SPATIAL PATTERNS IN TEXAS LOTIC FISH COMMUNITIES

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Michael S. Lane, B.S.

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Michael S. Lane

APPROVED BY

______________________________
George Guillen, Ph.D., Chair

______________________________
Cindy Howard, Ph.D., Committee Member

______________________________
Dongmin Sun, Ph.D., Committee Member

______________________________
Ju H. Kim, Ph.D., Associate Dean

______________________________
Zbigniew Czajkiewicz, Ph.D., Dean
DEDICATION

For my father, Andrew Lane (1960-2014).

Thank you for always pushing me towards greatness and being a superb role model. Your guidance is cherished and will never be forgotten.
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This project was a real undertaking and I am extremely grateful for all the help I received throughout the entire process.
Spatial patterns in Texas lotic fish communities have been influenced and restructured by anthropogenic modification. These modifications and pursuant changes are well documented in the literature. The need for a statewide (and national) assessment to evaluate the status of freshwater streams using fish communities and their habitats was most recently addressed by the EPA National Rivers and Streams Assessment (NRSA). During the summers of 2013 and 2014, the Environmental Institute of Houston (EIH) used a probabilistic sampling design to survey various reaches of rivers and streams across Texas, sampling fish at nearly all sites through electrofishing. Utilizing GIS software (ArcMap), a watershed analysis was conducted relative to these sites. The relationship between major river basins, land use/land cover type and fish communities was examined. Fish communities exhibited distinct patterns with regards to richness and diversity gradients across Texas, however the number of sample sites in some basins was insufficient to conduct an analysis of factors affecting fish communities within these watersheds. Land use patterns across the major basins were reflected by delineated
watersheds relatively well, with some bias associated with specific watersheds. Relationships between fish communities and land use/land cover did provide significant evidence ($P < 0.05$) that diversity was lower in drainages with higher agricultural and developed land use, but higher in drainages with more forested land. Broad scale spatial analysis of Texas lotic fish communities indicated that land use was as much as 33.9% correlated with fish community structure. The data further suggests that other variables such as physical habitat, water quality, and flow should be incorporated into future analyses in order to better predict variability in fish community structure not captured by land use/land cover data. Additionally, expanding the number of sample sites in some basins and including additional sampling gear (such as seines) would improve the ability to accurately assess fish community structure in future surveys.
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INTRODUCTION

On a global scale, modern freshwater ecosystems have been influenced by industrialization (Smith and Darwall 2006), urbanization (Walsh et al. 2005), and increased water demands for agriculture (Allan 2004). Texas freshwater ecosystems have also been subjected to the same suite of anthropogenic stressors. Amidst the development of many (ongoing) contemporary activities including reservoir development, increased freshwater diversions, additional wastewater loads, and changing land use, the freshwater systems of the state have exhibited significant changes in hydrology, water quality, and geomorphology. Anthropogenic activities continue to modify Texas rivers and streams which can disrupt native aquatic organism assemblages and threaten the survival of sensitive freshwater species (Higgins 2009).

The concern for the United States' imperiled freshwater ecosystems, specifically rivers and streams, has been highlighted by the implementation of the Environmental Protection Agency's (EPA) National Rivers and Streams Assessment (NRSA). This comprehensive study utilizes a broad variety of sampling methodologies that incorporate physical, chemical, and biological data to determine the status and health of the nation's rivers and streams and what major stressors affect them. The goal of the NRSA program is to determine whether or not the condition of the United States' rivers and streams is improving and in what ways we can best protect and restore them.

Fish Communities and Historical Context

A major component of the NRSA program is the collection of river and stream fish community data. The fish community data collected for NRSA in the state of Texas and the
subsequent analysis of Texas lotic fish communities serves as the basis for this thesis project. Fish serve as a useful taxa group when assessing stream health for several reasons. Fish are sensitive to anthropogenic influences and their response to various forms of anthropogenic stress can be easily monitored (Adams et al. 1996; Argent and Carline 2004). Fish also exhibit diverse life histories (Argent and Carline 2004; Heino et al. 2013), thus they occupy a variety of lotic habitats (Aadland 1993; Vannote et al. 1980). In conjunction with their diverse life histories, fish occupy multiple trophic levels, utilize various substrate types for spawning, and respire through water, therefore their communities are structured by a broad suite of environmental conditions (Argent and Carline 2004). Some fish exhibit tremendous longevity and their populations and communities can reveal long-term effects of environmental stress (Karr 1981). In summary, fish populations and communities are ideal metrics for evaluating the health and condition of rivers and streams.

Texas has a very diverse composition of freshwater fishes, with 268 nominal species in 49 families (Hubbs et al. 2008). Of the portion of these species that are considered widely distributed, 91% of them find range terminations southwesterly, while only 9% find range terminations northeasterly (Conner and Suttkus 1986). This coincides with the fact that there is a longitudinal gradient in species richness, decreasing from east to west. The Sabine and Neches drainages contain entirely lowland fish species which exhibit strong similarities with drainages to the east, notably the Calcasieu. From the Trinity drainage westward there is an increasing number of upland species (while still decreasing in total species richness). Conner and Suttkus (1986) suggested that the Nueces drainage marked the southwestward terminus for most, if not all of the eastern lowland/Mississippi Valley ichthyofaunal elements.

In regards to Texas' many species, an alarming 44% of them are considered imperiled (Hubbs et al. 2008). Trends documented From the Anderson et al. (1995) study include
decreases in the relative proportions of cyprinids, ictalurids, percids, catostomids, and cyprinodontids over three decades, while poeciliids, atherinopsids, and clupeids all showed increases. Additionally, there were greater changes in the eastern drainages than those in the western portion of the state (Anderson et al. 1995). Hubbs et al. (1997) described these changes to include regional homogenizing of assemblages, greater faunal overlap among river systems, as well as increased variation in assemblages within drainages. Reservoir construction, pollution (i.e. waste dumping, fertilizers in runoff, etc.), dredging, intrusion by saline water, and the introduction and proliferation of exotic species have all been implicated as major factors responsible for changes in fish assemblages (Anderson et al. 1995).

