ASSESSMENT OF WATER QUALITY, BENTHIC COMMUNITY STRUCTURE AND MICROBIAL INDICATORS IN CATTARAUGUS CREEK, ZOAR VALLEY, NY.

By

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ABSTRACT

Assessing the ecological health of rivers has become one of the most important environmental goals worldwide. This study of Cattaraugus Creek in Zoar Valley, western New York State, aims to identify spatial and temporal trends in physical and chemical characteristics and benthic community structure, and to establish unified baseline data for this previously little-studied site. Sampling was conducted during 2003 – 2004 in the two main branches of Cattaraugus Creek, and in the combined reach below their confluence. Water quality parameters (temperature, conductivity, pH, dissolved oxygen, turbidity, chlorophyll *a*, fecal coliform count) were measured either by YSI 6600 Multiparameter probe, or by standard wet chemistry methods. Macroinvertebrates were sampled with either a 0.3 x 0.3-m Surber sampler, or 5-cm diameter core tubes, depending on substratum. Principal Components Analysis (PCA) of water quality variables suggested a greater influence of sampling season than of river branch/reach on water quality. Turbidity and fecal coliforms were especially strongly associated with spring 2004 samples. Benthic macroinvertebrate community structure (density, richness, diversity, EPT richness, and FBI) appeared to be more influenced by substratum type (riffle-cobble, bedrock, pool-soft sediment) than by river reach. Benthic community metrics generally suggested moderate water quality. All of the microbial indicators evaluated (heterotrophic bacteria, fecal coliforms, and *E. coli)* were highly correlated with suspended solids. Fecal coliforms and *E. coli* numbers in Cattaraugus Creek met the US EPA standards dictated for recreational purposes. Long-term monitoring of this system will further assess possible stresses on ecosystem health, and should guide watershed management practices.

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CHAPTER I

INTRODUCTION AND BACKGROUND

Restoring and maintaining the chemical, physical, and biological integrity of the Nation's waters and the continued use of water for recreation purposes are important barometers of how effectively we are meeting the objectives of the Clean Water Act (CWA). Therefore, assessing the ecological health of rivers and streams is an increasingly important water management issue worldwide. In the United States, the Environmental Protection Agency monitors water quality to characterize waters, identify changes or trends over time, and comply with pollution regulation standards. Through the STORET (short for STOrage and RETrieval), a database that includes biological and physical data collected by state environmental agencies, such information is available to the public. In addition, environmental policies increasingly focus on the enhancement of ecological functions across large geographical areas and thus call for biomonitoring tools that assess these functions at large spatial scales (Statzner, 2001).

Water Quality Assessment and Biomonitoring

Water quality assessments include a wide range of parameters that determine or at least estimate the condition of a stream at any given time. From system variables (total dissolved solids, electrical conductivity and pH, ions, cations, hardness, alkalinity, and conductivity) to nutrients (nitrate and phosphate), and toxic substances (pesticides and metals) water quality assessments can identify and explain differences between regulated and non-regulated sites (spatial discrimination) and between sampling periods(temporal discrimination).

Water resource developments are also responsible for unprecedented impacts to riverine ecosystems, most of which emanate from alterations to the natural hydrological regime (Rosenberg *et al.*, 2000). Recognition of the escalating hydrological alteration of rivers on a global scale and resultant environmental degradation, has led to the establishment of the science of environmental flow assessment, whereby the quantity and quality of water required for ecosystem conservation and resource protection are determined (Tharme, 2003). However, there are to date no simple water quality models that relate changing water quality conditions to an altered and reduced flow regime. (Scherman *et al.*, 2003).

Water quality assessments also include biomonitoring and biological data as indicators of both pollution and environmental stresses. Benthic communities have been extensively used because they spend part of their life cycle in the river bottom. Therefore, any environmental change will be expressed in spatial and temporal changes, less diverse communities and finally deformities of organisms. For example, Diggins and Stewart (1998) established that benthic community structure and sediment chemistry can contribute to a comprehensive site evaluation in a study of the lower Buffalo River, New York. Diggins and Stewart (1998) determined that chironomid density and mean richness declined significantly along a gradient of increasing trace elements levels such as Zn, Cu, Hg, Mn, Pb and Cd. They also found high chironomid mouthpart deformities, suggesting that larvae are stressed to some degree due to the exposure to trace elements. A historical review of 18 benthic macroinvertebrate and water quality studies of the Buffalo River, New York, Area of Concern, (Diggins and Snyder, 2003) revealed dramatic changes in family richness, oligochaetes abundance, and chironomid abundance over time. In addition, dissolved oxygen, total suspended solids and summer temperature showed trends, biological recovery,

water and sediment quality improvement and biological responses drawn from the analysis of the benthic macroinvertebrate community.

The River Continuum Concept (Vannote, 1981), largely developed specifically for naturally undisturbed river ecosystems in North America (Lorenz *et al.*, 1997), suggests that the biotic stream community adapts its structural and functional characteristics to abiotic variables such as flow velocity and temperature. Hydraulic conditions such as suspended load, bed load movement, turbulence, velocity profile and substratum interactions affect the distribution, morphology, behavior and ecological success of lotic biota (Gore *et al.*, 2001). As a result, a typical response is exhibited by the distribution of organic matter and macroinvertebrate functional feeding groups. Biological communities can reflect overall ecological integrity, and communities often integrate the stresses over time to provide an ecological measure of environmental conditions. In addition biological processes may be responsible for marked seasonal changes in the dynamics of the stream benthic population, and ultimately in community structure (Bunn and Davies, 2000). Therefore, temporal and spatial variations in environmental health are often reflected in gradients and zonation of benthic macroinvertebrates, which form a dominant structural characteristic of the river ecosystem (Lorenz *et al.*, 1997). Nevertheless, only by linking physico-chemical parameters resulting from classical approaches of water quality assessment and data and biological biomonitoring can requirements be adequately elucidated and translated into resource quality objectives (Scherman *et al.*, 2003).

Study Area

Zoar Valley of Cattaraugus Creek is an important tributary to Lake Erie in western New York State (Figure 1). Located in the middle reaches of the creek, the Zoar Valley Canyon is one of the most outstanding and impressive natural ecosystems in the northeastern United States. It encloses perhaps the premier old-growth broadleaf forest in the region (Hunt *et al.* 2002; Diggins and Kershner, 2005).

Zoar Valley is considered a Multiple Use Area since there has not been assignedto a long term land use category. Zoar Valley is under the jurisdiction of the New York State Department of Environmental Conservation (NYS DEC) Region 9. Swimming, fishing, rafting and hunting are some of the recreational activities in the area and its surroundings. Zoar Valley is considered the second deepest walled canyon in New York State.

Cattaraugus Creek from Zoar Valley downstream to Lake Erie is a 5^{th} or 6^{th} order stream, depending on stream order assignments within headwater reaches (Hunt *et al.*, 2002). Following the longer of the two major branches (Main Branch), Cattaraugus Creek is approximately 80 km in length. Cattaraugus Creek originates at Java Lake in Wyoming County, and flows west about 80 Km into Lake Erie. Typical low-flow discharge below the confluence is <5.0 m^3/s, but floods can exceed 700 m^3/s (US Geological Survey, 2005). The watershed of Cattaraugus Creek encompasses 1430 km^2, in which land cover includes forests, wetlands, agricultural lands, and small villages. The watershed of the South Branch is less developed and more forested than that of the Main Branch (NYS Office of Technology, 2005).

Cattaraugus Creek river bottom is mainly composed of sand/gravel bar, cobble shore and few pools. Some of the threats and stream systems disturbances of the creek are chemical pollutants and geomorphological changes associated with elevated flood levels and erosive forces (Hunt *et al.*, 2002). A preliminary reconnaissance survey allowed identifying study sites. At the commencement of the present study it was assumed that substrates were relatively stable However, high flow events altered the hydrology dramatically during the course of the study.

Zoar Valley has an intact riparian ecosystem with unique aquatic riverine communities. Diggins and Kershner (2005) describe the ecology of Zoar Valley's canyon-bottom old-growth woodlands in detail.

Objectives

The objectives of the present study were (1) to catalogue physico-chemical and biological parameters, (2) assess possible microbial indicators of water quality, and (3) characterize the benthic community structure, within each of two major branches of Cattaraugus Creek (Main Branch and South Branch), and in the Combined Reach below their confluence (Figure 2). The results will allow a more complete assessment of environmental health in Cattaraugus Creek, and will aid in the development of future watershed management practices.

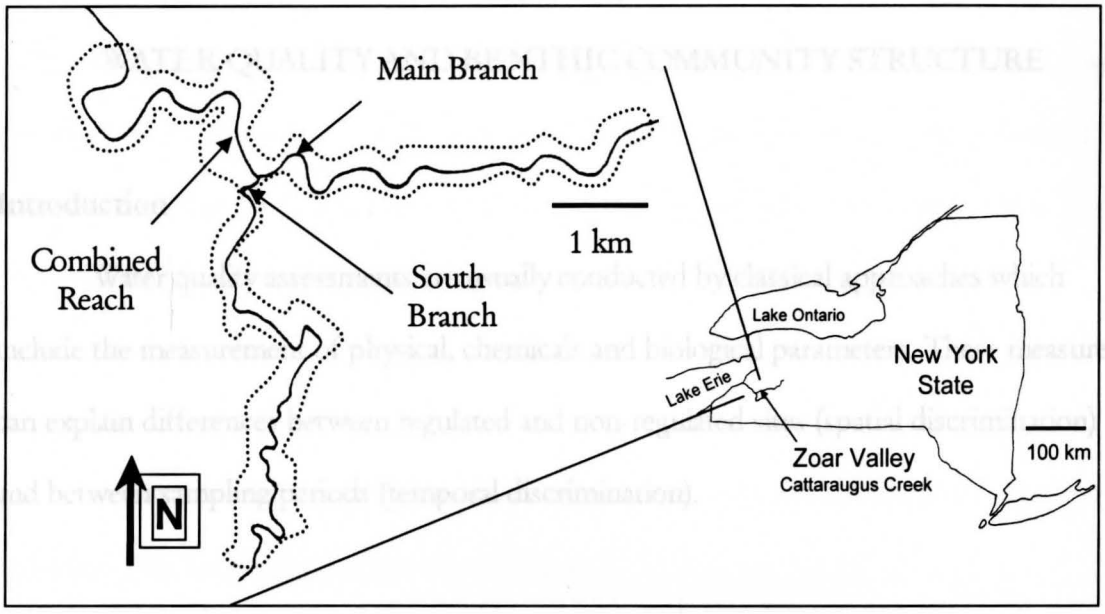

Figure 1. Location of Cattaraugus Creek study site and detail of Zoar Valley Canyon showing locations of sampled stream reaches. Dotted line indicates approximate boundaries of canyon

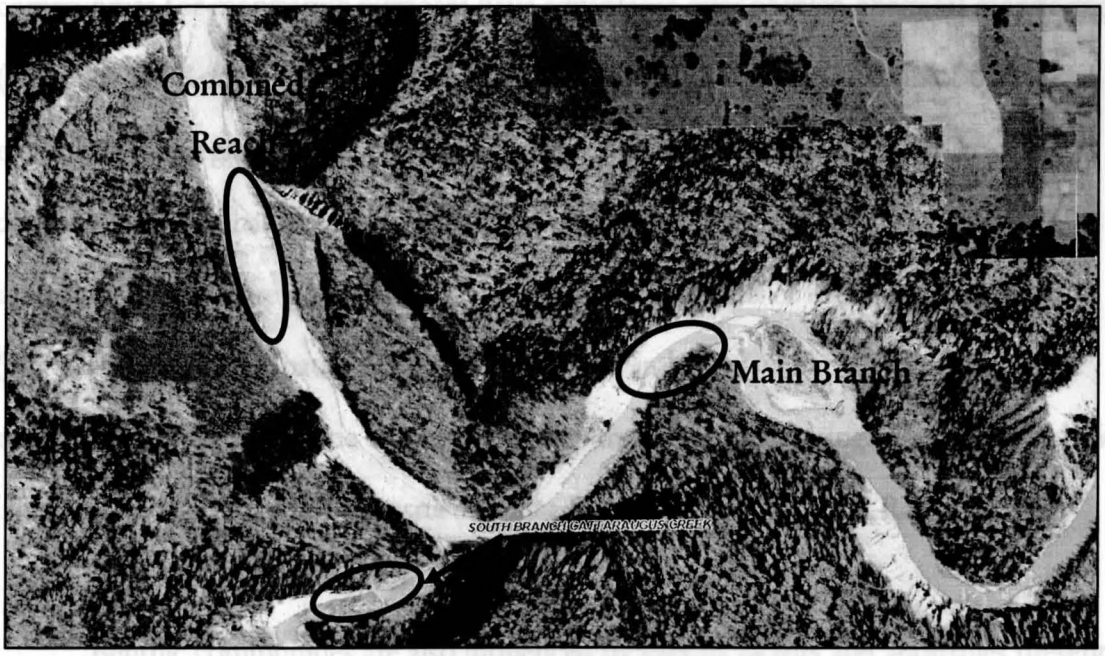

Figure 2. Ortho-satellite picture (NYS Office of Technology, 2002) showing the study sites at the South Branch, Main Branch and Combined Reach in Cattaraugus Creek., Zoar Valley, NY.

