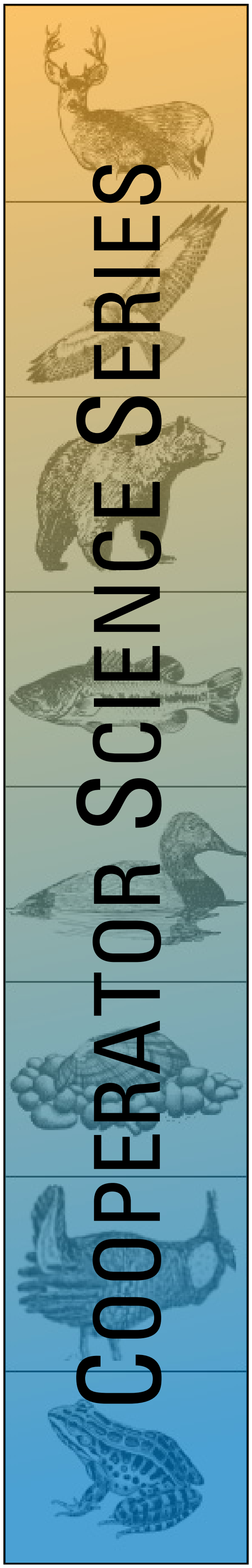

U.S. Fish and Wildlife Service

Evaluating habitat associations of a fish assemblage at multiple scales in a minimally disturbed stream on the Edwards Plateau, central Texas

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EVALUATING HABITAT ASSOCIATIONS OF A FISH ASSEMBLAGE AT MULTIPLE SCALES IN A MINIMALLY DISTURBED STREAM ON THE EDWARDS PLATEAU, CENTRAL TEXAS

Final project report to Texas Parks and Wildlife Department and the U.S. Geological Survey

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Abstract

Understanding how environmental factors operating at different spatial scales within a watershed structure instream habitat is essential for accurately quantifying fish habitat associations and developing effective means for assessing stream conservation and restoration activities. In this study, we used a combination of side scan sonar surveys, imagery collected by an unmanned aerial vehicle, and instream surveys of fishes and physicochemical conditions to evaluate the effect of physicochemical and habitat variables at various spatial scales, e.g., micro-mesohabitat, mesohabitat, riffle-run-pool complex, stream reach, on fish assemblage habitat associations in the South Llano River, a spring-fed second order stream on the Edwards Plateau in central Texas. We found that the micro-mesohabitat scale and the riffle-run-pool complex scale had the greatest explanatory power. Many of the fishes endemic to the streams of the Edwards Plateau, such as Guadalupe bass *Micropterus treculii* and Texas logperch *Percina carbonaria*, exhibited associations with similar physicochemical and landscape variables. Our results suggest that conservation and restoration efforts targeting single species, such as the Guadalupe Bass Restoration Initiative, can benefit a suite of species. However, our results did not demonstrate incontrovertibly that a single species, such as Guadalupe bass, can serve as an indicator of the status of the stream fish assemblage as a whole. These findings will help provide data on the habitat use patterns of a fish assemblage in a relatively undisturbed Edwards Plateau stream and potentially help prioritize future restoration efforts for other streams in the region.

Introduction

Applying landscape ecology principles to riverine systems has spawned a holistic perspective (Wiens 2002; Palmer et al. 2010), where researchers are recognizing the influences that scale may have on fish assemblage structure (Wang et al. 1997; Fitzpatrick et al. 2001; Wang et al. 2003; Benda et al. 2004; Gido et al. 2006; Wehrly et al. 2006). Understanding the complexity of a riverine ecosystem at multiple scales is essential for accurately quantifying fish-habitat associations since riverine fish population distribution and abundance is ultimately determined by both fine and coarse scale phenomena (Poff 1997; Allan 2004). Modeling the factors influencing fish assemblages at different scales has produced very different levels of accuracy depending on whether the focus was placed upon smaller scales (Gorman 1988; Gido and Propst 1999; Lammert and Allan 1999; Wang et al. 2003; Bouchard and Boisclair 2008) or broader scales (Wang et al. 1997; Fitzpatrick et al. 2001; Benda et al. 2004; Gido et al. 2006; Wehrly et al. 2006) resulting in differing conclusions as to which scale was more important in determining assemblage structure. Regardless, there is agreement that fish assemblages are impacted by factors at multiple scales (Poff 1997), so understanding the impacts of scale and the dynamic nature of the lotic systems on subsequent habitat associations is a critical step towards effectively managing, conserving, and restoring fish populations because anthropogenic disturbances, such as agriculture, urbanization, and road development (Naiman et al. 1995; Allan 2004; Walsh et al. 2005) have the potential to act at multiple spatial and temporal scales. Attempts to address these disturbances at the wrong scale may at best result in temporary improvements and at worst result in wasted resources that might have been better applied at different scales.

The importance of approaching riverscape and watershed conservation from the appropriate scale is illustrated in the rivers and streams of central Texas. The disturbance of riverine systems is a primary threat for the native fish populations and endemic species located throughout the state, and while the proximate mechanisms of disturbance vary, the ultimate factor is growing human populations. The state of Texas is projected to experience a 20 – 25% increase in human populations over the next 10 – 15 years (Murdock et al. 2002) resulting in changes in land use, water demand, and water quality. Some of the greatest areas of growth are expected to occur around the urban centers of Austin, San Antonio, and San Marcos along the I-35 corridor in central Texas and the Edwards Plateau ecoregion (Murdock et al. 2002). For the Edwards Plateau region, which is characterized by high biodiversity and high endemism (Bowles and Arsuffi 1993), an increase in urban growth is expected to lead to a higher demand on surface and ground water resources resulting in decreased flows, decreased water levels, and physicochemical changes to regional streams. These disturbances have the potential to threaten the desirable qualities in the region and threaten the integrity of Edwards Plateau fish populations (Gorman and Karr 1978; Garrett et al. 1992; Hubbs 1995; Edwards et al. 2004), including the official freshwater fish of Texas, Guadalupe bass *Micropterus treculii*.

The Guadalupe Bass Restoration Initiative (GBRI) was implemented to help restore and conserve the endemic Guadalupe bass in the streams and rivers of the Edwards Plateau region. This fish is recognized as a species of greatest conservation need (Warren et al. 2000; Hubbs et al. 2008; Jelks et al. 2008; TPWD 2012) for the state of Texas, requiring specific habitat types and conditions like an undisturbed matrix of run and pool habitats with adequate flows (Perkin et al. 2010). This quality may render them particularly sensitive to habitat disturbance thus potentially making them a viable indicator species to help detect anthropogenic disturbances and predict changes in stream condition (Scott 2006).

However, the assumption that only one environmentally sensitive species like the Guadalupe bass can represent all the current factors and future issues in a riverine ecosystem is unlikely to be confirmed (Dale and Beyeler 2001), thus other environmental indicators are needed for effective and long-term management success. Establishing a target or "benchmark" (Hughes et al.1986; Raven et al. 2010) in order to measure success in conservation and restoration is critical. This involves quantifying fish habitat preferences in a regional riverine system that not only supports the regional fish assemblage but also represents the typical habitats that would be found in the region. Preferably, targets would be developed from pristine lotic systems within the region. However, very few systems remain untouched within the Edwards Plateau ecoregion due to anthropogenic pressures (Benke 1990), therefore minimally disturbed systems may be the best available option.

The South Llano River, located on the Edwards Plateau, is still considered to be a minimally disturbed system (Stoddard et al. 2006) and is one of the few remaining unregulated rivers in Texas. This stream is also characterized as an ecologically healthy river supporting a fish assemblage typical for the region, including several species endemic to the Edwards Plateau ecoregion (Linam et al. 2002; Hubbs et al. 2008). These qualities have made it the focal watershed for the GBRI and created an opportunity to develop a target or "benchmark" for restoring streams and rivers throughout the Edwards Plateau. Therefore, the objective of this study was to assess the fish assemblage habitat relationships in the South Llano River at multiple scales to determine what factors exert the most influence in structuring a relatively undisturbed fish assemblage in the Edwards Plateau ecoregion. Ultimately, this information could potentially provide the appropriate standard or "benchmark" (Hughes et al. 1986) for other river ecosystems in the Edwards Plateau region and provide information for managers regarding potential repercussions that may come with any future alterations to instream and riparian habitats at multiple spatial scales in the region.

Methods

<u>Study area</u>

The study area is located within the Colorado River Basin in west-central Texas on the Edwards Plateau (Linam et al. 2002) or commonly known throughout the state as the "Texas Hill Country". A majority of the 10 million hectares of the karst plateau (Edwards et al. 2004; Heilman et al. 2009) overlays the Edwards Aquifer which supplies water to approximately one half of all the springs in the state (Brune, 1981) and serves as a significant water supply for adjacent urban areas, such as San Antonio, San Marcos, and Austin. The Edwards Plateau is often characterized as an important area for conservation for Texas since it is associated with high levels of biodiversity and endemism with over 90 species of various fauna identified as endemics in the region (Bowles and Arsuffi 1993), including Guadalupe roundnose minnow *Dionda nigrotaeniata,* Texas logperch *Percina carbonaria*, and Guadalupe bass (Hubbs et al. 1991).

Our research was conducted on the South Llano River, a small spring-fed, second order tributary located in the Colorado River Basin located approximately 175 km northwest of San Antonio. It is approximately 88 km in length from its headwaters in Edwards County, Texas to its confluence with the North Llano River in Kimble County near Junction, Texas. Our study area consisted of a 39-km stretch beginning at Mclean Ranch and ending at Lake Junction Dam in Junction (Figure 1). Despite being spring fed, a majority of the headwater reaches above 700 Springs Ranch remained dry throughout the study while the downstream stretches maintained a constant flow. However, due to the current drought conditions a decreasing trend in annual discharge was observed throughout 2012-2013 (Figure 2) with an overall mean discharge of 1.4 $m^3/s \pm 0.34$.

Compared to most watersheds on the Edwards Plateau the South Llano has relatively low levels of human impact mainly due a low population density within its watershed (Linam et al. 2002). The most disturbed areas are found in the lower reach of the river. As a result of the dam this reach is characterized by deep, homogenous pools with undercut banks. Fragmentation through the construction of road crossings is also present throughout the extent of the river. The localized impacts of these crossings to the channel morphology are apparent. While their influence on the transport of nutrients and sediment is reasonably well understood (Heitmuller and Asquith 2008; Heitmuller 2009), the impact of road crossings on the connectivity of fish and macroinvertebrate populations has not been investigated. Despite the anthropogenic disturbances the South Llano is still considered to be ecologically intact (Linam et al. 2002), composed of the typical habitats that are found in Hill Country streams and supports high levels of endemic fish species found throughout this region (Hubbs et al. 1991).

Characterizing instream habitat availability

We followed the protocols described by Kaeser and Litts (2010) to map instream habitats. Briefly, a Humminbird 988c SI side scan sonar unit (Humminbird, Eufaula, Alabama) with the transducer mounted off the starboard bow of a canoe was used to capture sonar images of the stream bottom. Each sonar image recorded was automatically tagged with a waypoint by the side scan sonar unit. Each image and waypoint was captured on an MMC/SD card installed into the Humminbird control head prior to the surveys. In addition, a Garmin 78sc handheld GPS (Garmin International, Olathe, KS) was connected directly to the control head to record and was placed near the transducer to maximize accuracy (Kaeser and Litts 2010). The handheld GPS was set to collect trackplots at 3-s intervals during the survey.

Once the entire study area was mapped, each sonar image was prepared for importation into ArcMap v. 10 (ESRI, Redlands, California). HumminbirdPC v4.1.8 (Humminbird, Eufaula, Alabama) software was used to import the images and captured waypoints into a PC. Each image was then cropped and manually clipped to any adjacent sonar images with IrfanView v. 4.30 (Irfan Skiljan, Austria, Europe) in order to eliminate any overlap between the images. Both the trackplot and waypoint data were imported into ArcMap 10 and visually inspected to ensure they lined up with the river channel. For organizational purposes, any duplicate trackplots due to irregular speeds or changes in direction by the canoe were removed to display a uniform line segment.

A network of control points was created for georeferencing purposes on each sonar image using the Generate Control Point tool, through a custom toolbar (VBA code) developed by Kaeser and Litts (Kaeser and Litts 2010) called Sonar Tools. Once georeferenced, the clipped sonar images were transformed into a mosaic raster layer using the Rectify Tool, another component of the Sonar Tools toolbar and again inspected to ensure correct alignment with the river. Different substrate classes were defined by digitized lines, converted to polygons, and then assigned a micro habitat class (Kaeser and Litts 2010). The substrate classification included seven primary classes: bedrock, cobble, gravel, sand, unidentifiable rocky, submerged aquatic vegetation (SAV) and unidentifiable substrates (Kaeser and Litts 2010; Table 1).

The complex mixture of the primary classes of substrates was identified both through sonar imagery and observation in the field, necessitating the use of substrate sub-classes (Barnhardt et al. 1998; Kendall et al. 2005); Kaeser and Litts 2010) These sub-classes were defined by their dominant (≥ 50% of the area) and subordinate (≤ 50% of the area) constituent substrate types (Barnhardt et. al. 1998). For example, if an area that was predominately gravel substrate but also included cobble, then that area would be labeled “GRco” (Barnhardt et. al. 1998). In addition, instream structures ≥ 100 mm in length, such as boulders and large woody debris, were identified and assigned to separate polygon classes.

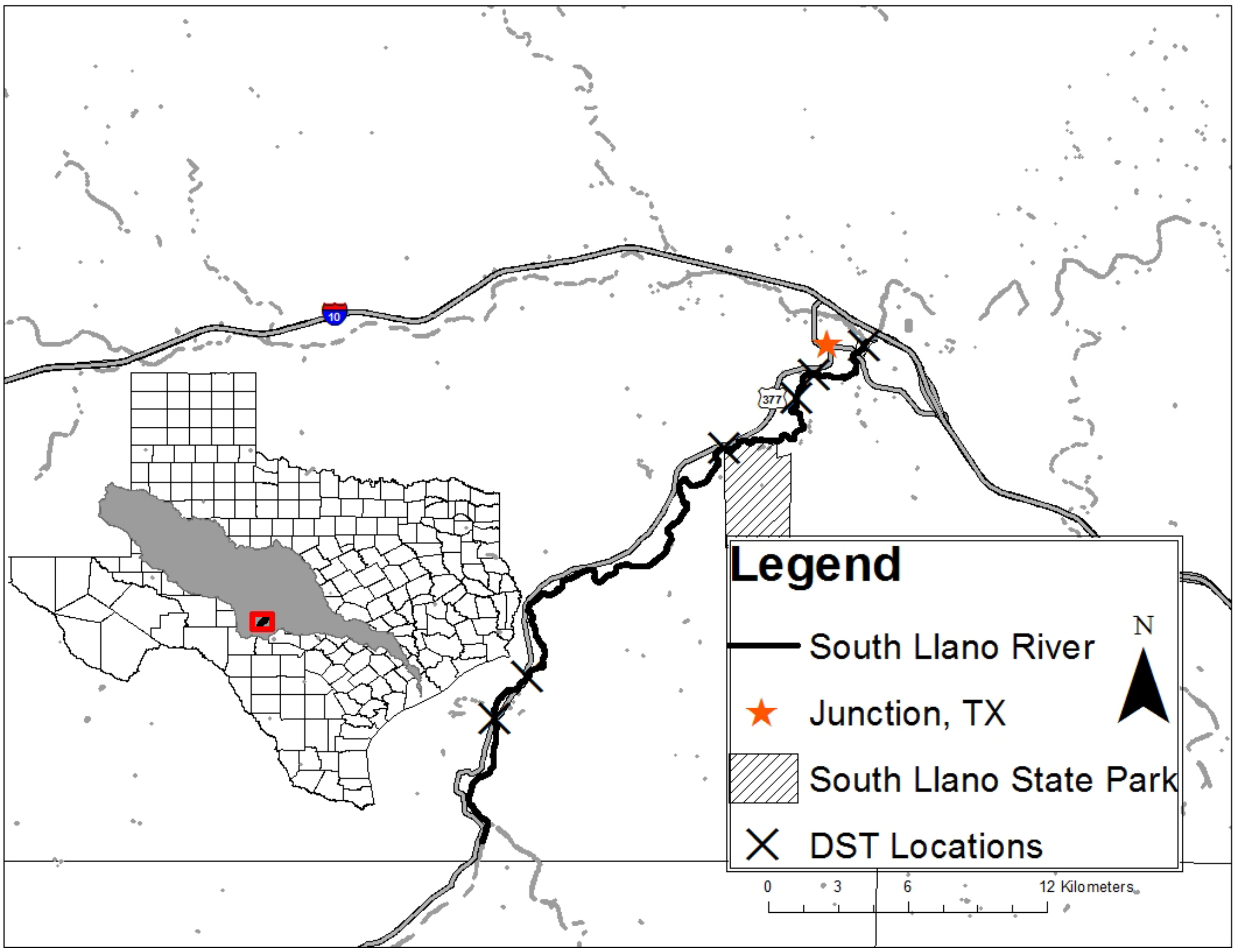

Figure 1. Map of the South Llano River study area in Kimble County, Texas. Portion of the river surveyed is represented by a bold black line. Inset map illustrates the relative location of the South Llano River within the Colorado River Basin. A total of six data storage tags (DSTs) were typically located at public access areas of the river, e.g., road crossings.

