Studies of Benthic Macroinvertebrate Use for Biomonitoring of Mid-Atlantic Highland Streams

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ABSTRACT

Research was conducted in three areas of water quality assessment. Long term ecological monitoring data from Shenandoah National Park (SNP) were analyzed and a protocol for data analysis was presented. Streams in SNP were found to be comparable to the best that can be found in the Blue Ridge ecoregion. Land use in SNP (mostly for recreational purposes) does not appear to be causing impairment to the macroinvertebrate assemblages. Streams in the SNP were found to recover quickly from disturbance.

The Macroinvertebrate Aggregated Index for Streams (MAIS) was found to have an overall classification efficiency (CE) of 86% in the Ridge and Valley ecoregion, and an overall CE of 91% in the Central Appalachians ecoregion. Refinement of the MAIS for use in the Blue Ridge ecoregion resulted in an increase of the overall CE to 78%. The CE for reference sites in the Blue Ridge was 75%, and the CE for degraded sites was 87%.

An intensive study of a stream (Peak Creek) with suspected heavy metal impairment showed that capping of an industrial waste site has resulted in improvements to the macroinvertebrate assemblages. The source of the impairment was not linked solely to heavy metals, but was found to be a mixture of pollution sources and environmental stress.

Dedicated to my wife Kerry and to my two wonderful children, David and Katherine.

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CHAPTER 1: INTRODUCTION

Biological monitoring, or biomonitoring, is the use of living organisms to determine the condition of the environment. The advantages of biomonitoring versus physical or chemical monitoring are: (1) biomonitoring reflects overall ecological integrity (i.e., physical, chemical and biological); (2) it provides a holistic measure of environmental condition by integrating stresses over time; and (3) the public better understands living organisms as measures of a "healthy" environment (Plafkin et al. 1989).

In streams, biomonitoring can be done with benthic macroinvertebrates, fish, or periphyton, but benthic macroinvertebrates are generally the assemblage of choice. They have several characteristics that make them particularly useful for biomonitoring. (1) Benthic macroinvertebrates occur in almost all types of freshwater habitats. (2) There are many taxa of benthic macroinvertebrates, and among these taxa there is a wide range of sensitivity to pollution and environmental stress. (3) They have mostly sedentary habits so they are likely to be exposed to pollution or environmental stress. (4) Their life cycles are sufficiently long that they will likely be exposed to pollution and environmental stress, and the community will not recover so quickly that the impact will go undetected. (5) Sampling the benthic macroinvertebrate assemblage is relatively simple and does not require complicated devices or great effort. (6) Taxonomic identification is almost always easy to the family level and usually relatively easy to the genus level. (Voshell et al. 1997).

The practice of using organisms to measure the condition of the environment has been around for many years. The basis for modern day biological monitoring of streams has roots in the Saprobien system developed in Germany in the early 1900s (Cairns and Pratt 1993). It was noticed that many organisms were always absent and only certain organisms were always present when there was contamination by sewage. This knowledge led to the development of simple indicator organism lists, based solely on presence or absence of taxa. Analysis of the fauna based on presence or absence evolved into a consideration of all organisms, not just indicator organisms, and to analyze the indicator macroinvertebrates as part of the overall benthic community, or least the macroinvertebrate assemblage (Rosenberg and Resh 1996). These types of studies were especially well suited to detect a specific type of pollution, and it was known how the assemblage would respond to that type of pollution. The usual study design was to compare the stream or reach suspected of being impaired to a stream or reach that was not subjected to the specific type of pollution. Decisions about impaired water quality were made by using statistics to determine if the assemblages in the two streams or reaches were different from one another. This approach resulted in the need to find reference streams or reaches for every study, and to obtain enough replicate samples to give the test sufficient statistical power.

The increased cost of carrying out the needed replicates was one of the major reasons leading to the development of the Rapid Bioassessment Protocols (RBPs) by the United States Environmental Protection Agency (USEPA) (Plafkin et al. 1989, Barbour et al. 1999). Requirements of the Clean Water Act (CWA) required states to report on the condition of their waters, especially identifying the impaired waters (Barbour et al. 1999). The need for water quality agencies to conduct high numbers of water quality assessments in a short time further increased the implementation of rapid bioassessment techniques.

One of the developments that came out of this need to conduct large numbers of assessments was the regional reference approach. Instead of finding reference streams for every water quality assessment, agencies can select the minimally impaired streams ahead of time, and conduct analyses of the streams to determine what the reference condition is for a particular region. This same reference condition can be used in multiple studies, and is not as costly as the site-specific approach (Hughes and Larsen 1988).

Along with the idea of regional reference conditions came multimetric indices, two advances that have helped agencies to fulfill their goals of assessing water quality (Barbour et al. 1996). One of the first applications of a multimetric index was the Index of Biotic Integrity (IBI), developed for fish (Karr 1991, Karr et al. 1986). The IBI was a multimetric index that incorporated multiple aspects from the fish assemblage of a site into one easy to interpret index value (Karr et al. 1986). The index worked well because fish collection data from each site were compared to the expected values for that site which had been derived from extensive research in reference streams. The expected values will change from site to site along a stream, depending on the stream drainage area at each site. Modifications of this process became a model for the development of multimetric indices involving benthic macroinvertebrates (Barbour et al. 1995).

The benthic macroinvertebrate indices have been created by different agencies for many different regions in recent years (OEPA 1987, Shackleford 1988, OEPA 1989, Kerans and Karr 1994, Barbour et al. 1996, Smith and Voshell 1997, Stribling et al. 1998, Gerritsen et al. 2000). These multimetric indices have been developed using varying levels of taxonomic resolution, various types of metrics, and various numbers of metrics. One thing they all have in common is that they are a tool to be used in water quality assessment programs using benthic macroinvertebrates. One need of these indices is that they be validated and refined using new data as a method of confirmation that the index is accurately detecting impairment.

While considerable advances have been made in the field of biological assessment in the last 20 years (Barbour et al 1996), the state-of-the-art in bioassessment has not reached a point that is equivalent to the techniques used in physical and chemical monitoring. Biomonitoring tools are continuously being developed, modified, and tested to provide quicker and more accurate assessments of our waters. The reference condition is being assessed and refined for various regions and scale throughout the country. Research into macroinvertebrate assemblage response to specific contaminants continues to be studied within the different regions (Clements 2000). The data interpreting tools (e.g., multimetric indices) used for analyses remain an area of active analyses and refinement.

I conducted research into three areas of water quality assessment in order to: 1) analyze the long term ecological monitoring data of the Shenandoah National Park to determine the condition of the park's streams; 2) analyze and refine a multimetric index (the Macroinvertebrate Aggregated Index for Streams) developed for use in Mid-Atlantic Highland streams; and 3) examine the macroinvertebrate assemblages in a stream with suspected heavy metal perturbation.

The specific objective of these research areas were:

Shenandoah National Park (SNP)

a) select a suite of metrics that will be effective for long-term biomonitoring of benthic macroinvertebrates in SNP streams;
b) ascertain the ecological condition of streams in SNP as compared to other similar streams in the Blue Ridge ecoregion;

c) determine if the ecological condition of any streams differs within SNP, and, if so, which ones are different and are the differences related to any of the likely causative factors in SNP
d) if the ecological condition of SNP streams compares favorably with that of streams outside SNP and if the ecological condition of streams is consistent within SNP, decide if the current benthic macroinvertebrate biomonitoring protocol in SNP and recommended metrics would be effective at discerning impaired ecological condition if significant pollution or environmental stress did occur in SNP.

Macroinvertebrate Aggregated Index for Streams (MAIS):

a) determine if the classification efficiency of the MAIS in the Ridge and Valley and the Central Appalachian ecoregions is repeatable and accurate
b) ascertain if the MAIS in the Blue Ridge Mountains can be modified in order to obtain classification rates similar to the rates observed during the development stage for the MAIS in the other 2 ecoregions.

Site specific analysis of a stream (Peak Creek) suspected of heavy metal disturbance

a) to determine if the benthic macroinvertebrate assemblages at the downstream Peak Creek sites differ from reference site assemblages
b) decide if spatial trends of the macroinvertebrate assemblages at the sites along Peak Creek comparable to previous studies
c) if the downstream sites are shown to be different from the reference condition, is heavy metal contamination the cause, and is a gradient of contamination observed

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CHAPTER 2: Long Term Biomonitoring with Benthic Macroinvertebrates in Shenandoah National Park, Virginia

INTRODUCTION

The National Park Service (NPS) of the U. S. Department of Interior has the difficult charge of providing for the nation's enjoyment of its national parks while preserving the parks unimpaired for the enjoyment of future generations (Keiter 1988). The NPS policy of not allowing any consumptive uses of flora, fauna, or minerals in national parks helps to accomplish part of this goal. However, unavoidable natural events, such as fires, floods, earthquakes, and volcanoes, can cause catastrophic changes to the natural resources contained in national parks. Human activities that take place outside park boundaries can also induce undesirable changes to the natural resources within national parks. For example, contaminants can be transported into national parks through the atmosphere, and rivers in national parks can be degraded by flowing through private lands used for agriculture, forestry, mining, manufacturing, or urban development before they enter park boundaries. Non-native species of plants and animals brought into the U. S. can invade national parks and displace native species, thereby changing the structure and function of park ecosystems. The dual mission of NPS to manage national parks for enjoyment and preservation requires a delicate balancing act. Permissible activities that account for the unique enjoyment of national parks by visitors, such as driving motorized vehicles to scenic areas, hiking, fishing, horseback riding, and camping, can lead to degradation of the very natural resources that the visitors come to enjoy. The tradition of providing lodging, food, and other amenities within national parks further complicates the preservation part of the NPS mission.

NPS has long recognized the importance of monitoring the condition of the natural resources under its jurisdiction, including the living organisms (Runte 1987, Stottlemyer 1987, Commission on Research and Resource Management Policy in the National Park System 1989, Franklin 1989). Monitoring gives park managers the ability to detect changes and make important decisions on land use (Segar et al. 1987). Biological monitoring (usually abbreviated as "biomonitoring") involves the systematic sampling of biological assemblages to determine if changes are taking place, especially from anthropogenic sources (Karr et al. 1986). By taking field measurements of natural, living assemblages, scientists can determine whether the biota is being impaired by pollution or other human activities. The advantages of biomonitoring versus physical or chemical monitoring are: (1) biomonitoring reflects overall ecological integrity (i.e., physical, chemical and biological); (2) it provides a holistic measure of environmental condition by integrating stresses over time; and (3) the public better understands living organisms as measures of a "healthy" environment (Plafkin et al. 1989).

In streams, biomonitoring can be done with benthic macroinvertebrates, fish, or periphyton, but benthic macroinvertebrates are generally the assemblage of choice. They have several characteristics that make them particularly useful for biomonitoring. (1) Benthic macroinvertebrates occur in almost all types of freshwater habitats. (2) There are many different taxa of benthic macroinvertebrates, and among these taxa there is a wide range of sensitivity to all types of pollution and environmental stress. (3) They have mostly sedentary habits so they are likely to be exposed to pollution or environmental stress. (4) The duration of their life history is sufficiently long such that they will likely be exposed to pollution and environmental stress, and the community will not recover so quickly that the impact will go undetected. (5) Sampling the benthic macroinvertebrate assemblage is relatively simple and does not require complicated devices or great effort. (6) Taxonomic identification is almost always easy to the family level and usually relatively easy to the genus level.

In order to have effective monitoring of Shenandoah National Park (SNP), Virginia, NPS personnel began planning the Long-Term Ecological Monitoring System (LTEMS) in 1984. The resulting multifaceted LTEMS includes an aquatic component that focuses on biomonitoring of benthic macroinvertebrates in SNP streams, but also includes other biological, physical, and chemical measurements (Ravlin et al. 1990). Data collection for the aquatic component of the LTEMS began in 1986. Since 1986, 17 core sites have been sampled at least once per year, and in 1995 SNP personnel began to cycle in other sites along with the original 17, with the goal of eventually sampling every permanent stream within park boundaries. The first 6 years of macroinvertebrate data (1986-1992) were analyzed in a previous report (Smith and Voshell 1994), but very little of the physical and chemical data were available in the LTEMS computer database at the time of those statistical analyses. Thus, there have been no comprehensive analyses of the entire LTEMS database on macroinvertebrates and related biological, physical, and chemical measurements. In addition, a standard protocol for analyzing and interpreting the aquatic component of the LTEMS has not been developed. Since the original development of the LTEMS, SNP personnel have incorporated land use information and various other important data related to the LTEMS into a geographical information system (GIS) database.

This project was undertaken in response to these data analysis needs and advancements in available information technology at SNP. The overall goals of this project were to analyze the data on benthic macroinvertebrates assemblages that have been collected in SNP as part of the aquatic component of the LTEMS; investigate any relationships between these data and GIS data on physical features, land use, invasive species, natural catastrophes, and resource management practices; and provide SNP with methods for future data analysis. The specific objectives of this project were to:

1) select a suite of metrics that will be effective for long-term biomonitoring of benthic macroinvertebrates in SNP streams;

2) ascertain the ecological condition of streams in SNP as compared to other similar streams in the Blue Ridge ecoregion;

3) determine if the ecological condition of any streams differs within SNP, and, if so, which ones are different and whether the differences are related to any of the likely causative factors in SNP; and

4) if the ecological condition of SNP streams compares favorably with that of streams outside SNP and if the ecological condition of streams is consistent within SNP, decide if the current benthic macroinvertebrate biomonitoring protocol in SNP would be effective at discerning impaired ecological condition if significant pollution or environmental stress did occur in SNP.

Study Area

Shenandoah National Park is located 120 km west of Washington, D.C. in the north-central area of Virginia, USA (Fig. 2.1). The approximately 80,000-ha park lies entirely within the Blue Ridge ecoregion (Omernik 1987, 1995), and the majority of the park is forested and very mountainous. The underlying geology of SNP consists mainly of 5 formations: Catoctin, Pedlar, Old Rag Granite, Hampton (Harpers), and Erwin (Antietam) (Gathright 1976). Streams in the Hampton and Erwin geological formations have very low alkalinity (<20 ueq/L) (Dise 1984), and these streams have been shown to contain different benthic macroinvertebrate assemblages compared to streams in the other geological formations (Smith and Voshell 1994). The streams in the park are typically high gradient, cool, and well oxygenated, with substrate composed mainly of pebble and cobble along with boulders and outcrops.

SNP was officially established in 1935. In the year before establishment, there were still 465 families living on land that would become part of SNP. Prior to the formation of SNP, much of the land had been heavily used for agriculture, logging, and mining. Most of the timber in the area of the future SNP had been cut approximately every 30 years (Lambert 1989). Since becoming a national park, much of the land has returned to a natural state, with deciduous forest covering most of the area. The streams in SNP now appear to be in good condition, without any obvious signs of the previous land use, and most have reproducing populations of native brook trout. In 1976, Congress designated 32,000 ha of the SNP as wilderness area (Lambert 1989). However, there are a number of environmental concerns in SNP at the present time.

Acid deposition from the atmosphere has been documented to be lowering the pH of SNP streams in the Hampton and Erwin geological formations because their low alkalinity gives them very little, or no, buffering capacity (Ravlin et al. 1990). Analysis of the first 6 years of sampling data (1986-1992) documented that the benthic macroinvertebrate assemblages were different in those streams but could not link observed differences to specific environmental variables (Smith and Voshell 1994). At the time the LTEMS was initiated, the non-native gypsy moth (*Lymantria dispar*) was invading SNP from the north. In 1988, 16,000 acres of SNP were defoliated, and in 1989 a total of 43,000 acres were defoliated (Ravlin et al. 1990). There has been no in-depth analysis of the LTEMS database to investigate possible effects of defoliation by gypsy moth on benthic macroinvertebrates in SNP streams. In recent years, hemlocks, which are often the dominant overstory trees in stream riparian zones, are being killed by another exotic insect species, hemlock woolly adelgid (*Adelges tsugae*). There have been several major fires and floods in SNP. An extensive network of unpaved roads winds through SNP for management activities, fire control, and rescue operations. Although visitors to SNP cannot drive vehicles on these roads, sediment enters streams as a result of erosion on these steep, unpaved roads. There are also several major paved roads that pass through park boundaries and are open to the public. These include Skyline Drive, which runs the length of the park from Front Royal at the north end 168 km south to Rockfish Gap, and US Routes 211 and 33, both of which proceed east/west across the narrow width of the park. There are visitor centers, restaurants, lodges, cottages, gift stores, and gas stations located along Skyline Drive. US Routes 211 and 33 are salted by the Virginia Department of Transportation during winter storms to prevent icing. In addition, there are over 800 km of hiking trails in SNP, including some horse trails, and a 152-km stretch of the Appalachian Trail. Trails are another potential source of sediment in SNP streams.

METHODS

Data Acquisition

SNP LTEMS Database

The following information is a synopsis of the SNP LTEMS protocol for benthic macroinvertebrates. This information was summarized from Voshell and Hiner (1990) and the SNP Field Season Summary Reports for the LTEMS Benthic Macroinvertebrate Monitoring Program (internal documents). Each SNP site consists of a 100-m long stream section, and habitat measurements are made at 10-m interval transects. Measurements taken at each site are listed in Table 2.1.

Macroinvertebrate sampling was stratified random (randomized within riffles) and quantitative, using either a Portable Invertebrate Box Sampler (PIBS) or a Surber Sampler. The PIBS was the primary sampling device, with the Surber Sampler being used only when the water was not deep enough to properly use the PIBS. Only one type of sampling device was used at a site on the same date. Both of the sampling devices were fitted with a 350-μm mesh catch net. Three replicate samples were collected from different locations in the stream at each of the sites. The sampling device was placed on the stream bottom with the catch net situated in the downstream direction. Each cobble and pebble inside the sampling frame was brushed with a small brush to remove any macroinvertebrates that were attached. After brushing, each of these rocks was visually inspected and any remaining clinging organisms were removed with forceps. After removal of the larger rocks, a small rake was used to disturb the top 10 cm of substrate that remained in the sampler (mostly sand and gravel), causing any macroinvertebrates inhabiting that area to be washed into the catch net. Samples were transferred from the catch net to a plastic storage bag, preserved with undiluted ethanol, and transported to the laboratory for processing.

The macroinvertebrate samples were sorted in the lab by washing the samples in a 355-μm mesh soil sieve, transferring the sample to a shallow pan, and picking out all the organisms. The organisms were preserved in 70% ethanol and stored for subsequent identification.

Benthic macroinvertebrates were identified in the lab to the following taxonomic levels: class (Oligochaeta and Turbellaria), family (Mollusca), and genus (Insecta, excluding Chironomidae which were identified to family). It was not always possible to identify early instar insects to genus, and in these cases the organisms were left at family level.

Data from the SNP LTEMS were acquired in the form of a Microsoft Access database that contained macroinvertebrate, habitat, physical, and chemical data from 1986 through 2000.

Comparative Data from Other Streams in Blue Ridge Ecoregion

Macroinvertebrate sample data from 32 normal acid-neutralizing capacity (ANC) sites (ANC ≥ 50) and 13 low ANC sites (ANC < 20) were selected from the Virginia Tech historical database (maintained by the aquatic entomology program of J. Reese Voshell, Jr., Virginia Tech). These data were used to determine how SNP sites compared to other sites outside of SNP within the Blue Ridge ecoregion. The macroinvertebrate samples from the database were identified to genus, and were collected in the mid 1990's. Samples were collected with a 1-m kickscreen, after which the samples were sub-sampled to approximately 200 individuals.

Geographical Information System (GIS) Data

An Arcview geodatabase file was acquired from SNP for use in investigating the LTEMS monitoring data. This file included information on: drainage size per site; roads and trails (people and horse); forest type; historical land use information (number of buildings at the time the land was purchased by the state government); defoliation due to gypsy moths; forest fires; and geologic formation. A theme was created to determine the amount of area in each sampling site drainage that was an intersection of the stream buffer zone (stream buffer set at 30 meters) and roads and trails (road and trail buffer area set at 3 meters). The sites with a greater percentage of immediate stream buffer area overlapping with roads and/or trails were hypothesized to be different from sites with less overlap. The GIS data were used to investigate any potential cause and effect relationships between the environment and the monitoring data.

Data Analysis

Metric Selection

Thirty-eight metrics were considered in the preliminary analysis of how benthic macroinvertebrate data could be used effectively for biomonitoring SNP streams. These candidate metrics have been shown to be effective in assessing the biological integrity of streams (Ohio EPA 1988, Resh and Jackson 1993, Kerans and Karr 1994, Barbour et al. 1995, Barbour et al. 1996, Barbour et al. 1999). Some metrics (e.g., percent *Leuctra* out of total Plecoptera) were selected because they measured an organism tolerant of low pH, and should therefore be sensitive to decreasing pH from acid deposition (Townsend et al. 1983, Kimmel et al. 1985, Mackay and Kersey 1985, Simpson et al. 1985, Smith et al. 1990, Rosemond et al. 1992). An overriding criterion for metric selection was to ensure that metrics measured different aspects of the macroinvertebrate assemblage (richness, composition, balance, tolerance, trophic, and habit measures) in order to maximize the amount of ecological information (Barbour et al. 1995). For each category, metrics were selected that provided the best separation of reference and impaired conditions and were not redundant with other metrics. In the case of SNP streams, reference and impaired conditions consisted of the normal and low ANC streams, respectively. Metric redundancy was determined by stepwise discriminant analysis and correlation analysis. Redundant metrics were eliminated when one of a pair of metrics exhibited a correlation above 0.9 and the scatterplot of the two metrics showed a linear relationship (Barbour et al. 1996). At least one metric from each of the six ecological categories was included in the final list, making six metrics the minimum number of final metrics and no maximum number was planned. Having a diverse group of metrics results in an index that is sensitive to a broad range of stressors, and represents an array of assemblage characteristics (Karr and Chu 1997). Some metrics were chosen not for what they show now, but for what they may show in SNP streams if impairments from certain events occur in the future (e.g., sedimentation from trails).

Comparison of Streams in SNP to Similar Streams Outside of SNP

In order to evaluate the present ecological condition of SNP streams, it was necessary to compare them to streams outside of park boundaries. The Blue Ridge ecoregion is a very narrow, mountainous region, and in Virginia much of the land is under federal jurisdiction; either in SNP managed by the NPS, or Washington and Jefferson National Forest managed by the U. S. Forest Service. The result is that most of the streams are in good ecological condition, compared to streams that flow through private lands, and neither the SNP LTEMS database or the Virginia Tech Aquatic Entomology Program historical database contained instances of Virginia Blue Ridge streams that were severely impaired. The sites in these databases that were designated as impaired were all subjected to one type of perturbation, atmospheric acid deposition. Although acid deposition changes the structure of the benthic macroinvertebrate assemblage, in the stages where pH stays around 4.5-5.0, the diversity of the assemblage is not appreciable altered. Benthic macroinvertebrate data were acquired from sites identified as acid deposition impaired outside the SNP boundaries (identified in this study as low ANC and normal ANC sites), but within the Blue Ridge ecoregion, and compared to low alkalinity and normal alkalinity sites within SNP). The different ANC type streams in SNP were shown in a previous study of the park to have significantly different benthic macroinvertebrate assemblages (Smith and Voshell 1994).

The difference in sampling methods between the SNP data and these data made measures of proportional abundance the best metrics to use in the comparisons, and richness measures the least desirable due to potential sub-sampling effects (Barbour and Gerritsen 1996, Courtemanch 1996

The SNP sites were then compared to the non-SNP using Multivariate Analysis of Variance (MANOVA) on the collective metrics and Analysis of Variance (ANOVA) for general linear models with the follow-up Tukey-Kramer multiple comparison test (due to unbalanced data) on the individual metrics (SAS, version 8.02, SAS Institute Inc., Cary, NC, USA).

Comparison of Streams Within SNP

The aquatic LTEMS data and SNP GIS data were used to investigate if any streams within the park differ and the likely reasons for the differences. The LTEMS program originally started out with benthic macroinvertebrate sampling twice a year, spring and summer, but in 1997 this was decreased to only spring sampling. Therefore, only the spring data set was used in these analyses.

Streams within SNP were compared spatially and temporally. Spatial analysis was complicated by the fact that some streams had been sampled many times since 1986, while others had been sampled only once in recent years. Using all of these data would bias the interpretation of spatial trends in the entire SNP toward spatial trends in the streams that had been sampled many times. The first step of the spatial analysis therefore, was to select one site visit for each of the SNP sites to create a spatial data set. Rules followed to select site visits for inclusion were: only one visit per site; only site visits from the spring sampling period; and the site visit must have occurred within the 1995 to 2000 time period with selection of the year based on the progression 1998-1997-1999-1996-2000-1995. This method allowed for the inclusion of the maximum number of sites within the same sampling season, while eliminating problems that might arise from including repeated measures at the same site in different years or having the data collected from greatly different times. The spatial data set included macroinvertebrate metrics, GIS information, and the site chemical and physical measurements. GIS and site chemical-physical measurements are referred to as site variables.

Spatial analysis included looking for groupings of sites by cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) on the metrics with standardized mean = 0 and variance = 1 for each metric, and UPGMA cluster analysis on the standardized site variables (SAS, version 8.02, SAS Institute Inc., Cary, NC, USA). The cubic clustering criterion (CCC) and Hotelling's pseudo t^2 values were used to help determine meaningful clusters (Johnson 1998). Principal components analysis (PCA) was used as an exploratory technique to investigate any groups that may be formed from the creation of new variables that summarize the initial variables (SAS, version 8.02, SAS Institute Inc., Cary, NC, USA). Pearson correlation analyses were used to explore relationships between the macroinvertebrate taxa and site variables and between metrics and site variables. MANOVA was used to investigate the hypothesized cause-and-effect relationships between various factors and the metric data (SAS, version 8.02, SAS Institute Inc., Cary, NC, USA). The hypothesized cause-and-effect relationships included: localized floods (Hurricane Fran - September 1996, rapid snow melt - January 1996, localized storm - June 1995); fires; gypsy moth defoliation;

woolly adelgid defoliation; historical land use; current human activities (roads and trails); and underlying geology.

