FINAL REPORT

USING BENTHIC MACROINVERTEBRATES TO IDENTIFY CAUSES OF FISH KILLS IN THE SHENANDOAH RIVER (2006 and 2007)

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EXECUTIVE SUMMARY

Detailed studies of benthic macroinvertebrates in the Shenandoah River and its tributaries were done in 2006 and 2007. A total of 324 benthic samples were taken, and a total of 179,360 macroinvertebrate organisms were identified and enumerated. The overall purpose of these studies was to determine what the macroinvertebrate assemblage indicated about the biological condition of the Shenandoah River watershed in relation to the fish kills that have been occurring since 2004. There were two major questions: (1) do macroinvertebrate assemblages differ spatially within the watershed; and (2) what environmental variables are responsible for observed differences in macroinvertebrate assemblages? It was anticipated that comparisons of macroinvertebrate assemblage data with measurements of environmental variables would suggest testable hypotheses for causes of the fish kills.

In 2006, the study was confined to "large river" sites on the Shenandoah River and its major tributaries, with one site in the James River basin. In 2007, an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River was undertaken to examine the effects of specific land uses and environmental stressors. A lesser number of large river sites were also studied in 2007. The observed biological condition of the Shenandoah River, as indicated by benthic macroinvertebrates, was also compared to that of two other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region. Some information about long-term temporal changes in the biological condition of the Shenandoah River was obtained by comparing the present benthic macroinvertebrate assemblages with those observed about 40 years ago. There were eight specific objectives, which are listed below along with a summary of the most important findings.

1) Characterize the benthic macroinvertebrate assemblages in the large river sections of the Shenandoah River system and analyze for differences and similarities in biological condition among the sections.

The benthic macroinvertebrate assemblage was sampled at 11 large river sites in May and August 2006 and 8 sites in May 2007. Nine sites were located where fish kills had occurred previously, and the other two sites were designated as reference sites because there had been no fish kills as of 2006. The fish kill sites were in the Shenandoah River watershed and included sites on the Main Stem (1), North Fork (4), South Fork (3), and major South Fork tributaries (South River, 1; North River, 1). One reference site was in the Shenandoah River watershed (Cedar Creek), and one was in the James River watershed (Cowpasture River). Six replicate samples were taken at each site on all dates. The assemblage was analyzed in terms of the presence of taxa, abundance of taxa, and metrics that summarize the structure and function of the assemblage. Analyses included counts and biomass of invertebrates.

Combining May and August 2006 samples at all sites, 116 taxa were collected. The numbers and kinds of benthic macroinvertebrate taxa do not appear to be unusual at any of the sites that were investigated in 2006. The overall abundance of organisms was very high at sites in the Shenandoah River watershed. The two reference sites (Cowpasture and Cedar) had more taxa than other sites. Two sites on the South Fork (Whitehouse and Front Royal) had somewhat

fewer taxa than the other Shenandoah River sites. Many of the dominant taxa are fairly sensitive to environmental stress.

The taxonomic composition of the benthic macroinvertebrate assemblage was analyzed simultaneously among sites using ordination, a multivariate method used to assess similarities among sample sites with respect to macroinvertebrate composition and density. In addition, the benthic macroinvertebrate assemblage data were condensed into 11 metrics that represented different ecological characteristics. Univariate ANOVA tests were performed for each metric among all sites. The multivariate and univariate techniques both indicated that the two reference sites were different from the nine fish kill sites, with slightly better biological condition at the reference sites. The Cowpasture River assemblage was more strongly different from the other sites than Cedar Creek. When data from the New River were added to the analyses, the benthic macroinvertebrate assemblage there was different from the assemblage at any of the Shenandoah or Cowpasture sites; however, the macroinvertebrate assemblage did not indicate any appreciable difference in the biological condition of the New River. There was no grouping or similarity pattern among site groups that emerged from the analyses. Since the greatest number of fish kills in 2006 occurred in the lower North Fork (Woodstock, Strasburg), it was hypothesized that the macroinvertebrate assemblage at the North Fork site grouping might be different from the other site groupings, but the benthic macroinvertebrate results do not support that supposition. Analyses of benthic macroinvertebrate assemblages have not distinguished any sites on the Shenandoah River with significant reduction in biological condition, nor suggest that any Shenandoah River sites are significantly different from other sites in the basin.

2) Determine the environmental variables responsible for benthic macroinvertebrate assemblage structure in the large river sections.

Ordination (DCA and CCA) and linear regression both suggested that nutrients and substrate conditions influenced macroinvertebrate assemblages in large rivers although no sites appeared differentially influenced compared to others. Available environmental variables at the large river sites included nutrients, periphyton, substrate composition, heavy metals, and the various chemicals measured with passive samplers. There were an appreciable number of significant relationships with moderately strong coefficients of determination. Most of the significant and strong relationships were with various measures of nitrogen and phosphorus compounds, suggesting nutrient concentration as a primary determinant of assemblage structure and function.

Various forms of nitrogen and phosphorus nutrients were positively related to several top taxa densities in many cases. *Optioservus*, in particular, was significantly less abundant in the presence of high nitrate concentration. Conversely, it is possible that nutrient enrichment stimulates primary production which, in turn, stimulates secondary production and invertebrate abundance. Similarly, substrate size and suitability for epilithic primary production appears to be important as quantified by relationships to AFDM and substrate size (*i.e.*, cobble). We speculate that epilithic primary production is stimulated by nutrient enrichment and cascades upward trophically to influence the macroinvertebrate assemblage at sites where nutrient concentrations are higher. In addition, more epilithic primary production creates more microhabitat on rock surfaces for macroinvertebrates.

Heavy metal and passive sampler results were inconclusive, which is at least partially an artifact of small sample sizes available for statistical analyses.

3) Characterize the benthic macroinvertebrate assemblages in a set of subwatershed tributaries that reflect the predominant types of land use within the Shenandoah River system and analyze for differences and similarities in biological condition among the subwatersheds.

After sampling the large river sites in 2006, it became apparent that it would be very difficult to explain any observed differences in benthic macroinvertebrate assemblage structure because all the large river sites are subjected to all of the land uses and all of the potential environmental stressors in the Shenandoah River basin. Thus, we decided to conduct an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River that could be categorized according to predominant land uses. We speculated that by narrowing the spatial scale of analysis within the Shenandoah River basin we might be able to pinpoint impairment or influence at a smaller more manageable spatial scale or scope. Twenty-six Shenandoah River tributaries were selected using GIS, and various land use variables were calculated, including aspects such as: wetland, forest, pasture/hay, crops, developed land, dairies, beef operations, poultry houses, acres in nutrient management, animal feeding operations, and municipal sewage treatment plants.

Preliminary DCA ordination analysis showed that Passage Creek was a major outlier, being different from other tributary sites based on abundances of *Prosimulium*, a taxa not found in other streams. We removed this site and taxa from a subsequent DCA analysis which allowed us to use a higher level of resolution in detecting differences and relationships between site separation and taxa. The resulting DCA clearly distinguished groups of sites that were characterized by different key taxa. One group of sites was characterized by abundant Planariidae and *Cheumatopsyche*. A second group was comprised of a disproportionate number of Chironomidae. A third group was numerically dominated by *Chimarra*, *Ephemerella*, and *Macaffertium/Stenonema*.

Statistical analyses suggest that tributary sites within the Shenandoah River basin are somewhat distinct based on macroinvertebrate densities. Further, sites likely differ with respect to environmental conditions shown to be significantly related to taxonomic structure.