**Land Use Trends**

Aquatic ecosystems are strongly influenced by their surroundings at multiple scales (Allan et al. 1997; Fausch et al. 2002; Schlosser 1991; Townsend et al. 2003) and it has been consistently recognized that rivers and streams are affected by the watersheds they flow through (Hynes 1975; Vannote et al. 1980). The natural condition of streams and their associated biota have evolved in response to the surrounding topography, geology, and soils (Conner and Suttkus 1986; Heino et al. 2013). Anthropogenic modification however continues to restructure stream communities through modification of hydrology, land use and cover, and pollutant loadings (Anderson et al. 1995). As natural processes have been interrupted, fauna (such as fish species) have been forced to adapt at an unprecedentedly fast rate compared to the evolutionary timespan in which they had evolved (Heino et al. 2013). At a large scale, land cover alteration is perhaps the biggest driver of habitat and biota degradation. The two most prevalent land disturbances have historically arisen from activities associated with agriculture and urbanization (Allan 2004).
Agricultural activities represent a major portion of the economy in Texas. The cattle industry and agriculture are both priorities for Texans' river water usage, not to mention the importance of the river valleys' fertile soils in farming (Stewart et al. 1993). Agricultural water demands have put a strain on aquatic ecosystems through water withdrawals and diversions for irrigation and watering of livestock. Beyond water usage agriculture has influenced rivers and streams through increased loading from nonpoint source pollution and riparian zone removal (Allan 2004). Additionally, sedimentation in rivers and streams is a major issue attributed to agricultural activities (Walser and Bart 1999; Zimmerman et al. 2003). Wang et al. (1997) reported that agricultural lands support fewer sensitive fish species than streams in forested catchments. While agriculture makes up the largest portion of land cover in many disturbed catchments (as compared to urban land use), recent trends in Texas show significant land conversion from private farm, ranch or forest lands to urban uses (Anderson et al. 2014).

Urban land use is typically a low percentage of total drainage area, yet its effects are disproportionately large both locally and at larger scales (Paul and Meyer 2001). Urbanization leads to a myriad of effects on lotic systems, many of which are considered detrimental to freshwater fish species (Klein 1979; Miltner et al. 2004; Walsh et al. 2005; Wang et al. 2000). The development of new subdivisions and municipalities often creates geometric landforms over the natural geography of an area which requires modifications of adjacent meandering rivers and streams. Historically, developers and municipalities have typically dealt with this problem by straightening/channelizing adjacent streams and rivers. This process of removing meanders in a stream is called “rectification” and was widely practiced in the past to reduce flood risks in urban areas (Langhammer and Vajskebr 2003). Rectification creates straighter streams containing little, if any of the former instream or riparian habitat, and highly variable, “flashier” fluctuations in stream discharge (Walsh et al. 2005). The other major contributor to
flashiness has to do with increased and more rapid runoff conveyance as a result of increased impervious surfaces in surrounding urban land. These impervious surfaces also serve as a vector for increased amounts of nonpoint pollutants in runoff (Paul and Meyer 2001).

Due to the multidimensional nature of stream ecology and the interaction of disturbances, it is often very difficult to match a response to the appropriate stressor (Allan 2004). Historically, agricultural and urban land use impacts have been studied at a local scale, however Wang et al. (1997) pointed out that most of these effects occur at a larger watershed scale. They noted that very few studies have investigated land use effects on rivers and streams at the catchment scale. Until recently, this approach was considered very difficult (Heino et al. 2013), but as Argent and Carline (2004) discussed, the advancement of geographic information systems (GIS) has significantly promoted the study of land use effects on rivers and streams on a large scale. The use of this software has promoted studies that have reviewed the effects scale when measuring the influence of land use on fish communities (Allan 2004; Lammert and Allan 1999).

Research Needs and Objective

The need for this research arises out of the lack of recent spatial and temporal analysis of Texas lotic fish communities on a large scale. Published statewide historical trends have only been compiled as recently as 1986 (Anderson et al. 1995; Hubbs et al. 1997), and the EPA’s NRSA 2008-2009 and 2013-2014 NRSA collections for Texas have only been examined at larger regional and national scales. One objective of this study was to examine historical trends in Texas freshwater fishes and compare those results with those of the most recent collections (2013-2014).
Another objective of this study was to characterize Texas freshwater fish communities and determine potential watershed scale factors affecting their distribution. The necessity to examine rivers and streams at a large spatial scale has been prompted by researchers (Heino et al. 2013; Wang et al. 1997). GIS technologies were utilized to address this objective.

The final objective of this study was to determine the status of freshwater fishes in Texas streams and rivers relative to NRSA sampling protocols and to make recommendations for the future NRSA surveys based on this study’s findings. This was an extremely important aspect to this research as the EPA is using the NRSA data to analyze the entire nation’s rivers and streams to eventually be applied towards management practices.
METHODS

Site Selection

Sites for this study were selected by the EPA using a probabilistic survey design. Nationwide, 900 sites classified as Strahler order 1-4 and 900 sites classified as Strahler order 5 and greater were chosen for sampling. The sample frame was derived from the National Hydrography Dataset (NHD) from 1:100,000 scale maps, thus further reconnaissance utilizing satellite imagery was required for some sites to determine if they were targets (EPA 2013a; EPA 2013b). Sixty-eight of the selected sites were within Texas. Texas NRSA sampling was contracted to the Texas Commission on Environmental Quality (TCEQ) who then subcontracted the sampling to the Environmental Institute of Houston (EIH).

Study Area

The study area for NRSA encompassed the 48 continental United States, however the focus of this study was limited to the state of Texas. Texas is unique in that its large area extends into three physiographic provinces including the Gulf Coastal Plain, the Central Lowlands, and the Great Plains (Fenneman 1938). In Texas, the Great Plains province is subdivided into the Pecos Valley, Llano Estacado, Edwards Plateau, and Central Texas while the Osage Plains subdivision of the Central Lowlands overlaps from the Oklahoma border. The western edge of the Coastal Plain is defined by the border between Cretaceous and older rocks as far south as Waco, on the Brazos River; below this point however, between the Brazos and Colorado rivers, there is an outcropping of Lower Cretaceous rocks that is included in the Central Texas subdivision of the Great Plains (Conner and Suttkus 1986). South of the Colorado River the Coastal Plain boundary is defined by the Balcones Escarpment (Figure 1). The Coastal
Plain includes an inner zone of eroded scarps and lowlands that slopes gently onto an outer zone of terraced deltaic and alluvial plains. In upper section of the Great Plains, known as the High Plains (Pecos Valley and Llano Estacado), the Ogallala Formation and several other fluviatile deposits dominate the topography. These deposits were once even more widespread in the Great Plains province, but over time were removed from the Edwards Plateau and Central Texas subdivisions. On the Edwards Plateau, the removal of this “fluviatile veneer” exposed Cretaceous limestone that, in some places, exhibits karst topography (Conner and Suttkus 1986). The most significant relief in these regions is found along major stream valleys and along the Balcones Escarpment. The last region, the Osage Plains of the Central Lowlands is eastward-
sloping, consisting of flatlands, depressions, low escarpments, and exposures of primarily Pennsylvanian and Permian rocks (Conner and Suttkus 1986).