In addition to spatial trends within SNP, temporal analyses were conducted on data from the original 17 core sites in the SNP LTEMS. The temporal data set was used to investigate any trends occurring within SNP over the entire14 years that the project has been in place. Analysis was a site-by-site graphical analysis of the metrics by year.

Comparison of SNP Streams to Known Impaired Streams Outside of SNP

The comparisons of SNP streams to other streams within the Blue Ridge ecoregion and comparisons of streams within SNP did not demonstrate what information the LTEMS biomonitoring program would provide if there were serious pollution or environmental stress in SNP streams (due to the general lack of impairment other than low ANC streams and acid deposition). In order to gain additional insight into the performance of the SNP LTEMS biomonitoring program under extreme environmental problems, 5 additional sites were selected from the Virginia Tech Aquatic Entomology Program historical database that were impaired by something other than acid deposition (Table 2.2). The streams selected were similar in size to SNP streams and were selected from the nearby Ridges and Valleys ecoregion. This analysis consisted of comparing metric values for single site visits at the impaired sites outside of SNP to box-and-whisker plots of the same metrics at all SNP sites.

RESULTS

Metric Selection

Twelve metrics from six different categories were chosen from the candidate metrics (Table 2.3). Four of the final metrics are expected to increase in value in response to increasing perturbation, while the rest will decrease. The 12 selected metrics should provide a variety of useful information about the benthic macroinvertebrate assemblages in SNP streams.

Comparison of Streams in SNP to Similar Streams Outside of SNP

The 4 categories of sites (Blue Ridge low ANC, Blue Ridge normal ANC, SNP low ANC, and SNP normal ANC) were significantly different from each other using MANOVA on the metrics listed in Table 2.4 (Wilks' Lambda = 0.0562 , F value = 12.06, p <0.0001). MANOVA showed the SNP and Blue Ridge normal ANC sites to be significantly different (Wilks' Lambda = 0.1574, F value= 23.56, p <0.0001), as did the comparison of the SNP normal ANC to the Blue Ridge low ANC sites (Wilks' Lambda = 0.1779, F value =12.13, p <0.0001). ANOVA showed the SNP and Blue Ridge normal ANC sites were not statistically different for 6 of the 10 metrics (Table 2.4). Three of the remaining metrics showed the SNP normal ANC streams to be of superior ecological condition (% Hydropsychidae/Trichoptera, % intolerant, and HBI) compared to the Blue Ridge normal ANC sites.

MANOVA analysis of SNP and Blue Ridge low ANC sites showed them to be significantly different (Wilks' Lambda = 0.1651, F value =12.13, p <0.0001), as did comparison between the SNP low ANC and Blue Ridge reference sites (Wilks' Lambda = 0.1100, F value = 50.59, p <0.0001). Analyzed separately, the metrics showed the SNP low ANC sites were not significantly different from the Blue Ridge low ANC sites for 5 of the 10 metrics, and not different from the normal ANC streams for 6 of the 10 metrics (Table 2.4).

Percent Ephemeroptera and % scrapers were greater in the SNP and Blue Ridge normal ANC streams, and % shredders and % 5 dominant taxa were less than in the other streams (Table 2.4). The SNP streams had less dominance in Trichoptera by the filter-feeding family Hydropsychidae, as well as more intolerant organisms and lower HBI values.

Comparison of Streams Within SNP

The spatial set consisted of 89 sites: 28 identified as being located in low ANC streams (located in the Hampton and Erwin geological formations) and 61 were from normal ANC streams. Classification of the 89 sites using cluster analysis of the 12 metric values resulted in 4 groupings of sites; one cluster of 21 low ANC sites, one cluster of 1 low ANC site, one cluster of 4 normal ANC sites, and the remaining cluster of 6 low ANC sites and 57 normal ANC sites. The clusters were analyzed to determine if other site variables were leading to the separation of sites, but the classification appeared to be most consistent with the ANC type division. The different types of natural disturbance did not seem to be related to the site separation.

MANOVA showed the low ANC streams to be significantly different from normal ANC streams (Wilks' Lambda = 0.2405, F value = 20.00, p <0.0001), with only % EPT, HBI, and % intolerant organisms not significantly different at p=0.05. The low ANC type streams had higher metric values for % shredder, % 5 dominant taxa, and % *Leuctra*/Plecoptera. The values were lower in the low ANC type streams for % scrapers, % haptobenthos, % Hydropsychidae/Trichoptera, # taxa, # EPT, and % Ephemeroptera (Table 2.5).

Cluster analysis of a reduced environmental variable set (due to missing values for the rest of the variables) and other site characteristic variables resulted in a similar separation of the different ANC type streams. Principal component analysis (PCA) resulted in the first 2 eigenvalues accounting for only 63.13% of the total variation in the data. Plotting principal component 1 versus principal component 2 and using ANC type as the plot symbol showed a clear separation of the 2 ANC type streams (Fig. 2.2). An outlier was detected in the dataset (Fig. 2.2), and it was determined that this was the Big Meadows effluent site. This site was located directly in the effluent stream, with little to no natural water, so it was removed from further analyses. MANOVA analysis of the 2 ANC types using the reduced environmental set showed the 2 ANC type streams to be significantly different (Wilks' Lambda =0.1704, F value = 30.03, p <0.0001). All environmental variables tested were different between normal and low ANC type streams at p=0.05 (Table 2.6). All variables tested were higher at the normal ANC sites, with the exception of temperature ($\bar{x}$=14.2 C for low ANC streams, $\bar{x}$=13.2 C normal ANC), and amount of defoliation by gypsy moths (Table 2.6).

Number of taxa (r = 0.53, p < 0.0001), # EPT (r = 0.57, p < 0.0001), % scrapers (r = 0.48, p < 0.0001), % haptobenthos (r = 0.26, p = 0.0128), and % Ephemeroptera (r = 0.66, p < 0.0001) were all positively correlated with pH. Percent shredders (r = -0.63, p < 0.0001), % 5 dominance (r = 0.63, p < 0.0001), and % *Leuctra*/Plecoptera (r = 0.24, p = 0.02) were all negatively correlated with pH. Other relationships indicated by correlation were weak (r^2 values ranged from 0.05 to 0.16), with the exception of # EPT increasing with discharge (r = 0.49, p < 0.0001), and % Ephemeroptera decreasing with increased gypsy moth defoliation (r = -0.43, p < 0.0001).

There were 189 taxa present in the SNP spatial data set. Correlations between the individual taxa and environmental variables were weak (all r values ≤ 0.55). The only taxon with an r value > 0.5 was the snail family Ancylidae, which increased in abundance with both increasing conductivity (r = 0.55, $p < 0.0001$) and increasing dissolved solids (r = 0.54, $p < 0.0001$).

Gypsy moth defoliation did not result in any differences in the macroinvertebrate assemblages (MANOVA F value = 1.43, $p = 0.1693$), or the environmental variables (F value = 1.42, $p = 0.1761$). Fire history in the site drainages did not result in differences in the macroinvertebrate assemblages (F value = 1.43, $p = 0.1693$) or the environmental variables (F value = 0.63, $p = 0.8102$) in the spatial data set; nor did recent flood events in the drainages result in differences in either (F value = 1.20, $p = 0.2999$; F value = 1.32, $p = 0.2277$).

Streams with no trails or roads intersecting their 30-m stream buffer areas appeared to have different environmental attributes from those streams with trails and roads in their buffer zone (F value = 2.60, $p = 0.0310$), but no differences in their macroinvertebrate assemblages (F value = 1.63, $p = 0.1016$). Separate MANOVA analyses by ANC type did not show differences between these stream types in either the low ANC site environmental variables (F value = 1.07, $p = 0.4060$), nor in the normal ANC streams (F value = 1.07, $p = 0.4060$). Sites with concentrations of hemlock forests did not differ from sites without hemlock concentrations in either assemblage (F value = 1.41, $p = 0.2368$) or environmental attributes (F value = 0.85, $p = 0.5977$) (analysis was restricted to the normal ANC sites due to no hemlock concentrations in the low ANC streams (Table 2.6)).

Temporal analysis of the LTEMS sites indicated the macroinvertebrate assemblages are highly variable over time (Figs. 2.3-2.6), with similar fluctuations occurring in low ANC streams (Fig. 2.3A) as occurred in the normal ANC streams (Fig. 2.3B). Most of the metric values indicated a worsening of the biotic integrity at the sites in the period from 1987 to 1990 and then a rise back to 1987 levels, similar to what was shown by % intolerant organisms (Fig. 2.6). Individual metrics by year for each site also showed the wide range of year-to-year variability (Fig. 2.7). The 1995 flood effects, not observed with MANOVA or when all the LTEMS normal ANC site temporal data were analyzed (Fig. 2.6B), was easily observed when a single site was analyzed (Fig. 2.7B).

Comparison of SNP Streams to Known Impaired Streams Outside of SNP

The badly impaired streams used for comparison to the SNP low and normal ANC sites show examples of metric values that could be expected if serious perturbation occurred in SNP (Figs. 8-10). Percent EPT (Fig. 2.8A), % intolerant organisms (Fig. 2.9C), % haptobenthos (Fig. 2.9D), and % shredders (Fig. 2.10A) were all greater in the SNP streams while HBI was less (Fig 9B). Ephemeroptera were more abundant in the SNP normal ANC streams than in the impaired streams, although, the abundance was similar between the impaired streams and the SNP low ANC streams due to the lower pH (Fig. 2.8B). Conversely, there was more overall dominance by few taxa in the impaired streams and the SNP low ANC streams than in the SNP normal streams (Fig. 2.9A). Dominance in Trichoptera by Hydropsychidae was generally much higher in the impaired streams (Fig. 2.8C). Dominance in Plecoptera by *Leuctra* was intended to show continued effects of acidification, and the SNP low ANC streams showed them in higher proportions. Abundance of scrapers was variable between the impaired streams, showing both higher values due to organic enrichment and lower values due to disturbance then observed in SNP.

DISCUSSION

Metric Selection

We selected a fairly diverse group of metrics for use in SNP that will allow park personnel to monitor changes and make timely management decisions. The metrics selected (Table 2.3) have been shown to respond to various environmental factors, and will allow for the detection of disturbance or recovery of the macroinvertebrate assemblages over time. The richness measures, number of taxa and number of EPT, provide insight into the diversity of the assemblage (Resh et al. 1995). These richness measures should help indicate sites that are being affected by acidification and inorganic sedimentation, as richness has been shown to decrease with acidification (Wiederholm 1984) and sedimentation (Hellawell 1986).

Composition measures provide insight into the contribution of major taxonomic categories of organisms (percent Ephemeroptera and percent EPT) relative to the whole assemblage. Percent Ephemeroptera has been shown to be especially sensitive to acidification (Mackay and Kersey 1985, Smith et al. 1990, Feldman and Connor 1992), and percent EPT measures the relative abundance of three orders of insects that are generally sensitive to and indicative of disturbance (Wallace et al. 1996). These composition measures may detect subtle changes in the assemblage that the richness metrics do not, as in the case of decreased relative abundance of EPT, but no or little change in overall number of EPT taxa.

Balance metrics (percent 5 dominant taxa, percent Hydropsychidae/Trichoptera, and percent *Leuctra*/Plecoptera) are relative measures that provide insight into the evenness of the assemblage. A high value of percent 5 dominant taxa is a measure of redundancy, and high values equate to lowered diversity (Plafkin et al. 1989).

The tolerance measures (modified HBI and percent intolerant organisms) provide information about the sensitivity of the assemblage to various types of perturbation (Hilsenhoff 1987). The modified HBI used in this study followed the guidelines set forth by Hilsenhoff (1982, 1987, 1988), but used tolerance values developed for organisms in Virginia streams (Voshell, unpublished data). Percent intolerant organisms measures the relative abundance of the extremely pollution intolerant organisms (tolerance values ≤ 3), and in a stable assemblage without disturbance this should vary little over time.

Trophic metrics (percent scrapers and percent shredders) are also relative abundance measures and measure the abundance of organisms that obtain their food by a specific feeding mechanism. Scrapers and shredders are considered specialized feeders that should be well represented in healthy streams, but they are sensitive to perturbation (Barbour et al. 1996). The relative abundance of scrapers should naturally be higher than shredders in mid-order streams (orders 4-6), and this trend is reversed in small order streams (orders 1-3) (Vannote et al. 1980).

Habit, or mode of existence, describes how an organism moves about, conceals itself, or attaches itself to the substrate (Cummins and Merritt 1996). The habit metric, percent haptobenthos, measures the relative abundance of those organisms that require clean, firm substrate, which are the clingers and crawlers (Smith and Voshell 1997). This decreases in response to several types of perturbation, such as sedimentation, nutrient enrichment, and organic loading.

Comparison of Streams in SNP to Similar Streams Outside of SNP

The similarity between the SNP normal ANC sites and the Blue Ridge reference sites, as well as the superior ecological condition suggested by some assemblage metrics (Table 2.4) indicates that the streams in SNP are comparable to the best that can be found in the Blue Ridge ecoregion. Land use in SNP (mostly for recreational purposes) does not appear to be causing impairment to the macroinvertebrate assemblages found at the sites sampled, or at least impairment no worse than what occurs at reference sites outside the park boundaries. The low ANC streams were similar to the Blue Ridge impaired streams; however, the disturbance to both types of streams is atmospheric acid deposition, which results in greater observed response in streams of lower buffering capacity. Metrics that measured organic enrichment to the streams indicated that both types of SNP sites were of superior environmental quality. A potential reason that haptobenthos were apparently more abundant in the Blue Ridge streams (Table 2.4) is the differences between sampling protocols. SNP samples were taken with PIBS or Surber samplers, for which the top layer of stream substrate was agitated thoroughly, resulting in the collection of a fair number of Chironomidae (burrowers) in the sample. The whole sample was then identified with no subsampling. The method for the samples outside SNP used a 1-m kickscreen with subsampling, which may have resulted in lower numbers of Chironomidae.

Comparison of Streams Within SNP

The taxa and environmental variable correlations indicated that no one environmental variable strongly affects the taxa distribution, or that the ranges of the environmental variables found in the park were not sufficiently extreme to affect distribution. Environmental variables and metrics both differed between the low and normal ANC streams. The low ANC sites within SNP were found to differ greatly from the normal ANC sites, but this is the only easily understood separation of sites. Environmental variables differed between the stream types; however, the lower pH at the low ANC sites in the park appears to be the cause of the differences detected in the macroinvertebrate assemblages, because the other environmental variables were not correlated with the macroinvertebrates.

It was hypothesized that historical land use, indicated by buildings per drainage at the time the land was purchased, might still be affecting the stream assemblages. SNP land was in heavy use prior to becoming a national park, and Harding et al. (1998) demonstrated that even after 47 years of riparian recovery the benthic assemblages are still not similar to the pre-disturbance condition. One possible explanation for why we did not detect differences in SNP stream other than between ANC types was that the streams in the park are primarily very high gradient streams that were able to transport the silt or contaminants from past land use rapidly out of the system. Additionally, they have all recovered to a similar degree for the same period of time.

Drainage areas with gypsy moth defoliation, fires, floods, and concentrations of hemlock forests were also not shown to differ within the park. It is possible differences were not detected because, in the case of gypsy moth defoliation, there has been apparent recovery since the disturbance occurred (Fig. 2.6). Floods did not cause the sites affected to form distinct groupings, but were evident in the graphical analyses of the affected sites in the time series data (Fig. 2.7B).

Hemlock forested drainage areas may still show differences over time, as defoliation by hemlock woolly adelgid increases. However, the areas of the park that have hemlock forests are small compared to the rest of the drainage, and may not result in differences in the benthic macroinvertebrate assemblage even after the defoliation occurs.

Stream drainages affected by fires do not appear to show long term effects in SNP. This may be partly due to the low intensity of the recent fires in the park. The most recent large-scale fire in the park was the Pinnacle/Old Rag fire, which encompassed 14% of SNP area, consuming a total of 9234 hectares (NPS SNP 2002). The SNP effects summary listed the fire as having consumed mainly leaf litter, and the large trees were largely not affected (NPS SNP 2002). The aquatic macroinvertebrate assemblages are affected by fires in large part due to increased runoff and sedimentation after a fire (Minshall et al. 2001). Additionally, the observed effects of the fire may be delayed by some years until a large storm or spring snowmelt occurs in the area (Minshall et al. 2001).

The LTEMS data showed that the macroinvertebrate assemblages vary greatly from year to year. These differences are probably not due to any type of sampling effort differences, since SNP has had the same employee involved in the sampling since 1991. These yearly variations may have hampered interpretation of the spatial data set, because it was created by compiling data from a 5-year span. However, the inclusion of the multiple years of data, which included natural yearly variations, allowed us to draw a more accurate range of the macroinvertebrate assemblage structure in SNP. Natural resource managers need to have an understanding of natural variation in order to better understand when differences in assemblages occurring over time is expected variation, or anthropogenic in nature. Therefore, SNP personnel conducting analysis of the biomonitoring data should do a within year analysis of the data, as well as a temporal analysis. Analyses of both types of data sets will allow the researcher to determine if a change observed from a previous year is specific to a single site or group of sites, or if it is a natural yearly variation.

Comparison of SNP Streams to Known Impaired Streams Outside of SNP

The interest in comparing the SNP sites to the severely impaired sites was to investigate if the metrics selected would be effective at detecting impaired ecological condition if significant environmental stress occurred within the park. Both the low ANC and the normal ANC streams were of superior ecological condition when compared to sites that are impaired by agriculture and urbanization. The normal ANC sites showed aquatic assemblages far different from the impaired streams. The low ANC streams were only similar to the impaired streams for % Ephemeroptera (Fig. 2.8B) which is probably a result of the lower pH levels found in those park streams, and % scrapers (Fig. 2.10B) which may also be a result of the lower pH. The streams impaired by cattle grazing (Table 2.2) were used to examine what might happen to the ecological condition of the SNP streams if the road, hiking trail, and horse trail use in the park were to increase to a point where they were adversely affecting the stream habitat. At the current levels of use, these factors do not appear to be having an adverse affect on the macroinvertebrates.

Some analyses were hampered by missing data, and it is our recommendation that when sites are visited for the first time, that all the environmental measures be taken at that time. We further recommend that the core LTEMS sites continue to be visited to facilitate detecting long-term trends. The long-term trends of recovery or distress may not be evident for years.

Our results illustrate what was intuitively expected from the SNP streams: they have ecological condition as good or better than others in Blue Ridge ecoregion due to the current land management practices. The only indication of appreciable alteration of ecological condition in SNP streams involves effects of acid deposition in low ANC streams; other disturbances do not appear to have had noticeable long-term effects on the streams. The monitoring system of the SNP could be revised over time, as additional data are gathered, but the metrics selected appear to meet the current monitoring needs. The protocol for SNP should be transferable to other parks, forests, and wilderness areas interested in monitoring water quality and changes over time, because the same general principals in place in the SNP will be the same outside the park boundary.

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TABLE 2.1. Physical and chemical measurements taken at each site. Present day method used by the SNP and previous methods used are listed.

Variable measured	Sampling method/information
Temperature (°C)	Currently - Hydrolab multiprobe unit
	Other methods - long-stem thermometer, YSI Model 33 meter
Dissolved oxygen (DO, mg/l)	Currently - Hydrolab multiprobe unit
	Other methods - YSI Model 58 meter, Hach kit
Total dissolved solids (TDS, g/l)	Hydrolab multiprobe unit
pH	Currently - Hydrolab multiprobe unit
	Other methods - Fisher Accumet 640 meter
Conductivity (μmhos)	Currently - Hydrolab multiprobe unit
	Other methods - YSI Model 33 meter
Discharge (m^3/s)	Normally - Marsh-McBirney flow meter and cross sectional area (Gore 1996)
	Under extreme low flow - capture flow (SNP Bag and Bucket method) and measure actual discharge
	Infrequently - Float Method (Gore 1996)
Stream width	Measured at each transect
Riparian cover amount	Observed at each transect, cover categories: 0-33%, 34-66%, and 67-100% covered.
Substrate size	Dominant substratum noted at 0.3-m intervals along each transect, substrate categories: bedrock, boulder (> 25 cm, movable), cobble (6-25 cm), pebble/gravel (0.2-6 cm), and sand (<0.2 cm)
Pool-riffle ratio	Observed for the whole 100-m section

TABLE 2.2. Impaired sites from the Ridge and Valley ecoregion selected for comparison to Shenandoah National Park sites.

Stream Location	Source of Impairment
Mill Creek tributary (MCT) Montgomery County, VA	Cattle grazing with free access to stream, no trees, riparian zone comprised only of grasses
Ogden Stream (OS) Rockbridge County, VA	Cattle grazing with free access to stream, no trees, riparian zone comprised of heavily grazed grasses, prominent bank slumping, much bare soil on banks of stream, substratum comprised of 80% sand and silt
Peak Creek (PK) Pulaski County, VA	Urbanization effects, industrial waste runoff, sedimentation effects, channelization of the stream in sections
Piney Creek (PC) Grayson County, VA	Cattle grazing with free access to stream, no trees, riparian zone comprised of heavily grazed grasses, bank slumping and eroded gullies present
Upper Wilson Creek (UWC) Rockbridge County, VA	Cattle grazing with free access to stream, sparse shrubs and trees, riparian zone comprised mainly of heavily grazed grasses, eroded and unstable banks, substratum comprised of 43% sand and silt

TABLE 2.3. Metrics and expected response to increasing perturbation in streams in the Blue Ridge Mountain ecoregion.

Measure category	Metric	Definition	Expected response to perturbation
Richness	Number of taxa	The total number of taxa in the macroinvertebrate assemblage	Decrease
Richness	Number of EPT	Total number of taxa in the orders Ephemeroptera (E, mayflies), Plecoptera (P, stoneflies), and Trichoptera (T, caddisflies)	Decrease
Composition	% EPT	Relative abundance of insects in the orders Ephemeroptera, Plecoptera, and Trichoptera	Decrease
Composition	% Ephemeroptera	Relative abundance of Ephemeroptera	Decrease
Composition	% Hydropsychidae/ Trichoptera	Percent abundance of insects in the family Hydropsychidae divided by the total number of caddisflies	Increase
Composition	% *Leuctra*/ Plecoptera	Percent abundance of the low pH tolerant stonefly *Leuctra* divided by the total number of stoneflies	Increase
Balance	% 5 dominant taxa	Relative abundance of the 5 most abundant taxa	Increase
Tolerance	HBI (modified Hilsenhoff Biotic Index)	Weighted sum of total taxa by pollution tolerance values	Increase
Tolerance	% Intolerant organisms	Percent abundance of macroinvertebrates with tolerance values of 0, 1, 2, or 3	Decrease
Trophic	% Scrapers	Relative abundance of the functional feeding group containing scrapers	Decrease
Trophic	% Shredders	Relative abundance of the functional feeding group containing shredders	Decrease
Habit	% Haptobenthos	Relative abundance of macroinvertebrates requiring clean, firm, course substrates (crawlers and clingers)	Decrease

TABLE 2.4. ANOVA of site categories showing F values and *p*-values. Tukey-Kramer multiple comparison results are shown as a line connecting means that are not significantly different. BR-NORM= Blue Ridge normal ANC, BR-LOW= Blue Ridge low ANC sites, SNP-Norm= Shenandoah National Park non-low ANC sites, SNP-Low= Shenandoah National Park low ANC sites.

Metric	F-value (p-value)	Site Category Mean			
% EPT	2.67 (0.0528)	BR-NORM 65.8	BR-LOW 62.4	SNP-Low 59.3	SNP-Norm 55.2
% Ephemeroptera	18.89 (<0.0001)	SNP-Norm 28.9	BR-NORM 25.0	SNP-Low 10.2	BR-LOW 9.6
% Hydropsychidae/ Trichoptera	28.11 (<0.0001)	BR-NORM 67.5	BR-LOW 44.5	SNP-Norm 25.1	SNP-Low 22.1
% Leuctra/Plecoptera	6.07 (0.0008)	SNP-Low 68.1	SNP-Norm 52.8	BR-LOW 49.0	BR-NORM 38.2
% 5 Dominant taxa	29.08 (<0.0001)	SNP-Low 87.3	BR-LOW 76.1	SNP-Norm 71.8	BR-NORM 65.2
HBI (modified Hilsenhoff Biotic Index)	17.11 (<0.0001)	BR-NORM 4.1	BR-LOW 3.6	SNP-Norm 3.3	SNP-Low 2.8
% Intolerant organisms	20.05 (<0.0001)	SNP-Low 56.8	SNP-Norm 52.1	BR-LOW 42.0	BR-NORM 27.7
% Scrapers	15.90 (<0.0001)	BR-NORM 16.1	SNP-Norm 15.4	SNP-Low 4.4	BR-LOW 4.2
% Shredders	29.44 (<0.0001)	SNP-Low 38.3	BR-LOW 29.5	SNP-Norm 13.7	BR-NORM 10.5
% Haptobenthos	9.79 (<0.0001)	BR-NORM 83.3	BR-LOW 78.0	SNP-Norm 69.4	SNP-Low 67.2

TABLE 2.5. Summary of means for the two SNP ANC type streams for each of the metrics (N=28 for low ANC, N=61 for normal ANC). Comparison *p*-values for ANC types are from SAS general linear models ANOVA due to unbalanced data. SNP Normal ANC = Shenandoah National Park non-SNP Low ANC sites, SNP Low ANC = Shenandoah National Park SNP Low ANC sites.