4) Determine the types of land use and environmental variables responsible for benthic macroinvertebrate assemblage structure in the subwatersheds.

The most informative results of these studies came from using linear regression to assess relationships between macroinvertebrate metrics and top taxa with land use and environmental variables. Regressions supported ordination results and this combination of statistical conclusions strengthens our findings. There were many significant regressions, with macroinvertebrates usually responding positively to increased forest cover and negatively to increases in agricultural or developed area. Non-insect taxa, however, were more abundant in less forested areas having higher agricultural activity in the watershed. The number of dairy farms, cattle farms, and poultry houses was frequently a predictor of reduced biological

condition as quantified by macroinvertebrate metrics. There was not a clear trend for any one of these agricultural land uses to be more important than any others. In addition to summary metric responses, several top taxa responded significantly to land use variables. Planariid worms demonstrated the clearest responses: positively to cropland and negatively to forest cover. Similarly, planariid worms responded positively to poultry houses, animal feeding operations, and nutrient management plans for poultry waste.

A large number of environmental variables were also compared to summary macroinvertebrate metrics and top taxa in tributary streams, which produced an appreciable number of significant and moderately strong relationships. Several forms of nitrogen and phosphorus produced the most relationships. Typically, higher concentrations of nutrients induced a negative taxonomic response, except for planariids, which responded positively. Generally, higher nutrients led to reduced taxa richness, EPT, sensitive taxa, and clingers.

Heavy metals in sediments and clam tissue were also related to macroinvertebrate structure and function as quantified by metrics and top taxa abundance. The most ecologically and statistically significant results showed that higher clam and sediment lead concentrations predicted negative responses in several metrics.

5) Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to that of some other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region.

We were able to make reasonable comparisons to the New River in West Virginia and the Susquehanna River in Pennsylvania. Both drain larger watershed areas than the Shenandoah River, but they have similar rocky bottoms, support smallmouth bass populations, and have not suffered fish kills like the Shenandoah River. Summary taxa lists from the three rivers were analyzed with Jaccard's coefficient, and the benthic macroinvertebrate fauna of the Shenandoah River was not very similar to that of either the Susquehanna or New Rivers, which were also not similar to each other. However, the nature of the difference in the fauna does not indicate that the biological condition of the Shenandoah River is bad. There are appreciably more taxa in the Shenandoah River (68) than the Susquehanna (43) or New (35). Many of the additional taxa in the Shenandoah belong to the insect orders Ephemeroptera, Plecoptera, and Trichoptera, which are mostly comprised of sensitive species. In summary, comparison of the benthic macroinvertebrates in the Shenandoah River to two other somewhat similar rivers in the mid-Atlantic region provides no evidence that the biological condition of the Shenandoah is lower than what would be expected.

6) Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to the historical biological condition of the same sections.

In order to consider long-term temporal differences in the biological condition of the Shenandoah River, we made comparisons to benthic macroinvertebrate data collected by Eugene Surber in the 1960s. He used quantitative methods that made it possible to compare results at seven large river sites sampled in 2006: three on the South Fork (Lynnwood, Whitehouse, Front

Royal), three on the North Fork (Mt. Jackson, Woodstock, Strasburg), and one on the Main Stem (Berryville). Seven of eleven assemblage-level metrics were significantly different between 1960s and 2006 data, and six of those seven metrics indicated better biological condition in 2006. The Bray-Curtis coefficient showed little to moderate similarity between 1960s and 2006 assemblages because there were higher densities and more taxa collected in 2006. Some of the additional taxa are sensitive to environmental stressors, and many of the taxa with much higher densities in 2006 are ones that benefit from the additional algae and fine detritus associated with increased nutrients. The number and types of taxa found in 2006 suggest improvemed biological condition relative to the 1960s.

7) Determine benthic macroinvertebrates that can be identified reliably and feasibly to the species level and can serve as indicator species for the range of biological condition observed in the Shenandoah River system.

We thought that a group of related benthic macroinvertebrates that could be identified to species might demonstrate differential, predictable responses to environmental stressors in the Shenandoah River better than the entire assemblage identified to genus and higher taxonomic levels. Adult riffle beetles (Elmidae) were common in the benthic samples and could be reliably identified, and, thus, were selected as the best candidate species.
In total we collected 14 species of adult elmid beetles. We conducted similar analyses as we did with assemblage-level information including ordination and regression. Elmid adults were useful in detecting differences among sites and relationships with land-use and environmental variables. However, differences among sites generally agreed with assemblage-level analyses, and regression relationships were generally weaker with elmid species data. It does not appear that increased taxonomic resolution of elmid species provides any additional information about the biological condition of the Shenandoah River and its tributaries.

8) Conduct *in situ* toxicity tests with the Asian clam (*Corbicula fluminae*) in the large river sections.

Results from the in-situ bioassays with the Asian clam in June and August 2006 showed significantly lower growth and significantly higher mortality at some sites in comparison to reference sites. However, the sites with significantly different growth or mortality did not follow a consistent spatial pattern and did not correspond with the sites with the greatest fish kills in 2006.

Conclusions

Analyses of benthic macroinvertebrate assemblages have not distinguished any large river sites in the Shenandoah basin with significant reduction in biological condition. The benthic macroinvertebrate assemblages exhibit very high densities and a great deal of taxonomic richness. There are some significant differences among sites, but there are no spatial patterns that correspond with a particular section, such as a fork, or to areas where fish kills have been more prevalent. The benthic macroinvertebrate assemblages at the large river sites fall within the range of what would be expected in similar rivers in the mid-Atlantic region and our comparisons with data from the 1960s show that biological condition has improved temporally.

However, the benthic macroinvertebrate assemblages have become somewhat out of balance, both taxonomically and ecologically. The taxa with exceptionally high densities show strong relationships with various forms of nitrogen and phosphorus that act as nutrients for plant growth. It is likely that high nutrients provide abundant food for macroinvertebrates that are scrapers, collector-filterers, and collector-gatherers. In addition, heavy growth of plant material on solid stable substrate creates excellent microhabitat for macroinvertebrates. While the present benthic macroinvertebrate assemblages at the large river sites do not indicate much, if any, impairment of biological condition, further increases in nutrient concentrations will eventually lead to lower dissolved oxygen, and reduced quantity and quality of food and microhabitat.

Moreover, the results of our benthic macroinvertebrate assemblage analyses at large river sites suggest that macroinvertebrate assemblages are not experiencing any influences similar to fishes. These results lend support to the hypothesis that the fish kills are primarily being caused by a factor specific to fish, probably a biological pathogen. However, results of fish pathology studies seem to indicate a diverse array of fish health problems, including parasites such as trematodes. Some trematodes use snails as intermediate hosts, and snails are one of the taxa whose density has increased greatly and is strongly related to increased nutrients. Other numerically dominant macroinvertebrates may also be involved in the life cycles of trematodes and other parasites that eventually infect fish.

Analyses of tributary streams according to subwatersheds were more informative than the large river studies for elucidating the factors responsible for macroinvertebrate assemblages. Unlike the large river sites, the biological condition of tributary sites ranged from good to poor. Assemblages in these smaller tributaries were strongly related to agricultural land use, including dairy, beef, poultry, and crops. Nutrients derived from land use were the driving force for determining macroinvertebrate assemblages. There was no clear evidence that toxic contaminants of any kind were a major influence on the benthic macroinvertebrate assemblages. It was obvious in some tributary streams that high nutrients had caused an appreciable decrease in biological condition. If nutrient concentrations continue to increase in more tributary streams, the impaired biological condition of the tributaries will eventually be manifested in the large river sections of the Shenandoah basin.

