Effectiveness of Macroinvertebrate-based Biotic Indexes in Assessing Steam Water Quality in Sycamore Creek, IN

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Introduction

Stream water quality is affected by changes in the stream's watershed e.g. agriculture, clear-cutting, destruction of wetlands, and urbanization. These developments cause changes in water chemistry, biota, and/or physical characteristics of the stream (Allan 1995). To determine the influence of these changes on a stream's water quality frequent and constant monitoring is necessary (Barbour et al. 1999). There are many programs available to monitor stream water quality. The best methods combine different types of monitoring programs to reflect all aspects of the stream.

One group of monitoring programs are the Rapid Bioassessment Protocols (RBPs). RBPs use a biological indicator to infer data about stream water quality. RBPs were introduced on a national level in the mid to late 1980s (Barbour et al. 1999). There are three main types of RBPs for streams—fish surveys, periphyton surveys, and macroinvertebrate surveys. Methods for these surveys are outlined in the Environmental Protection Agency's (EPA) *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition.* The macroinvertebrate survey is most commonly used because it requires little expertise or equipment (Barbour et al. 1999).

Currently the EPA encourages the use of RBPs because they provide quick and valid results while being cost effective, time efficient, and minimally invasive (Barbour et al. 1999). Since organisms collected for RBPs spend much of their life cycle in streams, RBPs are less influenced by daily changes and more reflective of overall stream water quality (Barbour et al. 1999). Compared to other monitoring methods, RBPs are more likely to detect significant changes in water quality based on changes in the biota, avoiding the ambiguity of daily and seasonal changes in temperature and chemical reactions (Nerbonne and VonDracek 2001).

Typically, the results of a macroinvertebrate survey are interpreted using several indexes. Macroinvertebrates indexes often describe a taxon in terms of its tolerance of environmental conditions. Tolerance or intolerance is defined as the ability of an organism to withstand pollution stressors; it is generally expressed as a numerical value in an index (Cook 1976). The type of gills present in the taxon, the taxon's biological oxygen demand (BOD), and the taxon's resilience to disturbance, all affect the tolerance value (Cook 1976).

The most common indexes used to evaluate stream water quality are the Hilsenhoff Family Biotic Index (HFBI) and the Invertebrate Community Index (ICI). For each taxon (usually family) a tolerance value is assigned. Using the following equation:

HBI=$\frac{n_i \, a_i}{N}$ Equation (1)

Where

n is the number of individuals of the i^{th} taxon;

~~a~~" is the index (tolerance) value of that taxon

N is the total number of individuals in the sample assigned an index value

a HFBI score is calculated (Hilsenhoff 1988). Scores are split into 5 categories ranging from no impairment to severely impaired (Hilsenhoff 1988). In the HFBI an increasingly high score indicates lower water quality. The index values (a_i) used in Equation (1) are related to how well the family can tolerate organic pollutants, increased nutrient and sediment loads and dissolved oxygen (DO) limitations (Mandaville 2002). Since HFBI uses both richness and abundance in its analysis, it is weighted toward the most abundant taxa. An abundance of one highly tolerant taxon is as influential to the score as a small number of organisms in multiple tolerant taxa. The most important assumption in the HFBI is that all samples have over 100 organisms (Hilsenhoff 1988). Recent evidence shows only 20 organisms are necessary for accurate results (Mandaville

2002). The HFBI also assumes that the collection method is a multi-habitat approach in a wadeable stream. The HFBI consistently underestimates pollution in severely polluted streams, but accurately identifies low to moderate pollution. Finally, HFBI does not always recognize the effects of pollution due to heavy metals, because this may result in mutation of organisms rather than death (Hilsenhoff 1988).

The ICI, developed by the Ohio EPA (OEPA), is another type of RBP. The ICI uses a multimetric approach, rather than a ratio calculation. Ten metrics are summed together including the % intolerant taxa, % tolerant taxa, different forms of richness and abundance, drainage area, and type of waterbody to determine the water quality of the stream (OEPA 1987). In each metric the site is given a score between 0 and 6, where 6 implies the highest water quality. Total scores are categorized as acceptable (not significantly impaired), somewhat acceptable (some impairment), and unacceptable (impaired). As well the top 10% of stream are categorized as exceptional (OEPA 1987). Score ranges are determined by geographical region to accommodate for differences in topography and climate. Scores ranges were developed based on reference sites in Ohio. In SW Ohio (the most similar area in climate and topography to central Indiana) OEPA considers a small wadeable stream acceptable if the score is above 30, and exceptional if the score is above 46. The ICI is applicable for that samples were collected in the summer using a Hester-Dendy sampler in riffles or shallow runs in headwaters, wadeable, or non-wadeable stream. The watershed must be larger than 10 km^2 to use the ICI (OEPA 1987).

Other metrics can be used to help categorize the composition of the sample site. A site with a higher percentage of Ephemeroptera, Plecoptera, and Trichoptera (% EPT) and low percentage Chironomidae (% Chironomidae) is highly correlated with high quality water (Barbour et al. 1999).

When using RBPs to assess water quality, multiple indexes must be used to quantify the stream water quality. Each index responds strongly to a different characteristic of water quality. The minimal recommended number of indexes is four, but more indexes give higher accuracy. Multiple indexes allows for an outlier to be discounted in the conclusions (Barbour et al. 1999).