CHAPTER II

WATER QUALITY AND BENTHIC COMMUNITY STRUCTURE

Introduction

Water quality assessments are usually conducted by classical approaches which include the measurement of physical, chemicals and biological parameters. These measures can explain differences between regulated and non-regulated sites (spatial discrimination) and between sampling periods (temporal discrimination).

The use of aquatic communities as indicator systems or as criteria for conservation and restoration has stimulated research (Statzner *et al.*, 2001), especially because the resident biota integrates and responds to a wide range of chemical and physical dynamics. Biotic communities for monitoring purposes have advantages over physico-chemical monitoring (Soininen and Kononen, 2004) because information on the global situation of the environment integrated over time can be obtained (Rodrigues *et al.* 2001).. Due to their diversity of forms, habits and responses to environmental stresses, benthic macroinvertebrates are good candidates for the bioassessment of ecosystem integrity (Statzner *et al.*, 2001). Spatial and temporal changes in habitats and community structure reflect how abiotic and biotic conditions of the stream are functioning. Thus, their inclusion in aquatic ecosystem health assessment is clearly warranted (Yoder and Rankin, 1995)

Benthic communities are also influenced by various factors and anomalies present at any given time. Abiotic factors such as pollution, disturbance in the riparian ecosystem, agricultural run off and excessive nutrient loading make the benthic macroinvertebrate

respond by showing differences in their community structure. Therefore, specific interactions among species may occur so species adapt to environmental changes and match the physical environment (Usseglio-Polatera *et al.*, 2000). Nevertheless, physico-chemical parameters should not be excluded from the picture. Physico-chemical monitoring is important to identify and quantify the potential causes of effects on the biota.

Study Site

A description of Zoar Valley, Cattaraugus Creek, is detailed in Chapter I. Study area and study sites are shown in Figure 1 and Figure 2.

Methods

Physico-Chemical and Biological Parameters

Water quality sampling was conducted in Cattaraugus Creek during 2003 – 2004, divided temporally into the seasons: summer 2003, fall 2003, spring 2004, and summer 2004.

Three water samples were collected from each river reach making a total number of nine samples on each date. All samples were collected between 10:00 am and 2:00 pm. Sampling locations were identified through visual landmarks after considering weather conditions and accessibility.

Because there was little reason to expect water quality to vary with underlying substratum in the high-energy flow regime characterizing my study site, water quality was assessed at only one station within each river reach (a riffle/cobble zone). Temperature (°C), pH, conductivity ($\mu S\ cm^{-1}$), dissolved oxygen (DO $mg\ l^{-1}$), turbidity (NTU), and chlorophyll

($\mu g\ l^{-1}$) were measured in the field using a Multiparameter probe (Model 6600 YSI, Yellow Springs, Ohio). Ten readings of 30 seconds each one were taken randomly at each site.

Fecal Coliform Enumeration

Fecal coliforms were enumerated using the fecal coliform membrane filtration technique (APHA, 1999). Triplicate samples taken at each branch were collected using a 100 ml sterile bottle. When sampling occurred within 24 hours of storm events and run off 10 ml water samples were collected in sterile 15 ml tubes to avoid "*too numerous to count*" (TNTC) data. Samples were transported to the lab, and processed within 24 hours. Fecal coliform were detected using the membrane filtration technique, incubating the filters placed on sterile plates with m-FC agar for fecal coliforms. Plates were incubated inverted at 44.5 $\pm$ 0.5 °C for 24 hours and colonies that were various shades of blue were counted as fecal coliform. Colonies were counted under a magnifier with light source.

Benthic Sampling

Benthic macroinvertebrates were collected for a period of 4 seasons: summer 2003, fall 2003, spring 2004, and summer 2004. South Branch, Main Branch and Combined Reach were monitored. Samples were taken from three different substratum types within each stream reach, visually identified as dominant within Zoar Valley: 1) riffle-cobble, 2) continuous bedrock pavement, and 3) pools containing soft sediment. Unexpectedly high water flows during spring and summer of 2004 precluded collection of macroinvertebrate samples from pools during these seasons. Three replicates were taken arbitrarily.

Macroinvertebrates were collected from riffle and bedrock substrata using a standard 0.3 x 0.3-m Surber sampler with a 500 μm mesh net. We agitated and/or manipulated the substratum for a uniform time of two minutes for each replicate. Sampling of bedrock substrata differed slightly from that of riffles, as we sampled only along joints in the rock, which was the only place macroinvertebrates were present in measurable numbers.

Macroinvertebrates were collected from soft sediment pools using a 5-cm diameter core tube, which was pushed into the substratum and capped from underneath manually. Core tube contents were transferred to a 2 mm sieve, and then to the 500 μm mesh net of the Surber sampler, to achieve similar retention of material to that of riffle and bedrock samples. All benthic samples were returned to the laboratory where organisms were removed, sorted and preserved in 70% ethanol.

Macroinvertebrates were identified to family according to Pennak (1953), Pecharsky *et al.* (1990), Merritt and Cummings (1996), NYS DEC (2004), and/or US EPA (2004). The total number of benthic samples that were taken during the length of the present study is shown in Table 1.

Table 1 Sampling desing for 2003 - 2004. Number of benthic samples taken per season at each branch. Sampling method used and range of high water mark (m) observed.

				Number of benthic samples				
branch / season				summer 2003	fall 2003	spring 2004	summer 2004	total
	Substratum	Water depth (range in m)	Sampling method					
South branch								**93**
	Bedrock	(0.3 - 0.7)	Surber	12	12	6	6	
	Riffles	(0.2 - 0.4)	Surber	12	12	6	6	
	Pools	(0.6 - 1.5)	Core tube	12	9	0	0	
Main branch								**93**
	Bedrock	(0.3-0.6)	Surber	12	12	12	12	
	Riffles	(0.3-1.2)	Surber	12	12	6	6	
	Pools	(0.8-1.2)	Core tube	12	9	0	0	
Combined reach								**81**
	Bedrock	(0.2 - 1)	Surber	12	12	6	6	
	Riffles	(0.4-0.6)	Surber	9	9	6	6	
	Pools	(0.8-1.4)	Core tube	9	6	0	0	

Data Analysis

Physico-chemical and biological parameters were investigated using three basic approaches:

1. For water quality parameters a Principal Component Analysis (PCA) was carried out to (1) *reduce* the number of variables and (2) to *detect structure* in the relationships between variables.

2. For water quality parameters and benthic community structure, one- way Multiple ANOVA (Analysis of Variance) test (Type III sum of squares and a significance level of 0.05) and Pearson correlation were performed.

   Descriptive statistics (mean, standard deviation and standard error) of physico-chemical variables during the 2003-2004 period of study were considered as representative of the mean per branch of each sampling date.

3. For benthic community structure a combination of indexes was used to evaluate the biotic integrity of communities.

   - Total abundance of macroinvertebrates, and of each family
   - Total invertebrate density expressed as number of organism per m^2
   - Family richness expressed as number of families
   - EPT richness (Ephemeroptera [mayfly], Plecoptera [stonefly], Trichoptera [caddisfly] expressed as number of these three families per sample

- Community diversity index (Shannon-Weiner H') determined by the following formula:

$$H' = -\sum_{i=1}^{s} (p_i)\ (\log_2 p_i)$$

where:

H = Index of species diversity,

s = Number of species

$\mathbf{p_i}$ = Proportion of total sample belonging to $\mathbf{i^{th}}$ species

- Hilsenhoff Field Biotic Index. The pollution tolerances of the various macroinvertebrates included in the biotic index are based upon the macroinvertebrates' tolerance to dissolved oxygen concentrations in water. The organisms are separated into four categories of pollution tolerance: sensitive, semi-sensitive, semi-tolerant, and tolerant to pollution (Table 2).

Table 2 Interpretation of Field Biotic Index Scores- FBI- (Hilsenhoff, 1988) based upon the macroinvertebrates' tolerance to dissolved oxygen concentrations in water.

FBI	Water Quality	Degree of Organic Pollution
0.00-3.5	Excellent	no apparent organic pollution
3.51-4.5	very good	possible slight organic pollution
4.51-5.50	Good	some organic pollution
5.51-6.50	Fair	fairly significant organic pollution
6.51-7.50	fairly poor	significant organic pollution
7.51-8.50	Poor	very significant organic pollution
8.51-10.00	very poor	severe organic pollution

The metric calculates the average pollution tolerance of the stream using the formula

$$FBI = \frac{\Sigma[(TV_i)(n_i)]}{N}$$

where:

TV_i = tolerance value for each family [Soil & Water Conservation Society of Metro Halifax, 2004]

n_i = the number of individuals in the family,

N = the total number of individuals in the collection.

Results

Physico-Chemical Parameters

Several physico-chemical parameters in Cattaraugus Creek showed notable temporal and spatial variation (Table 3), suggesting a dynamic system, but others appeared more stable.

Higher water temperature values characterized summer seasons. Values ranged from 20.1 °C to 23.6 °C. Lower temperature values characterized the fall season with the lowest temperatures of 9.8 to 11.1 °C.

Measured pH values during the four sampling seasons did not vary too much, ranging from 8.32 to 8.75. Chlorophyll values presented a wider range, varying from 3.7 ug/l to 14.4 ug/l. Interestingly both extremes values corresponded to summer seasons.

Variability in dissolved oxygen (DO) in Cattaraugus Creek suggests there may be some oxygen demand, but not necessarily enough to be considered a stressed stream system. Concentrations during the two summers reflected only 75% to 92% saturation, but super-saturation levels of up to 113% were recorded during spring 2004. The highest dissolved oxygen concentrations, as expected, corresponded to low temperatures in fall 2003.

Turbidity in particular was highly variable temporally, ranging from 3.6 NTU during low flow in summer 2003 to 52.9 NTU after precipitation events in Spring 04 (Table 3).

Values in Cattaraugus Creek were higher than the values reported for the Mahoning River, OH, during spring and summer, which only ranged from 4.5 to 10.3 NTU (Xu and Leff 2004).

Fecal coliform numbers per 100 ml ranged from 6.9 CFUs in fall to 150.6 CFUs in spring. In general higher values were reported in spring which corresponded to high discharges after storm events.

Conductivity values ranged from 289 μScm^{-1} to 445 $\mu S\ cm^{-1}$ measured in spring and summer, respectively.

Table 3 Physico-chemical and biological parameters of Cattaraugus Creek (sampling period 2003 - 2004) Mean in left -hand columns, SE in right-hand

Season	River reach	Temperature °C		pH		Dissolved O_2 mg/L		Conductivity µS/cm		Turbidity NTU		Chlorophyll µg/L		Fecal Coliforms CFUs	
Summer 03	South branch	21.5	1.6	8.53	0.08	6.4	0.2	309	26	3.6	1.4	14.4	0.0	78.8	72.4
(n=12)	Main branch	23.6	1.6	8.33	0.10	6.1	0.1	376	55	9.1	3.2	14.4	0.0	39.8	37.4
	Combined reach	22.5	1.1	8.43	0.10	8.2	1.4	445	101	9.9	7.7	12.7	1.7	43.3	39.6
Fall 03	South branch	10.9	3.6	8.75	0.03	9.9	1.4	311	8.8	16.5	5.1	5.6	2.9	41.7	30.8
(n=15)	Main branch	11.1	4.2	8.56	0.06	9.9	1.4	425	6.5	8.5	3.1	7.1	2.8	6.9	2.8
	Combined reach	9.8	2.4	8.62	0.02	10.0	1.0	391	7.5	20.9	5.3	6.9	2.1	31.8	22.3
Spring 04	South branch	21.1	1.0	8.39	0.08	9.5	0.1	289	16.6	53.0	16.2	3.9	0.7	85.4	70.8
(n=9)	Main branch	20.1	1.3	8.33	0.06	9.5	0.3	398	12.7	47.6	20.4	6.1	0.3	150.6	137.6
	Combined reach	22.2	1.2	8.38	0.08	9.3	0.1	362	16.3	53.3	18.3	4.3	1.1	78.0	67.9
Summer 04	South branch	22.0	2.3	8.43	0.01	7.7	1.2	338	4.3	24.9	1.8	7.8	3.4	25.8	6.0
(n=12)	Main branch	20.1	1.6	8.32	0.01	6.6	1.8	431	9.5	24.6	1.2	3.7	0.4	45.0	8.9
	Combined reach	21.9	2.0	8.41	0.01	7.4	1.4	420	10.1	23.3	1.7	5.9	2.6	20.2	4.1

As one can see in Figure 3 and 4 physico-chemical parameters seemed to follow the same pattern at the South Branch, Main Branch and Combined Reach, suggesting that water quality parameters were not influenced by river reach. Confirming that supposition, a one - way Multiple ANOVA test (Table 4) showed that six of the seven water quality parameters - dissolved oxygen ($p = 0.825$), water temperature ($p = 0.985$), pH ($p = 0.063$), turbidity ($p = 0.421$), chlorophyll ($p = 0.958$) and fecal coliforms ($p = 0.903$)- were not significantly different among the branches. Only for conductivity did values differ significantly ($p < 0.01$).

