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Plum Run: A Stream Evaluation Based on Macroinvertebrate Community Structure

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PLUM RUN: A STREAM EVALUATION
BASED ON MACROINVERTEBRATE COMMUNITY
STRUCTURE

By

Danielle DiFederico

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biology

West Chester University of Pennsylvania

2007

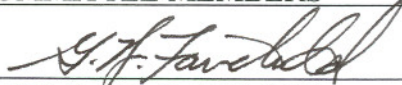
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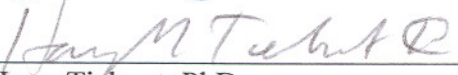
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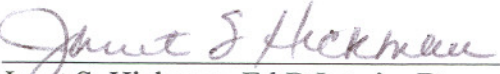
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I. INTRODUCTION

Methods of Bioassessment

Aquatic macroinvertebrates are commonly used in the assessment of water quality in streams for several reasons: 1) they respond more quickly to environmental stresses than do fish, 2) they are more easily identified than attached algae (periphyton), 3) there are large numbers of species, allowing for the integration of many individual responses, and 4) their relatively sedentary nature makes them easy to collect (Resh & Rosenberg, 1993; Barbour, 1999). There are disadvantages to using invertebrates as well: 1) large numbers of samples are needed to make accurate population assessments, 2) they do not respond to all kinds of impacts, 3) assemblages vary seasonally and spatially in taxonomic composition, in both impaired and unimpaired streams (Resh & Rosenberg, 1993; Linke, 1999), and 4) macroinvertebrate communities can be affected by factors other than water quality such as substrate and current velocity (Merritt & Cummins, 1996).

Although macroinvertebrate assessments are usually preferred for the above reasons, algae and fish studies are also used to assess water quality. Periphyton (benthic algae attached to stream substrates) are often used with macroinvertebrate assessments (Barbour, 1999). Periphyton is an important food source for many invertebrates (Allan, 1995), and is affected by light, temperature, current, substrate, floods, water chemistry and grazing (Allan, 1995). Very high periphyton biomass can impair invertebrate assemblages by decreasing levels of dissolved oxygen, particularly at night (DEP, 2005). Dissolved oxygen levels usually decrease at night due to the absence of algal

photosynthesis, which produces oxygen, and to continued respiration, which consumes oxygen. Therefore, a severe increase in algal biomass will further decrease levels of dissolved oxygen at night. Very high periphyton densities can also modify rock surfaces, leaving invertebrates unable to attach or forage successfully.

Fish are also used to assess water quality for several reasons: 1) they are more recognized and appreciated by the public, 2) they are easily identified in the field, 3) there are large quantities of resources and references on their life history and ecology, and 4) they are usually secondary consumers, so their biomass is partly dependent on the abundances of organisms that serve as their food (Ohio EPA, 1987). Recent water quality studies based on fish community data have often used multimetric approaches such as the index of biotic integrity (IBI). The IBI was originally developed by Karr (1981) for use in midwestern states, and has been modified to reflect regional differences in fish faunas outside the Midwest (Schleiger, 2000).

Many indices and metrics have been developed to relate the structure of aquatic invertebrate assemblages to ambient water quality (Table 1). These metrics (described further in the results section) include the Hilsenhoff Biotic Index (HBI), % EPT, % Chironomids, Shannon Diversity Index, and % tolerant taxa.

Table 1. Definitions of potential metrics and predicted direction of metric response to increasing perturbation (Barbour, 1999).

Metric	Definition	Predicted response to stream impairment
No. invertebrate taxa	Measures the overall richness of the macroinvertebrate assemblage.	Decrease
No. EPT	Number of taxa in the insect orders Ephemeroptera, Plecoptera, and Trichoptera.	Decrease
% EPT	Total abundances of Ephemeroptera, Plecoptera, and Trichoptera species divided by the total number of invertebrates per sample.	Decrease
% Filterers	Abundances of species that filter FPOM (fine particulate organic matter) from either the water column divided by the total number of invertebrates per sample.	Variable
% Grazers and Scrapers	Abundances of invertebrate species that scrape or graze periphyton divided by the total number of invertebrates per sample.	Decrease
% Chironomidae	Number of midge larvae divided by the total number of invertebrates per sample	Increase
Hilsenhoff Biotic Index	Uses tolerance values to weight abundance in an estimate of overall pollution. Originally designed to evaluate organic pollution	Increase

As with the IBI often used to evaluate fish communities, multimetric analysis incorporating several of the above metrics has become standard practice in analyzing macroinvertebrate data (Norris & Georges, 1995; Rosenberg & Resh, 1996). A commonly used multimetric approach is the Macroinvertebrate Aggregated Index for Streams (MAIS), which incorporates 10 metrics into a single numerical evaluation of stream habitat quality (Smith & Voshell, 1997).

As an alternative to the multimetric approach, multivariate analysis uses statistical ordination techniques to summarize differences in invertebrate community structure among sites. Rosenberg and Resh (1996) describe four common methods of multivariate analysis: 1) direct gradient analysis where invertebrate abundances are related to environmental variables, 2) inference, where environmental variables are deduced from species composition, 3) indirect gradient analysis where differences in species

composition among sites are secondarily interpreted in relation to environmental gradients, and 4) “constrained ordination” where axes of variation in community composition are computed based on their fit to accompanying environmental data. Norris and Georges (1995) describe some of the weaknesses of multivariate analysis, including 1) elimination of variables with missing data, 2) lack of significance testing, 3) an assumption that predictive environmental variables are measured, and 4) the need for a large database of reference sites with which test sites can be compared.

Both multimetric and multivariate approaches usually depend on the taxonomic structure of the invertebrate community, such as the abundances of invertebrate families or genera. Alternatively, invertebrate abundances may be tabulated in terms of the functional guilds represented by the organisms collected (Norris & Georges, 1995). Invertebrate functional guilds, or feeding roles, are classified based both on the kind of food consumed and how it is acquired. Functional groups are usually broken down into shredders, filterers, predators, scrapers (or grazers), and collectors (or gatherers). Shredders consume coarse particulate organic matter (CPOM) such as leaf litter and are typically found in smaller, forested streams, or in larger streams with accumulations of CPOM in depositional areas (Cushing & Allan, 2001). Common shredders are members of the dipteran family Tipulidae (cranefly larvae) and the plecopteran family Leuctridae (stonefly nymphs). Predators are invertebrates that feed on other animals. There are many different adaptations. Common predators are dragonfly nymphs and most species of stonefly nymphs. Scrapers, or grazers, have specialized mouth parts used to remove algae from rock surfaces. They are found mostly in areas where light is adequate for

algal growth (Cushing & Allan, 2001). Common scrapers are larvae of the trichopteran genera *Glossosoma* and *Neophylax*. Collectors (gatherers) are the most common functional group. These invertebrates have different mechanisms for gathering fine particulate organic matter (FPOM) throughout the stream. Common collectors are nymphs of the mayfly genera *Baetis* and *Callibaetis*. Filterers are often described as a division of the collector guild, but they obtain their food by gathering suspended particles from the water column. Common filterers are the trichopteran family Hydropsychidae (caddisflies) and the dipteran family Simuliidae (black flies).

A disadvantage of using functional guilds instead of taxonomic structure is that the feeding methods of some species change as they mature: (e.g., later instars of Tanypodine chironomids, and larval hydrophilid beetles). This makes assignment to a specific feeding group difficult (Norris & Georges, 1995). The evaluation of functional guild structure, however, can provide valuable information about stream quality that may not be immediately evident based solely on taxonomic composition.

Environmental Influences on Invertebrate Community Structure

Macroinvertebrate assemblages may respond to a range of stream and watershed characteristics, considered further below: (1) stream habitat, (2) riparian vegetation (as canopy density), (3) physicochemical conditions, (4) land use, and (5) composition of the fish community. My study focused on the response of macroinvertebrate assemblages in riffle habitats to the first 3 characteristics. Riffle habitats were selected because the majority of stream quality work has been done using riffle habitats. Using pool habitats could be problematic as they usually contain fewer and difficult-to-identify invertebrates.

Also there is little literature to extrapolate results of pool habitats to stream quality (Personal communication with Dr. John Jackson of Stroud Water Research Center). Data regarding land use and fish community composition are included as inferential and supplemental data. This information can also be incorporated in future studies of Plum Run if needed.

Stream Habitat: Differences in stream geomorphology and size contribute strongly to habitat diversity, which in turn generally enhances the diversity of invertebrate communities (Cushing & Allan, 2001). For example, water velocity usually increases with gradient and is negatively related to depth. A stream's gradient helps to determine the alternation of riffles and pools in small streams. Pools usually have slower currents, which allow finer particles to settle to the bottom; in contrast, the steeper gradients and fast currents of riffles transport finer sediments downstream, leaving coarser substrate behind. Substrate type and water velocity in turn strongly influence the kinds of invertebrates present.

Stream order is a classification system used to describe stream size and position within stream networks. A first order stream is permanently flowing and has no upstream tributaries. Two first order streams joining together form a second-order stream (Allan, 1995). Third order streams occur below the confluence of two second-order streams, etc. Stream order thus provides a convenient "shorthand" for summarizing stream size and position within a stream network; both of these attributes may affect the invertebrate community.

For example, Paller, Specht and Dyer (2006) examined the effect of stream size on macroinvertebrate taxa at 27 sites in 12 first through fourth order streams in South Carolina. Their results indicated that stream width was positively related to total number of taxa, number of EPT taxa and total number of organisms. A study by Heino and others (2005) of 27 riffle sites in the River Kiiminkijoki in Finland, likewise showed an increase in invertebrate diversity with increasing stream size.

Riparian Vegetation: Streamside (riparian) vegetation cover fluctuates from urban to forested areas. Riparian forests provide food for stream organisms in the form of woody debris, leaves, flowers, and terrestrial insects (Klapproth & Johnson, 2000). For smaller (\leq fourth order) streams, the organic debris of riparian vegetation is usually an essential energy input (Wheeler, Angermeier, & Rosenberger, 2005). Riparian areas also retain fine sediment, metals and nutrients from runoff (Wheeler et al., 2005; Nerbonne & Vondracek, 2001). Trees stabilize stream banks; reduce bank erosion and increase the diversity of stream habitats when they fall into or drop limbs into streams (Klapproth & Johnson, 2000; Wheeler et al., 2005). These fallen limbs and trees strongly influence channel features by causing small-scale depositional and erosional microhabitats within the larger stream segment, thereby influencing the range of substrates available to invertebrates (Cushing & Allan, 2001).

In stream segments without riparian forest cover, increased light penetration can increase stream temperatures, reduce oxygen levels, and stimulate the growth of algae (Klapproth & Johnson, 2000; Roy, Faust, Freeman, & Meyer, 2005). Whereas trees

effectively intercept runoff generated during precipitation events, their absence may lead to greater temporal variation in flow and nutrient content (Rios & Bailey, 2006).

In effect, if streamside vegetation is altered, the invertebrate community is ultimately affected through changes in substrate, water chemistry and food resources (Weigel, 2003). Rios and Bailey (2006) showed the effects of riparian tree cover on macroinvertebrate communities at 33 sites on the Upper Thames River catchment in southwestern Ontario. Their study included three spatial scales: the outflow reach (a segment length equal to 10X the width of the stream channel), the stream network buffer (a 30 m buffer on each side of the stream network), and the whole basin (the 3500 km² study area of the Upper Thames River). The results showed that macroinvertebrate taxon richness and Simpson's diversity increased with increased tree cover at the outflow reach scale. Simpson's equitability decreased with increased agricultural use within the stream network buffer. Agriculture in this study was defined as agricultural drain systems, livestock grazing and other agricultural uses. In contrast with the stream buffer network and outflow reach scale results; land use at the whole basin scale did not significantly affect the macroinvertebrate community.

In effect, decreasing riparian vegetation should cause a decrease in invertebrate diversity, and cause a shift in functional guild structure from a community dominated by shredding invertebrates to one dominated by scrapers (Roy et al., 2005). Stream segments with little or no canopy cover should also have higher temperatures, increased algae, increased chlorophyll-*a*, and more variable dissolved oxygen than closed-canopy sites.

Physicochemical effects: The water chemistry of a stream can be influenced by precipitation, geology and associated soils, and by point and non-point source inputs of human origin. Below I discuss several physicochemistry parameters known to influence macroinvertebrate communities.

Water temperature can have a strong effect on the metabolism and life history attributes of invertebrates. Populations of some invertebrate species in warmer climates can complete a life cycle in less than a year, compared to populations of the same species in colder climates that need more than one year to complete the life cycle (Allan, 1995). Temperature can influence invertebrate body size, fecundity, development, growth, resource consumption, and egg hatching (Allan, 1995). Water temperature is modified by riparian vegetation, overland runoff and other anthropogenic inputs such as sewage treatment plants. The effluent from sewage treatment plants is warm and can increase the temperature of the water downstream of the plant. In a study by Wheeler (2005), runoff from impervious surfaces was estimated to increase stream temperatures by 0.25°C for every 1% increase in impervious area. Sweeney (1986) examined the impact of water temperature on larval development of the winter stonefly *Soyedina* (family Nemouridae). His study found that increasing temperature significantly accelerated growth rates and reduced the development time of the larvae.

A pH of less than 5 or greater than 9 is considered detrimental to most aquatic organisms (Voshell, 2002). Low pH can lower the density and diversity of aquatic macroinvertebrates and cause a species shift in the community towards more tolerant taxa (Keener & Sharpe, 2005).

Conductivity is a measure of the ability of water to pass an electrical current, and is directly proportional to ion concentration (Allan, 1995). The major ions expected in most regions are the cations Ca^{2+} , Mg^{2+} , Na^+ and K^+ , and the anions HCO_3^- , CO_3^{2-} , Cl^- and SO_4^{2-} . Rock weathering accounts for the majority of calcium and magnesium found naturally in streams, resulting in widely ranging conductivity values within stream networks crossing multiple bedrock types. Human-induced increases in conductivity can be brought about by sewage inputs and road salt (Allan, 1995). Wheeler et al. (2005) cite a study of a Pennsylvania stream by Weber and Reed (1976) that showed a 20-30 fold increase in stream conductivity during winter when the ice with road salt melted and ran into the stream. Because most solutes are in ionic form, total dissolved solids (TDS) and conductivity are strongly correlated as descriptors of ion concentration.

Dissolved oxygen is usually near saturation in small streams (Cushing, 2001). Concentrations of dissolved oxygen may decline at night in the absence of photosynthesis, and decrease in warm water owing to effects of temperature on oxygen solubility. An increase in organic pollution increases the biological demand for oxygen by decomposers and thus reduces oxygen levels in the water.

Sediment and nutrient loading can cause substantial impacts on stream invertebrates. An increase in the amount of sediments in a stream affects invertebrate communities by reducing both food and habitat (Nerbonne & Vondracek, 2001). Streams in watersheds dominated by impervious surfaces often experience considerable runoff and consequent heavy sediment loading. Increased suspended sediments decrease light penetration, reducing photosynthesis and dissolved oxygen levels (Allan, 1995).

Sediments may also introduce toxins and abrasive suspended materials into streams (Lemly, 1982; Klapproth & Johnson, 2000) and may, like excessive periphyton accumulation, render rock surfaces unsuitable for habitation by some kinds of invertebrates. In a 1996 survey by USEPA, sediment was the most common agricultural pollutant, contributing to 50% of impaired streams (Wheeler et al., 2005).

Nutrients such as phosphorus enter streams mostly through soil erosion and sewage inputs (Cushing, 2001). Increased phosphorus in a stream can stimulate the growth of algae in the presence of adequate light. As discussed earlier, high algal densities can intensify the daily fluctuation in levels of dissolved oxygen and modify rock surfaces, impairing invertebrate assemblages.

Watershed Effects (land use): Urbanization can be described as development within a watershed in which previous land uses of rural areas are changed (Kemp & Spotila, 1997; Wheeler et al., 2005). Urbanization can impact stream communities by increasing the prevalence of impervious surface areas. Impervious surface is generally defined as any material (e.g., roads, sidewalks, rooftops, compacted soil) that prevents the infiltration of water into the soil (Arnold, Gibbons, & James, 1996; Wheeler et al., 2005). Stream degradation and altered macroinvertebrate communities can occur at relatively low levels (~10%) of imperviousness (Arnold et al., 1996; Wheeler et al., 2005). Based on a map of the Brandywine watershed prepared in 1998, the Plum Run drainage basin had 11-13% impervious cover (Chester County Water Resources Authority). A study by Robson, Spence and Beech (2006) of a 374-ha catchment in England showed strong effects of

increased impervious surface area on stream invertebrates, including declines in the number of taxa.

Increased amounts of impervious surface, usually associated with urbanization in a watershed, can accentuate temporal variation in stream flow. According to Wheeler et al. (2005), approximately 20% paving of the watershed can cause up to 10-fold increases in flood frequency. This increase in flooding erodes stream banks and deepens the channel, thus changing the geomorphology of the stream. Impervious surface, in addition to modifying the quantity of water in stream channels, also modifies water chemistry by increasing the amount of contaminants that enter a stream. These contaminants are a leading cause of streamwater impairment (Robson et al., 2006), resulting in altered water chemistry and increased sedimentation, leading to a decrease in the diversity or abundance of sensitive invertebrates (Lieb & Carline, 2000; Roy et al., 2003).

Water quality may be affected by other (non-urban) land uses as well. Land use categories described in this thesis include agriculture (pasture or cropland), forested land (deciduous, evergreen and mixed), water (streams, canals, lakes, reservoirs, bays and estuaries), wetlands, barren lands (beaches, quarries, strip mines) and rangeland (mostly grasses) (USGS, n.d.). Agricultural land use is a leading source of water pollution and, according to a USEPA water quality study, is a contributing factor for 70% of impaired streams (Nerbonne & Vondracek, 2001). By contrast, watersheds with predominantly forested land tend to retain water and contaminants, reducing the impacts of runoff on stream systems (Roy et al., 2003).

Fish: The effect of fish predation on macroinvertebrates in streams is poorly understood, can vary greatly, and is often inconsistent (Williams, Taylor & Warren, 2003; Dahl, 1999; Gibson, Ratajczak & Grossman, 2004). According to Dahl (1999), some of this variation can be due to differences in feeding habits. Fish feeding on invertebrates associated with the streambed, for example, may have more of an impact on benthic macroinvertebrates than drift-feeding fish, which may consume proportionally more terrestrial insects that fall into the stream. Culp (1986) used containers with different densities of salmon fry and allowed invertebrates to colonize the containers; his results showed that density, biomass and size distribution of invertebrates in the drift and the benthos were unaffected by the fish.

