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Assessing effects of land use on streams along the Natchez Trace Parkway using rapid bioassessment protocol techniques

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ASSESSING EFFECTS OF LAND USE ON STREAMS ALONG THE NATCHEZ TRACE PARKWAY USING RAPID BIOASSESSMENT PROTOCOL TECHNIQUES

By

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THE NATCHEZ TRACE PARKWAY USING RAPID

BIOASSESSMENT PROTOCOL TECHNIQUES

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Stream quality along the Natchez Trace Parkway was evaluated by hydrologic unit code 4 (HUC4) watersheds and habitat assessment scores as a broad and local scale, respectively. Water chemistry parameters and rapid bioassessment techniques for habitat and fish and invertebrate communities were sampled in 18 streams and six HUC4 watersheds. Forest, agriculture, and developed land use had little variation at HUC4 level; land use impacts could not be determined. Turbidity and TSS were important factors determining habitat scores and created a “boundary” separating southern and northern watersheds. A latitudinal trophic shift was observed of fish omnivore-insectivore-piscivore in southern watersheds to generalist-insectivore-herbivore species in northern watersheds. Fish families were correlated significantly to the water chemistry matrix. Fish species were correlated significantly with the habitat matrix using Canonical Correspondence Analysis. Management implications differ considering the function of

these two scales. Only turbidity and percentage cobble substrate were significant at both scales.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Water quality has been declining due to anthropogenic impacts since the Industrial Revolution (Trombulak and Frissell 2000; Long and Plummer 2004). Land converted for growing populations and their needs has caused concern for natural resource managers and watershed protection (Trombulak and Frissell 2000; Long and Plummer 2004; Snyder et al. 2005). Water quality monitoring, such as Rapid Bioassessment Protocols (RBP), is a way of tracking these changes and their impacts in aquatic ecosystems. Rapid Bioassessment Protocols outlined by the Environmental Protection Agency (EPA) offer methods of assessing instream habitat, water quality, and fish and macroinvertebrate communities of streams. These metrics are scaled relative to reference streams and conditions to evaluate the quality of a sampled stream (Barbour et al. 1999).

Several studies suggest that land practices can influence stream quality (e.g., Hawkins et al. 1982; Schueler 1994; Bryce et al. 1999; Ometo et al. 2000; Fajardo et al. 2001; Timm et al. 2001; Coulter et al. 2004; Kyriakeas and Watzin 2006; Pinto et al. 2006; Burcher et al. 2008; Bonner et al. 2009; Infante et al. 2009). Agricultural, urban, and forested landscapes are often used to analyze land use impacts (e.g., Jones et al. 2001; Coulter et al. 2004; Long and Plummer 2004). Landscape metrics can be used to predict sediment and nutrient loading of streams. Riparian vegetation is influential in filtering runoff. Forest land use tends to have lower levels of turbidity and suspended solids because of riparian vegetation. Waste and fertilizer runoff from agricultural land practices are associated with high levels of nitrogen and phosphorous (Hem 1985; Edwards et al. 2000; Jones et al. 2001; Fajardo et al. 2001). Fecal coliform contamination from grazing land can be particularly high after rain events (Baudart et al. 2000; Edwards et al. 2000). High levels of turbidity and specific conductance are generally found in urban and livestock agricultural areas (Schueler 1994; Long and Plummer 2004). Impervious surfaces found in urban land use is a predictor variable of stream quality (Schueler 1994). Pollutant loads associated with impervious surfaces can include runoff from fertilizers, pesticides, and vehicles. The more land covered by impervious surfaces, the higher the risk of runoff into waterbodies. Forested areas, especially riparian vegetation, reduce impacts of this runoff.

Land use also can impact biotic parameters within the aquatic ecosystem. Fish and macroinvertebrate communities can serve as biotic indicators of stream quality. Human disturbance, as with agricultural and developed land, can cause poor water quality and displace many pollution-intolerant organisms. Pollution intolerant families and taxa richness decrease with increasing organic pollution (Barbour et al. 1992; Timm et al. 2001; Camargo et al. 2004). Agricultural and urban or developed land uses tend to negatively affect macroinvertebrate and fish assemblages whereas forested land is considered a more pristine land use, allowing for more pollution intolerant taxa and robust diversity (Lammert and Allan 1999; Sawyer et al. 2004).

The Natchez Trace Parkway (NTP) is a national park located in Mississippi, Alabama, and Tennessee. Approximately 100 streams run through the park boundary; however, very little data have been reported on streams within the park. Physicochemical parameters and macroinvertebrates have never been studied on the entire length of the park nor has there been research at the watershed level for invertebrate communities of the NTP. The NTP has a narrow boundary (average approximately 200 meters wide); therefore, the influence on adjacent land uses and land uses within the watersheds can greatly impact the water quality of streams in the park boundary. This study advances the field of stream ecology by assessing biotic and abiotic parameters of streams in a national park rarely studied. It also provides baseline data to the National Park Service on physicochemical parameters, land use, and macroinvertebrate communities for streams along the entire NTP as well as contributing to fish community data. This research gives insight into the aquatic ecosystems within the park boundary, a largely untapped resource, and offers stream quality analysis to assess watersheds within the region.

Goals and Objectives

My goal is to determine invertebrate and fish community responses to water chemistry, instream habitat, and agricultural, developed, and forested land uses within watersheds of the Natchez Trace Parkway (NTP) boundary. This thesis represents a subset of a larger project funded by the National Park Service to develop a water chemistry database in 44 streams on the NTP. Thirteen of these sites and five additional wadeable sites were selected for more intensive sampling, including a Rapid

Bioassessment Protocol (RBP) (Barbour et al. 1999) for habitat and the RBP sampling techniques for fish and macroinvertebrate communities. The thesis is constructed of two chapters, separating abiotic and *Escherichia coli* from biotic (invertebrate and fish) parameters, and a final conclusion chapter (IV) that summarizes results and discusses important management implications. In chapter II, I investigate the hypothesis that land use at a watershed level and habitat assessment scores at the instream level will influence physicochemical parameters in streams along the NTP. I sampled water chemistry, *E. coli*, and assessed habitat to determine possible impact in 18 streams of the NTP. I use ANOVA tests to determine differences across watersheds and linear regression tests to determine differences across habitat scores. In chapter III, I investigate the hypothesis that land use at the watershed level and habitat assessment scores at the instream level will influence macroinvertebrate and fish assemblages in streams along the NTP in chapter III. I use ANOVA tests to determine differences in invertebrate and fish richness, trophic level, biotic index values, percentage Ephemeroptera, Plecoptera, and Trichoptera (EPT), and fish catch per unit effort across watersheds. I use linear regression tests to determine differences of these variables among habitat assessment scores. I use multivariate analysis to look at correlations of invertebrate families and genera and fish families and species with physicochemical matrices at an instream scale.

CHAPTER II

ASSESSING LAND USE EFFECTS ON WATER QUALITY PARAMETERS IN STREAMS OF THE NATCHEZ TRACE PARKWAY

Land use practices can influence water quality (Hawkins et al. 1982; Schueler 1994; Fajardo et al. 2001; Timm et al. 2001; Bonner et al. 2009; Infante et al. 2009). Abiotic parameters, or physicochemical parameters, include data on water chemistry and instream habitat. No physicochemical research has been conducted over the entire length of the Natchez Trace Parkway (NTP). Water quality is a growing concern for stream managers, and for a national park that is visited as considerably as the NTP, the aesthetic beauty is also of concern. Streams along the NTP need to be monitored to conserve the integrity of the park and protect them from adjacent land uses.

Forested, agricultural, and urban land uses are typically evaluated for impact analysis. Parameters most often impacted by land use are suspended solids and turbidity because of erosion and sedimentation from vegetation removal (Jones et al. 2001; Meador and Goldstein 2003; Long and Plummer 2004; Larsen et al. 2009). Riparian vegetation is useful in reducing nutrient loading and sediment yield (Heathwaite et al. 1998; Jones et al. 2001; Snyder et al. 2005). Fertilizers and animal wastes are often sources of nitrate and phosphate; therefore, watersheds with a large percentage of agricultural land use generally have high concentrations of these nutrients (Hem 1985;

Edwards et al. 2000; Fajardo et al. 2001; Jones et al. 2001). Phosphorous is typically a limiting nutrient in freshwater systems with concentrations increasing with increasing soil erosion (Edwards et al. 2000). Rain events wash fecal coliform into the stream and can significantly increase coliform contamination in streams (Baudart et al. 2000; Edwards et al. 2000).

Impervious surfaces in urban or developed land use can be an influential variable in predicting stream quality at the watershed level (Snyder et al. 2005). Vegetation slows runoff and soil allows infiltration of precipitation, whereas concrete carries runoff to lower elevations, taking pollutants along with it to streams. This runoff often contains fertilizers used on lawns and golf courses and sewage wastes, adding excess nutrients to streams. Catchment areas containing more impervious areas have higher levels of pollutants and specific conductance than other land uses (Schueler 1994).

Goals and Objectives

I investigate the hypothesis that watersheds and land use at the instream level, indicated by habitat assessment scores, will influence physicochemical parameters in streams of the Natchez Trace Parkway. I measure for differences in water chemistry, *Escherichia* coli, and habitat across watersheds and instream levels. Habitat was assessed following Barbour et al. (1999).

Methodology

Study Site

The Natchez Trace Parkway is a 444-mile road crossing through Mississippi, Alabama, and Tennessee. Approximately 100 streams flow within the park boundary, ranging from large rivers to streams commonly referred to as drainage ditches. This thesis represents a subset of a larger project funded by the National Park Service to develop a water chemistry database in 44 streams on the Natchez Trace Parkway. Thirteen of these sites and five additional wadeable sites were selected for more intensive sampling, using a Rapid Bioassessment Protocol (RBP) (Barbour et al. 1999) for habitat. Sites were selected by a stratified random method to encompass the length of the NTP and provide three replicate samples per watershed (Figure 2.1). Six HUC4 watersheds were identified within the NTP boundary: Mississippi (0806), Pearl (0318), Tombigbee (0316), Tennessee (0603), Lower Cumberland (0604), and Upper Cumberland (0513). Mississippi, Pearl, and Tombigbee watersheds are referred as southern watersheds during some analysis. Similarly, Tennessee, Lower Cumberland, and Upper Cumberland watersheds are referred as northern watersheds.

Thirteen of the original 44 streams had six sampling periods (October 2007 – April 2009) and the five additional streams had two sampling periods (June – July 2008 and February and April 2009). Water quality parameters and *E. coli* were sampled once per stream site by season: April, July, October, and January as spring, summer, fall, and winter seasons, respectively.

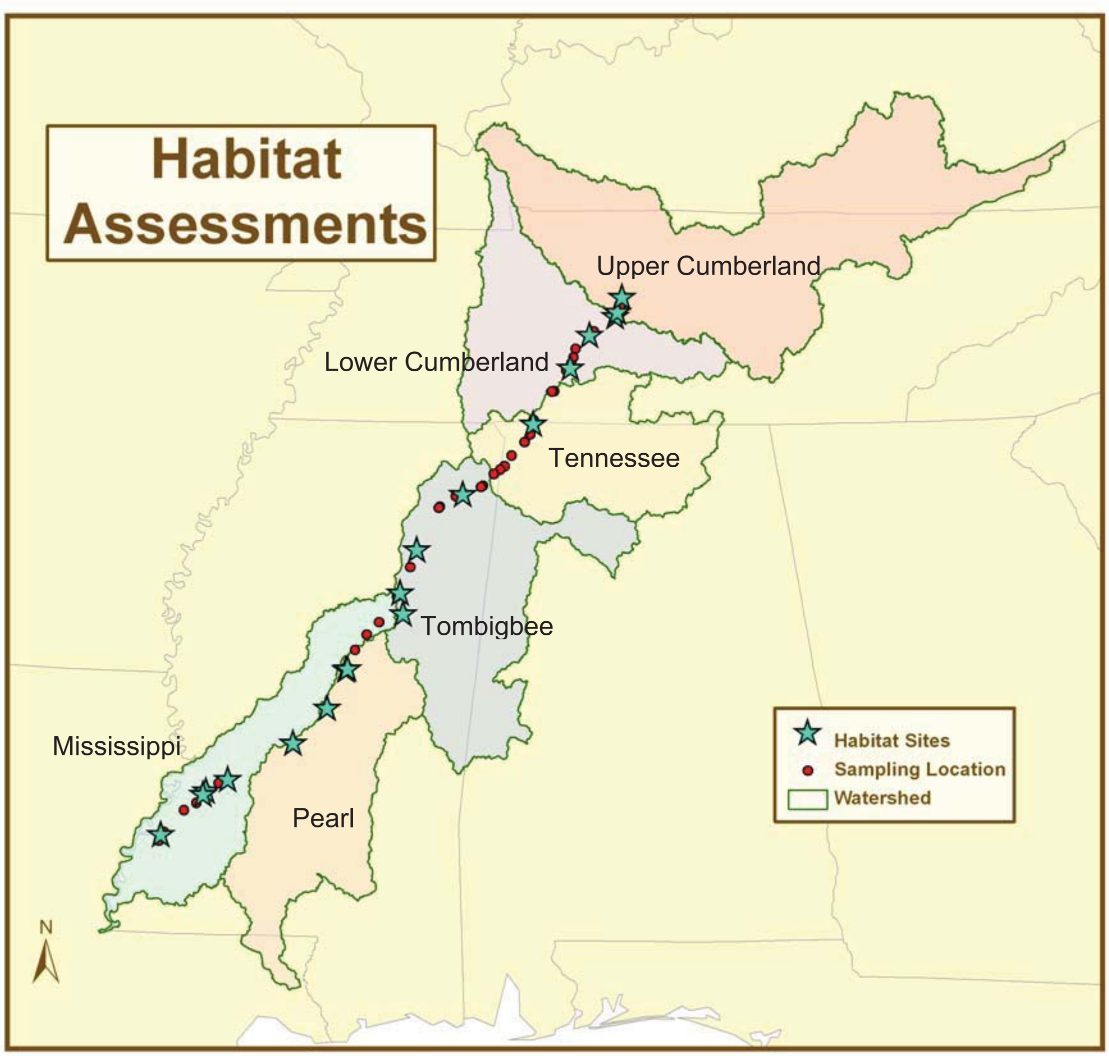


Figure 2.1 Sampling locations along the Natchez Trace Parkway (October 2007 – April 2009). The red dots represent the 44 water chemistry sites sampled for the National Park Service whereas the blue stars represent the 18 sites chosen for this project. Six Hydrologic Unit Code 4 watersheds are labeled.

Field Procedures

Water chemistry and fecal *E. coli* were sampled in 18 streams of the Natchez Trace Parkway. Nutrient samples were collected in vials using a 25 millimeter (mm) syringe filter. Other water samples were taken directly from the stream. An *E. coli* water sample was collected in 100 milliliter (mL) IDEXX bottles (IDEXX 2007a) and one 800 mL sample was collected to calculate total suspended solids and alkalinity (Wetzel and Likens 1991) for each stream. Dissolved oxygen (mg L^{-1} and % saturation), pH, water temperature (˚C), and specific conductance (µS cm^{-1}) were measured using an YSI 556 handheld multi-probe meter (YSI Environmental, Yellow Springs, OH, USA). Turbidity (Nephelometric Turbidity Units (NTU)) was measured using a LaMotte 2020 Turbidimeter.

Habitat assessments (Barbour et al. 1999) were conducted in June-July 2008. One sample was taken per stream. Scores ranged from 0 - 200, with the score of 0 representing highly disturbed sites with poor stream quality and 200 as a pristine site with excellent stream quality. Two assessment forms were used depending upon the characteristics of the stream: one for high gradient streams and one for low gradient streams. High gradient streams are characterized by clear, flowing streams with coarse substrates and high structural complexity whereas low gradient streams are characterized more by pools, heavy erosion, and fine substrates. I used high gradient forms for sites within the Tennessee, Lower Cumberland, and Upper Cumberland watersheds and low gradient forms for sites within the Mississippi, Pearl, and Tombigbee watersheds. Visual observations of canopy cover were estimated within the sampling reach for each stream.

A modification to RBP was necessary to sample within the park boundary. The protocol suggests sampling 100 meters (m) from a bridge or roadway; however, many sites had limited accessibility past a 20 m distance from the road. To standardize methodology, I took water chemistry samples approximately 6 m from culverts. Only wadeable sites were selected, thus the deeper rivers were excluded from the assessment. Two streams initially chosen for RBP, Hurricane and Pigeon Roost creeks, were removed from the final analysis due to the lack of water during summer sampling and bridge construction, respectively.

