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RESEARCH REPORT

Developing greater understanding of functional response of stream fishes to natural and anthropogenic landscape gradients across large regions.

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1 Acknowledgements

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became my friends, supported me, and helped me with everything from daily life stuff to technical issues; it was truly wonderful to have met each of you.

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Abstract

Cumulative distribution functions were used to quantitatively measure homogeneity or departure of central tendencies between landscape variables for all stream reaches at multiple spatial scales for an entire Freshwater Ecoregion versus just the streams reaches within that Ecoregion that have biological samples associated with the reach. For further research this method was used to help select a study area from a large landscape database to evaluate regional differences in fish assemblages. In a pilot study I analyzed data from 969 sites across the highly agriculturally influenced Middle Missouri Freshwater Ecoregion to identify key landscape factors that explained stream fish assemblage patterns and to evaluate the relative influence and relationship of landscape factors and fish assemblage functional metrics. The first axis of the CCA explained a large amount of variance between landscape variables and functional fish metrics. More sensitive species (i.e., intolerant species and species listed by the endangered species act as threated or endangered), migratory species and piscivores more strongly associated with mean elevation in the local catchment, local and network catchment groundwater contribution to a streams baseflow, and percent forest in the network catchment. There was no association of any of functional fish metrics with landscape variables representing high levels of anthropogenic influence like proportions of road crossing in the network catchment, percent of pasture/hay and cultivated crops, percent of impervious surfaces, percent developed lands or population density in the network catchment. Describing the associations between landscape variables and fish functional metrics across the Middle Missouri Freshwater Ecoregions is a first step to developing a large scale regional understanding of the major landscape factors influencing fish assemblage structure across large regions. Further analysis across different regions of the conterminous U.S. will highlight specific regional differences in landscape variables that influence fish assemblage structure.

3 Introduction

Our understanding of how human alterations to landscapes affect freshwater systems has advanced greatly in recent history, and this knowledge has helped to slow degradation of aquatic systems and facilitated the prescriptive rehabilitation of many of US freshwater ecosystems in key locations, allowing for improvements in small areas. Unfortunately, across large regions, trends in degradation of aquatic ecosystems and loss of freshwater biodiversity have continued, as both indirect and direct human impacts on freshwater ecosystems continue to occur throughout the United States due to large-scale changes in land use (Helfman 2007, Jelks et al. 2008, Tockner et al. 2009).

There is inherent natural variability across different regions of the US, and landscape-level anthropogenic disturbances and their impacts also vary widely throughout the Nation. With regionally-different underlying natural landscape variables like lithology, land cover and climate; fluvial fish assemblages with similar membership in one region may respond to anthropogenic disturbances in a different manner than fish assemblages in another region. These differences across regions may render one locale more sensitive to biodiversity loss or fish assemblage compositional change from the same magnitude of anthropogenic disturbance in the landscape.

The few continental bioassessment studies of aquatic ecosystems in the US have detailed major challenges that exist in assessing aquatic conditions across a continental landscape (Paulsen et al. 2008, Herlihy et al. 2008, Esselman et al. 2013). One issue is with differing regional quality of minimally-disturbed sites, which help to evaluate or set a baseline for comparison of habitat degradation, due to different levels of human activity in the landscape both within and across major regions of the continent. A second issue stems from differences in natural characteristics across regions and how similar levels of human impacts may have different effects on aquatic habitats.

These problems increase the challenges of describing differing regional responses of fish across biogeographic regions. These regional differences seen in aquatic assemblage composition, species' response, and stream condition may be mediated by differences in regional land cover or lithology and a changing response with increased anthropogenic stressors. Differences across geographic regions may render one locale more sensitive to biodiversity loss from the same magnitude of anthropogenic disturbance even for regions with the same potential for supporting given organisms and species pools (Utz et al. 2010). High levels of anthropogenic stressors in the landscape may decouple the predictive aquatic assemblages theorized by Vannote et al. (1980) and switch to a less predictive more stochastic assemblage structure (Larsen and Ormerod 2013). This may also be confounded by varying threshold response between species to the same anthropogenic disturbances (Huggett 2005). Also declines in the biomass of fish species has been seen to be associated more strongly with additive effects from multiple anthropogenic pressures (Schinegger et al. 2013). In degraded habitats, some community metrics like relative abundance and species richness can mask ecologically important shifts in species composition if there are increases in tolerant taxa while sensitive taxa decline (Walters et al. 2003, Walters et al. 2005, Walsh et al. 2005). Research conducted in aquatic habitats, including streams draining landscapes dominated by anthropogenic disturbances, support this idea. For example, Utz et al. (2010) has described distinct patterns of fish population reduction and species loss due to land use change (e.g. urban, agriculture) in contiguous physiographic regions in Maryland. Similarly, Meador et al. (2005) has described regionally distinct fish species compositional change along urban gradients in Boston and Birmingham streams.

With increasing spatial extent, there is a need when evaluating fluvial landscapes to control for natural abiotic variation that can confound interpretation of biological assessment (Pont et al. 2006). However, there is little described about the major landscape-scale controls that may be influencing different regional responses in fluvial fish assemblages (Schmutz et al. 2007, Utz et al. 2010). There have been recent calls for further investigation into the interregional comparisons of landscape stressor sensitivity to fish assemblage response and regionally mediated landscape-stream interactions (e.g. Walsh et al. 2005, Utz et al. 2010). Due to the aforementioned complexities, there is a need to better understand regionally-distinct differences in landscape stressor sensitivity of fish to natural and anthropogenic landscape gradients, including response of both individual species and taxa summarized by functional traits (Walsh et al. 2005, Utz et al. 2010). A more comprehensive examination of regional differences using individual taxa and functional traits is warranted because response differences among or across regions could have major implications for ecological assessments and their applications for management and conservation of freshwater fishes. Based on this need, the major goals of this research will be, first to select a future study area of 4-5 Freshwater Ecoregions for further analyses and second to evaluate natural and anthropogenic environmental variation and characterize relationships between natural and anthropogenic landscape variables and functional fish metrics in the Middle Missouri Freshwater Ecoregion as a pilot study that informs further research in evaluating regional differences across and among a group of Freshwater Ecoregions.