Invertivores, primarily insectivores, are the dominant fish trophic guild of most North American surface waters. As the invertebrate food source decreases in abundance and diversity due to habitat degradation, there is a shift from insectivorous to omnivorous fish species.

Habitat disturbances that affect invertebrate populations may also affect fish populations (Lemly & Crawford, 1982). Even a few kilometers of unshaded stream channel are enough to make a perceptible difference in temperature and determine whether some fish species, including trout, can occupy that stream section (Cushing & Allan, 2001). Therefore, habitat can either directly affect the invertebrate community, or indirectly affect it through changes in abundance or species composition within the fish community.

The River Continuum Concept: The River Continuum Concept (RCC) is a comprehensive and broadly accepted paradigm often used to describe a stream's ecosystem from its source to its mouth, and includes predicted shifts in invertebrate functional feeding groups (Vannote et al., 1980). The concept incorporates channel size, riparian vegetation and macroinvertebrate diversity among many stream attributes. In narrow headwater streams (stream orders 1-3) with riparian canopies, for example, respiration is expected to exceed primary production as a result of increased leaf fall from streamside trees (augmenting respiration associated with leaf decomposition) and shading (decreasing photosynthesis). In these stream orders, shredders and collectors are expected to dominate the invertebrate community due to the increased vegetative debris in the stream (Cushing & Allan, 2001; Vannote et al., 1980). Fish species are few and small in size. In downstream sections (stream orders 4-6), primary production is predicted to exceed respiration. Increased stream width permits less shading of the streambed by riparian vegetation; therefore, higher temperatures and light levels often promote algal growth. Here collectors dominate the invertebrate community, shredder populations decrease, and grazers (scrapers) increase (Cushing & Allan, 2001). Fish diversity and size are expected to increase downstream, accompanying the greater availability of deep pools.

Plum Run

Here I describe the physical, chemical, watershed and biotic features at 14 sites within the stream network of Plum Run (Fig. 1, Appendix B). Plum Run is a tributary of the Brandywine Creek in Chester County, Pennsylvania, with a 9.6 km² watershed.

Plum Run is composed of two main branches (east and west), each with various tributaries. The East Branch of Plum Run comes out from an underground stormwater pipe near High Street by West Chester University's new Performing Arts Center. The East Branch then passes through the Robert B. Gordon Natural Area (GNA) next to the university's South Campus. The West Branch emerges from an underground pipe on the west side of New Street adjacent to university parking lot F. The two branches join near the intersection of Route 52 and Tigue Road, and the stream empties into the Brandywine Creek near Lenape.

Plum Run Watershed

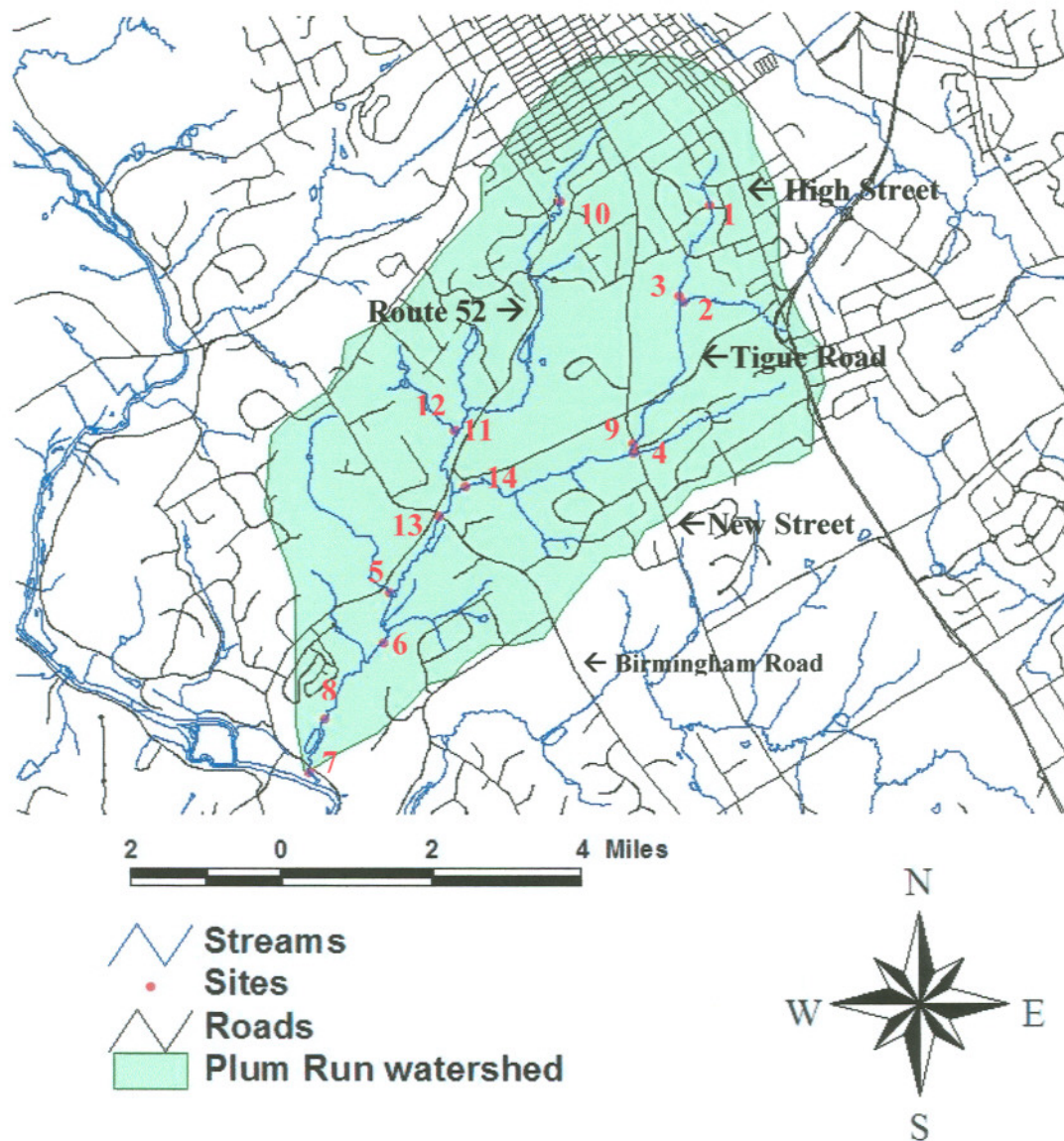


Figure 1. Plum Run watershed, with 14 sampling sites starred.

Plum Run was designated an “impaired” stream by the Pennsylvania Department of Environmental Protection (PADEP) in 1997, and is included in the Clean Water Act section 303(d) list of impaired waters. The principal causes of impairment listed by PADEP were considered to be siltation from agriculture, and effects of urban runoff/storm sewers (WRAS, 2003). Designated uses for aquatic life in Plum Run are listed as Warm Water Fishery (WWF) and Migratory Fishery (MF). The designation of WWF provides minimum protection for streams in Pennsylvania (Penn Future, 2006).

According to The Pennsylvania Bulletin [26 Pa.B. 2659], the Storm Water Management Act requires Pennsylvania counties to plan and implement stormwater plans for designated watersheds. These plans address the impacts of development on existing stormwater runoff levels and recommend measures to control accelerated runoff. At the time of this study, Plum Run was not a designated watershed for an Act 167 plan.

Objective and Predictions

This project is intended as a base model for future studies of the watershed. These studies could be through university classes, the township, or other organizations. It was also the intention that this study be helpful in a restoration plan for the stream. The study focus was to relate variation in the invertebrate community of Plum Run to differences in environmental characteristics of the 14 sites sampled. I will evaluate the following predictions:

Stream Habitat: My measurements of stream geomorphology included stream width, depth, stream order, gradient and discharge. I predict that stream size will be positively related to macroinvertebrate density and richness. As predicted by the RCC, maximum

richness is reached in mid-order streams (Paller et al., 2006). Since all stream segments within the Plum Run stream network are no larger than fourth order, macroinvertebrate density and richness should increase with increasing order.

Riparian Vegetation: I predict that sites with greater riparian canopy cover will have lower amounts of periphyton chlorophyll-*a*, lower stream temperatures, and exhibit oxygen levels that are driven more by exchange with the atmosphere than by photosynthesis. These habitat characteristics in turn will affect the macroinvertebrate community by maintaining a high proportion of shredders and fewer scrapers. By contrast, I predict that sites with little riparian forest cover will have a higher percentage of pollution tolerant taxa, as well as a shift in the feeding guilds toward more algal scrapers when compared to sites with riparian canopy cover.

Physicochemical: I measured temperature, dissolved oxygen, specific conductance, total dissolved solids (TDS), pH, total phosphorus and chlorophyll-*a*. As mentioned above, sites with higher chlorophyll- *a* counts are predicted to have a higher percentage of invertebrates that feed on algae. Sites with higher specific conductance (and also higher TDS) are predicted to have communities of more pollution tolerant invertebrates. Higher levels of total phosphorus may be connected with sites that have higher levels of chlorophyll- *a* since, as mentioned previously, increased phosphorus can stimulate the growth of algae.

I predict that dissolved oxygen levels will be near saturation at all sites since measurements were taken during June when photosynthesis and respiration are likely to be less important than later in the summer. However, nutrient-enriched sites with

particularly long stretches without riparian shading upstream may have higher oxygen levels owing to increased photosynthesis.

II. METHODS

Study Sites: Four sites were chosen on the East Branch of Plum Run (1,3,9,14), two on the West Branch (10, 11), four on the main stem (6,7,8,13), and four on tributaries (2,4,5,12). Sites were chosen after reviewing a map of the watershed and observing multiple locations in the field. Only two sites were chosen on the West Branch because after initial observation there appeared to be too little difference between sites 10 and 11 to warrant establishing another site between them. A 50-m sampling reach was defined for each of the sites. The 50-m boundaries were chosen to include reaches containing both riffles and pools suitable for invertebrates and fish that could be used for sampling. All 14 sites (starred in Figure 1 and listed in Appendix B) were visited in March 2005, and were added to a GIS database using a Global Positioning Trimble Pro-XR backpack unit. All sampling and measurements were done during base flow conditions.

Physicochemical Conditions: Water chemistry measurements were obtained for each site, at various times during the day, between June and August 2005 using a Yellow Springs Instrument (YSI) 6600 datasonde (Table 2). All measurements were taken at base flow, at locations where water depth allowed submersion of the sonde probes (>15 cm). YSI measurements included temperature ($^{\circ}\text{C}$), specific conductance ($\mu\text{S}/\text{cm}$), total dissolved solids (g/L), dissolved oxygen (% saturation and mg/L), and pH. TDS and pH were averaged over two sampling dates (Table 2).

Total phosphorus concentrations were determined from water samples taken during the first sampling date at each site. These samples were sent to PADEP for analysis.

Table 2. Dates and times of the physicochemical measurements per site.

Site #	Date/Time #1 & TP sample	Date/Time #2	D.O. & Temp measurements
1	6/15/05 at 10:30 am	6/28/05 at 10:38 am	8/24/05 at 10:45 am
2	6/14/05 at 9:30 am	6/22/05 ~10:30 am	8/24/05 at 10:05 am
3	6/14/05 at 2:00 pm	6/22/05 at 11:15 am	8/24/05 at 10:25 am
4	6/20/05 at 12:03 pm	6/29/05 at 9:11 am	8/24/05 at 9:40 am
5	6/21/05 at 9:15 am	6/30/05 at 10:54 am	8/23/05 at 11:20 am
6	6/21/05 at 10:16 am	6/30/05 at 1:23 pm	8/23/05 at 11:38 am
7	6/16/05 at 9:25 am	6/30/05 at 3:42 pm	8/23/05 at 1:15 pm
8	6/15/05 at 9:00 am	6/30/05 at 9:11 am	8/23/05 at 12:35 pm
9	6/20/05 at 11:45 am	6/29/05 at 9:05 am	8/24/05 at 9:25 am
10	6/21/05 at 1:11 pm	6/28/05 at 9:24 am	8/23/05 at 8:47 am
11	6/20/05 at 9:10 am	6/24/05 at 10:14 am	8/23/05 at 9:34 am
12	6/20/05 at 9:20 am	6/24/05 at 10:20 am	8/23/05 at 9:57 am
13	6/16/05 at 11:30 am	6/23/05 at 11:32 am	8/23/05 at 10:21 am
14	6/21/05 at 12:07 pm	6/23/05 at 9:07 am	8/24/05 at 9:05 am

Stream Habitat: Stream width at each site was measured at 6 locations, each 5 m apart within a 25 m segment, and averaged. Three depths were taken along each of the 6 transects used to measure width (n=18), and averaged to obtain mean depth.

WCU undergraduate student Danielle Varnes, under the direction of Dr. Tim Lutz (Department of Geology and Astronomy), performed additional geomorphological

analyses at all 14 sites during the summer of 2005. At each site measurements of the position of the deepest part of the channel and its elevation were made relative to a local coordinate system using an optical transit and stadia rod. Measurements were made at points along the channel, extending as far as was practical given the available lines of sight. The median channel length over which measurements were made was approximately 80 meters. Water depth was also measured. From the measurements a profile of the channel and water surface was constructed for each site (Appendix B). Channel length was estimated using the map coordinates; gradient was calculated as the difference in water surface elevation at the upstream and downstream ends of the profile divided by the channel length.

Canopy density/vegetation composition: Four measurements of canopy cover were taken using a concave densiometer at two random locations within the 50-meter reach defining each site. At both locations, canopy estimates were obtained facing upstream, downstream, right and left bank. The extent of riparian vegetation was based on visual estimates from the 25 m segment used for width and depth analyses, and approximated to the nearest meter. If the extent was less than 30 meters it was approximated to the nearest meter. If the extent was greater than 30 meters, ">30" was recorded.

Flow Measurements: A pygmy-Gurley flowmeter was used to take flow rate measurements at each site on two dates (8/23/05 and 8/24/05). A transect was established across the stream at a location with adequate depth to submerge the flowmeter, and with simple hydraulic characteristics (fairly uniform flow and few obstructions). Flow estimates were obtained from multiple segments within the stream

cross-section defined by the transect, and summed. To obtain discharge rate, all measurements were taken during base flow.

Periphyton Abundance: Periphyton was collected for chlorophyll-*a* analysis on the same days as the invertebrate sampling (6/23/05-6/30/05) using a PEP (Pennsylvania Epilithic Periphyton) sampler designed by PADEP (Fig. 2). Six rocks were chosen from riffles within the 50-meter reach at each site. Rocks with flat surfaces were selected to ensure the watertight seal needed to effectively use the PEP sampler. An area of 118 cm² was scrubbed from each of the 6 rocks. The rocks were scrubbed 4 times with a hard-bristled brush, each time removing the material with 30 cc of rinsewater with a 60-cc syringe. The slurries from each of the 6 rocks were combined and a 40 ml aliquot from the combined volume was filtered through a Whatman GF-C glass fiber filter with 1 ml of MgCO₃. The filters were ground in 15 mL of hot 90% ethanol, placed in a centrifuge tube and stored in the refrigerator (in the dark) overnight. The samples were centrifuged for 10 minutes and decanted into a 1-cm cuvette. The samples were read with a Perkin Elmer Lambda 35 Dual Beam UV/VIS spectrophotometer at 665 and 750 nm. The reading at 750 is subtracted from the reading at 665 nm to obtain E665b'. The samples were then acidified with 1 N HCL and again read at 665 and 750 nm. The reading at 750 nm was again subtracted from the reading of 665 nm to obtain E665a'. Chlorophyll-*a* and phaeophytin (a decomposition product of chlorophyll-*a*) were computed in µg/cm² using calculations based on Nusch (1980):

$$\mathbf{Chl-a} = 29.6 * (E665b' - E665a') * (15) * (VSAMP / VFILT) / A \quad (1)$$

$$\mathbf{Phaeo} = 20.8 * (E665a') * (15) * (VSAMP / VFILT) / A - \mathbf{Chl-a} \quad (2)$$

Where A = the area of the substrate (117.81 cm^2), $VSAMP$ = the volume of the sample (720 cm^3), and $VFILT$ =the volume of the filtration (40 cm^3).



Figure 2. Photo of PEP sampler designed by PADEP
(Photo provided by Alan Everett, PADEP)

Watershed Analysis: WCU Geography graduate student Mike McGeehin, under the direction of Dr. Joan Welch, used ArcGIS (Environmental Systems Research Institute, Inc.) to compute subbasin areas upstream of each sampling site, and the percentage of total area of 11 land uses within each subbasin (a subbasin, or subwatershed, is a subdivision of a larger watershed). Land use variables were % pasture, % community service (the university and other institutional and public facilities), % parking (parking lots, independent from buildings), % low residential housing, % multiple family (MF) residences, % recreation (parks), % vacant (unpaved, undeveloped land), % wooded area, % commercial land (private business and retail), % water (ponds and other waters not part of Plum Run), and % highway transportation.

Fish sampling: Spatial variation in the fish assemblage within the stream network was evaluated in May 2005 with the help of two staff members of the Philadelphia Water Department and four WCU undergraduates. All 14 sites were sampled using a Smith-Root LR24 backpack electrofishing unit. Two passes of a 50-meter reach were typically performed at each site. Very small stream size and the increased probability of capturing all fish present at site 1 justified the decision to limit electrofishing to a single pass. An estimated 100-m of stream was sampled at site 4 with a single pass; dense vegetation precluded continuous electrofishing of the reach. Fish were typically identified to species on site and returned to the stream. In several instances where the identity was uncertain, fish were preserved in 70% ethanol for later identification in the lab. Total fish were expressed as densities/m² of stream surface.

Invertebrate sampling.

Pilot Study: Qualitative samples of invertebrates were collected on March 7th, 9th, and 10th, 2005. Invertebrates were collected using a kick screen (~1.5 mm mesh) at two riffle locations within each site. Invertebrates were randomly removed from the screen until approximately 100 individuals were collected. The actual counts were 123-252 individuals. The invertebrates were identified to genus or family. Because of the large mesh size of the kickscreen used, and the semi-quantitative nature of the sampling, the data are not formally evaluated in the Results section, but are included in Appendix A to provide a taxonomic summary of the assemblage during early spring.

June Study: Aquatic invertebrates were sampled quantitatively between 6/23/05-6/30/05. One-ft² Surber samples (250- μ m mesh) were obtained from each of four riffles

within the 50-meter reach at each site and combined into one sample. All invertebrates and debris collected within the Surber sampler were washed into 250- μ m mesh sieves, then transferred to polybottles and preserved in 90% ethanol.