Laboratory Procedures

Nutrients, *E. coli* colonies estimates, alkalinity, and total suspended solids were analyzed in the laboratory at Mississippi State University. All water samples excluding *E. coli* samples were refrigerated and analyzed within 28 days. Nutrients were measured using a Dionex DX-500 ion chromatograph. Anions nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) were analyzed using method 4110B (ASTM 2005), and cations ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) were analyzed using the ASTM D6919-03 method (ASTM 2003). Total dissolved nitrogen and phosphorous as phosphate were calculated by taking the sum of each affiliated nutrient. First, the molecular mass of the nutrient was divided by the molecular mass of the compound and then the nutrient concentration collected in parts per million (ppm) was multiplied. For example:

$$[(14.01 \text{ gmol}^{-1} \text{ N}) / (62.01 \text{ gmol}^{-1} \text{ NO}_3)] \text{ 10 ppm NO}_3 = 2.26 \text{ ppm NO}_3\text{-N}$$

$$[(14.01 \text{ gmol}^{-1} \text{ N}) / (46.01 \text{ gmol}^{-1} \text{ NO}_2)] \text{ 3 ppm NO}_2 = 0.91 \text{ ppm NO}_2\text{-N}$$

$$[(14.01 \text{ gmol}^{-1} \text{ N}) / (18.05 \text{ gmol}^{-1} \text{ NH}_4)] \text{ 5 ppm NH}_4 = 3.88 \text{ ppm NH}_4\text{-N}$$

$$\text{Total Nitrogen} = 7.05 \text{ ppm}$$

Fecal *E. coli* water samples were kept on ice and analyzed within 24 hours (IDEXX 2007a). Contamination was estimated (number of colonies 100 mL^{-1}) by using the IDEXX 51-well Quanti-Tray Most Probable Number (MPN) Table (IDEXX 2007b). The MPN Table used to estimate colonies had a maximum of 200.5 colonies 100 mL^{-1} water sample, therefore if samples reached this level they were designated as > 200.5. Lower 95% confidence limit fecal *E. coli*, provided on the MPN Table, was used to analyze contamination because most total fecal *E. coli* samples reached the 200.5 colony maximum.

Alkalinity (ANC) was calculated following the Gran Alkalinity Titration procedures (Wetzel and Likens 1991) using a Mettler Toledo meter and pH probe. The initial pH was measured and recorded for 50 mL of each water sample. I used an automatic pipette to add appropriate increments of 0.10N HCl to the water sample to achieve a pH of 3.9-4.1. The Gran Function (F1) value was calculated for each sample. Gran Function is the measure of accumulated protons in the initial sample or as the acid is added. The formula used is:

$$F1= [\text{volume of sample (L)} + \text{volume of acid added (L)}] * 10^{-pH}$$

The data from pH 4 to pH 3.5 was plotted in a graph in which the F1 value (y-axis) is regressed against the volume of acid added (x-axis). The slope and y-intercept from the regression equation are used to calculate the x-intercept by:

$$\text{X intercept} = -1 \text{ (Y intercept / slope)}$$

Alkalinity was then calculated for each sample:

$$\text{Alkalinity} = [(0.1 \text{ N} * \text{x-intercept (L)}) / \text{sample volume (L)}] * 1_E^{6}$$

Total suspended solids (TSS) were calculated after filtering the water sample through a motor-powered vacuum (i.e., Maul et al. 2004). One hundred milliliters of deionized water was drawn through a 47 mm filter. The filter was then placed in a drying oven for 24 hours, weighed on an electronic balance, and the weight was recorded. Each water sample was shaken to bring sediment into solution. I measured 100 mL and promptly filtered each sample. The sides of the filter holder were rinsed to remove sediment residue for the next water sample. The filter was again dried, weighed, and recorded. TSS was calculated by taking the difference of the filter weight (mg) after the water sample was filtered and the filter weight (mg) before the water sample was filtered and dividing by amount of sample (L) filtered.

Analysis

Water quality determined at two scales: one at a broad scale, using HUC4 watersheds ($n = 6$) and one at a local scale, using habitat assessment scores as a local land use proxy ($n = 18$). Land use information was acquired through the 2001 National Land Cover Data as part of the Multi-Resolution Land Characteristic Consortium (MRLC 2001) for the six HUC4 watersheds. I reclassified land use (e.g., Jones et al. 2001) as *forest*, *developed*, and *agriculture* (Appendix A and B; Figure 2.1).

Data were checked for normality using PAST (Hammer et al. 2001) and log transformed when Shapiro-Wilk (Shapiro and Wilk 1965) P-values were less than 0.05. Analysis was performed using JMP software (JMP 2007). Analysis of variance (ANOVA) and Tukey-Kramer Honestly Significant Difference (HSD) tests ($\alpha = 0.05$)

were used to analyze HUC4 watersheds for physicochemical parameters and *E. coli*, and linear regressions were used to determine relationships between habitat assessments and physicochemical parameters and *E. coli.*

Results

The overall difference of land use among watersheds was not significant (chi-squared P = 0.42). Land use composition did not vary enough to identify a high and low value of land use (i.e., agriculture, forest, developed) per watershed. Analysis of land use impacts at the HUC4 watershed scale was greatly weakened due to this fact, therefore analysis was by HUC4 watershed rather than the land use.

Table 2.1 Percentage developed, forest, and agricultural land uses within Hydrologic Unit Code (HUC) 4 watersheds of the Natchez Trace Parkway (MS: Mississippi, PL: Pearl, TB: Tombigbee, TN: Tennessee, LC: Lower Cumberland, and UC: Upper Cumberland).

HUC4	Developed (%)	Forest (%)	Agriculture (%)
MS	4.76	49.82	18.7
PL	6.74	47.58	18.51
TB	5.49	48.03	22.57
TN	8.05	41.65	35.71
LC	4.77	59.59	24.47
UC	8.2	58.47	26.12

Forest covered slightly more area in Lower and Upper Cumberland watersheds (60 % and 58 %, respectively), whereas Tombigbee (48 %) and Tennessee watersheds (42 %) had the least amount of forested land (Table 2.1 and Figure 2.2). The least *developed* land was in Mississippi and Lower Cumberland watersheds (both 5 %) whereas Upper Cumberland (8 %) and Tennessee watersheds (11 %) had the most. *Agricultural* practices covered more area in the Tennessee watershed (36 %) and less area in Mississippi and Pearl watersheds (both 19 %).

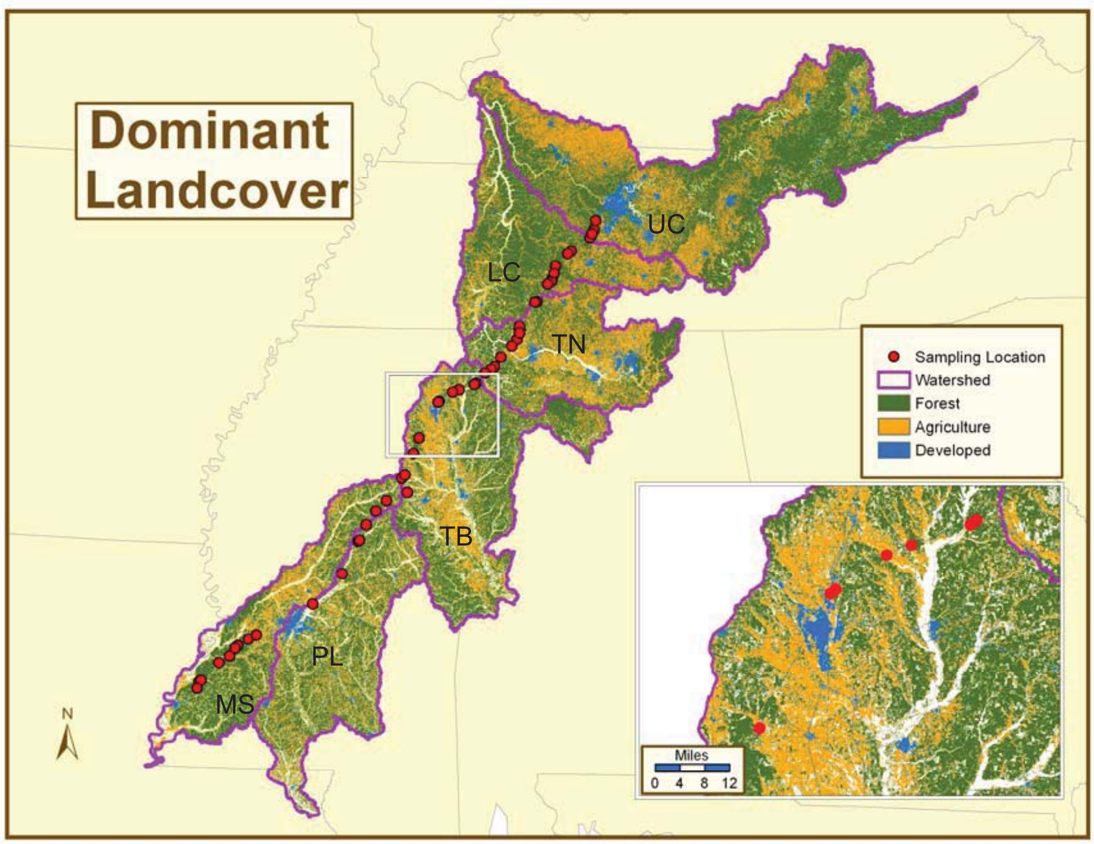


Figure 2.2 Forest, agriculture, and developed land uses on the Natchez Trace Parkway were analyzed at the HUC4 watershed level (MS: Mississippi, PL: Pearl, TB: Tombigbee, TN: Tennessee, LC: Lower Cumberland, UC: Upper Cumberland). Inset zooms in on the three land uses defined.

Water Chemistry Parameters

Several water chemistry parameters varied among watersheds (Table 2.2). ANOVA tests showed significant differences in dissolved oxygen (DO) mg L^{-1} between watersheds ($P = 0.05$); however, Tukey-Kramer HSD analysis ($\alpha = 0.05$) did not show significant differences between watersheds. Mean DO concentrations were greatest in the Upper Cumberland (11 mg L^{-1}) and Lower Cumberland watersheds (10 mg L^{-1}) and least in the Pearl watershed (7 mg L^{-1}). Average pH differed significantly between watersheds ($P = 0.03$), but Tukey-Kramer analysis did not show significant differences. The Lower and Upper Cumberland watersheds had the greatest average pH (7.31 and 7.54, respectively) whereas the Pearl and Tennessee watersheds had the least average (6.27 and 6.31, respectively).

Table 2.2 ANOVA results of water chemistry averages for HUC4 watersheds of the Natchez Trace Parkway (October 2007 – April 2009). Bold font signifies significant values.

HUC4	Water Temp (˚C)	Cond. (µS cm^{-1})	DO (mg L^{-1})	pH	Turbidity (NTU)	Fecal E.coli (colonies 100 mL^{-1} sample)	ANC (mg L^{-1})	TSS (mg L^{-1})
MS	16.78	169.14	8.09	7.01	38.28	136.72	89.06	44.75
PL	17.24	42.51	7.34	6.27	25.56	89.81	26.77	14.56
TB	13.97	215.83	9.84	7.10	51.84	58.39	59.27	38.17
TN	13.96	25.28	9.61	6.31	2.80	104.26	10.34	3.18
LC	13.27	131.39	10.09	7.31	2.56	42.73	94.31	3.43
UC	12.10	213.61	10.77	7.54	1.93	105.90	115.59	2.56
(p-value)	0.19	**<0.01**	**0.05**	**0.03**	**<0.01**	0.07	**0.02**	0.18
(r^2)	0.42	**0.74**	**0.57**	**0.61**	**0.78**	0.54	**0.63**	0.43

Total suspended solids (TSS) and turbidity generally decreased with increasing latitude. Turbidity differed significantly among watersheds ($P < 0.01$). The Pearl watershed tended to have the greatest average turbidity (52 NTU), whereas Upper Cumberland (2 NTU), and Lower Cumberland and Tennessee watersheds (both 3 NTU) tended to be the least. Mississippi and Tombigbee watersheds tended to have the greatest mean TSS (45 and 38 NTU respectively) and Upper Cumberland, Lower Cumberland, and Tennessee watersheds (all 3 NTU, respectively) tended to be least.

Mean alkalinity ($P = 0.02$) was significantly greater in the Upper Cumberland watershed (116 mg L^{-1}) compared to the Tennessee watershed (10 mg L^{-1}). Conductivity differed significantly among watersheds ($P < 0.01$). The Upper Cumberland was significantly greater than Tennessee ($P < 0.01$) and Pearl watersheds ($P = 0.04$). The Tennessee watershed was significantly less than Tombigbee ($P = 0.01$) and Mississippi watersheds ($P = 0.03$). Average conductivity was least in the Tennessee (25 mg L^{-1}) and Pearl watersheds (43 mg L^{-1}) whereas the Tombigbee and Upper Cumberland watersheds (216 and 214 mg L^{-1} respectively) had the greatest.

Average nitrate (NO_3^-) concentration ($P = 0.50$) (Table 2.3) tended to be greater in the Lower Cumberland watershed (3 mg L^{-1}). The least average concentrations tended to be in Mississippi and Pearl watersheds (both 0.2 mg L^{-1}). Total nitrogen (TN) ($P = 0.86$) also tended to have a greater mean concentration in the Lower Cumberland watershed (0.61 mg L^{-1}) whereas Mississippi and Pearl watersheds (both 0.08 mg L^{-1}) tended to have the least. Nitrite (NO_2^-) ($P = 0.38$) tended to have the greatest average concentration in the Tombigbee watershed (0.02 mg L^{-1}). The least average concentrations tended to be in Upper Cumberland, Tennessee, and Pearl watersheds (all < 0.001 mg L^{-1}). Average ammonium (NH_4^+) (P = 0.59) tended to be greatest in Mississippi (0.05 mg L^{-1}) and Pearl watersheds (0.05 mg L^{-1}) whereas the Tennessee watershed (0.005 mg L^{-1}) had the least.

Table 2.3 Mean nutrient concentrations analyzed by HUC4 watersheds of the Natchez Trace Parkway (October 2007 – April 2009). Bold font signifies significant values.

HUC4	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	PO_4 (mg L^{-1})	SO_4 (mg L^{-1})	TN (mg L^{-1})	NH_4 (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Ca (mg L^{-1})
MS	0.01	0.16	0.11	5.46	0.08	0.05	3.61	6.27	15.68
PL	0.00	0.19	0.00	2.31	0.08	0.05	1.13	1.08	3.43
TB	0.02	0.58	0.01	13.52	0.16	0.03	2.57	7.05	25.72
TN	0.00	0.71	0.01	2.32	0.16	0.00	0.74	0.93	3.31
LC	0.00	2.60	0.19	6.66	0.61	0.03	1.09	2.58	27.89
UC	0.00	0.41	0.05	28.51	0.11	0.01	0.77	6.36	37.72
(p-value)	0.38	0.49	0.37	**0.01**	0.86	0.60	0.06	**0.03**	**<0.01**
(r^2)	0.33	0.28	0.33	**0.72**	0.14	0.24	0.54	**0.62**	**0.77**

Phosphate (PO_4^-) mean concentrations (P = 0.37) tended to be greatest in Lower Cumberland and Mississippi watersheds (0.19 and 0.11 mg L^{-1}, respectively). The Pearl watershed tended to have the least average PO_4^- concentration, < 0.001 mg L^{-1}. Mean sulfate (SO_4^-) concentrations differed significantly among watersheds (P = 0.01). The Upper Cumberland watershed had significantly greater SO_4^- levels than the Pearl watershed (P < 0.01). Average concentrations were greatest in Upper Cumberland (29 mg L^{-1}) and Tombigbee (14 mg L^{-1}) and least in Pearl and Tennessee watersheds (both 2 mg L^{-1}).

Magnesium (Mg^+) mean concentrations (P = 0.03) differed significantly among watersheds; however Tukey-Kramer tests did not find significance at α = 0.05. Average concentrations were greatest in Tombigbee (7 mg L^{-1}) and Upper Cumberland and Mississippi watersheds (both 6 mg L^{-1}) and least in Tennessee and Pearl watersheds (both 1 mg L^{-1}). Average calcium (Ca^{+}) concentrations ($P = 0.002$) differed significantly among watersheds. Mean concentrations in the Upper Cumberland were significantly greater than Tennessee and Pearl watersheds (both $P = 0.005$, respectively). Average Ca^{+} concentrations in the Tombigbee watershed was also significantly greater than the Tennessee and Pearl watersheds (both $P = 0.02$, respectively). Potassium means ($P = 0.06$) tended to be least in Tennessee (0.7 mg L^{-1}) and Upper Cumberland watersheds (0.8 mg L^{-1}) and greatest in Mississippi (4 mg L^{-1}) and Tombigbee watersheds (3 mg L^{-1}).

Habitat Parameters

Instream parameters, except in cobble substrate composition, did not show significant differences among watersheds (Table 2.4). Percentage cobble ($P < 0.001$) was significantly greater in the northern watersheds (Tennessee, Lower Cumberland, and Upper Cumberland) compared to the southern watersheds (Mississippi, Tombigbee, and Pearl) (all $P < 0.001$). Coarse substrates, percentage bedrock, boulder, cobble, and gravel, tended to be greatest in northern watersheds whereas percentage sand, silt, and clay were greatest in southern watersheds. Bedrock ($P = 0.09$) was only noted in Lower Cumberland (42 %) and Upper Cumberland watersheds (15 %). Average boulder substrate ($P = 0.27$) composed only 7 % of Lower Cumberland and 4 % of Tennessee and Upper Cumberland watersheds. Percentage gravel averages ($P = 0.14$) tended to be least in Mississippi and Pearl watersheds (3 and 7 %, respectively) and greatest in Upper Cumberland (38%), Tennessee (29 %) and Lower Cumberland watersheds (28 %).

Sand ($P = 0.11$) tended to compose more average percentage channel substrate in Mississippi (61 %) and Pearl watersheds (48 %), whereas the least percentage sand composition was in Lower Cumberland (1 %) and Upper Cumberland watersheds (3 %). Silt ($P = 0.40$) tended be greater on average in Mississippi (29 %) and Tombigbee watersheds (20 %). The Lower Cumberland watershed tended to have the least percentage silt (5 %). Upper Cumberland and Tennessee watersheds also tended to have very low silt composition (both 6 %). Mean clay ($p= 0.05$) percentage composition tended to be greatest in Pearl (33 %) and Tombigbee watersheds (30 %). Clay was not part of the substrate composition in Lower Cumberland or Tennessee watersheds.