Metric	*p*-value	Site category Mean metric value	
Number of taxa	<0.0001	SNP Low ANC 27.86	SNP Normal ANC 36.54
Number of EPT	<0.0001	SNP Low ANC 17.18	SNP Normal ANC 22.95
% EPT	0.7794	SNP Low ANC 59.29	SNP Normal ANC 60.17
% Ephemeroptera	<0.0001	SNP Low ANC 10.16	SNP Normal ANC 32.21
% Hydropsychidae/ Trichoptera	0.0138	SNP Low ANC 21.73	SNP Normal ANC 32.56
% Leuctridae/ Plecoptera	0.0204	SNP Normal ANC 56.41	SNP Low ANC 68.85
% 5 dominant Taxa	<0.0001	SNP Normal ANC 71.61	SNP Low ANC 86.93
HBI (modified Hilsenhoff Biotic Index)	0.1566	SNP Low ANC 2.82	SNP Normal ANC 3.03
% Intolerant organisms	0.6330	SNP Normal ANC 55.60	SNP Low ANC 57.06
% Scrapers	<0.0001	SNP Low ANC 4.75	SNP Normal ANC 15.72
% Shredders	<0.0001	SNP Normal ANC 14.45	SNP Low ANC 38.39
% Haptobenthos	0.0269	SNP Low ANC 67.34	SNP Normal ANC 73.91

TABLE 2.6. Summary of means for the two SNP ANC type streams for each of the environmental variables (N=26 for low ANC, N=61 for normal ANC). Comparison *p*-values for ANC types are from SAS general linear models ANOVA due to unbalanced data. SNP Normal ANC = Shenandoah National Park non-SNP Low ANC sites, SNP Low ANC = Shenandoah National Park SNP Low ANC sites.

Environmental variable	*p*-value	Site category Mean variable value	
Discharge (M^3/second)	0.0014	SNP Low ANC 0.04	SNP Normal ANC 0.12
Temperature (°C)	0.0221	SNP Normal ANC 13.2	SNP Low ANC 14.2
Dissolved oxygen (DO, mg/l)	<0.0001	SNP Low ANC 9.2	SNP Normal ANC 9.9
Total dissolved solids (TDS, g/l)	0.0004	SNP normal ANC 0.012	SNP low ANC 0.019
pH	<0.0001	SNP Low ANC 5.4	SNP Normal ANC 6.8
Conductivity (µmhos)	0.0005	SNP Low ANC 18.2	SNP Normal ANC 28.7
Drainage size at each site (hectares)	0.0412	SNP Low ANC 518.8	SNP Normal ANC 847.8
Percent stream buffer/trails + road buffer overlap (%)	0.0010	SNP Low ANC 0.46	SNP Normal ANC 1.19
Percent Hemlock dominated hectares/total hectares (%)	0.0034	SNP Low ANC 0.0	SNP Normal ANC 13.5
Historical houses per hectare for each site's drainage area	0.0002	SNP Low ANC 0.03	SNP Normal ANC 0.08
Percent of drainage that was defoliated by gypsy moths (since 1986) (%)	<0.0001	SNP Normal ANC 21.8	SNP Low ANC 63.2

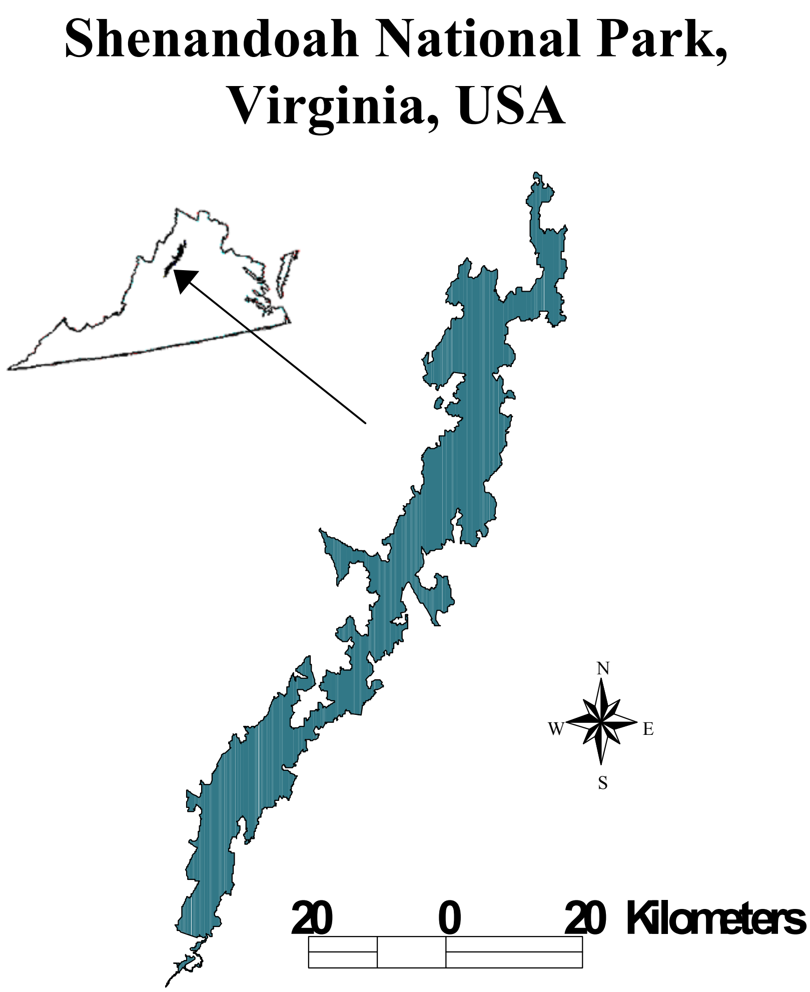


FIG. 2.1. Location of the Shenandoah National Park within Virginia, USA (scale shown is for the park).

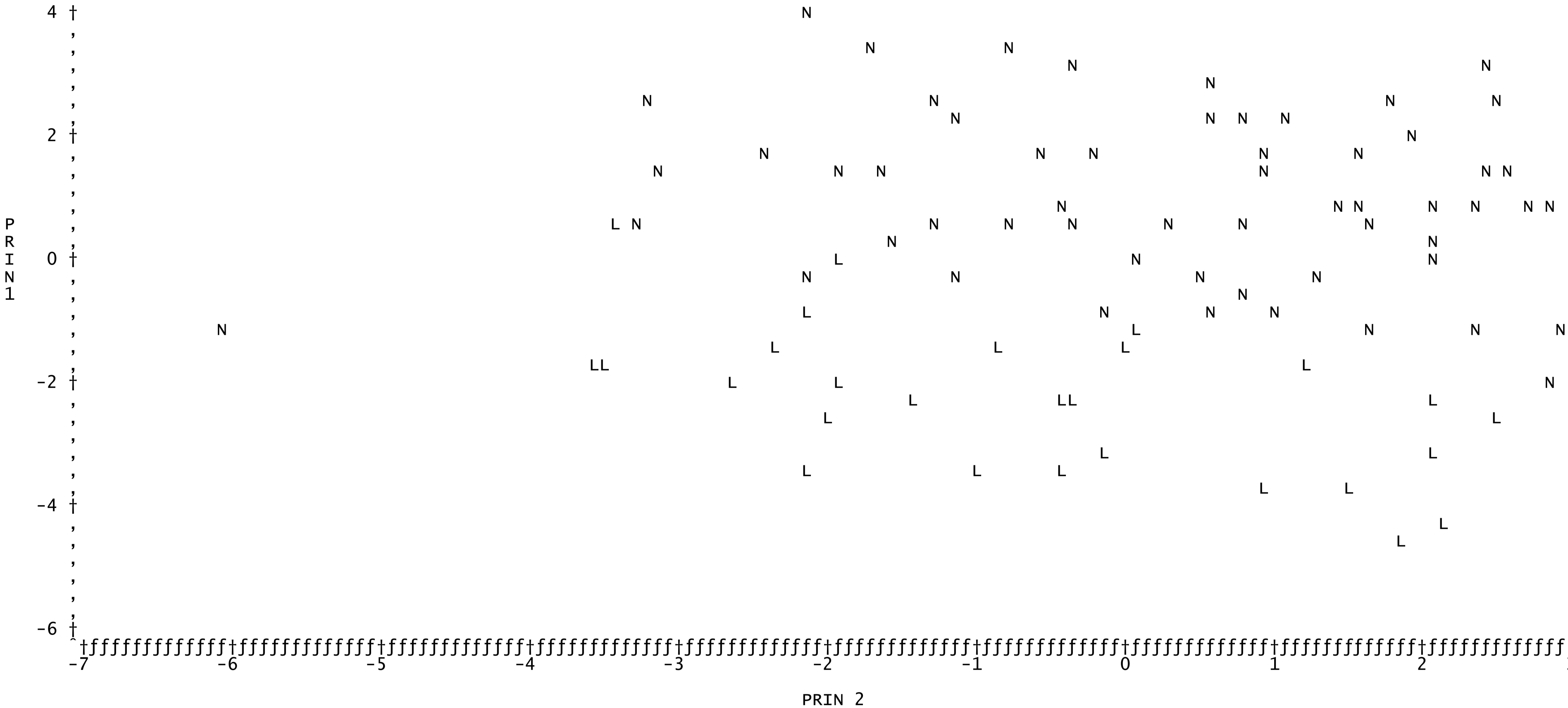
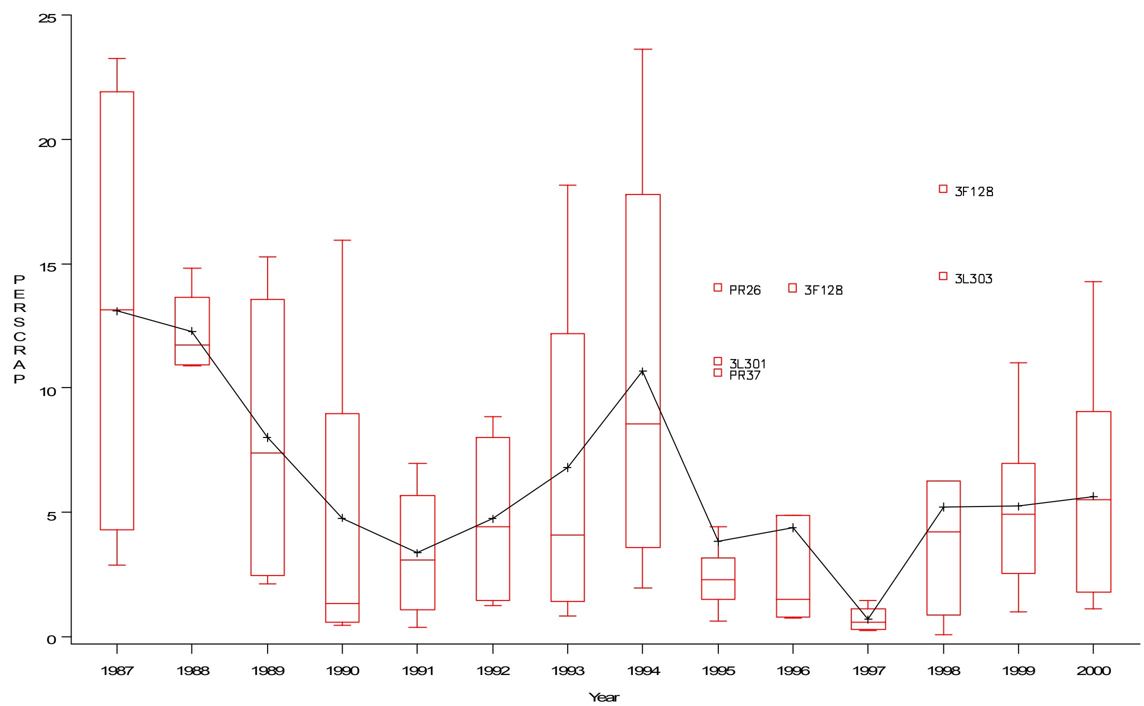


FIG. 2.2. Plot of principal component 1 (PRIN 1) versus principal component 2 (PRIN2) for the spatial data set of metric values. The symbols used to indicate the sites refer to the ANC type of the streams, L = low ANC streams, and N = normal ANC streams

A



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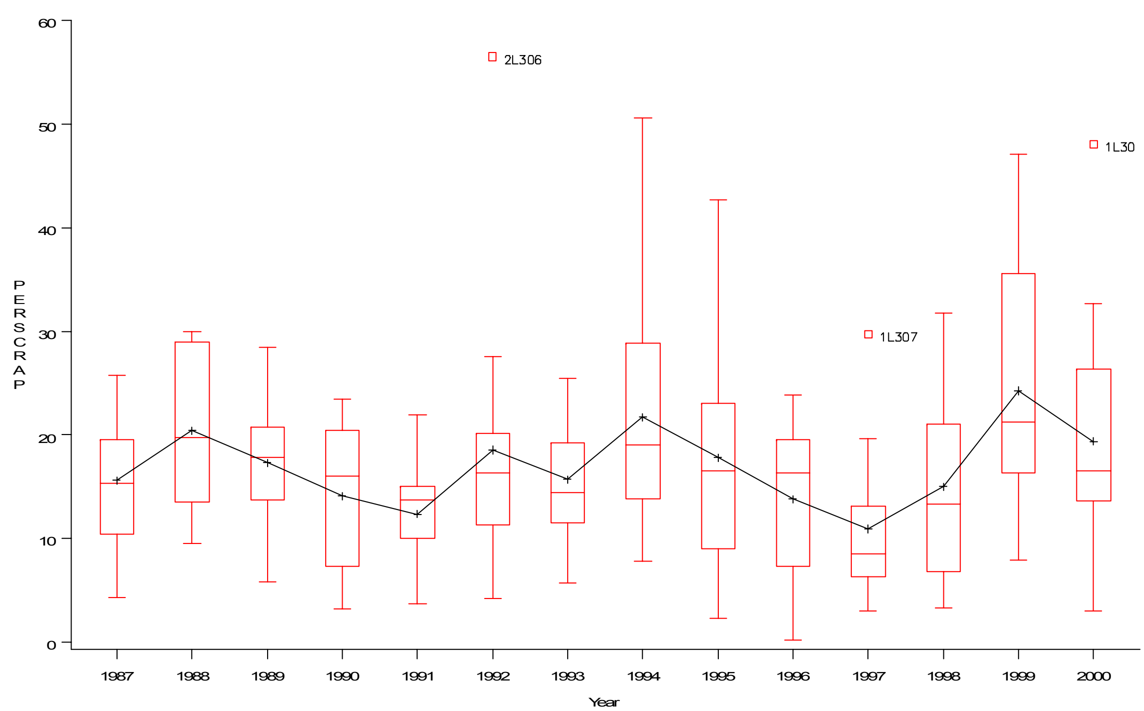
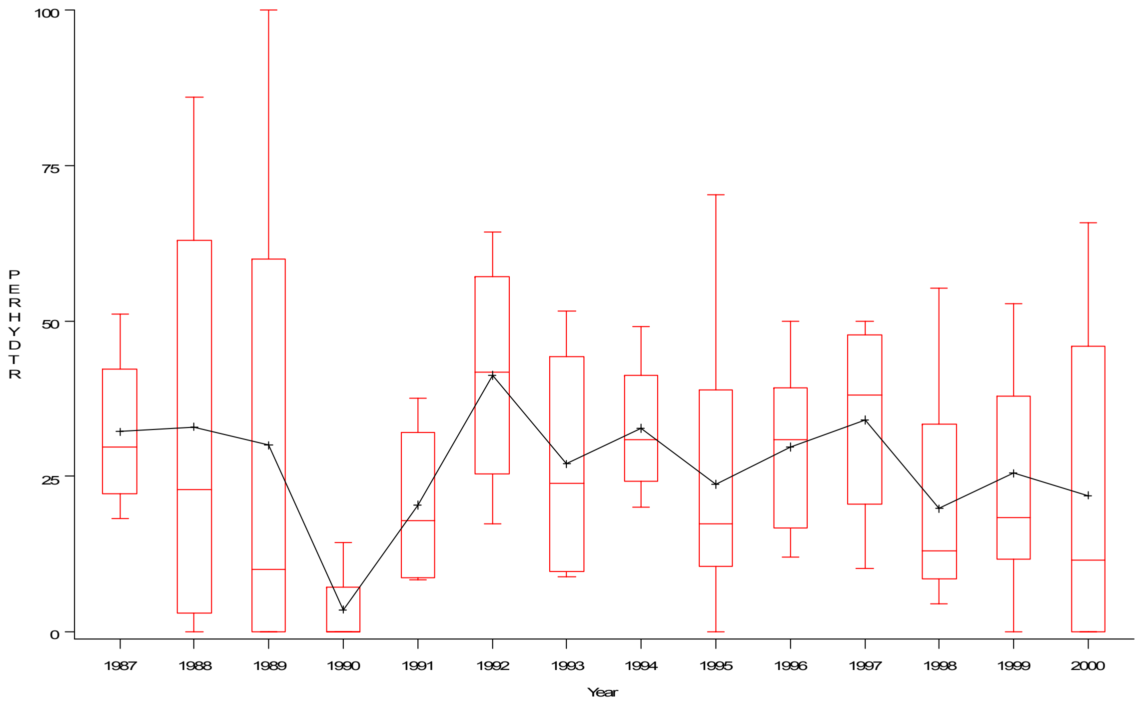


FIG. 2.3. Box plot of percent scrapers (PERSCRAP) for spring samples by year. Fig. A is for sites in the Hampton/Erwin geologic type, and Fig. B is the non-Hampton/Erwin sites. The trend line is connecting the means of each year. Site codes are shown for outlier data.

A



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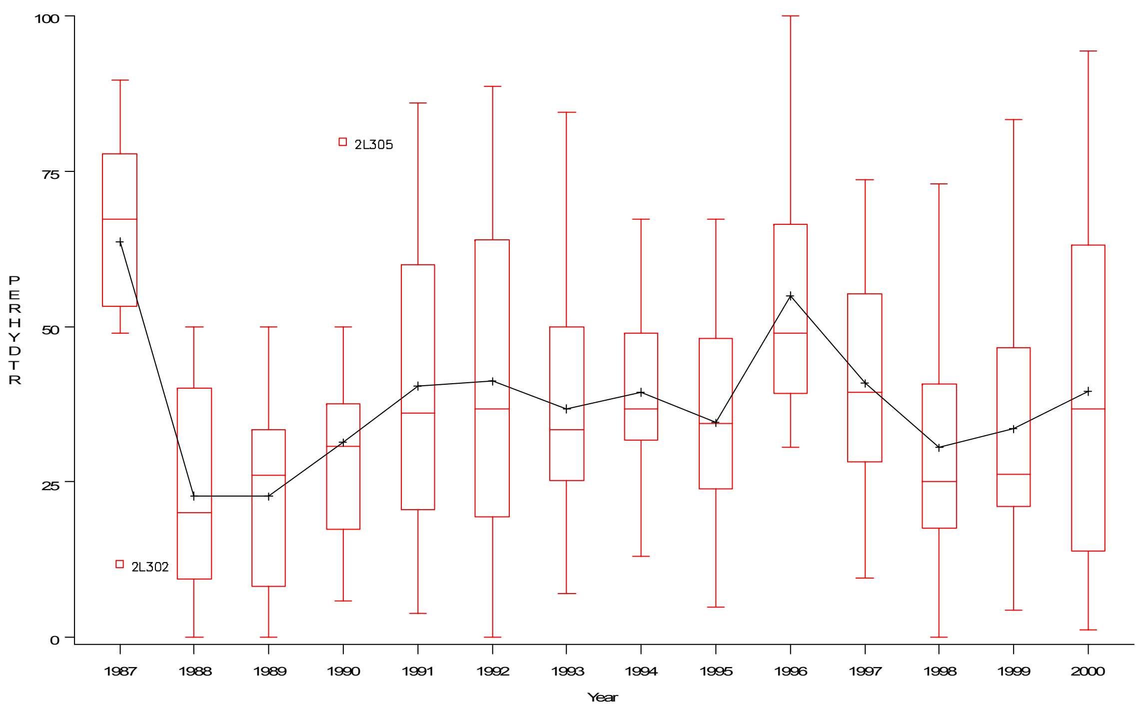
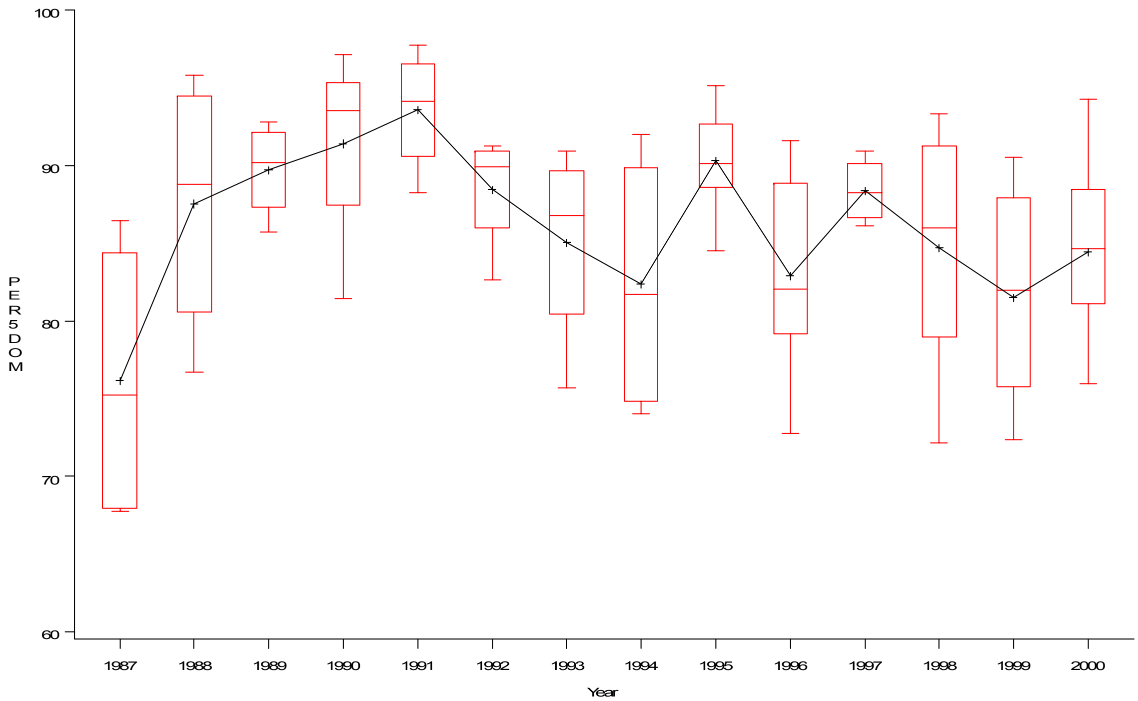


FIG. 2.4. Box plot of percent Hydropsychidae/total Trichoptera (PERHYDTR) for spring samples by year. Fig. A is for sites in the Hampton/Erwin geologic type, and Fig. B is the non-Hampton/Erwin sites. The trend line is connecting the means of each year. Site codes are shown for outlier data.

A



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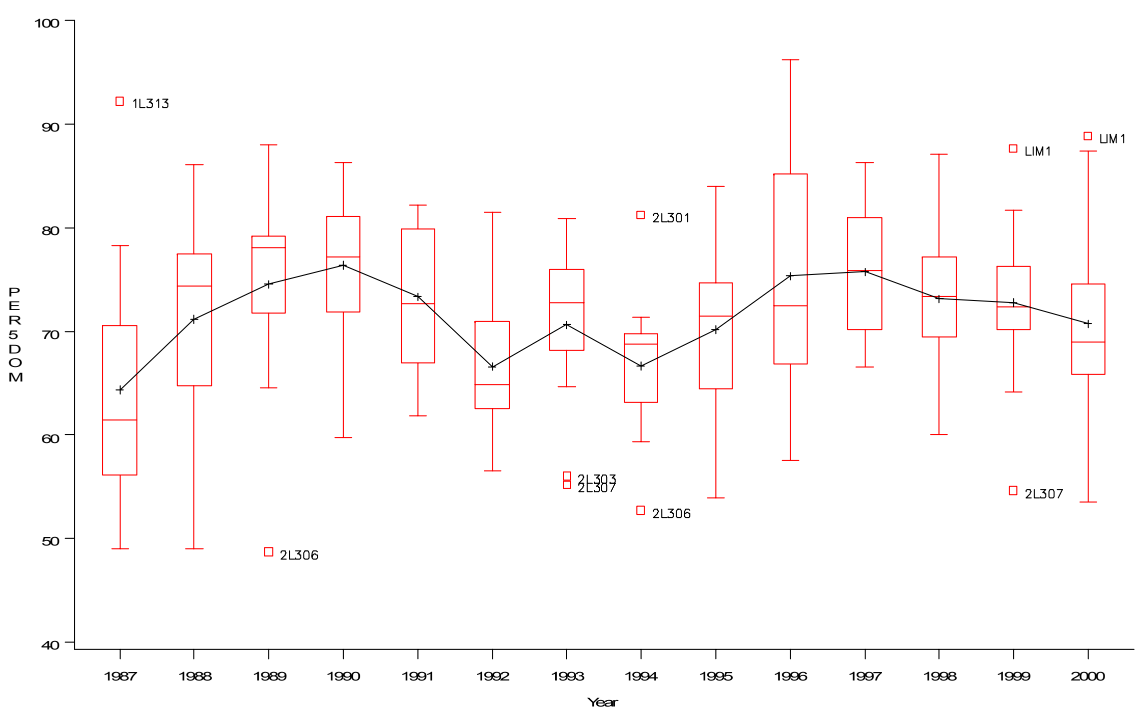


FIG. 2.5. Box plot of percent 5 dominant (PER5DOM) for spring samples by year. Fig. A is for sites in the Hampton/Erwin geologic type, and Fig. B is the non-Hampton/Erwin sites. The trend line is connecting the means of each year. Site codes are shown for outlier data.