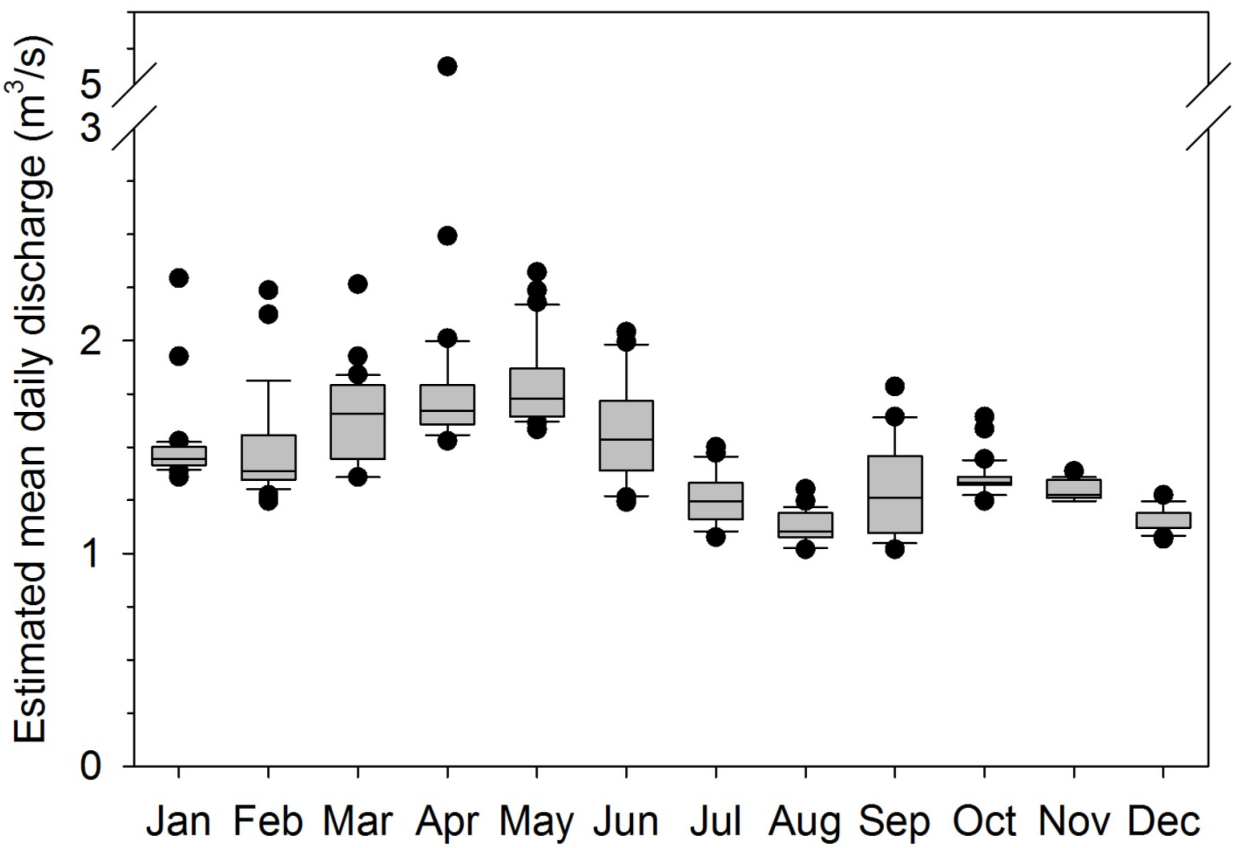

Figure 2. Estimated monthly median, Q_{10}, Q_{25}, Q_{75}, and Q_{90} daily discharges in the South Llano River, Texas during 2012.

Table 1. Substrate types and their definitions used to classify substrate within the South Llano River, Texas study area using side scan sonar imagery collected in October 2011.

Substrate Type	Definition
Bedrock (Br)	Substrate composed of solid sheets of karsts bedrock
Cobble (Co)	Substrate composed of 299 mm ≥ size ≥ 60 mm particles
Gravel (Gr)	Substrate composed of 59 mm ≥ size ≥ 2 mm particles
Sand (Sa)	Substrate composed of particles size < 2 mm
Unidentified (Un)	≥75% of area cannot be classified from images
Unidentified Rocky (UnR)	≥75% of area cannot be classified from images but is in a predominately rocky area

We delineated mesohabitats, i.e. pools, runs, riffles, through a combination of aerial photos provided by Texas Parks and Wildlife and the raw sonar images collected by the side scan sonar unit, recognizing that these designations were potentially subject to the influence of stream discharge. Pools and runs were classified by the changes in depth. Depth was recorded on each sonar image in two ways: 1) the depth directly underneath the transducer in meters 2) a "depth band" which changed in width depending on the depth of the channel. The latter allowed us to identify subtle changes in depth which helped determine when runs ended and pools started. Due to a majority of the riffles being very shallow the transducer was raised out of the water to prevent damaging the unit. As a result a majority of the riffle habitats were not captured with sonar imagery but each one was noted in a field note book and georeferenced with a handheld GPS unit. The riffle locations were then cross-referenced through the aerial images in ArcMap 10. The aerial photos were collected by unmanned aerial vehicles by Texas Parks and Wildlife in October and November 2011 with a resolution of approximately 103x86 cm per pixel. Once identified, each mesohabitat was digitized, converted to polygons, and then assigned a mesohabitat class in ArcMap 10.

The micro-mesohabitat scale consisted of variables describing the patch of a particular substrate type and mesohabitat type combination from which fishes were sampled. In addition to metrics such as the size of the habitat patch, distance to nearest neighboring patch, etc. physicochemical variables were included in this scale. After fish sampling was completed at each site, water temperature, current velocity, dissolved oxygen (DO), conductivity, turbidity, canopy cover, stream width and distance from the nearest bank were recorded. The distance from the nearest bank was taken with a handheld Hawkeye Digital Sonar H22PX (NorCross, Orlando, Florida). Current velocity was measured with a Global Water Flow Probe FP211 (Global Water, Sacramento, California). An Oakton TN-100 Portable turbidimeter (Oakton, Vernon Hills, Illinois) was used to measure turbidity at each sample site. An YSI Model 85 Handheld System was used to measure the conductivity while an YSI Model 95 Handheld System was used to assess the dissolved oxygen levels (YSI, Yellow Springs, Ohio). In addition to on site measurements, a continual recording of temperature and depth was captured with DST Milli data storage tags (DSTs; Star-Oddi Marine Device Manufacturing, Reykjavik, Iceland) placed near accessible public access points like bridges and road crossings along the stretch of the study area (Figure 1). Data was offloaded from each DST during each seasonal sampling period.

Based off the mesohabitat classifications, the next spatial scale termed pool-run-riffle complexes was generated in ArcMap 10. Pool-run-riffle complexes were composed of the mesohabitat located at each sampling site and all of the other upstream and downstream mesohabitats between it and its nearest upstream and downstream neighbor. For example, if the mesohabitat at a sample site was a pool habitat then the riffle and run habitats located between the next upstream and downstream pool habitats would be selected as part of the pool-run-riffle complex for that site. These complexes were selected, exported, and assigned to a separate feature class in the geodatabase. For the reach scale, a total of seven reaches were identified in ArcMap 10 within the study area. A reach was defined as a stretch of river that was uninterrupted by any type of man-made or natural barriers, e.g., road crossings or a waterfall (Figure 3) and ranged in length from approximately 2.4-10.4 rkm. Each reach was digitized into polygons in ArcMap 10 and stored as separate feature classes in the geodatabase. For the largest spatial scale, landcover data from Phase 4 of the Texas Ecological Systems Project acquired from Texas Parks and Wildlife (available online at: https://www.dropbox.com/sh/3dzdedqbi8n8s9h/y3scyoM8PZ) were used to quantify the various floodplain habitats within the South Llano River watershed. For each sampling site, any upstream portion of the landscape that fell within a 50 meter buffer around the river was exported into a separate shapefile for further analysis. In addition to the habitat data, eroded or cut banks located within the study area were recorded using a handheld GPS. Waypoints were taken at the beginning (upstream) and end (downstream) of each eroded stretch and then imported into ArcMap 10 to be digitized and measured for length in meters.

Verification of the substrate types identified through the sonar imagery was conducted by traveling back through the study area and recording the observed substrates and depth approximately every 130 meters. Ground proofing deep pools consisted of using a NavRoute underwater camera (NavRoute Technologies, Miami, Florida) to determine the substrate type and recording the depth with a handheld Hawkeye Digital Sonar H22PX (NorCross, Orlando, Florida). These sites were ultimately used to verify the correct substrate classifications and depths for each raw sonar image used in the classification map. In addition, a subset (25%) of the habitat features, such as boulders and large woody debris, were selected for groundtruthing. Each structure in this subset was located, marked with a waypoint, and its location verified through the substrate classification layer.

In order to calculate a variety of metrics for each habitat scale, the micro-mesohabitat, mesohabitat, complex, reach and landscape layers were then converted to a raster dataset in ArcMap 10 and imported into FragStats 4.1 (McGarigal et al. 2012; Table 2). Additional calculations of other habitat variables were manually calculated in ArcMap 10 using the Measurement Tool. These variables include reach sinuosity, distance to nearest micro-meso patch, length-width ratios of complexes and reaches, total length of upstream eroded banks, sum of eroded upstream banks within each reach, distance to nearest eroded upstream bank, and distance to nearest barrier.

<u>Collection of fish data</u>

ArcMap 10 was used to randomly select 70 sample locations according to the proportions of classified substrate types and mesohabitats in the final mosaic layer. Fish surveys were conducted seasonally at each site location over a full year (n = 4). Fish were collected using a 3.96 x 1.22 x 2.00 m bag seine with a 0.5-cm mesh. Due to habitat variation in length and width, each transect was ≤ 25-m in length at each site. Each transect was sampled from upstream to downstream in one pass and ArcMap 10 was used to determine the lengths of each transect after a GPS waypoint was taken at the start and end of each one. However, six out of the 70 sites were too deep for effective sampling with a seine. Six alternative sites with similar substrate types and mesohabitats were chosen near public access points and sampled with a boat electrofisher along 50-m transects. Public access points were chosen due to the lack of boat ramps and private land accessibility in a majority of the South Llano River. The electrofishing settings were set at pulsed DC at 120 pulses per second (Hz), adjusted duty cycle between 60 and 80% to maintain approximately 4 amps. After each transect was sampled a small number of vouchers were taken from each species and were euthanized in a 0.06% MS-222 solution and fixed in a 10% formalin solution (Nickum et. al. 2004). The remaining individuals were released after being identified to species and measured to the nearest mm total length.

<u>Data analysis</u>

Canonical correspondence analysis

Canonical correspondence analysis (CCA; Ter Braak 1986) was performed using CANOCO v. 5 (Microcomputer Power, Ithaca, New York) to evaluate the fish habitat associations. This multivariate technique is commonly used in community ecology (McCune and Grace 2002) and combines both the ordination step in correspondence analysis and multiple regression (Ter Braak 1986; McGarigal et al. 2000). Using this constrained ordination helps determine the relationships between the species and the measured environmental variables (Khattree and Naik, 2000) through the construction of a biplot (Ter Braak 1986).

Prior to analysis, the habitat data and environmental measurements data were evaluated for normality through SAS 9.2 software package (SAS Instituite, Cary, North Carolina) by evaluating stem leaf plots and normal probability plots. For a majority of the variables, the

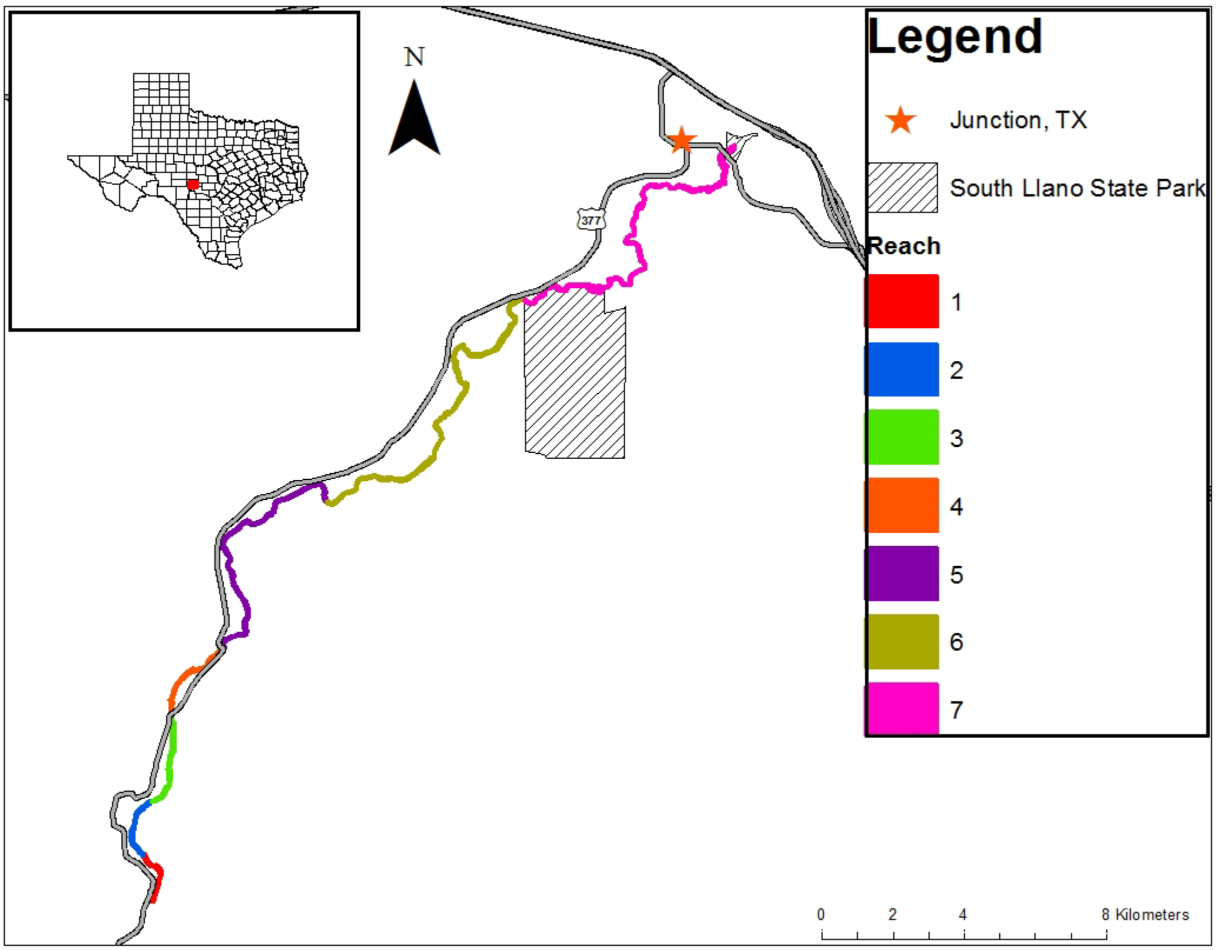

Figure 3. Map of the South Llano River, Texas illustrating the seven study reaches delineated within the study area. Each study reach was defined as river segment that was bounded by any type of man-made or natural barriers at both the upstream and downstream end.

Table 2. List of habitat metrics calculated across the micro-mesohabitat, mesohabitat, complex, reach, and landscape scales from data collected in the South Llano River, Texas. All metrics with (*) were calculated in Fragstats 4.1. All others were manually measured or calculated in ArcMap 10.

