SPATIAL GRADIENTS AND FOOD WEB STRUCTURE OF GROUNDWATER COMMUNITIES

by

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DEDICATION

This thesis is dedicated to my fiancé, Jessica, who has been supportive of me every step of the way in my graduate school journey. Her advice, understanding, love, and encouragement have made this thesis possible.

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LIST OF ABBREVIATIONS

Abbreviation	Description
BSEA	Barton Springs Segment, Edwards Aquifer
CLA	Chemolithoautotrophy
DI	Deionized
DO	Dissolved Oxygen
EA	Edwards Aquifer
EtOH	Ethanol
FPOM	Fine Particulate Organic Matter
FWSWI	Freshwater Saline Water Interface
HCl	Hydrochloric Acid
HDPE	High Density Polyethylene
IRMS	Isotope Ratio Mass Spectrometer
L	Liter
MANOVA	Multivariate Analysis of Variance
MCMC	Markov Chain Monte Carlo
NSEA	Northern Segment, Edwards Aquifer
OM	Organic Matter
PC	Principal Component
PCA	Principal Component Analysis
POM	Particulate Organic Matter

RDA	Redundancy Analysis
SAEA	San Antonio Segment, Edwards Aquifer
SRP	Soluble Reactive Phosphorus
TDS	Total Dissolved Solids
TEF	Trophic Enrichment Factor
TN	Total Nitrogen
TP	Total Phosphorus

ABSTRACT

The Edwards Aquifer (EA) and its springs in Texas are some of the most biologically unique and diverse subterranean-surface coupled systems on the planet, containing many endemic and endangered crenic and hypogean species. However, relatively little is known about the spatial patterns of diversity and trophic ecology of groundwater dependent species in the northern (Barton Springs) segment of the EA. Microbial chemolithoautotrophy (MCLA) is an important source of organic matter (OM) for some aquatic organisms in the southern EA, but the importance of MCLA is unknown in the northern EA. We examined spatial patterns of geochemistry, stable isotope values of particulate OM, and stygofaunal community structure across the northern EA. We also examined the diet of the endangered crenic Barton Springs salamander (*Eurycea sosorum*) and subterranean Austin Blind salamander (*Eurycea waterlooensis*) at Eliza Springs (a major spring) using stable isotopes. We found spatial variation in geochemistry in the northern EA, but OM isotopic composition did not vary with geochemistry, suggesting that MCLA was not a major contributor to OM production. Dietary mixing models found that copepods were the most important diet item for both salamander species, with ~60% of both species' diets consisting of hypogean organisms. Isotopic niche analyses indicate substantial overlap between salamander species, which has conservation implications for these endangered species, as well as ecosystem functioning of the EA as a whole.

I. SPATIAL GRADIENTS AND FOOD WEB STRUCTURE OF GROUNDWATER COMMUNITIES

Introduction

The structure and function of aquatic food webs is often complex due to the variability of biophysical and hydrogeochemical processes (Polis and Winemiller, 1996; Peipoch et al. 2019; Venarsky et al. 2014), the prevalence of size-structured interactions (Hairston and Hairston, 1997), and the frequency of trophic cascades (Bartrons et al. 2018). In aquatic systems, feeding relationships and trophic interactions are indicative of and linked to critical ecological processes, including energy flow, community stability, and ecosystem functioning (Carrasco et al. 2019). Energy flows and dominant organic matter (OM) sources for freshwater communities influence feeding interactions and are often a function of hydrology (Vannotte et al. 1980; Covich et al. 1999); however, due to their down gradient positions they are often strongly linked to surrounding terrestrial catchments (Shurin et al. 2006) and some aquatic food webs are highly dependent upon inputs of terrestrial OM (Wallace et al.1997; Meyer et al. 2000). Most of our knowledge of the structure and function of aquatic food webs and the OM supporting communities has come from lake (Carpenter et al. 1988; Cole et al. 2000) and river ecosystems (Vannotte et al. 1980; Jepsen and Winemiller 2002), but comparatively little is known about the trophic ecology of groundwater communities (but see Hutchins et al. 2016; Alvear et al. 2020).

Groundwater ecosystems often exhibit consistent year-round geochemistry and low or highly variable resource availability (Hutchins et al. 2016). Organic matter inputs

to groundwater ecosystems are hypothesized to come as terrestrial vegetation inputs from the surface that are relativity low-quality and are spatio-temporally variable. Thus, groundwater communities are expected to be largely energy (resource) limited with food webs populated by generalist consumers (Culver and Pipan 2009). Due to limited surface connectivity, photosynthetically derived detritus is limited, making allochthonous organic matter (OM) an unreliable basal energy source for groundwater food webs (Vernarsky et al. 2014). However, there is a growing body of research indicating that some groundwater ecosystems produce autochthonous OM via chemolithoautotrophy (CLA) that greatly influence trophic complexity, food web interactions, and biological diversity of subterranean communities (Sarbu et al. 1996; Baron et al. 2002; Kumaresan et al. 2013; Hutchins et al. 2016). Autochthonous OM via CLA provides a more spatio-temporally consistent and higher quality OM base for groundwater organisms, thereby supporting diverse groundwater communities with greater trophic diversity than previously hypothesized (Hutchins et al. 2016).

The Edwards Aquifer (EA) is a regional karst aquifer in south-central Texas formed in Cretaceous carbonates of the Edwards Formation (Maclay and Small, 1983). The EA is partitioned into three regions along fault lines and hydrologic flow paths: the southwestern San Antonio segment (SAEA), the Barton Springs segment (BSEA), and the Northern segment (NSEA) (Figure 1). The EA is considered a global biodiversity hotspot for groundwater, and spring associated fauna (Gibson et al. 2008; Hutchins, 2016; Hutchins et al. 2021). Previous work in the SAEA determined that the primary OM sources utilized by stygobiont communities (surface-derived OM or subterranean microbial-derived OM) varied spatially across the aquifer in relation to proximity to a

freshwater – saline water interface (FWSWI) (Hutchins et al. 2016). The FWSWI is a region along the eastern boundary of the EA where freshwater and saline water (containing high dissolved solids and H_2S and low dissolved oxygen) mix (Engel and Randall, 2011). As a result of the steep geochemical gradients of freshwater and saline water, microbial CLA occurs and is thought to be important to groundwater ecosystems because it provides an additional more spatio-temporally consistent basal energy source which promotes increased trophic complexity and biological diversity (Hutchins et al. 2016). Consequently, the diversity of stygobionts (obligate subterranean aquatic organisms) varies at different locations across the SAEA, with the highest stygobiont diversity and trophic complexity observed at locations nearest to the FWSWI (Hutchins et al. 2014; Hutchins et al. 2016). Although we know a great deal of the role of CLA in the food webs in the SAEA, considerably less is known about the importance of CLA in other segments of the EA. Thus, there is a need to determine if CLA is an important driving factor in other EA segments diversity and OM use in this regionally important aquifer.

I examined the importance of CLA in stygobiont community composition and food web structure of the BSEA for this thesis. The phreatic zone, vadose zone, and spring openings of the BSEA contains >16 endemic species of stygobitic invertebrates as well as two endemic and endangered salamanders (the Barton Springs salamander, *Eurycea sosorum*, and the Austin blind salamander, *Eurycea waterlooensis*) (Krejca and Reddell 2019). The BSEA (Figure 1) covers an area of around 400 km^2 ranging from the city of Kyle, TX (Hays County) to the Colorado River in the city of Austin, TX (Travis County) and provides water for around 60,000 people in the region (Krejca and Reddell

2019). Like the SAEA, a FWSWI runs along the eastern boundary of the BSEA and are a variety of site types (springs, wells, and recharge creeks). There are 4 major springs (Eliza Springs, Main Barton Springs, Old Mill Springs, and Upper Barton Springs) at the northern end of the segment which have different proportions of saline water contribution to their flow paths (Hunt et al. 2019). Different saline water contributions to the terminal springs in the BSEA indicate flow paths differences among springs which may lead to variation in the relative importance of OM sources to stygobiont and spring orifice communities. Although the saline water contribution to all the major springs is limited, Old Mill Springs has the greatest contribution of the Saline-Line flow path (estimated 3% saline water contribution to the spring) of the four springs (Hunt et al. 2019). Upper Barton Springs is solely supplied by the Sunset Valley flow path with no saline water contribution (Hunt et al. 2019). Eliza Springs and Main Barton Springs are supplied by a mixture of all three primary flow paths (Manchaca, Sunset Valley, and Cold Springs flow routes) where saline contribution is dependent upon hydrologic flow rates (Eliza = 0.5% saline contribution; Main Barton 1% to 6% saline contribution; Hunt et al. 2019).

The Barton Springs salamander (*E. sosorum*) is a spring associated, perennibranchiate salamander that has been collected at four springs in the Barton Springs Complex (Eliza Springs, Main Barton Springs, Old Mill Springs, and Upper Barton Springs) and the Barton Springs pool (Chippindale et al. 1993). More recently, the Barton Springs salamander have also been collected at seven springs (Emerald Spring, Bello Spring, Pearly's Spring, Ben McCulloh Spring, Stuart Spring, Spillar Ranch Spring 2, and Backdoor Spring) in Onion Creek, Bear Creek, and Barton Creek (Devitt and Nissen 2017). The Barton Springs salamander exhibits dorsal blotching and mottling and

has a rounded snout (Chippindale et al. 1993). The Austin blind salamander (*E. waterlooensis*) is a stygobitic and groundwater-associated perennibranchiate salamander that is often found at three springs in the Barton Springs Complex (Eliza Springs, Main Barton Springs, and Old Mill Springs) and the Barton Springs pool (Hillis et al 2001). Although the Austin blind salamander is found in the vicinity of spring orifices in the Barton Springs complex, it is assumed to be adapted for a primarily subterranean existence because it lacks skin pigmentation and external eyes (Hillis et al. 2001).

The four specific research goals of this thesis were: (1) to determine spatial patterns of geochemistry and particulate OM origin (surface OM *versus* CLA-generated OM) at locations across the BSEA; (2) evaluate spatial patterns of stygofaunal assemblage composition across the BSEA; (3) investigate relationships between stygofaunal assemblage composition at sites across the BSEA and spatial patterns in geochemistry and OM origin in the BSEA; and (4) evaluate if CLA derived OM contributes to food webs at one of the major springs in the BSEA (Eliza Springs) by determining the food web structure and dietary composition of the two endemic salamanders (i.e., *Eurycea sosorum* and *Eurycea waterlooensis*) at this location. To investigate these research goals, I tested the following hypotheses:

- (H_1) There will be substantial geochemical gradients across the BSEA with CLA derived OM being present at sites proximate to the FWSWI [as indicated by the stable isotope values of particulate OM (POM)]
- (H_2) There will be substantial variation in stygofaunal assemblage structure among sites, with stygofaunal composition depending on site type (i.e., well, spring orifice, recharge creek) and distance from the FWSWI.