INTRODUCTION

Each spring since 2004, extensive fish kills have occurred in the Shenandoah River drainage. These fish kills have tended to occur at low rates but have lasted for extended periods over great distances. The magnitude of the fish kills has varied spatially each year. In 2004, the fish kills affected nearly the entire length of the North Fork of the Shenandoah River. In 2005, over 100 miles of the South Fork of Shenandoah River were impacted. In 2006, the fish kills were focused in the North Fork, but also occurred in a portion of the South River and the main stem Shenandoah River. In 2007, fish kills spread to the James River basin, including the Cowpasture River, Maury River, and main stem James, and also occurred throughout the Shenandoah River.

Benthic macroinvertebrates are key organisms for assessing the biological condition of freshwaters because: (1) there are many species within the natural assemblages of streams that

fill a wide variety of ecological niches and perform important ecosystem functions, especially in large, relatively shallow, rocky streams such as the Shenandoah River; (2) different species demonstrate a wide range of sensitivity to stressors; (3) they tend to be largely sedentary organisms that do not move away from sudden episodes of poor water quality or toxic substances; (4) their life history is sufficiently long (most require about 1 year for development from egg to adult) that their absence will be noticeable with a reasonable sampling schedule (*e.g.,* biannually); (5) some species are intermediate hosts for important fish pathogens that occur in some Shenandoah River fishes; and (6) their small body size and habitat spatial scale make it possible to take replicate samples, which facilitates detailed statistical analyses for elucidating relationships between organisms and environmental variables.

Beginning March 1, 2006, scientists at Virginia Tech were contracted by the Virginia Department of Game and Inland Fisheries (DGIF) to make a broad assessment of benthic macroinvertebrates in the Shenandoah River basin in coordination with other studies being conducted to explain the fish kills. The overall purpose of this study was to determine what the macroinvertebrate assemblage indicated about the biological condition of the Shenandoah River watershed. The two major questions were: (1) do macroinvertebrate assemblages differ spatially within the watershed; (2) what environmental variables are responsible for observed differences in macroinvertebrate assemblages? It was anticipated that comparisons of macroinvertebrate assemblage data with measurements of environmental variables would suggest testable hypotheses for causes of the fish kills. This report includes analyses and interpretations of samples taken in 2006 and 2007. In 2006, the study was confined to "large river" sites on the Shenandoah River and its major tributaries, with one site in the James River basin. In 2007, an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River was undertaken to examine the effects of specific land uses and environmental stressors. A lesser number of large river sites were also studied in 2007. The observed biological condition of the Shenandoah River, as indicated by benthic macroinvertebrates, was also compared to that of two other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region. Some information about long-term temporal changes in the biological condition of the Shenandoah River was obtained by comparing the present benthic macroinvertebrate assemblages with those observed about 40 years ago.

The specific objectives of this study were to:

1. Characterize the benthic macroinvertebrate assemblages in the large river sections of the Shenandoah River system and analyze for differences and similarities in biological condition among the sections;
2. Determine the environmental variables responsible for benthic macroinvertebrate assemblage structure in the large river sections;
3. Characterize the benthic macroinvertebrate assemblages in a set of subwatersheds that reflect the predominant types of land use within the Shenandoah River system and analyze for differences and similarities in biological condition among the subwatersheds;
4. Determine the types of land use and environmental variables responsible for benthic macroinvertebrate assemblage structure in the subwatersheds;

5. Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to that of some other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region.
6. Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to the historical biological condition of the same sections;
7. Determine benthic macroinvertebrates that can be identified reliably and feasibly to the species level and can serve as indicator species for the range of biological condition observed in the Shenandoah River system;
8. Conduct *in situ* toxicity tests with the Asian clam (*Corbicula fluminae*) in the large river sections.

METHODS

Study Sites

Large river

A field reconnaissance was conducted throughout the North Fork, South Fork, and Main Stem of the Shenandoah River in April 2006 to establish benthic macroinvertebrate sampling locations and develop sampling protocols. Nine benthic macroinvertebrate sampling sites were established in riffle areas in the immediate vicinity of previous fish kills and near locations where the Virginia Department of Environmental Quality (DEQ) was taking water quality samples. In addition, "reference" sites (sites where there had been no fish kills) were established on Cedar Creek and Cowpasture River. The locations of these sites are shown in Fig. 1, and information about geography, watershed size, and abbreviations for site names is summarized in Table 1.

Prior to the fish kills that began in 2004, no comparable benthic macroinvertebrate data were available from our fish kill sites, and as such, no pre-fish kill data were available for comparison. Therefore, we chose to use similar sites where there have been no reported fish kills as of 2006 as reference sites for comparison to the fish kill sites. The selection of reference sites for this study was challenging because locations in the basin where there had been no reported fish kills drain smaller watershed areas than fish kill sites. Based on discussions with DEQ, DGIF, and our best professional judgment, a large tributary to the North Fork Shenandoah (Cedar Creek) and the Cowpasture River in the James River basin were selected as reference sites. There had been no reported fish kills at these reference sites prior to and during our benthic studies in spring 2006. However, in 2007 and 2008, the fish kills became more extensive and included both reference sites. Thus, Cedar Creek and Cowpasture River can only be considered nominal reference sites.

Previous data from the New River in West Virginia and the Susquehanna River in Pennsylvania were added to our analyses as additional benchmarks for comparison. Also, historical data from the Shenandoah River collected by Eugene W. Surber in the 1960s were used for temporal comparisons.

Tributaries

After sampling the large river sites in 2006, it became apparent that it would be very difficult to explain any observed differences in benthic macroinvertebrate assemblage structure because all the large river sites would be subjected to all of the land uses and all of the potential environmental stressors in the Shenandoah River basin. Thus, we decided to conduct an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River that could be categorized according to predominant land uses. Twenty-five Shenandoah River tributaries were were selected using GIS, ranked based on poultry operations, dairy operations, and sewage treatment plants (STPs) within the watershed. Flowlines for the entire Shenandoah River watershed were obtained from the National Hydrography Dataset. The 78 subwatersheds (6^{th} level Hydrologic Unit) for the Shenandoah River in Virginia were obtained from the U.S. Department of Agriculture National Resources Conservation Service. These subwatersheds range from 9,800 to 39,800 acres in size, and typically represent the watershed of individual tributaries draining into the Shenandoah River. Locations of permitted poultry operations and STP discharges were obtained from DEQ, and locations of confined animal feeding operations were obtained from the Virginia Department of Conservation and Recreation (DCR). The subwatersheds were ranked for poultry operations based on both the number of poultry houses and the percentage of acreage in a nutrient management plan for poultry litter. Each poultry category was assigned a score of 1-5 based on quantile ranges of the data , and the scores were added to create the final poultry rank (2-10). The dairy operation rank (1-5) was determined using quantile ranges of the number of dairies in each subwatershed. The STP rank for each subwatershed was based on the presence or absence of a discharge. Subwatersheds were first classified based on the poultry rank, followed by dairy, then STP presence or absence. Reference sites (4) had the lowest poultry and dairy ranks and no STP discharges. Downstream (DS) sites typically drained one more subwatershed than the upstream sampling site on the same tributary. These sites were targeted based on the addition of one or more STP discharges. Specific ranks were not calculated for these sites, but the influence of confined animal feeding operations (CAFO) was anticipated to be similar to the upstream sites. The factorial sampling design is presented in Fig. 2. Finally, the land use for each of the 25 tributaries was quantified in more detail by calculating 20 variables using GIS and information from DEQ and DCR. All tributary sampling sites are shown in Fig. 3, and tributary names and site codes are listed in Table 2. Land use variables and codes are listed in Table 3.