Prior to identification, most samples were first passed through a splitter (Figure 3) to reduce counting effort to 221-676 total invertebrates. The splitter was a cylindrical tube with a 250- μ m mesh screen at the bottom. The screen had a black line drawn down the center. One side of the line (right or left) was chosen randomly and the sample was poured into the splitter. Only invertebrates that fell on the chosen side of the line were identified. The sample at Site 1 was not split. Site 2 was split once, and half was identified. Samples from the remaining sites were split twice and $\frac{1}{4}$ of the original sample was identified. Invertebrates were then separated from the sediment and organic debris and typically identified to genus. Flatworms, nematodes and oligochaetes were identified to order; chironomids and baetid mayflies were identified to family.



Figure 3. Sample splitter (250- μ m mesh)
(photo taken by Danielle DiFederico)

Data Analysis

Data were analyzed using SPSS version 15.0 unless otherwise specified. A variety of metrics were used to examine the invertebrate data. Because values of many of the invertebrate metrics used in the study are sensitive to the number of organisms examined, invertebrate numbers used for computing these metrics were reduced to random subsets of 200 individuals selected from the original data using an auto-resampling routine developed in SAS (Statistical Analysis System) by Dr. Charles Dow at the Stroud Water Research Center in Avondale, Pennsylvania. Each sample was randomly subsampled 1000 times. At the end of each subsampling, metrics were calculated. The 1000 individual estimates of each metric were then averaged for each site.

The MAIS (Macroinvertebrate Aggregated Index for Streams) is a multimetric index incorporating the values of 10 metrics (described in more detail below): Ephemeroptera Richness, EPT Richness, Intolerant Taxa Richness, % Ephemeroptera, % EPT, % 5 Dominant Taxa, Simpson Diversity, HBI, % Scrapers, and % Haptobenthos. The MAIS score was based on family-level taxonomy of a 200-invertebrate sample. Based on the MAIS calculation, sites were classified based on scores potentially ranging from 0-20: <6 = poor; $6-13.1$ = fair; >13.1 = good (Smith, 1997).

Ephemeroptera richness is the number of mayfly species per sample. EPT richness is the number of taxa within the insect orders Ephemeroptera, Plecoptera and Trichoptera (mayfly, stonefly and caddisfly). Intolerant taxa richness is the number of invertebrate families, per sample, with tolerance values of ≤ 5 .

Percent Ephemeroptera is the abundance of all mayflies per sample divided by the total number of invertebrates in that sample. Percent EPT is the number of invertebrates in the orders Ephemeroptera, Plecoptera and Trichoptera divided by the total number of invertebrates in that sample.

Percent 5 dominant taxa is the combined percent composition of the 5 numerically most abundant taxa per sample. Simpson Diversity integrates species richness and evenness to measure general diversity. It is expected to decrease in response to disturbance (Smith, 1997):

$$D = 1 - \sum p_i^2 \quad (1)$$

where D is the diversity index, p_i is the proportion of individuals in the i th species, and Σ means “sum of” (Allaby, 1998).

The Hilsenhoff Biotic Index (HBI) was calculated as the mean weighted tolerance value of taxa present in the sample (with tolerances based on PADEP values used in Unassessed Waters surveys, and weighting based on taxon abundances) (Barbour, 1999):

$$HBI = \sum_i n_i a_i / N \quad (2)$$

where n_i = the number of individuals of taxon i , a_i = the tolerance value for taxon i , and N = the total number of individuals in the sample.

Percent scrapers is the percent composition of invertebrates that have mouthparts designed to scrape periphyton from the substratum. Percent haptobenthos is the percentage of organisms that cling or crawl on rock surfaces. They require clean, rough, firm substrates (Smith, 1997). They are associated with, but do not live within, substrates such as snags, roots, brush, or large rocks (Neuswanger, 1982).

Another metric often used in water quality bioassessment, but not included in the MAIS, is the Shannon Diversity Index. Shannon Diversity Index (H') is an equation where the proportion of each species i is first multiplied by its natural logarithm ($\ln p_i$). The resulting products are summed across species, and multiplied by -1 (Beals, 2000):

$$H' = -\sum_{i=1}^S p_i \ln p_i \quad (3)$$

A major purpose of the study was to relate the invertebrate assemblage to three groups of habitat variables: 1) stream size, 2) canopy cover and associated water chemistry and 3) land use. Because of the large number of interrelated variables comprising each group, methods were developed to summarize their covariation, producing a smaller number of variables that could then be related to the invertebrate community as described below.

Stream size was jointly described by 6 variables: gradient, stream order, depth, width, discharge and percent riffles. These were strongly inter-correlated. Principal Components Analysis (PCA) was therefore used to create a single, composite variable (PCA axis 1) that captured much of the variation among sites expressed by the original variables. PCA1 was then used as a surrogate to describe effects of stream size on the invertebrate community.

Inspection of canopy cover at the 14 sites indicated that sites could be described as either having “closed” canopies (with tree cover $\geq 80\%$ based on canopy densiometer readings), or “open” canopies (with densiometer readings $< 80\%$). Mean estimates of water temperature, dissolved oxygen saturation, chlorophyll- a , pH, total phosphorus and

specific conductance were then compared between open and closed canopy sites using a series of two-sample t-tests.

Principal components analysis was also used to summarize differences in land use among the subwatersheds. Cononical axes PCA1 and PCA2 were related to the 11 land use categories and used in a 2-dimensional ordination plot summarizing differences among subwatersheds.

Spearman rank correlation analysis and linear regression were used to measure the relationship between the invertebrate community and environmental variables. Spearman correlation is a nonparametric rank statistic used to measure the strength of the relationship between two variables (Weisstein, 2002). Linear regression illustrates the relationship between two variables by fitting a linear equation to observed data:

$$Y = a + bX \quad (4)$$

Where X is the explanatory (in this case environmental) variable and Y is the dependent variable (in this case a measure of invertebrate community structure).

III. RESULTS

Three major groups of environmental variables are first evaluated: 1) stream habitat, 2) riparian canopy cover and its effects on water physicochemistry, and 3) subbasin characteristics within the larger Plum Run watershed. Macroinvertebrate community metrics, taxonomic structure and functional guild composition are then related to stream size, riparian cover and water physicochemistry. In the final section, densities and species abundances of fish in the Plum Run stream network are also briefly related to variation in the macroinvertebrate community.

Stream Habitat: The 14 sampling locations encompassed a range of stream channel sizes and water flow, with smaller sites typically occupying higher ground on the periphery of the watershed. Both headwater sites (1, 10) had the same elevation of 117.35 m (Fig. 4). The furthest downstream site (7) had an elevation of 53.34 m, resulting in an overall elevational change from source to mouth of 64 m.

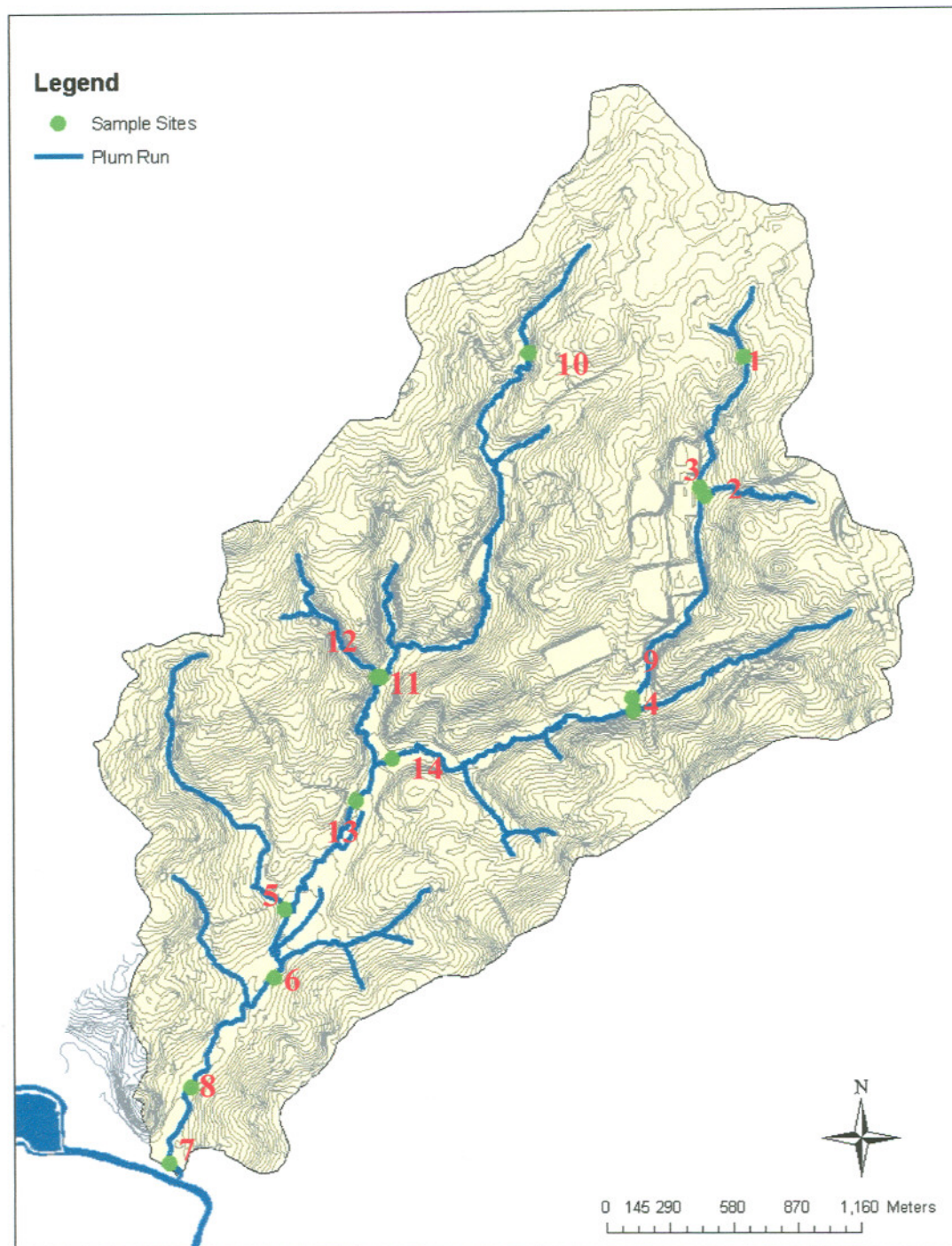


Figure 4. Plum Run watershed, showing elevation and site locations. Five foot contour lines indicating stream gradients for the watershed. The highest elevation is 146 meters; the lowest is 51.8 meters. (provided by Dr. Gary Coutu, West Chester University Department of Geography and Planning).

Headwater sites with smaller widths, depths and discharge were also characterized by higher gradients and consequently high areal percentages of riffle habitat (Table 3).

Table 3. Variation among sites in variables related to stream habitat. Highest and lowest numbers are in bold.

Site #	Mean Width (m)	Mean Depth (cm)	% Riffles	Gradient (m/m)	St. Order	Discharge (L/sec)
1	1.2	5.65	33	0.017	2	0.25
2	1.5	6.51	78	0.032	1	1.23
3	2.2	12.4	50	0.009	2	16.25
4	1.6	7.9	67	0.038	4	5.05
5	1.2	8.9	23	0.014	1	3.44
6	3.8	20.2	17	0.004	4	31.70
7	3.5	15.2	25	0.003	4	49.49
8	3.4	21.2	26	0.004	4	52.85
9	3.1	12.9	34	0.012	2	11.62
10	2.1	9.78	20	0.011	1	6.69
11	2.8	8.93	61	0.014	3	9.47
12	0.9	6.7	72	0.047	2	3.99
13	3.8	14.5	79	0.012	4	35.67
14	3.0	11.3	36	0.009	3	14.99

Depth, width, order, and discharge were all positively correlated, increasing with distance downstream (Table 4). All four variables were negatively correlated with gradient and percent riffle habitat, both of which were greatest at upstream sites.

Table 4: Correlations among variables describing stream size. * = $p < 0.05$, ** = $p < 0.01$

	Depth	Width	% Riffles	Gradient	Order	Discharge
Depth	1					
Width	0.844**	1				
% riffles	-0.456	-0.263	1			
Gradient	-0.695**	-0.734**	0.690**	1		
Order	0.636*	0.693**	0.012	-0.271	1	
Discharge	0.887**	0.810**	-0.292	-0.616*	0.734**	1

Principal Components Analysis (PCA) was used to create a smaller number of composite variables capturing much of the covariation among the six variables related to stream size. The first axis of the PCA (PCA1) explained 67% of the variation in the original variables; PCA1 was positively related with depth, width and discharge, and negatively related to stream gradient (Table 5). PCA2 explained an additional 21.2% of the total variation and was largely determined by the proportion of riffle habitat.

Table 5. Correlations of the first two axes of a principal components analysis describing stream size with the six variables from which they were derived.

Size Variable	PCA 1	PCA 2
Depth (cm)	0.946	0.022
Width (m)	0.921	0.158
% Riffles	-0.498	0.797
Gradient (m/m)	-0.912	0.470
Stream Order	0.710	0.589
Discharge (L/sec)	0.918	0.212

Similarities in size among sites are shown as a scatterplot of PCA2 vs. PCA1 in Figure 5. Downstream sites 6, 7 and 8 occur at the right side of the plot, whereas smaller headwater stream segments (e.g., sites 1, 2, 4, 5 and 12) occupy the left side of the figure. The relatively high proportion of riffle habitat at sites 4, 12 and 13 is consistent with their position near the top of the figure.

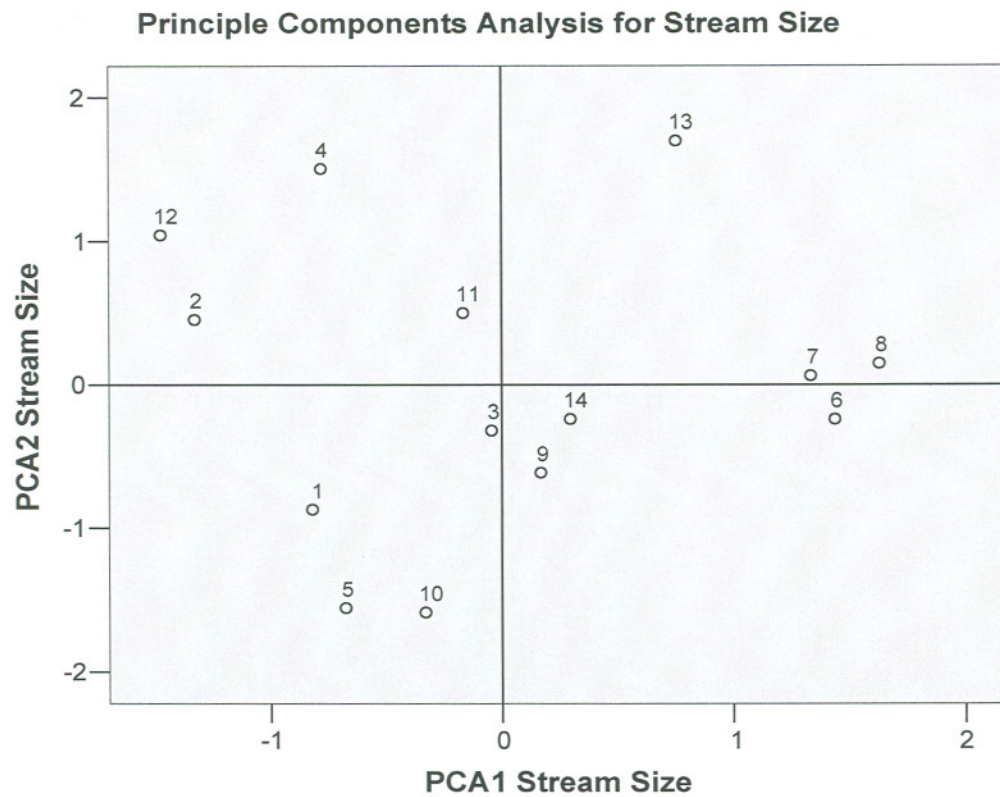


Figure 5. Principal components ordination of stream size. Relationships of the first two principal components axes to the original variables are shown in Table 4.

Regression analysis was used to relate cumulative watershed area vs. discharge. 92% of the variation in discharge was explained by watershed area (Figure 6). Sites 6, 7, 8 and 13 (at the base of the watershed near the confluence with Brandywine Creek) had the highest discharge values within the stream network. The slope of the regression indicated that approximately 4.8 L/sec of base flow was added to the stream for each 100 ha of added watershed area.

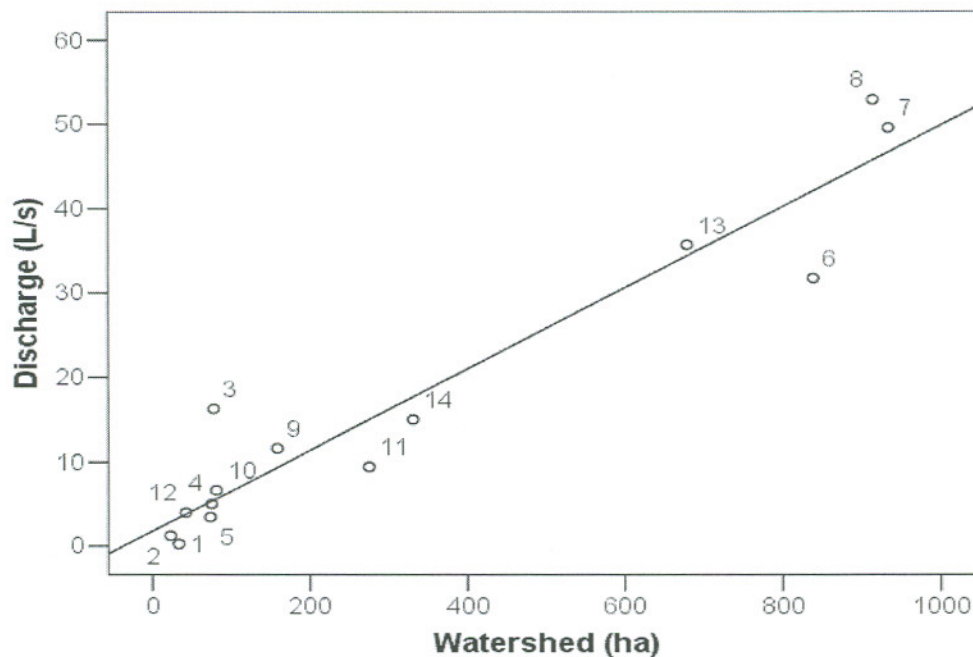


Figure 6. Discharge of 14 sites vs. watershed size in hectares (ha).
 $\text{Discharge} = 1.8 + 0.048(\text{watershed area})$. $p = 0.001$.

