FINAL PROJECT REPORT (DRAFT)

Refinement and Validation of Habitat Quality Indices (HQI) and Aquatic Life Use (ALU) Indices for Application to Assessment and Monitoring of Texas Surface Waters

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Statement of Research Problem

To support its responsibilities of monitoring and setting standards for surface water quality, the Texas Commission for Environmental Quality (TCEQ) has adopted Aquatic Life Use Standards (ALUS) that rely on indices of biotic integrity (IBIs) and habitat quality indices (HQIs). These tools allow natural resource managers and regulators to assess the status of ecological systems for evaluation of trends and compliance with established water quality standards. Following several years of research by the Texas Parks and Wildlife Department (TPWD), the fish IBI used to assess Texas streams underwent major revision to reflect more accurately the major faunal differences among the states diverse geologic, climatic, and faunal regions (Linam et al. 2002). This IBI was created to assess the ecological condition of wadeable streams in Texas. No such metric currently exists for dealing with smaller (headwater springs, isolated wetlands) and larger (rivers, lakes) freshwater systems in the state.

A recent analysis of the correspondence between the fish IBI and the TCEQ's HQI revealed a very low and negative correlation between these indicators of ecological condition of streams (Kleinsasser et al 2004). A variety of factors could explain this lack of correspondence, including i) errors in one or both of these metrics, ii) poor matching of spatial scales of assessment, and iii) poor correspondence between temporal dynamics of environmental disturbances and habitat or biotic responses. The fish IBI has been researched more extensively than the HQI used by TCEQ, therefore the latter index required immediate study. The issue of scale and resolution, both in terms of space (geography, watershed position, siting within stream reach) and time (seasons, hydroperiod, time elapsed since last major disturbance) should be evaluated quantitatively in order to design and evaluate the validity and reliability of these assessment tools. This research project was designed to fill these critical information gaps. In order to complement ongoing research designed to refine Tiered Aquatic Life Uses (TALU) in Texas (U.S. EPA in collaboration with representatives of state natural resource agencies), the project focused on the region corresponding to the Subhumid Agricultural Plains (SAP) in Texas (Central Great Plains, Central Texas/Oklahoma Plains, Texas Blackland Prairies). Variable scales of resolution were examined for environmental and geographic variables and analyzed for correspondence with the fish IBI and HQI metrics.

Project objectives-

1) Describe and evaluate key metrics of stream habitat in relation to position within stream reach, watershed, and landscape (geology/soils, topography).

2) Describe and evaluate fish assemblage structure for use as biotic indicators of ecological status.

3) Statistically analyze fish and habitat metrics for potential sensitivity to aquatic life uses as well as variation in temporal and spatial scales of analysis.

4) Using quantitative methods, identify the most useful (sensitive, reliable) metrics (individual elements, suites of elements, or aggregate variables derived from multiple elements) in terms of potential for creation of improved HQI and IBI for wadeable streams in the SAP.

METHODS

Study Area

Data were collected from 64 perennial, wadeable streams in the Brazos and Trinity River basins within the Cross Timbers, Blackland Prairies, and East Central Texas Plains ecoregions (Figures 1-3). The Cross Timbers ecoregion (ECO 29) is a mosaic of forest, woodland, savanna, and prairie and is currently used mostly for rangeland and pastureland. The Texas Blackland Prairies ecoregion (ECO 32) is a disjunct ecological region distinguished from neighboring regions by fine-textured, clayey soils. This region was historically tallgrass prairie and now contains a higher percentage of cropland than adjacent ecoregions. In addition, large areas of the ecoregion are being converted to urban and industrial uses. The East Central Texas Plains ecoregion (ECO 33) was historically covered by post oak savanna and now is used primarily for pasture and rangeland (Griffin et al. 2004). The study sites were selected to provide broad geographic coverage, a range of landscape features (including land use), and representation of a range of stream habitat conditions. Existing TCEQ Surface Water Quality Monitoring (SWQM) stations were used as study sites where possible.

Data Collection

We sampled the 64 streams in the summers (June, July, and August) of 2006 and 2008. Due to heavy rains and flooding in 2007, only 46 streams were surveyed and sampling was extended through September and October. At each stream site, a 160-500 m study reach was designated for fish collection and local habitat measurements. Reach length was determined based upon the wetted width of the stream (approximately 40 times the average width). Study reach selection, fish collection, and habitat measurements were performed following the protocols of TCEQ SWQM Procedures (TCEQ 2003, 2004).

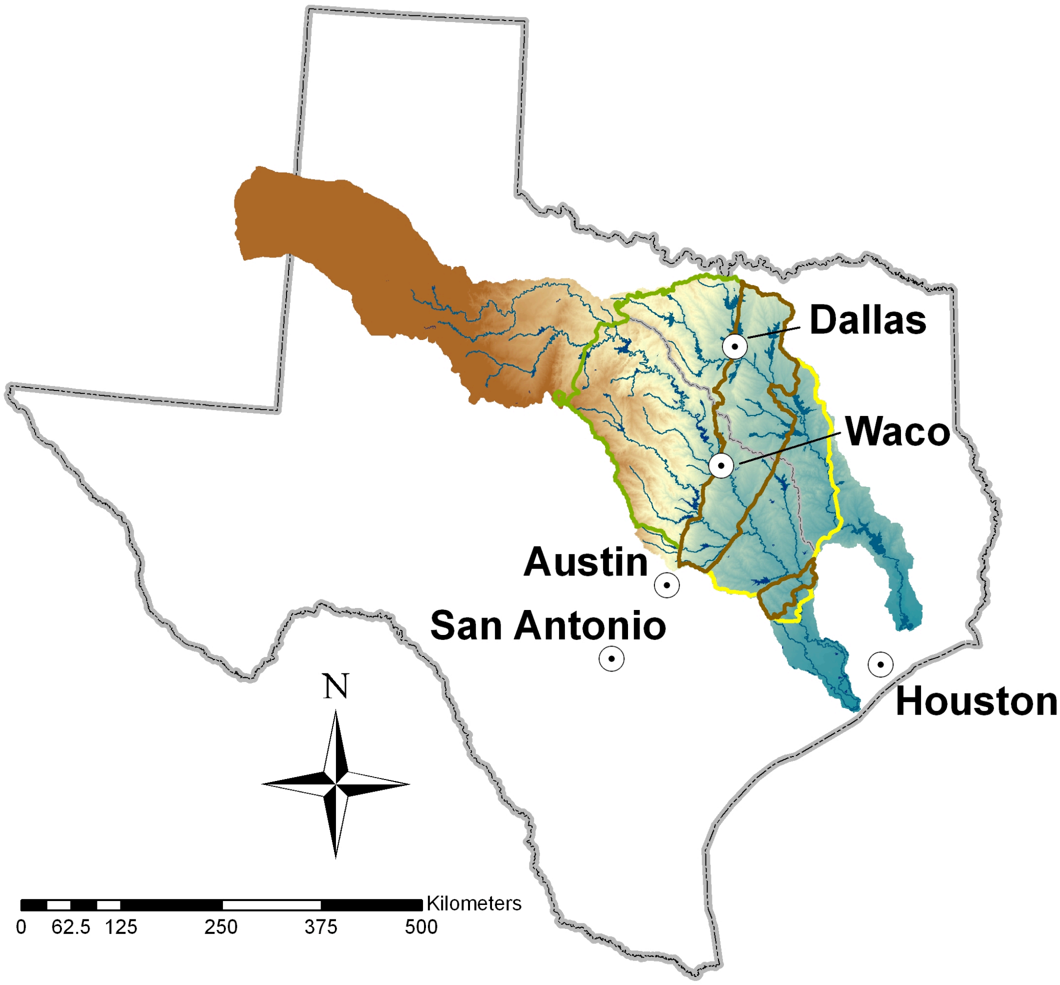


Figure 1. Map showing the study region in the Brazos and Trinity watersheds. Colored lines delineate ecoregion boundaries (green = Cross Timbers, brown = Texas Blackland Prairies, yellow = East Central Texas Plains).

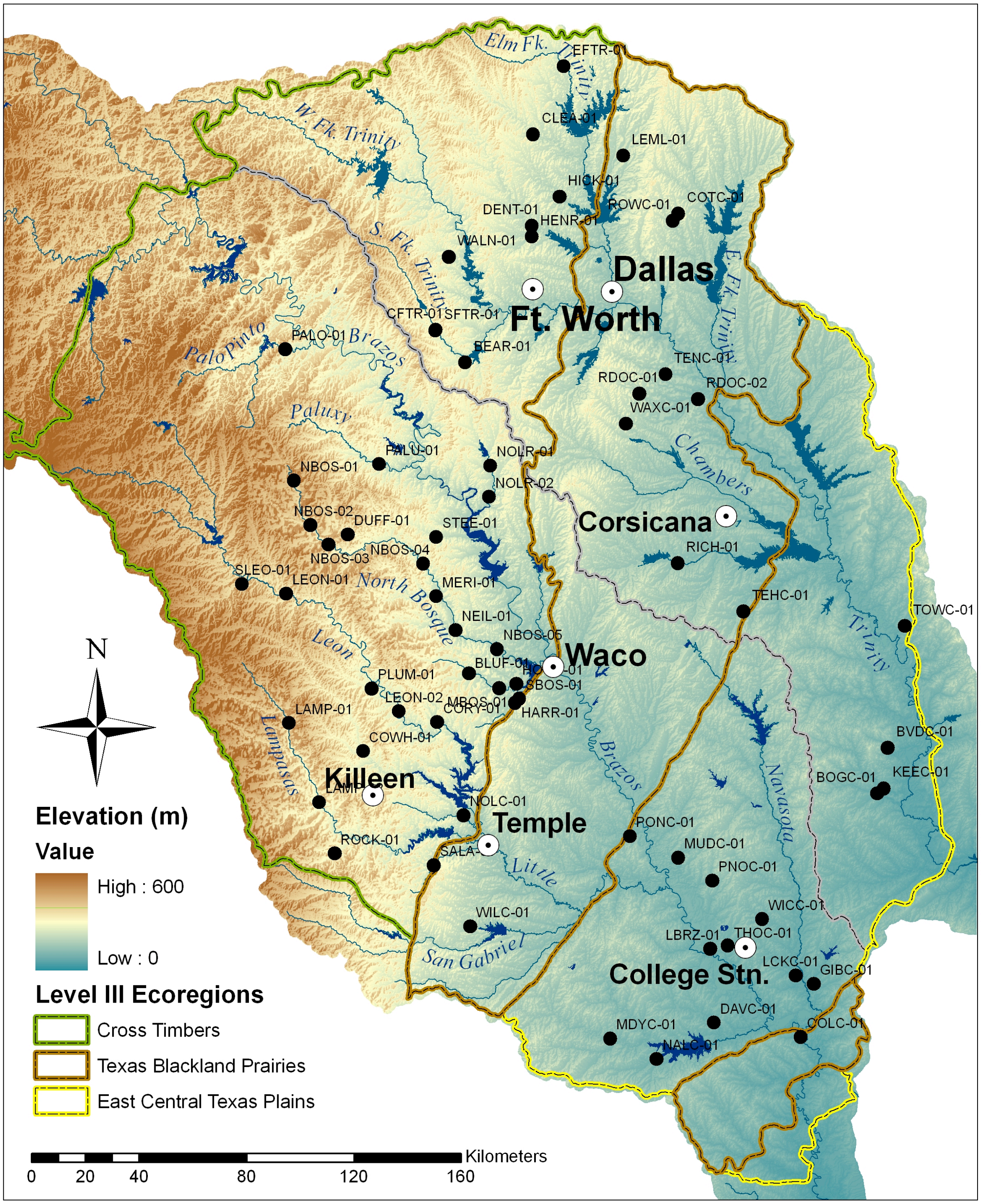


Figure 2. Map showing the 64 study sites and elevation gradients in the watersheds.

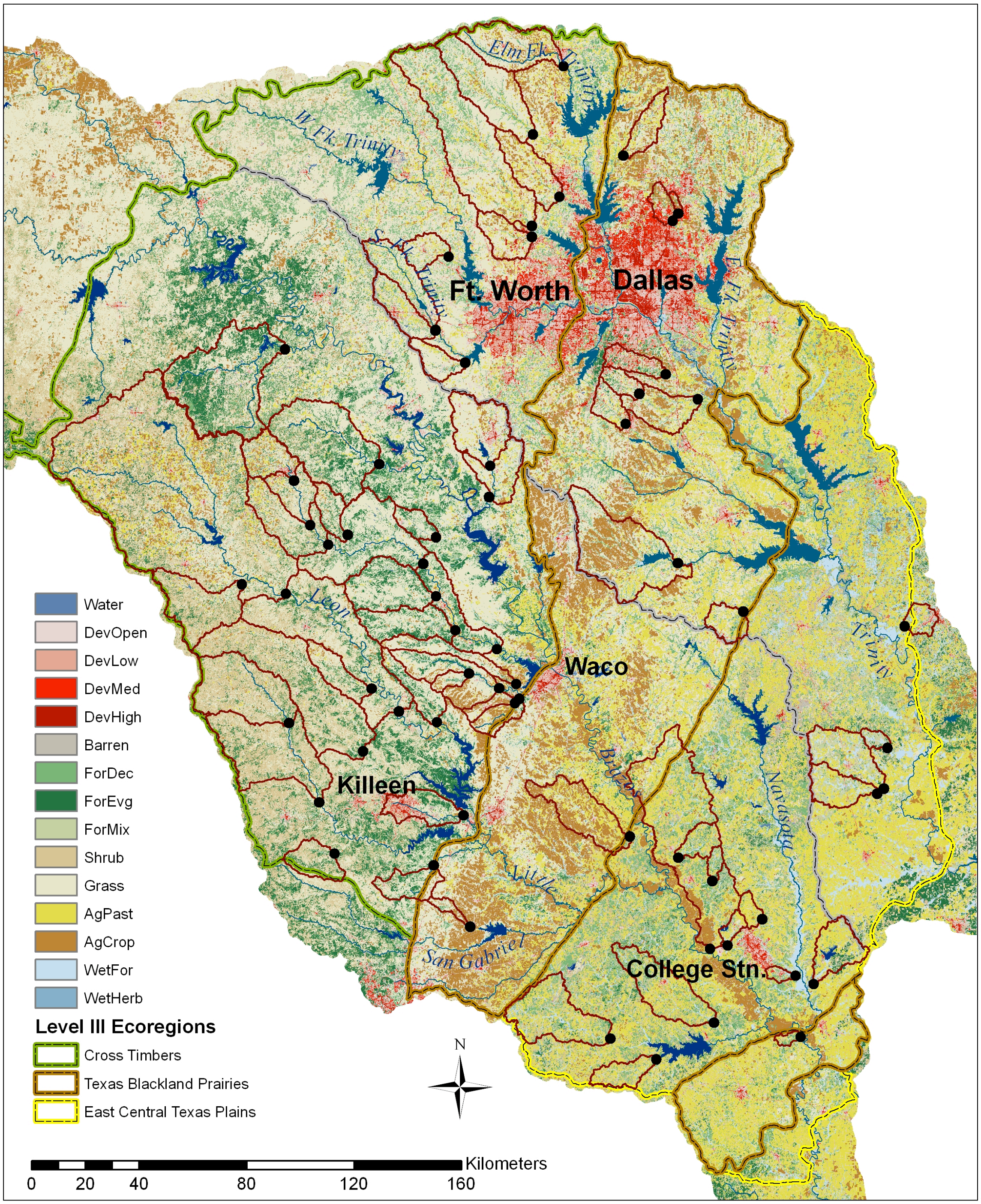


Figure 3. Map showing the 64 study sites and land-cover characteristics of the study region.

Fish sampling

Within each study reach, all available habitats were sampled using a backpack electrofisher (Smith-Root Model LR-24) and seine net (15' x 6' or 6' x 6'). Crews of 3-4 people electrofished each study reach in a single upstream pass with a minimum effort of 900 seconds. The reach was then sampled with a seine net with a minimum of six 10-m hauls. Sampling continued beyond the minimum effort until all habitats were sampled and no new species were captured within the study reach. Collected fishes were identified, separated into juvenile and adult age classes, counted, and either released into the habitat or preserved in 10% buffered formalin for later identification. Numerical abundance of each fish species was recorded for each study reach and sampling event for analyses of patterns in fish community structure.

Local-scale environmental variables

At each study site, we measured 57 habitat variables (Table A1) including substrate composition, instream cover, wetted width, depth, canopy cover, bank slope, riparian buffer width, instantaneous dissolved oxygen, conductivity, and pH on the same dates as fish sampling. We made these measurements at 5 to 6 evenly spaced transects (depending on reach length). Some measurements, such as number of riffles, maximum pool depth, stream sinuosity, and composition of riparian vegetation, were summarized for the entire study reach. Discharge (in ft^3/sec) was also measured along a representative transect within each reach using a portable electromagnetic flow meter (Marsh-McBirney Flo-Mate Model 2000).

Landscape variables

Twenty-three landscape variables describing spatial relationships (coordinates), physical characteristics and topography, land use, and distribution of disturbance points (outfalls and dams) were calculated for each site (Table A2). Watershed boundaries for each sample site were automatically digitized in ArcGIS 9.2 with the ArcHYDRO 9 extension using a 1:24,000 scale digital elevation model (DEM) expressed as a 30 m raster, available from the U. S. Geological Survey. Mean slope and elevation were calculated for each watershed using the digital elevation model. Mean annual precipitation was calculated for each watershed from a polygon coverage of average monthly and annual precipitation for the climatological period 1961-90. This dataset was obtained from USDA-NRCS. Number of wastewater outfalls and cumulative outfall (mgd) were calculated for each watershed based on the TCEQ municipal and industrial wastewater outfall shapefile available from http://www.tceq.state.tx.us/gis/sites.html. The cumulative outfall metric was based on cumulative amount of permitted discharge upstream of a site. Landcover class percentages were calculated for each watershed using National Land Cover Database (NLCD 2001) available from http://www.mrlc.gov/nlcd_multizone_map.php. All GIS analysis was performed with ArcGIS 9.2 (ESRI, Redlands, CA.).

Data Analysis

Relationships between IBI and HQI

Index of biotic integrity (IBI) scores for each site were computed using the fish IBI metrics for assessing Texas streams (Linam et al. 2002). All IBI scores were calculated based on ecoregion specific scoring criteria to account for ecoregional differences in fish communities (Linam et al. 2002). The habitat quality index (HQI) score used by TCEQ (TCEQ 2005) was also calculated for all sites. We examined the relationship between IBI and HQI scores with linear regression. Initially, linear relationships were examined for all sites and all years combined and for all sites separated by year. Subsets of sites according to ecoregion and year were then used to evaluate relationships between IBI and HQI scores that may have been obscured by ecoregional differences in fish communities and in-stream habitat and by temporal differences associated with extreme variation in hydrologic regimes between years. All regression coefficients and significance ($P \leq 0.05$) were calculated using the linear model (lm) procedure in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing).

We also evaluated the relationship between Aquatic Life Use (ALU) designations based on IBI and HQI scores. ALU designations were calculated for each sampling event based on IBI ranges calibrated for each ecoregion and corresponding HQI values (Table 1). We used a confusion matrix approach to determine the percentage of occurrences where ALU designations were in agreement betweeen both indices. This approach also allowed a more specific analysis of what proportion of occurrences within each ALU designation determined by the IBI that HQI agreed. This analysis was initially computed for all years and ecoregions combined, but was also computed for all sites separated by year. Additionally subsets of sites according to ecoregion and year were used to evaluate agreement in ALU designation between the two indices within ecoregions.

Table 1. Ecoregion specific IBI ranges for each aquatic life use designation and corresponding HQI ranges.

	Aquatic Life Use			
Index	**Exceptional**	**High**	**Intermediate**	**Limited**
Index of Biotic Integrity				
ECO 29	≥ 49	41 - 48	35 - 40	< 35
ECO 32	≥ 49	41 - 48	35 - 40	< 35
ECO 33	≥ 52	42 - 51	36 - 41	< 36
Habitat Quality Index	26 - 31	20 - 25	14 - 19	≤ 13

Environmental-Fish community structure linkages

We used non-metric multidimensional scaling (NMS) to estimate relationships between fish community structure (numerical abundance of species) and environmental variables. NMS is a distance based procedure that ordinates study units based on rank dissimilarities (Minchin 1987, Clarke 1993, Legendre and Legendre 1998). Sites that are close to each other in ordination plots have similar species composition and relative proportions of each species whereas sites that are spaced farther apart have dissimilar species composition and/or relative proportions of each species. Because it avoids assumptions of linearity, NMS is considered well suited for analyzing patterns in community structure without some of the problems associated with other commonly used methods such as correspondence analysis (McCune and Grace 2002). We used Bray-Curtis dissimilarity (BCD) as the distance measure, a coefficient that has been repeatedly demonstrated to be robust for ecological community data (Faith and Norris 1989). A two-dimensional solution was used for all analyses as stress values (a measure of agreement between BCDs and the configuration of the ordination) were relatively low and did not substantially decrease when additional axes were included in ordinations. Before running ordinations on the data sets, fish species occurring at only one site within an ecoregion were excluded, and abundances were log transformed. Variables from the landscape, environmental and IBI matrices with high skewness (> 1) were also log transformed. Ordinations were performed in PC-Ord version 5.2 (MjM Software, Gleneden Beach, OR, U.S.A.).

We performed ordinations on all sample sites within each year (n = 64, 2006 and 2008; n = 46, 2007) and three subsets of sites according to ecoregion (n = 38 for Eco 29; n = 11 for Eco 32; n = 15 for Eco 33) for 2006 and 2008 datasets. Additionally, we performed ordinations on all years combined within ecoregions for sites with three years of data (n = 28 for ECO 29; n = 6 for ECO 32; n = 12 for ECO 33). Ordinations of all sites combined within each year were used to assess ecoregional differences in fish community structure. Site symbols were coded based on respective ecoregions and separation of sites grouped by ecoregion was assessed visually in ordination space. Subsets of sites were used to examine potential relationships between community structure and environmental variables that may have been obscured by ecoregional differences in fish community structure and/or environmental variables. Ordinations of sites with three years of data within each ecoregion were used to assess temporal changes in fish community structure associated with contrasting hydrological conditions and its effect on fish community structure-environmental relationships.