Scale	Metric	Description
Micro-mesohabitat	Area*	Patch area (m^2)
	Perimeter*	Patch perimeter (m)
	Perimeter to Area Ratio*	Ratio of patch perimeter to area
	Contiguity*	Averages the spatial connectivity of cells within a patch
	Nearest Neighbor	Distance to nearest neighboring patch (m)
Mesohabitat	Area*	Patch area (m^2)
	Perimeter*	Patch perimeter (m)
	Perimeter to Area Ratio*	Ratio between area of patch and patch perimeter
	Contiguity*	Average of the spatial connectivity of cells within a patch
	Proportion of Substrates*	Proportions of each substrate type within each meso habitat patch.
Riffle-run-pool complex	Area*	Patch area (m^2)
	Perimeter*	Patch perimeter (m)
	Perimeter to Area Ratio*	Ratio of patch perimeter to area
	Contiguity*	Average of the spatial connectivity of cells within a patch
	Proportion of habitat*	Proportions of micro-meso and mesohabitats patches within complex
	Length to Width Ratio	Ratio of patch length to patch width
Reach	Area*	Patch area (m^2)
	Perimeter*	Patch perimeter (m)
	Perimeter to Area Ratio*	Ratio of patch perimeter to area
	Contiguity*	Average of the spatial connectivity of cells within a patch
	Proportion of habitat*	Proportions of micro-meso and mesohabitat patches within each reach
	Length to Width Ratio	Ratio of patch length to patch width
	Sinuosity	Length of actual path of river divided by shortest length between upstream and downstream extents
Landscape (riparian buffer)	% shrubland*	Proportion of shrubland within 50-m buffer
	% floodplain forest*	Proportion of forest within 50-m buffer
	% grassland*	Proportion of grassland within 50-m buffer
	% barren land*	Proportion of barren land within 50-m buffer
	% urban land*	Proportion of low-intensity urban development within 50-m buffer
	% agriculture*	Proportion of agriculture land within 50-m buffer

appropriate transformations, typically log or square root transformations, were applied to improve homogeneity of variances and to help dampen the effects of outliers in the dataset (McCune and Grace, 2002). Once the transformations were applied, all variables were standardized to a standard deviation of one and a mean of zero. In CCA a high amount of correlation amongst variables can cause an arch effect or quadratic relationship amongst the first and second axes (Jongman et al. 1995). To prevent this, all variables were assessed through Pearson's correlation analysis and any variables that were highly correlated ($r > 0.70$, $P < 0.01$; McGarigal et al. 2000) were considered for removal from the analysis to reduce multicollinearity amongst variables (McGarigal et al. 2000). In order to decide which variables were to be eliminated from the analysis, an alternative to the one-way ANOVA called the Kruskal-Wallis test was conducted with each habitat variable as the dependent variable and the species abundance as the main effect (McGarigal et al. 2000). The variable with the greatest among-group variance (F-value) was ultimately kept in the analysis while the others were eliminated (Noon 1981).

The remaining variables for each scale were then entered into a principle component analysis (PCA) in order to remove the least informative variables from the dataset (Khattree and Naik 2000) and to further reduce the number of variables to prevent any unstable results in the CCA that usually arise when number of variables match or exceed the number of sampling units (Ter Braak 1986). Variable selection through the PCA results was based on the cumulative proportion of total variance method (Jolliffe 1973; Jolliffe 1986; Khattree and Naik 2000). This method involved selecting as many variables as the number of principle components selected. For this dataset, all the principle components that contributed to explaining ≥ 90% of total variation were considered for variable selection (Khattree and Naik 2000). For each principle component, the variable with the largest coefficient value was selected for the final analysis. Fish relative abundance measures were used in each partial and full CCA. Prior to analysis, all species that occurred in less than 5% of the sample units were removed from the analysis in order to decrease noise within the dataset (McCune and Grace 2002). In order fulfill the unimodal assumption of CCA, the species relative abundance data were square root transformed (McCune and Grace 2002).

To evaluate the influences of environmental and habitat variables by scale, a total of five spatial scales were used in the CCA analysis: micro-mesohabitat, mesohabitats, riffle-run-pool complexes, reach, and landscape scale. For each scale, one partial CCA was conducted using the fish relative abundance data and the variables selected through PCA as described above. An additional CCA was executed using the relative abundance data with habitat variables from all five scales in order to assess which scales explain the largest amount of variance of each species. Furthermore, the significance of each canonical axis was evaluated by Monte Carlo permutation test using 500 iterations. Any axes that were not found to be statistically related to assemblage structure ($P < 0.05$) were removed from the analysis because these axes were considered to not explain any more variation than random (Legendre et al. 2010). Additionally, a temporal aspect was included in the analysis to evaluate any changes in habitat associations over time. For each season collected (e.g. summer, fall, winter, spring), a CCA was executed at each scale using the relative abundance data.

Cluster analysis

The polythetic agglomerative hierarchical clustering (PAHC) technique was used to determine any species-to-species associations in relative abundance within the fish community. This type of analysis identifies classes or groups of similar variables, e.g., abundance of species, and arranges them into clusters by first placing each variable into their own separate cluster or group, then grouping all the variables together into a hierarchy of larger clusters until all variables are included into one single cluster (McGarigal et al. 2000). The pattern of the clustering technique was illustrated through a dendrogram using the Ward's minimum-variance linkage fusion method (Ward 1963) based on a Euclidean distance matrix (McCune and Grace 2000; McGarigal et al. 2000). This method determines cluster distances using the error sum of squares (McCune and Grace 2000). Ward's linkage was ultimately chosen in the analysis over the other common linkage methods, e.g., average linkage, single linkage, because it has a tendency to chain less frequently than the other linkage methods (McCune and Grace 2000) and performed the highest in terms of accounting for the most variance by possessing the highest squared multiple correlations for the clusters chosen to be in the analysis (McGarigal et al. 2000). All operations in the cluster analysis were performed in SAS 9.2 software package.

Choosing the number of significant clusters for the analysis was based on a combination of criteria which included observing peaks of the cubic clustering criterion, pseudo F-statistic (Khattree and Naik 2000; McGarigal et al. 2000), and visually identifying the major inflection point or "steep slope" in each scree plot provided by the SAS output (Khattree and Naik 2000). Furthermore, when using the Ward's method in SAS the user is cautioned that clusters can be highly influenced by any outliers in the dataset (SAS 2008). To control for this we omitted points with low estimated probability densities from the analysis (SAS 2008) which can be automatically executed with the TRIM statement. It was recommended to use at most a 10% TRIM when using the Ward's method (SAS 2008) but for this analysis a 1% TRIM was sufficient.

For comparison purposes, the environmental and habitat variables selected for the CCA as described above were also used in the hierarchal cluster analysis along with the relative abundances of all the species. In order to assess the species-to-species associations across different scales a cluster analysis was executed on the relative abundance data for each separate scale, e.g., micro-mesohabitat, mesohabitat, riffle-run-pool complexes, reach and landscape. Once the cluster analysis was completed, we identified the physicochemical and habitat variables appropriate to that scale that might be influencing the observed separations. We used a Kruskal

Wallis test, a non-parametric version of the ANOVA, to test the null hypothesis that the means of each environmental and habitat variable within the selected cluster pair did not differ (McGarigal et al. 2000; Ott and Longnecker 2010). The results were used to identify environmental and habitat variables that might be important factors driving or correlated to observed species associations (McGarigal et al. 2000). The level of significance was set at $\alpha = 0.05$ for all tests.

Variance partitioning

Variance partitioning was quantified using CANOCO 5. The variance partitioning method (Borcard et al. 1992) involves using partial canonical ordinations which can quantify independent effects of the explanatory variables at each individual scale and identifying the residual variation by using explanatory variables from the others scales as covariables. Ultimately, the partial canonical ordinations reveal the total percentage of the variance explained by each spatial scale and calculating the variance between spatial scales thus revealing the strength of interactions between them.

Due to software restrictions, only three spatial scales could be used in the variance partitioning analysis. Therefore, the spatial scales selected for the analysis were based off the partial CCA results. The three spatial scales that explained the highest amount of variation in the species data were selected and used in the analysis.

Results

Fish assemblage composition

A total of 3,402 individual fishes encompassing 25 species was captured over the duration of the study (Table 3), with the largest number of species captured in the spring sampling period followed by the summer, fall, and winter. Both blacktail shiner *Cyprinella venusta* and Texas shiner *Notropis amabilis* were the most abundant species captured in the study accounting for over 55% of the total catch. Seasonally, blacktail shiner was the most abundant species encountered, except during spring when Texas shiner dominated the catch. Species diversity was highest during the summer and spring sampling periods (Table 3). A full reporting of the relative abundance and occurrence of species in each substrate type, mesohabitat, and micro-mesohabitat type are included in Appendices 1-4.

The majority of fishes captured were < 100 mm TL. However, both largemouth bass and grey redhorse were the exceptions to the trend, averaging 153.2 ± 128.3 mm TL (mean ± STD) and 197.2 ± 122.9 mm TL (mean ± STD) over all four seasons respectively (Table 5). A majority

Table 3. Number of individuals of each fish species captured per season and in total from the South Llano River during 2012. Percent total is the relative contribution of each individual species to the total abundance captured. Rank indicates the relative position of each species according to their total abundance.

Species	Summer	Fall	Winter	Spring	Total Abundance	% Total	Rank
blacktail shiner (BTS)	309	222	187	286	1004	29.56	1
Texas shiner (TXS)	49	127	172	544	892	26.26	2
mimic shiner (MS)	184	7	2	54	247	7.27	3
longear sunfish (LS)	75	37	22	53	187	5.50	4
Guadalupe roundnose minnow (DI)	0	113	25	21	159	4.68	5
redbreast sunfish (RBS)	23	76	15	40	154	4.53	6
Guadalupe bass (GB)	70	47	5	24	146	4.30	7
central stoneroller (CSR)	58	31	6	10	105	3.09	8
western mosquitofish (GAM)	33	27	6	12	78	2.30	9
Rio Grande cichlid (RGC)	37	27	10	1	75	2.21	10
bluegill (BG)	23	19	8	24	74	2.18	11
gray redhorse (GRH)	20	19	12	13	64	1.88	12
orangethroat darter (OTD)	2	11	29	14	56	1.65	13
Texas logperch (TLP)	6	13	13	14	46	1.35	14*
largemouth bass (LMB)	22	12	6	6	46	1.35	14*
redear sunfish (RES)	9	5	0	0	14	0.41	16
channel catfish (CC)	7	4	2	0	13	0.38	17
gizzard shad (GSD)	3	2	6	1	12	0.35	18
greenthroat darter (GTD)	3	1	4	2	10	0.29	19
green sunfish (GS)	0	0	4	1	5	0.15	20
warmouth (WM)	0	1	3	0	4	0.12	21
common carp (CCP)	0	2	0	0	2	0.06	22*
flathead catfish (FHC)	2	0	0	0	2	0.06	22*
longnose gar (LG)	0	0	0	1	1	0.03	24*
river carpsucker (RC)	0	1	0	0	1	0.03	24*
Total	935	804	537	1121	3397		

* - *denotes a tie in ranks*

Table 4. Results of Kruskal-Wallis tests comparing the mean total lengths of selected fish species collected from the South Llano River, Texas during winter (Wi), spring (Sp), summer (Su) and fall (Fa) 2012. Only the species that were captured at ≥ 5% of all sampled sites were used in this analysis. Species abbreviations are provided in Table 3. Bold values indicate any test that resulted with *P* < 0.05 and — indicates no comparison of length was available between the two seasons.

	***P*-value for Kruskal-Wallis test**														
Comparison	**BTS**	**TXS**	**MS**	**LS**	**DI**	**RBS**	**GB**	**CSR**	**GAM**	**RGC**	**BG**	**GRH**	**OTD**	**TLP**	**LMB**
All	**<0.01**	**0.02**	**<0.01**	0.11	**<0.01**	0.15	**<0.01**	**0.01**	**0.05**	0.53	**<0.01**	**<0.01**	0.48	0.46	**<0.01**
Su-Sp	**<0.01**	0.70	**<0.01**	—	—	—	**<0.01**	**0.02**	0.30	—	0.32	**<0.01**	—	—	**<0.01**
Su-Fa	**<0.01**	0.26	**0.04**	—	—	—	**<0.01**	0.18	0.07	—	**<0.01**	**<0.01**	—	—	**<0.01**
Su-Wi	0.08	0.24	**0.05**	—	—	—	**<0.01**	**0.02**	0.28	—	**<0.01**	**<0.01**	—	—	**<0.01**
Wi-Sp	**<0.01**	**0.03**	0.38	—	**<0.01**	—	0.95	0.40	**0.05**	—	**0.02**	**<0.01**	—	—	0.75
Wi-Fa	**<0.01**	0.58	**0.04**	—	0.27	—	0.77	0.08	0.94	—	0.85	**0.03**	—	—	0.12
Fa-Sp	0.96	**<0.01**	**<0.01**	—	**<0.01**	—	0.48	0.13	**0.01**	—	**0.02**	0.36	—	—	0.11

Table 5. Seasonal and overall number of individuals, mean total length (TL) and length range of fish species captured from the South Llano River, Texas during 2012. Key to species abbreviations is in Table 3.

	Summer			Fall			Winter			Spring			Overall		
Species	***n***	**Mean TL (mm)**	**Range (mm)**	***n***	**Mean TL (mm)**	**Range (mm)**	***n***	**Mean TL (mm)**	**Range (mm)**	***n***	**Mean TL (mm)**	**Range (mm)**	***n***	**Mean TL (mm)**	**Range (mm)**
BTS	309	61.1	35-124	222	66.6	36-101	187	58.9	33-98	286	67.1	23-80	1004	63.7	33-124
TXS	49	50.4	30-70	127	51.1	30-67	172	50.3	17-64	544	47.4	33-111	892	48.7	17-80
MS	184	53.2	40-70	7	57.4	52-64	2	46	40-48	54	49.4	30-60	247	52.4	30-70
LS	75	67.4	30-142	37	80.3	21-149	22	67.9	37-130	53	73.8	30-164	187	71.7	21-164
DI	—	—	—	113	55.1	42-70	25	53.2	42-70	21	62.6	49-89	159	55.8	42-89
RBS	23	105.6	49-179	76	96.9	21-207	15	93.9	44-161	40	80.9	34-158	154	94	21-207
GB	70	59.1	35-211	47	130.7	55-347	5	123.2	72-176	24	134.3	68-351	146	96.1	35-351
CSR	58	54.3	33-100	31	56.8	45-73	6	64.3	53-82	10	60.4	51-69	105	56.1	33-100
GAM	33	41.5	20-54	27	44.3	25-53	6	44.3	40-48	12	40.3	36-46	78	42.5	20-54
RGC	37	60.0	21-247	27	50.3	25-180	10	48.1	32-80	1	57.0	—	75	54.8	21-247
BG	23	71.7	36-139	19	51.7	26-143	8	40.6	25-71	24	63.3	13-113	74	60.6	13-143
GRH	20	107.7	50-415	19	253.6	110-439	12	164.6	88-371	13	268.8	136-432	64	197.2	50-439
OTD	2	44.5	44-45	11	46.3	39-67	—	—	—	14	44.0	37-54	56	43.2	30-67
TLP	6	81.7	55-117	13	91.1	65-120	13	78.5	62-115	14	85.4	54-114	46	84.5	54-120
LMB	22	78.8	40-327	12	169.1	58-390	6	261	70-394	6	276.5	95-396	46	153.2	40-396
RES	9	67.8	48-107	5	76	89-59	—	—	—	—	—	—	14	70.7	48-107
CC	7	87.9	21-417	4	442	268-515	2	305	268-515	—	—	—	13	230.2	21-540
GSD	3	317	270-396	2	311.5	301-322	6	307.3	245-420	1	408	—	12	318.8	245-420
GTD	3	42.0	35-46	1	48	—	5	39.2	36-46	2	30	15-45	10	39.1	15-48
GS	—	—	—	1	116	—	4	79	36-129	1	113	—	5	85.8	36-129
WM	—	—	—	—	—	—	3	126.3	99-165	—	—	—	4	123.8	99-165
RC	—	—	—	2	617.5	610-625	—	—	—	—	—	—	2	617.5	625-610
FHC	2	154.5	49-260	—	—	—	—	—	—	—	—	—	2	154.5	49=260
CCP	—	—	—	1	535	—	—	—	—	—	—	—	1	535	—
LG	—	—	—	—	—	—	—	—	—	1	553	—	1	553	—

of the species captured in the study showed a substantial difference in seasonal lengths, particularly when comparing lengths between the summer and spring sampling. A total of five species did not show a large fluctuation of length between seasons which included: longear sunfish *Lepomis megalotis*, redbreast sunfish *Lepomis auritus*, Rio Grande cichlid *Herichthys cyanoguttatum*, orangethroat darter *Etheostoma spectabile*, and Texas logperch *Percina carbonaria* (Table 4).