Benthic Macroinvertebrate Sampling

In 2006, we sampled exclusively at large river sites. Benthic macroinvertebrates were sampled in spring 2006 during high baseflow conditions (May 11-13) and late summer 2006 during low baseflow conditions (August 15-17). In 2007, we sampled smaller tributaries between March 24 and April 23 and large river sites between May 22 and May 24. All sampling was stratified to riffle areas and was quantified. Six replicate benthic samples were taken with a D-frame dip net (500 µm) by disturbing a standard area of stream bottom (0.09 m^2). Contents of the net were preserved with 95% ethanol.

In the laboratory, benthic samples were sorted in their entirety (no subsampling) and individuals were identified (mostly to genus), enumerated, and measured to the nearest mm. Published

length-mass regressions were used to estimate invertebrate biomass in each sample (Benke *et al.* 1999). Specimens of emergent adult mayflies, adult riffle beetles, and snails were identified to species.

Environmental variables

Periphyton

During the August 2006 benthic collection, periphyton samples were collected from riffles in conjunction with benthic macroinvertebrate samples. Six cobbles were randomly collected from each study site and frozen until analysis. In the laboratory, periphyton was removed from each cobble with a wire brush and deionized water, and the resultant slurry was subsampled for chlorophyll *a* and epilithic biomass analysis. Epilithic subsamples were split and analyzed for chlorophyll *a* and epilithic biomass. The epilithic fraction was filtered onto preweighed glass fiber filters (0.45-µm) and dried to a constant weight at 60°C. After dry weights were obtained, filters were ignited at 550^{o}C for 24 hours, desiccated, and reweighed to obtain ash-free dry mass (AFDM). Chlorophyll *a* was extracted with 90% acetone and then analyzed with a spectrophotometer after correcting for pheophytin following the methods of Lorenzen (1967).

Inorganic substrate

Field estimates of inorganic substrate and deposited sediment occurred in conjunction with the August 2006 benthic collection. Bottom substrate was visually estimated within a 1 m^2 area of streambed at areas immediately adjacent to benthic macroinvertebrate sample locations. The proportion of stream bottom composed of one of four standard size classes (cobble, pebble, gravel, sand) was estimated. Additionally, the proportion of the stream bottom surface covered by fine deposits was visually estimated.

Chemistry

At large river sites, DEQ measured an array of water quality parameters that could potentially act as stressors to the biota (primarily pH, temperature, and various forms of nitrogen and phosphorus). All analyses were performed on samples from the water column. Water quality samples were taken frequently during spring and summer of 2006 and 2007 from all large river sites where macroinvertebrates were sampled. A random temporal sampling design provided water quality measurements from each site approximately every other day during the five usual working days (no weekend samples). For tributary sites sampled in 2007, DEQ provided all available data from their regular ambient water quality sampling program. We screened those data and used only those that were close enough spatially and temporally to provide meaningful comparisons with our macroinvertebrate data. After screening the DEQ data, there were no water quality data available for some of the tributary sites where benthic macroinvertebrate samples were collected. For comparison to large river and tributary macroinvertebrate data, which were collected on only a few dates in 2006 and 2007, it was necessary to condense the water quality data, which were collected on multiple dates, to a single value for each macroinvertebrate sampling date. Using the available data (which varied with each 'sample set') we calculated the average parameter value for the 2-week period preceding sample collection

and the maximum parameter value during the same period to generate two metrics (mean and maximum) for each water quality parameter.

In 2007, other variables were measured at the large river and tributary sites as part of a separate grant from the Virginia Environmental Endowment, including body burdens of metals in mollusk tissue, sediment metal concentrations, and estrogenic activity in sediment. Asian clams were collected during the spring sampling period for analysis of heavy metal body burdens (As, Cd, Cr, Pb, Se, and Hg). Immediately after collection, clams were placed in well water for 24 hr in order to clear their gut contents. The well water was tested by the Virginia Tech Soils Testing Laboratory for concentrations of the metals of interest and all were below the instrument detection limits. After 24 hr, the mollusks were killed by freezing. Prior to analysis, shells were removed and the tissue was freeze dried and homogenized. One sample was analyzed per site; the number of organisms pooled varied according to their size (average of 20). Clam tissue was analyzed at the College of William and Mary, through an agreement with the Virginia Institute of Marine Science (VIMS). The same laboratory analyzes metals in fish tissue for the DEQ. Results are reported as μg/g dry wt.

Sediment was collected from depositional areas of each sampling site for analysis of 23 target analyte list (TAL) metals. Sediment was collected using a stainless steel scoop, homogenized in a stainless steel pot, and placed in pre-cleaned glass jars. Sediment samples were analyzed by Hampton-Clarke Veritech Labs in Fairfield, NJ. Results are reported as mg/kg dry weight (equivalent to μg/g).

Sediment was also analyzed for estrogenic activity using a bioluminescent yeast estrogen screen (BLYES). Yeast (*Saccharomyces cerevisiae*) is bioengineered to contain the human estrogen receptor, plasmid-bound estrogen response elements, and plasmid-bound luminescence reporters (Sanseverino et al 2005). When an estrogen-like compound binds to the receptor, the luminescence reporters produce light. Specific compounds are not quantified, but the data provide an indication of the potential for sediment constituents to contribute to endocrine disruption in organisms. Sediment was collected as stated above and was freeze-dried and homogenized prior to analysis. Sediment was extracted with solvents, and the extracts were subjected to solid phase extraction (SPE) to remove some of the co-extracted organic material. The SPE extract was subjected to the BLYES assay. The assay was performed at the U.S. Geological Survey (USGS) laboratory in Leetown, WV, using a protocol developed at the facility with permission from the Center for Biotechnology at the University of Tennessee. Standards of 17β-estradiol (E2) were analyzed and used to construct a standard curve. Data are reported as pg/g dry wt. estradiol equivalents (E2Eq). Additionally, dried sediment was analyzed for concentrations (%) of total organic carbon (TOC) and total nitrogen (TN) by the Nutrient Analysis Laboratory at VIMS.

In 2007, DEQ provided data from passive samplers at most of the large river sites, except Mt. Jackson and Woodstock on the North Fork (DEQ 2008). Two types of passive samplers were deployed at each site. Semipermeable membrane devices (SPMDs) were deployed to sequester hydrophobic organic chemicals, and polar organic chemical integrative samplers (POCIS) were deployed to sequester more hydrophilic polar organics. Samplers were deployed for 42 days from late March to early May.

Comparison to Other Similar Large Rivers

In addition to examining for differences in benthic macroinvertebrates within the Shenandoah River, it was also the objective of this study to consider the biological condition of the Shenandoah River in comparison to other similar bodies of water in the mid-Atlantic region. It is difficult to find comparable data for large rivers, but we were able to make reasonable comparisons to the New River in West Virginia (Voshell et al. 1990, 1991) and the Susquehanna River in Pennsylvania (Jackson et al. 1994).

Comparison to Historical Shenandoah River Data

In order to consider the trend in the biological condition of the Shenandoah River over time, we made comparisons to benthic macroinvertebrate data collected by Eugene W. Surber in the 1960s (Surber 1965, 1967, 1969). He used quantitative methods that made it possible to calculate and compare the same measures of assemblage structure and function based on density that we used in this study as well as biomass. Surber reported biomass as wet weight determined from specimens recently blotted to remove excess fluid. We divided his wet weights by 4.12 to convert them to comparable dry weights determined in this study (Mason et al. 1983).