A

B

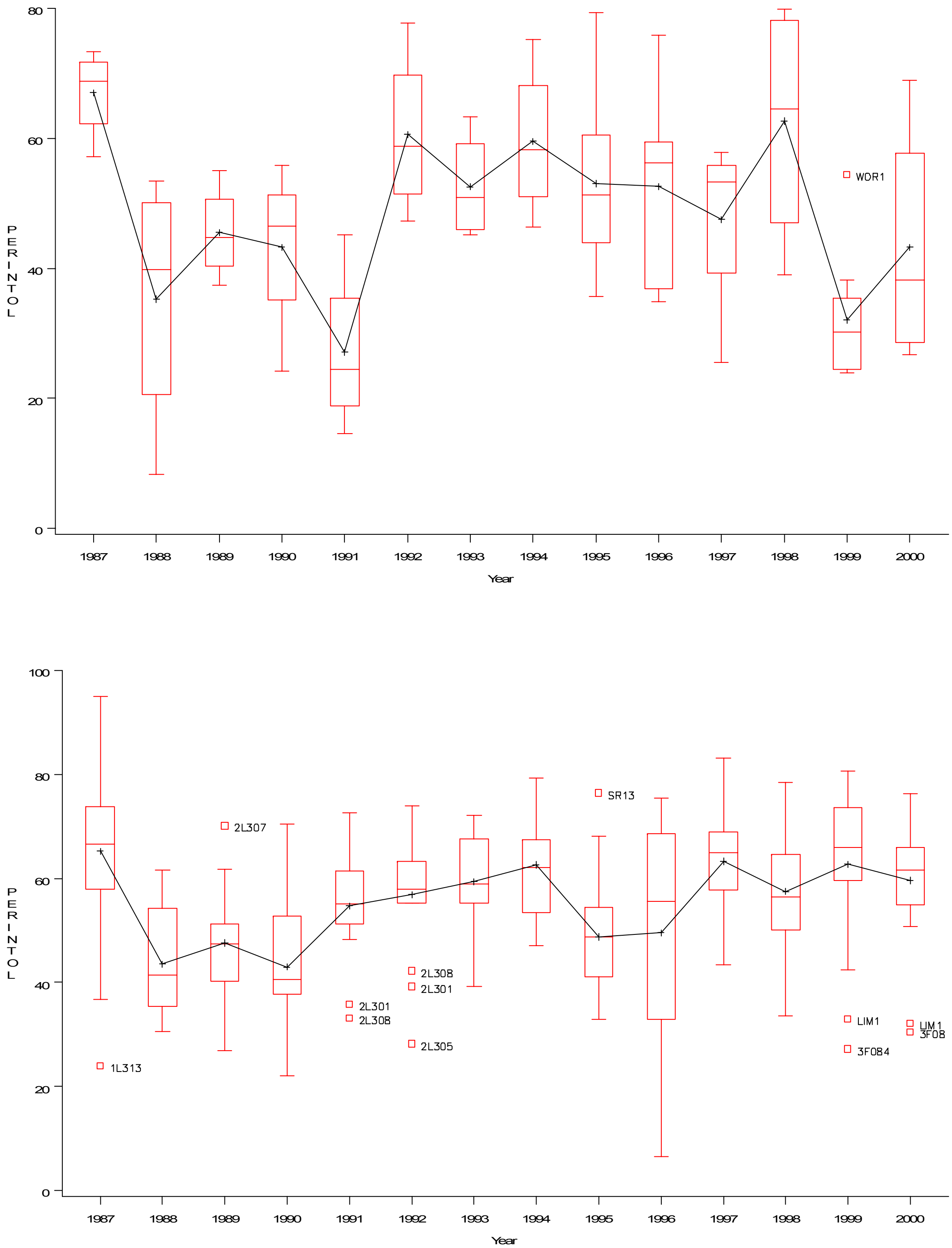


FIG. 2.6. Box plot of percent intolerant organisms (PERINTOL) for spring samples by year. Fig. A is for sites in the Hampton/Erwin geologic type, and Fig. B is the non-Hampton/Erwin sites. The trend line is connecting the means of each year. Site codes are shown for outlier data.

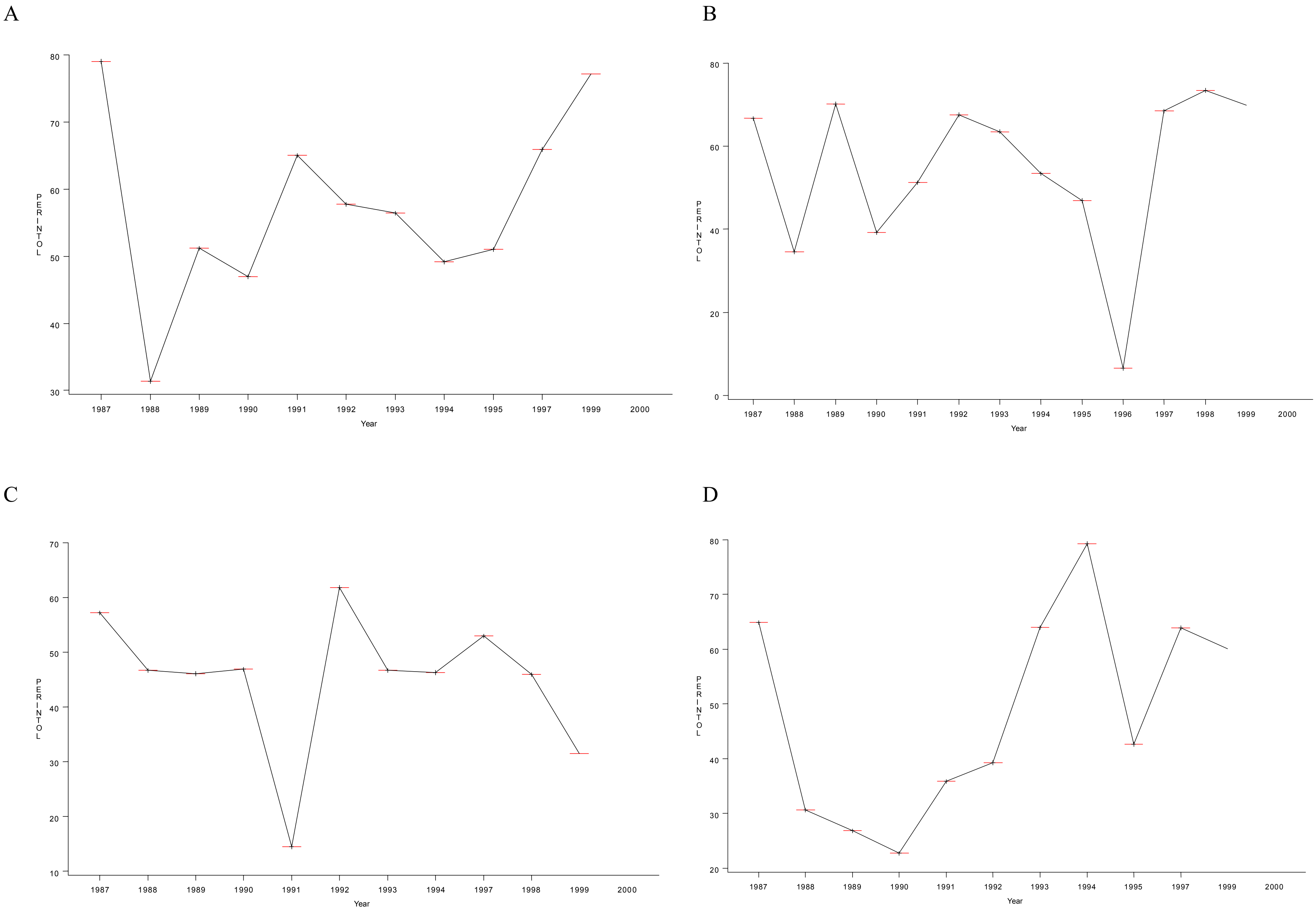


FIG. 2.7. Percent intolerant organisms at representative sites (A) HazelRiver-lower, normal ANC (B) StauntonRiver-lower, normal ANC, flooded after 1995 sample was taken (C) TwomileRun-lower, low ANC and (D) WhiteOakCanyon-lower, normal ANC, flooded after 1995 sample was taken, scoured by rapid snow melt in January 1996, and flooded by a hurricane after the 1996 sampling season.

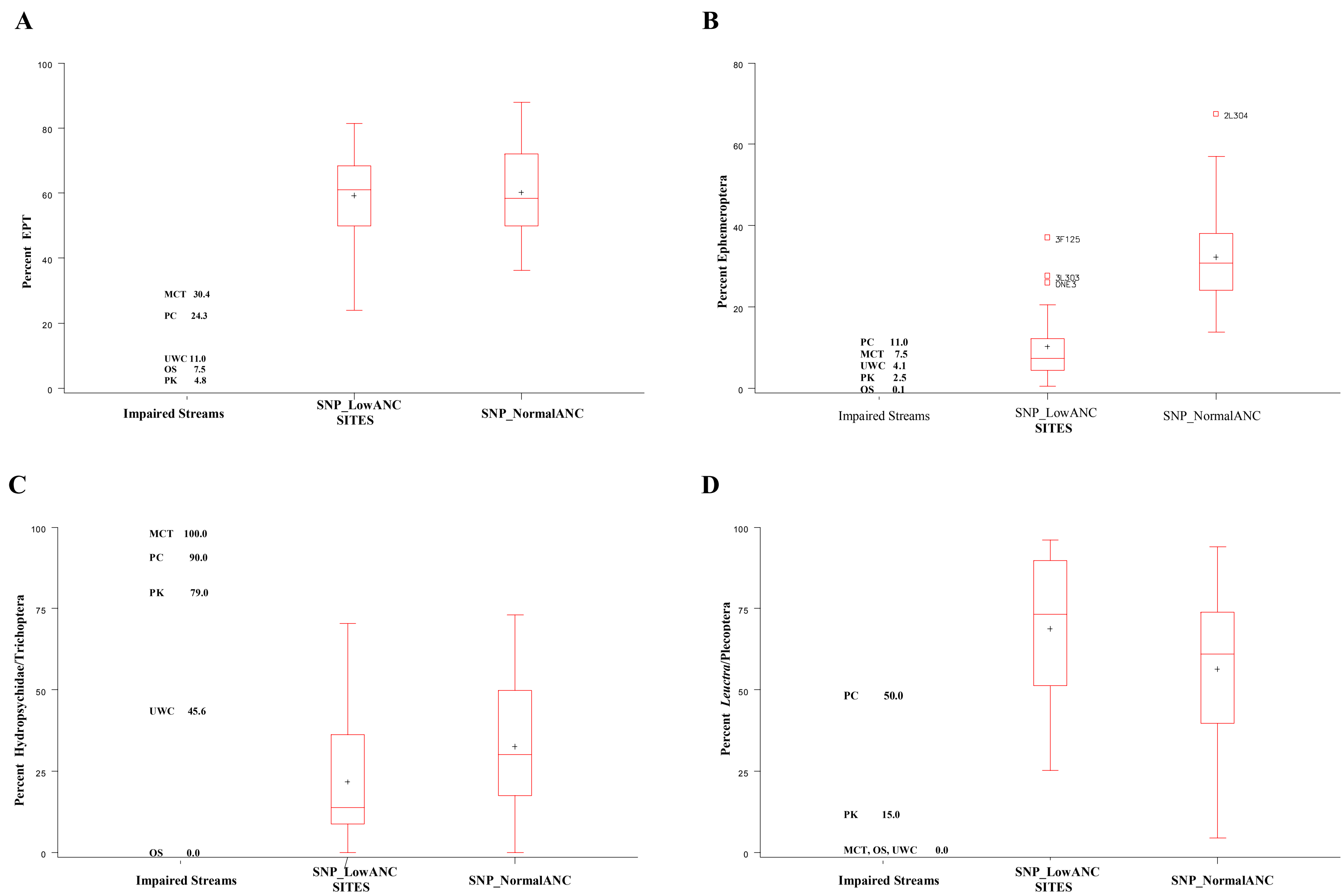


FIG. 2.8. Box plots of (A) percent EPT (B) percent Ephemeroptera (C) percent Hydropsychidae of Trichoptera and (D) percent *Leuctra* of Plecoptera for the SNP low (SNP_LowANC) and normal ANC (SNP_NormalANC) streams, and the 5 non-Blue Ridge ecoregion comparison streams (MCT= Mill Creek tributary, UWC= Upper Wilson Creek, PK= Peak Creek, OS= Ogden Stream, PC= Piney Creek).

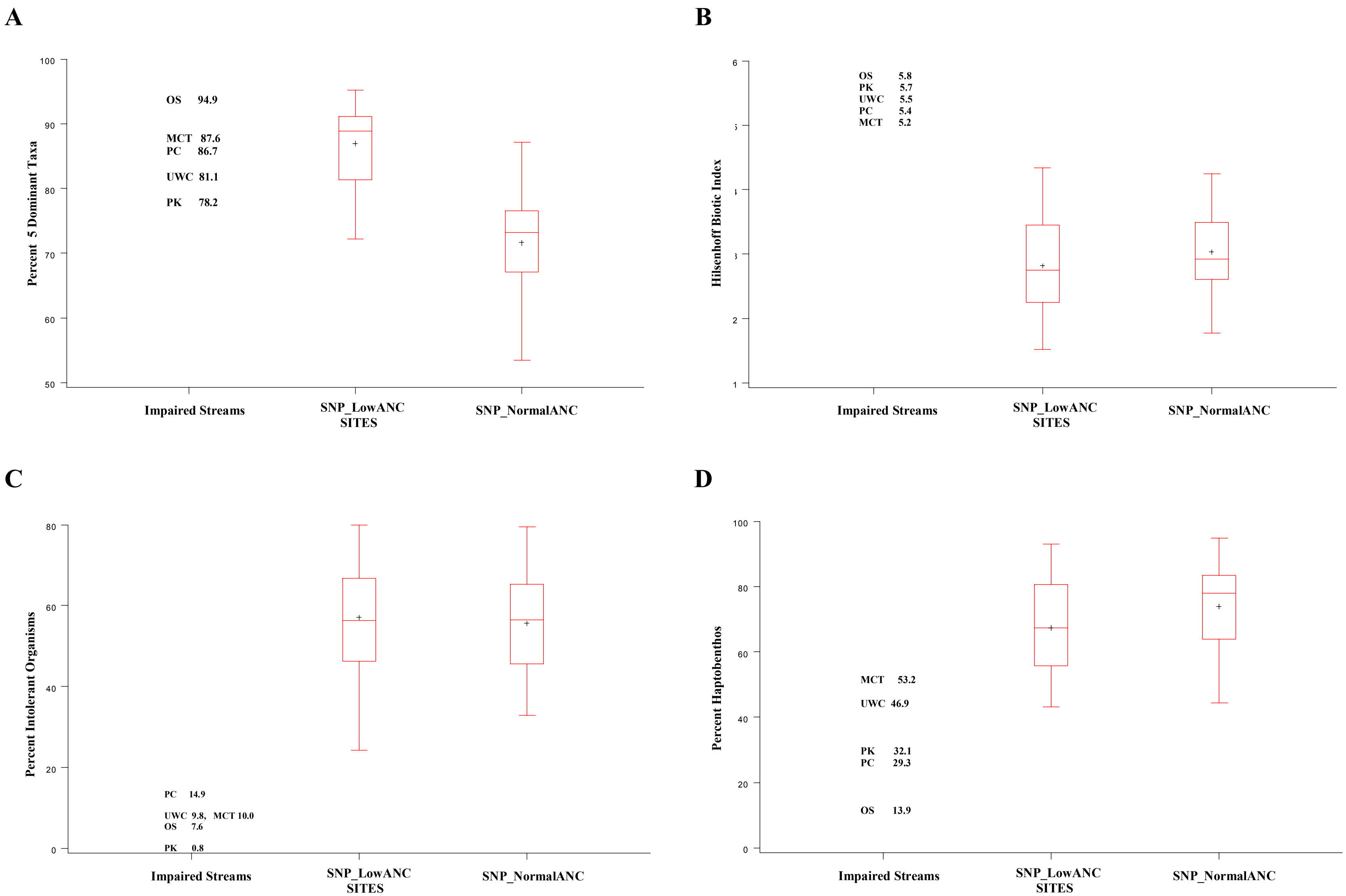
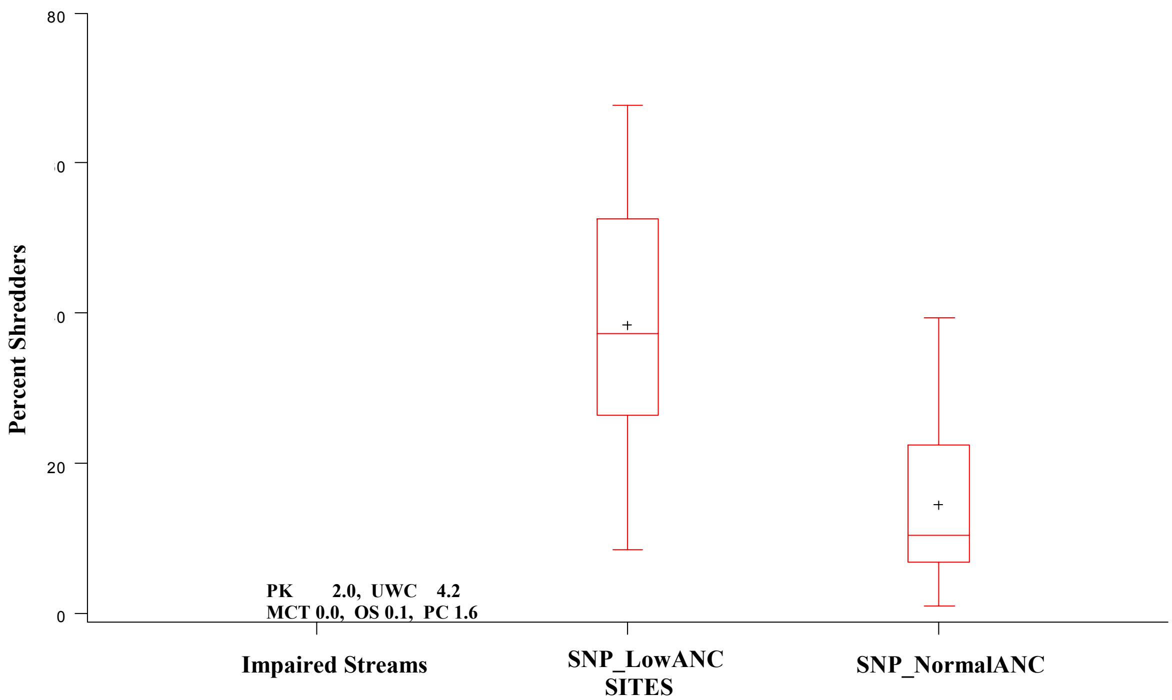


FIG. 2.9. Box plots of (A) percent 5 dominant taxa (B) HBI (C) percent intolerant organisms and (D) percent haptobenthos for the SNP low (SNP_LowANC) and normal ANC (SNP_NormalANC) streams, and the 5 non-Blue Ridge ecoregion comparison streams (MCT= Mill Creek tributary, UWC= Upper Wilson Creek, PK= Peak Creek, OS= Ogden Stream, PC= Piney Creek).

A



B

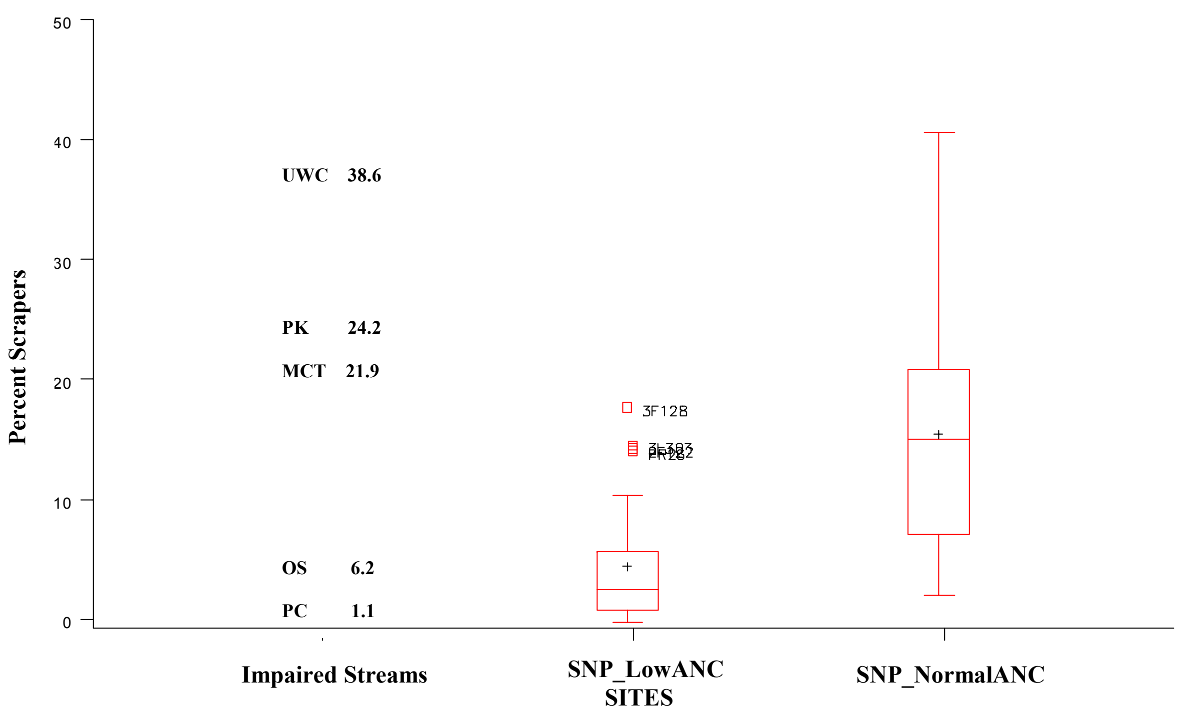


FIG. 2.10. Box plots of (A) percent shredders and (B) percent scrapers for the SNP low (SNP_LowANC) and normal ANC (SNP_NormalANC) streams, and the 5 non-Blue Ridge ecoregion comparison streams (MCT= Mill Creek tributary, UWC= Upper Wilson Creek, PK= Peak Creek, OS= Ogden Stream, PC= Piney Creek.

CHAPTER 3: Analyses of the Macroinvertebrate Aggregated Index for Streams (MAIS), a Biomonitoring Tool for Mid-Atlantic Highland Streams

INTRODUCTION

Biomonitoring involves the systematic sampling of communities to determine if changes are taking place or have taken place, especially from anthropogenic sources (Karr et al. 1986). It is especially effective in detecting changes in aquatic habitats because the organisms that live in the water often have a reduced ability to avoid any disturbance to their environment. Cairns and Pratt (1993) described biomonitoring as "surveillance using the responses of living organisms to determine whether the environment is favorable to living material". Aquatic communities serve as monitors of the environmental quality and can indicate habitat alteration, as well as both episodic and cumulative pollution (Plafkin et al. 1989).

The need for measuring impact to aquatic communities stems from the United States 1972 Clean Water Act, which has a goal of restoring and protecting the biological integrity of the nation's streams (Davis et al. 1996) and was a response to growing public concern for water pollution. Biological integrity is the ability of a system to "support and maintain a balanced, integrated, and adaptive community with a biological diversity, composition, and functional organization comparable to those of natural aquatic ecosystems in the region" (Karr and Dudley 1981, Karr et al. 1986). The Clean Water Act is the major federal law that requires states to eliminate discharge of pollutants into waters and to restore biological integrity to impacted waters. States are required to assess the quality of their waters and report the information (305b reporting) to the United States Environmental Protection Agency (USEPA), who then submits a report to the United States Congress (Barbour et al. 1999). Methods that measure the biological integrity of the biological community in a stream are therefore needed to fulfill the Clean Water Act's biological integrity related goals.

Historically, the biological component of monitoring programs used quantitative sampling methods that required many replicates and rigorous statistical analyses. These traditional methods of monitoring streams were expensive and time consuming, so methods began to be developed that allowed states to monitor their waters in a faster, more cost-effective way (Karr 1981, Plafkin et al. 1989, Rosenberg and Resh 1993).

One of the first cost effective methods developed was the Index of Biotic Integrity (IBI), which is a method of assessing the biotic integrity of a site using fish (Karr 1981). The IBI is a multi-metric index that incorporates different aspects from the fish assemblage of a site into three categories and twelve different metrics (Karr et al. 1986). Metric values obtained from samples taken at a site are compared to expected values for that site. Each metric is given a numerical score depending on how they differ from values expected at reference sites (Karr et al. 1986). The values assigned to each metric are tallied, and the total is assigned to a category, which describes the biotic integrity of the site and indicates the overall condition of the community (Plafkin et al. 1989). This method does not require many replicates or detailed statistical analyses during the decision making process because the statistical analysis has been moved to the index development stage, where the reference conditions were determined. An important step in the index development is determining the reference condition, which is based on conditions that exist in streams with minimal human disturbance in a region (Hughes 1995) and is determined from the aggregate of multiple reference streams (Gibson et al. 1996, Barbour et al. 1996).

The original IBI was developed for use in warmwater streams in Indiana and Illinois, and the reference condition of that index was for that area of the country (Karr 1981). Reference conditions are different for all areas and waterbody types, so the reference condition for one region may not be correct for another region (Faush et al. 1984, Hughes et al. 1986). This problem has been addressed by the development of modified versions of the IBI for use in different areas, and many different versions of the IBI have been and continue to be developed (Simon and Lyons 1995, Simon 1999, Barbour et al. 1999). The adjustments to the IBI can involve the addition, deletion, or modification of metrics that are more sensitive to regional fish assemblages (Miller et al. 1988). Such modifications allow the IBI to be used as a tool for identifying degradation of streams within any particular region.