One of the disadvantages of using RBPs is that they can be affected by other factors besides water quality. Competition theory, niche theory, and disturbance theory are still play large roles in streams, although it is difficult to quantitatively measure their effect on taxa's populations. Classifying macroinvertebrates by their guilds, such as feeding or habitat, can help one infer information about interspecies competition for food and niches (Barbour et al. 1999). Disturbance theory, as applied in lotic systems, implies that constant disturbances (natural and anthropogenic) including but not limited to flooding, erosion, severe precipitation events, snow melt, tree falls etc has an effect on the biota present in the stream (Ward 1998). A survey of macroinvertebrate assemblages in streams of different disturbance level by McCabe and Gotelli showed that Chironomidae was unaffected by disturbance, however other taxa, especially EPT taxa were severely affected by disturbance in terms of abundance, but not in terms richness (2000). The best method to measure disturbance in an area is field notes and observations unless the study is manipulative.

Sometimes to ensure significance of a difference in results, the composition of macroinvertebrates at two sites is compared using a similarity index. The advantage of a similarity index is that it can be evaluated for statistical significance. The simplest similarity index available is a presence/absence test, usually Sørensen index. It is calculated by:

$$S=\left(\frac{2C}{A+B}\right)*100 \qquad \text{Equation (2)}$$

Where

A is the number of taxa unique to site 1

B is the number of taxa unique to site 2

C is the number of taxa shared by sites 1 and 2

The Sørensen index shows the percentage of overlap in the taxa present between two sites (Wolda 1981). The percentage is significant regardless of the value. The Bray-Curtis Similarity Index (also known as the Czekanowski's Quantitative Index) gives a quantitative measurement of the similarity of two sites, using abundance as well as presence/absence (Bloom 1981). It is calculated by:

$$S=\frac{\sum|n_{1i}-n_{2i}|}{\sum|n_{1i}+n_{2i}|} \qquad \text{Equation (3)}$$

Where

n_{1i} is the number of occurrences of taxa i at site 1

n_{2i} is the number of occurrences of taxa i at site 2

The Bray-Curtis Similarity Index can be tested for significance using a Pearson Product-Moment Correlation Coefficient (Bloom 1981). A correlation test (r) between two sites is also used to determine similarity between two sites: a Pearson Product-Moment Correlation Coefficient can be used to determine the correlations significance (Bloom 1981). All three methods of measuring similarity are acceptable; depending on what statistic is desired and what type of data is given (Wolda 1981).

Stream water quality is also related to the substrate and available habitat, which can be quantified through macroinvertebrates habitat guild. There are 8 habitat guilds: skaters, planktonic, divers, swimmers, clingers, sprawlers, climbers, and burrowers. The most commonly found guilds in wadeable streams are the clingers, sprawlers, swimmers, and burrowers (Brigham et al. 1985). Clingers prefer to attach to a surface, while sprawlers prefer to stay near rooted plants. Swimmers prefer the open water, and burrowers prefer to live in the sediment (Meixer and Bain, 1999). The percentage of each guild present in the stream is not directly correlated to the overall water quality of the stream, but it does characterize differences between streams in terms of available habitat and geomorphology of the stream (Brigham et al. 1985).

Although macroinvertebrate sampling is one of the most common ways to monitor streams and determine stream water quality, it is not the only way. Chemical monitoring is, historically, one of the oldest ways to monitor stream water quality (Barbour et al. 1999). The chemical compounds most often monitored when assessing stream water quality are: DO, Dissolved Organic Carbon (DOC), pH, alkalinity, NH_4^+, NO_3^-, Total Dissolved Nitrogen (TDN), and Soluble Reactive Phosphorous (SRP) (Allan 1995). These analytes are often chosen to monitor stream water quality because they control the biota and the chemical processes within the stream. DOC, alkalinity (primarily HCO_3^-), nitrogen (NH_4, NO_3,,and TDN) and SRP play a large role in determining the biota present in the stream (Wetzel 2001).DO and DOC are required for respiration and photosynthesis, a control for biota (Allan 1995). pH (the activity of free hydrogen ions) effects the solubility of nutrients via le Chatelier's principle and all acid/base reactions within the stream (Wetzel 2001). For all of these analytes, the EPA has constructed a 5-category guideline system to categorize stream water quality based on the analytes' concentration or result.

Conductivity is also often measured in a stream study because it tells about the ionic nature of the stream. Since ions play an important role in chemical reactions, this may also help characterize a stream (Wetzel 2001).

Chemical data can help characterize a stream by describing it in terms of concentrations and activities. However, chemical data is highly affected by precipitation events because these event change concentration. Changes in temperature also affect chemical data because temperature affects reaction rates (Barbour et al. 1999). Chemical data is often called a snapshot analysis, because it only reflects conditions on the sampling date (Barbour et al. 1999). Data taken after a precipitation event or during melting will not accurately reflect the stream under standard conditions. As well, lingering ice and snow, or high humidity can lower accuracy on instruments (Allan 1995). The sensitivity of the collection means that there may be a high signal to noise ratio associated with this data.

Physical steam attributes are included in many methodologies, because they provide useful information. Physical attributes of a stream are the abiotic, non-chemical factors, including turbidity (the clarity of the water), geomorphology, surrounding watershed practices, geographic location, climate, discharge, substrate size and stability, and water velocity. All of these components can be observed or calculated in the field; however many have more precise methodologies in a laboratory. When stream water quality is the primary goal of the study, topography, climate, and watershed practices are important to explaining the chemical and biological observations (Barbour et al. 1999). Stream bottom morphology and water velocity are well correlated to the biota present in the stream (Brigham et al. 1985). Although physical data is useful, there is no index that uses only physical characteristics to predict the water quality of a stream. As such, physical monitoring gives a context for biological and chemical data, but it is not a predicative measurement.