Indicator Species

Concurrent with May 2006 benthic sampling, recently emerged subimago and adult mayflies were collected from streamside vegetation. Representative snails were also hand-picked from stones at each study site for consideration as indicator species. All of these specimens were preserved separately in 70% ethanol for later identification to species in the laboratory. Adult riffle beetles (Elmidae) contained in the replicate benthic macroinvertebrate samples were also identified to species and enumerated.

In-Situ Toxicity Tests

Approximately 700 Asian clams, 8-12 mm in length, were collected from the New River at Eggleston, Giles Co., VA. As clams were collected, they were placed in a holding tank at the Freshwater Mussel Lab, operated by the Department of Fisheries and Wildlife Sciences at Virginia Tech. Clams were prepared for deployment to all study sites on June 20, 2006. Preparation for deployment involved measuring clams and placing them in mesh bags (5 clams per bag, 10 bags per site) (Soucek et al. 2000). Immediately after clams were packaged in mesh bags, they were placed in a cooler with water from the holding tank and driven to the study sites. Two riffles were selected at each study site for placement of mesh bags. A 2.5-ft length of rebar was driven into the streambed at each riffle. Five mesh bags were secured to the rebar. This resulted in 10 mesh bags at each study site, or 50 clams at each site.

The 30-day in situ toxicity test with Asian clams that was initiated on June 25, 2006 evolved into a 60-day study because of high flows throughout July and early August, so results from this test are preliminary. Because of the unsuccessful retrieval of clams after 30 days, a new 30-day in situ test was initiated on August 16, 2006. Again, high flows prevented terminating the second

experiment at 30 days, but we were able to retrieve the Asian clams successfully on September 23 (38 days). Upon retrieval, clams were counted for mortality and measured for growth.

Data Analysis

General descriptors

Data from benthic macroinvertebrate samples were organized into four separate data sets for statistical analysis: Large River Spring 2006, Large River Summer 2006, Large River Spring 2007, and Tributary Spring 2007. Raw data (densities of individual taxa) were summarized into 11 metrics that represent different ecological characteristics of assemblage structure and function (Table 4). For metric calculation, each taxon was assigned a pollution tolerance value (PTV), functional feeding group (mode of acquiring food based on morphology and behavior), and habit (how the organism moves or maintains its position in its environment; also called mode of existence). Assignments to these categories were made based on a synthesis of published literature (*e.g.,* Brigham *et al.*, 1982; Barbour *et al.*, 1999) and 30 years of data and professional experience in the aquatic entomology program at Virginia Tech. PTVs are commonly reported on a scale of 0 to 10, with 0 indicating very tolerant. In this study, taxa with PTVs of 0-2 were considered sensitive while taxa with PTVs of 8 – 10 were considered tolerant.

Ordination

Macroinvertebrate taxa composition was analyzed among sites simultaneously using multivariate methods for each data set. Detrended correspondence analysis (DCA), using PC-ORD (McCune and Mefford, 1999), without downweighting or axis rescaling, was used to ordinate benthic samples in species space using taxa densities. Only numerically dominant taxa (those that comprised > 0.2% of total macroinvertebrate abundance) were included in DCA to reduce variability in the dataset (Gauch, 1982). This analysis was used to determine similarity in taxa composition in benthic samples among the study sites during each sampling period and within each spatial category (within large river sites and within tributary sites).

Similarly, Canonical Correspondence Analysis (CCA) was used where possible to determine which, if any, environmental factors contributed to taxonomic ordination. CCA essentially overlays a second matrix onto the same taxa matrices used in DCA and calculates the percentage of total variance in the dataset explained by the second matrix by axes (typically 2). In some cases, CCA was not possible due to limited environmental data.

Linear regression

Linear relationships between benthic macroinvertebrates (metrics and individual densities for the most abundant taxa) and environmental variables were examined using regression analysis. Environmental variables were treated as independent variables and macroinvertebrate metrics and individual densities for the most abundant taxa were considered dependent variables. Habitat variables (*e.g.*, epileptic material, inorganic substrate) were available only for the August 2006 data. As such, 16 environmental variables, which included watershed size, measures of water chemistry, and habitat, were available to analyze relationships with macroinvertebrates in

August 2006, while only 12 environmental variables (watershed size and water chemistry variables) were available to analyze relationships with macroinvertebrates in May 2006. Environmental variables considered for 2007 large river and tributary data sets included DEQ nutrient data, passive sampler concentrations, and metal concentrations in stream sediments and Asian clam tissue. Regressions were tested for significance with $\propto = 0.05$. We used the coefficient of determination (R^2) for interpreting the strength of relationships in significant regressions. We considered $R^2 > 0.3$ to be ecologically relevant and $R^2 > 0.5$ to be especially meaningful.

Analysis of Variance (ANOVA)

ANOVA and Holm-Sidak tests were performed for each metric and the most abundant individual taxa to analyze differences in assemblage structure and function among the study sites. We hypothesized that the reference sites (Cedar and Cowpasture) might be different from the nine sites where fish kills had occurred during 2004-06 and that the North Fork sites might be different from other sites because the fish kills were greater there in 2006. ANOVA and Holm-Sidak tests compared each site to all other sites and evaluated the hypothesis that at least one site mean for a given metric or taxon differed among the other sites. Using the six replicates collected at each site allowed us to consider all possible groupings.

RESULTS AND DISCUSSION

Large River Assemblage Structure and Function

Taxa present

A list of all taxa collected at each of the 11 study sites in May and August 2006 is presented in Table 5. Combining May and August 2006 samples at all sites, 116 taxa were collected. Of that total, 106 taxa were collected in May versus 82 taxa in August. Thirty-one taxa were collected only in May, while five taxa were collected only in August.

The highest numbers of taxa were collected at the two reference sites: 72 at Cedar Creek and 67 at Cowpasture River. The lowest numbers of taxa were collected at two South Fork sites: 44 at Front Royal and 48 at Whitehouse. The remaining sites, including all of the North Fork sites, had intermediate numbers of taxa that were rather uniform among sites, ranging from 54 to 58. Thirty-five taxa occurred at almost all sites (defined as 10 or 11) in either May or August 2006. Forty-five taxa occurred at only a few sites (defined as 1 or 2) in either May or August 2006.

The presence or absence of particular taxa did not demonstrate any consistent trends among sites, either for individual sites or groupings of sites according to large tributaries, forks, main stem, or reference. This table of data did not reveal any trend for certain taxa to be either present or absent at the North Fork study sites below Burnshire Dam where the most severe fish kills were reported in 2006 (Woodstock and Strasburg).

General descriptors

Initial analyses of density by taxonomic composition to discern patterns among sites were restricted to numerically dominant taxa or 'top taxa'. Data from each analysis set are presented collectively in Table 6. Dominant taxa were determined by ranking the taxa at each site according to density from high to low and including only taxa that comprised more than 0.2% of total density for all taxa at a site. In May 2006, there were 36 top taxa, while in August 2006 there were 28 top taxa. In May 2007, the large river sites had 41 top taxa.