Considering season as a factor the one way-Multiple ANOVA test (Table 4) showed that, of the seven water quality parameters measured, five exhibited seasonal variation over the study period. Water temperature ($p < 0.01$), dissolved oxygen ($p = 0.002$), pH ($p < 0.01$), turbidity ($p = 0.001$) and chlorophyll ($p = 0.007$) were different among the sampling seasons.

A correlation matrix was developed to see if the seven water quality parameters under study were related (Table 6). The Pearson correlation was high for turbidity and fecal coliforms ($r = 0.678$, $p < 0.05$). Water temperature was correlated with dissolved oxygen ($r = 0.485$, $p < 0.05$), pH ($r = 0.438$, $p < 0.05$) and chlorophyll ($r = 0.469$, $p < 0.05$). Turbidity values were also associated in less degree with pH ($r = 0.381$, $p < 0.01$) and chlorophyll ($r = 0.336$, $p < 0.01$)

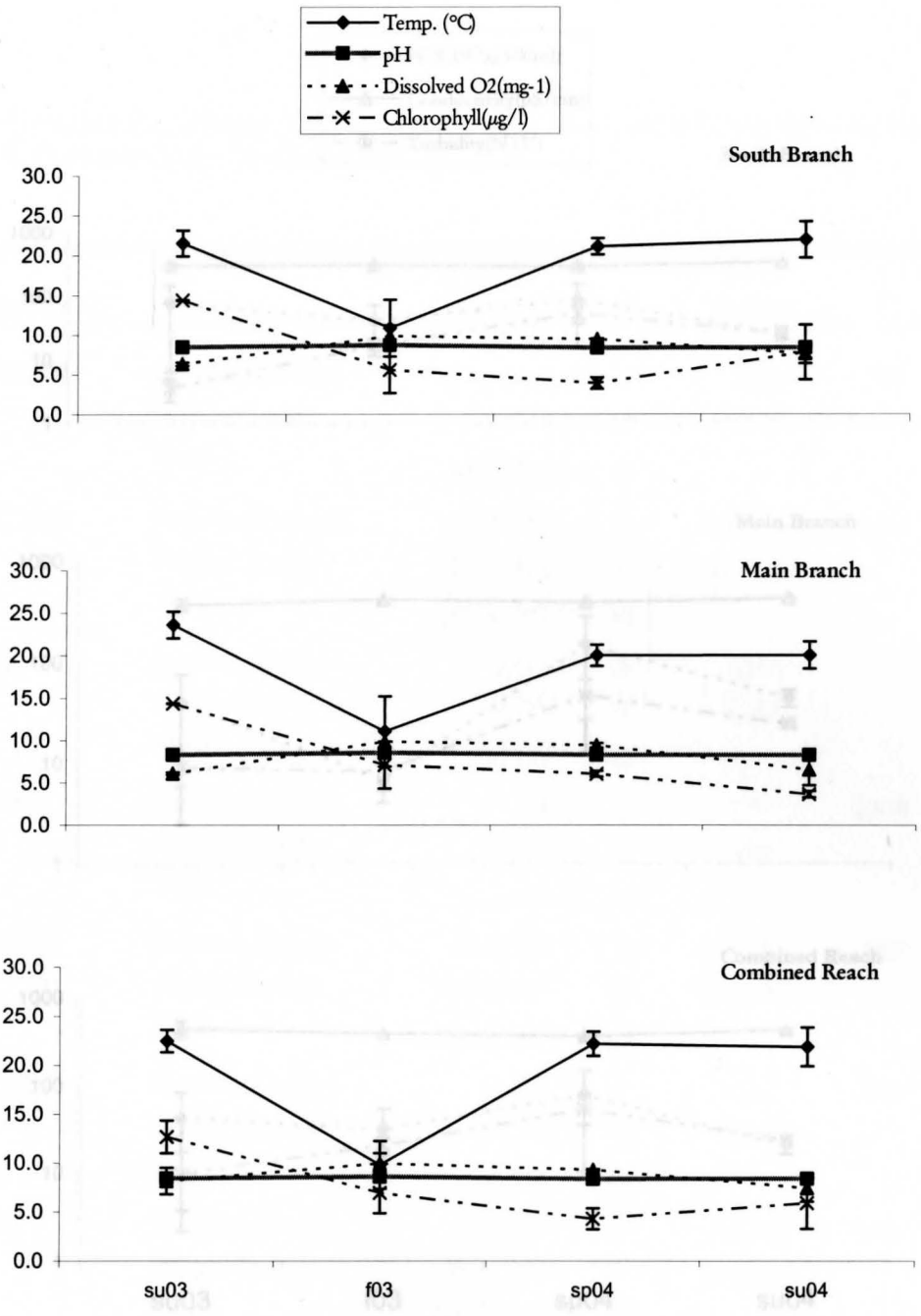

Figure3 Water temperature (°C), pH, dissolved oxygen (mg/L) and chlorophyll (μg/L) mean values and standard errors measured in Cattaraugus Creek.

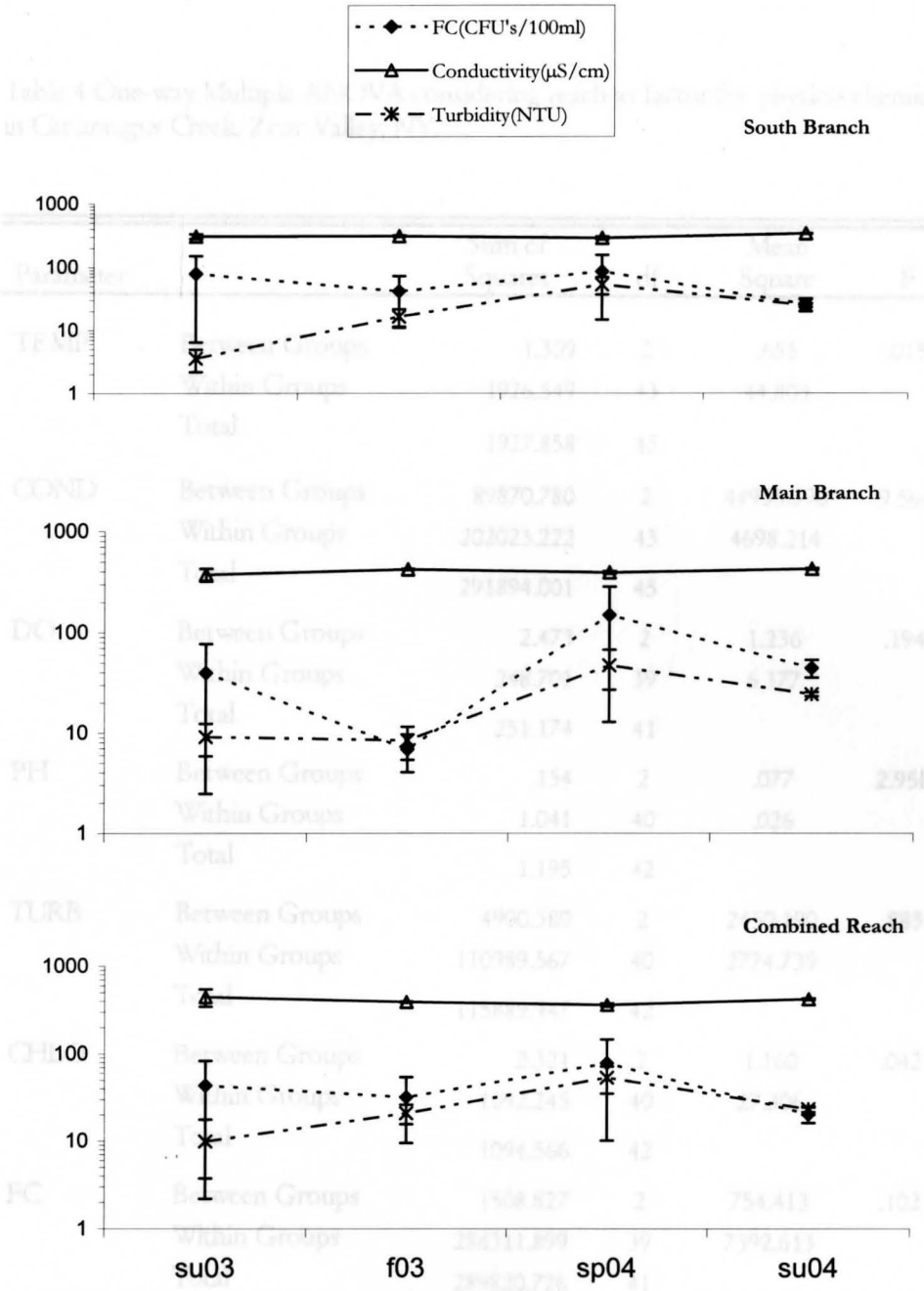

Figure 4 Fecal coliforms(CFUs / 100 ml), Turbidity (NTU) and Conductivity (µS/cm) values for Cattaraugus Creek presented in a logaritmic scale for better visualization of seasonal trends

Table 4 One-way Multiple ANOVA considering reach as factor for physico-chemical parameters in Cattaraugus Creek, Zoar Valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
TEMP	Between Groups	1.309	2	.655	.015	.985
	Within Groups	1926.549	43	44.803		
	Total	1927.858	45			
COND	Between Groups	89870.780	2	44935.390	9.564	.000
	Within Groups	202023.222	43	4698.214		
	Total	291894.001	45			
DO	Between Groups	2.473	2	1.236	.194	.825
	Within Groups	248.701	39	6.377		
	Total	251.174	41			
PH	Between Groups	.154	2	.077	2.958	.063
	Within Groups	1.041	40	.026		
	Total	1.195	42			
TURB	Between Groups	4900.380	2	2450.190	.883	.421
	Within Groups	110989.567	40	2774.739		
	Total	115889.947	42			
CHLO	Between Groups	2.321	2	1.160	.042	.958
	Within Groups	1092.245	40	27.306		
	Total	1094.566	42			
FC	Between Groups	1508.827	2	754.413	.102	.903
	Within Groups	288311.899	39	7392.613		
	Total	289820.726	41			

Table 5 One-way Multiple ANOVA considering season as factor for physico-chemical parameters in Cattaraugus Creek, Zoar valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
TEMP	Between Groups	1170.229	2	585.115	33.209	.000
	Within Groups	757.629	43	17.619		
	Total	1927.858	45			
COND	Between Groups	8801.447	2	4400.723	.668	.518
	Within Groups	283092.555	43	6583.548		
	Total	291894.001	45			
DO	Between Groups	66.469	2	33.235	7.017	.002
	Within Groups	184.704	39	4.736		
	Total	251.174	41			
PH	Between Groups	.542	2	.271	16.610	.000
	Within Groups	.653	40	.016		
	Total	1.195	42			
TURB	Between Groups	33157.545	2	16578.772	8.016	.001
	Within Groups	82732.402	40	2068.310		
	Total	115889.947	42			
CHLO	Between Groups	239.240	2	119.620	5.594	.007
	Within Groups	855.326	40	21.383		
	Total	1094.566	42			
FC	Between Groups	38004.894	2	19002.447	2.943	.065
	Within Groups	251815.832	39	6456.816		
	Total	289820.726	41			

Table 6 Correlation matrix between physico-chemical and biological parameters in Cattaraugus Creek, Zoar Valley, NY.

		TEMP	COND	DO	PH	TURB	CHLO	FC
TEMP	Pearson Correlation	1	.146	-.485(**)	-.438(**)	.095	.469(**)	.073
	Sig. (2-tailed)	.	.332	.001	.003	.546	.002	.652
	N	46	46	42	43	43	43	40
COND	Pearson Correlation	.146	1	.009	.012	.017	-.065	-.118
	Sig. (2-tailed)	.332	.	.956	.938	.914	.677	.469
	N	46	46	42	43	43	43	40
DO	Pearson Correlation	-.485(**)	.009	1	.116	-.014	-.268	-.018
	Sig. (2-tailed)	.001	.956	.	.481	.935	.099	.915
	N	42	42	42	39	39	39	36
PH	Pearson Correlation	-.438(**)	.012	.116	1	-.381(*)	-.001	-.138
	Sig. (2-tailed)	.003	.938	.481	.	.012	.996	.416
	N	43	43	39	43	43	43	37
TURB	Pearson Correlation	.095	.017	-.014	-.381(*)	1	-.336(*)	.678(**)
	Sig. (2-tailed)	.546	.914	.935	.012	.	.028	.000
	N	43	43	39	43	43	43	37
CHLO	Pearson Correlation	.469(**)	-.065	-.268	-.001	-.336(*)	1	-.017
	Sig. (2-tailed)	.002	.677	.099	.996	.028	.	.919
	N	43	43	39	43	43	43	37
FC	Pearson Correlation	.073	-.118	-.018	-.138	.678(**)	-.017	1
	Sig. (2-tailed)	.652	.469	.915	.416	.000	.919	.
	N	40	40	36	37	37	37	42

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Because the data showed a degree of correlation, a data reduction analysis_ Principal component analysis_ was performed. The total variance of the seven water quality parameters studied is shown in Table 7.

