# IMPACTS OF LAND USE ON THE HABITAT AND MACROINVERTEBRATE ASSEMBLAGE OF THE TRIBUTARIES OF THE LOWER BRAZOS RIVER

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IMPACTS OF LAND USE ON THE HABITAT AND MACROINVERTEBRATE

ASSEMBLAGE OF THE TRIBUTARIES OF THE LOWER BRAZOS RIVER

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**ABSTRACT**

IMPACTS OF LAND USE ON MACROINVERTEBRATE DIVERSITY IN THE TRIBUTARIES OF THE LOWER BRAZOS RIVER

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Ecological research on stream and river ecosystems aims to gain an understanding of the dynamic and complex impacts of environmental factors on biotic communities within riverine landscape (riverscape). Human activities significantly impact both terrestrial and aquatic ecosystems. Local environmental factors are known to influence the structure and function of stream ecosystems and biodiversity. The patterns of benthic macroinvertebrate distribution are related to the natural and human influenced variation of environmental factors. Ecologists assess stream physical and biological conditions in response to human land use activities using ecological indicators. This study is an assessment of the ecological condition of the Lower Brazos River Watershed. Macroinvertebrate assemblage structure was compared from 33 sites within the 6 subbasins differing in land use type and degree. Land use within the subbasins was measured using data from the National Land Use Database. Benthic macroinvertebrate samples were collected and a variety of physiochemical variables were measured. Multivariate analysis grouped the subbasins using habitat variables and macroinvertebrate assemblages. Decreasing habitat heterogeneity resulted in a decline of diversity and richness of organisms. Differences in the richness and diversity of the macroinvertebrate assemblages are attributed to habitat structure and land use. This study highlights the importance of considering both local habitat and landscape parameters of watersheds in stream biological assessments to understand the response of benthic communities to disturbances.

# CHAPTER I

## THE INFLUENCE OF LAND USE ON THE MACROINVERTEBRATE DIVERSITY IN THE TRIBUTARIES OF THE LOWER BRAZOS RIVER

### INTRODUCTION

Ecological research on stream and river ecosystems aims to gain an understanding of the dynamic and complex impacts of environmental factors on biotic communities within riverine landscape (riverscape) (Ward 1998). Riverscape is an intricate mosaic of highly connected habitat types and environmental gradients (Ward et al. 2002), which are characterized by a nested hierarchal network of scales from the level of the microhabitat to the basin (Wiens 2002; Allan 2004; Johnson and Host 2010). The distribution of benthic communities is determined by the interaction between habitat mosaic features and other environmental gradients (Robinson et al. 2002; Moore and Palmer 2005).

Human activities significantly impact both terrestrial and aquatic ecosystems (Resh et al. 1988; Poff et al. 1997; McKinney 2002; Sweeney et al. 2004). Landscapes are altered through anthropogenic land use, most notably urbanization and agriculture (Moore and Palmer 2005; McTammany et al. 2007), which cause chemical, hydrological, geomorphic, and erosional changes, as well as affect many other physical processes in running water ecosystems (Malmqvist and Rundle 2002; Roy et al. 2005; Chadwick et al. 2006; Burcher et al. 2007). However, ecologists do not have full understanding how aquatic biodiversity is impacted by multiple disturbance events, such as pollution, flow modification, and aquatic habitat degradation or destruction (Dudgeon et al. 2006; Kreutzweisr et al. 2008), and how the biological integrity of benthic communities is disrupted by land use disturbances (Karr and Chu 1999).

Urbanization exerts especially severe damage on aquatic ecosystems due to the considerable array of types and sources of negative impacts on watersheds (Paul and Meyer 2001; Walsh et al. 2007; Von Schiller et al. 2008). Impervious areas in watersheds and altered riparian zones change result in hydrological regime alteration, amplify surface pollutant runoff, and increase suspension of fine particles as well as sediment load in stream channels, (Roy et al. 2003; Burcher and Benfield 2006; Walters et al. 2009), which reduce water quality and degrade habitat quality (Harding et al. 1998; Roy et al. 2005). Suspended particles increase turbidity, reduce mean particle size and fill interstitial spaces in the benthos leading to decreased habitat suitability and changes in the benthic communities (Sponseller and Benfield 2001; Burcher et al. 2008; Roy et al. 2003). Agricultural practices on the landscape not only increase nutrient loads and suspension of particles through surface runoff of manure and fertilizer, but also degrade riparian areas by vegetation alteration and increase algae growth through increased sunlight and temperature in the stream (Kauffmen and Krueger 1984). Deforestation for crops and grazing can increase the loading of fine sediment to rivers through increased erosion (Malmqvist and Rundle 2002). Such disturbances in riparian zones and in stream channels influence allochthonous input, reduce habitat quality, cause concomitant changes in macroinvertebrate assemblages, and impact the structure and function of lotic ecosystems (Minshall 1988).

Local environmental factors are known to influence the structure and function of stream ecosystems and biodiversity (Cummins 1974; Poff 1997). The patterns of benthic macroinvertebrate distribution, especially insects, are related to the natural variation of environmental factors (Poff 1997; Ward et al. 2002). Benthic habitats consist of many variables, but current velocity, substrate type, particle size, and food resources are the most important in the organization of the macroinvertebrate assemblage (Rabeni and Minshall 1977; Palmer et al. 2000). The change of physical heterogeneity of riverine ecosystems at temporal and spatial scales is also important to benthic macroinvertebrate diversity (Meyer 1990; Malmqvist 2002). Our understanding on dynamic and complex riverine systems is still limited, especially on the interactive relationship of physical habitats, benthic diversity, and species traits, such as morpho-behavioral groups, trophic behavior, pollution tolerance, body size and mobility, in a variety of heterogeneous mosaic range (Poff 1997; Allan 2004).

This is the study of the ecological condition of the Lower Brazos River Watershed. The aim of this study is to investigate the relationship between stream abiotic characteristics and macroinvertebrate communities, as well as the influence of land use patterns on the benthic assemblages of the tributaries of the Brazos River. The specific objectives of this study are: to identify the habitat and land use gradients within the Lower Brazos River Watershed and compare them among the subbasins, to quantify the benthic macroinvertebrate diversity within the watershed and assess their differences among the subbasins, and to analyze the relationship between the macroinvertebrate assemblage and the environmental gradients and quantify the variation explained by local habitat, geographic characteristics, land use patterns and season.

# METHODS

*Site description*

The Brazos River is the eleventh longest river in the United States and the longest river in Texas. From its headwaters in New Mexico to its mouth at the Gulf of Mexico, the entire watershed of the Brazos River spans 116,000 $km^2$. The Lower Brazos Watershed is defined as the Brazos River south of the Waco, Texas (Figure 1). The Lower Brazos River is a turbid and meandering floodplain river. The study was conducted on thirty-three sites during Spring (February through May 2008), Summer (June through September 2008), and Winter (November 2008 through January 2009) in the tributaries of the Lower Brazos River. The study area is comprised of the six subbasins that drain into the Brazos River and encompasses about 41,000 $km^2$. This area traverses three physiographic regions: the Edwards Plateau, the Blackland Prairie, and the Gulf Coastal Plains (TCEQ 2002).

Six subbasins were sampled: the Central Brazos River subbasin (CW), the Lower Brazos River subbasin (LB), the Yegua Creek subbasin (YG), the Navasota River subbasin (NR), the Little River subbasin (LR) and the Lampasas River subbasin (LM). Within the Central Watershed subbasin and the Lower Brazos subbasin, study sites were conducted on smaller tributaries. Within the other four major tributaries, Yegua Creek, Little River, Navasota River, and the Lampasas River, study sites were nested, and these tributaries were sampled repeatedly along the longitudinal gradient (Table 1).

The Central Brazos River subbasin consisted of six small tributaries (CW1-CW6) and drains 7,019 km$^2$. The Lower Brazos River subbasin consisted of eight small tributaries (LB1-LB8) and drains 5,379 km$^2$. The Yegua Creek subbasin consisted of three sites (YG1-YG3) and drains 3,408 km$^2$. The Navasota River subbasin consisted of six sites (NR1-NR6) and drains 5,789 km2. The Little River subbasin (excluding the Leon and the Lampasas River tributaries) consisted of 7 sites (LR1-LR7) and drains 6,083 km$^2$. The Lampasas River subbasin consisted of 3 sites (LM1-LM3) and drains 3,890 km$^2$. The majority of the sites lie within the Gulf Coastal Plain physiographic region, however, the Lampasas River in its entirety and the North and South Fork San Gabriel Rivers (LR4 and LR5) are located on the Edwards Plateau.

*Land cover classification*

Watershed land use patterns were classified as the percentages of the same land use categories using land cover/land use (LULC) data, which were generated from Labay (2010). Briefly, LULC was quantified using land data obtained from USGS National Land Cover Database (NLCD) 2001. The NLCD classified land use into 15 categories for the Lower Brazos River Watershed. Data were reclassified following the methods of Anderson et al. (1976) into five land use categories (urban, agricultural, forest, grassland, and wetland) at three spatial scales that are commonly used to relate land cover to ecosystem functioning (Allan 2004). The levels considered were reach buffer (100 m buffer 2km upstream from the sampling site), riparian buffer (100 m buffer of whole stream upstream from the sampling site), and the entire catchment upstream from site (cumulative area upstream from site) using ArcGIS (ArcView 9.3, 2007) and ArcHydro

(Maidment 2002). A Spearman rank correlation matrix was utilized in the statistical package R (R Development Core Team) to reduce the effects of autocollinearity and correlation among the land-use categories in the three nested spatial scales (Labay 2010). To reduce the variables used in the multivariate ordination, any significant correlation ($p \leq 0.05$) resulted in the omission of the category at the smaller spatial scale. The goal of reducing the variables is to understand what combination of land-use categories contribute most to the macroinvertebrate assemblage.

*Stream characterizations*

*Physiochemical parameters*. Environmental variables were measured at each site during each season. Parameters measured were dissolved oxygen concentration (mg/L), conductivity (µS/cm), pH and temperature (°C) using a calibrated YSI 556 Handheld multiparameter meter (YSI Incorporated, Yellow Springs, OH, U.S.A.).

*Physical habitat characteristics*. Habitat variables were measured along transects where benthic macroinvertebrate samples were collected**.** Channel width was measured across each transect. Habitat physical parameters, including water depth, dominant substrate type, filamentous algae, percent silt, and current velocity were measured at five points equidistant across a transect. Dominant substrate class (clay, sand, gravel, pebble, cobble, boulder and bedrock) at each point was categorized as 1-7, respectively (Bain et al. 1985). Average current velocity was measured at 60% depth using a Marsh-Mcbirney Flo-Mate 2000 electromagnetic flow meter (Hach Company, Fredrick, MD, U.S.A.). Filamentous algae and silt were evaluated by estimating surface cover percentage present. Vegetation canopy cover was determined by using a canopy densitometer (Wildco, Inc.)

in the middle of the transect facing up stream. Mean annual flow was estimated for each site using the USGS National Hydrogeography Dataset and ArcGIS following the methods of (Labay 2010).

*Sampling methods*

*Benthic primary producer biomass*. Algal biomass (as chlorophyll *a*, $mg/m^2$) was sampled at each site throughout all seasons. Six rocks or sticks were collected from a single habitat and the substrate was brushed vigorously with a nylon bristle brush and rinsed with 50 mL of DI water. Samples were kept in the dark until returning to the laboratory where the slurry was filtered through a 1µm glass fiber filter. Filters were placed 10 mL of 90% HPLC grade acetone for three hours in the refrigerator at 3˚C. The samples were brought to room temperature and chlorophyll α concentration was measured with a fluorometer (Turner Triology Inc.). The surface area of the substrate was measured using aluminum foil to precisely measure the area that was scrubbed. Aluminum foil was dried and weighed in the lab. The weights of foil from each in-stream substrate were plotted against a standard weight-area conversion line to obtain the sampled surface area (Sponseller et al. 2001).

*CPOM and FPOM*. Organic matter was separated from the benthic invertebrate samples (for both the summer and the winter samples) and divided into two size categories: coarse organic particulate matter (CPOM; >1 mm) and fine organic particulate matter (FPOM; <1 mm). The samples CPOM and FPOM were dried for at 60 ˚C for least 48 hours, weighed, and ashed in a muffle furnace at 450˚C for 5 hours. The samples cooled for 12 hours and reweighed to determine the Ash Free Dry Mass (AFDM).

<u>*Macroinvertebrates*</u>. Benthic macroinvertebrates were collected using both qualitative and quantitative methods, depending on season; due to high water levels, spring sampling methods differed from summer and winter sampling. During the spring sample collection, the macroinvertebrates were collected from three riffle, three run, and one bank habitat within the reach when different geomorphic unites were present, otherwise five samples were taken from the same habitat. The samples were collected were taken using a modified kick net with a bag (500 µm mesh net; 1 m × 1 m.) This allowed for more water to flow pass through the net. The bank samples were collected by multiple sweeps a larger modified dip net (500 µm mesh net) along the bank for 10 m. During summer and winter sampling, macroinvertebrates were collected using a Hess sampler (500 µm mesh net; 0.3m × 0.4m; Wildco). At each site, five benthic macroinvertebrate samples using the Hess sampler were collected. The bank sample was collected with multiple sweeps of a dip net (500 µm mesh net; 0.305 m × 0.254 m; Wildco) along a 10 m section of the reach.

All samples were fixed with 70% ethanol and brought back to the laboratory for processing. Samples were all hand-picked using a dissecting microscope at 15× magnification. Macroinvertebrates were identified to genus, using traditional keys (Thorp and Covich 2001; Merritt and Cummings 2008) except for individuals in the Chironomidae family which were identified to the tribes, *Chironomini*, *Orthocladinae*, and *Tanypodinae*. Non-hexapod invertebrates were at least identified to Order, except for Gastropoda and Decapoda, which were identified to family level, and bivalves in Unionidae were identified to species. Individual numbers of all taxa were counted. All taxa were assigned to the following functional feeding groups: scrapers, shredders, collector-gatherers, collector-filterers, and predators using Merritt and Cummings (2008) and Thorp and Covich (2001). All samples for each site were pooled for analysis and the pooled data was used to calculate a variety of diversity metrics. Taxon (species) richness is defined as the total number of taxa present at each site.

*Community structure and composition:* Macroinvertebrate assemblage structure across sites, subbasins, and season were characterized by species richness (S), the abundance of species ($n_i$) , diversity (H'), and species evenness (j) indices calculated using PRIMER (version 6; Clarke and Warwick 2001). Multiple measures of biodiversity were calculated using the following three indices: Shannon-Weiner index which measures diversity considering both richness and evenness, Margalef's diversity index which takes species richness and sampling size and effort into account, and Simpson's diversity index which considers species richness and relative abundance. The indices are described as the following equations:

The Shannon-Weiner index:

$$H' = -\sum_{i=1}^{S} P_i \cdot \ln P_i$$

where $P_i$ is the proportion of individuals of a given species.

Margalef's diversity index: $d = (S-1)/\ln N$

where S is the total number of species and N is the total number of individuals.

Simpson's diversity index:

$$\Delta = [\sum \quad n_i \cdot (n_i - 1)]/N(N-1) \ ,$$

where $n_i$ is the total number of individuals belonging to i species and N is the total number of individuals.

Species evenness was calculated using Pielou's evenness index (Pielou 1966) as:

$$J' = \ H'/\ln(S),$$

where S is the total number of species and $H'$ is the Shannon-Weiner index.

*Statistical Analysis.*

*Physical habitat:* Multivariate ordination was used to analyze stream physical habitat variables and catchment variables. Principle Component Analysis (PCA) was used to assess the spatial and temporal patterns of habitat variance (Gauch 1982). Ordinal data (i.e., water depth, current velocity, and vegetation canopy cover) were z-score transformed (Krebs 1999, Williams and Bonner 2006) and categorical variables (i.e., season) were represented by dummy variables (Zar 2010). Twenty-seven variables were used in the habitat PCA. Generated PCA biplots and variables demonstrated patterns across sites, subbasins and seasons.