Depending on the season, gear type may have influenced the calculated diversity index. For the winter collections, the electrofishing samples tended to be more diverse than those collected by seine (t_{14} = -2.68, P = 0.02). However, a similar comparison for the spring collections found no difference in the mean Shannon Diversity Index between the seine and electrofishing samples (t_{13} = -1.41, P = 0.18). Additionally, average species richness for winter seining sites was almost half that of winter electrofishing sites, while in the spring the two gears were separated by less than one species on average.

Groundtruthing side scan sonar substrate classifications

We accurately classified substrate from side scan sonar imagery for 315 of the 349 (90.3%) groundtruthing sites. A majority of the misidentifications occurred when classifying karst bedrock as gravel-sand substrates since both appeared as relatively bright and smooth substrates in the sonar imagery. Additionally, other misidentifications occurred with submerged aquatic vegetation patches. In some of the sonar imagery, these patches appeared as blurred, dark patches that were originally labeled as unknown. Each misidentified substrate was corrected in the final instream habitat map.

Habitat availability in the South Llano River

The final instream habitat map revealed a longitudinal shift from the upstream portions of the South Llano River that are predominately riffle and run habitats with coarse substrates to downstream sections that consisted of pool dominated reaches with finer substrate compositions (Figure 4). The instream habitat was dominated by karst bedrock in the headwaters while the majority of the substrates of the lower reaches were finer and primarily gravel-sand (Figure 4). The substrate of the first two stream reaches was > 98% karst bedrock, with a more diverse mixture of substrate classes appearing within the third stream reach. Cobble-gravel substrates and submerged aquatic vegetation were most common within the middle reaches of the river while finer substrates like gravel-sand dominated the substrate (>50%) in the lowest reaches approaching the dam in Junction. As expected, the proportion of mesohabitat accounted for by pools increased in the more downstream reached; however, proportions of pools peaked with reach 4 and then decreased back down to 50% by the final reach (Figure 4). While stream reach 1 was dominated by run habitats (63%), this mesohabitat was generally less common throughout

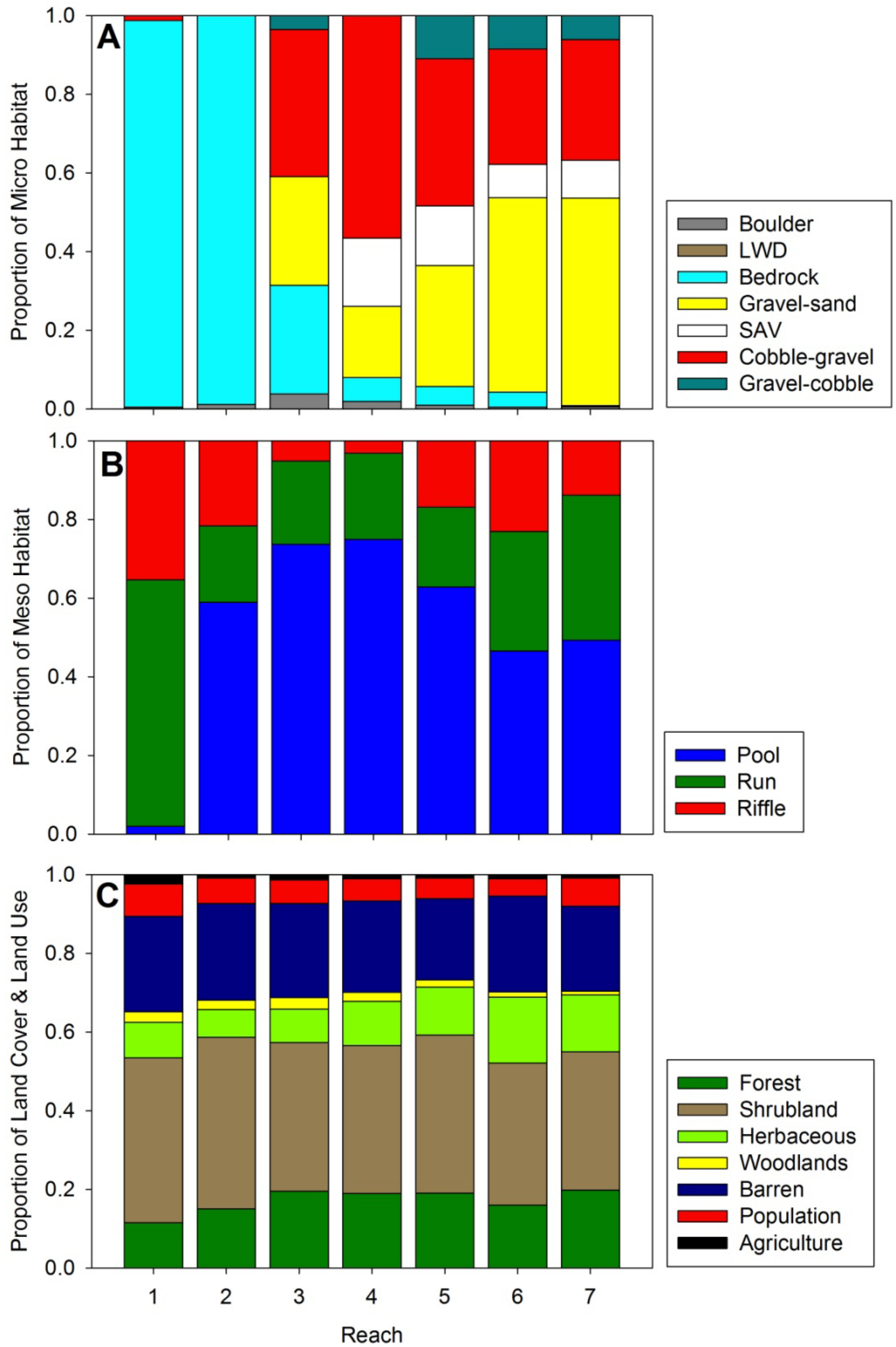

Figure 4. Proportions of instream substrate classes (A), mesohabitat classes (B), and riparian land cover and land use classes (C) in each of the reaches of the South Llano River as delineated in Figure 3. Instream substrate classes were determined from side scan sonar surveys conducted in 2011. Mesohabitat classifications were determined from side scan sonar and aerial imagery collected in 2011. Riparian land use and land cover were determined from the Texas Ecological Systems Classifications dataset.

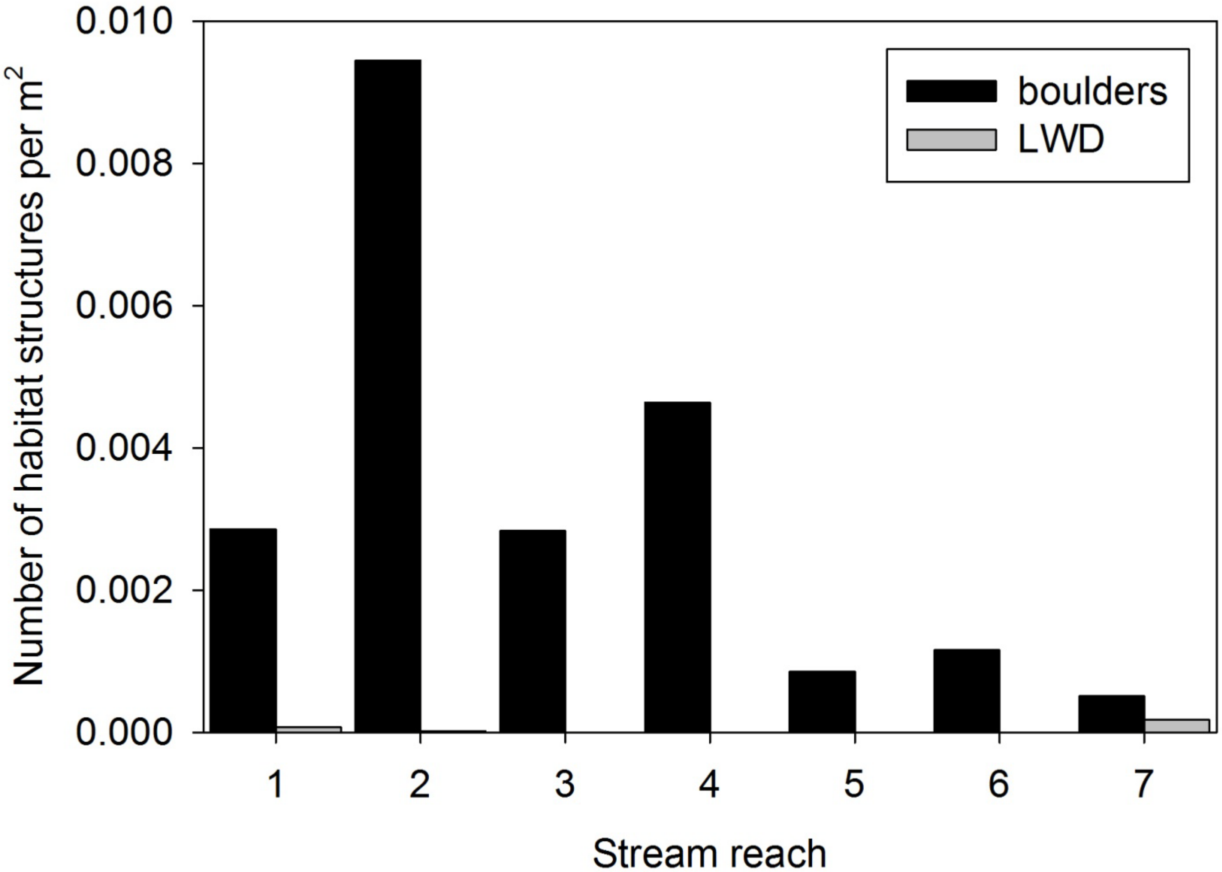

Figure 5. Occurrence per m^2 of boulders and large woody debris (LWD) as determined by side scan sonar and aerial imagery collected in 2011 within each of the stream reaches of the South Llano River. Stream reaches correspond to those delineated in Figure 3.

the remainder of the river. However, it did account for an increasing proportion of the mesohabitat between stream reaches 2 (19%) to stream reach 7 (37%). Boulders were more common on a per unit area basis in the upstream reaches than in those further downstream (Figure 5). Large woody debris was relatively rare throughout the river, and only comprised a meaningful component of instream habitat in stream reach 7.

In general, the riparian buffer habitat along the South Llano River primarily consisted of shrubland habitats (38%), such as ashe juniper *Juniperus ashei* shrubland, followed by forested (19%) and barren (24%) land cover classes (Figure 4 C). Other common land cover classes included herbaceous vegetation (15%) and woodland (3%). Impervious cover and other land cover classes associated with urbanization ranged between 5-8% along the South Llano River. It was highest along stream reach 1 (8%) due to the proximity of a large ranch and stream reach 7 (7%) due to the reach occurring within the city limits of Junction. Proportion of urbanized land cover classes, primarily defined by the presence of anthropogenic structures and coverage of impervious surfaces (Ludeke et al. 2012), remained at or near 5% for the remainder of the South Llano River. Although agricultural land use accounted for a smaller proportion of the riparian buffer than urbanized land cover classes, it exhibited a similar pattern. Agricultural land, defined as cropland that is fallow for some portion of the year and can include overgrazed pastures (Ludeke et al. 2012), use was highest in stream reaches 1 (2%) and 7 (1%) and was at low levels along the rest of the river corridor (< 1%).

Variable selection

We eliminated highly correlated variables (r = 0.70) from each scale as recommended by McGarigal et al. (2000) and used PCA to select the most informative variables prior to canonical correspondence analysis. This resulting in the retention of 67%, 55%, 39%, 19% and 43% of the variables from the micro-mesohabitat scale, mesohabitat scale, run-riffle-pool complex scale, reach scale, and landscape scale datasets, respectively (Table 6). Based on these remaining variables, the partial CCA conducted for each scale indicated only a single significant axis for the reach and landscape scales (Table 7) suggesting that the remaining axes at this scale should not be used for accurate interpretation. Ultimately, the reach and landscape scales were dropped in any further canonical correspondence analysis.

Cluster analysis

There was consistent separation between species that were associated with pools and those associated with riffles/runs at every spatial scale except at the landscape level (Figure 6). Cluster 1 at each scale generally contained species that were associated with lower current velocities and pool habitats, such as bluegill, largemouth bass, and redbreast sunfish. Cluster 2 was typically occupied by fishes associated with higher current velocities and riffle or runs, such as orangethroat darter, central stoneroller, and mimic shiner (Figure 6). Additional clusters

Table 6. Variables at each spatial scale identified by principle component analysis as being informative describing the structure of stream fish assemblages in the South Llano River, Texas. Variables were selected through the cumulative proportion of total variance method.

Scale	Variable	Abbreviation
Micro-meso habitat	Contiguity	mm_Contig
	Nearest neighbor	mm_nn
	Temperature	Temperature
	Current velocity	Current Velocity
	Turbidity	Turbidity
	% Canopy cover	Canopy Cover
	Width of channel	Width
	Distance to nearest bank	Distance
	Conductivity	Conductivity
	Depth	Depth
Mesohabitat	Contiguity	m_Contig
	Proportion of boulder	m_BO
	Proportion of woody debris	m_LWD
	Proportion of bedrock	m_BR
	Proportion of aquatic vegetation	m_SAV
	Proportion of cobble-gravel	m_CoGr
Riffle-run-pool complex	Area	c_Area
	Contiguity	c_Contig
	Proportion of riffle cobble-gravel	c_RiCg
	Proportion run boulder	c_RnBO
	Proportion riffle boulder	c_RiBO
	Proportion pool gravel-sand	c_PoGs
	Proportion run aquatic vegetation	c_RnSAV
	Proportion riffle aquatic vegetation	c_RiSAV
	Proportion pool aquatic vegetation	c_PoSAV
	Proportion pool woody debris	c_PoLWD
	Proportion run gravel-cobble	P_RnGc
Reach	Perimeter-area ratio	r_PA
	Proportion pool	r_Pool
	Proportion riffle	r_Riffle
	Proportion run cobble-gravel	r_RnCg
	Proportion pool cobble-gravel	r_PoCg
	Proportion pool aquatic vegetation	r_PoSAV
Landscape (riparian buffer)	Proportion of forest	Forest
	Proportion of barren land	Barren
	Proportion of vegetation	Vegetation

Table 7. Results from testing individual canonical axes to determine if axes represent variation that can be distinguished from random. Axes that resulted in $P > 0.05$ were determined to not explain variation more than random and were not used in the analysis. Additionally, the results also highlight the percentage of variation explained by each axis followed by the percentage of the explained variation by each axis.

Scale	Axis	*F*-value	*P*-value	% species data explained	% explained variation
Micro-meso habitat	1	10.5	0.002	4.42	37.81
	2	6.5	0.002	2.78	23.75
Mesohabitat	1	7.4	0.002	3.12	44.44
	2	4.3	0.004	1.78	25.26
Riffle-run-pool complex	1	7.9	0.002	3.34	34.48
	2	5.1	0.002	2.10	21.66
Reach	1	4.7	0.002	1.95	41.91
	2	2.1	0.398	0.85	18.32
Landscape (riparian buffer)	1	3.3	0.002	1.37	51.19
	2	2.0	0.078	0.81	30.15
Micro-Meso + Meso + Complex	1	12.3	0.002	5.62	24.90
	2	8.8	0.002	3.85	17.60

typically consisted of a single species, e.g., Texas shiner and Guadalupe roundnose minnow, with the exception of the mesohabitat scale (Figure 6B) which included a two-species cluster in addition to the two large multi-species clusters.

At the micro-mesohabitat scale, species associated with Cluster 1 were found in deeper ($P = 0.004$) and more shaded ($P = 0.02$) pool habitats with predominately gravel-sand substrates ($P = 0.01$; Table 9). Cluster 2 contained species that were primarily associated to habitats with higher current velocities ($P = 0.01$) and submerged aquatic vegetation within run habitats ($P = 0.03$; Table 8). Additionally, the two single-species clusters containing Texas shiner and Guadalupe roundnose minnow were loosely associated with pool habitat types (Figure 6A) based on the linkage distances to Cluster 1. At the meso-scale, each multi-species cluster (Figure 6B) was associated with different mesohabitat variables. Cluster 1 was associated with pool habitats ($P = 0.03$) with high contiguity ($P = 0.004$) and relatively high frequencies of large woody debris ($P = 0.01$; Table 8). Similarly, Cluster 3 was also was associated with pool habitats but instead of being linked to large woody debris, species in this cluster were associated with pool habitats containing a combination boulders ($P = 0.05$) and aquatic vegetation ($P = 0.05$; Table 8). In contrast, species located in Cluster 2 were found in run habitats associated with higher current velocities ($P = 0.03$).