- (H_3) Because both *Eurycea* species occur at the same spring orifice habitat, they will likely derive a majority of their diets from groundwater or spring orifice obligate organisms and will occupy a similar feeding niche.

Methods

The BSEA and study sites

There are five main sub-basins in the Barton Springs watershed (Barton Creek, Williamson Creek, Slaughter Creek, Bear Creek, and Onion Creek) that provide water (and surficial OM inputs) to the aquifer when creeks are flowing (Figure 1 and Table 1). Sampling sites in the five creeks were located along the western boundary of the BSEA (recharge zone). I sampled 22 accessible wells that intersect the EA in various locations across the BSEA (i.e., deep-confined, shallow-confined, recharge, unconfined, and saline sites) at various distances from the FWSWI (Table 1). I also sampled four spring sites at the northeastern end of the BSEA in the Barton Springs complex (i.e., Eliza spring, Old Mill spring, Main Barton spring, and Upper Barton spring). Sites were sampled from October 2019 to May 2021.

Water sample collection and processing

Water sampling at wells consisted of measuring well volume by using a Solinst® water level meter (Model 101), a measurement of pipe diameter, and the total depth of each well. Prior to sample collection, double the well volume was purged using a purge pump and water samples were collected after pH, temperature (°C), conductivity (µS/cm), and dissolved oxygen (DO) stabilized (approximately 30 minutes to 5 hours,

depending on well volume and depth). Geochemical parameters were measured with a Eureka Manta 30+ water quality multiprobe sonde. Conductivity was converted to total dissolved solids (TDS) in mg/L (Rusydi, 2018), which is routinely used to characterize fresh versus saline water in the EA, by dividing values by 0.67 (salinity ratio; Stein et al. 2019). Bulk water samples were collected at each site in 1-L bottles that were acid-washed, triple rinsed with deionized (DI) water, and rinsed twice with sample water for aqueous geochemistry and stable isotope analyses in the joint Aquatic Ecology Lab (e.g., major ions, stable isotopes of hydrogen and oxygen in liquid water, and total and dissolved nutrients).

At the recharge creek sites, water and POM samples were collected using surface grab samples to fill a 20-L carboy which had been pre-rinsed with DI water and then rinsed with water from the site of the sample and transported back to Texas State University. In-stream geochemical data (i.e., pH, temperature, conductivity, and DO) were collected with a Eureka Manta 30+ water quality multiprobe sonde. During the study period, I was unable to collect geochemical data and water for POM samples from Slaughter and Williamson creeks because of no surface flow due to lack of rainfall. Water and POM samples from spring openings were collected by grab sampling at the spring orifice using a 20-liter car boy. Geochemistry at spring sites was measured by placing the Eureka Manta 30+ sonde into the spring orifice.

Water and POM samples from all sites were processed within 48 h of collection. Water was filtered through pre-ashed Pall A/E filters to measure cations [e.g., Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}), anions (e.g., F^-, Cl^-, and Br^-), and nutrients (e.g., ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), total nitrogen (TN), total

phosphorus (TP), and soluble reactive phosphorus (SRP)]. Filtered (dissolved nutrients) and unfiltered (total nutrients) water samples were placed in clean HDPE (High Density Polyethylene) bottles, acidified, and held at 4°C until analysis. Dissolved SO_4^{2-}, NH_4^+, and DO were measured in the field using a CHEMetrics® V-2000 multi-analyte photometer. Dissolved ion concentrations were analyzed using Dionex ICS-1600 ion chromatographs (Bannockburn, IL). Values for $\delta^{18}O$ and $\delta^{2}D$ in liquid water were measured on a Picarro Cavity Ring-Down Spectrometer L2130-I Analyzer with Vaporization Module A0211 (Santa Clara, CA). Alkalinity as total titratable bases dominated by bicarbonate was measured via end-point titration with 1.6 N sulfuric acid. NO_3^- and TN were quantified on a Varian Cary 50 UV-Vis spectrophotometer using second-derivative spectroscopy (Crumpton et al. 1992). TN was assessed as NO_3^- after digestion with alkaline potassium persulfate. Concentration of SRP was measured as phosphate using the ascorbic acid method (Wetzel and Likens, 2000). TP was determined with the ascorbic acid method after digestion with potassium persulfate and analysis with the molybdenum blue method (Wetzel and Likens, 2000). NH_4^+ was quantified using the sodium hypochlorite method (Wetzel and Likens, 2000).

Invertebrate and salamander collection

Each well site was visited at least once over the study period and invertebrates were collected at all wells except anoxic wells. Wells were sampled with bottle traps, modified from Fenolio et al. (2017), and 100 μm aperture phreatobiological nets (i.e., plankton nets modified for deployment in groundwater boreholes) put into the well borehole. Bottle traps were constructed of a plastic bottle to create an easy entry and

difficult exit for stygobionts. At each well, a single bottle trap was baited with materials representative of proteins, carbohydrates, and fats (e.g., a frankfurter, peanuts, raw potato, cheese, and a poly-cotton mophead material) and the trap was lowered down and collected after 48 – 72 h. Trap contents (i.e., stygobitic invertebrates) were preserved with 95% ethanol (EtOH). Traps were constructed to prevent organisms from directly accessing bait so organisms would not consume bait and potentially alter isotopic values of tissue. After trap retrieval, a phreatobiological net was lowered down well bores and pulled back up (Hancock and Boulton, 2009). A total of five tows of the plankton net were performed at each site and net contents were preserved with 95% EtOH.

Invertebrates (and salamanders) at spring openings (Eliza spring, Old Mill spring, Main Barton spring, and Upper Barton spring) were collected by placing 100-μm aperture drift nets over spring outflow points. Some nets had sample cups attached to the ends to reduce turbulence and provide refuge for salamanders and fragile invertebrates. Drift nets and pipe traps were deployed for 12-24 h. Staff from the City of Austin (COA) group checked nets every 12 h on most days to check for salamanders in the net. Upon net removal, samples were examined to remove and release live, juvenile salamanders. Adult salamanders were processed for tail clips in the field and released by the COA group. Adult salamanders were identified to species on site with the help of photos from the COA group.

Invertebrate samples from springs and wells (both surface and groundwater taxa) were sorted and identified using a Nikon dissection microscope to the lowest practical taxon using keys (e.g., Holsinger and Longley, 1980; Merritt et al. 2019) and various reference images of stygobites from Pete Diaz (United States Fish and Wildlife Service,

San Marcos Aquatic Resources Center, San Marcos, Texas). Chironomidae were identified to subfamily (Chironominae, Orthocladiinae, and Tanypodinae). Cyclopoid copepods were a relatively abundant organism found across multiple sites. To determine if cyclopoid copepods captured in nets at the spring sites were epigean or hypogean, I haphazardly examined 100 individuals from Eliza Springs to determine if they possessed an eye spot or not (lack of eye spot suggests hypogean origin; Hose et al. 2016). Examination indicated that a clear majority of individuals (n = 98 out of 100) did not have an eye spot.

Stable isotope analysis

Particulate OM for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis was collected by filtering water onto pre-weighed 47 mm diameter Whatman GF/F filters (nominal pore size = 0.7 µm). Filters were dried at 60°C for 48 h followed by the measurement of FPOM (Fine Particulate Organic Matter) mass using a microbalance. Filters were then bisected, and halves designated for $\delta^{13}C$ were placed in a hydrochloric acid (HCl) fumigation chamber for at least 8 hours to remove inorganic C. After fumigation, filters were dried at 60°C for 24 h.

Preserved, sorted, and identified invertebrate samples from each site were used to obtain $\delta^{13}C$ and $\delta^{15}N$ values of invertebrate communities. The number of taxa and individuals analyzed for each taxon at a site was determined by rarity and sample mass needed for isotopic analysis. When feasible, single individuals were used for analysis, but small taxa (e.g., copepods, isopods, and some amphipods) used for isotopes were a composite of n = 2 to as many as 4,050 individuals. Salamander tail clips were also used

for $\delta^{13}C$ and $\delta^{15}N$ analysis. Animal samples were dried at 60°C for 48 h and ground to a fine powder using a clean mortar and pestle. The $\delta^{13}C$ and $\delta^{15}N$ values were measured at the University of California Davis (UCD) Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). PeeDee Belemnite (PDB) was used for the $\delta^{13}C$ standard and atmospheric N_2 for the $\delta^{15}N$ standard.

Analysis of environmental gradients and stygobiont community composition

I assessed spatial patterns in POM isotopic composition, nutrient concentrations, and geochemistry among locations in the BSEA using principal component analysis (PCA). The initial data set contained 21 nutrient and geochemical variables from four spring sites, 19 wells, and three recharge creeks (Figure 2A, Table 3). To reduce multicollinearity, a Pearson correlation matrix was used to exclude correlated variables ($|r| \geq 0.70$) prior to analysis. Data were *z*-score transformed prior to analyses. The final PCA data set included 16 water quality, ionic, and stable isotope variables for water but not POM (water temperature, pH, DO, conductivity, alkalinity, DO, F^-, K^+, Mg^{2+}, Ca^{2+}, δ^2D of water, $\delta^{18}O$ of water, TP, NO_3^-, and NH_4^+) and a dummy variable that categorized site types (i.e., Spring, Low DO Well, High DO Well, and Recharge Creek). Wells were classified as “Low DO Wells” (field DO concentrations <1.9 mg/L) or “High DO Wells” (field DO concentrations >3.6 mg/L). We then ran a second PCA which included the sites which had both environmental data and the POM stable isotope values (n = 16 sites had both data sets) to examine how POM isotopic values ($\delta^{13}C$ and $\delta^{15}N$; which may be

reflective of OM sources to groundwater fauna; Hutchins et al. 2016) varied with nutrients and geochemistry. All PCAs were run in SPSS, Version 27.0.

Redundancy analysis (RDA) was used to examine stygobiont community composition relative to spatial patterns in nutrients and geochemistry across the BSEA (Tables 2 and 3). Data were Hellinger transformed (Legendre and Gallagher, 2001) and taxa were chosen based on presence at sites where environmental data were also collected (Table 1). Initially, there were a large number of environmental variables relative to the number of sites, so I reduced the number of environmental variables to avoid variable-to-sample ratios that would hinder the analysis (Borcard et al. 2018). A Pearson correlation matrix was used to exclude variables that were highly correlated ($|r| > 0.70$). I was able to collect invertebrates from 19 sites, and the final data set contained 19 taxa and 10 environmental predictors (water temperature, pH, alkalinity, Ca^{2+}, NO_3^-, NH_4^+, SO_4^{2-}, TN, TP, and SRP) for the RDA. Significance of the overall RDA model was determined with a permutation test ($n = 999$, $\alpha = 0.05$) and axes were corrected using the R^2_{adj} (Legendre and Legendre, 2012). The RDA was completed using R version 4.1.1 (R Core Team 2021) with the R package 'vegan' (Oksanen et al. 2020).

