

BENTHIC MACROINVERTEBRATE METACOMMUNITY STRUCTURE OF THE
GUADALUPE RIVER BASIN, TX

by

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
CHAPTER	
I. INTRODUCTION	1
II. METHODS.....	5
III. RESULTS	12
IV. DISCUSSION.....	17
APPENDIX SECTION.....	32
LITERATURE CITED	38

LIST OF TABLES

Table	Page
1. Site names, the major river they are located on, and the coordinates for sample sites in the Guadalupe River Basin	24
2. Total number of taxa found in each stream of the Guadalupe River Basin	25

LIST OF FIGURES

Figure	Page
1. The Guadalupe River Basin outlined in black in Texas with study sample sites along each major river: Guadalupe, Comal, Blanco, and San Marcos Rivers in 4 Ecoregions: Edwards Plateau, East Central Texas Plains, Texas Blackland Prairies, and Western Gulf Coast Plains	26
2. Principal components analysis (PCA) of 4 variable groups used in the study	27
3. Functional feeding groups of BMI across the Guadalupe River Basin	28
4. Redundancy Analysis (RDA) biplots of 4 variable groups used in the study with genus level taxa identification.....	29
5. Redundancy Analysis (RDA) biplots of 4 variable groups used in the study with family level taxa identification.....	30
6. Variance Partitioning Redundancy Analysis (RDA) for variables sets compared with space.....	31
7. Average Shannon's Diversity Index (H') of each river in the Guadalupe River Basin.....	31

ABSTRACT

Benthic macroinvertebrates (BMI) are widely used as bio-indicators for local in-stream quality. However, local community structure can be affected by smaller scale (local) environmental conditions and larger scale processes. I assessed the abundance and diversity of BMI and their relationship with local in-stream conditions, regional patterns of land-use/land cover (LULC), and large scale physiographic gradients across the Guadalupe River System, a large basin (3,256 km²) in Central Texas. Macroinvertebrates, water quality, and habitat conditions across 28 sites in the Guadalupe River and its main tributaries were sampled. Highest species diversity occurred near the headwaters, and decreased downstream. Pollution tolerance levels increased downstream and followed an increasing agriculture gradient. Landscape factors explained a large proportion of variation in macroinvertebrate community structure (38%), but 16% of it was spatially structured (shared with spatial factors latitude and longitude) and 4% was explained by spatial factors alone. Local environmental factors were strongly correlated with landscape factors and explained similar amount of variation as landscape factors. My study highlights the importance of incorporating physiographic gradients when examining local and regional diversity and composition of BMI communities, especially in large complex watersheds. My results will help develop more effective monitoring programs for larger river systems.

I. INTRODUCTION

Biological monitoring is a widely accepted survey methodology to evaluate the ecological health of rivers and streams (Barbour *et al.* 1999). Benthic macroinvertebrates (BMI) are often used as bioindicators for environmental health and quality for a variety of reasons. BMI are relatively abundant and easy to sample, have different tolerances to changes in pollutants and water quality, and have a low mobility, thus the composition and diversity of BMI communities are thought to reflect local conditions (Metcalf 1989, Cairns & Pratt 1993, Barbour *et al.* 1999). Community composition also reflects environmental conditions integrated over longer periods of time rather than measurements of physico-chemical conditions that represent snapshots in time (Barbour *et al.* 1999).

Macroinvertebrate community composition and diversity at a given location a certain point in time is a function of processes which occur at a variety of spatial scales (Parsons *et al.* 2003). Recently, it has been suggested, that the examination of BMI communities within and across riverine drainages should be performed within a metacommunity context (Brown *et al.* 2011). A metacommunity is generally defined as a set of communities composed of interacting species which are spatially connected via dispersal (Wilson 1992, Leibold *et al.* 2004). One of the principle assumptions of BMI biomonitoring is that local communities are primarily controlled by local environmental conditions. However, according to metacommunity theory, whether a community composition is an accurate reflection of local environmental conditions (as assumed in BMI monitoring) depends on dispersal rates. For example, high dispersal (or mass effects) can override the importance of local factors, while limited dispersal may prevent

species from reaching suitable habitat patches (Winegardner *et al.* 2012). Many aquatic insects have high dispersal abilities due to their terrestrial winged adult stage, hence regional aspects must be considered when examining the metacommunity structure of BMI (Welborn *et al.* 1996, Brown *et al.* 2011, Cañedo-Argüelles *et al.* 2015).

Although the metacommunity theory is highly relevant to riverine BMI communities, its application to these systems presents several substantial challenges. In riverine systems, habitat does not occur in discrete patches; habitat patches are linearly distributed along a continuous dendritic network which strongly dictates the dispersal of organisms, e.g., through larval drift (Brown *et al.* 2011). Macroinvertebrate communities are also complex with a large number of species occupying different trophic levels and organisms show ontogenetic changes in dispersal behavior (Brown *et al.* 2011). Many aquatic insects, for example, can actively seek out suitable patches as winged adults across terrestrial habitat, but drift passively during their larval stage depending on flow in the river (Cañedo-Argüelles *et al.* 2015).

A number of studies have examined the effect of a wide range of both local environmental and regional factors on these BMI metacommunity structures. Several studies determined that a diversity of local and regional factors can be important in influencing macroinvertebrate distribution in riverine networks, including river width and depth, velocity, conductivity, pH, dissolved oxygen (DO), temperature, substrate, chlorophyll a, dissolved organic carbon (DOC), ammonium, phosphorus, non-volatile suspended solids, surface run off, macrophytes, substrate, slope, precipitation, land cover, and riparian and catchment scale land cover (Gore, J.A. 1978, Corkum, L.D. 1989, Allan 2004, Mykrä *et al.* 2007, Vanschoenwinkel *et al.* 2007, Grönroos *et al.* 2013, Dallas &

Drake 2014). However, the relative importance of local versus regional factors in influencing BMI community composition and diversity can vary spatially across landscapes. For example, Brown and Swan (2010) showed that local abiotic factors are more important and have a larger effect on macroinvertebrate communities in the headwaters of a watershed. In contrast, regional dispersal effects were found to be more important for metacommunity structure at mainstem sites where high dispersal can override local effects (Brown and Swan 2010). Typically, in a riverine network, higher diversity can be found at confluence points in a network and in the lower reaches of networks, and that community composition in headwaters differ more than elsewhere in the network (Altermatt 2013). Indeed, environmental heterogeneity increases downstream in riverine mainstems, which offers a greater habitat range for macroinvertebrates (Brown and Swan 2010, Cañedo-Argüelles *et al.* 2015). Thus, in order to design effective monitoring plans, it is important to identify the crucial parameters that determine the distribution of BMI and to understand the relative importance of local vs. regional factors, and land use vs. physiographic gradients.

The purpose of this study is to analyze and evaluate BMI communities in the Guadalupe River Basin in central Texas in a metacommunity context and to identify the parameters which most strongly affect their distribution. The Guadalupe River Basin encompasses a pronounced regional physiographic gradient, including four ecoregions (i.e., the Edwards Plateau, the Texas Blackland Prairies, the East Central Texas Plains, and the Western Gulf Coastal Plain) and a variety of land use patterns, which are likely affect local environmental conditions and the spatial distribution of macroinvertebrates. All ecoregions can be characterized by homogeneity associated with both abiotic – soils,

vegetation, geology, climate, and physiography (Omernik 1987, Griffith *et al.* 2006), and biotic factors, including algal coverage. The relatively large scale of the basin, the regional variation in environmental conditions, ecoregions and land use), and the overall BMI diversity across the drainage presents a unique opportunity to examine the relative importance of local and regional factors in structuring BMI communities. My general predictions were: (1) Species diversity will be greater in the Lower Guadalupe (mainstem) portion of the drainage when compared to headwater and tributary sites, (2a) spatial factors will be more important when determining community composition than local environmental factors, and (2b) landscape-level factors (regional physiographic and land use/land cover patterns) will explain more variation in BMI community composition between sites than local abiotic conditions.

II. METHODS

Study Area

The Guadalupe River Basin is a relatively large river system that spans 3,256 km² across central and southeastern Texas (Fig. 1). The greater Guadalupe watershed contains four major rivers systems: the Comal, the Guadalupe, the Blanco, and the San Marcos. The Comal and the San Marcos rivers are highly influenced by spring flows, while the Guadalupe and Blanco are less spring-influenced. The greater Guadalupe basin spans across four EPA Level III ecoregions: the headwaters of the Guadalupe reside in the Edwards Plateau (EP) and the mainstem of the Guadalupe then crosses the Texas Blackland Prairies (TBP), the East Central Texas Plains (ECTP), and the Western Gulf Coastal Plains (WGCP) before discharging into the Gulf of Mexico. The headwaters in the EP is dominated by karst limestone geology and surface waters flow in a south-easterly direction into the mainstem and to the other ecoregions in the drainage. Due to its position in a karst landscape, many headwaters and stream reaches are strongly spring-influenced, containing clear water with high physicochemical stability. Moving across the basin in a south-easterly direction, the drainage crosses the TBP, which is dominated by clays and silty soils and contains larger fraction of cropland and urban space. The ECTP is largely composed of mostly savanna and is used mostly for pasture. The WGCP is topographically flat and lies adjacent to the Gulf of Mexico and land use includes mostly cropland and urban areas. Rainfall varies substantially across the basin, ranging from 558.8 - 863.6 mm/yr in the EP to 660.4 - 939.8 mm/yr in the WGCP.

In this study, I collected data on BMI and environmental conditions at 28 sites across the basin between June 2015 and October 2015, with each individual river basin

within the drainage having between 2 – 16 sampling sites; the number of sites sampled per river is dependent upon the total length of the river in the greater Guadalupe drainage (Fig. 1; Table 1). Individual sampling sites were selected to encompass the range of physiographic conditions and LULC types which are present in the basin.