Approximately 60 metrics were calculated to summarize macroinvertebrate assemblages for spring and summer 2006 data (separately). These metrics were categorized according to the following ecological characteristics: density, richness/diversity/evenness, composition, tolerance, trophic, and habits. We eliminated redundant metrics (*i.e*., metrics that summarized similar aspects of macroinvertebrate assemblages) using Pearson product-moment correlation. Redundancy analysis is important to avoid repeating information already summarized by other metrics and to ensure accurate depiction of patterns by multivariate ordination techniques. Eleven metrics emerged as non-redundant, or unique, and were considered in further analyses (Table 4). These eleven metrics were calculated for each sample period: May 2006 (Table 7), August 2006 (Table 8), and may 2007 (Table 9).

Ordination

May 2006. Distinct site separation occurred along two axes in DCA for large river top taxa. Cowpasture River was separated from all other sites due to densities of *Rithrogena, Perlesta, Antherix,* Ceratopogonidae*, Chimarra, Protoptila, Optioservus, and Drunella tuberculata*. Strasburg, North River, and Woodstock formed a separate group, and this was attributed to densities of Planariidae, *Berosus*, and *Leptoxis carinata*.

DEQ nutrient measurements were available for spring 2006 including several nitrogen and phosphorus compounds. CCA ordination of taxa using nutrients as the second matrix revealed separation of Mount Jackson, Berryville, Harriston, and Front Royal from other sites due to higher concentrations of total phosphorus, orthophosphate, and lower ammonia (Fig. 4). The first two axes accounted for 36% of variation in the dataset. In this ordination, Cowpasture, White House, and Mount Jackson were separated from other sites, which clustered together. Nutrients explain the broad separation of these (Cowpasture, White House, and Mount Jackson) sites from other sites, the separation among other sites is not explained by nutrient information.

August 2006. Ordination (DCA) of top taxa by density indicated that Strasburg, Front Royal, White House, and Woodstock were separated from other sites due largely to higher densities of *Protoptila* and *Tricorythodes*. Berryville, Harriston, and Cowpasture were separated due to lower densities of *Isonychia* and *Macaffertium/Stenonema*. Lower densities of *Optioservus*, *Promoresia,* and *Leptoxis carinata* accounted for separation of the remaining sites.

CCA of taxa with DEQ nutrient data suggested that Berryville, Cowpasture, and Cedar Creek were different from other sites due to higher ammonia and total phosphorus and lower total nitrogen and nitrate concentrations (Fig. 5). Lynnwood and North River formed another separate

group based on differences in the same nutrients. Strasburg, Woodstock, and Whitehouse were different from other sites due to concentrations of total phosphorus, nitrate plus nitrite-nitrogen, and ammonia. Essentially, there appear to be two subtle nutrient gradients among sites but the absolute differences in nutrient concentrations still qualify as being moderate to high for all sites. We don't believe the subtle differences in these nutrient concentrations are of much value in explaining differences among sties.

CCA of taxa by substrate characteristics indicated that Woodstock, Lynnwood, North River, Whitehouse, and Harriston formed a distinct group separate from other sites due to higher proportions of cobble and higher epilithic biomass (AFDM) (Fig. 6). Other sites were fairly scattered, but differed not only by lower cobble and lower AFDM but also higher proportions of deposited sediment and gravel. This taxa versus substrate ordination suggests that some sites (Cowpasture, Cedar Creek, Strasburg, Berryville, and Mount Jackson) are characterized as having relatively smaller substrata (gravel, depositional sediments such as silt or detritus), whereas other sites (Harriston, Whitehouse, Lynnwood, Woodstock, and North River) were comprised of larger substrata having more epilithic biomass than other sites.

May 2007. Because only limited DEQ nutrient data (4 sites), passive sampler data (6 sites), and clam tissue/sediment heavy metal data (4 sites) were available for some large river sites, ordination analyses were not particularly useful in assessing differences among sites due to environmental conditions. CCA ordination suggested ammonia and phosphorus concentrations were important in separating sites but the absolute difference in concentrations among sites was very low (*e.g.,* nitrate concentration differed by 1 ppm at most among sites). Ordination using heavy metal and passive sampler data did not reveal patterns useful to determining differences among sites based on taxonomic composition. Generally, ordinations of May 2007 macroinvertebrate top taxa were weaker than 2006 analyses because fewer large river sites were sampled to facilitate extensive sampling in tributaries.

ANOVA

May 2006. In general, reference sites had higher Simpson's diversity index and the percent modified EPT indices, Cedar Creek had higher Total richness, although Mount Jackson actually had higher percent modified EPT index than reference sites. Reference sites also had higher percent non -insect taxa. This could be due to higher densities of pleurocerid snails that are algal grazers and were generally higher at North Fork sites. Similarly, reference sites typically had lower percent sensitive organisms, but some sites had very high percentages of sensitive organisms including Berryville, Front Royal, Harriston, and Lynnwood. North Fork sites did not adhere to a distinct pattern with respect to sensitive organisms. Reference sites also had higher percent modified scrapers with the exception of North River and Mount Jackson, which were similar to reference sites. Percent collector-gatherers, percent collector-filterers, and percent modified clinger metrics did not show a distinct pattern among the forks of mainstem. Percent Crawlers was significantly higher at reference sites, Mount Jackson, and North River.

August 2006. In general, New River sites had much lower density than Shenandoah River sites. Total richness, Simpson's diversity Index, percent modified EPT, and percent modified clingers were different between New River and Shenandoah sites but with no particular pattern. Percent

non-insects was higher in the New River except for Lynnwood, which was similar to New River. Other metrics showed no particular patterns with respect to the New River.

For each sampling season there were many significant ANOVAs, but no apparent patterns emerged to suggest that particular Shenandoah River sections were distinctly different. Shenandoah sites, in general, differed taxonomically from the Cowpasture River and Cedar Creek, but these differences could likely be the result of other factors than simple taxonomic composition.

Multimetric index

The Virginia Stream Condition Index (VSCI) is a multimetric index based on benthic macroinvertebrate assemblages that DEQ uses for monitoring biological condition of streams. The range of possible values for the VSCI is 0 to 100. The criterion for acceptable biological condition is a score ≥ 61, whereas a score ≤ 60 indicates impaired biological condition. VSCI scores for the 11 sites sampled in 2006 are plotted in Fig. 7. All scores for the August samples are well above the acceptable criterion and generally uniform. There is a much greater spread in the scores for the May samples, with two sites scoring below the acceptable criterion (Harriston and Berryville). In addition, five sites scored in the 60s just above the acceptable criterion in May (Lynnwood, Whitehouse, Front Royal, Woodstock, and Strasburg). Four sites in May had VSCI scores very close to those for August, all of which were well above the acceptable criterion (North River, Mount Jackson, Cedar Creek, and Cowpasture River).

The individual metrics that compose the multimetric VSCI are presented in Tables 10 and 11 for May and August, respectively. The VSCI was also calculated for two sites on the New River and compared to the August values from the Shenandoah River. Lower VSCI scores in May as compared to August are largely due to three individual metrics: percent top 2 Dominant Taxa, percent Chironomidae, and percent Ephemeroptera. Higher values for the first two metrics indicate lower biological condition, whereas, higher scores for percent Ephemeroptera indicate higher biological condition. The trend for these three metrics was pronounced at Harriston and Berryville in May, leading to the VSCI score at those two sites being slightly below the criterion for acceptable biological condition.

The VSCI scores indicate that the biological condition of the Shenandoah River compares favorably to the New River, for which major fish kills have not been reported. The reference sites for this study demonstrated biological condition consistently well above the acceptable criterion. Considering the sites according to forks and main stem, showed only minor differences. The only sites for which the VSCI fell slightly below acceptable were on the South Fork (Harriston) and the Main Stem (Berryville) in May. All three North Fork sites had very high VSCI scores in August. In May, The two North Fork sites with the greatest fish kills in 2006 (Woodstock, Strasburg) exhibited lower VSCI scores than in August, but the scores remained above the acceptable criterion. The most upstream North Fork site (Mount Jackson) had similarly high VSCI scores in May and August.