Riparian Vegetation: Canopy cover varied widely among sites. Eight sites with at least 80% canopy cover were labeled “closed” (Table 6); the remaining six sites were considered “open.” The site with the highest percent canopy cover was site 2, a headwater tributary of the East Branch passing through the Gordon Natural Area; site 2 was dominated by American Beech, Red Maple, Norway Maple, Tulip Poplar, Privet and Spice Bush. The Radley Run golf course (site 8) and Fox Hill Farm (site 11) had zero canopy cover. The width of forested riparian cover adjacent to the stream bank was likewise greater at closed-canopy sites, with greatest values at sites 2, 4 and 9 in the Gordon Natural Area and at site 14 on the lower East Branch.

Table 6. Canopy cover percentages (highest and lowest % are in bold) and the width of forested

buffer on the left and right banks as viewed by an observer in the stream.

Site #	Canopy Open/Closed	% Canopy	Riparian Right Bank (m)	Riparian Left bank (m)
1	Open	51.45	5	0
2	Closed	96.25	>30	>30
3	Closed	95.5	5	>30
4	Closed	88.2	>30	>30
5	Open	76.45	10	5
6	Open	36.75	>30	>30
7	Closed	93.3	10	>30
8	Open	0	0	0
9	Closed	88.9	>30	>30
10	Closed	94.1	5	>30
11	Open	0	0	0
12	Open	48.5	2	1
13	Closed	90.4	5	>30
14	Closed	94.1	>30	>30

Water Physicochemistry:

Six parameters were used in the physicochemistry analysis (Table 7). Site 7, below the golf course, had the highest temperature and pH but had the lowest percent saturation of dissolved oxygen.

Table 7. Physicochemistry variables at 14 sites within the Plum Run stream network during summer 2005. Highest and lowest numbers are in bold.

Site #	Temp (°C)	D.O. (mg/L)	D.O. (%)	Conductance (µS/cm)	TDS (g/L)	pH	TP (µg/L)	Chl-a (µg/cm ²)
1	19.0	11.50	124.0	522	0.365	7.2	39	0.346
2	17.7	9.59	100.8	324	0.250	7.0	30	0.549
3	16.1	9.34	94.8	333	0.256	7.1	33	0.475
4	15.6	9.52	95.7	252	0.201	6.9	27	0.556
5	17.5	9.66	103.9	249	0.190	7.1	27	1.540
6	18.8	9.64	106.6	286	0.204	7.1	38	0.366
7	22.3	7.78	91.7	388	0.245	7.7	27	0.326
8	21.7	11.23	130.8	346	0.242	7.2	32	0.536
9	16.3	9.92	104.4	305	0.153	7.0	66	1.553
10	16.2	9.00	94.4	566	0.420	7.1	27	1.174
11	18.8	10.23	113.2	367	0.268	7.2	18	0.549
12	19.9	8.67	97.8	217	0.155	7.4	22	1.479
13	17.3	9.69	104.1	340	0.249	7.5	33	1.533
14	15.3	9.95	102.6	319	0.247	7.4	63	0.787

Sites 1 and 10 had the two highest values of specific conductance and total dissolved solids (TDS), both measures of total solute concentration. Sites 1 and 10 are close to the surface origins of the East Branch and West Branch of Plum Run, respectively, and the high ion concentrations may reflect the high proportion of stormwater runoff and absence of biological uptake within the pipes feeding each branch.

Total phosphorus (TP) concentrations at sites 9 and 14 on the East Branch were considerably higher compared to other sites at the time of sample collection (Figure 7). These high levels, based on samples taken June 20 and 21, 2005, are likely due to sewage overflow from a manhole located next to the sewage lift station on the South Campus. Raw sewage overflow into Plum Run just above site 9 was reported on both days in a letter from the Pennsylvania Department of Protection to West Chester University (Gillespie, 2005). The nutrient plume provided by the sewage was evident as far downstream as site 14, raising the TP levels there as well. Further downstream, site 6 was also sampled on June 21. The TP level there was not as high. This is probably due to dilution from the West Branch, phosphorus adsorption onto sediment particles, and incorporation into microbial biomass. Site 8 had been sampled on June 15 and sites 7 and 13 had been sampled on June 16; measurements at these sites thus preceded the spill.

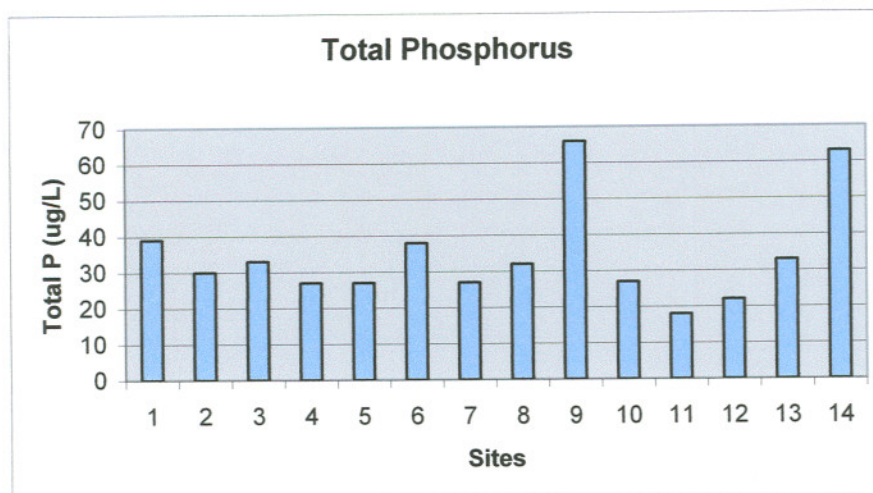


Figure 7. Total phosphorus concentrations at 14 sites in Plum Run.

As expected, percent saturation of dissolved oxygen was positively correlated with values expressed as mg/L, and specific conductance was positively correlated with TDS (Table 8). Other correlations among physicochemistry variables were weak, probably reflecting differences in weather conditions among sampling dates.

Table 8. Pearson Correlations among physicochemistry variables.

	Chl- <i>a</i> μg/L	Temp (°C)	D.O. (mg/L)	D.O. (%)	Conductance (mS/cm)	pH	TP	TDS (g/L)
Chl- <i>a</i>	1							
Temp	-0.301	1						
D.O. (mg/L)	-0.176	-0.038	1					
D.O. (%)	-0.217	0.383	0.900**	1				
Conductance	-0.340	0.106	0.172	0.181	1			
pH	0.038	0.568*	-0.335	-0.046	0.139	1		
TP	0.152	-0.408	0.263	0.091	-0.205	-0.052	1	
TDS	-0.296	-0.066	0.206	0.141	0.981**	-0.001	-0.175	1

* = $p < 0.05$, ** = $p < 0.01$

Percent saturation of dissolved oxygen was consistently higher at open canopy sites ($p=0.02$) (Table 9). Temperature was likewise significantly elevated at open sites

($p=0.003$). Specific conductance, pH, TP and chlorophyll-*a* were not affected by the presence or lack of canopy.

Table 9. Mean, standard error and significance of physicochemistry variables based on one tailed t-tests. Significant values ($p<0.05$) are in bold.

	Canopy Open	Canopy Closed	p
Specific Conductance	Mean 330.9 SE 44.6	Mean 340.5 SE 38.04	0.44
Temperature	Mean 19.28 SE 0.576	Mean 17.09 SE 0.795	0.03
D.O. (% sat)	Mean 112.7 SE 5.14	Mean 98.56 SE 1.75	0.02
pH	Mean 7.21 SE 0.036	Mean 7.20 SE 0.099	0.47
TP	Mean 29.3 SE 3.5	Mean 38.2 SE 5.8	0.25
Chl-<i>a</i>	Mean 0.80 SE 0.23	Mean 0.87 SE 0.17	0.41

Temperature was not just affected by canopy, but also slightly but not significantly affected by stream width (Figure 8).

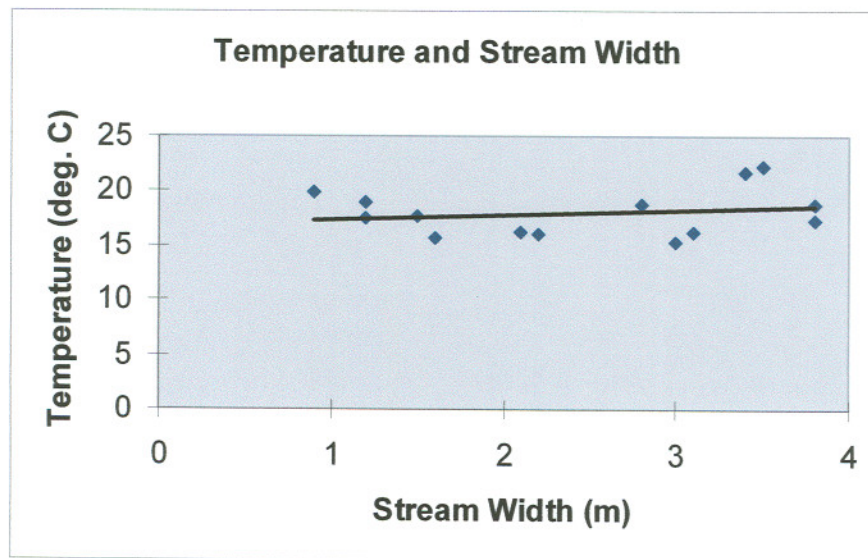


Figure 8. Linear relationship between stream temperature and stream width. $Y=17.01+0.42(\text{stream width})$. $p=0.50$, $R^2=0.04$.

Watershed Description: Major land use types within the Plum Run watershed are shown in Figure 9.

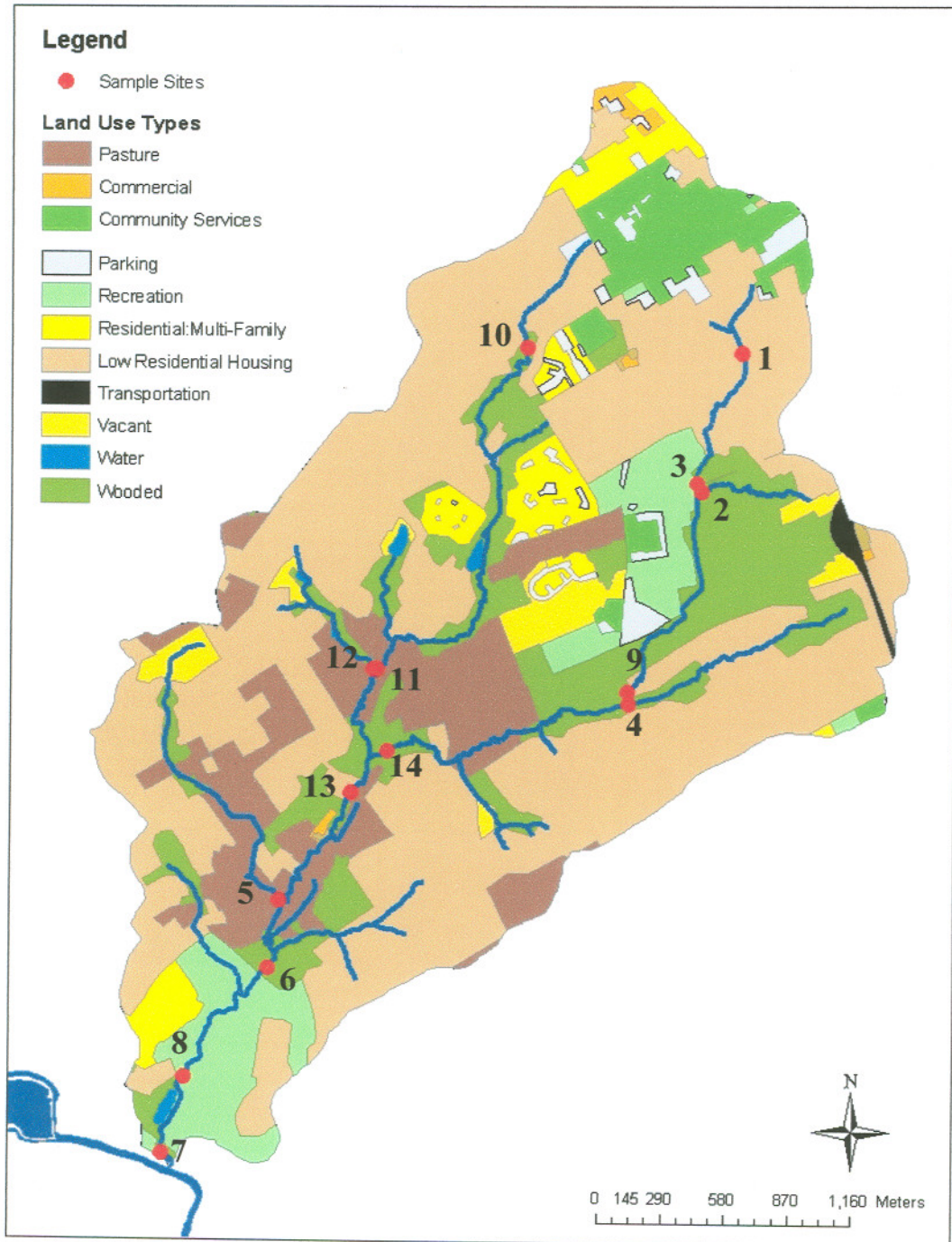


Figure 9. Major land use types within the Plum Run watershed

The most abundant land uses were low residential housing, wooded and pastureland (Figure 10). Low residential housing occupied half (50%) of the watershed, dominated most subbasins, and appears as light brown polygons occupying the majority of Figure 9. The second largest land use was wooded (16%) and appears as dark green polygons. Pasture (12%), shown in Figure 9 as dark brown polygons, occurred closest to sites 5, 11, 12, and 13. Percentages of total watershed area comprised of other land uses are shown in Figure 10.

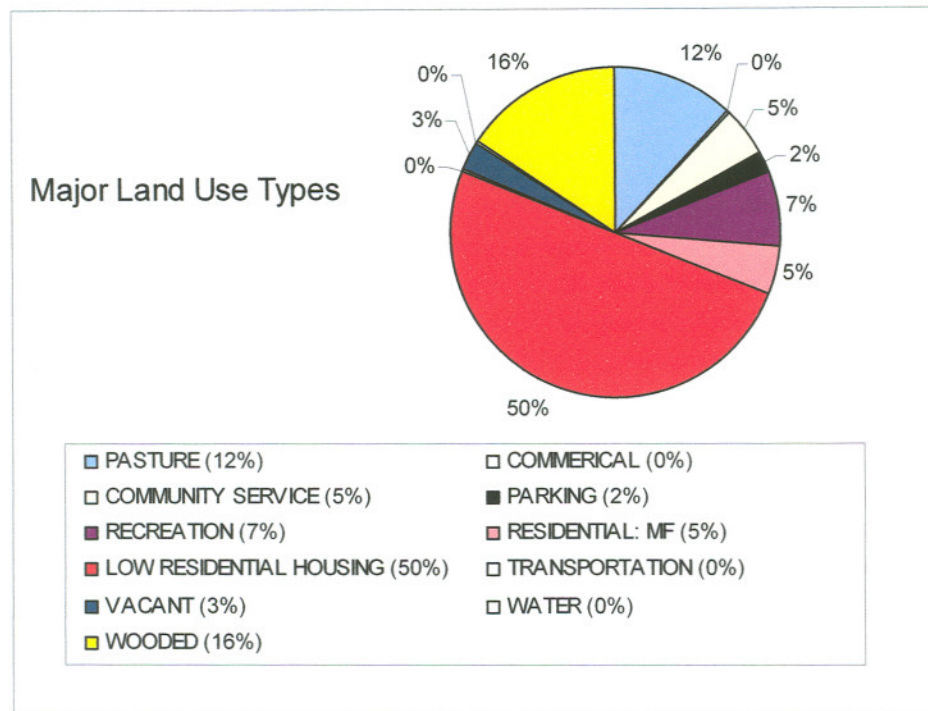


Figure 10. Land use percentages within the Plum Run watershed.

The proportions of major land uses within the subbasins upstream of individual sites are shown in Table 10. Low residential housing dominated the land upstream of ten of the 14 sites. Subbasins of the other four sites were primarily wooded and recreation. Wooded land use was the primary land use potentially affecting the stream at sites 2 and

9, located in the Gordon Natural Area. Recreation was the primary land use affecting sites 7 and 8; the subwatersheds of both sites were dominated by the Radley Run golf course. Community service, while only 5% of the entire watershed, was a large portion of the land affecting headwater sites 1 and 10; community service areas consisted mostly of university property (Figure 9).

Table 10: Land use percentages. PS=pasture, CS=community service, PK=parking, RC=recreation, MF=multiple family residence, LR=low residential housing, VC=vacant, WD=wooded. Boxes left blank are 0%. Highest % in bold.

Site	PS	CS	PK	RC	MF	LR	VC	WD
1		34.45%	9.08%	0.71%		55.76%		
2			2.63%			36.38%	10.36%	37.86%
3		0.92%	2.66%	12.95%		77.77%		5.70%
4		1.39%	0.05%	1.12%		82.50%	0.68%	13.04%
5	33.33%					52.84%	7.82%	6.01%
6	24.63%		0.23%			58.41%		15.99%
7				53.94%	4.74%	21.15%		15.73%
8	5.91%			38.36%	9.84%	37.39%		8.50%
9	0.68%	4.25%	6.73%	22.23%		4.84%	3.94%	57.35%
10		31.25%	7.05%	0.69%	15.46%	40.95%		1.31%
11	10.43%		3.03%	1.57%	11.27%	52.92%	3.79%	16.04%
12	22.27%					65.25%	3.78%	7.81%
13	30.04%					45.23%		24.73%
14	22.83%	1.29%	1.08%	4.69%		43.74%	4.75%	21.63%

Correlations among land use variables are shown in Table 11. Percent commercial land, water, and highway transportation were not included because they each comprised 1% or less of the total watershed. Community service was positively correlated with parking, which is expected, reflecting the abundance of parking lots on university property.

Table 11: Pearson correlations of land use variables as described in Table 10 (* = $p < 0.05$, ** = $p < 0.01$).