Goals of Study

This study investigates two sites along the same stream with different macroinvertebrate assemblages but similar water chemistry and physical characteristics. Chemical factors, physical attributes, and ecological observations are analyzed to determine their role in causing these differences. This type of study leads to better understanding of the effectiveness of macroinvertebrate indexes and the usefulness of RBPs in a scientific investigation. It provides insight into how biota, water chemistry, and physical attributes affect each other and ecology's role in monitoring streams using biological indicators.

Methods

Study Site

This study was conducted along Sycamore Creek, a perennial, second-order stream in Bradford Woods, central Indiana (Figure 1). The stream runs north-south. The watershed is 34.02 km^2. Oak hickory forests cover more than 45% of the land in the watershed and agriculture covers 40% of the upper reaches of the watershed.

Topography in the area includes moderate to steep slopes with shale bluffs. During low flow, the majority of the water in the stream comes from seeps along the stream. Sycamore Creek also exhibited flashy hydrology, herein defined as a rapid pulse in discharge after a precipitation or melting event. During a flash, the majority of the water in the stream is believed to come from surface runoff based on observations

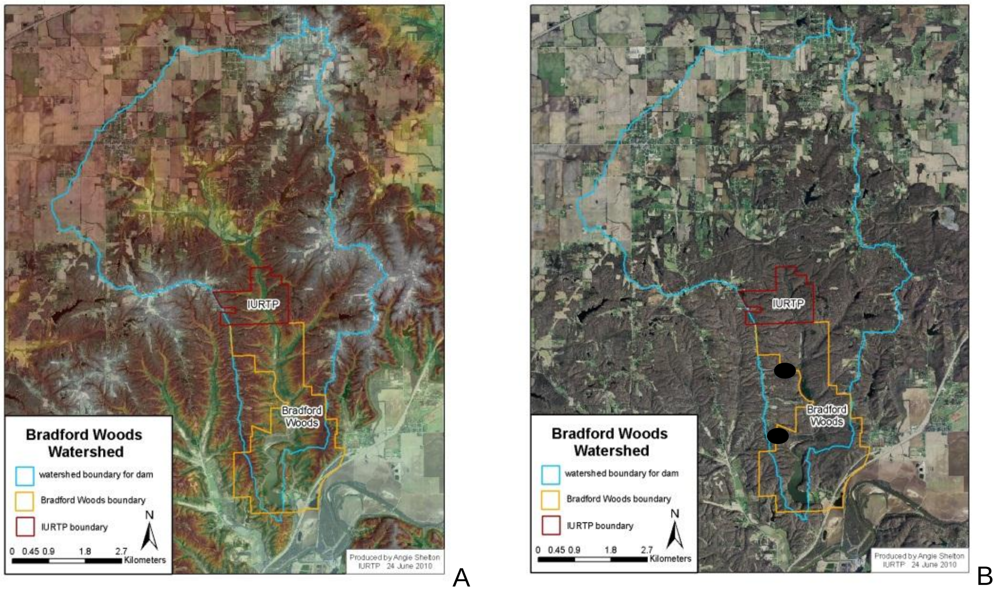

Figure 1: Watershed Maps of Sycamore Creek. These maps show the topography (A) and land used (B) within the watershed. The watershed is 34.02 Km^2 and 45% of the land is covered by oak hickory forest. 40% of the land (mostly the upper regions) is agriculture. The sites are marked with black dots. The top dot is SC, bottom is BR. Maps were prepared using GIS software by CRES.

Data were collected at two sites, Scout Camp (SC) and Bridge (BR) marked in Figure 1. SC is 1.5 km N of BR. At each site a 100 m reach was marked for sampling. Both reaches were at least 30 m from the nearest road or bridge.

SC was surrounded by 20 m cliffs and 5 m soil banks. Woody vegetation and sedges cover both banks. The stream flow is generally channelized with little meandering. The streambed is wider than deep. There are often gravel bars in the stream; however these were covered by water when the stream flashed. There is an island with a large downed tree whose branches stretched into the stream and created riffles and snags.

BR had 20 m cliffs on one side of the stream. On the other side the bank is slightly elevated from the stream. Woody vegetation grows on both sides, but grasses are dominant closer to the bank. There is a high amount of meandering in the channel of the stream. The stream flow has a strong riffle pool pattern. Through the year, four medium-to-large trees fell into the stream, and created snag habitats.

Routine Stream Sampling

Bi-weekly sampling occurred from January 2010- March 2011. A Hach Conductivity Meter (model 44600) was used to sample temperature (˚C) and conductivity (μS) in field. At each site a 50 ml sample was collected at the thalweg between a riffle and a pool in a dark Nalgene bottle for TP and DOC analysis. This process was repeated with a clear 50 ml bottle for NH_4 and NO_3, to which 2 drops of 0.1 N HCL were added. Additionally, a 100 ml sample of water was collected in a dark Nalgene bottle for alkalinity. Samples were stored on ice during transportation. Additionally 2 DO measurements were taken in February and March using an YSI 85 DO Meter in field.

In the laboratory, alkalinity was measured by titration until the sample reached a pH of 4.5 using an OakIon pH meter. Results were expressed as mg/L $CaCO_3$, pH was measured using the same meter. Turbidity was measured using a Hach turbidity meter (model 2100P) with 3 replicates using a detection limit of 0.001 NTU.