Similar to the micro-meso scale, the fish assemblage clustered into four groups at the pool-run-riffle complex scale. A majority of the species was associated with pool habitats (Cluster 1), while the remaining species in Cluster 2 were associated with riffle and run habitats (Figure 6C). Specifically, Cluster 2 contained species that were associated with higher proportions of riffle-cobble-gravel ($P = 0.01$), run-boulder ($P = 0.01$), and run-gravel-cobble habitats ($P = 0.03$; Table 8). Additionally, species in Cluster 2 were also found within pool habitats with hard structure like large woody debris ($P = 0.04$). Species in Cluster 1 were mainly associated with pool-run-riffle complexes with relatively large areas ($P = 0.01$). The two single species clusters, containing Texas shiner and Guadalupe roundnose minnow, were loosely associated with pool habitats based off the linkage distances to Cluster 1 (Figure 6C).

At the reach scale, species associated within Cluster 1 were more common in reaches containing a higher percentage of pool habitats with larger proportions of aquatic vegetation ($P = 0.02$; Table 8) suggesting that these species may prefer deeper, low flow velocity habitats with soft structure. In terms of species, Cluster 2 had the most diverse cluster in the dataset which included associations to higher proportions of riffle habitats throughout each reach ($P = 0.005$) and reach perimeter-to-area ratio ($P = 0.005$; Table 8). There was only one single species cluster at the reach scale that included Guadalupe roundnose minnow which is very dissimilar from the other two clusters according to the distance measure (Figure 6D).

Like a majority of the other scales, the landscape scale produced two multi-species clusters and one single-species cluster (Figure 6E). However, unlike the other scales, the usual

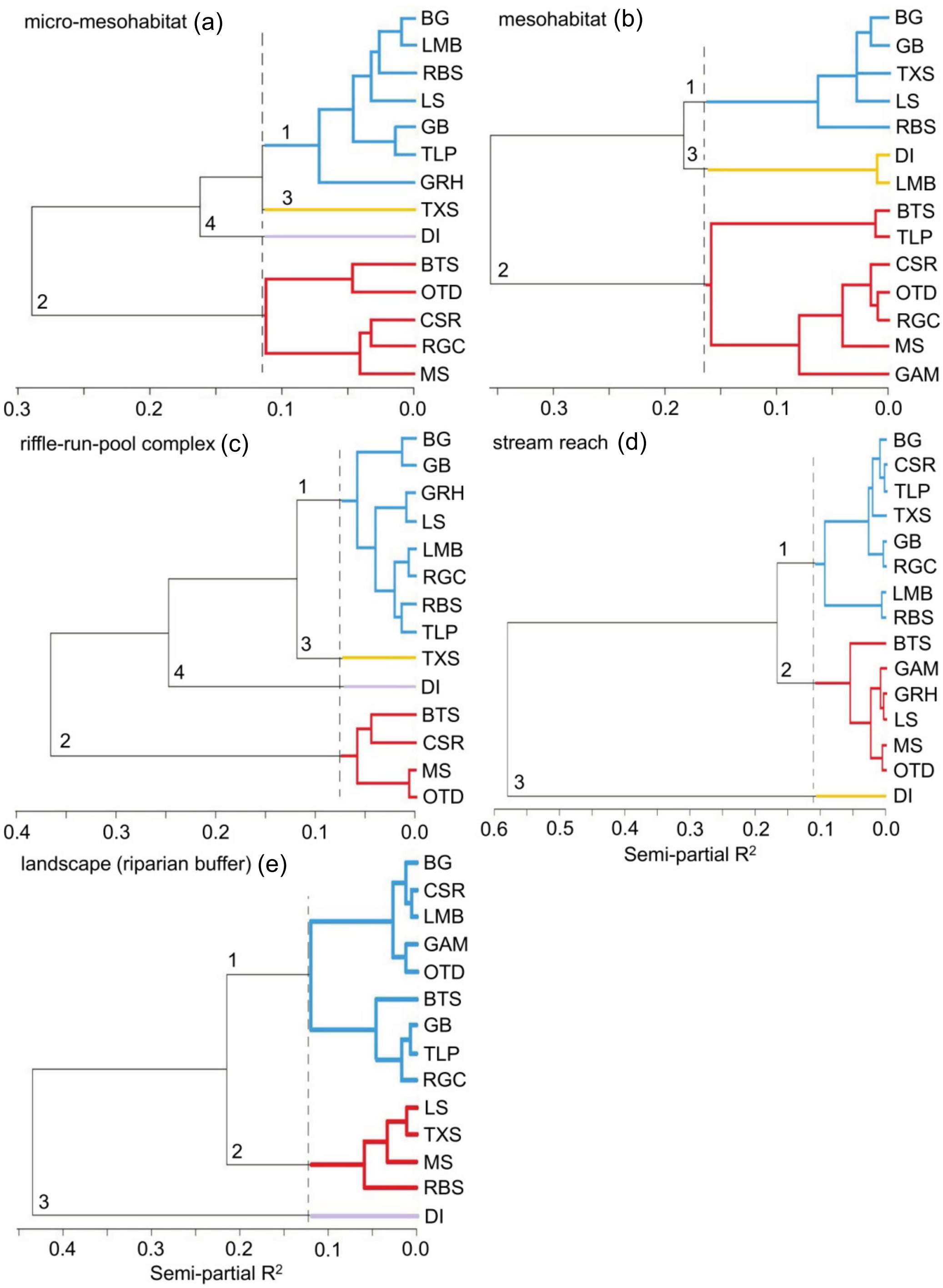
micro-mesohabitat (a)
BG
LMB
RBS
LS
GB
TLP
GRH
TXS
DI
BTS
OTD
CSR
RGC
MS
1
2
3
4
0.3
0.2
0.1
0.0
mesohabitat (b)
BG
GB
TXS
LS
RBS
DI
LMB
BTS
TLP
CSR
OTD
RGC
MS
GAM
1
2
3
0.3
0.2
0.1
0.0
riffle-run-pool complex (c)
BG
GB
GRH
LS
LMB
RGC
RBS
TLP
TXS
DI
BTS
CSR
MS
OTD
1
2
3
4
0.4
0.3
0.2
0.1
0.0
stream reach (d)
BG
CSR
TLP
TXS
GB
RGC
LMB
RBS
BTS
GAM
GRH
LS
MS
OTD
DI
1
2
3
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Semi-partial R2
landscape (riparian buffer) (e)
BG
CSR
LMB
GAM
OTD
BTS
GB
TLP
RGC
LS
TXS
MS
RBS
DI
1
2
3
0.4
0.3
0.2
0.1
0.0
Semi-partial R2

Figure 6. Hierarchal dendrogram of fish species associations in the South Llano River, Texas at the micro-mesohabitat scale (A), mesohabitat scale (B), riffle-run-pool complex scale (C), the reach scale (D), and landscape (riparian buffer) scale (E) using Ward's method and Euclidean distance. Distances are measured in semi-partial R-squared with smaller distances between linkages representing higher associations between each species.

Table 8. Based on the results from the hierarchical cluster analysis, each multi-species cluster was tested through the Kruskal-Wallis test to determine if any of the variables were significantly associated with any of the clusters. Any test that resulted with p > 0.05 was not considered significant. * = Variables associated with Cluster 1. † = Variables associated with Cluster 2. ^ = Variables associated with Cluster 3.

Scale	Cluster	Variable	Mean rank	Mean rank	χ^2	$P > \chi^2$
Micro-mesohabitat	Cluster 1 (BG-GRH) vs. Cluster 2 (BTS-RGC)	Width of bank*	8.9	3.2	7.2	0.007
		Depth*	9.0	3.0	8.1	0.005
		Contiguity*	8.9	3.2	7.2	0.007
		Pool-gravel-sand*	8.7	3.4	6.3	0.012
		% canopy cover*	8.6	3.6	5.5	0.019
		Flow†	4.3	9.6	6.3	0.012
		Run-aquatic-vegetation†	4.6	9.2	4.8	0.028
Mesohabitat	Cluster 1 (BG - RBS) vs. Cluster 2 (BTS - GAM)	Contiguity*	10.0	4.0	8.1	0.005
		Proportion of woody debris*	9.8	4.1	7.2	0.007
		Pool*	9.6	4.3	6.3	0.012
		Run†	3.8	8.4	4.8	0.028
	Cluster 1 (BG - RBS) vs. Cluster 3 (DI - LMB)	Proportion of boulders^	3.0	6.5	3.8	0.053
		Proportion of SAV ^	3.0	6.5	3.8	0.053
	Cluster 2 (BTS - GAM) vs. Cluster 3 (DI - LMB)	Contiguity^	4.0	8.5	4.2	0.040
		Proportion of SAV^	4.0	8.5	4.2	0.040
		Proportion of cobble-gravel^	4.0	8.5	4.2	0.040
Riffle-run-pool complex	Cluster 1 (BG - TLP) vs. Cluster 2 (MS - OTD)	Complex area*	2.8	8.4	6.5	0.012
		Proportion of riffle-cobble-gravel†	10.5	4.5	7.4	0.007
		Proportion of run-boulder†	10.3	4.6	6.5	0.011
		Proportion of run-gravel-cobble†	9.8	4.9	4.8	0.027
		Proportion of pool-woody-debris†	9.5	5.0	4.2	0.042
Stream reach	Cluster 1 (BG - RBS) vs. Cluster 2 (BTS - OTD)	Proportion of pool-aquatic vegetation*	9.8	4.5	5.4	0.020
		Proportion of riffle habitat†	4.8	11.2	8.1	0.005
		Perimeter-area ratio†	4.8	11.2	8.1	0.005
Landscape (riparian buffer)	Cluster 1 (BG - RGC) vs. Cluster 2 (LS - OTD)	Proportion of forest†	5.4	10.5	4.7	0.031
		Proportion of barren land*	8.7	3.3	5.4	0.021

grouping of fish species with associations to pool or riffle/run habitat characteristics was not observed at the landscape scale. Rather this scale resulted with a mixture of both groups belonging to Cluster 1 and Cluster 2. Specifically, the species found in Cluster 1 were associated with barren landscapes ($P = 0.02$) without a major cover type while species in Cluster 2 were associated with a forested land area ($P = 0.03$) which includes hardwoods like oaks and junipers. The cover type herbaceous floodplain vegetation was not different between these two groups.

Canonical correspondence analysis

Canonical correspondence analysis supported the results of the cluster analysis, indicating two large divisions of species clusters generally associated with pool and riffle/run habitat characteristics (Figure 7). The relative abundance of species associated with riffle or run habitat was strongly correlated with higher current velocity at the micro-meso habitat scale. The relative abundance of species associated with pool habitats were correlated with the occurrence of deeper and wider portions of the river with greater canopy cover and micro-meso habitat contiguity (Figure 7A).

A similar separation of species existed at the mesohabitat scale. Separation of species along the first axis was primarily associated with pool habitat characteristics and with riffle/run habitat characteristics (Figure 7B). The relative abundance of species associated with riffle/run habitats was correlated with higher proportions of coarse substrates, such as bedrock and cobble-gravel (Figure 7B). Habitat contiguity remained a major influence on the relative abundance of pool-associated species, but the proportion of structured habitat within a pool, such as large woody debris, was also an important factor affecting relative abundance for a majority of these species (Figure 7B). Guadalupe roundnose minnow stood out as being the sole species for which submerged aquatic vegetation was a primary requirement (Figure 7B).

At the riffle-run-pool complex scale, the total area of the complex and the availability of structured habitat and coarse substrates were the factors most highly correlated with the composition of the fish assemblage (Figure 7C). While there were still two primary groups of species, there was no clear grouping comprised of pool-associated species. Species associated with complexes containing higher proportions of coarse substrate riffles and runs with a greater number of boulders were those captured almost exclusively from riffle and run habitats. Species associated with larger complexes with higher proportion of submerged aquatic vegetation were captured from all three types of mesohabitats (Figure 7C). Interestingly, the position of Guadalupe bass near the origin of all the vectors suggests its relative abundance is not correlated to habitat characteristics at this spatial scale (Figure 7C).

The relative abundance of species associated with riffle/run habitat seemed to be more strongly correlated with variables from finer spatial scales, while factors at coarser spatial scales seemed

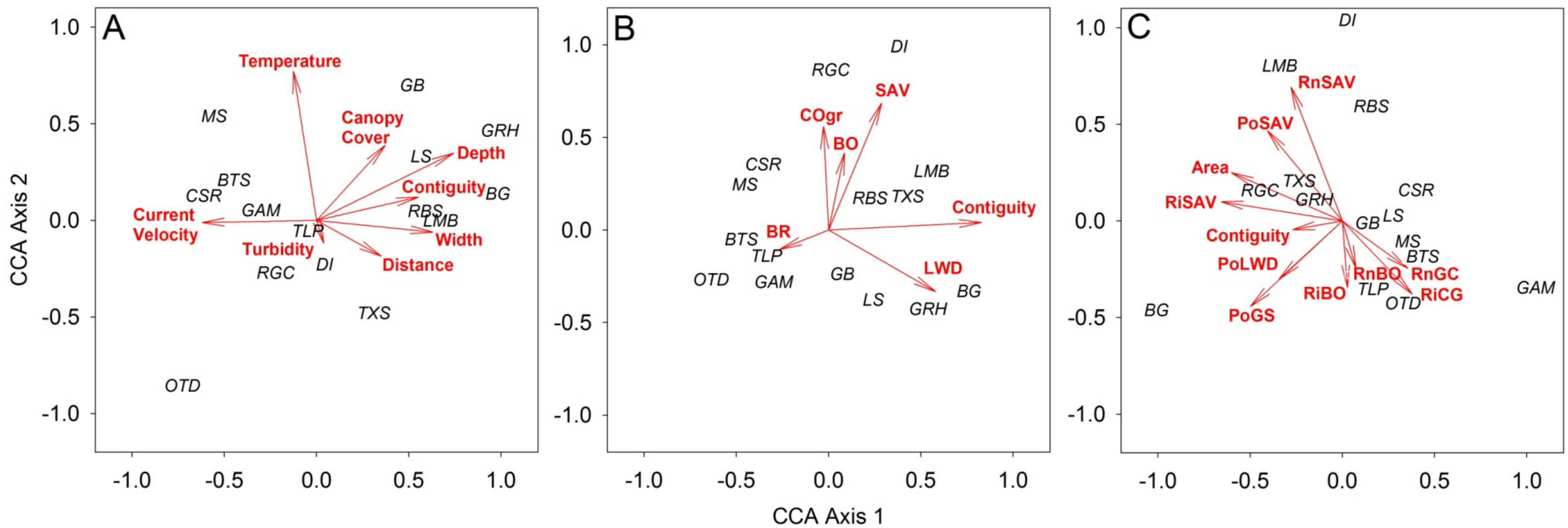

Figure 7. Canonical correspondence analysis biplots of stream fish species scores at the micro-mesohabitat scale (A), mesohabitat scale (B), and the riffle-run-pool complex scale (C) in the South Llano River, Texas during 2012. For all panels, the species scores are represented by the abbreviations listed in Table 3. Environmental variables at each scale are indicated by arrows and abbreviations are listed in Table 6. For panel A, CCA Axis 1 represents 37.8% of the explained variance with an eigenvalue of 0.25. CCA Axis 2 describes 23.8% of the explained variance with an eigenvalue of 0.16. In panel B, CCA Axis 1 represents 44.4% of the explained variance with an eigenvaue of 0.18. CCA Axis 2 describes 25.3% of the explained variance with an eigenvalue of 0.10. For panel C, CCA Axis 1 represents 34.5% of the explained variance with an eigenvalue of 0.1912. CCA Axis 2 describes 21.7% of the explained variance with an eigenvalue of 0.12.