	% CS	% PK	% RC	% MF	% LR	% VC	% WD	% PS
% CS	1							
% PK	0.914**	1						
% RC	-0.065	0.187	1					
% MF	0.381	0.302	-0.191	1				
% SF	-0.142	-0.349	-0.156	-0.363	1			
% VC	-0.620*	-0.500	-0.264	-0.294	-0.427	1		
% WD	-0.568*	-0.266	0.321	-0.269	-0.457	0.611*	1	
% PS	-0.505	-0.643*	0.249	-0.128	0.061	0.430	-0.193	1

Variation in land use among the subbasins characterizing individual sites was summarized using PCA. PCA1 explained 40.4% of the variation in the original variables; PCA1 was positively related to % community service, % parking, % recreation and % MF residence, and negatively related to % pasture, % low residence, % vacant and % wooded (Table 12). Because community service was so variable (range 0.92% - 34.45%), it strongly affected the computation of PCA1. PCA2 explained an additional 24% of the variation in the original variables; PCA2 was positively related to % community service, % parking, % recreation, % MF residence, % vacant and % wooded, and was negatively related to % pasture and % low residence.

Table 12. Correlations of original variables with the first two principal components describing land use within the subbasins of 14 sites.

Land Variable	PCA 1	PCA 2
% pasture	-0.614	-0.446
% community service	0.944	0.003
% parking	0.886	0.343
% recreation	0.091	0.491
% MF residence	0.485	0.063
% low residence	-0.036	-0.808
% vacant	-0.796	0.330
% wooded	-0.549	0.770

Land use patterns were the most dissimilar for first order stream sites (1, 2, 4, 5, 9, 10, 12), as shown by their occurrence on the periphery of a scatterplot of PCA1 vs. PCA2 (Figure 11). Sites 1 and 10 were farthest to the right on PCA1, consistent with the high % community service and parking in their subbasins (both sites were close to the university). Sites 2 and 9 occurred at high values for PCA2, near the top of the figure, indicating the high % wooded and recreational components of their subbasins; both sites were located within the Gordon Natural Area. Sites 5 and 12 had low values for both PCA1 and PCA2 as a consequence of the large amounts of pastureland surrounding them. By contrast, the much larger subbasins of downstream sites (especially sites 6, 7, 8, 13) more nearly reflected land use patterns within the entire watershed, and those sites thus occur near the center of Figure 11.

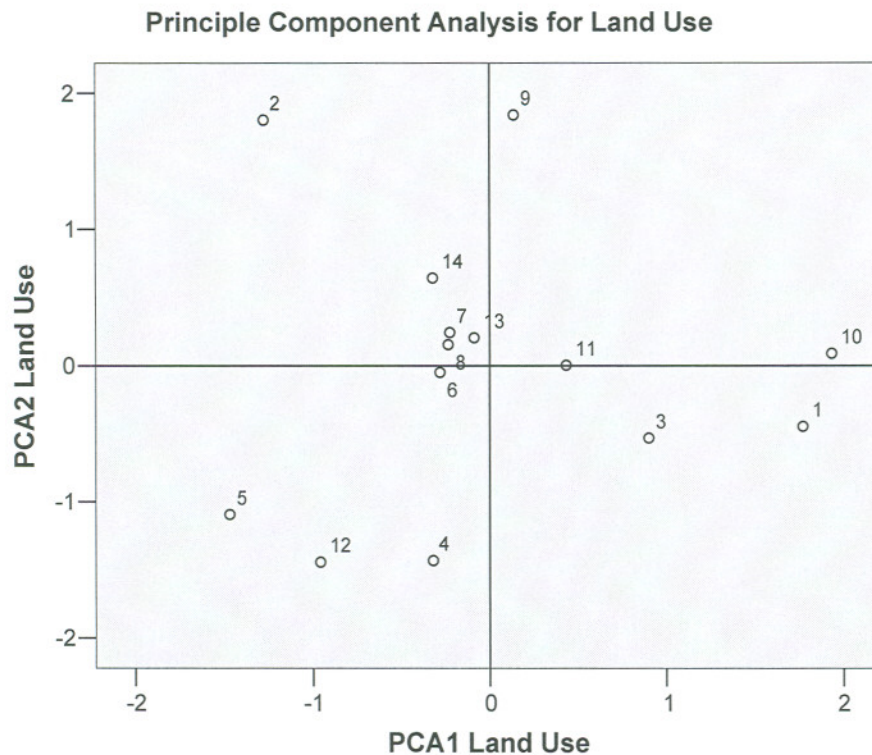


Figure 11. Principal components ordination of 14 sites, based on the percentages of 8 land uses (Table 11).

Macroinvertebrate Pilot Study

Kickscreen samples of the invertebrates in early March were not accompanied by concurrent environmental measurements, and provided only qualitative relative abundance. They nonetheless provide valuable taxonomic information relevant to the interpretation of invertebrate densities in June (below), and are therefore included in Appendix A.

June Study of Macroinvertebrates

Distributions of Individual Taxa: Invertebrate taxa collected at the fourteen sites in June are shown in Table 13. The caddisfly *Hydropsyche*, the mayfly family Baetidae, and midges of the family Chironomidae were present at all 14 sites. *Hydropsyche* was most abundant at Site 7 (downstream of the golf course) with an estimated 871 individuals/m² (Table 13). Baetidae was the most abundant taxon at Site 4, with 2452 individuals/m². Chironomids were the most abundant taxon found overall. The amphipod *Gammarus*, the second most abundant taxon, was found primarily at downstream sites (especially sites 6, 7, 8 and 13), but was also a dominant component of the community at site 10 in the upper West Branch. The stonefly *Leuctra* was very abundant at Site 2 (1097 individuals/m²) replacing the mayfly *Ameletus* (found in March) as the dominant taxon. Site 10 showed the lowest taxonomic richness (13 taxa) while site 2 showed the highest (28 taxa).

Table 13. Invertebrates collected during June 2005. Numbers indicate densities (invertebrates/m²). Values >100 are rounded to the nearest integer. Highest densities are in bold.

Family	Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nematoda			5	43	11								140		21
Turbellaria		140						11		11			97		
Cambaridae			11										11		
Asellidae	Caecidotea	220		97							97		53		
Crangonyatidae	Crangonyx						11				11				
Gammaridae	Gammarus	3				226	1021	3881	710		720	806	441	860	613
Collembola					11									11	
Baetidae		51	204	247	2452	97	108	53	54	376	43	118	269	140	301
Ephemereidae	Ephemerella								11						11
Tricorythidae	Tricorythodes								21						
Odonata	Unknown												11		
Calopterygidae	Calopteryx			11											
Plecoptera	Unknown		43												
Peltoperlidae	Tallaperla		5												
Nemouridae	Anphinemura		11												
Leuctridae	Leuctra		1097	86	183		21			54				21	43
Perlidae	Eccoptura				21					54					11
Veliidae	Microvelia	5													
Veliidae	Rhagovelia					11									
Philopotamidae	Unknown									11					
Philopotamidae	Chimarra	67				11	21		11	11		75	75	108	
Philopotamidae	Dolophilodes		140		75	21	11			505				43	21
Philopotamidae	Wormaldia		16								11				
Hydropsychidae	Unknown		32	11	301	21	161	280	11	108	108	54	97	64	11
Hydropsychidae	Ceratopsyche	11													
Hydropsychidae	Cheumatopsyche	245	21	97		21	183	140	54	32	97	419	150	108	86
Hydropsychidae	Diplectrona		38												
Hydropsychidae	Hydropsyche	113	5	32	11	151	785	871	376	226	204	226	172	161	129
Rhyacophilidae	Rhyacophila				11	11				11					
Glossosomatidae	Glossosoma		11	53	226	75.3	32			118		11		54	54
Hydroptilidae	Leucotrichia						53							32	

Table 13 (continued)

Family	Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Uenoidae	Neophylax		70	11	43	11				151			64	21	
Pyrilidae	Acentria	3													
Dytiscidae	Hydroporinae	3													
Hydrophiloidea	Tropisternus					11									
Psephenidae	Ecopria	21	5												
Psephenidae	Psephenus						43					54	11	75	11
Dryopidae	Helichus		5												
Elmidae	Unknown		5			97									
Elmidae	Optioservus	21	43	86	64	634	366	53	409	54		129		140	108
Elmidae	Stenelmis	78		54	11	172	591	194	527			151	75	194	11
Elmidae	Promoresia		5												
Elmidae	Oulimnius		16	21	21.5	194	21	11	43	32				75	75.3
Ptilodactylidae	Anchytarsus					97									
Diptera	Unknown		16												
Chironomidae		492	758	1527	1151	409	548	344	1871	1548	1548	4634	688	1204	1108
Empididae	Chelifera		5		11		11		10						
Empididae	Hemerodromia										11				
Simuliidae	Prosimulium		27	21	237	32	11			204		43			
Simuliidae	Simulium	13	16	21	108	75		21	21	355	312	108		32	
Tipulidae	Antocha	21		366	11	32	97	32	151	11		430	11	269	32
Tipulidae	Dicranota		27	527	11	548	11		54	129				43	87
Tipulidae	Tipula	19			11				11		54				11
Tipulidae	Pedicia			11											
Physidae								21							
Number of taxa		19	26	19	21	22	20	13	16	19	12	14	16	20	19
Total Density		1530	2661	3398	5108	3011	4118	5924	4344	4021	3043	7269	2376	3677	2774

Invertebrate water quality metrics were computed to examine the invertebrate data after auto-resampling (Table 14).

Table 14. Multiple metrics for 14 sites of June invertebrates with highest and lowest values in bold type

Site #	MAIS	HBI	% EPT	% Chironomids	Shannon (H')	% Tolerant
1	8	5.8	31.9	32.0	2.07	79.8
2	12	3.0	63.5	28.5	1.83	35.0
3	8	4.8	15.8	45.1	1.93	56.7
4	12	4.5	65.0	22.6	1.71	38.4
5	11	4.4	13.9	13.6	2.47	33.3
6	12	5.3	33.5	13.4	2.16	67.9
7	8	5.9	22.5	5.8	1.23	94.2
8	10	5.3	12.5	43.1	1.77	70.4
9	11	4.9	41.2	38.5	2.14	62.2
10	6	6.0	15.2	44.9	1.65	96.5
11	6	5.7	12.4	63.7	1.39	86.7
12	10	5.7	34.8	28.9	2.23	76.9
13	11	5.1	20.6	32.7	2.21	67.7
14	10	5.3	24.1	40.1	1.95	72.1

Because the MAIS score integrates the values of 10 metrics, it was used as the primary means of summarizing the relationship between the invertebrate metrics and the environmental data. Figure 12 shows a highly significant ($p = 0.008$) relationship between MAIS and specific conductance; higher specific conductance levels resulted in lower MAIS scores.

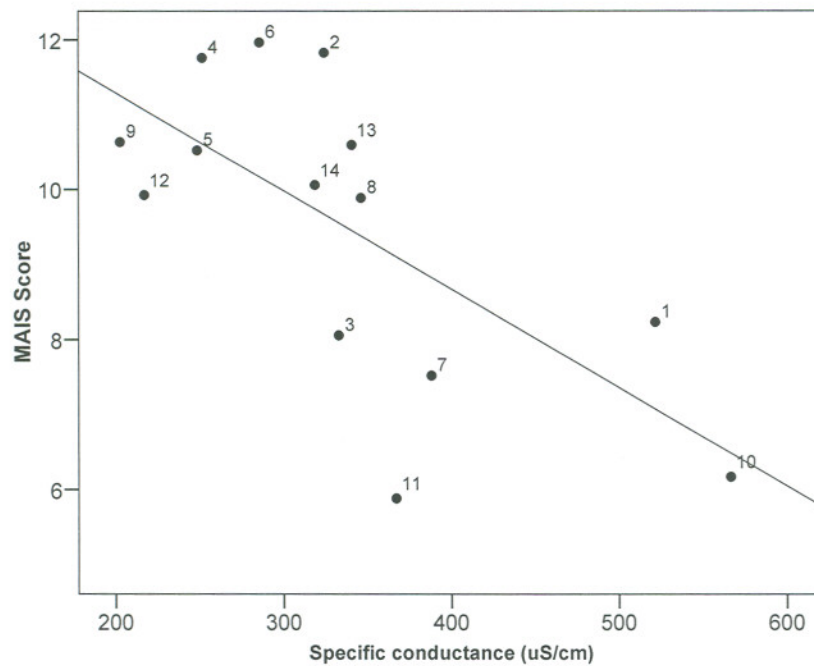


Figure 12. Relationship of specific conductance to MAIS score. $Y=13.91-0.013(\text{conductance})$. $p=0.008$, $R^2=0.46$

The MAIS index also varied significantly with land use (Figure 13). Subbasins with high amounts of urbanization (high % parking, high % recreation, and high % multiple family residence) were associated with low MAIS scores (site 10), whereas sites with forested subbasins (site 2) typically had high MAIS scores (Figure 11 and Table 10).

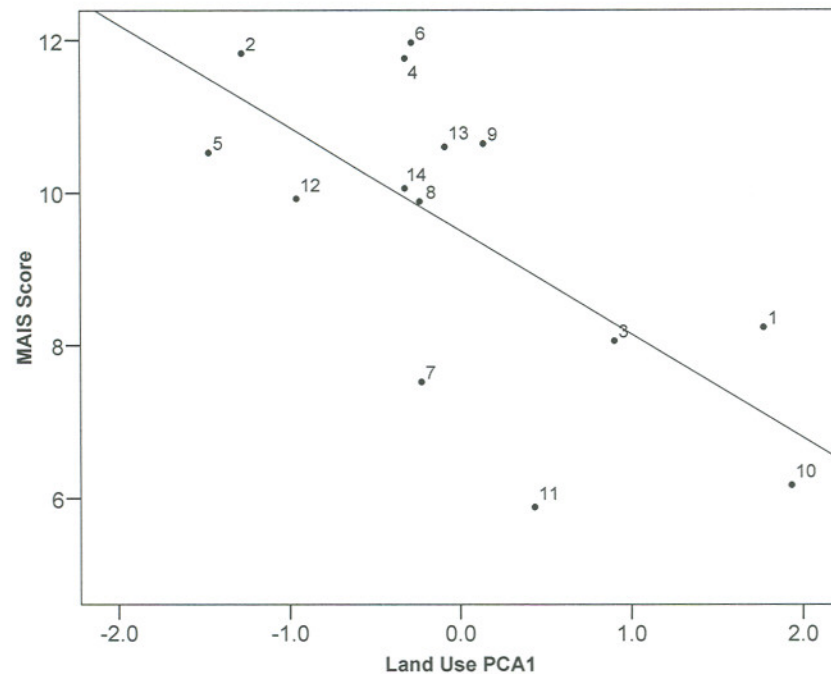


Figure 13. Relationship of Land Use PCA1 to MAIS score.
 $MAIS = 9.5 - 1.4(Land\ Use)$. $P=0.008$, $R^2=0.45$

Based on the MAIS index, site 11 in the West Branch was the only site considered to have “poor” water quality (site 11 also had the lowest % EPT and the highest % chironomids).

The metrics for site 1 (Table 14) generally fall within the mid to high range, indicating low to fair habitat quality near the origin of the East branch. The MAIS and Hilsenhoff indices both categorize site 1 as “fair.”

Site 10 near the origin of the West Branch likewise indicated very low habitat quality based on the MAIS index. Site 10 also had the highest percent of tolerant species, and had a Hilsenhoff biotic index value (HBI) that indicated the worst habitat conditions

among all sites included in the study (whereas high values for the MAIS index indicate high water quality, a high HBI score indicates low water quality).

Sites with higher MAIS values were 2 and 4, although these values were considered “fair.” Site 2, one of the sites considered of higher quality based on the MAIS index, also had a rating of “excellent” based on Hilsenhoff’s (1988) HBI. The high relative abundances of sensitive stonefly, mayfly and caddisfly taxa in the March samples at site 2 (see Appendix A) are also consistent with the view that this tributary of the East Branch within the Gordon Natural Area is of particularly high quality.

As predicted, total invertebrate densities increased significantly at downstream sites (Figure 14). Water quality (as evidenced by both physicochemistry and invertebrate metrics) was not consistently related with overall densities. For example, sites 1 and 10, scored as having poorer water quality, had highly variable invertebrate densities; site 2, with superior water quality, had approximately the invertebrate densities that might be predicted for a tributary stream of its size. Site 11 at the top of figure 14 had a much higher density of invertebrates than the other sites owing to particularly high density of chironomids.

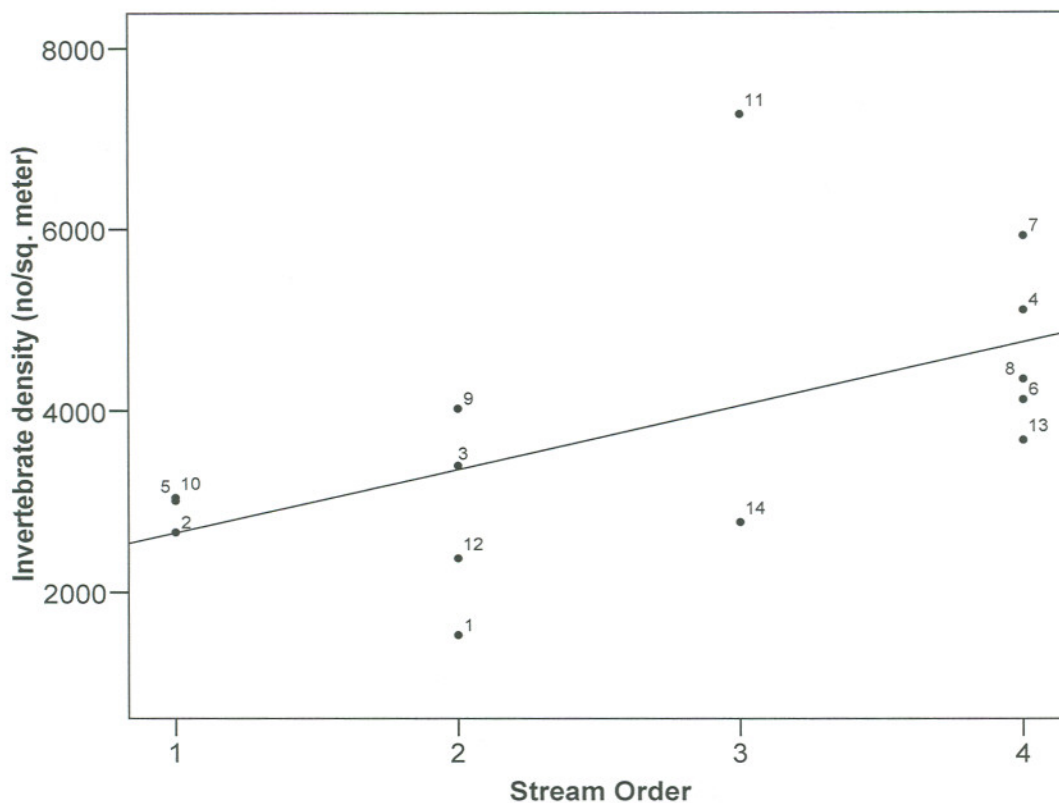


Figure 14. Relationship of stream order to invertebrate density.
 $Y=1955+699(\text{stream order})$. $p=0.036$, $R^2=0.32$

Relationship of Dominant Invertebrate Taxa to Environmental Variables: Five of the most abundant and widespread taxa within the stream network were chosen for analyses of the relative importance of the environmental variables: Chironomidae, *Antocha*, *Cheumatopsyche*, *Optioservus* and *Hydropsyche*. Correlation coefficients summarizing their relationship with environmental parameters are shown in Table 15. These taxa had a total abundance of ≥ 10 individuals and were found in at least 11 of the 14 sites. Of the five taxa considered, the majority (Chironomidae, *Antocha*, and *Cheumatopsyche*) are considered more tolerant taxa (tolerance values ≥ 6). The stream size variables depth, width, percent riffles, gradient, and discharge influenced the

distribution of *Hydropsyche*, showing that this genus was more typical of larger, downstream sites. As previously shown, the MAIS index was negatively related to specific conductance.