Within these physiographic provinces exists a region known as the “western Gulf Slope” which is essentially the range of the Texas (and western Louisiana) coast encompassing the major drainages between the Mississippi River basin and Rio Grande basin. Due to the distinctiveness of the Mississippi and Rio Grande as distributional barriers for fish, this range of intermediate drainages forms a convenient unit for ichthyogeographic study (Hubbs 1957). A closer examination of the topography and climate of these watersheds provides more background on their characteristics. Similarities can be seen between the Nueces, San Antonio, Guadalupe, and Trinity rivers as they all arise on sedimentary rocks, Coastal Plain clays, sands, and soft limestones. Between these drainages, the Brazos and Colorado rivers flow across the same aforementioned terrain, however their headwaters rise on clays, sands, gravels, and caliches of the Llano Estacado. Conversely, the Neches and Sabine rivers to the east only flow over Coastal Plain clays, sands, and limestones. These most eastern Texas drainages are confined to a humid subtropical zone, whereas drainages from the Brazos River westward flow from semiarid steppes in their upper reaches to dry/dry-subhumid zones in their lower reaches (Conner and Suttkus 1986). These climatic zones translate into an overall east to west pattern of increasing aridity and decreasing annual precipitation (Carr 1967).

As previously mentioned, 68 of the nationally selected sample sites fell within Texas. However, due to endangered species permit limitations fish collections were only permitted at 64 of these sites. These sites ranged in size from zero order headwater streams to seven order major rivers. Additionally, these sites exhibited a random distribution and encompassed nearly
all of the state’s major river basins. There was one site in the Canadian River drainage, five sites in the Red River drainage, four sites in the Sabine River drainage, five sites in the Neches River drainage, ten sites in the Trinity River drainage, fourteen sites in the Brazos River drainage, ten sites in the Colorado River drainage, four sites in the Guadalupe River drainage, one site in the San Antonio River drainage, four sites in the Nueces River drainage, three sites in the Rio Grande drainage, and three sites classified as coastal drainages. Thus, the majority of the (Texas) sites sampled for the 2013-2014 National Rivers and Streams Assessment lie in the western Gulf Slope region. Due to the limited number of sample sites in “extralimital” drainages (defined by Conner and Suttkus (1986) as those drainages outside the western Gulf Slope), several of these sites (Rio Grande, Canadian and Red drainages) were not included in the study area analyzed in during this study. Additionally, to simplify analysis and add precision to the characterization of western Gulf Slope drainages in Texas, coastal drainages have been removed from the study area. These coastal drainages were those that discharged directly into a bay system rather than into one of the major basins, and by removing them statistical variability was reduced. The adjusted study area included 52 of the 64 fish collection sites, however one Trinity River drainage stream yielded no catch, thus it was excluded from the dataset reducing the final set of sites to 51. Lastly, since there was only one site in the San Antonio drainage, this drainage was combined with the Guadalupe drainage for analysis based on proximity and a shared delta and named “SanGuad” (Figure 2). For specific locations and details of each sample site see Table 5 in the Appendix.
Figure 2. Map of study area including sample sites (red dots) and major river basins.

Field Sampling

The National Rivers and Streams Assessment involved comprehensive stream surveys. These surveys included characterizing the physical, chemical, and biological aspects of each sample site (USEPA 2013a; USEPA 2013b). Field crews characterized physical habitat(s): measured dimensions, estimated sediment size and type, characterized riparian vegetation, and counted woody debris presence. Additionally, water quality and flow were measured utilizing a YSI multiparameter sonde and SonTek Flowtracker, respectively) and water, benthic invertebrate, and fish samples were collected. Data collected in relation to fish community characterization was the primary focus of this study.
Using the coordinates provided by the EPA, target sampling sites were located with Google Earth prior to travel, and precisely located with GPS units upon arrival. These precise locations were referred to as “X-site(s)” and served as the midpoint of the sampling reach for each site (USEPA 2013a; USEPA 2013b). If the channel was dry at the X-site, it was examined 75 meters (m) upstream and downstream for isolated pools; the EPA required that water be in at least 50% of the length of the channel to consider a site “sampleable”.

In order to lay out the sampling reach, the wetted width of the channel was measured at five locations within approximately five channel widths upstream and downstream of the X-site. These channel widths were averaged, and this average was multiplied by 40 to find the total reach length; the total reach length was then divided by ten to separate the sampling reach into ten equal subreaches separated by transect lines A-K. If the average channel width was <4 m, then 150 m was used as the minimum reach length, and if the average channel width was >100 m, then four kilometers was used as a maximum reach length. Based on average channel widths, the rivers and streams that were sampled were classified as small (<12.5 m), medium (12.5-25 m), or large (>25 m). Small stream sampling reaches were between 150-500 m in length and required that all 10 subreaches be fished in their entirety (from bank to bank) beginning at transect A. Medium stream sampling reaches were between 500-1000 m in length and large stream sampling reaches were between 1000-4000 m in length; both medium and large streams required that each subreach be fished in an 8 m swath from the bank, alternating banks every two subreaches. Additionally, if 500 fish were captured within the first 500 m of the sampling reach of medium streams or first five subreaches of large streams then sampling effort was considered complete and terminated (USEPA 2013a; USEPA 2013b).

Fish collections were done primarily by electrofishing as mandated by the EPA, while seining was only implemented where electrofishing was found to be inadequate. Sample sites
were classified as wadeable or “boatable” (non-wadeable) and the main difference in (fish) sampling procedures was how the sampling reach was oriented relative to the stream’s direction of flow and the gear used (Figure 3 and Figure 4). For wadeable streams, electrofishing was most often conducted with a barge shocking unit (Smith-Root RCB-6B junction box with a 2500 watt generator), and occasionally with a Smith-Root LR-24 backpack shocking unit (<500 watts) on sites where logistics prevented the use of the barge. At boatable sites, electrofishing was primarily conducted with an electrofishing boat (Smith-Root GPP with 5000 watt generator) and if access prevented the launching of this heavy boat a smaller aluminum boat was utilized with the barge shocking equipment. While electrofishing, EPA guidance was followed when “irruptive species” were encountered. Irruptive species were defined as species of fish which are found in locally (extremely) abundant “patches”. When these species were encountered, sampling crews would quickly move through these patches to avoid skewing relative abundance (USEPA 2013a; USEPA 2013b).