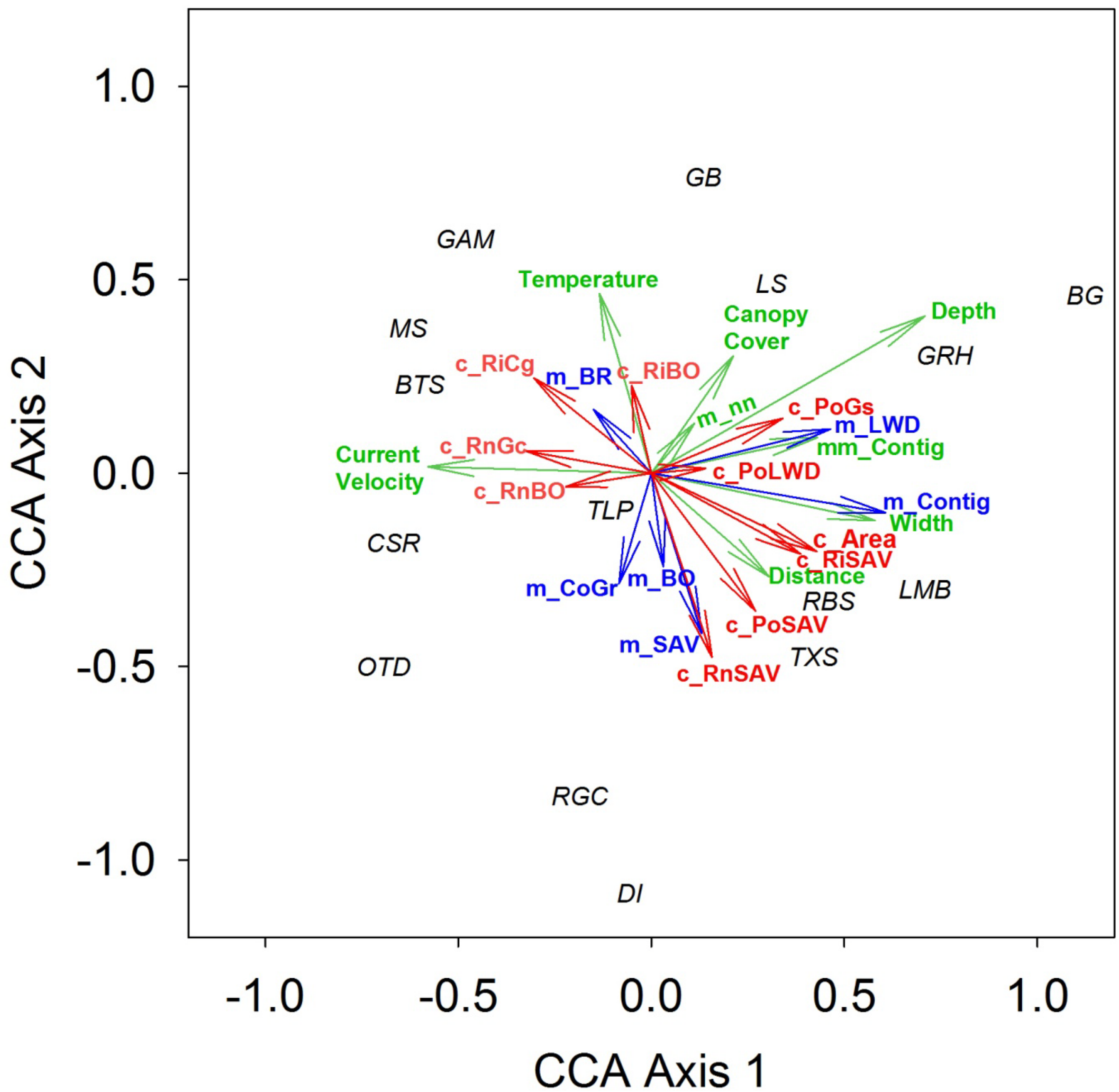

Figure 8. Canonical correspondence analysis biplot of stream fish species scores including a composite of variables from the micro-mesohabitat (green), mesohabitat (blue), and riffle-run-pool complex (red) scales in the South Llano River, Texas during 2012. Axis 1 represents 24.9% of the explained variance with an eigenvalue of 0.32. Axis 2 describes 17.6% of the explained variance with an eigenvalue of 0.22. Descriptions of the abbreviations for species names can be found in Table 3 and the environmental variables can be found on Table 6.

to have more influence on the relative abundance of species associated with pool habitats (Figure 8), though this was more of a general trend with exceptions than a consistent pattern. For example, bluegill *Lepomis macrochirus* relative abundance was strongly influenced by depth at the micro-mesohabitat scale, while Texas logperch was most associated with the number of boulders and proportion of cobble-gravel substrate at the mesohabitat scale. Overall, the 28 variables across all scales accounted for 22.6% of the total variation in the species relative abundance data (Table 9). The micro-meso scale explained approximately 8% of the variation, followed by 6.5% of the variation explained by the complex scale variables, and only 2.9% explained by the mesohabitat scale variables. Furthermore, the percent variation explained by all three scales combined was rather low at 1.2% suggesting that there is little correlation among variables at the different spatial scales.

Seasonal variation in habitat associations

In general, the relative abundance of most species tended to be correlated with the same micro-meso and meso habitat scale variables throughout the year (Figure 9, Figure 10). However for several species there seemed to be seasonal differences in the variables correlated with their relative abundance, likely due to a combination of ontogenetic shifts in habitat use and capture probability. For example, spring habitat associations at the micro-meso and meso scale for a majority of the pool species involved deep and wide sections of river with a high proportion of coarse substrate and submerged aquatic vegetation (Figures 11D; Figure 12D). However, both Guadalupe bass and longear sunfish shifted from primarily pool habitats in the winter to occupying deeper runs in close proximity to banks with relatively dense canopy cover in the spring (Figure 11D; Figure 13D).

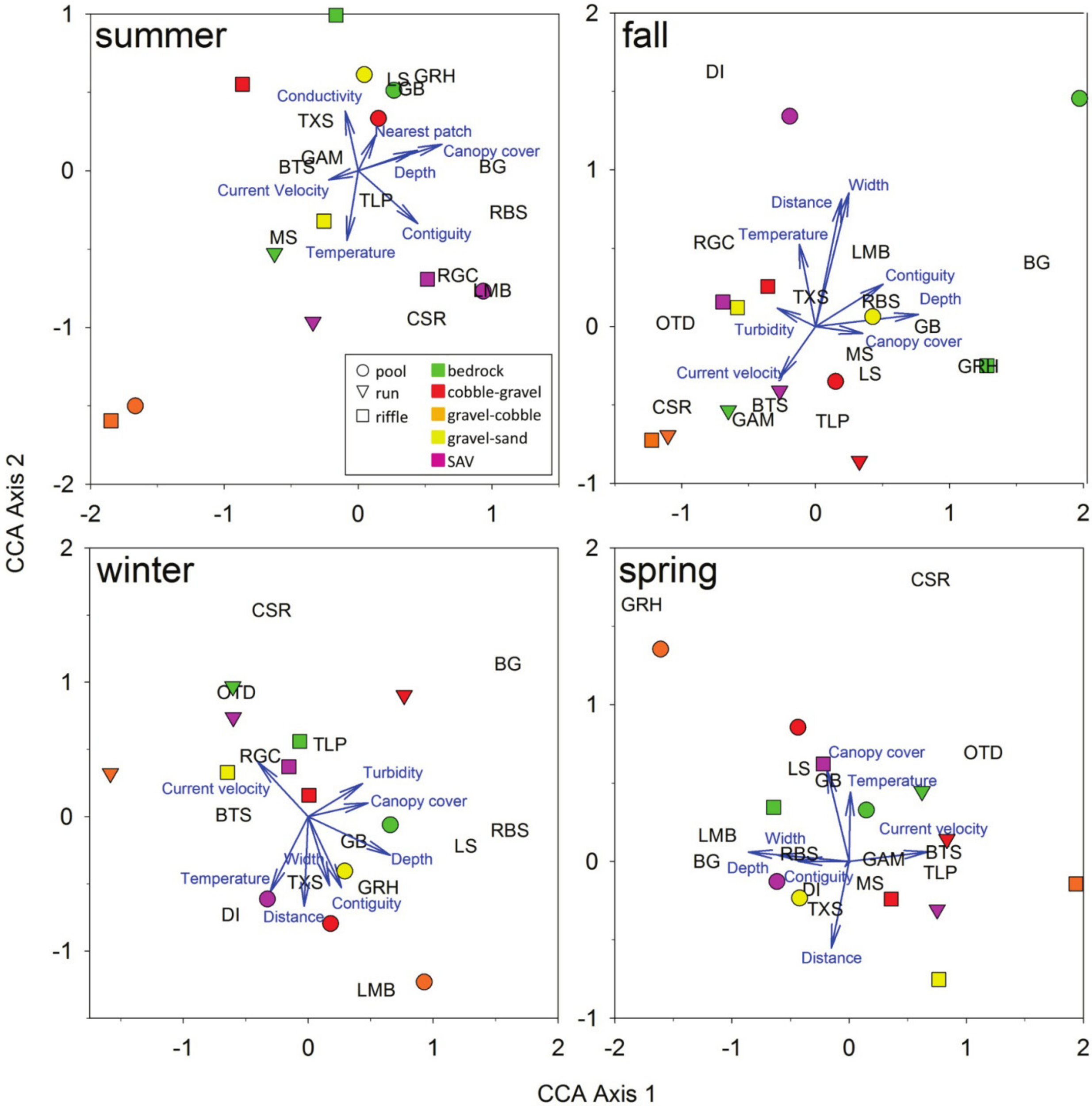

Figure 9. Canonical correspondence analysis triplots representing seasonal stream fish species and site scores at the micro-mesohabitat scale in summer fall, winter, and spring 2012 in the South Llano River, Texas. For each mean site score, the mesohabitats are represented by the shape of symbol while substrate types are represented by color. CCA Axis 1 represents 45.8%, 34.7%, 30.4%, and 44.3% of the explained variance in summer, fall, winter, and spring, respectively, while CCA Axis 2 describes 21.1%, 22.0%, 22.4%, and 17.8% of the explained variance in panels in summer, fall, winter, and spring, respectively. Eigenvalues for CCA Axis 1 ranged from 0.33, 0.42, 0.46, and 0.35 and eigenvalues for CCA Axis 2 ranged from 0.15, 0.26, 0.34 and 0.14 for summer, fall, winter, and spring, respectively.

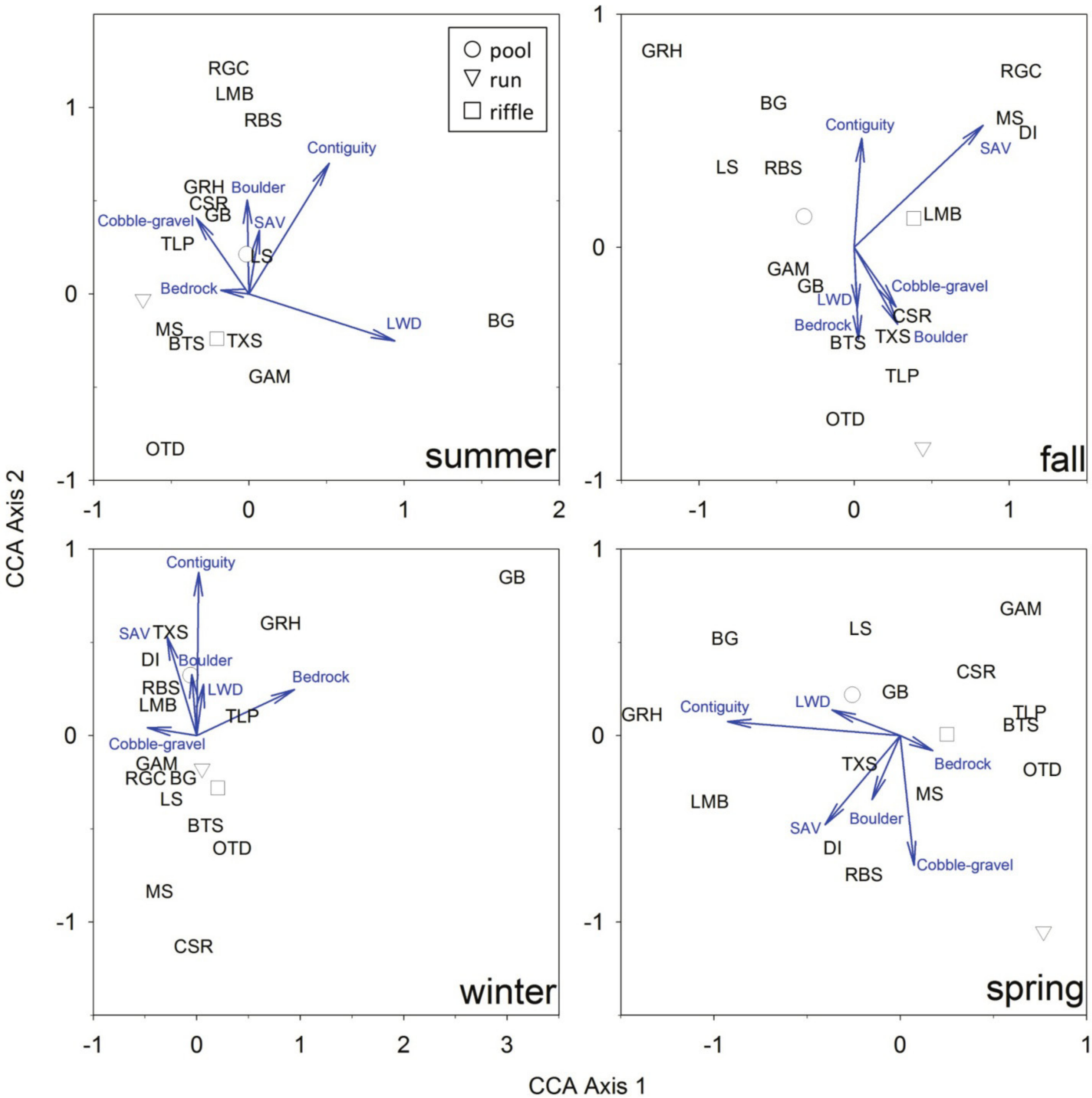

Figure 10. Canonical correspondence analysis triplots representing seasonal stream fish species and site scores at the mesohabitat scale in the South Llano River during summer, fall, winter, and spring 2012. The mean score of each mesohabitats are represented by the shape of symbol. Abbreviations for the fish species are listed in Table 3 and environmental variable abbreviations are listed in Table 6. CCA Axis 1 represents 54.6%, 40.1%, 31.3% and 44.3% of the explained variance in summer, fall, winter, and spring respectively, while CCA Axis 2 describes 19.1%, 26.5%, 26.0% and 17.8%. Eigenvalues for CCA Axis 1 were 0.48, 0.32, 0.29, and 0.35 for summer, fall, winter, and spring respectively. Eigenvalues for CCA Axis 2 were 0.17, 0.211, 0.25, and 0.14 for summer, fall, winter, and spring respectively

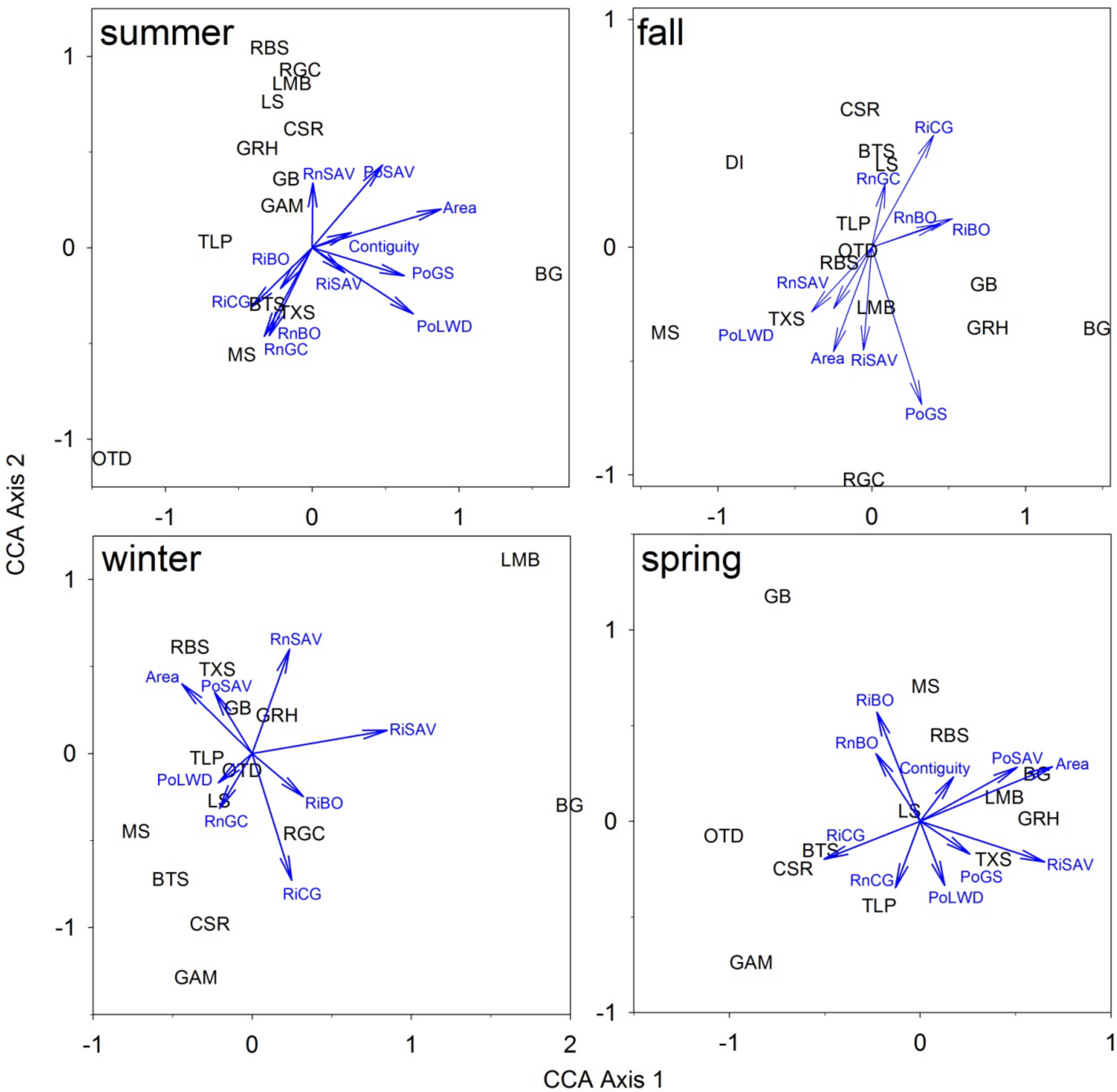

Figure 11. Canonical correspondence analysis biplots of seasonal stream fish species at the riffle-run-pool complex scale in the South Llano River, Texas during summer, fall, winter, and spring 2012. For all panels, the species scores are represented by the species abbreviations in Table 3, while environmental variables abbreviations are listed on Table 6. CCA Axis 1 represents 40.2%, 25.4%, 30.6% and 26.7% of the explained variance in summer, fall, winter, and spring, respectively. CCA Axis 2 describes 20.1%, 26.1%, 19.7% and 20.2% of the explained variance in summer, fall, winter, and spring, respectively. Eigenvalues for CCA Axis 1 ranged from 0.46, 0.29, 0.54, and 0.23 and 0.23, 0.24, 0.35, and 0.17 for CCA Axis 2 during summer, fall, winter, and spring, respectively.