In the ordination of water quality variables shown in Figure 5A the first and second principal components (PCs) explain 69.9% of data variance (Table 7). PC 1 is explained by mainly of water temperature loading of 0.87 and pH (0.93) while PC2 is more a representation of dissolved oxygen (0.75), turbidity (0.76) and chlorophyll(0.81). Loadings of turbidity and fecal coliforms were closely associated, and oriented towards spring 2004 samples. In Figure 5B, the first and third PCs explain 56.1% of water quality data variance. The third PC was almost completely explained by loading of conductivity (0.92).

Table 7 Total Variance explained based on data reduction by Principal Component Analysis

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.652	37.887	37.887	2.652	37.887	37.887
2	2.244	32.062	69.949	2.244	32.062	69.949
3	1.207	17.249	87.198	1.207	17.249	87.198
4	.587	8.391	95.589			
5	.219	3.134	98.723			
6	.070	1.001	99.724			
7	.019	.276	100.000			

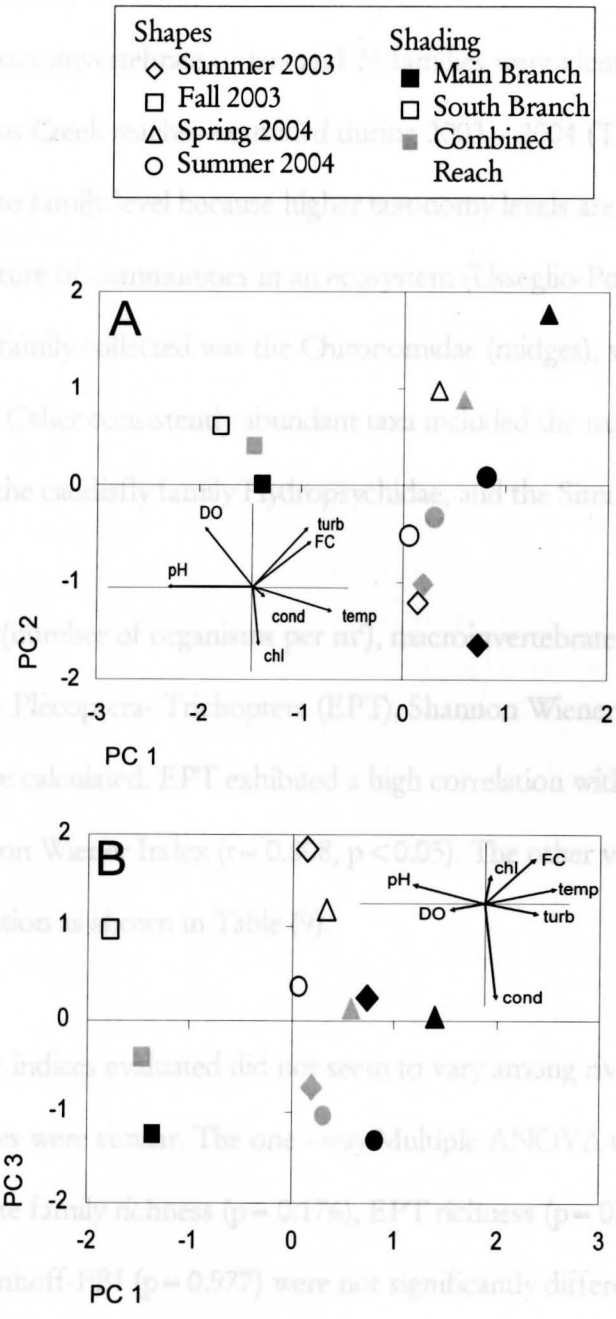

Figure 5. Ordination plots of principal components (PC 1, PC 2, PC 3) extracted from water quality variables temperature (temp), dissolved oxygen (DO), pH, conductivity (cond), turbidity (turb), chlorophyll *a* (chl), and fecal coliform count (FC). Shapes identify data points temporally, by sampling season. Shading identifies data points spatially, by stream reach. Vector arrows on inset plots denote relative loadings of original water quality variables.

Benthic Community Structure

Eight macroinvertebrate orders and 24 families were identified and catalogued in the three Cattaraugus Creek reaches examined during 2003 - 2004 (Table 8). Macroinvertebrates were identified to family level because higher taxonomy levels are more suitable for studying functional structure of communities in an ecosystem (Usseglio-Polatera et al. 2000). The most abundant family collected was the Chironomidae (midges), which were predominant in pool sediments. Other consistently abundant taxa included the mayfly families Baetidae and Heptageniidae, the caddisfly family Hydropsychidae, and the Simuliidae (blackflies).

Density (number of organisms per m^2), macroinvertebrate family richness, Ephemeroptera- Plecoptera- Trichoptera (EPT), Shannon Wiener Index and Field Biotic Index (FBI) were calculated. EPT exhibited a high correlation with richness ($r = 0.867$, $p < 0.05$) and Shannon Wiener Index ($r = 0.808$, $p < 0.05$). The other variables also showed lower values of correlation as shown in Table (9).

The five indices evaluated did not seem to vary among river reaches. As one can see in Figure 6, values were similar. The one -way Multiple ANOVA test showed that macroinvertebrate family richness ($p = 0.176$), EPT richness ($p = 0.104$), and diversity-H'-($p = 0.596$) and Hilsenhoff-FBI ($p = 0.977$) were not significantly different among river reaches (Table 10). Only density ($p = 0.019$) differed significantly between the Combined reach and the Main branch.

Table 2. Abundance by family (m^2) of zoobenthos catalogued per substratum type during the length of the study in Cattaraugus Creek, Zoar Valley, NY

		Order	Coleoptera	Diptera				Ephemeroptera										Odonata	Plecoptera	Trichoptera				Gastropoda	Plesiophora	
		Family																								
Season	Substrate	Reach	Elmidae	Psephenidae	Anthericidae	Athericidae	Chironomidae	Simuliidae	Tipulidae	Ameletidae	Baetidae	Caenidae	Ephemerellidae	Heptageniidae	Isonychiidae	Leptophlebiidae	Potamanthidae	Anisoptera	Capniidae	Perlidae	Dipseudopsidae	Hydropsychidae	Psychomyiidae	Rhyacophilidae	Physidae	Tubificidae	All taxa
Summer 03	Bedrock	C	0	0	25	0	59	1	0	5	3	6	0	5	2	0	0	0	0	0	0	29	1	0	0	0	49
		M	1	0	0	0	28	0	0	0	6	7	3	2	1	0	0	0	0	0	0	44	2	0	0	1	65
		S	0	0	2	0	51	3	0	4	6	11	0	3	0	0	0	0	0	0	0	45	1	0	0	0	70
	Riffle	C	6	0	0	1	7	0	0	0	0	2	0	21	0	1	0	0	0	9	0	3	1	0	0	0	37
		M	0	0	0	0	9	1	0	0	5	2	1	12	0	0	0	0	0	2	0	19	1	0	0	0	42
		S	5	0	0	0	95	1	0	3	2	9	4	26	6	0	8	2	0	17	2	56	2	0	0	0	136
	Pool	C	0	0	0	0	594	0	0	0	0	42	0	0	42	0	0	0	0	0	0	0	0	0	0	0	84
		M	42	0	0	0	298	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		S	0	0	0	0	1062	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0	0	0	42
Fall 03	Bedrock	C	2	0	4	0	70	2	1	0	28	19	0	15	2	0	6	1	0	1	0	144	0	0	1	0	216
		M	4	0	8	0	20	0	0	1	6	2	0	2	0	0	0	0	0	0	0	61	0	0	0	0	71
		S	0	0	3	0	25	3	1	1	1	2	0	7	0	0	0	0	0	4	0	31	1	1	0	0	48
	Riffle	C	6	0	3	0	6	0	1	0	0	2	1	19	5	0	1	0	0	1	0	20	0	0	0	0	48
		M	1	0	2	0	12	0	3	1	1	0	0	9	0	0	0	0	0	2	0	23	0	0	0	0	36
		S	3	3	3	0	44	0	0	1	5	6	1	37	1	0	7	0	0	3	0	32	0	0	0	0	93
	Pool	C	0	0	0	0	2336	0	0	0	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	680	722
		M	0	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		S	0	0	0	0	1020	0	42	0	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	42	84

Table 2 continued. Abundance by family (m^2) of zoobenthos catalogued per substratum type during the length of the study in Cattaraugus Creek, Zoar Valley, NY

		Order	Coleoptera	Diptera						Ephemeroptera									Odonata	Plecoptera		Trichoptera					Gastropoda	Plesiophora	
		Family																											
Season	Substrate	Reach	Elmidae	Psephenidae	Anthericidae	Athericidae	Chironomidae	Simuliidae	Tipulidae	Ameletidae	Baetidae	Caenidae	Ephemerellidae	Heptageniidae	Isonychiidae	Leptophlebiidae	Potamanthidae	Anisoptera	Capniidae	Perlidae	Dipseudopsidae	Hydropsychidae	Psychomyiidae	Rhyacophilidae	Physidae	Tubificidae	All taxa
Spring 04	Bedrock	C	0	0	0	0	13	0	0	20	17	19	0	13	2	2	0	0	17	0	0	6	0	0	0	0	94
		M	0	0	0	0	11	0	0	0	2	4	0	7	35	0	0	0	0	0	0	33	7	2	0	0	91
		S	4	0	2	7	13	0	2	19	74	26	0	6	0	54	24	0	0	4	26	31	4	2	0	0	269
	Riffle	C	0	0	0	0	0	0	0	0	0	2	0	2	0	0	2	0	2	6	0	24	0	0	0	0	37
		M	0	0	0	0	4	0	0	0	35	0	0	30	6	7	0	0	0	2	2	15	4	0	0	0	100
		S	0	0	0	0	4	0	0	4	28	0	0	52	0	2	0	0	0	7	0	7	2	0	0	0	102
Summer 04	Bedrock	C	11	2	2	2	174	70	0	19	6	13	0	15	2	0	0	2	0	7	0	80	0	0	0	0	143
		M	2	0	4	7	194	137	0	17	17	11	2	2	2	13	0	0	0	2	0	35	4	0	0	0	104
		S	6	0	0	9	102	24	0	7	26	20	2	6	0	0	4	0	2	9	0	96	0	7	0	0	180
	Riffle	C	0	0	0	2	72	20	0	35	4	6	0	0	0	0	0	0	0	4	0	96	0	0	0	0	144
		M	0	2	0	0	61	31	2	6	11	13	0	31	2	0	11	0	0	11	0	54	0	0	0	0	139
		S	4	0	4	6	26	0	2	17	22	6	0	7	0	22	11	0	2	2	0	94	0	0	0	0	183

Table 9 Correlation Matrix between Benthic Macroinvertebrate Indices for Cattaraugus Creek, Zoar Valley, NY.