We used rotational vector fitting to relate environmental variables, HQI and IBI metrics (Tables A3 and A4) to gradients in fish community structure quantified by the NMS ordinations (Faith and Norris 1989). Vector fitting was used to find the direction of the maximum correlation for each environmental variable. Vectors represent the direction and magnitude of the correlation between environmental variables and fish community structure. For example, the direction of a vector in a plot implies that sites in that direction have a higher value for the environmental variable and that changes in fish community structure (quantified by the NMS ordination axes) are correlated with increasing values for the environmental value. Vector fitting was performed on all within-ecoregion ordinations. Significance ($P \leq 0.05$) of each environmental vector was estimated using 1,000 random permutations of the data. Vector fitting was performed using the ECODIST package in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing).

RESULTS

We collected a total of 60 fish species over the study period, with richness per site ranging from 6 to 26 species (Table 2). While diversity at some sites was similar across the three years, many sites showed considerable variability between years. IBI scores ranged from 28 to 56 with a median score of 44, and scores were generally higher for sites in the Cross Timbers ecoregion. Streams in the Cross Timbers ecoregion had rockier substrates and more riffles, while sites in the East Central Texas Plains and Blackland Prairies had higher proportions of mud and silt substrates and more woody debris. HQI scores ranged from 10.5 to 27, with higher average scores in the Cross Timbers stream sites. IBI and HQI scores varied between years for all sites (Table 2). Inter-annual variation in scores was generally more pronounced for sites sampled in 2007, with some having higher scores and others having lower scores during the wet year.

Table 2. Abundance (N), species richness (S), IBI scores, and HQI scores for the stream sites surveyed for this study. TCEQ SWQM station IDs are given in parentheses below the study site abbreviations.

Site	**Site Abbrev.**	**Eco-region**	**Basin**	**Year**	**N**	**S**	**IBI**	**HQI**
Bear Creek	BEAR-01	29	Trinity	2006	512	14	46	21.5
	(13624)			2007	359	11	40	24
				2008	849	19	56	25
Beaver Dam	BVDC-01	33	Trinity	2006	118	12	41	18
Creek	(20381)			2007	102	14	39	16.5
				2008	129	13	37	17
Bluff Creek	BLUF-01	29	Brazos	2006	922	10	48	17.5
	(11832)			2007	178	9	36	25
				2008	629	10	44	27
Boggy Creek	BOGC-01	33	Trinity	2006	142	10	37	14.5
	(20383)			2008	200	17	51	21
Clear Creek	CLEA-01	29	Trinity	2006	234	9	36	13.5
	(10859)			2007	234	19	44	20
				2008	450	17	42	22
Clear Fork	CFTR-01	29	Trinity	2006	403	20	50	18.5
Trinity	(17445)			2007	270	15	48	17
				2008	415	20	54	18.5
Coles Creek	COLC-01	32	Brazos	2006	577	16	54	15
	(20389)			2007	238	19	52	18.5
				2008	144	15	44	18.5
Coryell Creek	CORY-01	29	Brazos	2006	790	13	46	18
	(11804)			2007	113	12	42	20
				2008	558	13	46	18.5
Cottonwood	COTC-01	32	Trinity	2006	143	6	34	18.5
Creek	(20377)			2007	118	10	32	16.5
				2008	184	9	34	20.5
Cowhouse	COWH-01	29	Brazos	2006	796	12	48	21
Creek	(11805)			2007	443	13	46	20
				2008	818	15	52	20.5
Davidson	DAVC-01	33	Brazos	2006	199	16	47	18
Creek	(20388)			2008	69	15	35	19
Denton Creek	DENT-01	29	Trinity	2006	1364	25	52	15.5
	(20391)			2008	407	17	50	18.5
Duffau Creek	DUFF-01	29	Brazos	2006	316	16	48	14.5
	(20392)			2007	210	14	42	24
				2008	961	14	52	20

Table 2 Continued. Abundance (N), species richness (S), IBI scores, and HQI scores for the stream sites surveyed for this study. TCEQ SWQM station IDs are given in parentheses below the study site abbreviations.

Site	Site Abbrev.	Eco-region	Basin	Year	N	S	IBI	HQI
Elm Fork Trinity	EFTR-01 (11030)	29	Trinity	2006	1064	20	50	20.5
				2007	257	12	44	18.5
				2008	566	18	48	22
Gibbons Creek	GIBC-01 (11756)	33	Brazos	2006	120	15	45	14
				2007	204	22	45	19
				2008	110	14	43	18
Harris Creek	HARR-01 (20393)	29	Brazos	2006	1551	14	48	17.5
				2007	155	15	40	21
				2008	477	14	46	23
Henrietta Creek	HENR-01 (16825)	29	Trinity	2006	465	19	50	18.5
				2008	556	18	54	19.5
Hickory Creek	HICK-01 (13687)	29	Trinity	2006	411	22	52	14.5
				2008	360	15	48	19
Hog Creek	HOG-01 (20394)	29	Brazos	2006	574	10	48	20.5
				2007	305	13	46	19.5
				2008	1793	16	50	22
Keechi Creek	KEEC-01 (20382)	33	Trinity	2006	42	8	35	16
				2008	69	20	43	16
Lampasas River (site 1)	LAMP-01 (15770)	29	Brazos	2006	748	15	48	25
				2007	220	13	32	19
				2008	2109	15	48	19.5
Lampasas River (site 2)	LAMP-02 (16404)	29	Brazos	2006	940	15	52	21.5
				2007	220	17	46	23
				2008	967	20	56	19
Lick Creek	LCKC-01 (20387)	33	Brazos	2006	79	13	35	14.5
				2007	160	14	43	18.5
				2008	93	10	33	16.5
Little Brazos River	LBRZ-01 (11591)	33	Brazos	2006	329	12	35	19
				2007	473	20	45	20.5
				2008	192	15	41	23.5
Little Elm Creek	LELM-01 (13617)	32	Trinity	2006	365	13	34	10.5
				2008	65	15	34	14.5
Leon River (site 1)	LEON-01 (11933)	29	Brazos	2006	629	15	46	18
				2008	1172	13	44	19
Leon River (site 2)	LEON-02 (17501)	29	Brazos	2006	1230	15	48	21.5
				2008	1194	14	46	22

Table 2 Continued. Abundance (N), species richness (S), IBI scores, and HQI scores for the stream sites surveyed for this study. TCEQ SWQM station IDs are given in parentheses below the study site abbreviations.

Site	Site Abbrev.	Eco-region	Basin	Year	N	S	IBI	HQI
Middle Bosque River	MBOS-01 (17612)	29	Brazos	2006	994	15	56	18.5
				2007	251	14	44	21
				2008	478	14	52	23.5
Meridian Creek	MERI-01 (14908)	29	Brazos	2006	199	13	48	20
				2007	49	6	36	25
				2008	520	15	52	21
Middle Yegua Creek	MDYC-01 (11840)	33	Brazos	2006	91	10	35	12
				2007	107	14	41	14
				2008	442	12	43	10.5
Mud Creek	MUDC-01 (16402)	33	Brazos	2006	67	9	39	17.5
				2007	150	11	43	17.5
				2008	50	14	37	17
Nails Creek	NALC-01 (16885)	33	Brazos	2006	417	20	45	18.5
				2007	209	18	45	20.5
				2008	175	13	39	18
North Bosque River (site 1)	NBOS-01 (11963)	29	Brazos	2006	231	8	42	19
				2007	259	10	44	21
				2008	358	11	44	23
North Bosque River (site 2)	NBOS-02 (15123)	29	Brazos	2006	1615	17	48	18.5
				2007	130	12	32	22.5
				2008	763	15	40	19
North Bosque River (site 3)	NBOS-03 (11961)	29	Brazos	2006	907	11	52	21
				2007	406	12	48	22.5
				2008	1259	15	48	21
North Bosque River (site 4)	NBOS-04 (11958)	29	Brazos	2006	462	16	52	21.5
				2008	2574	16	52	23
North Bosque River (site 5)	NBOS-05 (11951)	29	Brazos	2006	1039	16	52	23
				2008	1178	20	52	22.5
Neils Creek	NEIL-01 (11826)	29	Brazos	2006	728	12	40	18.5
				2007	228	10	40	20
				2008	567	14	52	22.5
Nolan Creek	NOLC-01 (11904)	29	Brazos	2006	257	14	44	17
				2007	305	13	42	18.5
				2008	495	13	40	19
Nolan River (site 1)	NOLR-01 (11972)	29	Brazos	2006	468	15	52	20.5
				2007	176	11	46	20.5
				2008	577	14	46	20.5

Table 2 Continued. Abundance (N), species richness (S), IBI scores, and HQI scores for the stream sites surveyed for this study. TCEQ SWQM station IDs are given in parentheses below the study site abbreviations.

Site	Site Abbrev.	Eco-region	Basin	Year	N	S	IBI	HQI
Nolan River (site 2)	NOLR-02 (11967)	29	Brazos	2006	1337	20	46	21
				2007	782	21	46	21.5
				2008	876	17	48	23
Palo Pinto Creek	PALO-01 (16408)	29	Brazos	2006	269	13	36	21
				2008	189	16	46	23
Paluxy River	PALU-01 (14481)	29	Brazos	2006	666	16	52	20
				2007	302	13	46	22
				2008	950	17	50	22
Plum Creek	PLUM-01 (11806)	29	Brazos	2006	684	14	54	17.5
				2007	91	16	44	24
				2008	162	12	46	22
Pin Oak Creek	PNOC-01 (20385)	33	Brazos	2006	160	9	39	18
				2007	170	14	43	22.5
				2008	83	13	37	17
Pond Creek	PONC-01 (20384)	33	Brazos	2006	1065	17	41	21
				2007	519	10	41	19.5
				2008	690	10	41	16.5
Red Oak Creek (site 1)	RDOC-01 (20379)	32	Trinity	2006	338	12	40	18.5
				2007	206	15	42	19.5
				2008	176	13	36	21.5
Red Oak Creek (site 2)	RDOC-02 (17506)	32	Trinity	2006	74	8	32	11
				2007	127	14	38	15.5
				2008	124	9	36	11.5
Richland Creek	RICH-01 (18344)	32	Trinity	2006	409	12	46	15.5
				2008	32	11	34	16.5
Rocky Creek	ROCK-01 (18332)	29	Brazos	2006	741	10	46	23.5
				2007	242	10	46	23
				2008	554	10	42	23
Rowlett Creek	ROWC-01 (20378)	32	Trinity	2006	27	6	30	16.5
				2008	40	6	28	16
Salado Creek	SALA-01 (12053)	29	Brazos	2006	1240	11	44	19.5
				2007	646	14	44	22.5
				2008	1089	15	48	21
South Bosque River	SBOS-01 (17228)	29	Brazos	2006	2103	24	56	17
				2007	663	26	54	18
				2008	444	22	54	17

Table 2 Continued. Abundance (N), species richness (S), IBI scores, and HQI scores for the stream sites surveyed for this study. TCEQ SWQM station IDs are given in parentheses below the study site abbreviations.

Site	**Site Abbrev.**	**Eco-region**	**Basin**	**Year**	**N**	**S**	**IBI**	**HQI**
South Fork Trinity	SFTR-01 (17454)	29	Trinity	2006	203	16	42	16
				2007	324	13	42	21
				2008	204	13	42	19.5
South Leon River	SLEO-01 (20390)	29	Brazos	2006	1547	12	44	16.5
				2007	320	15	42	19.5
				2008	1602	15	48	19
Steele Creek	STEE-01 (11835)	29	Brazos	2006	510	10	50	17
				2008	558	14	50	22.5
Tehuacana Creek	TEHC-01 (18572)	32	Trinity	2006	195	12	38	16.5
				2008	208	13	46	19
Tenmile Creek	TENC-01 (17840)	32	Trinity	2006	564	8	34	21
				2008	346	9	34	20.5
Thompson Creek	THOC-01 (16397)	33	Brazos	2006	187	6	35	12
				2007	239	15	47	14
				2008	9	6	35	16.5
Town Creek	TOWC-01 (10706)	33	Trinity	2006	125	11	41	14.5
				2007	136	19	39	15.5
				2008	166	11	41	16.5
Walnut Creek	WALN-01 (10853)	29	Trinity	2006	492	14	44	16
				2008	409	15	42	18
Waxahachie Creek	WAXC-01 (20380)	32	Trinity	2006	125	13	44	17.5
				2007	224	16	48	22
				2008	35	10	36	19.5
Wickson Creek	WICC-01 (20386)	33	Brazos	2006	73	7	37	17
				2007	135	16	45	17
				2008	55	10	37	17
Willis Creek	WILC-01 (11573)	32	Brazos	2006	512	10	44	17
				2007	43	10	38	18
				2008	268	7	38	14.5

Relationships between IBI and HQI

There was a significant but weak relationship between IBI scores and HQI scores for all sampling events combined (years = 2006, 2007, 2008 and Ecoregion = 29, 32, and 33) (Figure 4). Examining differences in this relationship between years showed significant weak relationships for dry years (2006 and 2008) (Figures 5 and 7). There was no relationship between IBI scores and HQI scores in 2007, one of the wettest years on record (Figure 6). While these differences are interesting, examination of the distribution of sites by ecoregion within each scatterplot reveals that the pattern may be driven more by ecoregional differences in IBI and HQI than a relationship between IBI and HQI. Boxplots of HQI and IBI scores by ecoregion confirm ecoregional differences in IBI and HQI scores (Figure 8). In all three years, the Cross Timbers had higher overall HQI scores than the TX Blackland Prairie and East Central TX Plains ecoregions. Cross Timbers ecoregion IBI scores were considerably higher than TX Blackland Prairie and East Central Texas Plains ecoregion in 2006 and 2008 but not 2007. After separating the dataset by ecoregion and year, there were no significant relationships between IBI and HQI scores (Figures 9-17).

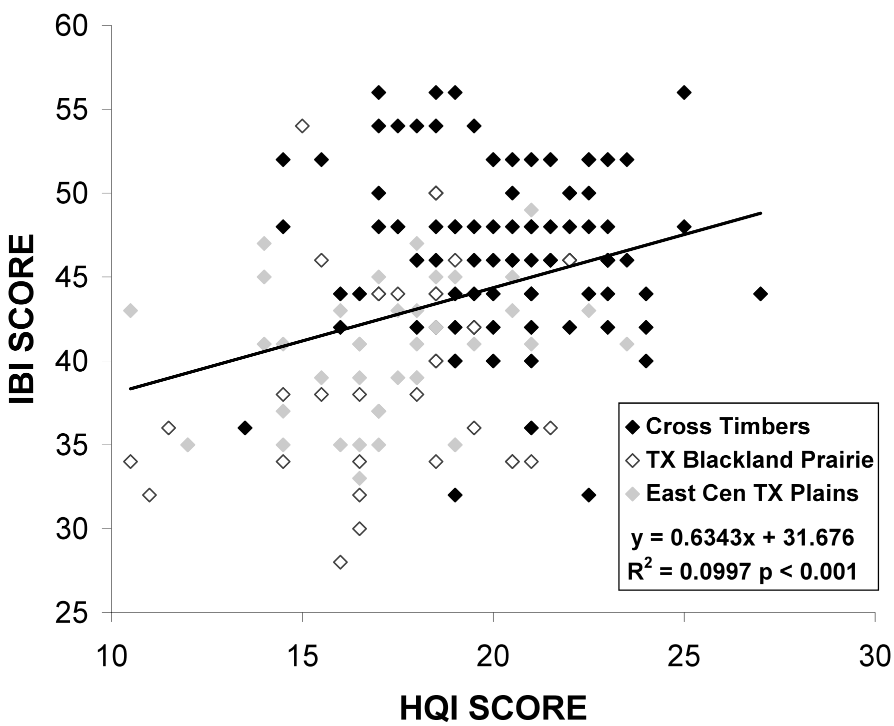


Figure 4. Linear trend between HQI and IBI scores for 174 sampling events.

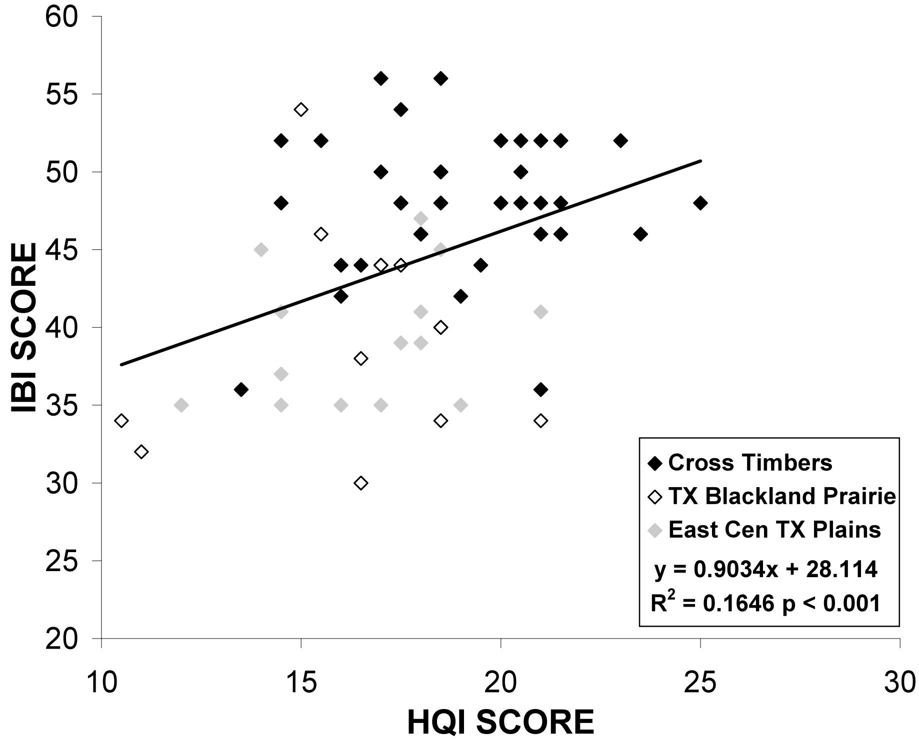


Figure 5. Linear trend between HQI and IBI scores for 64 sampling events conducted in 2006.

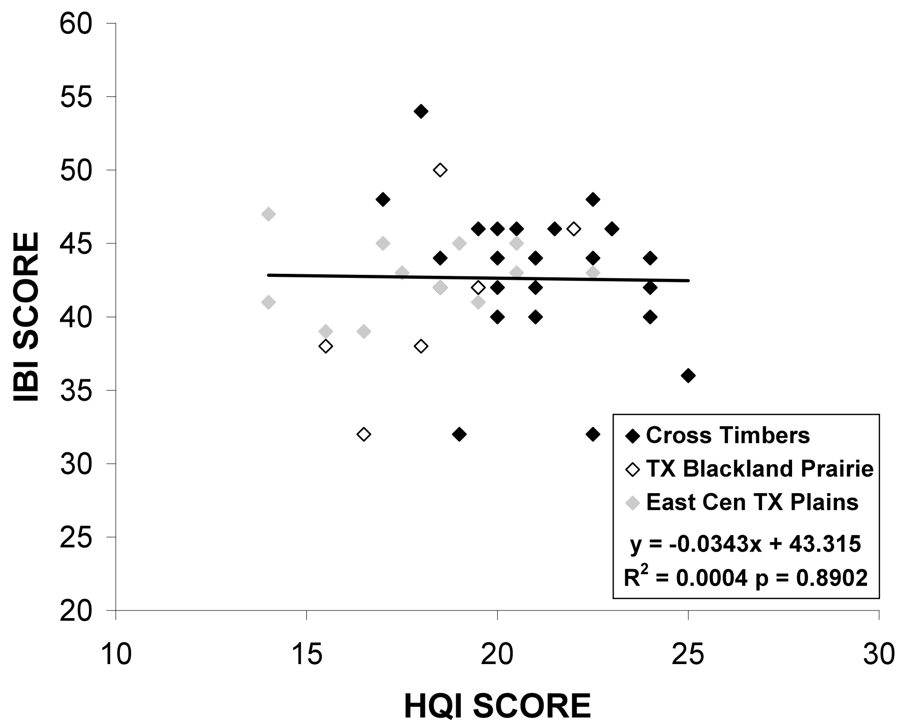


Figure 6. Linear trend between HQI and IBI scores for 46 sampling events conducted in 2007.

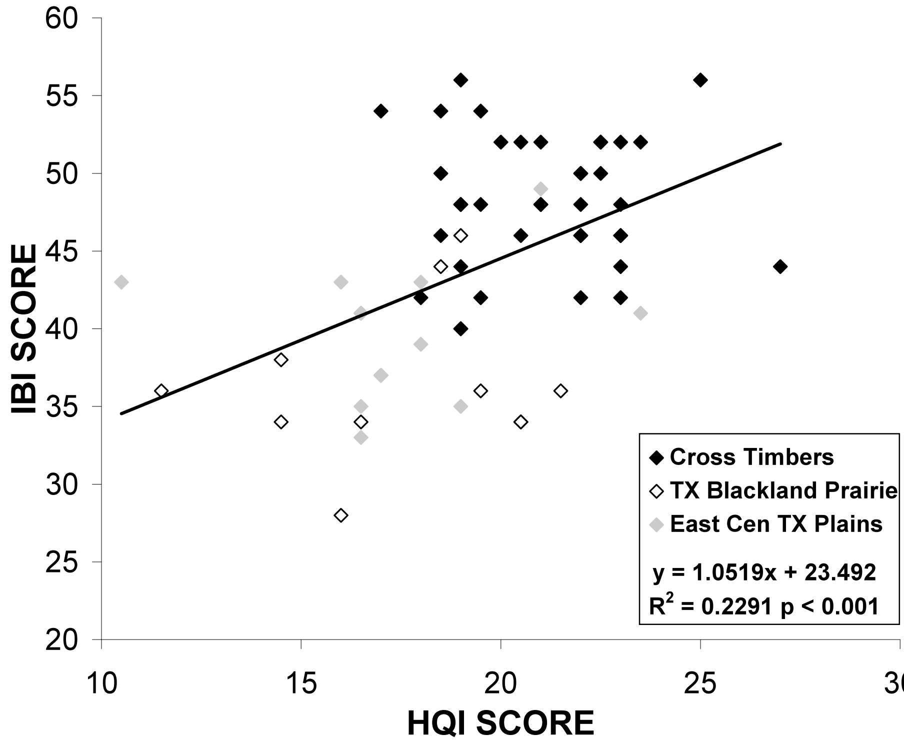


Figure 7. Linear trend between HQI and IBI scores for 64 sampling events conducted in 2008.