Field Methods

At each sampling location, environmental data and macroinvertebrates were collected within a riffle. Riffles are the most ideal mesohabitat to sample when evaluating BMI metacommunities as it has been shown to consistently contain higher macroinvertebrate diversity (Brown & Brussock, 1991, Barbour *et al.* 1999). In addition, many environmental monitoring programs focusing on BMI communities focus on riffle areas (TCEQ 2014). At each site, I measured pH, temperature (°C), dissolved oxygen (DO; mg/L), conductivity ($\mu\text{/cm}^2$), using a multiparameter probe (YSI 556). I also measured water velocity (m/s) using a Hach Company flow meter (FH950) at 60% of the depth. At each sampling location, I visually estimated the percent sediment size composition using a modified Wentworth scale (Wentworth 1922) and percent algae cover using a viewing window. Duplicate water samples were taken at each sampling location using clean 2-L brown Nalgene bottles which were rinsed three times with site water before sample collection. Water samples were kept in a cooler with ice until transport to the lab at Texas State University, where samples were filtered and preserved within 48 hours of collection. Samples were stored at -20° C until analysis.

Macroinvertebrate samples were collected using a 500- μm aperture Hess sampler (surface area = 0.46 m^2). At each sampling site, three Hess samples were collected from within the larger riffle area. During Hess sampling, sediments were agitated for a 2-

minute interval. Samples were sieved in a 125 μm sieve in the field to reduce small particles and organic matter (OM) and preserved in 95% ethanol (EtOH) in Whirlpack bags until sorting in the lab.

Laboratory Methods

Water samples were filtered to determine the concentration of dissolved cations and anions, ammonium (NH_4^+), soluble reactive phosphorus (SRP, assumed to be PO_4^{3-}), total suspended solids (TSS), non-volatile suspended solids (NVSS), and suspended chlorophyll *a*. Chlorophyll *a* samples were filtered onto Gelman A/E glass-fiber filters, frozen, extracted with acetone for 8 hours in the dark, and measured using a Turner Designs Trilogy fluorometer. Dissolved nutrients were determined from water filtered through pre-ashed Gelman A/E filters. Nutrient concentrations were measured with a Varian Cary 50 UV-Vis spectrophotometer. SRP was determined with the molybdenum blue method (Wetzel and Likens 1991). NH_4^+ was determined with the phenate method (Solorzano 1969). NO_3^- was determined with second derivative UV spectroscopy (Crumpton *et al.* 1992). DOC samples were filtered through pre-ashed Whatman GF/F filters, and the filtrate was analyzed on a Shimadzu TOC-VCSH total organic carbon analyzer. TSS and NVSS concentrations were determined by filtering water onto precombusted and pre-weighed Gelman A/E filters and drying filters at 60°C for 48 h and reweighing to determine TSS. Filters were subsequently ashed and reweighed to calculate NVSS (Knoll *et al.* 2003).

Macroinvertebrate samples were picked under a stereomicroscope (Nikon SMZ745T) and identified down to the lowest practical taxonomic level (typically genus)

using relevant several taxonomic keys (Merritt *et al.* 2008, Diaz 2014, P. Diaz, US Fish and Wildlife Conservation Office, unpublished data). A total of 59 macroinvertebrate taxa were identified including 6 non-insect taxa (Table 2). These taxa were identified down to order, while all other taxa were identified down to the genus-level (except Diptera, which was identified to family). Hirudinea, Trombidiformes, *Hydropsyche*, *Ochotrichia*, *Mayatrichia*, *Triaenodes*, *Nectopsyche*, *Polycentropus*, *Atopsyche*, *Tropicus*, *Haliphus*, *Petrophila*, *Neoelmis*, *Stenelmis*, *Pubraphia*, *Enochrus*, *Lutrochus*, *Limnocoris*, Tipulidae, and Stratomyidae were excluded from analysis because they contained <5% of taxa at all sites (Zhao *et al.* 2017).

Data Analysis

Land cover data was downloaded from USGS and overlaid on sample site locations in ArcGIS v10.4 using the National Land Cover Database (2011 version). LULC was determined as percent composition among 20 categories: developed open space, developed low intensity, developed medium intensity, developed high intensity, open water, perennial ice/snow, barren land (rock/sand/clay), deciduous forest, evergreen forest, mixed forest, dwarf scrub, shrub/scrub, herbaceous grassland, herbaceous sedge, lichens, moss, pasture/hay, cultivated, woody wetlands, and emergent herbaceous wetlands (National Land Cover Database 2011 Product Legend; <https://www.mrlc.gov/nlcd2011.php>). Three spatial scales were examined based on Allan 2004 and Becker *et al.* 2014: (1) reach scale with land cover in a 100-m buffer on either side of the river with a 2km buffer upstream from each site; (2) riparian scale with land cover in a 100-m buffer for total distance upstream for each site; and (3) catchment scale

with land cover for the whole watershed upstream of the lowest site. I followed the procedure outlined in Becker *et al.* (2014) to combine all land use/land cover into 8 categories, keeping the forest covers separate: urban, cultivated, evergreen forest, deciduous forest, mixed forest, rangeland, wetlands, and open water. Barren land was removed from any analyses because it made up <1% of the coverage area (Dodds & Oakes 2008, Becker *et al.* 2014). Ecoregions for each site were determined based upon US Environmental Protection Agency Level-III Ecoregions and downloaded from the EPA (Griffith *et al.* 2004) and overlaid across the Guadalupe River Basin in ArcGIS. Estimates of river slope were generated using a digital elevation model (DEM), and distances between sites were evaluated by using a river network map in ArcGIS. Precipitation data was obtained Texas Parks and Wildlife Department and reported as the mean annual rainfall from 2000-2010 at each sample site.

I grouped all predictor variables into four different groups: (1) spatial (latitude and longitude of each site), (2) local environmental conditions (specific conductance, temperature, dissolved oxygen, pH, velocity, depth, channel width, TSS, NVSS, Chlorophyll *a*, nutrient concentrations, % algal coverage, and substrate size distributions), (3) physiographic factors (ecoregion of each site, mean slope for each site, and annual precipitation), and land use land cover (LULC of each site at the three aforementioned scales). Prior to analysis, I averaged values obtained from duplicate water samples for each analyte from each site prior to analysis. BMI data from the three riffle samples at each sampling location was pooled prior to analysis.

I initially ran principal components analysis (PCA) to explore each of the predictor set gradients across the drainage and to determine patterns of variation in the

predictor variable sets. In order to avoid issues with multicollinearity in subsequent analyses, predictor variables which were highly correlated were removed from the predictor data sets. Mean, maximum, minimum, and point slope estimates were highly correlated, so mean slope was selected for further analyses. TSS and NVSS were also highly correlated, thus TSS was used in further analyses. I also ran a Pearson correlation matrix for each group of predictor variable data set, and it revealed that the riparian and catchment scales for LULC percent coverage were highly correlated for nearly all variables, so I removed the riparian LULC scale from further analyses.

A redundancy analysis (RDA) was used to determine variation composition (Legendre and Legendre 1998) and to assess any specific correlations among spatial data, physiographic factors, and local factors across the watershed with the relation to BMI community composition. This analysis explains several different variations: (1) the variation of each set of variables, (2) the variation of each set of variables after reducing the effects of the other variables (e.g., the amount of variation of BMI after removing the effect of river basins), and (3) the variation between shared sets of variables.

Randomizations calculated the significance of the variances, but only for types (1) and (2) (Legendre and Legendre 1998). Variables were not transformed, but species were Hellinger transformed to reduce the weight given to rare species (Legendre & Gallagher 2001). Significance was inferred at $\alpha \leq 0.05$.

In order to determine how much variation in community composition could be explained by local vs. regional factors, and land use vs. physiographic gradients, I did the following Pairwise Variance Partitioning Comparisons: (1) space vs. LULC, (2) space vs. physiographic factors, (3) LULC vs. physiographic factors, (4) space vs. local

environmental factors, (5) local environmental factors vs. physiographic + LULC combined (large scale factors) (Table 3). It should be noted that all four groups of variables could not be tested together, as high collinearity would have resulted in negative R^2 values for shared variation. All analyses were performed in R (version 3.4.0) using the package *vegan*.

Tolerance values and functional feeding groups (FFG) were determined using the identification key by Merritt, Cummins, and Berg (2008). As a wide range of tolerance values was found in every site, percent tolerance value was calculated by taking the sum of all taxa with a tolerance value <6 and dividing by the sum of all taxa with a tolerance value ≥ 6 (Harrison, 1996, TCEQ 2014). I combined similar functional feeding groups (FFG) (facultative and non-facultative) until I had four: collectors, shredders, predators, and scrapers. An Index of Biotic Integrity (IBI) was calculated according to Harrison (1996) and TCEQ (2014) to determine the aquatic life use index (ALU). IBI assesses the degradation of fish and BMI assemblage due to water quality changes and report them as either exceptional, high, intermediate, and limited (Harrison, 1996, TCEQ 2014).

III. RESULTS

Environmental variables

When examining study site gradients, the PCA for local environmental variables accounted for 40.8% of the variation among sites on the first 2 components (Fig. 2a, b). PC 1 explained 25.7% of the variation among sites and showed a gradient that ranged from the southeast portion of the water to the northwest (downstream to upstream). It stated that high total suspended solids (TSS), non-volatile suspended solids (NVSS), Chl *a*, ammonium (NH₄⁺), and SRP were found in sites that had a small sediment size (sand and silt), while sites with more gravel and a higher velocity were located in the lower Guadalupe River (mainstem). Benthic algal coverage was highest on boulder and cobble substrate, as well as having a high DO concentration closer upstream in the Blanco, Comal, and upper Guadalupe Rivers.