		DENSITY	RICHNESS	EPT	H'	FBI
DENSITY	Pearson Correlation	1	.082	-.008	-.049	.360(**)
	Sig. (2-tailed)		.224	.903	.464	.000
	N	222	222	222	222	222
RICHNESS	Pearson Correlation	.082	1	.896(**)	.867(**)	-.148(*)
	Sig. (2-tailed)	.224	.	.000	.000	.028
	N	222	222	222	222	222
EPT	Pearson Correlation	-.008	.896(**)	1	.808(**)	-.346(**)
	Sig. (2-tailed)	.903	.000	.	.000	.000
	N	222	222	222	222	222
H'	Pearson Correlation	-.049	.867(**)	.808(**)	1	-.224(**)
	Sig. (2-tailed)	.464	.000	.000	.	.001
	N	222	222	222	222	222
FBI	Pearson Correlation	.360(**)	-.148(*)	-.346(**)	-.224(**)	1
	Sig. (2-tailed)	.000	.028	.000	.001	.
	N	222	222	222	222	222

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

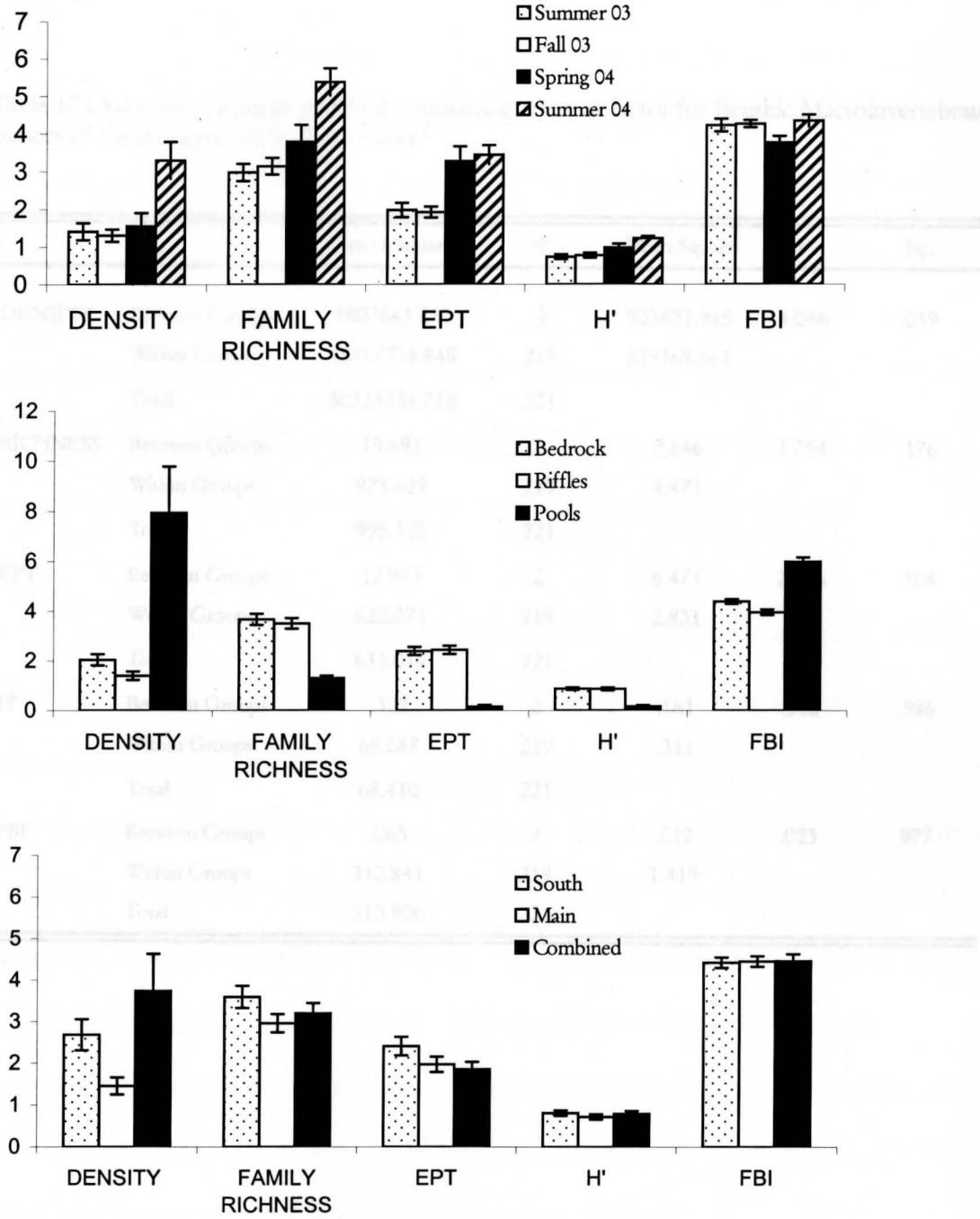

Figure 6 Benthic macroinvertebrate community structure presented as organism density (per 0.01m2), family richness, EPT family richness (Ephemeroptera, Plecoptera, Trichoptera), family diversity (Shannon-Weiner H') and FBI- Hiselhofff Field of Biotic Index. The small areal unit for density allowed all data to be presented on the same y-axes. Data from all replicates averaged (SE) by A) stream reach, B) substratum type, and C) sampling season. Only data from riffles and bedrock were included in panel C because macroinvertebrates were not sampled from pools during 2004.

Table 10 One – way Multiple ANOVA considering reach as factor for Benthic Macroinvertebrate Indices of Cattaraugus Creek, Zoar Valley

		Sum of Squares	df	Mean Square	F	Sig.
DENSITY	Between Groups	1807643.890	2	903821.945	4.046	.019
	Within Groups	48917714.848	219	223368.561		
	Total	50725358.738	221			
RICHNESS	Between Groups	15.691	2	7.846	1.754	.176
	Within Groups	979.629	219	4.473		
	Total	995.320	221			
EPT	Between Groups	12.943	2	6.471	2.286	.104
	Within Groups	620.071	219	2.831		
	Total	633.014	221			
H'	Between Groups	.322	2	.161	.518	.596
	Within Groups	68.088	219	.311		
	Total	68.410	221			
FBI	Between Groups	.065	2	.032	.023	.977
	Within Groups	310.841	219	1.419		
	Total	310.906	221			

The macroinvertebrate indices evaluated did appear to be more influenced by season as shown in Table 11, which suggests some temporal change in benthic macroinvertebrate community structure. The one-way Multiple ANOVA test showed that family richness ($p < 0.01$), EPT richness ($p < 0.01$), Shannon Wiener Index ($p < 0.01$) and Hilsenhoff Index ($p = 0.04$) were significantly different among the sampling seasons. It is important to mention that even though samples were taken during two summers, the test was performed independently because the physical characteristics of the river changed dramatically from one year to the next one. Also skills in the collection of the aquatic organism and its corresponding sorting and identification in the laboratory improved with time.

Benthic community variables also appeared to be strongly influenced by substratum type (Figure 6). Diversity H', EPT richness, and family richness were notably higher in the bedrock and riffle substrata, whereas organism density and Hilsenhoff values were higher in pools. A one-way Multiple ANOVA test (Table 12) was performed considering substratum type as factor. The analysis showed that density of organism ($p < 0.01$), family richness ($p < 0.01$), EPT taxa richness ($p < 0.01$), diversity H' ($p < 0.01$) and Hilsenhoff index ($p < 0.01$) from bedrock and riffle substratum types were significantly different from pools. Expectedly, the multiple comparisons analysis also showed that density ($p = 0.303$), family richness ($p = 0.571$), EPT richness ($p = 0.827$), and Shannon Wiener index H' ($p = 0.915$) in riffles were not statistically different from those on bedrock.

Table 11 One – way Multiple ANOVA considering season as factor for Benthic Macroinvertebrate Indices of Cattaraugus Creek, Zoar Valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
DENSITY	Between Groups	769217.615	3	256405.872	1.119	.342
	Within Groups	49956141.124	218	229156.611		
	Total	50725358.738	221			
RICHNESS	Between Groups	210.210	3	70.070	19.456	.000
	Within Groups	785.109	218	3.601		
	Total	995.320	221			
EPT	Between Groups	142.421	3	47.474	21.096	.000
	Within Groups	490.592	218	2.250		
	Total	633.014	221			
H	Between Groups	11.368	3	3.789	14.482	.000
	Within Groups	57.042	218	.262		
	Total	68.410	221			
FBI	Between Groups	18.069	3	6.023	4.484	.004
	Within Groups	292.837	218	1.343		
	Total	310.906	221			

Table 12 One – way Multiple ANOVA considering substratum type as factor for Benthic Macroinvertebrate Indices of Cattaraugus Creek, Zoar Valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
DENSITY	Between Groups	10755941.977	2	5377970.988	29.467	.000
	Within Groups	39969416.761	219	182508.752		
	Total	50725358.738	221			
RICHNESS	Between Groups	143.905	2	71.952	18.507	.000
	Within Groups	851.415	219	3.888		
	Total	995.320	221			
EPT	Between Groups	140.587	2	70.293	31.262	.000
	Within Groups	492.427	219	2.249		
	Total	633.014	221			
H'	Between Groups	13.743	2	6.871	27.527	.000
	Within Groups	54.668	219	.250		
	Total	68.410	221			
FBI	Between Groups	98.414	2	49.207	50.714	.000
	Within Groups	212.492	219	.970		
	Total	310.906	221			

Discussion

In general water temperature, pH, dissolved oxygen and chlorophyll values from the South and Main Branches and Combined Reach followed a common pattern(Figure 4) showing limited spatial and temporal variability in Cattaraugus Creek during the study.

Variability in dissolved oxygen (DO%) in Cattaraugus Creek suggests there may be some oxygen demand, but not necessarily enough to be considered a stressed stream system. The percentage of dissolved oxygen is a crucial factor of aquatic life. A decreased percentage of oxygen saturation leads to anaerobic conditions. A high percentage of oxygen saturation in the surface layer in summer can suggest increased organic production due to the large quantities of total phosphorus and organic nitrogen in some rivers, caused either by more intensive growth of plankton or by waste water pollution (Stambuk-Giljanovic, 1999). However, that appears not to be the case in Cattaraugus Creek where levels of dissolved oxygen were consistent in the two summers evaluated.

With respect to fecal coliforms and turbidity, the South Branch, Main Branch and Combined Reach all followed a seasonal pattern (Figure 4). This is supported by the association of fecal coliforms and sediments described by Crabill *et al.* (1999), who suggested that disturbance of sediments can serve as a source for fecal matter. In fact, higher values of turbidity and fecal coliforms were observed after storm events in spring season. Flow rates were not measured in the field. However, high values of fecal coliforms also corresponded to high flow rates reported by the USGS (2005). Several fecal coliform counts in Cattaraugus Creek were elevated, but still low compared to some reports from other western New York

streams. For example, Greer *et al.* (2002) recorded occasional extreme fecal coliform counts up to 104,000 per 100 ml in Cazenovia Creek, a Niagara River tributary.

The Principal Component Analysis showed that data points aggregate on the ordination plot much more distinctly by sampling season than by river reach. Data for the Main Branch, which is suspected of being affected by runoff from upstream agricultural use (Hunt *et al.*, 2002), plot farthest out along this apparent turbidity/coliform gradient (Figure 5A). Anthropogenic inputs can also cause decreases in dissolved oxygen levels (Rodriguez-Capitulo *et al.*, 2001), but in Cattaraugus Creek oxygen concentrations generally remained high.

Aggregation of data points by season was not as apparent in Panel 5B of Figure 5 as it was for Principal component 1 and 2 in Panel 5A of Figure 5. This may be because Principal component 3, which represented mainly conductivity, appeared less influential in the ordination of the other six water parameters.

Overall the physico chemical parameters evaluated represent a glimpse of a complex hydrological system which not only follows a spatial and temporal pattern but also interacts with the riparian ecosystem that supports it. Spink *et al.* (1998) suggest that information about floods and nutrient dynamics can provide a clearer picture of the hydrological conditions about a given site. It was found that in Cattaraugus Creek only a long term monitoring is likely to explain such a variation and correlation within parameters. These first-year data probably cannot yet discriminate a temporal trend from inherent year-to-year variability.

Family richness and EPT richness values in Cattaraugus Creek generally corresponded to moderate water quality according to the biological assessment profile from the Quality Assurance Work Plan for Biological Stream Monitoring in New York State (see Greer *et al.*, 2002). Low EPT values in pools may not necessarily indicate low water or sediment quality, but perhaps simply that this metric is not appropriate for soft sediment zones where these insect orders are not usually prevalent. In the fine-sediment-dominated lower reaches of the Buffalo River, NY, chironomid (order Diptera) abundance and/or generic richness reflected temporal patterns in water quality (Diggins and Snyder, 2003) and spatial patterns in sediment contamination (Diggins and Stewart, 1998) – patterns that would not have been revealed by EPT richness, as few if any of the EPT taxa occurred in this river. However, it appears that siltation might be a stressor which is causing such low EPT. Further and more accurate study reporting EPT to genus may raise values of this index.

According to the Hilsenhoff Field Biotic Index the South branch, Main branch and the Combined reach of Cattaraugus Creek were categorized as "good" water quality which also means that there is "*some organic pollution*"(Lander University, 2005) in the creek. In streams with good water quality, both sensitive and tolerant species will be found. Yet, with increased organic pollution (from nutrients found in fertilizers, sewage, and other sources) dissolved oxygen levels within the stream are expected to fluctuate more extremely and fewer pollution sensitive organisms will be found. Applying the Hilsenhoff index to bedrock and riffles substrates showed a "very good" water quality, which also means "*possible slight organic pollution*". The Hilsenhoff index assigned just a "fair" classification for pools substratum, which indicates *fairly significant organic pollution.* Macroinvertebrates that can tolerate lower oxygen levels will become more prevalent. As organic pollution continues to

increase, some pollution tolerant macroinvertebrates will become dominant and will be able to support large populations within the stream. Dissolved oxygen values in water were constant among substratum type; however oxygen availability may decline in sediments.

Biological monitoring studies typically emphasize the importance of spatial differences and tend to neglect temporal scales (Bunn and Davies, 2000). However, seasonal trends for the benthic community of Zoar Valley provide a good example of temporal patterns. Biological processes (reproduction, survival, adaptation, etc.) may be responsible for marked seasonal changes in the dynamics of the stream population, and ultimately in community structure (Bunn and Davies, 2000). The sites studied though, present a narrow range of variation, which means that they support communities with a relatively similar benthic community composition for a given period. Therefore when benthic community structure is grouped only according to seasons, a temporal pattern becomes visible.