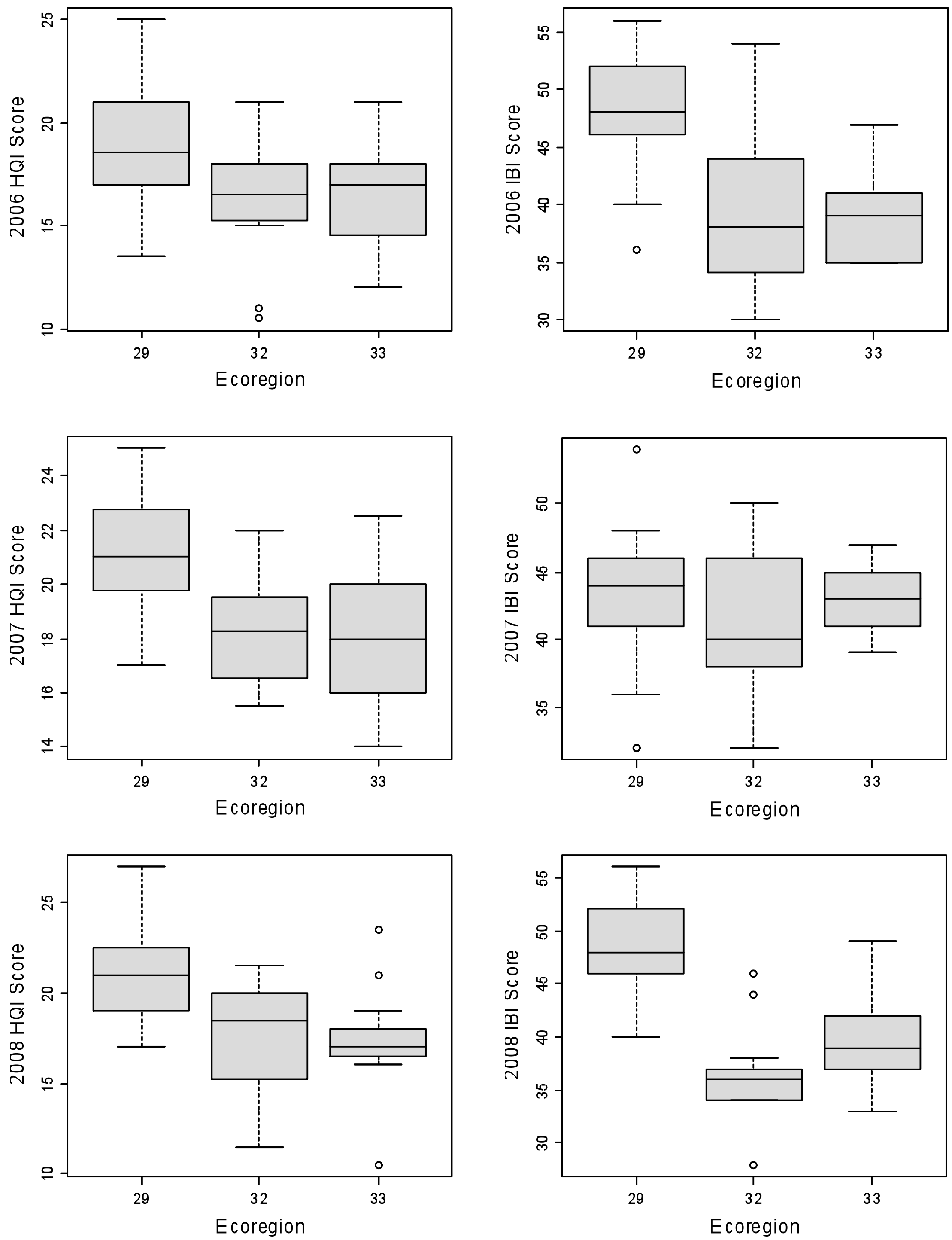


Figure 8. Distribution of HQI and IBI scores by Ecoregion for each year. 29 = Cross Timbers, 32 = TX Blackland Prairie, 33 = East Central TX Plains.

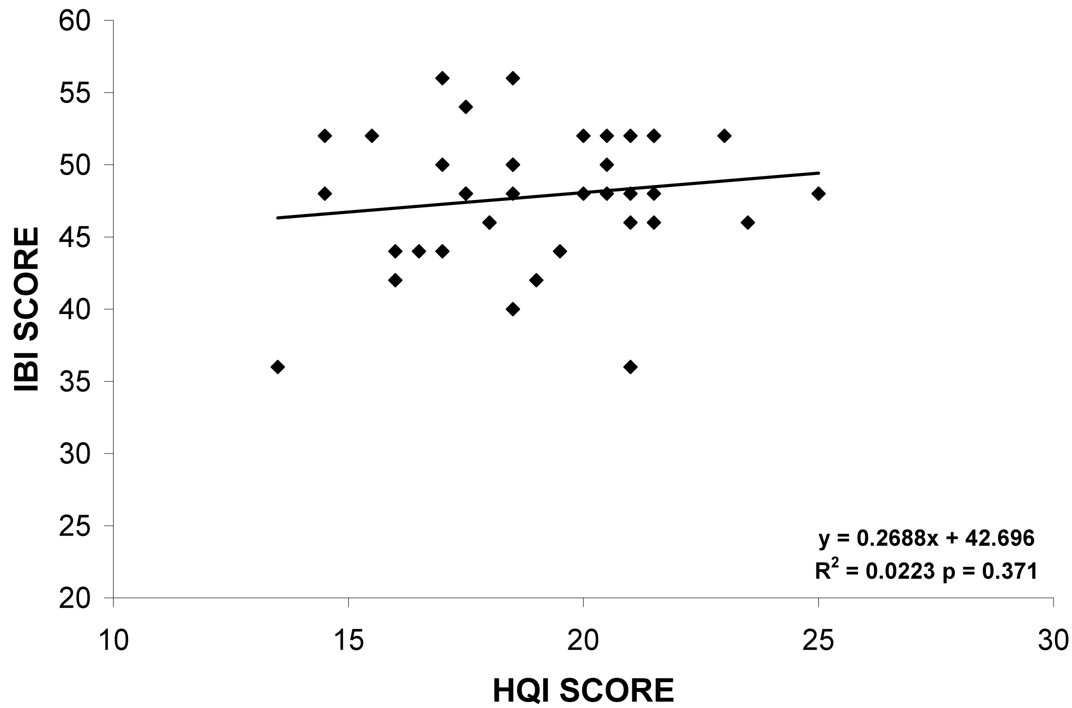


Figure 9. Linear trend between HQI and IBI scores for 38 sampling events conducted in the Cross Timbers ecoregion during 2006.

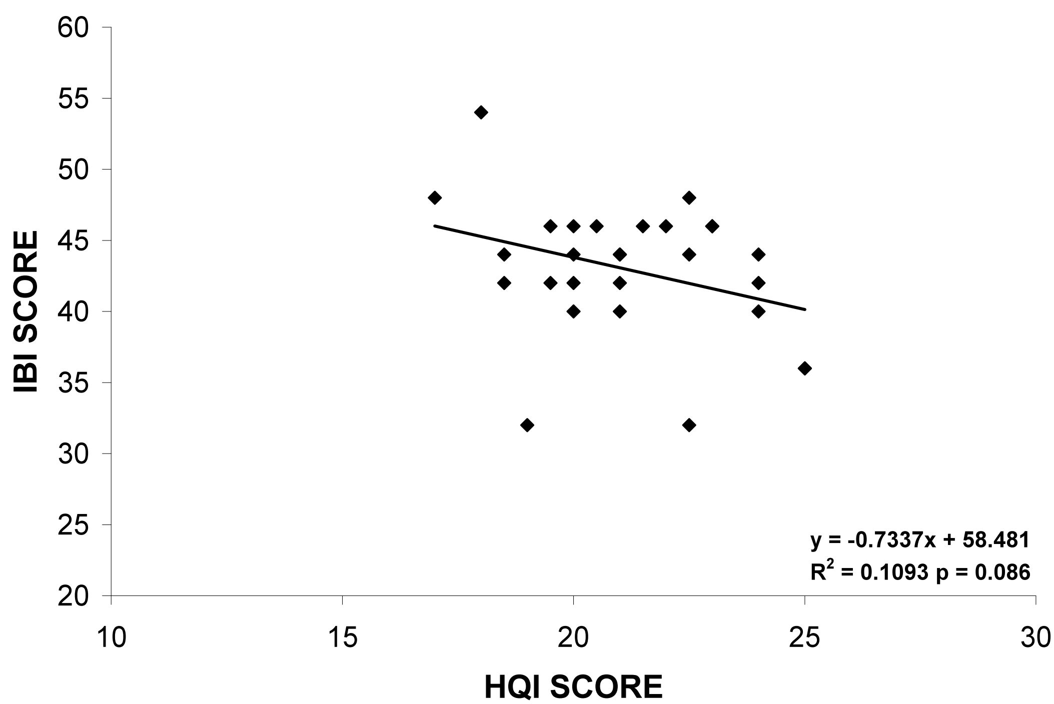


Figure 10. Linear trend between HQI and IBI scores for 28 sampling events conducted in the Cross Timbers ecoregion during 2007.

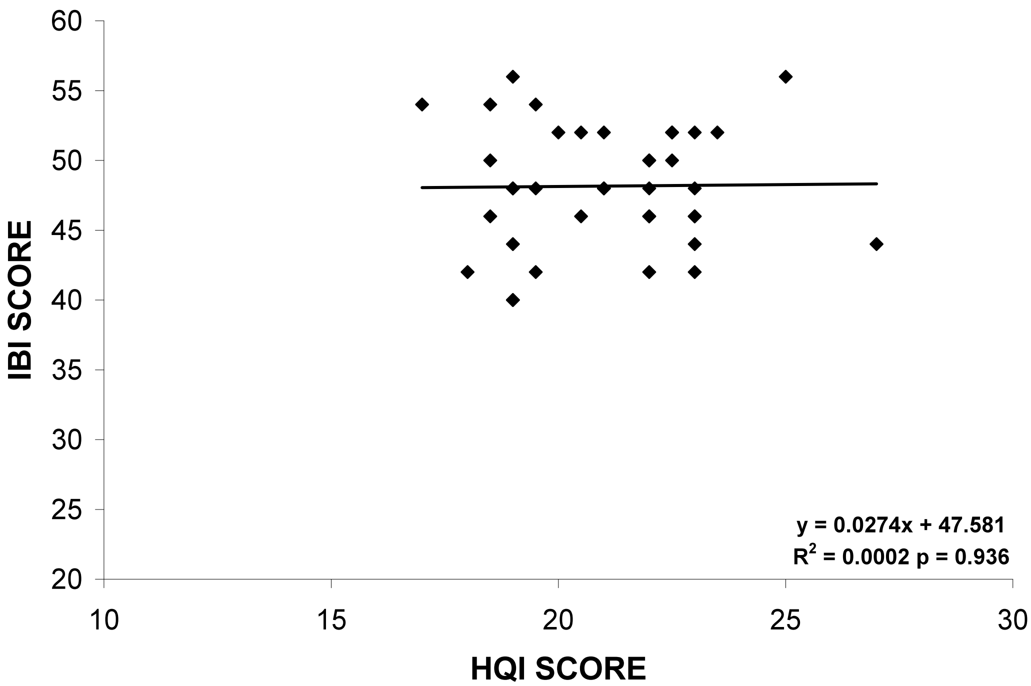


Figure 11. Linear trend between HQI and IBI scores for 38 sampling events conducted in the Cross Timbers ecoregion during 2008.

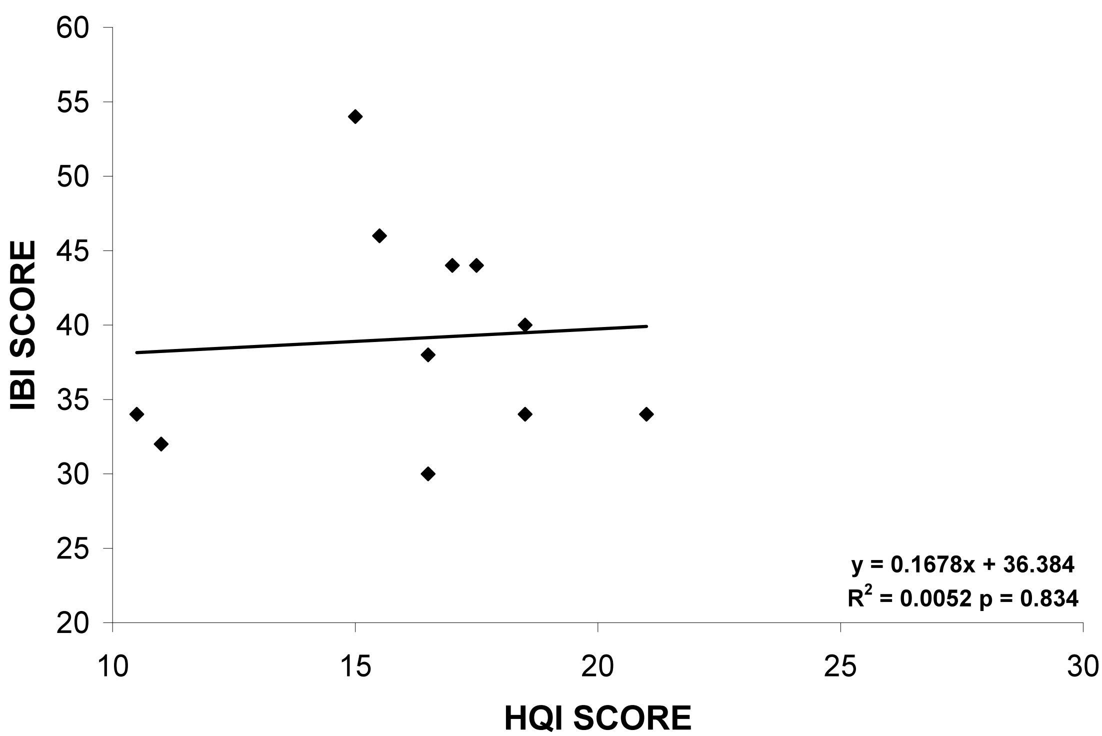


Figure 12. Linear trend between HQI and IBI scores for 11 sampling events conducted in the Blackland Prairie ecoregion during 2006.

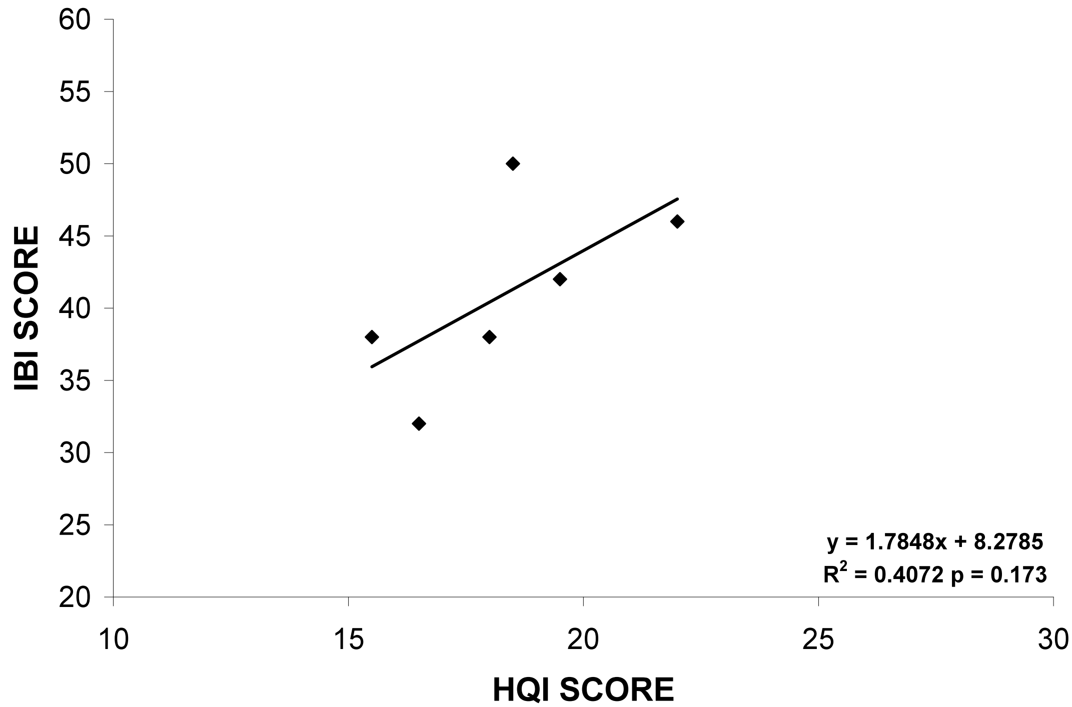


Figure 13. Linear trend between HQI and IBI scores for 6 sampling events conducted in the Blackland Prairie ecoregion during 2007.

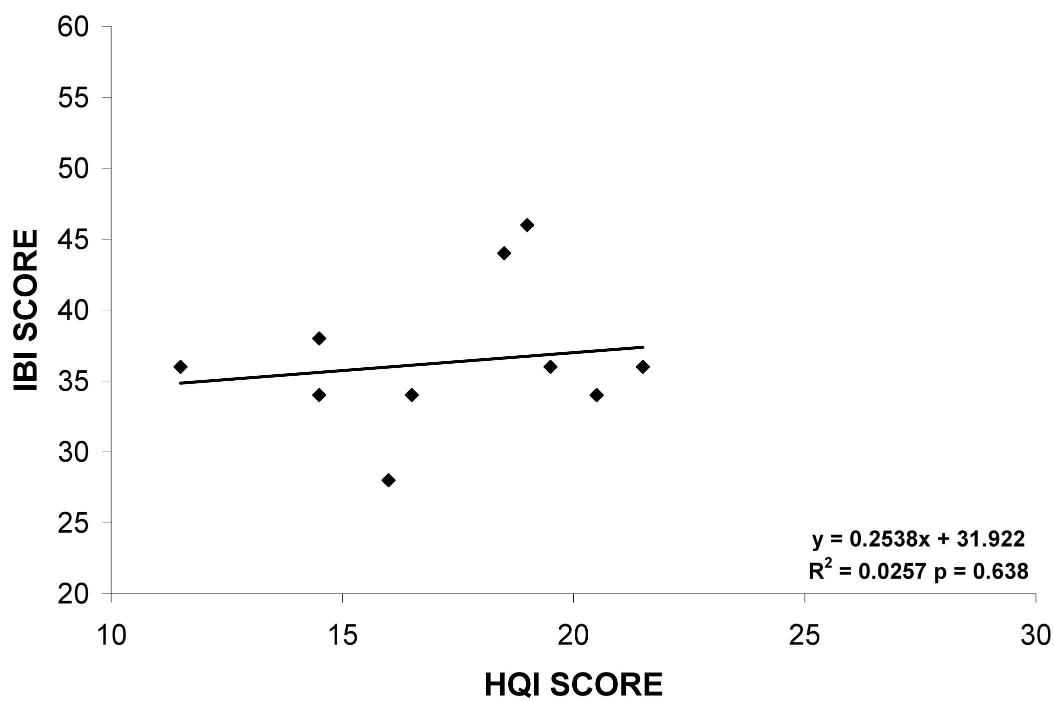


Figure 14. Linear trend between HQI and IBI scores for 11 sampling events conducted in the Blackland Prairie ecoregion during 2008.

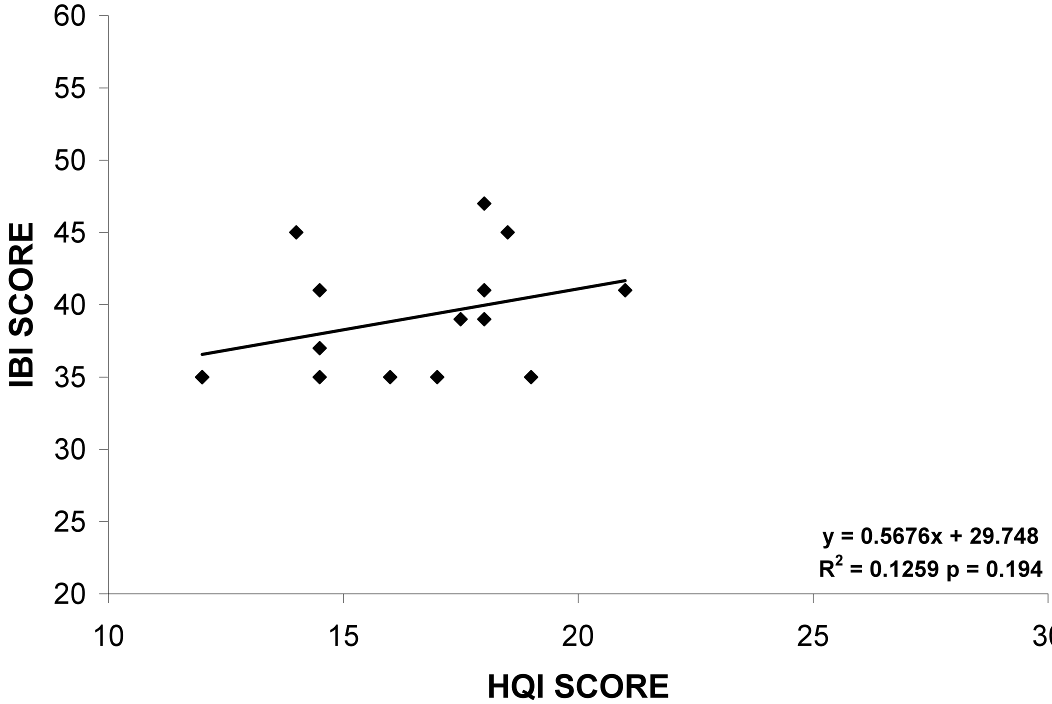


Figure 15. Linear trend between HQI and IBI scores for 15 sampling events conducted in the East Central Texas Plains ecoregion during 2006.