Regional variables

The PCA for physiographic variables accounted for 80.7% of the variation among sites on the first 2 components (Fig. 2c, d). PC 1 explained 65.2% of the variation alone and showed that slope decreased and precipitation increased with longitude and ecoregion from the northwest to the southeast, an upstream-downstream gradient in the water basin. The PCA for LULC variables explained 56.0% of the variation among sites on the first 2 components (Fig. 2g, h). PC 1 explained 34.4% of the variation among sites and showed urban and evergreen forest cover at the reach and riparian scales were more prevalent in the middle portion of the basin, covering the TBP and ECTP, while rangeland at the reach and riparian scale and evergreen forest cover at the catchment

scale were higher in the upper part of the basin in the Edwards Plateau. The lower portion of the watershed in the WCTP contained open water, agriculture, and wetlands at all 3 scales. One site, the headwaters of the San Marcos River, was an outlier, most likely because its catchment size LULC was so small.

Macroinvertebrate spatial patterns

The lowest diversity was observed in the Blanco river at the furthest downstream site with both Shannon's and Simpson's Indices for diversity at 1.12 and 2.01 respectively. Diversity indices indicated the highest diversity 90 km downstream from the headwaters of the Guadalupe (2.25 and 7.23, for Shannon and Simpson's diversity index respectively), San Marcos (0 km from the headwaters; 2.23 and 6.54), Blanco (31.9 km from the headwaters; 1.57 and 3.06) and Comal (0 km from the headwaters; 2.10 and 5.77). Macroinvertebrate density ranged from 456 individuals per square meter (Upper Blanco) to 13986 individuals per square meter (mid-Guadalupe, 7.13 km from the San Marcos influx), with the highest densities found in the middle of the river then generally decreasing downstream. After running an ANOVA to determine the difference of diversity between rivers, it was determined that the Blanco had a considerably lower diversity than the rest of the basin, which did not differ (p -value = 0.0337) (Fig. 6). Chironomids were in low abundance throughout much of the basin, but high amounts (39%) were found on the Guadalupe, just upstream of the San Marcos influx, approximately 19 kilometers from the first site after the influx of the San Marcos River. Coleopterans were present at every site (less than 10%), but highest in the upper and lower (mainstem) Guadalupe, and the lower San Marcos Rivers (15-30%) and the Comal River had the greatest concentration of Coleopterans with 42% at the spring head.

Plecopterans were mainly found in the lower section of the mainstem of the basin, but they were also present within the first 127 km of the Guadalupe from the headwaters, and then again at the site 92 km upstream from the first site after the San Marcos tributary, where they become present once more.

Functional feeding groups (FFG) were examined and found to vary throughout the basin. I found that the majority of collector-filterers were in the Blanco River and the headwaters of the Guadalupe and Comal Rivers (Fig. 3). Scrapers made up the majority of the Guadalupe (both upper and the mainstem), and the entirety of the San Marcos River (Fig. 3). Collector-gatherers were present in roughly equal amounts throughout the basin. Shredders were only heavily present in the headwaters of the San Marcos River, where they made up 41% of the community composition as compared to the rest of the watershed (0-3%) (Fig. 3). Predators did not change noticeably across the basin, but the Blanco had consistently higher percentages of collectors (73-84%), while scrapers were highest in the both the upper and lower Guadalupe (14-39%) (Fig. 3).

A general decrease of % Tolerance values occurred downstream, where values <6 were less tolerant and those ≥ 6 are more tolerant to water quality degradation (Harrison, 1996, TCEQ 2014). The most downstream sites of the Blanco River had the highest value (7.5), while mid-Guadalupe (just before the San Marcos influence) had the lowest (0.3). This helped aid in determining the ALU for each site. Overall, the Guadalupe has an intermediate Aquatic Life Use (ALU), with high ALUs mid-river. The most downstream site of the Blanco was the only site that had a limited ALU (20).

BMI community patterns vs. large scale and small scale factors and spatial data

When examining genus-level taxa, landscape factors explained 38% of the variation in macroinvertebrate community composition, but 16% of those factors were spatially structured (shared variation with space) (Fig. 6a). Landscape factors included physiographic and LULC factors and, when tested separately, LULC alone explained the same amount of variation in BMI community composition as all landscape factors combined (22%), whereas physiographic factors only explained 1% alone (pure effect) (Fig. 6a). All effects were significant ($p < 0.05$).

Local environmental factors at the genus-level explained slightly less than landscape factors (36%) and 17% was shared with spatial factors. Spatial factors alone explained little additional variation when compared with landscape or LULC (4%), local environmental (2%) or physiographic factors (6%) factors. (Fig. 6a). Similar patterns were found in family-level taxa (Fig. 6b).

When comparing LULC reach vs. catchment scale factors, catchment scale explained slightly more (29%) of the variation in community composition vs. 24% explained by reach scale, while 15% of the variation was shared between catchment and reach-scale LULC factors when run against space.

Spatial differences as apparent in the biplots (Fig. 4) were associated with certain genera. The net-spinning caddisfly *Chimarra* was more abundant in the Blanco River, which is an area with evergreen forest cover at a catchment scale and has a steeper slope. The stonefly *Neoperla* was most abundant in the mainstem, which also contained high mixed forest cover at a reach scale (Fig. 4g, h). Chironomids and other pollution tolerant taxa were more prevalent in the areas of the watershed that contained a higher percentage of rangeland and agriculture (Fig. 4g, h). Similar patterns were seen when examining

BMI identified at the family level (Fig. 5), with Philopotamidae being more abundant in the Blanco River and Perlidae in the Lower Guadalupe mainstem.

IV. DISCUSSION

To the best of my knowledge, this is the first study that looks at variation in community composition of macroinvertebrates across a watershed in Texas. In contrast to my first prediction the highest alpha diversity was found closer to the spring sites (headwaters) in each reach, rather than in the mainstem (Lower Guadalupe). The importance of headwaters for stream network biodiversity was suggested by Finn *et al.* (2011) based on a meta-analysis of biodiversity of streams across the globe. The study however focused on beta-diversity (among-site variation) as alpha-diversity usually increases along streams. Headwaters generally have been found to have lower diverse communities because these communities do not have environmental factors that occur at a wide range, unlike mid-sized streams (Vannote *et al.* 1980). In addition to a greater range of habitats in mainstem, the drift of macroinvertebrates may increase the abundance and number of species downstream (Brown and Swan 2010, Altermatt 2013, Cañedo-Argüelles *et al.* 2015). A possible explanation for the opposite finding in our system of higher alpha diversity near the springs may be due to the potential overlap and presence of two ecological communities (species with affinity to spring systems and riverine communities, called ecotones (Ghiselin 1977). Most non-insect taxa in the study, many of which may have lower dispersal abilities as compared to insects, resided in the headwaters. Spring-fed systems are unique, especially in Central Texas in that they remain stable in their environment. While habitat in other parts of the stream network may have to be re-colonized after drying out or being flooded, spring-systems may provide a refugia especially for species with low dispersal abilities. The sites near the headwaters could also have a higher diversity due to fewer predators, as well as the

presence of taxa found only in the spring headwaters, which cannot handle the less stable environment of the mainstem and contain a larger amount of intolerant species, where the mainstem contains more tolerant species. However, it is important to note that the site that contained the highest species diversity for the basin was not at the spring head (as Comal, San Marcos, or Blanco River which also had high diversity), but rather approximately 90.1 km from the Guadalupe River spring head (upper Guadalupe). The upper reaches (Blanco and Upper Guadalupe) are mostly boulder and cobble substrate, which may offer better refugia from predators in these areas. The upper Guadalupe had larger deciduous and evergreen forestry coverage, whereas the San Marcos tributary had more urban land use cover, which may explain the difference in diversity between these areas as only species tolerant of urban impact may occur in the San Marcos tributary.

The impact of differences in land use have been shown by other studies. For example, Sponseller *et al.* (2001) found that large scale spatial factors, such as LULC, may have an effect on macroinvertebrate assemblages downstream and cause diversity to decrease with the percentage of non-forest coverage. Forest cover at small streams has been shown to remove 90% of particulate matter that could accumulate from the surrounding agricultural land, thus having a large impact on macroinvertebrate communities (Sponseller *et al.* 2001, Peterjohn & Correl 1984). EPT generally decline with increased urbanization, while Chironomids and Simuliids, because of their high pollution tolerance, tend to increase (Feld & Hering 2007). However, the site in the Guadalupe River Basin with the lowest Shannon's and Simpson's diversity index (1.12 and 2.01 respectively) was located downstream in the Blanco River, just before it enters the San Marcos River. This site also contained the highest percentage of wetland

coverage at the reach scale. Wetlands in this basin were shown to have a higher velocity (Fig. 2), which might have affected the passive dispersal of the BMI at this site since dispersal is controlled by local factors (Tomanova *et al.* 2007, Grönroos *et al.* 2013, Padail *et al.* 2014). This might have caused the more passive dispersers in this site to be swept past this site and lowering the diversity.

I found that landscape variables such as evergreen forests at the reach scale, and agriculture and wetlands at the catchment scale explained a significant proportion of variation in community composition in the Guadalupe River basin (Fig. 4c, d, g, h, Fig. 6a). This could at least partly be also attributed to differences in ecoregion and large-scale biogeographic differences. For example, Plecopterans (generally a scraper) became most abundant in the Guadalupe River after the confluence with San Marcos tributary. This could be due to these areas containing the most algae cover (stoneflies major food source) or biogeographic differences.

Most of the forest cover was upstream, in the Edward's Plateau ecoregion, where the slope is higher and the precipitation is lower. As the water flows downstream towards the mainstem, however, the slope decreases and the precipitation increases as the forest cover is replaced with agricultural land and wetlands (Fig. 4c, d, g, h). Most of the evergreen forest cover is in the headwaters and most of the agriculture and wetland coverage is in the lower, wetter regions (Fig. 4g, h), which can influence much of the local factors (Atkinson *et al.* 2012, Sponseller *et al.* 2001, Wang *et al.* 1997). My data shows that areas that receive more precipitation contain a larger amount of ammonium (NH_4^+), which could be due to the surrounding wetlands and agricultural input. These

areas also contained the least amount of taxa (Fig. 4). This could be because those taxa that are intolerant cannot exist there.

