Environmental Factors Determining the Pre-Restoration Benthic Macroinvertebrate Assemblage In A Stream Used By Cattle

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(ABSTRACT)

I investigated the baseline benthic macroinvertebrate community in relation to the environmental conditions in a section of Smith Creek, north of Harrisonburg, VA, prior to restoration. Quantitative benthic macroinvertebrate and environmental samples were collected in April and September 2006 from the Bruce Farm (BR) section of Smith Creek and the nearby Mixed Use (MU) section of Mountain Run. BR had been heavily used for cattle grazing for decades and suffered from sediment, nutrients, and lack of a forested riparian zone. MU had a forested riparian zone, but still received nutrient and sediment inputs from upstream cattle grazing. Visual habitat assessments were performed in September 2006 and were compared to quantitative measures. Benthic macroinvertebrate densities and taxa richness were greater at BR (total density for combined seasons = 52,438; taxa richness for both seasons = 84) than MU (total density for combined seasons = 3,982 and taxa richness for both seasons = 63). Biological environmental variables related to nutrients and growth of plants on rocks (ash-free dry mass, chlorophyll *a,* epilithic biomass) influenced the benthic macroinvertebrate assemblage more than physical environmental variables related to the substrate composition (% fines, % gravel, Trask's sorting coefficient). Visual habitat estimates were not as effective as quantitative measures of habitat for explaining the benthic macroinvertebrate assemblage.

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Introduction

To be compliant with the Clean Water Act, the U.S. Environmental Protection Agency (EPA) has been working with states and tribes to assess the 3.7 million miles of streams and rivers in the U.S. As of 2002, 19% of streams and rivers had been assessed (EPA Wadeable Streams Assessment, 2002). Of those stream miles assessed, 45% were classified as impaired by the states. The report focused on three main regions of the country and nine ecoregions. Results indicated that nitrogen, phosphorus and sediment posed the greatest risk to flowing waters in the U.S. The Eastern Highlands Region, which includes Virginia, scored highest in impairment for most categories. The Eastern Highlands had the greatest loss of expected taxa, greatest proportion of stream length with high phosphorus and nitrogen, the greatest proportion of stream length in poor condition according to the Macroinvertebrate Index, and the greatest proportion of stream length with high riparian disturbance. The Eastern Highlands Region was equal with other regions for the greatest percent of stream length in poor condition for streambed sediment and for riparian vegetation in poor condition. Within the Southern Appalachian Ecoregion, 55% of stream length was in poor condition according to the Macroinvertebrate Index, 41% of stream length had high concentrations of phosphorus and nitrogen, 33% of stream length had high levels of riparian disturbance caused by human activities, and 27% of stream length was poor because of streambed sediment. The Wadeable Streams Assessment indicated that streams with high concentrations of nutrients or large amounts of sediment were more likely to have a poor macroinvertebrate assemblage.

Agriculture is the major human activity responsible for nonpoint-source pollution in the U.S., causing impairment in 48% of rivers and streams that have been assessed (U.S. EPA, 2000). In Virginia, agriculture also causes most of the impairment identified in the Wadeable Streams Assessment. In Virginia, there are 8.5 million acres of land used for agriculture (Pease, 2000), and at the beginning of 2008 there were 1,570,000 head of cattle (USDA, 2008).

The riparian zone is the area of land on either side of a stream that serves as the interface between the terrestrial and aquatic environment. It provides a variety of important functions, such as regulating runoff during rain events, uptake and cycling of nutrients so they do not enter the water, providing allochthonous inputs for food and habitat, shading to keep water temperatures low, filtering runoff inputs, and stabilizing banks to prevent erosion. Cattle grazing and trampling reduce the amount and change the composition of the vegetation in a riparian zone, thereby reducing or even eliminating the aforementioned important functions. (Belsky et al., 1999; Chambers et al., 2006; Clary and Kinney, 2002; Flenniken et al., 2001; Green and Kauffman, 1995).
Stream banks, the narrow areas immediately adjacent to the channel, are especially vulnerable to cattle grazing and trampling. Bare, compacted stream banks are more likely to erode and introduce sediment into the stream channel. Natural stream processes influencing channel shape are slow, and recovery from stream bank erosion caused by cattle may take years if not decades (Agouridis et al., 2005). Cattle grazing and

trampling impact the hydrology of stream areas by compacting soil, reducing soil drainage capability, and by altering the microtopography of the area. Cattle grazing has been found to straighten microchannels that drain the riparian area, creating a decreased drainage density (Flenniken et al., 2001).

In addition to physical changes, cattle also cause increased nutrient concentrations. Cattle often congregate in streams to stay cool and to access drinking water, resulting in urine and feces directly deposited in the stream. Waste deposited on the surrounding land is washed into the stream during rain events. A forested riparian zone is important for nutrient uptake from upland agricultural activities and may reduce nutrient input into the stream (Fail et al., 1988). Nitrogen is not well retained in soils (Chambers et al., 2006), so the riparian zone plays an important role in nitrogen uptake. Low levels of excess nutrients in streams may stimulate primary production and provide food for aquatic organisms, but high levels of nutrients will trigger an overproduction of algae that may lead to an oxygen deficit as it decomposes (Belsky et al., 1999). Abundance and diversity of aquatic organisms may flourish with small nutrient inputs, but high nutrient inputs will have a detrimental effect on both abundance and diversity (Chambers et al., 2006).

Cattle grazing also alters the benthic macroinvertebrate assemblage. There may be an increase in some organisms, while others decrease. Usually, the assemblage shifts from a diverse assemblage of organisms with low tolerance for pollution to a less diverse assemblage of organisms that are generalists tolerant of environmental stressors such as nutrients, sediment, warmer water, and less stable habitat (Delong and Brusven, 1998). The non-vegetated riparian zone that results from cattle grazing leads to reduced allochthonous inputs, and increased algae production because of higher nutrient concentrations and increased sunlight, all of which leading to a change in the assemblage structure (Delong and Brusven, 1998).

In forested systems, allochthonous inputs provide the main source of food for benthic macroinvertebrates. In systems lacking a forested riparian zone, the stream receives more sunlight and little input from vegetation outside of the stream. This results in an increase of primary productivity in the form of algal growth. Boothroyd et al. (2004) found that periphyton biomass was lowest at sites with a forested riparian zone and highest in areas that had been clearcut. In areas with agriculture and/or cattle grazing, this is often accompanied by an increase in nutrients, which results in excess algal growth. In streams lacking a forested riparian zone, macroinvertebrates that consume leaves (shredders) make up a disproportionably small component of the assemblage (Delong and Brusven, 1998).

Cattle grazing alters habitat available to benthic macroinvertebrates. Habitat usually shifts from a heterogeneous mix of coarse substratum to a more homogenous fine substratum. This shift is caused by erosion as well as direct damage from the cattle in the stream. Riffles become choked with sediment eliminating important niches for benthic macroinvertebrates. Often existing habitat is less stable because the lack of a forested riparian zone leads to more high flow events that move even large substrates. Even moderate rain events will have a dramatic impact on stream flows without a forested riparian. Lack of a forested riparian zone also means lack of large woody debris that provides habitat diversity. Braccia and Voshell (2006) found that, of several environmental factors, sediment had the greatest impact on the macroinvertebrate community in small streams that ran through fields used for cattle grazing.

The Clean Water Act (CWA) is the primary legislation for protecting the condition of the country's water bodies. Water bodies that have a significant level of pollution are listed as impaired and must have a Total Maximum Daily Load (TMDL) plan developed to restore the water body to meet its intended use. TMDL plans are a management tool to assess the total input of point and non-point pollution that a waterway can receive and meet non-impairment goals. The TMDL program manages for specific pollutants at the watershed level. States, localities, or non-governmental groups can implement TMDLs, though the final document must be approved by the USEPA. In areas with significant agricultural land use, conservation efforts are important for reducing the non-point source inputs, which is necessary to meet many TMDLs.

The EPA has recommended several best management practices (BMPs) for minimizing the impact of agriculture and cattle grazing on streams including: conservation buffers, irrigation water management, grazing management, animal feeding operations management, and erosion and sediment control (USEPA, 2007B). These are voluntary measures that farmers can undertake in order to reduce their impact on local water systems.

The Conservation Reserve Program (CRP) is run by the U.S. Department of Agriculture with the goal of reducing non-point source pollution. CRP is a voluntary program that provides financial incentives to farmers to implement conservation measures to address erosion, water quality, soil, and other environmental issues. This program is useful for helping states meet their TMDL goals for pollution associated with agriculture. The Conservation Reserve Enhancement Program (CREP) is part of CRP and specifically focuses on improving water quality by offering farmers a financial incentive for removing livestock or eliminating cultivation along the stream and restoring a riparian zone. CREP is administered at the state level.

Stream restoration is often used to meet Clean Water Act goals. Stream restoration has been defined as assisting the recovery of ecological integrity in a degraded watershed system by reestablishing the processes necessary to support the natural ecosystem within a watershed. In order to reestablish ecological processes, stream restoration often emphasizes the establishment of improved hydrologic and geomorphic processes in a degraded watershed system and replacing lost, damaged, or compromised elements of the natural system. Because both technical and societal constraints often preclude full restoration of ecosystem structure and function, rehabilitation is sometimes distinguished from restoration and used as a more practical goal. The scope of stream restoration projects includes, but is not limited to: erosion control, nutrient reduction, toxics reduction, reforestation, reshaping channels, in-stream structures, daylighting streams, removal of non-native species and weeds, reintroduction of native species, and habitat and range improvement for targeted species (Wohl et al., 1995).

Stream restoration is being implemented more often to address water quality problems. Restoration projects may be implemented by many different organizations, including local not-for-profit groups, various environmental businesses, and governmental agencies. Because of the variety of people participating in stream restoration, it is difficult to collect information on techniques and results. An estimated $14-15 billion has been spent on restoration efforts since 1990, averaging more than $1 billion per year, though this is probably a low estimate (Bernhardt et al., 2005). There has been little documentation on restoration efforts and results. The lack of documentation means that it is difficult to assess the success of projects and to improve upon restoration techniques.

Restoration projects may have very different goals, and therefore will have different approaches, such as stream stabilization, habitat restoration, or the return of native biota. Bohn and Kershner (2002) recommend the watershed analysis procedure to take a holistic approach to determine land use and restoration efforts that will benefit the entire watershed rather than localized efforts. Bond and Lake (2005) identified five issues that should be considered for restoration projects: (i) barriers to colonization, (ii) temporal shifts in habitat use, (iii) introduced species, (iv) long-term and large-scale processes, and (v) inappropriate scales of restoration. USEPA (2007A) explained 17 guiding principles for aquatic restoration, which emphasized restoring ecological integrity through passive restoration whenever possible and natural fixes and bioengineering when more aggressive measures need to be taken.

It is clear that ecological integrity is one of the main objectives of stream restoration and that restoring ecological integrity must include the biological components of aquatic ecosystems. Projects with the goal of restoring biota must consider the relationships and processes between the cause of degradation, the biota, and how recovery will take place (Adams, Ryon and Smith, 2005). Many restoration projects focus on restoring physical habitat, however there are several other factors that will also influence the biota and must be considered (Bond and Lake, 2005). Thus far, the emphasis of most stream restoration projects has been on physical habitat restoration, with the assumption that the biological restoration necessary for the recovery of ecological integrity will happen by means of what has been dubbed the Field of Dreams Hypothesis (Bond and Lake, 2003; Palmer et al. 1997; Palmer et al. 2005). This title is in reference to a popular movie in which a farmer heard voices telling him “if you build it, they will come”.

Unfortunately the Field of Dreams Hypothesis has never been proven because the necessary quantification of prerestoration conditions and monitoring of the results of ongoing restoration activities have not been done. Monitoring restoration projects helps to understand the regeneration process and can identify strategies that are successful and those that are not. Often, restoration projects that are monitored do not examine the biota in connection to the environmental factors. Monitoring and assessment are critical to understand if restoration projects have achieved their goals (Kondolf and Micheli, 1995). In addition to monitoring, pre-restoration conditions must be understood in order to effectively design a restoration project and to understand how the ecosystem has changed as a result of the restoration effort. It is critical to understand the relationships among the baseline conditions in order to assess the changes caused by restoration. Monitoring is necessary for most of the restoration guiding principles proposed by USEPA (2007A).

Only 10% of projects are either assessed or monitored (Bernhardt et al., 2005). Projects with engineering goals are less likely to conduct a pre-restoration assessment or include monitoring than the projects with goals related to improving the biological community (Bash and Ryan, 2002). Monitoring should include biological, chemical and physiological measures to effectively understand the restoration process (Adams, Ryon and Smith, 2005). Benthic macroinvertebrates are ideal for biomonitoring because their assemblages can be related to water quality and specific types of degradation and they reflect the status of the riparian zone.

The Environmental Protection Agency's Rapid Bioassessment Protocol, Chapter 5 Habitat Assessment and Physicochemical Parameters, is used to assess stream habitat for aquatic organisms. The RBP is often used to gauge progress of stream restoration and available habitat for biota. However, there have been few studies examining how well the visual estimates of the RBP reflect the actual conditions in the stream or how the RBP compares to more rigorous quantitative habitat measures. The RBP is advantageous because it requires little equipment, but is prone to error because it relies on visual estimates which may vary from scientist to scientist. Quantitative measures, such as granular sieve analysis to classify mineral substrate, or chlorophyll analysis, are time consuming and require specialized equipment and procedures.

An opportunity arose to begin long-term biomonitoring of a stream reach that was degraded from cattle grazing and was to be restored passively by conservation programs. A number of scientists are collaborating on studies of water chemistry, microbiology, fluvial geomorphology, aquatic ecology, plant ecology, fish populations, and macroinvertebrate assemblages. The overall purpose of this thesis was to investigate the prerestoration benthic macroinvertebrate assemblage in Smith Creek.

This study had three objectives: (1) Describe the prerestoration benthic macroinvertebrate assemblages in the reach of Smith Creek being restored; (2) Quantify the environmental factors responsible for the observed prerestoration benthic macroinvertebrate assemblages; (3) Compare the effectiveness of measurements versus visual estimates of substrate composition for explaining macroinvertebrate assemblages.