Multimetric indices similar to the IBI have been developed for benthic macroinvertebrates. The Invertebrate Community Index was developed by the Ohio Environmental Protection Agency (OEPA) for Ohio streams (OEPA 1987, 1989), and Arkansas developed a 7 metric index for biomonitoring in Arkansas streams (Shackleford 1988). The USEPA has developed protocols for assessing the biological integrity of streams, known as Rapid Bioassessment Protocols (RBPs) (Plafkin et al. 1989, Barbour et al. 1999), and many states have adopted these RBPs in their water quality monitoring programs (Davis et al. 1996). Florida, (Barbour et. al. 1996), Maryland (Stribling et al. 1998), and West Virginia (Gerritsen et al. 2000) have recently developed multimetric indices for assessing the condition of streams within their respective state (Barbour et al. 1996). In the original RBPs, the USEPA provided three precisely structured protocols for benthic macroinvertebrates that could be immediately implemented anywhere (Plafkin et al. 1989). The only difference in the three protocols was the intensity of sampling and level of taxonomic identification. In the revised version of the RBPs

(Barbour et al. 1999), the USEPA recognized that the “one size fits all” approach was not valid for biomonitoring protocols. Instead of providing precisely structured protocols, the revised version of the RBPs is a guidance document that provides detailed instructions on how to develop an effective biomonitoring protocol for a particular state or region. The emphasis of the revised RBPs is on multimetric index development in accordance with the ecoregion concept. While the revised RBPs have greater scientific credibility, the development of biomonitoring protocols is left up to states and government agencies.

The development of a valid biomonitoring protocol is an expensive, time-consuming process, requiring advanced scientific expertise. In response to this need, Smith and Voshell (1997) developed a multimetric index for benthic macroinvertebrates for use in wadeable streams in the Mid-Atlantic Highlands that they called the Macroinvertebrate Aggregated Index for Streams (MAIS).

The MAIS was developed using family-level macroinvertebrate data and followed the six-step multimetric index development framework of Barbour et al. (1995; 1996), which is almost identical to the five-step approach subsequently recommended by the USEPA in the revised RBPs (Barbour et al. 1999). Family-level benthic macroinvertebrate data are less expensive to analyze and the results are obtained more quickly than those obtained with genus/species level identification, which is especially important to agencies with large numbers of streams to assess (e.g., State Water Resource Agencies) (Gerritsen et al. 2000, Lenat and Resh 2001). Family level identification of benthic macroinvertebrates has been shown to be effective at achieving accurate bioassessments (Bowman and Bailey 1998), and is especially well suited to the use of biotic indices and tolerance values (Bailey et al. 2001). The MAIS was developed for use in Mid-Atlantic Highland streams (specifically the Blue Ridge, Central Appalachians, and the Ridge and Valley ecoregions) using data assembled by Virginia Tech from within EPA Region 3 (Smith and Voshell 1997).

The MAIS metric selection process utilized both reference and impaired streams. The status of each site was determined by the agency that provided the data. The nine metrics included in the index were: % 5 dominant taxa, modified Hilsenhoff Biotic Index (HBI), % haptobenthos (relative abundance of clingers + crawlers), Ephemeroptera-Plecoptera-Trichoptera (EPT) Index, # Ephemeroptera, % Ephemeroptera, Simpson Diversity Index (SDI), # intolerant taxa, and % scrapers. The range of possible scores for the MAIS was 0-18; with 0 representing the highest deviation from the reference condition and 18 the highest expected condition.

Different index scoring criteria were developed for each of the three ecoregions to achieve better accuracy (Kerans et al. 1992), and the data used during metric selection were subsequently used to determine the classification efficiency of the metric in each of the ecoregions. The MAIS appeared to work well in the Ridge and Valley (ecoregion 67), correctly classifying 90% of the sites used during index development, while misclassifying 5% of the reference sites and 21% of the impaired sites (Smith and Voshell 1997). The classification rate was not as good in the Central Appalachians (ecoregion 69), correctly classifying 79% of the total sites and misclassifying 24% of reference sites and 17% of impaired sites, however the results in the Central Appalachians were still considered acceptable for bioassessment use (Smith and Voshell 1997).

Misclassification rates were highest in the Blue Ridge ecoregion (66) (aka Blue Ridge Mountains). There, the MAIS correctly classified72% of the total sites, while misclassifying 23% of reference sites and 46% of impaired sites. The high misclassification rate of the impaired sites may have been due to low number, type, and designation of impacted sites in the database for that ecoregion (Smith and Voshell 1997). Smith and Voshell (1997) were not able to refine the MAIS in the Blue Ridge Mountains nor validate the accuracy of the MAIS in the other two ecoregions because of a lack of additional data.

The overall goal of my study was to complete the necessary analyses to determine whether the MAIS is an effective tool for biomonitoring streams. The specific objectives of this study were: (1) to determine the classification efficiency of the MAIS in the Ridge and Valley and the Central Appalachian ecoregions and (2) refine the MAIS for use in the Blue Ridge Mountains ecoregion with the goal of obtaining classification rates similar to the rates observed during index development in the other two ecoregions.

METHODS

MAIS development

The MAIS development process utilized by Smith and Voshell (1997) followed the guidelines for multimetric index development described by Barbour et al. (1995; 1996). The six steps are summarized below. The specifics of the index development steps that follow were described in detail in Smith and Voshell (1997).

Step 1 - Stream Classification. The first step in development was to determine what regional scale to use for developing the index. The regional scale used was the ecoregions described by Omernik (1987; 1995), specifically the Level 3 ecoregions, which are areas that have similar climate, vegetation, soils, and physical geography. The ecoregions of interest were all located in the Mid-Atlantic Highlands region: Blue Ridge Mountains, Central Appalachians, and Central Appalachian Ridges and Valleys (aka. Ridge and Valley). Subregions were analyzed as a possibility for classification scale, but were not found to improve the classification analysis. Additionally, combining the ecoregions into one large region resulted in decreased classification rates compared to using ecoregion levels.

Step 2 – Data Acquisition. This step involved gathering data from various sources, including: Virginia Tech in-house data (25 samples), Maryland (51 samples), Pennsylvania (53 samples), United States Forest Service (USFS, 101 samples), Virginia (25 samples), and West Virginia (200 samples). Ecoregion designation for each site was determined, as well as subregion, using maps provided by J.M Omernik (USEPA – Corvallis) (Table 3.1). The data were restricted to riffle samples that had been collected by semi-quantitative methods (e.g., D-frame net samples, not Surber samples), and the sample period was restricted to June through October. The data were mixed as far as sub-sampling practices, but because the metrics were based mainly on proportional abundance metrics, all data regardless of sub-sample size were included. However, qualitative samples without abundances were not used.

The biological condition of each site was assigned by enlisting the help of the various agencies that provided the data (Table 3.1). The condition was assigned based on the agency's knowledge of the presence or absence of physical or chemical sources of perturbation, not on the results of benthic macroinvertebrate biomonitoring. Condition categories included reference, impacted, and unknown, but only the samples of reference or impacted status were used in the analysis.

Macroinvertebrates in the samples were assigned ecological information (pollution tolerance value, functional feeding group, habit) based on the researchers' previous studies in Mid-Atlantic Highland streams, as well as by consulting pertinent text (eg., Merrit and Cummins 1996). An additional category of habit type, crawlers, was created to describe those insects which “move about regularly, but slowly, on or within spaces in solid substrata that is relatively clean” (Smith and Voshell 1997).

Step 3 – Data evaluation and metric calibration. A battery of 69 metrics was evaluated using various graphical and statistical techniques to determine a smaller list of 10 candidate metrics. Techniques used to evaluate metrics included: boxplots of reference site scores compared to impaired site scores to identify metrics that could not differentiate between the site types, plots of normality, summary statistics, separation statistics ((reference mean-impaired mean)/pooled standard deviation), and stepwise discriminant analysis to eliminate redundancy. Metrics were also selected to maintain a balanced representation of six ecological categories: richness, composition, balance, tolerance, trophic/feeding group, and habit. Richness, tolerance, and trophic metrics are all commonly used in biomonitoring. Habit metrics were not commonly used at the time the MAIS was developed but have become more common since listed by Barbour et al. (1999). The tolerance values ranged from 0 to10, with 0 being most intolerant of disturbance. Measures of intolerant taxa involved those taxa whose tolerance values ranged from 0 to 5. Composition metrics in the MAIS project involved those metrics that measured the relative abundance of a taxon or select taxa compared to the whole assemblage. Balance metrics included diversity and evenness metrics, top dominance metrics, as well as metrics that were similar to the composition metrics, with the distinction that they were of some particular significance to pollution studies.

Discriminant analysis, using both resubstitution and cross validation, was used to examine the stream classification ability of the list of 10 candidate metrics. The 10 metrics were found to have an overall error rate of 7-10%, depending upon the validation type, and misclassifications were distributed approximately evenly between reference and impact sites.

Metric calibration involved testing to see if ecoregions provided adequate grouping for the macroinvertebrate data, or if some other grouping would be better. Stepwise discriminant analysis was used to select important metrics for the subregions, and canonical variate analysis was then used to select new bioregions, which were groupings of the subregions that were clustered together when the canonical variates were graphed. The results indicated that the Level 3 ecoregion classification for the streams provided the best regional frame for development of the index and the criteria.

Step 4 – Metric transformation. This step allows metrics of different scale and different responses to perturbation to be combined by standardizing the metrics on a similar scale. After analyzing six methods of standardization (transformation), four of the methods were determined to be useful by analyzing the data. The selected method (Method 2) utilized data from reference and impacted sites. This method used box plots of the reference and impacted site scores for each of the candidate metrics, and assigned the lowest score (0) to metric values ≤ the lower fence for the reference sites, the highest score (2) to metric values greater than the upper quartile of them impact sites, and the middle score (1) to metric values that fell between the two limits. This method resulted in the sites with the best biological condition receiving a high value, and those with the poorest condition receiving a low value.

Step 5 – Multimetric index development. Once the scores were transformed, selected metrics were aggregated into different indices. The various indices were then analyzed to determine which method of transformation and which aggregated group of metrics provided the best separation of reference and impaired sites. Box plots showing the median, interquartile range, and upper and lower fences were used to examine the ability of the proposed index to separate the reference and impacted sites. Indices with overlapping interquartile ranges were not used as they would not be good for management purposes.

Step 6 – Biocriteria Development. The final step was to develop the criteria for discrimination of impaired sites. The approach used to determine the boundaries and biocriteria thresholds utilized both reference and impacted data, with the cutoff between impaired sites and non-impaired occurring between the Poor and Good categories (Table 3.2). Different thresholds were developed for each of the three ecoregions in the study, as indicated by analyses in the previous steps. Biocriteria thresholds were developed for the indices that performed well in the previous step's box and whiskers analysis. Once the biocriteria were determined, the criteria were applied to the macroinvertebrate data to test the discrimination ability of the various metrics developed. The multimetric index that had the lowest misclassification rate (eg. sites classified as Good by the index, but which were identified as impacted) was determined to be the best index. The resulting index ranged from 0 (worst condition) to 18 (best condition) and included the following metrics: % 5 dominant taxa, modified Hilsenhoff Biotic Index (HBI), % haptobenthos (relative abundance of clingers + crawlers), Ephemeroptera-Plecoptera-Trichoptera (EPT) Index, # Ephemeroptera, % Ephemeroptera, Simpson Diversity Index (SDI), # intolerant taxa, and % scrapers. Cutoff levels for assigning scores are shown in Table 3.3.

The classification rates were highest in the Ridge and Valley ecoregion, correctly classifying 95% of reference sites, 79% of impaired sites, and 90% overall. The Central Appalachian region correctly classified 76% of the reference sites, 83% of the impaired sites, and 79% overall. The classification rates were lowest for the Blue Ridge Mountains ecoregion, with the MAIS correctly classifying 77% of the reference sites, but only 55% of the impaired sites, and 72% overall. The reasons for the low rates in the Blue Ridge Mountains were thought to be due to low numbers of impaired sites compared to reference sites, and the single type of impact represented in the data – atmospheric acidification.

Validation and Refinement of MAIS

Data Acquisition

New data were obtained for use in the validation and refinement stages. Benthic macroinvertebrate data collected since 1994 (the last year of data in the original dataset) were obtained from: the water quality divisions in Maryland, Virginia, and West Virginia; the USFS in Virginia; the National Park Service Shenandoah National Park (SNP); the Environmental Monitoring and Assessment Program (EMAP) study of the Mid-Atlantic Highland; and additional data gathered in biomonitoring studies by various students and programs at Virginia Tech. Additionally, stream habitat assessment and water chemistry data were acquired with the macroinvertebrate data to allow ecological condition decisions for each site based upon empirical data instead of the professional judgment method employed in the original data sets. Some of the data provided had recently been used in studies by the donor organizations (Maryland, EMAP, and West Virginia) and the ecological conditions had already been determined by those agencies using empirical methods. The criteria (Table 3.4) in this study used to determine the status of the sites with no designation were an amalgamation of the levels set by the different agencies within EPA region 3 (Stribling et al. 1998, USEPA 2000, Gerritsen et al. 2000).

Data were entered into Microsoft Access, and a modified version of the Ecological Data Application System (EDAS) developed by Tetra Tech was used to facilitate data management and metric determination (Tetra Tech, Inc. 1999). Macroinvertebrate ecological information (habit, trophic/feeding group, tolerance value) used was from the Virginia Tech aquatic entomology program (maintained by the aquatic entomology program of J. Reese Voshell, Jr., Virginia Tech).

Level 3 ecoregion membership was determined for each site (if not provided) by entering the site coordinates into Arcview, and selecting the ecoregion using a layer provided by the USEPA (USEPA 2001).

Validation

New data were used to test the ability of the MAIS to discriminate between reference and impaired streams in each of the Ridge and Valley and Central Appalachian ecoregions. The MAIS was calculated for the sites in these ecoregions, and box and whisker plots were created to examine the separation of the index values between reference and impaired site classes. Classification rates were determined for each of the regions by determining the number of sites that were correctly classified by the MAIS into the same ecological condition derived from non-biological methods. The placements of sites into the different levels of ecological categories of the MAIS were also determined to examine the responsiveness of the index to different levels of degradation.

Refinement for the Blue Ridge

The new data were used to refine the MAIS for use in the Blue Ridge Mountains ecoregion, in order to decrease misclassification rates, especially for impaired sites. It was determined that it would be best if the initial metrics selected for use in the index would remain unchanged, and that the refinement would come during index development steps 4-6. The number of degraded sites was still small in the new data set, therefore a different method of metric transformation based only on reference sites was utilized.

Metric transformation followed the steps described by Barbour et al. (1996), which were a modification of methods developed for the fish IBI (Karr et al. 1986). The lower reference site quartile for each metric whose values decrease in response to disturbance was used as the level for assigning the highest value; all scores equal to or greater than this quartile received a 2. This same quartile was bisected to obtain the minimum level for the middle category (scoring a 1), which would encompass values below the reference quartile, but equal to or greater than the bisect. Values lower than the bisect value received the lowest score (0). Values that increase with disturbance (% top 5 dominant taxa and HBI) were treated differently. In the case of % top 5 dominant taxa, the upper quartile was used to set the threshold level for the highest score, and the amount above that was bisected to obtain the other categories of scores. The HBI upper quartile was also used to set the threshold for the highest score, but 6 was selected as the cutoff between the next two categories based on relevance from other studies of pollution tolerance (Hilsenhoff 1988, Lenat 1993).

The metric scores were aggregated to obtain the MAIS value, and box-and-whiskers plots of the reference and impaired sites were examined to see if the metric provided good separation of the stream types. Overlapping interquartile ranges would indicate poor separation. Because the metric transformation step was based solely on reference site data, the different levels of assessment were also derived only from reference site data. The cutoff between impaired and unimpaired (or unacceptable and acceptable ecological condition) was set as the lower quartile of reference site MAIS scores , and the cutoffs for the other categories were placed at the 5th

percentile and the 50th percentile or median (Table 3.5). Reference and impaired sites were used to determine the classification efficiency of the refined MAIS by determining how many sites were correctly placed in their known ecological condition categories (reference or degraded). The placement of the sites by the MAIS into the different ecological condition categories was also determined.

RESULTS

Data from 3150 sites were assembled from the various agencies, and assigned to the different ecoregions. After the reference or impaired designation process, 256 sites were usable from the Ridge and Valley ecoregion (176 reference and 80 impaired), 307 sites were usable from the Central Appalachian ecoregion (104 reference and 203 impaired), and 49 additional sites were added to the Blue Ridge Mountains ecoregion data set (35 reference and 14 impaired). The majority of sites collected were not usable because they were not able to be placed into either the reference category or the impaired category.

Validation

Box-and-whisker plots of the metric values in the Central Appalachian ecoregion showed a clear separation between the reference and impaired sites, with no overlap of the interquartile ranges (Fig. 3.1). The overall classification rate (86%) was similar to what was observed when the index was created, with the misclassifications being more evenly distributed compared to the original data set, and with the best classification falling in the impaired data set (Table 3.6).

Graphical analysis of the Ridge and Valley ecoregion showed a similar pattern of good separation between reference and impaired site classes (Fig 3.2). Classification rates for Ridge and Valley sites were better in every category than in the original data, improving from an overall classification efficiency of 79% to 91% (Table 3.6). Classification was best in the reference set, but only several percentage points higher than with degraded sites.

Distribution of the sites within the different ratings of assessment did not result in any impaired sites being classified into the top category of Very Good, nor were any reference sites classified in the Very Poor category (Table 3.7).

Refinement for the Blue Ridge

The cutoff levels determined using only reference sites (Table 3.8) produced boxplots that clearly separated reference from degraded sites, but with a much larger interquartile range for the degraded sites (Fig. 3.3). Since the boxplots indicated a strong discriminatory power, the biocriteria and assessment classes were determined (Table 3.9). These levels and cutoffs resulted in classification efficiencies (Table 3.10) higher than those originally found by Smith and Voshell (1997). The best classification was in the impaired class of streams (87%), which was only 54% using the original method of transformation and data set. Examination of the distribution of the reference and degraded sites into the assessment classes resulted in no degraded sites being classified as Very Good (Table 3.11). However, 3 of the reference sites were classified as very poor.

DISCUSSION

Validation

The classification rates of the MAIS in both the Ridge and Valley and the Central Appalachian ecoregions were similar to those found in the original work. The classification rates obtained in the validation stage as well as in the original development were equal to or better than classification rates described as being indicative of high discriminatory power by other researchers. Stribling et al. (1998) found overall classification efficiency rates of 87% and 88% for their two regions, and validation testing with a new data set resulted in classification efficiencies of 72% and 82%, for the same respective regions. Gerritsen et al. (2000) reported classification efficiency rates of 85% for reference sites and 92% for impaired sites, resulting in an overall successful classification rating of 90%. The repeatability of an index to achieve similar high classification ratings from one data set to the next is one indication of good precision.

Natural seasonal variability may result in some of the misclassification error seen with the reference sites (Barbour et al. 1996), and mistakes in collecting or interpreting the physical/chemical data on a stream may also result in some of the error. While it is the goal of everyone developing multimetric indices to obtain a 100% classification efficiency, misclassifications of these types will always be present. Repeated testing and refinement of index using new data from known impaired and reference sites will improve confidence in the index, and in its ability to correctly identify streams in different ecological condition categories (Gerritsen et al. 2000).

Refinement for the Blue Ridge Mountains

Refinement of the MAIS by using a different method of metric transformation resulted in a much greater classification rate for impaired sites (Table 3.10), 87% in the testing of the refinement method compared to 54% obtained during metric development (Smith and Voshell 1987). The refinements also resulted in non-overlapping interquartile ranges, which is expected of metrics that can repeatedly differentiate reference from impaired streams. The authors attributed the original impaired site error to the low number of impaired sites relative to reference sites, and the singular type of perturbation represented in the data, atmospheric acid deposition. The new data suggest that the high error rate may not have been due to the singular type of perturbation, but perhaps due to the different types of acid depositional impaired streams.

Mid-Atlantic Highland streams may be acidic consistently throughout the year with low pH values and acid neutralizing capacity (ANC) values below zero, and are known as chronically acidic (USEPA 2000). These streams would probably have shown true degraded status and resulted in higher classification rates if they were solely the ones included in the MAIS development. Another category of Mid-Atlantic Highland streams affected by acid deposition are those streams with ANC <50, which results in these streams being susceptible to short term acidification, known as episodically acidic streams (USEPA 2000). Since the streams included in MAIS development were not identified as impaired using empirical methods, it is possible that many of the streams identified as impaired by acid deposition were not experiencing any acidification degradation, but were susceptible to it due to low ANC values.

Studies of streams in the Shenandoah National Park in Virginia (Shenandoah National Park Inventory and Monitoring Division, unpublished data) have shown that while the assemblage composition of low ANC streams (<20 *u*eq/L) may differ from the streams of higher

ANC values, the assemblages of benthic macroinvertebrates are not consistent with what is considered impaired. Additionally, the EMAP studies of Mid-Atlantic Highland streams have found that chronically acidic streams are not that common in the Highlands, involving less then 4% of the stream miles (USEPA 2000). We do no believe that separate criteria are necessary for the different ANC type streams because the low pH levels of chronically affected streams is not natural, and will be detectable with this index.

The classification rate for reference sites was lower than the impaired sites rate (87% vs. 75% respectively), but was similar to the rate obtained during index development (Table 3.10). While the overall classification rate improved only slightly, the classification rate for impaired sites was substantially improved at only a minor loss to the classification efficiency of reference sites. The original authors did not attempt to have one classification rate better than the other, and believed an evenly distributed error was best (Smith and Voshell 1987). Error rates obtained from this refinement are much more evenly distributed than during development. The error rates that resulted from this refinement are the best rates that could be achieved at this time, and will result in fewer errors being made misidentifying impaired sites as non-impaired. While the classification of the reference sites might be improved by moving the cutoff points around, the impaired sites classification rate would suffer from such an adjustment. The MAIS will perform very well as a rapid bioassessment tool for indicating sites that are of concern to water quality agencies, and subsequently which sites warrant further analyses.

Recommendations for the MAIS

The family level MAIS, as presented by Smith and Voshell (1987) and as modified in this study, is a robust index that can be used for assessing the biological condition of streams located throughout the Blue Ridge Mountains, Central Appalachians, and Ridge and Valley ecoregions. Further refinements may be indicated as additional data are added to the database. The family level identification and regionalisation results in a cost effective index that is usable over a wide area. The addition of data to the database will allow for continued validation and refinement of the index.

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Table 3.1. Breakdown of sample numbers used in data analysis for creating the MAIS, showing division by ecoregion, and ecological condition category as defined by the agency that provided the data.

Ecoregion	Reference sites	Impaired sites	Unknown condition	Total
Blue Ridge Mountains	35	11	13	59
Ridge and Valley	60	28	55	143
Central Appalachians	25	23	129	177

Table 3.2. Criteria for the rating system for classification of sites with the MAIS. Abbreviations used: RQ3 = the upper quartile for reference sites, IQ1 = the lower quartile for impacted sites. (Smith and Voshell 1997).

Category	Very Good	Good	Poor	Very Poor
Criteria	≥ RQ3	> average of impact mean and reference mean, <RQ3	≤ average of impact mean and reference mean, > IQ1	≤ IQ1

Table 3.3. Cutoff levels used during index development to assign scores to each of the metrics of the MAIS (Smith and Voshell 1997).

Metric	0	1	2
EPT	≤2	>2 – 7	>7
% 5 most dominant taxa	100	79.13 - <100	<79.13
HBI	≥5.56	4.22 - <5.56	<4.22
# Ephemeroptera taxa	0	>0 - 3	>3
% haptobenthos	≤51.98	>51.98 – 83.26	>83.26
% Ephemeroptera	≤0.1	>0.1 – 17.515	>17.515
# intolerant taxa	≤1	>1 - 9	>9
% scrapers	≤0.1	>0.1 – 10.7	>10.7
Simpson Diversity Index	≤0.656	>0.656 – 0.8225	>0.8225

Table 3.4. Reference and impaired criteria for the MAIS validation.

Measurement	Criterion
Reference condition (all must be met)	
Dissolved oxygen	≥ 5.0 mg/L
Acid neutralizing capacity (ANC)	> 50 μeq/L
PH	between 6.0 and 9.0
Conductivity	<500 μmhos/cm
Urban land use	< 20% of drainage
Habitat assessment scores	≥ 11 for sediment deposition and epifaunal substrate categories; no channel alteration; No scores < 6
Impaired condition (one must be met)	
Dissolved oxygen	< 4 mg/L
ANC	< 0
PH	<4
Habitat assessment scores	< 7 for sediment deposition, epifaunal substrate, or channel alteration categories as well as a total habitat score <120
Nitrogen and phosphorus	> 750 ppb nitrogen AND > 100 ppb phosphorus
Conductivity	> 1000 μmhos/cm

Table 3.5. Criteria for the rating system for classification of sites with the MAIS in the Blue Ridge Mountains. RQ1 = the lower quartile for reference sites.

Category	**Very Good**	**Good**	**Poor**	**Very Poor**
Criteria	≥ median	RQ1 to <median	≥ 5th percentile to <RQ1	< 5th percentile

Table 3.6. Index classification efficiencies (correctly classified/total in the category) from the validation the index development for the Central Appalachian and Ridge and Valley ecoregions. MIS=misclassifications, CEV=classification efficiency from validation, CED= classification efficiency from development.

	N	MIS	CEV	CED
Ridge and Valley				
Reference sites	176	25	86%	95%
Degraded sites	80	10	88%	79%
Total	256	35	86%	90%
Central Appalachians				
Reference sites	104	6	94%	76%
Degraded sites	203	21	90%	83%
Total	307	27	91%	79%

Table 3.7. Distribution of ecological condition categories for each of the Central Appalachian and Ridge and Valley ecoregions.

Ecoregion	Status	
MAIS ecological category	Degraded	Reference
Ridge and Valley		
Very Good	0	60
Good	10	91
Poor	44	25
Very Poor	26	0
Central Appalachians		
Very Good	0	52
Good	21	46
Poor	84	5
Very Poor	98	0

Table 3.8. Quartiles and metric scores for the MAIS metrics in the Blue Ridge Mountains ecoregion derived using only reference site values.