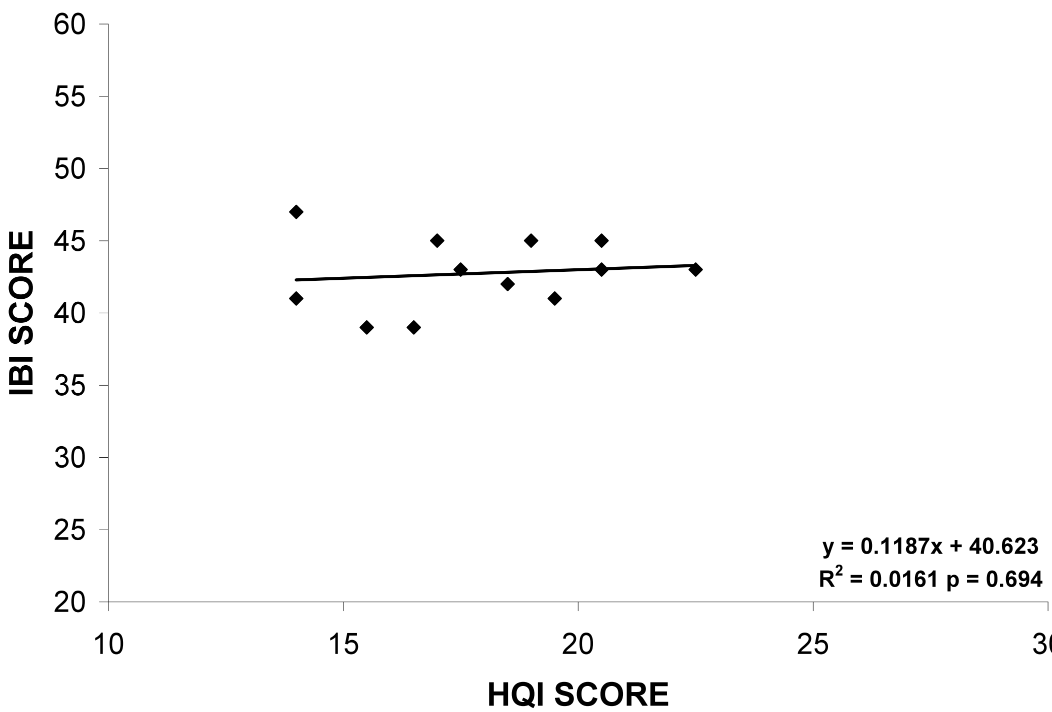


Figure 16. Linear trend between HQI and IBI scores for 12 sampling events conducted in the East Central Texas Plains ecoregion during 2007.

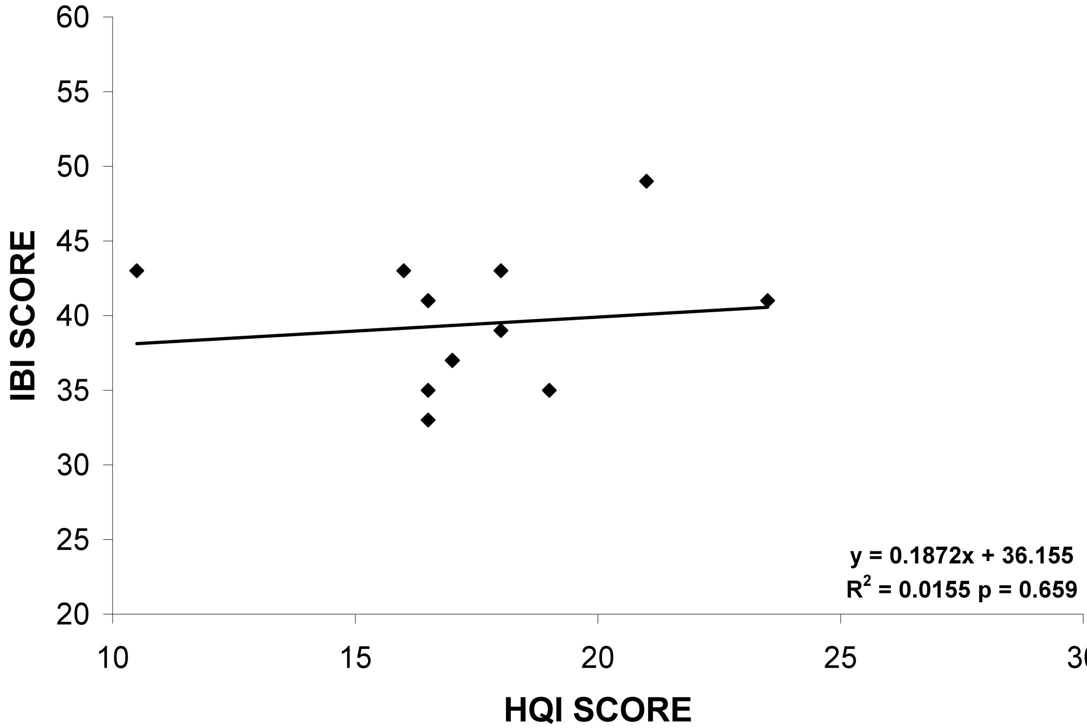


Figure 17. Linear trend between HQI and IBI scores for 15 sampling events conducted in the East Central Texas Plains ecoregion during 2008.

Relationships between IBI and HQI Aquatic Life Use designations

Forty percent of sampling events during the three year study had aquatic life use (ALU) designations assigned based on IBI scores that corresponded with use designations based on HQI values (Table 3). The best agreement in ALU assignment between the two indices occurred for High and Intermediate designations (49 and 69% respectively). Thirty-four sampling events met the criteria for exceptional ALU designation based on IBI scores, but no sampling event ever scored an exceptional rating based on HQI scores (Table 3). IBI and HQI based ALU designations were in agreement for 31, 54 and 38 % of the sampling sites in 2006, 2007, and 2008, respectively (Table 3). Within a given year, higher percent-agreement values were primarily associated with high and intermediate ALU designations, whereas exceptional and limited ALU designations generally had lower and higher use designations, respectively, based on HQI values. One exception was in 2006 when limited ALU designations had a 36 % agreement rate (Table 3).

Table 3. Aquatic Life Use values for study sites (all ecoregions, all years) based on IBI and HQI. % Agreement represents the percentage of occurrences that HQI ALU designations agreed with ALU designation determined by IBI. Total % Agreement is the percent of occurrences where ALU designations determined by IBI and HQI were in agreement across all sites in all use designations. Shaded cells represent those where all occurrences would lie if there were perfect agreement between the two indices.

	Aquatic Life Use - IBI			
Aquatic Life Use-HQI	**Exceptional**	**High**	**Intermediate**	**Limited**
All years				
Exceptional		1		
High	18	40	9	4
Intermediate	16	40	25	14
Limited		1	2	4
% Agreement	0	49	69	18
Tot. % Agreement	40			
2006				
Exceptional				
High	7	8	2	1
Intermediate	9	18	8	6
Limited			1	4
% Agreement	0	31	73	36
Tot. % Agreement	31			
2007				
Exceptional				
High		19	5	1
Intermediate	2	11	6	2
Limited				
% Agreement	0	63	55	0
Tot. % Agreement	54			
2008				
Exceptional		1		
High	11	13	2	2
Intermediate	5	11	11	6
Limited		1	1	
% Agreement	0	50	79	0
Tot. % Agreement	38			

Table 4. Aquatic Life Use values for Cross Timbers (ECO 29) study sites based on IBI and HQI. % Agreement represents the percentage of occurrences that HQI ALU designations agreed with ALU designation determined by IBI. Total % Agreement is the percent of occurrences where ALU designations determined by IBI and HQI were in agreement across all sites in all use designations. Shaded cells represent those where all occurrences would lie if there were perfect agreement between the two indices.

	Aquatic Life Use - IBI			
Aquatic Life Use-HQI	**Exceptional**	**High**	**Intermediate**	**Limited**
ECO 29				
Exceptional				
High	7	8	1	
Intermediate	8	12	1	
Limited			1	
% Agreement	0	40	33	NA
Tot. % Agreement	24			
ECO 29				
Exceptional				
High		15	5	1
Intermediate	1	5		1
Limited				
% Agreement	0	75	0	0
Tot. % Agreement	54			
ECO 29				
Exceptional		1		
High	11	12		
Intermediate	5	7	2	
Limited				
% Agreement	0	60	100	NA
Tot. % Agreement	37			

Within the Cross Timbers ecoregion, 24, 54 and 37 % of sites' ALU designations based on IBI and HQI scores were in agreement during 2006, 2007 and 2008 (Table 4). In all three years, sites that met criteria for exceptional ALU based on the IBI always had HQI scores that did not meet exceptional ALU criteria. Higher percent agreement values were observed for High and Intermediate ALU designations (Table 4). Similar results were observed for the Texas Blackland Praire ecoregion in which percent agreement values were 36, 50 and 18 % for the 2006, 2007 and 2008 sampling seasons, respectively (Table 5). Higher % agreement values were generally observed for High and Intermediate ALU designations with one exception. In 2006, there was a

40 % agreement rate for Limited ALU designations (Table 5). East Central Texas Plains (ECO 33) had higher overall percent agreement rates than the other two ecoregions with values for all years around 50% (Table 6). Similar to the first two ecoregions, higher percent agreement rates were observed for High and Intermediate ALU designations, although it should be noted that no site met criteria for Exceptional ALU based on either index in any year.

Table 5. Aquatic Life Use values for Texas Blackland Prairie (ECO 32) study sites based on IBI and HQI. % Agreement represents the percentage of occurrences that HQI ALU designations agreed with ALU designation determined by IBI. Total % Agreement is the percent of occurrences where ALU designations determined by IBI and HQI were in agreement across all sites in all use designations. Shaded cells represent those where all occurrences would lie if there were perfect agreement between the two indices.

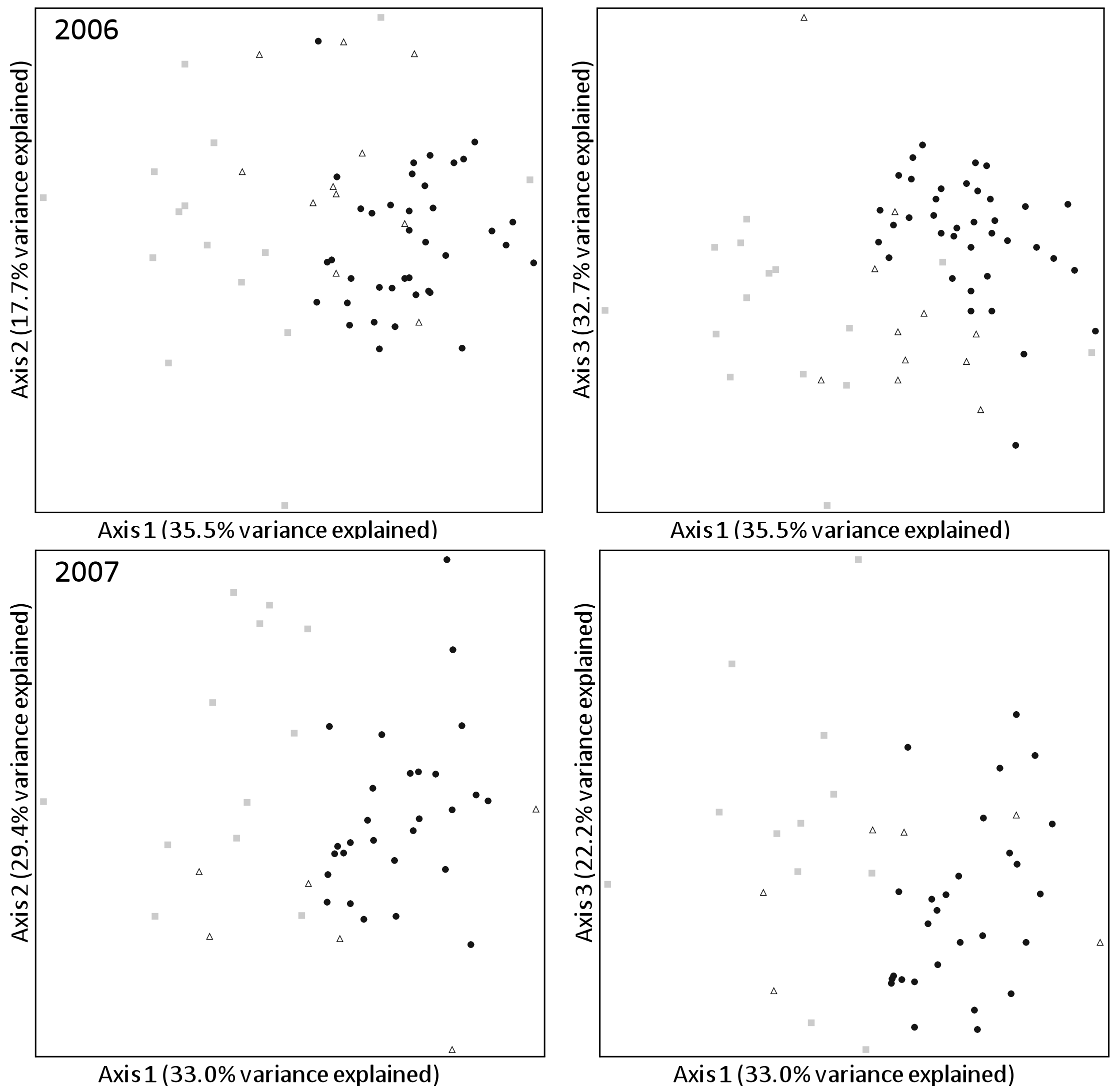
	Aquatic Life Use - IBI			
Aquatic Life Use-HQI	**Exceptional**	**High**	**Intermediate**	**Limited**
2006				
Exceptional				
High				1
Intermediate	1	3	2	2
Limited				2
% Agreement	0	0	100	40
Tot. % Agreement	36			
2007				
Exceptional				
High		1		
Intermediate	1	1	2	1
Limited				
% Agreement	0	50	100	0
Tot. % Agreement	50			
2008				
Exceptional				
High			1	2
Intermediate		2	2	3
Limited			1	
% Agreement	NA	0	50	0
Tot. % Agreement	18			

Table 6. Aquatic Life Use values for East Central Texas Plains (ECO 33) study sites based on IBI and HQI. % Agreement represents the percentage of occurrences that HQI ALU designations agreed with ALU designation determined by IBI. Total % Agreement is the percent of occurrences where ALU designations determined by IBI and HQI were in agreement across all sites in all use designations. Shaded cells represent those where all occurrences would lie if there were perfect agreement between the two indices.

	Aquatic Life Use - IBI			
Aquatic Life Use-HQI	**Exceptional**	**High**	**Intermediate**	**Limited**
2006				
Exceptional				
High			1	
Intermediate		3	5	4
Limited				2
% Agreement	NA	0	83	33
Tot. % Agreement	47			
2007				
Exceptional				
High		3		
Intermediate		5	4	
Limited				
% Agreement	NA	38	100	NA
Tot. % Agreement	58			
2008				
Exceptional				
High		1	1	
Intermediate		2	7	3
Limited		1		
% Agreement	NA	25	88	0
Tot. % Agreement	53			

Relationships between fish community structure, environmental variables, and IBI metrics

The NMS analysis of all sites for 2006 identified three axes that explained 85.9% of the variation in fish assemblage structure among sites (stress = 14.75, instability = 0.0283, 500 iterations). For 2007, ordination of fish species abundance at the 46 sites sampled yielded three axes that explained 84.6% of variation (stress = 14.13, instability = 0.0316, 500 iterations). The NMS on 2008 fish community data for all sites required only two major axes to explain 86.4% of variation (stress = 16.68, instability = 0.0341, 500 iterations) (Figure 18). For all three years, stream sites in the Cross Timbers ecoregion and the East Central Texas Plains separated clearly along the community structure gradient in the primary NMS axis. Sites in the Texas Blackland Prairies were intermediate along axis 1 in 2006, and these sites generally separated from the other two ecoregions along axis 2 in 2007 and 2008.



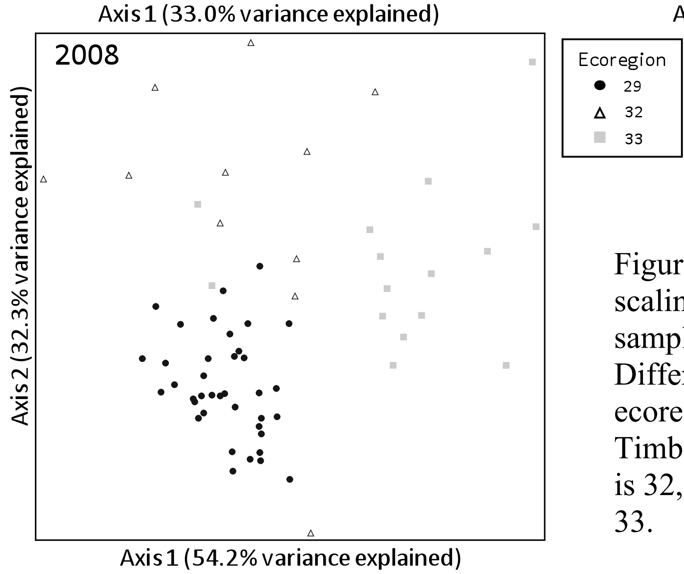


Figure 18. Nonmetric multidimensional scaling (NMS) ordination of all sites sampled in 2006, 2007, and 2008. Different symbols represent the three ecoregions in the study area. Cross Timbers is 29, Texas Blackland Prairies is 32, and East Central Texas Plains is 33.

Cross Timbers Ecoregion (Ecoregion 29)

Ordination of fish species composition at 36 sites in 2006 identified two major axes that explained 70.6 % of the the original distances in n-dimensional space (stress = 22.34, instability = 0.0233, 500 iterations). 2008 fish species composition data sorted 38 sites along two major axes that explained 73.3 % of the original distances in n-dimensional space (stress = 20.93, instability = 0.0282, 500 iterations). Several landscape variables were significantly correlated with fish community structure in both years including pasture, precipitation, watershed size, number of dams, WWTP outfalls, development, total agriculture, and canopy percent (Figure 19, Table A5). In stream habitat variables that were correlated with fish community structure for both years included mud/silt, coarse woody debris within the wetted channel, and erosion potential (Figure19, Table A6). Several relationships between in stream habitat and fish community structure varied between years. Discharge, flow status, pool width, velocity depth regime, number of riffles, leaf packs, artificial cover, exposed soil, and habitat type were correlated with fish community structure during 2006, a dry year (Figure 19, Table A6). In 2008 these habitat variables showed no relationship with fish community structure. However, depth, percent gravel or larger, percent gravel, microalgal cover, large woody debris, bank slope, tree and grass cover within the riparian, aesthetic rating, and specific conductivity were significantly correlated with fish community structure in 2008 (Figure 19, Table A6). Despite repeatable patterns in some of the landscape and in stream habitat variables, no HQI metrics were significantly correlated with fish community structure in both 2006 and 2008 (Figure 19, Table A7). Riffle scores, flow scores and HQI scores were correlated with fish community structure in 2006 but not 2008. This corresponds with several raw habitat variables related to flow and riffles that were correlated with fish community structure in 2006 but not 2008. In 2008, the only HQI measure that was correlated with fish community structure was bank scores (Figure 19, Table A7).

Relationships between Index of Biotic Integrity (IBI) metrics and fish community structure varied with year. In 2006 richness, native cyprinid richness, percent intolerant species, percent stonerollers, richness scores, and cyprinid scores were significantly correlated with fish community structure (Figure 20, Table A8). Percent tolerant, percent invertivores, percent piscivores, number caught per seine haul, percent nonnative, sunfish score, tolerance score, invertivore score, piscivore score, and seine score were significantly correlated with fish community structure in 2008. No IBI metric was correlated with fish community structure in both years.

Figure 19. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between environmental variables and fish community structure. See Table A1-A3 for explanation of abbreviated environmental variable names.

While no repeatable relationships between IBI metrics or scores and fish community structure were observed, several species responded positively or negatively along environmental gradients associated with ordination axes for both years. Yellow bullhead catfish abundance was positively associated with axis 2 which represented a gradient of decreasing watershed size (Figure 21). In both 2006 and 2008, central stonerollers, blacktail shiners and orangethroat darters showed consistent negative correlations with axis 1 which represented an environmental gradient of increasing pasture and mud/silt substrates (Figures 22-24). Red shiner and bluntnose minnow abundances decreased within fish communities along axis 2 in 2006 and increased along axis 1 in 2008 (Figures 25-26). This shift corresponds with the relationship between WWTP outfalls and number of dams and community structure shifting from axis 2 to 1 between years (Figure 19). These species appear to respond positively to habitat alterations associated with flow and nutrients from WWTPs.