Materials and Methods

Study Area

Smith Creek is a third order stream within the Valley and Ridge physiographic province (Fig. 1). It lies within the 5,539-ha Smith Creek subwatershed (Hydrologic Unit Code 510172), which is in Rockingham County, north of Harrisonburg, in western Virginia (Hudy and Shiflet, in press). The underlying geology of the subwatershed is southern limestone/dolomite valleys and low rolling hills. The headwaters of Smith Creek originate in the George Washington and Jefferson National Forest, and then the stream flows through a mixture of agricultural and residential land after leaving the National Forest. It is a pool riffle stream with an average gradient of 1.74 % and an average wetted width of 7.1 m. Most of the overall subwatershed is classified as forested (61%) or agricultural (38%) (Hudy and Shiflet, in press). The riparian areas along Smith Creek (100 m each side) are similarly classified as forested (56%) or agricultural (42%) for the overall subwatershed (USGS 2004; Thieling 2006). The 3.14-km section of Smith Creek that is being restored and is the subject of this study is located on the Bruce Farm. This section has been used for agriculture, especially cattle grazing, for decades. There are few riparian trees (only 113 trees greater than 10 cm diameter breast height; Hudy and Shiflet, in press), so the stream channel is almost completely exposed to full sun as it flows through a pasture that was heavily grazed by cattle until winter 2006. The banks in the BR section had been severely eroded, with undercuts and bank slumping. There were also numerous depositional areas that had formed sand bars and islands within the channel. In February 2006, all cattle were removed from the 3.14-km section of Smith Creek on the Bruce Farm. Sapling trees were planted in April 2006 as part of CREP.

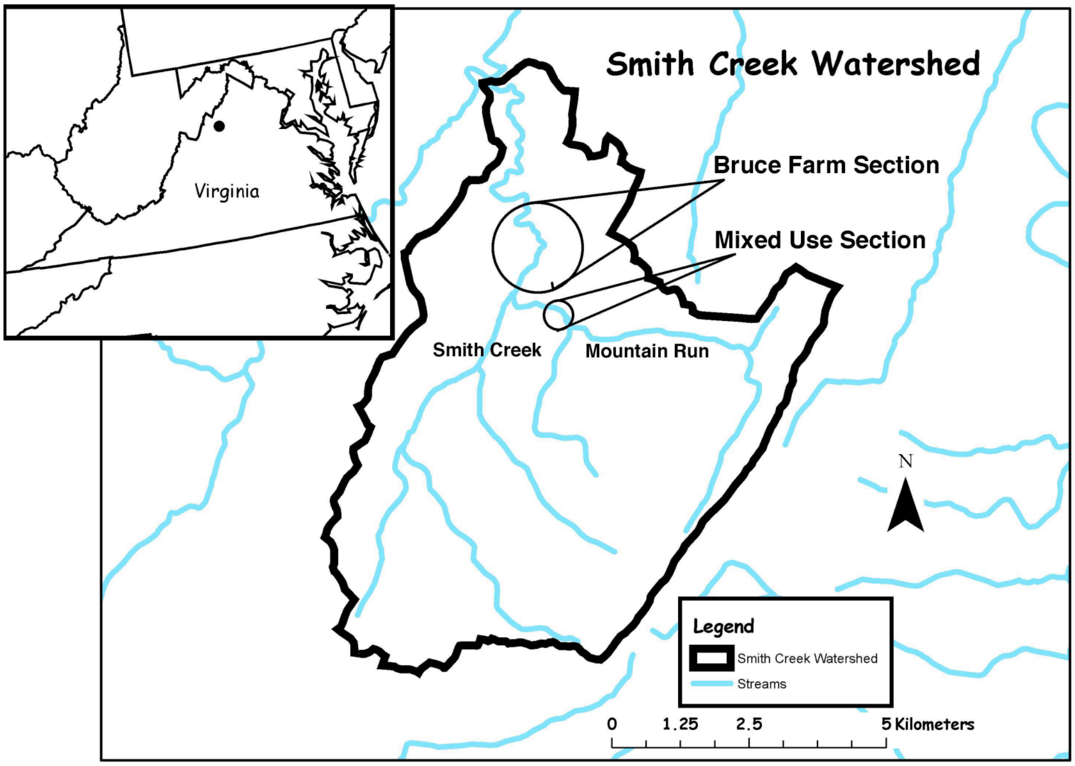

Figure 1. Smith Creek study area.

For comparison with pre-restoration conditions at the Bruce Farm section of Smith Creek, a study area was established on Mountain Run, a nearby tributary of Smith Creek (Fig. 1). Mountain Run is similar to the Bruce Farm section of Smith Creek in size and gradient, and it also flows through an area of intensive cattle grazing. However, the study area on Mountain Run was a 1.1-km section that has forested riparian zones approximately 10 m wide on both sides. This study area is called the Mixed Use section. There are mature deciduous trees that completely shade the stream channel from April-May until leaf abscission in October-November. In addition, the stream banks and channel are stable with minimal sources of erosion and sedimentation within the section. Although cattle are fenced out of the steam within the forested Mixed Use section, there is intensive grazing immediately upstream of the section as well as in pastures just outside of the forested riparian zone. Thus, there are ample sources for nutrients and sediment to enter the Mixed Use section, at least from upstream. Mountain Run does have lower acid neutralizing capacity, an average of 55.56 μeq/L, compared to Smith Creek, which has an average of 181.35 μeq/L. This is due to the underlying geology of the two streams and can influence primary productivity

The Virginia Department of Environmental Quality (VADEQ) has listed Smith Creek and Mountain Run as impaired for fecal coliform and *E. coli* bacteria for the entire length of each stream (VADEQ, 2008B). Benthic impairment is also indicated by VADEQ, and a TMDL was developed in 2004 (VADEQ, 2008B). The benthic impairment is primarily from sediment. Agricultural land use in the watershed is mostly responsible for the bacteria and sediment problems. Rockingham County is the largest livestock producing county in Virginia, with 25% of dairy cattle in VA located within the county (Virginia

Places, 2007). Cattle outnumber people in Rockingham County, with 121,000 cattle (USDA, 2008) and 72,564 people (USCB, 2007) in 2006. Table 1 summarizes the physical and chemical properties of Smith Creek and Mountain Run.

Table 1. Physical and chemical properties of Bruce Farm and Mixed Use reaches.

	Bruce Farm		Mixed Use	
	Mean	Range	Mean	Range
Riffle gradient (m/m)	0.03	0.01-0.07	0.04	0.02-0.07
pH	8.2	7.7-9.1	7.7	6.5-8.4
Acid neutralizing capacity (ueq/L)	181.4	87.4-317.7	55.6	32.6-113.6
Discharge (L/min)	5135	1173-15,403		
Temperature (^{o}C)	15.7	4.8-24.6	14.8	4.3-23.2
Nitrate – N	1.77	0.76-2.94	0.25	0.06-1.18
Ammonia – N (ppm)	0.03	0.00-0.12	0.01	0.00-0.05
Phosphorous (ppm)	0.25	0.08-0.57	0.19	0.09-0.40
Conductivity (uS)	377.9	113.0-1102.0	117.2	67.0-450.0
Turbidity (NTU)*	13.9	0.0-120.0	9.7	0.2-72.0
Bankfull width (m)	10.3	8.7-12.2		
Depth (cm)	12.2	4.3-27.7	11.5	7.0-21.7
Current velocity (m/s)	0.44	0.07-1.51	0.60	0.08-5.51

*includes storm flows

Field Sampling

The sampling design for benthic macroinvertebrates involved a total of nine 300-m reaches, two riffles within each reach, and three random replicate samples within each riffle. Sampling was stratified to shallow, rocky areas of riffles with current. There were six sampling reaches within the Bruce Farm (BR) restoration section of Smith Creek and three sampling reaches within the Mixed Use (MU) section of Mountain Run. Samples were collected twice, in April and September of 2006, for a total of 54 samples in each season and 108 samples for the entire study. Although all cattle were removed during the winter of 2006, there did not appear to have been any changes in the environmental conditions of Smith Creek and its riparian areas when the first macroinvertebrate samples were taken in April 2006. There was still ample evidence of cattle grazing, including hoof prints, trampled trails, manure, and closely cropped grass. Therefore, the data from 2006 are considered to be representative of the pre-restoration conditions of Smith Creek.

Benthic macroinvertebrates, epilithic biomass, benthic organic matter, and inorganic substrate were sampled with a modified stovepipe sampler that was 30 cm in diameter (Cummins, 1962; Merritt and Cummins, 1996; Braccia and Voshell, 2006). The sampler was inserted approximately 10 cm into the bottom substrate, and then the stones lying on the surface were removed and set aside. Visible macroinvertebrates, benthic organic matter, and inorganic substrate were removed by hand and placed in a plastic bag. As those materials were removed, the water within the sampler was stirred to suspend the fine benthic matter, and a hand pump was used to collect 15 liters of water from within the sampler. That water was pumped through a 1-mm sieve, and macroinvertebrates and material retained on that sieve were added to the benthic sample. A 250-ml subsample of the 15 liters of water was collected for a measurement of fine benthic organic matter (FBOM), and the remainder was filtered through a 63-μm sieve. The material retained on that sieve was then added to the benthic sample. The FBOM sample was put on ice and transported to the lab for analysis. The surface stones were examined for benthic macroinvertebrates, which were removed and added to the benthic sample. The surface stones were separated into standard size classes (Bunte and Abt, 2001), weighed and discarded. The benthic sample was then preserved in 95% ethanol for later processing in the laboratory. For a measurement of epilithic biomass, a small cobble was selected randomly from the immediate vicinity of the sampler, placed in a ziplock bag inside of a black plastic bag, and then put on ice for later processing in the laboratory (Steinman et al., 2006). Three velocity and depth measurements were taken in close proximity to each benthic sample. Velocity was measured with a digital Marsh–McBirney flow meter.

In September 2006, habitat variables were also assessed by means of visual estimates using modifications of the Rapid Bioassessment Protocol (RBP) (Barbour et. al., 1999). The RBP habitat estimates were scaled down to consider only the area of each benthic macroinvertebrate sample. RBP habitat estimates that were not applicable to the sample scale, such as riparian zone characteristics, were not used. Two researchers collaborated on the scoring of each habitat characteristic.

Laboratory Analysis

Benthic samples were washed over a series of stacked sieves (16 mm - 63 μm). Material on the sieves ≥ 250 mm was sorted by hand to remove all macroinvertebrates. Macroinvertebrates were identified to the lowest practical taxonomic level, usually genus, and enumerated. Organic material on the sieves was retained and hand sorted into four categories (wood, leaves, periphyton, and grass), dried at 60° C, and then weighed. Inorganic material on the sieves was set aside for dividing into standard size categories (Bunte and Abt, 2001) and weighing. Inorganic material >16 mm was hand sorted into standard size classes (Bunte and Abt, 2001) and weighed. Inorganic material <16 mm was placed in trays to dry. Once the material was dry, it was placed on a series of stacked sieves and separated into standard size classes with a shaker, and then the inorganic matter in each size class was weighed.

The FBOM sample was analyzed using the methods described in (Wallace et al., 2006). The sample volume was measured then filtered through a pre-weighed glass-fiber filter. The filter was dried at 60° C in an oven and weighed. The filter was then ashed at 550° C in a muffle furnace and re- weighed. The epilithic sample was analyzed for ash free dry mass (AFDM) and chlorophyll *a* using the method described in Steinman et al. (2006). The sample cobble was scrubbed with a wire brush, and the resulting slurry was washed into a graduated cylinder to measure the volume. A subsample was collected and filtered through a pre-weighed glass-fiber filter.

Table 2. List of environmental variables measured in this study, with explanations.

Environmental Variable	Code for Analysis	Unit	Description
Biological			
Fine Benthic Organic Matter	FBOM	g AFDM m^{-2}	Deposited benthic organic matter < 1mm obtained from benthic water subsamples from within each benthic sample
Autotrophic Index	AI	none	AFDM (mg m^{-2}) / chlorophyll *a* (mg $^{m-2}$)
Chlorophyll *a*	ChlA	mg m^{-2}	Chlorophyll *a* extracted from epilithic material that was collected from a surface cobble collected with each benthic sample
Wood	Wood	g DM m^{-2}	Portion of CBOM (based on dry weight) composed of decomposing wood
Grass	Grass	g DM m^{-2}	Portion CBOM (based on dry weight) composed of grasses
Epilithic Biomass	Epil	g DM m^{-2}	Portion of CBOM (based on dry weight) composed of epilithic biomass
Leaves	Leav	g DM m^{-2}	Portion of CBOM (based on dry weight) composed of decomposing deciduous leaves
Miscellaneous	Misc	g DM m^{-2}	Portion of CBOM (based on dry weight) composed of unidentifiable plant material
Coarse Benthic Organic Matter	CBOM	g DM m^{-2}	Deposited benthic organic matter ≥ 1 mm from within each benthic sample
Physical			
Velocity	Vel	m/s	Average flow (n=3) at the substrate water interface of the sample location
Depth	Dep	cm	Average water depth (n=3) at the sample location
% Fines	%Fin	%	Proportion by weight of substreate sized <2mm within each benthic sample
% Fines no Cobble	%FNC	%	Proportion by weight of substreate sized <2mm within each benthic sample, excluding cobble from the total weight
% Gravel	%Gra	%	Proportion by weight of substreate sized <16, >=2mm within each benthic sample
% Pebble	%Peb	%	Proportion by weight of substreate sized <64, >=16mm within each benthic sample
% Cobble	%Cob	%	Proportion by weight of substreate sized <256, >= 64mm within each benthic sample
D50	D50	g	Median particle size determined from substrate size class weights obtained through granular sieve analysis.
Fredle Index	Fred	none	Geometric skewness as the ratio of geometric mean to geometric sorting
Trask's Sorting Coefficient	Tras	none	Substrate size homogeneity within each benthic sample (heterogeneity >1)

Chlorophyll was extracted from the filters using 90% acetone. Chlorophyll concentrations were determined using a spectrophotometer with readings taken at 664 and 750 nm before and after the addition of HCl. The filters were dried, weighed, ignited at 550° C, and weighed again to determine the ratio of organic matter to inorganic matter present. A list of environmental variables, with explanations, is provided in Table 2.

Data Analysis

Statistical analyses were performed using SAS 9.1 unless otherwise stated. Approximately 30 benthic macroinvertebrate assemblage metrics were calculated (Barbour et al. 1999). These prospective metrics were placed into one of five ecological categories (trophic, habits, pollution tolerance, diversity, richness). To reduce metric redundancy, Pearson product-moment correlations were performed among metrics those that were significantly ($p < 0.05$) and strongly ($r > 0.7$) correlated were eliminated, except that at least one metric was retained in each ecological category. This process produced a list of 12 metrics. Some commonly used metrics, such as % Shredders, were not used for this study because the values were so low that they were not useful in discerning relationships between environmental variables. Densities were also calculated for all individual taxa. Benthic macroinvertebrate metrics, densities, and environmental variables were tested for normality using the Shapiro-Wilk test. The metrics were generally found to be non-normal. Metrics that were percentages were transformed using an arcsine square root transformation. Metrics that were counts were transformed using a log (x+1) transformation. Taxa densities and environmental variables were found to be normal and no transformations were necessary.