Table 9. Independent (1-3) and confounded (4-7) components of explained variance in stream fish assemblage data across all three spatial scales in the South Llano River, Texas from collections made in 2012. Component 1 represents variance explained by micro-mesohabitat scale variables alone. Component 2 represents the variance explained by mesohabitat scale variables alone. Component 3 represents the variance explained by riffle-run-pool complex scale variables alone. Component 4 displays the variance explained by both micro-mesohabitat and mesohabitat scales while component 5 represents the variance explained by both the mesohabitat and riffle-run-pool complex scales. The variance explained by micro-mesohabitat and riffle-run-pool complex scales is component 6 while component 7 represents the variance explained by all three scales together.

Component number	Component	Variation	% species variance explained	% of variance accounted for by each component
1	Micro-Mesohabitat	0.45	35.0	7.9
2	Mesohabitat	0.17	12.9	2.9
3	Riffle-run-pool complex	0.37	29.0	6.5
4	Micro-Mesohabitat x Mesohabitat	0.09	7.0	1.6
5	Mesohabitat x Riffle-run-pool complex	0.06	4.6	1.0
6	Micro-Mesohabitat x Riffle-run-pool complex	0.06	4.8	1.1
7	Micro-Mesohabitat x Mesohabitat x Riffle-run-pool complex	0.09	6.7	1.5
	Total Explained:	1.29	100.0	22.6
	All Variation:	5.70	-	100.0

Discussion

Fish assemblage composition

We encountered 24 of the 45 species reported from the South Llano River (Hendrickson and Cohen 2010), plus one previously unreported species during this study. Red shiner *Cyprinella lutrensis,* speckled chub *Macrhybopsis aestivalis,* burrhead chub *Macrhybopsis marconis*, golden shiner *Notemigonus crysoleucas*, Tamaulipas shiner *Notropis braytoni*, sand shiner *Notropis stramineus*, pugnose minnow *Opsopoeodus emiliae*, fathead minnow *Pimephales promelas*, bullhead minnow *Pimephales vigilax*, quillback *Carpiodes cyprinus*, spotted sucker *Minytrema melanops*, yellow bullhead *Ameiurus natalis*, Mexican tetra *Astyanax mexicanus*, blackstripe topminnow *Fundulus notatus*, plains killifish *Fundulus zebrinus*, inland silverside *Menidia beryllina*, sailfin molly *Poecilia latipinna*, smallmouth bass *Micropterus dolomieu,* spotted bass *Micropterus punctulatus*, black crappie *Pomoxis nigromaculatus*, and logperch *Percina caprodes* have been reported from the South Llano River, but were not encountered in the present study. Most of the above species, with the exception of golden shiner, spotted sucker, yellow bullhead, and spotted bass, have not been reported from recent surveys using electrofishing or seines (Higgins 2005; T.B. Grabowski, *unpublished data*), which suggests that most of the species missing from this study are minor components of the fish assemblage or even simply spurious records, e.g., Tamaulipas shiner and spotted bass. We also captured common carp *Cyprinus carpio* during this study. This species had not been previously reported from this system (Hendrickson and Cohen 2010). However, the species has been encountered by other researchers in the past (P.T. Bean, *pers. comm.*) and specimens may not have been retained or deposited with the Texas Natural History Collections.

Generally, our estimates of the relative abundances of the species encountered were consistent with those reported from other surveys (Higgins 2005; Hendrickson and Cohen 2010, Grabowski, *unpublished data*). However, there were some notable exceptions. Our sampling methodology likely underestimated the relative abundance of several species, most notably larger and more mobile species such as channel catfish *Ictalurus punctatus*, flathead catfish *Pylodictis olivaris*, longnose gar *Lepisosteus osseus*, river carpsucker *Carpiodes carpio*, and common carp, and these species comprised the majority of those that were excluded from analysis due to low sample sizes. Larger representatives of several species, such as gray redhorse and Guadalupe bass, were also likely underrepresented but sufficiently represented to be included in the analysis. Electrofishing surveys of the South Llano River conducted for a related study during 2012-2013 suggest that these species, particularly channel catfish, gray redhorse, and Guadalupe bass, can make up a substantial component of the fish assemblage (T.B. Grabowski, *unpublished* *data*). If numbers of these species were sufficient to include in the analysis, habitat associations and species-to-species associations could change as a result. The relative importance of the various spatial scales in terms of explaining the variance in the species data, particularly for the larger instream scales like the pool-riffle-run complex scale, would also likely shift as the abundance of these larger, more mobile species are more likely to be influenced by habitat characteristics at larger scales (Gowan and Fausch 1996; Schlosser and Kallemeyn 2000; Fausch et al. 2002). The underrepresentation of these species in our samples was likely due to their ability to evade the seine and/or tendency to inhabit habitats that were largely inaccessible or difficult to effectively sample with a seine. This effect seemed to be more pronounced during winter based upon the paired electrofishing and seine samples, likely due to the movement of individuals to deeper, more thermally stable water and a decrease in the vulnerability of young-of-year associated with growth or ontogenetic habitat shifts (Bayley and Herendeen 2000). Similar seasonal changes in the efficiency of seines have been previously described (Allen et al. 1992).

<u>Instream habitat availability in the South Llano River</u>

Our estimates of the substrate composition and the spatial distribution of substrate types in the South Llano River generated from side scan sonar surveys were consistent with those from more traditional, on the ground assessments conducted by Heitmueller (2009). The South Llano River is dominated by coarse gravel substrates, with bedrock comprising a large proportion of the substrate in the upstream reaches of the study area. Substrate composition at the sampling sites remained consistent during this study, but it should be noted that variations in flow, particularly large flood pulses, have the potential to move substrate materials. Follow-up studies in the South Llano River should prioritize re-surveying substrates to ensure the spatial distribution of substrate patches has not shifted. While the instream habitat map generated by our side-scan sonar surveys remained accurate throughout the duration of the study, the same was not true for submerged aquatic vegetation. We observed directly and through follow-up side-scan sonar surveys of select river segments that percent cover of the submerged aquatic vegetation substrate class fluctuated seasonally due to the spring and summer growth and winter dieback of American water-willow *Justicia americana* and other species. On a seasonal basis, the influence of submerged aquatic vegetation on fish assemblage structure is likely underestimated in the summer and overestimated in the winter. However, it is not clear how the seasonal fluctuations in submerged aquatic vegetation coverage affected the combined-season estimates of its influence on fish assemblage structure.

The methodology described by Kaeser and Litts (2010) seemed to provide an effective means of classifying substrate and other instream habitat features in the South Llano River. Our groundtruthing efforts suggest an overall accuracy rate of approximately 90.0% in assigning substrate classes. This high level of accuracy is fairly typical for this approach (Kaeser and Litts 2010) and was likely enhanced by the relative low diversity of fine substrate classes, such as mud or silt, in the South Llano River (Heitmueller 2009). However, our procedure for the assignment of mesohabitat classifications, i.e., riffle, run, pool, could be refined to better account for the dynamic nature of riverine systems. Mesohabitats are typically delineated through a combination of factors, including gradient, turbulence, bed roughness, and flow velocity (Bisson et al. 1982; Hawkins et al. 1993). While our approach was sufficient for the purposes of this study, several factors need to be considered during the interpretation of our results and in future applications of this methodology. The first consideration is that in many cases, the boundaries between mesohabitats are somewhat arbitrary (Poole et al. 1997) and may represent transitional habitat. To attempt to minimize the potential of samples being taken from transitional habitat, transects were centered within the micro-mesohabitat patches sampled. The second consideration is that the designation of mesohabitats is highly dependent upon flow conditions. Our sonar and aerial surveys were conducted during the low flows at the height of the drought experienced by Texas in 2011 and flows remained low and relatively stable throughout the duration of the study. However, mesohabitat classifications may have fluctuated had collecting occurred under more variable flow conditions altering the boundaries of both mesohabitats and riffle-run-pool complexes. A more quantitative means of designating mesohabitats and complexes would likely provide results that were more consistent through time and across streams, but would require a substantially larger investment of time during the habitat mapping stage of a project.

Influence of scale on species-habitat associations

Our results suggest that variables at the micro-meso scale are the most important in structuring the fish assemblage of the South Llano River when considering instream and riparian habitats at multiple spatial scales. Specifically, the variables at micro-mesohabitat scale, such as current velocity, depth, canopy cover, and the size and distance between micro-mesohabitat types, i.e., contiguity, tended to be the most informative for explaining the variance in the South Llano River fish assemblage data. This was not unexpected given that the majority of our samples were comprised of relatively small-bodied cyprinids, juvenile centrarchids, and percid darters. These species tend to be relatively sedentary and may not exhibit large amounts of movement (Gerking 1959). Thus the composition and characteristics of their habitat at this fine scale would be expected to play a major role in determining their occupation and abundance in this habitat. However, the micro-mesohabitat variables would also be expected to exhibit a similar, but potentially less pronounced, influence on more mobile, larger-bodied fishes as these fishes would likely move between similar habitats or use a range of habitat types over their larger home ranges.

The riffle-run-pool complex scale accounted for approximately 30% of the total explained variance in the fish assemblage data, suggesting that many of the fish species could be heavily influenced by habitats at the complex scale. Many species undergo ontogenetic habitat shifts, require various habitats to complete their life history, or require larger home ranges and as a result, larger spatial scales could exert considerable influence on the distribution and abundance of these fishes (Schlosser 1991; Schlosser and Angermeier 1995; Fausch et al. 2002). For example, most catostomids, like the grey redhorse, are known to travel relatively long distances within rivers (Lucas and Baras 2001). Depending on the species, the length of the river, and order of the river, an individual could travel 500 to 3,000 meters upstream in the spring prior to spawning (Matheney and Rabeni 1995; Lucas et al. 2001). Additionally, some findings have suggested that home ranges of riverine fish are much larger than previously anticipated, even for some smaller-bodied species (Skalski and Gilliam 2000). This further supports the idea that some species in South Llano River could be exploiting relatively large sections of the river, presumably at the complex or higher spatial scale. Furthermore, there are likely linkages between the variables measured at the riffle-run-pool complex scale and the response of specific micro-mesohabitat characteristics, such that the values recorded at finer spatial scales are heavily influenced or determined largely by factors at higher scales.

Our results suggest that mesohabitat scale variables, intermediate to the micro-meso and complex scales and related to the pool, riffle, or run that a given sample location was located in, were not particularly influential in explaining variation in fish assemblage structure. Furthermore, the two largest scales, reach and the riparian buffer, were both inconclusive in terms of results for both the cluster analysis and canonical correspondence analysis. Ultimately, we were not able to conduct a CCA or variance partitioning for both the reach and landscape scale due to the lack of variation explained by the CCA axes. One of the key underlying assumptions of the hierarchical nature of riverine systems is that processes at larger spatial scales will influence fish assemblages at smaller spatial scales (Frissell et al. 1986). However, the variables at our riparian buffer scale explained little of the observed variance in the fish samples relative to the other scales. This is a surprising observation considering the important roles that riparian buffers can play in determining the quality and composition of instream habitats, including natural filtering mechanisms that reduce sedimentation (Schlosser and Karr 1981), contribution of allochthonous matter for biota nourishment and habitat enhancement (Hauer et al. 2003, France et al. 1996), and reduction of bank erosion (Beeson and Doyle 1995). The low variance associated with the riparian buffer scale may be a function of the relatively low anthropogenic disturbance found within our 100 meter riparian buffer. Past studies examining fish assemblages in lotic systems at multiple scales have shown that finer spatial scales tend to have greater explanatory power when the surrounding landscape is either minimally disturbed (Debano and Schmidt 1989; Wang et al. 2003; Wang et al. 2006) or highly disturbed (Stauffer et al. 2000; Heitke et al. 2006; Diana et al. 2006; Gido et al. 2006). This trend may be due to a lack of variation in the predictor variables at the riparian or landscape level as a result from low heterogeneity of land use and cover types across the landscape mosaic (Lammert and Allan 1999; Johnson and Host 2010), which was apparent in the South Llano River watershed. A lack of explanatory power in higher spatial scales can be a function of sites being located within similar subcatchments that possessed relatively homogeneous land uses and levels of anthropogenic disturbance (Lammert and Allan 1999).

However, it should be noted that the literature provides conflicting viewpoints as studies involving multiple spatial scales in both minimally disturbed (Debano and Schmidt 1989; Wang et al. 2003; Wang et al. 2006) and heavily disturbed watersheds (Stauffer et al. 2000; Heitke et al. 2006; Diana et al. 2006; Gido et al. 2006) have produced contrasting results regarding the scale with the greatest influence on biotic assemblages. For instance, Esselman and Allan (2010) found that watersheds associated with low levels of anthropogenic disturbance in Belize show more explanatory power with landscape scale variables versus local scale factors. Their findings suggest that some undisturbed catchments may have strong enough natural gradients to overcome local scale influences on fish assemblages. In contrast, Heitke et al. (2006) found a correlation between disturbance in a land use buffer and index of biotic integrity (IBI) scores in agriculturally dominated watersheds in Iowa (Heitke et al. 2006). These findings illustrate the lack of understanding we have of the impacts of landscape heterogeneity and the roles they play in different regions of the world (Johnson and Host 2010), and may indicate that there may be a high degree of system specificity. Furthermore, results can vary between studies in the same system with slight alterations to the data resolution and study design (Allan 2004). For example, an assessment of the biotic integrity of fish and macroinvertebrate assemblages of River Raisin in southeastern Michigan found that instream habitat variables were more informative than those from scales at the subcatchment level (Lammert and Allan 1999). This was in contrast to a previous study on the same watershed which found that larger scale land use variables were more informative (Roth et al. 1996). The major differences between these two studies stemmed from sampling design and spatial scope. The latter study included a larger area of subcatchments and more widely spaced sampling sites, which potentially introduced greater amounts of variance or contrast amongst the variables at the subcatchment scale (Lammert and Allan 1999).