Fish collected while sampling were typically identified in the field using regional taxonomic guides (Thomas et al. 2007) and taxonomic keys (Hubbs et al. 2008) after each subreach was fished. If identification timeliness or uncertainty were issues, smaller specimens were preserved in 10% formalin and brought back to the laboratory for identification. These specimens were transferred to 70% ethanol and identified between field sampling events. Unidentified specimens or suspect identifications were removed from the dataset prior to analysis.
Figure 3. Sample reach layout for wadeable streams as prescribed by the EPA. The diagram includes stream size classifications and instructions for fish collection at each stream size. Note the direction of flow relative to transects A-K. This figure was reproduced from (USEPA 2013b).
Figure 4. Sample reach layout for non-wadeable streams as prescribed by the EPA. The diagram includes stream size classifications and instructions for fish collection at each stream size. Note the direction of flow relative to transects A-K. This figure was reproduced from (USEPA 2013a).
Data Analysis

Fish Community Analysis

Fish communities were characterized by calculating total species abundance (N), species richness (S), and number of families for each site, basin, and Texas as a whole. Additionally, species diversity (H’) was calculated for sample sites. Utilizing Minitab 17 software, boxplots were constructed to display species richness (S) and diversity (H’) for major river basins (Sabine, Neches, Trinity, Brazos, Colorado, SanGuad, and Nueces). To further characterize the major river basins, the abundance of each species was totaled by basin and entered into PRIMER 6 software. In PRIMER, abundance data was fourth root transformed as recommended by Warwick (1988) to reduce variance. Similarity in fish communities using transformed abundance data between basins was evaluated using the Bray-Curtis similarity index. The resulting resemblance matrix was utilized as input for cluster analysis to produce a dendrogram displaying the similarity between major basins by percent similarity. The SIMPROF analysis procedure was performed to test for significant structure in the data.

In addition to characterizing Texas lotic fish communities based upon the 2013-2014 NRSA fish collections, the data was compared to 2008-2009 NRSA fish collection data and less recent historical data. Several approaches were used. First, sites that were sampled during both NRSA surveys were compared by computing the percent change between fish assemblages. The percent change was calculated as the reciprocal of the percent similarity index developed by Renkonen and Whittaker (Gorman and Karr 1978). The percent change results for each site were then averaged by basin. Further comparisons were made between the 2008-2009 and 2013-2014 datasets utilizing multivariate analysis. In this analysis, all sites from each NRSA survey within the major basins were included to provide a larger number of sample points and an alternative perspective on this historical comparison. The species abundance (N)
data for each of these sample sites was entered into PRIMER on separate worksheets for each basin. Each sites’ data was entered with the year(s) sampled as a factor. Similar to the previously described cluster analysis, the data was first fourth root transformed and then compared using a Bray-Curtis similarity index. Then, each resemblance matrix was plotted through non-metric multidimensional scaling (NMDS) and analyzed through a one-way analysis of similarity (ANOSIM) testing to see if the fish assemblages were significantly different in each basin between NRSA surveys. In order to observe more long-term trends in freshwater fishes of Texas, the data was also compared to data collected by Clark Hubbs and others in 1953 and 1986 (Anderson et al. 1995). The unavailability of raw data from Anderson et al. (1995) precluded in depth comparisons, but relative proportions of major families were able to be extracted from their figures. Bar charts were produced with SigmaPlot 13 to compare the relative proportions of the most prevalent families in each major basin between 1953, 1986, and 2013-2014.

GIS Analysis

After conducting fish community analysis, GIS software (Esri ArcMap 10.2 and 10.3) was utilized to conduct a series of watershed analyses. The main goals of these analyses were to delineate watersheds relative to each sample site as well as calculate the areas of each land use classification within each of these delineated watersheds.

The watershed analysis began after digital elevation models (DEMs) were obtained for each of the major river basins from DEMs of Texas and Louisiana (Louisiana was needed to delineate the Sabine River watershed). After DEMs were obtained, a series of ArcMap tools were utilized to complete watershed analysis for each basin. First, the ‘Fill (Spatial Analyst)’ tool was run to fill in and remove any topographic “sinks” so that there are no issues determining where the flow accumulates later. Next, the ‘Flow Direction (Spatial Analyst)’ tool was run on
the filled raster layer in order to code the directions in which water will flow when it precipitates on land. Afterwards, the next step was to run the ‘Flow Accumulation (Spatial Analyst)’ tool to produce the accumulated flow for each raster cell. Upon the completion of this step, the stream network was visible as lightly colored lines across a black landscape. The sample sites were then “snapped” into place on the stream network utilizing the ‘Snap Pour Point (Spatial Analyst)’ tool. The final step was to then run the ‘Watershed (Spatial Analyst)’ tool, which produced a layer of various colored polygons representing the contributing drainage area upstream of each sample site (Figure 5). Since each raster cell represented an area of 30 x 30 m (900 m²), the contributing drainage area could be calculated by multiplying the cell counts within each polygon by 900 m². When there were smaller, separately delineated watersheds upstream of a larger watershed, it was important to still include the smaller watershed’s drainage area within the larger watershed’s drainage area. This was done by simply adding these smaller drainage areas into the appropriate larger watersheds’ drainage areas when necessary.
Figure 5. An example of the ‘Watershed Analysis’ output in ArcMap for sample sites in the Colorado River basin. Note that in order to accurately calculate the upstream drainage area for some sample sites, other delineated watersheds may need to be included i.e. the series of connected watershed polygons on the mainstem Colorado River.

Once the watersheds upstream of each site were delineated, they were layered over top of the National Land Cover Database (NLCD) land cover map in order to approximate the area of each land cover class in each watershed (Homer et al. 2015). Each basin’s watershed layer was run through the ‘Raster to Polygon (Conversion)’ tool in order to “vectorize” them. Polygons were not simplified during this conversion in order to maintain as much precision as possible. After vector layers of each watershed in each basin were created, the ‘Project (Data Management)’ tool was used to convert the coordinate system because each watershed was
originally delineated in a geographic coordinate system (NAD 1983) whereas the NLCD land cover map was created in a projected coordinate system (Albers Conical Equal Area). Any miscellaneous polygons that were not originally grouped into the vectorized watersheds were “dissolved” into the appropriate watersheds utilizing the ‘Dissolve (Data Management)’ tool. Lastly, the ‘Tabulate Area (Spatial Analyst)’ tool was run in order to tabulate the area of each land cover class in the delineated watersheds.

Land Use Analysis

After the completion of GIS analysis, land cover class areas were output into tables for each basin and were grouped by the following classes: open water, developed - open space, developed - low intensity, developed - medium intensity, developed - high intensity, barren land, deciduous forest, evergreen forest, mixed forest, shrub/scrub, grassland/herbaceous, pasture/hay, cultivated crops, woody wetlands, and emergent herbaceous wetlands. To simplify analysis, these classes were grouped into the following classes: water (open water), developed (developed - open space, developed - low intensity, developed - medium intensity, and developed - high intensity), barren (barren land), forest (deciduous forest, evergreen forest, and mixed forest), shrubland (shrub/scrub), herbaceous (grassland/herbaceous), planted/cultivated (pasture/hay and cultivated crops), and wetlands (woody wetlands and emergent herbaceous wetlands). Additionally, the area (m²) of each land cover class were converted to percentages for each sample site, and then averaged across basins. The percentages of each land cover class in the entirety of each major basin (encompassing the delineated watersheds and beyond) were also examined.