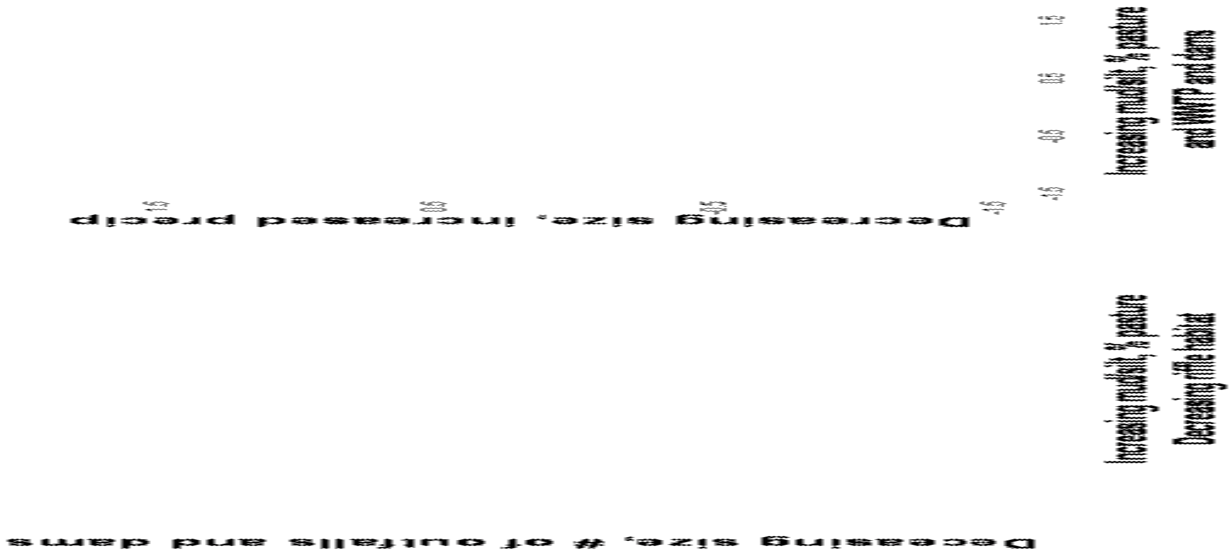


Figure 20. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between IBI variables and fish community structure. Axes 1 and 2 are labeled based on dominant environmental gradients identified in Figure 1. See Table A4 for explanation of abbreviated IBI variable names.

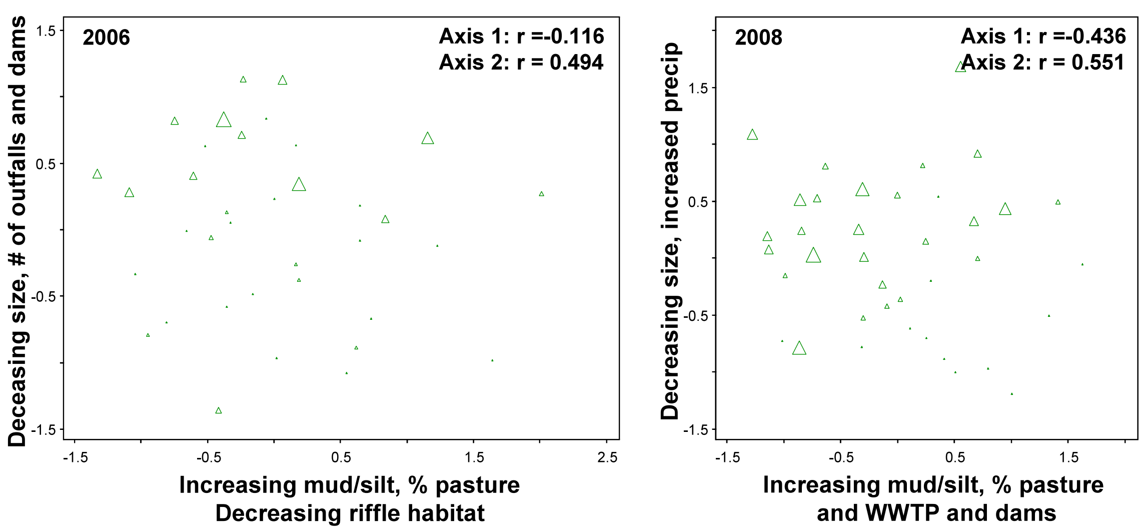


Figure 21. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of yellow bullhead catfish (*Ameirus natalis*). Axis 1 and 2 correlation coefficients with yellow bullhead abundance are displayed in upper right.

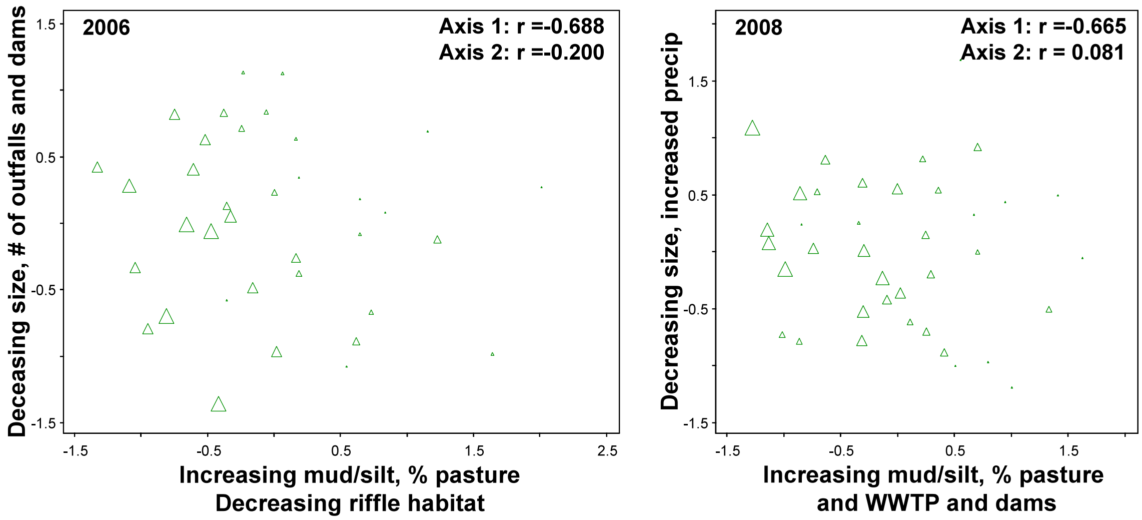


Figure 22. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of central stonerollers (*Campostoma anomalum*). Axis 1 and 2 correlation coefficients with central stoneroller abundance are displayed in upper right.

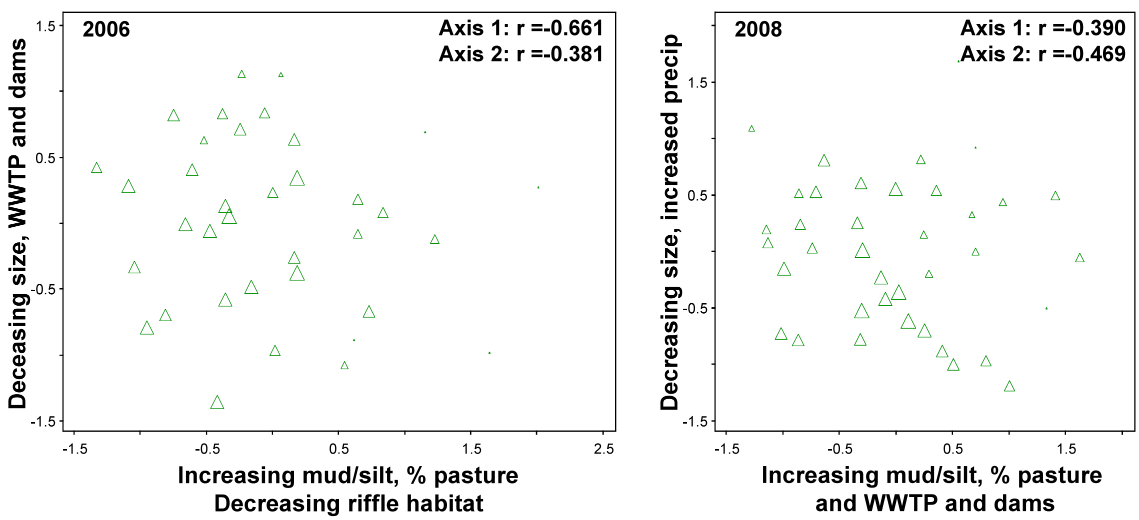


Figure 23. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of blacktail shiner (*Cyprinella venusta*). Axis 1 and 2 correlation coefficients with blacktail shiner abundance are displayed in upper right.

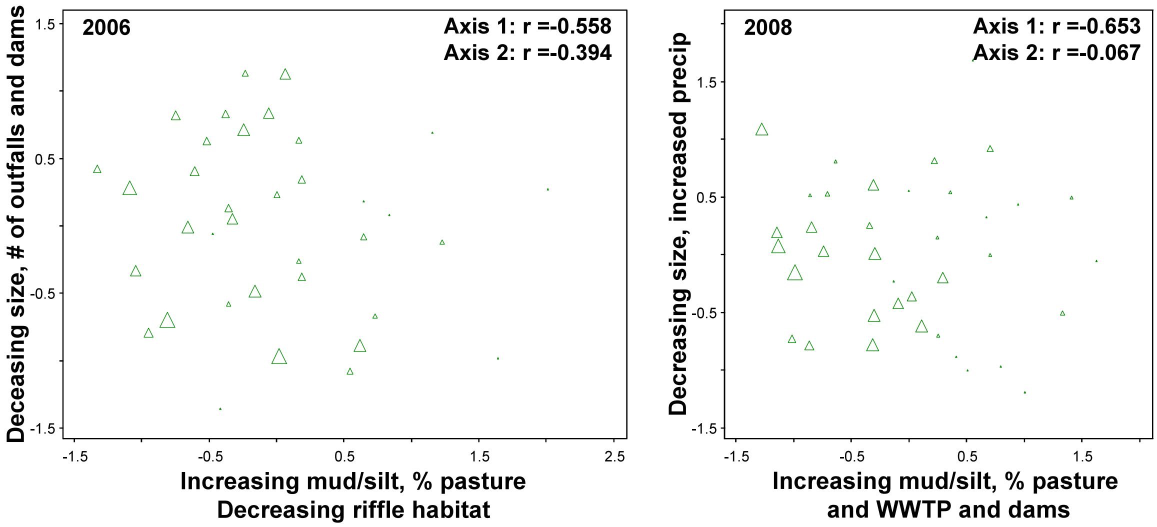


Figure 24. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of orangethroat darter (*Etheostoma spectabile*). Axis 1 and 2 correlation coefficients with orangethroat darter abundance are displayed in upper right.

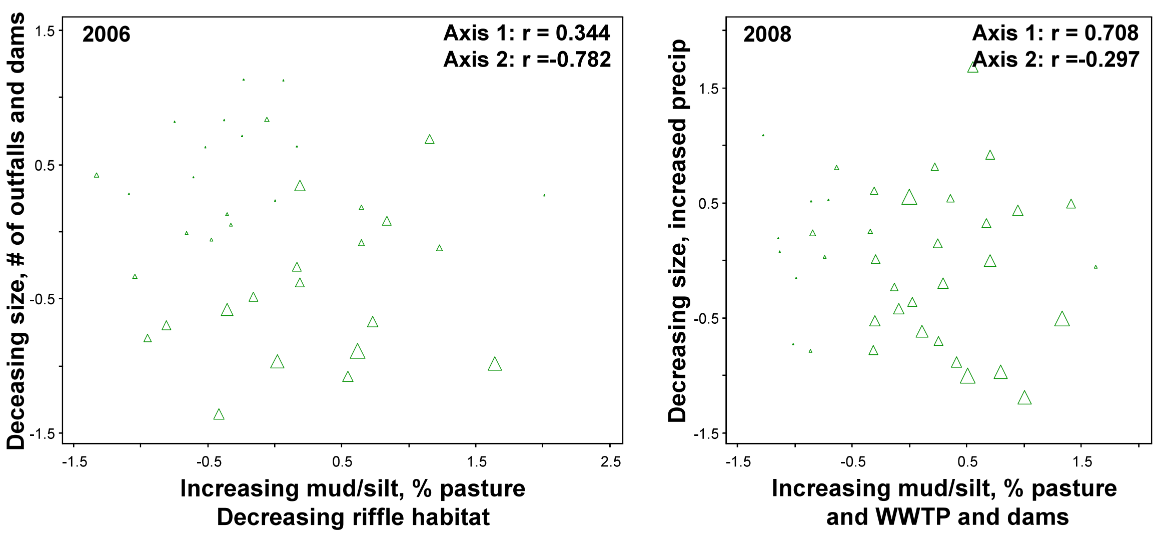


Figure 25. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of red shiner (*Cyprinella lutrensis*). Axis 1 and 2 correlation coefficients with red shiner abundance are displayed in upper right.

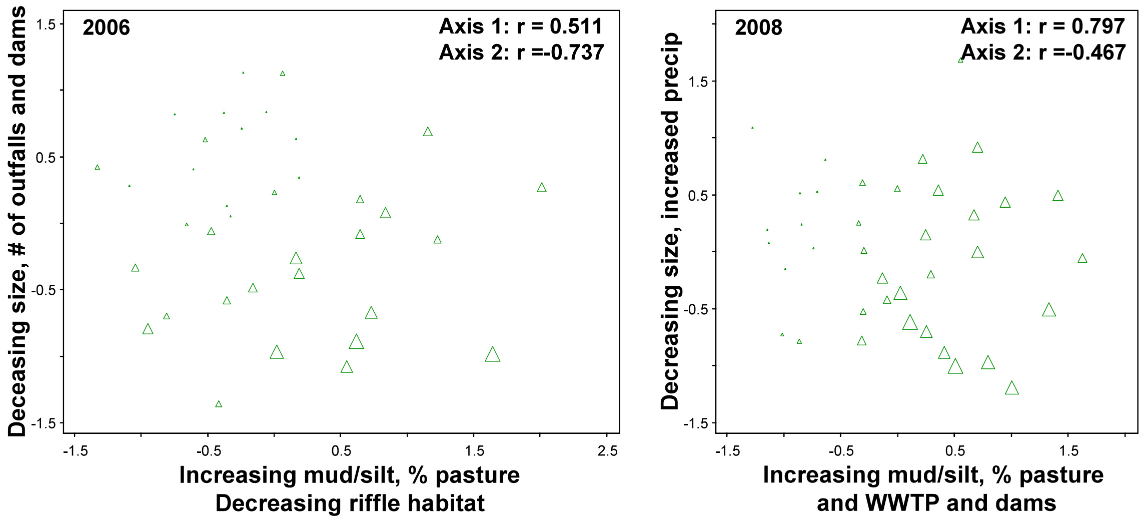


Figure 26. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 29 for 2006 and 2008. Size of points indicates abundance of bluntnose minnow (*Pimephales vigilax*). Axis 1 and 2 correlation coefficients with bluntnose minnow abundance are displayed in upper right.

Texas Blackland Prairie Ecoregion (Ecoregion 32)

NMS ordination of the fish abundance data for the 11 sites in Ecoregion 32 for 2006 resulted in two axes that explained 89.5% of variation, but the solution was not considered statistically useful compared to randomized runs. However, repeated analyses resulted in generally consistent ordinations (mean stress = 14.622). For 2008, the sites sorted in multidimensional species-space along two axes that explained 85.6% of variation (stress=11.39, instability = 0.03216, 500 iterations). Percent of impervious area was significantly correlated with fish community structure for both years, and pasture and total developed were significant landscape variables for the 2006 data (Figure 27, Table A9). Number of riffles and percent cobble in substrate were the local-scale variables with a consistently significant relationship with community structure (Figure 27, Table A10). Discharge, flow status, amount of large woody debris, percent mud and silt, percent riparian shrub, habitat type, and thalweg depth were also significantly correlated with species composition in 2008. The number of root wads along the bank was an additional significant variable in 2006. Among HQI metrics, the riffle score was significantly correlated with fish community structure in both years (Figure 27, Table A11). Substrate score and flow score were also significantly correlated for the 2008 data set.

None of the metrics used for IBI scoring were significantly correlated with fish community structure for both years. In 2006, metrics related to species richness and abundance of sunfish species were significant. In 2008, only the percentage of non-native species had a significant correlation with community structure (Figure 28, Table A12). As in the Cross Timbers ecoregion, several individual species had positive or negative relationships with the environmental axes revealed in the NMS ordinations. Yellow bullhead catfish were negatively correlated with axis 2 in 2006 which was associated with increasing impervious cover. In 2008, abundance of this species had a negative correlation with axis 1 which represented a gradient of increasing mud/silt (Figure 29). Central stonerollers and blacktail shiners showed negative correlations with axis 1 in 2006 (Figures 30-31) which indicated that these species were positively associated with number of riffles and negatively with pasture. In 2008, they showed a negative correlation with the increasing mud/silt gradient along axis 1. Red shiners were positively associated with axis 2 in 2008 (Figure 32), which represented a gradient of increasing impervious cover and higher discharge. Bullhead minnows and blackstripe topminnows were positively correlated with axis 2 in 2006, indicating that they were more abundant in sites with more development and impervious cover, but this relationship did not hold in 2008 (Figures 33 and 34).

Figure 27. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites for Ecoregion 32 in 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between environmental variables (top), IBI metrics (bottom) and fish community structure. See Tables A1-A3 for explanation of variables corresponding to abbreviations.

Figure 28. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites for Ecoregion 32 in 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between IBI metrics and fish community structure. See Table A4 for explanation of variables corresponding to abbreviations.

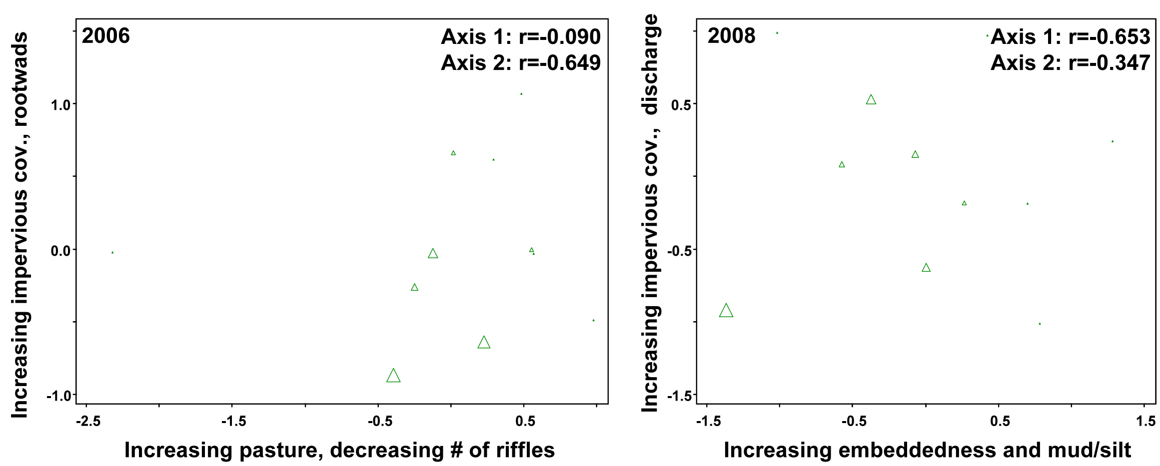


Figure 29. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of yellow bullhead catfish (*Ameiurus natalis*). Axis 1 and 2 correlation coefficients with yellow bullhead abundance are displayed in upper right.

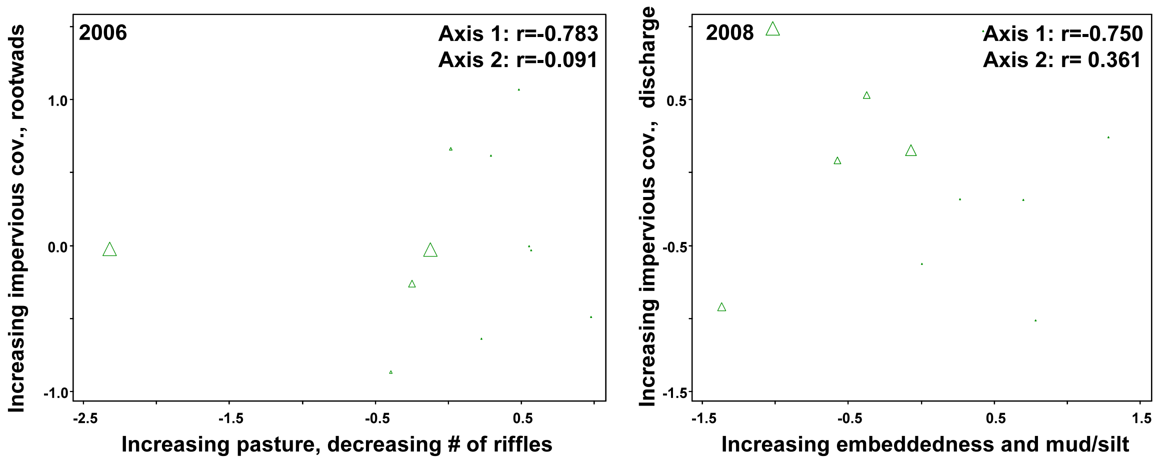


Figure 30. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of central stonerollers (*Campostoma anomalum*). Axis 1 and 2 correlation coefficients with central stoneroller abundance are displayed in upper right.