VSCI scores for the Shenandoah and New Rivers should be interpreted cautiously because the watershed areas at the study sites (Table 1) are much larger than the sites in the database used to

develop the VSCI. Excluding the reference sites on Cedar Creek and Cowpasture River, the watershed areas of the Shenandoah sites ranged from 131,411 ha (≈ 500 square miles) at Mount Jackson to 746,877 ha (≈ 2,900 square miles) at Berryville. In contrast, the database for the probabilistic reference sites used to develop the VSCI contained only 3 sites with a watershed area ≥500 square miles and the site with the largest watershed area in the database was only 656 square miles. The sites on the New River in West Virginia drain an even larger watershed (≈ 6,600 – 6,700 square miles). Only the reference sites on Cedar Creek and Cowpasture River had watershed areas (157 and 318 square miles, respectively) that were somewhat representative of the database for the probabilistic reference sites.

Biomass

General descriptors. As was done for density, the biomass of the benthic macroinvertebrate assemblage was examined primarily according to dominant taxa (Table 12). Biomass was only measured in May. The grand mean for total biomass in all May samples (n = 66) was 6354 mg dry mass per m^2. The two sites with the highest mean biomass were Whitehouse and Woodstock, while the two sites with the lowest mean biomass were Harriston and Berryville. There did not appear to be any pattern for forks, reference, or fish kill sites.

Ordination. Detrended correspondence analysis explained 31% of the variation in May 2006 invertebrate biomass data. Most of the site separation was along axis 1, which explained 23% of the variation. The groupings that emerged from the ordination based on biomass data supported the results from the ordinations based on density data. For instance, ordinations based on density also indicated that Cowpasture had a different invertebrate composition, and some of the taxa that that separated Cowpasture based on density data (*Rithrogena*, *Acroneuria*, *Corydalus*, *Chimarra*), also separated Cowpasture based on biomass data.

Metrics. Comparisons among various site groups using metrics based on biomass provided very similar results as comparisons using metrics based on density. The most effective use of the biomass data was for comparing historical data from the 1960s with 2006 data.

Large River: Relationships Between Macroinvertebrates and Environmental Variables

Generally, linear regression was used to assess relationships between macroinvertebrate metrics and top taxa with environmental variables. We used linear regression as a follow up analysis to CCA, where ordination suggested key environmental variables to use in regressions and helped guide decisions about which environmental variables might be most important in determining assemblage structure. CCA in essence was used as a screen to guide regression analysis, but we did not restrict ourselves to relationships indicated by CCA. We also relied on our professional expertise in aquatic ecology to choose other regression analyses that had the potential to be ecologically meaningful for the objectives of this study. The types of environmental variables selected changed with sampling time (May 2006, August 2006, and May 2007).

May 2006

Only DEQ nutrient data were available for spring 2006 analysis. Many relationships were significant (*i.e.*, $p > 0.05$) but we chose to focus on relationships where the coefficient of determination (R^2) was greater than 0.3, or more than 30% of the variation in the dependent variable (macroinvertebrate metric or taxon) was explained by the independent variable (environmental parameter).

Five significant regressions fit our criteria during May 2006 (Table 13). Higher total phosphorus concentrations caused a negative response with percent modified EPT index across sites ($p=0.029$, $R^2=0.428$). Similarly, Simpson's diversity index was negatively influenced by ammonia ($p=0.038$, $R^2=0.397$), total Kjeldahl nitrogen ($p=0.031$, $R^2=0.420$), and total phosphorus ($p=0.039$, $R^2=0.395$). Percent Crawlers was also lower when ammonia concentration was higher ($p=0.05$, $R^2=0.362$). Otherwise, no ecologically significant relationships with assemblage metrics existed during May 2006.

Regarding individual taxa, *Optioservus* responded negatively to the maximum N-N concentration ($p=0.003$, $R^2=0.636$) during May 2006. No other top taxa responded significantly to May 2006 environmental conditions.

August 2006

A greater number of significant relationships were detected when we compared August 2006 environmental variables with metric and taxonomic responses (Table 14). Total density and percent non-insect metrics were higher with higher epilithic biomass (AFDM). Higher nitrogen concentrations (as estimated by different nitrogen parameters including N-N, Nitrate, etc.) were associated with higher density, Simpson's diversity, and percent modified clingers. Similarly, higher phosphorus led to higher diversity and percent modified clingers. Lastly, chlorophyll-a concentration had a positive effect on the percent sensitive organisms.

Regarding individual taxa, *Corbicula* responded positively to substrate size, nitrate concentration, total nitrogen, ammonia, and total phosphorus in August 2006. The amount of epilithic AFDM had a positive effect on *Baetis*, *Leptoxis*, *Optioservus*, and Planariidae but a negative effect on *Hydropsyche* in August 2006. Cobbles, which are larger more stable substrata, positively influenced *Isonychia*, while gravel had a negative effect on *Isonychia*, *Hydropsyche*, and Planariidae. Deposited sediments, or silt, had a positive effect on *Tricorythodes*.

In general, biological materials on the substrate (algae and microbes as reflected by epilithic chlorophyll a and AFDM) better predicted macroinvertebrate responses than the physical nature of the substrate (cobble, gravel, fines). The explanation lies in the increased food and microhabitat provided by the growths of biological materials on the substrate, which are stimulated by nutrients.

Several macroinvertebrate summary metrics and top taxa were significantly related to DEQ nutrients (Table 15). *Stenelmis* and *Leptoxis* densities responded positively to nitrite, nitrate-nitrogen, and ammonia. *Baetis* and *Macaffertium/Stenonema* densities were higher with higher Kjeldahl nitrogen, phosphorus, and orthophosphate. *Hydropsyche* were more abundant with higher N-N. All of these relationships can be explained by the nutrients stimulating growths of biological materials on the substrate, which increased food and microhabitat for these particular macroinvertebrates. We also assessed relationships between passive sampler variables and clam/sediment metals, but these analyses were weakened by lack of data for large river sites in 2007. In general, there were no obvious relationships between macroinvertebrates and metals or other potential contaminants, nor were there any signs that these chemicals were affecting biological condition at the large river sites. Typically, concentrations of contaminants were at or slightly above detection limits and we do not know whether macroinvertebrates would respond at these levels.