All species data were fourth root transformed to standardize the distribution. A Bray-Curtis similarity matrix (Bray and Curtis 1957) was calculated for species abundance at each site, season, and subbasin. These matrices were tested with analysis of similarities (ANOSIM; Clarke and Green 1988; Clarke 1993). A one-way ANOSIM with sampling period as a factor was performed to assess seasonal effects on macroinvertebrate assemblages (α=0.05; 9,999 permutations), with sites as a factor to assess similarities among macroinvertebrate assemblages between sites (α=0.05; 9,999 permutations) and with subbasin as a factor to test similarities among macroinvertebrate assemblages of the subbasins of the Lower Brazos Watershed (α=0.05; 9,999 permutations). Multidimensional scaling (MDS) was used to represent dissimilarities within the watershed and between subbasins in a two-dimensional ordination space.

Canonical Correspondence Analysis (CCA) is a direct gradient analysis that can identify the influence of environmental factors on biotic assemblages. CCA was used to examine the relationships between the macroinvertebrate community parameters and the environmental variables using the program CANOCO 4.5 (ter Braak and Smilauer 2002). The CCA focused on inter-sample distances using Hill's scaling which allows distances between groups containing nominal data to be interpreted. In order to explore the species-environment relationship, this analysis included the most influential local habitat variables detected from the PCA, such as subbasins and geography variables, as well as the season, and the ten land-use variables retained from the Spearman-rank correlation reduction technique (ter Braak 1986). Species abundance data were $\log_{10}(x+1)$ transformed to normalize and equalize the variance, rare species were downweighted and ordinal environmental variables were z-score transformed (ter Braak and Similauer 2002). Species scaling is in standard deviation units (SD-units) and the distances between samples represent species turnover (β-diversity) (ter Braak and Similauer 2002). To test significance of the model ($p < 0.05$) of the variation explained, a Monte Carlo randomization test (1000 permutations) was performed on each CCA model (Palmer 1993).

Since constrained ordination (such as CCA) is limited in its ability to explicitly test on the pure or unique effect of environmental variables on benthic community assemblages, partial canonical correspondence analysis (pCCA) for variance partitioning (Borcard et al. 1992) was performed to assess the pure effects of land-use, local habitat, season, and geography effects on macroinvertebrate community response variables. This analysis tests the relationship of the groups of variables and the response of macroinvertebrate communities.

CCA was also used also to examine the relationship between habitat and land use on the functional feeding groups of benthic macroinvertebrate assemblage. Functional feeding group abundances were $\log_{10}$ (x+1) transformed and environmental variables were z-score transformed and focused on inter-species distances.

The influence of land use (urban and agriculture) on the aquatic habitat was explored using a two sample t-test assuming equal variance on the PCI scores. With aquatic habitat showing natural variation between the subbasins the streams were grouped into Eastern and Western streams by location. Land use was defined as agriculture and medium plus high urbanized areas at the catchment scale.

## RESULTS

*Land use Characterization*

Land use in the Lower Brazos Watershed varied among the tributaries of the sub-basins and different spatial scales (reach scale, riparian scale, and catchment scale) (Table 2). At the catchment level, land-use in the Lower Brazos Watershed was predominantly grassland (47%) and forest (30%). Urban area and agriculture land use were 8% and

10%, respectively. Among subbasins, the Lower Brazos (LB) subbasin has the most urban (14%) land use, and the Central Brazos (CW) subbasin had the most agriculture (23%). The ten original categories retained from the Spearman rank correlation are listed in Table 3.

*Habitat Characterization*

Physical characteristics of the aquatic habitat among the subbasins varied along a west to east gradient (Table 4). The western sites, the Lampasas and Little River subbasins, were characterized by coarser substrates (pebble and cobble), higher current velocity and more abundant riffle habitat. The eastern sites, the Central, Lower Brazos, Navasota, and Yegua Creek subbasins were associated with finer substrates and silt, increased water depth, and greater prevalence of run habitat. In total, four principle component axes (PC) explained 44% of the variation in the physical habitat of the sites in the Lower Brazos watershed (Figure 2). PCI (16.0% of total variation) represented a substrate-stream flow gradient with riffle (-0.75), pebble (-0.63), substrate coarseness (-0.60), run (0.59), silt (0.62), and clay (0.80) having the strongest loadings along the axis. PCII (10.9% of total variation) described the steam morphology and physiochemical gradient with depth (-0.52), width (-0.50), discharge (-0.43), conductivity (0.54), pH (0.68), and dissolved oxygen (DO) (0.73) having the strongest loadings along the axis.

The Central and the Lower Brazos subbasins were similar in physical habitat structure. Both subbasins consisted of small tributaries that were sampled once above the confluence of the Brazos River. Among the six sites within the Central (CW) subbasin, the streams were shallow (23.11 cm ± 2.24 (SE)) and narrow (6.15 m ± 1.04) with a current velocity of 15.22 cm/s ± 5.57. The benthic substrate was comprised of a fine substrate mostly clay (43%) and high a high silt percentage (65%). The eight lower Brazos subbasin sites were also shallow (32.54 cm ± 7.78) but wider (8.84 m ± 1.68) with a current velocity of 10.88 cm/s ± 3.85. The benthos was comprised of mostly clay (40%) and sand (36%) and had a high silt percentage. The Yegua and the Navasota subbasins were also similar in physical habitat structure. These rivers are slow moving with fine substrate. The six sites of the Navasota subbasin were relatively deep (71.61 cm ± 13.18) and wide (16.71 m ± 2.60) with a current velocity of 13.35 cm/s ± 4.56. The benthos was comprised of clay (55%). The silt percentage in this river was high (85%). The three sites of the Yegua subbasin were moderately deep (43.91 cm ± 18.38) and wide (12.51 m ± 4.38) with a current velocity of 11.59 cm/s ± 2.44. The benthic habitat was comprised of clay (33%) and cobble (24%). These sites were only moderately silty (57%). The Lampasas subbasin is a tributary of the Little River that is within its entirety on the Balcones Uplift. It was wide (16.39 m ± 4.33) and shallow (22.81 cm ± 0.71) with a current velocity of 23.88 ± 4.45. The substrate was primarily pebble (27%) and bedrock (26%). These sites were only moderately silty (54%). The Little River subbasin was moderately deep (30.02 cm ± 7.63) and wide (11.04 m ± 1.95) with a current velocity of 22.04 ± 5.05. The benthos was primarily bedrock (33%), gravel (17%), and cobble (16%). These sites were also only moderately silty (40%).

The t-test of the PC-I scores showed a significant difference between high intensive land use and low intensive land use in both Eastern and Western streams (Figure 3). Intensive land use was calculated by the addition of both agriculture and urbanization land use at the catchment scale. High intensive land use was defined as land use more than 20%, while low intensive land use was defined as land use less than 20%. In the Western streams $p < 0.001$ (t-statistic = 4.76; d.f. = 49); and in the Eastern streams $p = 0.025$ (t-statistic = -2.23; d.f. = 46).

*Macroinvertebrate Community Characterization*

A total of 187,651 macroinvertebrates were collected from all sites across the three sampling seasons, representing 181 taxa from five different phyla. Within the class Hexapoda, nine orders, 57 families, and 125 genera were present in study sites across three sampling seasons (Appendix I); eighty-two percent of all invertebrates were Hexapods. Among aquatic insect orders (Class Hexapoda), Diptera were the most abundant (41%), followed by Ephemeroptera (26%), Trichoptera (16%), and Coleoptera (13%). Plecoptera taxa were least abundant (0.70%). From the phylum Mollusca, six Unionidae species were identified (Appendix 1).

The most common taxa collected were Chironominae (a tribe of Chironomidae) which were present all 33 sites cross three sampling seasons (found at 96 sites of the 99: 97% of all sampling events). Chironominae were also the most abundant taxon group and comprised 17.5% of all individuals found. Other common taxa with relative abundance over 5% included *Cheumatopschye* (8%), *Tricorythodes* (7%), *Hyallela* (6.5%), Tanypodinae (5%), and Orthocladinae (5%). Rarer taxa included the five of the six Unionid species: *Amblema plicata*, *Lampsilis teres*, *Quadrula apiculata*, *Quadrula houstonensis* (listed under the state of Texas as a species of concern), and *Tritogonia verrucosa*, as well as the shrimp species *Macrobrachium ohione*. Invasive snail taxa present were *Marisa* (the ramshorn snail), *Pomacea* (the apple snail), and *Melanoides* all of which are commonly found in the aquarium trade.

An analysis of similarity (ANOSIM) showed three major groupings among the subbasins of the Lower Brazos Watershed (Table 5). The three major groupings were the Lampasas River and the Little River subbasins, the Lower Brazos and the Central Brazos subbasins, and the Yegua and the Navasota subbasins. There were differences among the three seasons in the macroinvertebrate assemblages ($R$ = 0.134; $p$ < 0.01), and there were differences among the sites ($R$ = 0.441; $p$ < 0.01). Non-metric multidimensional scaling (MDS) highlighted two major groupings (Figure 4). The Lampasas and the Little River subbasins macroinvertebrate assemblages separated from the rest of the subbasins.

*Macroinvertebrate-Environmental Variable Relationships*

Canonical Correspondence Analyses (CCA) detected the significant effect of spatial and temporal environmental variables on benthic macroinvertebrate assemblages. CCA results show that habitat parameters, season, site, and land-use explained 52.8% of the variability of the macroinvertebrate community (total inertia = 1.97) (Figure 5). The CCA resulted in a significant model as indicated by the Monte Carlo Test ($F$ = 1.989; $p$ <0.001). Canonical Correspondence Axis I reflected a West to East land use gradient with easting (-0.33), forest (3) (-0.29), and run (-0.23), wetland (3) (0.24), grassland (3) (0.28), and precipitation (0.30) having the highest loadings. Canonical Correspondence Axis II was comprised of a seasonal and local habitat gradient with velocity (-0.19), run (-0.09), drainage area (-0.09), Winter (0.09), Summer (0.12), and Silt (0.13) having the greatest loadings. The more cosmopolitan taxa were the Elmid *Stenelmis,* the Baetid

*Fallceon, and* the Mollusc *Corbicula* sp.; these taxa showed no strong relationship with any environmental variables. The Leptophlebids *Travarella* and *Thraulodes,* the Philopotamid *Chimarra,* the Pshephenid *Psephenus,* the Elmids *Hexacylleopus and Microcylleopus, Planaria,* and the Leptohyphid *Vacupernis* were associated with higher forest catchments and the western catchments and consequently, the Little River and the Lampasas subbasins. The sensitive taxa of Ephemeroptera, Plecoptera, and Trichoptera (EPT) were more associated with the Western subbasins, but were present in the Eastern subbasins (Figure 6). Trichopteran taxa were mainly present in the Western subbasins. Plecopteran taxa are mainly absent in this drainage, because the natural distribution range of this order in limited in Texas. The Epehmeropteran taxa were present throughout all subbasins. The shrimp *Palomonetes*, the bivalve *Sphaeridae* and the Dipteran *Simuliidae* were associated with higher precipitation, percent grassland, and percent wetland. The variance partitioning by partial canonical correspondence analysis (pCCA) showed that shared effects were 19.1% of the variation in the macroinvertebrate assemblage. Among pure effects on the assemblage composition, geographic characteristics contributed 8.2%, local habitat contributed 12.5%, season contributed 3.8%, and land-use contributed 12.0%.

The variables of land use in the CCA model explained 16.6% variation of the diversity indexes in three axes (Table 6) of the macroinvertebrate assemblage (Figure 7). The significance of the model was validated with a Monte Carlo test with 9,999 permutations (F = 1.97; p = 0.04). The strongest loadings on Axis I were forest (1) (-0.47), agriculture (3) (-0.39), grassland (1) (-0.29), wetland (2) (0.32), grassland (3) (0.43), and wetland (3) (0.79). The strongest loadings on Axis II were grassland (1) (-

0.68), urban (3) (-0.66), wetland (3) (-0.33), forest (2) (0.09), forest (3) (0.59), and forest (1) (0.63). Margalef's diversity index was mostly associated with forest cover. Shannon's diversity index, Pielou's evenness index, and Simpson's diversity index were associated with wetland and grassland. Species richness was associated with grassland, forest and wetland.

The first four axes of the CCA model with local habitat and land use variables explained 47.7% of the variability of the functional feeding groups of the macroinvertebrate community (Figure 8). The significance of the model was validated with a Monte Carlo test with 9,999 permutations ($F = 27.400p < .001$). Axis I consisted of a habitat and season gradient with velocity (-0.63), riffle (-0.51), substrate coarseness (-0.40), winter (0.335), pool (0.38), and silt (0.42) having the strongest loadings. Axis II consisted of a land use gradient with wetland (3) (-044), wetland (2) (-0.34), agriculture (2) (-0.34), channel width (0.40), forest (2) (0.24), and forest (1) (0.43) having the strongest loadings. The trophic community structure was not distinctly associated with any subbasin, but was linked to specific resources and habitat structure. The shredders in the community were associated with increased channel width and velocity, and forest at all three spatial scales. The collector-filterers in the community were highly associated with riffle habitat and coarse substrate, as well as wetland and agricultural land use. The predators and collector-gatherers were associated with clay, silt, and run habitat, as well as grassland, wetland, and forest land use. Scraper density had no strong associations.

# DISCUSSION

The main objective of this study was to assess the relationship of the macroinvertebrate assemblages and the environmental variables of the Lower Brazos Watershed. The results indicate that a substantial portion of the observed variability of the macroinvertebrate communities can be attributed to both in stream habitat and land use gradients. More specifically, both macroinvertebrate and in stream habitat responded to land-use changes, highlighting the significant impact of human land-use disturbances on lotic biodiversity.

## *Taxonomic diversity and similarity patterns*

The comparison of the six subbasins showed there were two main groupings of the macroinvertebrate assemblages, which can be described as the Eastern and the Western communities. Biodiversity (Shannon's index) in the Western subbasins ranged from 2.20 to 3.15, and taxon richness ranged from 59 to 97 taxa. In the Eastern subbasins, the diversity ranged from 1.15 to 3.09, and richness ranged from 23 to 63 taxa. The assemblages within the Western drainages are comprised of more sensitive taxa that require riffle habitats which include individuals within the Trichoptera, Ephemeroptera, and Coleoptera orders. The Lampasas River and the Little River subbasin communities were comprised of high abundances of the Ephemeropteran genera, *Travarella, Thraulodes,* and *Isonychia*, the Trichopteran genera, *Chimarra, Ocetis*, and *Hydropsyche,* and the Coleopteran genera, *Microcyllepeous, Hexacyllepeous, Neolemis* and *Psephenus.* The Eastern drainages were comprised of individual that are mostly generalists. The Central, Lower, Navasota River, and Yegua River subbasins communities consisted of increased abundances of taxa within the Odonata, Diptera, Hempitera, and the Non-hexapoda orders. Within these rivers, there were high abundances of the Odonata genus, *Progomphus*, the Hemipertan genus, *Neocorixia*, the Dipteran genus, *Chaoborous*, the Decapoda genus, *Palaemonetes*, and the Bivalvia genera, *Sphaeriidae*. The uniqueness of these assemblages is primarily associated to the combination of habitat structure and land use patterns as has been documented in other studies (Richards et al. 1996; Quinn et al. 1997; McTammany et al. 2007; Walsh et al. 2007).

There were longitudinal shifts in the physical characteristics of the streams that influenced biodiversity. According to the River Continuum Concept (RCC; Vannote *et al.* 1980), biodiversity exhibits a unimodal pattern where the maximum species richness is found in the middle reaches of a river network. This study was not specifically intended to focus on the variation of biodiversity along the longitudinal gradient; however three tributaries were sampled repeatedly along the longitudinal gradient. Using the drainage size as a surrogate for stream order, the taxa richness pattern displayed supported the predictions of the RCC (Figure 9). This is unexpected as the RCC has been shown not to depict low gradient rivers (Meyer 1990). There were two sites that were below their expected taxon richness. The intermediate Yegua subbasin site (YG2), and the Navasota site (NR5), both mid-sized streams, had unexpectedly low taxa richness compared with sites located upstream and downstream. This could be attributed to anthropogenic disturbance within the river as both of these sites are located just downstream of dams and give light to the serial discontinuity theory (Edwards 1978; Ward and Stanford 1983; Malmqvist 2002; Malmqvist and Rundle 2002; Ward et al. 2002).