Compared to many watersheds on the Edwards Plateau, the South Llano River watershed has relatively low levels of anthropogenic disturbance due to low population densities, minimal water withdrawals for agricultural purposes, and relatively low grazing pressure from livestock (Linam et al. 2002). A majority of the disturbed areas are concentrated in the lower reach of the river, particularly in proximity to the dam and associated reservoir in Junction, Texas (Linam et al. 2002). Throughout the remainder of the watershed, we observed consistently low levels of anthropogenic disturbances within the riparian buffer, e.g., 6.0 ± 0.01 % low-intensity urban and 1.1 ± 0.4 % agricultural land cover. Therefore, it is reasonable to conclude that restoration and conservation efforts in the South Llano River focused on the micro-meso habitat and riffle-run-pool complex scales would have the greatest impacts on the fish assemblage. However, fish habitat and assemblages are ultimately being structured by both large and small scale processes simultaneously (Frissell et al. 1986, Rowe et al. 2009) and it is crucial to not discount the larger scales or underestimate their importance to the fish assemblage in the South Llano River. Furthermore, it is important to note that this study represents a single year of data collection and that it occurred during one of the most extreme droughts recorded in the state. Even though the South Llano River maintained a relatively constant flow during the study period, it is not clear if our results would change under more typical conditions.

Management implications and considerations for the future

The effectiveness of stream restoration efforts is too often limited by a lack of criteria for defining and assessing success (Kondolf 1995; Palmer et al. 2005) and restoration activities are often undertaken without a full understanding of the desired target state of the stream in terms of community composition and ecological functionality (Palmer et al. 2005). Furthermore, many restoration projects are designed to restore relatively small portions of riverine and riparian habitat without understanding the landscape scale questions driving species occupation or use of such habitats, much less the impact to the riverine system as a whole (Palmer et al. 2005). Other projects are targeted towards a single species of conservation concern with the assumption that benefits will be seen throughout the system. However, this assumption that the condition of a single species in a stream system can serve as an indicator for the health of the entire system is rarely evaluated. Without ecologically-based criteria by which to measure success, restoration activities are unlikely to meaningfully address the underlying causes of habitat degradation and result in minimal or temporary improvements in the health of the stream. Our results provide a snapshot image of how the fish assemblage of a minimally-disturbed, spring-fed stream on the Edwards Plateau is structured in relation to instream habitat at various scales and characteristics of its watershed. In general, our results represent a potential target state to which the health and status of similar systems in the Colorado River Basin, such as the North Llano River, James River, San Saba River, and Pedernales River, can be compared. The species relationships and species-habitat relationships are also broadly applicable across the Edwards Plateau ecoregion as a starting point for developing watershed-specific guiding images for restoration.

Overall, the majority of species collected from the South Llano River seemed to be relatively plastic in their habitat use despite displaying clear associations to specific habitat characteristics at various spatial scales. The fishes of the South Llano River could be loosely classified as pool "specialist" species and riffle/run "specialist" species and these groupings remained consistent across multiple spatial scales. Guadalupe roundnose minnow represented an exception to this as it was associated exclusively with a specific habitat type, i.e., submerged aquatic vegetation. Based on past findings, these fish are known to be herbivorous (Wayne 1979) and like other small herbivores, this species may also be relying on the vegetation for evading predation by using it as cover (Camp et al. 2012) thus making the presence of submerged aquatic vegetation essential for their survival. Submerged aquatic vegetation was an important habitat component for other species even if they did not exhibit the same exclusivity as Guadalupe roundnose minnow. Largemouth bass was another species that was frequently found near aquatic vegetation in our study, regardless of scale. This is not unusual since selecting habitat with high structural complexity (Savino and Stein 1982) is a common trend for this species. There was also evidence of aquatic vegetation being an important seasonal factor for various species like the Guadalupe bass and Rio Grande cichlid which exhibited ties to this habitat type in runs and pools during summer. While these findings suggest that undertaking restoration activities aimed at increasing the structural complexity of instream habitats may prove beneficial, it should be noted that associations with boulders and large woody debris were relatively weak at multiple spatial scales. Seasonally there were stronger associations with woody structure involving the Texas shiner and sunfish like the redbreast sunfish. Additionally, seasonal habitat associations at the meso scale suggest that the Guadalupe bass may be using woody structure during their spawning periods which typically occur in spring as well as between late summer and early fall (Edwards 1997). Specifically, Guadalupe bass tend to be associated with woody debris and with slower current velocities, near canopy cover during these seasons. This is an interesting conclusion since this association to woody debris has not been recorded for Guadalupe bass during these periods before this study and this spawning behavior is typically seen with largemouth bass (Ross 2001; Hunt and Annett 2002).

Based on the results of this study and those of Groeschel (2013), it is difficult to state unequivocally that Guadalupe bass can serve as an ideal indicator for the health of streams on the Edwards Plateau. Guadalupe bass seems to be sensitive to low flow conditions, exhibiting growth that is correlated to annual stream discharge, but a relationship between growth and habitat characteristics at various scales is not clear (Groeschel 2013). At both the micro-meso and riffle-run-pool complex scale, Guadalupe bass were associated with a suite of species with similar habitat associations, including largemouth bass, bluegill, redbreast sunfish, longear sunfish, Texas logperch, gray redhorse, and to a lesser extent Rio Grande cichlid and Texas shiner. However, the habitat associations of Guadalupe bass described both in this study and by Groeschel (2013) suggested a greater usage of pool and deep run habitats than previously reported (Edwards 1997; Perkin et al. 2010). It is unclear if this was because drought conditions prevalent on the South Llano River during the course of these studies forced Guadalupe bass to occupy pool and deep run habitats with greater frequency. Alternatively, the extensive stocking of Guadalupe bass may have resulted in individuals spilling over from preferred habitats into other habitat types. The bias of our gear for smaller individuals potentially may have exacerbated these observations and made the associations with these habitats seem stronger than they might otherwise be. This is supported to some extent by the finding that Guadalupe bass habitat associations at multiple spatial scales vary by age class (Groeschel 2013). Though further work is necessary to determine whether our results are representative of "normal" conditions, our results suggest that the distribution and abundance of Guadalupe bass could be indicative of a large proportion of the fish assemblage in streams on the Edwards Plateau.

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Appendices

Appendix 1. Average relative abundance of stream fish species in the micro-mesohabitat types sampled in the South Llano River during 2012. Species abbreviations are described in Table 3. Substrate types are abbreviated as follows: BR = bedrock, CoGr = cobble-gravel, GrCo = gravel-cobble, GrSa = gravel-sand, and SAV = submerged aquatic vegetation.

		Pool					Run					Riffle			
Species	***n***	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**	**BR**	**CoGr**	**GrCo**	**SAV**
BTS	1004	0.56	0.30	0.09	0.25	0.04	0.32	0.28	0.82	0.22	0.09	0.44	0.34	0.27	0.24
TXS	892	0.10	0.16	0.50	0.37	0.24	0.18	0.16	0.03	0.29	0.31	0.48	0.27	0.04	0.35
MS	247	0.12	0.03	0.00	0.02	0.16	0.06	0.10	0.00	0.15	0.04	0.04	0.10	0.50	0.06
LS	187	0.00	0.07	0.00	0.07	0.02	0.14	0.03	0.00	0.07	0.05	0.00	0.05	0.00	0.05
DI	159	0.00	0.05	0.00	0.01	0.37	0.00	0.10	0.00	0.02	0.04	0.00	0.00	0.00	0.00
RBS	154	0.00	0.11	0.00	0.05	0.08	0.05	0.04	0.00	0.01	0.04	0.00	0.07	0.00	0.02
GB	146	0.10	0.07	0.03	0.05	0.04	0.13	0.02	0.00	0.02	0.06	0.00	0.03	0.00	0.03
CSR	110	0.00	0.04	0.00	0.01	0.00	0.00	0.10	0.01	0.02	0.04	0.02	0.02	0.00	0.03
GAM	78	0.00	0.02	0.00	0.03	0.00	0.00	0.02	0.00	0.00	0.19	0.00	0.00	0.00	0.00
RGC	75	0.00	0.01	0.00	0.01	0.02	0.00	0.02	0.00	0.04	0.03	0.00	0.02	0.02	0.11
BG	74	0.02	0.03	0.00	0.05	0.02	0.00	0.03	0.00	0.00	0.03	0.00	0.01	0.00	0.00
GRH	64	0.01	0.02	0.13	0.03	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.06	0.00	0.00
OTD	56	0.02	0.01	0.00	0.01	0.00	0.00	0.02	0.12	0.02	0.02	0.00	0.01	0.08	0.09
LMB	46	0.00	0.03	0.03	0.01	0.01	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00
TLP	46	0.07	0.02	0.00	0.01	0.00	0.05	0.00	0.01	0.01	0.02	0.00	0.01	0.08	0.00
RES	14	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CC	13	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00
GSD	12	0.00	0.01	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GTD	10	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00
GS	5	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WM	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CCP	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FHC	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LG	1	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 2. Proportions of observations of stream fish species occurring in the micro-mesohabitat types sampled in the South Llano River during 2012. Species abbreviations are described in Table 3. Substrate types are abbreviated as follows: BR = bedrock, CoGr = cobble-gravel, GrCo = gravel-cobble, GrSa = gravel-sand, and SAV = submerged aquatic vegetation.

		Pool					Run					Riffle			
Species	***n***	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**	**BR**	**CoGr**	**GrCo**	**SAV**
BTS	1004	0.06	0.16	0.00	0.19	0.00	0.01	0.15	0.06	0.20	0.02	0.03	0.10	0.01	0.00
TXS	892	0.01	0.10	0.01	0.34	0.04	0.01	0.08	0.00	0.17	0.08	0.03	0.10	0.00	0.00
MS	247	0.05	0.04	0.00	0.06	0.01	0.01	0.23	0.00	0.31	0.04	0.01	0.12	0.10	0.00
LS	187	0.00	0.17	0.00	0.32	0.02	0.03	0.06	0.00	0.22	0.06	0.00	0.09	0.00	0.01
DI	159	0.00	0.15	0.00	0.04	0.30	0.00	0.38	0.00	0.06	0.06	0.00	0.01	0.00	0.00
RBS	154	0.00	0.32	0.00	0.22	0.10	0.01	0.13	0.00	0.01	0.06	0.00	0.14	0.00	0.01
GB	146	0.08	0.21	0.00	0.26	0.04	0.04	0.12	0.00	0.08	0.10	0.00	0.06	0.00	0.00
CSR	110	0.00	0.17	0.00	0.06	0.01	0.00	0.48	0.01	0.09	0.08	0.01	0.06	0.00	0.00
GAM	78	0.00	0.14	0.00	0.22	0.00	0.00	0.08	0.00	0.01	0.55	0.00	0.00	0.00	0.00
RGC	75	0.00	0.05	0.00	0.16	0.04	0.00	0.14	0.00	0.31	0.09	0.00	0.08	0.01	0.00
BG	74	0.03	0.11	0.00	0.51	0.04	0.00	0.18	0.00	0.01	0.10	0.00	0.02	0.00	0.00
GRH	64	0.02	0.19	0.00	0.42	0.00	0.00	0.05	0.00	0.00	0.03	0.00	0.29	0.00	0.00
OTD	56	0.04	0.06	0.00	0.13	0.00	0.00	0.15	0.17	0.17	0.08	0.00	0.02	0.08	0.00
LMB	46	0.00	0.23	0.00	0.25	0.07	0.00	0.34	0.00	0.02	0.07	0.00	0.02	0.00	0.00
TLP	46	0.17	0.26	0.00	0.17	0.00	0.05	0.02	0.00	0.07	0.10	0.00	0.12	0.04	0.00
RES	14	0.00	0.07	0.00	0.07	0.22	0.00	0.57	0.00	0.07	0.00	0.00	0.00	0.00	0.00
CC	13	0.00	0.38	0.00	0.15	0.00	0.00	0.00	0.00	0.31	0.08	0.08	0.00	0.00	0.00
GSD	12	0.00	0.33	0.59	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GTD	10	0.00	0.00	0.00	0.09	0.00	0.00	0.55	0.09	0.00	0.27	0.00	0.00	0.00	0.00
GS	5	0.00	0.00	0.00	0.60	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WM	4	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CCP	2	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FHC	2	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50	0.00	0.00
LG	1	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC	1	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 3. Average relative abundance and proportion of observations of stream fish species in the substrate classes (microhabitat types) sampled in the South Llano River during 2012. Species abbreviations are described in Table 3. Substrate types are abbreviated as follows: BR = bedrock, CoGr = cobble-gravel, GrCo = gravel-cobble, GrSa = gravel-sand, and SAV = submerged aquatic vegetation.

		Relative abundance					Proportion of observations				
Species	***n***	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**	**BR**	**CoGr**	**GrCo**	**GrSa**	**SAV**
BTS	1004	0.49	0.29	0.50	0.29	0.10	0.10	0.40	0.07	0.38	0.05
TXS	892	0.24	0.19	0.12	0.34	0.33	0.05	0.28	0.01	0.52	0.14
MS	247	0.07	0.07	0.16	0.07	0.05	0.06	0.39	0.10	0.37	0.07
LS	187	0.03	0.05	0.00	0.08	0.05	0.03	0.33	0.00	0.54	0.10
DI	159	0.00	0.06	0.00	0.00	0.13	0.00	0.53	0.00	0.11	0.36
RBS	154	0.01	0.06	0.00	0.03	0.06	0.01	0.57	0.00	0.26	0.16
GB	146	0.08	0.03	0.00	0.04	0.06	0.09	0.38	0.00	0.35	0.15
CSR	110	0.01	0.04	0.01	0.01	0.04	0.01	0.70	0.01	0.17	0.11
GAM	78	0.00	0.01	0.00	0.01	0.00	0.00	0.22	0.00	0.23	0.55
RGC	75	0.00	0.02	0.01	0.03	0.04	0.00	0.27	0.01	0.47	0.25
BG	74	0.01	0.05	0.00	0.03	0.03	0.02	0.32	0.00	0.52	0.13
GRH	64	0.01	0.03	0.04	0.02	0.00	0.02	0.53	0.00	0.42	0.03
OTD	56	0.01	0.07	0.08	0.01	0.03	0.03	0.23	0.25	0.30	0.18
LMB	46	0.00	0.02	0.01	0.01	0.02	0.00	0.59	0.00	0.27	0.14
TLP	46	0.04	0.01	0.03	0.01	0.02	0.17	0.33	0.12	0.24	0.10
RES	14	0.00	0.01	0.00	0.00	0.00	0.00	0.64	0.00	0.14	0.22
CC	13	0.01	0.01	0.00	0.00	0.00	0.06	0.38	0.00	0.46	0.08
GSD	12	0.00	0.00	0.04	0.00	0.00	0.00	0.34	0.58	0.08	0.00
GTD	10	0.00	0.00	0.01	0.00	0.01	0.00	0.55	0.09	0.09	0.27
GS	5	0.01	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.60	0.00
WM	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
CCP	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
FHC	2	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
LG	1	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
RC	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00

Appendix 4. Average relative abundance and proportion of observations of stream fish species in the mesohabitat types sampled in the South Llano River during 2012. Species abbreviations are described in Table 3.

		Relative abundance			Proportion of observations		
Species	***n***	**pool**	**run**	**riffle**	**pool**	**run**	**riffle**
BTS	1004	0.28	0.30	0.31	0.41	0.41	0.18
TXS	892	0.32	0.23	0.32	0.50	0.35	0.15
MS	247	0.03	0.10	0.13	0.17	0.58	0.25
LS	187	0.06	0.05	0.04	0.50	0.39	0.11
DI	159	0.05	0.06	0.00	0.48	0.51	0.01
RBS	154	0.06	0.03	0.05	0.60	0.25	0.15
GB	146	0.06	0.03	0.02	0.59	0.33	0.08
CSR	110	0.02	0.05	0.02	0.24	0.67	0.09
GAM	78	0.02	0.04	0.00	0.35	0.65	0.00
RGC	75	0.01	0.03	0.03	0.26	0.54	0.20
BG	74	0.03	0.01	0.00	0.68	0.29	0.03
GRH	64	0.03	0.01	0.03	0.63	0.08	0.29
OTD	56	0.01	0.02	0.02	0.23	0.57	0.20
LMB	46	0.02	0.01	0.00	0.55	0.43	0.02
TLP	46	0.02	0.01	0.01	0.60	0.24	0.16
RES	14	0.00	0.01	0.00	0.36	0.64	0.00
CC	13	0.00	0.00	0.00	0.54	0.38	0.08
GSD	12	0.01	0.00	0.00	1.00	0.00	0.00
GTD	10	0.00	0.01	0.00	0.09	0.91	0.00
GS	5	0.00	0.00	0.00	0.60	0.40	0.00
WM	4	0.01	0.00	0.00	1.00	0.00	0.00
CCP	2	0.00	0.00	0.00	1.00	0.00	0.00
FHC	2	0.00	0.00	0.00	0.00	0.50	0.50
LG	1	0.00	0.00	0.00	1.00	0.00	0.00
RC	1	0.00	0.00	0.00	1.00	0.00	0.00