & T. Pipan. 2019. The Biology of Caves and Other Subterranean Habitats. Oxford University Press, 336 pp.

Edwards Aquifer Authority. 2019. San Marcos National Fish Hatchery and Technology Center, Uvalde National Fish Hatchery, and Inks Dam National Fish Hatchery-Refugia Edwards Aquifer habitat conservation plan year 1 workplan. https://www.edwardsaquifer.org/wp-content/uploads/2019/02/04-19-12RefugiaWP.pdf

Esquilin, R., B. C. Lillibridge, D. M. Mahula, J. Mireles, J. R. Hoyt & G. Schindel. 2001. Edwards Aquifer hydrogeologic report for 2000, San Antonio, TX, v+102 pp.

Groschen, G. E. 1996. Hydrogeologic factors that affect the flowpath of water in selected zones of the Edwards Aquifer, San Antonio region, Texas, U.S. Geological Survey, Water-Resources Investigations Report 96-4046, Austin, TX, vi+73 pp.

Hawes, R. S. 1939. The flood factor in the ecology of caves. J. Anim. Ecol. 8:1–5.

Hutchins, B. T., A. S. Engel, W. H. Nowlin & B. F. Schwartz. 2016. Chemolithoautotrophy supports macroinvertebrate food webs and affects diversity and stability in groundwater communities. Ecol. 97:1530–1542.

Hutchins, B. T., J. R. Gibson, P. H. Diaz & B. F. Schwartz. 2021. Stygobiont diversity in the San Marcos artesian well and Edwards Aquifer groundwater ecosystem, Texas, USA. Diversity 13:234. https://doi.org/10.3390/d13060234

Longley, G. 1981. The Edwards Aquifer: Earth's most diverse groundwater ecosystem? Int. J. Speleol. 11:123–128.

Maclay, R. W. 1995. Geology and hydrology of the Edwards Aquifer in the San Antonio area, Texas. U.S. Geological Survey, Water-Resources Investigations Report 95-4186, Austin, TX, v+64 pp.

Mammola, S., P. Cardoso, D. C. Culver, L. Deharveng, R. L. Ferreira, C. Fišer, D. M. P. Galassi, C. Griebler, S. Halse, W. F. Humphreys, M. Isaia, F. Malard, A. Martinez, O. T. Moldovan, M. L. Niemiller, M. Pavlek, A. S. P. S. Reboleira, M. Souza-Silva, E. C. Teeling, J. J. Wynne & M. Zagmajster. 2019. Scientists' warning on the conservation of subterranean ecosystems. Biosci. 69:641–650.

Mammola, S., E. Lunghi, H. Bilandžija, P. Cardoso, V. Grimm, S. I. Schmidt, T. Hesselberg & A. Martínez. 2021. Collecting eco-evolutionary data in the dark: impediments to subterranean research and how to overcome them. Ecol. Evol. 11:5911–5926.

Moore, L. & E. Kalish. 2015. Uvalde National Fish Hatchery- Uvalde TX. Living New Deal. Department of Geography, University of California, Berkeley. https://livingnewdeal.org/sites/uvalde-national-fish-hatchery-uvalde-tx/ [accessed 10/3/2023.2023].

Nissen, B. D., T. J. Devitt, N. F. Bendik, A. G. Gluesenkamp & J. R. Gibson. 2018. New occurrence records for stygobiontic invertebrates from the Edwards and Trinity aquifers in west-central Texas, USA. Subterr. Biol. 28:1–13.

Perez, K. E., N. Noreika, C. Norris, M. Kelly, M. Lopez, C. Ortega, S. Ruiz Sandoval, S. Gonzalez & W. Nowlin. 2022. Population density and reproductive seasonality of *Tryonia cheatumi* (Gastropoda: Cochliopidae), the Phantom Tryonia. Freshw. Moll. Biol. Conserv. 25:82–90.

Secutti, S. & E. Trajano. 2021. Reproduction, development, asymmetry and late eye regression in the Brazilian cave catfish *Ituglanis passensis* (Siluriformes, Trichomycteridae): evidence contributing to the neutral mutation theory. Subterr. Biol. 38:91–112.

Snyder, G. L. 2004. Well Testing Report. Uvalde National Fish Hatchery "Burkett" and "Spurgeon" production wells Uvalde County, Texas. URS Corporation, 16 pp.

Stejneger, L. 1896. Description of a new genus and species of blind tailed batrachians from the subterranean waters of Texas. Proc. U.S. Natl. Mus. 18: 619–621.

Strenth, N. & G. Longley. 1990. Reproductive patterns of the subterranean shrimp *Palaemonetes antrorum* Benedict (Decapoda, Palaemonidae) from Central Texas. Stygologia 5:221–224.

Texas Department of License and Regulation. 2000. Well Report, Wilson Well. Texas Water Development Board, Austin, TX, 2 pp.

Texas Water Development Board. 1958. Well Report, Burkett Well, YP 69 50.402, 4 pp.

Texas Water Development Board. 1966–2004. Well Report, Spurgeon Well. YP 69 50.501. Texas Water Development Board, Austin, TX, 31 pp.

Texas Water Development Board. 2021. Explanatory report for the proposed desired future conditions of the Leona Gravel Aquifer groundwater management Area 10. Texas Water Development Board, Austin, TX, 59 pp.

Verhaegen, G., K. von Jungmeister & M. Haase. 2021. Life history variation in space and time: environmental and seasonal responses of a parthenogenetic invasive freshwater snail in northern Germany. Hydrobiologia 848:2153–2168.

Wayne, N. L. 2001. Regulation of seasonal reproduction in mollusks. J. Biol. Rhythms 16:391–402.

Weck, R. G. 2022. Life history observations of the Illinois state endangered Enigmatic Cavesnail, *Fontigens antroecetes* (Hubricht, 1940) made under simulated cave conditions. Subterr. Biol. 43:185–198.

Welder, F. A. & R. D. Reeves. 1962. Geology and ground-water resources of Uvalde County, Texas. Texas Water Commission Bulletin 6212, Austin, TX, vi+246 pp.

Wells, J. 2000. National fish hatcheries: authority needed to better align operations with priorities. U.S. General Accounting Office, GAO/RCED-00-151, Washington, D.C., 23 pp.