Metric	Quartile			Metric Score		
	25%	Median	75%	0	1	2
# Ephemeroptera taxa	4	4	5	<2	2-3	≥4
# EPT taxa	10	12	15	<5	5-9	≥10
% Ephemeroptera organisms	19.5	26.3	39.4	<9.8	9.8-19.4	≥19.5
Modified HBI	3.40	3.80	4.28	>6.0	4.29-6.0	≤4.28
# intolerant taxa	14	17	21	<7	7-13	≥14
Top 5 dominant taxa	66.5	73.9	79.7	>89.7	79.4-89.7	≤79.3
Simpson diversity index	0.83	0.86	0.89	<0.42	0.42-0.82	≥0.83
% scrapers	14.6	23.1	35.1	<7.7	7.7-15.2	≥15.3
% haptobenthos	71.9	82.4	88.1	<37	37.0-72.9	≥73.0

Table 3.9. Biocriteria and levels for the MAIS in the Blue Ridge Mountains ecoregion using only reference sites.

	Unacceptable		Acceptable	
	Very Poor	Poor	Good	Very Good
Reference site quartiles	≤5%	6-24%	25-49%	≥50%
MAIS score	<11	11-14	15-16	≥17

Table 3.10. Classification efficiency of the MAIS in the Blue Ridge Mountain ecoregion from method using only reference sites. CER =classification efficiency from refinement, CED = classification efficiency from development.

Sites	N	Misclassification from refinement	CER	CED
Reference	68	17	75%	77%
Degraded	23	3	87%	54%
Total	91	20	78%	72%

Table 3.11. Distribution of ecological condition categories for the Blue Ridge Mountains ecoregion.

	Status	
MAIS ecological category	Degraded	Reference
Blue Ridge Mountains		
Very Good	0	35
Good	3	16
Poor	6	14
Very Poor	14	3

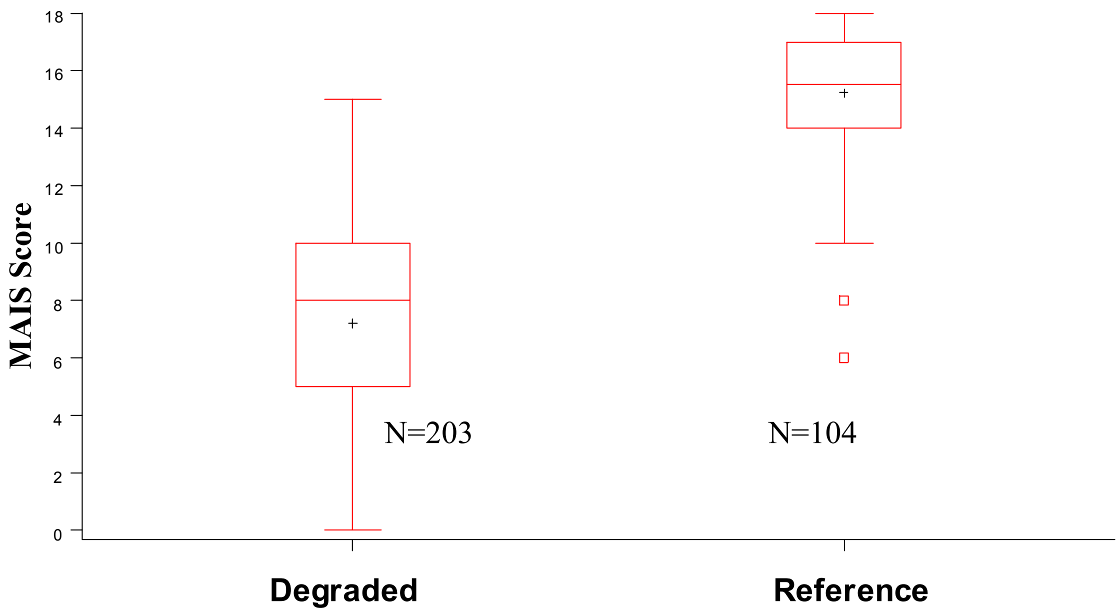

FIG. 3.1. Comparison of MAIS scores for reference and degraded sites in the Central Appalachian ecoregion.

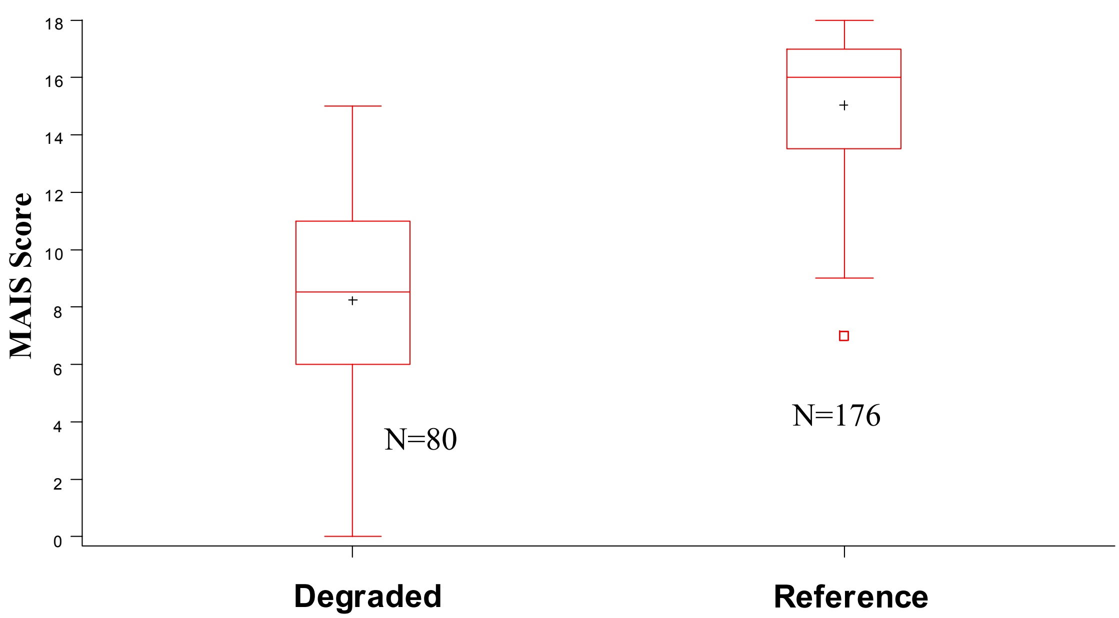

FIG. 3.2. Comparison of MAIS scores for reference and degraded sites in the Ridge and Valley ecoregion.

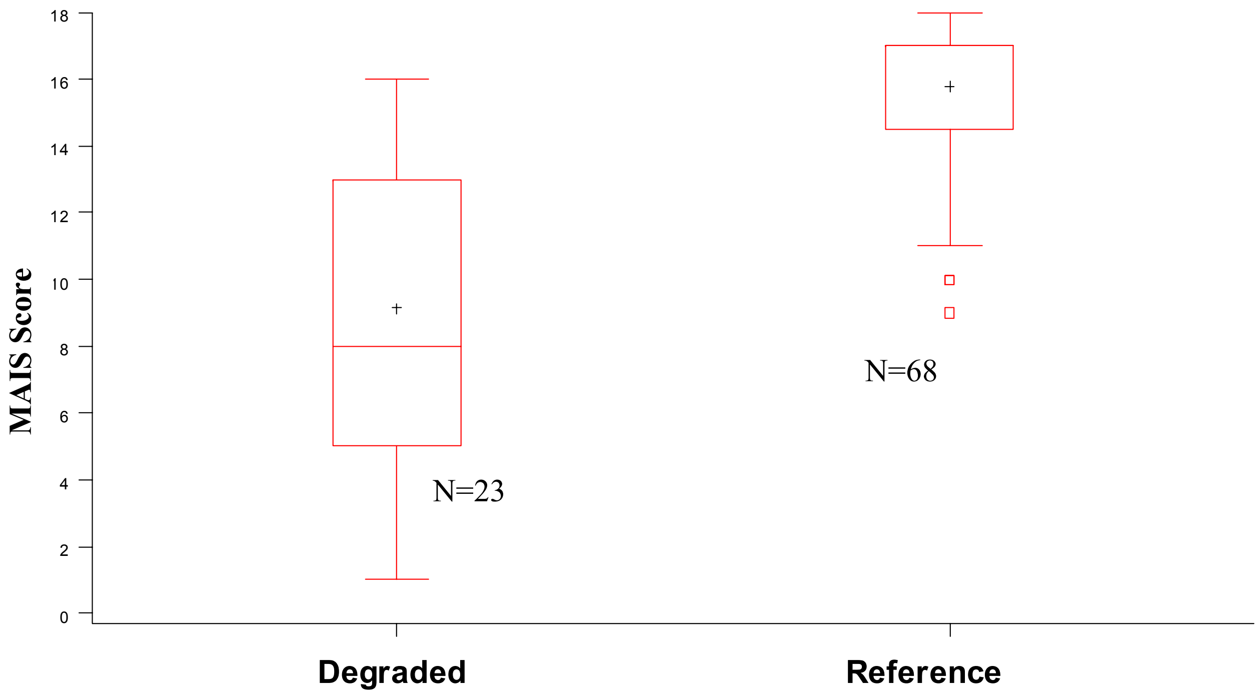

FIG. 3.3. MAIS scores for reference and impaired sites in the Blue Ridge Mountains ecoregion.

CHAPTER 4: Effects of heavy metal pollution on benthic macroinvertebrates in Peak Creek in Pulaski, Virginia

INTRODUCTION

Peak Creek is a tributary of the New River in southwest Virginia and is in the Central Appalachian Ridges and Valleys ecoregion (Omernik 1987). This mountain stream begins in the George Washington and Jefferson National Forest, and then flows through the town of Pulaski before it drains into an arm of Claytor Lake, which is an impoundment of the New River. Peak Creek is a 3rd order stream as it enters the town of Pulaski and becomes a 4th order stream when it is joined by Tract Fork in the town. The catchment area of Peak Creek is 263 km^2, and its stream length is 39 km.

There are multiple sources of contaminants within the catchment, attributable to both historical and present day factors. Iron and coal were mined historically throughout the watershed, and there were three iron furnaces located in the town of Pulaski. Iron furnaces were used to remove impurities from iron ore, and the process resulted in a waste product (slag). Waste slag from these furnaces was used as fill for road building and other construction projects throughout the town. A sewage treatment plant discharged effluent into Peak Creek until 1996, when a larger regional facility went into operation. The ecological condition of Peak Creek has been degraded in the reach within the town of Pulaski by channelization to reduce flooding. This action has produced a nearly homogeneous channel lined with cobbles. Currently, Magnox Pulaski Inc., located in the town of Pulaski, has a permit allowing discharge of various pollutants, including Fe, Cr, Cu, Zn, sulfates, Na, and decreased pH (VPDES Permit No. VA0000281).

A major source of contamination is attributed to a former industrial site of Allied Signal (also known as Allied Chemical), which manufactured sulfuric acid and ferric sulfide (Willis 1989). When the plant ceased manufacturing operations in 1976, extensive piles of spoils were left uncovered, which allowed the loose material laden with various heavy metals (Cd, Pb, Cr, Zn, Cu, and Ni), to erode and be transported into Peak Creek, especially during storms (Willis 1989). The problem was intensified when a shopping center with an asphalt parking lot was constructed up slope from the spoils. During heavy rains, runoff from the shopping center was funneled directly through the waste piles into Peak Creek. In 1989, chemical analyses of the stream sediments found levels of Cu, Pb, and Zn that were all above the 99th percentile for sediments of streams in Virginia (Willis 1989). An accompanying biological survey reported virtually no aquatic life at stations downstream of the waste pile runoff (Willis 1989). In the early 1990's, the waste piles were capped with soil to contain the toxic substances, however, heavy rains flowing over the impervious parking lot breeched the cap in several places. During the late 1990's, cursory biomonitoring was done in Peak Creek by students in a Freshwater Biomonitoring class and others conducting undergraduate research at Virginia Tech (unpublished data, Voshell et. al. 1997). Results of these macroinvertebrate studies showed an appreciable improvement in the ecological condition of Peak Creek, as compared to the results of Willis (1989), but not enough improvement to make the ecological condition of Peak Creek comparable to the ecological condition expected for streams in this ecoregion.

The focus of this project was to address the following questions:

1. Do the benthic macroinvertebrate assemblages at the downstream Peak Creek sites differ from reference site assemblages?
2. Are the spatial trends of the macroinvertebrate assemblages at the sites along Peak Creek comparable to previous studies?
3. If the downstream sites are different from the reference condition, is heavy metal contamination the cause, and is a gradient of contamination observed?

METHODS

To examine the ecological condition of the macroinvertebrate community of a southwestern Virginia stream, six sampling stations were selected on a target stream (Peak Creek) suspected to by impaired by heavy metal contamination, and one additional site selected on each of two nearby reference streams. The sites were sampled four times over one year, with macroinvertebrate, surface water, and sediment pore water samples undergoing analyses.

Study Site

Six sites were selected along Peak Creek (Fig. 4.1). There was one reference site (PK1) upstream of the town and any major sources of impairment, and the remaining sites were spread out downstream and designated as PK2, PK3, PK4, PK5, and PK6. PK2 was located downstream from Magnox Pulaski Inc., and PK3 was in the middle of the Pulaski town area. PK4 was located in the vicinity of the Allied Signal site, PK5 was located in the area where the runoff from a parking lot would wash through the Allied Signal spoils piles, and PK6 was the final site situated below all possible sources of impairment. Additional reference sites were located in nearby watersheds that matched PK1 for water hardness, drainage area, stream size, and stream morphology. These reference streams were (Big) Stony Creek (BS) located in Giles County VA, and Little Walker Creek (LW) located in Pulaski County VA. A single reference site was located on each.

Each study site consisted of a stream section of approximately 20 meters containing riffle habitat and more depositional areas with minimal velocity immediately upstream. All of these streams experience large variations in flow through the year and are predominantly erosional in character. Samples were collected in August 1998, December 1998, April 1999, and August 1999. On each date, the benthic macroinvertebrate assemblage was sampled, and pertinent physical and chemical parameters were measured.

Sampling and Measurements

Ten to fourteen days before each sampling event *in situ* pore water samplers (peepers (Hesslein, 1976)), were placed at each site to collect sediment pore water used in water chemistry assessment. Peepers consisted of 5-ml polypropylene vials (2.5 cm H x 2.0 cm D) filled with deoxygenated, Type I reagent-grade water and sealed with a 0.4 m polycarbonate membrane filter held in place with an open top cap. Eight peepers were contained in a 30 centimetre length of leached PVC pipe (25.4 mm D) with 1 centimetre holes drilled through the sides every 3 cm. The vials were spaced and held in place with nylon bolts through additional 0.7 centimetre holes every 3 centimetres. The pipe was tied to a rock with string and buried horizontally just underneath the surface of the substrate. The peeper membranes were 1-3 centimetre below the surface. On sampling days the peepers were retrieved and rinsed with site water. One peeper sample was immediately fixed for dissolved oxygen determinations using a micro-Winkler technique. The remaining peepers were placed in a plastic bag on ice packs for transport back to the laboratory.

Individual peepers were randomly chosen for analysis of pH, dissolved organic carbon (DOC), and total ammonia. Water from the remaining peepers was combined and pore water for metal analysis was filtered through a 0.4-µm mesh polycarbonate membrane filter or a GHP Acrodisc GF syringe 0.45-µm filter, then acidified with 1% HNO_3. DOC was analyzed using a Model 1100 TOC analyzer, and total ammonia was determined using the automated phenate method on a Technicon Autoanalyzer.

Two grab samples of overlying water were collected into acid-washed polyethylene bottles, placed on ice, and transported to the laboratory for analysis where the pH was determined. Samples for metals were filtered through a 0.4-µm polycarbonate membrane filter or a GHP Acrodisc GF syringe 0.45-µm filter and acidified with 1 % trace pure HNO_3

Fifteen sediment core samples were collected in the more depositional areas of the sampling reach for chemical analyses. Each core was collected in a 5-cm diameter cellulose acetate butyrate resin tube 12 cm in length. Cores were inserted into the sediment to a depth of 4 cm by hand. The top of the tube was sealed with a polyethylene end cap. The core was then carefully tilted upstream and sealed with a rubber stopper covered by polyethylene plastic wrap (Glad Wrap). The liner tubes were sealed in Zip-lock bags, placed in a cooler at ambient temperatures, and transported to the laboratory.

For oxygen sensitive analyses, sediment and interstitial water in an intact core were decanted in a glove bag under a nitrogen atmosphere. Material was homogenized, and a subsample collected for determination of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM).

AVS was determined by the modified diffusion method (Leonard et al. 1996). A 30-ml wide-mouth glass jar was affixed to the inside of 500-ml wide-mouth septa-seal jar with silicon sealer. A 10-ml aliquot of sulfide antioxidant buffer (SAOB) was added to the small jar and 50 ml of 1 N HCl was added to the large jar. A 5-cm^3 sediment sample was added to the jar and the jar quickly capped. Then the jar was placed on a shaker at 120 rpm for 1 hour. Sulfide was determined in the SAOB using an ion specific electrode. The material remaining in the flask was filtered through layered glass fiber (GF-C) over a 0.45 µm polycarbonate membrane filter within 24 h. SEM concentrations were determined on the filtrate. The material retained by the glass fiber filter was used to estimate organic carbon content of the sediment. The acid treatment removed carbonates that might otherwise be driven off at higher ashing temperatures. Subsequent weight loss after combustion at 550 ^{o}C for 1 h was used to estimate an index of organic carbon content of the sediment using a conversion factor of 0.5 % C/1.0 % weight loss after combustion (e.g., Luoma and Bryan 1981).

Metal concentrations in surface water, pore water, and SEM were determined using inductively coupled plasma spectroscopy (ICAP, Jarrell-Ash Model 61 or Spectroflame FTMOA85D). Where ICAP detection limits were not sufficient to bracket chronic water quality criteria or levels associated with toxicity in previous tests, graphite furnace atomic absorption spectroscopy (AAS, Perkin-Elmer Model 1100) was used. Metals analyzed include Al, Cd, Co, Cr, Cu, Pb, Ni, Na, Se, Zn, Fe, and Mn. After initial tests, this group of metals was reduced to those showing elevation at any site (2x mean in reference sites) or those possibly affecting the bioavailability of other metals. For quality assurance, duplicates, spikes and purchased unknowns were analyzed along with samples.

The particle size composition of the sediment was determined on composite samples by wet-sieving sediment samples and weighing fractions separated by 2-mm and 63-µm sieves.

Habitat assessments were performed at each of the sites on the sampling day using the methods described by Barbour et al. (1999) for high-gradient streams. Surface water temperature, dissolved oxygen, and conductivity were measured in the field.

Macroinvertebrate assemblage structure was determined by standard methods for rapid bioassessment protocols in accordance United States Environmental Protection Agency (USEPA) guidelines (Barbour et al. 1999). A 0.3-m wide D-frame dip net with a custom fitted 500-µm mesh catch net was used to collect macroinvertebrates from a uniformly estimated surface area. In riffle areas, a 0.09-m^2 area in front of the net was scrubbed by hand for 15 seconds, and the organisms were allowed to wash into the catch net. This procedure was repeated in a different area of the riffle (both D-frame net samples were taken within an area of 1 m^2), and the contents of the two nets were combined to form one composite sample of 0.18 m^2. In depositional areas, the net was moved through the top 2 cm of sediment for a linear distance of 0.5 m for 15 seconds. This was repeated in a different location (within an area of 1 m^2), and the contents of the two passes with the net were combined for a sample of 0.30 m^2. All samples were preserved in 95% ethanol in the field and transported to the laboratory for further processing.

Each of the eight macroinvertebrate samples per station/date (4 for each habitat type) was processed independently. Organisms were sorted from the organic detritus and mineral sediments by hand, and then the macroinvertebrates were identified to genus, with the exception of Nematoda, Platyhelminthes, Oligochaeta, Gastropoda, Bivalva, and Hydracarina which were generally identified to family.

Data analysis

Macroinvertebrate assemblage metrics that reliably distinguish impact in Mid-Atlantic Highland streams (Smith and Voshell 1997) and others which were sensitive to heavy metal impact were calculated on the data. These metrics included: % 1 dominant taxon; % Chironomidae; modified Hilsenhoff Biotic Index (HBI); number of Ephemeroptera, Plecoptera, and Trichoptera Taxa (#EPT); % haptobenthos (percent abundance of macroinvertebrates requiring clean, coarse, firm substrates); number of Ephemeroptera taxa; % collector-filterer; % scrapers; % intolerant taxa (determined as taxa with tolerance values ranging from 0 to 3 inclusive); % tolerant taxa (determined as taxa with tolerance values ranging form 8 to 10 inclusive); and % facultative taxa.

Additionally, the MAIS, a multimetric index incorporating different aspects of the benthic macroinvertebrate assemblage, was determined. The MAIS (Smith and Voshell 1997) is a family-level multimetric index created for use in Mid-Atlantic Highland streams, and was developed following methods described by Barbour et. al. (1995, 1996) and in accordance with current USEPA protocols (Barbour et. al., 1999). The MAIS is composed of 9 metrics aggregated into one final score that ranges from 0 (most impaired) to 18 (least impaired), and the score falls into one of the following categories in the Ridge and Valley ecoregion: Very Poor (0-6), Poor (7-12), Good (13-16), and Very Good (17-18) (Smith and Voshell 1997). The Very Poor and Poor categories are considered to be unacceptable biocriteria, and the Good and Very Good are considered acceptable according to Smith and Voshell (1997).

Variation among sites was analyzed using one-way analysis of variance (ANOVA). If the ANOVA indicated significant effects ($p<0.05$), Ryans Q multiple range test was use to test for differences between the sites. The ANOVA test and the Ryans Q test were performed using SAS (SAS Institute Inc. 1999).

Site grouping was investigated using two methods of multivariate analyses: an agglomerative cluster analysis that used an unweighted pair-group method with arithmetic averages (UPGMA) using SAS (SAS Institute Inc. 1999), and the divisive two-way indicator species analysis called TWINSPAN (Hill 1979) using PC ORD (MjM Software 1997). These methods determined similarities or groupings of sites based upon the presence and absence of macroinvertebrate taxa. The cluster analysis starts from the separate sites and begins to form larger clusters of sites based upon the similarity or dissimilarity of taxa between the sites or clusters. TWINSPAN is a divisive method of analysis that starts with all sites as one group, then divides this group into two smaller groups, and then analyzes each of the smaller groups to determine if these can also be subdivided into smaller groups. One difference between the two methods is that divisive methods put more importance on the large differences between sites, while agglomerative cluster analysis puts more importance on the local similarity of the sites (Jongman et. al., 1995).

Canonical correspondence analysis (CCA) using Canoco 4.0 (GLW-CPRO 1998) of the taxa with the environmental variables was used to investigate the importance of the various environmental variables for site separation. The significance of the environmental variables was determined using forward selection of the variables and 999 Monte Carlo permutations.

RESULTS

Most of the pools at our sites more closely resembled runs than pools; they were shallow, gravel bottomed habitats nearly devoid of organic matter. The three streams in the study were fairly high gradient, and although Peak Creek was not high gradient where it passed through the town of Pulaski, it still did not have well developed pools at the majority of the sites due to the channelization effects. The assemblages observed at the eight sites were fairly similar, with little to no discernable differences. The Chironomidae were most prevalent in pool samples, but analyses of the number of Chironomidae taxa and a biotic index of Chironomidae tolerance values did not show distinct patterns (Fig. 4.2). This lack of difference was thought to be a related to the nature of the pools in this study, and not a result of any differences in water quality between the sites. After various analyses showing little to no differences in the pool data from site to site, the pool data were not included.

Water temperature was similar among the 3 streams for all sampling dates, with temperature being slightly higher at all sites on Peak Creek below PK1. Dissolved oxygen was also similar among the streams and was at or near saturation. The pH of surface water ranged from 7.1 to 8.4, and was slightly more alkaline at the more downstream Peak Creek sites (means = 7.4 at PK1 and 8.1 at PK6). Total ammonia was undetectable (<0.05 mg NH_3-N/L) in all but one sample.

Several surface water quality parameters (hardness, conductivity, and Na concentration) changed immediately downstream of the permitted effluent above Site Peak 2 (Fig. 4.3). Zinc was the only metal occasionally found in surface water at concentrations above the EPA's hardness adjusted concentration (Table 4.1).

In addition to the 8 metals analyzed from the sediment samples (Table 4.2), Al, Cr, and Se were analyzed on one or more sampling dates. These metals did not approach levels of concern in sampled sediments and are not reported. Other characteristics of sediments are summarized in Table 4.3. These stream sediments were dominated by gravel. Organic carbon content ranged from 0.41-1.79%. AVS concentrations were low relative to SEM.

Pore water concentrations of Cu, Cd, Pb, and Zn were occasionally above the EPA's hardness adjusted chronic criteria (Table 4.4). The April 1999 sample from site Peak 6 exceeded the chronic criterion for Zn by more than an order of magnitude (1,864 μg/L), although Zn levels were much lower in the other 3 samples. DO was ≥1.93 mg/L. Total ammonia concentrations were usually detectable, but <1.00 mg NH_3-N/L. DOC ranged from <1 to 18.2 mg/L.

Total habitat scores were higher at all three reference sites as compared to the other sites, although the scores at LW were only marginally higher than at the Peak Creek sites downstream from PK1 (Table 4.5). Most of the parameter scores did not change from one date to the next, with some exceptions. The values at BS changed because the site location was moved to a more upstream site that was more similar to the Peak Creek sites, and this change was made after the first collection had already occurred. Once the site was relocated, there were no changes between dates other than channel flow status that changed at all sites due to seasonal variation. Changes at PK2, between dates, are attributed to a bridge construction project that started after the first collection had been made. The major difference that occurred at LW between dates was the lack of the slow-deep velocity-depth regime for the last 3 dates.