*Physical habitat*

Local physical habitat is a well researched determinant of macroinvertebrate community structures (Townsend et al. 2003; Allan 2004; Johnson et al. 2007). Physical habitats within each of the subbasins are distinctly related to the geological history of the region. Western catchments are located within the uplifted, limestone dominated Edwards Plateau region and have higher gradients, faster current velocity, coarser substrate, and more riffle habitats. The Lampasas and the Little River sites have the greatest habitat heterogeneity and stable in stream conditions which contribute to the greatest macroinvertebrate biodiversity. On the other hand, Eastern catchments are located on the gulf coast plain and are characterized by finer substrate, higher water depths, and slower current velocity. Higher percentage of fine sediment and low flow is not ideal for high diversity of benthic aquatic invertebrates. The Navasota River displayed the greatest beta-diversity due to longitudinal changes along the river. The headwaters (NR6) displayed high seasonal variation in habitat complexity, ranging from a lotic system in spring to a lentic series of pools during summer and winter sample seasons. Also, downstream of this site, the habitat of site NR5 had low habitat suitably due to a dam. NR4 and NR1 had riprap riffles which increased the habitat heterogeneity and also the invertebrate biodiversity. The high beta-diversity in streams with rather low habitat suitability can be attributed to the intermediate stages between high connectivity and disconnectivity which have been associated with high species richness (Ward et al. 1999; Ward et al. 2002; Ward and Standford 2006). Low precipitation in 2008 was a factor contributing to the lotic-lentic status of many of the streams with in the Eastern subbasins.

Another conceptual framework proposes the idea of communities existing through 'filters' (Poff 1997). The filters operate at different spatiotemporal scales and determine what taxa can persist in the community (Angermieir and Winston 1998). Environmental filters include variables such as temperature, flow regime and bed composition. Chessman and Royal (2004) demonstrated that each of the aforementioned environmental variables were most important in excluding taxa depending on the river characteristics. For example, flow regime is most important in non-perennial rivers, but in perennial rivers temperature or substrate may be more important. Seasonal changes reflected variation in temperature and flow in the Lower Brazos tributaries and filtered at the local habitat scale, and the macroinvertebrates were able to quickly respond to the changes in available habitat and decreasing habitat (Palmer et al. 2000; Robinson et al. 2002). Substrate and channel composition were spatially a stronger filter and represented the differences between the subbasins. At all scales, filters are important. Sandin and Johnson (2000) found that though large-scale factors were important in macroinvertebrate assemblages in different ecoregions in New Zealand, but also noted that local-scale variables were important. Another similar framework, the River Environment Classification (Snelder et al. 2004) also focused on the importance of environmental filters from the broadest scale, climate in that study, to the most local considered in that study, land use and land cover, the largest scale in this study. The strength of classification was low in the REC, but the authors noted the importance of local habitat, arguably the most important within the lifecycle most macroinvertebrates.

The temporal dynamics on the environmental gradients in the Lower Brazos tributaries was responsible for a small percentage of the compositional changes. Seasonality had a rather low influence on the macroinvertebrate assemblage during this study period. The different sampling techniques between spring and the other two seasons may have contributed to increasing the seasonal difference (Cao et al. 2002). Many macroinvertebrates have a rather short life span and are present throughout the year at different stages in their life cycle. This coupled with the rather mild seasonal changes in the region explains the constant presence of many species.

*Land use impact*

Land use at the catchment scale, the largest in this analysis, was the primary contributor of the variation of the macroinvertebrate community structure, as this pattern is found in many studies (Allan et al. 1997; Vinson and Hawkins 1998; Sliva and Williams 2001; Sponseller and Benfield 2001; Sponseller et al. 2001; Walsh et al. 2007). In this study, land use variables were the second most influential group of variables that influenced the biotic assemblage. However, forest and grassland at the site level were important in the determining biodiversity. In this study, higher diversity was associated with wetland and forest habitat, which represents low intensity land use in the natural form within this region. There has been considerable documentation of the declines in water quality, habitat, and biological assemblages due to landscape influences of agriculture (Roth et al. 1996; Richards et al. 1996; Wang et al. 1997; Sponseller et al. 2001), urban or impervious area (Walsh et al. 2007), and lack of an intact riparian corridor (Gregory et al. 1991; Stauffer et al. 2000). Many of the sites that are degraded due to agriculture and urban land uses are within the Central and Lower Brazos subbasins. The rich soils of this region have been converted from prairie habitat to cropland, and furthermore the region is in close proximity to increasingly developed suburban areas of Houston. Alternatively the differences could be attributed to the smaller catchment size of the streams in these basins. Smaller streams are highly coupled with their landscape and are more vulnerable to human activities (Meyer and Wallace 2001). This is in contrast to other sites containing high amounts of catchment level urban (LR7) and agriculture (YG3). This supports that land use is a main driver in community composition. There are relatively diverse communities within the watershed even with some levels of human impaction.

*Summary*

This study highlights the importance of considering both local habitat and landscape parameters of watersheds in stream biological assessments to understand the response of benthic communities to disturbances. While it is important to have an inventory of the biodiversity of an aquatic ecosystem (Dudgeon et al. 2006), it is becoming increasingly more important to understand the factors that are influencing the biodiversity and macroinvertebrate assemblage. Impacts of anthropogenic land use and water use in the Brazos River (Vogl and Lopes 2009) combined with changing climate in the region (Nielsen-Gammon 2009) there is reason to be concerned that human actions will influence the biodiversity of the Lower Brazos River.

Table 1. Location and general information of the 33 sampling sites of the tributaries of the Lower Brazos River. See Fig. 1 for a geographic distribution of the sites.

| Code | Stream Name | Watershed | County | Latitude | Longitude | Elevation (ft) | Watershed Area (ha) | Discharge ($m^3/s$) |
|---|---|---|---|---|---|---|---|---|
| **CW1** | Old River | Central | Burleson | 30.4040264°N | 96.3140678°W | 55 | 306.24 | 46.2417 |
| **CW2** | Thompsons Creek | Central | Brazos | 30.6008885°N | 96.4435228°W | 67 | 144.44 | 21.81059 |
| **CW3** | Little Brazos River | Central | Brazos | 30.6409039°N | 96.5206297°W | 63 | 1153.27 | 174.14114 |
| **CW4** | Big Creek | Central | Falls | 31.2567854°N | 96.8597668°W | 98 | 808.07 | 122.01777 |
| **CW5** | Deer Creek | Central | Falls | 31.2648098°N | 97.0320237°W | 110 | 231.12 | 34.898 |
| **CW6** | Tehuacana Creek | Central | McLennan | 31.5639615°N | 97.0481453°W | 114 | 482.98 | 47.85165 |
| **LB1** | Big Creek | Lower | Fort Bend | 29.3784390°N | 95.6024479°W | 14 | 415.67 | 77.03 |
| **LB2** | Bullhead Bayou | Lower | Fort Bend | 29.6066179°N | 95.6866399°W | 22 | 55.11 | 4.03585 |
| **LB3** | Allens Creek | Lower | Austin | 29.7039007°N | 96.1289913°W | 39 | 61.18 | 11.338 |
| **LB4** | Irons Creek | Lower | Waller | 29.8267771°N | 96.0363805°W | 37 | 137.69 | 25.51533 |
| **LB5** | Mill Creek | Lower | Austin | 29.8694630°N | 96.1550180°W | 37 | 1031.16 | 191.09029 |
| **LB6** | Clear Creek | Lower | Waller | 30.0544433°N | 96.0580244°W | 47 | 147.02 | 27.24472 |
| **LB7** | Caney Creek | Lower | Austin | 30.0621125°N | 96.2090383°W | 53 | 123.03 | 22.80008 |
| **LB8** | New Year Creek | Lower | Washington | 30.1657452°N | 96.2232700°W | 46 | 433.84 | 65.50841 |
| **LM1** | Lampasas River | Lampasas | Bell | 31.0018555°N | 97.4918558°W | 148 | 3422.03 | 238.70839 |
| **LM2** | Lampasas River | Lampasas | Bell | 30.9723781°N | 97.7782011°W | 206 | 3096.59 | 216.00678 |
| **LM3** | Lampasas River | Lampasas | Lampasas | 31.0794292°N | 98.0158551°W | 254 | 2115.05 | 147.53866 |
| **LR1** | Little River | Little River | Milam | 30.8254215°N | 96.7435651°W | 79 | 19688.32 | 1849.5091 |
| **LR2** | Big Elm Creek | Little River | Milam | 30.9030406°N | 96.9790851°W | 95 | 818.48 | 91.29443 |
| **LR3** | San Gabriel | Little River | Williamson | 30.6943662°N | 97.2787716°W | 134 | 1917.25 | 237.80125 |
| **LR4** | San Gabriel | Little River | Williamson | 30.6373391°N | 97.5724726°W | 138 | 1506.12 | 186.80789 |
| **LR5** | North San Gabriel | Little River | Williamson | 30.7031423°N | 97.8773021°W | 177 | 525.52 | 65.18178 |
| **LR6** | South San Gabriel | Little River | Williamson | 30.6207162°N | 97.8609248°W | 182 | 274.34 | 34.02739 |
| **LR7** | Brushy Creek | Little River | Williamson | 30.5261307°N | 97.5664998°W | 261 | 439.24 | 54.4801 |
| **NR1** | Navasota River | Navasota | Grimes | 30.4183477°N | 96.1064750°W | 48 | 5680.97 | 1131.45795 |
| **NR2** | Navasota River | Navasota | Grimes | 30.5707004°N | 96.1664846°W | 56 | 4840.86 | 964.13686 |
| **NR3** | Navasota River | Navasota | Grimes | 30.7203727°N | 96.1676675°W | 64 | 4322.42 | 860.88108 |
| **NR4** | Navasota River | Navasota | Leon | 31.1694968°N | 96.2986485°W | 87 | 2440.75 | 486.11562 |
| **NR5** | Navasota River | Navasota | Limestone | 31.5124660°N | 96.4510747°W | 113 | 806.21 | 160.56947 |
| **NR6** | Navasota River | Navasota | Limestone | 31.7018385°N | 96.7223061°W | 146 | 154.96 | 30.86252 |
| **YG1** | West Yegua Creek | Yegua | Lee | 30.2912744°N | 96.9604991°W | 53 | 277.73 | 24.55637 |
| **YG2** | Yegua Creek | Yegua | Washington | 30.3215025°N | 96.5073441°W | 61 | 2625.42 | 232.13048 |
| **YG3** | Yegua Creek | Yegua | Washington | 30.3681099°N | 96.3431751°W | 104 | 3394.93 | 300.16799 |

Table 2. Land use relative abundance across the watershed and within subbasins at the three spatial scales: 2-km upstream of site (level 1), total riparian upstream of site (level 2), and total catchment upstream of site (level 3).

| | Urban | Forest | Grass | Agriculture | Wetland |
|---|---|---|---|---|---|
| **Catchment Scale (Level 3)** | | | | | |
| Lower Brazos Watershed | 8% | 30% | 47% | 10% | 4% |
| Central Brazos | 8% | 18% | 45% | 23% | 5% |
| Lampasas | 2% | 68% | 29% | 1% | 0% |
| Little River | 8% | 38% | 40% | 11% | 2% |
| Lower Brazos | 14% | 15% | 54% | 10% | 6% |
| Navasota River | 6% | 25% | 60% | 3% | 4% |
| Yegua Creek | 5% | 45% | 39% | 2% | 7% |
| **Riparian Scale (Level 2)** | | | | | |
| Lower Brazos Watershed | 4% | 27% | 32% | 4% | 26% |
| Central Brazos | 3% | 15% | 38% | 10% | 32% |
| Lampasas | 3% | 45% | 34% | 4% | 11% |
| Little River | 5% | 41% | 31% | 2% | 18% |
| Lower Brazos | 8% | 15% | 37% | 4% | 35% |
| Navasota River | 2% | 37% | 30% | 1% | 23% |
| Yegua Creek | 1% | 12% | 14% | 1% | 35% |
| **2km Reach Scale (Level 1)** | | | | | |
| Lower Brazos Watershed | 4% | 20% | 29% | 4% | 40% |
| Central Brazos | 3% | 11% | 41% | 8% | 33% |
| Lampasas | 5% | 36% | 22% | 0% | 30% |
| Little River | 7% | 35% | 24% | 4% | 26% |
| Lower Brazos | 4% | 8% | 32% | 7% | 48% |
| Navasota River | 2% | 19% | 24% | 0% | 53% |
| Yegua Creek | 4% | 14% | 23% | 0% | 52% |

Table 3. National Land Cover Data (NLCD 2001) categories with reclassification scheme. Categories retained from Spearman rank correlation (Labay 2010) are indicated with reclassification code and level code.

| Original categories | Reclassified categories | Spatial scales: Local (100m buffer, 2km upstream) | Spatial scales: Riparian (100m buffer, total upstream) | Spatial scales: Catchment (cumulative area upstream) |
|---|---|---|---|---|
| Developed, Open Space | Urban | | Urban (2) | Urban (3) |
| Developed, Low Intensity | | | | |
| Developed, Medium Intensity | | | | |
| High Intensity, Residential | | | | |
| Deciduous Forest | Forest | Forest (1) | Forest (2) | Forest (3) |
| Evergreen Forest | | | | |
| Mixed Forest | | | | |
| Shrub/Scrub | | | | |
| Grasslands/Herbaceous | Grassland | Grassland (1) | | Grassland (3) |
| Pasture/Hay | | | | |
| Cultivated Crops | Agriculture | | | Agriculture (3) |
| Woody Wetlands | Wetland | | Wetland (2) | Wetland (3) |
| Emergent Herbaceous Wetlands | | | | |

Table 4. Physical stream characteristics for the 33 sites in the tributaries of the Lower Brazos River Watershed. Values are the means of the three seasons.

| Stream | Channel Width (m) | Depth (cm) | Velocity (cm/s) | Dominant Substrate | Canopy Coverage (%) | Silt Percentage | Filamentous algae (%) | Chlorophyll *a* (µg/cm$^2$) |
|---|---|---|---|---|---|---|---|---|
| **CW1** | 5.30 | 21.77 | 7.62 | clay | 51 | 55 | 1 | 526.61 |
| **CW2** | 5.59 | 33.00 | 20.50 | sand | 19 | 67 | 1 | 881.09 |
| **CW3** | 10.39 | 19.22 | 37.58 | clay | 23 | 63 | 9 | 1027.96 |
| **CW4** | 7.00 | 21.98 | 3.92 | clay | 18 | 98 | 1 | 329.15 |
| **CW5** | 3.75 | 20.10 | 7.38 | clay | 14 | 110 | 18 | 501.71 |
| **CW6** | 4.88 | 22.59 | 14.34 | clay | 36 | 114 | 13 | 466.51 |
| **LB1** | 12.54 | 73.63 | 16.41 | clay | 22 | 14 | 2 | 575.66 |
| **LB2** | 4.27 | 10.89 | 1.31 | clay | 6 | 22 | 0 | 777.07 |
| **LB3** | 4.05 | 22.11 | 3.57 | sand | 63 | 39 | 20 | 809.55 |
| **LB4** | 16.04 | 48.17 | 0.00 | clay | 7 | 37 | 33 | 403.40 |
| **LB5** | 8.32 | 20.22 | 21.51 | sand | 1 | 37 | 3 | 1028.86 |
| **LB6** | 8.65 | 20.22 | 16.62 | sand | 51 | 47 | 3 | 941.83 |
| **LB7** | 4.62 | 23.80 | 2.24 | clay | 60 | 53 | 14 | 419.02 |
| **LB8** | 12.23 | 41.31 | 25.38 | clay | 59 | 46 | 9 | 776.76 |
| **LM1** | 12.13 | 22.20 | 20.09 | gravel/pebble | 77 | 148 | 9 | 520.88 |
| **LM2** | 13.63 | 22.27 | 31.15 | gravel | 13 | 206 | 16 | 243.94 |
| **LM3** | 23.41 | 23.98 | 20.40 | bedrock | 0 | 254 | 57 | 761.61 |
| **LR1** | 19.51 | 46.66 | 34.54 | gravel/pebble | 0 | 79 | 4 | 642.73 |
| **LR2** | 6.83 | 20.83 | 18.50 | clay | 32 | 95 | 3 | 631.95 |
| **LR3** | 8.70 | 29.27 | 26.55 | pebble/cobble | 9 | 134 | 4 | 774.30 |
| **LR4** | 14.47 | 18.93 | 20.75 | bedrock | 26 | 138 | 39 | 1284.83 |
| **LR5** | 9.80 | 11.62 | 3.07 | bedrock | 13 | 177 | 2 | 277.76 |
| **LR6** | 5.77 | 18.88 | 12.35 | bedrock | 4 | 182 | 8 | 500.32 |
| **LR7** | 12.21 | 63.93 | 38.56 | bedrock | 48 | 261 | 3 | 860.61 |
| **NR1** | 14.52 | 45.34 | 30.45 | clay/boulder | 4 | 48 | 0 | 372.52 |
| **NR2** | 17.42 | 62.80 | 16.09 | sand | 36 | 56 | 0 | 33.31 |
| **NR3** | 22.54 | 86.79 | 5.56 | clay | 8 | 64 | 0 | 317.18 |
| **NR4** | 19.92 | 95.56 | 15.61 | clay | 28 | 87 | 0 | 775.20 |
| **NR5** | 19.64 | 106.75 | 1.12 | clay | 1 | 113 | 0 | 1007.97 |
| **NR6** | 6.20 | 32.41 | 11.24 | sand | 60 | 146 | 0 | 536.54 |
| **YG1** | 7.96 | 35.26 | 10.17 | cobble | 92 | 104 | 0 | 352.35 |
| **YG2** | 19.56 | 73.13 | 9.07 | clay | 0 | 61 | 2 | 585.16 |
| **YG3** | 10.01 | 23.36 | 15.51 | sand | 8 | 53 | 3 | 223.65 |