Principal Components Analysis (PCA) was run utilizing Canoco 4.5 software in order to assess land use variation between watersheds. Principle Component (PC) scores and loadings were plotted utilizing SigmaPlot in order to group scores by basin and observe how they
ordinated relative to land cover types (displayed as loadings). PC scores were grouped by basin and compared through a one-way analysis of variance (ANOVA) to detect for significant differences. Tukey pairwise comparisons ($\alpha = 0.05$) were made post-hoc in order to determine which basins differed from each other. In addition to ANOVA, PC scores were utilized for regression analysis. Scores for PC1 and PC2 were each regressed against diversity ($H'$) and richness ($S$) scores for each basin individually and then all sites sampled as a whole to assess significant relationships between land use and fish communities.

A final analysis between land use and associated fish communities was done through the BEST procedure in PRIMER. This procedure selects a subset of environmental variables (in this case drainage area of each land use type) that maximize rank correlation ($\rho$) between them and biotic variables (in this case fish communities) utilizing (dis)similarity matrices by analyzing all combinations of environmental variables (Clarke et al. 2014). In PRIMER, the fish community data was fourth root transformed and compared using a Bray-Curtis similarity index as in the previous analyses. On a separate worksheet, the land use areas were entered and Log $(X+1)$ transformed due to the wide range of drainage area sizes associated with the sample sites, which resulted in a wide range of land use areas. Then, the BEST procedure was run utilizing all combinations of land use areas, Spearman rank correlation method, and Euclidean distance for the environmental data similarity matrix. The best combination of land use areas and their correlation with the fish community data were the output. Additionally, NMDS ordinations of the fish community data and the (subset of) land use data were created in PRIMER as a visual supplement to the BEST results.
RESULTS

Fish Communities

A total 29,378 individuals were collected from 51 sample sites across the western Gulf Slope drainages of Texas (seven major basins) over the course of two summer field seasons. These individuals consisted of 22 families and 94 different species. The two most abundant families were Cyprinidae and Centrarchidae (14,768 and 9,683 individuals, respectively). The most abundant species were Cyprinella lutrensis (6,687 individuals), Pimephales vigilax (4,610 individuals), Lepomis macrochirus (3,727 individuals), Lepomis megalotis (2,531 individuals), and Gambusia affinis (1,803 individuals). The following species were also found at the majority of the sample sites: C. lutrensis (30 sites), P. vigilax (34 sites), L. macrochirus (45 sites), L. megalotis (45 sites), and G. affinis (44 sites). Other frequently captured species included Lepomis cyanellus (1,153 individuals, 35 sites) and Micropterus salmoides (434 individuals, 34 sites). The abundance of fishes was correlated with the species richness ($r^2 = 0.6395$) in the major basins and the most individuals, species, and families were collected from the Brazos River basin (Table 1).

<table>
<thead>
<tr>
<th>Basin</th>
<th># of Sites</th>
<th># of Individuals</th>
<th># of Species</th>
<th># of Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabine</td>
<td>4</td>
<td>741</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Neches</td>
<td>5</td>
<td>1041</td>
<td>46</td>
<td>11</td>
</tr>
<tr>
<td>Trinity</td>
<td>9</td>
<td>4929</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>Brazos</td>
<td>14</td>
<td>12691</td>
<td>61</td>
<td>16</td>
</tr>
<tr>
<td>Colorado</td>
<td>10</td>
<td>8422</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>SanGuad</td>
<td>5</td>
<td>695</td>
<td>38</td>
<td>13</td>
</tr>
<tr>
<td>Nueces</td>
<td>4</td>
<td>859</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>Overall</td>
<td>51</td>
<td>29378</td>
<td>94</td>
<td>22</td>
</tr>
</tbody>
</table>
Species richness (S) and diversity (H’) differed among sample sites, ranging from 2 to 25 and 0.390 to 2.703, respectively. Similarly, these metrics differed between basins as well. Comparisons of the fish communities in overall basins found that total richness (S) ranged from 32 (Nueces) to 61 (Brazos), and overall diversity (H’) ranged from 2.111 (Brazos) to 2.891 (Neches). Accordingly, average species richness (S) ranged from 13.5 (Nueces) to 18 (Neches) and average diversity (H’) ranged from 1.654 (Trinity) to 2.032 (Neches). These means were projected in addition to the medians, first and third quartile ranges, upper and lower limits, and outliers on boxplots (Figure 6 and Figure 7).

**Figure 6.** Boxplot of site species richness (S) by major basin. The crosshair symbols represent mean richness, while the horizontal lines represent median richness. The upper box represents the third quartile (Q3) while the lower box represents the first quartile (Q1). The whiskers represent the highest and lowest values within the upper and lower limits.
Hierarchical cluster analysis revealed basin relationships based on fish assemblage similarity. The Brazos and Trinity basins were the most similar at 62.9%, followed by the Sabine and Neches at 61.1%. Next, the group average of the Trinity and Brazos was 60.2% similar to the Colorado basin. The SanGuad and Nueces were 55.2% similar, and their group average was 49.3% similar to the group average of the Trinity/Brazos and Colorado. Lastly, the Sabine and Neches were most dissimilar from the other five basins as their group average was only 45.7% similar to the group average of the Trinity/Brazos/Colorado and SanGuad/Nueces (Figure 8). SIMPROF analysis identified three significant clusters of basins (Sabine/Neches, Trinity/Brazos/Colorado, and SanGuad/Nueces).
**Figure 8.** Dendrogram portraying the outcome of cluster analysis. Basins are linked based on the group average method utilizing fourth root transformed fish abundance data. Fish assemblage similarity is indicated by the length of each branch linking the basins together. The main clusters are indicated by orange linkages and separated by the black linkages.

A large variation in average percent change between the major basins was observed when comparing only paired sites sampled during both 2008-2009 and 2013-2014 (Figure 9). The percent change by basins were as follows: Sabine (41.5%, n=2), Neches (74.2%, n=2), Trinity (35.6%, n=4), Brazos (60.8%, n=3), Colorado (54.3%, n=2), SanGuad (41.9%, n=2), and Nueces (64.7%, n=2). Overall, the percent change in fish assemblage between the two NRSA events was 51.7% (n=17).
Figure 9. Interval plot representing the average percent change in fish assemblages for sites in each basin between the 2008-2009 and 2013-2014 NRSA surveys. The points represent means and the bars represent standard error.

Multivariate analysis on all abundance (N) data from both NRSA surveys offered an alternate perspective on this historical comparison. Utilizing all the sample sites’ (from within the major basins) data and not just paired sites allowed for a total of 84 sample points instead of 17. One-way ANOSIM with year(s) sampled as the factor provided that there were almost no significant differences in the fish assemblages within most basins between the two NRSA surveys. The Colorado basin, however, did show a significant difference in the fish assemblages observed between 2008-2009 and 2013-2014 ($P = 0.032$, Global $R = 0.227$, n=17). MDS provided a visual of how each basin's sample points fell in space relative to one another, and the Colorado sites clearly showed the highest affinity for grouping by year(s) sampled (Figure 10).
Figure 10. NMDS plot of Colorado River basin sites sampled for two NRSA surveys. The groupings are not completely distinct, but there is a significant difference between the year(s) sampled.