Dietary analysis of Eliza Springs salamanders

I examined $\delta^{13}C$ and $\delta^{15}N$ values of invertebrates and salamanders at Eliza Springs, Old Mill Springs, Main Barton Springs, and Upper Barton Springs to qualitatively examine how food web structure varies among spring sites. However, I examined the most probable food sources for the Barton Springs and Austin blind salamanders at Eliza Springs via stable isotope dietary mixing models (Parnell et al.

2010). Dietary mixing models estimated the proportional contributions (using posterior probability estimates) of prey items for both salamanders using a Bayesian approach. Salamander diets were only modeled at Eliza Springs because it was the only site with an adequate number of salamander samples that could be identified to species.

Prior to conducting dietary models, I conducted an *a priori* aggregation for $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources to reduce the number of dietary sources using multivariate analysis of variance (MANOVA; Flaherty and Ben-David 2010). Reducing and combining potential dietary sources were performed to avoid an underdetermined mixing model (Phillips and Gregg 2003; Phillips et al. 2005). *A priori* comparison of isotopic values and subsequent combining identified five potential dietary items that were used in mixing models for both salamander species at Eliza Springs: (1) chironomids + psephenids, (2) cyclopoid copepods, (3) *Dugesia* sp. + *Lirceolus* spp., (4) *Hyalella azteca*, and (5) *Stygobromus* sp. + *Stygobromus flagellatus* (hereafter referred to as "*Stygobromus*" in mixing models). Dietary mixing models used uninformed priors and contained a MCMC (Markov Chain Monte Carlo) chain length of 300,000, a burn-in of 200,000, thinned by 100, and the MCMC contained 3 chains. Model performance was assessed using the Gelman-Rubin diagnostic and burn-in was determined to be a sufficient size using the Geweke diagnostic. I used trophic enrichment factors (TEFs) for freshwater organisms from Caut et al. (2009): $1.33‰ \pm 0.454$ for ^{13}C and $2.75‰ \pm 1.637$ for ^{15}N. The Bayesian biotracer mixing model was completed using R version 4.1.1 (R Core Team, 2021) package 'MixSIAR' (Stock et al. 2018).

I further examined resource partitioning and niche overlap between organisms at Eliza Springs using a dietary niche model with bulk stable isotope values ($\delta^{13}C$ and $\delta^{15}N$)

in a Bayesian isotopic niche model (Swanson et al. 2015). This approach estimates isotopic niche regions, percent niche overlap, and niche region sizes for the taxa in question. I completed two niche model analyses: one on the niche area overlap (overlap probability) of the two salamander species and the second on the niche area overlap of eight invertebrate taxa (e.g., annelids, cyclopoid copepods, *Dugesia* sp., *Hyallela azteca*, *Lirceolus hardeni*, *Lirceolus* sp., *Phreatodrobia nugax*, and *Stygobromus* spp.). Niche overlap probabilities were calculated using MCMC draws (n = 10,000) and a present mean probability (±95% credible interval) of species "A" occurring in the niche space of species "B", and vice-versa. The niche model was completed using R version 4.1.1 (R Core Team 2021) package 'nicheROVER' (Swanson et al. 2015).

Results

Geochemical, nutrient, and POM stable isotope variation in the BSEA

The PCA using nutrient and geochemical variables indicated several spatial patterns across the BSEA, particularly in relation to site type (Figure 2A). Principal components (PC) 1 and 2 cumulatively explained 51.79% of the variation among sites. The first PC axis (30.12 % of variation explained) generally described a gradient of sites with higher DO, Mg^{2+}, Ca^{2+}, and conductivity (categorized as High DO wells and Spring sites) to well sites with lower DO and higher conductivity and NH_4^+ (categorized as Low DO wells). The second PC axis generally described a gradient from recharge creeks with lower pH and more enriched δ^2D values to a combination of Low and High DO Well sites with higher Mg^{2+}, Ca^{2+}, and alkalinity.

The PCA examining environmental conditions and POM isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) revealed similar environmental gradients among sites; particularly the differences among High DO Wells, Low DO Wells, and Recharge creeks. However, the loadings for POM $\delta^{13}C$ and $\delta^{15}N$ on the first two principal components did not show much, if any, explanatory power (explain < 0.5% of variation) and are thus not presented in Figure 2B. POM $\delta^{15}N$ was more enriched in the recharge creek sites (Recharge creek mean ± SD = 7.55 ± 2.01 *vs.* wells and spring mean ± 1 SD = 3.74 ± 3.08). However, POM $\delta^{13}C$ values showed little variation among site types in the BSEA and had a comparatively small loading on the PCA, indicating that $\delta^{13}C$ of POM did not vary substantially across environmental gradients in the BSEA. Consequently, these results contrast with what was found in the SAEA where it was possible to identify potential OM sources for stygobionts using POM isotope ratios (Hutchins et al., 2016).

Spatial variation in community composition in stygobiont community structure in the BSEA

The RDA examining variation in stygobiont communities in response to nutrients and geochemistry (Figure 3) indicated several spatial patterns across the BSEA, particularly in the relationship between a smaller set of taxa at specific sites in the BSEA. The first two RDA axes accounted for 50.20% of variance (R^2_{adj} = 0.29, p = 0.05). RDA 1 generally described a gradient in which all spring sites and a wide distribution of well sites across the BSEA were characterized by higher alkalinity and Ca^{2+} while a group of wells near the western recharge zone were characterized by higher water temperatures, and greater TN, TP, and NO_3^- concentrations (Figure 3A). Cyclopoid copepods were

associated with the higher alkalinity of springs and wells, but mites were associated with wells close to the recharge zone (Figure 3B). RDA 2 described a gradient in which springs and wells across much of the BSEA exhibited higher alkalinity, and greater Ca^{2+}, pH, TN, TP, and NO_3^- concentrations, while well sites closer to the eastern FWSWI exhibited greater SO_4^2 and NH_4^+ concentrations. The groundwater snail, *Phreatodrobia micra* was also associated with a smaller group of wells near the FWSWI. It is critical to note that isotopic composition of POM did not substantially explain variation among sites and therefore had little explanatory power for stygobiont community composition in the BSEA.

Dietary composition of salamanders at Eliza Springs

Dietary mixing models for both salamander species using the same five putative dietary sources for both salamander species (i.e., chironomids + psephenids, cyclopoid copepods, *Dugesia* sp. + *Lirceolus* spp., *H. azteca*, and *Stygobromus*) indicated that of the invertebrates analyzed, cyclopoid copepods had the greatest dietary contribution to the Barton Springs and Austin blind salamander (Figure 4). Groundwater cyclopoid copepods comprised nearly a third (29%) of the Austin blind salamander diet with *Stygobromus* (21%) and chironomids + psephenids (20%) representing the next greatest dietary contributors. Similarly, cyclopoid copepods contributed to almost a third of the diet to the Barton Springs salamander (26%), with *Stygobromus* being the next largest contributor to *E. sosorum* diets (24%). However, *Dugesia* sp. + *Lirceolus* spp. was the third largest dietary source for the Barton Springs salamander (19%). Stygobitic

invertebrates cumulatively contributed >60% to the diets of both salamander species at Eliza Springs. Model performance resulted in a Gelman diagnostic <1.05.

Niche overlap in salamanders and invertebrates at Eliza Springs

Both salamander species at Eliza Springs had similar and overlapping isotope values ($\bar{x} \pm 1$ SD) for $\delta^{13}C$ (Barton Springs salamander = -34.02 ± 1.45‰, Austin blind salamander = -33.81 ± 0.96‰) and $\delta^{15}N$ (Barton Springs salamander = 9.07 ± 0.91‰, Austin blind salamander = 8.74 ± 0.91‰; Figure 5). Niche overlap estimates indicated that the isotopic niche space of the Austin blind salamander was relatively more restricted and was almost entirely contained within the niche space of the Barton Springs salamander, resulting in a mean 91% probability (95% credible interval = 58 – 100%) of an individual of Austin blind salamander occurring in the niche space of an individual Barton Springs salamander (Table 4). However, the niche space of an individual Barton Springs salamander was substantially larger than that of an individual Austin blind salamander, resulting in a 40% probability (95% credible interval = 21 – 63%) of an individual occurring in the nested niche space of the Barton Springs salamander (Table 4).

In contrast to salamanders at Eliza Springs, the invertebrate community showed substantially more interspecific variation in isotopic values (Figure 5). In general, the degree of niche overlap among the eight taxa was low (<2% niche overlap probability; Table 4), indicating a high degree of niche specialization amongst the various invertebrates at Eliza Springs, with the exception of a few taxa. As indicated by my *a priori* isotope analysis for salamander diet sources, the extent of niche overlap estimates

between the flatworm *Dugesia* sp. and unidentified *Lirceolus* spp. was high (97%; Table 4). Further, the extent of niche overlap between *Dugesia* sp. and *L. hardeni* was 33%; the extent of niche overlap between *Dugesia* sp. and *Stygobromus* sp. was substantially lower (11%). The only invertebrate in the community with >10% probability of niche overlap was amphipod *H. azteca* with a 23% probability of occurring niche space of *L. hardeni* and a 17% probability of occurring in the niche space of annelids.

Discussion

Geochemical, nutrient, and POM stable isotope variation in the BSEA

Consistent with my first hypothesis, there were several geochemical gradients that exist across the sites I examined in the BSEA. The primary geochemical gradient was a recharge zone-to-FWSWI gradient, where sites closer to the recharge zone were associated with higher DO, NO_3^-, alkalinity, and more enriched δ^2D values, whereas sites nearer the FWSWI were associated with greater conductivity, Mg^{2+}, TP, but lower DO. Among the sites examined by this study, five wells (Dowell, Kai Frech, Sweeney, Old San Antonio 1, and United Gas) near the FWSWI either intersect the FWSWI or are within 1 km of the FWSWI. Low DO along the FWSWI is expected because it is a sulfide-rich, anoxic saline water source (Lambert et al. 2010). However, the higher DO, NO_3^-, alkalinity, and more enriched aqueous δ^2D values originate in the recharge zone and as concentrations decrease water mass is transported closer to the FWSWI and geochemical changes may be due to the combined effects of microbial decomposition of photosynthetic OM (Katz et al. 2004) and denitrification (Arango et al. 2007; Burgin and Hamilton, 2007). The secondary geochemical gradient represented a contrast between a

combination of springs and wells to recharge creeks in which springs and wells were generally associated with higher NO_3^-, alkalinity, and DO while creeks were associated with higher TP and water temperature. This geochemical and nutrient contrast is likely present because creek sites are more immediately influenced by changing surface conditions (i.e., air temperature, evaporation) and inputs of surface runoff of suspended particulates, and nutrients, whereas spring and well sites are more likely to be buffered from changes to ambient surface conditions and less immediately influenced by microbial decomposition surficial photosynthetic OM inputs. It is important to note that stable isotopes for water and water temperature may only be important because of the season when samples were collected.