Water samples for phosphorous were filtered using Millipore Membrane Filters 0 .45μm HA and water samples for NH_4 and NO_3 were filtered using GF/F 25 mm filters by Whatman. Samples were frozen until they could be analyzed. SRP, NH_4, and NO_3 were analyzed on a Lachat flow injection analyzer. DOC and TDN were analyzed using Shimadzu TOC analyzer. NH_4-N was analyzed using a detection limit 0.01 mg/L. NO_3 –N was analyzed using a detection limit of 0.001 mg/L. A detection limit of 0.001 mg/L was for TDN and DOC. SRP was analyzed using a detection limit of 5 μg/L. DO had a detection limit of 0.5 mg/L.

Macroinvertebrate Collection Method

SC and BR were sampled 6 times between January - March 2011 for macroinvertebrates. Methods were adapted from *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish - Second Edition* (Barbour et al 1999). Samples were collected using a D-frame kick-net with a 5-jab method per site.

Macroinvertebrates were picked in the field for 20 minutes using a tweezers and preserved in 80% Ethyl Alcohol. In the laboratory, macroinvertebrates were identified to lowest order possible using a dissecting microscope. All insects were identified to family using a dichotomous key from Peckarsky et al. (1990).

Stream Bottom and Habitat Type

Data were collected on March 24, 2011. Stream bottom type was categorized as bedrock, sand, small rock, or large rock every 5 m. At these intervals habitat type was also categorized as downed trees, riffle, pool, or open water. Stream bottom and habitat type were collected as percentages based on observation. Results were graphed as percentages of the whole.

Statistical Analysis

Analyte data was analyzed for significance with either a Student's T-test or a paired T-test (Temperature, SpC, pH only). Significance was determined if $p < 0.05$. Any analyte concentration below significance level was not used in the analysis.

Results

Macroinvertebrate Counts

Six macroinvertebrate samples were taken at each site over the collection period. Appendix 1 shows the abundance of each family collected at each site. The most commonly collected families were Chironomidae (Order Diptera: tolerant), Ephemerillidae (Order

Ephemeroptera: Intolerant), Hydropsychidae (Order Trichoptera: Intolerant), and Perlodidae (Order Plecoptera: Intolerant). A total of 18 taxa were captured at both SC and BR. Total organism counts at SC were 464 and at BR total organism counts were 373. Average number collected per sample was 67 organisms.

Macroinvertebrates Indexes and Similarity

Data from the macroinvertebrate study were applied to in four indexes: HFBI, ICI, % EPT taxa and % Chironomidae (Table 1). Data were averaged for each sample independently. Interpretations of indexes were based on details in Barbour et al. (1999) (HFBI, % EPT, % Chironomidae) and OPEA 1987b (ICI).

The HFBI showed that SC had no organic pollution with a score of 3.06. BR had a score of 4.78, implying a slight to moderate organic pollution. The ICI for SC was 36, which is higher than BR at 23. SC was above the acceptable range, and BR was below the acceptable range, implying that BR has been moderately impacted by pollution. The % EPT was higher at SC than Br, which indicates higher water quality at SC. The % Chironomidae was higher at BR, which indicates lower water quality at BR. Since the data at each site gave consistent interpretations, the difference in water quality is significant (Barbour et al. 1999). It is likely that SC has higher

Table 1: Macroinvertebrate index results. All results for SC imply low amounts of pollutants in the stream. At BR all results indicate that there is some organic pollution affect the stream. The results are consistent for each site, but different between the sites.

Index		SC	BR
HFBI	**Value**	3.09	4.78
	Interpretation	Organic Pollution unlikely	Some Organic Pollution present
ICI	**Value**	36±4.6	23±2.1
	Interpretation	Above Acceptable level for stream. Indicates little to no pollution present	Below Acceptable level for stream Indicates some pollution present
%EPT	**Value**	77.32	16.73
	Interpretation	High quality water	Low quality water
%Chironomidae	**Value**	15.04	68.34
	Interpretation	High Quality water	Low Quality water

water quality than BR based only on the RBPs indexes.

Figure 2 shows a distribution of the percent of families found within each sample. Figure 2 shows that while both sites had similar families present, SC was not dominated by any particular family and its top four families (Hydropsychidae, Chironomidae, Perlodidae, and Ephemerillidae) were relatively even, between 20 % - 30%. Chironomidae (63%) followed by Perlodidae (30%) were the dominant taxa at BR.

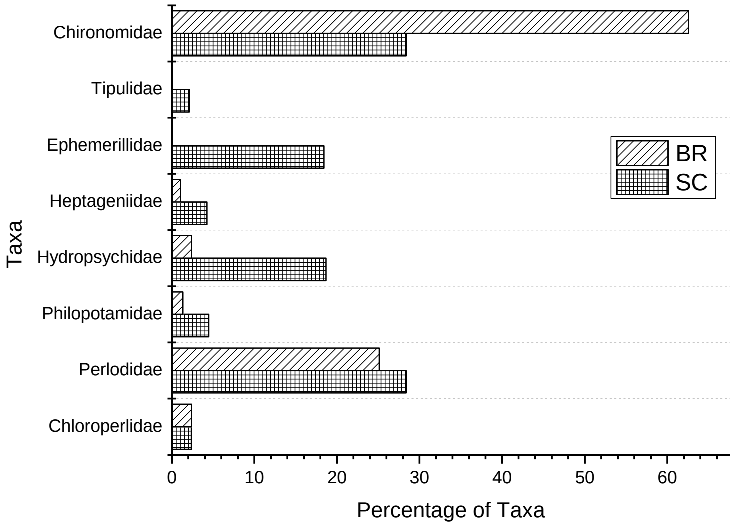

Figure 2: The percentage of taxa in the sample (for taxa that represented over 1% of the sample). This figure shows that in comparison to other taxa at BR Chironomidae is dominant (68%). At SC, Chironomidae, Perodidae Ephemerillidae and Hydropschidade dominate almost evenly.