This research will describe the major natural landscape factors and anthropogenic factors structuring fluvial fish assemblages across the Middle Missouri World Wildlife Fund Freshwater Ecoregion (wwf-feow, Abell et al. 2008). From here after I will refer to the World Wildlife Fund Freshwater Ecoregions as Freshwater Ecoregions. I used these Freshwater Ecoregions as spatial descriptive units because they incorporate major ecological and evolutionary patterns of freshwater fishes are based on the composition and distribution of freshwater fish species, and having boundaries determined by watersheds, which act as natural dispersal barrier for freshwater species (Matthews 1998, Abell et al. 2008). A river network based on the National Hydrography Dataset Plus (NHDplusV1) was also incorporated as hierarchical spatial units that incorporated biological data at a river reach extent, but also integrated a river reaches' local and network catchment in a nested fashion to relate landscape factors (Wang et al. 2011). These units together should comprise the spatial frameworks work of this study to describe landscape factors at multiple spatial extents.

Major study objectives include: 1) Use quantitative methods to select a subset of a large landscape database to withdraw the best set of data to evaluate regional difference in fish assemblage 2) Identify major structural relationships within both landscape variables and fish functional metrics in the Middle Missouri Freshwater Ecoregion. 3) Characterize major relationships between landscape variables and fish functional groups that are likely defining assemblage structure in the Middle Missouri Freshwater Ecoregion. A pilot study was conducted on one Freshwater Ecoregion to explore analytical methodologies to describe major patterns in the datasets and associations between landscape variables and function fish metrics. Successful analytical techniques can be standardized and later be applied across multiple Freshwater Ecoregions.

4 Methods

4.1 Data summary

The 1:100,000-scale National Hydrography Dataset Plus (NHDPlusV1) streams layer was used as a base layer for geographic representation of streams reaches and their catchments (USEPA & USGS, 2005). We define a stream reach in this study as a section of river in the NHDPlusV1 that extends 1) from the stream origin to the first downstream confluence or junction with a lake or reservoir, 2) from an upstream confluence or lake/reservoir outflow to the next downstream confluence or lake/reservoir junction, or 3) from an upstream confluence or lake/reservoir outflow to the river mouth where it meets with lake/reservoir or estuary (Brenden et al. 2006, Esselman et al. 2011). Catchments summarizing information over two spatial extents are used in this analysis based on the NHDPlusV1, “Local catchments” include all land that drains directly into an individual stream reach without being transported via other fluvial pathways represented in the NHDPlusV1, and “network catchments” include all land upstream of and draining into a given reach via fluvial pathways and including the local catchment.

A variety of landscape data have been summarized within local and network catchments and attributed to corresponding NHDPlusV1 stream reaches. Land cover types were summarized from the National Land Cover Dataset (30m x 30m grid size, NLCD, Homer et al. 2004) and the major classes of land cover data used for this analysis include developed lands and pasture/crop lands. Surficial lithology were summarized by local and network catchments, attributed to each reach (Soller and Reheis 2004, Cress et al. 2010) and major categories were grouped by substrate size (e.g. fine and coarse) and used in analysis. Other human disturbances in the landscape that available for the conterminious U.S. were summarized by local and network catchments and attributed to each reach were: road density, road crossings and length, and percent of impervious surfaces. Elevation and slope were attributed to the reach and network catchment from the National Elevation Data (NED, Gesch 2007). All landscape data used in analysis for the Middle Missouri Freshwater Ecoregion are list with descriptions in Table 1.

Table 1. A list of landscape variables that were used in analysis of the Middle Missouri Freshwater Ecoregion study area with variable codes, variable descriptions and descriptive statistics (i.e., mean, median, minimum, maximum, standard deviation and the 10th and 10th percentiles).

Landscape Variables	Description	mean	median	minimum	maximum	stdv	10%	90%
Developed_c	Developed land (% network catchment)	5.70	4.10	0.00	92.22	9.16	1.60	7.42
Forest_c	Forested land (% network catchment)	6.84	2.29	0.00	97.07	11.72	0.09	17.44
Pasture_Crops_c	Pasture/Hay & Cultivated Crops (% network catchment)	49.75	53.17	0.00	94.88	29.57	4.52	87.42
coarseC	Coarse Lithology (% network catchment)	9.48	0.00	0.00	100.00	23.18	0.00	30.69
AREASQKMC	Network catchment area (km^2)	5515.31	163.07	1.57	208652.97	20483.53	16.49	7122.28
SLOPE	Mean local catchment slope (degrees)	2.42	2.07	0.00	22.96	2.25	0.46	4.44
ELEV_MEAN	Mean local catchment elevation (meters)	598.94	423.88	195.32	3134.90	425.45	289.36	1165.36
GWINDEX	Groundwater Index (% local catchment groundwater contribution to baseflow)	38.94	35.00	12.00	85.68	18.89	19.00	69.00
GWINDEXC	Groundwater Index (% network catchment groundwater contribution to baseflow)	39.87	36.41	12.20	84.20	18.73	19.41	68.87
ROAD_CROSSC	Road crossings (# network catchment)	68.40	55.00	0.00	147.00	52.45	6.00	128.00
POPDENSC	Population density (#/km^2 network catchment)	16.96	2.29	0.00	1285.77	93.01	0.52	12.95
temp	Air temperature (°C x 10 Mean annual local catchment)	102.54	103.86	1.94	129.93	17.72	81.96	123.27
AREAWTMAP	Area weighted average precipitation (ml Mean annual network catchment)	689.88	700.36	344.53	1113.56	179.00	428.06	927.03

Data characterizing stream fish assemblages were assembled and referenced to particular stream reaches in the NHDPlusV1. Assemblages were sampled by state and federal programs using methods determined to be comparable (Esselman et al. 2011) for wadeable streams (e.g. steams or rivers d 10,000 km^2, Wang et al. 2011, Esselman et al. 2011). Fish data used in analysis included specimens identified to species, naming standardized to Integrated Taxonomic Information System (ITIS) codes (ITIS 2013). Fish assemblage structure will be used in this research as a response metric to describe independent landscape variables. The fish assemblages were characterized by functional metrics, incorporating metrics describing trophic structure, habitat preference, reproductive guild, and levels of tolerance (Lyons 1992, Matthews 1998, Barbour et al. 1999, Frimpong and Angermeier 2009). Trophic metric followed Lyons (1992), tolerance metrics followed Barbour et al. (1999), and threatened and endangered fish species followed U.S. Fish & Wildlife (2013). Proportion of individuals in each sampling event and percent taxa at a sampling event were calculated for functional metrics representing the fish assemblage structure at sampling locations. Fish family richness and species richness were also calculated for each sampling event. All fish functional metrics that were used in analysis for the Middle Missouri Freshwater Ecoregion are listed with descriptions in Table 2.