Although there were strong geochemical and nutrient gradients in the BSEA, I did not find strong evidence of CLA generated OM for the stygobiont food webs in the BSEA. The POM $\delta^{13}C$ isotopic values in the BSEA (observed $\delta^{13}C$ = - 33 to -27) were not widely variable among sites (including low DO wells in the saline zone) and the POM $\delta^{13}C$ isotopic values were not widely deplete due to strong enzymatic discrimination effects from chemoautotrophy; the literature reports $\delta^{13}C$ of OM generated via CLA ranging from -35 to values less than -55‰ (Sarbu et al. 1996; Opsahl and Chanton 2006). Indeed, in the SAEA, $\delta^{13}C$ of POM was -28‰ and signatures of invertebrates using CLA were as low as -45‰ (Hutchins et al. 2016). It is important to note that I did not observe systematic variation among site types and isotopic values of POM did not vary with nutrient and geochemical variables across the BSEA.

The lack of isotopic evidence that POM across the BSEA was not derived via CLA does not eliminate the possibility that CLA is actively occurring and can be an

important process in this portion of the EA. First, sites at which I collected POM may have not had active microbial CLA occurring (but it occurs at other unsampled sites in the BSEA or has other metabolic pathways) or the suspended POM material I collected is more indicative of surface OM inputs and not OM generated via CLA. In many subterranean systems, active microbial CLA is often associated with attached biofilms (Hutchens et al. 2003; Engel and Randall 2011) and the lack of phreatic biofilm accessibility and sampling in my study did not allow me to assess if biofilm CLA varied spatially and with geochemical conditions across the BSEA. Also, it is important to note that I was not able to obtain many invertebrates from sites adjacent to the FWSWI to complete a similar comparison as Hutchins et al. (2016) in regards to taxa a proximity to the FWSWI. Lastly, it may not be particularly surprising that isotopic values were not indicative of CLA because the groundwater hydrologic conditions in the BSEA were not conducive (low-flow conditions) to generating a strong signal for CLA detection. The BSEA groundwater flow velocity in the BSEA has been estimated at 9 km/d during the highest flow conditions (Smith et al. 2005), which is a relatively fast flow rate across the aquifer segment when compared to the SAEA where strong CLA signals were detected (Hutchins et al 2016). Groundwater flow velocity in the SAEA has been estimated at 3 km/d during the highest flow conditions (Smith et al. 2005). Faster groundwater travel times across the BSEA may limit the amount of hydrologic exchange along the FWSWI and reduce the amount of time for water-biofilm-rock interactions. However, it is critical to note that the sampling period for this study was during a historically low-flow period (Hunt et al. 2019). Lower BSEA aquifer levels and spring discharge would likely lengthen hydrologic travel times and increase potential for FW-SW exchange and time

for water-biofilm-rock interactions. This combination of factors makes the potential for detecting CLA isotopic signals in the suspended POM more likely during this study.

Spatial variation in community composition in stygobiont community structure in the BSEA

I found variation in stygofaunal assemblage structure amongst sites, with the occurrence and density of a limited number of taxa dependent upon site type and distance from the FWSWI (Figure 3). Geochemical and nutrient gradients across aquifers have been shown to cause spatial variation in stygofaunal assemblages (Humphreys, 2009; Shapouri et al. 2016). Although I captured invertebrates at all springs and oxic wells that were sampled, none of the anoxic wells contained invertebrates, indicating that the presence of DO > 1 mg/L and lower salinity water was a primary factor controlling stygobiont occurrence and abundance. Prior studies have found that groundwater and subterranean species frequently have restricted distributions and are often collected at a single or limited number of sites (e.g., Larned 2012). However, in the present study, a few taxa were widespread in the BSEA (2 of more than 20 taxa were widespread and occurred at more than 10 sites). However, the higher groundwater flow rates and hydrologic connectivity across this regional aquifer may contribute to the relatively high number of taxa that occur at multiple sites (Hutchins et al. 2021).

Although the sparse nature of most of the stygobitic taxa in the BSEA, the densities of some taxa varied substantially with site type and/or environmental conditions. For example, cyclopoid copepods and mites were found throughout the BSEA (occurred at more than 10 out of 22 sites), but the number of cyclopoid copepods

captured was associated with sites with higher pH, higher Ca^{2+} concentration, and greater alkalinity; these conditions largely corresponded to spring sites and multiple well sites. In addition, aquatic mites exhibited association with sites with higher TN, TP, and NO_3^- concentrations, which corresponded to wells near the recharge zone. Finally, the groundwater obligate snail, *P. micra*, was associated with a limited number of well sites near the FWSWI with higher NH_4^+ and SO_4^{2-} concentrations (Old San Antonio 1 and Old San Antonio 2).

Dietary composition of salamanders at Eliza Springs

This study found the Barton Springs salamander and the Austin blind salamander derive most of their diets from groundwater obligate or spring orifice associated organisms. For both species, >60% of the diets was comprised of groundwater and interstitial prey (i.e., chironomids + psephenids, cyclopoid copepods, *Dugesia* sp. + *Lirceolus* spp., *H. azteca*, and *Stygobromus*), suggesting that groundwater and orifice-associated taxa are key dietary items for these at-risk conservation-priority salamanders in the Barton Springs complex. One key finding of this study is that cyclopoid copepods was the largest diet contributor and *Stygobromus* was the second most important diet item for both species. Copepods were numerically dominant members of the invertebrate community of spring orifices (see above), many salamanders are opportunistic predators that show limited selectivity toward prey items (Gillespie 2013), and copepods are often used as a food source for captive salamanders (Zabierek and Gabor, 2016), thus it is not particularly surprising that copepods were determined to have the largest contribution to salamander diets. Cyclopoid copepods comprising most of the Austin blind salamander's

diet is also not unexpected because a wild-caught specimen was observed defecating the remains of copepods, amphipods, ostracods, and plant material after being collected (Hillis et al. 2001).

Gillespie (2011) conducted a stable isotope-based study on the Barton Springs salamander and found that the flatworm *Dugesia* was the largest dietary contributor. However, Gillespie (2011) examined only three possible diet sources (*Dugesia* sp., *H. azteca*, and chironomids) for the Barton Springs salamander, all of which are more cosmopolitan surface water organisms that exhibit body sizes larger than some of the prey examined here. In the present study, I considered a more diverse suite of potential dietary items that included both smaller bodied and subterranean prey that occur at spring orifices and found that inclusion of these prey items likely represents a more complete and accurate assessment of the diet of the Barton Springs salamander. Furthermore, I found that some of the prey items considered by Gillespie (2011) and the prey I examined had overlapping isotopic values (i.e., *Dugesia* and *Stygobromus*), indicating that the exclusion of groundwater and smaller-bodied prey may have led to identifying some prey items as greater dietary contributors than they are in reality. The results of this study and that of Gillespie (2011) highlight the critical nature of identifying, a priori or posteriori consolidating, and then utilizing all appropriate potential dietary items for isotopic dietary mixing models and that the exclusion of harder to collect or rare items from mixing models can lead to erroneous results if these items are indeed used by the consumer in question (Phillips et al. 2014).

Niche overlap in salamanders and invertebrates at Eliza Springs

Niche overlap models clearly indicate the Barton Springs salamander and the Austin blind salamander occupy a similar feeding niche at Eliza Springs. Relatively opportunistic consumers positioned at the top of a food web, like the two salamanders examined here, show limited variation in isotopic composition (Fry 2006). However, the more important finding of this study is the difference in niche size and asymmetrical differences in the degree of niche overlap between the two species. The Barton Springs salamander may indicate a broader diet due to the inhabitation of surface water and groundwater. The observed niche overlap and differences in niche space between salamander species may be partially due to the small sample size. The small sample size of the Barton Springs salamander (n = 6) and the Austin blind salamander (n = 4) were somewhat limited because of low flow conditions, and low sample size in niche modelling can cause the model to be less likely to account for individual or prey item variation (Newsome et al. 2009). Thus, future attempts should be made to increase sample size for both species to increase niche modelling accuracy and if there are temporal changes or shifts in diet-tissue fractionation patterns related to food quality (Newsome et al. 2009).

Diet similarity may also cause substantial isotopic niche space overlap amongst these species because they are consuming similar food sources whether they share the same habitat or not. The Barton Springs salamander is known to inhabit surface waters but is inferred to move back and forth between surface and groundwater habitats (Bendik et al. 2021). Conversely, the Austin blind salamander is clearly a groundwater adapted species assumed to spend a majority of their lives in subterranean habitats (Hillis et al.

2001), but it is often observed and captured at spring orifices in the Barton Springs complex. The most likely explanation for the amount of niche overlap and similarity of diets could be due to the collection of all salamanders for this study occurring at spring orifices and that individuals examined in this study are consuming a similar suite of spring orifice- and groundwater-dependent invertebrates. In addition, these results also suggest that the Austin blind salamander may be similarly migrating back and forth between deeper phreatic habitats and spring orifices and their associated interstitial spaces to feed. Alternatively, Austin blind salamander individuals collected for this study were at spring orifices because they were unintentionally discharged from deeper phreatic habitats and were effectively trapped at these locations and were unable to migrate back below the surface. Regardless of the mechanism by which both species are found at the spring orifices, members of the genus *Eurycea* are known to occupy interstitial spaces and associate with cobbles and gravel in streams and caves to avoid predation (Edgington and Taylor, 2019) and it is likely that the high degree of dietary overlap (i.e., a majority of both species' diets composed of the same interstitial and groundwater-dependent prey) at spring orifices is due to the fact that they are occupying the same habitat around orifices. Indeed, spring orifices serve as an ecotone between surface and phreatic habitats and contain trophically complex invertebrate communities composed of surface dwelling, spring orifice-dependent, and subterranean-dependent taxa (Nair et al. 2021).