The MAIS values were higher at the reference sites (LW, BS, and PK1) and consistently in the Good Biological Condition Category (Fig. 4.4). PK2 through PK6 had lower MAIS values, and were never above a Biological Condition Category rating of Poor, unacceptable biocriteria rating.

Cluster analysis of the taxa resulted in the three reference sites (LW, BS, and PK1) being grouped together in all four seasons (Fig. 4.5). The cluster analysis identified two major groupings of sites for three of the four seasons, with the exception of December 1998 when PK3 formed its own separate cluster (Fig. 4.5B). The major groupings seem to be consistent with the suspected impairment status of the sites, i.e., one cluster was a reference site grouping and the other a grouping of suspected impaired sites. There was a likely impaired site grouped in the reference cluster in three of the four seasons, but only one site per season. The same site was never repeated in two successive seasons(Fig. 4.5). None of the reference sites were ever grouped in the cluster of sites suspected to be impaired.

The analysis with TWINSPAN produced results similar to those from the cluster analysis. The impaired sites formed two groupings of sites in each season, and the splitting off of these groups was the last division in the analysis. PK5 and PK6 were in the same cluster all four times, PK3 and PK4 together in the opposite impaired site cluster three of the four times, PK2 shifted back and forth somewhat, but was clustered with PK5 and PK6 three of the four times (Table 4.6). The reference sites separated out first, forming a single cluster only one time, but reference sites never formed a group with a non-reference site. Analysis of the taxa important for group separation showed that the positive taxa at the reference sites were generally intolerant of disturbance.

Ephemeroptera richness was consistently higher at the reference sites compared to the downstream sites (Table 4.7). HBI and Number of EPT taxa per site also differed between sites, but only separated reference from impaired sites three out of the four seasons. Percent scrapers and percent haptobenthos were different between sites, but showed no clear pattern of site association from season to season. Percent Chironomidae and percent collector-filterer taxa were each significantly different among sites three of the four seasons, but showed no distinguishable pattern (Table 4.7). Percent 1 dominant taxon was only significantly different two of four times.

Percent intolerant taxa and percent facultative taxa clearly separated the reference from impaired sites, but no distinction was seen among impaired sites (Fig. 4.6). Percent tolerant taxa was not significantly different between sites, but does increase at PK2. This showed that intolerant taxa were replaced by facultative taxa at PK2, and the intolerant taxa never reappeared in comparable numbers at the downstream Peak Creek sites.

Canonical correspondence analysis (CCA) did not show the metals explaining a significant amount of variability among the riffle assemblages of Peak Creek. Of the 35 environmental variables measured, conductivity explained 0.42 of the 1.67 variance, embeddedness 0.24, sand 0.21, gravel 0.19, vegetation right bank 0.18, sediment deposition 0.16, bank stability right bank 0.12, and temperature 0.11, with the remainder being split up between the other variables. Nickel was the only metal to explain a portion of the variability of the benthic macroinvertebrate assemblages (0.11), but the levels of Ni were below the PEL (Ingersol et al. 1996) and far below the observed effects levels found in other studies (Warnick and Bell 1969, Nebeker et al. 1984, Kemble et al. 1994).

DISCUSSION

Our data showed that the biological condition at each of the sites below PK1 was severely impaired, but between those impaired sites there was not much difference. The percent of pollution intolerant organisms declined downstream from PK1 and was replaced by organisms facultative to pollution. While the downstream Peak Creek sites showed a distinct difference from the reference sites, there were no differences among the downstream sites. None of the analyses showed a consistent pattern of differences between sites PK2 through PK6. This result was somewhat surprising since the major impact was thought to occur at or near the Allied Signal site (sites PK4 and/or PK5). These finding do not agree with Willis (1989) who indicated that the worst damage to the biological integrity was occurring near the PK5 site.

The benthic macroinvertebrate assemblages found at and downstream from the industrial waste piles (PK4 and PK5) were much different than the ones noted by Willis (1989). The biological condition at those sites was found to be impaired, but not to the same degree as in 1989, when Willis noted that there were no benthic macroinvertebrates at one of the sites (Willis 1989). The major change that occurred near or upstream from those sites was the capping of the waste piles, and this action appears to have been beneficial to the aquatic community.

Cluster analysis grouped the reference sites together in all occasions, as expected. The impaired sites also grouped together, for the most part, but no discernable sub-groups of impaired sites are seen with the agglomerative methods. The lack of sub-groups indicated that the taxa at the sites in the vicinity of the Allied Signal area were not distinctly different from the nearby sites. TWINSPAN suggested a clear separation of the reference sites from the PK2-PK6 sites, also what was expected when a group of impaired sites was compared to reference condition sites. However, the taxa at PK2 through PK6 were not sufficiently different to produce any clear clustering of the sites.

Our macroinvertebrate and chemistry data were not similar to the general findings of other researchers who have shown heavy metal affects on benthic macroinvertebrate assemblages (Chadwick et. al., 1986, Clements et. al., 1988, Clements, 1994, Clements and Kiffney 1994). The concentrations of Cu and Pb in the sediment samples increased at PK4 and remained at elevated levels at the downstream sites. The levels of these metals at those sites were above the PEL in a toxicity study (Ingersoll et. al., 1996), but these sites do not exhibit any noticeable differences in biological condition. This was not consistent with what other researchers have found when aquatic macroinvertebrates are being affected by heavy metal, because the assemblage structure would have differed at these sites if the heavy metals were having a detrimental affect (Clements 1991, Winner et. al., 1980, Clements et. al., 1992, Beltman et. al., 1999).

The water hardness, concentration of Na, and conductivity of the surface water all increased at PK2 and remained elevated at the rest of the downstream sites, coinciding with the deterioration in biological condition. While the levels of Na and conductivity observed were not thought to be causing impairment, they may be one of the reasons that the elevated levels of Cu and Pb were not noticed to be causing changes in the benthic macroinvertebrate assemblages. The stream receives multiple sources of pollution and environmental stress as it enters and progresses through the Town of Pulaski. This resulted in organisms considered facultative or tolerant of perturbation replacing the intolerant organisms found at the upstream site (Fig. 4.6), and these organisms may be tolerant of the increased metal concentration.

The concentrations of metals found in the water (Tables 4.1 and 4.4) are lower than the levels found by other researchers in other streams contaminated with heavy metals (Clements et. al., 1988, Moore et. al., 1991, Clements and Kiffney 1994, Beltman et. al., 1999). The lower concentrations may be the reason why metal contamination effects have not been noticed. Additionally, the intolerant taxa found at PK1 were replaced by facultative and tolerant organisms at the other Peak Creek sites, and may explain why heavy metal impacts are not detected (Clements and Kiffney 1994).

The concentration of copper in the sediment was higher at sites PK4 through PK6, coinciding with the location of the Allied Signal waste piles. The concentrations at PK4-PK6 were all above the PEL (Ingersoll et. al., 1996), but were lower than the concentrations shown to have effects by Beltman et. al., (1999). The concentrations were higher at PK4 and decreased downstream, indicating the source of the metal contamination was at or between PK4 and PK3. However, because none of the our data indicated distinct differences between the assemblages at PK2-PK3 and the ones at PK4-PK6, it does not appear that the Cu concentrations in the sediment are affecting the macroinvertebrates.

A potential cause for the impaired biological condition found in Peak Creek was generalized urbanization, which encompasses myriad anthropogenic disturbances occurring around the stream near and in the town of Pulaski. Effects of urbanization include the loss of allochthonous organic matter (Richards and Host 1994), toxic components of the sediment (Pitt and Bozeman 1980, Medeiros et. al., 1983), homogeneity of substrate (Richards and Host 1994), and nutrients in runoff (Hachmoller et. al., 1991). Site specific effects of heavy metal contamination, which were once observed near the Allied Signal site, were not observed in this study. The consistent decrease in measures of the macroinvertebrate assemblage that was observed at and downstream from PK2 indicated that the permitted discharge of Magnox Pulaski Inc. might be having a substantial effect on the benthic macroinvertebrates. The assemblage at and downstream from PK2 had less intolerant organisms and more facultative organisms, which was evident in the increased HBI values at these downstream sites compared to reference sites. Ephemeroptera taxa was one group that dropped significantly at PK2 and remained in low numbers at the rest of the downstream sites.

In conclusion, benthic macroinvertebrate assemblages were observed to be impaired at the sites that are located in and below the town limits. The impairment was not to the degree that was noted in studies conducted in the late 1980's, indicating that a capping of an industrial waste pile has led to some recovery of the stream assemblage. The elevated levels of heavy metals observed in this study do not appear to be the sole cause of the decreased ecological condition.

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Table 4.1. Concentrations of dissolved metal in surface water from Peak, Little Walker, and Stony Creeks (µg/L). Values marked with an asterisk exceed the hardness adjusted chronic water quality criterion. LW= Little Walker Creek, BS = (Big) Stony Creek.

Station	Sampling Date	Cd	Co	Cu	Fe	Mn	Na	Ni	Pb	Zn
LW	8-98	<0.5		<2	55	<1.0	2,044	31	<1.0	39
	12-98	<0.5		<2	60	<1.0	2,902	<6	<1.0	<4
	4-99	<0.5	<4	<2	61	<1.0	2,975	<12	<1.0	37*
	8-99	<0.2	3.4	<1.2	55	2.5	4,085	<16	<2.0	74*
BS	8-98	<0.5		<2	16	<1.0	702	27	<1.0	126*
	12-98	<0.5		<2	77	5.7	572	<6	<1.0	<4
	4-99	<0.5	<4	<2	45	<1.0	1,013	<12	<1.0	28*
	8-99	<0.2	<3.4	<1.2	134	<0.2	1,648	<16	<2.0	35
Peak Creek 1	8-98	<0.5		<2	354	<1.0	1,189	14	1.3*	<4
	12-98	<0.5		<2	82	5.7	1,344	<6	<1.0	<4
	4-99	<0.5	<4	<2	60	1.1	1,860	<12	<1.0	31
	8-99	<0.2	<8.5	<1.2	179	<0.2	1,904	<16	<2.0	73*
Peak Creek 2	8-98	<0.5		<2	129	<1.0	60,420	11	<1.0	<4
	12-98	<0.5		<2	11	17	65,170	<6	1.1	<4
	4-99	<0.5	<4	<2	51	4.2	17,030	<12	<1.0	50
	8-99	<0.2	<8.5	<1.2	68	46	92,200	<16	5.7	178*
Peak Creek 3	8-98	<0.5		<2	68	<1.0	85,030	12	4.6*	<4
	12-98	<0.5		<2	24	22.7	60,360	<6	1.8	52
	4-99	<0.5	<4	<2	41	6.7	18,260	<12	<1.0	49
	8-99	<0.2	<8.5	<1.2	73	17.4	78,800	<16	<2.0	66
Peak Creek 4	8-98	<0.5		<2	61	<1.0	80,750	11	<1.0	<4
	12-98	<0.5		<2	25	17	56,760	<6	<1.0	<4
	4-99	<0.5	<4	<2	45	8	18,580	<12	<1.0	47
	8-99	<0.2	<3.4	<1.2	67	30.8	77,600	<16	<2.0	50
Peak Creek 5	8-98	<0.5		<2	74	<1.0	62,780	24	1.6	<4
	12-98	<0.5		<2	38	45.3	44,570	<6	<1.0	44
	4-99	<0.5	<4	<2	83	21.1	18,270	<12	<1.0	39
	8-99	<0.2	<3.4	<1.2	75	30.3	66,500	16	<2.0	82
Peak Creek 6	8-98	<0.5		<2	74	<1.0	58,430	27	<1.0	<4
	12-98	<0.5		<2	52	5.7	41,810	<6	<1.0	52
	4-99	<0.5	<4	<2	78	17.9	18,700	<12	<1.0	60
	8-99	<0.2	4.9	<1.2	67	14.3	68,500	19	<2.0	85

Table 4.2. Concentrations of simultaneously extracted metal (SEM in µg/g dry wt), sum of SEM in µm/g, and Zn TU in µg/g dry wt. Values marked with an asterisk are above the Probable Effects Level (Ingersoll et al. 1996). LW= Little Walker Creek, BS = (Big) Stony Creek.

Station	Sampling Date	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn	ΣSEM	Zn TU
LW	8-98	0.33	2.4	3.5	1,889	91	4	10	32	0.7	94
	12-98	<0.01	2.2	3.2	3,313	181	5	7	39	1.3	78
	4-99	<0.01	3.6	4.8	14,666	102	8	3	38	0.8	64
	8-99	0.90	3.0	3.4	3,557	114	5	11	37	0.8	118
BS	8-98	0.50	7.6	<0.1	1,785	917	10	18	45	1.0	141
	12-98	<0.01	2.8	0.6	1,005	198	3	4	22	0.8	41
	4-99	<0.01	4.8	0.8	2,268	267	5	4	31	0.6	52
	8-99	0.50	3.9	0.7	1,033	199	4	8	25	0.5	75
Peak Creek 1	8-98	0.54	8.0	1.9	10,409	461	6	176*	1,811*	29.0	2,670
	12-98	<0.01	1.6	36.0	2,696	309	9	22	251	8.0	425
	4-99	<0.01	2.9	7.4	3,206	273	2	8	52	1.0	108
	8-99	1.90	4.3	6.3	7,494	419	6	28	49	1.1	240
Peak Creek 2	8-98	0.48	6.7	17.3	6,274	582	5	89*	1,575*	24.9	2,030
	12-98	<0.01	3.6	4.1	2,400	370	2	44	1,015*	31.8	1,225
	4-99	<0.01	8.9	8.7	8,320	484	6	56	1,096*	17.4	1,374
	8-99	1.50	4.7	8.2	2,772	340	5	98*	6,413*	98.8	6,917
Peak Creek 3	8-98	0.81	8.2	21.1	9,286	724	8	89*	11,367*	174.7	11,838
	12-98	<0.01	3.5	9.6	2,652	423	2	29	2,919*	90.8	3,071
	4-99	<0.01	7.3	9.8	8,939	432	8	54	2,095*	32.8	2,366
	8-99	1.20	5.3	4.9	4,045	445	5	30	4,088*	62.8	4,267
Peak Creek 4	8-98	1.14	9.7	462.3*	9,454	251	5	125*	1,404*	29.4	2,969
	12-98	<0.01	3.9	152.6*	610	253	4	74	935*	29.4	1,592
	4-99	<0.01	8.8	132.5*	9,060	319	6	60	1,948*	32.5	2,500
	8-99	2.10	6.8	198.1*	7,128	271	5	92*	3,872*	62.9	4,763
Peak Creek 5	8-98	0.88	31.1	135.5*	7,687	427	8	218*	1,132*	20.6	2,433
	12-98	<0.01	9.3	76.5	6,381	667	6	237*	1,149*	36.9	2,393
	4-99	<0.01	9.6	50.0	10,320	792	5	74	960*	16.0	1,404
	8-99	2.50	7.7	112.1*	9,288	468	4	296*	6,288*	99.4	7,940
Peak Creek 6	8-98	0.61	10.0	117.3*	7,303	365	5	167*	1,446*	24.9	2,469
	12-98	<0.01	4.6	83.7	7,518	346	4	128*	949*	30.2	1,709
	4-99	<0.01	7.2	75.1	7,985	456	4	82*	798*	19.9	1,330
	8-99	2.50	8.7	121.6*	9,952	566	5	324*	1,133*	20.9	2,933

Table 4.3. Chemical and physical characteristics of sediment core samples from Peak (Peek Creek1-6), Little Walker (LW), and Stony Creeks (BS). TOC = total organic carbon.

Station	Sampling Date	TOC (%)	Acid-volatile sulfide (μmol/g)	Gravel (%)	Sand (%)	Silt and Clay (%)
LW	8-98	0.76	<0.01	93.0	6.8	0.2
	12-98	1.44	0.07	74.1	23.5	2.5
	4-99	0.99	<0.02	96.2	2.9	0.9
	8-99	0.98	0.06	91.0	8.3	0.7
BS	8-98	0.76	0.06	95.9	4.0	0.1
	12-98	0.41	0.06	52.3	47.5	0.2
	4-99	0.52	<0.02	75.8	23.9	0.3
	8-99	0.38	<0.02	72.9	26.9	0.2
Peak Creek 1	8-98	0.98	1.61	95.0	4.6	0.3
	12-98	1.79	0.03	89.0	10.3	0.7
	4-99	1.08	<0.02	93.7	5.5	0.8
	8-99	0.98	<0.02	96.4	3.1	0.5
Peak Creek 2	8-98	1.58	2.27	85.0	14.4	0.6
	12-98	1.59	<0.01	86.8	11.0	2.2
	4-99	1.04	<0.02	87.8	10.6	1.6
	8-99	0.48	<0.02	88.6	10.7	0.7
Peak Creek 3	8-98	1.58	3.66	91.8	8.0	0.2
	12-98	1.65	<0.01	90.2	8.8	1.0
	4-99	1.06	0.35	96.2	3.1	0.7
	8-99	1.04	<0.02	96.6	2.9	0.5
Peak Creek 4	8-98	0.65	1.38	96.3	3.5	0.1
	12-98	1.55	0.05	87.7	11.0	1.3
	4-99	1.06	<0.02	93.6	5.7	0.7
	8-99	0.57	0.75	90.9	8.5	0.6
Peak Creek 5	8-98	1.07	4.42	83.6	15.4	1.0
	12-98	1.57	0.13	90.7	7.8	1.4
	4-99	1.54	6.10	91.5	7.0	1.5
	8-99	1.02	1.49	94.0	5.4	0.6
Peak Creek 6	8-98	1.24	2.43	82.2	16.5	1.3
	12-98	1.47	0.64	61.5	35.4	3.1
	4-99	1.10	<0.02	89.4	8.8	1.8
	8-99	1.06	1.64	95.9	3.4	0.7

Table 4.4. Concentrations of dissolved metal in pore water from Peak, Little Walker, and Stony Creeks (µg/L). Values marked with an asterisk exceed the hardness adjusted chronic water quality criterion. LW= Little Walker Creek, BS = (Big) Stony Creek.

Station	Sampling Date	Cd	Co	Cu	Fe	Mn	Na	Ni	Pb	Zn
LW	8-98	<0.5		3.8*	<5	<1.0	3,523	40	<1.0	<4
	12-98	<0.5		<2.0	25	<1.0	2,977	<6	<1.0	84*
	4-99	<0.5	<4	<2.0	4	<1.0	3,239	<12	<1.0	35
	8-99	<0.2	<8.5	<1.2	<3	<0.2	7,100	<16	<2.0	<2
BS	8-98	0.8		<2.0	<5	<1.0	1,055	--	--	39*
	12-98	2.0*		12.2*	52	17.0	1,704	8	<1.0	157*
	4-99	<0.5	<4	<2.0	6	<1.0	1,607	<12	<1.0	58
	8-99	<0.2	<8.5	<1.2	<3	<0.2	2,310	<16	<2.0	<2
Peak Creek 1	8-98	0.5		9.6*	573	23.3	1,410	37	<1.0	10
	12-98	0.8		7.6*	19	17.0	1,762	<6	<1.0	88
	4-99	<0.5	75	<2.0	<1	<1.0	2,962	<12	<1.0	46
	8-99	<0.2	4.3	<1.2	<3	<0.2	3,317	<16	<2.0	<2
Peak Creek 2	8-98	0.8		19.2*	122	<1.0	48,260	36	<1.0	39
	12-98	0.6		<2.0	16	17.0	47,110	<6	<1.0	48
	4-99	<0.5	<4	<2.0	<1	<1.0	21,500	<12	<1.0	54
	8-99	<0.2	<3.4	<1.2	<3	<0.2	54,500	<16	<2.0	<2
Peak Creek 3	8-98	<0.5		<2.0	32	<1.0	44,960	36	<1.0	19
	12-98	<0.5		<2.0	16	<1.0	44,440	<6	2.1	56
	4-99	<0.5	<4	<2.0	<1	<1.0	22,830	<12	<1.0	38
	8-99	<0.2	<3.4	<1.2	<3	<0.2	51,300	<16	<2.0	<2
Peak Creek 4	8-98	<0.5		3.8	55	<1.0	39,800	35	<1.0	19
	12-98	<0.5		<2.0	13	<1.0	38,730	<6	3.0*	72
	4-99	<0.5	<4	<2.0	<1	<1.0	23,270	<12	<1.0	46
	8-99	<0.2	<8.5	2.8	<3	<0.2	54,800	<16	<2.0	<2
Peak Creek 5	8-98	<0.5		<2.0	32	220.9	30,520	40	<1.0	<4
	12-98	<0.5		<2.0	13	<1.0	38,300	<6	1.6	76
	4-99	1.1	<4	<2.0	<1	<1.0	22,070	<12	<1.0	601*
	8-99	<0.2	<8.5	<1.2	<3	<0.2	62,600	<16	<2.0	<2
Peak Creek 6	8-98	<0.5		<2.0	42	511.6	24,150	--	--	10
	12-98	<0.5		<2.0	22	<1.0	36,960	<6	3.0	80
	4-99	<0.5	<4	<2.0	<1	<1.0	22,490	<12	<1.0	1,864*
	8-99	<0.2	<8.5	2.5	<3	<0.2	58,100	<16	<2.0	<2

Table 4.5. Mean habitat assessment values showing the standard deviation (in parentheses). The mean value is for the 4 sampling dates, and the total shown is the sum of the averages. Habitat methods from Barbour et. al. (1999). PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

	Site							
Habitat Parameter	LW	BS	PK1	PK2	PK3	PK4	PK5	PK6
Epifaunal substrate, available cover	14.8 (1.3)	17.3 (1.5)	13 (0.0)	10 (0.0)	13 (0.0)	13 (0.0)	13 (0.0)	13 (0.0)
Embeddedness	18 (0.0)	17.5 (1.0)	18 (0.0)	8.5 (4.7)	13 (0.0)	13 (0.0)	13 (0.0)	13 (0.0)
Velocity-Depth Regime	14 (2.0)	18 (0.0)	18 (0.0)	18 (0.0)	13 (0.0)	18 (0.0)	18 (0.0)	18 (0.0)
Sediment Deposition	18 (0.0)	17.5 (1.0)	18 (0.0)	11.8 (5.3)	18 (0.0)	18 (0.0)	18 (0.0)	18 (0.0)
Channel Flow Status	13 (1.4)	13.3 (1.7)	12 (1.2)	12 (1.2)	12 (1.2)	12 (1.2)	12 (1.2)	12 (1.2)
Channel Alteration	18 (0.0)	18.3 (0.5)	18 (0.0)	18 (0.0)	13 (0.0)	18 (0.0)	18 (0.0)	18 (0.0)
Frequency of Riffles (bends)	13 (0.0)	17.8 (0.5)	11 (0.0)	11 (0.0)	15 (0.0)	11 (0.0)	8 (0.0)	11 (0.0)
Bank Stability (left bank)	9 (0.0)	9.3 (0.5)	9 (0.0)	9 (0.0)	9 (0.0)	6 (0.0)	7 (0.0)	7 (0.0)
Bank Stability (right bank)	9 (0.0)	9 (0.0)	9 (0.0)	9 (0.0)	9 (0.0)	9 (0.0)	7 (0.0)	7 (0.0)
Vegetative Protection (left)	9 (0.0)	8.5 (1.0)	9 (0.0)	2 (0.0)	9 (0.0)	5 (0.0)	7 (0.0)	7 (0.0)
Vegetative Protection (right)	8 (0.0)	7.8 (2.5)	9 (0.0)	7 (0.0)	9 (0.0)	9 (0.0)	7 (0.0)	7 (0.0)
Riparian Vegetative Zone (left)	7 (0.0)	10 (0.0)	8 (0.0)	2 (0.0)	4 (0.0)	3 (0.0)	9 (0.0)	7 (0.0)
Riparian Vegetative Zone (right)	2 (0.0)	8 (4.0)	8 (0.0)	2 (0.0)	4 (0.0)	9 (0.0)	9 (0.0)	7 (0.0)
Total Average Habitat Score	152.8	172.3	160	120.3	141	144	146	145

Table 4.6. TWINSPAN separation of sites showing three positive and negative taxa important for group separation. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

	August 1998	December 1998	April 1999	August 1999
First Separation	**PK1 and BS**	**BS**	**BS**	**PK1, LW, and BS**
	+ Stilocladius	+ Leuctra	+ Wormaldia	+ Acroneuria
	+ Stempellinella	+ Hexatoma	+ Paraleptophlebia	+ Stenonema
	+ Parametriocnemus	+ Paraleptophlebia	+ Microtendipes	+ Isonychia
	- Stenelmis	- Corydalus	- Planariidae	- Cheumatopsyche
	- Dicrotendipes	- Stenelmis	- Caenis	- Cricotopus/Orthocladius
	- Corydalus	- Optioservus	- Cricotopus	- Hydropsyche
Second Separation	**LW**	**PK1 and LW**	**PK1 and LW**	**PK2, PK5, and PK6**
	+ Serratella	+ Stenonema	+ Drunella	+ Simulium
	+ Macrostemum	+ Isonychia	+ Isonychia	+ Planorbidae
	+ Leucrocuta	+ Oemopteryx	+ Tanytarsus	+ Nigronia
	- Cricotopus	- Diamesia	- Caenis	- Thienemannimyia grp.
	- Cardiocladius	- Physa	- Dicrotendipes	- Cricotopus/Orthocladius
	- Antocha	- Lymnaea	- Corbicula	- Helicopsyche
Third Separation	**PK2, PK4, PK5, and PK6**	**PK2, PK5, and K6**	**PK2, PK3, and PK4**	**NA**
	+ Planorbidae	+ Ancyllidae	+ Amphinemura	
	+ Optioservus	+ Acroneuria	+ Tvetnia	
	+ Synorthocladius	+ Chimarra	+ Thienemanniella	
	- Sweltsa	- Thienimanniella	- Berosus	
	- Planaridae	- Microtendipes	- Lanthus	
	- Cricotopus	- Psephenus	- Corydalus	
Remaining Group	**PK3**	**PK3 and PK4**	**PK5 and PK5**	**PK3 and PK4**