However, the results of this study indicate a clear relationship between the benthic macroinvertebrate community and habitat. The metrics analyzed (family richness, FBI, EPT index and diversity) indicated that the substratum types, with its respective abiotic characteristics, seems to be the main factor determining habitats and benthic community structure along the stream. Metrics of river health as mentioned by Usseglio-Polatera and Beisel (2002) are measures of structure and function that can be compared between sites, but more important is their ability to explain geographical variations. However, one must consider that the bottom heterogeneity may influence the biodiversity at each substratum type (Beisel *et al.*, 2000). More heterogeneous substratum type such as riffles and bedrock provide a more diverse environment than homogeneous substrate. In Cattaraugus Creek,

aggregations of very adapted families such as Chironomidae, Elmidae, Tipulidae, Isonychiidae, Perlidae and Tubificidae characterize the soft sediment substratum type. Similar findings regarding the river bottom are explained by Beisel *et al.* (2000) and Rodrigues *et al.* (2001) who found that muddy and sand-silt substrates hold fewer adapted species than heterogeneous substrates such as gravel, cobbles and rocks with more taxonomic groups. In addition, the unstable physico-chemical characteristics of streams (Soininen and Könönen, 2004), which make them unique and complex ecosystems, also influence the benthic community distribution.

Conclusions

This study suggests that water quality in Zoar Valley varies less by river reach than by sampling season. Thus it appears that possible differences in land use between the Main Branch and South Branch watersheds do not necessarily translate into obvious spatial trends in water quality. Benthic community structure within Cattaraugus Creek also did not differ substantially among reaches, but instead reflected natural zonation of the substrata. Stream systems, however, respond to environmental changes at multiple spatial and temporal scales. Therefore, long-term monitoring is needed to fully assess environmental dynamics within Zoar Valley and Cattaraugus Creek, and to implement best management practices for this important natural resource.

Further research in Zoar Valley and Cattaraugus Creek should include nutrient loads (phosphate, nitrate, and ammonia), flow rates, and upstream watershed land use. A more refined statistical approach such as the co-inertia analysis to check for relationships between benthic communities and physico-chemical variables should also be developed.

CHAPTER III

MICROBIAL INDICATORS OF WATER QUALITY

Introduction

Bacterial communities and their interaction with other stream biota have been characterized and reported for disturbed and undisturbed streams. In fact, microbial communities along with other organisms not only support the intricate network of a river ecosystem but also are generally responsive to environmental changes. Microbial assemblages can remove inorganic nutrients, heavy metals, dissolved organic carbon, particulate organic matter and suspended solids from the water column and sediment. As well they can play a key role in supporting food webs and influencing global climate change through their role in methanogenesis (Adamus and Brandt, 1990). In some cases assemblage-level measurements, including CFUs (coliform forming units), appear to be sensitive to environmental stresses; and those changes are appropriate for documenting gross levels of change (Lemke and Leff, 1999) when studying anthropogenically disturbed streams. Thus bacteria are potentially useful indicators of water quality because of their species diversity and ability to rapidly respond to changing environmental condition (Lemke *et al.*, 1996). Community structure and mechanisms that allow individual organisms to survive and actively grow can indicate the degree of pollution in a stream.

There are a number of techniques frequently used as microbiological indicators of water quality. Assessments include heterotrophic plate counts (HPC), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), sulfite-reducing clostridia (SRC), *Pseudomonas aeruginosa*, and *Salmonella spp.* (Howard *et al.*, 2004; Shibata *et al.*, 2004; Crabill *et al.*, 1999,

Mendoza *et al.*, 2004). Generally, bacterial indicators have been limited to the measurement of the abundance of the fecal coliform group, and species tolerance of chemical pollution has rarely been monitored (Lemke *et al.*, 1996).

From microbiological indicators such as sorbitol-fermenting bifidobacteria, bacteriophages of *Bacteriodes fragilis*, *Crystosporidium*, *Coprostanol* and others to complex procedures such as the fluorometric assay of β – D- galactosidase activity (Howard *et al.*, 2004; Shibata *et al.*, 2004; Crabill *et al.*, 1999; Mendoza *et al.*, 2004; Fernandez - Molina *et al.* ,2004) studies try to find the best and less intrusive method for analysis. On the other hand, more conservative assessments consider that classic indirect chemical indicators of fecal contamination along with fecal coliform counts are probably more predictable, useful and flexible to apply (Fernandez-Molina *et al.*, 2004). Possible relationships of human health risk with fecal coliform contamination can be drawn from simple fecal coliform analysis as shown in the study conducted in Vilanos stream, Montefrio (Granada, Spain) (Fernandez-Molina *et al.*, 2004) which determined a possible relationship between fecal counts and presence of hepatitis A virus in water.

Fecal Coliform contamination

The primary source of microbial pollution in agricultural watersheds such as that of Cattaraugus Creek is often fecal matter generated from livestock production. This loading can come both from direct point sources such as feedlots, and from non-point sources such as grazed pastures and rangelands. However, there are also non-agricultural sources of microbial loading in rural areas, including wildlife and failing septic systems (Jamieson *et al.*, 2004). Given these scenarios, pollution assessment for fecal matter is not an easy task. For

instance, waterfowl were significant contributors to fecal pollution, accounting for approximately 67% of the fecal coliform loading in a coastal embayment (Jamieson *et al.*, 2004).

There is also an association between fecal coliforms and transported suspended sediments. Crabill *et al.* (1999) suggest watershed studies should consider the potential pollution risk from sediments because disturbance of these sediments can release reservoirs of fecal matter. Crabill *et al.* (1999) in a study conducted at Oak Creek, Arizona, found that water quality was generally first impaired due to resuspension of bacteria into the water column from polluted sediments after being disturbed by the rolling caused by increased water currents after storm events. Second fecal coliform numbers were correlated with sediments since fecal pollution can be transported in a culturable stage through aquifers.

The inclusion of heterotrophic bacteria is also important because of its high level of agreement with total coliforms and fecal coliforms in the evaluation of water quality of rivers with fecal contamination (Fernandez-Molina *et al.*, 2004).

E. coli as indicator of water quality

Fecal contamination of aquatic environments is a concern nationwide in the U.S. Its association with and negative effects on human population and ecosystems are being studied more often. According to the New York State Department of Health (2005), "water pollution caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens". The presence of these pathogens can be detected by testing for "indicators" of fecal contamination such as total coliforms, fecal coliforms and

Escherichia coli. Total coliforms include bacteria that are found in the soil water that has been influenced by surface water, and in human or animal waste. Fecal coliforms are the group of the total coliforms that are considered to be present specifically in the gut and feces of warm-blooded animals. *E. coli* is a member of the indigenous fecal flora of warm-blooded animals and its presence indicates fecal contaminations and the possible presence of enteric pathogens (APHA, 1999).

Among the classic indicators of fecal pollution, total coliforms and fecal coliforms were recommended for study by the US Environmental Protection Agency in 1976. However, in 1986, the US EPA modified its guidelines to specify the use of *E. coli* as the indicator of choice when monitoring water quality. The reason for this new policy is that research has shown that *E.coli* densities are more strongly correlated with different pathogens that have the potential to infect humans (USEPA, 2005). Because *E. coli* survives in river water for a shorter period than other coliforms (Baudišová, 1997) it is generally not found growing and reproducing in the environment. Its presence is therefore the most immediate indicator of fecal pollution and the possible presence of pathogens (NYSDOH, 2005).

In addition, several studies have shown positive correlations of *E. coli* with turbidity, suspended solids and agricultural run off (Shibata *et al.*, 2004; AB Razak and Christensen, 2001; Maul and Cooper, 2000; Chigbu *et al.*, 2004; Crabill *et al.*, 1999; Arienzo *et al.*, 2000; Field *et al.*, 2003; Baudišová, 1997).

Hunt et al. (2002) suggest there is unnaturally high turbidity in Cattaraugus Creek, with agricultural runoff suspected as the cause. Stream bank erosion and the influences of

agricultural land use upstream have garnered Cattaraugus Creek a classification as "stressed" (NYS DEC, 1996). Additionally, there have been anecdotal reports of whitewater rafters contracting bacterial infections during spring high water flows (J. Broyles, Cattaraugus Creek Watershed Advisory Committee). It is not known, however, if coliform loading to this stream is abnormally high and, if so, what is the source. Therefore, a first preliminary assessment of *E. coli* as indicator of fecal contamination in Cattaraugus Creek was conducted.

Objective

The purpose of this study was to assess *E. coli* as an indicator of water quality in Cattaraugus Creek, Zoar Valley.

Study Area

The study area is described in Chapter I . Location and study areas are shown in Figure 1 and Figure 2 of Chapter I.

Methods

Physico Chemical Parameters

Triplicate water samples were collected at each site (Main branch, South branch and Combined Reach). Samples were taken randomly. 1000-ml Nalgene bottles were previously acid washed and rinsed with distilled water before use. The samples were collected from the water column in midstream as grab samples just below the surface of water. The bottles were stored and transported in ice and analyzed for the following analyses within 48 hours after collection:

- Suspended solids. Suspended solids were determined according to APHA (1999). A well mixed water sample (250 ml) collected for suspended sediment analysis was passed through glass fiber filters. Material retained by the filter was classified as suspended sediment while material passing through the filter was considered dissolved. Filtration occurred within 24 h of collection and no longer than 72 h after collection. Glass fiber filters and the residue retained on the filter were dried to a constant weight at 103 to 105° C. The increase of the weight of the filter represents the total suspended solids expressed in mg/L.

  mg total suspended solids/L = (A-B) *1000 / sample volume in mL (1)

  where:

  A: weight of the filter + dried residue, mg

  B: weight of filter, mg

- Nitrate. Nitrate concentrations were determined by the Cadmium Reduction Method (APHA 1999) over a range 0.01 to 1.0 mg NO_3^- N /L. A UV-Visible Spectophometer (Thermo Spectronic Biomate 5) was used for absorbance readings. Standards curves were drawn to relate the measured quantity (absorbance) to concentration of the sample.

- Phosphorus. Phosphorus was analyzed in its two fractions: Total phosphorus and Dissolved reactive phosphorus. Total phosphorus was analyzed by the Persulfate Digestion Method (APHA 1999) followed by a colorimetric Ascorbic Acid Method (APHA 1999). Samples were analyzed without filtration. Dissolved reactive

phosphorus includes phosphates that respond to the colorimetric test without preliminary hydrolysis or digestion of the sample. For that analysis the Ascorbic Acid colorimetric method (APHA, 1999) was conducted without Persulfate Digestion. Samples were filtered previously by using a glass fiber membrane filter. A UV-Visible Spectophometer (Thermo Spectronic Biomate 5) was used for absorbance readings. Standards curves were drawn to relate absorbance to phosphate concentration of the sample

- Velocity. A FP101-FP201 Global Flow Probe (Global Water) was used to measure current velocity. Triplicate measurements were taken when the weather conditions and accessibility allowed it. Otherwise a single reading was recorded at each branch on every sampling day.

- Temperature (°C), dissolved oxygen (DO mg/L), turbidity (NTU), and chlorophyll (µg/L) were measured in the field using a Multiparameter probe (Model 6600, YSI Yellow Springs, Ohio) as detailed in Chapter II of this thesis.

Biological and Microbial Parameters

- Chlorophyll. Phytoplankton was assessed by looking at chlorophyll *a* concentration and pheophytins. The Spectrophotometric Determination of Chlorophyll (APHA, 1999) was performed using 500 ml water sample. Samples were kept at -4 °C at dark until analysis in the laboratory. Samples were processed within 24 hours. A modified 90% acetone was used (9:1 acetone: distilled water). A UV-Visible Spectophometer (Thermo Spectronic Biomate 5) was used for absorbance readings.

- Fecal Coliform Enumeration. Fecal coliforms were enumerated using the fecal coliform membrane filtration technique (APHA , 1999) as described in Chapter II of this thesis.

- *Escherichia coli* Test. The *E. coli* procedure (APHA, 1999) was used as a confirmatory test after a prior presumptive test for total coliform bacteria such as the membrane filter method describe above. Using a sterile loop, blue colonies resulting from the fecal enumeration method were inoculated in EC-MUG medium which was prepared with sterile water and IDEXX's Colilert 18R reagents. Tubes were incubated at 35 ± 0.5 °C for 24 hours. Test tubes showing a yellow color were positive for fecal coliform and the ones that fluoresced under UV light were positive for *E. coli*. A positive control of a known *E. coli* culture and a negative control of a thermotolerant *Kleibsella pneumoniae* culture were used as controls.