In contrast to the findings of the salamander niche modelling, results for the eight invertebrate taxa indicated a much higher degree of niche specialization at Eliza Springs. The base of food webs and lower trophic level consumers in food webs typically exhibit a great degree of isotopic variation than upper-level consumers because of differential use

of basal OM sources that are isotopically distinct (Fry 2006). A high degree of trophic complexity and niche specialization in the Eliza Springs community is similar to the high degree of isotopic variation found amongst invertebrate communities in the SAEA (Hutchins et al. 2016; Nair et al. 2021). In the present study, estimates of percent niche overlap among invertebrates was low (<2% overlap in niche space) between most species pairs, even when those pairs were within the same larger taxonomic group (i.e., *H. azteca* and *Stygobromus* spp. are both amphipods). Nair et al. (2021) assessed the degree of niche overlap in spring orifice communities at two larger spring complexes in the Edwards and Edwards-Trinity Aquifer systems and found similar levels of specialization and lack of niche overlap among taxonomic pair groups, and the overall general lack of overlap amongst many of the taxa examined by this study indicates a high degree of trophic specialization (Ercoli et al. 2019).

Conclusions

Results from this study suggest that although there are clear nutrient and geochemical gradients across the BSEA, it appears that the POM isotope data are not useful for determining if CLA does not contributes to OM collected at sites. Additional research is needed to determine if and where CLA is present and whether the OM generated via CLA is utilized by stygobitic and spring orifice communities. The nutrient and geochemical gradients across the BSEA lead to spatial variation in the occurrence and density of cyclopoid copepods, mites, and *P. nugax*, but most taxa do not show relationships with site types or geochemical conditions. The stochastic nature of collecting rare taxa make distribution and occurrence models difficult and require large

sampling efforts to collect an adequate number of individuals. Dietary analyses of the two co-occurring salamanders at Eliza Springs indicate that both species are largely reliant upon the spring orifice and stygobiont food sources. The Barton Springs Salamander and Austin blind salamander occupy similar feeding niches, but additional research is needed to understand the seasonal variation in niche overlap at Eliza Springs as well as the degree of niche overlap variation that exists between springs.

Table 1. Geographical coordinates (˚) of spring, creek, and well sites in the BSEA. Water for geochemistry, including POM = WG; water for isotopes = WI; invertebrate samples = I; microbial samples = M.

Site Name	Samples Collected	Latitude (˚)	Longitude (˚)
Spring			
Eliza	WG, WI, I, M	30.26428	-97.7701
Main Barton	WG, WI, I, M	30.26351	-97.771
Old Mill	WG, WI, I, M	30.26351	97.76811
Upper Barton	WG, WI, I, M	30.26348	-97.7741
Creek			
Barton	WG, WI, I, M	30.27403	-97.8446
Bear	WG, WI, I, M	30.15543	-97.9397
Onion	WG, WI, I, M	30.08328	-98.0082
Well			
Bliss Spillar	WG, WI, I, M	30.14721	-97.8969
Circle C	WG, WI, I, M	30.1867	-97.8492
Dowell	WG, WI, I, M	30.14281	-97.8108
Greenbelt	WG, WI, I, M	30.26101	-97.8176
Gregg	WG, WI, I, M	30.00861	-97.8961
Holiday Inn	WG, WI, I, M	30.23487	-97.8141
Hoskins	WG, WI, I, M	30.06635	-97.9445
Kai (Frech)	WG, WI, M	30.25631	-97.7696
Lovelady	WG, WI, I, M	30.21035	-97.7816
McCoys	WG, WI, I, M	30.14048	-97.8387
Negley	WG, WI, I, M	30.03851	-97.8862
Old San Antonio 1	WG, WI, I, M	30.13202	-97.8126
Old San Antonio 2	WG, WI, I, M	30.13082	-97.8169
Republic of Texas	WG, WI, I, M	30.25111	-97.8372
Ruby Road	WG, WI, I, M	30.08833	-97.915
Sweeney	WG, WI, I, M	30.05208	-97.8334
Target	I	30.23222	-97.7928
United Gas	WG, WI, I, M	30.09229	-97.7894
Whirlpool Cave	WG, WI, I, M	30.21556	-97.8472
Wyldwood	WG, WI, I, M	30.17606	-97.8709

Latitude and Longitude coordinates for spring, spring, and well sites in the BSS of the EA. Water for geochemistry, including POM = WG; water for isotopes = WI; invertebrate samples = I; microbial samples = M

Table 2. Stable isotopes (δ13C, δ15N) means, standard deviations, range, and sample size for salamanders and invertebrates from Barton Springs, Edwards Aquifer Texas, USA

Site	Taxon	$\delta^{13}C‰$	$\delta^{15}N‰$
Eliza Springs			
	Eurycea sosorum	-34.02±1.45 (-36.87, -32.3) N = 6	9.07±0.91 (7.24, 9.87) N = 6
	Eurycea waterlooensis	-33.81±0.96 (-35.4, -32.88) N = 4	8.74±0.91 (7.24, 9.58) N = 4
	Annelids	-31.45±0.72 (-32.47, -30.91) N =3	4.92±0.37 (4.39, 5.2) N = 3
	Cyclopoid Copepods	-36.93±0.40 (-37.46, -36.21) N =6	3.01±0.29 (2.54, 3.36) N = 6
	Dugesia sp. (Flatworms)	-34.07±0.67 (-35.03, -32.47) N = 6	7.06±0.77 (5.91, 7.89) N = 6
	Hyallela azteca	-32.16±0.64 (-33.18, -31.17) N = 9	5.35±0.32 (4.72, 5.82) N = 9
	Lirceolus sp.	-34.42±0.17 (-34.65, -34.19) N = 4	6.60±0.25 (6.31, 6.99) N = 4
	Lirceolus bisetus	-33.93 N = 1	6.13 N = 1
	Lirceolus hardeni	-33.45±0.99 (-34.07, -32.53) N = 3	6.48±0.99 (5.07, 7.26) N = 3
	Mites	-34.07±0.29 (-34.35, -33.78) N = 2	10.35±0.12 (10.23, 10.46) N = 2
	Phreatodrobia nugax	-29.54±0.20 (-29.76, -29.27) N = 3	8.14±0.06 (8.06, 8.21) N = 3
	Stygobromus sp.	-33.19±0.33 (-33.49, -32.73) N = 3	9.26±0.28 (8.89, 9.58) N = 3
	Stygobromus bifurcatus	-29.98 N = 1	11.7 N = 1
	Stygobromus flagellatus	-32.63±0.49 (-33.12, -32.14) N = 2	9.25±0.16 (9.09, 9.41) N = 2

	Stygobromus russelli	-30.80±1.89 (-32, -26.62) N = 5	10.775±0.70 (10.06, 11.8) N = 5
	Chironomidae	-36.05±0.58 (-36.78, -35.35) N = 3	5.79±0.11 (5.65, 5.91) N = 3
	Psephenidae	-34.31±0.68 (-35.76, -33.33) N = 12	3.82±0.30 (3.23, 4.13) N = 12
	FPOM	-32.41±1.44 (-34.87, -31.2) N = 4	4.81±0.44 (4.07, 5.23) N = 4
Old Mill Springs			
	Annelids	-26.3±1.96 (-28.26, -24.34) N = 2	13.48±0.89 (12.59, 14.36) N = 2
	Cyclopoid Copepods	-35.91±0.47 (-36.37, -35.44) N = 2	5.73±0.37 (5.36, 6.1) N = 2
	Hyallela azteca	-32.31±0.36 (-32.92, -31.9) N = 5	4.734±0.22 (4.48, 5.12) N = 5
	Ingolfiellidae	-26.01 N = 1	12.71 N = 1
	Lirceolus sp.	-29.1625±0.50 (-29.78, -28.63) N = 4	5.67±0.21 (5.36, 5.89) N = 4
	Mites	-34.84±0.51 (-35.35, -34.33) N = 2	9.36±0.26 (9.1, 9.61) N = 2
	Stygobromus sp.	-27.44 N = 1	8.03 N = 1
	FPOM	-27.66±0.18 (-28.98, -27.02) N = 4	5.79±0.18 (5.58, 6.08) N = 4

Main Barton Springs			
	Annelids	-25.93±0.02 (-25.96, 25.91) N = 3	7.67±0.35 (7.3, 8.14) N = 3
	Cyclopoid Copepods	-36.61±0.36 (-36.97, -36.25) N = 2	5.47±0.18 (5.29, 5.64) N = 2
	Dugesia sp. (Flatworms)	-31.81±0.06 (-31.86, -31.75) N = 2	9.86±0.3 (9.56, 10.16) N = 2
	Lirceolus sp.	-31.42 N = 1	7.5 N = 1
	Mites	-34.00±0.12 (-34.17, 33.84) N = 4	9.11±0.11 (8.93, 9.23) N = 4
	Eurycea spp.	-38.32±0.16 (-38.49, -38.16) N = 2	7.86±0.14 (7.72, 8.00) N = 2
	FPOM	-31.09±0.94 (-32.66, -30.24) N = 4	5.1±0.50 (4.45, 5.71) N = 4
Upper Barton Springs			
	Cyclopoid Copepods	-31.89 N = 1	6.83 N = 1
	Dugesia sp. (Flatworms)	-31.79±0.42 (-32.38, -31.48) N = 3	7.78±0.31 (7.4, 8.16) N = 3
	Lirceolus sp.	-22.47 N = 1	7.54 N = 1
	FPOM	-29.48 N = 1	2.58 N = 1

Bulk stable isotope values (δ13C, δ15N) for salamander/invertebrate taxon and FPOM (Fine Particulate Organic Matter) collected from the 4 main springs of the Barton Springs complex. Isotopic mean and standard deviation, range of isotope values (presented in parentheses), and sample size (N) are presented for each taxon at each spring site.

Table 3. Bulk stable isotope μ (mean) and σ (standard deviation) and range of values at each of the 12 Wells, 3 Creeks, and 4 spring sites in the BSEA.