Table 15. Significant Spearman correlations of invertebrate variables to environmental variables.

	MAIS	Invert. Density	Antocha	Hydropsyche	Cheumatopsyche
Depth		0.604 p=0.022		0.706 p=0.005	
Width		0.622 p=0.018		0.592 p=0.026	
Order		0.668 p=0.009			
Discharge		0.631 p=0.016	0.597 p=0.024	0.625 p=0.017	
% Riffles				-0.546 p=0.044	
Gradient				-0.614 p=0.019	
Sp. Cond	-0.688 p=0.007				
Temp				0.556 p=0.039	
pH					0.535 p=0.049

Functional Guilds: Collectors made up the highest percentage of invertebrates for every site except sites 2 and 5 (Table 16). The invertebrate community at Site 2 had the highest percentage of shredders (2.5 fold greater than at any other site). This is expected, as site 2 also had the highest canopy density. Site 5, an open-canopy tributary site of the main stem near the base of the watershed, had the highest percentage of scrapers and predators. The two most abundant taxa in site 5 were *Optioservus* (Family Elmidae; a scraper), and *Dicranota* (Family Tipulidae; a predator).

Table 16. Functional feeding groups of all 14 sites. Highest percentage for each group is in bold.

Site	% shredders	% filterers	% predators	% scrapers	% collectors
1	2.81%	29.35%	9.67%	7.91%	50.26%
2	42.98%	11.36%	1.03%	6.40%	38.22%
3	16.14%	5.38%	16.14%	6.65%	55.70%
4	3.79%	14.32%	0.84%	7.16%	73.89%
5	3.23%	10.39%	19.35%	39.43%	27.60%
6	0.54%	25.54%	0.27%	27.99%	45.65%
7	0.00%	22.00%	0.18%	4.73%	73.09%
8	0.25%	10.67%	0.00%	23.82%	65.26%
9	1.39%	34.72%	5.28%	8.06%	50.56%
10	1.83%	21.25%	0.37%	0.00%	76.56%
11	0.00%	12.07%	0.00%	4.77%	83.16%
12	0.00%	19.91%	4.27%	4.27%	71.56%
13	0.00%	14.08%	1.17%	15.25%	69.50%
14	0.78%	8.59%	3.52%	8.98%	78.13%

Shredders were predicted to increase at sites with higher canopy cover. Figure 15 suggests a slight, but not significant, positive relationship between shredder abundance and canopy cover ($p = 0.20$). The absence of a stronger relationship could be explained by the June sampling date when much of the leaf litter from the previous fall is gone, leaving shredders less likely to populate closed canopy sites during the summer season.

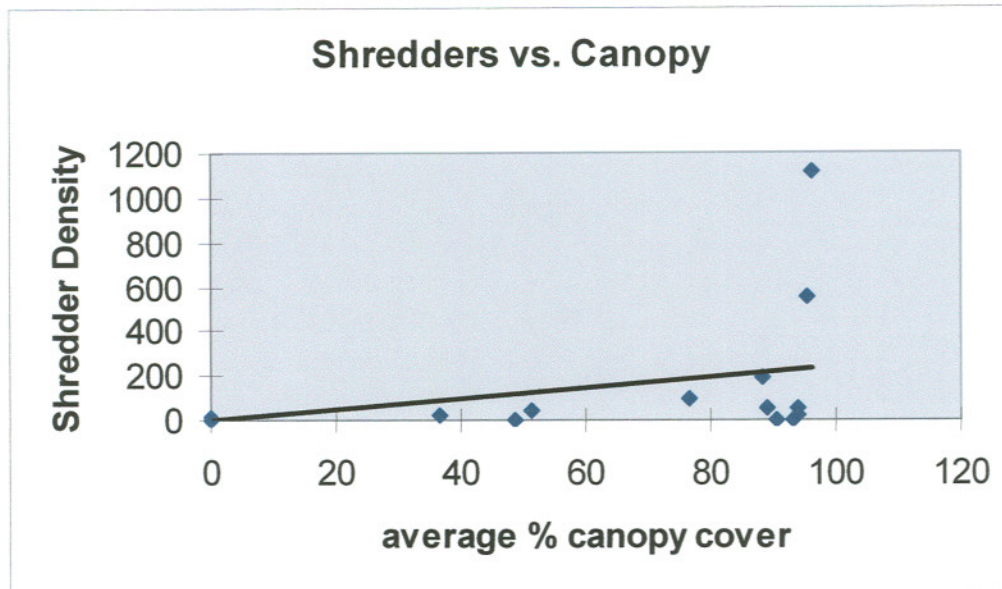


Figure 15. Relationship between densities of shredders (SD) and percent canopy cover. $SD = -69.45 + 3.28(\text{percent canopy})$. $p=0.20$, $R^2=0.13$

Scrapers were predicted to increase in sites with higher chlorophyll-*a*. This was clearly not the case ($p = 0.89$) (Figure 16).

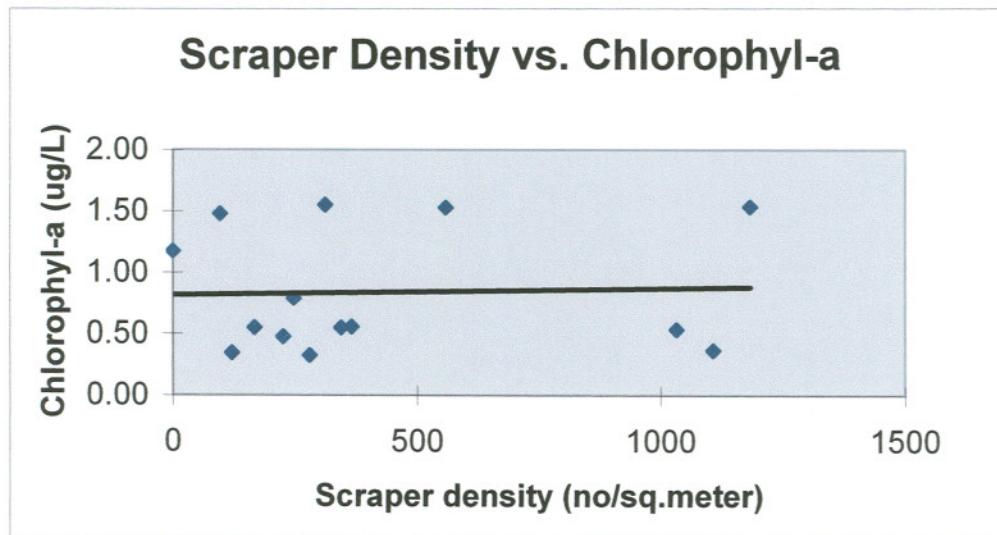


Figure 16. Linear relationship between densities of scrapers and chlorophyll-*a* levels. $Y = 403.76 + 32.94(\text{chlorophyll-}a)$. $p=0.89$, $R^2<0.01$

Fish

The abundances of fish species collected at the fourteen sites are shown in Table 17. Species from the families Cyprinidae, Centrarchidae, Catastomidae, Ictaluridae, Percidae, and Anguillidae were found. Cyprinids (minnows) and Centrarchids (sunfish) were the most abundant fishes within the stream network.

Species richness was negatively related to distance from Brandywine Creek (Figure 17), which may serve as a seasonal refugium particularly for larger fish species (Butler & Fairchild 2005). Site 7 (downstream of the golf course) showed the highest species richness, while Site 1 showed the lowest richness.

Table 17. Fish species collected from Plum Run in May 2005. Numbers are densities/m² of stream surface

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(Centrarchidae)														
<i>Lepomis macrochirus</i> (Bluegill)							0.12							
<i>L. auritus</i> (Redbreast Sunfish)							0.06	0.05						
<i>L. cyanellus</i> (Green Sunfish)							0.01	0.01						
<i>L. gibbosus</i> (Pumpkinseed)							0.14	0.02						
<i>Ambloplites rupestris</i> (Rock bass)						0.04	0.12	0.20	0.01		0.01		0.01	
<i>M. salmoides</i> (Largemouth Bass)							0.01							
(Cyprinidae)														
<i>Semotilus atromaculatus</i> (Creek Chub)			0.11	0.04	0.28	0.08		0.01	0.15	0.10	0.15	0.50	0.02	0.36
<i>S. corporalis</i> (Fallfish)						0.02	0.01	0.03	0.01					
<i>Clinostomus funduloides</i> (Rosyside Dace)					0.08				0.15		0.09			0.01
<i>Exoglossum maxillingua</i> (Cutlips Minnow)						0.06		0.08					0.01	
<i>Luxilus cornutus</i> (Common Shiner)						0.02	0.09	0.06						
<i>Rhinithys atratulus</i> (Blacknose Dace)	0.10	0.25	0.22	0.15	0.57	0.20			0.41	0.01	0.68	0.73	0.33	0.28
<i>R. cataractae</i> (Longnose Dace)						0.02							0.02	0.01
<i>Cyprinella analostana</i> (Satinfin Shiner)					0.07									
<i>C. spiloptera</i> (Spotfin Shiner)							0.01							
<i>Notropis hudsonius</i> (Spottail Shiner)							0.09							
(Catastomidae)														
<i>Catostomus commersoni</i> (White Sucker)					0.02	0.04	0.28	0.06	0.03		0.06		0.01	0.09
(Ictaluridae)														
<i>Ameiurus natalis</i> (Yellow Bullhead)				0.02				0.02						
(Percidae)														
<i>Etheostoma olmstedii</i> (Tessellated Darter)					0.03	0.14	0.01	0.14			0.01		0.02	0.07
<i>Perca flavescens</i> (Yellow Perch)							0.01							
(Anguillidae)														
<i>Anguilla rostrata</i> (American Eel)					0.02									
TOTAL DENSITY	0.10	0.25	0.33	0.19	1.08	0.60	0.95	0.66	0.78	0.11	1.00	1.23	0.39	0.82
TOTAL SPECIES	1	1	2	2	8	9	13	11	6	2	6	2	7	6

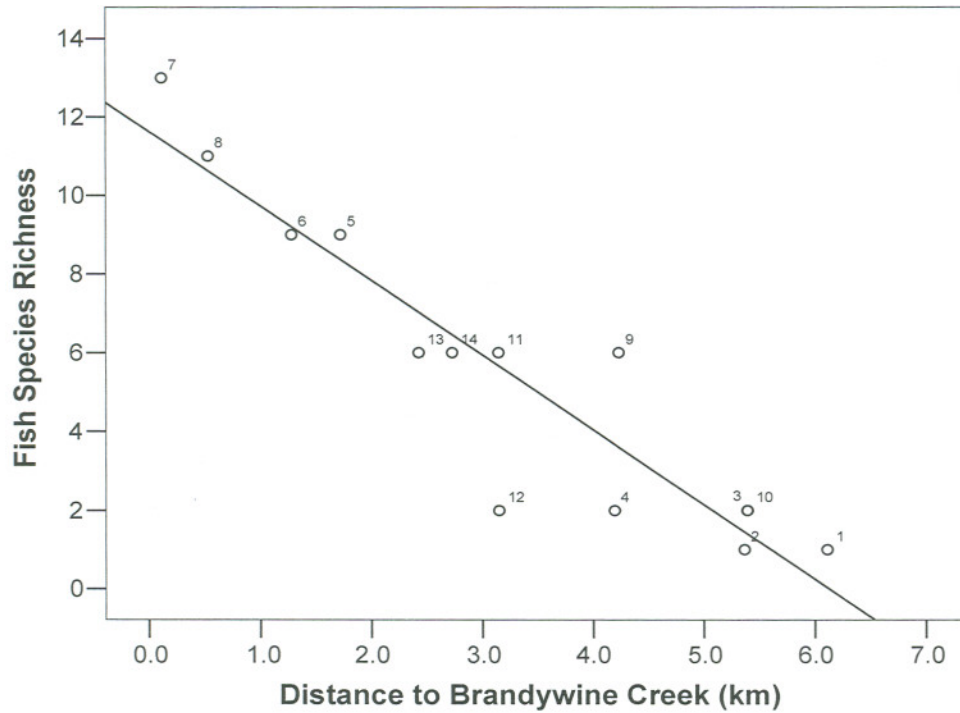


Figure 17. Fish species richness (S) in relation to site distance (D) from Brandywine Creek. $S=11.8-1.9(D)$. $R^2=0.87$, $p<0.001$

Most fish in Plum Run are considered predominantly invertivores (Cooper 1983), and thus their abundances might be expected to negatively impact the macroinvertebrate community. Because I didn't record size differences among fish or examine gut contents, inclusion of fish sampling results in this study is meant solely to provide inferential evidence of potential interactions among fish and invertebrate taxa.

IV. DISCUSSION

Plum Run is unusual in that its two main tributaries both originate in an urban setting. Thus, stream habitat impairment may be more likely in headwater sections of the stream network than is typical of streams in the region. The stream network, however, includes segments with widely varying water quality, both as a consequence of downstream amelioration and the presence of smaller tributaries. Personnel from the Pennsylvania Department of Environmental Protection (PADEP) have performed previous assessments of portions of the Plum Run stream network. A survey of three sites (near sites 10, 13 and 7 in the present study – see Figure 1) in 1979 suggested “generally good” conditions in that portion of the stream network (Strekal, 1979). A later appraisal in October 1997 using PADEP’s Unassessed Waters protocol approximately 10 meters downstream of the PA Rte.100 bridge over Plum Run (near site 7), however, formed the basis of the current designation of the stream network as “impaired” (19971023-1320-GLW, 1997). Ten families of invertebrates were found, with net-spinning caddisfly larvae of the family Hydropsychidae being the most abundant (a result similar to the surveys of sites 6, 7 and 8 reported here). The study by PADEP attributed the impairment to “agricultural uses, urban runoff and new housing developments.” PADEP evaluation of three additional sites on unnamed tributaries to Plum Run (entering Plum Run upstream of site 6) in December 2000 indicated generally high invertebrate diversity and good conditions (Boyer, 2001). This evaluation was done in order to assess the potential damage of channelization to that area. The assessment showed a diversity of invertebrates indicative of good water quality, including many EPT taxa. It was

recommended that alternative measures be taken, as the proposed channelization would cause “an adverse impact to the benthic community in the stream.” In effect, the results of earlier surveys, consistent with the present results, support the view that the Plum Run network is in reality a composite of stream segments with varying water quality.

Sampling methods frequently result in incomplete descriptions of the invertebrate community, because only a portion of the taxa can be caught (Ostermiller & Hawkins, 2004), and because natural variability over time and within even short distances in streams cannot be controlled (Resh & Jackson, 1993). Temporal change in the response of invertebrates to their environment is not simple to interpret, and may reflect daily, seasonal or annual events (Jackson & Füreder, 2006). The spatial distribution of invertebrates can be due to many factors such as flow, drift (passive downstream movement), the presence of predators, substrate type, and food resources for both the larvae and adult stages (Closs, Downes, & Boulton, 2004). Most invertebrates have seasonally-cued life cycles, and sampling date can thus cause large changes in invertebrate abundances (Sporka, Vlek, Bulankova & Krno, 2006). Long-term studies have a greater probability of overcoming daily or seasonal effects and thereby allow detection of more gradual environmental change (Jackson & Füreder, 2006). Studies extending over many years thus provide the possibility of separating changes that are natural from those caused by human involvement (Voelz, Zuellig, Shieh & Ward, 2005). The present study, while emphasizing spatial variability in Plum Run, cannot address questions related to such seasonal or interannual variation.

Many stream studies incorporate a reference or control for comparison. Comparisons can be temporal (before vs. after an identified environmental perturbation) or spatial (among sites within the same stream network or among different stream systems) (Reynoldson et al., 1997). However, streams in many regions are so modified that available reference sites may not provide truly unimpacted conditions, and comparisons between study and reference sites may thus fail to convey the full extent of habitat impairment (Chessman & Royal, 2003). The following sections will discuss the apparent direct and indirect effects of environmental variables on variation in the aquatic invertebrate community observed in the Plum Run network.

Stream Habitat: Larger downstream sites were expected to support a wider diversity of invertebrate taxa (Clenaghan, Giller, O'Halloran & Hernan, 1998). The Plum Run study did show that increasing stream depth, width, order, and discharge were positively correlated with total invertebrate density, and were associated with increased abundances of some taxa (for example the caddisfly *Hydropsyche*). Paller et al. (2006), in a study of South Carolina upper coastal plain streams, found that increasing stream width was associated with increased total taxon richness, EPT richness, and total number of organisms; as in Plum Run, Trichoptera were more abundant in larger streams in their study.

Watershed Effects (land use): The relationship between land use and MAIS score showed that subbasin land usage has a significant effect on water quality. Several other qualitative inferences can be drawn from the land use data. First, wooded areas made up 16% of the watershed, with the largest portions concentrated around the Gordon Natural

Area (GNA) on the East Branch. The higher abundance of sensitive invertebrates found in the stream in the GNA, especially at site 2, is most likely due to the persistence of this large, wooded tract over many decades (Rios & Bailey, 2006).

Second, community service makes up 5% of the watershed and is the fourth highest percentage of land use. The largest portions of these areas are concentrated around West Chester University at the headwaters of Plum Run (just upstream of sites 1 and 10). The low abundance of sensitive invertebrates at the headwaters and the low taxa richness at site 10 are most likely due to runoff from the parking lots, construction, and roadways throughout the university area.