Table 2. A list of fish functional metrics that were used in analysis of the Middle Missouri Freshwater Ecoregion study area with metric codes, metric descriptions and descriptive statistics (i.e., mean, median, minimum, maximum, standard deviation and the 10th and 10th percentiles).

Fish functional metrics	Description	mean	median	min	max	sd	10%	90%
TE_PTAX	Proportion of all taxa that are threatened and endangered	11.51	9.09	0.00	100.00	13.06	0.00	20.00
INTOL_PIND	Proportion of all individuals that are intolerant	2.40	0.00	0.00	100.00	11.28	0.00	2.86
NATIVE_PIND	Proportion of individuals that are native	97.31	100.00	0.00	100.00	9.76	94.47	100.00
GEN_PIND	Proportion of all species that are generalist	12.07	3.10	0.00	100.00	18.59	0.00	39.00
INV_PIND	Proportion of all indiviudals that are invertivore	35.87	33.76	0.00	100.00	24.10	5.45	70.78
OMNI_PIND	Proportion of all individuals that are omnivore	38.31	33.33	0.00	100.00	28.43	3.26	80.86
PISC_PIND	Proportion of individuals that are piscivores	6.49	0.71	0.00	100.00	15.79	0.00	18.50
LITH_PIND	Proportion of individuals that are lithophilic	10.72	3.03	0.00	100.00	17.04	0.00	35.15
LOTC_PIND	Proportion of individuals that are lotic	40.10	38.46	0.00	100.00	29.18	1.50	80.87
RHEO_PIND	Proportion of individuals that are rheophilic	43.24	43.21	0.00	100.00	29.27	0.48	84.15
WCOL_PIND	Proportion of individuals that are water column	68.77	72.79	4.93	100.00	26.15	29.57	100.00
VAGIL_PIND	Proportion of indivuiduals that are migrating (vagile)	1.88	0.00	0.00	100.00	11.40	0.00	0.36
FISH_RICH	Fish family richness	10.83	11.00	1.00	31.00	5.56	4.00	18.00
FAM_RICH	Fish species richness	4.19	4.00	1.00	10.00	1.80	2.00	7.00

In a landscape environmental database for the conterminous U.S. descriptive statistics were calculated to measure the central tendencies and dispersion of variables for each Freshwater Ecoregion and this data was used as a first step in a selection process to elucidate areas in the conterminous U.S. that have a balance of natural environmental gradients, minimally disturbed areas, but also have a variety of anthropogenic stressors (Wang et al. 2011, R Core Team 2013). Descriptive statistics were used to characterize natural variability and anthropogenic stressors for all stream reaches by Freshwater Ecoregions for the conterminous United States (Abell et al. 2008). The descriptive statistics that were calculated were minimum, maximum, mean, standard deviation, the ten percentile and ninetieth percentile values. These statistics were calculated for all landscape data summarized in local and network catchments within each of 44 Freshwater Ecoregions (Figure 1).

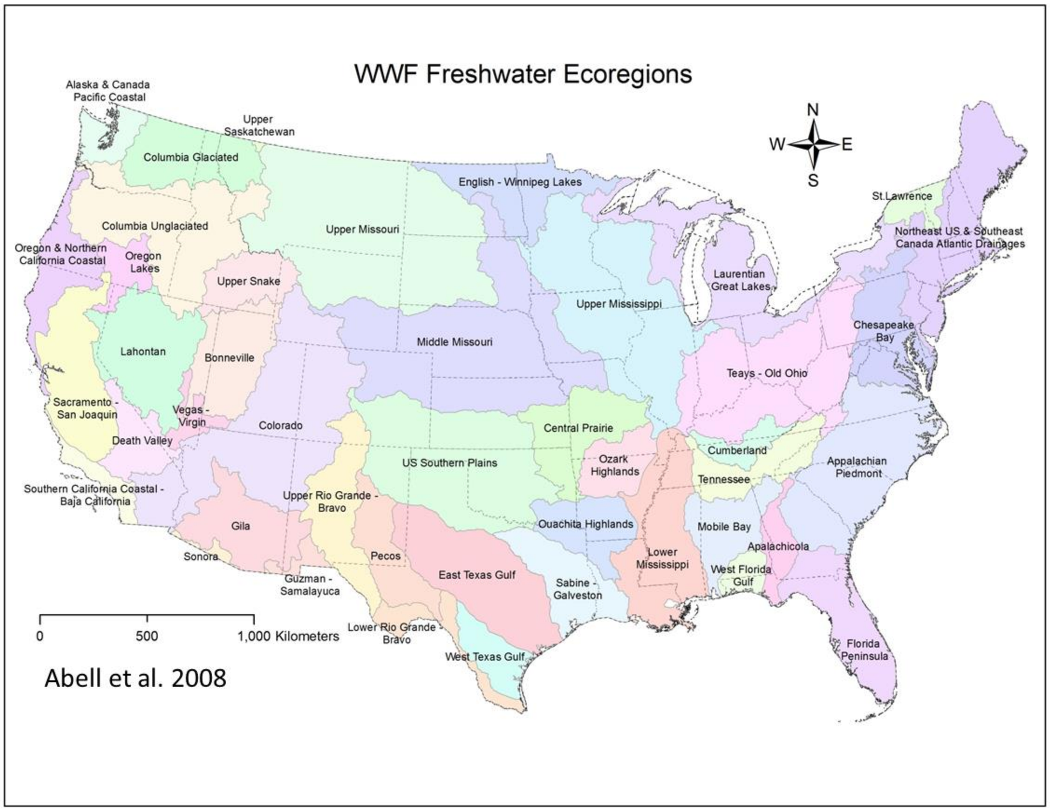

Figure 1. A map of the World Wildlife Fund Freshwater Ecoregions for the conterminous United States of America.

The natural parameters that statistics were calculated for were: drainage area, maximum elevation, precipitation, air temperature, groundwater index, gradient, coarse and fine lithology size classes (Wolock 2003, PRISM Climate Group 2004, Soller and Reheis 2004, USEPA and USGS 2005, Gesch 2007, Cress et al. 2010). The anthropogenic stressor parameters that statistics were calculated for were: urbanization, agriculture, road density, imperviousness surfaces and road crossings (Homer, 2004).