The final historical comparisons were made between the NRSA 2013-2014 dataset and data collected by Clark Hubbs and others (Anderson et al. 1995). Relative proportions of the dominant families in each basin across three sampling periods (1953, 1986, and 2013-2014) varied greatly over time. The fish collections in the eastern basins (Sabine, Neches, Trinity, and Brazos) exhibited a reverse trend in the Cyprinidae family from what Anderson et al. (1995) had observed, which was an overall decline in cyprinids from 1953 to 1986. The proportions indicate an overall increase from 1986 to 2013-2014 with proportions in the Neches [45.9% (2013-2014) versus 8.8% (1986) or 30.7% (1953)], Trinity [43.5% (2013-2014) versus 9.3% (1986) or 35.6% (1953)], and Brazos [73.2% (2013-2014) versus 43.6% (1986) or 59.1% (1953)] exceeding the historical data’s for either sampling period. Conversely, this study’s dataset showed agreement with Anderson et al. (1995) in regards to Centrarchidae, continuing the increasing trends in
proportion of this family in the Sabine and Brazos basins and the decreasing trend in the Neches basin (Figure 11). In the western basins (Colorado, SanGuad, and Nueces), Anderson et al. (1995) observed increases in the relative proportions of cyprinids in the SanGuad and Nueces basins, while this study’s data showed a major decline in cyprinids, with proportions lower than either of the historical records. Additionally, recent NRSA data indicated large increases in the relative proportions of centrarchids in all three western basins, and was the dominant family represented in the Colorado (54.7%), SanGuad (38.4%), and Nueces (67.9%) basins (Figure 12).
Figure 11. Bar charts displaying the overall relative proportions of dominant families in the major eastern basins for three sampling periods: 1953 (dark gray), 1986 (light gray), and 2013-2014 (black).
Land Use

Land cover areas relative to delineated watersheds varied considerably across the major basins of Texas. The dominant land cover transitioned from forest in the east to shrubland in the west. The highest proportion of urban (developed) land use existed in Trinity basin watersheds (35.9%), while the highest proportion of agricultural (cultivated) land use existed in
Brazos basin watersheds (26.7%). The proportion of developed land in the delineated Trinity watersheds was substantially greater than the proportion of developed land in the overall basin, which was only 15.0%. On the other hand, the proportion of cultivated land in the delineated Brazos watersheds (26.7%) represented the overall basin (31.4%) quite accurately (Table 2).
**Table 2.** Summary of land use/land cover percentages by basin based on data from the drainage areas upstream of the NRSA sample (fish collection) sites in Texas.

<table>
<thead>
<tr>
<th>Basin</th>
<th>Drainage Area</th>
<th>km²</th>
<th>Water</th>
<th>Developed</th>
<th>Barren</th>
<th>Forest</th>
<th>Shrubland</th>
<th>Herbaceous</th>
<th>Cultivated</th>
<th>Wetlands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabine</td>
<td>20040.79</td>
<td></td>
<td>2.91</td>
<td>19.81</td>
<td>0.54</td>
<td>31.72</td>
<td>11.13</td>
<td>5.50</td>
<td>21.22</td>
<td>7.16</td>
</tr>
<tr>
<td>Neches</td>
<td>6141.03</td>
<td></td>
<td>1.72</td>
<td>5.13</td>
<td>0.17</td>
<td>36.77</td>
<td>7.20</td>
<td>1.18</td>
<td>24.49</td>
<td>23.35</td>
</tr>
<tr>
<td>Trinity</td>
<td>42686.82</td>
<td></td>
<td>0.97</td>
<td><strong>35.87</strong></td>
<td>0.11</td>
<td>10.37</td>
<td>1.84</td>
<td>31.84</td>
<td>18.26</td>
<td>0.73</td>
</tr>
<tr>
<td>Brazos</td>
<td>100009.64</td>
<td></td>
<td>0.67</td>
<td>7.07</td>
<td>0.33</td>
<td>15.28</td>
<td>22.95</td>
<td>25.81</td>
<td><strong>26.65</strong></td>
<td>1.24</td>
</tr>
<tr>
<td>Colorado</td>
<td>70991.02</td>
<td></td>
<td>0.23</td>
<td>11.71</td>
<td>0.10</td>
<td>3.61</td>
<td><strong>63.65</strong></td>
<td>9.78</td>
<td>10.71</td>
<td>0.20</td>
</tr>
<tr>
<td>SanGuad</td>
<td>16572.77</td>
<td></td>
<td>0.59</td>
<td>9.77</td>
<td>0.30</td>
<td>17.96</td>
<td><strong>36.70</strong></td>
<td>8.69</td>
<td>23.50</td>
<td>2.49</td>
</tr>
<tr>
<td>Nueces</td>
<td>40761.18</td>
<td></td>
<td>0.14</td>
<td>2.43</td>
<td>0.26</td>
<td>21.02</td>
<td><strong>64.15</strong></td>
<td>6.32</td>
<td>4.74</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Principal Components Analysis (PCA) explained over 77% of total variation in the land use characteristics of sample sites’ watersheds, however for graphical representation, the first two principal components represented 47.6% of total variation. PC axis 1 (PC1) accounted for 27.1% of total variation with strong positive loadings for water (1.31), forest (1.20), barren (1.06), and agriculture (1.02) and a strong negative loading for shrubland (-1.27). PC axis 2 (PC2) accounted for 20.5% of total variation with strong positive loadings for forest (1.21) and wetland (1.11), and strong negative loadings for herbaceous (-1.73) and agriculture (-1.29). Based on their PC1 and PC2 scores, the Sabine and Neches basin sites possessed high amounts of forests and wetlands within their respective watersheds, whereas Trinity and Brazos basin sites were associated with high amounts of agriculture, herbaceous and developed land, and the Colorado and Nueces sites were associated with developed land and shrubland. Sites within the San Antonio and Guadalupe basins were clustered near the origin and did not appear to be strongly associated with any particular land use (Figure 13). Basins differed along PC1 (F_{6,50} = 13.88, P < 0.01) and PC2 (F_{6,50} = 8.63, P < 0.01). Along PC1, Sabine and Neches did not differ, Trinity, Brazos, SanGuad, and Nueces sites did not differ, and Colorado and Nueces sites did not differ (Table 3). Along PC2, Sabine, Neches, Colorado, SanGuad, and Nueces sites did not differ, Sabine, Brazos, Colorado, and SanGuad sites did not differ, and Trinity, Brazos, and SanGuad sites did not differ (Table 3).
Figure 13. PCA biplot ordination of sites grouped by basin (points) relative to land use (loading arrows). Percent variation explained by each PC is listed on the axes.