- Heterotrophic Bacteria. The heterotrophic plate count (HPC) is a procedure for estimating the number of live heterotrophic bacteria in water. Water samples (15 ml) were collected in sterile sample tubes and transported to the laboratory. Nutrient agar plates were inoculated with 0.1 ml of water from serial dilutions of triplicates water samples. The spread plate method (APHA, 1999) was used rather than the pour plate method since it causes no heat shock and all colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Heterotrophic CFUs were enumerated after incubating for 48 h at 35 °C.

All materials including sample bottles and instruments were sterilized prior to the analysis to prevent contamination. Blank samples were analyzed in order to confirm that there was no cross contamination.

Data Analysis

The following statistical approaches were performed in order to analyze physico-chemical parameters, biological parameters and microbial indicators.

1. Descriptive statistics (mean and standard deviation) of physico-chemical variables during the late spring / early summer 2004 were considered as representative of the mean per branch of each sampling date.

2. For microbial indicators, a Principal Component Analysis (PCA) was carried out.

3. For microbial indicators of water quality, one - way Multiple ANOVA (Analysis of Variance) test (Type III sum of squares and a significance level of 0.05) and Pearson correlation were performed.

Results

Unfortunately due to water contamination in the laboratory, nitrate and phosphate concentrations measurements were not trust worthy. Consequently, those results are not presented in this study.

Water quality parameters followed a seasonal pattern explained previously in Chapter II. During the sampling period (June 3 to July 7 2004) turbidity mean values (± SD) ranges from 17.2 (±3.6) to 86.6 (±2.0) NTU while suspended solids ranged from 3.2 (±3.0) to 82 (±13.5) mg/L (Figure 7). Concurrently measured trends in heterotrophic bacteria, fecal coliforms and numbers of *E. coli* are presented in Figure 8. HPC mean values (± SD) ranged from 8167 (± 1159) to 133 (±58) CFUs per ml. FC mean values (± SD) ranged from 426 (± 18.6) to 5.0 (7.0) CFUs per 100 ml showing great variability in a short period of time. Typically high FC counts were found after storm events that at the same time correlated with high flows reported by USGS (2005). Numbers of *E. coli* (mean values) ranged from 2 - 156 per 100 ml.

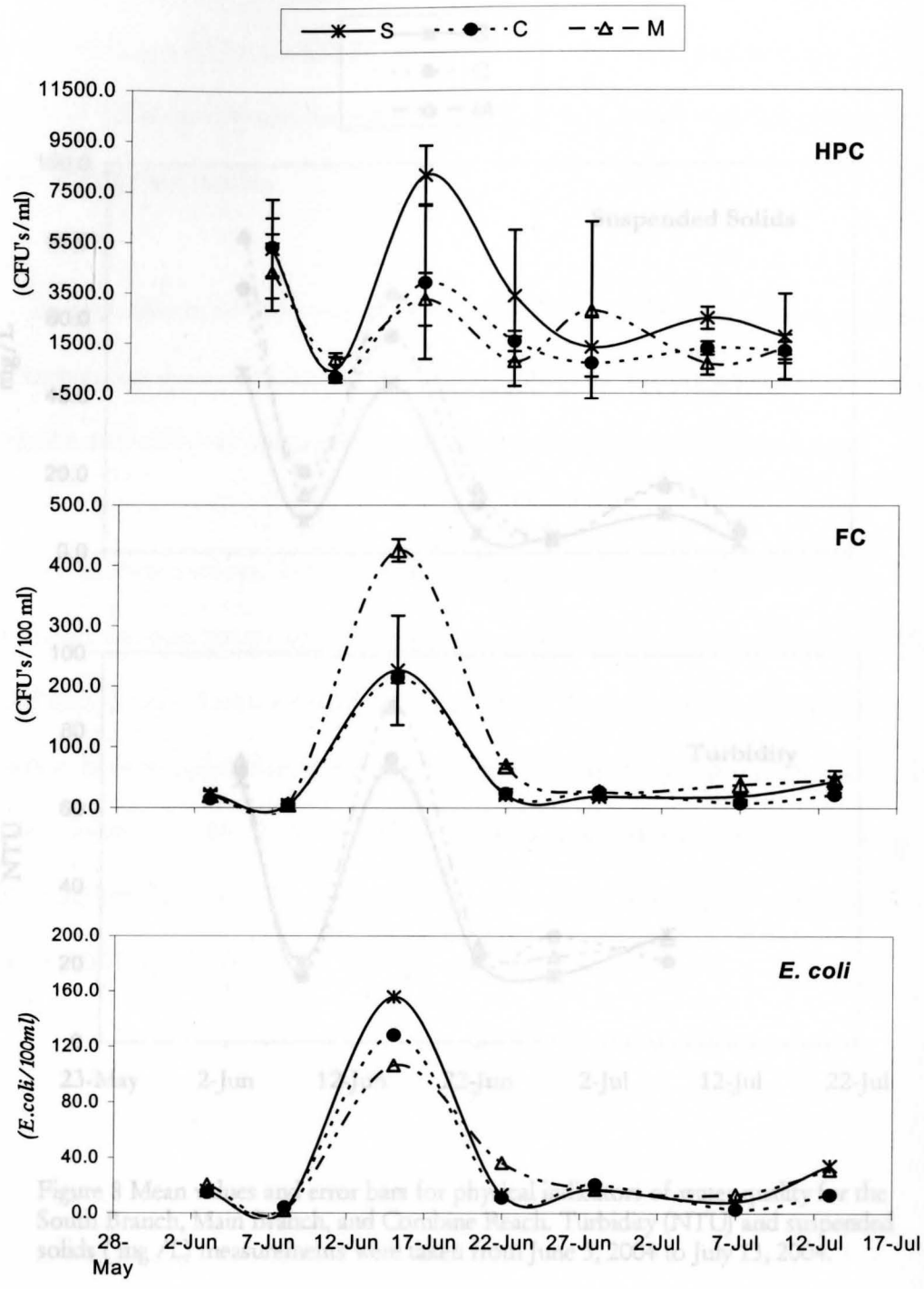

Figure 7 Mean values and error bars of microbial indicators for the South Branch, Main Branch, and Combine Reach. Heterotrophic bacteria (CFUs/ ml), fecal coliforms (CFUs /100 ml), and *E. coli* numbers 100 ml^{-1} measurements were taken from June 3 th, 2004 to July 13th , 2004.

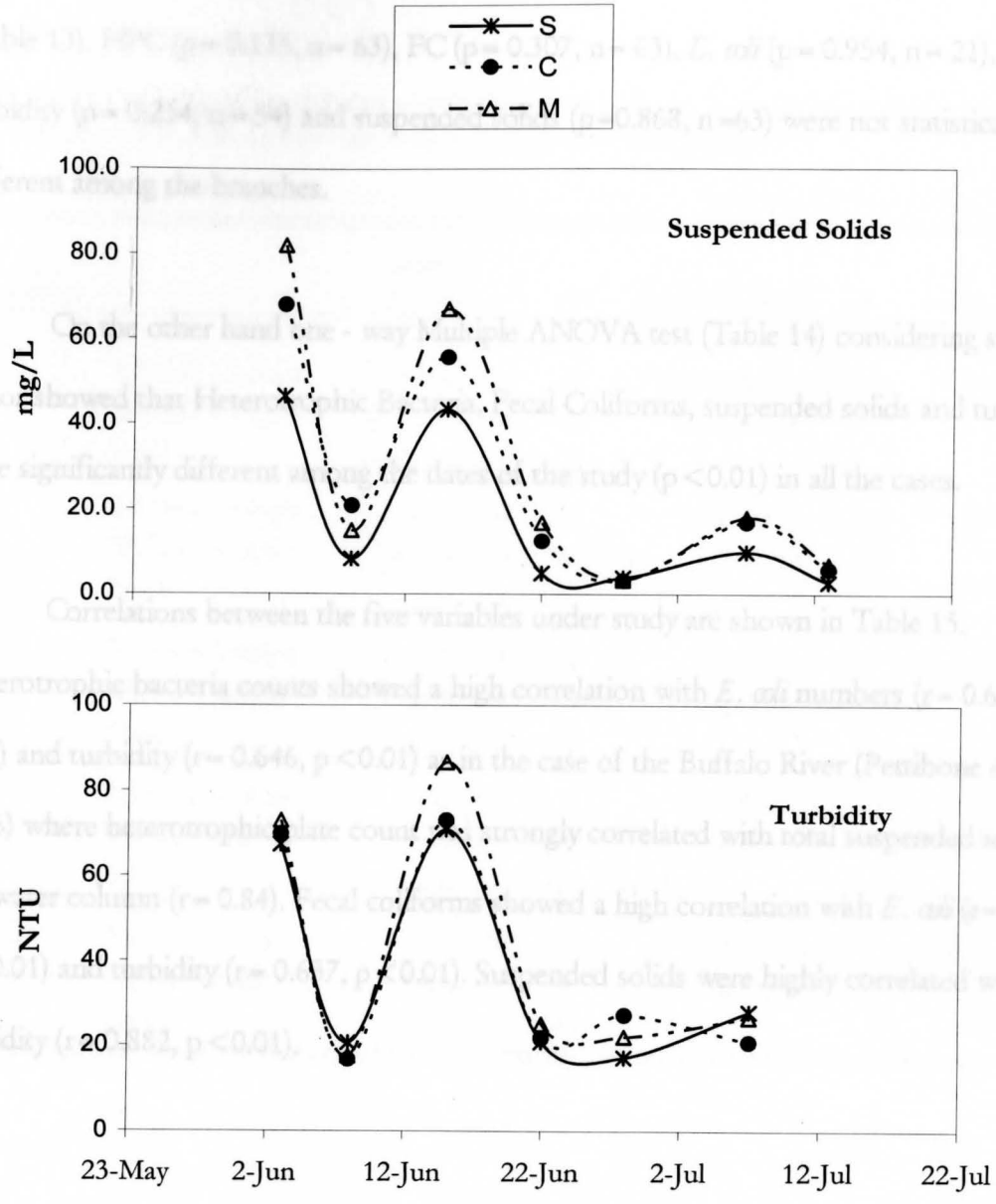

Figure 8 Mean values and error bars for physical indicators of water quality for the South Branch, Main Branch, and Combine Reach. Turbidity (NTU) and suspended solids (mg /L) measurements were taken from June 3, 2004 to July 13, 2004.

Considering reach as a factor a one-way Multiple ANOVA test was performed (Table 13). HPC ($p = 0.135$, $n = 63$), FC ($p = 0.307$, $n = 63$), *E. coli* ($p = 0.954$, $n = 21$), turbidity ($p = 0.254$, $n = 54$) and suspended solids ($p = 0.868$, $n = 63$) were not statistically different among the branches.

On the other hand one - way Multiple ANOVA test (Table 14) considering season as factor showed that Heterotrophic Bacteria, Fecal Coliforms, suspended solids and turbidity were significantly different among the dates of the study ($p < 0.01$) in all the cases.

Correlations between the five variables under study are shown in Table 15. Heterotrophic bacteria counts showed a high correlation with *E. coli* numbers ($r = 0.620$, $p < 0.01$) and turbidity ($r = 0.646$, $p < 0.01$) as in the case of the Buffalo River (Pettibone *et al.* 1996) where heterotrophic plate count was strongly correlated with total suspended solids in the water column ($r = 0.84$). Fecal coliforms showed a high correlation with *E. coli* ($r = 0.776$, $p < 0.01$) and turbidity ($r = 0.637$, $p < 0.01$). Suspended solids were highly correlated with turbidity ($r = 0.882$, $p < 0.01$).

Table 13 One- way Multiple ANOVA considering reach as factor for microbial indicators and suspension-particle indicators in Cattaraugus Creek , Zoar Valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
HPC	Between Groups	22047936.508	2	11023968.254	2.069	.135
	Within Groups	319651428.571	60	5327523.810		
	Total	341699365.079	62			
FC	Between Groups	25344.857	2	12672.429	1.205	.307
	Within Groups	631062.571	60	10517.710		
	Total	656407.429	62			
E. coli	Between Groups	193.011	2	96.505	.047	.954
	Within Groups	36587.468	18	2032.637		
	Total	36780.479	20			
SS	Between Groups	1819.876	2	909.938	1.442	.245
	Within Groups	37868.510	60	631.142		
	Total	39688.386	62			
TURB	Between Groups	193.778	2	96.889	.142	.868
	Within Groups	34844.972	51	683.235		
	Total	35038.750	53			

Table 14 One- way Multiple ANOVA considering season as factor for microbial indicators and suspension-particle indicators for Cattaraugus Creek, Zoar Valley, NY.