Paired *t*-tests on metrics and taxa densities were used to determine if there were differences between seasons. Analysis of variance (ANOVA) was performed on each environmental variable, metric, and taxa density to identify differences among the reaches for a given season.

All tests were performed with an α-value of 0.05 unless otherwise noted.

Before using multivariate statistics, taxa and environmental variables were reduced to help eliminate noise in the data. Taxa comprising less than 0.2% of the total abundance were deemed to be rare and were eliminated from the multivariate analyses. The list of environmental variables was reduced with step-wise discriminant analysis.

Principal Components Analysis (PCA) was performed on the densities and environmental variables to identify patterns in reach groupings. Canonical correspondence analysis (CCA) with PCORD 4.25 was used to determine if there were relationships between any environmental variables and taxa densities. Based on the significant results of CCA, regression analysis was used to further explore relationships between environmental variables and metrics as well as individual taxa densities. Bonferroni adjustments for repeated tests were used; however, results are also reported with significant *p*-values of < 0.05.

Results

Description of Benthic Macroinvertebrate Assemblage

It was first necessary to determine if all data from the study could be pooled for analysis, or if the spring data and summer data (April versus September, respectively) should be analyzed separately. This was done by using paired *t*-tests to analyze densities of 16 individual taxa that appeared to be abundant in at least one season, density of total organisms, and 12 assemblage metrics that represented a variety of ecological information. Nine of the 16 taxa densities were significantly different between April and September (Table 3). Of those nine densities, six were significantly higher in September and three were significantly higher in April. Density of total organisms was not significantly different between seasons. Of the 12 assemblage metrics, 9 were significantly different between April and September (Table 4). Since more than half of the taxa densities and three-fourths of the assemblage metrics were significantly different, it was concluded that the data from each season should be analyzed separately. It is most likely that the seasonal differences reflect the natural life cycles of the invertebrates, especially the insects.

Table 3. Comparison of densities (organisms/m^2) for selected dominant taxa between April and September samples at all reaches (paired *t*-tests; n = 54)

Taxa	Mean		*p*-value
	April	September	
Oligochaeta	640.4	30.7	<.0001
Pleuroceridae	134.1	191.8	0.1508
Baetis complex	39.4	694.8	<.0001
Ephemerella	1310.3	7.9	<.0001
Maccaffertium	25.9	86.0	<.0001
Hydropsychidae	1405.5	7,404.9	<.0001
Cheumatopsyche	1122.5	5,544.4	<.0001
Hydropsyche	283.0	1,860.5	<.0001
Elmidae	1584.9	1,643.0	0.6615
Optioservus	1368.7	1,346.9	0.5811
Stenelmis	177.6	277.7	0.1301
Psephenus	78.5	124.7	0.1015
Antocha	815.4	1760.4	0.7821
Chironomidae	10,565.7	9,678.7	<.0001
Hemerodromia	100.6	138.7	0.9262
Simulium	78.5	554.5	0.0002
Total organisms	17,550	23,127.1	0.3909

Table 4. Comparison of metrics for benthic macroinvertebrate assemblage between April and September samples at all reaches (paired *t*-tests; n = 54)

Metrics	Mean		*p*-value
	April	September	
Number of Total Taxa	23.9	23.7	0.2828
Number of EPT Taxa	11.7	98	<.0001
Simpson Diversity Index	0.6	0.7	<.0001
Hilsenhoff Biotic Index	5.3	5.6	<.0001
Number of Sensitive Taxa	5.6	3.2	<.0001
% Diptera and Non-insects	70.3	45.6	<.0001
Number of Clinger Taxa	12.6	13.1	0.3101
% Clingers (minus Hydropsychidae)	16.8	24.6	<.0001
Number of Crawler Taxa	7.3	5.2	<.0001
% Collector Filterers	10.1	41.3	<.0001
% Predators	2.9	2.1	0.0051
% Scrapers	10.4	12.2	0.3301

A total of 158,882 organisms belonging to 91 taxa were collected during this study. A greater number of taxa occurred in April than September (Table 5). More taxa were found at the Bruce Farm (BR) reaches than the Mixed Use (MU) reaches (84 versus 63), though twice as many samples were taken at BR.

Table 5. List of all taxa collected, with mean densities (organisms/m^2)(±1 SD). "Rare" denotes taxa that comprised less than 0.2% of the total abundance for a sampling period. "A" and "S" indicate taxa that were rare in April and September, respectively.

	Bruce Farm Reaches		Mixed Use Reaches	
	April	September	April	September
Platyhelminthes: Turbellaria				
Planariidae$^{Rare:A}$	23.6 (4.1)	246.2 (31.6)	2.3 (0.5)	1.5 (0.3)
Nemertea$^{Rare:A,S}$	8.2 (1.9)	18.3 (1.6)	0.8 (0.2)	45.7 (3.3)
Nematoda$^{Rare:A,S}$		0.8 (0.2)		
Annelida				
Oligochaetaa$^{Rare:S}$	953 (122.4)	43.8 (9.2)	15.2 (1.6)	4.6 (1.2)
Hirudinea$^{Rare:A,S}$	0.4 (0.2)			
Mollusca: Gastropoda				
Ancylidae$^{Rare:A,S}$	3.4 (0.9)	49.9 (7.7)		
Physa$^{Rare:A,S}$	0.8 (0.2)	1.5 (0.5)	0.8 (0.2)	
Planorbidae$^{Rare:A,S}$	0.8 (0.3)	0.4 (0.2)		
Pleuroceridae	161.9 (19.1)	26.6 (17.4)	78.5 (6.8)	54.2 (3.1)

	Bruce Farm Reaches		Mixed Use Reaches	
	April	September	April	September
Mollusca: Bivalvia				
Corbicula fluminea[Rare:A,S]	14.5 (2.6)	21.3 (3.8)	0.8 (0.2)	
Arthropoda: Chelicerata				
Hydracarina	19 (19.9)	78.9 (5.6)	4.4 (6.7)	3.5 (0.4)
Arthropoda: Crustacea				
Cambaridae[Rare:A,S]	1.1 (0.3)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)
Lirceus[*Rare:A,S*]	14.5 (3.0)	55.6 (1.5)	0.8 (0.2)	
Arthropoda: Atelocerata				
Colembolla[Rare:A,S]	1.1 (0.3)	39.6 (3.4)		1.5 (0.3)
Ephemeroptera	2032.7	200.8	215.1	142.9
Baetidae				
Baetis complex	24.6 (2.2)	14.4 (53.9)	7.1 (1.9)	75.4 (3.8)
Caenidae				
Caenis[Rare:A,S]		1.7 (1.4)		0.8 (0.2)
Ephemerellidae				
Ephemerella[Rare:S]	1886.9 (14.3)	11.4 (1.5)	157.0 (9.6)	0.8 (0.2)
Eurylophella[Rare:A,S]	3.5 (0.8)		4.6 (1.0)	
Serratella[Rare:A]	36.6 (5.3)	73.9 (4.9)		3.5 (0.4)
Heptageniidae				
Epeorus[Rare:A,S]	34.7 (3.5)		29.7 (4.2)	1.5 (0.3)
Maccaffertium[Rare:A]	13.7 (1.7)	12.1 (6.2)	5.3 (4.8)	55.6 (3.6)
Stenacron[Rare:A,S]	2 (0.6)	2.7 (0.5)		
Isonychiidae				
Isonychia[Rare:A,S]	3.4 (0.6)	41.5 (2.9)	3.8 (0.5)	2.3 (0.5)
Leptophlebiidae[RareA,S]	0.4 (0.2)	7.2 (0.8)	2.3 (0.5)	0.8 (0.2)
Habrophlebiodes amer.[Rare:A,S]		0.4 (0.2)		
Paraleptophlebia[Rare:A,S]	0.4 (0.2)		1.5 (0.5)	
Tricorythidae				
Tricorythodes[*Rare:A,S*]	26.7 (3.3)	35.4 (5.0)	3.8 (0.8)	2.3 (0.4)
Odonata	3.4		5.3	
Coenagrionidae				
Argia[Rare:A,S]	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	
Gomphidae	0.4 (0.2)		0.8 (0.2)	2.3 (0.4)
Gomphus[Rare:A,S]	0.8 (0.2)		1.5 (0.3)	0.8 (0.2)
Lanthus[Rare:A,S]	1.5 (0.5)	0.8 (0.2)	2.3 (0.5)	4.6 (1.3)

	Bruce Farm Reaches		Mixed Use Reaches	
	April	September	April	September
Plecoptera	368.6		207.3	5.3
Chloroperlidae				
Sweltsa[Rare:A,S]	43.6 (4.3)		13.7 (1.9)	0.8 (0.2)
Leuctridae				
Leuctra[Rare:A,S]	2.3 (0.6)		0.8 (0.2)	0.8 (0.2)
Nemouridae				
Amphinemura[Rare:S]	264.8 (25.9)		163.9 (8.5)	
Soyedina[Rare:A,S]			2.3 (0.5)	
Perlidae				
Acroneuria[Rare:A,S]	0.4 (0.2)		0.8 (0.2)	3.8 (0.6)
Perlesta[Rare:A,S]	0.4 (0.2)			
Perlodidae				
Isoperla[Rare:A,S]	57.2 (6.0)		25.9 (2.3)	
Hemiptera	0.4	3.9		1.5
Corixidae[Rare:A,S]	0.4 (0.2)	2.0 (0.8)		1.5 (0.3)
Veliidae[Rare:A,S]		2.0 (0.8)		
Megaloptera	3.8	30.1	3.5	3.8
Corydalidae				
Corydalus cornutus[Rare:A,S]	7.6 (1.0)	14.5 (1.8)		2.3 (0.5)
Nigronia[Rare:A,S]	3.8 (0.7)	15.6 (1.5)	3.5 (0.5)	1.5 (0.3)
Trichoptera	683	10832.6	439.5	287
Brachycentridae				
Micrasema[Rare:A,S]	11.5 (1.4)	2.6 (3.5)		1.5 (0.3)
Glossosomatidae				
Glossosoma[Rare:A,S]	3.4 (1.0)	0.8 (0.2)	23.6 (1.8)	1.7 (1.3)
Helicopsychidae				
Helicopsyche[Rare:A,S]	33.5 (4.0)	58.3 (11.8)		
Hydropsychidae				
Cheumatopsyche	155.5 (99.8)	7826.2 (371.1)	266.7 (9.5)	98.8 (38.8)
Diplectrona[Rare:A,S]			0.8 (0.2)	
Hydropsyche	393.6 (39.2)	2711.5 (132.3)	61.7 (3.2)	158.5 (11.4)
Hydroptilidae				
Agraylea[Rare:A,S]		7.2 (1.4)		
Hydroptila[Rare:A,S]	45 (2.4)	192.4 (14.4)	2.3 (0.5)	0.8 (0.2)

	Bruce Farm Reaches		Mixed Use Reaches	
	April	September	April	September
Ochrotrichia[Rare:A,S]	1.1 (0.5)			
Lepidostomatidae				
Lepidostoma[Rare:A,S]	0.4 (0.2)		3.5 (0.7)	
Leptoceridae				
Oecetis[Rare:A,S]		2 (0.5)		
Limnephilidae				
Goera	6.9 (1.0)	12.6 (1.3)		
Pycnopsyche[Rare:A,S]	0.4 (0.2)	0.4 (0.2)		
Philopotamidae				
Chimarra[Rare:A]	1.7 (1.0)	3.1 (4.6)	39.6 (4.2)	9.7 (13.4)
Polycentropodidae				
Neureclipsis[Rare:A,S]	1.5 (0.5)			
Polycentropus[Rare:A,S]	0.4 (0.2)	1.1 (0.5)	5.3 (0.8)	6.9 (0.9)
Psychomyiidae				
Lype[Rare:A,S]		0.8 (0.2)		
Psychomyia[Rare:A,S]	12.2 (0.7)	13.3 (1.3)	10.0 (1.1)	7.6 (0.6)
Rhyacophilidae				
Rhyacophila[Rare:A,S]	0.8 (0.2)		2.3 (0.4)	0.8 (0.2)
Uenoidae				
Neophylax[Rare:A,S]	15.2 (3.6)	0.4 (0.2)	23.6 (2.6)	0.8 (0.2)
Coleoptera	454.5	2553.7	67	118.2
Dryopidae				
Helichus[Rare:A,S]		0.4 (0.2)		
Elmidae				
Dubiraphia[Rare:A,S]	49.9 (4.5)	8.4 (1.4)		
Optioservus	21.2 (153.2)	1955.1 (15.3)	13.6 (6.5)	13.3 (8.3)
Oulimnius[Rare:A,S]	0.4 (0.2)		8.4 (2.4)	3.5 (0.5)
Microcylloepus[Rare:A,S]	0.8 (0.2)	1.5 (0.4)		
Promoresia[Rare:A,S]	2.7 (0.8)	16.4 (2.2)		
Stenelmis	265.2 (25.3)	414.2 (25.9)	2.3 (0.5)	4.6 (0.7)
Psephenidae				
Ectopria[Rare:A,S]	16.4 (1.3)	16.8 (1.2)		
Psephenus herricki	97.2 (12.9)	138.7 (23.3)	41.2 (3.1)	96.8 (4.3)
Ptilodactylidae				
Anchytarsus	0.4 (0.2)		1.5 (0.3)	

	Bruce Farm Reaches		Mixed Use Reaches	
	April	September	April	September
Hydrophilidae				
*Berosus*Rare:A,S	0.4 (0.2)	0.4 (0.2)		
Diptera	16383.2	17098.5	1831.8	434
Athericidae				
*Atherix*Rare:A,S	2.7 (0.7)	5.7 (1.0)	0.8 (0.2)	0.8 (0.2)
Blephariceridae				
*Blepharicera*Rare:A,S			10 (1.7)	
Ceratopogonidae	7.2 (1.3)	0.8 (0.2)	2.3 (0.5)	
*Atrichopogon*Rare:A,S		0.4 (0.2)		
*Bezzia*Rare:A,S		3.5 (0.9)		
*Culicoides*Rare:A,S				0.8 (0.2)
*Forcipomyia*Rare:A,S				1.5 (0.3)
Chironomidae	15011.47 (461.8)	14327.6 (598.8)	1674.3 (59.7)	381.4 (25.4)
StratiomyidaeRare:A,S		0.4 (0.2)		
Simuliidae				
*Prosimulium*Rare:A,S	1.5 (0.7)			
Simulium	19 (7.9)	82.4 (91.4)	17.5 (2.1)	22.9 (2.4)
TipulidaeRare:A,S	0.4 (0.2)			
Antocha	1196.5 (45.2)	2631.8 (165.3)	53.3 (3.2)	17.5 (1.2)
*Hexatoma*Rare:A,S	25.5 (3.8)	21.3 (1.6)		
*Tipula*Rare:A,S	2.3 (0.6)	1.1 (0.3)	0.8 (0.2)	
Muscidae				
*Limnophora*Rare:A,S			0.8 (0.2)	
Empididae				
*Chelifera*Rare:A,S			3.5 (0.5)	
Hemerodromia	116.6 (9.3)	23.5 (11.7)	68.6 (5.6)	9.1 (1.0)
TabanidaeRare:A,S				
MEAN TOTAL DENSITY	21131.87	31305.7	2874.5	1107.6
TOTAL TAXA EACH SEASON	73	64	55	46
TOTAL TAXA BOTH SEASONS	84		63	

Macroinvertebrate density was high at the BR reaches, especially in September (Table 5). Density was over 7X higher at BR than MU in April and approximately 28X higher at

BR than MU in September. The most abundant organisms in the benthic macroinvertebrate assemblage were insects in the order Diptera (true flies), primarily Chironomidae (non-biting midges). Chironomidae exhibited much higher density at BR than MU in both seasons. Tipulidae (crane flies) were another group of abundant Diptera, especially *Antocha* at BR on both dates. There were relatively few taxa of Diptera, most of which were rare in occurrence.