Table 4.7. ANOVA of site categories showing F-value. (* indicates significance at p =0.05) Ryans Q multiple comparison results are shown as a line connecting means that are not significantly different. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

Season	Metric	F-value	Stations
August 98	% 1 Dominant	14.57 *	PK5 PK2 PK6 PK3 PK4 BS PK1 LW
	% Chironomidae	1.87	Not significant
	HBI	16.67 *	PK3 PK2 PK5 PK4 PK6 PK1 LW BS
	# EPT	23.20 *	BS LW PK1 PK2 PK3 PK6 PK4 PK5
	% Haptobenthos	3.85 *	PK5 LW PK6 PK4 PK2 BS PK3 PK1
	# Ephemeroptera	30.31 *	LW BS PK1 PK3 PK2 PK6 PK4 PK5
	% Collector-Filterer	2.17	Not significant
	% Scrapers	4.85 *	PK6 PK5 LW PK1 PK4 PK2 PK3 BS
December 98	% 1 Dominant	2.61	Not significant
	% Chironomidae	4.56 *	PK3 PK6 BS PK4 PK1 LW PK2 PK5
	HBI	35.43 *	PK2 PK3 PK4 PK5 PK6 LW PK1 BS
	# EPT	27.93 *	BS LW PK1 PK6 PK3 PK2 PK4 PK5
	% Haptobenthos	12.81 *	LW BS PK5 PK1 PK6 PK4 PK2 PK3
	# Ephemeroptera	82.63 *	BS LW PK1 PK2 PK3 PK4 PK5 PK6
	% Collector-Filterer	2.78 *	PK4 PK6 LW PK5 PK2 BS PK1 PK3
	% Scrapers	7.95 *	LW PK5 PK1 PK2 PK6 PK4 PK3 BS
April 99	% 1 Dominant	1.97	Not significant
	% Chironomidae	6.52 *	PK3 PK2 PK4 PK6 PK1 PK5 BS LW
	HBI	21.23 *	PK6 PK2 PK4 PK5 PK3 PK1 LW BS
	# EPT	15.67 *	PK1 BS LW PK3 PK4 PK2 PK6 PK5

	% Haptobenthos	22.38 *	BS LW PK1 PK5 PK4 PK6 PK2 PK3
	# Ephemeroptera	25.69 *	LW PK1 BS PK2 PK3 PK4 PK5 PK6
	% Collector-Filterer	10.88 *	BS PK1 LW PK5 PK4 PK6 PK3 PK2
	% Scrapers	7.03 *	LW PK5 PK4 PK6 PK3 PK2 PK1 BS
August 99	% 1 Dominant	7.29 *	PK3 PK5 PK2 PK6 PK4 PK1 LW BS
	% Chironomidae	4.52 *	BS PK3 PK2 PK4 LW PK1 PK6 PK5
	HBI	43.80 *	PK2 PK3 PK4 PK5 PK6 PK1 LW BS
	# EPT	14.34 *	BS LW PK1 PK4 PK3 PK6 PK5 PK2
	% Haptobenthos	7.28 *	PK6 PK5 LW PK1 PK4 BS PK3 PK2
	# Ephemeroptera	28.65 *	BS LW PK1 PK4 PK3 PK6 PK5 PK2
	% Collector-Filterer	3.39 *	PK3 PK1 PK4 PK2 PK5 PK6 BS LW
	% Scrapers	10.63 *	LW PK5 PK6 PK4 PK1 PK3 PK2 BS

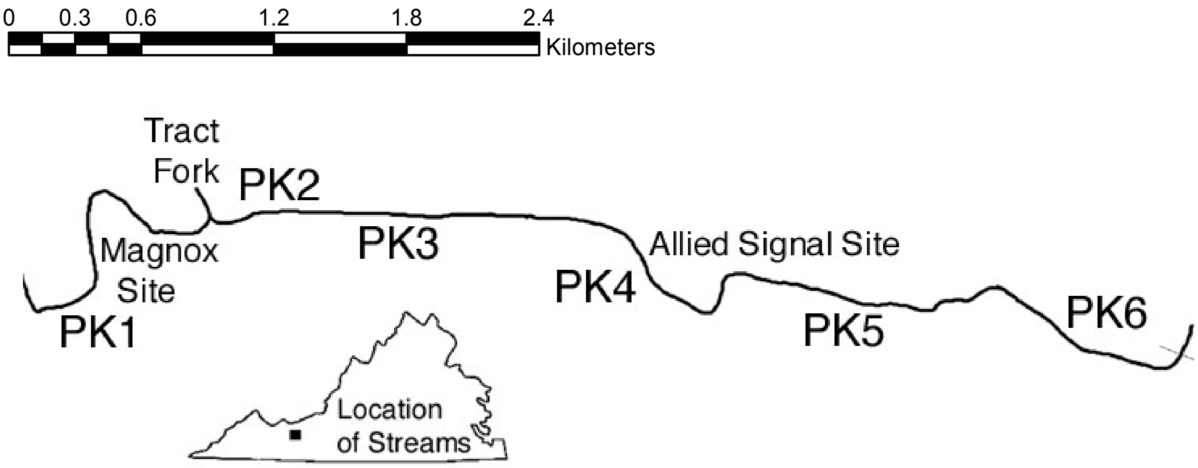

FIG. 4.1. Map showing locations of sampling sites in Pulaski, Virginia, USA (PK1 is located upstream). PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

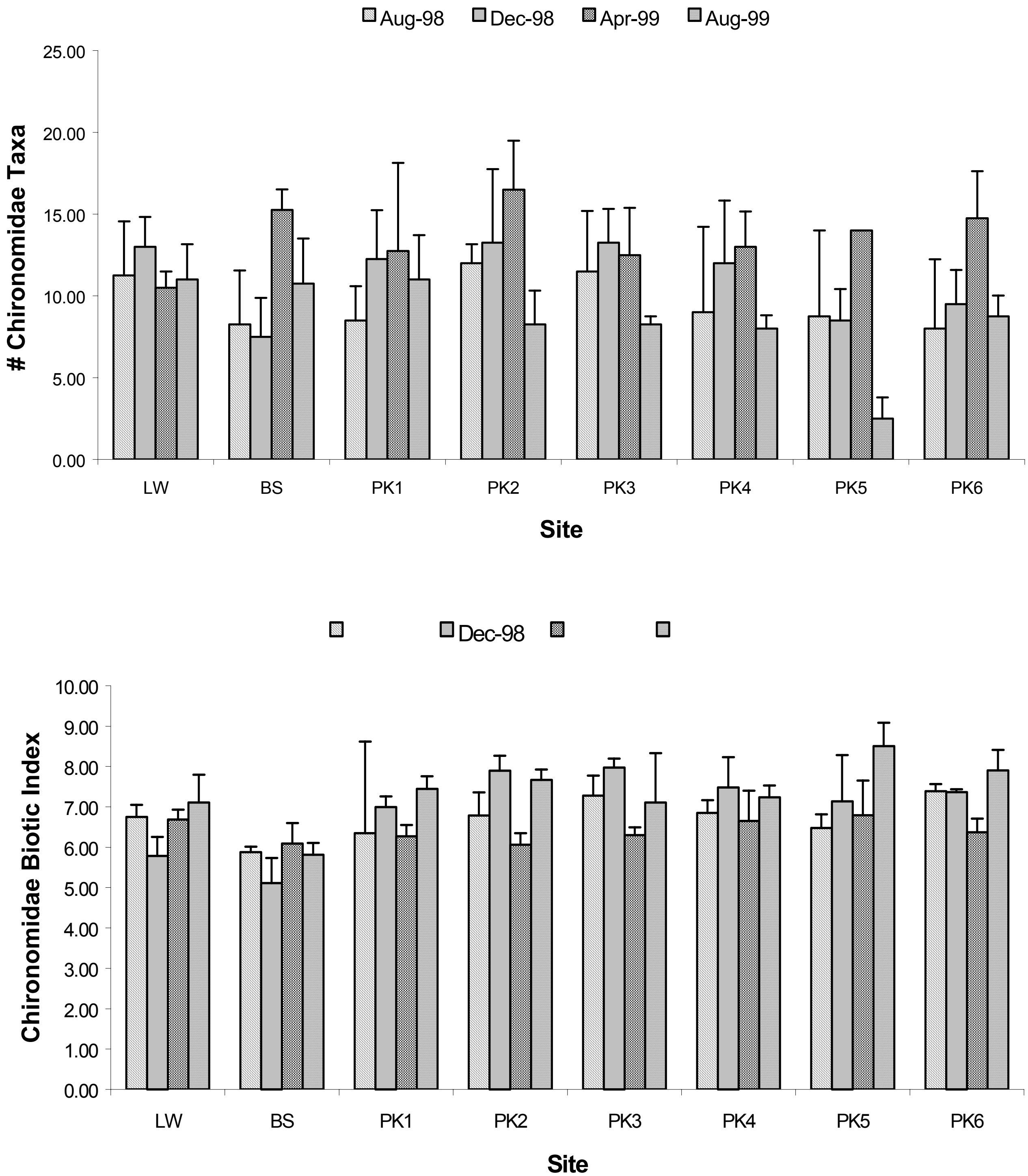

FIG. 4.2. Mean (n = 4, +1 SE) measures of the Chironomidae assemblages in pool samples. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

A

B

C

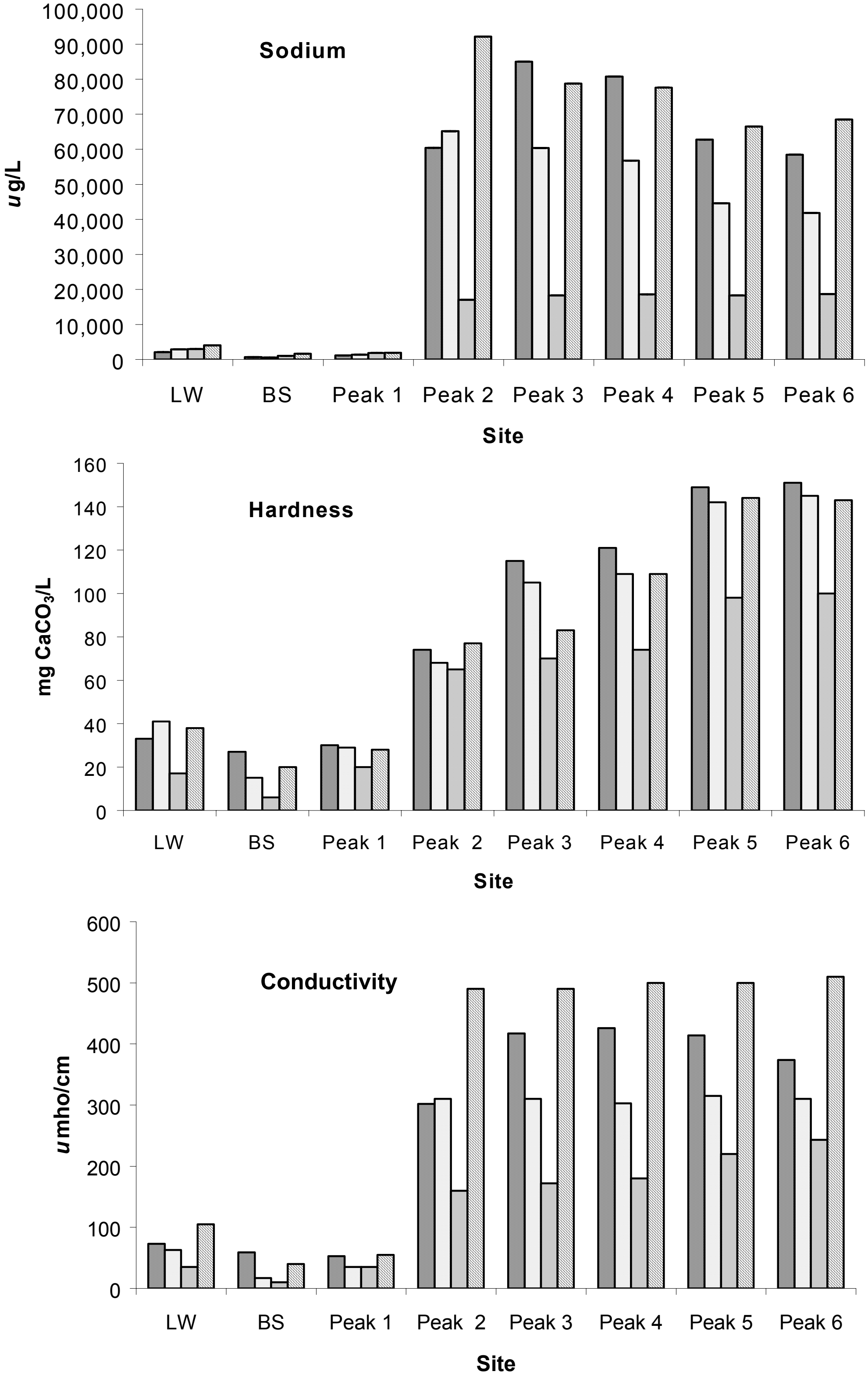

FIG. 4.3. Chemical characteristics of surface water. Bars represent the four sample dates; August 1998, December 1998, April 1999, and August 1999, respectively. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

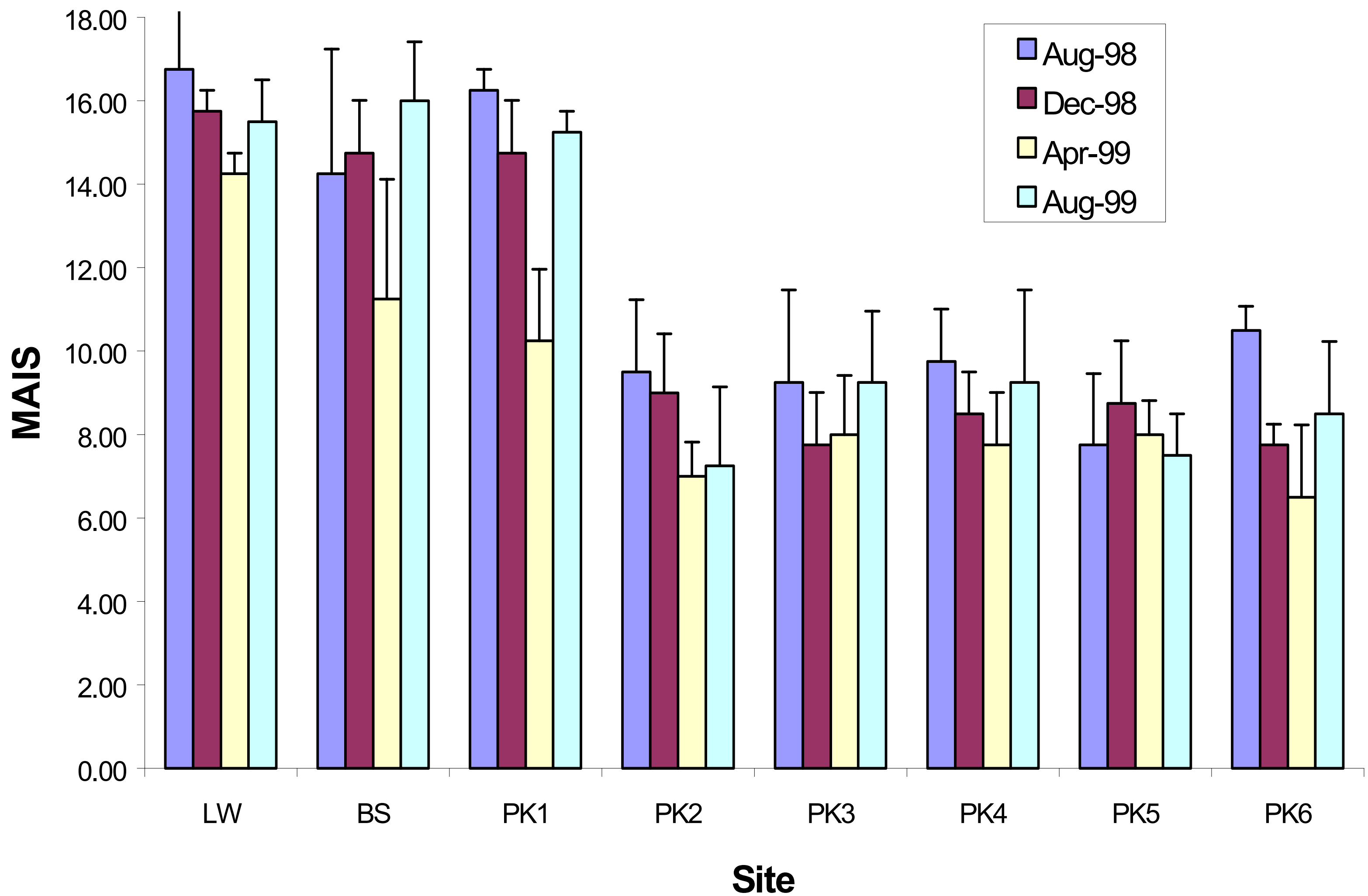

FIG. 4.4. MAIS values for the four sample dates. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek.

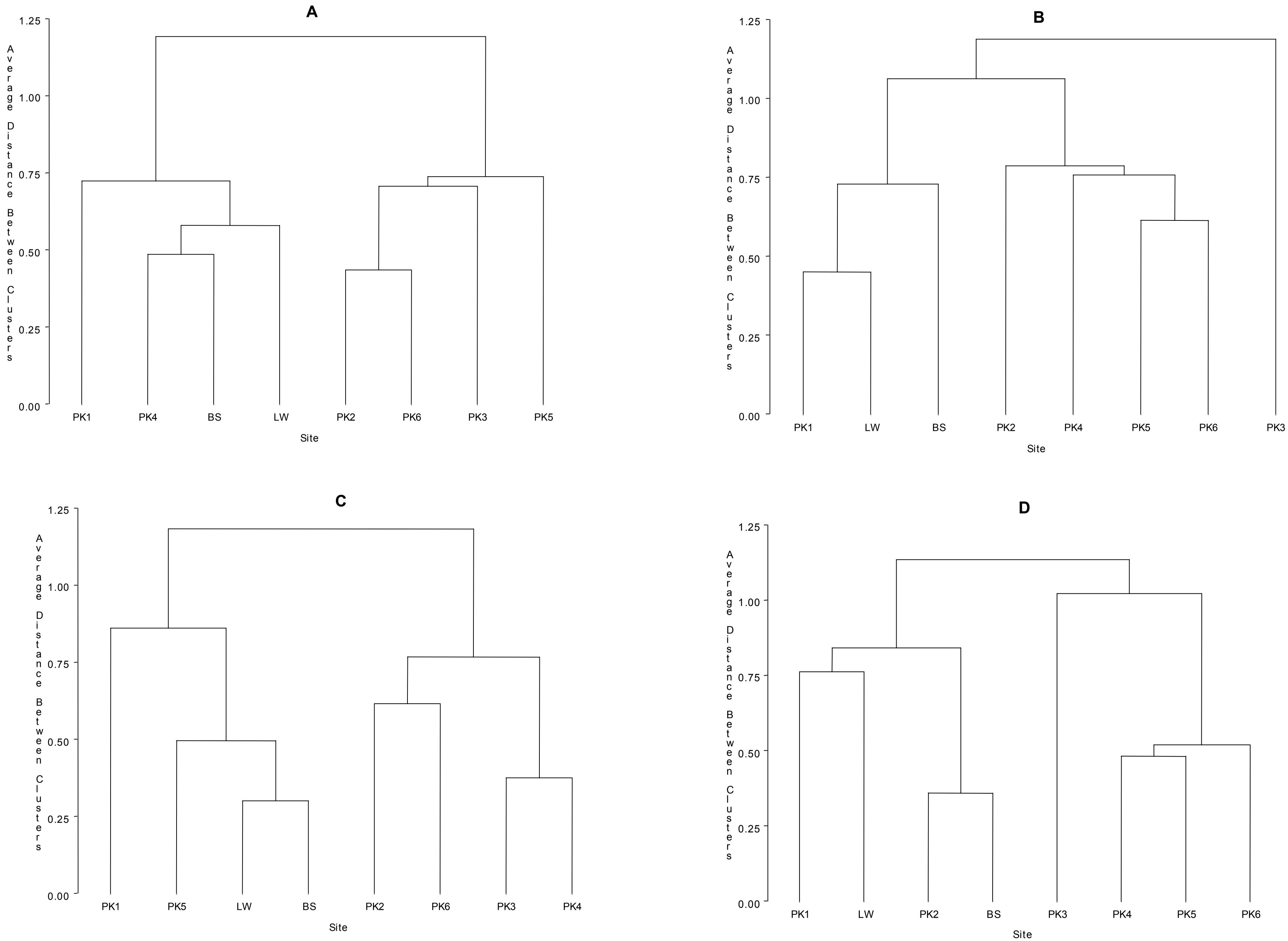

FIG. 4.5. UPGMA cluster analysis of sites, .PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek. A = August 1998, B = December 1998, C = April 1999, and D = August 1999.

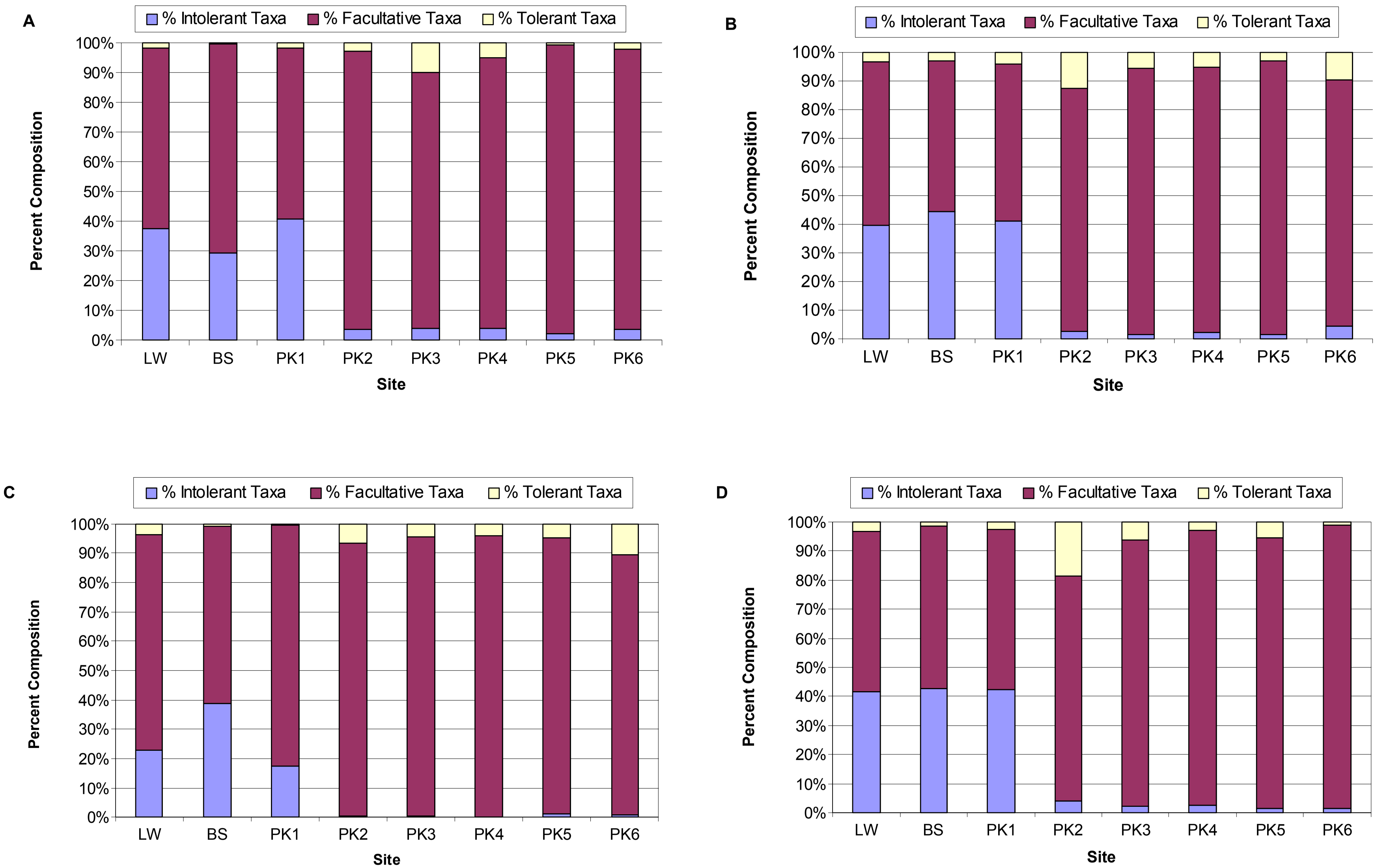

FIG. 4.6. Percent composition of intolerant (0-3), facultative (4-7), and tolerant (8-10) organisms. PK1-6 = Peak Creek sites, LW= Little Walker Creek, BS = (Big) Stony Creek. A = August 1998, B = December 1998, C = April 1999, and D = August 1999.

VITA

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I was born in Newport, Vermont, USA in 1966. I graduated from the University of Vermont in 1988 with a BS in Agricultural Economics. In 1995 I started my MS in Biology at Eastern Kentucky University, conducting my research under the guidance of Dr. Guenter Schuster. Upon completion of my MS in 1997, I started my Ph.D. in Entomology at Virginia Polytechnic Institute and State University, working with Dr. J. Reese Voshell, Jr. I married Kerry Lee Flynn in 1990, and during my MS studies we became the proud parents of David (born in 1996) and Katherine (born in 1997).