Table 5. ANOSIM global and pair-wise tests illustrating significance of macroinvertebrate communities between sampling periods, sites and subbasins.

| | | | R | *P* value |
|---|---|---|---|---|
| **Sampling Period** | | | | |
| Global test | | | 0.134 | <0.01 |
| Pairwise Tests: | | | | |
| Spring | vs. | Summer | 0.174 | <0.01 |
| Summer | vs. | Winter | 0.073 | .008 |
| Spring | vs. | Winter | 0.155 | <0.01 |
| **Site** | | | | |
| Global Test: | | | 0.441 | < 0.01 |
| **Subbasin** | | | | |
| Global Test: | | | 0.286 | < 0.01 |
| Pairwise Tests: | | | | |
| Central Brazos | vs. | Lampasas | 0.521 | < 0.01 |
| Central Brazos | vs. | Little River | 0.398 | < 0.01 |
| Central Brazos | vs. | Lower Brazos | 0.011 | 0.329 |
| Central Brazos | vs. | Navasota | 0.173 | < 0.01 |
| Central Brazos | vs. | Yegua | 0.391 | < 0.01 |
| Lampasas | vs. | Little River | -0.018 | 0.555 |
| Lampasas | vs. | Lower Brazos | 0.295 | 0.050 |
| Lampasas | vs. | Navasota | 0.462 | < 0.01 |
| Lampasas | vs. | Yegua | 0.637 | < 0.01 |
| Little River | vs. | Lower Brazos | 0.333 | < 0.01 |
| Little River | vs. | Navasota | 0.568 | < 0.01 |
| Little River | vs. | Yegua | 0.744 | < 0.01 |
| Lower Brazos | vs. | Navasota | 0.079 | .038 |
| Lower Brazos | vs. | Yegua | 0.193 | 0.052 |
| Navasota | vs. | Yegua | 0.028 | 0.325 |

Table 6. Watershed, subbasin, and site macroinvertebrate assemblage characteristics.

| Site Code | Total Species (*S*) | Total individuals (N) | Pielou's evenness (J') | Shannon diversity (H') | Margalef's diversity (d) | Simpson's diversity (Δ) |
|---|---|---|---|---|---|---|
| Lower Brazos Watershed | 181 | 187,651 | 0.649 | 3.37 | 14.83 | 0.938 |
| Central Brazos | 93 | 24,544 | 0.587 | 2.66 | 9.10 | 0.892 |
| CW1 | 61 | 2,433 | 0.652 | 2.68 | 7.70 | 0.893 |
| CW2 | 46 | 4,088 | 0.587 | 2.25 | 5.41 | 0.840 |
| CW3 | 57 | 7,552 | 0.696 | 2.81 | 6.27 | 0.917 |
| CW4 | 43 | 3,972 | 0.306 | 1.15 | 5.07 | 0.460 |
| CW5 | 48 | 1,433 | 0.622 | 2.41 | 6.47 | 0.866 |
| CW6 | 53 | 5,264 | 0.539 | 2.14 | 6.07 | 0.805 |
| Lampasas River | 99 | 49,668 | 0.657 | 3.03 | 9.06 | 0.898 |
| LM1 | 87 | 11,447 | 0.702 | 3.13 | 9.20 | 0.932 |
| LM2 | 82 | 14,583 | 0.695 | 3.06 | 8.45 | 0.916 |
| LM3 | 77 | 24,332 | 0.533 | 2.32 | 7.53 | 0.745 |
| Little River | 123 | 75,520 | 0.666 | 3.21 | 10.86 | 0.932 |
| LR1 | 61 | 13,199 | 0.535 | 2.20 | 6.32 | 0.835 |
| LR2 | 77 | 4,318 | 0.651 | 2.83 | 9.08 | 0.893 |
| LR3 | 78 | 9,825 | 0.688 | 3.00 | 8.38 | 0.929 |
| LR4 | 97 | 20,949 | 0.586 | 2.68 | 9.65 | 0.863 |
| LR5 | 59 | 3,265 | 0.594 | 2.42 | 7.17 | 0.844 |
| LR6 | 79 | 6,991 | 0.720 | 3.15 | 8.81 | 0.931 |
| LR7 | 72 | 21,808 | 0.709 | 3.03 | 7.11 | 0.925 |
| Lower Brazos | 117 | 17,700 | 0.628 | 2.99 | 11.86 | 0.911 |
| LB1 | 51 | 3,540 | 0.504 | 1.98 | 6.12 | 0.776 |
| LB2 | 38 | 1,916 | 0.570 | 2.07 | 4.90 | 0.822 |
| LB3 | 59 | 2,561 | 0.569 | 2.32 | 7.39 | 0.810 |
| LB4 | 47 | 1,401 | 0.648 | 2.49 | 6.35 | 0.846 |
| LB5 | 60 | 2,513 | 0.673 | 2.76 | 7.54 | 0.897 |
| LB6 | 60 | 3,293 | 0.527 | 2.16 | 7.28 | 0.727 |
| LB7 | 45 | 857 | 0.568 | 2.16 | 6.52 | 0.762 |
| LB8 | 50 | 2,217 | 0.713 | 2.79 | 6.36 | 0.897 |
| Navasota River | 91 | 7,567 | 0.674 | 3.04 | 10.08 | 0.917 |
| NR1 | 45 | 1,551 | 0.672 | 2.56 | 5.99 | 0.876 |
| NR2 | 43 | 510 | 0.682 | 2.57 | 6.74 | 0.817 |
| NR3 | 46 | 1,460 | 0.588 | 2.25 | 6.18 | 0.804 |
| NR4 | 63 | 1,060 | 0.747 | 3.09 | 8.90 | 0.917 |
| NR5 | 35 | 525 | 0.671 | 2.39 | 5.43 | 0.857 |
| NR6 | 40 | 2,565 | 0.565 | 2.08 | 4.97 | 0.783 |
| Yegua Creek | 72 | 5,631 | 0.541 | 2.32 | 8.22 | 0.831 |
| YG1 | 53 | 2,221 | 0.587 | 2.33 | 6.75 | 0.837 |
| YG2 | 23 | 168 | 0.571 | 1.79 | 4.29 | 0.624 |
| YG3 | 53 | 3,297 | 0.507 | 2.01 | 6.42 | 0.754 |

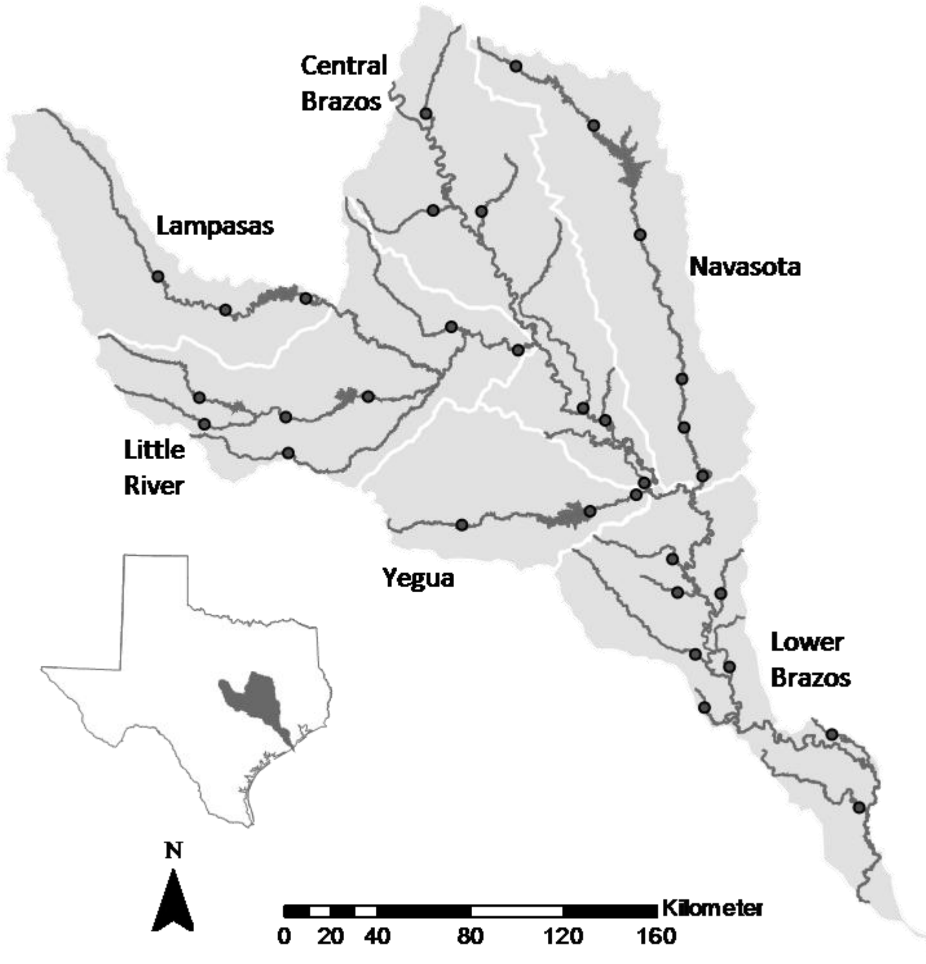


Figure 1. Map of the study sites of the Lower Brazos Watershed.

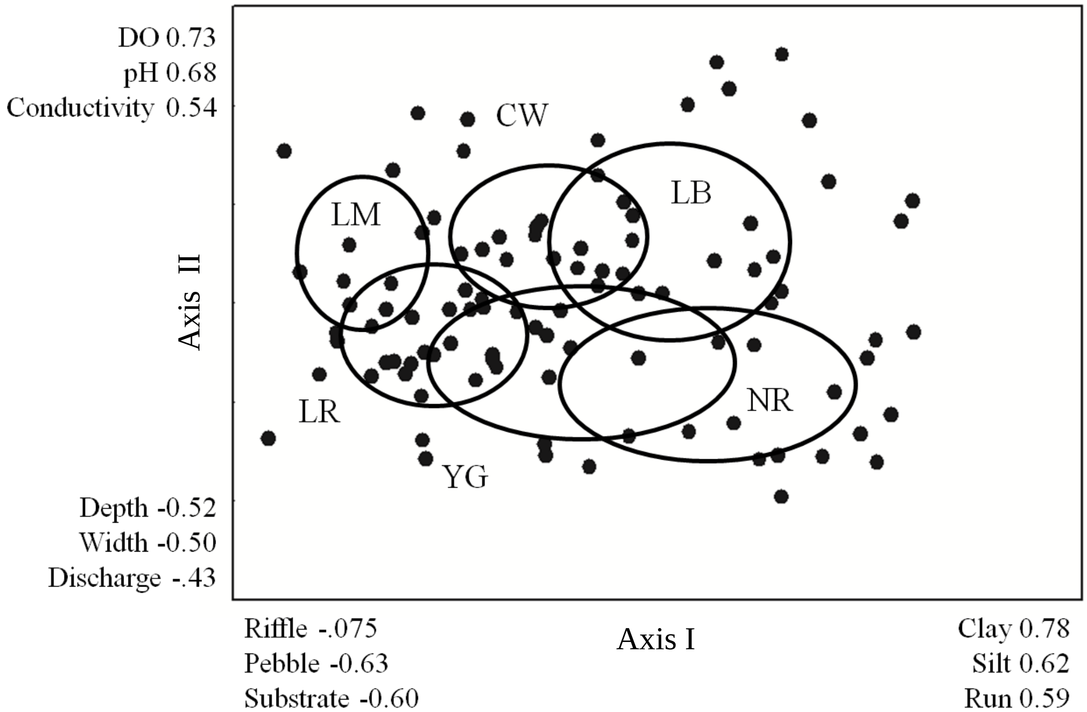


Figure 2. Biplot diagram resulting from principle component analysis (PCA) displaying sample scores. Principal component (PC) axis I explained 16% of the total variation and PC axis II explained 10.9%. Central Brazos (CW), Lampasas (LM), Little River (LR), Lower Brazos (LB), Navasota (NR), and Yegua (YG) subbasin groupings are enclosed in 1 standard deviation of the group's mean sample score.

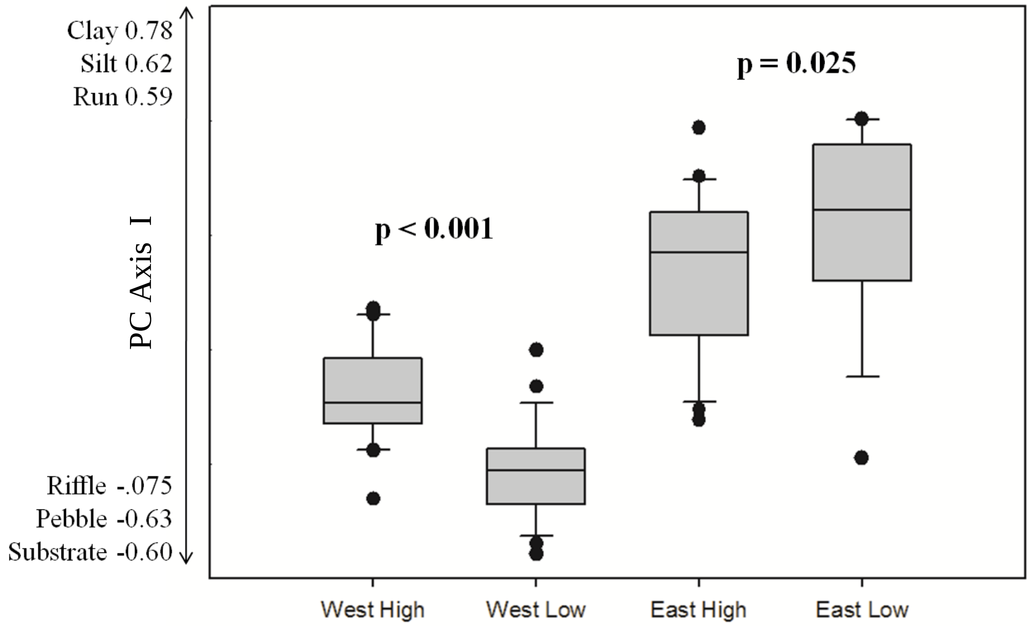


Figure 3. Boxplot of land use influence on the habitats of the Eastern and Western streams. Error bars represent 95% confidence intervals.