Vannote *et al.*'s (1980) River Continuum Concept (RCC) states that headwaters are generally influenced by the riparian zone because they have the most interface with the surrounding landscape and therefore contain more shredders due to the large amount of coarse particulate matter from the large influx of allochthonous material (Jiang *et al.* 2011). The Guadalupe Basin has headwaters in the Blanco and Upper Guadalupe that are surrounded by evergreen forests and rangeland, yet contained hardly any shredders (Fig. 3). The mainstem in the Lower Guadalupe is surrounded by open water, wetlands, and agriculture, and contained slightly higher percentages of shredders than the rest of the basin (0-3%) (Fig. 3). This could also be due to width of the streams in the headwaters. The RCC states that the headwaters are small enough that the shade of a single tree could expand across the width of the creek. However, the width of the headwaters in this system were larger (10-30 m), so the forest cover couldn't cover the entire width of the spring. This could allow the sun to penetrate the bottom of the stream and allow the algae to grow, which is the opposite of the RCC proposed by Vannote *et al.* (1980). The headwaters of the San Marcos River rests heavily on urbanized and mixed forestry areas (Fig. 2g, h). Coincidentally, this is also the site that contained the majority of shredders (41%), which partly agreed with the RCC because of the spring food source, and could also be because of the larger substrate that resides there (Jiang *et al.* 2011). Collectors and predators increased more in the mainstem of the Guadalupe, especially downstream of the San Marcos tributary, and could be due to the amount of finer substrate and more agricultural landcover (Jiang *et al.* 2011). FFGs can change drastically across a region,

especially longitudinally and with slope (Heino *et al.* 2002, Tomanova *et al.* 2007), but it is important to remember, however, that the RCC is made for species richness, not genus or family richness, and was developed for eastern and north-eastern parts of the United States, not the south with a vastly different landscape setting affecting physico-chemical conditions (Statzner & Higler 1985).

Many other studies (Cornell & Lawton 1992, Cottenie 2005, Vanschoenwinkel 2007, Zhang *et al.* 2014) have examined the importance of large scale, space, and local factors on macroinvertebrate metacommunities. While landscape factors were an important group of variables, there was also a significant overlap/correlation with local environmental factors. This makes sense as landscape factors across larger spatial factors will affect the local environmental conditions. For example, presence of wetlands can affect flow rates by reducing the frequency of floods because they provide water storage, and absence of forest coverage can affect substrate and temperature by providing shade and a barrier from the surrounding land-use entering the stream (Atkinson *et al.* 2012, Sponseller *et al.* 2001). Temperature generally increased downstream and correlated with a decrease in forest cover (0-24.11%) (Fig. 2a, b). The headwater of the San Marcos is spring-fed, which experiences little temperature variation year-round. Therefore, higher temperatures during summer when sampling occurred could only occur further away from the spring, The Lower Guadalupe River was also dominated by sandy substrate, which has been found to be low in fine particulate organic matter (FPOM) and therefore can contain the most macroinvertebrate abundance (Hawkins *et al.* 1982).

It has been stated that macroinvertebrates communities are related to local factors at small spatial scales (Heino *et al.* 2003, Vilmi *et al.* 2016). But my study shows that

both reach and catchment scale seemed to play a role in structuring the BMI community composition which agrees with the discovering of mussel communities (Atkinson *et al.* 2012) in Oklahoma and Arkansas and what Feld & Hering (2007) discovered about grassland/shrubland at the catchment scale. However, another study (Hawkins *et al.* 2000) found that community variation of macroinvertebrates was primarily controlled by local factors. Substrate is an important factor for macroinvertebrates and LULC cover at the reach scale was found to be a more important factor for substrate than catchment scale (Lammert & Allan 1999, Arbuckle & Downing 2002).

Our study found that both environmental and spatial factors played a role for the distribution of BMI. In contrast, other studies have stated that environmental factors are the most important driving force of macroinvertebrate community composition (Moritz *et al.* 2013), and that environmental gradients affect species community similarly independent with regards to position in the drainage (Heino *et al.* 2015).

Macroinvertebrate communities are important because they reflect the overall integrity of rivers and streams (Barbour *et al.* 1999). However, these communities can change drastically within the same watershed, especially between the headwaters and the mainstem of a river system due to changes in environmental factors (Brown & Swan 2010). Because of this, especially in large watersheds that contain a large amount of physiographic variation due to ecoregions, landscape factors and their influence on the local environmental factors need to be considered when performing BMI monitoring. Due to the high dispersal abilities of most aquatic insects, these macroinvertebrates can occupy the wide range of habitat that these local environmental factors create as stream order increases (Brown and Swan 2010, Cañedo-Argüelles *et al.* 2015) across different

ecoregions. This habitat difference can be somewhat attributed to the characteristics that are continuously changing from the headwaters to the mouth of the river, which can be reflected by the macroinvertebrate community composition (Vannote *et al.* 1980).

It seems that the scale factors that influence macroinvertebrate metacommunity variance is not uniform across the globe and differs with river systems. This study shows how important it is to determine the important driving factors of macroinvertebrate metacommunities because it highlights how different variables influence separate aspects of a watershed that drives BMI community composition. In the future, it would also be important to look at temporal aspects to determine how these communities change over time in response to the surrounding area.

TABLES

Table 1: Site names, the major river they are located on, and the coordinates for sample sites in the Guadalupe River Basin. Sites with an asterisk indicate sites where different microhabitats within riffles were also sampled.

River	Site	Latitude	Longitude	Ecoregion
Blanco	Blanco SP (BSP)	30.091446	-98.424527	EP
	Hwy 32 Bridge (Hwy 32)	30.020586	-98.330192	EP
	River Park (RP)	30.036898	-98.222908	EP
	G W Haschke (GWH)	29.990151	-98.199709	EP
	5 Mile Dam	29.937423	-97.895566	TBP
	River Rd	29.985228	-98.109433	EP
	Uhland Rd	29.894941	-97.902391	TBP
Comal	Comal Run	29.714103	-98.136483	TBP
	Comal Springs Run 3	29.713642	-98.136911	TBP
Guadalupe	River Bluff	29.946325	98.038756	EP
	Comfort	29.972356	-98.833456	EP
	474 Upper	29.894353	-98.670483	EP
	Rockin R River Rd	29.763706	-98.140193	EP
	Hwy 337	29.727833	-98.111568	TBP
	Cypress Bend Park (CBP)	29.713146	-98.106237	TBP
	FM 1117	29.536395	-97.881062	TBP
	Lake Wood Rec Area (LWR)	29.470057	-97.49112	ECTP
	Gonzales (GZ)*	29.48433	-97.48	ECTP
	Hochheim*	29.321589	-97.307042	TBP
	Cuero	29.150067	-97.316133	TBP
	DS of Cuero	29.058958	-97.252025	ECTP
	HWY 77	28.831306	-97.060653	WGCP
	Victoria (G)*	28.822229	-97.017445	WGCP
	HWY 59	28.74927	-97.00016	WGCP
San Marcos	Spring Lake Dam (SLD)	29.890197	-97.933845	TBP
	Martindale	29.849589	-97.857099	TBP
	Fentress	29.753256	-97.780298	TBP
	Luling TIFP	29.667881	-97.700478	ECTP

Table 2: Total number of taxa found in each stream of the Guadalupe River Basin. Baetidae was only identified down the family level for all except 3 sites because of the large amount and time constraints. The Upper Guad is separated from the Lower Guad from the influx of the San Marcos River

Order	Family	Genus	Blanco	San Marcos	Comal	Upper Guad	Lower Guad
Oligochaeta			114	7	33	153	6
Amphipoda	Hyaletellidae	Hyaletella	2	456	93	47	0
Hirudinea			0	0	1	1	0
Gastropoda			3	17	47	100	81
Planaria			39	32	18	121	0
Trombidiformes			3	1	0	1	0
Ephemeroptera	Baetidae		216	401	65	1007	602
		Pseudocloeon	0	0	0	0	101
		Camelobaetidius	0	0	0	0	50
		Falleon	0	0	0	0	455
		Baetis	0	0	0	0	6
	Leptophlebiidae	Thraulodes	381	822	9	564	418
		Traverella	130	178	2	167	3167
		Neochoropteres	56	87	0	102	106
	Leptoxyphidae	Tricorythodes	47	131	18	240	875
		Leptoxyphes	5	38	0	73	62
	Caenidae	Caenis	5	17	0	18	25
	Heptageniidae	Stenacron	352	2	0	22	14
	Isonychidae	Isonychia	788	185	13	528	94
Trichoptera	Hydropsychidae	Smicridea	88	103	2	65	67
		Cheumatopsyche	561	170	40	467	2604
		Hydropsyche	0	0	0	0	53
	Hydroptilidae	Hydroptila	3	0	122	3	319
		Ochotrichia	0	0	0	3	5
		Mayatrichia	0	0	0	13	9
	Leptoceridae	Oecitis	0	0	0	1	15
		Trienodes	0	0	0	0	3
		Nectopsyche	0	2	0	3	0
	Polycentropodidae	Polycentropus	3	1	0	4	2
	Philopotamidae	Chimmara	4728	183	120	303	0
	Hydrobiosidae	Atopsyche	8	9	6	0	0
Plecoptera	Perlidae	Neoperla	0	0	0	14	640
Odonata	Gomphidae	Phylogomphoides	6	13	0	6	79
	Corduliidae	Somatachlora	27	14	3	6	0
	Coenargionidae	Argia	215	18	15	52	4
Coleoptera	Psephenidae	Psephenus	3	9	143	10	0
	Elmidae	Phanocerus	0	1	1	39	0
		Hexacyllopus	72	213	46	256	1235
		Mycrocyllopus	2	24	125	5	0
		Neelmis	0	0	9	0	36
		Heterlemis	5	126	0	7	139
		Stenelmis	0	0	0	5	0
		Pubraphia	0	0	1	0	0
	Hydrophilidae	Enochrus	0	0	0	0	1
	Lutrochidae	Lutrochus	1	6	0	0	0
	Heteroceridae	Tropicus	0	0	0	0	0
	Haliplidae	Haliplus	0	0	0	0	0
Hemiptera	Naucroidae	Ambrysus	28	42	16	12	50
		Limnocoris	0	0	0	1	0
Lepidoptera	Crambidae	Petrophila	0	0	0	0	1
		Paraponyx	1	3	27	6	29
Megaloptera	Coryalidae	Corydalus	43	35	5	25	140
Diptera	Chironomidae		344	142	30	293	972
	Simuliidae		40	44	2	81	4
	Tipulidae		0	2	0	0	1
	Empididae		3	2	0	7	25
	Ceratogonidae		0	0	0	1	5
	Stratiomyidae		1	2	0	0	0