The order Trichoptera (caddisflies) was the second most abundant component of the benthic macroinvertebrate assemblage. Most of their abundance was accounted for by two genera, *Cheumatopsyche* and *Hydropsyche*, in the family Hydropsychidae (common netspinners). Density of Hydropsychidae was slightly higher at BR than MU in April, but was much higher at BR than MU in September. Many Trichoptera taxa other than Hydropsychidae were collected in this study, many of which are considered to be sensitive to environmental stressors. Four genera of these other Trichoptera had appreciably higher density at BR than MU (*Micrasema*, *Helicopsyche*, *Hydroptila*, *Goera*), while the reverse was true for three genera (*Glossosoma*, *Chimarra*, *Neophylax*). Of these seven genera, *Hydroptila* exhibited conspicuously higher density at BR than MU.

The insect orders Coleoptera (water beetles) and Ephemeroptera (mayflies) occurred at about equal density and were the third most abundant groups of macroinvertebrates. Within the Coleoptera, Elmidae (riffle beetles), especially *Optioservus* and *Stenelmis*, accounted for most of the high density. *Psephenus herricki* in the family Psephenidae (water pennies) also occurred at an appreciable density. All of these water beetles were much more abundant at BR than MU, especially in September.

Among the Ephemeroptera, one genus, *Ephemerella*, in the family Ephemerellidae (spiny crawlers) accounted for most of the density in April. *Ephemerella* occurred almost exclusively in April, and density was much greater at BR than MU. The genus *Seratella* was the most abundant in September, and also occurred at higher density at BR than MU.

The only other groups that occurred at moderately high density were non-insect taxa: Oligochaeta (aquatic earth worms), Planariidae (flat worms), and Pleuroceridae (snails). All of these non-insect taxa were much higher at BR than MU. Oligochaeta and Pleuroceridae had their highest density in April, whereas Planariidae had their highest density in September.

Plecoptera (stoneflies) demonstrated much lower density and richness than what would be expected in a pristine stream comparable to Smith Creek. The only stonefly with moderately high density was *Amphinemura*, which had higher density at BR than MU and was only collected in April. With the exception of *Amphinemura* in April, all Plecoptera taxa were considered rare for analyses.

The Virginia Stream Condition Index (SCI) was calculated for each sample at BR and MU. The maximum score at BR was 55.89, the minimum was 27.95, and the average score was 44.86. All BR samples were classified as impaired (score < 61.3) according to the SCI. The maximum score at MU was 64.2, the minimum was 37.83, and the average was 49.86. Though MU scored slightly higher than BR, all but one sample at MU were classified as impaired (VADEQ, 2008b).

Table 6. Comparison of assemblage metric means for Bruce Farm and Mixed Use reaches in April and September (paired *t*-tests; n = 54)

	April			September		
	BR	MU	*p*-value	BR	MU	*p*-value
Taxa Richness	26.2	19.3	<.0001	28.0	15.2	<.0001
Number of EPT Taxa	12.4	10.3	0.0052	11.2	7.1	<.0001
Simpson's Diversity Index (SDI)	0.6	0.7	0.0003	0.7	0.7	0.2543
Hilsenhoff Biotic Index (HBI)	5.4	5.1	0.0402	5.6	5.5	0.006
Number of Sensitive Taxa	5.6	5.5	0.2375	3.7	2.3	0.0003
% Diptera and Non-insects	73.8	63.1	<.0001	56.2	24.4	<.0001
Number of Clinger Taxa	13.3	11.2	0.0024	14.4	10.5	<.0001
% Clingers - Hydropsychidae	15.8	18.8	0.1338	23.1	27.7	0.083
Number of Crawler Taxa	8.5	5.1	<.0001	7.0	1.7	<.0001
% Collector-Filterers	8.5	13.3	0.0015	34.4	55.3	<.0001
% Predators	1.8	5.3	<.0001	1.1	4.1	<.0001
% Scrapers	9.6	12.0	0.233	9.4	17.8	0.001

Several statistical techniques were used to examine spatial differences among the nine reaches. First, two-sample *t*-tests were used to compare the means for the 12 selected assemblage metrics in the BR section versus the MU section in April and September (Table 6). The majority of the metrics were significantly different between the two streams in both seasons.

In April, nine of the twelve metrics were significantly different between the BR and MU sites. Number of Sensitive Taxa, % Clingers-Hydropsychidae, and % Scrapers were the only metrics that were not significantly different between the two sites in April. Four of the significantly different metrics portrayed BR as having better water quality. Taxa Richness, Number of EPT, Number of Clinger Taxa, and Number of Crawler Taxa, were significantly greater at BR than MU. Five metrics indicated better water quality at MU, including Simpson's Diversity Index, Hilsenhoff Biotic Index, % Predators, % Diptera and Non-Insects, and % Scrapers.

In September, ten of the twelve metrics were significantly different between the BR and MU sites. Only SDI and % Clingers – Hydropsychidae were not significantly different. As with April, about half of the significantly different metrics tended to portray BR as having better environmental conditions, including Taxa Richness, Number of EPT Taxa, Number of Sensitive Taxa, Number of Clinger Taxa, Number of Crawler Taxa, and % Collector-Filterers. Other metrics tended to portray better environmental conditions at MU, including HBI, % Diptera and Non-insects, % Scrapers, and % Predators.

The metrics that indicated better environmental conditions at BR were mostly measures of richness, whereas the measures that indicated better environmental conditions at MU were based on relative abundance. EPT taxa are sensitive to environmental stressors. Clinger and crawler taxa require microhabitats that are relatively free of fine sediment. Collector-filterers exhibited lower relative abundance at BR, possible indicating less suspended organic matter that comes from nutrient enrichment. The HBI is a biotic index that produces a lower score when sensitive taxa are more abundant. Diptera and non-insects tend to include taxa that are tolerant of environmental stressors. Scrapers require a healthy, productive, but thin layer of nutritious algae on rock surfaces.

Table 7. Comparison of assemblage metrics among nine reaches in April 2006 (1-way ANOVA; n = 6). Reaches with the same superscript were not significantly different.

	BR1	BR2	BR3	BR4	BR5	BR6	MU1	MU2	MU3
Number of EPT Taxa	14.50^{A}	12.67^{AB}	12.17^{AB}	12.67^{AB}	11.50^{AB}	10.17^{B}	10.5^{AB}	10.00^{AB}	8.83^{AB}
HBI	5.21^{BC}	5.31^{AB}	5.41^{AB}	5.40^{AB}	5.58^{AB}	5.62^{A}	5.21^{BC}	5.14^{BC}	4.04^{C}
Num of Sensitive Taxa	6.83^{A}	5.50^{A}	6.33^{A}	6.33^{A}	3.83^{A}	4.33^{A}	6.50^{A}	4.83^{A}	4.50^{A}
Taxa Richness	28.67^{A}	27.33^{AB}	26.33^{ABC}	26.67^{A}	23.17^{ABCD}	23.50^{ABCD}	20.33^{BCD}	18.50^{CD}	15.83^{D}
SDI	0.59^{BC}	0.63^{ABC}	0.63^{AB}	0.59^{ABC}	0.58^{ABC}	0.55^{C}	0.66^{AB}	0.65^{AB}	0.62^{A}
% Clingers - Hydropsychidae	16.60^{A}	17.00^{A}	17.87^{A}	16.01^{A}	18.71^{A}	15.89^{A}	15.64^{A}	18.16^{A}	18.51^{A}
Number of Clinger Taxa	15.00^{A}	13.83^{A}	13.33^{A}	13.00^{A}	12.00^{A}	12.33^{A}	11.17^{A}	11.17^{A}	9.17^{A}
Number of Crawler Taxa	10.17^{A}	9.17^{AB}	8.50^{AB}	8.67^{AB}	7.33^{AB}	6.33^{BC}	5.50^{C}	4.33^{C}	4.50^{C}
% Scrapers	13.11^{AB}	9.76^{AB}	10.76^{AB}	11.25^{AB}	5.62^{AB}	7.08^{B}	8.31^{AB}	11.01^{AB}	13.85^{A}
% Collector-Filterers	3.82^{A}	8.84^{BC}	8.91^{ABC}	13.24^{AB}	10.39^{ABC}	8.45^{BC}	11.5^{AB}	12.00^{ABC}	13.46^{A}
% Predators	2.43^{BC}	1.80^{BC}	2.92^{BC}	1.05^{BC}	0.97^{C}	2.23^{C}	7.56^{A}	4.17^{BC}	2.89^{AB}
% Diptera and Non-insects	69.17^{BC}	70.66^{B}	72.85^{B}	68.30^{BC}	77.45^{B}	81.31^{A}	66.62^{BC}	65.81^{BC}	46.87^{C}

ANOVAs were performed and followed by Tukey's post hoc test if there were significant differences among sites. Tukey's post hoc test was used for further analysis of spatial groupings among the three MU and six BR reaches in April and September (Tables 7 and 8). Since there were 12 individual metrics and 9 reaches, it was difficult to distinguish consistent groupings. Upstream BR reaches (BR1-3) frequently grouped together, but not exclusively so. Downstream BR reaches (BR5-6) also often grouped together, although BR6 also frequently grouped with the MU reaches. This is likely because BR6 has a narrow riparian zone with a few trees, unlike the other BR reaches. The three MU reaches usually grouped together but almost never exclusively separate from the BR reaches.

Table 8. Comparison of assemblage metrics among nine reaches in September 2006 (1-way ANOVA; n = 6). Reaches with the same superscript were not significantly different.

	BR1	BR2	BR3	BR4	BR5	BR6	MU1	MU2	MU3
Number of EPT Taxa	13.00^{A}	10.67^{ABC}	11.83^{AB}	10.17^{ABCD}	11.17^{AB}	10.33^{ABCD}	6.50^{D}	7.00^{CD}	7.67^{BCD}
HBI	5.57^{A}	5.58^{A}	5.57^{A}	5.69^{A}	5.67^{A}	5.61^{A}	5.49^{A}	5.51^{A}	5.40^{A}
Number of Sensitive Taxa	4.00^{AB}	3.83^{AB}	4.67^{A}	3.50^{ABC}	3.50^{AB}	2.83^{ABC}	2.33^{BCD}	1.67^{C}	2.83^{ABC}
Taxa Richness	31.67^{A}	27.33^{A}	29.33^{A}	25.50^{A}	27.17^{A}	2683^{A}	15.83^{B}	14.17^{B}	15.67^{B}
SDI	0.69^{A}	0.67^{A}	0.71^{A}	0.76^{A}	0.79^{A}	0.67^{A}	0.76^{A}	0.73^{A}	0.72^{A}
% Clingers - Hydropsychidae	19.56^{A}	20.15^{A}	21.89^{A}	25.71^{A}	28.95^{A}	22.27^{A}	26.81^{A}	25.76^{A}	30.62^{A}
Number of Clinger Taxa	15.00^{A}	13.67^{AB}	15.17^{A}	13.50^{AB}	15.17^{A}	13.83^{AB}	11.17^{AB}	10.00^{B}	10.33^{B}
Number of Crawler Taxa	9.00^{A}	6.50^{A}	7.00^{A}	6.33^{A}	7.33^{A}	5.83^{A}	1.17^{B}	1.67^{B}	2.17^{B}
% Scrapers	8.46^{AB}	4.98^{B}	10.30^{AB}	8.43^{AB}	12.21^{AB}	11.81^{AB}	20.33^{A}	12.57^{AB}	20.40^{A}
% Collector-Filterers	29.65^{B}	28.51^{B}	27.60^{B}	46.05^{AB}	44.55^{AB}	29.81^{B}	53.35^{A}	57.78^{A}	54.77^{A}
% Predators	1.16^{B}	1.02^{B}	1.07^{B}	1.18^{B}	0.91^{B}	1.35^{B}	5.07^{A}	3.53^{A}	3.73^{A}
% Diptera and Non-insects	61.23^{AB}	66.15^{A}	59.86^{AB}	5145^{AB}	42.24^{BC}	56.02^{AB}	26.50^{CD}	25.89^{CD}	20.90^{D}

Since univariate ANOVAs with 12 assemblage metrics provided only limited insight into spatial patterns among the nine reaches, a multivariate ordination technique, Principal Components Analysis (PCA), was performed on densities of all common taxa. Rather than classifying groups of reaches, ordination arranges the reaches in all possible gradients (axes) of how they relate to one another based on "species space," then chooses and displays the best two gradients (axis 1 and axis 2). Multivariate ordination techniques, such as PCA, offer the advantage of integrating information on all of the taxa simultaneously rather than interpreting many individual univariate analyses.