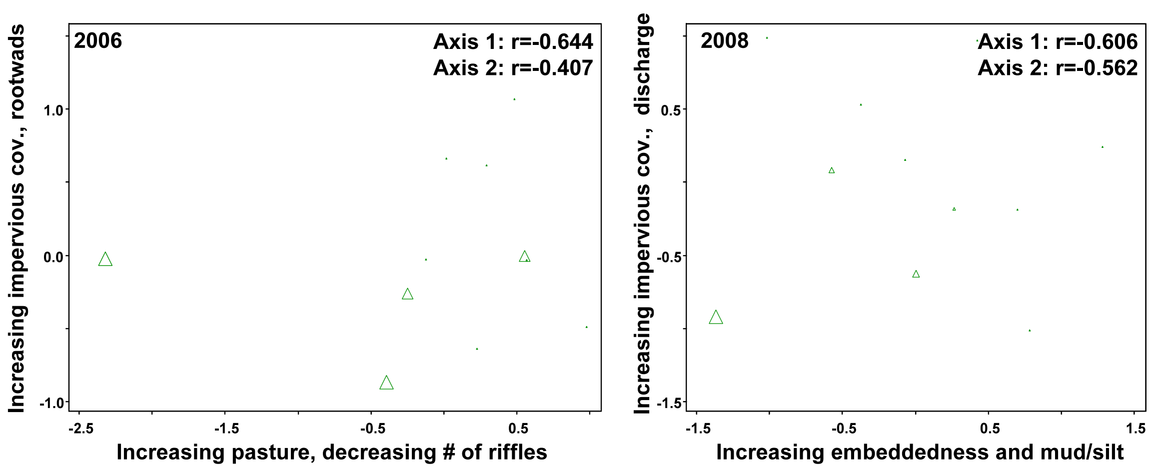


Figure 31. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of blacktail shiner (*Cyprinella venusta*). Axis 1 and 2 correlation coefficients with blacktail shiner abundance are displayed in upper right.

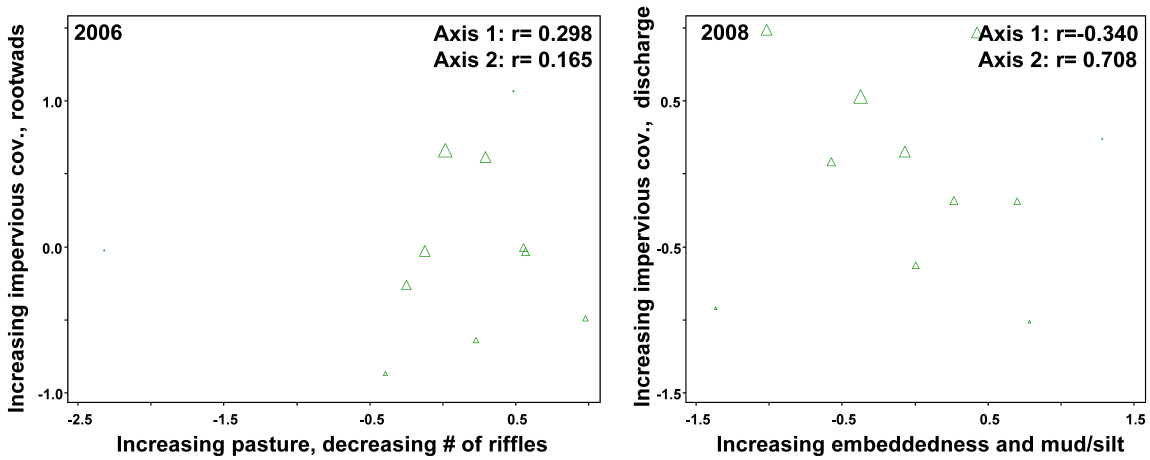


Figure 32. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of red shiner (*Cyprinella lutrensis*). Axis 1 and 2 correlation coefficients with red shiner abundance are displayed in upper right.

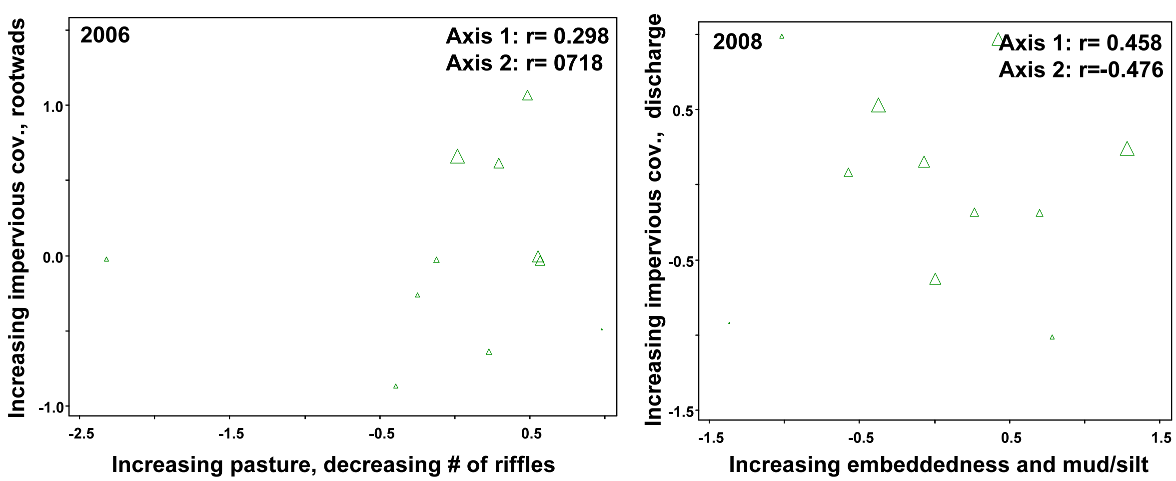


Figure 33. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of bullhead minnow (*Pimephales vigilax*). Axis 1 and 2 correlation coefficients with bullhead minnow abundance are displayed in upper right.

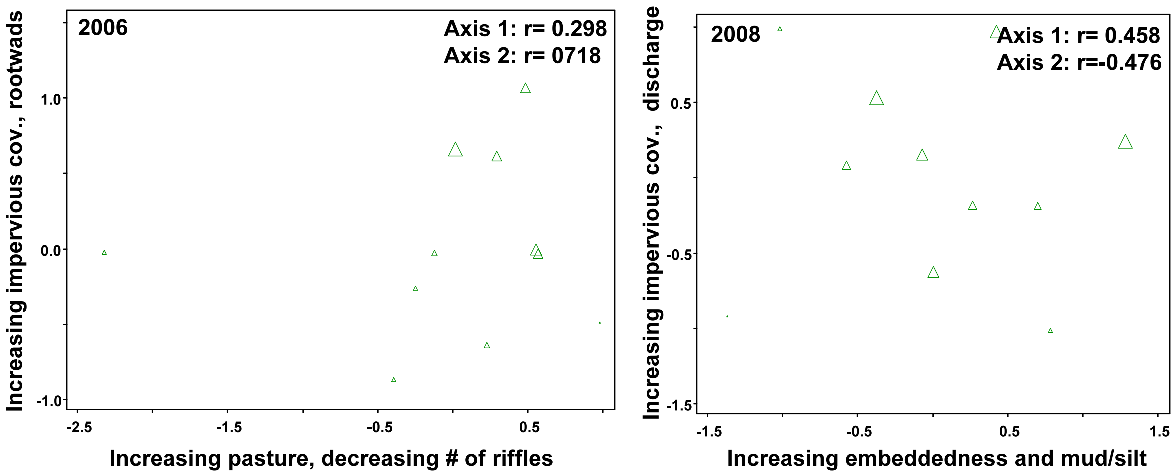


Figure 34. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 32 for 2006 and 2008. Size of points indicates abundance of blackstripe topminnow (*Fundulus notatus*). Axis 1 and 2 correlation coefficients with blackstripe topminnow abundance are displayed in upper right.

East Central Texas Plains Ecoregion (Ecoregion 33)

NMS ordination of the fish abundance data for the 15 sites in the East Central Texas Plains for 2006 resulted in two axes that explained 74.1% of variation (stress = 10.20, instability = 0.03525, 500 iterations). For 2008, the sites sorted in along two axes that explained 89.3% of variation (stress=10.20, instability = 0.03201, 500 iterations). Five landscape-scale variables showed consistently significant relationships with fish community structure in both years: watershed slope, row crop production, percent wetland, total area used for agriculture, and percent canopy cover (Figure 35, Table A13). Total forest cover and pasture were also significant in 2006, while precipitation, percent reservoir, and area covered in water were significant in 2008. Amount of small woody debris as cover and habitat type were the two local-scale variables that were significantly related to fish species composition in both years (Figure 35, Table A14). In 2006, number of stream bends, large woody debris, artificial cover, number of cover types, and percent riparian grass were also significantly correlated with community structure. In 2008, percent of cobble in substrate, percent of mud/silt, filamentous algae as cover, leaf packs, percent boulders and other rocky cover types, coarse woody debris on the bank, percent “other” riparian vegetation, canopy cover, algae abundance, dissolved oxygen, and pH were significant. None of the metrics used to calculate HQI score were significantly correlated with fish community structure in either year (Table A15).

Tolerance score, percent tolerant species, and percent piscivorous species were the IBI metrics that showed a consistent, significant correlation with species composition for both years in Ecoregion 33 (Figure 36, Table A16). Sunfish metrics (percent sunfish species and sunfish score) were also significant for 2006. In 2008, species richness, percent native cyprinids, percent benthic invertebrate feeders, percent intolerant species, percent captured with seine, number of minutes of sampling with electrofisher, richness score, and cyprinid score had significant correlations with fish community structure.

Red shiners and western mosquitofish had positive correlations with axis 1 in 2006 and 2008 (Figures 37 and 42), so they were more abundant in sites within more agricultural landscapes with fewer forests and wetlands. Blacktail shiners and ribbon shiners showed the opposite relationship along the agricultural gradient, and they were associated negatively with increasing mud and silt and decreasing dissolved oxygen along axis 2 in 2008 (Figures 38 and 39). Ribbon shiners were also positively associated with axis 2 in 2006, which represented a gradient with increasing woody debris and higher channel sinuosity. Like blacktail shiners and ribbon shiners, bullhead minnows were negatively associated with the gradient of increasing mud/silt and reservoir influence and decreasing instantaneous D.O. (axis 2) in 2008 (Figure 40). Blackstripe topminnows were negatively associated with the agricultural gradient (axis 1) in both years (Figure 41).

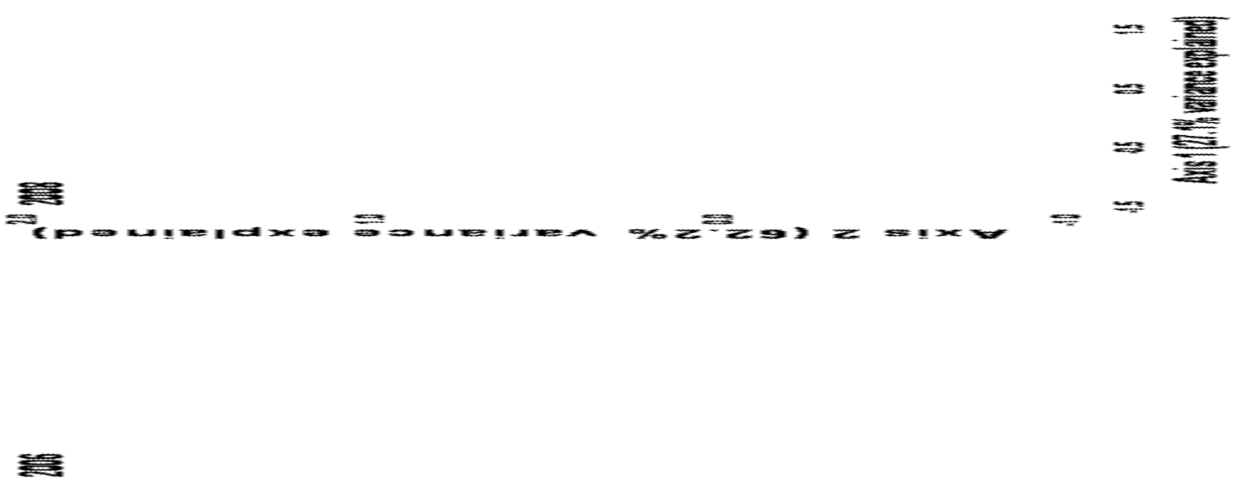


Figure 35. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites for Ecoregion 33 in 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between environmental variables and fish community structure. See Tables A1-A3 for explanation of variables corresponding to abbreviations.

Figure 36. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites for Ecoregion 33 in 2006 and 2008. Environmental vectors show the direction and magnitude of correlations within the ordination space between IBI metrics and fish community structure. See Table A4 for explanation of variables corresponding to abbreviations.

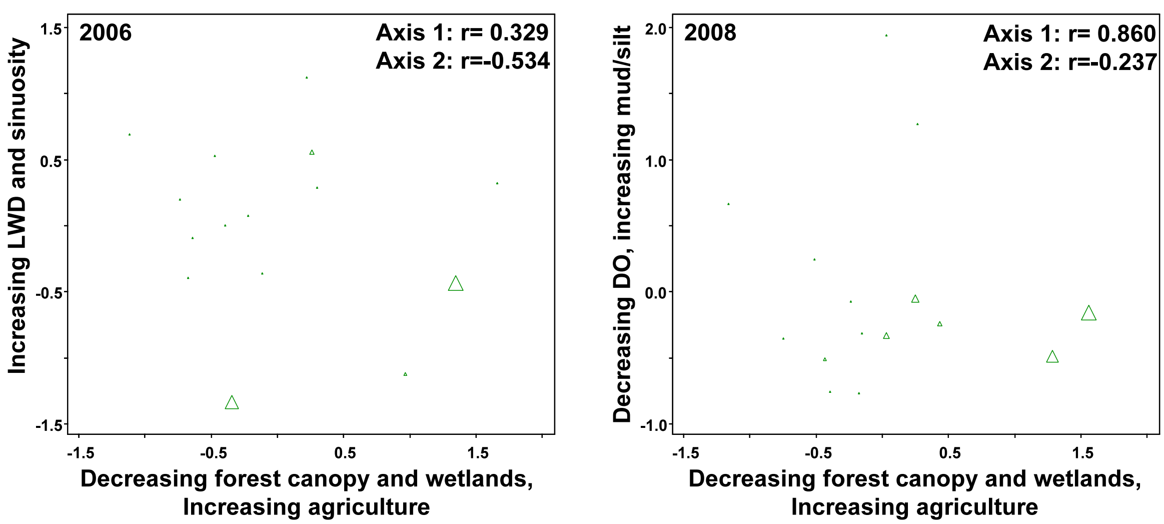


Figure 37. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 33 for 2006 and 2008. Size of points indicates abundance of red shiner (*Cyprinella lutrensis*). Axis 1 and 2 correlation coefficients with red shiner abundance are displayed in upper right.

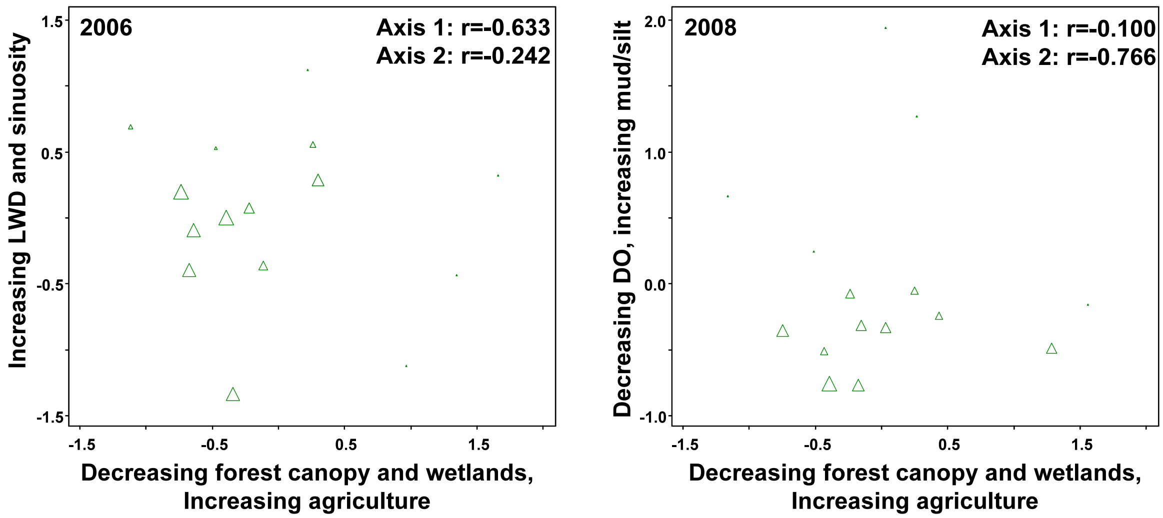


Figure 38. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 33 for 2006 and 2008. Size of points indicates abundance of blacktail shiner (*Cyprinella venusta*). Axis 1 and 2 correlation coefficients with blacktail shiner abundance are displayed in upper right.

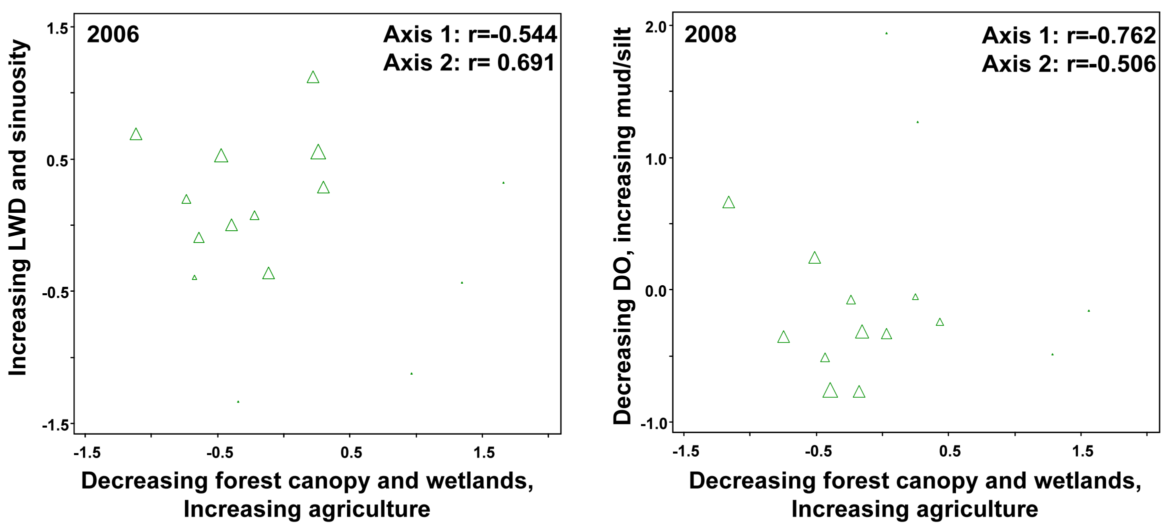


Figure 39. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites sites in Ecoregion 33for 2006 and 2008. Size of points indicates abundance of ribbon shiner (*Lythrurus fumeus*). Axis 1 and 2 correlation coefficients with ribbon shiner abundance are displayed in upper right.

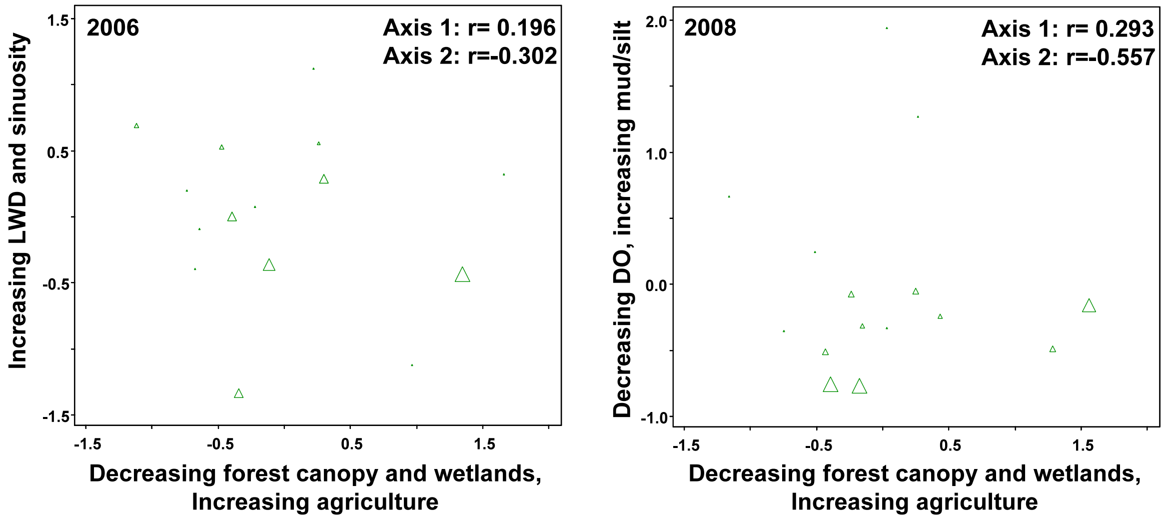


Figure 40. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 33 for 2006 and 2008. Size of points indicates abundance of bullhead minnow (*Pimephales vigilax*). Axis 1 and 2 correlation coefficients with bullhead minnow abundance are displayed in upper right.

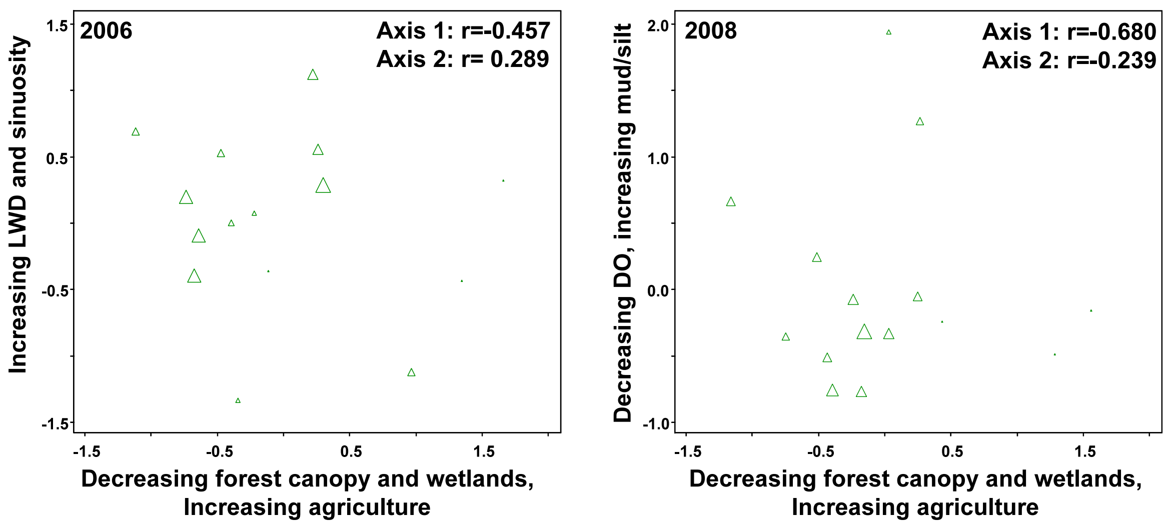


Figure 41. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 33 for 2006 and 2008. Size of points indicates abundance of blackstripe topminnow (*Fundulus notatus*). Axis 1 and 2 correlation coefficients with blackstripe topminnow abundance are displayed in upper right.