FIGURES

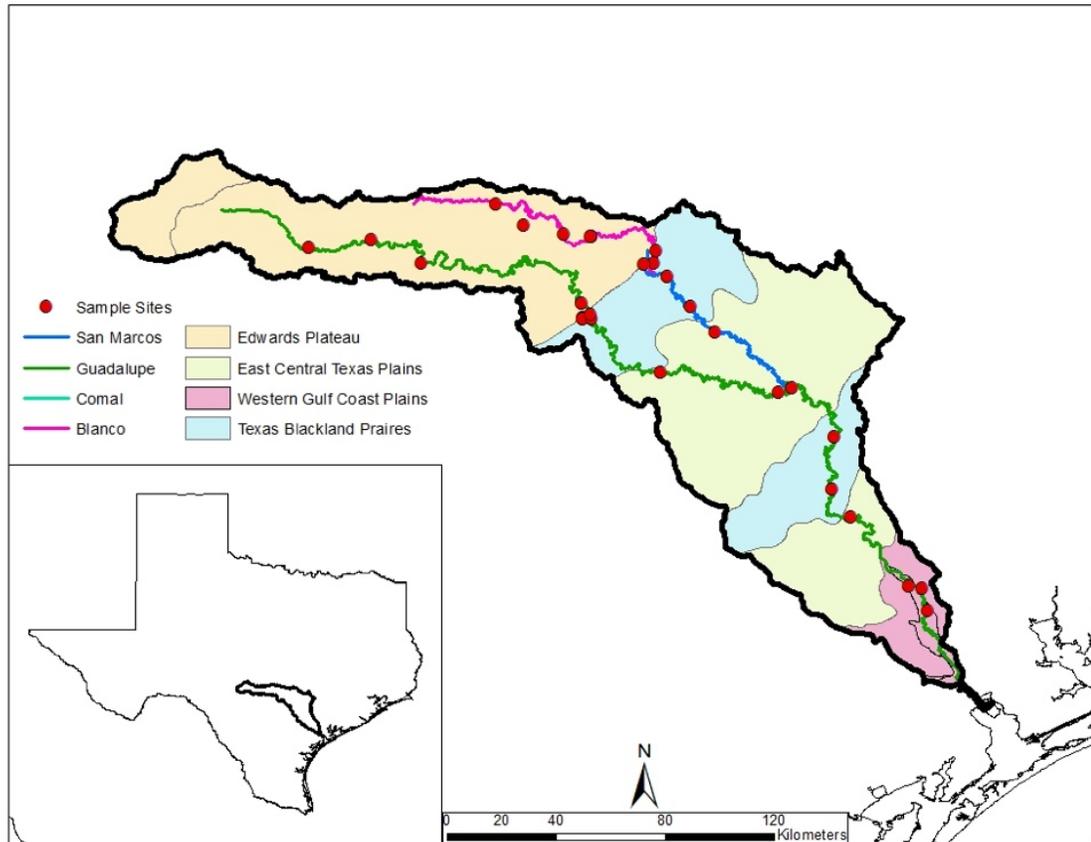


Figure 1: The Guadalupe River Basin outlined in black in Texas with study sample sites along each major river: Guadalupe, Comal, Blanco, and San Marcos Rivers in 4 Ecoregions: Edwards Plateau, East Central Texas Plains, Texas Blackland Prairies, and Western Gulf Coast Plains

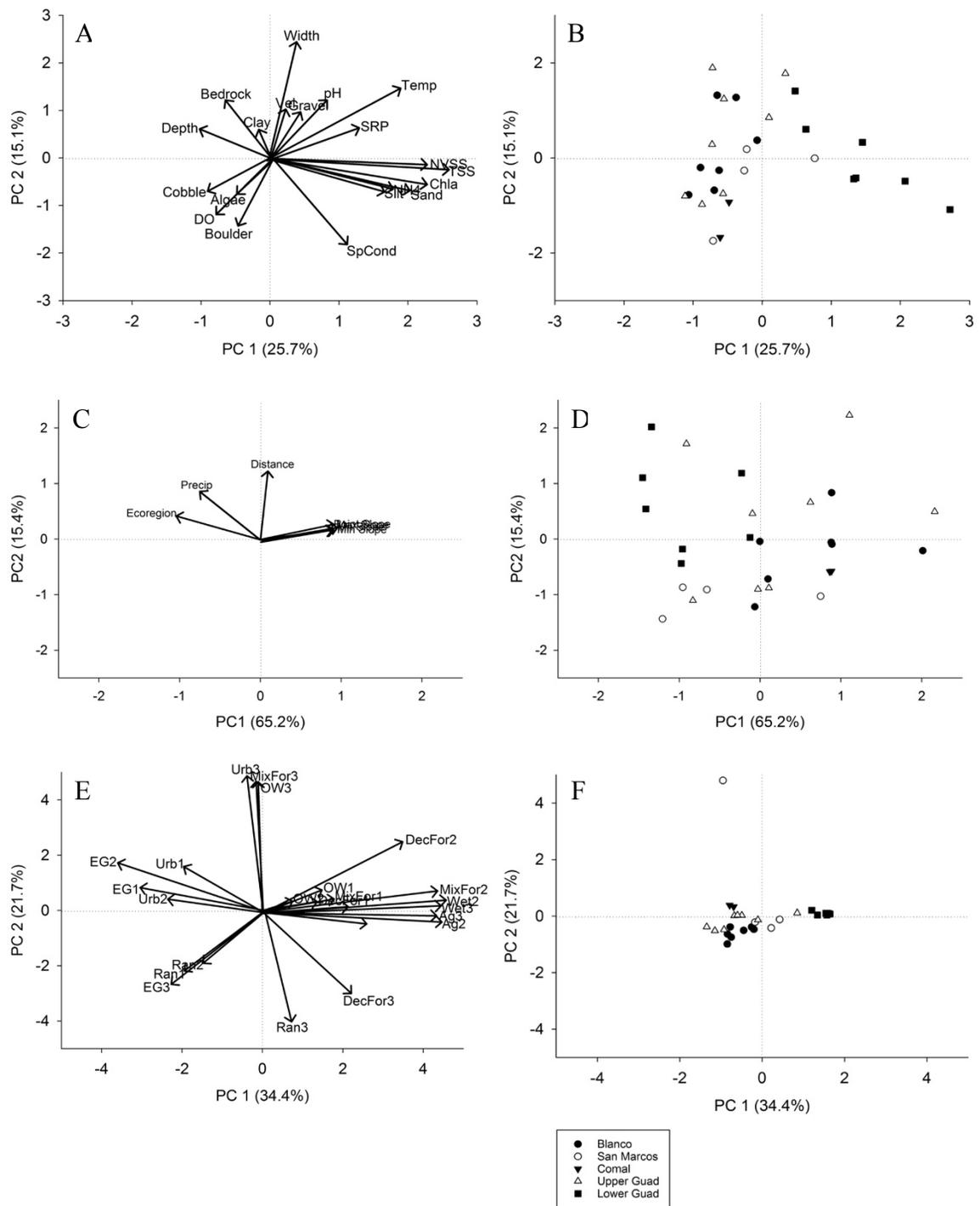


Figure 2: Principal components analysis (PCA) of 4 variable groups used in the study. A-Relationships among local factors. B-Ordination of sites with local factors. C-Relationship among physiographic factors. D-Orientation of sites with physiographic factors. E-Relationship of LULC factors at all 3 scales. F-Orientation of sites with LULC at all 3 scales. Space not shown because only 1 PC extracted