Average depth ($P = 0.60$) and width ($P = 0.62$) did not differ significantly among watersheds. Streams tended to be deeper in Mississippi (0.4 m) and Tombigbee watersheds (0.3 m), whereas Tennessee, Upper Cumberland, and Lower Cumberland watersheds (all 0.2 m, respectively) tended to have the least average depths. Stream width tended to be greater in Mississippi (5 m) and Lower Cumberland watersheds (4 m).

Instream parameters had fewer significant differences among habitat scores. There was a significant negative correlation between assessments scores and TSS ($P = 0.04$) (Table 2.7). Greater habitat assessment scores were associated with lesser TSS levels. Average water temperatures ($P = 0.60$) tended to be greater in sites with lesser habitat assessment scores. Conductivity ($P = 1$) means were not correlated with habitat assessment scores. DO averages ($P = 0.33$) showed a general trend of greater concentrations found in greater habitat score sites. Mean pH (P = 0.30) was generally greater in sites with greater habitat assessment scores. Turbidity averages (P = 0.02) were significantly correlated negatively with habitat assessment scores. There was a trend of lesser mean lower limit fecal *E. coli* estimates (P = 0.44) in sites with greater habitat scores. Alkalinity averages (P = 0.91) were not correlated with assessment scores.

Table 2.4 Instream parameters for HUC4 watersheds of the Natchez Trace Parkway (June – July 2008). Bold font signifies significant values.

HUC4	Bedrock (%)	Boulder (%)	Cobble (%)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Avg Depth (m)	Avg Width (m)
MS	0.00	0.00	0.00	3.00	61.33	29.00	6.67	0.43	4.79
PL	0.00	0.00	0.00	6.67	48.33	11.67	33.33	0.28	3.52
TB	0.00	0.00	0.00	11.67	38.33	20.00	30.00	0.33	3.01
TN	0.00	4.33	47.67	28.67	13.67	5.67	0.00	0.15	3.62
LC	42.00	7.00	17.33	28.33	0.67	4.67	0.00	0.16	4.06
UC	15.00	4.33	32.67	37.67	2.67	6.00	1.67	0.16	2.79
(p-value)	0.09	0.27	**<0.01**	0.14	0.11	0.40	0.05	0.60	0.62
(r^2)	0.51	0.38	**0.97**	0.47	0.49	0.32	0.56	0.24	0.23

Linear regression analysis did not show significant correlations among habitat assessment scores and nutrients; however, habitat scores tended to be less in sites with greater mean NO_2^- concentrations (P = 0.31). Average nitrate and total nitrogen concentrations (P = 0.75 and P = 0.95, respectively) did not differ significantly among habitat assessment scores. Lesser mean concentrations of NH_4^+ (P = 0.05) were found in greater scoring sites. Mean phosphate concentrations (P = 0.45) were generally greater in higher scoring sites. Sulfate (P = 0.83) and calcium (P = 0.59) concentration averages were not significant among habitat assessment scores. Mean potassium (P = 0.11) and magnesium concentrations (P = 0.70) were generally lesser in higher scoring habitat assessment sites.

Table 2.5 Linear regression results of habitat scores for water chemistry parameters on the Natchez Trace Parkway (October 2007 – April 2009). Habitat scores listed in latitudinal order with most southern stream reported first. Bold font signifies significant values.

HUC4_Habitat Score	Water Temp (˚C)	Cond. (µS cm^{-1})	DO (mg L^{-1})	pH	Turb (NTU)	Fecal *E. coli* (colonies 100 ml^{-1} sample)	ANC (mg L^{-1})	TSS (mg L^{-1})
MS_114	18	303	10	7.65	35	119	166	36
MS_117	16	93	9	6.85	16	152	43	11
MS_97	16	111	5	6.53	64	139	58	88
PL_141	22	57	8	6.86	39	103	20	22
PL_96	14	37	9	6.07	22	97	51	12
PL_141	16	34	5	5.88	17	70	10	9
TB_123	12	385	9	6.89	9	37	25	12
TB_102	14	138	10	7.38	11	64	117	13
TB_113	16	125	10	7.02	135	74	36	90
TN_164	14	28	9	6.52	6	160	11	5
TN_105	14	19	10	5.89	2	116	3	2
TN_155	14	30	10	6.51	1	37	17	2
LC_156	13	55	10	7.01	1	10	35	1
LC_151	15	49	9	6.79	4	36	26	5
LC_172	12	291	11	8.14	2	81	222	4
UC_119	10	183	11	7.64	2	63	115	2
UC_146	11	177	11	7.48	2	140	108	3
UC_145	16	281	11	7.5	2	116	123	2
(p-value)	0.60	0.80	0.33	0.30	**0.02**	0.44	0.91	**0.04**
(r^2)	0.02	0.00	0.06	0.07	**0.30**	0.04	0.00	**0.24**

Canopy cover was listed as four levels: open, partly open, partly shaded, and shaded (Table 2.5). Observations of shaded canopy cover tended to increase with latitude. The northern watersheds, Lower Cumberland and Upper Cumberland, tended to have the most shaded of all watersheds. All sites within the Upper Cumberland watershed were shaded.

Table 2.6 Canopy cover observations sampled June – July 2008 (MS: Mississippi, PL: Pearl, TB: Tombigbee, TN: Tennessee, LC: Lower Cumberland, UC: Upper Cumberland). Canopy level in increasing order is open, partly open, partly shaded, and shaded.

HUC4_Stream Name	Canopy Cover
MS_Mud Island Creek	Partly Shaded
MS_Big Sand Creek	Partly Shaded
MS_14 Mile Creek	Shaded
PL_Baker Creek	Shaded
PL_9 Mile Creek	Open
PL_Jaybird Creek	Shaded
TB_Old Field Creek	Partly Shaded
TB_Chuquatonchee Creek	Partly Shaded
TB_Donivan Slough	Shaded
TN_Burcham Branch	Partly Shaded
TN_Cooper Branch	Shaded
TN_Glenrock Branch	Partly Open
LC_Jack's Branch	Partly Open
LC_Chief Creek	Shaded
LC_Jackson Falls	Shaded
UC_Burns Branch	Shaded
UC_Garrison Branch	Shaded
UC_Little East Fork Creek	Shaded

Instream parameters, percentage cobble ($P = 0.02$) and gravel ($P = 0.03$), were correlated negatively with habitat assessment scores (Table 2.6). Percentage average bedrock ($P = 0.09$), boulder ($P = 0.06$), and cobble substrates also tended to be greatest in higher scoring sites. There was a trend of greater percentages of sand ($P = 0.15$), silt ($P = 0.38$), and clay ($P = 0.16$) in lower scoring sites. Average width and depth did not show significant differences among habitat assessment scores ($P = 0.45$ and $P = 0.06$, respectively). There was a trend with low scores in wider and deeper streams.

Table 2.7 Linear regressions for habitat assessment scores and instream parameters of streams along the Natchez Trace Parkway (June – July 2008). Habitat scores listed in latitudinal order with most southern stream reported first. Bold font signifies significant values.

HUC4_ Habitat Score	Avg Depth (m)	Avg Width (m)	Bedrock (%)	Boulder (%)	Cobble (%)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)
MS_114	0.34	2.33	0	0	0	1	94	5	0
MS_117	0.10	6.33	0	0	0	8	90	2	0
MS_97	0.85	5.70	0	0	0	0	0	80	20
PL_141	0.30	2.81	0	0	0	20	20	10	50
PL_96	0.30	4.40	0	0	0	0	45	10	45
PL_141	0.25	3.36	0	0	0	0	80	15	5
TB_123	0.15	2.33	0	0	0	0	25	25	50
TB_102	0.10	5.20	0	0	0	5	85	10	0
TB_113	0.73	1.50	0	0	0	30	5	25	40
TN_164	0.07	3.54	0	0	6	56	32	6	0
TN_105	0.17	3.17	0	0	82	10	7	1	0
TN_155	0.21	4.17	0	13	55	20	2	10	0
LC_156	0.21	4.50	56	20	10	10	2	2	0
LC_151	0.17	4.67	0	0	30	65	0	5	0
LC_172	0.10	3.00	70	1	12	10	0	7	0
UC_119	0.15	3.33	45	3	33	10	3	6	0
UC_146	0.11	4.17	0	0	20	70	5	5	0
UC_145	0.21	0.87	0	10	45	33	0	7	5
(p-value)	0.06	0.45	0.09	0.06	**0.02**	**0.03**	0.15	0.38	0.16
(r^2)	0.20	0.04	0.17	0.21	**0.29**	**0.28**	0.12	0.05	0.12

Discussion

At the HUC4 level, watersheds along the Natchez Trace Parkway are primarily forested. Several studies have shown that riparian buffers and forested areas help reduce nutrient loading and sediment yield (e.g., Heathwaite et al. 1998; Jones et al. 2001; Snyder et al. 2005; Larsen et al. 2009); however, forested areas are only able to buffer a stream if they are adjacent to the stream. From a broad HUC4 scale, it is unknown whether the forested area is found in the riparian zone along the NTP. Watersheds in the NTP also have moderate percentages of agricultural land. It is possible that water samples with significantly different physicochemical parameters such as turbidity were taken during or recently after crops were harvested, land was tilled, or if livestock had access to the stream channel upstream from the sampling locations. It also is possible that Best Management Practices were used in agricultural land more in watersheds where these parameter values were least. It was difficult to analyze land use impacts at a HUC4 scale because there was little variation in percentage developed, forest, and agriculture land uses among HUC4 watersheds.

More detailed descriptions of land use categories also would be beneficial for determining impacts. Farms raising pigs and chickens do not fit the *Pasture/Hay* classification, for example. There is no class given for such land use, although it is a prevalent industry and a source of organic pollution that can negatively affect physicochemical parameters (Timm et al. 2001). Waste runoff from this unaccounted practice could alter the significance of agricultural impacts on water quality parameters, nutrients in particular. In 1997, Scorecard (2005), a pollution information database, estimated 111,386 cattle in counties of the Tennessee HUC4 watershed and approximately 2,236,696 hog, poultry, and sheep in this same area. This non-cattle group resulted in an estimated 1,954,764 pounds of nitrogen per year in waste and 551,657 pounds of phosphorous per year in waste whereas cattle accounted for 8,260,000 pounds of nitrogen per year in waste and 2,146,000 pounds of phosphorous per year in waste (Scorecard 2005). Despite this oversight, fecal *E. coli* was not statistically significant among HUC4 watersheds or habitat assessment scores.

Urban and agricultural areas are linked with high levels of turbidity and specific conductance (Jones et al. 2001; Long and Plummer 2004). Watersheds in the Long and Plummer (2004) study had notably high and low forest vs. agriculture land uses; therefore, the land use impacts were more detectable. Watersheds in the current study had a poor breakdown of land use composition, making it difficult to determine effects due to the miniscule land use changes.

Turbidity differed significantly among HUC4 watersheds. Levels were significantly greater in Mississippi, Pearl, and Tombigbee watersheds, although these had comparatively lesser percentages developed and agricultural land uses. Turbidity was associated with TSS, which also tended to be greater in these watersheds. At the habitat assessment score scale, turbidity and TSS differed significantly. This created a separation approximately at the Mississippi state line. All streams found within HUC4 watersheds encompassing Mississippi (Mississippi, Pearl, and Tombigbee watersheds) had significantly greater turbidity and TSS levels. This, coincidentally, is also the "boundary" used to mark the separation of low-gradient and high-gradient habitat assessment forms used.

Conductivity differed significantly among HUC4 watersheds. Upper Cumberland, Tombigbee, and Mississippi watersheds had significantly greater conductivity averages. Unpublished data collected by the NPS also showed a Mississippi versus non-Mississippi shift in turbidity averages in select streams along the NTP. In July – August 2008, data loggers recorded an average 71.39 NTU in Mississippi, Pearl and Tombigbee watersheds compared to 14.2 NTU in Tennessee, Lower Cumberland, and Upper Cumberland watersheds.

Average DO differed significantly among HUC4 watersheds. Lower and Upper Cumberland watersheds generally had greater DO averages. The Pearl watershed generally had the least DO average. This was primarily because of Jaybird Creek (see Table 2.6 for list of HUC4 watersheds and stream names). This site has large amounts of woody debris in the channel and evergreen trees in the riparian community, which probably accounted for the low DO concentration. Another study also observed low DO concentrations, characteristic of the Pearl watershed and region (Hashim 2005).

Alkalinity, Ca^+, and Mg^+ differed significantly among watersheds. Although ANC is not synonymous with water hardness, which is related to Ca^+ and Mg^+ concentrations, sampled Ca^+ or Mg^+ parameters could be forms of carbonate used to increase total alkalinity (Wetzel 2001). The Tennessee watershed had significantly less ANC and Ca^+ than the Lower Cumberland watershed. The Tennessee watershed tended to have the least average Mg^+. Also, greater pH is often associated with high levels of bicarbonate alkalinity (Wetzel and Likens 1991; Wetzel 2001). The Upper Cumberland watershed tended to have greater pH levels and ANC.

Average pH differed significantly among HUC4 watersheds. Land use is more relevant in explaining mean pH if land uses are unclassified. In general, pH increased with increasing latitude. Evergreen forest covered more area in Pearl, Tombigbee, and Mississippi watersheds where pH tended to be least. DO saturation increases with decreasing temperature (Long and Plummer 2004), and lesser DO averages tended to be found in these southern watersheds. Catchment vegetation can define nutrient composition of a waterbody such as low pH associated with coniferous forest (Thomsen and Friberg 2002; Irfanullah 2009).

Percentage cobble substrate differed significantly among HUC4 watersheds and habitat assessment scores. The presence of cobble marked an invisible “boundary” just as habitat assessment forms, turbidity, and TSS. Only Tennessee, Lower Cumberland, and Upper Cumberland sites contained cobble.

Streams in the northern portion of Pearl watershed along the NTP had fine sediment, primarily sand, silt, and clay, with approximately 20-25 percentage gravel (Hashim 2005). In the current study, the Pearl watershed had predominantly fine particle substrate, primarily sand and clay with minor amounts of gravel. Percentage gravel differed significantly among habitat assessment scores. Only one site in the Pearl watershed had gravel (20 %), falling very close to the observation by Hashim (2005). Greater percentage gravel was found in greater scoring habitat assessment sites.

Scale is an important variable in ecological studies (Levin 1992; Lewis et al. 1996; Herlihy et al. 2006; Wang et al. 2006; Díaz-Varela et al. 2009). Variation can occur within a watershed, and given the scale, can be misrepresented as a homogeneous rather than heterogeneous structure. As window size increases, variation decreases in homogenous environments (Levin 1992). It is possible that the HUC4 scale viewed portions of the region with similar land use composition whereas a coarser, HUC2 or finer, HUC8 scale might detect land use variability. Díaz-Varela et al. (2009) observed that using various sizes of windows to give multiple scales of a location can result in different landscape patterns. Smaller windows discern local landscape variation that is otherwise undetectable with larger windows. The HUC4 watershed scale might have been too large of a window, masking land use differences among sampling sites. This is a scale-response problem (Díaz-Varela et al. 2009) that probably affected results of physicochemical parameters along the NTP because stream quality characteristics will vary with scale (Levin 1992). The pattern may be operating at one scale whereas the mechanism may be operating at another scale (Levin 1992). Nutrients were not only entering and cycling in the 10-15 m stream reaches sampled in the current study, for example, therefore creating conclusions at that scale may not be appropriate.

Management decisions need to be based on the proper temporal and spatial scale. Seasonal variation of land practices such as springtime fertilizer application on agricultural land could cause peaks in NO_3^-, NO_2^-, NH_4^+, and PO_4^- concentrations and increase the overall mean for affected watersheds. Changes and shifts associated with land practices might not have been apparent in the months sampled. Also, five of the sites were only sampled two times, once in summer during the habitat assessment and once in spring or winter for the fish assessment. Sample size could be increased to better assess variability in water quality.

Hydrologic Unit Codes can be used as a watershed scale with varying sizes of HUC8 or cataloging unit (fine) to HUC2 or regional unit (coarse) (Seaber et al. 1987). Watersheds are hydrologic and political boundaries used to address human impacts at specific streams or waterbodies (Omernik and Bailey 1997). There is a false assumption that all levels of HUC are true watersheds (Omernik and Bailey 1997). Seaber et al.

(1987) stated that only four out of eleven HUC6 watersheds in Tennessee were true topographic watersheds (Figure 2.3).

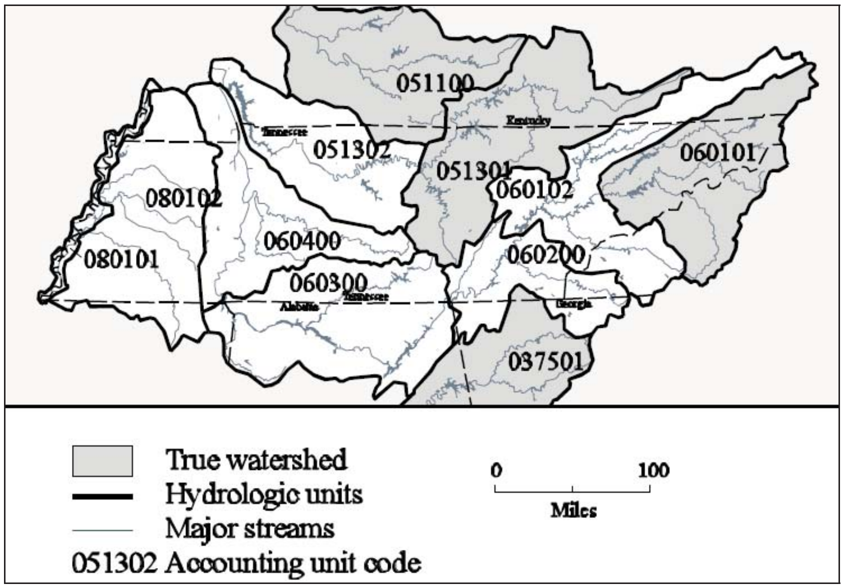


Figure 2.3 Only four out of eleven HUC6 watersheds are true watersheds in Tennessee (Seaber et al. 1987; Omernik and Bailey 1997).