In April (Fig. 2), the first two axes of the PCA explained 52.1% of the relationships among the reaches based on taxa, with 37.2% of that being explained by axis 1 (vertical). The MU reaches are clearly arranged together on one end of the gradient, and BR 1 lies on the other end. BR3, BR2, BR5, and BR 6 are arranged in intermediate positions along the gradient created by axis 1. BR4 appears to be more influenced by the gradient along axis 2. Using the intraset correlation coefficients, the taxa most responsible for the gradient along axis 1 (Eigenvalues > 0.25) in April are: *Optioservus, Ephemerella, Chironomidae, Stenelmis. Hemerodromia, Psephenus, Amphinemura, and Isoperla.*

In September (Fig. 3), the first two axes of the PCA explained 55.1% of the relationships among the reaches based on taxa, with 40.9% of that being explained by axis 1 (vertical). Again, the MU reaches are clearly arranged together on one end of the gradient, and BR 2 lies on the other end. BR3, BR4, BR1, and BR 6 are arranged in intermediate positions along the gradient created by axis 1. BR5 appears to be more influenced by the gradient along axis 2. It is interesting to note that BR6 lies closest to the MU reaches for April and September, similar to what was noted in the results of ANOVA. Using the intraset correlation coefficients, the taxa most responsible for the gradient along axis 1 (Eigenvalues > 0.25) in September are: *Cheumatopsyche*, *Optioservus, Chironomidae, Hemerodromia, Hydropsyche, Serratella, Baetis, Stenelmis, Antocha.*

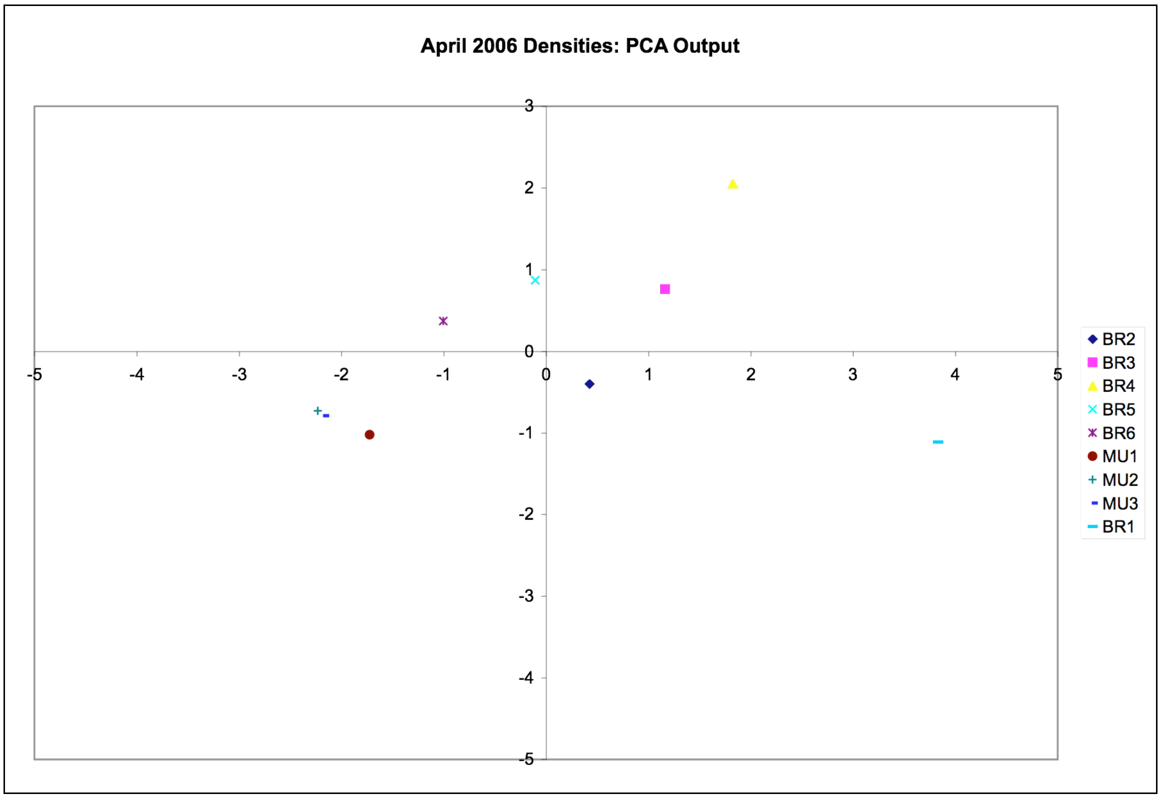

Figure 2. Principal Components Analysis on April 2006 densities. Axis 1 is vertical; axis 2 is horizontal.

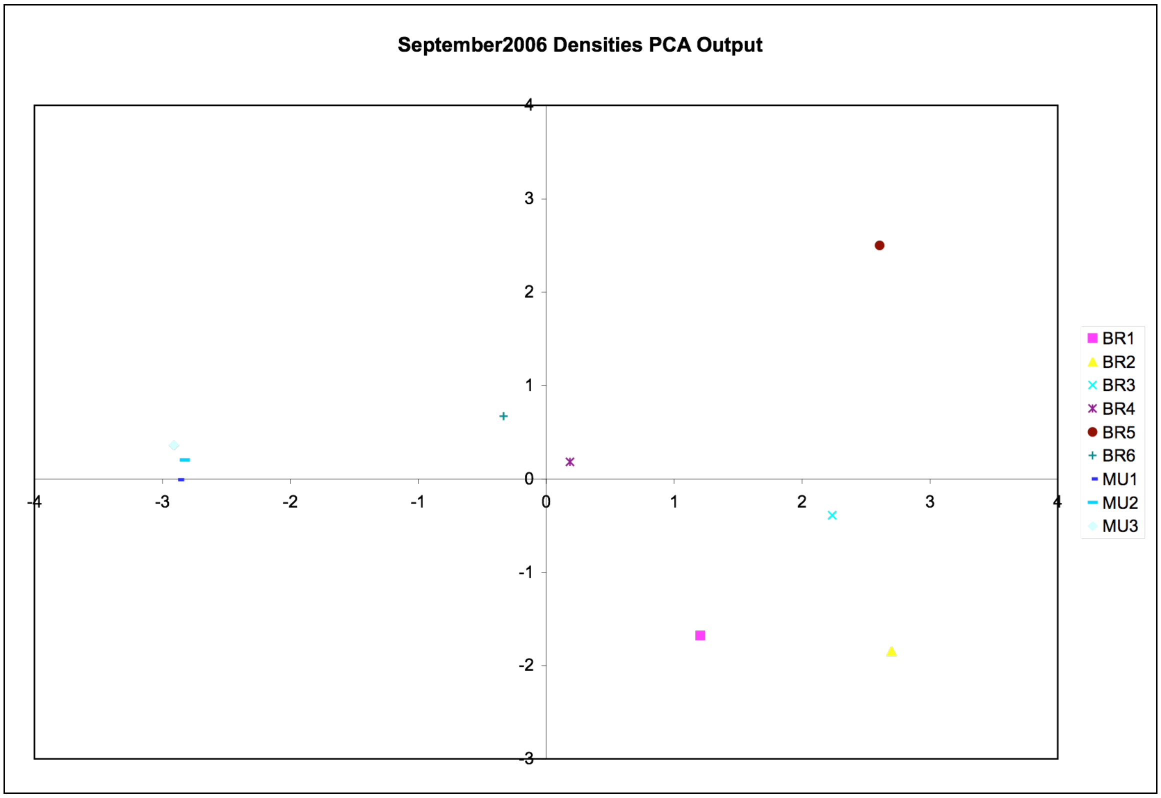

Figure 3. Principal Components Analysis on September 2006 densities. Axis 1 is vertical; axis 2 is horizontal.

Relationships of environmental factors and benthic macroinvertebrate assemblages.

An array of environmental measurements, both biological and physical, was taken in an attempt to explain the benthic macroinvertebrate assemblage (Table 9). Among the biological variables, measures of organic matter related to primary productivity (AFDM, chlorophyll *a*, epilithic biomass) were greater at BR than MU for both seasons.

Among the physical variables, some of the measures of inorganic substrate demonstrated some trends between BR and MU. MU had a greater proportion of coarse substrate, especially cobble, while BR had a higher proportion of fine substrate. This was consistent for percents, weights, and D values. There was little difference between seasons. The two indices of inorganic substrate, the Fredle Index and Trask's Sorting Coefficient, varied little between the two stream sections and between seasons.

We used a multivariate technique, Canonical Correspondence Analysis (CCA), to identify the primary environmental variables that structure the assemblage (Fig. 4 A and B and Tables 10, 11). The results of the CCA for September data were more meaningful than April and will be emphasized. For the April data, the first 3 axes generated by CCA explained approximately 25% of the taxa-environmental variable relationship, but the Monte Carlo permutation procedure indicated that the results of the first two axes, which accounted for 18% of the relationship, were not significant ($p > 0.05$).

Table 9. Summary of environmental measurements (means ±1 SD; n = 54)

	Bruce Farm		Mixed Use	
	April	September	April	September
Biological				
FBOM	24.8 (20.9)	18.1 (14.9)	16.1 (11.6)	29.2 (19.6)
AFDM	98.1 (55.1)	63.3 (25.2)	44.6 (20.4)	17.8 (11.6)
Autotrophic Index	14.3 (14.0)	2.9 (1.2)	12.8 (9.6)	5.6 (3.8)
Chlorophyll A	8.8 (5.1)	25.6 (13.4)	4.8 (3.4)	3.9 (2.2)
Wood	34.6 (58.5)	37.9 (63.0)	11.5 (16.8)	7.4 (12.5)
Leaves	0 (0)	18.3 (22.0)	0 (0)	7.5 (10.4)
Grass	1.2 (1.9)	1.4 (2.2)	0.5 (0.7)	0.1 (0.1)
Epilithic Biomass	30.1 (25.0)	16.8 (15.4)	0.1 (0.3)	0.2 (0.3)
Miscellaneous	4.1 (8.6)	0 (0)	2.2 (3.9)	0 (0)
Total CBOM	76.4 (57.9)	74.4 (70.0)	21.1 (20.6)	15.2 (16.1)
Physical				
Velocity	0.6 (1.1)	0.4 (0.2)	0.7 (1.4)	0.5 (0.9)
Depth	12.7 (4.0)	11.7 (3.1)	11.9 (4.0)	11.0 (2.0)
Total Inorganic Weight	8333.5 (3420.7)	11785.9 (16488.1)	10745.1 (3064.0)	10969.8 (3123.8)
% Fines	3.0 (3.0)	3.4 (2.7)	1.2 (0.7)	2.8 (2.6)
% Fines no Cobble	5.9 (5.9)	7.1 (7.2)	3.2 (1.8)	7.0 (5.5)
% Gravel	10.5 (8.2)	10.9 (7.2)	8.0 (2.5)	9.4 (5.3)
% Pebble	40.5 (24.4)	37.2 (21.1)	29.9 (7.5)	26.6 (12.8)
% Cobble	46.0 (26.9)	48.4 (26.2)	60.8 (8.5)	61.2 (16.9)
Fines (g)	212.1 (213.4)	268.2 (233.5)	137.5 (107.5)	280.4 (241.5)
Gravel (g)	745.4 (502.4)	841.1 (416.2)	831.2 (332.1)	948.6 (414.4)
Pebble (g)	2909.9 (1476.2)	2913.5 (1358.9)	3184.0 (1221.1)	2695.6 (1005.4)
Cobble (g)	4417 (3365.9)	7763.2 (17075.6)	6592.4 (2245.0)	7045.3 (3338.3)
D16	26.2 (16.1)	24.3 (15.5)	29.3 (8.1)	27.3 (12.7)
D25	37.4 (19.9)	39.1 (18.8)	44.0 (10.2)	45.1 (18.0)
D50	78.1 (91.4)	71.6 (27.4)	86.5 (20.2)	83.0 (24.7)
D75	93.8 (30.5)	100.4 (29.8)	129.5 (36.4)	111.9 (27.5)
D84	104.8 (31.5)	112.3 (29.1)	143.1 (43.3)	122.0 (27.2)
Fredle	32.7 (18.0)	32.0 (16.5)	37.6 (9.0)	36.4 (15.2)
Trask	2.3 (0.8)	2.4 (0.6)	2.2 (0.4)	2.3 (0.6)

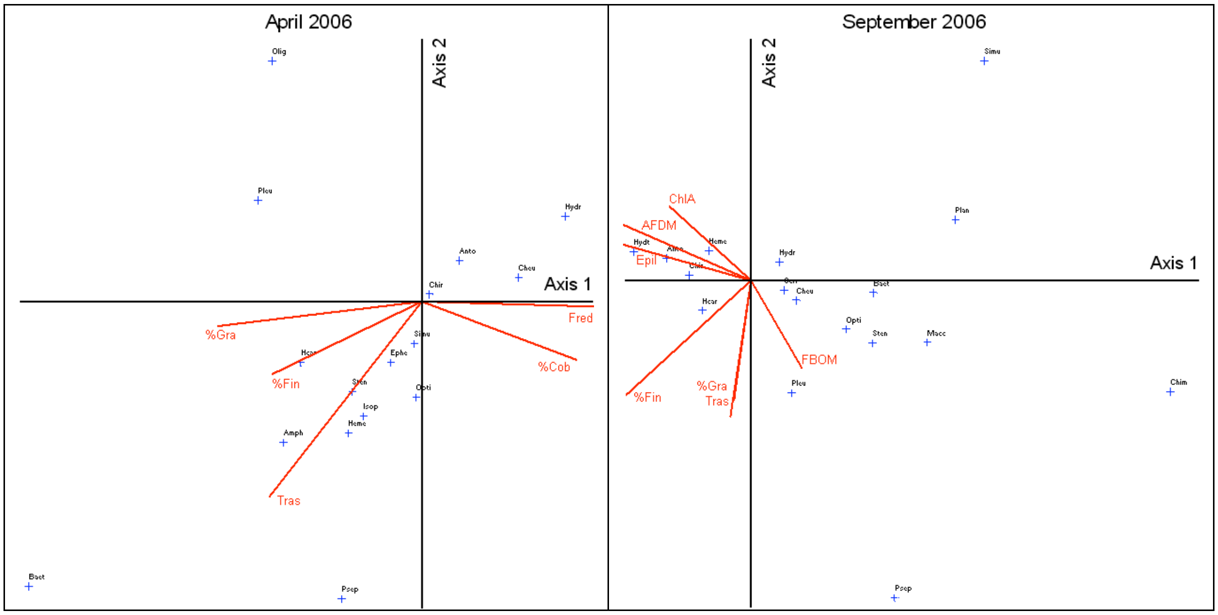

Fig. 4. Results from CCA for (A) April 2006 and (B) September 2006. Environmental variable codes are listed in Table 11. Taxa codes: Olig = Oligocheata, Pleu = Pleuroceridae, Plan = Planariidae, Hcar = Hydracarina, Ephe = *Ephemerella*, Macc = *Maccaffertium*, Baet = *Baetis Complex*, Serr = *Serratella*, Amph = *Amphinemura*, Isop = *Isoperla*, Sten = *Stenelmis*, Opti = *Optioservus*, Psep = *Psephenus*, Simu = *Simulium*, Heme = *Hemerodromia*, Chir = *Chironomidae*, Anto = *Antocha*, Chim = *Chimarra*, Hydt = *Hydroptila*, Hydr = *Hydropsyche*, Cheu = *Cheumatopsyche.*

The CCA for the September data showed good relationships between taxa densities and environmental variables. The first three axes explained 32.2% of the relationships, with axis 1 explaining 18.8% and Axis 2 explaining 8.7% (Table 10). The Monte Carlo permutation procedure indicated that Axes 1 and 2 were both significant. Axis 1, which explained the majority of the variance, was largely determined by biological variables related to plant growth on the rocks (AFDM, epilithic biomass, chlorophyll *a*). The relationships represented on axis 2, which explained less than half as much of the variance as axis 1, were mostly due to two physical substrate variables (% gravel and Trask's sorting coefficient) and one biological variable (FBOM). Percent fines, which is a physical substrate variable, was also an important environmental variable, but its influence was equally split between axis 1 and axis 2. Although FBOM involves biologically derived organic matter, its influence on the benthic macroinvertebrates is likely to involve the physical composition of the substrate much like fine inorganic sediment. All of these relationships were negative. From CCA it was concluded that the plant growth on the rocks was the primary factor determining the benthic macroinvertebrate assemblage (axis 1), with fine sediments in the substrate being second in importance (axis 2).