4.2 Data analysis

A second step in selection was to compare trends in the landscape environmental data by Freshwater Ecoregions between all stream reaches and reaches that have a biological fish assemblage sample events. This step was used to select Freshwater Ecoregions with a well distributed number of biotic sample sites where major natural and anthropogenic landscape parameters were well representative across the entire freshwater ecoregion. Cumulative distribution function (cdf) were calculated for environmental parameters for reaches that have biotic samples and then compared with the cdf for the same environmental parameter for all reaches by Freshwater Ecoregions. Cumulative distribution function were calculated for some major natural and anthropogenic landscape parameters that have been shown to influence fish assemblage structure (Wang et al. 2001, Wang et al. 2003, Allan 2004). The major landscape parameters that cdf were calculated for were: percent agriculture, percent urban, percent fine and course lithology, catchment area, and mean elevation at the network catchment extent (e.g. catchments between 10 km^2 and 10,000 km^2). The cdf were calculated for all Freshwater Ecoregions that had two hundred plus biotic samples (i.e., 17 ecoregions in the database). The area between the cdf curves (i.e., the difference of the two cdf area under the curve) was calculated as a measure of difference or departure between landscape parameters for streams reaches that have a biotic sample events and landscape parameters for all stream reaches in an entire Freshwater Ecoregion (R Core Team 2013, Ekstrom 2013). A ranking and summary scoring of the departure between the two cdf curves for calculated parameters was used to finalize a selection of freshwater ecoregions that have a balance of natural environmental gradients, pristine areas, but also have a variety of anthropogenic stressors and are most likely to have a set of biological sampled stream reaches that are representative of the range of natural and anthropogenic stressors seen for the entire Ecoregion. The 5 highest ranking Freshwater Ecoregions from the cdf evaluation were then selected as the study region. But only one Freshwater Ecoregion (i.e., Middle Missouri) for this research was used as a pilot project in the final analyses to characterize relationships between landscape variables and fish functional metrics.

The landscape variables and fish functional metrics were evaluated for missing values, zero inflation, and outliers. Metrics were removed if they did not have a site occurrence of e 10% (Wang et. al. 2003). Landscape variables were transformed and evaluated for assumptions of normality. Continuous data were transformed with natural log +0.01, percentage and proportional data were transformed with Arcsin square root and count data were transformed with square root +0.01. Transformed landscape variables and functional metrics were evaluated for normality visually with Q-Q plots, histograms and boxplots, checked for skewness and kurtosis and poorly preforming variables and metrics were removed.

Pearson product-moment correlation analysis was conducted on both the landscape variables and functional metrics to investigate the associations between variables and metrics. In any highly correlated pair of variables (i.e., correlation coefficient >0.7) one of the variables, usually the least interoperable or poorest preforming variable would be removed to reduce redundancy in the dataset.

As a pilot study, one of the top 5 Freshwater Ecoregions selected for the study region was chosen for some preliminary analysis exploring landscape relationships with functional metrics. The Middle Missouri Freshwater Ecoregion was selected for these further analyses. For the Middle Missouri Freshwater Ecoregion a principle component analysis (PCA) was conducted on both landscape variables and fish functional metrics. The PCA analyses were performed with SPSS (IBM Corp. 2012). These analyses were used to characterize the structural dimensionality of groups of landscape variables and groups of functional metrics. This describes the variables or metrics that explain the highest degree of variability in each of the two datasets (i.e., landscape variables and functional metrics). By removing variables that describe only a small amount of variance in you dataset you can structurally simplify your data and will likely improve the interpretability of any further analysis.

A Pearson product-moment correlation analysis was preformed between the linear combinations of factor site scores resulting from both the landscape variables PCA and the fish functional metrics PCA. Correlation coefficients were evaluated for strength of relationships between variables and metrics that weighted heavily on each axes of the two independent PCA (i.e., landscape variables PCA and fish functional metrics PCA axes).

Partial constrained ordination was used to determine the unique effect of group explanatory variables on community metrics (Borcard et al. 1992, Borcard et al. 2011). The analysis was a multi-step process using a Canonical Correspondence Analysis (CCA) or redundancy analysis (RDA). The CCA and RDA are direct gradient analyses that use a matrix of predictor variables (i.e., environmental variables) to describe variation in a matrix of response variables (e.g. fish functional metrics). A detrended correspondence analysis (DCA) was run to determine the appropriate ordination technique (CCA or RDA) for further analysis (ter Braak 1995; Esselman and Allan 2010; Pool et al. 2010). The gradient length of functional metrics along the first DCA axis allows for an estimation if the functional response to environmental data is more linear or unimodal. Larger gradient lengths (>2) equals a unimodal response and suggest a CCA analysis and gradient lengths (<2) equals a linear response and suggest a RDA analysis (ter Braak 1995; Esselman and Allan 2010; Pool et al. 2010).

A CCA was preformed between the landscape variables and fish functional metrics after the gradient length was determined. For each of the two datasets (e.g. landscape variables and fish functional metrics) a bi-plot was created to display correlations between the locations of functional metrics and environmental variables. Correlations between landscape factors and fish functional metrics were interpreted visually from the bi-plot. The direction and length of environmental vectors in relation to community metrics were interpreted as having stronger correlation with the community points found on the same axis as vectors. The analysis for the DCA and CCA were performed with CANOCO 4.5 and a bi-plot was graphed from the results of the CCA for the functional metric versus landscape variables with the computer package CANOCO 4.5 (ter Braak and Smilauer 2002).

5 Results

5.1 Study Region

The Middle Missouri Freshwater Ecoregion was chosen as a study region to perform some preliminary analyses to characterize the major relationships between landscape factor that are structuring local fish assemblages. The Middle Missouri Freshwater Ecoregion covers 594 079 km^2 and encompasses portions of 9 states (i.e., Colorado, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Wyoming, Figure 2). The Middle Missouri land cover is primarily comprised of pasture/hay and cultivated crops (46%), grasslands (29%), with a smaller percentage in forest (9%) and developed (8%), with the remainder (<8%) in wetland, water, shrub and barren land cover (Homer et al. 2004). Streams at the collection sites ranged from 1st to 8th order (Strahler 1957), and basin area of study reaches ranged from 0.2 to 208 652 km^2. Collections at 969 localities between 1990 to 2010 were sample in wadeable streams in the Ecoregion. Fourth-four species of fish representing 33 genera in 12 families were collected from the 969 sites in the Middle Missouri Freshwater Ecoregion.