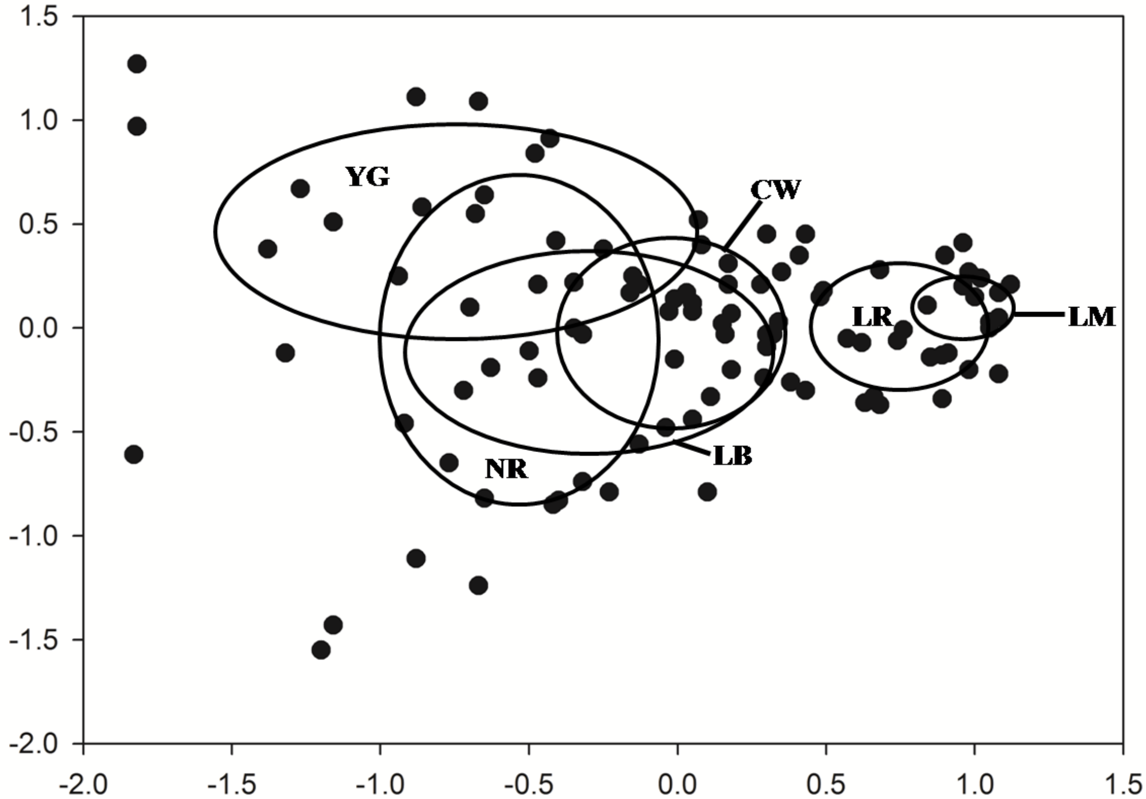


Figure 4. Multidimensional scaling (MDS) biplot diagram of the Lower Brazos River Watershed macroinvertebrate assemblages showing subbasin groupings enclosed in one standard deviation.

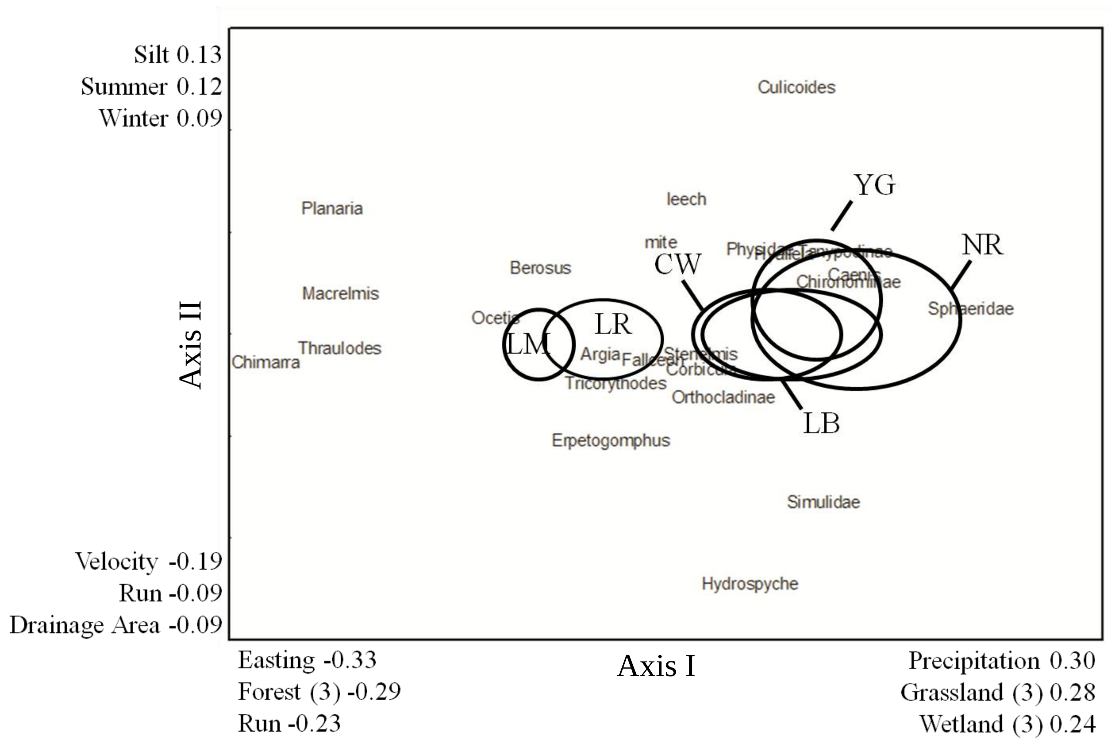


Figure 5. Biplot diagram resulting from canonical correspondence analysis (CCA) displaying sample and species scores. CCA explained 52.8% of the total variance; Axis I explained 21.2% and Axis II explained 9.7%. Central Brazos (CW), Lampasas (LM), Little River (LR), Lower Brazos (LB), Navasota (NR), and Yegua (YG) subbasin groupings are enclosed in 1 standard deviation of the group's mean sample score.

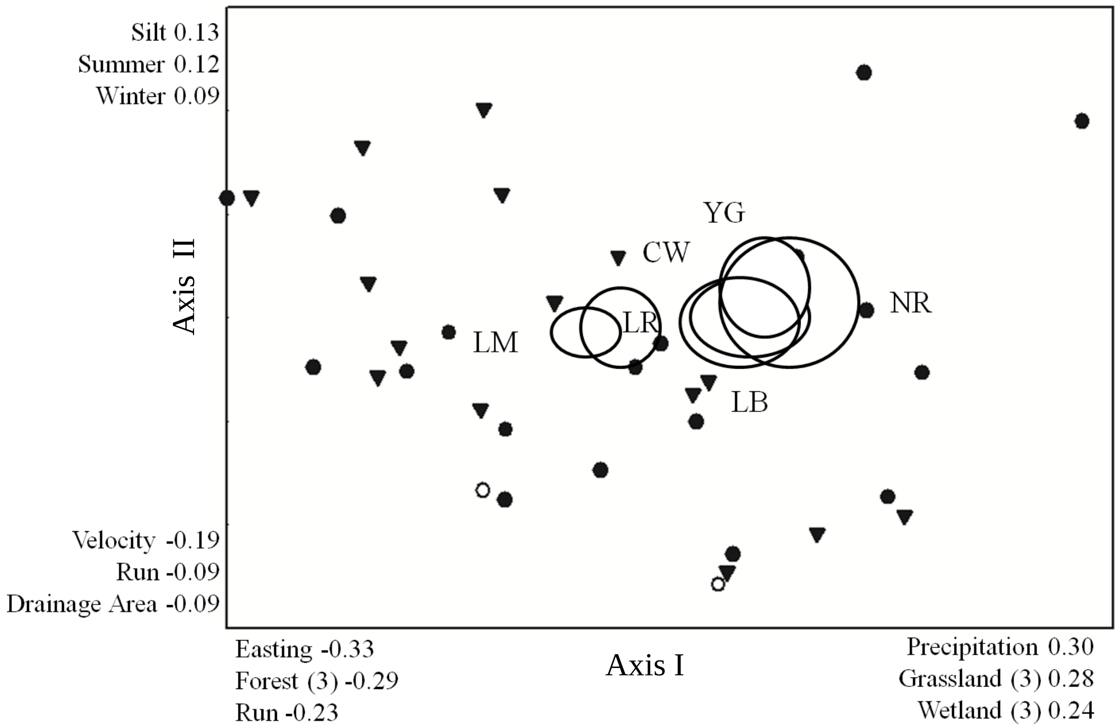


Figure 6. Biplot diagram resulting from canonical correspondence analysis (CCA) displaying sample and Ephemeroptera (●), Plecoptera(○), and Trichoptera (▼) taxa scores. The CCA explained 52.8% of the total variance; Axis I explained 21.2% and Axis II explained 9.7%. Central Brazos (CW), Lampasas (LM), Little River (LR), Lower Brazos (LB), Navasota (NR), and Yegua (YG) subbasin groupings are enclosed in 1 standard deviation of the group's mean sample score.

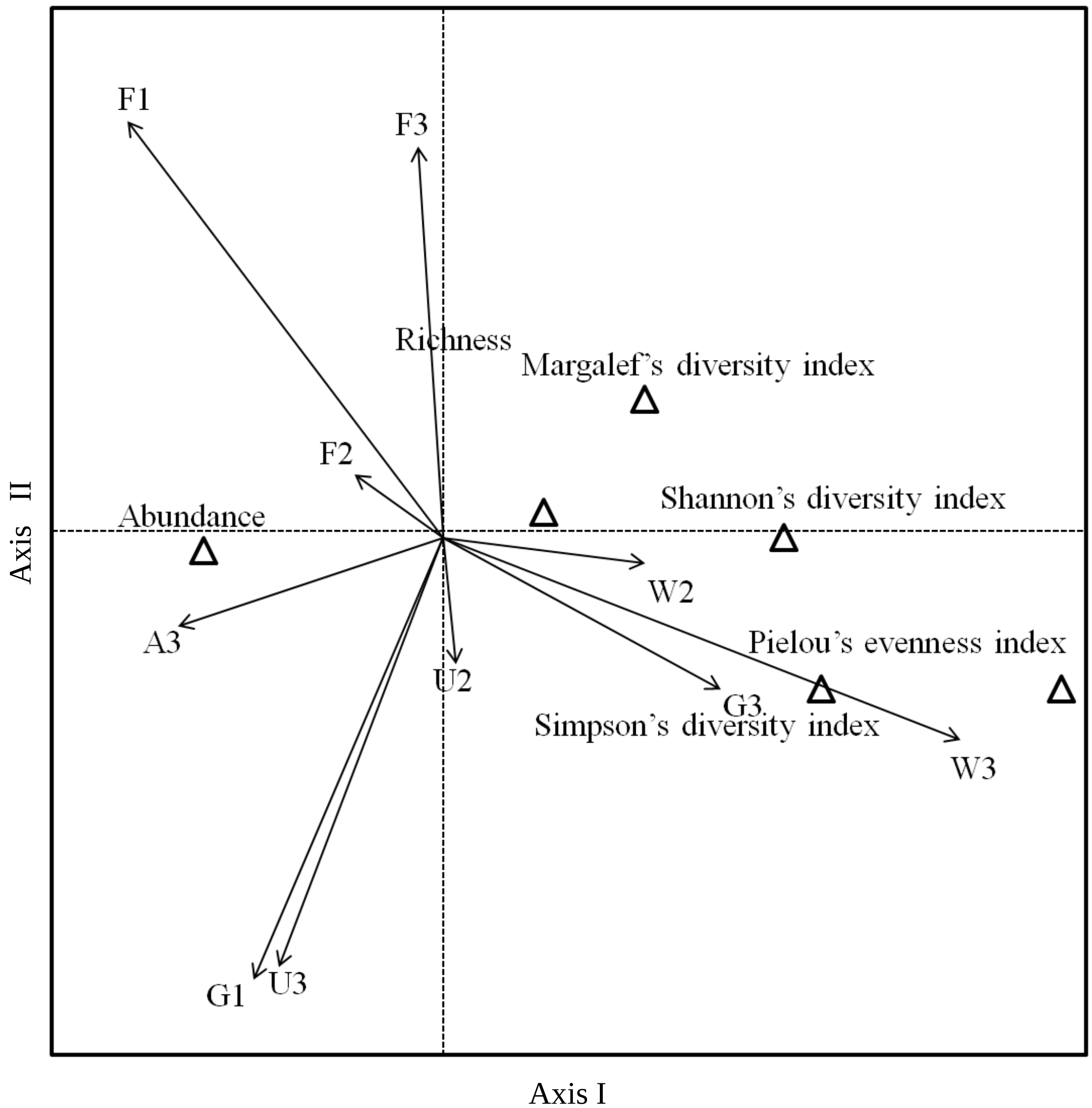


Figure 7. Biplot diagram resulting from canonical correspondence analysis (CCA) displaying diversity indices and land use. The CCA explained 16.7% of the total variance; Axis I explained 15.3% and Axis II explained 1.1%. Land use is agriculture (A), forest (F), grassland (G), urban (U), and wetland (W). Numbers represent the different spatial scales of land use: (1) is 2km upstream of site; (2) is total riparian upstream of site; (3) is total catchment upstream of site.

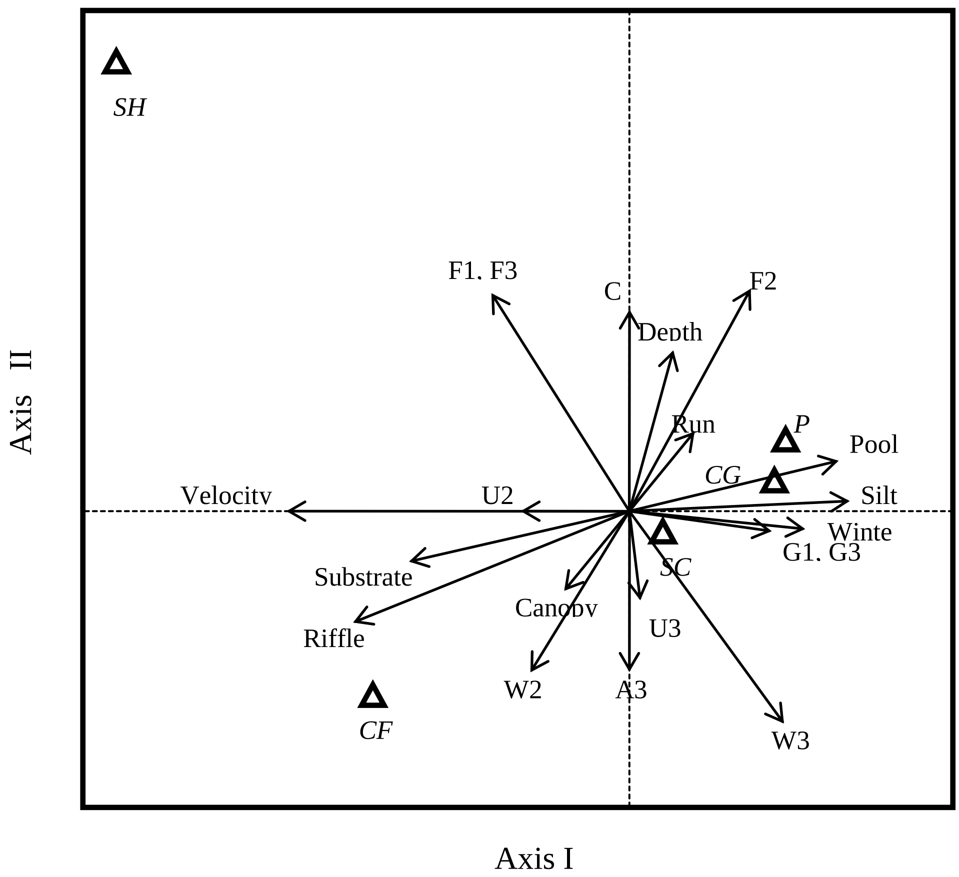


Figure 8. Biplot diagram resulting from canonical correspondence analysis (CCA) displaying trophic groups. The CCA explained 47.7% of the total variance; Axis I explained 27.1% and Axis II explained 15.9%. Functional feeding groups are collector-filterers (CF), collector-gatherers (CG), predators (P), scrapers (SC) and shredders (SH).