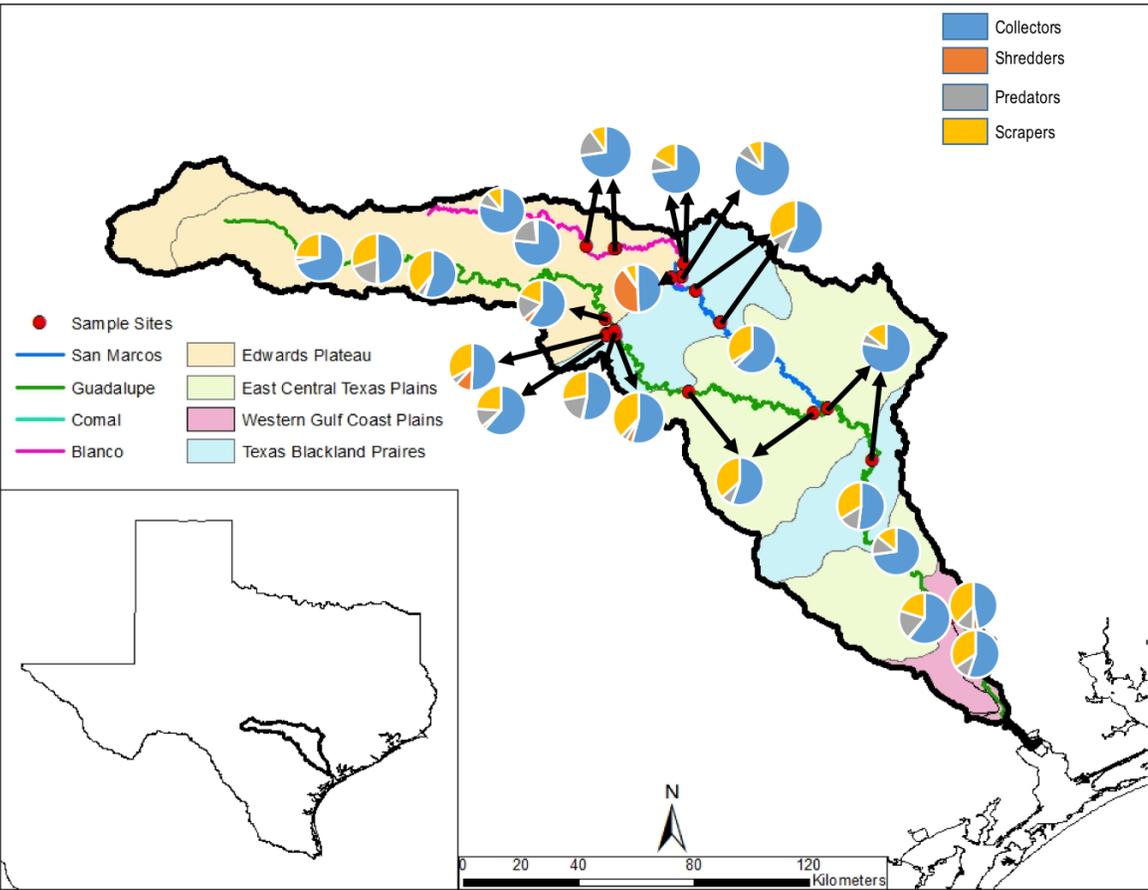


Figure 3: Functional feeding groups of BMI across the Guadalupe River Basin

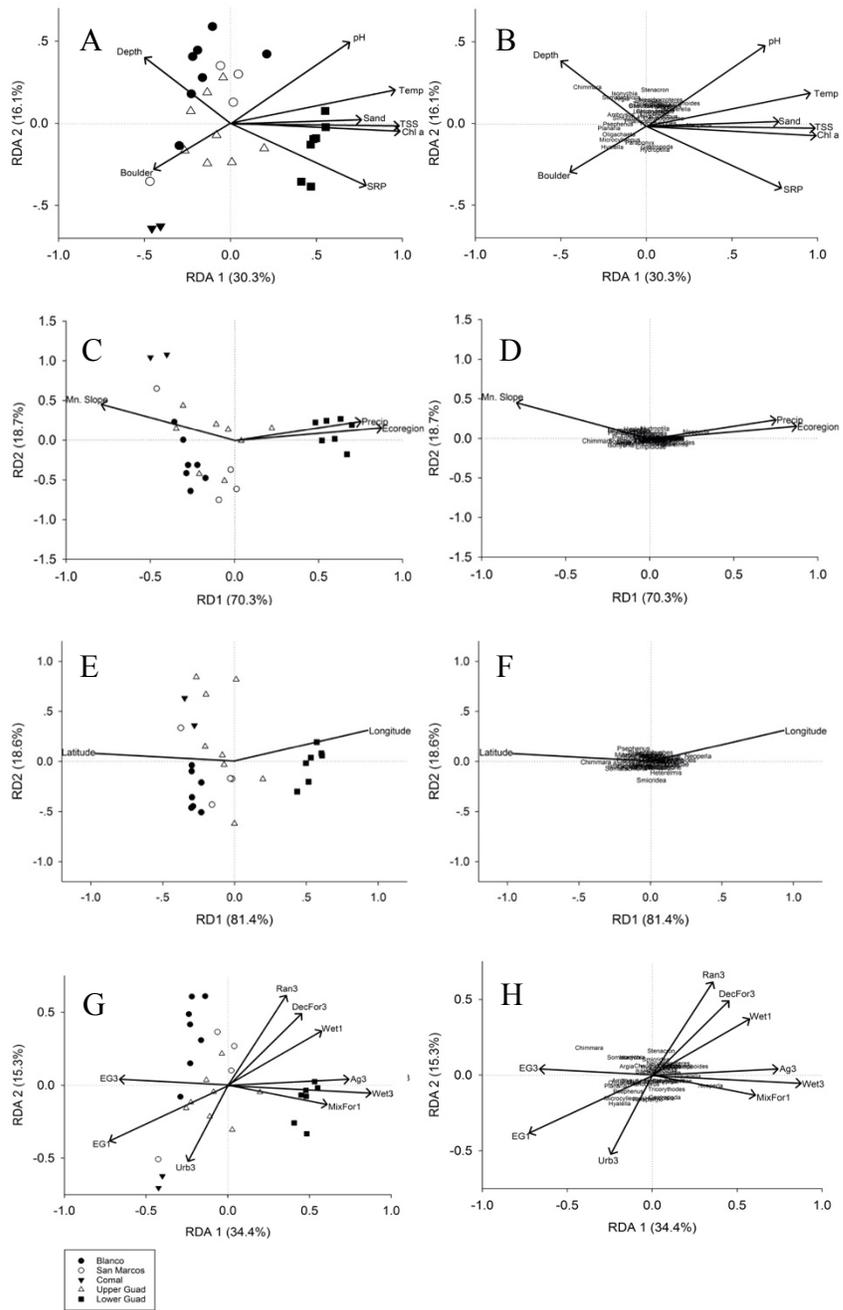


Figure 4: Redundancy Analysis (RDA) biplots of 4 variable groups used in the study with genus level taxa identification. A-biplot of sites in for significant local factors. B-biplot of genus taxa for significant local factors. C-biplot of sites for significant physiographic factors. D-biplot of genus taxa for significant physiographic factors. E-biplot of sites for significant space factors. F-biplot of family taxa for significant space factors. G-biplot of sites for significant LULC factors. H-biplot of genus taxa for significant LULC factors

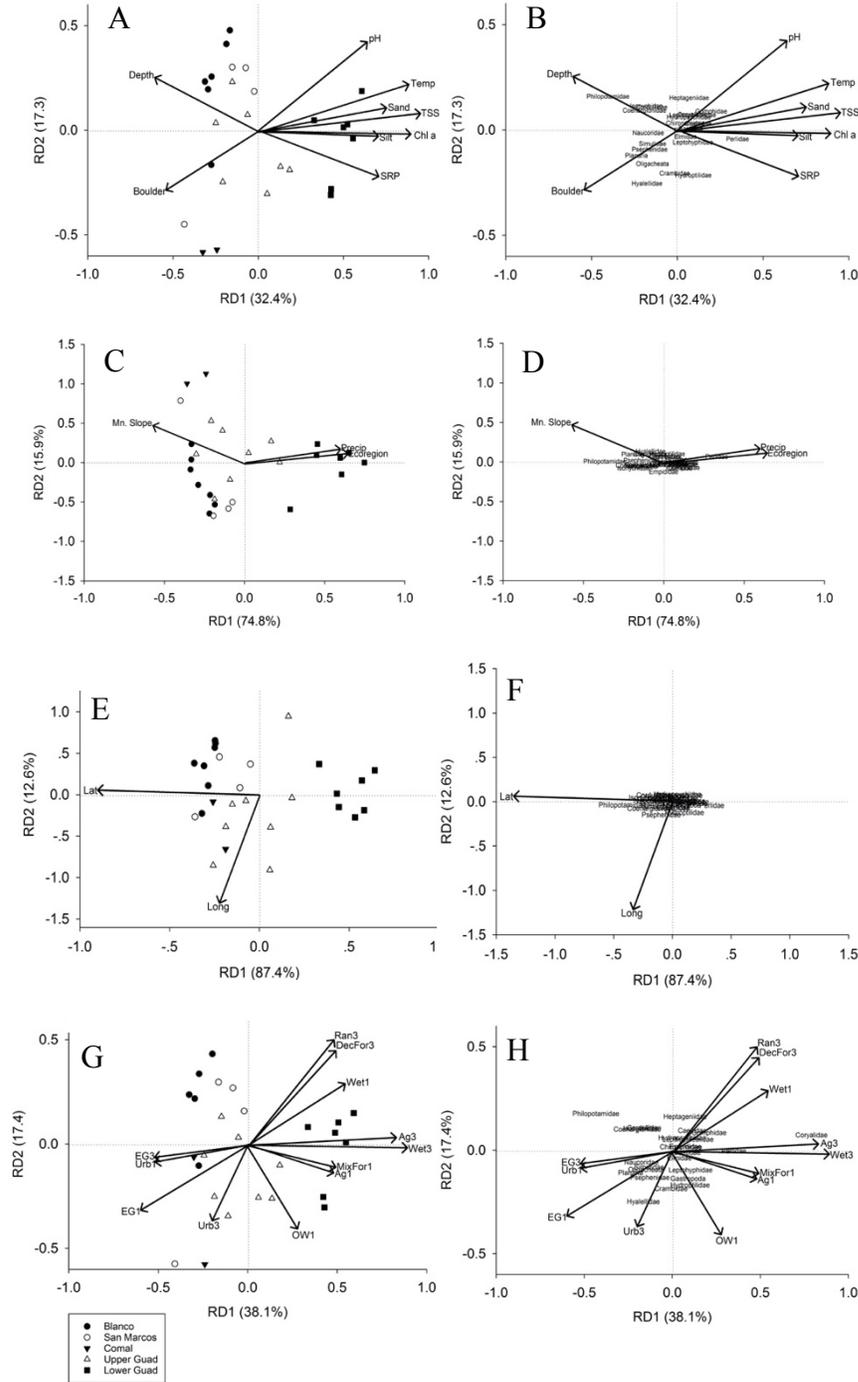


Figure 5: Redundancy Analysis (RDA) biplots of 4 variable groups used in the study with family level taxa identification. A-biplot of sites in for significant local factors. B-biplot of family taxa for significant local factors. C-biplot of sites for significant physiographic factors. D-biplot of family taxa for significant physiographic factors. E-biplot of sites for significant space factors. F-biplot of family taxa for significant space factors. G-biplot of sites for significant LULC factors. H-biplot of family taxa for significant LULC factors