Site Type	Site	$\delta^{13}C‰$	$\delta^{15}N‰$
Well			
	Wyldwood	-28.07±0.24 (-28.25, -27.74) N = 3	1.00±0.42 (0.42, 1.40) N = 3
	Circle C	-32.36±0.31 (-32.75, -32.01) N = 3	4.15±0.54 (3.42, 4.72) N = 3
	Dowell	-34.50±0.54 (-35.23, -33.95) N = 3	3.63±0.13 (3.51, 3.81) N = 3
	Hoskins	-28.76±0.57 (-29.50, -28.13) N = 3	6.13±0.12 (6.01, 6.30) N = 3
	Kai Frech	-27.03±0.12 (-27.18, -26.89) N = 3	4.77±0.45 (4.35, 4.57) N = 3
	Holiday Inn	-33.33±1.31 (-34.64, -32.02) N = 2	6.72±0.19 (6.54, 6.91) N = 2
	Old San Antonio 1	-27.05±0 N = 2	3.55±0.31 (3.23, 3.86) N = 2
	Old San Antonio 2	-27.59±0.42 (-28.01, -27.17) N = 2	2.08±0.07 (2.01, 2.15) N = 2
	Negley	-29.52±0.24 (-29.77, -29.28) N = 2	6.07±0.84 (5.23, 6.91) N = 2
	United Gas	-30.56±0.23 (-30.79, -30.33) N = 2	4.62±0.29 (4.33, 4.91) N = 2
	Bliss Spillar	-28.60±0.49 (-29.09, -28.11) N = 2	2.02±0.17 (1.86, 2.19) N = 2
	Sweeney	-25.79±0.45 (-26.23, -25.34) N = 2	2.02±0.17 (-8.66, -2.61 N = 2

Creek			
	Barton	-30.41±0.03 (-30.44, -30.37) N = 2	9.45±0.367 (8.49, 9.92) N = 3
	Bear	-30.56±0.04 (-30.60, -30.53) N = 2	8.40±0 N = 1
	Onion	-29.72±0.04 (-29.76, -29.69) N = 2	5.31±0.06 (5.25, 5.37) N = 2
Spring			
	Old Mill	-27.66±0.78 (-28.98, -27.02) N = 4	4.84±0.14 (4.68, 5.03) N = 4
	Upper Barton	-29.48±0 N = 1	4.27±0 N = 1
	Eliza	-32.41±1.44 (-34.87, -31.20) N = 4	5.04±0.58 (4.06, 5.56) N = 4
	Barton Pool	-31.09±0.94 (-32.66, -30.24) N = 4	6.30±0.16 (6.16, 6.57) N = 4

Bulk isotopes values (δ13C, δ15N) for FPOM (Fine Particulate Organic Matter) at 12 Wells, 3 Creeks, and 4 Spring sites in the BSEA.

Table 4. Posterior estimates and 95% credible intervals of probabilistic niche overlap (%) amongst salamanders and invertebrates from Eliza Springs.

Site	Group	Species A	Species B	Overlap Probability (%)
Eliza Springs				
	Chordata	*Eurycea sosorum*	*Eurycea waterlooensis*	40.82 (21-63)
	Chordata	*Eurycea waterlooensis*	*Eurycea sosorum*	91.85 (58-100)
	Annelida	Annelids	Cyclopoid Copepods	0.05 (0-1)
			Dugesia sp.	0.00 (0-0)
			Hyallela azteca	0.62 (0-2)
			Lirceolus hardeni	0.78 (0-2)
			Lirceolus sp.	0.00 (0-0)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	0.00 (0-0)
	Copepoda	Cyclopoida	Annelids	0.15 (0-2)
			Dugesia sp.	0.02 (0-0)
			Hyallela azteca	0.03 (0-0)
			Lirceolus hardeni	0.00 (0-0)
			Lirceolus sp.	0.00 (0-0)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	0.00 (0-0)
	Platyhelminthes	*Dugesia sp.*	Annelids	0.00 (0-0)
			Cyclopoid Copepods	0.02 (0-0)
			Hyallela azteca	0.11 (0-1)
			Lirceolus hardeni	32.47 (8-61)
			Lirceolus sp.	96.87 (76-100)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	11.17 (0-96)
	Amphipoda	*Hyallela azteca*	Annelids	17.14 (0-67)
			Cyclopoid Copepods	0.02 (0-0)
			Dugesia sp.	0.10 (0-2)
			Lirceolus hardeni	22.81 (6-43)
			Lirceolus sp.	0.03 (0-0)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	0.00 (0-0)

	Isopoda	*Lirceolus hardeni*	Annelids	4.50 (0-14)
			Cyclopoid Copepods	0.00 (0-0)
			Dugesia sp.	8.90 (2-16)
			Hyallela azteca	9.83 (4-19)
			Lirceolus sp.	0.55 (0-6)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	0.11 (0-2)
	Isopoda	*Lirceolus sp.*	Annelids	0.00 (0-0)
			Cyclopoid Copepods	0.00 (0-0)
			Dugesia sp.	18.51 (5-41)
			Hyallela azteca	0.00 (0-0)
			Lirceolus hardeni	0.00.59 (0-7)
			Phreatodrobia nugax	0.00 (0-0)
			Stygobromus sp.	0.00 (0-0)
	Gastropoda	*Phreatodrobia nugax*	Annelids	0.00 (0-0)
			Cyclopoid Copepods	0.00 (0-0)
			Dugesia sp.	0.00 (0-13)
			Hyallela azteca	0.00 (0-0)
			Lirceolus hardeni	0.00 (0-4)
			Lirceolus sp.	0.00 (0-0)
			Stygobromus sp.	0.00 (0-0)
	Amphipoda	*Stygobromus sp.*	Annelids	0.00 (0-0)
			Cyclopoid Copepods	0.00 (0-0)
			Dugesia sp.	1.78 (0-13)
			Hyallela azteca	0.00 (0-0)
			Lirceolus hardeni	0.29 (0-4)
			Lirceolus sp.	0.00 (0-0)
			Phreatodrobia nugax	0.00 (0-0)

Posterior estimates and 95% credible intervals (in parentheses) are presented for each species pair and group by utilizing μ (mean) and Σ (variance). Posterior probabilities are bi-directional and indicate the probability of an individual of Species A niche being found within the niche of Species B, and vice versa. Niche overlaps were calculated using α=0.95.

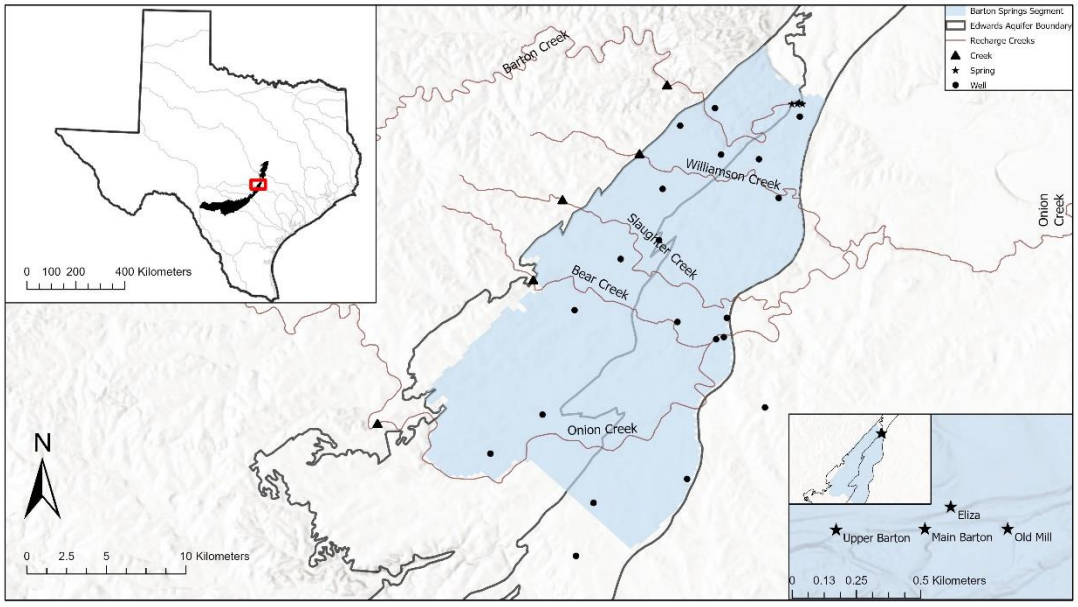

Figure 1. Map of the creeks, wells, and springs sampled within the Barton Springs Segment of the Edwards Aquifer in south-central Texas