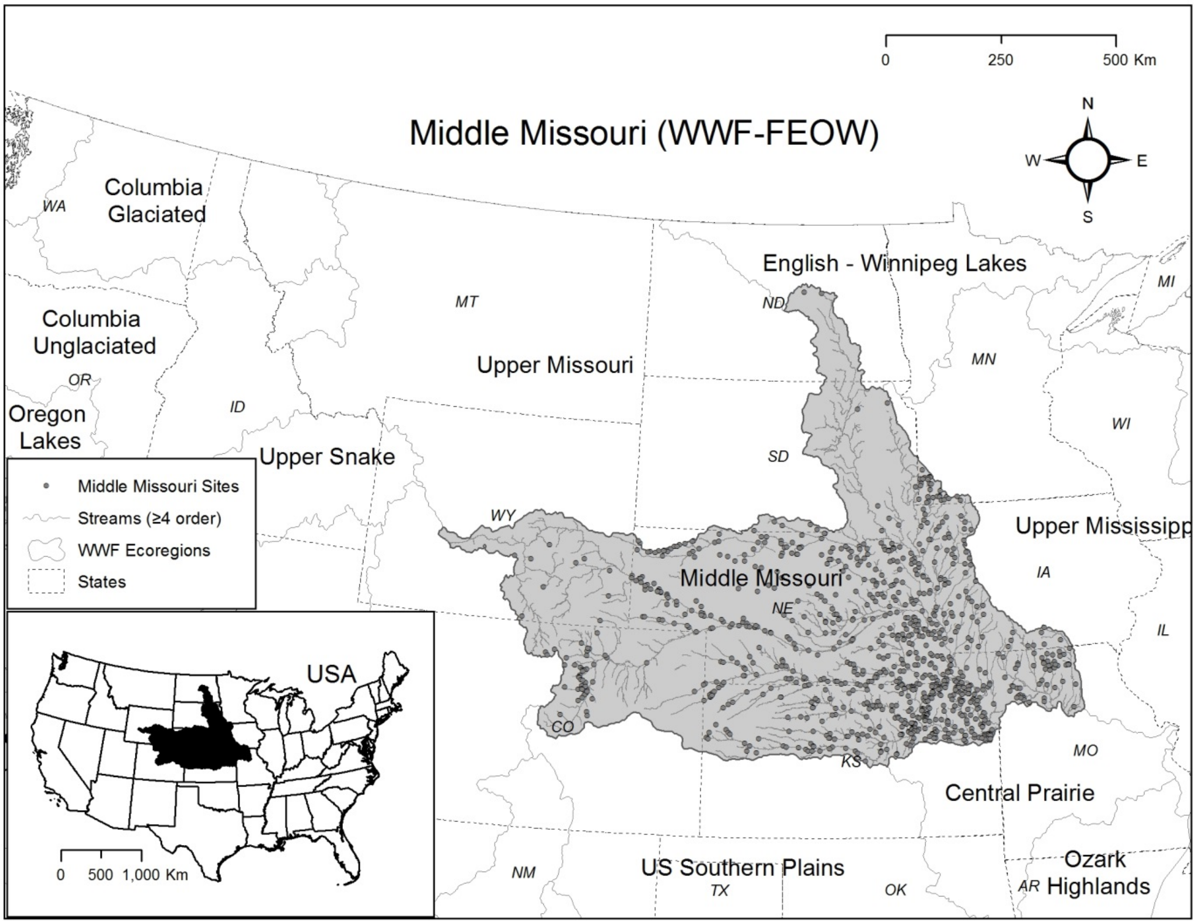

Figure 2. (This figure will be replaced by one that is much better!!! It is a place holder for now). Analysis was conducted on one of the five Freshwater Ecoregions selected for the regional analysis study region as a pilot study. The study area analyzed in this research was the Middle Missouri World Wildlife Freshwater Ecoregion.

5.2 Study Area Selection Process

In a selection process to develop a final analytical study area seventeen Freshwater Ecoregions that had two hundred plus sampling events on different stream reaches were evaluated with cumulative frequency distributions (Figure 1). The cdf were used to characterize the dispersion or departure between landscape variables for stream reaches that have biotic sampling events associated with them versus the entire stream reaches within a Freshwater Ecoregion. The landscape variables that were evaluated with cdf were percent agriculture, percent urban, percent fine and course lithology, catchment area, and mean elevation at the network catchment extent (e.g. catchments between 10 km^2 and 10,000 km^2). For each landscape variable cdf were calculated and graphed for all stream reaches and for streams reaches with associated biotic samples in a Freshwater Ecoregion. The area between the curves (abc) was calculated (i.e., that is the difference between the two cdf area under the curve (auc)) and compared between all landscape variables and across all seventeen Freshwater Ecoregions. For the Middle Missouri Freshwater Ecoregion cdf the abc value for percent of Pasture/Hay and Cultivated Crops land use in a catchment was 3.45 (Figure 2). For the Middle Missouri Freshwater Ecoregion cdf the abc value for percent of developed land use in a catchment was 1.66 (Figure 3).

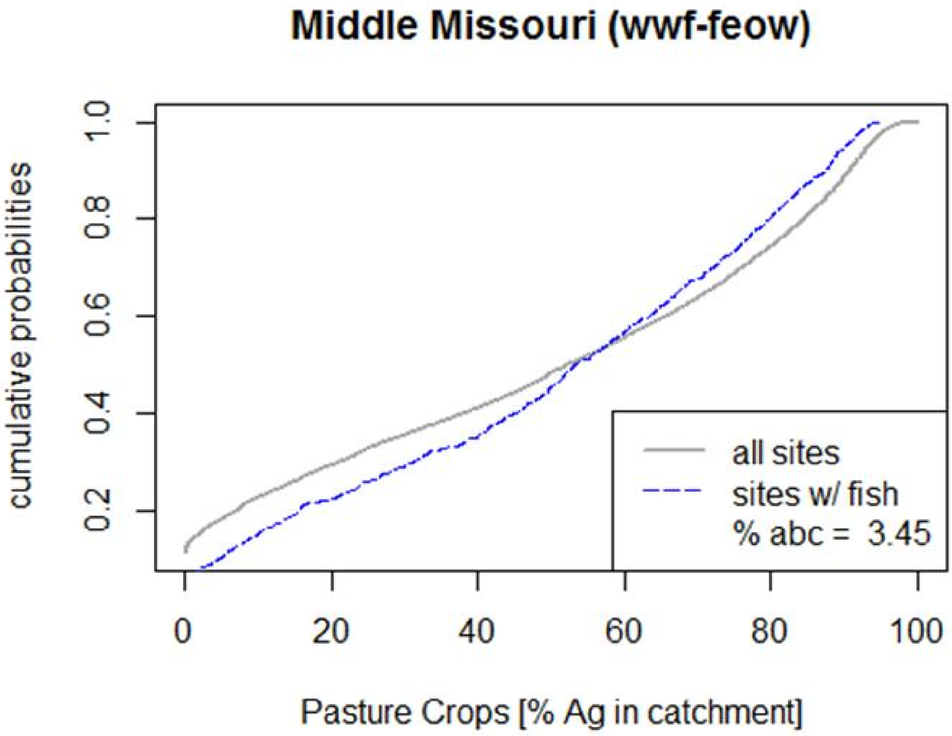

Figure 2. Graph of cumulative distribution function for the percent of Pastrue/Hay and Cultivated Crops land use between all stream reach and stream reach with an associated biological fish assemblage collection for the Middle Missouri Freshwater Ecoregion. The area between the curves for the two cdf is also given in the legend.