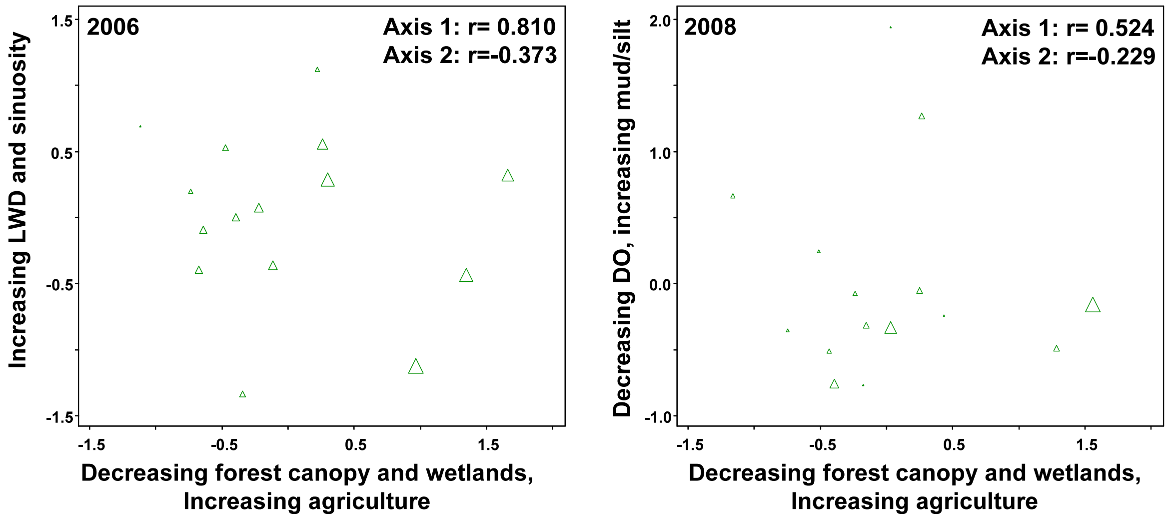


Figure 42. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in Ecoregion 33 for 2006 and 2008. Size of points indicates abundance of western mosquitofish (*Gambusia affinis*). Axis 1 and 2 correlation coefficients with western mosquitofish abundance are displayed in upper right.

Potential alternative IBI metrics

Based on the results from the previous section, we began developing alternative or additional metrics that may increase the sensitivity of the IBI to environmental gradients. Within the Cross Timbers ecoregion, NMS ordinations revealed robust relationships between fish community structure and an environmental gradient associated with increasing pasture and mud/silt substrates as well as increasing WWTP influences (Figure 19). Several species responded positively or negatively to this gradient (Figures 21-26). Using this information we developed four candidate metrics which were % herbivore (*C. anomalum*), % darter (*E. spectabile*), % intolerant cyprinids (*C. anomalum, C. venusta, N. vollucellus*), and % tolerant cyprinid (*C. lutrensis, P. vigilax*). Tolerant and intolerant designations differed for some species from established tolerance group classification for Texas fishes (Linam and Kleinsasser 1998). Specifically, *C. anomalum* and *C. venusta* were considered intolerant and *P. vigilax* was considered tolerant instead of intermediate. These alternative tolerance group designations were based on these species' response within fish communities to negative environmental gradients within the study area (King et al. 2009). *Notropis vollucellus* and *C. lutrensis* responsed as would be expected based on established tolerance group designations for these two species. We found that the first three metrics responded negatively to NMS axis scores for 2008 suggesting that these metrics capture a decrease in these species as communities change in response to increasing pasture, mud/silt, and WWTP influences (Figure 43). In contrast, the % tolerant cyprinids metric increased along NMS axis 1 (Figure 43). To assess how these metrics responded directly to an environmental gradient, we assessed relationships with % Pasture and found that the first three metrics all had negative relationships with % Pasture, whereas % tolerant cyprinids showed a positive trend (Figure 44). These results suggest that using multivariate analyses to identify both species and environmental gradients provides a basis for developing more sensitive biological metrics that reflect changes in communities in relation to changing environmental conditions. Data from more sites representing a wider range of environmental variation is needed to develop similar metrics for the additional two ecoregions.

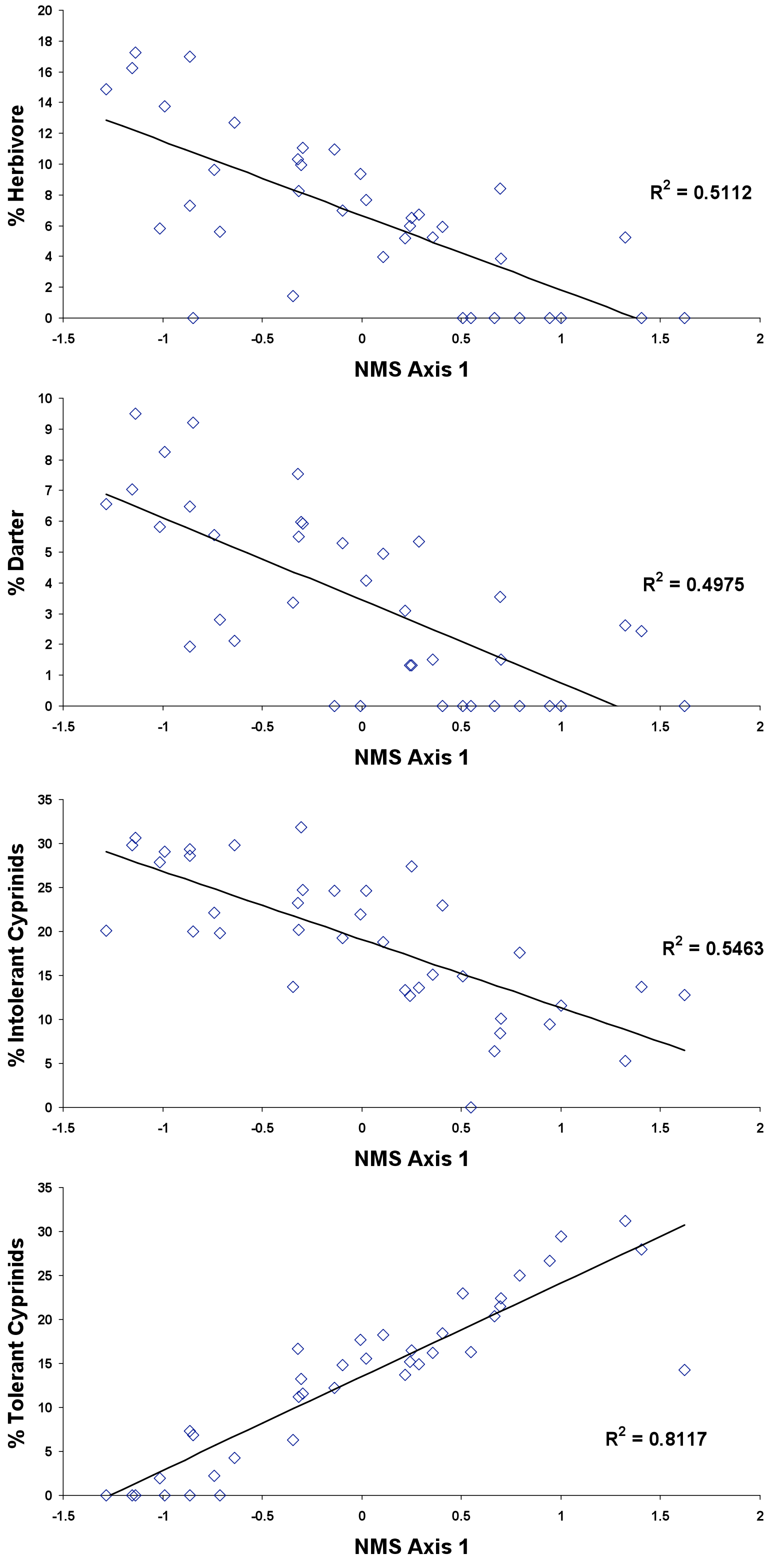


Figure 43. Relationships between alternative metrics and NMS axis-1 scores for the Cross Timbers Ecoregion in 2008.

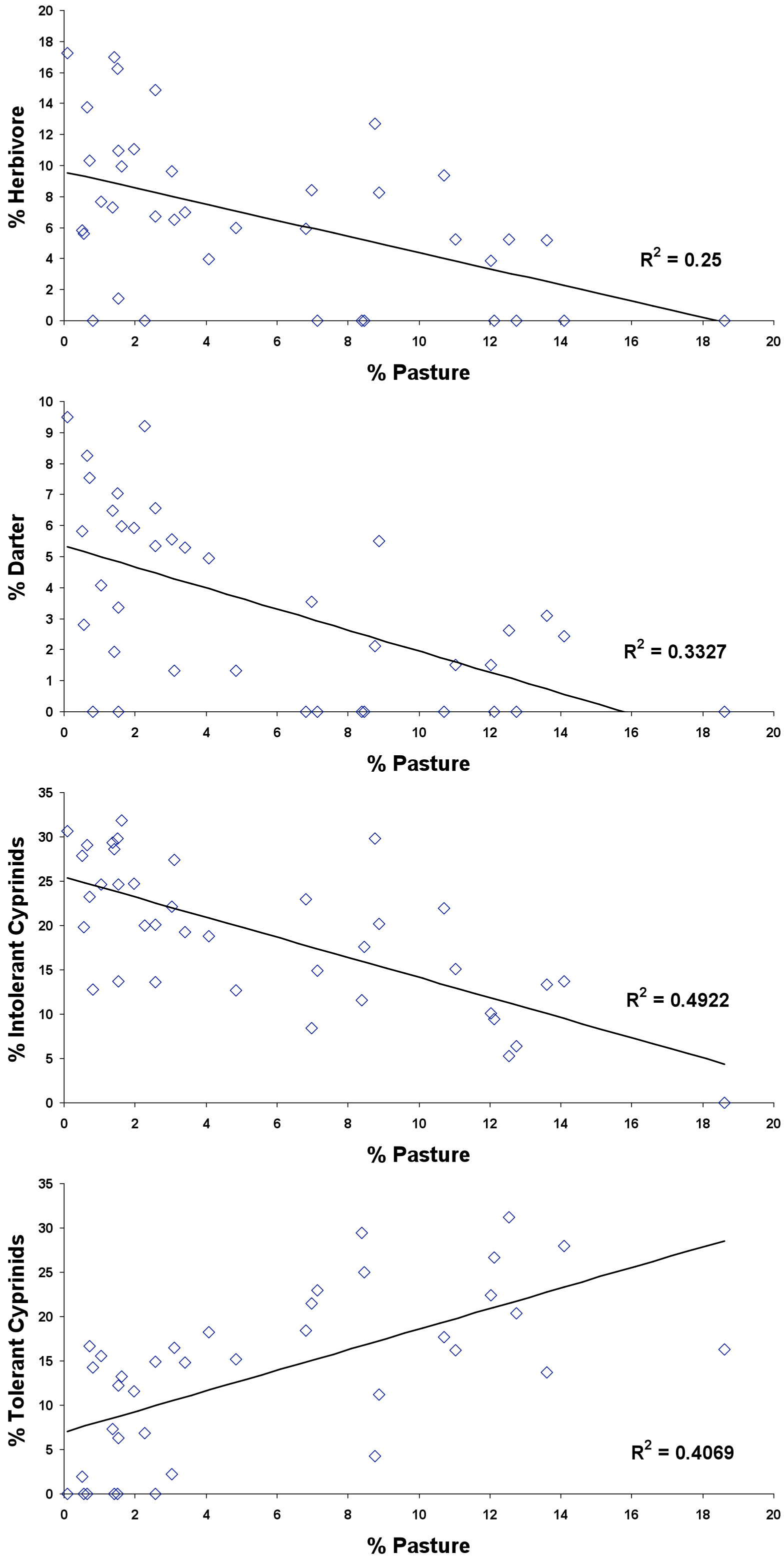


Figure 44. Relationships between alternative metrics and % Pasture for the Cross Timbers Ecoregion in 2008.

Shifts in community structure associated with continued flooding in 2007

In 2007, central Texas experienced one of the wettest years in recorded history, with the majority of that rain occurring during the critical sampling period. Sampling was continually postponed throughout the summer due to high flow. The majority of sampling did not occur until September and larger sites were omitted due to unwadeable conditions. However, a total of 46 sites (ECO 29 N= 28, ECO 32 N= 6, and ECO 32 N= 12) was sampled and provided an opportunity to examine the effects of sustained high water on central Texas fish communities.

Despite the high variability in flow between the 3 years of sampling, the dominant environmental gradient comprised of increasing mud/silt substrates associated with increased pasturelands within catchments from the Cross Timbers ecoregion (ECO 29) was preserved. A non-metric multidimensional ordination of 28 sites across 3 years identified two major axes that explained 71.4 % of the the original distances in n-dimensional space (stress = 17.55, instability = 0.0253, 500 iterations). Axis 1 was most strongly correlated with pasture, mud/silt substrates, latitude, and number of dams. Axis 2 was primarily explained by flow status and discharge (Figure 45 and Table A17). Sites show a clear pattern between years of shifting along axis 2 in the direction of increased flow for 2007 and shifting back in 2008. Successional vector fitting for each site between years indicate that these shifts were fairly uniform across sites (Figure 46). These results provide evidence that fish communities were influenced by the high-water year, but also that shifts in community structure associated with hydrologic regimes did not affect the response of fish community structure to the dominant environmental stress gradient.

Fish communities in the TX Blackland Prairie ecoregion (ECO 32) did not respond uniformly to changes in hydrology between the 3 years. The ordination identified two axes that explained 89.5% of the original distances in n-dimensional space (stress = 11.85, instability = 0.0341, 500 iterations). Fish community structure was related to increased pasture and forest cover and decreasing row-crop cover and substrate quality on axis 1. Increasing development and artificial cover were related to fish community structure on axis 2 (Figure 47). Flow status or discharge was not highly correlated with community structure on either axis. Fish community structure was fairly stable between years within a site. Only two sites, COTC-01 and RDOC-02, shifted substantially in species space among years. These sites were associated with the development axis and may have been more influenced by hydrology associated with high amounts of urban runoff during 2007. These results indicate that fish community structure may be driven more by landscape factors and embedded local habit features and less by annual variation in precipitation.

Differences in hydrology between years did not have a large effect on fish community structure in the East Central TX Plains ecoregion. NMS ordination identified two axes that explained 76.3 % of the original distances in n-dimensional space (stress = 20.24, instability = 0.027, 500 iterations). Fish community structure on axis 1 was related to shifting land-use practices from forested areas with more wetlands to agricultural areas. Fish community structure on axis 2 was related to an environmental gradient of decreasing instantaneous D.O. and increasing amounts of surface water and reservoirs (Figure 48, Table A17). Similar to the Blackland Prairie fish communities, structure in the East Central TX Plains was not strongly influenced by differing hydrologic regimes between years. Successional vectors between years within sites did not move in a consistent direction between time periods. Nor was the magnitude of change between years very large for most sites. Similar to results observed in the Blackland Prairie, fish community structure in the East Central TX Plains seemed to be more related to dominant landscape gradients within the ecoregion and were not affected greatly by the widely differing hydrologic regimes experienced across the three years during the study.

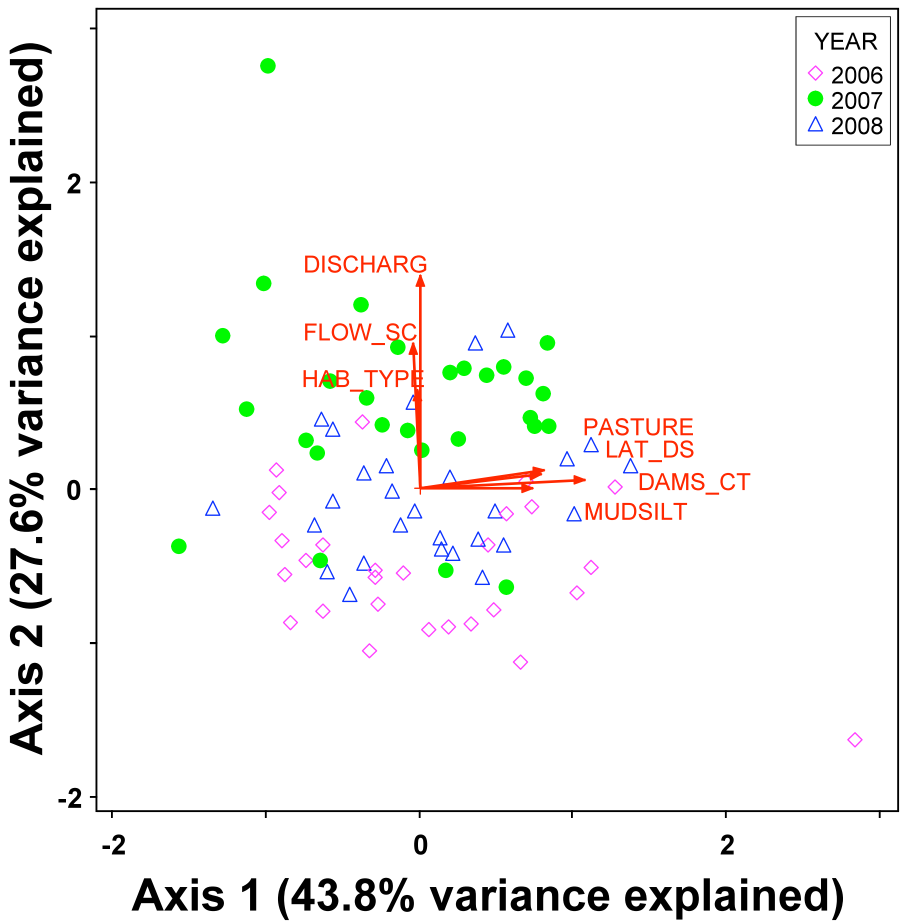


Figure 45. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in the Cross Timbers ecoregion for three years combined. Sites are color coded according to year. Environmental vectors show the direction and magnitude of correlations within the ordination space between environmental variables and fish community structure. See Tables A1-A3 for explanation of variables corresponding to abbreviations.

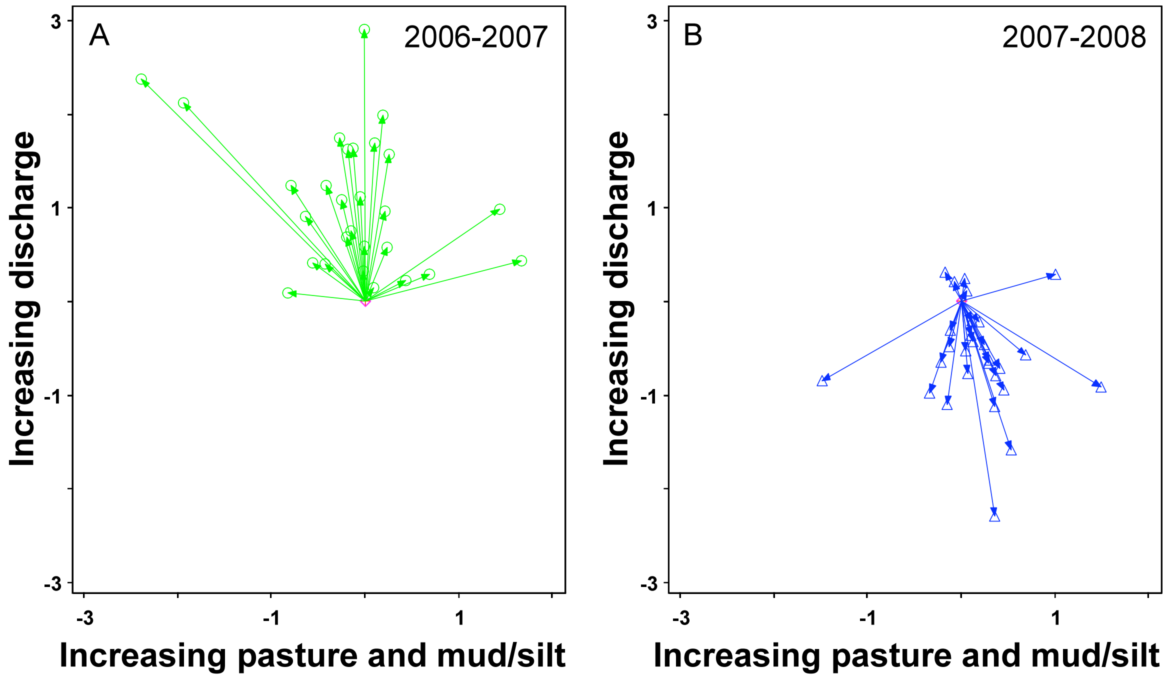


Figure 46. Nonmetric multidimensional scaling (NMS) ordination of sampling sites in the Cross Timbers ecoregion with successional vector fitting illustrating inter-annual differences in fish community structure. All sites are centered to 0 for the preceding year. Arrows indicate the direction and magnitude of change in species space for each site between 2006 and 2007 (A) and between 2007 and 2008 (B). Environmental gradients on axes 1 and 2 are defined based on Figure 43.