While watersheds are useful in explaining contaminants carried by water, an inaccurate scale for watershed boundaries could invalidate analysis. For example, hydrology would not follow the boundary of an HUC4 watershed if it was not a true watershed; therefore, any management decisions based upon this false watershed would be inaccurate. Streams not included in the delineation of this HUC4 watershed could carry fish, nutrients, or sediment an agency was trying to regulate, and although the agency could think they were fulfilling the regulation, the problem would still appear due to the erroneous information.

Conclusion

This project observed that land use, as indicated by habitat assessment scores, influenced physicochemical parameters in streams of the Natchez Trace Parkway. At the HUC4 watershed scale, physicochemical parameters also showed significant differences. Land use practices can influence water quality (Hawkins et al. 1982; Schueler 1994; Fajardo et al. 2001; Timm et al. 2001); however, those impacts are only detectable when a comparison can be made. Land practices were determined at the HUC4 watershed level and classified as forest, developed, and agriculture. Land cover at this scale was too similar among watersheds to relate water chemistry, *E. coli*, and habitat assessment scores to land uses. Forested land covered the most area in all watersheds (42 - 60 %) followed by agriculture (19 - 36 %). Habitat assessment scores were used as a local, land use proxy. Scores were quantified by instream parameters such as riparian vegetation and substrate composition. Land use at this scale did show variability in water quality parameters among habitat assessment scores.

Hydrologic Unit Code 4 watersheds showed significant differences with conductivity, DO, pH, turbidity, ANC, SO_4^-, Mg^+, Ca^+, and percentage cobble. Comparisons could be made at the watershed level, but because land uses were so similar, distinctions could not be made by land uses. Two watersheds, Upper Cumberland and Tennessee, did show relationships between their relatively “high” land covers and physicochemical parameters. The Upper Cumberland watershed had significantly greater conductivity, ANC, and Ca and significantly less turbidity. This watershed had one of the greatest forest cover percentages relative to other HUC4 watersheds in the park, and the buffering ability of vegetation probably helped reduce turbidity. The Tennessee watershed had the greatest percentage agriculture cover relative to other HUC4 watersheds along the NTP. It also had significantly less conductivity, turbidity, alkalinity, and Ca^{+} and significantly greater percentage cobble substrate.

Habitat assessment scores showed significant differences in TSS, turbidity, and percentage cobble and gravel. Higher scoring sites had less TSS, except in Cooper Branch and Burns Branch in the Tennessee and Upper Cumberland watersheds, respectively. Higher scoring sites also had lesser mean turbidity except in Cooper Branch and Burns Branch. Cobble substrate was greatest in higher scoring sites except in Cooper Branch and Burns Branch, and gravel generally composed more substrates in higher scoring sites.

A different scale should be implemented to assess regional impacts. Watersheds at the HUC4 level was possibly too coarse or too fine of a scale to observe land use variability within designated watershed boundaries. Canopy cover and riparian vegetation are important variables for aquatic fauna (Hawkins et al. 1982; Timm et al. 2001; Wetzel 2001; Camargo et al. 2004) and would be beneficial to better quantify for analysis rather than categorize in broad groups. This project gives insight into the quality of streams along the NTP, but it did not provide an assessment of land use impacts at a regional level. A better understanding of scale and ecology is needed to better assess stream quality along the Natchez Trace Parkway.

CHAPTER III

ASSESSING LAND USE EFFECTS AND WATER QUALITY PARAMETERS ON FISH AND INVERTEBRATE COMMUNITIES IN STREAMS OF THE NATCHEZ TRACE PARKWAY

Fish assemblages (Ross 1994; Johnston and Phillips 1999; Johnston 2007) and macroinvertebrate communities (Hashim 2005; Hashim and Jackson 2008) have been studied on the Natchez Trace Parkway (NTP). However, these taxa have never been studied to determine relationships with land use in watersheds of the NTP. Furthermore, little is known about macroinvertebrate communities in streams across the entire length of the park. Water chemistry offers a snapshot of the quality of a stream. Macroinvertebrate assemblages indicate disturbances over time and can provide short-term insight, due to their short lifespans, to cumulative impacts of localized stress (Barbour et al. 1999). Fish communities, on the other hand, offer long-term indications of environmental stress because of their relatively long lifespans (Barbour et al. 1999; Grabarkiewicz and Davis 2008). By assessing water quality using these biotic parameters, land use impacts may be better understood for streams in the Natchez Trace Parkway.

Land use effects have been shown to influence invertebrate and fish communities (e.g., Lammert and Allan 1999; Nerbonne and Vondracek 2001; Meador and Goldstein

2003). Impacts of land uses on water quality from nonpoint source pollution are associated commonly with urban and agricultural land. Increased levels of nitrate, turbidity, conductivity, and suspended solids are a few issues linked with these land practices (Edwards et al. 2000; Jones et al. 2001; Long and Plummer 2004). Changes in these parameters could be harmful for aquatic ecosystems. Nutrient enrichment is a damaging effect from fertilizer usage on agricultural land (Edwards et al. 2000; Jones et al. 2001). It could impact invertebrate and fish communities by altering the growth of aquatic vegetation, which influences predator-prey interactions and herbivore food abundance, and/or lead to low DO (Meador and Goldstein 2003; Camargo et al. 2004; Maul et al. 2004).

Abiotic parameters are the underlying factors in determining the biotic integrity of a stream. They are essential to understanding the quality and communities of a stream. Standards for water chemistry parameters such as dissolved oxygen (DO) have been placed to maintain stream quality and support aquatic life. The Environmental Protection Agency set a minimum DO concentration of 5 milligrams per liter (mg L^{-1}) because research has supported that diverse aquatic life is not sustainable below this concentration. This parameter is one of many that can influence the presence and abundance of particular organisms. Other factors can shape community structure directly (i.e., temperature) or indirectly (i.e., riparian vegetation).

Riparian vegetation provides allochthonous material, a food source for many aquatic organisms (Hawkins et al. 1982). Canopy cover and overhanging vegetation can affect other parameters such as water temperature, which in turn additionally influences presence and abundance of biota, especially organisms with narrow temperature tolerance ranges. Invertebrate trophic groups can be determined by instream parameters. For example, shredders, which feed on leaf litter, are invertebrates that are generally less abundant in areas lacking canopy and more abundant in areas with deciduous canopy; scrapers, which feed on periphyton, are expected to be more abundant in areas without canopy (Hawkins et al. 1982; Timm et al. 2001; Camargo et al. 2004).

Invertebrate and fish communities can be analyzed by taxa richness, trophic levels, habits, and pollution tolerance levels. These parameters are affected by water chemistry as well as habitat composition and adjacent land uses. Pollution tolerance levels can indicate stream quality by the presence of pollution-intolerant organisms (Sawyer et al. 2004; Kyriakeas and Watzin 2006). In general, disturbed areas show an increase in pollution-tolerant invertebrates such as oligochaetes and some chironomids and a decrease in intolerant species such as Ephemeroptera, Plecoptera, and Trichoptera (EPT) (Barbour et al. 1999; Kyriakeas and Watzin 2006).

It is useful to categorize assemblages into trophic groups to further interpret land use effects. Trophic groups of fishes respond to levels of forest cover, for example, generalist species are more abundant than specialists in less forested land (Burcher et al. 2008). Agricultural land use may negatively affect herbivorous organisms such as macroinvertebrate scrapers due to herbicide runoff reducing their food source, periphyton (Camargo et al. 2004; Kyriakeas and Watzin 2006). Trophic levels and pollution tolerance levels are often associated with one another. Many macroinvertebrate collector-gatherers and collector-filterers are categorized as tolerant and can be more abundant in polluted streams whereas scrapers and shredders are generally more intolerant of pollution, thus are not expected to be abundant in disturbed sites (Kerans and Karr 1994; Barbour et al. 1999; Camargo et al. 2004; Kyriakeas and Watzin 2006).

While fish and macroinvertebrate assemblages are often studied to determine responses to environmental conditions, taxa congruence has rarely been studied in stream ecosystems (Heino et al. 2005). An example of taxa congruence is high family richness in fish and invertebrate communities in a particular sampling site. Minimum requirements for water chemistry and instream habitat are similar in organisms with high congruence. Macroinvertebrate richness is influenced by stream size, acidity, and water color whereas fishes were most influenced by stream size, moss cover, and slope (Heino et al. 2005).

Little is known of water quality tolerances of benthic macroinvertebrates. Flow rate, pH, conductivity, and nutrient concentrations can influence the abundance and composition of invertebrate communities (Timm et al 2001; Camargo et al. 2004). Impacts on invertebrate and fish communities can be determined by understanding the effects of land use on physicochemical parameters as addressed in Chapter I.

Goals and Objectives

My objective was to investigate the following two hypotheses: 1) that land use affects the composition of benthic macroinvertebrate and fish assemblages by assessing taxa richness, trophic groups, and pollution tolerance levels from Rapid Bioassessment Protocol (RBP) samples (Barbour et al. 1999), and 2) that fish and invertebrate community attributes are correlated with water quality and instream habitat variables.

These biotic parameters will be used as response variables for land uses and their associated water quality parameters.

Study Site

See Chapter II for site locations and stream selection information. Rapid Bioassessment Protocol sampling techniques (Barbour et al. 1999) were performed in 18 streams in June – July 2008 for invertebrates and February and April 2009 for fishes.

Methodology

Field Procedures

Benthic macroinvertebrates were collected in June – July 2008 following the multi-habitat approach outlined in the EPA Rapid Bioassessment Protocol (Barbour et al. 1999). A D-frame dipnet was used to sample invertebrates in a 10-15 m reach for 18 streams. Three reaches were sampled to assess representative flow rates and substrates. The invertebrates were removed from the net, placed into labeled Whirl-paks® with 10% formalin or 70% ethanol, and transported to the Mississippi State University laboratory for identification and analysis.

Fish samples were collected using a Smith-Root backpack shocking unit (i.e., Burcher et al. 2008) in February and April 2009. A 25-30 minute timed sample was taken in 18 streams within the same 10-15 meter reach where macroinvertebrate samples were collected and habitat assessments were performed. Moving upstream, one scientist operated the backpack shocker while another gathered fishes with a dipnet. Fish samples were preserved in 10% formalin and transported to the laboratory for identification and analysis.

Laboratory Procedures

A modified, fixed-count subsampling technique was implemented using a grided sieve with 36 quadrants to assess benthic macroinvertebrates. In each sample, quadrants were selected from the tray using a random number generator until I reached the goal of 200 specimens. If the quadrant was not complete, I completed it which resulted in over 200 specimens for many of my samples. All organisms visible under 2X magnification within the subsample were processed except for Brachyzoa. Time efficiency was the major contributing factor to the taxa level of identification. Chironomidae, in particular, and time-inefficient and require specialized training to identify (Rabeni and Wang 2001). Keys were used to identify aquatic insects as low as the genus level using Merritt et al. (2008) and other invertebrates (Phyla Annelida and Nematoda, Classes Turbellaria, Bivalvia, Ostracoda, and Gastropoda, Subclass Copepoda, Orders Decapoda, Amphipoda, Isopoda, and Cladocera, and Suborder Hydracarina) as low as the suborder level using Pennak (1989).

Fish identification keys were used from respective states—Inland Fishes of Mississippi (Ross 2001), Fishes of Alabama and the Mobile Basin (Mettee et al. 1996), and Fishes of Tennessee (Etnier and Starnes 1993). Fishes were identified to species with a few exceptions of young of year *Lepomis spp*. and one juvenile lamprey.

Analysis

As in Chapter II, analysis was performed for biotic response variables among HUC4 watersheds and RBP habitat assessment scores. Relative abundance was used for invertebrate data. Taxa richness was analyzed at the family and species level for fishes. Invertebrate richness was calculated for genera, families, and higher level taxa (phyla, class, subclass, order, suborder levels). Percentage Ephemeroptera, Plecoptera, and Trichoptera orders (EPT) was calculated for invertebrates relative to total specimens collected. Fish abundance was expressed as CPUE, the total number of fishes collected in the 20 minutes timed sample.

Invertebrate pollution tolerance levels ranged from 0 to 10, with 0 being the most intolerant and 10 being the most tolerant to pollution (Barbour et al. 1999; Merritt et al. 2008). These pollution tolerance levels were then used to calculate the Hilsenhoff Biotic Index (BI) (Hilsenhoff 1977) for each stream to acquire an overall value of stream quality as indicated by the invertebrate communities.

$$BI = \sum TV_1N_1 / \text{Total N}$$

Where TV_1 is tolerance level of the ith taxa, N_1 is abundance of the ith taxa, and Total N is total number of organisms in the sample. BI scores are least with greater abundances of pollution-intolerant organisms in a sample. Only organisms with listed tolerance values were calculated with TV and N. Some organisms did not have a pollution tolerance level listed either due to poor documentation or insufficient taxonomic resolution and were reported as N/A or unlisted. These tolerance levels were only included in the Total N.

Fish pollution tolerance levels were designated as tolerant, intermediate, intolerant (Barbour et al. 1999). Some fish taxa also did not have listed pollution tolerance levels. Unlisted or N/A were reported for these organisms. I modified the Hilsenhoff BI to give fish pollution tolerance levels a numeric value. Like with invertebrates, intolerant fishes were given a value of 0. Intermediate species were classified as 1 and tolerant species were classified as 2. Having pollution intolerant taxa denoted as 0 gives the most weight to intolerant species since they indicate stream quality more aptly than pollution tolerant species. As with invertebrates, lower BI scores indicate an abundance of pollution intolerant organisms and should be found in higher quality streams. The Hilsenhoff formula was then calculated for fishes with the same equation used with invertebrates.

Trophic groups for fishes included insectivores, herbivores, piscivores, and generalists and collector-gatherer, collector-filterer, scraper, shredder, predator, parasite, piercer/scraper/collector-gatherer, collector-gatherer/predator, and parasite/predator for invertebrates. Trophic levels are influenced by an invertebrate's lifestage. For example, an organism may be a collector-gatherer as a larva and predator as a pupa or adult. As with pollution tolerance levels, several specimens did not have a trophic level designated, therefore the "unlisted" or "N/A" group was added for analysis.

Data were checked for normality using PAST (Hammer et al. 2001) and log transformed when Shapiro-Wilk (Shapiro and Wilk 1965) P-values were less than 0.05. Univariate analyses were performed using JMP software (JMP 2007). Analysis of variance (ANOVA) and Tukey-Kramer Honestly Significant Difference (HSD) tests (α =

0.05) were used to analyze HUC4 watersheds (n = 6) for pollution tolerance, trophic level, richness, and biotic index scores. Linear regression was used to analyze the relationship between pollution tolerance, trophic level, richness, and biotic index scores and habitat assessments scores (n = 18).

Canonical Correspondence Analysis (CCA) was performed in PC-ORD version 4.01 (McCune and Mefford 1999) to determine relationships between biotic and abiotic parameters. Relationships between matrices were tested using Monte-Carlo randomization tests with 200 permutations, and when a significant relationship was detected, biplot overlays were graphed using linear combination scores (McCune and Grace 2002). Main matrices were datasets with biotic parameters. Fish matrices included family and species level, and invertebrate matrices included higher taxa (phylum, class, subclass, order, and suborder) and genus level. The secondary matrix was either the water chemistry matrix or habitat matrix. The water chemistry matrix contained water chemistry, nutrients, and fecal *E. coli* parameters. *E. coli* was included with abiotic parameters, although it is a biotic variable. The habitat matrix included substrate type, average depth, average width, and habitat score. Parameters were log transformed to improve linearity when necessary.

Because there were 18 sites, or dependent variables, biotic variables had to be reduced within the dataset to ≤ 17 parameters. For example, if there were 21 fish species present, at least four species had to be consolidated. To do this, I created an *Other* category where uncommon species, based upon a percentage of total number of species or other taxon, were grouped. Fish species comprising less than 2 % of the overall total for all streams were consolidated into the *other* category. Invertebrate orders making up less than 1 %, families less than 0.3 %, and genus comprising less than 2.5 % of the total abundance were consolidated and labeled as *other*. All other matrices contained less than 18 variables and were not further grouped.

Results

Invertebrate Assessment

There were 4,160 invertebrate specimens identified from 18 streams along the Natchez Trace Parkway (Appendix C). Non-insect taxa (i.e., Decapoda and Cladocera) were identified to 2 phyla, 4 classes, 1 subclass, 12 orders, and 1 suborder. There were 47 identifiable aquatic insect families and 77 genera.

Average family richness did not differ significantly among watersheds (P = 0.16; Table 3.1). Family richness tended to be greatest in the Tennessee watershed (n = 18) and least in the Mississippi watershed (n = 12). Average genus richness (P = 0.64) tended to be greatest in the Upper Cumberland watershed (n = 11) and least in Pearl and Mississippi watersheds (n = 6 and 7, respectively).