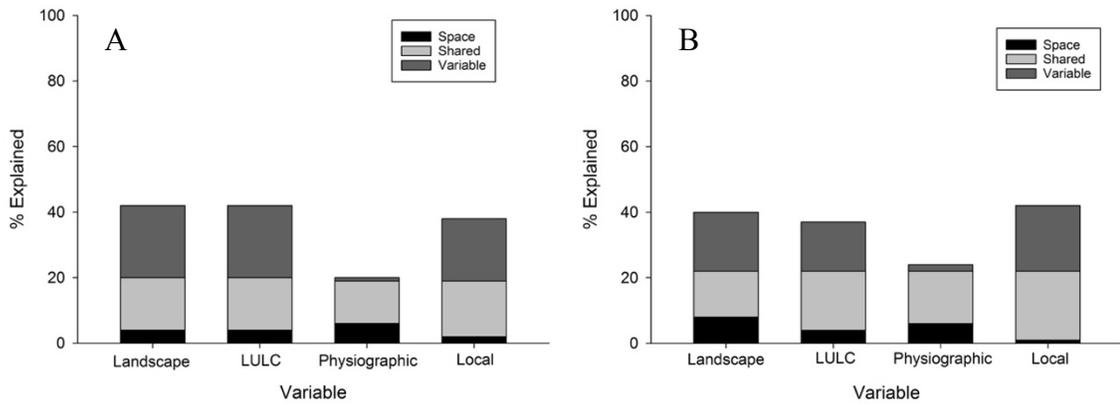


Figure 6: Variance Partitioning Redundancy Analysis (RDA) for variables sets compared with space. Landscape factors include physiographic and LULC variables. A- Genus-level taxa. B-Family-level taxa.

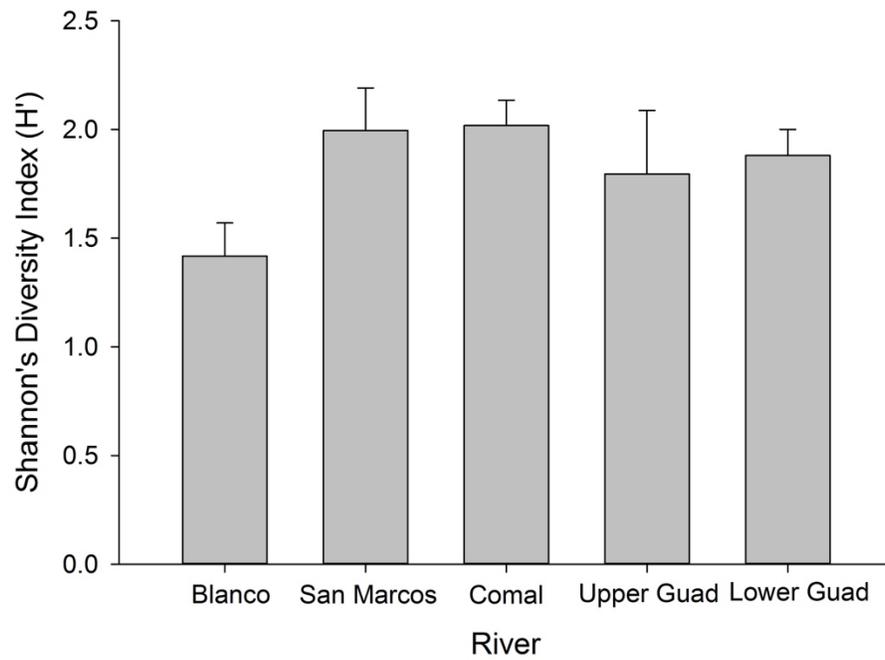


Figure 7: Average Shannon's Diversity Index (H') of each river in the Guadalupe River Basin

APPENDIX SECTION

Table A1: RDA predictor values for LULC factors. Only evergreen forest, mixed forest and wetlands at the reach scale were significant, while all LULC except for open water were significant for the catchment scale

Predictor	RDA1	RDA2	R ²	P-value
OW1	0.24138	-0.97043	0.1974	0.067
Urb1	-0.99974	0.02266	0.2012	0.051
DecFor1	0.99993	0.01148	0.1492	0.13
EG1	-0.80697	-0.59059	0.5667	0.001
MixFor1	0.96829	-0.24983	0.2114	0.038
Ran1	-0.36255	0.93196	0.153	0.116
Ag1	0.92147	-0.38845	0.1689	0.088
Wet1	0.85967	0.51084	0.3186	0.008
OW3	-0.37981	-0.92507	0.1177	0.214
Urb3	-0.3508	-0.93645	0.2021	0.041
DecFor3	0.70777	0.70645	0.2481	0.023
EG3	-0.99876	-0.04973	0.2495	0.025
MixFor3	-0.31796	-0.94811	0.1308	0.165
Ran3	0.53866	0.84252	0.3772	0.004
Ag3	0.99998	0.00691	0.7029	0.001
Wet3	0.99118	-0.13254	0.8329	0.001

Table A2: 2-way ANOVA results for species richness and % composition of EPT, Diptera, and Coleoptera within riffles. Factors are depth (shallow and deep) and velocity (slow and fast). Significant values are marked with an * ($\alpha \leq 0.05$)

Metric	F _D	F _V	F _{D*V}	P _D	P _V	P _{D*V}
Species Richness	12.961	5.103	2.870	0.001*	0.029*	0.097
% EPT	0.041	5.098	2.087	0.841	0.029*	0.156
% Diptera	0.307	24.284	3.078	0.582	<0.001*	0.086
% Coleoptera	0.000	10.635	0.791	0.985	0.002*	0.379

Table A3: 2-way ANOVA results for cross-classified factors (depth (shallow and deep) and velocity (slow and fast)) for family-level taxon. Significant values are marked with an * ($\alpha \leq 0.05$)

Taxon	F _D	F _V	F _{D*V}	P _D	P _V	P _{D*V}
Oligochaeta	0.160	0.534	0.401	0.691	0.469	0.530
Gastropoda	2.540	2.608	2.158	0.118	0.113	0.149
Baetidae	5.915	2.465	5.065	0.019*	0.124	0.029*
Leptophlebiidae	11.241	20.179	5.192	0.002*	<0.001*	0.028*
Leptohyphidae	17.451	4.356	1.461	<0.001*	0.043*	0.233
Caenidae	0.244	8.890	0.047	0.624	0.005*	0.830
Heptageniidae	0.951	2.473	1.363	0.335	0.123	0.249
Isonychidae	7.495	0.612	0.008	0.009*	0.438	0.927
Hydropsychidae	2.781	9.538	1.536	0.102	0.003*	0.222
Hydroptilidae	7.458	6.615	2.469	0.009*	0.014*	0.123
Leptoceridae	0.007	0.002	0.394	0.933	0.969	0.533
Polycentropodidae	0.000	2.000	0.000	1.000	0.164	1.000
Perlidae	7.892	9.565	0.087	0.007*	0.003*	0.770
Coenagrionidae	0.095	5.297	0.157	0.760	0.026*	0.693
Gomphidae	13.267	0.587	1.886	0.001*	0.448	0.177
Cordulidae	1.000	1.000	1.000	0.323	0.323	0.323
Elmidae	12.180	17.621	2.056	0.001*	<0.001*	0.159
Crambidae	8.230	2.902	0.205	0.006*	0.096	0.653
Naucoridae	0.355	5.041	0.355	0.555	0.030*	0.555
Chironomidae	11.394	4.378	7.144	0.002*	0.042*	0.011*
Ceratopogonidae	4.013	0.274	0.274	0.051	0.603	0.603
Empididae	0.958	5.388	2.035	0.333	0.025*	0.161
Tipulidae	0.034	0.034	0.034	0.854	0.854	0.854
Coryalidae	29.218	40.908	32.439	<0.001*	<0.001*	<0.001*

Table A4: 2-way ANOVA results for cross-classified factors (depth (shallow and deep) and velocity (slow and fast)) for family-level taxon. Significant values are marked with an * ($\alpha \leq 0.05$)