Covariation of land use with geology and topography (for example the occurrence of agriculture on fertile soils or of woodlots on steeper slopes) may often make it difficult to separate natural impacts from impacts caused by humans (USGS, n.d.; Nerbonne & Vonrecek, 2001). Within the Plum Run stream network, it was predicted that sites with more “urbanized” subbasins would have fewer invertebrate taxa due to increased runoff (Robson et al., 2006). Lenat and Crawford (1994) compared the effects of different land uses (forested, agricultural and urban) on three streams in North Carolina. The urban stream showed low invertebrate richness and abundance compared to the forested and agricultural streams. The invertebrate community shifted from one dominated by Ephemeroptera in the forested stream to a community dominated by Chironomidae, in the agricultural stream, and by Oligochaeta in the urban stream. Their study concluded that land use “strongly influenced” the macroinvertebrate community. In Plum Run most sites were dominated by Chironomidae; however, sites with more wooded settings (sites

2, 3, 4 and 9) had high densities of the Ephemeropteran family Baetidae. Oligochaeta densities were low throughout Plum Run, and no sites were dominated by this taxon.

Roy and others (2003) examined the relationship between catchment land cover and macroinvertebrates in 30 streams in Georgia. Urban land cover explained 29-38% of the variation in some macroinvertebrate metrics and was positively correlated with specific conductance. The authors found that macroinvertebrate communities at sites in drainages with more than 15-20% urban land cover ranged from “fair to fairly poor” based on the Hilsenhoff Biotic Index (HBI) applied to family-level identifications. As stated earlier, stream degradation and altered macroinvertebrate communities can occur at relatively low levels (~10%) of imperviousness (Arnold & Gibbons, 1996; Wheeler et al., 2005). Based on these studies and the fact that the Plum Run drainage basin had 11-13% impervious cover based on a map of the Brandywine watershed prepared in 1998 (Chester County Water Resources Authority), much of Plum Run’s impairment is most likely due to urbanization. This view is consistent with the 1997 assessment by PADEP that part of Plum Run’s impairment is due to urban runoff and new housing developments.

Riparian Vegetation: Compared to canopied sites, stream segments with little or no canopy cover were predicted to have higher temperatures, increased algae, and dissolved oxygen levels exceeding 100% saturation as a consequence of high levels of photosynthesis during daylight hours. These changes are likely to favor some invertebrate taxa over others. I expected invertebrate trophic guild composition to shift from dominance by shredding invertebrates to include more scrapers. Highly sensitive

taxa such as stoneflies, which occupy streams with low temperatures and high dissolved oxygen, were expected to decrease or vanish in open canopy sites (Peckarsky, 1990).

My study of Plum Run compared macroinvertebrate communities at open vs. closed canopy sites, based on percentage canopy cover. Similarly, a study of six streams in the Cascade Mountains of Oregon by Hawkins, Murphy, and Anderson (1982) looked at the importance of riparian vegetation to the structure of macroinvertebrate assemblages. Their study showed that a simple open vs. closed canopy contrast among study sites was insufficient to show differences in some macroinvertebrate communities, specifically shredders. Likewise Roy and others (2005) in a study of five small suburban streams (basin area of 10-20 km²) in Georgia, found no differences in macroinvertebrate assemblage “integrity” between open sites and forested sites at what the study referred to as “reach-scale” (defined in the study as 200 m). Instead, the study suggests that the assemblages were more likely influenced either by catchment-scale factors such as land cover or reach-scale habitat quality. The reasons for the results of Roy’s study may be similar to this study; negligible differences in invertebrate communities were most likely the response to minimal differences in habitat between sites.

Canopy cover significantly affected the densities of only two taxa: oligochaetes and the caddisfly genus *Cheumatopsyche*; oligochaete densities increased with increasing canopy cover and *Cheumatopsyche* densities decreased. Closed canopy sites contained slightly, but not significantly, higher densities of invertebrates. Roy and others (2005) found that percent canopy cover explained less than half of the variation in EPT density.

In Plum Run, sampling sites located near private residences (sites 1, 5, 10, 11, 12) all had lawns as streambank vegetation. Grass-covered banks tend to have lower width-to-depth ratios because grass roots hold only the thin upper stratum of soil while allowing erosion of the soil below; this produces “entrenched” channels that are more narrow and deep (Nerbonne & Vondracek, 2001). These channels have higher water velocity, resulting in greater sediment transport. Sediments then settle when they get to slower, wider sections downstream (Nerbonne & Vondracek, 2001).

By contrast, trees and other woody vegetation may help stabilize banks and decrease erosion, as well as providing food and habitat for invertebrates (Klapproth & Johnson, 2000; Wheeler et al., 2005). Riparian buffers of 50-100 meters are often recommended for protecting streams (Cushing & Allan, 2001). In the Plum Run watershed sites 2, 4, 6, 9, and 14 had riparian vegetation of > 30 meters on both right and left banks. Sites 3, 7, 10 and 13 had > 30 meters of riparian vegetation on the left bank only. Therefore several sites that are >30 meters may have an adequate riparian buffer width, but exact measurements need to be made to ensure that they are at least 50 meters. Most of these sites that may have adequate buffer widths are located in the Gordon Natural Area (sites 2, 4 and 9).

Riparian Cover and Water Physicochemistry: Water chemistry may vary within stream networks for reasons other than canopy cover. Even when there are documented correlations between variation in water chemistry and invertebrate distribution, the primary cause is difficult to pinpoint (Allan, 1995), owing partly to the multiplicity of interrelationships among variables. Also, other variables, not examined in this study,

may be influencing the results. For example, pesticides or herbicides from residential lawns or Radley Run golf course or pollutants in stormwater runoff could also be affecting the relationship between water chemistry and the invertebrate community.

Studies of unimpacted streams have shown widely varying specific conductance within a range of approximately 150-500 $\mu\text{S}/\text{cm}$, depending in part on the composition of the underlying bedrock (limestone streams, for example, typically have high specific conductance levels). Specific conductance values above this range often indicate highly impacted water quality that is not suitable for certain species of macroinvertebrates (USEPA, 2006). Specific conductance was highest at sites 1 (522 $\mu\text{S}/\text{cm}$) and 10 (566 $\mu\text{S}/\text{cm}$) presumably due to runoff from roads and university parking lots, which provide most of the flow to both stream segments. Runoff from urban areas may have large concentrations of inorganic pollutants, contributing to elevated specific conductance levels (Voelz et al., 2005). Water chemistry analyses of Plum Run by the PADEP (1979) similarly indicated high specific conductance (440 $\mu\text{S}/\text{cm}$) near site 10 at the origin of the west branch on New Street at the university. Use of specific conductance as a criterion for identifying stream impairment is currently under review by the Florida Department of Environmental Protection based on evidence of a significant reduction in sensitive taxa at high conductance levels (Florida, 2006).

Variation in periphyton abundance as chlorophyll-*a* was not significantly related to differences in the macroinvertebrate community in Plum Run. Even sites with little or no canopy cover showed low chlorophyll-*a* values. In fact the highest amount of

chlorophyll-*a* was found in a closed canopy site (site 9). By contrast, Roy and others (2005) found that chlorophyll-*a* was higher in open versus closed sites. The low periphyton biomass estimates in Plum Run could be a result of sediment scour, invertebrate grazing, or the sampling method used. Hawkins et al. (1982), who similarly used a wire brush to sample epilithic algae in six streams in Oregon, found lower chlorophyll-*a* levels than expected based on previous studies of streams in that area, and suggested that the reason might have been his inability to remove all algae from the rocks.

Fish: The fish community showed highly predictable distribution patterns in Plum Run. Larger sections of the stream closer to Brandywine Creek supported higher fish species richness, a predominance of larger-bodied fish, and more pool-dwelling species. The results showed little evidence of strong impact upon the invertebrate community by fish. As stated earlier, effects of fish predation vary greatly and are difficult to document (Gilliam, Fraser, & Sabat, 1989; Williams, Taylor & Warren, 2003; Dahl 1999).

Interpretation of invertebrate data: The metrics used to interpret the invertebrate data for this study were varied. The presence, absence or percentage of EPT taxa is a metric often used as an indicator of water quality (Rosenberg & Resh, 1996). However, absence of a particular species within these three orders of insects does not necessarily indicate environmental impairment (Johnson, 1993). Invertebrates do not respond to all impacts and therefore invertebrate studies can fail to indicate that a habitat is stressed (Rosenberg & Resh, 1993, Chapter 1). In Plum Run, EPT taxa were found throughout the stream network. The caddisfly *Hydropsyche* (order Trichoptera, family Hydropsychidae) and

the mayfly family Baetidae (order Ephemeroptera) were two taxa that were found at all 14 sites; both taxa are widespread in both impaired and unimpacted streams of Pennsylvania, and have tolerance values ≥ 4 (Hilsenhoff, 1988). Mayfly diversity declines as streams are degraded (Fore, 1998); however, mayflies from the family Baetidae are frequently found in moderate to poor water habitats.

Hydropsychidae are among the most common macroinvertebrates found in streams (Alexander & Smock, 2005). The hydropsychid caddisfly *Cheumatopsyche* was found at all but one site. This genus is one of the most tolerant within the order Trichoptera and is often a dominant taxon in degraded streams as a consequence (Alexander & Smock, 2005).

Three sensitive taxa of Trichoptera (*Glossosoma*, *Dolophilodes*, *Neophylax*) were found mostly at sites 2, 4 and 9 in the East Branch within the Gordon Natural Area. These same sites also showed the highest abundance and diversity of Plecoptera and Ephemeroptera. Plecopterans are generally associated with colder, well oxygenated water (Stewart & Harper, 1996). The combination of high % canopy cover and high % riffles make site 2 a good habitat for stoneflies. The shredder stonefly *Leuctra* was abundant at site 2, where the high percentage of coarse particulate organic matter (CPOM) may have helped to account for their high numbers (Merritt & Cummins, 1984; Peckarsky, 1990).

Upstream sites on the East Branch, with the exception of site 1, had lower HBI values and higher % EPT than the West Branch or main stem. Invertebrate taxa such as *Leuctra* (Family Leutridae, Order Plecoptera) and *Ameletus* (family Ameletidae, Order

Ephemeroptera) were both abundant at site 2. *Ameletus* was abundant in March but not present in the June sampling and *Leuctra* was abundant in the June sampling but not present in March. Site 4 contained high numbers of the mayfly family Baetidae. Site 9 also contained high numbers of Baetidae as well as the trichopteran *Dolophilodes* (family Philopotamidae). Many of these attributes of the invertebrate community are attributable to the higher percent riparian canopy cover of the east branch. In addition, the two tributaries sampled at sites 2 and 4 within the forested Gordon Natural Area probably contribute to the amelioration of downstream sites on the East Branch (Sponseller, Benfield & Valett, 2001).

All three sites (10, 11, and 12) had high HBI values. Site 11, the only “poor” site according to the MAIS calculation, was particularly conspicuous in having no woody riparian vegetation and exhibiting severe entrenchment of the stream channel. Site 12 was a very small tributary entering the West Branch just upstream of site 11, but its presence had no clear beneficial effect on site 11.

Just as the absence of EPT may not indicate that significant environmental requirements are not being met, the presence of tolerant taxa such as the family Chironomidae also does not always imply impairment. Chironomids were the most abundant taxa overall, and were found at every site along Plum Run. Chironomids are found in a wider variety of conditions than any other group of aquatic insects, often accounting for at least 50% of the total invertebrate species diversity (richness component) (Merritt & Cummins, 1984).

I was especially interested in possible effects of the Radley Run Golf Course on the invertebrate community. Site 8 was located within the golf course, and sites 6 and 7 were located upstream and downstream, respectively. It was predicted that any use of pesticides and fertilizers, along with the occasionally large quantity of grass clippings observed in the stream, would degrade the water quality and impact the in-stream biota. In a study by Winter and others (2002) comparing 6 streams flowing through operational golf courses with 7 forested streams in Toronto, Canada, temperatures were generally higher at golf course sites. Invertebrate taxa also differed considerably in 3 of the 6 golf course streams compared to the forested streams; the golf course sites had higher abundances of Turbellaria (flatworms), Isopoda, Amphipoda, Zygoptera (damselflies) and mites, whereas Ephemeroptera, Megaloptera, Culicidae (mosquitoes) and Plecoptera were more common at the forested sites. Winter concluded that these differences were due to management practices of the golf courses; for example, the dominance of amphipods at golf course sites was attributed their ability to consume grass clippings. In the Plum Run study, the average temperature was approximately 3°C higher on the golf course (site 8) and just downstream (site 7), compared to the forested site 6 directly upstream. This is most likely due to the lack of riparian vegetation to provide shading (Winter et al., 2002). The dominant taxon on the Radley Run golf course was Chironomidae, a tolerant taxon. The dominant taxa both upstream and downstream of the golf course were *Gammarus* followed by *Hydropsyche*.

Exposure to direct solar radiation, water temperature, dissolved oxygen, specific conductance, TDS, and chlorophyll-*a* were all higher at site 8 within the golf course

compared to site 6 directly upstream. Site 8 had 16 invertebrate taxa, compared to 20 taxa at site 6 directly upstream, and 13 taxa at site 7 directly below the golf course. The three sites had 9 taxa in common, and had similar HBI values (~5.3-5.9). However, the MAIS scores declined steadily from site 6 (MAIS = 12) to site 8 (MAIS = 10) to site 7 (MAIS = 8). The golf course site had lower % EPT, higher % chironomids, lower diversity, and 3% higher tolerant taxa.

Just downstream of the golf course the Radley Run Mews sewage treatment facility empties directly into Plum Run between sites 7 and 8 (Figure 18).

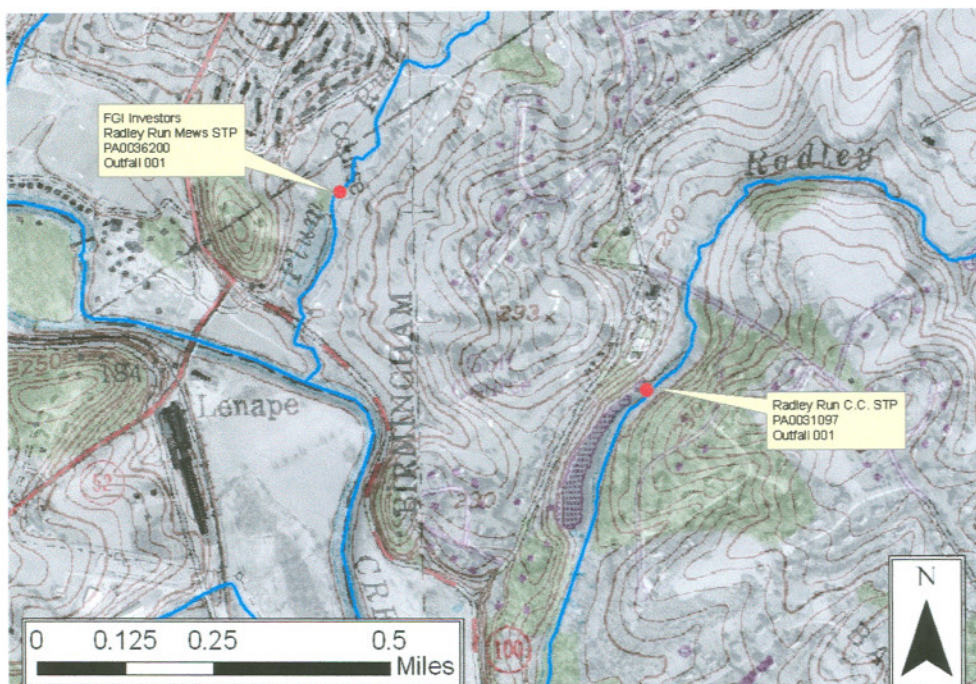


Figure 18. Location of Radley Run Mews sewage treatment plant as it enters into Plum Run. Also shown is the Radley Run Country Club sewage treatment plant as it enters into Radley Run (courtesy of Alan Everett, PADEP).

The effluent from the sewage treatment facility would be expected to affect site 7, the furthest downstream site. According to PADEP's discharge monitoring reports for

June 2005, the average flow is 0.012 million gallons/day or 0.526 Liters/second (Radley Run Mews Sewer Association, PA0036200, sampling date 7/29/2005; personal communication with Alan Everett of PADEP). The discharge at site 8 is 52.8 Liters/second (Table 4), which is 100x greater than the discharge entering from the Radley Run Mews facility. In effect Radley Run Mews contributes about 1% of the flow where it merges with Plum Run. Therefore, the decreased % dissolved oxygen, high temperatures, unusually high density of the amphipod *Gammarus*, and the sharp decline in MAIS score (from 12 at site 6 upstream of the golf course to 10 at site 7 downstream of the golf course) would suggest that the golf course has a sizable effect on this section of Plum Run.

V. RECOMMENDATIONS

The headwaters of both branches of Plum Run begin as impaired streams, originating underneath or near West Chester University and likely experiencing modifications of water chemistry and highly variable flow often associated with urban runoff. Headwater sites can contribute strongly to “ecological integrity” downstream, and should be a focus of maintenance (Heino et al., 2005; Saunders, Meeuwig & Vincent, 2002). Managing both water chemistry and fluctuations in water volume, both at the headwaters and in downstream areas of both branches may help restore stream integrity. In particular, riparian reforestation and stormwater management of the West Branch may be warranted.

Plum Run is considered to be an impaired stream based on sampling by PADEP at site 7 near its confluence with the Brandywine Creek. However, the results of my study suggest that the two major branches of Plum Run differ substantially in water quality. Many studies have shown the potential for substantial variation in stream quality among the smaller tributaries that collectively comprise small stream networks similar to that of Plum Run (Heino et al., 2005). The West Branch of Plum Run, having impacted headwaters and flowing through a more urbanized area, is in need of restoration and management. In future restoration planning, it may be reasonable to start with the West Branch first, for example the headwater at parking lot F, to see if improvement there leads to improvement of further downstream and main stem sites.

This study is largely based on a one-time, though analytically comprehensive, study performed during summer, although additional invertebrate data are also provided

based on qualitative sampling during the previous spring. Seasonal and interannual variation in discharge, water chemistry and watershed influences are all known to affect the invertebrate community, and the timing of field assessments may therefore influence the determination of whether or not a stream is judged impaired (Linke, Bailey & Schwindt, 1999). Continued sampling of the invertebrate community within the Plum Run stream network is needed to provide evidence of seasonal variability, and as a means of detecting longer-term trends associated with increased impairment or restoration. Plum Run also provides an opportunity for experimental research focused on particular ideas or relationships inferred in this study. Physicochemical analysis of sediments for particle size and chemical content (e.g., metals, pesticides, organic content), for example, would be particularly useful in future work. Such studies could be incorporated into university class activities or conducted by a non-profit conservation organization. It is my hope that this project will provide a solid basis for future restoration, management and ongoing biomonitoring of Plum Run.