Table 3. Grouping information using Tukey’s Method and 95% confidence. Means that do not share a letter are significantly different.
Another examination of the PC scores utilizing regression analysis and previously calculated fish community metrics [diversity (H') and richness (S)] provided insight into how land use areas may have affected fish communities. Regression analysis of PC1 and richness (S), PC1 and diversity (H'), and PC2 and richness (S) failed to detect any significant relationship between these variables in any of the major basins or Texas as a whole (all sample sites combined). Regression analysis between PC2 and diversity (H') did, however, detect two significant, yet weak relationships within the Brazos ($P = 0.037, r^2 = 0.313$) basin (Figure 14) and Texas ($P = 0.020, r^2 = 0.106$) as a whole (Figure 15).

**Figure 14.** Scatterplot of diversity (H’) versus PC2 scores for the Brazos River basin. The (red) trend line indicates the positive linear relationship between the two variables.
Comparison of land use and fish communities was conducted using the multivariate BEST method in PRIMER. After analyzing all possible combinations of land use data with regards to fish community structure, the procedure determined a subset of three of the eight land use types including open water, developed land, and forested land that was most associated with fish community structure. These three land cover types were able to explain 33.9% of the fish community structure in Texas lotic habitats. Alternatively, other subsets of land use types produced similar results (33.6% - 31.6%) with the fish community data and are summarized in Table 4. After the best subset of land use types was selected, NMDS ordinations were created utilizing the fish community data for each sample site (Figure 15) and the open water, developed land, and forested land cover data for each sample site (Figure 16) to graphically display the correlation between both data sets.
Table 4. BEST results summary from PRIMER utilizing the BIOENV method and Spearman rank correlation. The land use types in the selections column can be ascertained from the number coding in the variables column.

<table>
<thead>
<tr>
<th>Variables</th>
<th># of Variables</th>
<th>Correlation</th>
<th>Selections</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Water</td>
<td>3</td>
<td>0.339</td>
<td>1,2,4</td>
</tr>
<tr>
<td>2 - Developed</td>
<td>2</td>
<td>0.336</td>
<td>1,4</td>
</tr>
<tr>
<td>3 - Barren</td>
<td>1</td>
<td>0.330</td>
<td>2</td>
</tr>
<tr>
<td>4 - Forest</td>
<td>2</td>
<td>0.329</td>
<td>1,2</td>
</tr>
<tr>
<td>5 - Shrubland</td>
<td>2</td>
<td>0.322</td>
<td>2,4</td>
</tr>
<tr>
<td>6 - Herbaceous</td>
<td>1</td>
<td>0.321</td>
<td>1</td>
</tr>
<tr>
<td>7 - Agriculture</td>
<td>4</td>
<td>0.318</td>
<td>1,2,3,4</td>
</tr>
<tr>
<td>8 - Wetlands</td>
<td>4</td>
<td>0.316</td>
<td>1,2,4,6</td>
</tr>
</tbody>
</table>
Figure 16. (A) NMDS plot of fish community data displayed by basin. This figure is an ordination of all 51 sample sites distributed in multivariate space. (B) NMDS plot of the best subset of land use data displayed by basin. This figure is an ordination of how all 51 sample sites are distributed in multivariate space relative to the amount of open water, developed land, and forested land in their respective drainages. This ordination exhibited a 33.9% correlation with the ordination displayed in NMDS plot A.
DISCUSSION

Fish Communities

During this study there were several commonly (and constantly) recurring species found across most sample sites. The high abundance of cyprinid and centrarchid species can be attributed to their statewide distribution (Hubbs et al. 2008), as well as the sampling methodology used. Due to the primary use of electrofishing, it was unsurprising that high numbers of piscivorous, larger species (such as centrarchids) were captured as they are typically more prone to the effects of this type of sampling (Snyder 2003).

It was anticipated that a species [richness (S) and diversity (H')] gradient decreasing from east to west would be detected. However, the actual highest overall richness was observed in the Brazos River basin. This statistic should be reported cautiously as there were a greater number of sites sampled in this basin (n = 14) compared to anywhere else. Nonetheless, an examination of average richness and diversity supported the overall richness gradient and the previously reported species gradient described by Conner and Suttkus (1986). Across only five sample sites in the Neches basin, this drainage was found to have both the high average richness and diversity. The lack of a definitive species gradient can at least be attributed to the number of sample sites not being uniform across all basins. Additionally, the limited lengths of the sampling reaches in this study [as prescribed by the EPA (USEPA 2013a; USEPA 2013b)] likely limited the amount and variability of habitats sampled within each river or stream. Allan (2004) described the importance of habitat variation, implying that when habitat variety and variability are limited, diversity may be negatively influenced.
Conversely, there was a similarity gradient between basins as indicated by hierarchical cluster analysis. SIMPROF separated significant groups of basins, and based on spatial patterns described by Conner and Suttkus (1986) the groups confirmed distinctness in ichthyogeography across topographic and climatic zones. The Sabine and Neches basins are confined to eastern lowlands of Coastal Plain clays, sands, and limestones and a humid subtropical zone and were agreeably the most similar to one another and the most dissimilar to the rest of the major basins based on their species composition. Furthermore, while sharing a topographic transition from sedimentary rocks before reaching the Coastal Plain and a climatic transition from semiarid steppes in their upper reaches to dry/dry-subhumid zones the remaining basins showed east to west separation by percent similarities of fish fauna.