There was a significant difference among watersheds for average percentage EPT (P = 0.04); however, Tukey-Kramer analysis did not find significant differences among watersheds. Percentage EPT tended to be greatest in Mississippi and Upper Cumberland watersheds (n = 0.25 and 0.24, respectively). Pearl (n = 0.02) and Lower Cumberland watersheds (n = 0.03) tended to have the lesser percentage EPT.

Table 3.1 Invertebrate family richness, genus richness, Hilsenhoff Biotic Index (BI) scores, and percentage EPT for HUC4 watersheds of the Natchez Trace Parkway (June – July 2008).

HUC4	Habitat Score	Family Richness	Genus Richness	BI Score	% EPT
MS	109	12	7	1.33	25
PL	126	16	6	0.14	2
TB	113	13	9	1.35	24
TN	141	18	8	0.95	24
LC	160	15	9	0.14	3
UC	137	17	11	0.45	24
(p-value)	0.06	0.16	0.64	0.21	**0.04**
(r^2)	0.54	0.45	0.22	0.42	**0.58**

Average Hilsenhoff Biotic Index scores (P = 0.21) tended to be greater in Tombigbee (1.35) and Mississippi watersheds (1.33). Scores tended to be less in Pearl and Lower Cumberland watersheds (both 0.14). The dominant trophic level was collector-gatherer (Table 3.2). This trophic group was primarily composed of Chironomidae (1,517 out of 2,918 total specimens). Average collector-gatherers abundance showed no significant differences among watersheds (P = 0.21). They tended to be more abundant in Lower Cumberland and Pearl watersheds and less abundant in samples from the Tennessee watershed.

Average collector-filterers relative abundance (P = 0.22) tended to be more abundant in the Mississippi watershed and less abundant in the Upper Cumberland watershed. Average scraper abundance (P = 0.09) tended to be greater in Upper Cumberland watershed and less in the Mississippi watershed. Shredders (P = 0.70) tended to be more abundant in the Pearl watershed and less abundant in Mississippi and Tombigbee watersheds.

Table 3.2 Invertebrate trophic groups for HUC4 watersheds of the Natchez Trace Parkway (June – July 2008; Avg hab: average habitat assessment score, N/A: unlisted, c-g: collector-gatherer, c-f: collector-filterer, scrap: scraper, shred: shredder, pred: predator, para-pred: parasite/predator, par: parasite, c-g-pred: collector-gatherer/predator, pier-scrap-c-g: piercer/scraper/collector-gatherer). Bold font signifies significant values. All values are averages of total.

HUC 4	Avg Hab	N/A	c-g	c-f	scrap	shred	pred	par-pred	par	c-g-pred	pier-scrap-c-g
MS	109	3	129	66	5	0	4	3	0	0	2
PL	126	1	214	11	38	2	7	3	0	2	1
TB	113	0	166	12	19	0	20	1	0	2	0
TN	141	1	114	21	24	2	11	2	0	1	0
LC	160	2	219	24	43	1	9	5	0	0	0
UC	137	7	131	3	64	1	5	3	0	0	0
(p-value)	0.06	0.78	0.21	0.22	0.09	0.70	0.30	0.76	0.57	**0.03**	0.12
(r^2)	0.54	0.17	0.42	0.41	0.51	0.20	0.36	0.17	0.25	**0.61**	0.48

Predator abundance averages (P = 0.30) tended to be greater in the Tombigbee watershed and less in Mississippi and Upper Cumberland watersheds. The parasite/predator trophic group did not differ significantly among watersheds (P = 0.76). Averages tended to be greater in the Lower Cumberland watershed and less in the Tombigbee watershed. Average parasite abundance (P = 0.57) tended to be greater in Upper Cumberland and Tennessee watersheds. No parasites were collected in Mississippi, Pearl, Tombigbee, or Tennessee watersheds.

Average collector-gatherer/predators abundance differed significantly among watersheds (P = 0.03). Tukey-Kramer analysis showed abundance of this trophic group in the Tombigbee watershed was significantly greater than Mississippi and Upper Cumberland watersheds (both P = 0.04). Average piercer/scraper/collector-gatherer abundance did not differ significantly among watersheds (P = 0.12). Mississippi and Pearl watersheds had the only representative specimens for the Natchez Trace Parkway.

At a finer scale resolution, invertebrate family richness and genus richness (P = 0.19 and 0.93, respectively) were not correlated with habitat assessment scores (Table 3.3). Hilsenhoff BI scores (P = 0.08) and percentage EPT (P = 0.31) were not correlated with habitat scores either. Invertebrate parameters varied so much for habitat scores that there was no general trend detected. Also, no invertebrate trophic groups were correlated with habitat assessment scores (Table 3.4).

Table 3.3 Linear regressions for habitat assessment scores and average invertebrate family richness, genus richness, Hilsenhoff Biotic Index (BI) scores, and percentage EPT at each site (June – July 2008). Habitat scores listed in latitudinal order with most southern stream reported first.

HUC4_Habitat Score	Family Richness	Genus Richness	Hilsenhoff BI Score	% EPT
MS_114	15	8	1.77	30
MS_117	10	7	2.14	43
MS_97	12	5	0.14	2
PL_141	17	6	0.03	1
PL_96	16	7	0.32	4
PL_141	14	6	0.04	0
TB_123	11	5	2.34	40
TB_102	14	10	1.15	24
TB_113	15	11	0.37	7
TN_164	19	4	0.04	7
TN_105	17	11	2.49	23
TN_155	19	10	1.15	42
LC_156	16	14	0.26	8
LC_151	13	4	0.06	1
LC_172	16	8	0.14	1
UC_119	18	11	0.13	5
UC_146	21	14	0.80	51
UC_145	11	7	0.51	16
(p-value)	0.19	0.93	0.08	0.31
(r^2)	0.11	0.00	0.18	0.06

Table 3.4 Linear regressions of invertebrate trophic groups for habitat assessment scores (June – July 2008; N/A: unlisted, c-g: collector-gatherer, c-f: collector-filterer, scrap: scraper, shred: shredder, pred: predator, para-pred: parasite/predator, para: parasite, c-g-pred: collector-gatherer/predator, pier-scrap-c-g: piercer/scraper/collector-gatherer). Habitat scores listed in latitudinal order with most southern stream reported first. Values are total number of individuals represented by each trophic group.

HUC4_ Habitat Score	N/A	c-g	c-f	scrap	shred	pred	para-pred	para	c-g-pred	pier-scrap-c-g
MS_114	2	107	102	1	0	5	2	0	0	2
MS_117	8	135	47	2	0	3	1	0	0	4
MS_97	0	146	49	12	0	5	7	0	0	0
PL_141	0	166	29	27	1	11	3	0	0	0
PL_96	0	250	3	43	6	3	3	0	4	2
PL_141	2	226	0	43	0	6	2	0	1	0
TB_123	0	190	0	9	0	40	1	0	2	0
TB_102	1	155	34	21	1	4	2	0	2	0
TB_113	0	152	3	26	0	16	0	0	3	0
TN_164	0	178	4	27	0	6	2	0	1	0
TN_105	0	32	49	5	5	10	0	1	0	0
TN_155	3	132	9	40	0	16	3	0	1	0
LC_156	4	157	33	20	3	4	1	0	0	0
LC_151	0	157	36	100	1	6	15	0	0	0
LC_172	3	342	3	10	0	16	0	0	1	0
UC_119	1	170	3	116	2	2	0	1	0	0
UC_146	19	140	4	68	0	9	8	0	0	0
UC_145	0	84	1	8	1	3	0	0	0	0
(p-value)	0.26	0.21	0.38	0.30	0.13	0.32	0.92	0.25	0.78	0.09
(r^2)	0.08	0.10	0.05	0.07	0.13	0.06	0.00	0.08	0.01	0.16

There were important correlations in CCA between community attributes and the environmental matrix as indicated by biplot overlays; however, these relationships did not differ significantly according to randomization tests possibly due to low sample size. I chose to describe these relationships as they may still give insight to tendencies of invertebrate communities in environmental parameters.

Variation in invertebrate community composition at the order and higher taxa level could be explained by differences in water quality ($P = 0.39$; eigenvalue = 0.49; axis

1 = 23.5%, axis 2 = 17.0%; Figure 3.1a) and habitat variables (P = 0.13; eigenvalue = 0.47; axis 1 = 22.5%; axis 2 = 14.3%; Figure 3.1b) among streams. Coleoptera, Decapoda, Ephemeroptera, Hemiptera, Plecoptera, and % EPT were associated with greater DO and SO_4, whereas the opposite relationship was observed for Amphipoda, Hydracarina, Bivalvia, Annelida, Gastropoda, Copepoda, Cladocera, and Other. Isopoda showed a positive correlation with conductivity, Ca, pH, and TN, whereas Trichoptera and Diptera had a negative association with these factors. Trichoptera, Bivalvia, Annelida, and Diptera were associated with wider streams, greater percentages of sand substrate, lesser cobble substrates, and lower habitat assessment scores. Amphipoda, Coleoptera, Hemiptera, and Isopoda had the opposite relationship with these parameters. Percentage EPT, Ephemeroptera, and Plecoptera were correlated negatively with bedrock substrates whereas Gastropoda, Cladocera, Copepoda, and Hydracarina were correlated positively.

Invertebrate community composition at the genus level was correlated with fecal *E. coli*, pH, and alkalinity (ANC), although permutation analysis failed to detect significant linear relationship (P = 0.76; eigenvalue = 0.26; axis 1 = 24.4%; axis 2 = 13.2%; Figure 3.2a). *Simulium spp.*, a moderately intolerant Diptera, was correlated positively with fecal *E. coli* and correlated negatively with ANC and pH whereas, *Stenelmis spp.* and *Ectopria spp.*, moderately intolerant Coleopterans, had the opposite relationship with these water quality parameters.

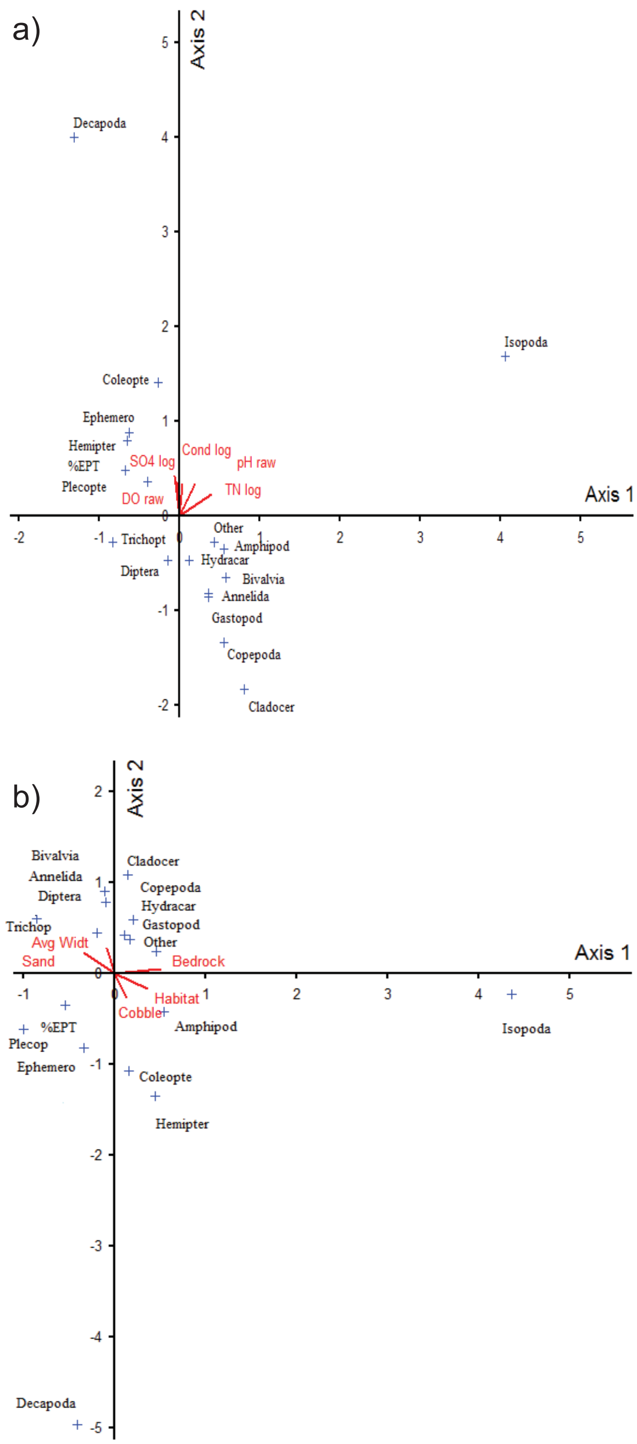


Figure 3.1 Distribution of higher taxa invertebrates for a) water chemistry variables and b) habitat parameters along CCA axes one and two (June – July 2008).

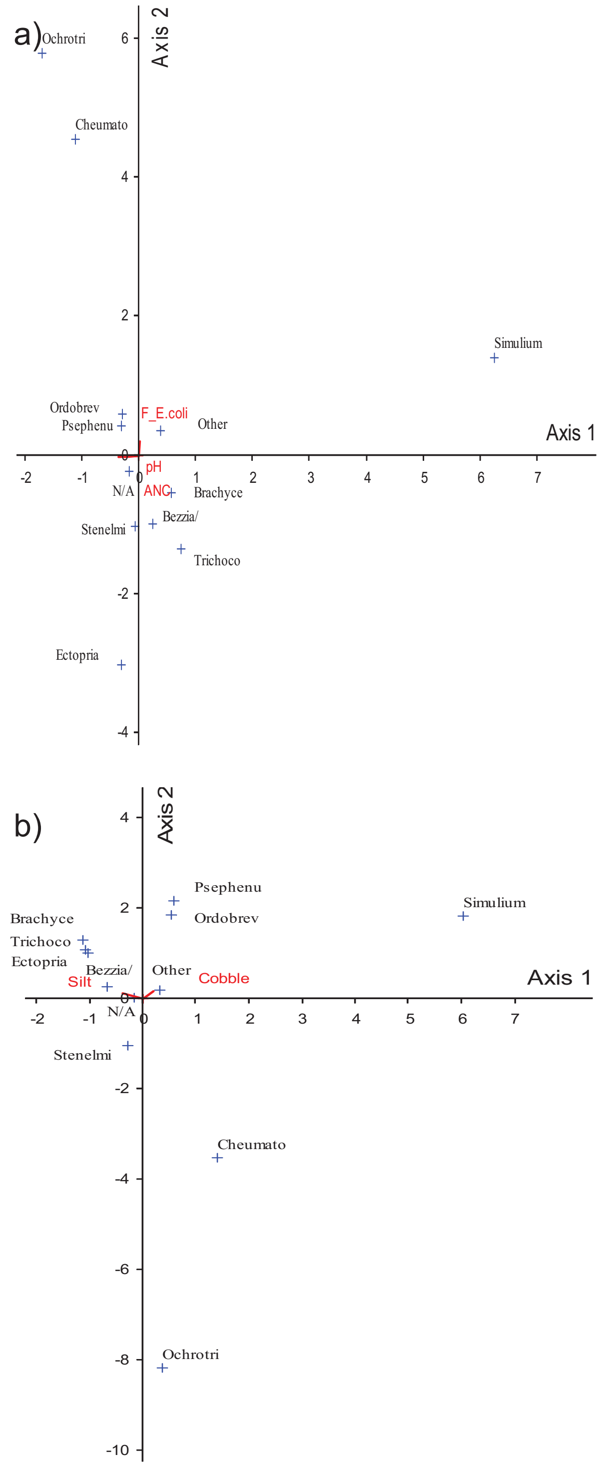


Figure 3.2 Distribution of invertebrate genera for a) water chemistry variables and b) instream habitat parameters along CCA axes one and two (June – July 2008).

There was no significant difference between invertebrate genera and habitat matrices (P = 0.08; eigenvalue = 0.32; axis 1 = 30.5%; axis 2 = 12.1%; Figure 3.2b). *Cheumatopsyche* was correlated negatively with silt substrate. *Psephenus*, *Ordobrevia*, and *Simulium* were associated with high percentages of cobble substrates, whereas *Stenelmis* was correlated negatively with cobble.

Fish Assessment

There were 1,459 fishes collected, representing 10 families and 63 species in 18 streams along the Natchez Trace Parkway (Appendix D). Species richness (P = 0.83) did not differ significantly among watersheds (Table 3.5), but tended to be greater in the Mississippi watershed (n = 11). The Lower Cumberland River watershed tended to have the least average richness (n = 7). Species richness tended to decrease with increasing latitude, except for the Pearl watershed.

Average catch per unit effort (CPUE) did not differ significantly among watersheds (P = 0.94). The least number of fishes tended to be caught in the Pearl watershed with 31 individuals on average, whereas the greatest was in the Mississippi watershed with an average of 110 fishes. Average BI scores were not significant among watersheds (P = 0.46). Lower scores tended to be in the Mississippi watershed (1.05), whereas greater scores were in Tennessee and Pearl watersheds (2.25 and 2.11, respectively).

Table 3.5 ANOVA analysis of average fish catch per unit effort (CPUE), species richness, and biotic index (BI) scores for HUC4 watersheds (February and April 2009; MS: Mississippi, PL: Pearl, TB: Tombigbee, TN: Tennessee, LC: Lower Cumberland, UC: Upper Cumberland watersheds).