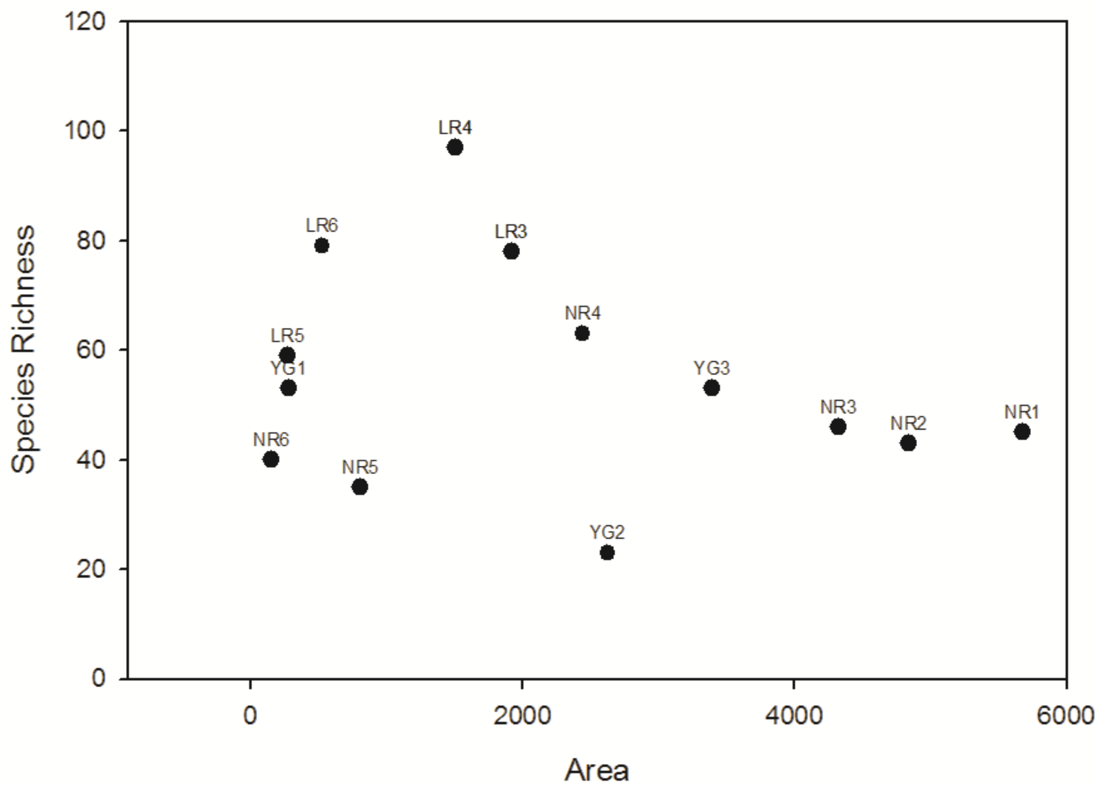


Figure 9. Species richness in relation to stream size. Stream size is represented by catchment area.

# CHAPTER II

## MACROINVERTEBRATE INTEGRITY IN THE LOWER BRAZOS RIVER: RESPONSE TO LAND USE DISTURBANCE

### INTRODUCTION

Land use and land cover change (referred to as land use from here on) affect stream ecosystems by altering the natural physical processes (Malmqvist and Rundle 2002). Urban and agriculture land use are by far the primary categories of anthropogenic land use, but other land uses such as mining, forestry operation, and recreation can have considerable negative impact on stream ecosystems (Price et al. 2003; Allan 2004; Pond et al. 2008; Zhang et al. 2009). This study focuses on three categories of land use (agriculture land, urban area, and natural land cover) in the Lower Brazos Watershed.

Intense human activities in the landscape have resulted in land disturbance, such as increased impervious surfaces, and caused changes in the hydrological processes, which impact river ecosystem health. Agriculture land use is associated with declines in water quality and alterations in habitat structure through inputs of pollution from non-point sources, increased sedimentation and nutrients from runoff, and increased temperature regime from removal of the riparian vegetation (Lenat and Crawford 1994). Urban land use increases impervious areas and degrades water quality and stream habitat through increased surface runoff, which leads to increased channel erosion, changed channel morphology, and erratic hydrology (Paul and Meyer 2001; Roy et al. 2005;

Burcher and Benfield 2006). The effects of agriculture and urban land use cause declines in the stream habitat quality and water quality, which decrease sensitive taxa and biodiversity (Lenat and Crawford 1994).

Ecologists assess stream physical and biological conditions in response to human land use activities using ecological indicators (Allan 2004; Kerans and Karr 2004; Mazor et al. 2006). Ward (2002) described the health of river as the ability of the river to structure the riverine landscape, maintain the interaction between landscape elements, thus supporting the biodiversity. Macroinvertebrates are often used to describe the health of a system because they are diverse in their form and habit, and respond in a predictable manner to ecological stressors (Rosenberg and Resh 1993). Biomonitoring employs indices calculated from multi-metric scores using characteristics that consider numerous biological species traits (Kerans and Karr 1994). Sensitive taxa which include many members of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are commonly used to measure stream health condition (Lenat and Barbour 1994). Other more detailed indices include the benthic index of biotic integrity (B-IBI), which is a grading system of river ecosystem-health based on benthic macroinvertebrates found at studying sites (Kerans and Karr 1994), and the invertebrate community index (ICI) (Ohio EPA 1988) which use a multi-metric approach of 10 metrics comprised of species richness, dominance, trophic level, and tolerance taxa. These indices are powerful tools in understanding river integrity and the influence of human disturbance (Karr 1991; Karr 1999; Mebane et al. 2003).

The tributaries of Lower Brazos River in Texas vary in their geomorphology and habitat structure. Benthic invertebrate assemblages are a function of natural habitat features which very across the watershed. The goals of this study are to provide ecological information for the biological assessment of the tributaries of the Lower Brazos Watershed with respect to land use. More specifically, the study objective is to analyze the relationship between environmental variables of habitats and watersheds and biological response variables to human land use.

## METHODS

*Study region*

Study sites were located in the Lower Brazos River Watershed, an area (41,000 $km^2$) in southeastern Texas, USA (Figure 10). The Lower Brazos River Watershed (LBRW) is defined as the Brazos River south of Waco, TX and empties into the Gulf of Mexico. The 33 sites chosen for this study were located in the six major subbasins of the LBRW. Precipitation is highest in the lower subbasins of the Gulf slope drainage and lowest in the Lampasas and Little River region.

The landscape in this region has been impacted by various anthropogenic disturbances, mostly agriculture, and urban land use. The northern portion of the study area is near Waco, TX, and the lower areas drain the cities of Bryan and College Station, and the suburban areas of Austin and Houston. Agriculture activity is the most intense in the central tributaries, the Navasota River and Yegua Creek due to the nutrient rich soil in this area. The Little River and Lampasas regions are within the limestone dominated Balcones uplift and are not as suited for agriculture. The study area represents a range of grassland and forest (natural), urban, and agriculture land use. The thirty-three sites within this study drained areas that ranged from 55.11 $km^2$ to 19,688 $km^2$. Urban land cover that reflects land use disturbance never exceeded 25%. The combined disturbance areas of urban land with agriculture land use was never more than 60%.

*Water physiochemical parameters.* Water parameters were collected at each sampling event from every site. Specific conductance, pH, and temperature were recorded with a calibrated YSI 556 multiparameter meter.

*Geomorphology and habitat.* Stream habitat and geomorphology was measured each season. Depth, velocity, silt, and substrate were measured at five points equidistance across five transects. Percent riffle and run was estimated for each site.

*Land use and land cover.* Land use and land cover data was calculated at the catchment scale for all sites using the National Land Use and Land Cover (LULC) database from 2001. High human disturbance was calculated from the catchment level percentages of urban and high intensity agricultural land use. Urban land use was the addition of high and low intensity urban. Agricultural land use was the addition of cultivated crops and Hay/Pasture. Natural land use was the addition of forest and grassland. The final land cover variable considered was the entire catchment all the way upstream of the site.

*Sampling methods*

*Macroinvertebrates.* Benthic macroinvertebrates were collected through three seasons (Spring, Summer and Winter) from February 2008 to December 2009. All sites were sampled each season, and those within the same subbasin on the same day. At each site macroinvertebrates assemblages were composed of six samples collected from available habitat using a timed kick net in the spring, and a Hess sampler summer and winter. All samples were stored in 70% ethanol and taken to the lab.

In the lab, samples were washed through 1 mm and 500 µm sieves to separate small macroinvertebrates from larger ones to increase sample picking efficiency. All invertebrates were picked under a dissecting microscope (15×) and preserved in 70% ethanol for later identification. Invertebrates were enumerated and identified to the lowest possible taxonomical level, often to genus, using traditional keys (Thorp and Covich 2001; Merritt and Cummins 2008). Chironomids were identified to tribes, and Simuliids were left at family level. Taxa were assigned to functional feeding groups (FFG) based on Texas Commission of Environmental Quality designations (TCEQ 2003).

*Trophic variables.* After all the invertebrates were picked from the samples, benthic organic matter (BOM) was quantified. Fine Particulate Organic Matter (<1mm to >500µm) and Coarse Particulate Organic Matter (>1mm) were dried at 50˚C for 48 hours and weighed. The organic matter was ashed at 500˚C for 4 hours and re-weighed to obtain ash free dry mass (AFDM). Algal biomass (chlorophyll α) was sampled by collecting six rocks or sticks from one habitat and scrubbing the whole surface vigorously. The slurry was immediately removed from the light. The surface scrubbed was measured with an aluminum foil cutout which was weighed in the lab. The slurry was filtered through a Whatman® GF/F filters (size .7µm, Whatman Inc, Clifton, NJ, USA), and immediately frozen. Chlorophyll α was extracted using 90% acetone and measured using a calibrate fluorometer.

*Biological Indices of benthic macroinvertebrate assemblages*

For each of the sites, invertebrate data from hess samples and kick nets were calculated for obtaining (1) Ephemeroptera, Plecoptera, and Trichoptera richness (EPT), (2) the Benthic Index of Biotic Integrity (B-IBI; Kerans and Karr 1994), (3) the Invertebrate Community Index (ICI; Ohio EPA 1989), and (4) the TCEQ Benthic Index of Biotic Integrity for (TB-IBI)(Table 7). Tolerance values for the Hilsenhoff Biotic Index which was part of the TB-IBI were obtained from the TCEQ invertebrate index.

*Data analyses*

The distribution of variables was checked for normality and transformed when necessary (Table 8). Percentage data were transformed using arcsin square-root, and the data of macroinvertebrate data were log(x+1) normalized. Least-squares regression was used to analyze the relationship between biological and environmental data and land use percentages (urban, agriculture, and forest/grassland). Biplots of biological indices and land use variables were analyzed to look for a potential threshold at which the biotic assemblages decline. Regressions were performed in the Statistical program R (R core development team 2010).

## RESULTS

Over 184, 000 macroinvertebrates from 178 taxa were collected. The most abundant taxa were in the family Chironomidae. The Ephemeropteran *Fallceon* and the Trichopteran *Hydropsyche* were commonly found in high abundance across all watersheds. The macroinvertebrate indices scores are listed in Table 9. Seasonally there were differences in the scores of the sites. Macroinvertebrate taxon richness ranged from 6 (LB5) taxa to 79 (LR4) in spring, 4 (YG2) taxa to 64 (LM1) in the summer, and 11 (NR3) taxa to 72 (LM1). The EPT richness at each site ranged from 0 (LB5) individuals to 24 (LR3) in the spring, 0 (YG2) individuals to 23 (LM2) in the summer, and 1(NR3, NR5, YG1, YG2) individual to 24 in the winter. The ICI result ranked LB5 as the most degraded site and LR2 as the least degraded in the spring, YG2 the most degraded and LR6 the least degraded in the summer, and YG2 the most degraded and LM2 and LR7 the least degraded in the winter. The B-IBI scored LB2 as the most degraded site and LM2 and LR2 as the least degraded sites in the spring, YG1 as the most degraded and LM3, LR4, and LR6 as the least degraded sites in the summer, and NR4 as the most degraded and LM2 and LR6 as the least degraded site in the winter. The T-BIBI ranked LB4 as the most degraded site and LM3 and LR2 as the least degraded sites in the spring, YG2 as the most degraded and LM1, LM2, LM3, and LR6 as the least degraded sites in the summer, and NR5 as the most degraded and LM2 as the least degraded site in the winter.

Biplots of the T-IBI and the B-BIBI with human land use (urban and agricultural land) reveal about a 20% threshold, above which there is a drop in the indices into limited/poor water quality (Figure 11 and 12, respectively). The Lampasas River sites (LM2 and LM3), South Fork San Gabriel River (LR6) and Brushy Creek (LR7) scored in the high to very high water quality levels representing very high biological integrity, while Yegua Creek (YG2) and Navasota River (NR5) both scored very low representing high influences of human disturbance. Richness, EPT, B-IBI, and T-BIBI were all significantly and positively related to total forest/grassland in the catchment and 100m buffer (Table 4-6). These same indices were negatively related to agriculture and total human land use.

The relationship with the physical habitat and geomorphic variables and land use varied among seasons (Tables 10, 11, and 12). There were significant negative relationships among stream habitat variables (riffle and substrate) and hay/pasture land use throughout all three seasons (spring, $p < 0.01$; both summer; winter, $p < 0.05$). In spring and winter seasons, there was a significant positive relationship with temperature and forest/grassland in the catchment and the buffer zone ($p < 0.01$), and a negative relationship with temperature and hay/pasture land use ($p < 0.01$). In the summer season, there was a significant relationship with BOM and human land use and forest in the catchment and buffer zone ($p < 0.01$).

The macroinvertebrate and land use disturbance relationship (Tables 10, 11, and 12) was strong throughout the seasons. Richness ($p<0.01$ for all seasons), EPT richness ($p<0.01$ for all seasons), and B-IBI ($p<0.05$ for all seasons) had strong negative relationships with increasing anthropogenic land use. The T-BIBI varied from significant only in the spring ($P<0.05$) to non-significant for the other seasons.

## DISCUSSION

Land use classifications had significant relationships with biotic indices. Between the seasons, higher EPT and taxa richness and B-IBI scores were significantly negatively related to human land use. Surprisingly, the regionally adapted T-BIBI and ICI did not show strong significant relationships with increasing human land use disturbance. Total agriculture was the strongest land use predictor across the seasons. Natural land use categories, both forest and grassland land cover at the catchment scale and the buffer scale were significantly related to higher EPT taxa and B-IBI scores. These results support the importance of an intact riparian buffer and natural land use on biological integrity (Richards et al. 1996; Roth et al. 1996).

Urban land use was minimal in the study area, with only one site draining more than 5% of urban area (LB2). The heavy agriculture in the area accounts for most human land disturbance. The relationship between the biotic indices and human land disturbance indicated a threshold around 20% land use which reflected poorer water quality which led to a decline in macroinvertebrate assemblage integrity.

Studies show that macroinvertebrate assemblages respond to land use disturbance through changes in habitat structure (Lammert and Allan 1999). There were seasonal differences in the relationships among land use and geomorphic and other environmental variables. Riffle percentage and substrate coarseness had a strong negative relationship with agriculture, and positive relationship with forest land cover. In the Lower Brazos Basin, the sites on the limestone uplifted portion of the Edwards Plateau have less agriculture than the sites that are located at the base of this uplifted region and have more fertile soil in the catchment area. There was a strong positive relationship between forest/grassland land cover and temperature in spring and winter. In the winter this relationship can be attributed to lower precipitation and spring influenced temperatures in the northwest regions of the Lower Brazos Watershed. In all, benthic macroinvertebrates responded to both changes in habitat structure and land use disturbance.

Lenat and Barbour (1994) and Wallace et al. (1996) found EPT richness to be the most reliable measure of water quality changes. This study found the EPT richness to be just as robust. Even with the near absence of the order Plecoptera in the study region in Texas, with only three genera from two families represented in this study in relatively low numbers, EPT richness was significant. The EPT taxa richness is sensitive to human land use disturbance. This supports the claim that simple indexes can be as effective as indices that require more metrics (Rosenberg and Resh 1993). Taking into account differences within genera, utilizing macroinvertebrate species traits as well as taxonomic-based assemblage metrics should provide insight into contributory mechanisms for stream impairments. Regional calibrations of the multi-metric approaches should theoretically differentiate land use and anthropogenic influences from natural forms of regional variability (Richards et al. 1997; Butcher el al. 2003). The B-IBI had more significant relationships with land use than the T-BIBI which is an unexpected result because the T-BIBI was developed specifically for Texas Rivers. Finally, the ICI showed little to no relationship with land use variables, suggesting that it should not be used to assess benthic macroinvertebrate integrity. The biotic integrity of Lower Brazos Watershed scored many streams in the intermediate category according to the B-IBI, but in the high category with the T-BIBI with increasing land use. The B-IBI had a much stronger significant relationship with land use disturbance than the T-BIBI, and perhaps may be a better indicator in this watershed.