Table 10. Summary of CCA results for macroinvertebrate taxa densities and environmental variables. *P*-values derived from Monte-Carlo permutation procedure.

	April			September		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Eigenvalue	0.035	0.033	0.026	0.08	0.037	0.02
% variance explained	9.4	8.6	7.0	18.8	8.7	4.8
Cumulative % variance explained	9.4	18	24.9	18.8	27.4	32.2
p-value	0.785	0.15	0.005	0.005	0.03	0.1

Table 11. Intraset correlation coefficients between environmental variables and CCA axes.

	September		
	Axis 1	Axis 2	Axis 3
FBOM	0.179	-0.348	0.047
AFDM	-0.57	0.28	-0.002
Chlorophyll *a*	-0.291	0.297	0.002
Wood	0.174	0.116	-0.24
Leaves	0.211	0.385	-0.558
Grass	-0.341	0.166	-0.563
Epilithic Biomass	-0.466	0.146	0.31
Velocity	-0.002	0.45	0.381
% Fines	-0.445	-0.456	0.097
% Gravel	-0.062	0.476	0.366
% Pebble	-0.287	0.052	0.529
% Cobble	0.289	0.132	-0.531
Fredle Index	0.129	0.231	-0.572
Trask Sorting Coefficient	-0.073	-0.545	0.026

Based on CCA results, six environmental variables were chosen for further analysis as dependent variables in univariate linear regressions: AFDM, chlorophyll *a*, epilithic biomass, % fines, % gravel, and Trask's sorting coefficient. The independent variables used in the regressions included 15 taxa that emerged as important in CCA and the group of 12 metrics that were found to be useful for summarizing the benthic macroinvertebrate assemblage. Regression analyses only involved the September data because of the weak results of CCA on the April data.

Table 12. Regression analysis of taxa densities versus environmental variables in September 2006. Bonferonni adjusted *p*-value = 0.0002. Bold indicates *r* value > 0.5

	AFDM	Chl*a*	Epilithic Biomass	% Fines	% Gravel	Trask
Psephenus	-0.31386					
	0.0208					
Stenelmis	**0.6291**	**0.6166**	**0.52966**	0.28796	0.38128	0.29876
	0.0001	**0.001**	**0.0001**	0.0347	0.0044	0.0282
Optioservus	**0.56714**	**0.60623**	**0.52432**	0.41749	**0.503**	0.43674
	0.0001	**0.0001**	**0.0001**	0.0017	**0.0001**	0.001
Hydropsyche	**0.55503**	**0.58182**				
	0.0001	**0.0001**				
Cheumatopsyche	**0.69587**	**0.69294**	**0.65593**			
	0.0001	**0.0001**	**0.0001**			
Baetis	**0.51432**	**0.56969**	0.46985			
	0.0001	**0.0001**	0.0003			
Maccaffertium		0.34144				
		0.0115				
Chironomidae	**0.7757**	**0.71678**	**0.66687**			
	0.0001	**0.0001**	**0.0001**			
Hemerodromia	**0.71998**	**0.66583**	**0.60576**			
	0.0001	**0.0001**	**0.0001**			
Pleuroceridae	0.30713	0.29606		40981	0.42786	0.37941
	0.0239	0.0297		0.0021	0.0012	0.0047
Antocha	**0.7235**	**0.69042**	**0.64237**			
	0.0001	**0.0001**	**0.0001**			
Simulium	**0.50442**	**0.5662**	0.4678			
	0.0001	**0.0001**	0.0004			
Planariidae	0.45657	**0.50972**	**0.65287**		0.28936	
	0.0005	**0.0001**	**0.0001**		0.0338	
Hydroptila	**0.68863**	**0.63078**	**0.71504**	0.29841	0.27381	
	0.0001	**0.0001**	**0.0001**	0.0284	0.0451	
Chimarra	-0.39464	-0.30581		-0.2717		
	0.0031	0.0245		0.0469		

The 90 regressions of individual taxa versus environmental variables (Table 12) produced 51 relationships that were significant at $p < 0.05$, 30 of which were also significant at the more conservative Bonferroni adjustment of $p < 0.0002$. All but one of these relationships involved biological environmental variables related to plant growth on rocks. The only significant relationship involving a physical environmental variable and density of a taxon was *Optioversus* versus % Gravel. Moderately strong relationships could be considered to be those with an r-value > 0.5 ($r^2 > 0.25$, thus explaining 25% of the variance). Eleven of the fifteen taxa had moderately strong relationships with one of more of the three biological environmental variables related to plant growth on rocks (usually all three). Strong relationships could be considered to be those with an r value >

0.71 ($r^2 > 0.50$, thus explaining 50% of the variance). Four of the fifteen taxa (Chironomidae, *Hemerodromia*, *Antocha*, *Hydroptila*) had strong relationships with one of more of the three biological environmental variables related to plant growth on rocks. Chironomidae in particular had a strong relationship ($r = 0.7757$) with AFDM from the chlorophyll analysis from the rocks as shown in Figure 5. Also notable in Figure 5 is the difference between BR and MU. Table 12 contains all regressions for which $p < 0.05$. Regressions that were significant with the Bonferroni adjustment are indicated by asterisks, and those that were moderately strong ($r > 0.5$) are in bold.

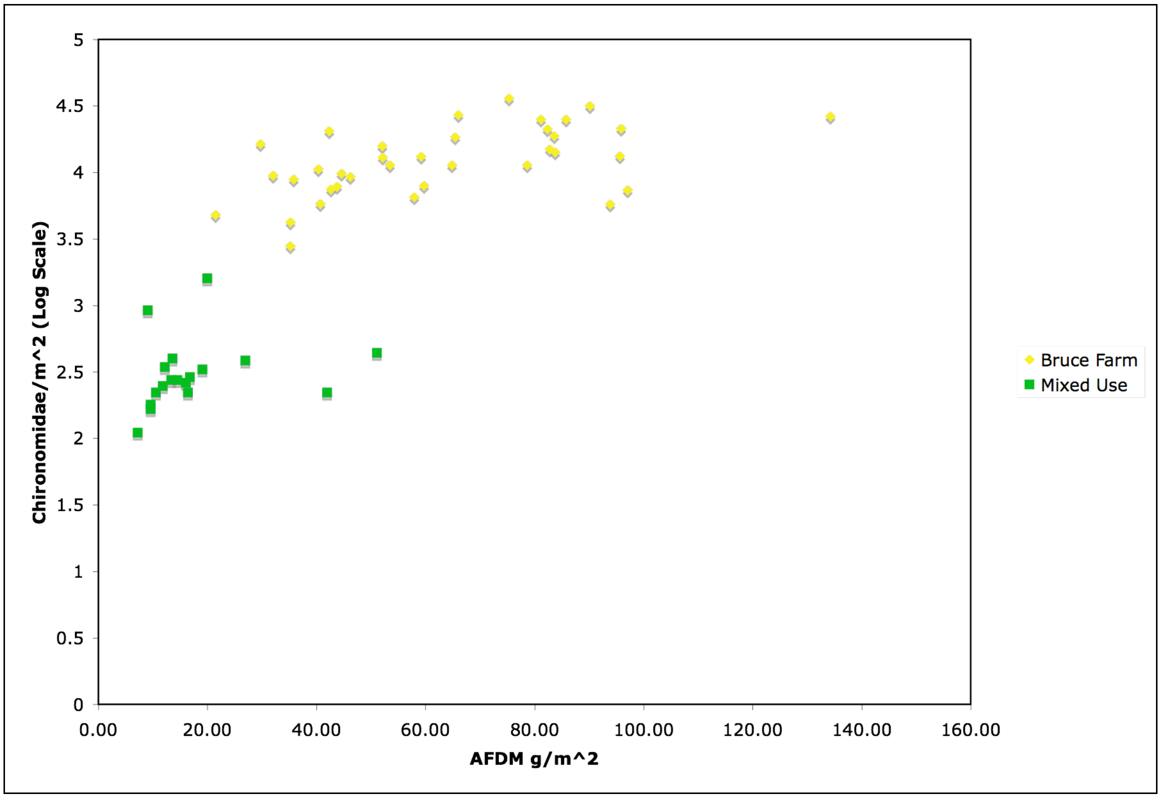

Figure 5. Regression analysis of Chironomidae versus AFDM in September 2006.

The 72 regressions of assemblage metrics versus environmental variables (Table 13) produced 48 relationships that were significant at $p < 0.05$, 21 of which were also significant at the more conservative Bonferroni adjustment of $p < 0.0003$. All but two of these relationships involved biological environmental variables related to plant growth on rocks. For example, Total Richness and Epilithic Biomass were significantly related ($r = 0.59005$) and the difference between BR and MU is apparent in Figure 6. The only significant relationships involving a physical environmental variable and a metric was Number Sensitive Taxa versus % Fines and % Scrapers versus Trask's Sorting Coefficient. Seven of the 12 metrics had moderately strong relationships ($r > 0.5$) with one of more of the three biological environmental variables related to plant growth on rocks. None of the metrics had a strong relationship ($r > 0.71$) with an environmental variable.

Table 13. Regression analysis of metrics versus environmental variables in September 2006. Bonferonni adjusted p-value = 0.0003.

	AFDM	ChlA	Epilithic Biomass	% Fines	% Gravel	Trask
#EPT	**0.51699**	**0.53004**	**0.52095**	0.36109	0.2789	0.26856
	0.0001	**0.0001**	**0.0001**	0.0073	0.0411	0.0496
HBI		0.31518		-0.28608	-0.34772	
		0.0203		0.036	0.01	
# Sensitive Taxa	0.44226	0.44606	**0.50546**	**0.52218**	0.31419	0.412
	0.0008	0.0007	**0.0001**	**0.0001**	0.0207	0.002
Total Richness	**0.61958**	**0.61115**	**0.59005**	0.32061	0.29269	
	0.0001	**0.0001**	**0.0001**	0.0181	0.0323	
SDI					0.35221	0.29161
					0.009	0.0324
% Clingers - Hydropsyche	-0.31645				0.30681	
	0.0197				0.024	
# Clinger Taxa	0.48422	**0.5111**	**0.50065**		0.29344	
	0.0002	**0.0001**	**0.0001**		0.0313	
# Crawler Taxa	**0.65233**	**0.58815**	**0.51394**	0.26818		
	0.0001	**0.0001**	**0.0001**	0.0499		
% Scrapers	-0.47465	-0.35328	-0.35677	0.38972	0.42683	0.48071
	0.0003	0.0088	0.0081	0.0036	0.0013	0.0002
% Collector Filterers	-0.499	-0.3749	-0.43151	-0.39767		
	0.0001	0.0052	0.0011	0.0029		
% Predators	**-0.57738**	**-0.56325**	-0.46381			
	0.0001	**0.0001**	0.0004			
% Diptera and Non-insects	**0.65703**	**0.54839**	**0.51509**			
	0.0001	**0.0001**	**0.0001**			

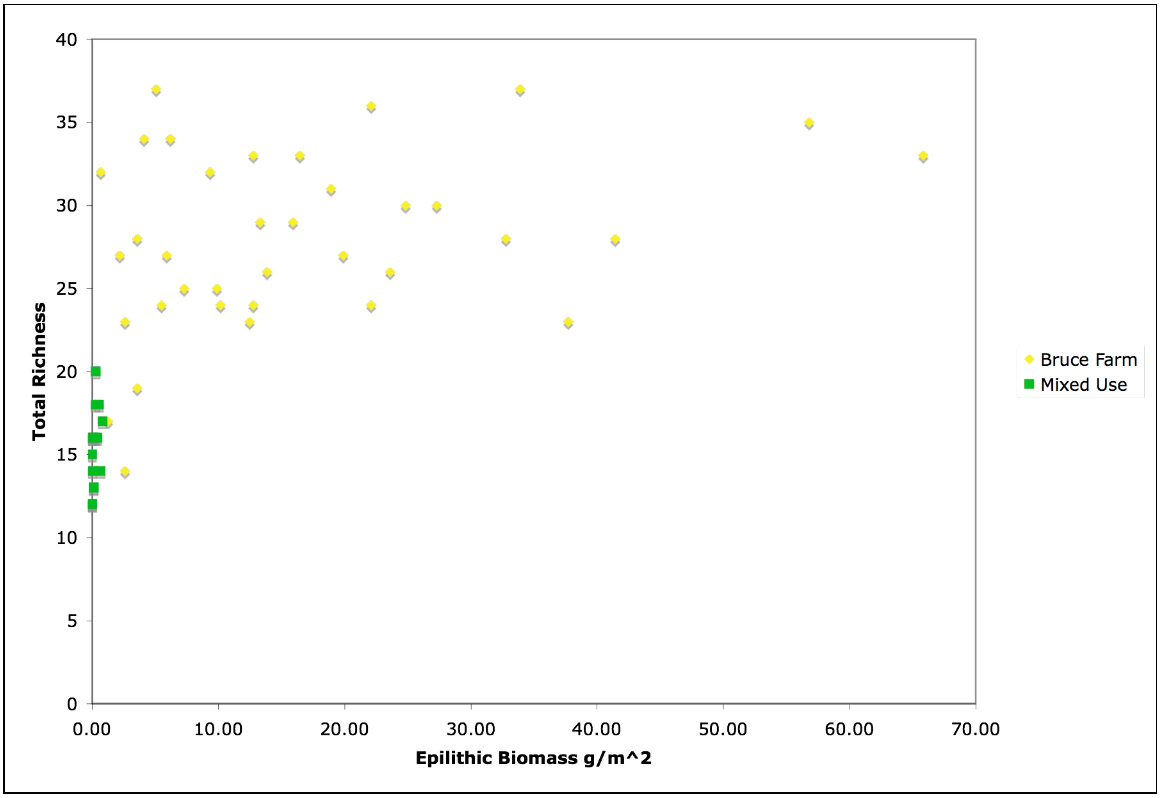

Figure 6. Regression analysis of Total Richness versus Epilithic Biomass for September 2006.