Similarity from macroinvertebrates was also calculated using the Sørensen index (Equation 2). A similarity of 68% was found between the taxa at SC and BR. A Bray-Curtis Similarity index (Equation 3) showed that the data were significantly different ($p < 0.05$). A correlation coefficient also showed that the two data sets were significantly different ($p < 0.001$).

Table 2 shows the habitat guilds of the macroinvertebrates found based on the taxa presence; not the abundance. Table 2 also shows the guilds habitat presence. SC has four habitat guilds present, and it was dominated by sprawlers. BR has three habitat guilds present, and it was dominated by crawlers.

Table 2: Habitat Guilds Present at SC and BR, percentage of the total sample and their preferred habitats. SC has the largest percentage of sprawlers and clingers. BR has the largest percentage of clingers and sprawlers.

Site	Guild	Sum	Percentage	Preferred habitat
SC	Burrower	9	1.92	Sandy, silty exposed bottoms
	Sprawler	246	52.45	Snags
	Clinger	132	28.14	Riffle areas
	Swimmers	3	0.64	Open Water
BR	Guild	Sum	Percentage	Preferred habitat
	Burrower	3	0.80	Sandy, silty exposed bottoms
	Sprawler	126	33.78	Snags
	Clinger	244	65.42	Riffle areas

Table 3: Analytes measured in laboratory. Average or median (pH) is shows, along with the sample number and the significance. Samples that were not above the detection limit were not included in the average or the t-test. NH4-N and Alkalinity were not included because of low sample numbers. Bolded font implies significant difference

Analyte	SC	BR	n (SC)	n (BR)
NO3 -N (mg/L)	1.444±0.802	1.059±0.816	30	30
TDN(mg/L)	1.089 ±0.383	0.846±0.467	24	22
SRP (µg/L)	10±4	8± 4	16	19
DOC(mg/L)	**2.487±0.639**	**1.809±0.397**	**24**	**24**
DO (mg/L)	12	12	2	2
Conductivity (µS)	509 ±136	477 ±110	28	28
pH	**8.36**	**8.2**	**22**	**22**
Temp. (°C)	11.81± 8.50	11.50 ± 8.00	28	28
Turbidity (NTU)	1.83±.87	2.46 ±1.16	26	26

Chemical Data

Nutrient data for NH_4-N, NO_3-N, SRP, TDN, and DOC, were analyzed in the laboratory from the frozen samples. Table 3 shows the average concentration and standard deviation, the number of samples and the significance (EPA 2000). The concentration of NH_4-N was rarely above the detection limit and therefore it was not included. The average concentrations of NO_3 –

N at SC (1.44±0.802 mg/L) and BR (1.06±0.816 mg/L) were not significantly different ($p > 0.05$). Both sites were near the 50th percentile determined by the EPA (2000). TDN showed similar results. At SC the average was 1.089±0.383 mg/L, and at BR the average was 0.846±0.467 mg/L, but the difference was not significant ($p > 0.05$). Both sites were slightly below the 50th percentile. SRP averaged 10±4 µg/L at SC and 8±4 µg/L ($p > 0.05$). The EPA rating for SRP was in the 10th percentile. DOC was significantly different between the two sites with SC averaging 2.487±0.639 mg/L-C and BR averaging 1.809 ± 0.397 mg/L-C ($p < 0.05$), however both sites were above the 10th percentile according to the EPA. pH was also significantly different. SC had a median pH of 8.36 while BR had a median pH of 8.20. This was on the basic side of the EPA's standard for stream pH (6.5-8.5) however it is still with the normal range (Barbour et al. 1999). Alkalinity was not included due to small sample size.

Table 3 also shows the analytes that were only analyzed in the field and that are not categorized by the EPA. Both sites had a DO of 12.0 mg/L ($p < 0.05$). Specific Conductivity was on average was 509±136 µS for SC and 477±110 µS at BR, however the difference was not significant ($p < 0.05$). The average for temperature at SC was $11.50 \pm 8.00\ ^{\circ}C$ and the average at BR was $11.81 \pm 8.50\ ^{\circ}C$. The difference in temperature was not significant ($p > 0.05$).

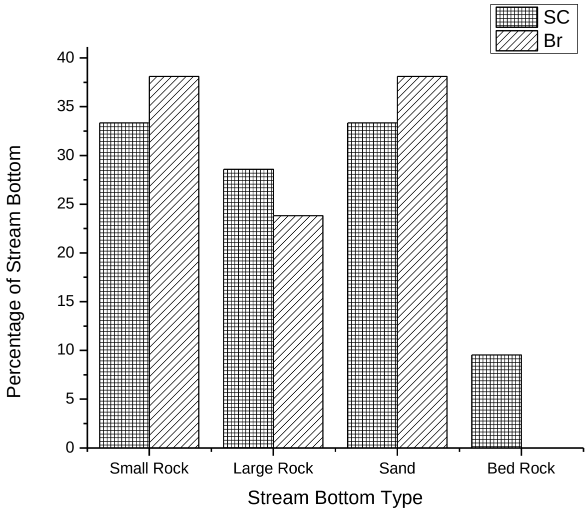

Figure 3: Percentage of Stream Bottom Types at SC and BR. SC had a slightly higher percentage of Large Rock and Bed Rock. BR had a slightly higher percentage of Small Rock and Sand.