Understanding the impact of human land use on stream ecosystems is imperative as urbanized areas grow with increasing population. Increased development in watersheds has a dramatic effect on stream biodiversity. Agriculture and urban land use have complex interactive mechanisms to influence the observed effects, making it difficult to determine the exact physical and chemical characteristics directly related to these land uses.  Changes in the physiochemical properties of streams from both urban and agriculture land disturbances negatively results in lower macroinvertebrate diversity and assemblages are dominated by pollution-tolerant organisms.  The results of this study indicate that human activities even at low land use levels will influence the biotic integrity of a riverine ecosystem.

Table 7. Metrics for (1) the TCEQ Benthic Index of Biotic Integrity (T-BIBI), (2) the Benthic Index of Biotic Integrity (B-IBI), and Invertebrate Community Index (ICI).

T-BIBI Metrics (Surber Samples)

1. Proportion of the 3 dominant taxa
2. Dipteran taxa richness
3. Ephemeropteran taxa richness
4. Intolerant taxa richness
5. Proportion of individuals as Ephemeropteran, Plecopteran, and Trichopteran (EPT) taxa
6. Proportion of individuals as Chironomids
7. Proportion of tolerant taxa
8. Proportion of individuals as grazers
9. Proportion of individuals as collector-gatherers
10. Proportion of individuals as filterers

T-BIBI Metrics (Kick net Samples)

1. Total taxa richness
2. Ephemeropteran taxa richness
3. Proportion of individuals as Chironomids
4. Proportion of dominant taxa (top three)
5. Proportion of individuals in dominant FFG
6. Proportion of individuals as predators excluding Chironomids
7. Ratio of intolerant to tolerant taxa
8. Proportion of Trichopteran as Hydropsychidae
9. Number of non-insect taxa
10. Proportion of individuals as collector-gatherers
11. Proportion of total number as Elmidae
12. Hilsenhoff biotic index

B-IBI Metrics

1. Total taxa richness
2. Ephemeropteran taxa richness
3. Trichopteran taxa richness
4. Plecopteran taxa richness
5. Proportion of *Corbicula*
6. Proportion of 2 most abundant taxa
7. Proportion of filterers
8. Proportion of scrapers
9. Proportion of predators excluding Chironomids
10. Total abundance

ICI Metrics

1. Total taxa richness
2. Ephemeropteran taxa richness
3. Trichopteran taxa richness
4. Dipteran taxa richness
5. Proportion of Ephemeropteran composition
6. Proportion of Trichopteran composition
7. Proportion predatory Chironomid composition
8. Proportion other Dipteran and non-insects
9. Percent tolerant organisms
10. EPT taxa richness

Table 8. Environmental variables across 6 subbasins used in the statistical analysis.

| Variable | Unit | Min | Mean | Max |
|---|---|---|---|---|
| Land Cover | | | | |
| Human Land Disturbance | Percent | <1.0 | 25.2 | 59.1 |
| Percent urban* | Percent | <1.0 | 1.6 | 24.1 |
| High intensity* | Percent | <1.0 | 1.6 | 24.0 |
| Low intensity* | Percent | <1.0 | <1.0 | 23 |
| Percent agriculture | Percent | <1.0 | 23.6 | 57.4 |
| Crop* | Percent | <1.0 | 9.8 | 39.7 |
| Hay/Pasture | Percent | 0 | 27.6 | 68.1 |
| Percent grassland and forest | Percent | <1.0 | 19.2 | 42.6 |
| Riparian natural buffer* | Percent | 3.9 | 42.4 | 96.0 |
| Habitat and Geomorphic Variables | | | | |
| Basin area† | Drainage area($km^2$) | 55.1 | 1935.7 | 19688.3 |
| Velocity | Flow at .6depth (cm/m) | 15.7 | 0 | 67.8 |
| Depth† | Water depth (cm) | 37.5 | 3.8 | 145.4 |
| Percent riffle* | Riffle area (%) | 0 | 29.3 | 100 |
| Percent Run* | Run area (%) | 0 | 58.5 | 100 |
| Substrate coarseness† | Coarseness (scale) | 1 | 1.8 | 6 |
| Silt percentage* | Percent | 0 | 63.2 | 100 |
| Environmental and Chemical Variables | | | | |
| Temperature | ˚C | 5.7 | 21.0 | 34 |
| pH | pH | 7.2 | 8.0 | 9.5 |
| Conductivity† | µS/cm | 156.0 | 753,5 | 2398.0 |
| BOM† | mg AFDM | 0.7 | 8.4 | 48.0 |
| Chlorophyll α | mg/$m^2$ | 24.0 | 611.1 | 2335.0 |

* Arcsin square-root transformed

† log (x) transformed

Table 9. Summary of the scores of macroinvertebrate assemblage for the 33 sites across three seasons. Richness values are totals of the benthic samples collected from each site. EPT richness is the taxon richness in insect orders Ephemeroptera, Plecoptera, and Trichoptera. The Invertebrate Community Index (ICI), Benthic Index of Biotic Integrity (B-IBI), and the Texas Commission of Environmental Quality Benthic Index of Biotic.

| | Indices | Min | Mean | Max |
|---|---|---|---|---|
| Spring | EPT | 0 | 9.1 | 24 |
| | ICI | 2 | 26.9 | 42 |
| | B-IBI | 12 | 26.2 | 40 |
| | T-BIBI | 19 | 34.8 | 45 |
| Summer | EPT | 0 | 10.6 | 23 |
| | ICI | 5 | 32.0 | 46 |
| | B-IBI | 12 | 24.5 | 38 |
| | T-BIBI | 17 | 36.3 | 45 |
| Winter | EPT | 1 | 10.1 | 24 |
| | ICI | 3 | 27.8 | 44 |
| | B-IBI | 14 | 25.9 | 38 |
| | T-BIBI | 19 | 34.0 | 47 |

Integrity (T-BIBI) are multi-metric indices described in Table 1.

Table 10. Spring season linear regression models with 2001 land use variables (n=33 sites); $r^2$ values reported, negative and positive symbols indicate the relationship of land cover variables and response variables. Macroinvertebrate variables are described in Table 7; Other variables are explained in Table 8.

| | Human Land Use | Urban | | | Agriculture | | | Forest/Grassland | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Total | High | Low | Total | Crop | Hay/Pasture | Total | Buffer |
| Macroinvertebrate Variables | | | | | | | | | |
| Richness | - 0.47*** | -0.00 | +0.01 | -0.01 | -0.38*** | -0.01 | -0.56*** | +0.41*** | +0.34*** |
| EPT | -0.42*** | -0.04 | -0.00 | -0.06 | -0.40*** | -0.00 | -0.57*** | +0.42*** | +0.29** |
| ICI | -0.13* | +0.00 | +0.00 | -0.01 | -0.09 | -0.01 | -0.14* | +0.11 | +0.10 |
| B-IBI | -0.34*** | -0.00 | +0.01 | -0.02 | -0.28** | +0.00 | -0.48*** | +0.30*** | +0.29** |
| T-BIBI | -0.19* | +0.00 | +0.03 | +0.00 | -0.13* | +0.00 | -0.32*** | +0.14* | +0.08 |
| Habitat and Geomorphic Variables | | | | | | | | | |
| Drainage | -0.15* | -0.19* | -0.13* | -0.13* | -0.25** | -0.02 | -0.12* | +0.22** | +0.14* |
| Velocity | -0.09 | -0.00 | -0.00 | +0.00 | -0.09 | +0.01 | -0.14* | +0.08 | +0.04 |
| Depth | +0.02 | -0.03 | -0.07 | -0.01 | +0.00 | -0.00 | +0.03 | -0.00 | -0.00 |
| Riffle | -0.20** | -0.04 | -0.01 | -0.04 | -0.20** | -0.01 | -0.21** | +0.22** | +0.18* |
| Run | +0.09 | +0.05 | +0.03 | +0.06 | +0.11 | +0.02 | +0.06 | -0.12* | -0.11 |
| Substrate | -0.17* | -0.05 | -0.02 | -0.05 | -0.20** | -0.00 | -0.22** | +0.24** | +0.22** |
| Silt | +0.06 | +0.01 | +0.00 | +0.01 | +0.06 | -0.02 | +0.16* | -0.07 | -0.03 |
| Environmental Variables and Other Variables | | | | | | | | | |
| Temperature | -0.35*** | -0.00 | +0.01 | -0.00 | -0.28** | -0.01 | -0.38*** | +0.28** | +0.26** |
| pH | -0.02 | +0.00 | +0.03 | +0.00 | -0.01 | -0.00 | -0.02 | +0.01 | +0.04 |
| Conductivity | -0.02 | -0.00 | -0.00 | -0.00 | -0.03 | +0.02 | -0.10 | +0.03 | +0.01 |
| BOM | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chlorophyll α | -0.01 | +0.12* | +0.12* | +0.12* | +0.00 | -0.00 | -0.01 | +0.00 | +0.00 |

*P <0 .05, **P<0.01, ***P<0.001

Table 11. Summer season linear regression models with 2001 NLUD land cover variables (n=33 sites); $r^2$ values reported, negative and positive symbols indicate the relationship of land cover variables and response variables. Macroinvertebrate variables are described in Table 3; Other variables are explained in Table 2.

| | Human Land Use | Urban | | | Agriculture | | | Forest/Grassland | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Total | High | Low | Total | Crop | Hay/Pasture | Total | Buffer |
| Macroinvertebrate Variables | | | | | | | | | |
| Richness | - 0.30*** | -0.05 | -0.01 | -0.05 | -0.29** | -0.10 | -0.19* | +0.30*** | +0.27** |
| EPT | -0.40*** | -0.04 | -0.00 | -0.04 | -0.38*** | -0.11 | -0.28** | +0.37*** | +0.32*** |
| ICI | -0.03 | +0.01 | +0.03 | +0.01 | -0.01 | -0.01 | -0.03 | +0.02 | +0.02 |
| B-IBI | -0.38*** | -0.03 | -0.00 | -0.04 | -0.42*** | -0.27** | -0.15* | +0.36*** | +0.35*** |
| T-BIBI | -0.08 | -0.04 | -0.02 | -0.03 | -0.10 | -0.02 | -0.06 | +0.11* | +0.10* |
| | | | | | | | | | |
| Habitat and Geomorphic Variables | | | | | | | | | |
| Drainage | -0.15* | -0.19* | -0.13* | -0.18* | -0.25 | -0.02 | -0.12* | +0.22** | +0.13* |
| Velocity | -0.05 | +0.01 | +0.02 | +0.02 | -0.04 | -0.01 | -0.04 | +0.02 | +0.00 |
| Depth | +0.06 | -0.01 | -0.05 | -0.00 | +0.02 | -0.01 | +0.13* | -0.04 | -0.03 |
| Riffle | -0.09 | -0.00 | +0.00 | -0.00 | -0.09 | +0.00 | -0.17* | +0.09 | +0.06 |
| Run | -0.00 | +0.12* | +0.13* | +0.11 | +0.01 | -0.03 | +0.00 | -0.01 | -0.04 |
| Substrate | -0.09 | -0.03 | -0.01 | -0.03 | -0.11 | +0.00 | -0.15* | +0.10 | +0.07 |
| Silt | +0.18* | +0.01 | +0.00 | +0.01 | +0.16* | +0.17* | +0.05 | -0.15* | -0.04 |
| | | | | | | | | | |
| Environmental Variables and Other Variables | | | | | | | | | |
| Temperature | -0.02 | -0.05 | -0.08 | -0.00 | -0.04 | -0.00 | +0.01 | +0.04 | +0.02 |
| pH | +0.05 | +0.04 | +0.02 | +0.04 | +0.07 | +0.00 | +0.07 | -0.05 | -0.04 |
| Conductivity | +0.12* | +0.08 | +0.03 | +0.10 | +0.16* | +0.19* | +0.02 | -0.14* | -0.10 |
| BOM | +0.22** | +0.01 | -0.00 | +0.03 | +0.19* | -0.01 | +0.04 | -0.22** | -0.27** |
| Chlorophyll α | -0.00 | +0.00 | +0.00 | -0.00 | -0.00 | +0.04 | -0.03 | +0.00 | +0.00 |

*P <0 .05, **P<0.01, ***P<0.001

Table 12. Winter season linear regression models with 2001 NLUD land cover variables (n=33 sites); $r^2$ values reported, negative and positive symbols indicate the relationship of land cover variables and response variables. Macroinvertebrate variables are described in Table 3; Other variables are explained in Table 2.

| | Human Land Use | Urban | | | Agriculture | | | Forest/Grassland | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Total | High | Low | Total | Crop | Hay/Pasture | Total | Buffer |
| Macroinvertebrate Variables | | | | | | | | | |
| Richness | - 0.27** | -0.05 | -0.01 | -0.06 | -0.27** | -0.00 | -0.35*** | +0.30*** | +0.24** |
| EPT | -0.23** | -0.02 | +0.00 | -0.02 | -0.21** | -0.02 | -0.23** | +0.22** | +0.16* |
| ICI | +0.00 | +0.05 | +0.07 | +0.03 | +0.00 | -0.03 | -0.04 | +0.00 | +0.00 |
| B-IBI | -0.18* | -0.01 | +0.00 | -0.02 | -0.14* | -0.00 | -0.24** | +0.17* | +0.16* |
| T-BIBI | -0.07 | -0.00 | -0.06 | -0.01 | -0.06 | +0.01 | -0.18* | +0.07 | +0.13* |
| Habitat and Geomorphic Variables | | | | | | | | | |
| Drainage | -0.15* | -0.19* | -0.13* | -0.18* | -0.25** | -0.02 | -0.12* | +0.22** | +0.14* |
| Velocity | -0.10 | +0.01 | +0.02 | +0.01 | -0.06 | -0.02 | -0.07 | +0.05 | +0.00 |
| Depth | +0.00 | +0.03 | +0.01 | +0.07 | +0.00 | -0.05 | +0.04 | -0.08 | -0.00 |
| Riffle | -0.10 | -0.01 | +0.00 | -0.01 | -0.09 | +0.00 | -0.17* | +0.10 | +0.05 |
| Run | +0.04 | +0.03 | +0.03 | +0.03 | +0.05 | -0.01 | +0.08 | -0.06 | -0.07 |
| Substrate | -0.00 | -0.04 | -0.02 | -0.04 | -0.13 | -0.00 | -0.12* | +0.15* | +0.12 |
| Silt | +0.12* | -0.01 | -0.01 | -0.02 | +0.07 | +0.04 | +0.08 | -0.06 | -0.02 |
| Environmental Variables and Other Variables | | | | | | | | | |
| Temperature | -0.32*** | -0.10 | -0.04 | -0.11 | -0.36*** | +0.00 | -0.29** | +0.36*** | +0.25** |
| pH | +0.02 | +0.07 | +0.06 | +0.09 | +0.04 | +0.00 | +0.01 | -0.03 | -0.03 |
| Conductivity | +0.01 | +0.16* | +0.15* | -0.14* | +0.07 | +0.05 | +0.00 | -0.05 | -0.05 |
| BOM | +0.00 | -0.15* | -0.15* | -0.11 | -0.01 | +0.04 | +0.01 | +0.01 | +0.00 |
| Chlorophyll α | +0.00 | +0.01 | +0.01 | -0.13* | +0.01 | -0.01 | +0.02 | -0.01 | -0.02 |

*P <0 .05, **P<0.01, ***P<0.001

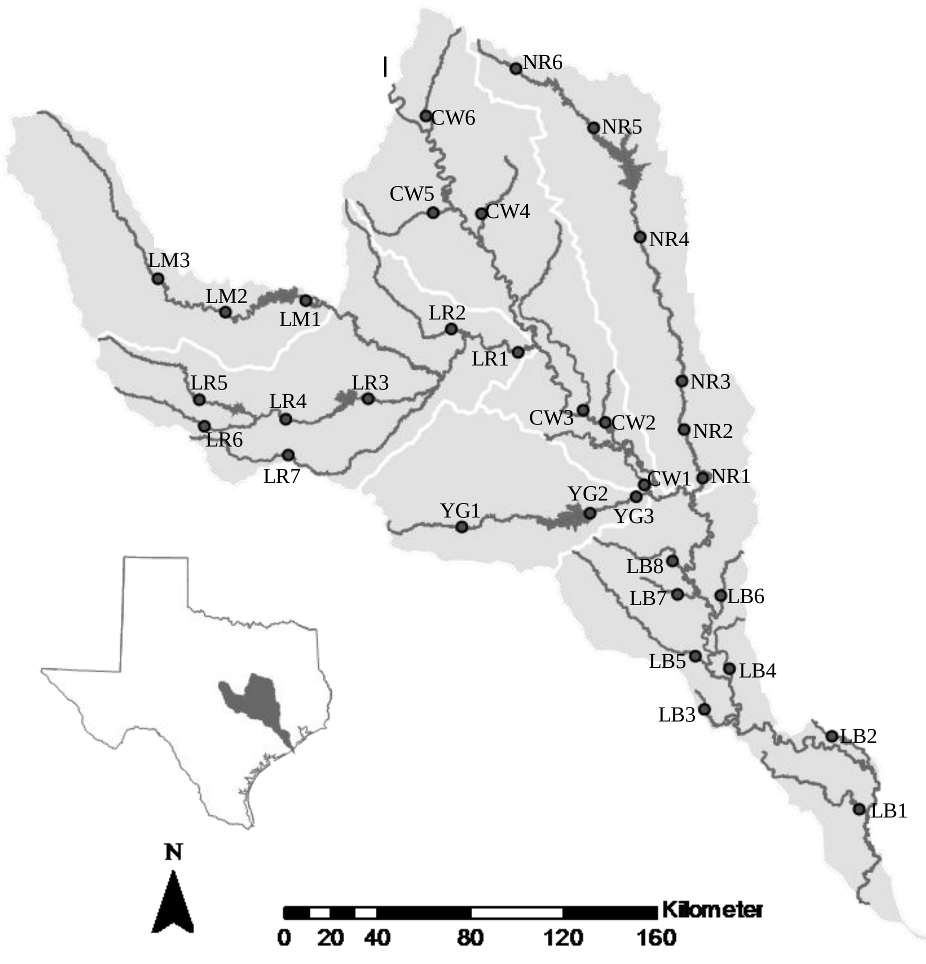


Figure 10. Map of the sites of the Lower Brazos Watershed.