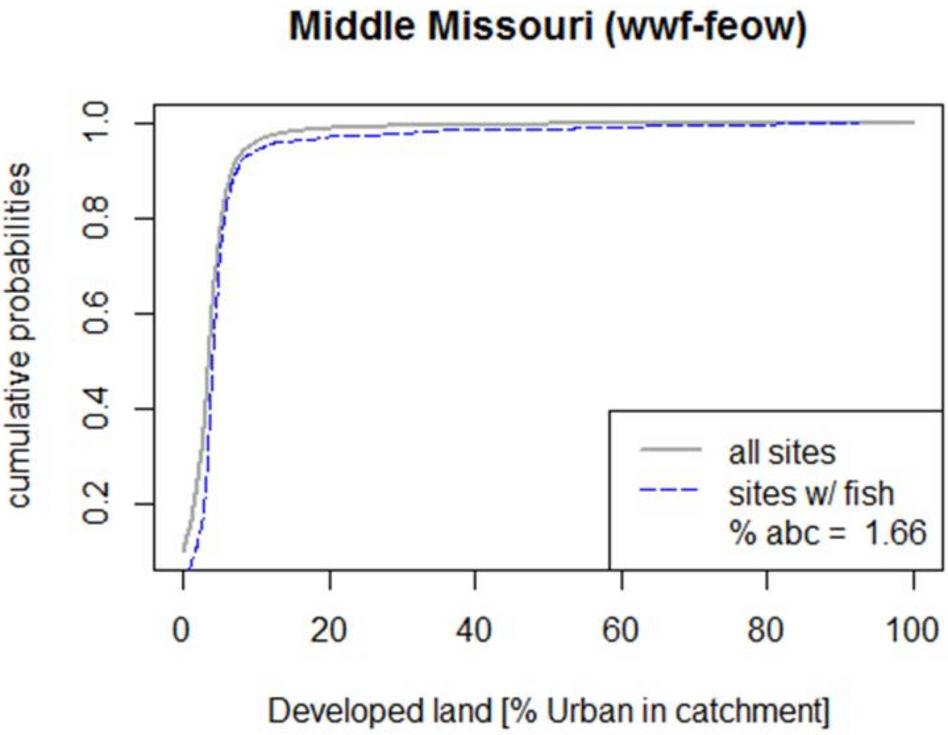

Figure 3. Graph of cumulative distribution function for the percent of Pastrue/Hay and Cultivated Crops land use between all stream reach and stream reach with an associated biological fish assemblage collection for the Middle Missouri Freshwater Ecoregion. The area between the curves for the two cdf is also given in the legend.

The abc value was ranked from smallest to largest for each of the landscape variables and then summarized across all variable classes for the seventeen Freshwater Ecoregions. The summarized values were sorted in ascending order, ranked across all variable classes and the top 5 Freshwater Ecoregions were select as the study area for further analysis. The top 5 Freshwater Ecoregions selected as the study area were: Chesapeake Bay, Upper Mississippi, Appalachian Piedmont, Middle Missouri and Laurentian Great Lakes (Figure 1). As a pilot study for this research report incorporated further analyses that were only preformed on the Middle Missouri Freshwater Ecoregion.

5.3 Principle Component Analysis Landscape Variables

The PCA on the Middle Missouri Freshwater Ecoregion of landscape variables resulted in four axes that explained 75.7% of the variation in sites (Table 3).

Table 3. — Principle component analysis results for fourteen landscape variables for the Middle Missouri Freshwater Ecoregion with weights assigned to each variable for each axes, and percentage of variance and cumulative variance in data explained by each axis. Total amount of variation explained was 75.7%. Landscape variable coding follows that in Table one.

Principal Component Analysis (PCA)	**axis 1**	**axis 2**	**axis 3**	**axis 4**
variance explained	35.92	16.84	14.62	8.31
cumulative variance explained	35.92	52.76	67.38	75.69

Landscape variable	**axis 1**	**axis 2**	**axis 3**	**axis 4**
GWINDEX	**0.92**	-0.11	0.05	-0.14
GWINDEXC	**0.90**	-0.08	0.13	-0.19
ELEV_MEAN	**0.89**	-0.07	0.07	0.14
coarseC	**0.52**	-0.32	0.16	-0.12
AREASQKMC	0.19	-0.19	**0.92**	0.00
ROAD_CROSSC	-0.05	0.00	**0.90**	-0.03
Developed_c	-0.07	**0.92**	-0.10	-0.06
IMPERVC	-0.11	**0.94**	-0.02	0.06
Forest_c	-0.13	0.12	-0.15	**0.90**
SLOPE	-0.16	-0.21	**-0.53**	0.35
POPDENSC	-0.27	**0.88**	0.11	0.06
temp	**-0.59**	0.14	0.23	-0.07
Pasture_Crops_c	**-0.65**	0.11	-0.05	**-0.53**
AREAWTMAP	**-0.79**	0.09	-0.37	0.16

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The first axis, explaining 35.9% of the variation in sites, was positively weighted by percent of local and network groundwater contribution to base flow, local mean elevation and the percent of coarse lithology in the network catchment. Axis one was negatively weighted by the area weighted average mean annual precipitation in the network catchment, the percent of pasture/hay and cultivated crop (i.e., agriculture) land use in the network catchment and local mean annual air temperature at the local catchment. Axis 2, explaining 16.8% of the variation in sites, was positively weighted by the percent of impervious surface in the network catchment, percent developed land use in the network catchment and population density in the network catchment. Axis 3, explaining 14.6% of variation in sites, was weighted positively by network catchment area and the number of road crossing in the network catchment and weighted negatively for local catchment slope. Axis 4, explaining 8.3% of variation in sites, was weighted positively by the percent of forested land use in the network catchment and weighted negatively for the percent of pasture/hay and cultivated crop land use in the network catchment.

5.4 Principle Component Analysis Functional Fish Metrics

A PCA on the Middle Missouri Freshwater Ecoregion of select fish traits yielded six axes explaining 83.2% of the variation across the study region (Table 4).