Physical Data

Stream bottom and habitat analysis was recalculated to show relative percentages of each type relative to the whole. Figure 3 shows the various types of percentage of stream bottom types. SC and BR were very similar in terms of stream bottom, but SC had a slightly higher percentage of bedrock and large rock, while BR a slightly higher percentage of small rocks and sand. Figure 4 shows the relative percentages of stream habitat at the SC and BR. SC had a higher percentage of open water and riffles, while BR had a higher portion of snags and pools.

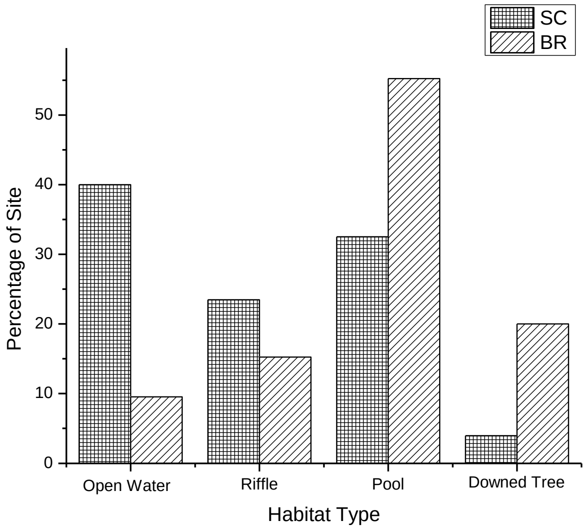

Figure 4: Percentage Habitat Types at SC and BR. BR had a higher amount of Pools and downed trees. SC had a higher amount of open water and downed trees.

Turbidity (the clarity of the water) was measured in the laboratory. The average turbidity for SC was 1.83±0.87 NTU. The average turbidity for BR was 2.46 ±1.16 NTU. Using a student's t-test, there was not a significant difference between these turbidities ($p > 0.05$)

Other Observations

At SC, the stream flowed continuously through the entire year, but there were periods where flow was not well connected due to both drought and ice. During the summer (May-August) there were periods where BR did not flow continuously due to drought. In winter, there were periods where ice stopped the flow of water between the pools (observed by researcher). Four trees were uprooted during the year at BR causing slumping in the stream bank. There was one large tree that had fallen recently at SC, but there was little slumping of the bank. Both sites experienced flashy hydrology.

Discussion

Macroinvertebrate Indexes and Assemblages

The results of each of the four indexes (HFBI, ICI, % EPT and % Chironomidae) were calculated based on the collected macroinvertebrates. They gave consistent results of water quality at SC and at BR (Table 1). All four indexes showed that SC had minimal organic pollution. At BR, the four indexes' results implied that the site had a slight to moderate degree of pollution. According to Barbour et al., an indication of pollution by four or more indexes is significant evidence of pollution in the area (Barbour et al., 1999). The macroinvertebrate data suggests pollution or high turbidity in the water at BR (Barbour et al. 1999).

Since SC and BR are 1.5 km apart, it was expected that the sites would have similar assemblages of macroinvertebrates. However, the macroinvertebrate assemblages at SC and BR did not appear similar (Figure 2). Three similarity tests and correlations tests were performed to determine if the difference was significant (Figure 2). The results show that the macroinvertebrate assemblages at SC and BR were 68% similar in terms of taxa present. When abundance was included in the similarity index, the distributions were not similar (Figure 2). The difference in assemblage seemed be caused by the high % Chironomidae and the low % EPT at BR. The % tolerant taxa (not including Chironomidae) were similar for BR and SC.

The indexes' and the macroinvertebrate assemblages' results imply that there is a difference in water quality between SC and BR. Since the assemblage is the strongest factor influencing the results of the indexes, the difference in macroinvertebrate assemblage should be the result of a difference in water quality between SC and BR. Water quality can be affected by three main factors: chemical parameters, turbidity, and trophic state (which describes the internal and external loading) (Barbour et al. 1999). Therefore, it is important evaluate the role each of these factors play in the water quality in the Sycamore Creek Watershed. It is also important to evaluate the ecology at both sites, since ecology can also explain the differences in assemblage and the results of the indexes irrespective of the water quality.

Factors that Directly Affect Water Quality: Chemistry, Turbidity, and Trophic State

The chemistry of a stream is affected by the concentration of nutrients (SRP, NH_4, NO_3, TDN, DOC) its alkalinity, pH, DOC, and DO content. The concentration of nutrients in a stream affects the trophic state of the stream, which affects the macroinvertebrate assemblage (Wang et al. 2007). A eutrophic stream generally has a lower DO concentration due to the amount of decomposition, which influences the macroinvertebrate assemblage (Dodds 2006). Although macroinvertebrates are most influenced by changes in SRP, changes in N (specifically TDN), and DOC can also affect the assemblage of macroinvertebrates (Dodds 2006). Wang et al. (2007) preformed a nutrient analysis and showed that abundance of intolerant macroinvertebrate taxa were significantly negatively correlated with increases in SRP NH_4, NO_3, and TDN.