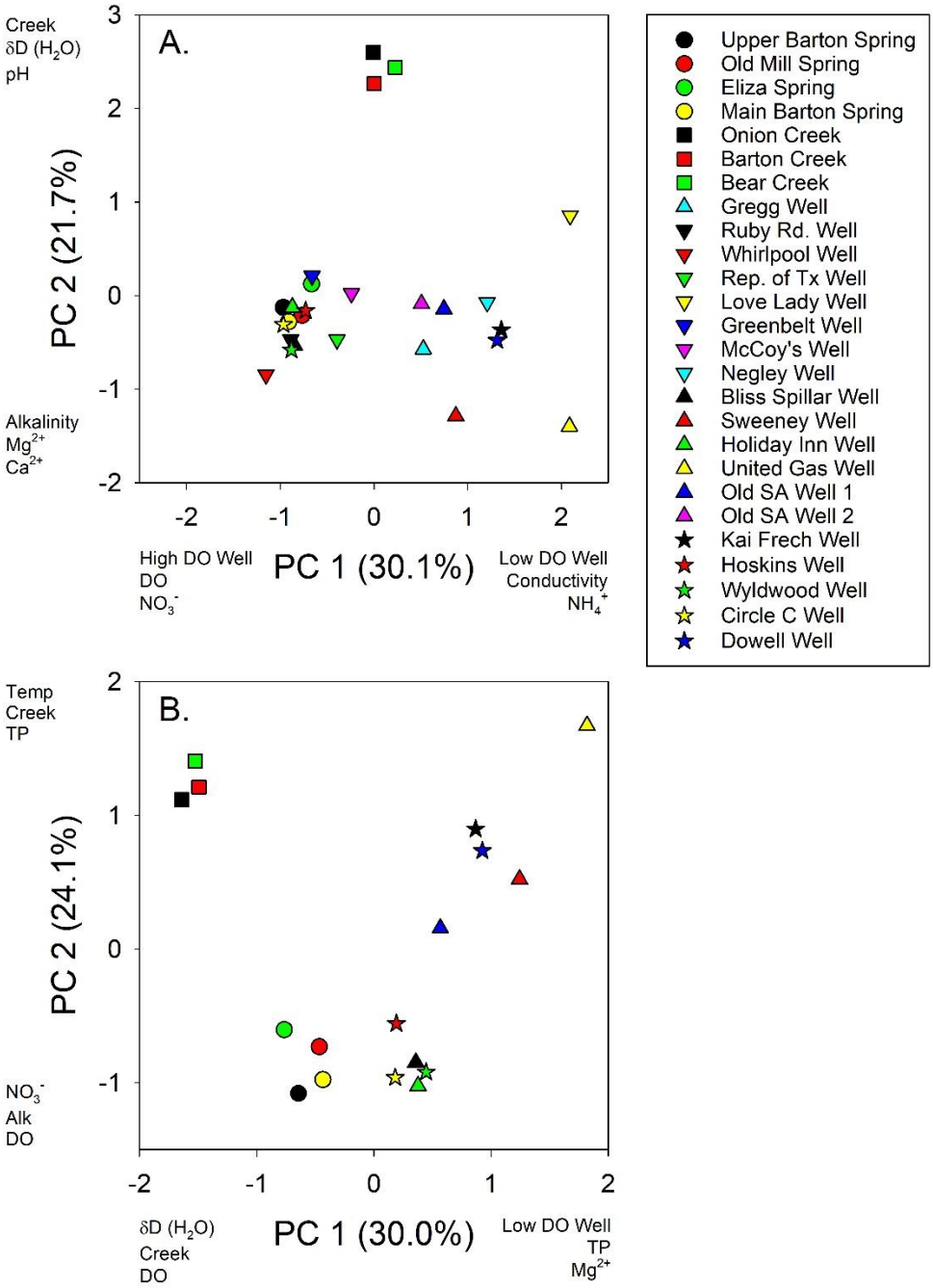

Figure 2. Principal Component Analysis (PCA) of environmental variables and sites withing the BSS of the EA. 2A. Data set including 16 water quality, ionic, and stable isotope variables (e.g., water temperature, pH, DO, conductivity, alkalinity, DO, F^-, K^+, Mg^{2+}, Ca^{2+}, δ^2H of water, TP, and NH_4^+) and four dummy variables that categorized site types (i.e., Spring, Low DO Well, High DO Well, and Recharge Creek). 2B. Data set including sites with both environmental data and POM stable isotope values

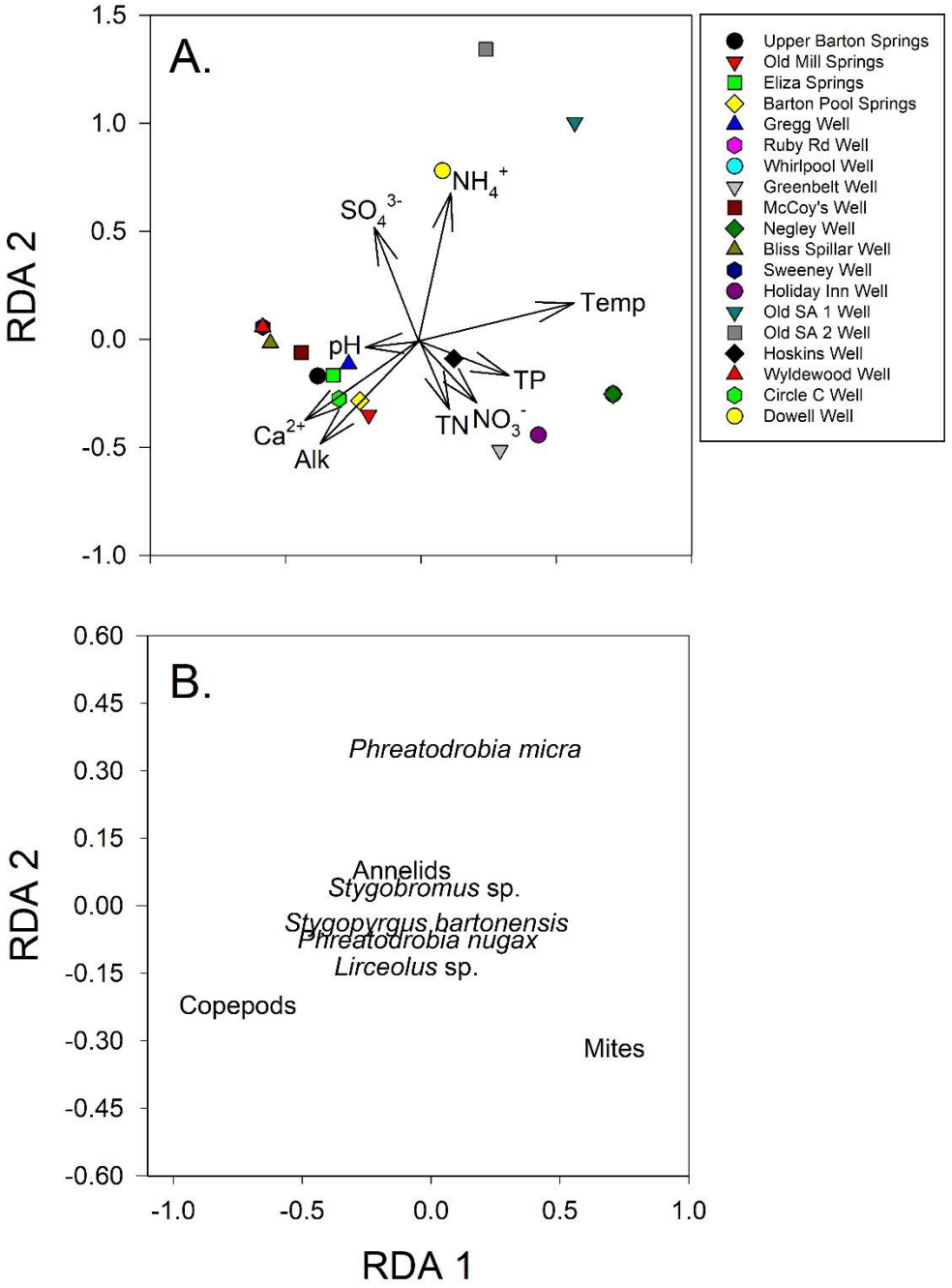

Figure 3**.** Redundancy Analysis (RDA) of environmental variables, sites, and taxonomic abundance within the BSS of the EA. 3A. Includes 8 envir. var. and all sites within the BSS where specimens were collected. Sites where no taxa were collected were 3 wells (Kai (Frech), Lovelady, and United Gas) and stream sites. The Target well was not included due to lake of water quality data. 3B. Includes the 8 taxa with the greatest species scores and 11 taxa are not included that had very low species scores (Platyhelminthes, Ostracods, Bathynellacea, Microcerberidae, *L. bisetus*, *L. hardeni*, *Cirolanides texensis*, Parabogidiella, *E. sosorum*, *E. waterlooensis*, and *Eurycea sp.*). P value = 0.05 and F statistic = 1.7256.

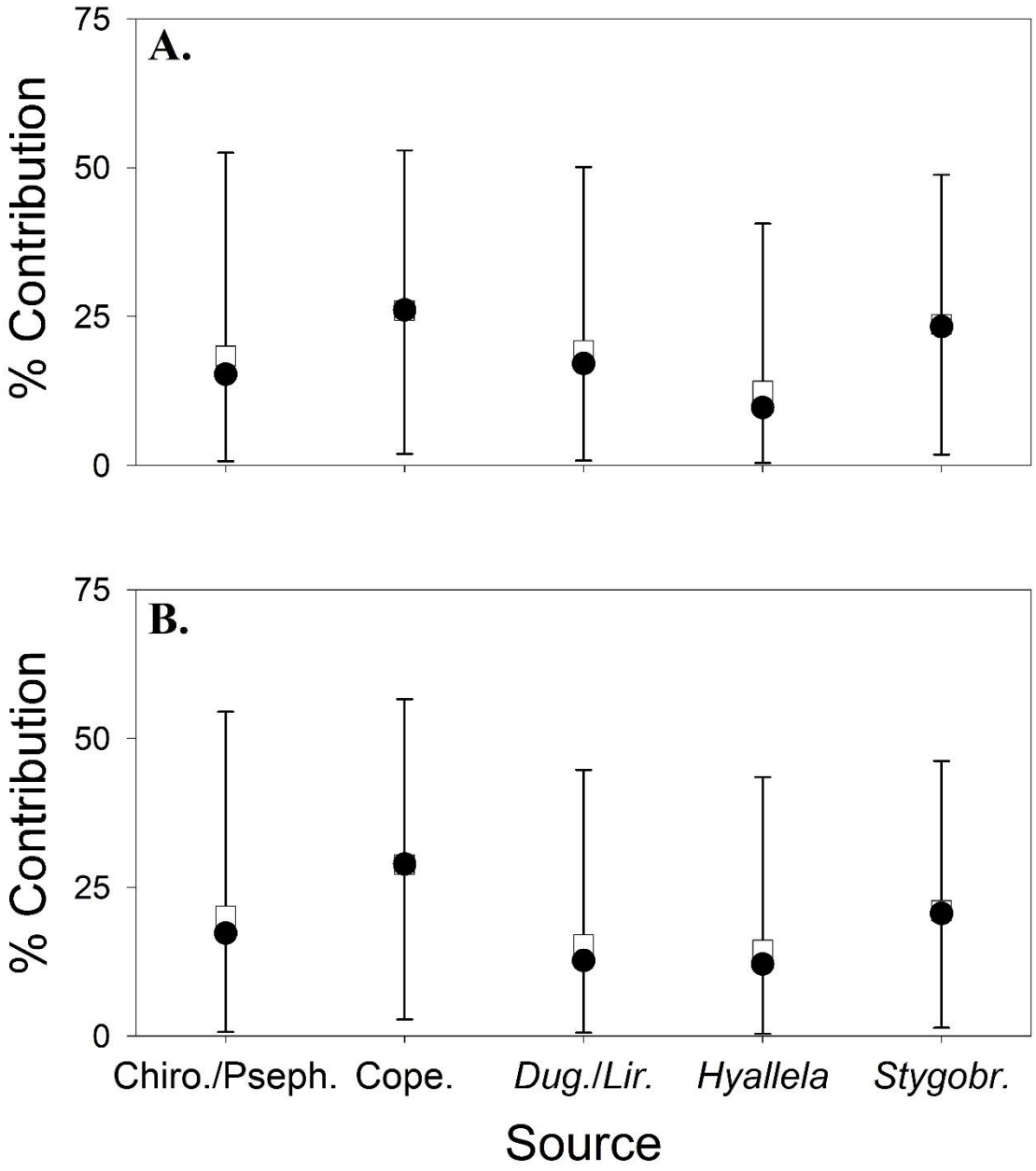

Figure 4. MixSIAR output of contributions of invertebrate sources (Chironomidae/Psephenidae, Cyclopoid Copepods, *Dugesia sp./Lirceolus sp.*, *Hyallela azteca*, and *Stygobromus sp.*) to the diets of *Eurycea sosorum* (4A) and *Eurycea waterlooensis* (4B). Open square = mean, filled circle = median, error bars = ±95% credible interval of the posterior probability distribution.