Visual Estimates Versus Measurements of Habitat Variables for Explaining Macroinvertebrate Assemblages

The RBP is used to quickly assess the quality of stream habitat in relation to the aquatic biota. The quantitative methods used to measure habitat variables in this study are more intensive and time consuming than the visual estimates used in the RBP, but may yield data that are more accurate and, thus, may better explain the relationships between habitat and benthic macroinvertebrate assemblage structure. Pearson product-moment correlation was first used to compare 9 habitat variables that were visually estimated with 15 related habitat variables that were measured (Table 14). The assumption was that quantitative measurement of habitat variables produced "correct" results, thus, estimated habitat variables must be correlated with measured variables in order to be effective.

In general, there was little correlation between estimated habitat variables and measured habitat variables that should be closely related. Epifaunal Substrate/Available Cover and the Total RBP Score correlated moderately strongly ($r > 0.5$) with % Cobble and the Fredle Index and had a moderately strong negative ($r > 0.5$) correlation with the other substrate measures (Table 14). Embeddedness estimates had a few weak correlations with measurements, most notably a negative correlation with % fines. Velocity/Depth estimates had a negative correlation with depth measurements and no correlation with velocity measurements. There was a weak negative correlation between Velocity/Depth estimates and measurements of % fines. The % Periphyton and % Macrophytes estimates had no significant correlation with measurements of epilithic biomass or chlorophyll *a*. The % Slime estimates correlated moderately strongly ($r > 0.5$) with measurements of AFDM, and weakly ($r < 0.5$) with FBOM (negatively), chlorophyll *a*, and epilithic biomass measurements. The % CPOM estimates correlated strongly ($r = 0.76129$) with measurements of leaves and weakly ($r = .38913$) with grass. Percent Large Woody Debris correlated weakly ($r = .27186$) with measurements of wood.

Table 14. Pearson product-moment correlation coefficients between estimated habitat variables (top row) and measured habitat variables (left column). Only significant results ($p < 0.05$) are included. Bonferroni adjusted p-value = 0.0002. Bold indicates $r > 0.5$.

	Epifaunal Substrate/ Available Cover	Embedd-edness	Velocity/ Depth	Total RBP Score	% Peri-phyton	% Macro-phytes	% Slimes	% CPOM	% LWD
FBOM							-0.2719		
							0.0467		
AFDM	**-0.5214**			-0.2806			**0.5485**		
	0.0001			0.0398			**0.0001**		
Chl*a*	-0.4277						0.4851		
	0.0013						0.0002		
Wood					0.3489				0.2719
					0.0097				0.0467
Leaves			0.4226	0.3566				**0.7613**	
			0.0015	0.0081				**0.0001**	
Grass								0.3891	
								0.0036	
Epilithic Biomass	-0.2704						0.4949		
	0.0480						0.0001		
Velocity									
Depth		-0.2694	-0.3925	-0.3382					
		0.0489	0.0033	0.0124					
% Fines	**-0.5742**	-0.3768	-0.2824	**-0.5543**					
	0.0001	0.005	0.0385	**0.0001**					
% Gravel	**-0.5219**			-0.3596					
	0.0001			0.0076					
% Pebble	**-0.6841**			**-0.5299**					
	0.0001			**0.0001**					
% Cobble	**0.7519**	0.2981		**0.5822**					
	.0001	0.0286		**.0001**					
Fredle	**0.6161**	0.3315		**0.5059**				0.2994	
	0.0001	0.0143		**0.0001**				0.0278	
Trask	-0.3650			-0.2818					
	0.0067			0.039					

In addition to examining the correlation between estimated and measured habitat variables, it is also important to determine whether estimated or measured habitat variables best explain the benthic macroinvertebrate assemblage. To examine this question, Pearson product-moment correlation was used to compare 9 estimated habitat variables with 12 assemblage metrics and the same estimated habitat variables with 15 dominant individual taxa. The best choice between estimated versus measured habitat variables would be the one that demonstrate the highest number of significant correlations. There were a number of relatively weak correlations between the estimated habitat variables and the metrics. Epifaunal Substrate/Available Cover was negatively correlated ($r < 0.5$) with Number of EPT, Number of Sensitive Taxa, Total Richness, Number of Crawler Taxa, and % Diptera and Non-Insects. The only positive and moderately strong correlation ($r = 0.51411$) was with % Collector-Filterers. Embeddedness positively correlated with the Hilsenhoff Biotic Index (r = 0.49626) and negatively correlated with % Predators ($r = -0.32385$). Velocity Depth had a weak correlation with % Clingers minus Hydropsychidae (r = 0.28058). The Total RBP Score correlated weakly ($r < 0.5$) with the HBI, % Collector-Filterers, and % Diptera and Non-Insects (negatively). Percent Periphyton correlated weakly ($r < 0.5$) with Total Richness, Number of Clinger Taxa, and # of Crawler Taxa. Percent Macrophytes had a weak ($r < 0.5$) negative correlation with Simpson's Diversity Index (SDI), and % Collector-Filterers and a weak positive correlation with % Diptera and Non-Insects ($r = 0.319$). Percent Slimes had the most correlations of any of the visually estimated habitat variables. It demonstrated a moderately strong positive correlation ($r > 0.5$) with Total Richness, Number of Crawler Taxa, and % Diptera and Non-Insects, and negative correlation with % Predators ($r = -0.61721$). Percent CPOM had a weak correlation with the HBI ($r = 0.29008$), and % LWD was not correlated with any metrics. The correlations between measured habitat variables and metrics were presented previously in Table 9.

Table 15. Pearson product-moment correlation coefficients between estimated habitat variables (top row) and metrics (left column). Only significant results ($p < 0.05$) are inclu
adjusted p-value = 0.0002. Bold indicates $r > 0.5$.

	Epifaunal Substrate/ Available Cover	Embeddedness	Velocity/ Depth	Total RBP Score	% Periphyton	% Macrophytes	% Slimes	% CI
Number of EPT Taxa	-0.34508						0.43127	
	0.0106						0.0011	
HBI		0.49626		0.29078			0.36891	0.290
		0.0001		0.0329			0.0061	0.033
Number of Sensitive Taxa	-0.33345						0.38185	
	0.0137						0.0044	
Total Richness	-0.37109				0.27561		**0.55438**	
	0.0057				0.0437		**0.0001**	
SDI						-0.37704		
						0.0049		
% Clingers - Hydropsyche			0.28058					
			0.0399					
Number of Clinger Taxa					0.27159		0.47121	
					0.047		0.0003	
Number of Crawler Taxa	-0.38266				0.27615		**0.61912**	
	0.0043				0.0432		**0.0001**	
% Scrapers							-0.43303	
							0.0011	
% Collector-Filterers	**0.51411**			0.37042		-0.30855	-0.49192	
	<.0001			0.0058		0.0232	0.0002	
% Predators		-0.32385					**-0.61721**	
		0.0169					**0.0001**	
% Diptera and Non-insects	-0.45461			-0.26904		0.319	**0.62946**	
	0.0006			0.0492		0.0187	**0.0001**	

The results of Pearson product-moment correlation analysis for the nine visually estimated habitat variables and 15 dominant taxa are presented in Table 16. Epifaunal Substrate/Available Cover was weakly negatively correlated ($r < 0.5$) with Elmidae, *Stenelmis*, *Optioservus*, *Cheumatopsyche*, Hydropsychidae, Chironomidae, *Hemerodromia*, Pleuroceridae, *Antocha*, Oligochaeta and Total Abundance. Embeddedness was weakly positively correlated ($r < 0.5$) with *Baetis* complex, *Maccaffertium*, and *Simulium*. Velocity/Depth was only weakly ($r = 0.27162$) correlated with *Simulium*. The Total RBP Score was weakly correlated with *Baetis* complex ($r = 0.35643$). Percent Periphyton was weakly correlated ($r < 0.5$) with Elmidae, *Stenelmis*, *Ephemerella*, *Baetis* complex, *Maccaffertium*, and *Simulium*. Percent Macrophytes had a negative correlation with *Psephenus* ($r = 0.29669$) and was positively correlated with Oligochaeta ($r = 0.36778$). Percent Slimes had the greatest number of moderately strong correlations of any visually estimated habitat variable. It was moderately strongly correlated ($r > 0.5$) with Elmidae, *Stenelmis*, *Cheumatopsyche*, Hydropsychidae, *Baetis* complex, Chironomidae, *Hemerodromia*, *Antocha*, *Simulium*, and Total Abundance as well as having weak correlations with five other taxa densities. The correlations between measured habitat variables and dominant taxa densities were presented previously in Table 10.

Table 16. Pearson product-moment correlations between estimated habitat variables (top row) and dominant taxa densities (left column). Only significant results ($p < 0.05$) are included. Bonferonni adjusted p-value = 0.0002. Bold indicates $r > 0.5$.

	Epifaunal Substrate/ Available Cover	Embeddedness	Velocity/ Depth	Total RBP Score	% Periphyton	% Macrophytes	% Slimes	% CPOM	% LWD
Psephenus						-0.29669	-0.32621		
						0.0294	0.0161		
Stenelmis	-0.32352				0.29235		**0.56541**		
	0.017				0.0319		**0.0001**		
Optioservus	-0.39341						0.49149		
	0.0033						0.0002		
Hydropsyche							0.44912		
							0.0007		
Cheumatopsyche	-0.27997						**0.66573**		
	0.0403						**0.0001**		
Baetis		0.29937		0.35643	0.32963		**0.6542**		
		0.0279		0.0082	0.0149		**0.0001**		
Maccaffertium		0.43251			0.34211		0.27674		
		0.0011			0.0113		0.0428		
Chironomidae	-0.42512						**0.73268**		
	0.0014						**0.0001**		
Hemerodromia	-0.42599						**0.65078**		
	0.0013						**0.0001**		
Pleuroceridae	-0.35894								
	0.0077								
Antocha	-0.48326						**0.69343**		
	0.0002						**0.0001**		
Simuliium		0.30069	0.27162		0.32647		**0.52664**	0.27325	
		0.0272	0.0469		0.016		**0.0001**	0.0456	
Planariidae					0.29103		0.37702		
					0.0328		0.005		
Hydroptila	-0.47588						**0.59209**		
	0.0003						**<.0001**		
Chimarra	0.3882		0.45361	0.37493		-0.28947	-0.35897		
	0.0037		0.0006	0.0052		0.0337	0.0077		

Tables 17 and 18 summarize the relationships between estimated and measured habitat variables and macroinvertebrates to assist with selecting the best approach for habitat assessment. Of the 12 assemblage metrics, 9 had more significant correlations with the measured variables than the estimated habitat variables. Only three metrics had more significant correlations with the estimated variables than the measured variables. Since there were fewer measured variables (6) than estimated variables (9), and hence different numbers of correlations for measured versus estimated, it is important to consider the proportions of total correlations that were significant (bottom of Table 17). From this perspective there were almost 4X as many significant correlations for measured variables and metrics than estimated variables and metrics. In addition to significant correlations, it is also important to consider the strength of the correlations. Using a criterion of $r > 0.5$ as moderately strong correlations, it can be seen at the bottom of Table 17 that there were more than 5X as many moderately strong correlations for measured variables and metrics than estimated variables and metrics.

Table 17. Summary of Pearson product-moment correlation coefficients between estimated and measured habitat variables and assemblage-level metrics. Values are the numbers of comparisons that meet the criterion of each column.

Metric	Estimated			Measured		
	Significant ($p < 0.05$)	Significant with Bonferroni adjustment ($p < 0.0002$)	Moderately strong ($r > 0.5$)	Significant ($p < 0.05$)	Significant with Bonferroni adjustment ($p < 0.0003$)	Moderately strong ($r > 0.5$)
Number of EPT Taxa	2			6	3	3
HBI	4	1		3		
Number of Sensitive Taxa	2			6	2	2
Total Richness	3	1	1	5	3	3
SDI	1			2		
0. % Clingers - *Hydropsyche*	1			2		
Number of Clinger Taxa	2			4	3	3
Number of Crawler Taxa	3	1	1	4	3	2
% Scrapers	1			6		
% Collector-Filterers	4	2	1	4		
% Predators	2	1	1	3	2	2
% Diptera and Non-insects	4	1	1	3	3	3
Number of Comparisons	29 (of 108)	7 (of 108)	5 (of 108)	48 (of 72)	19 (of 72)	18 (of 72)
% of Comparisons	17.60%	6.48%	4.63%	66.70%	26.39%	25.00%

A similar evaluation of the effectiveness of estimated versus measured habitat variables is presented in Table 18 for dominant taxa densities. Of the 15 taxa densities, 10 had more significant correlations with the measured variables than the estimated values. The other 5 taxa had more significant correlations with the estimated variables than the measured variables. Regarding the proportions of significant correlations, there were almost 2X as many significant correlations for measured variables and densities than estimated variables and densities. Considering the strength of the correlations, there were no moderately strong correlations between taxa densities and estimated habitat variables, while approximately one-third of the measured variables had moderately strong correlations with taxa densities.

Table 18. Summary of Pearson product-moment correlations between estimated and measured habitat variables and dominant taxa densities. Values are the numbers of comparisons that meet the criterion of each column.