However, the data from Sycamore Creek does not reflect the described relationships. Looking specifically at nutrients (SRP, NO_3, and TDN) at SC and BR there is no parameter that was significantly different. SRP was not significantly different between the two sites, so it is unlikely to affect the assemblage of macroinvertebrates. The NO_3 and TDN values were higher at SC, but the difference was not significant (Table 2). As well the more tolerant organisms were found in higher abundance at BR (Appendix 1, figure 2). Since the taxa with high tolerance were not found in higher predominance at SC, it is likely that the difference in NO_3 and TDN was not a strong factor in the difference in macroinvertebrate assemblages. Although there are some slight differences in nutrient concentrations, they are not strong enough to cause cascading effects through the food chain, and therefore do not significantly affect the macroinvertebrate assemblages (EPA 2000).

It is also important to consider other water chemistry parameters besides nutrient concentrations. DO was the same at both sites, so it is unlikely to be a strong factor in the difference of macroinvertebrate assemblages between SC and BR. DOC and pH were significantly different between SC and BR; however they were within the same ranges given by the EPA, implying that the difference, although significant, is not strong. SC had a more basic pH than BR and a higher DOC concentration (Table 2). A slightly more basic pH may allow more metals to be released at SC than BR. Since metals can be a limiting factor for biota, a more basic pH could cause a change in biota, specifically plants (Wetzel 2001). However, since the difference between the pH at SC and BR is categorically insignificant, it is unlikely to affect the water quality as strongly as the macroinvertebrate indexes indicate (EPA 2000, Barbour et al. 1999). The increased DOC concentration implies that there is more organic material decaying at SC than BR (Wetzel 2001). This could affect the types of feeding guilds present at SC as compared to BR. The difference is small compared to the possibilities shown in EPA (2000) but it could cause a difference in macroinvertebrate assemblages. It is hard to ensure the effect pH and DOC had the assemblages is negligible without further study.

A significant difference in turbidity between SC and BR could cause a difference in the macroinvertebrate assemblage. Alternative stable state theory implies that turbid waters support different macroinvertebrate assemblages than waters with less turbidity (Van de Meutter et al. 2005). In a turbid waterbody the assemblage usually includes a high level of tolerant taxa, and in a waterbody with low turbidity the assemblage includes more intolerant taxa. A high level of turbidity clogs an organism's gills; thereby causing a reduction in intolerant taxa within the waterbody (Van de Meutter et al. 2005).

Turbidity was not significantly different between SC and BR (Table 2). Turbidity was also low compared to the national acceptable turbidity levels (EPA 2000). Since there was no significant difference in turbidity between the two sites, it is unlikely to be the cause of a significant difference in the macroinvertebrate assemblage. As well, low turbidity is associated overall with a predominance of intolerant taxa. The data does not match the established pattern by the EPA. BR has low turbidity but the dominant taxa are not intolerant taxa (Figure 2, appendix 1). It is likely that some factor besides turbidity is causing the difference in macroinvertebrate assemblages between SC and BR.

Alternative stable state theory also asserts that trophic state, determined by combination nutrient concentrations and turbidity, could cause differences in assemblages of macroinvertebrates (Van de Meutter et al. 2005). A change in nutrient concentationsor turbidity can cause the stable state to shift, causing the macroinvertebrate assemblages to change (Van de Meutter et al. 2005). Turbidity data and nutrient concentrations data classify SC and BR as oligotrophic stream sites (Dodds 2006). Since both site are oligatrophic, it is unlikely that trophic state is causing the difference between macroinvertebrate assemblages and SC and BR.

Overall the data shows that chemistry, turbidity, and trophic state were not strong factors in explaining the difference between the macroinvertebrate assemblages at SC and BR. As well, it is unlikely that water quality was significantly different between the two sites. This means that macroinvertebrate indexes did not accurately represent the water quality at SC and BR. To determine the cause of the difference in macroinvertebrate assemblage, two other factors, habitat and disturbance were examined

Factors that Affect Macroinvertebrate Assemblage: Habitat and Disturbance

A change in stream bottom type and habitat types causes differences in macroinvertebrate assemblages, which causes changes in the macroinvertebrates measured by indexes (Brigham et al. 1985). Streams that have high amounts of sand and low amounts of snags often have low amounts of EPT, and misleading HFBI scores (Meixer and Bain, 1999). Habitat guilds are also well correlated with different types of stream bottoms and habitat types (Wang et. al. 2007).

Table 3 shows the macroinvertebrate assemblages for each site divided into habitat guilds. Both SC and BR had mostly sprawlers and clingers. SC had a higher amount sprawlers and BR had a higher amount of clingers. This implied the SC had a larger amount of snag habitat with loose or small sediments and BR had a larger amount of clingers with riffle areas in larger or small sediments.

Comparing the prediction from Table 3 with the stream bottom types (Figure 3) and habitat types (Figure 4) gives contrasting results. Figure 3 showed that overall SC ad BR had similar percentages of bottom type. Differences between SC and BR were within 5% in sand, small rock, and large rock, which is not an appreciable difference since there was only one habitat survey taken. Furthermore, the slight difference did not match the predictions from Table 3. BR had the higher amount of large rocks which is not the ideal habitat for clinger who prefer large rocks. SC had the higher amount of sand and small rocks, which is not ideal for sprawlers who prefer silty areas. Since there was an appreciable difference in habitat guilds but not in stream bottom types, it is unlikely that stream bottom type caused differences in the macroinvertebrate assemblages at SC and BR.