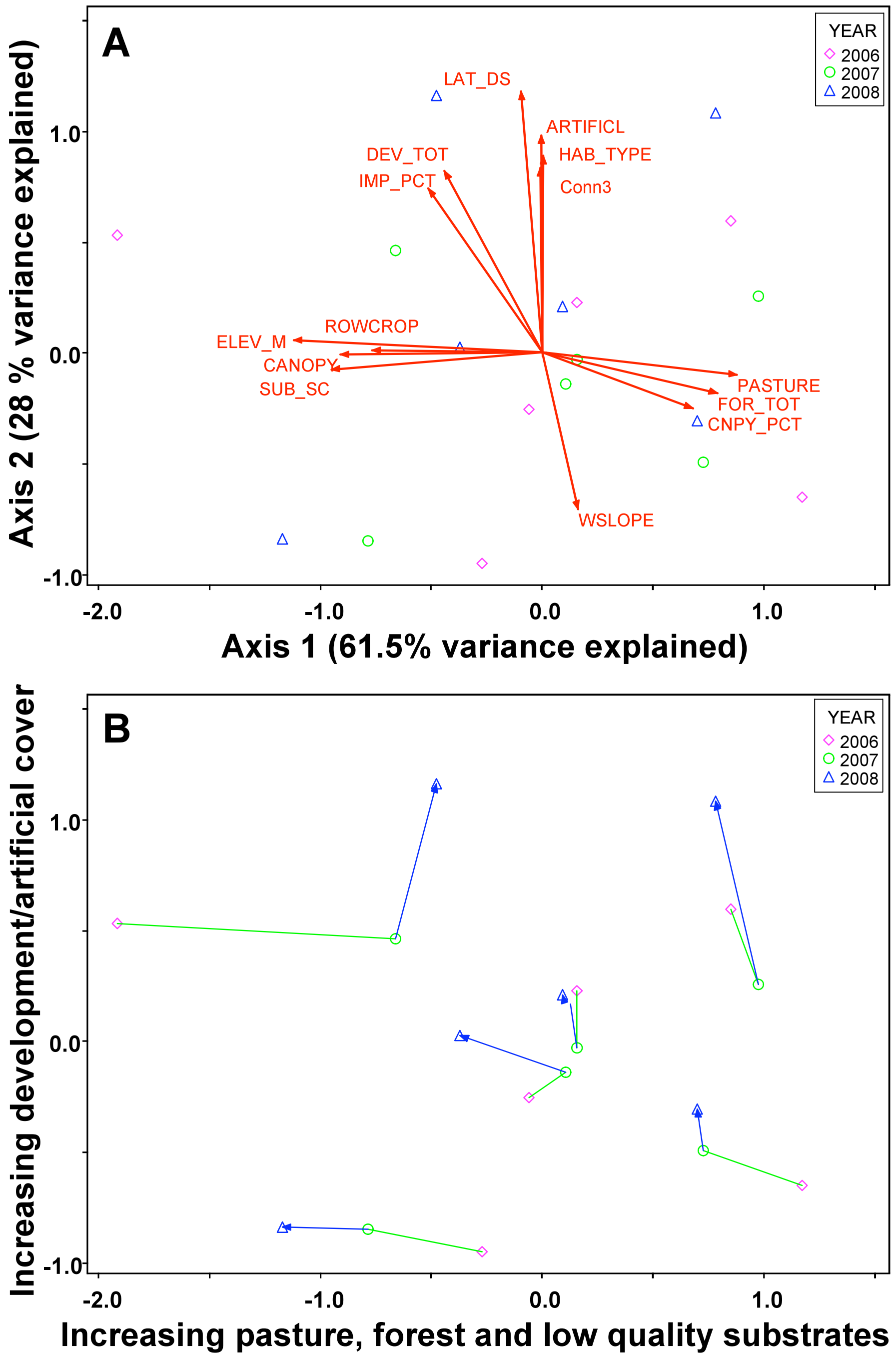


Figure 47. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in the TX Blackland Prairie ecoregion for three years combined. Sites are color coded according to year. Environmental vectors (A) show the direction and magnitude of correlations within the ordination space between environmental variables and fish community structure. Successional vectors (B) show the direction and magnitude of change in species space for each site between 2006 and 2007 (green) and between 2007 and 2008 (blue). See Tables A1-A3 for explanation of variables corresponding to abbreviations.

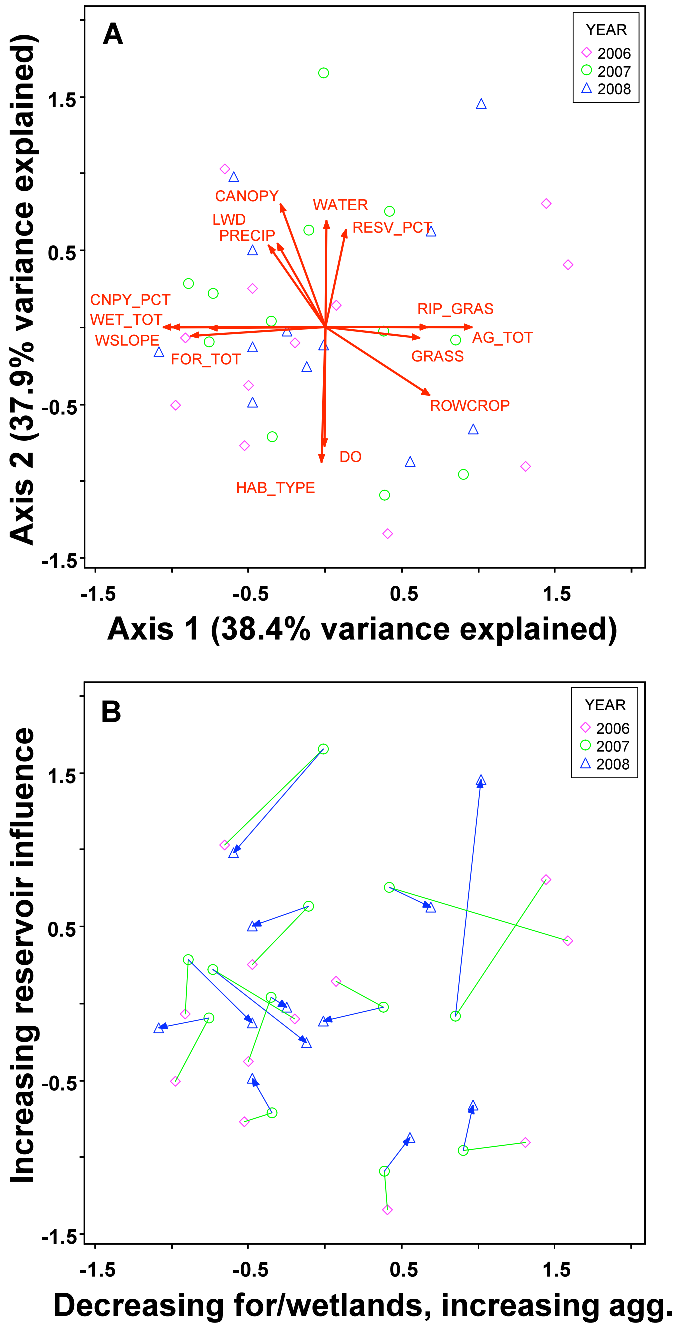


Figure 48. Nonmetric multidimensional scaling (NMS) ordination of fish sampling sites in the East Central TX Plains ecoregion for three years combined. Sites are color coded according to year. Environmental vectors (A) show the direction and magnitude of correlations within the ordination space between environmental variables and fish community structure. Successional vectors (B) show the direction and magnitude of change in species space for each site between 2006 and 2007 (green) and between 2007 and 2008 (blue). See Tables A1-A3 for explanation of variables corresponding to abbreviations.

CONCLUSIONS

IBI-HQI relationships

When all sampling events for all sites were combined, we found a weak significant correlation ($r^2 = 0.0997$, $p < 0.001$) between IBI and HQI scores for streams in our study region. This relationship remained when all sites were examined by year for 2006 and 2008, but there was no significant relationship between the two scores for sites sampled in 2007, the unusually wet year. Much of the correlation between scores appears to be driven by differences between ecoregions. That is, sites within the Cross Timbers tended to have higher scores for both indices, whereas sites within the other two ecoregions tended to have lower scores. Indeed, when we examined the relationship between the two indices for each ecoregion separately, there was no significant correlation.

The lack of a relationship between the two indices at the larger scale (all three ecoregions combined) when flows increased in 2007 indicates that temporal changes have an important effect on the metrics used for stream evaluation. Also, the insignificant relationship between the fish IBI and HQI at the ecoregion scale suggests that the most informative taxa and habitat variables differ within large drainage basins. These results support a recent analysis of Texas streams that revealed a poor match between current biotic and abiotic indicators used for stream health assessment (Kleinsasser et al. 2004).

IBI and HQI assessments are used to establish ALU designations for Texas streams. We did not find high levels of agreement between ALU designations based on the two indices. Agreement was better for High and Intermediate ALU designations. Sites that were classified as Exceptional based on IBI scores never received an Exceptional HQI score for any sampling event. In stream systems which often are impaired by multiple stressors one might expect that a stream may have Exceptional habitat but fish communities might still be degraded due to some other stressor (low D.O., chemical stressors, etc.). However, if fish are limited by habitat structure, one would expect that any site that has IBI scores meeting the Exceptional ALU criteria would also have HQI scores that supported Exceptional ALU designation. Similar results were reported by Kleinsasser et al. (2004) who sampled 91 sites across three ecoregions and found no sites indicating Exceptional ALU based on the HQI. These results suggest that the current IBI is overestimating ALU designations and/or the current HQI is not capturing or quantifying habitat features that support exceptional fish communities.

Ecoregional differences in Fish Community structure

Ordination of fish community structure for all sites by year revealed that sites within ecoregions consistently grouped together, resulting in a general geographic gradient along the primary NMS axis. Along the gradient from the Cross Timbers to the Blackland Prairies and the East Central Plains, habitats generally changed from rockier streams with more riffles to streams with soft substrate and more woody debris. Further examination of stream fish community structure in terms of species functional traits (e.g., using trophic and life-history traits for functional groupings of specimens) rather than taxonomic identity for characterization of community structure might be a useful next step for this research. This approach proved beneficial in clarifying the potential influence of environmental gradients on the structure of Texas stream fish assemblages (Hoeinghaus et al. 2006). By basing the analysis on functional "types" rather than taxa, the influence of historical biogeography was reduced and associations between community structure and environmental factors became more apparent.

Fish Community structure-environmental relationships

Despite poor relationships between IBI and HQI scores for sites within ecoregions, strong patterns in fish community structure were observed using ordination techniques. Fish species gradients were significantly correlated with several landscape and in-stream habitat variables in both 2006 and 2008. These relationships provide an opportunity to identify revised metrics that more accurately reflect fish community changes in response to environmental gradients related to landscape, habitat and water quality factors.

In the Cross Timbers Ecoregion fish community structure was correlated with natural (precipitation, watershed size) and anthropogenic (pasture, # of dams, WWTP outfalls, development, total agriculture, canopy percent) landscape factors during both years. In-stream habitat variables that were correlated with fish community structure within the Cross Timbers Ecoregion for both years included mud/silt, coarse woody debris within the wetted channel, and erosion potential. There also appeared to be a north-south spatial structure (latitude) underlying some of the variation in community structure. Although this may have been driven by small differences in species distributions between the Trinity and Brazos basins, an analysis completed for a separate study (Taylor and King, unpublished data) found similar environmental gradients (Pasture, mudsilt, WWTP outfalls) associated with community structure when only Brazos sites were analyzed. This suggests that dominant landscape and habitat gradients within the Cross Timbers Ecoregion are real, and correlations with latitudinal gradients are just an artifact of Trinity sites having more pasture than Brazos sites. Gradients in fish species composition were correlated with amount of impervious surface, number of riffles, and cobble substrates for both years within the TX Blackland Prairie ecoregion. In the East Central TX Plains ecoregion changes in fish community structure were correlated with shifts in landcover from more wetlands and forest canopy to row-crop agriculture. The amount of small woody debris within the stream decreased along this land-use gradient. These landscape and local habitat variables appear to be important factors related to fish community structure within each ecoregion. However, interpretations for the TX Blackland Prairie and East Central TX Plains ecoregions should be viewed with caution. Even though repeatable relationships were observed for both ecoregions, lack of water in 2006 inhibited the spatial distribution and number of sites, which in turn hampered analyses within ecoregions. These analyses would benefit from additional sampling within these ecoregions.

In contrast, our results indicate that IBI and HQI scores were not consistently related to fish community structure for both years in any ecoregion. No individual IBI metrics were consistently correlated with fish community structure within the Cross Timbers or TX Blackland Prairie ecoregions. In the East Central TX plains, only tolerant species and % piscivore metrics were related to fish communities in both years. Likewise, HQI metrics did not show consistent relationships with fish community structures. Riffle Score within the TX Blackland Prairie ecoregion was the only HQI metric correlated with fish community structure in both years. These results suggest that IBI metrics may not be responsive enough to changes in fish community structure to accurately identify habitat degradation when it occurs. Additionally, our results suggest that summarizing raw habitat data with HQI metrics may decrease the ability to quantify habitat features important to fish.

Identifying species or functional groups that are driving shifts in community structure in response to environmental gradients is an important step in deriving more sensitive IBI metrics. For example, several species declined or increased within communities along environmental gradients. *Campostoma anomalum*, *Cyprinella venusta*, and *Etheostoma spectabile* all declined along gradients of fish community change within the Cross Timbers Ecoregion that were correlated with % pasture, mud/silt substrates and bank erosion. *Cyprinella lutrensis* and *Pimephales vigilax* consistently increased abundance within communities in response to WWTP outfall metrics. Revising IBI metrics to reflect changes in community structure associated with environmental gradients could provide more sensitive biotic integrity measures than the current IBI. Potential new metrics for the Cross Timbers Ecoregion include %Herbivore (*C. anomalum*), % Darter (*E. spectabile*), % intolerant cyprinid (*C. anomalum* + *C. venusta* + *Notropis vollucellus*), and % tolerant cyprind (*C. lutrensis* + *P. vigilax*). These metrics had strong relationships with NMS axis 1 scores suggesting they reflect changes in fish community structure. Fairly good linear relationships between these metrics and % Pasture also suggest that they have the potential to capture changes in fish community structure associated with environmental gradients. Ironically, two opposing alternative metrics (% tolerant cyprinids and % intolerant cyprinids) would be combined into one metric (% Native cyprinids) using the current IBI and all diagnostic strength would be lost. These results demonstrate why IBI metrics do not show strong relationships with fish community composition.

Temporal effects on fish community structure-environmental relationships

Three field seasons with exceptionally different hydrologic regimes provided an opportunity to assess how fish community structure, IBI scores, and HQI scores varied temporally across hydrologically distinct years. The summer of 2006 has been characterized as one of the driest on record for central Texas. Several field sites were limited to perennial pools connected by hyporheic flow. Discharge in 2007 was exceptionally high, with multiple episodes of flooding across all three ecoregions. Most field collections during 2007were done before sites had sufficient time to recover according to TCEQ protocols. These sites were sampled, in part, because they never met those requirements at any time during the field season, and this was an opportunity to examine the effects of high flows on fish community structure, IBI scores, and habitat.

In all ecoregions, the dominant environmental gradient related to fish community structure was preserved across years. In the Cross Timbers ecoregion, fish communities shifted in ordination space in relation to differences in discharge between 2006 and 2007. However sites shifted back to communities that were similar in structure to 2006 when discharge decreased in 2008, suggesting that the extreme flow conditions of 2007 did not have permanent effects on fish community structure. For most sites in the TX Blackland Prairie and East Central TX Plains ecoregions, shifts in community structure between years that were unrelated to hydrology seemed to be minimal. Differences in response to high flows between the ecoregions could be associated with regional differences in landscape and in-stream habitat variables. For example, Cross Timbers streams, which tend to have more gravel and cobble substrates, could have benefited from the flushing of fine sediments during flood periods. In contrast, streams in the other ecoregions tend to be dominated by soft substrates and may not have experienced major substrate changes during high flow events. This is speculation, and the extensive datasets produced during this project could be analyzed further to examine this and other hypotheses regarding causal mechanisms for the faunal patterns.

Future Research Needs

This study identified several landscape and habitat variables that had strong relationships with fish community structure. Within the Cross Timbers ecoregion, we were able to identify fish species that responded to these variables and thereby produced changes in local fish community structure. More research is needed to identify species that have linear relationships as well as threshold responses to environmental stressors. This could pave the way for development of more sensitive biological metrics for measuring impairment within Texas streams. Drought conditions in 2006 limited the spatial distribution and number of sites sampled within the TX Blackland Prairie and East Central Texas Plains ecoregions. More sampling within these two ecoregions is needed to better quantify relationships between fish, landscape factors, and in-stream habitat. Existing data may exist for analysis of these two ecoregions. A recent TPWD study (Kleinsasser et al. 2004) collected fish and habitat data from 91 sites equally distributed between the Texas Blackland Prairies, East Central Texas Plains and South Central Plains ecoregions. Combining HQI and IBI data from this project with those from previous projects in the region might establish a dataset that could reveal gradients of anthropogenic stress with greater resolution.

Additionally, development and use of biological indices for Texas is potentially hampered by extreme variation in annual and seasonal hydrology. Nevertheless, our findings suggest that dominant environmental gradients underlying fish community structure were not greatly affected by inter-annual hydrological variation. However, our study only spanned three years, and long-term sampling at a subset of sites within each ecoregion would be beneficial as a follow-up to this work. Robust predictions of biotic response to anthropogenic environmental impacts will require improved understanding of long-term hydrological effects on fish communities. For example, findings from the Cross Timbers ecoregion suggests that aspects of fish assemblage structure respond to changes in hydrology and might yield a potential measure of long-term changes in hydrologic regimes. This is especially important in rapidly urbanizing areas where increased impervious surfaces and water use will change hydrologic regimes over time.

The datasets produced during this study will be analyzed in conjunction with datasets on nutrients and benthic algae that were gathered concurrently during the later stages of the project. These further data analyses may yield further evidence of how individual fish species and fish community structure respond to anthropogenic impacts to watersheds and stream habitat quality. Furthermore, the datasets from this project will be stored with the TCEQ and made publicly available via the Surface Water Quality Monitoring Information System (SWQMIS). This data and statistical associations revealed from our analyses should provide a basis for further research by other researchers in Texas.

Recommendations for HQI-IBI Refinement

The lack of a relationship between fish community structure, IBI and HQI scores does not mean that habitat is not an important factor regulating fish communities. Our results suggest that existing IBI and HQI metrics used by TCEQ to evaluate aquatic life use do not correspond with changes in fish community structure associated with anthropogenic stress gradients. We make the following specific recommendations for refinement:

1. Within specific ecoregions, datasets of adequate size (30 – 100 sites) that represent anthropogenic stress gradients need to be developed for establishing relationships between fish community structure and anthropogenic stress. Although this method

differs from the traditional reference reach approach, it has merit in regions that do not have reference sites to adequately characterize variation. This approach was effective for making recommendations for phosphorus nutrient criteria in the Cross Timbers Ecoregion (King et al. 2009). IBI and HQI metrics should be developed based on measures that correlate with changes in fish community structure. For example, in this report and in our separate nutrient indicators report (King et al 2009), we identified alternative fish metrics that corresponded with changes in fish community structure and were diagnostic of the two most prevalent stressors in wadeable streams in the Cross Timbers Ecoregion: nutrient loading and sedimentation. Mud-silt cover (analogous to %fines used by the EMAP protocol) was a habitat variable that correlated strongly with fish community structure in the Cross Timbers ecoregion and should be included as a component of the HQI. Embeddedness of hard substrate is another habitat variable that should be considered as a potential component. We also suggest that variables that never showed a reasonable correlation to fish community structure in any of the ecoregions should be removed from the HQI. Given that the purpose of the index is to express habitat in a biologically meaningful manner, these variables add noise to the index.

2. Currently, there is no quantitative understanding of whether the HQI protocols adequately capture reach-level habitat. Five to six transects across 160 – 500 m reaches results in a very small proportion of the reach being surveyed. EPA EMAP habitat protocols call for 11 transects for channel morphometrics, and further adds 10 more between each pair of transects to better characterize substrate particle sizes and embeddedness (EMAP reference). TCEQ might consider organizing a simple study in which multiple reaches of varying sizes are surveyed to determine the number of transects necessary to adequately characterize each habitat measure within a reach. The same study could be set up to examine variation in habitat measures across environmental gradients representing various degrees of degradation.

3. An alternative or potentially complimentary approach to recommendation #2 is a modified version of Hawkins et al. (2001) vegetation cover protocols, which yields a reach-wide assessment of aquatic macrophyte, macroalgae, and periphyton percentage cover and thickness of biofilm cover. The method entails walking a zigzag transect from one end of the reach to the other and visually assessing vegetation cover at each of 100 equally-spaced points located along the transect. This protocol was modified for the nutrient criteria project to include an index of sediment film thickness on stream substrate (sediment cover index), dominant substrate (percent of different particle sizes in the reach), an estimate of flow velocity (flow index), and water depth (King et al. 2009). This survey is rapid to deploy, could be further modified to include measures of instream cover within a defined radius of each sample point, and combined with reach-level data

(number of pools, riffles, ect. and transect data (width, depth, bank angle, erosion potential, etc.) for a more comprehensive habitat assessment.

4. Distilling habitat data into ten metrics with four categorical values probably masks some aspects of habitat degradation, and thus reduces sensitivity relative to environmental impacts. HQI scores need to be able to reflect relative positions along environmental gradients. We suggest increasing the number of bins (10 or 20) or moving to a continuous scale (0-100) for existing or future HQI variables. EPA rapid bioassessment protocols score each variable on a scale of 1 – 20 (reference).

5. Ultimately, HQI protocols and metrics need to be ecoregion specific. If fish community structure and species composition show ecoregional affinities and fish are limited by habitat quality, then it is reasonable to expect habitat structure to vary regionally. Therefore we should expect a habitat assessment protocols to account for ecoregional differences much like current IBI protocols do.

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