HUC4	Habitat Score	CPUE	Species Richness	BI Score
MS	109.33	110.33	11.33	1.05
PL	126.00	31.33	7.33	2.11
TB	112.67	109.33	9.67	1.26
TN	141.33	80.00	9.67	2.25
LC	159.67	88.33	7.00	1.93
UC	136.67	67.00	7.33	1.73
(p-value)	0.0640	0.8016	0.8313	0.4623
(r^2)	0.5424	0.1596	0.1466	0.2924

Insectivore was the dominant fish trophic group observed in streams during this study (Table 3.6). There was no significant difference in insectivores among watersheds (P = 0.83). The Tennessee watershed tended to have more insectivore species (n = 53), whereas Tombigbee (n = 22) and Upper Cumberland watersheds (n = 23) had the least.

Herbivore abundance differed significantly among watersheds (P = 0.03). Tukey-Kramer analysis did not show significant differences among watersheds. Herbivores tended to be more abundant in Lower Cumberland (n = 30) and Upper Cumberland watersheds (n = 26). The Pearl and Tombigbee watersheds did not have herbivore species represented in samples.

Table 3.6 ANOVA results for HUC4 watersheds by average fish trophic levels of the Natchez Trace Parkway (February and April 2009). Bold font signifies significant values.

HUC4	Avg Habitat Score	Avg N/A	Avg Insectivore	Avg Herbivore	Avg Piscivore	Avg Omnivore	Avg Generalist
MS	109	43	32	10	1	25	0
PL	126	2	28	0	1	0	0
TB	113	24	22	0	2	61	1
TN	141	6	53	14	0	0	7
LC	160	9	38	30	0	0	11
UC	137	10	23	26	0	6	2
(p-value)	0.06	0.89	0.83	**0.02**	0.53	0.05	0.06
(r^2)	0.54	0.12	0.15	**0.63**	0.27	0.56	0.54

There was no significant difference in average piscivore abundance among watersheds (P = 0.53). No piscivores were found in the Lower Cumberland or Upper Cumberland watersheds. The greatest abundance of piscivores tended to be in the Tombigbee (n = 2) and Pearl watersheds (n = 1). Average omnivores (P = 0.05) tended to be more abundant in the Tombigbee watershed (n = 61). No omnivores were found in the Pearl or Tennessee watersheds. Average generalist (P = 0.06) tended to be more abundant in the Lower Cumberland (n = 11), Tennessee (n = 7), and Upper Cumberland watersheds (n = 2). No representative generalists were collected in Mississippi or Pearl watersheds.

Table 3.7 Linear regression tests between fish catch per unit effort (CPUE), species richness, biotic index (BI) (February and April 2009) for habitat assessment scores (June – July 2008). Habitat scores listed in latitudinal order with most southern stream reported first.

HUC4_ Habitat Score	CPUE*	Species Richness	BI Score
MS_114	172	12	1.63
MS_117	133	14	1.83
MS_97	26	8	1.48
PL_141	23	4	2.00
PL_96	45	9	2.55
PL_141	26	9	2.08
TB_123	85	9	1.96
TB_102	237	17	1.44
TB_113	6	3	2.00
TN_164	25	7	1.76
TN_105	62	13	2.02
TN_155	153	9	2.68
LC_156	190	7	2.25
LC_151	63	13	2.02
LC_172	12	1	1.00
UC_119	88	9	2.25
UC_146	100	11	1.82
UC_145	13	2	1.92
(p-value)	0.52	0.06	0.83
(r^2)	0.03	0.20	0.00

* number of fish collected per 20-30 minute timed sample

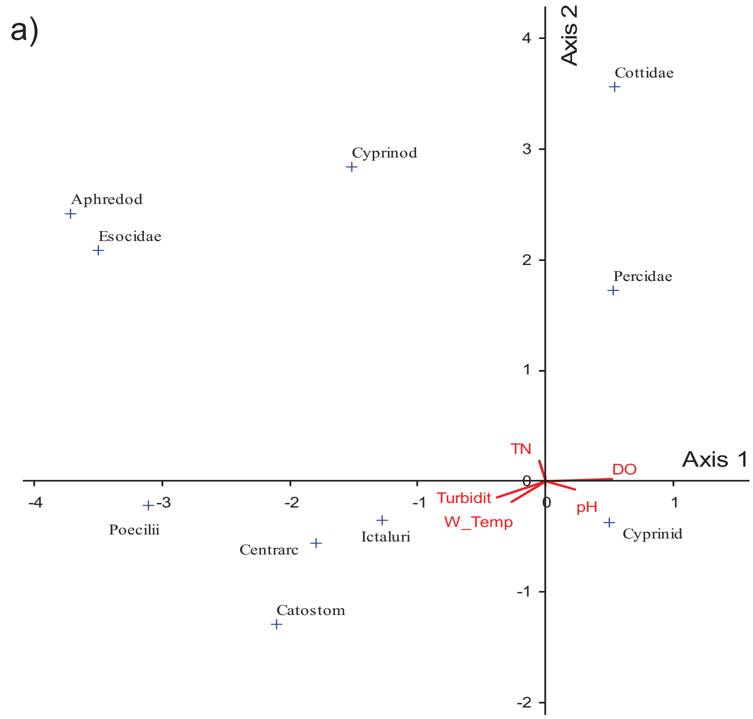
Linear regression tests showed no correlation between fish community parameters and habitat scores (Table 3.7). There was a no distinct trend in fish CPUE (P = 0.52), species richness (P = 0.06), or biotic index scores (P = 0.83). Fish trophic groups were not correlated with habitat assessment scores (Table 3.8); however, herbivores and generalists followed a trend with greater abundance in higher scoring sites. Omnivores showed a trend of greater abundance in low-medium scoring sites. Piscivore abundance showed a trend with mid-range habitat scores.

Table 3.8 Linear regressions for habitat assessment scores for each site (June – July 2008) and fish trophic groups (February and April 2009). Values represent total abundance of each trophic group. Habitat scores listed in latitudinal order with most southern stream reported first.

HUC4_ Habitat Score	N/A	Insectivore	Herbivore	Piscivore	Omnivore	Generalist
MS_114	101	28	0	2	41	0
MS_117	26	43	29	0	35	0
MS_97	1	25	0	0	0	0
PL_141	2	21	0	0	0	0
PL_96	5	40	0	0	0	0
PL_141	0	22	0	4	0	0
TB_123	12	6	0	1	65	1
TB_102	59	55	0	6	116	1
TB_113	0	5	0	0	1	0
TN_164	0	14	5	1	0	5
TN_105	12	27	8	0	0	15
TN_155	6	117	29	0	0	1
LC_156	12	68	88	0	0	22
LC_151	14	47	1	0	1	0
LC_172	0	0	0	0	0	12
UC_119	7	33	42	0	0	6
UC_146	23	37	23	0	17	0
UC_145	0	0	12	0	0	1
(p-value)	0.11	0.79	0.18	0.64	0.21	0.17
(r^2)	0.15	0.04	0.11	0.01	0.10	0.11

There was a significant relationship ($P < 0.01$; Eigenvalue = 0.38; Axis 1 = 37.9%; Axis 2 = 10.5%) between fish community structure at the family level and water chemistry parameters (Figure 3.3a). Cottidae and Percidae had a negative correlation with turbidity and water temperature whereas Ictaluridae, Poeciliidae, Centrarchidae, and Catostomidae were correlated positively with these variables, meaning they are tolerable of warm, turbid water. Turbidity and water temperature also show a positive correlation with streams in southern watersheds, Baker's Creek, Chuquatonchee Creek, and Big Sand Creek. Aphredoderidae, Esocidae, and Cyprinodontidae were correlated negatively with pH whereas Cyprinidae had the opposite relationship with these parameters. Ictaluridae, Centrarchidae, Catostomidae, and Poeciliidae were correlated negatively with DO whereas Cottidae and Percidae and correlated positively. Streams in northern watersheds generally have greater DO concentrations than southern watersheds. Fish families showed a significant relationship with the habitat matrix ($P = 0.10$; eigenvalue = 0.29; axis 1 = 28.8%; axis 2 = 17.5%; Figure 3.3b). Ictaluridae, Catostomidae, Esocidae, Centrarchidae, and Aphredoderidae were associated with high levels of silt, sand, and clay substrates as well as deeper streams. Also, they were correlated negatively with cobble and habitat assessment scores.

While the Monte-Carlo analysis of the relationship between the fish species and water chemistry matrix was not significant, there were important correlations as indicated by biplot overlays ($P = 0.08$; eigenvalue = 0.72; axis 1 = 23.3%; axis 2 = 22.0%; Figure 3.4a). *F. olivaceus*, *L. macrochirus*, *L. megalotis*, *P. notatus*, *C. camura*, and *Other* were correlated positively with potassium, turbidity, fecal *E. coli*, and water temperature whereas *C. funduloides*, *P. erythrogaster*, *C. anomalum*, *S. atromaculatus*, and *E. flabellare* had the opposite relationship with these parameters. There was a significant relationship with fish species and habitat matrix ($P < 0.01$; eigenvalue = 0.73; axis 1 = 23.9%; axis 2 = 19.0%; Figure 3.4b). *P. notatus* and *C. camura* were correlated positively with sand substrate and correlated negatively with habitat assessment scores. *F. olivaceous*, *L. megalotis*, *P. vigilax*, and *L. macrochirus* were associated with lesser percentages of cobble and boulder substrates.



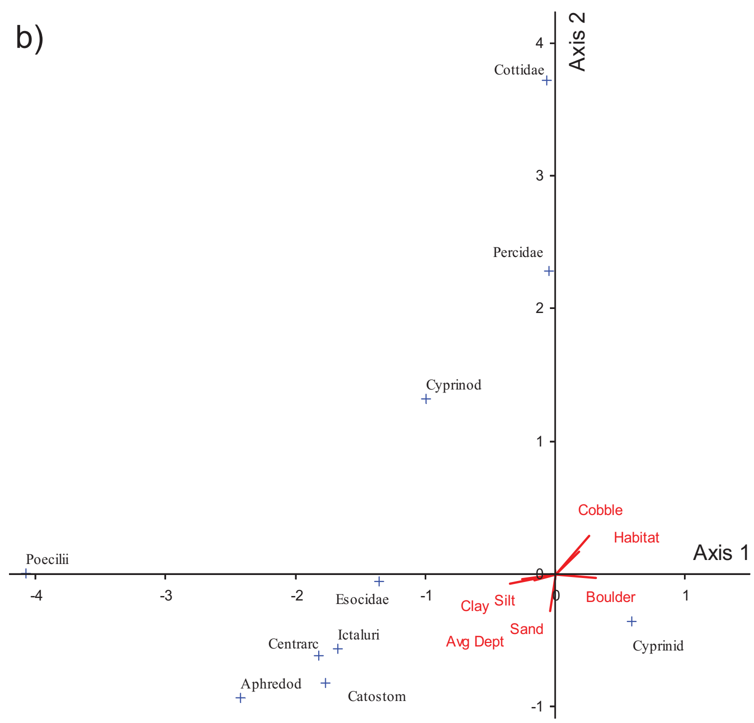


Figure 3.3 Distribution of fish families for a) water chemistry and b) instream habitat variables along CCA axes one and two (February and April 2009).

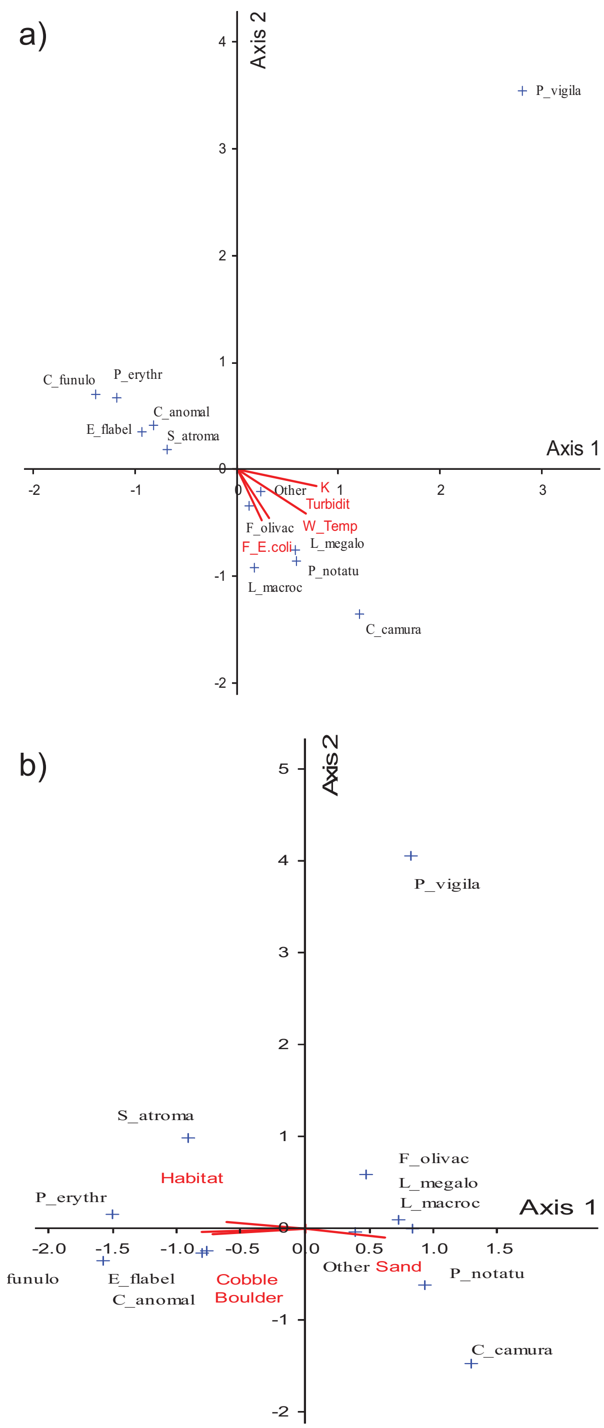


Figure 3.4 Distribution of fish species for a) water chemistry variables and b) instream habitat parameters along CCA axes one and two (February and April 2009).

Discussion

At the HUC4 scale, percentage EPT differed significantly among watersheds. Whiles et al. (2000) found that percentage EPT was the most effective metric in identifying impaired streams. In the current study, Pearl and Lower Cumberland watersheds had significantly lesser EPT than other watersheds along the NTP. These watersheds differed significantly in substrate composition, pH, conductivity, DO, turbidity, and alkalinity. Streams in the Pearl watershed were low-gradient streams, and although they tended to have greater habitat assessment scores, most stream parameters were poor. The Lower Cumberland watershed, however, had excellent water chemistry and tended to have the greatest habitat assessment scores. This leads to speculation that percentage EPT is influenced by confounding variables.

Budin et al. (2007) observed percentage EPT correlated negatively to turbidity and total suspended solids and correlated positively with DO. On the NTP, turbidity and DO in the Pearl watershed had the opposite relationship with EPT. Conductivity and pH at extreme values can also influence aquatic biota; distribution of EPT was not affected by moderate pH and conductivity (Bispo et al. 2006). Similar results were found in the NTP. Average conductivity and pH differed significantly among watersheds, but values were not extreme enough to influence EPT percentage.

Larsen et al. (2009) also found a negative relationship between fine sediments and percentage EPT and invertebrate taxa richness. In the current study, this observation was supported as percentage EPT was significantly less in Pearl and Tombigbee watersheds where turbidity was significantly greater and TSS and fine particle substrates tended to be greater. Invertebrate family and genus richness tended to be lesser in HUC4 watersheds with greater fine particle substrate, TSS, and turbidity. At the instream scale, invertebrate richness had less of a trend apparent among substrate size, TSS, and turbidity.

A previous stream study on the NTP was performed in the northern region of the Pearl watershed. Hashim (2005) found Ephemeroptera, Plecoptera, and Trichoptera were more abundant in moderate to high levels of DO, ANC, pH, and conductivity. This general relationship was observed in the current study for Mud Island and Chuquatonchee creeks and Garrison Branch, but often times average ANC negated this relative pattern. Streams within the Tennessee watershed had lesser conductivity and ANC values although percentage EPT was relatively high. At the HUC4 scale, the Tennessee watershed had relatively greater percentage EPT but lesser average conductivity, ANC, and pH. Only Mississippi and Upper Cumberland watersheds followed the relationship in Hashim (2005). Rosemond et al. (1992) found that Ephemeroptera and Trichoptera density was less in lesser pH values. The current study also found that average pH values tended to be greater in HUC4 watersheds with greater percentage EPT.

Percentage EPT and Hilsenhoff BI scores were strongly influenced by the overwhelming abundance of *Cheumatopsyche* (Trichoptera: Hydropsychidae) and *Brachycercus* (Ephemeroptera: Caenidae). Biotic index scores were influenced by their relative abundance because *Brachycercus* is moderately intolerant of pollution and *Cheumatopsyche* is moderately tolerant pollution (Barbour et al. 1999; Merritt et al. 2008). Two other groups influencing BI scores were unidentifiable genera or N/A and

Chironomidae. The BI score becomes skewed if a large portion of samples are listed as N/A pollution tolerance.

Average Hilsenhoff Biotic Index scores implied that Tombigbee and Mississippi watersheds tended to have more pollution-tolerant specimens indicating poor stream quality, whereas Pearl and Lower Cumberland watersheds tended to have more pollution-intolerant specimens indicating better stream quality. Helms et al. (2009) observed higher invertebrate BI scores associated with low total dissolved solids (TDS) and high DO and detritus. In the current study, habitat assessment scores at the watershed level tended to be less in Mississippi, Tombigbee, and Pearl watersheds; however, Hilsenhoff BI scores tended to be greatest in Mississippi, Tombigbee, and Tennessee watersheds where there was relatively high DO averages. At the stream scale, BI scores tended to be greater in greater habitat assessment scores, which tended to be in Tennessee, Lower and Upper Cumberland watersheds. The two scales represent different conclusions about the invertebrate pollution tolerance and biotic integrity of streams.