	Estimated			Measured		
	Significant ($p < 0.05$)	Significant ($p<0.0002$)	Moderately strong (r > 0.5)	Significant ($p < 0.05$)	Significant ($p< 0.0002$)	Moderately strong ($r > 0.5$)
Psephenus	2			1		
Stenelmis	3	1	1	6	3	3
Optioservus	2	1		6	4	4
Hydropsyche	1			2	2	2
Cheumatopsyche	2	1	1	3	3	3
Baetis	4	1	1	3	2	2
Maccaffertium	3			1		
Chironomidae	2	1	1	3	3	3
Hemerodromia	2	1	1	3	3	3
Pleuroceridae	1			5		
Antocha	2	1	1	3	3	3
Simulium	5	1	1	3	2	2
Planariidae	2			4		
Hydroptila	2	1	1	5	3	3
Chimarra	5			3		
Number of Comparisons	38 (of 135)	9 (of 135)	8 (of 135)	51 (of 90)	28 (of 90)	28 (of 90)
% of Comparisons	28.10%	6.67%	5.93%	56.70%	31.11%	31.11%

When assessing the validity of visual estimates, it is important to know if they accurately reflect the actual conditions in the stream. Therefore, covariance is useful to see if the estimated variables are related to the measured variables (Table 19). Covariance is similar to correlations, and two variables that have a high positive covariance will increase or decrease together. A negative covariance between two variables indicates that as one increases, the other decreases. Epifaunal Substrate/Available Cover and % cobble and the Fredle Index have a high positive covariance. Epifaunal

Substrate/Available Cover and % pebble, % gravel, % fines and the Trask Sorting Coefficient have a negative covariance. Embeddedness has a low negative covariance with % fines. Velocity/Depth (estimated) has a very low covariance with velocity (measured), and a low negative covariance with depth (measured). Percent Periphyton has a strong positive covariance with AFDM, chlorophyll *a*, and epilithic biomass. Percent Slimes strongly covaries with AFDM, chlorophyll *a*, and epilithic biomass and has a moderately negative covariance with the autotrophic index. Percent CPOM covaries strongly with all measures of coarse organic matter except % epilithic biomass which was weakly negative. Percent Large woody debris weakly negatively covaried with the wood measure.

Table 19. Covariance between estimated and measured habitat variables.

Estimated Variables	Measured Variables	Covariance
Epifaunal Substrate/Available Cover	% Cobble	74.05615
Epifaunal Substrate/Available Cover	% Pebble	-53.8025
Epifaunal Substrate/Available Cover	% Gravel	-14.0393
Epifaunal Substrate/Available Cover	% Fines	-6.21435
Epifaunal Substrate/Available Cover	Fredle Index	40.40157
Epifaunal Substrate/Available Cover	Trask Sorting Coefficient	-0.90693
Embeddedness	% Fines	-3.31819
Velocity/Depth	Velocity	0.139362
Velocity/Depth	Depth	-2.7316
% Periphyton	AFDM	20.92333
% Periphyton	AutotrophicIndex	-3.47354
% Periphyton	Chl*a*	20.33799
% Periphyton	Epilithic Biomass	6.510355
% Slimes	AFDM	405.5748
% Slimes	AutotrophicIndex	-24.9338
% Slimes	ChlA	176.7162
% Slimes	Epilithic Biomass	177.3578
% CPOM	wood	86.96719
% CPOM	leaves	119.7718
% CPOM	grass	5.856003
% CPOM	Epilithic Biomass	-2.43512
% CPOM	totalCBOM	210.1599
% LWD	Wood	-1.00549

Discussion

Benthic Macroinvertebrate Assemblage

The BR reaches were characterized by higher density and diversity of benthic macroinvertebrates than the MU reaches. There were some seasonal differences in the benthic macroinvertebrate assemblage, but most of those differences are more likely attributed to life cycles of the benthic macroinvertebrates, especially the insects, rather than changes in the condition of the streams. The higher density and high diversity at BR are probably caused by the abundance of plant growth on the rocks due to high nutrients combined with no shade. Parkyn et al. (2003) and Harding et al. (2006) identified similar patterns of periphyton associated with shaded and unshaded reaches and also found that the unshaded reaches had a higher abundance of macroinvertebrates. Other studies examining agricultural disturbance have found an increase in richness and abundance associated with the disturbance, though often pollution tolerant organisms dominate the community. Stone et al. (2005) found that some metrics that indicate pollution tolerance actually improved, indicating better water quality, as the amount of forested riparian zone decreased, though the increase was usually associated with less tolerant taxa. The rocks at BR were covered with a thick layer of epilithic biomass consisting of moss, algae, fungi, and microorganisms that, based upon observations, provided both food and habitat for the benthic macroinvertebrates. The rocks in MU lacked the overabundance of this material. Though both streams had comparable nutrient levels, MU was completely shaded from spring through autumn.

Nitrate levels at BR are at the 97^{th} percentile of total stream length in Virginia (VA Stream data citation), while nitrate levels at MU are at the 60^{th} percentile. Smith Creek has many miles of stream running through agricultural areas. Mountain Run is shorter than Smith Creek and runs through a mix of residential and agricultural areas, which is probably why the nitrate levels are lower. Total phosphorous levels at BR and MU are at the 98^{th} percentile of total stream length in Virginia. Chambers et al. (2006) found a strong positive correlation between benthic algal chlorophyll *a* concentrations and dissolved inorganic nitrogen concentrations ($r = 0.92$, $p < 0.001$, n = 9).

Measures of AFDM, chlorophyll *a* and epilithic biomass were much higher at BR than MU. The mats of this material that occurred at BR are often associated with high nutrient concentrations, such as wastewater treatment plant outfalls. Benthic macroinvertebrates use the epilithic biomass for habitat and food. Moutka and Syrjanen (2007) found that the loss of mosses during restoration caused by removal of substrate and heavy equipment operating in the stream resulted in large reductions in the macroinvertebrate community. Few macroinvertebrates feed on mosses, but they trap organic material that is used as food and they provide microhabitat refugia.

There were some weak relationships between substrate variables and assemblage metrics and individual taxa, but not as many as the relationships with measures of plant growth on rocks. However, other studies have found the percent of fines in the sediment to be very important. Stone et al. (2005) identified a relationship between decreased riparian cover, increased fines, and an increase in filterers. Percent fines was slightly higher at BR than MU, and slightly higher in September than April. Braccia and Voshell (2006b) found that the percent of fines and Trask's sorting coefficient were highly related to the macroinvertebrate assemblage. The relationship between % fines and the macroinvertebrates at BR and MU were much weaker than those with organic matter. Angradi (1999) found that the effect of fine sediments on metrics was subtle in a field experiment with a range of 0% to 30% fines and with sampling natural streams with a similar range in the percent of fines. Zweig and Rabeni (2001) determined that it was difficult to identify the impact of sediment on the macroinvertebrate assemblage because increased sediment is usually accompanied by other habitat changes caused by the primary disturbance. Zweig and Rabeni (2001) did find that as sediment increased, taxa richness decreased, though there was no threshold sediment level. Contrary to what was found in Smith Creek, Rabeni et al. (2005) found that taxa densities decreased and the community structure was altered with low levels of fine sediment. However, in this study it is likely that the extremely high levels of nutrients outweighed the influence of elevated levels of fine sediments. Multivariate analyses and individual regressions show a trend of diversity and abundance being more associated with the amounts of plant growth on rocks.

Most of the habit categories were well represented with the exception of sprawlers. Sprawlers were conspicuously under-represented and were lower in April than September. However, sprawlers occupy leaves or fine sediments (Merritt and Cummins, 1994), which are usually not associated with riffles, the area sampled for this study. Crawlers were more abundant in April and much lower in September at both sections. *Ephemerella* made up the majority of crawlers and they were present at much lower levels in September due to their life cycle. Burrowers were higher than would be expected in a riffle. Chironomidae are classified as burrowers and they utilized the epilithic biomass for habitat, which is why burrowers appear to be higher than expected.

Scrapers were very low at the BR reaches in comparison to MU and were the highest at MU in September. Epilithic biomass at BR made the rock surfaces unavailable for the algal growth that is the ideal food for scrapers. The epilithic biomass also made the surface much harder for scrapers to attach to. Rabeni et al. (2005) identified scrapers as being negatively affected by low levels of sediment. Shredder abundance probably reflects the different availability of leaf material in the two sections. The MU section is forested. Even though immediately upstream lacks a riparian area, in April there is likely to be some available leaf detritus from the previous autumn. However, the BR section lacks any forested riparian area, either within the section or upstream, hence the low number of shredders. Rabeni et al. (2005) found that collector-filterers were negatively impacted by fine sediment, and Ortiz et al. (2005) found that collector-filterers were negatively impacted by increased nutrients, however, collector filterers made up 34% of the community in Smith Creek in the September sampling period. Collector-filterers were slightly more abundant at the MU reaches in both seasons.

Many genera of Chironomidae are pollution tolerant. At BR, where total densities averaged over 10,000/m^2, Chironomidae comprised well over 50% of the organisms. The pollution tolerance of Chironomidae in less desirable conditions enables them to take advantage of the abundant organic matter resulting from high nutrients. Hence, they end up dominating the community. Chironomidae had positive relationships with measures of organic matter associated with the rocks. Chironomidae genera can occupy a diverse range of habitats and feed on an equally diverse range of organic matter (Merritt and Cummins, 2006). Therefore, they are usually found in every freshwater aquatic system. Their high abundance at BR indicates that there is a great availability of organic matter for food and that there is little competition for resources.

While Chironomidae were dominant, there were also several Ephemeroptera and Trichoptera taxa present in high numbers. Hall et al (2006) examined three rivers in the San Joaquin watershed and found they were dominated by a mix of pollution tolerant and intolerant organisms. *Hydropsyche* and *Cheumatopsyche*, pollution tolerant organisms, were more abundant at BR than MU. They appeared to build their burrows and nets in the mossy substance coating the rocks at BR. Burcher and Benfield (2006) examined macroinvertebrate communities in agricultural streams and suburban streams and found high abundances of Hydropsychidae at the agricultural streams. *Hydroptila*, another pollution tolerant Trichoptera, is often associated with periphyton and vascular plants located in the current (Merritt and Cummins, 1996; Unzicker et al., 1982). Their abundance and positive correlations with measures of organic matter associated with the substrate at BR indicates that the epilithic biomass was providing food and cover.

Ephemerella is relatively pollution intolerant (Merritt and Cummins, 1996); however it was very abundant in April. *Ephemerella* are crawlers and collector-gatherers. Their abundance is likely due to the availability of food trapped by the epilithic biomass and the microhabitat refugia provided by the tangles of this material. Plecoptera were present at both BR and MU in April, but absent at BR and present in low densities at MU in September. This is most likely a reflection of their life-cycle and not a change in conditions. Plecoptera emerge in the spring, and by September the next generation is either in the egg stage or has recently hatched, making them undetectable with the sampling methods used in this study.

Two genera of Diptera, *Hemerodromia* and *Antocha,* were also found in abundance at BR, particularly in September, and were positively correlated with measures of organic matter associated with the rocks. *Hemerodromia* are sprawlers or burrowers and may be either predators or collector-gatherers. *Antocha* are clingers that spin silk tubes in which they live, and they are collector-gatherers (Merritt and Cummins, 1996). The abundance of *Hemerodromia* and *Antocha* is likely related to the availability of habitat and food provided by the epilithic biomass. The epilithic biomass traps particles that collector gatherers may use, and it also provides habitat for smaller organisms that the predators feed on.

Though cattle were removed from the site in early 2006, few environmental changes had occurred by the April or September 2006 sampling periods. As the restoration of Smith Creek on the Bruce Farm progresses and the riparian zone develops into a mature forest, the stream will become more stable and more shaded. The stability will lead to less fines and better habitat for benthic macroinvertebrates. The shading will reduce the growth of epilithic biomass because of limited sunlight for photosynthesis. Allochthonous inputs will increase providing a new food source and large woody debris for habitat. The benthic macroinvertebrate assemblage will become more balanced with more scrapers and shredders than are currently present. More pollution intolerant organisms will also appear. Total abundance and diversity will likely decrease as a result of decreased nutrient levels, increased shade, and reduced epilithic biomass. The assemblage will be more evenly distributed between the various functional feeding groups. The community will also be more evenly distributed over the different orders, rather than being dominated by two or three taxa. Moutka and Syrjanen (2007) found that the benthic macroinvertebrate community remained variable in streams that had been restored 4-6 years previously and that streams that had been restored at least 8 years prior to sampling had communities similar to natural systems.

This study was performed to document the pre-restoration conditions of the Bruce Farm section of Smith Creek. By quantifying the benthic macroinvertebrate assemblage and the environmental factors responsible for the assemblage, scientists will better understand the processes that will be responsible for recovery and the overall success of the restoration, and to suggest improvements for other restoration projects. We suggest that this study provides a model for how benthic macroinvertebrates should be investigated in stream restoration projects. For example, by quantifying the relationships between the benthic macroinvertebrate assemblage and the different types of environmental variables acting as stressors, it is possible to rank their relative importance and prioritize restoration activities accordingly.

Measurements Versus Visual Estimates of Substrate Composition

It was difficult to perform a one-to-one comparison of the RBP estimated environmental variables and the measured environmental variables because there were not always direct relationships between them. For example, Epifaunal Substrate/Available Cover (an RBP estimate) may be influenced by the substrate present, leaf packs, large woody debris, and anything else that may provide habitat.

The visual estimates appear to describe the available habitat in a broad sense, but there are potential problems with the attempted quantification. The range of responses is bounded by the same numbers (usually 0 to 20), and within the range the responses are discrete, not continuous. Also, there may not be categories that accurately reflect the conditions in the streams. The intensive measurements can be adjusted in order to better understand the stream conditions. For example, the rocks at BR were coated in a thick mat of epilithic biomass that consisted of algae, mosses, fungi, and bacteria. This was characterized as % Slimes in the RBP visual estimates, which does not accurately reflect the material on the rocks. The epilithic biomass was accounted for in the chlorophyll *a* analysis and the CBOM analysis as part of the measured variables.

The measured variables had stronger relationships with the metrics and taxa densities than the RBP estimates because both the measured variables and the benthic macroinvertebrates were sampled together using the modified stove-pipe sampler. The measured variables accounted for every component within the pot, while the RBP estimates could not account for some measures such as FBOM.

There have been few, if any, studies assessing the accuracy of the RBP for assessing habitat conditions. Most studies on the RBP have focused on how it is applied. For example, Wang et al. (1996) found that visual habitat assessments varied between observers, which led to inconsistent results. The RBP estimates provided a general understanding of the habitat conditions at BR, but the measured variables provided a more complete understanding of the relationship between the benthic macroinvertebrate assemblage and the environmental conditions. The RBP estimates are useful for widescale biomonitoring to reveal suspected problems; however, detailed research to plan restoration activities or document recovery would benefit more from conducting intensive, quantitative environmental sampling such as performed in this study.

If only visual estimates had been performed, there would have been fewer moderately strong (r >0.5) relationships, and there would have been no strong (r > 0.7) relationships between the benthic macroinvertebrates and environmental conditions. Our understanding of how the assemblage was being shaped by the environmental conditions would be much less complete without the measured environmental variables. Nutrients and plant growth would not have emerged as primary factors, and it is likely that we would have concluded that fine sediment was the primary causative factor.

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