Parameter		Sum of Squares	df	Mean Square	F	Sig.
HPC	Between Groups	56459735.450	1	56459735.450	12.074	.001
	Within Groups	285239629.630	61	4676059.502		
	Total	341699365.079	62			
FC	Between Groups	84740.762	1	84740.762	9.042	.004
	Within Groups	571666.667	61	9371.585		
	Total	656407.429	62			
E. coli	Between Groups	5146.852	1	5146.852	3.091	.095
	Within Groups	31633.627	19	1664.928		
	Total	36780.479	20			
SS	Between Groups	20392.350	1	20392.350	64.466	.000
	Within Groups	19296.036	61	316.328		
	Total	39688.386	62			
TURB	Between Groups	13635.963	1	13635.963	33.130	.000
	Within Groups	21402.787	52	411.592		
	Total	35038.750	53			

Table 15 Correlation matrix for microbial indicators (HPC, FC, and *E. coli*) and physical parameters (suspended solids and turbidity) measured in Cattaraugus Creek, Zoar Valley, NY.

Parameter		TURB	HPC	FC	*E. coli*	SS
TURB	Pearson Correlation	1	.646(**)	.637(**)	.636(**)	.882(**)
	Sig. (2-tailed)	.	.000	.000	.005	.000
	N	54	54	54	18	54
HPC	Pearson Correlation	.646(**)	1	.359(**)	.620(**)	.555(**)
	Sig. (2-tailed)	.000	.	.004	.003	.000
	N	54	63	63	21	63
FC	Pearson Correlation	.637(**)	.359(**)	1	.776(**)	.481(**)
	Sig. (2-tailed)	.000	.004	.	.000	.000
	N	54	63	63	21	63
E. coli	Pearson Correlation	.636(**)	.620(**)	.776(**)	1	.438(*)
	Sig. (2-tailed)	.005	.003	.000	.	.047
	N	18	21	21	21	21
SS	Pearson Correlation	.882(**)	.555(**)	.481(**)	.438(*)	1
	Sig. (2-tailed)	.000	.000	.000	.047	.
	N	54	63	63	21	63

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tail

The data reduction analysis extracted one single factor which explained 70.01 % of the variability of the data (Table 16). The component matrix for this analysis is shown in Table 17

This finding showed that the four original variables (HPC, FC, suspended solids and turbidity) can be effectively explained by one single factor that represents the ordination of two highly associated parameters of microbial activity and two parameters that describe particle characteristics. This new factor was named as "microbial-particle" factor. Since the purpose of the study is try to understand how *E. coli* can be used as an indicator of fecal pollution in Cattaraugus Creek, a correlation analysis was performed to assess if the "microbial-particle" factor was correlated with *E. coli* numbers. Regression factors scores resulting from the data reduction analysis were used as values. Interestingly, *E. coli* numbers displayed a high correlation ($r = 0.706$, $p < 0.01$) with "microbial-particle" factor (Figure 9).

Table 16 Total variance for turbidity, suspended solids, heterotrophic bacteria and fecal coliforms Data reduction by Principal Component Analysis displayed one singlefactor which explained 70 % of the data variability.

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.801	70.013	70.013	2.801	70.013	70.013
2	.655	16.369	86.381			
3	.460	11.507	97.889			
4	.084	2.111	100.000			

Table 17 Component matrix of the variables studied heterotrophic bacteria, fecal coliforms, suspended solids and turbidity generated by Principal Component Analysis.

	Component 1
HPC	.753
FC	.722
SS	.889
TURB	.961

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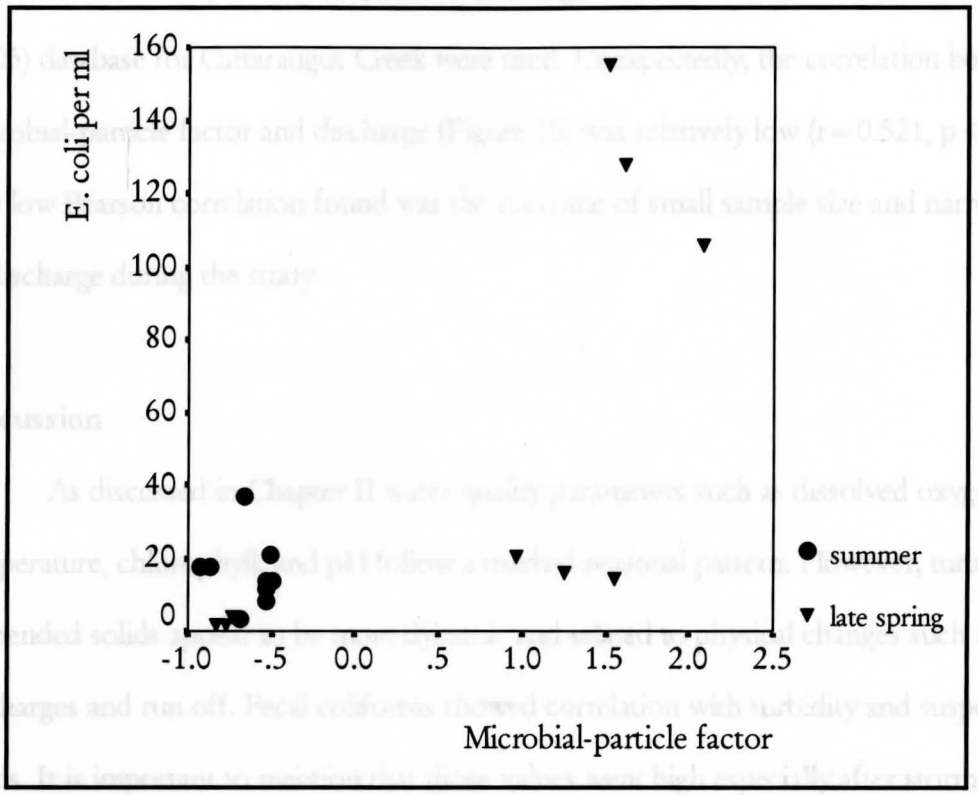

Figure 9 Correlation between "microbial-particle factor" and *E. coli* numbers per ml. Pearson correlation r = 0.71 indicates a high association. Clustering in data by season is apparent.

There is also a physical factor that has to be considered in the analysis: Flow rates. Velocity values were recorded for spring and summer 2004. However, discharge values were not measured directly in the field; instead discharge rates in m^3/s taken from the USGS (2005) database for Cattaraugus Creek were used. Unexpectedly, the correlation between the microbial-particle factor and discharge (Figure 10) was relatively low ($r = 0.521$, $p < 0.01$). The low Pearson correlation found was the outcome of small sample size and narrow range of discharge during the study.

Discussion

As discussed in Chapter II water quality parameters such as dissolved oxygen, water temperature, chlorophyll, and pH follow a marked seasonal pattern. However, turbidity and suspended solids appear to be more dynamic and related to physical changes such as higher discharges and run off. Fecal coliforms showed correlation with turbidity and suspended solids. It is important to mention that those values were high especially after storm events. Since the majority of enteric bacteria in aquatic systems are associated with sediments and these associations influence their survival and transport characteristics (Jamieson *et al.*, 2004) it was expected that high loading of sediment carried along the stream would cause adsorption of bacteria resulting consequently in more CFUs. Naturally, bacteria and sediment surfaces are negatively charged and repel each other. However, when high electrolyte concentration is present, repulsion is suppressed and by extra cellular mechanism (Jamieson *et al.*, 2004) bacteria adheres to surfaces. Future experiments should be conducted in order to analyze sediment-microorganisms associations.

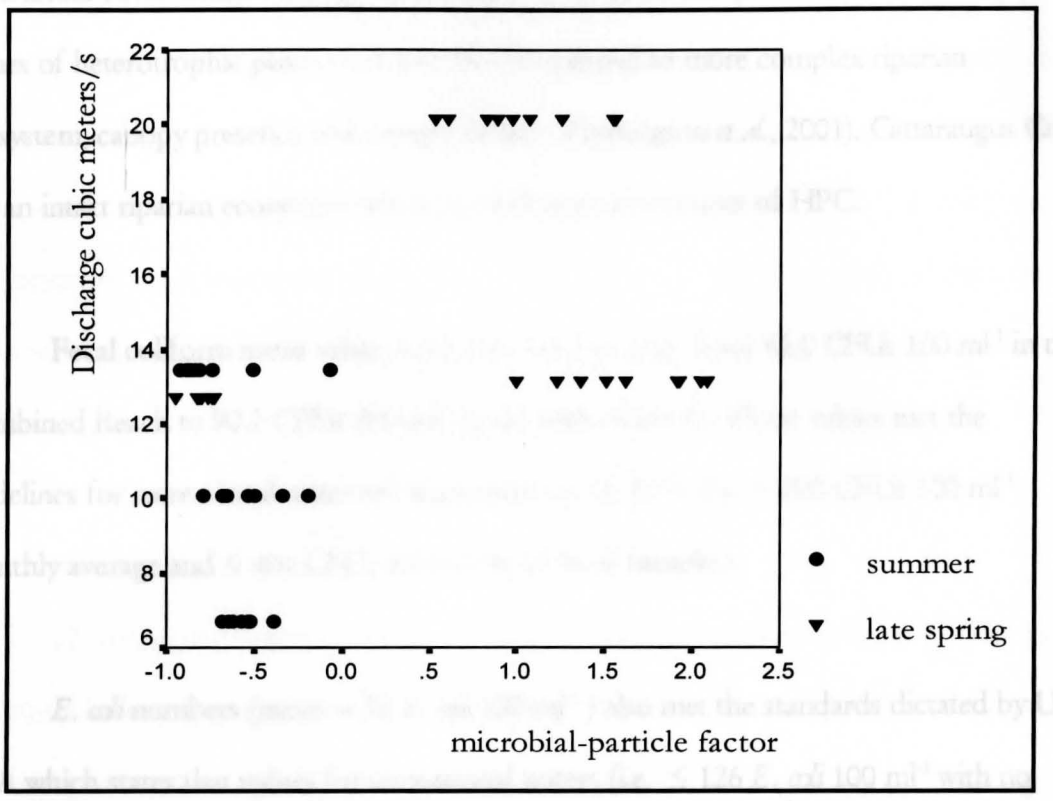

Figure 10 Simple scatter plot for "microbial -particle" factor and discharge in m^3/s. Clustering of data by season is apparent. Pearson correlation r = 0.521 indicates a relatively weak association.

Heterotrophic bacteria on average exhibited low values (from 2019 CFUs ml^{-1} in the Combined reach to 3262 CFUs ml^{-1} in the South Branch) compared to values observed at the Buffalo River which were approximately 37,000 CFUs ml^{-1} (Pettibone *et al.*, 1996). Low values of heterotrophic plate count bacteria correspond to more complex riparian ecosystem, canopy presence and canopy density (Pennington *et al.*, 2001). Cattaraugus Creek has an intact riparian ecosystem which can influence low counts of HPC.

Fecal coliform mean values were also low (ranging from 45.0 CFUs 100 ml^{-1} in the Combined Reach to 90.2 CFUs 100 ml^{-1} in the Main Branch). These values met the guidelines for recreational water recommended by US EPA (i.e. $\leq$ 200 CFUs 100 ml^{-1} monthly average and $\leq$ 400 CFUs 100 ml^{-1} in 10 % of samples).

E. coli numbers (mean = 32 *E. coli* 100 ml^{-1}) also met the standards dictated by US EPA which states that values for recreational waters (i.e. $\leq$ 126 *E. coli* 100 ml^{-1} with no sample exceeding 235 *E. coli.* 100 ml^{-1}).

Surprisingly, total suspended solids (24.3 mg^{-1}) appeared to be high. Pettibone, (1996) found that total suspended solids concentrations in the west bank of the Buffalo River after the effects of a ship passage were in average 20.8 mg^{-1}. In Cattaraugus Creek it can be speculated that agricultural run off may be causing these high values. After storm events, sediments are removed and carried along the stream, especially when high rates of discharge occur. While discharge increases dissolved solids decreases and suspended solids increases (Razak and Christensen, 2001).

The new "microbial-particle" factor also showed correlation with discharge rates ($r=0.521$, $p<0.01$). While significant, the only moderately strong coefficient may be explained by the narrow range of discharge values along the sampling dates- discharges values fluctuated between 6.68 m^3 /s and 20.12 m^3/s. An extensive sampling regarding water levels may bring a clearer explanation of the behavior of *E. coli* and the microbial-particle factor under different conditions of flow. However, since accessibility and feasibility were key factors in the development of this study, discharges correspond to periods when the creek was accessible and easy to cross (0.51 to 0.74 m USGS, 2005). Consequently, no fundamental conclusions could be drawn from this preliminary assessment.

Conclusions

Heterotrophic bacteria, fecal coliforms, turbidity, and suspended solids can be explained by a single factor denominated "microbial-particle" factor, which at the same time can be a predictor of *E. coli*. All of the microbial indicators evaluated were highly correlated with suspended solids.

Fecal coliforms and *E. coli* numbers in Cattaraugus Creek met the US EPA standards dictated for recreational purposes. However samples were not taken during high flows which would increase those values causing possible harmful health effects. Further research, then should include data collection when high discharges occur in order to have a better understanding of possible mechanisms of bacterial indicators (*E. coli* and Fecal coliforms) and run off events.

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