Taxa richness decreases with increasing perturbation (Barbour et al. 1999; Timm et al. 2001; Heino et al. 2007); however, in the current study, invertebrate family and genus richness and fish species richness did not differ significantly among habitat assessment scores. Studies often analyze assemblage structure and stream quality by parameters associated with anthropogenic disturbance, such as pH (e.g., Heino et al. 2007). Invertebrate richness has a positive relationship to stream width and pH (Rosemond et al. 1992; Heino 2002; Heino et al. 2007). In the current study, the pH in HUC4 watersheds did not differ but tended to be greater in Lower and Upper

Cumberland watersheds. Stream width tended to be greater in Mississippi and Lower Cumberland watersheds, but invertebrate family richness tended to be greater in the Tennessee watershed and genus richness tended to be greater in the Upper Cumberland watershed. Stream width tended to be greater in lesser scoring habitat assessment sites; invertebrate family and genus richness did not differ significantly or show any patterns among habitat scores.

Trophic group collector-gatherer/predator differed significantly among watersheds. This group was represented largely by Bezzia/Palpomyia, a burrowing Diptera. The Tombigbee watershed had a significantly greater abundance of this group possibly because it had significantly lesser levels of cobble substrate and tended to have greater percentages of clay, silt, and sand substrates, accommodating for burrowing organisms.

Low alkalinity, PO_4, and periphyton can be associated with low scraper abundance (Hashim 2005). In the current study, scrapers tended to be more abundant in Upper Cumberland, Lower Cumberland, and Pearl watersheds; however, alkalinity was significantly greater in the Upper and Lower Cumberland watersheds. Scrapers are influenced most by nutrient enrichment (Camargo et al. 2004), and in the current study, average PO_4 tended to be greatest in Lower Cumberland watershed. Scraper abundance tended to be greatest in Chief Creek and Burns Branch of Lower Cumberland and Upper Cumberland watersheds, respectively. Chief Creek tended to have lesser ANC and PO_4 averages. Because periphyton was not measured, this variable could have influenced scraper abundance in Burns Branch and affected the watershed average in Upper Cumberland.

Greater nitrogen concentrations are generally associated with greater scraper abundance, but in Whiles et al. (2000), nitrogen concentrations tended to be greater in sites with lesser scraper abundance. Nitrogen concentrations were remarkably greater than the current study. Total nitrogen, for example, ranged from 6.30-14.28 mg L^{-1} in Whiles et al. (2000) compared to 0.007-1.58 mg L^{-1} in the current study. Natchez Trace Parkway scraper abundance tended to be greater in sites with comparatively intermediate NO_2, NO_3, NH_4, TN, and PO_4 averages. The Lower Cumberland watershed tended to have greater nutrient enrichment and greater scraper abundance. Gastropods tended to make up a large portion of scrapers in Whiles et al. (2000). This is also the case in the current study for Chief Creek and Burns Branch, which accounted for the greater scraper abundance in Lower Cumberland and Upper Cumberland watersheds, respectively. Scrapers are also associated with high-gradient streams (Hawkins et al. 1982). Lower and Upper Cumberland watersheds do support this observation in the current study. Although the Pearl watershed has low-gradient streams, this watershed does have a relatively greater average habitat assessment score making these sites higher quality, low-gradient streams.

Pearl and Tennessee watersheds tended to have the greatest shredder density (n = 2 average, respectively). Heino (2009) discovered that shredder richness increased with increasing total phosphorous. This pattern was not supported in the current study as average phosphorous as phosphate tended to be least in streams and HUC4 watersheds with greatest shredder densities. Average phosphorous as phosphate tended to be least in the Pearl and Tennessee watersheds (both < 0.01 mg L^{-1}). At the instream scale, Nine Mile Creek and Cooper Branch tended to have the greatest shredder density (n = 6 and 5, respectively) and tended to have least average phosphorous as phosphate (both < 0.001 mg L^{-1}).

Shredder abundance can be influenced by the presence of riparian vegetation (Hawkins et al. 1982). Deciduous trees and overhanging vegetation offers sources of allochthonous material, a food source for many aquatic organisms in particular shredders (Hawkins et al. 1982; Wetzel 2001; Merritt et al. 2008). In the current study, shredders tended to be more abundant in the Pearl, Tennessee, Lower Cumberland, and Upper Cumberland watersheds. These were generally shaded sites, but the Upper Cumberland watershed was completely shaded. Nine Mile Creek (Pearl watershed) had the greatest abundance of shredders but a completely open canopy cover. Rosemond et al. (1992) found that greater shredder densities were associated with greater pH values. Cooper Branch, in the current study, tended to have the lesser pH average but the greatest shredder density. Likewise, Pearl and Tennessee watersheds had significantly lesser pH averages but tended to have the greatest shredder density. This could be misinterpreted because shredder density ranged zero to two for HUC4 watersheds and zero to six for instream sites.

Other studies found stream depth was correlated positively with predator and scraper invertebrates, and stream width was correlated positively with scraper richness (Heino 2000; Heino 2009). Predator densities were weakly associated with relatively intermediate stream depths in the current study. Old Field Creek tended to have the greatest predators density (n = 40) and an average depth of 0.15 m. At the HUC4 level, the Tombigbee watershed tended to have the greatest average predators (n = 20) with average stream depth of 0.33 m. Intermediate stream width is weakly associated with greater scraper densities. The Upper Cumberland watershed tended to have greater average scrapers (n = 64), but average stream width was least (2.79 m). Scraper density tended to be greatest in Chief Creek and Burns Branch (n = 100 and 116, respectively) and intermediate, average stream width (4.67 and 3.33 m, respectively). Average width was weakly correlated negatively with scraper density in the current study. Again, Burns Branch and Chief Creek tended to have the greatest scraper abundance, but average widths tended to be lesser (0.17 and 0.15 m, respectively). At the HUC4 scale, the Upper Cumberland tended to have the greatest average scraper density (n = 64), but the average width tended to be the least (2.79 m).

Invertebrate trophic groups can be relative because many are termed “facultative” such as facultative collector-filterer, meaning they are capable of feeding as a collector-filterer when resources do not allow the organism to feed as its primary trophic behavior (Merritt et al. 2008; Heino 2009). Most invertebrates have several optional trophic behaviors, which can shift during life stage transitions or limited food availability (Merritt et al. 2008). Organisms were classified in this study by the first listed trophic group (e.g., Barbour et al. 1999; Merritt et al. 2008). Many trophic groups were listed as a hyphenated group such as collector-gatherer-predator, representing particular organisms rather than a general group such as scraper. For these reasons, there are discrepancies in using trophic groups to analyze invertebrate community structure (Heino 2009). This is a limitation in the current study because the trophic groups used for analysis may or may not have been correct, or the classification used may not have grouped trophic behaviors properly. Fish, however, have fewer trophic groups and more studies have discerned their biology compared to invertebrates. Heino (2009) simplified analysis of invertebrate assemblages by using five main trophic groups: shredder, gatherer, filterer, scraper, and predator. This should have been considered in the current study.

Substrate composition was an important factor in predicting habitat score and invertebrate and fish structure. In general, percentage cobble and sand were the two most important substrates determining invertebrate and fish assemblage distribution. Habitat score was correlated positively to percentage cobble substrate in higher invertebrate taxa and fish family matrices. Habitat scores were correlated negatively with sand substrates in higher invertebrate taxa, fish family, and fish species matrices. Habitat scores were correlated negatively with clay and silt in the fish family matrix. Substrate was most important variable in determining macroinvertebrate presence and absence because fine substrates are associated with embeddedness, which negatively affects aquatic invertebrate recruitment, respiration, and refuge (Richards and Host 1994; Wetzel 2001; Merritt et al. 2008). Substrate composition was less important in influencing macroinvertebrate structure compared to canopy cover in Hawkins et al. (1982). This was supported in the current study as the only site with open canopy, Nine Mile Creek in the Pearl watershed, had a relatively low percentage EPT. At the HUC4 scale, canopy cover tended to be least in the Pearl watershed, and percentage EPT tended to be least.

Fish assemblage structure is associated with stream size (Heino et al. 2005). Fish species richness and CPUE in the current study tended to be greatest in the Mississippi watershed where the average width and depth tended to be greatest. The Upper Cumberland watershed tended to have the narrowest streams, least species richness, and relatively lesser CPUE. Dissolved oxygen is also an important factor determining fish structure (Sawyer et al. 2004). Fish family structure in the current study was influenced by DO, correlated positively with Cottidae and Percidae and correlated negatively with Ictaluridae, Centrarchidae, Poeciliidae, and Catostomidae. Ictaluridae, Catostomidae, and Centrarchidae have intermediate pollution tolerance levels overall and can be found in more degraded streams which is why they were also associated with greater silt, clay, and sand substrates.

Streams most abundant with generalists and omnivores can be regarded as having degraded quality as opposed to more specialized trophic categories such as herbivore, insectivore, and piscivore (Kerans and Karr 1994; Barbour et al. 1999; Grabarkiewicz and Davis 2008). Omnivores in the NTP were correlated positively with SO_4^-, Mg^+, and conductivity, and generalists were correlated negatively with turbidity. Conductivity and turbidity tend to be greater in developed and agricultural land (Jones et al. 2001; Meador and Goldstein 2003; Long and Plummer 2004). Generalists in the current study, however, were associated with lesser turbidity. As CCA observed, turbidity was correlated positively to habitat scores. Thus, generalists were found in sites with greater habitat assessment scores.

Fish trophic levels tended to shift from omnivore-insectivore-piscivore to generalist-insectivore-herbivore with increasing latitude. This could be attributed to the increase in habitat assessment scores, thus better stream quality (Kerans and Karr 1994). Sites in the northern NTP watersheds generally scored higher because high-gradient stream characteristics were present such as greater gravel and cobble substrates and canopy cover. The general increase in PO_4, NO_3, NO_2, and TN values in northern watersheds could also explain this trend. Increased nutrients, especially PO_4 could allow for more aquatic vegetation within the channel (Wetzel 2001; Hashim 2005). Herbivorous fishes were not collected in the streams of the Pearl watershed possibly because PO_4 levels were relatively untraceable.

Community transitions across sites or along reaches of a river have been a long sought after concept (Herlihy et al. 2006). Main factors are stream size, drainage basin, and landscape characteristics (Herlihy et al. 2006). Some research has found that drainage basins are the most important driving force in determining assemblage structure (Gilbert 1980); however, Omernik and Bailey (1997) showed that HUCs are often confused as drainages. This makes scale a delicate and sometimes controversial subject.

Scale at the HUC4 level may have affected analyses. As noted in Chapter II, HUC4 watersheds do not always represent true watersheds (Seaber et al. 1987; Omernik and Bailey 1997). Different conclusion may arise from using different scales. Lammert and Allan (1999) found that local rather than regional land use and habitat were better indicators of biota. In their study, fish assemblages showed more of an association with land use whereas macroinvertebrate assemblages were related more to instream habitat.

This was partly due to the fact that regional land use did not differ significantly among subcatchments and the sampling sites were fairly close together. Land use at the HUC4 watershed scale for the Natchez Trace Parkway had similar compositions of percentage forested, developed, and agricultural. Little variation between watersheds at this scale was not useful in assessing land use impacts on fish and invertebrate communities.

Brosse et al. (2003) observed that different environmental factors influence invertebrate structure at different scales. Invertebrate richness was most influenced by particle size at the instream scale, adjacent land use at the reach scale, and relief ratio at the catchment scale. This raises the question of which scale is appropriate to determine stream ecology characteristics such as assemblage structure and nutrient loading? Differences among instream and HUC4 watershed scales were evident in the current study. Percentage EPT and invertebrate and fish trophic levels were only significant at the HUC4 watershed level. This does not mean it is a more correct scale compared to the instream scale just because there were significant differences (Levin 1992). Levin (1992) assures that there is more than one mechanism to explain scale patterns by using krill studies as examples. Food resources and water movement can strongly influence krill population and distribution, and variance decreases with scale. Habitat assessment scores at the instream scale of the current study could also follow this observation. Perhaps the scale was too fine at the instream level to detect differences in invertebrate and fish assemblage structure. Canonical correspondence analysis shows that multiple water chemistry and habitat parameters influence assemblage structure. This analysis is more powerful and incorporates multiple parameters simultaneously, allowing for complexities of variables to be revealed compared to linear regressions. This could be why significance was detected in CCA and not in linear regressions. Also, the analysis was performed by habitat assessment scores in linear regressions and by streams in CCA.

Scale at the instream level was within a 10-15 m reach, which probably did not incorporate all stream structure variability. Analyzing data by habitat assessment scores gave an indication of localized stress on invertebrate structure and stream quality at an instream scale. Lloyd et al. (2006) discovered that autocorrelation, or similarity, of benthic invertebrate assemblages differed according to scale. Assemblages were significantly autocorrelated at small scale (0-6 kilometers (km)), negatively autocorrelated at large scale (20-40 km), and had no relationship at intermediate distances (6-12 and 12-20 km) in one stream. Another stream only had autocorrelation at one intermediate distance (12-20 km). This relates to the river continuum concept as trophic groups shift with productivity along the course of a stream (Vannote et al. 1980). Assemblages also showed seasonal variation as autocorrelation was apparent in only one of the sampling years or seasons for certain resolutions. Results from this study performed on two, relatively unimpacted streams in Australia, gives insight to the importance of identifying stream order, analyzing water velocity as an important variable. These two concepts could be incorporated as future implications for the current study.

Conclusion

Invertebrate and fish communities are important indicators of stream quality. This project identified that land use along the NTP, determined by habitat assessment scores at the instream scale, did not significantly affect the composition of benthic macroinvertebrate and fish assemblages. Trophic levels, taxa richness, and pollution tolerance levels as well as CPUE of fish were analyzed to assess stream quality biologically. Fish insectivores and invertebrate collector-gatherers dominated the overall community of streams sampled along the NTP. Only three variables were statistically significant among HUC4 watersheds: average herbivorous fishes, collector-gatherer-predator invertebrates, and percentage EPT. In invertebrate trophic groups, the collector-gatherer/predators were significantly greater in the Tombigbee watershed and percentage EPT tended to be least in Pearl and Lower Cumberland watersheds. In fish, herbivores were significantly more abundant in northern watersheds. There was a latitudinal shift in trophic groups from insectivore – omnivore – piscivore in southern streams to insectivore – generalist – herbivore in northern streams. Habitat scores did not show significant differences in community structure parameters.

In CCA, invertebrate higher taxa and genera were not correlated significantly to water quality or habitat matrices. Fish family distribution was correlated significantly to water chemistry parameters, with families such as Ictaluridae and Catostomidae correlated positively with water temperature and turbidity and correlated negatively with DO and cobble substrate. Sites with these conditions were found in Pearl, Tombigbee, and Mississippi watersheds where habitat scores tended to be less. Fish species were correlated significantly to habitat parameters. Variation in fish species was primarily related to habitat scores, bedrock, and sand.

This multimetric approach of RBP for invertebrate and fish communities allowed stream quality to be assessed across eighteen streams in six HUC4 watersheds of the NTP. Still little is known of water quality tolerances of benthic macroinvertebrates, but analysis from this study helped determined water chemistry and habitat characteristics preferred by invertebrates of this region as well as adding to the knowledge of fish community structure preferences in streams along the NTP. Future studies should analyze land use at a different scale and possibly sample periphyton to add more explanatory power to aquatic community dynamics.

CHAPTER IV

MANAGEMENT IMPLICATIONS

Land converted for growing populations and their needs has caused concern for natural resource managers and watershed protection agencies (Trombulak and Frissell 2000; Long and Plummer 2004; Snyder et al. 2005). Agricultural and urban land can cause increased amounts of sedimentation, turbidity, and nutrient runoff into streams, negatively affecting the stream health for aquatic biota (Timm et al. 2001; Camargo et al. 2004; Long and Plummer 2004; Larsen et al. 2009). Streams adjacent to forested areas have generally more pristine conditions allowing for more pollution intolerant taxa and robust diversity of aquatic fishes and macroinvertebrates (Lammert and Allan 1999; Sawyer et al. 2004). Streams along these land uses should be protected and monitored to at least maintain water quality and watershed condition. One approach to assessing stream quality for management decisions is through Rapid Bioassessment Protocols (RBP) outlined by the Environmental Protection Agency (Barbour et al. 1999; Herlihy et al. 2006).

When reviewing stream assessments, several points are should be taken into consideration. Results extrapolated from one particular spatial and temporal scale used may differ from another spatial and temporal scale. Water chemistry parameters such as water temperature and dissolved oxygen vary seasonally. Water temperature is greater and dissolved oxygen is lesser in the summer compared to winter. Invertebrate samples in were collected in June and July when most invertebrates were in larval state, a life stage before many emerge the stream to become terrestrial adults; if samples had been collected in the winter, invertebrates might still have been in the egg life stage (Wetzel 2001; Merritt et al. 2008).