Table 4. Principle component analysis results for fourteen fish functional metrics for the Middle Missouri Freshwater Ecoregion with weights assigned for variable for each axes, and percentage of variance and cumulative variance in data explained by each axis. Total amount of variation explained was 83.2%. Fish functional metric coding follows that in Table two. Bold variables show a strong component coefficient.

Principal Component Analysis (PCA)	**axis 1**	**axis 2**	**axis 3**	**axis 4**	**axis 5**	**axis 6**
variance explained	24.39	19.16	14.35	9.16	8.53	7.56
cumulative variance explained	24.39	43.55	57.90	67.06	75.59	83.15
Fish functional metric	**axis 1**	**axis 2**	**axis 3**	**axis 4**	**axis 5**	**axis 6**
INTOL_PIND	**0.93**	0.02	-0.05	-0.04	-0.04	0.09
VAGIL_PIND	**0.92**	-0.05	-0.14	-0.06	-0.03	0.06
TE_PTAX	**0.80**	0.03	0.21	-0.18	0.05	-0.07
PISC_PIND	**0.77**	-0.12	-0.10	0.03	-0.03	-0.46
LITH_PIND	0.18	**0.52**	0.38	-0.12	0.15	-0.13
WCOL_PIND	0.01	0.05	**-0.88**	-0.23	0.20	-0.09
NATIVE_PIND	-0.02	0.08	0.00	0.08	0.04	**0.96**
FAM_RICH	-0.07	-0.02	0.14	**0.93**	-0.02	-0.01
RHEO_PIND	-0.09	-0.11	**0.83**	0.20	0.11	-0.04
INV_PIND	-0.12	0.03	0.02	0.03	**0.97**	0.08
FISH_RICH	-0.13	-0.05	0.24	**0.90**	0.06	0.11
GEN_PIND	-0.13	**0.78**	-0.36	-0.15	-0.27	0.07
LOTC_PIND	-0.18	**0.85**	-0.01	0.07	0.12	0.19
OMNI_PIND	-0.23	**-0.64**	0.19	-0.03	**-0.59**	0.11

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

Axis 1, explaining 24.4% of the variation in sites, was weighted positively by low tolerance fish, vagile or migratory fish, fish species listed as threatened or endangered and piscivores. Axis 2, explaining 19.2% of the variation, was weighted positively by fish preferring lotic environments, generalists fish, and lithophilic species. The axis was weighted negatively by omnivorous. Axis 3 explained 14.4% of the variation in the sites; it was positively weighted by rheophilic species and negatively weighted by species that prefer the water column. Axis 4 explained 9.2% of the variation in the sites; it was positively weighted by richness in fish families and species. Axis 5 explained 8.5% of the variation in the sites; it was positively weighted by invertivores and negatively weighted by omnivorous. Axis 6 explained 7.6% of the variation in the sites; it was positively weighted by native fish of the region.

5.5 PCAs Factor Site Scores Correlation

Factor scores for each site resulting from the PCAs of landscape variables and fish functional metrics from the Middle Missouri Freshwater Ecoregion were not highly correlated (Table 5).

Table 5. Pearson product-moment correlation of liner axis combinations of site factor scores from PCAs of the landscape variables and fish functional metrics table three and table four. Variables codes with positive component scores from the PCAs are provided for interpretation of the correlation coefficients. Variable coding follows that of table one for landscape variables and table two for fish functional metrics. Bold values indicate moderate correlations between PCA axes.

		Landscape Variables			
		axis 1	axis 2	axis 3	axis 4
	variables with positive component scores	GWINDEX GWINDEXC ELEV_MEAN	IMPERVC Developed_c POPDENSC	AREASQKMC ROAD_CROSSC	Forest_c
Fish Traits					
axis 1	INTOL_PIND, VAGIL_PIND, TE_PTAX, PISC_PIND	**0.32**	-0.15	-0.18	**0.24**
axis 2	LOTC_PIND, GEN_PIND, LITH_PIND	**0.20**	0.06	**-0.22**	0.05
axis 3	RHEO_PIND	-0.13	-0.11	0.13	**0.23**
axis 4	FAM_RICH, FISH_RICH	**-0.20**	-0.07	**0.43**	0.05
axis 5	INV_PIND	0.02	-0.03	-0.08	0.02
axis 6	NATIVE_PIND	-0.19	0.07	-0.06	-0.24

There were some moderate positive correlations between landscape variables axis 1 and fish functional traits axis 1 with that of fish functional traits axis 1 and axis 2, highlighting a moderate correlation between the groundwater contribution to baseflow, local catchment mean elevation and sensitive species, vagile species, piscivores, generalists, lithophilic species and lotic species. Landscape variables axis 1 was moderately negatively correlated with family and species level fish richness. The strongest correlation was between landscape variables axis 3 and axis 4 from the fish functional traits, emphasizing a correlation between network catchment size and fish family and species diversity (r = 0.43). Landscape variables axis 3 was negatively correlated with fish functional traits axis 2. Landscape axis 4 having a strong weighting to sites with high percentages of forest land use in their network catchment were moderately correlated to fish functional traits axis 1 and axis 3 that had strong weightings to sites with high sensitive species, vagile species and piscivores for axis 1 and rheophilic species for axis 3.

5.6 Canonical Correspondence Analysis

Results from the DCA evaluation of the functional metrics gradient lengths for the Middle Missouri Freshwater Ecoregion was >2 (i.e., axis 1 gradient length 2.026) and suggested a more unimodal distribution across the gradient and a further analysis using a CCA. Results from CCA between landscape variable and fish functional metrics in the Middle Missouri Freshwater Ecoregions generated four ordination axes that together explained 22% of the total variation in fish functional metrics among sites, and 96% of the variation in functional metrics among sites along the functional metric-landscape gradient within the dataset.

The first CCA ordination axis described 67.2% of the variance among sites in between the functional metric and landscape variables of the Middle Missouri Ecoregion and had negative loadings of landscape variables that had network catchments with high levels of human pressures like developed land, pasture/hay and cultivated crops, population density, impervious surfaces, number of road crossings, higher mean annual air temperature in the local catchment and higher amounts of precipitation (Figure 4). There were no strong negative associations between fish functional metrics and landscape metric in the axis 1 gradient. Positive weightings on CCA axis 1 were characterized by catchments that had low human pressure with high percentages of forest in the network catchment, higher mean local catchment elevation, and greater amounts of groundwater contribution to baseflow for both local and network catchments (Figure 4). The fish functional metrics that had positive loading on axis 1 and were associated with lower human pressures were intolerant, threatened and endangered species, migratory species and piscivores. The higher population centers are in the eastern parts of this Freshwater Ecoregion, which were developed around some of the bigger rivers for access to water and transportation, also related to larger catchment sizes in the region. These patterns are also related to a precipitation gradient that increases as you move from the west to the east which corresponds with less agriculture in the arid west and more high intensity cultivated crops and road density in the east of the Ecoregion.