Taxon	F _D	F _V	F _{D+V}	p _D	p _V	p _{D+V}
Oligochaeta	0.160	0.534	0.401	0.691	0.469	0.530
Gastropoda	2.540	2.608	2.158	0.118	0.113	0.149
Pseudocloeon	1.282	13.921	0.746	0.264	0.001*	0.393
Camelobaetidius	1.230	5.021	1.230	0.274	0.03*	0.274
Fallceon	6.880	6.317	2.437	0.012*	0.016*	0.126
Baetis	2.198	0.377	2.198	0.145	0.542	0.145
Thraulodes	1.520	6.186	0.376	0.224	0.017*	0.543
Traverella	11.915	21.681	9.685	0.001*	<0.001*	0.003*
Neochoropteres	1.250	14.542	0.223	0.270	<0.001*	0.639
Tricorythodes	20.600	2.925	0.410	<0.001*	0.094	0.525
Leptohyphes	0.009	2.909	0.330	0.923	0.095	0.043*
Caenis	0.224	8.890	0.047	0.624	0.005*	0.830
Stenacron	0.951	2.473	1.363	0.335	0.123	0.249
Isonychia	7.495	0.612	0.008	0.009*	0.438	0.927
Smicridea	4.872	17.242	3.579	0.033*	<0.001*	0.065
Cheumatopsyche	2.207	8.316	1.068	0.145	0.006*	0.307
Hydropsyche	0.469	1.451	1.746	0.497	0.235	0.193
Hydroptila	6.111	6.162	2.239	0.017*	0.017*	0.142
Ochotrichia	2.018	0.101	0.101	0.162	0.752	0.752
Mayatrichia	4.713	8.110	2.232	0.035*	0.007*	0.142
Oecitis	0.322	1.002	0.034	0.573	0.322	0.854
Trienodes	4.316	1.439	1.439	0.044*	0.237	0.237
Nectopsyche	0.038	4.272	0.038	0.847	0.045*	0.847
Neoperla	7.892	9.565	0.087	0.007*	0.003*	0.770
Argia	0.095	5.297	0.157	0.760	0.026*	0.693
Phyllogomphoides	13.267	0.587	1.886	0.001*	0.448	0.177
Hexacylloepus	7.310	10.692	1.872	0.010*	0.002*	0.178
Heterelmis	4.812	0.906	0.960	0.034*	0.346	0.332
Microcylleopus	2.200	2.200	2.200	0.145	0.145	0.145
Paraponyx	8.070	2.778	0.166	0.007*	0.103	0.686
Ambrysus	0.355	5.041	0.355	0.555	0.030*	0.555
Chironomidae	11.394	4.378	7.144	0.002*	0.042*	0.011*
Ceratopogonidae	4.013	0.274	0.274	0.051	0.603	0.603
Empididae	0.958	5.388	2.035	0.333	0.025*	0.161
Tipulidae	0.034	0.034	0.034	0.854	0.854	0.854
Corydalus	29.218	40.908	32.439	<0.001*	<0.001*	<0.001*

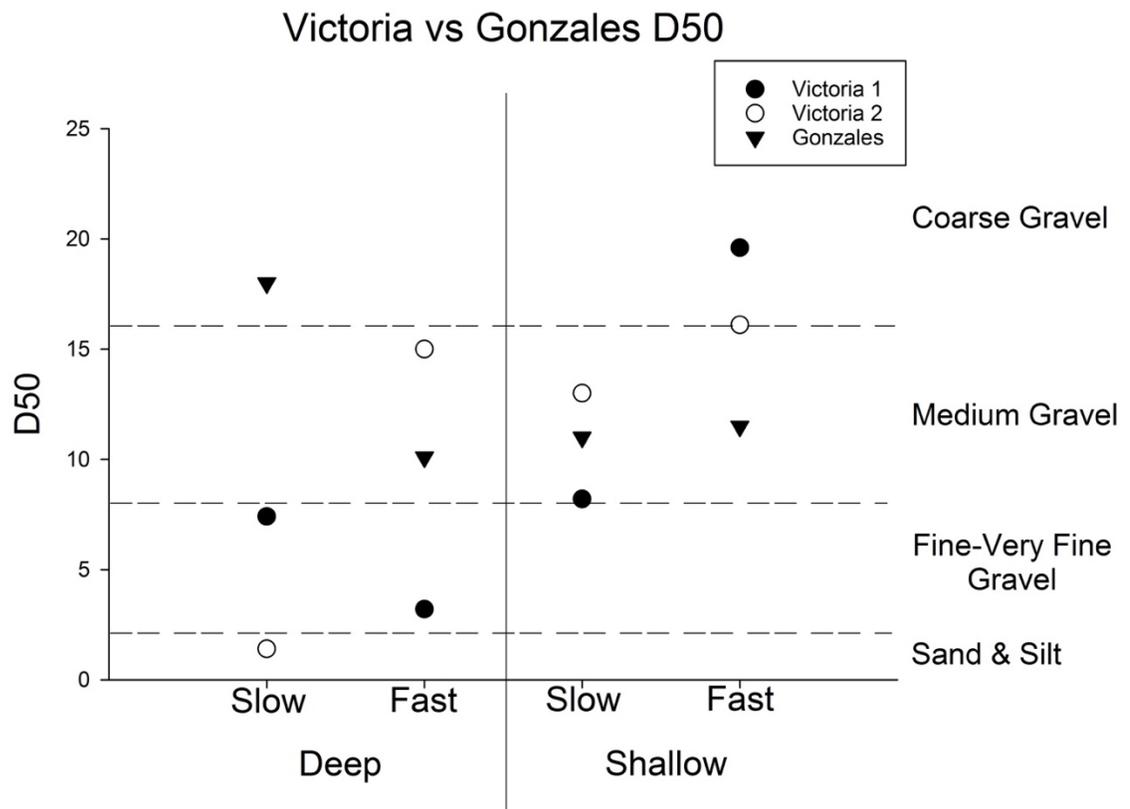


Figure A3: Median grain size (D50) of 4 riffle habitats: Gonzales and Victoria

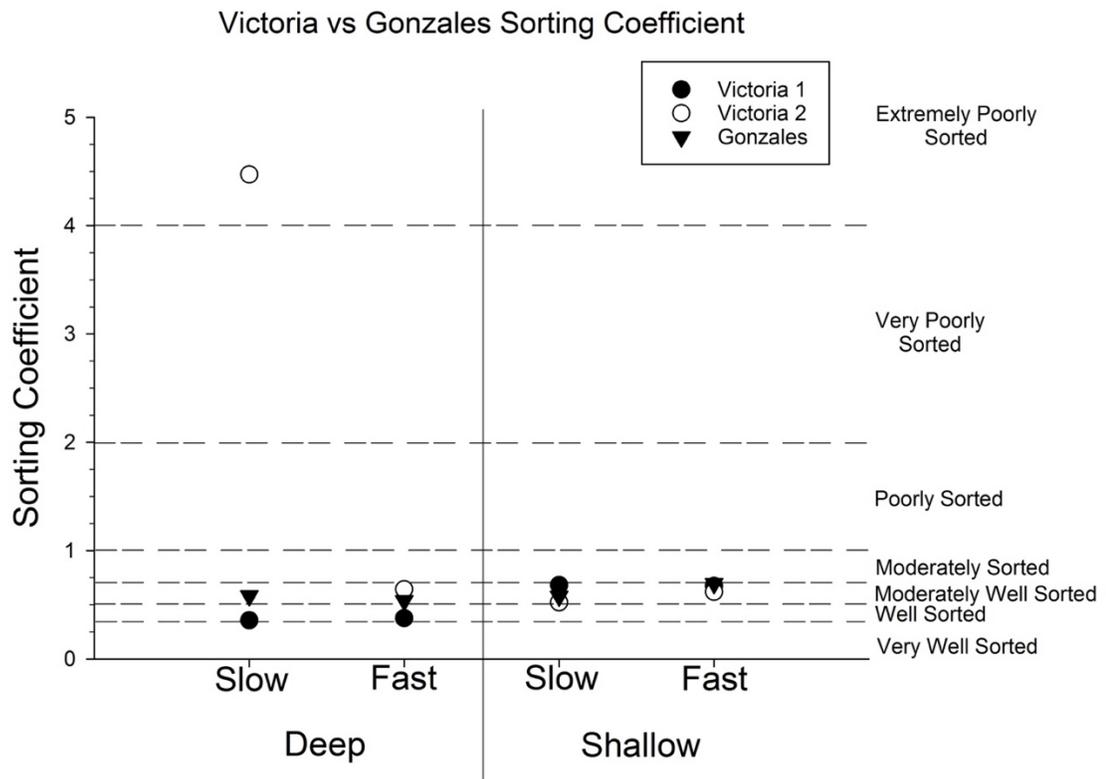


Figure A4: Sorting coefficient of 4 riffle habitats: Gonzales and Victoria

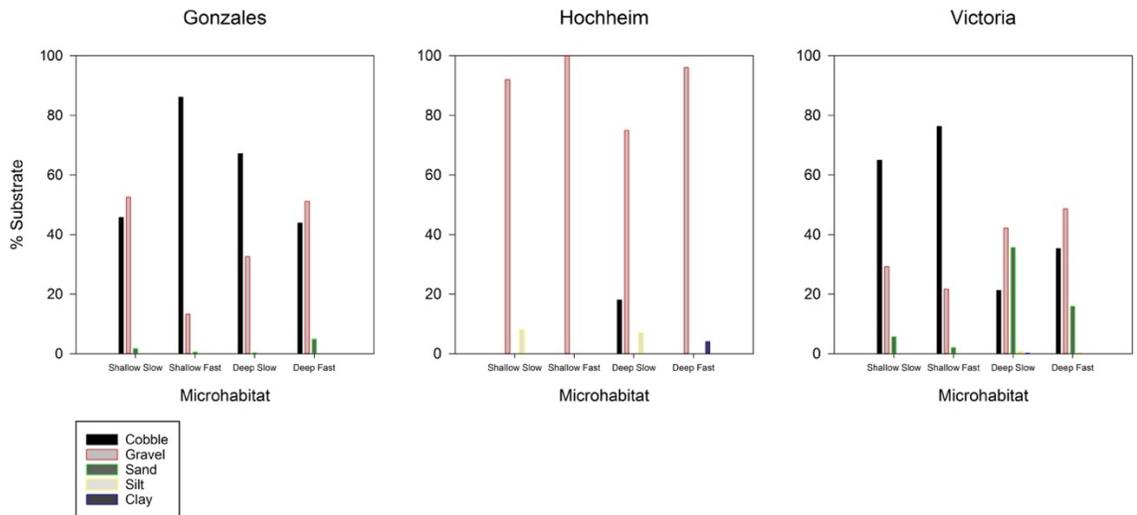


Figure A5: Sediment percentages for all 4 microhabitat riffles across 3 sample sites

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