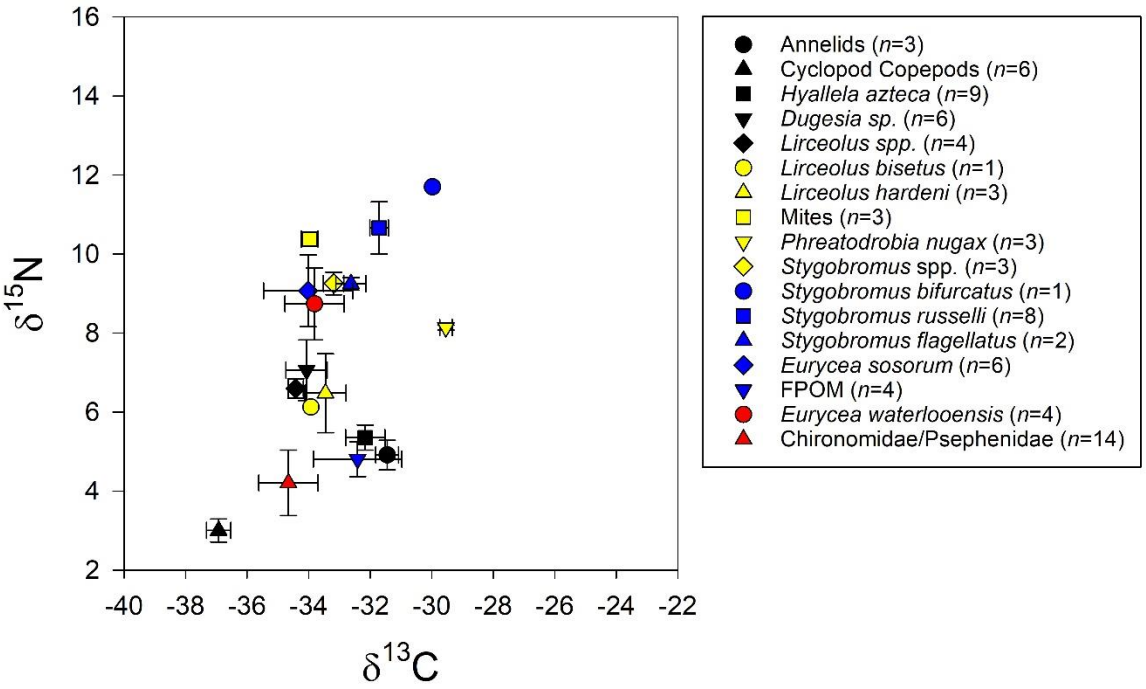

Figure 5. Isotope (δ13C, δ15N) biplot for Eliza Springs. Point = mean of each taxa, Bars = ±1 SD, FPOM = fine particulate organic matter. Sample size (N) is in parentheses in the figure legend.

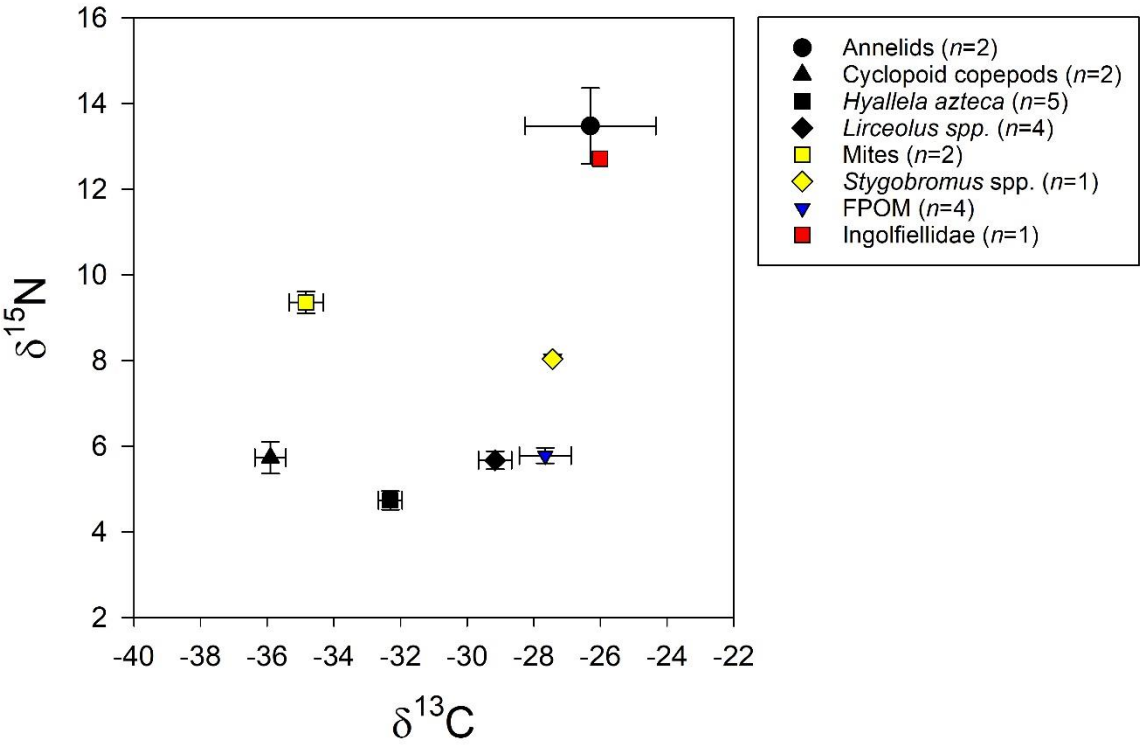

Figure 6. Isotope (δ13C, δ15N) biplot for Old Mill Springs. Point = mean of each taxa, Bars = ±1 SD, FPOM = fine particulate organic matter. Sample size (N) is in parentheses in the figure legend

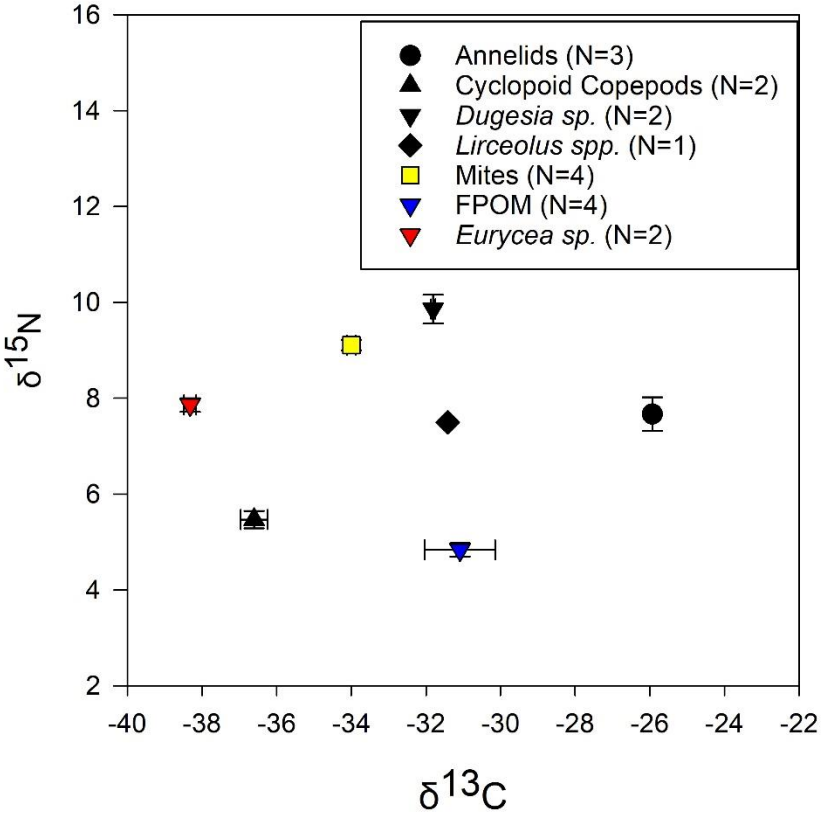

Figure 7. Isotope (δ13C, δ15N) biplot for Main Barton Springs. Point = mean of each taxa, Bars = ±1 SD, FPOM = fine particulate organic matter. Sample size (N) is in parentheses in the figure legend

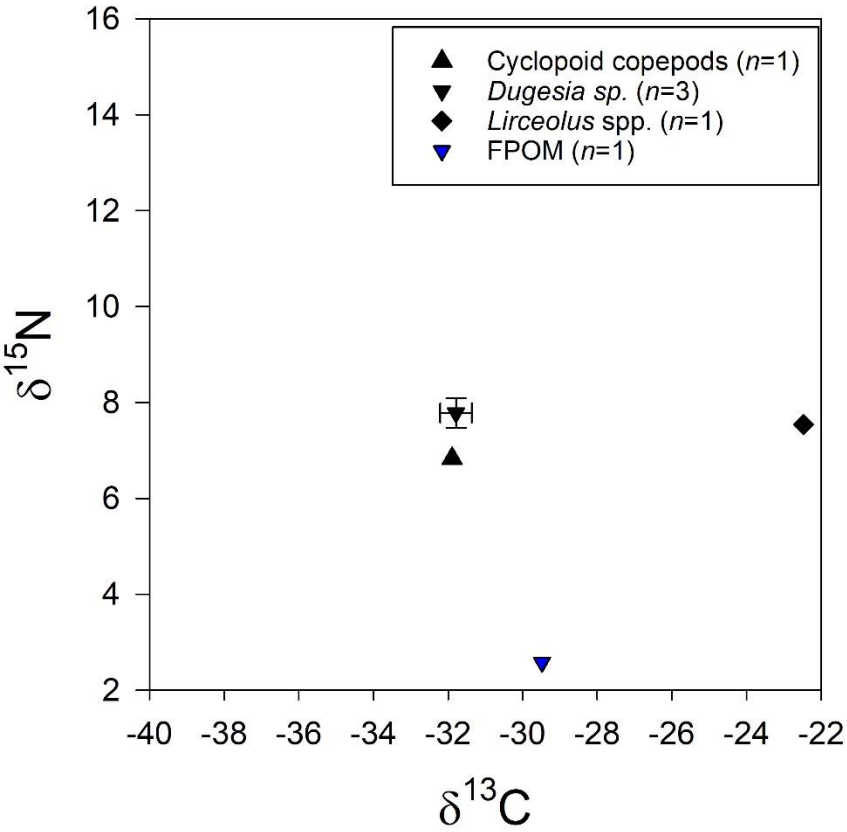

Figure 8. Isotope (δ13C, δ15N) biplot for Upper Barton Springs. Point = mean of each taxa, Bars = ±1 SD, FPOM = fine particulate organic matter. Sample size (N) is in parentheses in the figure legend

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