One objective of this study was to examine historical trends in Texas freshwater fishes and compare past results with those of the most recent collections (2013-2014). An examination of 17 paired sites that were sampled in 2008-2009 and then during this study in 2013-2014 revealed a 51.7% change in the fish communities between two NRSA events. Percent change in each basin varied substantially, however the subset of sample points that could be paired in each basin really limit the amount of conclusions that can be drawn from this analysis. The alternative approach to comparing the two NRSA events involved using additional unpaired sites, and therefore utilized all the fish community data collected in both surveys to examine each basins’ fish communities using multivariate statistical methods. NMDS analysis and ANOSIM found that only the Colorado basin had significantly different fish communities between 2008-2009 and 2013-2014 \( (P = 0.032) \). Colorado basin sites from 2013-2014 grouped together closely, with the exception of those in the Llano River drainage. With the exception of the Colorado, the data does not suggest there have been significant shifts in the fish communities of western Gulf Slope basins of Texas in recent years.
Prior to recent years, however, data collected by Clark Hubbs and colleagues and analyzed/summarized by Anderson et al. (1995) combined with this study’s findings provides a unique perspective on Texas freshwater fish communities over the past six decades. Due to the unfortunately limited availability of this historical data, this study’s analysis was limited to examining the relative proportions of dominant families in each major basin for three time periods (1953, 1986, and 2013-2014), rather than comparing their raw data from each sampling period. Nonetheless, the approximate three decade spacing between these sampling periods provides a unique opportunity to evaluate changes in the Texas freshwater fishes during evenly spaced time periods over a broad spatial scale. Similarly to the data collected for NRSA, Hubbs’ data collections revealed Cyprinidae and Centrarchidae as two of the most prevalent families in Texas lotic habitats. In several basins, however, the trends in the proportions of these families observed during this study contradicted those from (Anderson et al. 1995). While this may reflect true shifts in the abundances of these fishes, it is impossible to be conclusive as this study did not utilize the same sampling design, methodology, or therefore sampling efficiency as theirs. Patton et al. (1998) described these issues as problematic in using historical data to assess changes in Wyoming’s fish fauna. Additionally, gear bias plays is major confounding issue in the comparison as specimens in this study were primarily collected through electrofishing and Hubbs’ entire collections were done through seining. While the NRSA data available at this time is useful it may become more important as an early foundation for a new historical dataset as the assessment continues to occur every 4-5 years into the future. If possible, obtaining the raw data and sample site coordinates associated with the data analyzed in Anderson et al. (1995) and Hubbs et al. (1997) would ideally provide sufficient information for completing a better historical assessment of Texas freshwater fish communities on such a large spatial scale.
Similarly to the topographic, climatic, and species gradients, land use analysis indicated that the dominant land cover classes transitioned from forest to shrubland from east to west. The comparison of land use/land cover proportions between those in the delineated watersheds versus the overall river basins provided some insight into how accurately the sample sites’ watersheds represented their basins as a whole. While many of the delineated watersheds exhibited land use patterns very similar to the basins as a whole, one basin, the Trinity, did not follow this pattern and was notably skewed. While the overall Trinity basin was only found to contain 15% developed lands, this study’s sample sites averaged 35.9% developed land use, which can be attributed to the concentration of Trinity sites around the Dallas/Fort Worth metropolis. This bias in urbanized watersheds likely influenced the unexpectedly low species richness and diversity found within the basin. In fact, the Trinity basin had the lowest average richness and diversity of all of the more species diverse, eastern basins described by Anderson et al. (1995). This suggestion is reinforced by the fact that urban land use’s effects on aquatic life are disproportionately large even when it makes up a small percentage of the drainage area overall (Paul and Meyer 2001).

An examination of Texas’ major basins through PCA was instrumental in illustrating how these watersheds were classified relative to the major land use classes. With over 77% of total variation explained between all PCs, and 48% within the first two PCs (PC1 and PC2), this analysis provides a strong characterization of these basins’ land use/land cover. Based on known landscape features from field observation and previously reported land use/land cover characterizations provided by Conner and Suttkus (1986), the Sabine and Neches basins’ exhibited high associations with forest and wetland land cover, the Trinity and Brazos basins’ exhibited associations with agriculture, herbaceous, and developed (more so the Trinity) land
use/land cover. Finally, the Nueces basin exhibited an association with shrubland land cover. The ANOVA analysis reinforced the PCA results by indicating there were significant differences between groups of major basins based on their land use/land cover associations.

The second objective of this study was to characterize freshwater fish communities and determine potential factors affective their distribution, by specifically examining Texas rivers and streams at a broad spatial scale. In order to accomplish this, fish community structure was compared to land use/land cover characteristics quantified through GIS analysis. Utilizing the PC scores of land use and fish community metrics [i.e. diversity (H') and richness (S) scores], regression analysis was used to examine these relationships. The goal of these regressions was to discover clues to how land use/land cover potentially influenced fish species richness and diversity. It was hypothesized that there was an inverse relationship between these metrics and agricultural and developed land use. The first axis containing PC1 scores didn’t yield any significant results, which can be explained by the positive loading of agriculture (1.02) and a negative loading for developed land (-0.47), which essentially cancels out any additive effect of these two disturbed land cover types. In contrast, the PC2 scores did indicate some significant relationships when regressed against species diversity scores. Both the Brazos basin and Texas as a whole (all basins combined) showed a positive linear relationship between diversity scores and positive PC2 loadings. This was expected since unlike PC1, PC2 showed directional agreement between agricultural and developed loadings of -1.30 and -0.08, respectively. Furthermore, PC2 portrayed a strong positive loading for forest (1.21), indicating that diversity increased in drainages with more forested land and less agricultural or developed land. These statistics agree with the findings of Wang et al. (1997), who documented that declines in biological assemblages were associated with increased agricultural land use, and that streams
draining agricultural lands support fewer sensitive fish species than streams draining forested lands.

Another analysis conducted during this study attempted to evaluate the relationship of fish community structure to land use, attempting to determine which land use/land cover types were most correlated with the observed fish community structure in Texas lotic habitats. The BEST procedure in PRIMER analyzed all combinations of the eight major land cover classes and selected the most correlated subset(s) of these classes with regards to fish community structure. While open water, developed land, and forested land best explained this structure (33.9% correlation), it was apparent that there were a broad suite of alternative combinations close behind in rank. Nonetheless, these combinations all included at least one of these three land use types (open water, developed land, or forested land). With only about a third of the variance in fish community structure explained by these land cover classes, it is clear that the structure of Texas lotic fish communities is associated with a variety of other factors besides landscape features. This finding agrees with other research, because streams are generally affected by multiple interacting disturbances (Allan 2004). Furthermore, Allan (2004) noted the difficulty in matching specific responses to the associated stressor.

The final objective of this study was to determine the status of freshwater fishes in Texas streams and rivers using NRSA sampling protocols and to make recommendations for the future NRSA surveys based on this study’s findings. It is apparent that land disturbances have caused a decline in the condition of many Texas rivers and streams. However, analyzing only land use at a broad scale cannot identify specific factors responsible for declines, particularly at a local scale, with certainty. This study supports previous research documenting relationships between land use and measures of stream condition, ultimately providing solid evidence that the surrounding landscape and anthropogenic activity do in fact influence the health of lotic
ecosystems (Allan 2004). In Texas, NRSA has provided the foundation for a strategic sampling routine that can ultimately build a statewide database on fish community and associated environmental data allowing for both spatial and temporal analyses. The continued implementation of these surveys with expanded study areas (i.e. more sampling sites) and additional sampling methods (i.e. mandating seining in conjunction with electrofishing) would provide critical data needed to support responsive management of aquatic resources and to observe long-term changes in fish communities at relevant time scales. Future studies should aim to incorporate additional variables such as local physical habitat, flow, and water quality into analyses of stream condition based on fish communities.
LITERATURE CITED


Carr, J. 1967. The climate and physiography of Texas, Austin, TX.


Thomas, C., T. H. Bonner, and B. G. Whiteside. 2007. Freshwater fishes of Texas: a field guide. Texas A&M University Press, College Station, TX, USA.


APPENDIX

**Table 5.** Site specific details for all sample sites included in analyses including site name, Strahler stream order, major basin, latitude and longitude.

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<tr>
<th>Site ID</th>
<th>Site Name</th>
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