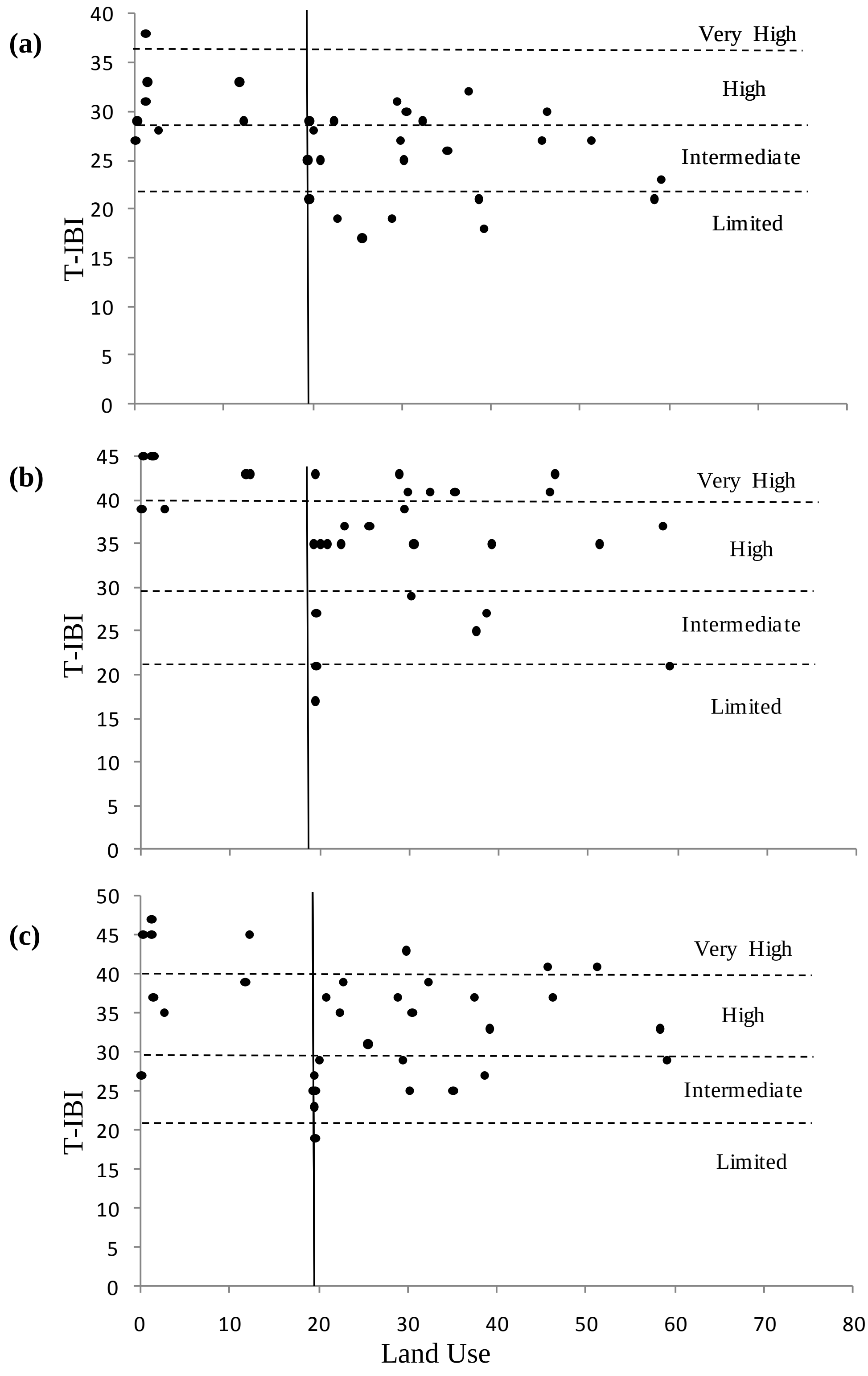


Figure 11. The relationship between the T-IBI adapted for Texas by TCEQ (Davis 1997) and the percentage of human land disturbance. The plots represent different seasons (a) Spring, (b) Summer, and (c) Winter. Higher biotic index values reflect higher water quality. The vertical line represents the suggested threshold which beyond sensitive taxa are lost.

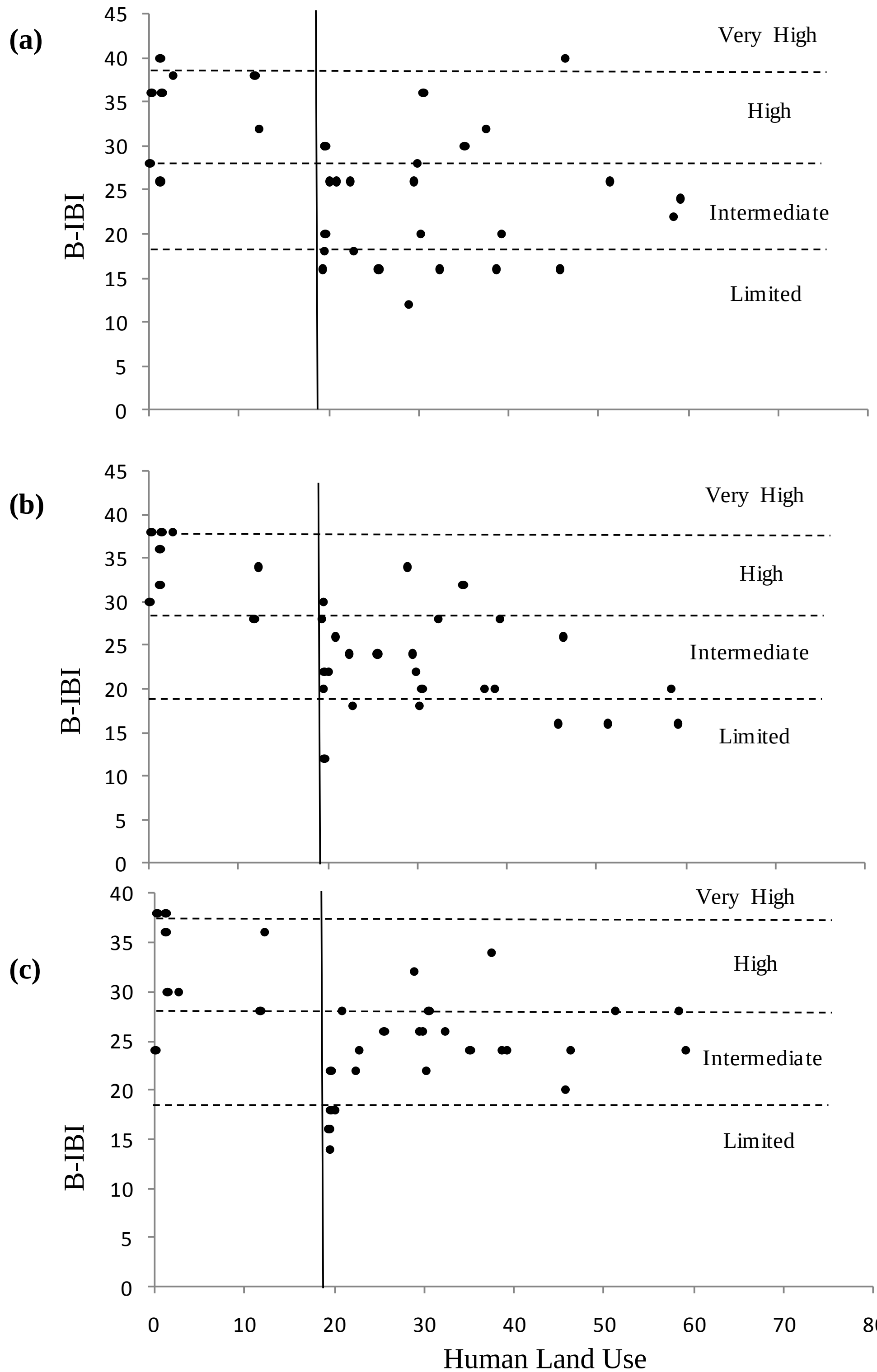


Figure 12. The relationship between the B-IBI (Kerans and Karr 1994) and the percentage of human land disturbance. The plots represent different seasons (a) Spring, (b) Summer, and (c) Winter. Higher biotic index values reflect higher water quality. The vertical line at 20% represent the suggested threshold which beyond sensitive taxa are lost.

Appendix I. Complete list of taxa found in the tributaries of the Lower Brazos River with functional feeding group assignment.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ephemeroptera** | | **Trichoptera** | | **Hemiptera** | | **Plecoptera** | |
| Apobaetis | Sc | *Cheumatopsyche* | Fc | *Ambrysus* | P | *Attaneuria* | P |
| Baetis | Sc | *Chimarra* | Fc | *Belostoma* | P | *Hydroperla* | P |
| *Caenis* | Sc | *Culoptila* | Sc | *Graptocorixa* | P | *Neoperla* | P |
| *Calliobaetis* | Gc | *Helicopysche* | Sc | *Hesperocorixa* | P | | |
| *Camelobaetis* | Sc | *Hydroptila* | Sc | *Limniporous* | P | **Leptidoptera** | |
| *Centropilum* | Sc | *Hydropysche* | Fc | *Limnocoris* | P | *Nympheulella* | Sh |
| *Cercobrachys* | Gc | *Ithytrichia* | Sc | *Lipogomphus* | P | *Petrophila* | Sc |
| *Fallceon* | Sc | *Leucotrichia* | Sc | *Microvelia* | P | | |
| *Heterocleon* | Sc | *Marillia* | Sh | *Neocorixa* | P | **Amphipoda** | |
| *Hexagenia* | Gc | *Mayatrichia* | Sc | *Notanecta* | P | Crangonyx | Gc |
| *Isonychea* | Fc | *Nectopysche* | Sh | *Pelocoris* | P | Gammarus | Gc |
| *Maccaffertium* | Sc | *Neurelipsis* | Fc | *Ranatra* | P | Hylella | Gc |
| *Neochoroterpes* | Gc | *Nyctiophlax* | Fc | *Rhagovelia* | P | | |
| *Plaudeus* | Sc | *Ocetis* | P | *Trepobates* | P | **Bivalvia** | |
| *Stenocron* | Sc | *Orchrotrichia* | Gc | *Trichocorixa* | P | *Corbicula sp* | Fc |
| *Stenonema* | Sc | *Oxyethira* | Sc | | | Sphaeridae | Fc |
| *Thraulodes* | Gc | *Polycentropus* | Fc | **Coleoptera** | | | |
| *Travarella* | Cf | *Scimidea* | Fc | *Acilius* | P | **Unionidae** | |
| *Tricorythodes* | Gc | *Trainodes* | P | *Agobetus* | P | *Amblema plicata* | Fc |
| *Vacupernis* | Gc | | | *Ancryonx* | Sc | *Lampsilis teres* | Fc |
| | | **Megaloptera** | | *Berosus* | P | *Quadrula apiculata* | Fc |
| **Odonata** | | *Corydalus* | P | *Copotomus* | P | *Q. houstonensis* | Fc |
| *Acanthagrion* | P | *Sialis* | P | *Cymbiodyta* | P | *Toxolasma texasesis* | Fc |
| *Anax* | P | | | *Cyphon* | Sc | *Tritogonia verrucosa* | Fc |
| *Aphylla* | P | **Diptera** | | *Dibolocetus* | Gc | | |
| *Argia* | P | *Aedes* | Fc | *Dineutus* | Sc | **Grastropoda** | |
| *Baesiaechna* | P | *Atherix* | P | *Dubriaphia* | Sc | Ancylinidae | Sc |
| *Boyeria* | P | *Bezzia* | P | *Eclichadidae* | Gc | Ampullariidae | Sc |
| *Brechmorhoga* | P | *Ceratopogon* | P | *Enochorus* | Gc | Hydrobiidae | Sc |
| *Calopteryx* | P | *Chaoborus* | P | *Gyretes* | P | Lynmaeidae | Sc |
| *Dromogomphus* | P | *Chironominae* | Gc | *Haplius* | P | Thiaridae | Sc |
| *Enallagma* | P | *Culex* | Fc | *Helichus* | Sc | Physidae | Sc |
| *Epitheca* | P | *Culicoides* | P | *Heterelmis* | Sc | Planorbidae | Sc |
| *Erpetogomphus* | P | *Dasyhelea* | Gc | *Heterostronata* | P | Plueroceridae | Sc |
| *Erythemis* | P | Ephyridae | P | *Hexacylloepus* | Sc | | |
| *Gomphus* | P | *Euporyphus* | Sc | *Hyrdrochus* | Gc | **Decapoda** | |
| *Hagenius* | P | *Forcipomyia* | Gc | *Laccophillus* | P | Cambaridae | P |
| *Hetaerina* | P | *Hemerodroma* | P | *Lateralus* | Sh | *Macrobrachium* | |
| *Ishnura* | P | *Monohelea* | Gc | *Lutrochus* | Gc | *ohione* | Gc |
| *Libellula* | P | *Nemotelus* | Gc | *Macrelmis* | Sc | *Palmonetes* | Gc |
| *Marcomia* | P | *Odontomya* | Gc | *Macronychus* | Sc | | |
| *Oligogomphus* | P | *Orthocladinae* | Gc | *Microcylleopus* | Sc | **Other Taxa** | |
| *Phyllogomphidae* | P | *Probezzia* | P | *Neoelmis* | Sc | Acrina | P |
| *Progomphus* | P | *Psychoda* | Gc | *Neoporus* | P | Anostraca | Gc |
| *Stomatochlora* | P | Sciomyzidae | Gc | *Optioservus* | Sc | Cladocera | Fc |
| *Stylurus* | P | *Serromyia* | P | *Ora* | Sc | Copepoda | Gc |
| *Sympetrum* | P | Simulidae | Fc | *Peltodytes* | Sh | Hirunidae | P |
| *Telebasis* | P | *Stratiomys* | Gc | *Psephenus* | Sc | Isopoda | Gc |
| *Tetragoneuria* | P | *Tabanus* | P | *Scirtes* | Sc | Nemtaoda | Gc |
| | | *Tanypodinae* | P | *Stenelmis* | Sc | Oligochaete | Gc |
| | | Tipulidae | Sh | *Tropisternus* | P | Ostracoda | Fc |
| | | | | *Uvarus* | P | Platyhelminthes | Gc |

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## VITA

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