Figure 4 showed that there was a difference in stream habitat distribution between SC and BR. Comparing the results of Figure 4 to the predictions from Table 3, there is a low amount of correlation. Table 3 shows that SC should have a large amount of downed trees and in stream plants to support the sprawler population. However, looking at Figure 4, downed tree habitat was a very small proportion at SC (< 5%). Table 3 also shows that BR should have a large amount riffles, but in Figure 4, BR actually had a high percentage of pool. There is a lack of correlation between habitat type and habitat guild. This implies that habitat type and presence was not a dominant factor in the difference in macroinvertebrate assemblages at SC and BR.

To evaluate disturbance, observational notes were used. Precipitation and melting events were identical between the sites because of their close proximity. Meandering stream bed was more common at BR than SC. There were more tree falling events at BR than at SC. These events also caused slumping of the stream bank at BR. Meandering, downed trees, and slumping can all be categorized as disturbances of BR. Comparatively, SC had relatively low levels of disturbance.

Comparing the results of the Sycamore Creek macroinvertebrate assemblages to the results found in McCabe and Gotelli, (2000), there are a large number of parallels. Both data sets showed that Chironomidae were dominant when there was a moderate to high level of disturbance in the area. As well, in areas with moderate to high disturbance the richness of EPT taxa did not change but the abundance of these taxa did. Both studies also showed an example of low % intolerant taxa even with a high level of disturbance. The agreement of results between this study at Sycamore Creek study to the McCabe and Gotelli (2000) study implies that disturbance could be a strong factor in understanding the changes in assemblages between SC and BR.

Other Considerations and Biases

When evaluating this study other considerations must be taken into account and possible biases must be mentioned. Although the season was controlled between SC and BR by always sampling on the same day, many of the studies used for comparison were completed during the summer. Therefore there may be differences in this study's results as compared to the background due to seasonal and temporal differences. An example of this is the large amount of Pelecoptera due to winter emergence (Peckarsky et al. 1990). This could have biased a site like BR to appearing more unpolluted. Likewise the low amount of taxa that overwinter in large stages could have biased BR to appear more polluted than it is in reality.

Some of the assumptions for HFBI and ICI were not met. In HFBI 100 organisms were needed for the sample, but the number of organisms collected from each site on average was 67 per sample. ICI also assumed that a Hester-Dendy sampler was used, but in this study, a D-net method was used. ICI also assumed that the samples were taken in summer, but these were taken in winter and early spring.

Many of the studies used for data comparison picked macroinvertebrates in the laboratory, but this study chose to pick macroinvertebrates in the field. There was also a preference given to sampling at riffles as opposed to pools due to ease of sampling, which may create a bias toward riffle clingers. Since BR has a high density of pool habitat, an analysis of the pool macro invertebrates may show higher water quality and allow for a more consistent comparison of the two sites. A better accounting of consistent sampling sites may increase consistency in the results A picking time longer than 20 minutes may have been necessary to obtain an appropriately diverse sample. Since macroinvertebrates were picked and identified by a new researcher, there was a learning curve associated with recognizing and identifying the macroinvertebrates, which may have led to inaccuracies in the data not seen in other studies. There was also a learning curve with physically using tweezers to grab mobile macroinvertebrates such as Chironomidae. This implies that larger organisms may have been picked preferentially over smaller organisms in this study, as compared to other studies.

Winter conditions had a strong impact in how this study was performed and could affect the results. It would have been preferable to measure DO weekly. However, this was not possible because the meter does not preform below freezing temperatures. Macroinvertebrates also froze during picking, which may have affected how they were picked.

There is also some bias associated with the classification of habitat and stream bottom analysis. These were only taken once, and one person took them. This implies that there could be a bias in the data due to limited sampling and due to the researchers opinion. There was no set definition of small or large rock. This problem could be corrected by using a gravelometer or sediment filtering pans.

Conclusion and Future Studies

This study demonstrates the importance of ecological theories in studying macroinvertebrates. While the water quality at SC and BR is most likely equivalent based on the chemical and physical data, there appears to be significant differences in assemblages, most likely caused by disturbance in the area, and to a lesser extent differences in habitat type, pH, and DOC.

Future studies should include a comparison of summer collections at Sycamore Creek to winter collections. This would allow this study to be compared to other studies, and determine if change seasonal variation is a factor affecting assemblage. Furthermore, an examination of colonization rates at SC and BR would give more of an explanation as to why Chironomidae dominates at BR and not at SC. This may be part of a competition for a resource that was not well observed in this study. An exploration of the feeding guilds and food sources present at each site may give further explanation of the differences between SC and BR. Different types of food sources available to create differing assemblages of macroinvertebrates between SC and BR.

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Appendix I: Full counts from SC and BR

Order	Family	SC	BR	Tolerant
Diptera	Tipulidae	9	2	Tolerant
	Chironomidae	120	234	Tolerant
Ephemoptera	Baetidae	2	0	Intolerant
	Caenidae	2	3	Intolerant
	Ephemerillidae	78	2	Intolerant
	Heptageniidae	18	4	Intolerant
Pelecoptera	Capniidae	0	2	Intolerant
	Chloroperlidae	10	9	Intolerant
	Leuctridae	3	0	Intolerant
	Perlidae	0	1	Intolerant
	Perlodidae	120	94	Intolerant
	Taeniopterygidae	0	2	Intolerant
Trichoptera	Hydropsychidae	79	9	Intolerant
	Philopotamidae	19	5	Intolerant
Amphipoda		3	3	n/a
Oligochaeta		1	1	n/a
Diptera pupae		0	2	n/a
Molussca		0	1	n/a

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