VI. APPENDIX A. Invertebrates collected at 14 sites on March 7th, 9th and 10th 2005.

Order	Family	Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ephemeroptera	Ameletidae	Ameletus		82												3
Ephemeroptera	Baetidae				1	129	28	5		2	3	2	4	1	21	88
Ephemeroptera	Ephemerellidae	Ephemerella					2	2							1	6
Ephemeroptera	Heptageniidae	Stenoma	1	1	1	1										2
Plecoptera	Unknown			1												
Plecoptera	Nemouridae	Prostoia		39		24	8			2	3		1		12	47
Plecoptera	Perlidae	Eccoptura					2							2		
Plecoptera	Perlidae	Phasganophora					3									
Plecoptera	Perlidae	Isoperla					1									
Plecoptera	Taeniopterygidae	Taenioma					1									
Trichoptera	Glossosomatidae	Glossosoma						1								
Trichoptera	Hydropsychidae	Cheumatopsyche	5		10		5	23	2	1	1	1	2	2	5	8
Trichoptera	Hydropsychidae	Diplectrona		2	2	1					1					
Trichoptera	Hydropsychidae	Hydropsyche	9		15	5	40	76	9	28	8	1	51	11	27	23
Trichoptera	Philopotamidae	Chimarra	7		12		12	30	1	24			2	8	7	32
Trichoptera	Philopotamidae	Dolophilodes			1	5	4			1	4		1			7
Trichoptera	Rhyacophilidae	Rhyacophila		3		1	1									
Trichoptera	Uenoidae	Neophylax		3												
Trichoptera	Limnephilidae	Pycnopsyche		1												
Diptera	Unknown												1			
Diptera	Chironomidae		86	1	152	3	19	8	41	55	97	130	126	16	95	16
Diptera	Tipulidae	Tipula	3	1	8	7	5	1	1	4	1	3		3	1	1
Diptera	Tipulidae	Dicranota		1	2	2	3	1	1							7
Diptera	Tipulidae	Helius			1											
Diptera	Tipulidae	Antocha					1			1			3		1	
Diptera	Empididae	Clinocera			2											
Diptera	Simuliidae	unknown														6
Diptera	Simuliidae	Stegopterna		2	2											
Diptera	Simuliidae	Simulium			18	2		2		4		1	11	18	1	7
Diptera	Simuliidae	Prosimulium			1	4	2									3
Isopoda	Asellidae	Caeciodotea	21		5				1		1		2	8		
Isopoda	Asellidae	Lirceus	1													
Amphipoda	Crangonyatidae	Stygonectes														5
Amphipoda	Gammaridae	Gammarus					86	20	90	52		29	44	66	30	3
Odonata	Gomphidae	Gomphus					1									
Odonata	Aeshinidae	Boyeria						1								
Coleoptera	Elmidae	Optioservus						1								
Coleoptera	Elmidae	Stenelmus					5	1								
Coleoptera	Dytiscidae	Agabus							1							
Coleoptera	Psephenidae	Psephenus								1					3	1
Megaloptera	Sialidae	Sialis		1												
Decapoda	Cambaridae						1						1			
Pulmonata	Physidae								1							
Oligochaeta					1				1				1			
Turbellaria											3		1			
Turbellaria											1					
TOTAL INDIV			133	138	233	181	232	175	149	174	123	167	250	135	217	252
TOTAL SPECIES			8	13	16	12	21	16	11	11	11	7	14	10	15	15

VII. APPENDIX B.**Locations of the 14 study sites in the Plum Run stream network.**

Site #	Location	Nearest Main Road	Latitude	Longitude
1	East Branch, headwater site	Oak Lane	39° 56' 47.39"	75° 35' 44.88"
2	East Branch tributary	Tigue Road	39° 56' 26.94"	75° 35' 53.18"
3	East Branch, GNA	New Street	39° 56' 28.37"	75° 35' 54.06"
4	East Branch tributary, GNA	New Street	39° 55' 55.72"	75° 36' 8.09"
5	West Branch tributary. Chesterdale Farm private property	Route 52	39° 55' 27.51"	75° 37' 15.99"
6	Main Stem, upstream of golf course	Route 52	39° 55' 17.21"	75° 37' 18.45"
7	Main Stem, downstream of golf course	Route 52	39° 54' 50.17"	75° 37' 39.60"
8	Main Stem, on Radley Run Golf Course	Route 52	39° 55' 1.39"	75° 37' 35.04"
9	East Branch, GNA	New Street	39° 55' 57.51"	75° 36' 8.24"
10	West Branch, headwater site	College Ave	39° 56' 48.97"	75° 36' 26.33"
11	West Branch, Fox Hill Farm private property	Route 52	39° 56' 1.59"	75° 36' 56.54"
12	West Branch tributary, Fox Hill Farm private property	Route 52	39° 56' 1.52"	75° 36' 57.07"
13	Main Stem, Strode Mill Art Gallery	Birmingham Road	39° 55' 43.29"	75° 37' 1.96"
14	East Branch, upstream of confluence	Route 52 and Tigue Road	39° 55' 49.43"	75° 36' 54.69"

VIII. APPENDIX C.

Photographs of stream origins and study sites, and geomorphological data (courtesy of Dr. Timothy Lutz, West Chester University Department of Geology and Astronomy).

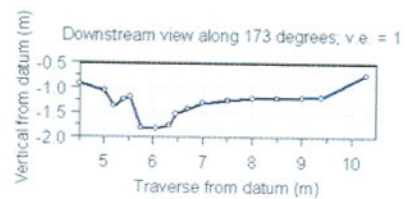
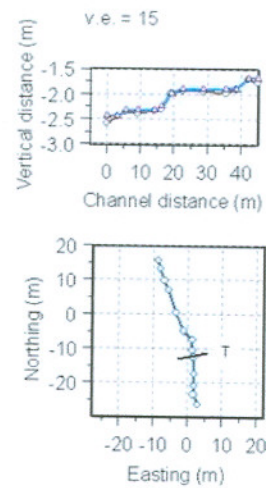


Origin of East Branch (Near High Street)

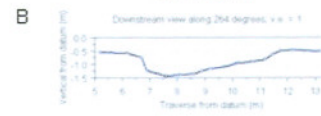
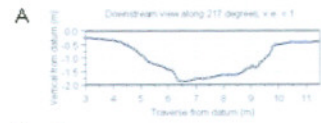
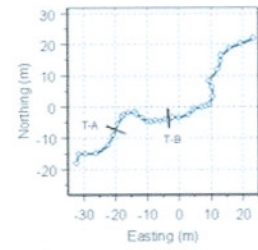
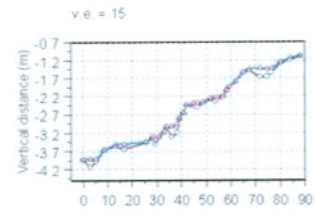


Origin of West Branch (WCU parking lot F)

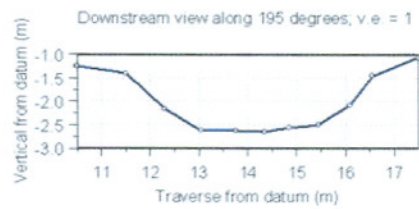
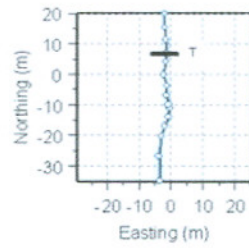
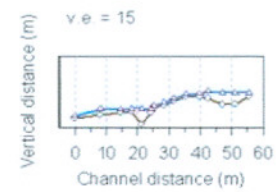
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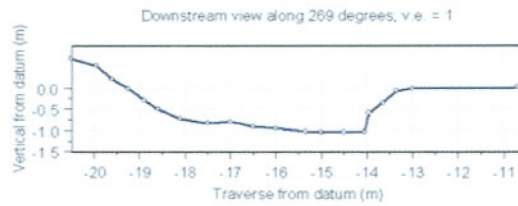
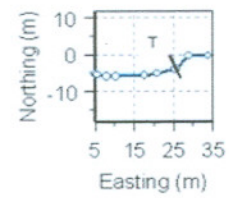
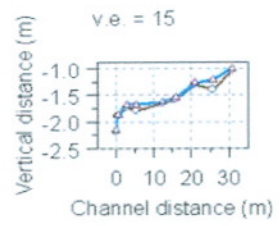
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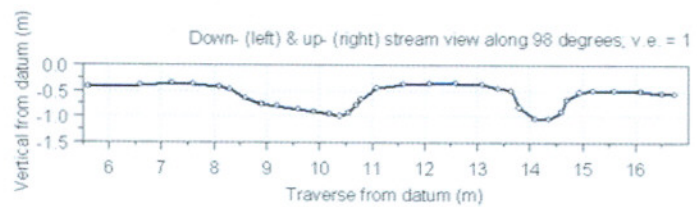
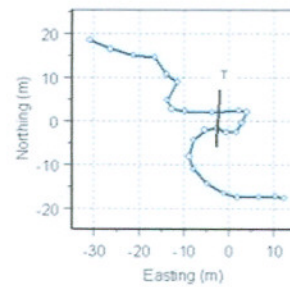
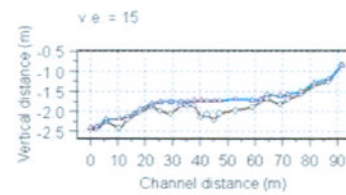
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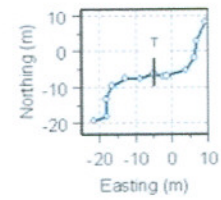
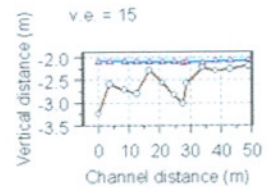
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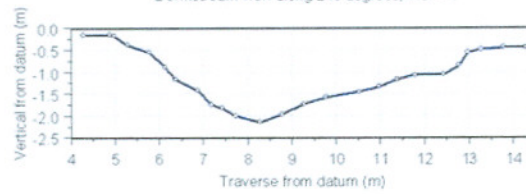
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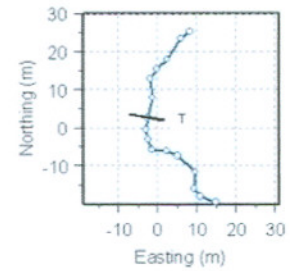
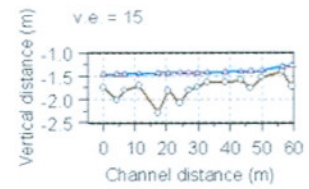
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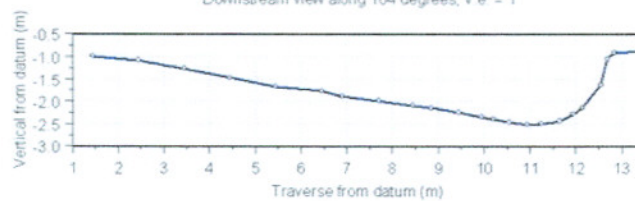
Downstream view along 245 degrees, v e = 1



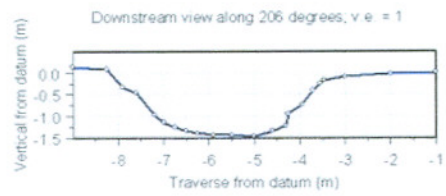
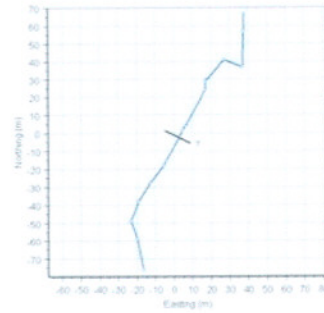
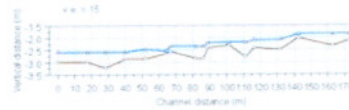
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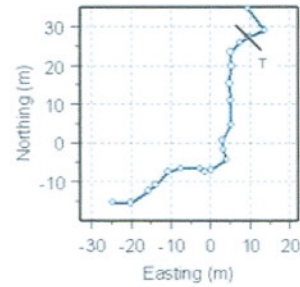
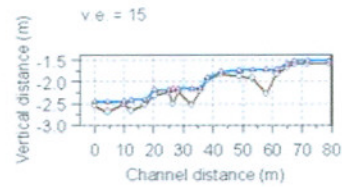
Downstream view along 164 degrees, v e = 1



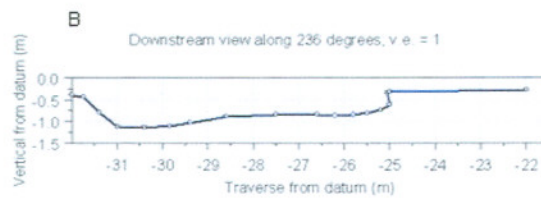
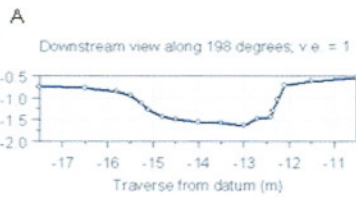
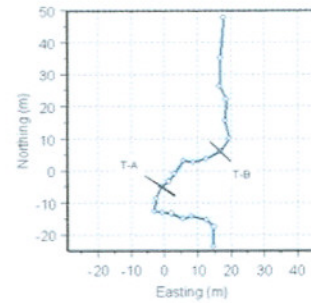
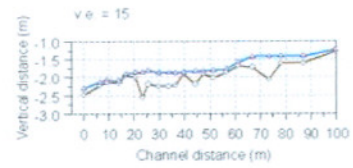
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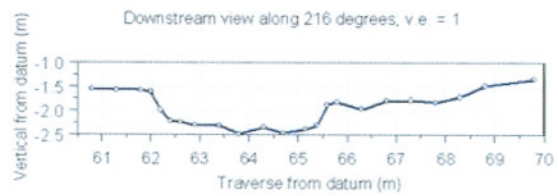
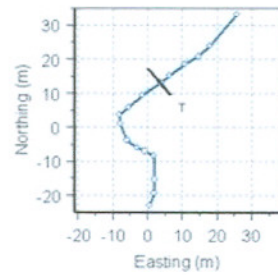
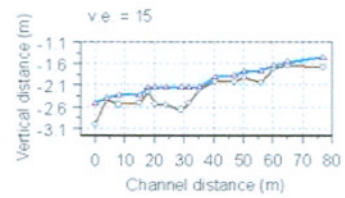
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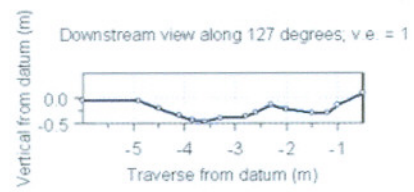
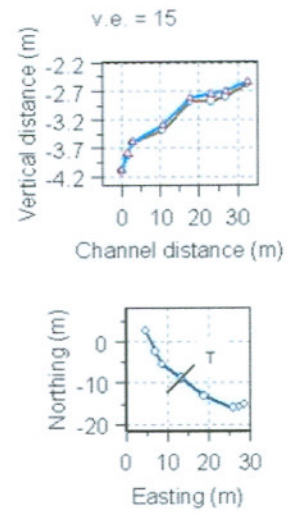
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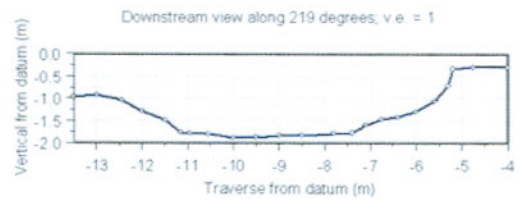
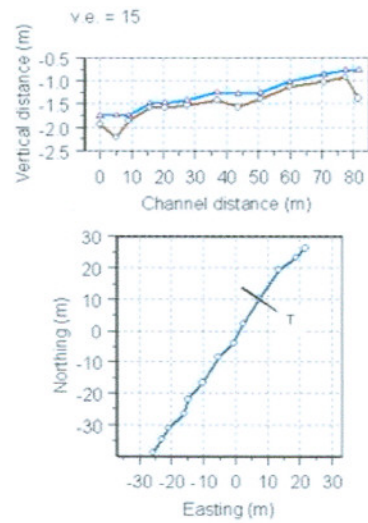
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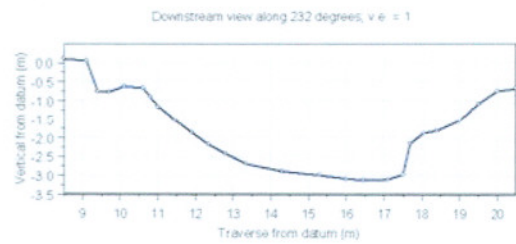
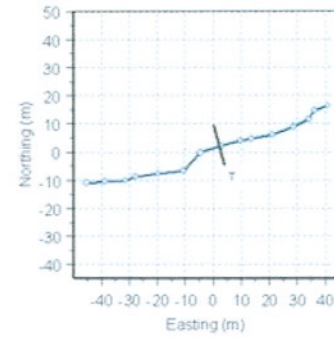
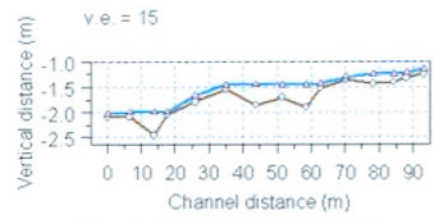
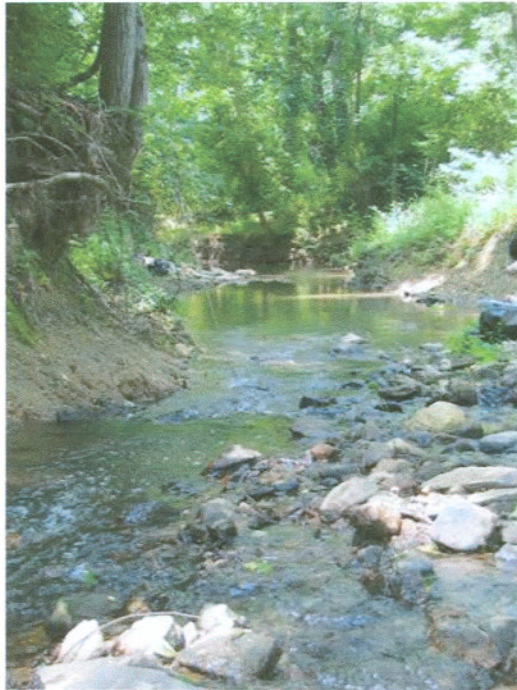
Site 12



Site 13



Site 14



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