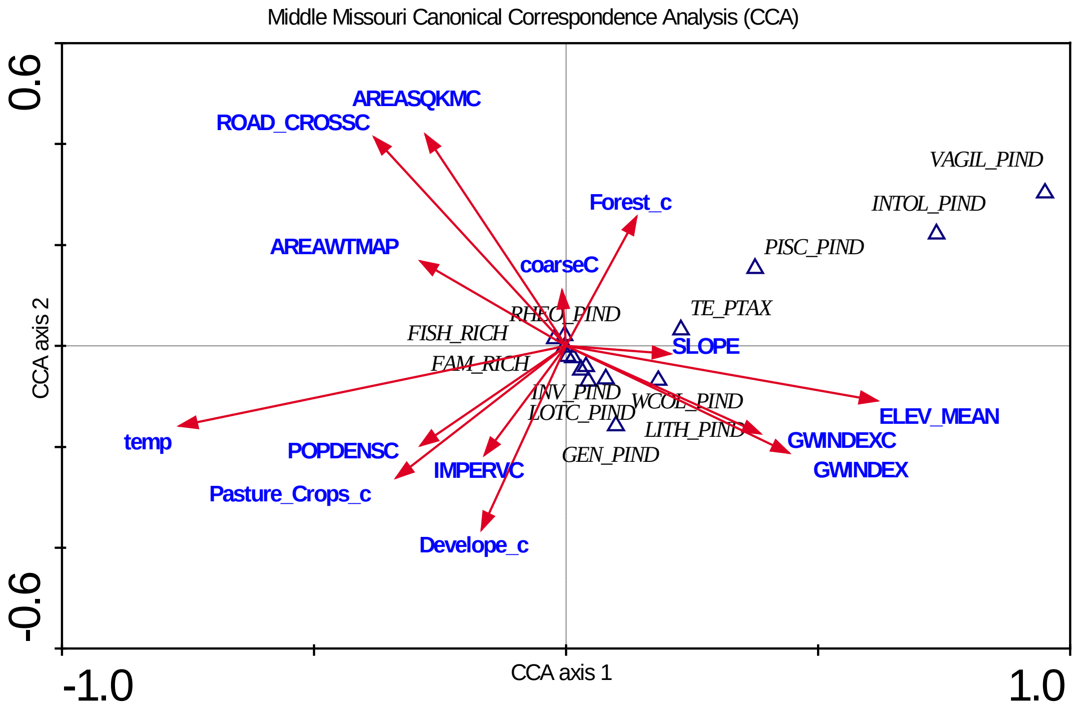

Figure 4. Biplot of fish functional metric scores and environmental variable vectors for canonical correspondence analysis (CCA) axis one and axis two from the CCA ordination of the sample sites in the Middle Missouri Freshwater Ecoregion. Fish community variables are points and the coding follows that in Table two and landscape variables are vectors by arrows and the coding follows that in Table one.

The second CCA axis described 19.8% more variance among sites between the functional metrics and the landscape variables and was distinguished primarily between sites with a higher groundwater contribution to baseflow with a negative weighting, which had an association of percent of generalist, lithophilic, lotic and water column fish species (Figure 4). Positive weightings on axis two were associated with larger network catchment area, higher percent forest in the network catchment and related more strongly with percent of piscivores, vagile and intolerant species of fish (Figure 4).

6. Discussion

Results from this study have described a quantitative method to use cdf to rank regional landscape datasets for their homogenization or departure with or from regional trends seen in an entire region related to areas of the region that have associated biological response data. This method can be used to evaluate if the areas of sampled locations within a region will hold similar empirical probabilities for landscape variables for the entire region. This method might be used to evaluate sampling completeness across environmental gradients within a region.

In the pilot study of Middle Missouri Freshwater Ecoregion there was no association between any of the functional fish metrics and anthropogenic influence landscape variables like percent pasture/hay and cultivated crops in the network catchment, developed land use in the network catchment and percent of impervious surfaces or population density. This follows what is seen in some previous studies that there is a negative effect on fish assemblages with increases of human activity in the local or network watersheds (Lyons 1996, Wang et al. 1997, Wang et al. 2003, Allan 2004). Mean elevation was seen in this study to have association with fish functional metrics, this might have been due to the fact that elevation was acting as a surrogate of geography in this low gradient region or a gradient from west to east with more minimally disturbed sites in the west and more human impacted sites in the east. There was an association between local and network groundwater contribution to baseflow and intolerant, threatened or endangered fish species along with migratory species and piscivores. Wehrly et al. (2003) showed and association between stream fish assemblage structure and groundwater accrual in low elevation Mid-western streams.

The CCA only characterizes groups of landscape variables that have strong influence or association with functional metrics of a fish assemblage. The method is limiting in its ability to be used as a tool to set specific management criteria for levels of specific anthropogenic that maybe causing structural assemblage changes. Maybe the use of Multivariate Regression Trees (MRT) may allow for a more management favorable analysis to present critical management levels of anthropogenic influences for criteria.

For further analysis I will try and categorize the differences across regions of anthropogenic influences on fish assemblage structure. A nested approach to the MRT might be used to constrain know major influences of longitudinal changes in assemblage structure (e.g. catchment area, elevation, gradient) across different regions and isolate the different regional levels of anthropogenic stressors on fish assemblages (Ouellette 2012). These analyses has the utility to compare large Freshwater Ecoregions in the Conterminous United States, but also using the same framework of Freshwater Ecoregions one could characterize the relative influence and importance of different anthropogenic stressors between areas in the European Union and the United States, describing major differences in human pressures in areas of Europe versus US.

Few studies have attempted to characterize regionally-distinct fish assemblage response to gradients of landscape human disturbance, yet this understanding is essential to develop large-scale policies and practices to protect and conserve aquatic ecosystems. Understanding the differential regional response to human influenced landscape will help inform future policies. This richer understanding about the complexity of landscape controls on aquatic systems will provide novel insights to enhance management opportunities in the face of changing ecosystems and improve our abilities to assess the biological integrity of fluvial ecosystems.

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