Spatial scale as noted in Chapters II and III can be misleading as well. Hydrologic unit code (HUC) watersheds may not be true watersheds (Seaber et al. 1987; Omernik and Bailey 1997). While this could have affected water chemistry and biological community results, land use composition was yet another problem with the HUC4 scale. Analysis at the HUC4 watershed scale did not show variation in agricultural, forested, or developed land use practices; therefore, it was a poor scale to evaluate land use impacts at a regional level. There are other ways to analyze an ecological study, and understanding the function and limitations of the scale is an important foundation in experimental design (Levin 1992; Woods et al. 1996; Merritt et al. 2008). For instance, watersheds offer hydrological characteristics of a region, marked by political boundaries. Ecoregions, a more holistic alternative to watersheds, offer climate, soil, vegetation, hydrology, geology, wildlife, land use, and physiographic characteristics of a region (Woods et al. 1996). Analysis in this study suggests the need for a better understanding of scale and land use. It is possible that another HUC level watershed would have land use variation. A coarser or finer scale might allow heterogeneity to be seen in the region as Levin (1992) and Díaz-Varela et al. (2009) have observed in experimenting with scale sizes. Another approach to assessing land use impacts in this region could involve using different land use practices and/or reclassifying land use differently as noted in Chapter II.

Another limitation to RBP is the use of regional reference conditions to rate streams. All streams in the southeastern United States are compared to reference streams in North Carolina. One must wonder how this might bias conclusions since climate, pollution, and geology of Mississippi differs from that of North Carolina. Regardless, regional pollution tolerance levels and trophic groups of fishes and invertebrates are all determined by this reference. Habitat assessments are either scored as high gradient or low gradient streams; therefore, a wetland, while it might be scored as excellent biologically because of high productivity and biodiversity, may rank lower in the habitat assessment because it lacks the riffle-run-pool variability characteristic of high gradient streams. It is difficult to categorize a waterbody in one of two groups or have a fair comparison when the reference stream is in another ecotone.

Rapid bioassessments serve as effective methods for assessing stream quality if they are sampling based on and analysis is founded on knowledge of local communities and systems (Whiles et al. 2000). Because water chemistry and invertebrates were never sampled across the entire NTP, there was little reference for water chemistry parameter averages or assemblage structure. This project helped gain a better understanding of the quality of streams along the NTP, but there is still much to be learned about the region including impacts and ecology of the environment.

Management implications would benefit with greater knowledge of how streams and regions vary in response to pollution and disturbances (Whiles et al. 2000; Wetzel

2001). Also, analysis incorporating several variables such as CCA and other multivariate techniques offer a better understanding of ecological interrelatedness of physical and biological parameters occurring from disturbances since many involve confounding variables (Wetzel 2001; Merritt et al. 2008). Adding periphyton to sampled metrics might add explanatory power to confounding factors of aquatic community dynamics such as the distribution and abundance of herbivores in streams.

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APPENDIX A

LAND USE CLASSIFICATION DEFININTIONS (MRLC 2001)

Forested Upland - Areas characterized by tree cover (natural or semi-natural woody vegetation, generally greater than 6 meters tall); tree canopy accounts for 25-100 percent of the cover.

- *Deciduous Forest* - Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75 percent of the tree species shed foliage simultaneously in response to seasonal change.

- *Evergreen Forest* - Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75 percent of the tree species maintain their leaves all year. Canopy is never without green foliage.

- *Mixed Forest* - Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75 percent of total tree cover.

Planted/Cultivated - Areas characterized by herbaceous vegetation that has been planted or is intensively managed for the production of food, feed, or fiber; or is maintained in developed settings for specific purposes. Herbaceous vegetation accounts for 75-100 percent of the cover.

- *Pasture/Hay* - Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20 percent of total vegetation.

- *Cultivated Crops* - Areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20 percent of total vegetation. This class also includes all land being actively tilled.

Developed - Areas characterized by a high percentage (30 percent or greater) of constructed materials (e.g., asphalt, concrete, buildings, etc).

- *Developed, Open Space* - Includes areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20 percent of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.

- *Developed, Low Intensity* - Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20-49 percent of total cover. These areas most commonly include single-family housing units.

- *Developed, Medium Intensity* - Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50-79 percent of the total cover. These areas most commonly include single-family housing units.

- *Developed, High Intensity* - Includes highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80 to100 percent of the total cover.

APPENDIX B

PERCENTAGE LAND USE COVER (MRLC 2001) RECLASSIFICATION (MS-MISSISSIPPI, PL-PEARL, TB-TOMBIGBEE, TN-TENNESSEE, LC-LOWER CUMBERLAND, UC-UPPER CUMBERLAND WATERSHEDS)

Land Use Classification	Reclassification	% Cover MS	PL	TB	TN	LC	UC
Developed, Open Space	Developed	3.95	4.95	4.16	5.16	3.82	5.47
Developed, Low Intensity	Developed	0.63	1.23	0.99	2.15	0.69	1.95
Developed, Medium Intensity	Developed	0.15	0.42	0.27	0.54	0.19	0.57
Developed, High Intensity	Developed	0.03	0.13	0.07	0.2	0.07	0.21
Deciduous Forest	Forest	25.99	10.55	22.93	32.26	52.5	50.69
Evergreen Forest	Forest	13.27	23.13	15.2	5.04	5.28	3.31
Mixed Forest	Forest	10.55	13.9	9.9	4.35	1.81	4.47
Pasture/Hay	Agriculture	11.48	16.08	14.33	25.54	17.51	17.96
Cultivated Crops	Agriculture	7.22	2.43	8.24	10.17	6.96	8.16

APPENDIX C

TOTAL NUMBER OF INVERTEBRATES COLLECTED ON THE NATCHEZ TRACE PARKWAY (JUNE – JULY 2008; MI-MUD ISLAND CREEK, BS-BIG SAND CREEK, 14-FOURTEEN MILE CREEK, BK-BAKER'S CREEK, 9-NINE MILE CREEK JB-JAYBIRD CREEK, OF-OLD FIELD CREEK, CQ-CHUQUATONCHEE CREEK, DS-DONIVAN SLOUGH, BB-BURCHAM BRANCH, CO-COOPER BRANCH, GB-GLENROCK BRANCH, JK-JACK'S BRANCH, CF-CHIEF CREEK, JF-JACKSON FALLS, BR-BURNS BRANCH, GR-GARRISON BRANCH, LE-LITTLE EAST FORK CREEK).

Phylum/Class/ Subclass/Order /Suborder	Family	Genus	Stream Name																	
			MI	BS	14	BK	9	JB	OF	CQ	DS	BB	CO	GB	JK	CF	JF	BR	GR	LE
Amphipoda						5	9				1	37	6			35	9		1	2
Annelida			19		46	23	10	126	2	2	3	2	16	6		4	8	15	14	7
Bivalvia			44		49	29	3			28		4				36	3			
Cladocera						19		22				9								
Coleoptera	Dytiscidae	*Derovatellus*						2												
Coleoptera	Dytiscidae	*Hydroporinae*															1			
Coleoptera	Dytiscidae																			
Coleoptera	Elmidae	*Dubiraphia*			1		3													
Coleoptera	Elmidae	*Neoelmis*															1			
Coleoptera	Elmidae	*Optioservus*										3		2				3	1	
Coleoptera	Elmidae	*Ordobrevia*													2				16	
Coleoptera	Elmidae	*Stenelmis*								14			1							6
Coleoptera	Elmidae								17						2					2
Coleoptera	Gyrinidae	*Dineutus*	1																	
Coleoptera	Gyrinidae	*Gyretes*																	3	
Coleoptera	Gyrinidae	*Gyrinus*																		1
Coleoptera	Haliplidae	*Peltodytes*					6													
Coleoptera	Hydrophilidae	*Helophorus*								1										
Coleoptera	Hydrophilidae	*Hydrobius*									1									
Coleoptera	Hydrophilidae	*Laccobius*								1										
Coleoptera	Hydrophilidae										1									
Coleoptera	Psephenidae	*Ectopria*															10		1	
Coleoptera	Psephenidae	*Psephenus*													1			1	12	
Coleptera	Curculionidae																			
Coleptera	Hydrainidae	*Hydraena*																		
Copepoda					4	13	8	28	2			1		6		36		1		
Decapoda											1	1	3	4			3		14	44
Diptera	Ceratopogonidae	*Bezzia/ Palpomyia*					4	1	2	1	3	1		1			1			
Diptera	Ceratopogonidae	*Dasyhelea*	1																	
Diptera	Ceratopogonidae	*Mallochohelea*	2		2															
Diptera	Ceratopogonidae	*Probezzia*											1				1			
Diptera	Ceratopogonidae			1	1	1	1											1	1	

Diptera	Chaoboridae	*Chaoborus*						1												
Diptera	Chironomidae		82	110	89	82	205	42	80	113	126	98	5	52	153	61	28	145	28	17
Diptera	Culicidae	*Mansonia/ Coquillettidia*										5						2		
Diptera	Cyclorraphous-Brachycera		1																	
Diptera	Dixidae	*Dixa*													1					
Diptera	Dolichopodidae												1							
Diptera	Empididae	*Clinocera*											1							
Diptera	Empididae	*Hemerodromia*								3					1					
Diptera	Empididae	*Neoplasta*											1							
Diptera	Empididae												1							
Diptera	Muscidae															1				
Diptera	Psychodidae	*Pericoma/ Telematopscopus*				1					9									
Diptera	Simuliidae	*Prosimulium*								1				8						
Diptera	Simuliidae	*Simulium*											42							
Diptera	Simuliidae																		1	
Diptera	Tipulidae	*Dicranota*													2					
Diptera	Tipulidae	*Limnophila*				1														
Diptera	Tipulidae					1														
Diptera										1								1		
Ephemeroptera	Baetidae	*Fallceon*												2						
Ephemeroptera	Baetidae	*Procloeon*		1																
Ephemeroptera	Baetidae		1	2			7			1			2	1				2	7	
Ephemeroptera	Caenidae	*Brachycercus*	3	1	5	2		1	77	34	9			57				1	34	
Ephemeroptera	Caenidae	*Caenis*								1										
Ephemeroptera	Helptageniidae	*Leucrocuta*													1					
Ephemeroptera	Heptageniidae	*Heptagenia*																	3	
Ephemeroptera	Heptageniidae	*Maccaffertium*												2						
Ephemeroptera	Heptageniidae								8	6			3	1				1	36	2
Ephemeroptera	Leptophlebiidae	*Choroterpes*																2	1	
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*												2					7	

Ephemeroptera	Leptophlebiidae																	1	3	
Ephemeroptera						1			12	4	2	14		2			2		31	11
Gastopoda			1	1	12	27	40	43	1		26	24		32	13	100		109	15	
Hemiptera	Belostomatidae	*Belostoma*									2									
Hemiptera	Corixidae	*Palmacorixa*																		2
Hemiptera	Corixidae	*Trichocorixa*						3	7		3									
Hemiptera	Corixidae								30								1			
Hemiptera	Gerridae	*Gerris*																	1	
Hemiptera	Naucoridae	*Cryphocricos*				1														
Hemiptera	Notonectidae	*Notonecta*															1			
Hemiptera	Veliidae	*Microvelia*				1											1			
Hemiptera	Veliidae	*Rhagovelia*	1																	
Hydracarina			2	1	7	3	3	2	1	2		2		3	1	15			8	
Isopoda					1	2	7	7			1	11				20	292	1		
Megaloptera	Sialidae	*Sialis*			1		1											1	1	
Nematoda													1					1		
Odonata	Coenagrionidae	*Argia*									1									
Odonata	Coenagrionidae	*Coenagrion/ Enallagma*														1				
Odonata	Coenagrionidae				1	7		1			7	4				3				
Odonata	Corduliidae	*Neurocordulia*												1		1				
Odonata	Corduliidae	*Somatochlora*							3		1									
Odonata	Corduliidae													2						
Odonata	Gomphidae	*Hagenius*																	3	
Odonata	Gomphidae	*Ophiogomphus*												1						
Odonata	Gomphidae	*Progomphus*		1																
Odonata	Gomphidae			1										1						
Ostracoda			1				1													
Plecoptera	Capniidae	*Allocapnia*											1			1				
Plecoptera	Leuctridae	*Leuctra*													1					
Plecoptera	Leuctridae	*Paraleuctra*													2					
Plecoptera	Nemouridae	*Amphinemura*											1							
Plecoptera	Perlidae	*Acroneuria*																		1

Plecoptera	Perlidae	*Attaneuria*											2							
Plecoptera	Perlidae	*Perlesta*													1					
Plecoptera	Perlidae											1								
Plecoptera	Taeniopterygidae												3							
Plecoptera													3	11					1	
Trichoptera	Glossosomatidae	*Agapetus*											1							
Trichoptera	Glossosomatidae	*Anagapetus*													1					
Trichoptera	Glossosomatidae	*Glossosoma*																2		
Trichoptera	Glossosomatidae													1						
Trichoptera	Hydropsychidae	*Ceratopsyche*																1		
Trichoptera	Hydropsychidae	*Cheumatopsyche*	56	37						5	2		6		3			2	3	1
Trichoptera	Hydropsychidae		2	1							1									
Trichoptera	Hydroptilidae	*Hydroptila*		1			3			1					4					
Trichoptera	Hydroptilidae	*Ochrotrichia*		21																
Trichoptera	Hydroptilidae		2	4			2													
Trichoptera	Leptoceridae	*Oecetis*	1																	
Trichoptera	Leptoceridae															1				
Trichoptera	Limnephilidae	*Limnephilus*																2		
Trichoptera	Limnephilidae	*Pycnopsyche*																		1
Trichoptera	Philopotamidae	*Chimarra*		8											2					
Trichoptera	Philopotamidae			1																
Trichoptera	Polycentropodidae	*Polycentropus*					1							1						
Trichoptera	Polycentropodidae												1							
Trichoptera	Psychomyiidae	*Lype*												2						
Trichoptera			1	8										3	2		1			
Turbellaria								1									12		2	
Abundance			221	200	219	219	314	280	242	219	200	217	102	204	193	315	375	295	248	97
Richness			8	7	5	6	7	6	5	10	11	4	11	10	14	4	8	11	14	7

APPENDIX D

TOTAL NUMBER OF FISHES COLLECTED ON THE NATCHEZ TRACE PARKWAY (FEBRUARY AND APRIL 2009; MI-MUD ISLAND CREEK, BS-BIG SAND CREEK, 14-FOURTEEN MILE CREEK, BK-BAKER'S CREEK, 9-NINE MILE CREEK, JB-JAYBIRD CREEK, OF-OLD FIELD CREEK, CQ-CHUQUATONCHEE CREEK, DS-DONIVAN SLOUGH, BB-BURCHAM BRANCH, CO-COOPER BRANCH, GB-GLENROCK BRANCH, JK-JACK'S BRANCH, CF-CHIEF CREEK, JF-JACKSON FALLS, BR-BURNS BRANCH, GR-GARRISON BRANCH, LE-LITTLE EAST FORK CREEK).

Fish Species	MI	BS	14	BK	9	JB	OF	CQ	DS	BB	CB	GB	JK	CF	JF	Burn	Garr	EF
	Stream Name																	
Ameiurus platycephalus			1															
Aphredoderus sayanus					3	3												
Campostoma anomalum		3									8	18	1	1		13	13	
Campostoma oligolepis										5								
Centrarchus macropterus						1												
Clinostomus funduloides										1	15	100	67			16		
Cottus carolinae											4	1		5		4	12	
Cyprinella camura	88																	
Cyprinella venusta		1						25										
Elassoma zonatum					1	3												
Erimyzon oblongus						5	1		4								1	
Esox americanus						2												
Etheostoma caeruleum												1		1			7	
Etheostoma crossopterum													12			5	2	
Etheostoma flabellare											1	14	1	18		3	1	
Etheostoma flavum												6		13				
Etheostoma nigripinne											7							
Etheostoma proeliare							4											
Etheostoma stigmaeum	1																	
Etheostoma virgatum																2	21	
Etheostoma whipplei		1					8	6										

Ethoestoma zonisturm								3										
Fundulus catenotus																10	3	
Fundulus notatus				1														
Fundulus olivaceus	2		1		9	6				1	3			8				
Gambusia affinis	2		5	5				1						1				
Hemitremia flammea											4							
Hybognathus nuchalis		26																
Hypentelium nigricans											1	1						
Ictalurus melas		1																
Ictalurus natalis	1	1	1											2				
Lepomis cyanellus		2	13				3			2								
Lepomis gulosus						2	1	4										
Lepomis macrochirus		1	3	15		2		26	1	10				7				
Lepomis megalotis	14	20	1		21	2	1	18						4				
Lepomis miniatus					2			1										
Luxilus chrysocephales		18									2							
Lythrurus ardens											1						13	
Lythrurus bellus								2										
Lythrurus fumeus					1													
Lythrurus fumeus/roseipinnis					3													
Lythrurus roseipinnis				2														
Lythrurus umbratilis								3										
Micropterus punctulatus	2																	
Micropterus salmoides										1								
Minytrema melanops			1															

Moxostoma poecilurum		13																
Notemigonus crysoleucas								1										
Notropis ammophilus								22										
Notropis longirostris	12	11																
Notropis texanus					1													
Noturus gyrinus							1	6										
Noturus miurus	2																	
Noturus nocturnus					4													
Percina caprodes														1				
Percina sciera	5							1										
Percina vigil	2																	
Petromyzontidae											1							
Phoxinus erythrogaster												11	87			29	10	12
Pimephales notatus		27												1				
Pimephales vigilax	41	8					65	115	1								17	
Pomoxis annularis								2										
Rhinichthys atratulus											13		6					
Semotilus atromaculatus							1	1		5	2	1	16		12	6		1
Abundance	**172**	**133**	**26**	**23**	**45**	**26**	**85**	**237**	**6**	**25**	**62**	**153**	**190**	**63**	**12**	**88**	**100**	**13**
Richness	**12**	**14**	**8**	**4**	**9**	**9**	**9**	**17**	**3**	**7**	**13**	**9**	**7**	**13**	**1**	**9**	**11**	**2**