Comparison to Other Similar Large Rivers

In addition to examining for differences in benthic macroinvertebrates within the Shenandoah River, it was also the objective of this study to consider the biological condition of the Shenandoah River in comparison to other similar bodies of water. It is difficult to find comparable data for large rivers, but we were able to make reasonable comparisons to the New River in West Virginia (Voshell et al. 1990, 1991) and the Susquehanna River in Pennsylvania (Jackson et al. 1994). Both drain larger watershed areas than the Shenandoah River, but they have similar rocky bottoms, support smallmouth bass populations, and have not suffered fish kills like the Shenandoah River. Summary taxa lists from the three rivers are presented in Table 16. Jaccard's coefficient was used to analyze the similarity of the three rivers based on the presence or absence of taxa. Values for this coefficient range from 0 to 1, with 1 indicating high similarity. The results were as follows:

Shenandoah compared to Susquehanna	0.3924
Shenandoah compared to New	0.3973
Susquehanna compared to New	0.3929

Thus, the benthic macroinvertebrate fauna of the Shenandoah River was not very similar to that of either the Susquehanna or New Rivers, which were also not similar to each other. However, the nature of the difference in the fauna does not indicate that the biological condition of the Shenandoah River is degraded. There are appreciably more taxa in the Shenandoah River (68) than the Susquehanna (43) or New (35). The two sites on the South Fork of the Shenandoah River with the lowest numbers of taxa (Front Royal with 44 and Whitehouse with 48) still had higher numbers of taxa than the Susquehanna or New Rivers. Many of the additional taxa in the Shenandoah belong to the insect orders Ephemeroptera, Plecoptera, and Trichoptera, which are mostly comprised of sensitive species. Within the Ephemeroptera, these include two members of the family Ephemerellidae: *Drunella turberculata* and *Ephemerella*. Within the Plecoptera, Chloroperlidae, *Leuctra*, *Agnetina*, and *Perlesta placida* only occur in the Shenandoah River. Among these three orders, Trichoptera contains the most taxa (9) that only occur in the

Shenandoah River. In addition, there are two sensitive genera of Coleoptera that only occur in the Shenandoah River: a riffle beetle, *Promoresia*, and a water penny, *Ectopria*. In summary, comparison of the benthic macroinvertebrates in the Shenandoah River to two other somewhat rivers in the mid-Atlantic region provides no evidence that the biological condition of the Shenandoah is lower than what would be expected.

Comparison to Historical Shenandoah River Data

In order to consider long-term temporal differences in the biological condition of the Shenandoah River, we made comparisons to benthic macroinvertebrate data collected by Eugene Surber in the 1960s (Surber 1965, 1967, 1969). He used quantitative methods that made it possible to compare total taxa richness and density, density of individual taxa, total biomass, and biomass of mollusks versus non-mollusks. We were able to compare seven of our sites sampled in 2006 to sites sampled previously by Surber: three on the South Fork (Lynnwood, Whitehouse, Front Royal), three on the North Fork (Mt. Jackson, Woodstock, Strasburg), and one on the Main Stem (Berryville). We used recent data collected in May 2006 and matched it with data collected by Surber at a similar time of year (April and June). This provided two replicate samples at each of the seven sites for the Surber data. We randomly chose two of our six replicate samples at each site in May 2006 for comparison.

In the 1960s Surber considered the North Fork to be "relatively unpolluted." He further stated: "Unlike the South Fork, the North Fork water is very clear throughout most of the year. Instead of abundant phytoplankton algae growths, the North Fork has been characterized by profuse growths of filamentous algae such a *Hydrodycton*." Although the South Fork from above Elkton to Front Royal received wastes, such as the Virginia Oak Tannery at Luray and inadequate sewage treatment plants at Stanley, Shenandoah, and Luray, the biota was "relatively unaffected by the wastes entering it." However, there were several large sources of pollution at Front Royal that seriously affected about one-third of the 34.9-mile section of the Main Stem in Virginia. These sources of pollution were: a viscose rayon plant (FMC Corp, Viscose Division), an Allied Chemical Company plant, a food processing plant (Old Virginia, Inc.), and an inadequate sewage treatment plant. The effects of pollution from these sources were sporadic among years for benthic macroinvertebrates and fish. Pollution from Front Royal caused serious fish kills in 1966, 1968, and 1969 and small kills in every year in late winter. Surber's macroinvertebrate data that were used for comparison were not associated temporally or spatially with any of the 1960s fish kills.

The same list of non-redundant macroinvertebrate assemblage metrics was calculated for the samples collected by Surber and compared to the samples from 2006 at each site (Table 17). Paired *t*-tests were used to assess differences in summary metrics between the historical samples and Virginia Tech samples. When compared as three site groups (North Fork, South Fork, Mainstem) no significant differences in metrics were detected. When considered as seven individual sites, several significant differences between dates emerged. Simpson's diversity index ($p=0.031$, $t=-36.501$), percent modified EPT ($p=0.003$, $t=-3.710$), percent scrapers ($p<0.001$, $t=-14.953$), and percent modified clingers ($p=0.004$, $t=-20.670$) were higher in 2006. All of the preceding metrics being significantly higher would indicate better biological condition in 2006. Percent Collector-gatherers ($p=0.006$, $t=19.274$), percent collector-filterers ($p=0.047$, t

=15.620), and percent crawlers (p=0.030, t =8.464) were lower in 2006. The first two of the three preceding metrics being significantly lower would also indicate better biological condition in 2006. In summary, four of the eleven assemblage metrics were not significantly between the 1960s and 2006, and of the seven metrics that were significantly different, six indicated better biological condition in 2006.

We also analyzed the densities of all individual taxa in the historical and Virginia Tech samples (Table 18). We began by calculating the Bray-Curtis similarity coefficient to assess the similarity of the entire assemblage of individual taxa (densities) at each site between the 1960s samples and the May 2006 samples.

	Site	Bray-Curtis Coefficient
NF		
	Mt. Jackson	0.447445
	Woodstock	0.345216
	Strasburg	0.694632
SF		
	Lynnwood	0.290172
	Whitehouse	0.437419
	Front Royal	0.539177
MS		
	Berryville	0.420468

General guidelines for interpreting Bray-Curtis coefficients of similarity are: > 0.7 indicates assemblages are similar; < 0.5 indicates assemblages are not similar; 0.5 – 0.7 indicates no conclusions about similarity. Almost all of the Bray-Curtis coefficients above indicate that the assemblages sampled in May 2006 were not similar to those sampled at the same sites in the 1960s. No site had similar benthic macroinvertebrate assemblages; Strasburg and Front Royal exhibited the most similarity but only fell in the inconclusive zone. However, the lack of similarity does not indicate a decline in biological condition in 2006 as compared to the 1960s, and actually can be interpreted as better biological condition in 2006.

There were considerably more taxa in 2006 as compared to the 1960s (65 versus 52, respectively; Table 18). Most of the additional taxa that appeared on the list in 2006 are somewhat sensitive to environmental stressors. This is especially true for most of the Ephemeroptera (*e.g.*, *Maccaffertium/Stenonema*, *Isonychia*), Plecoptera (*e.g.*, *Leuctra*, *Agnetina*), and Trichoptera (*e.g.*, *Brachycentrus*, *Protoptila*, *Helicopsyche*, *Lepidostoma*, *Chimarra*). A notable exception to the trend of more sensitive taxa is the conspicuous increased density of Planariidae (flatworms), which are very tolerant of environmental stressors. There were also conspicuous increases in densities of quite a few taxa that are considered facultative to environmental stressors: *Leptoxis carinata* (snail), *Baetis*, *Ephemerella*, *Tricorythodes*, *Corydalus cornutus*, *Cheumatopsyche*, *Hydropsyche*, Hydroptilidae, Chironomidae, and Elmidae. With the exception of *Corydalus cornutus*, the increased density of these taxa can be attributed to increased production of algae, either periphyton or plankton, stimulated by increased nutrients. Some feed on periphyton on rocks (*Leptoxis carinata*, *Baetis*, Hydroptilidae, Elmidae), some feed on algae or detritus suspended in the water (*Cheumatopsyche*, *Hydropsyche*), while others feed on fine detritus that is derived from dead algae and deposited on the bottom (Planariidae, *Tricorythodes*, Chironomidae). Most of the

aforementioned taxa also increase because the excess plant growth on rocks stimulated by increased nutrients creates an especially favorable microhabitat for hiding from predators and coping with fast current. The invertebrate predator *Corydalus cornutus*, has likely increased in density because of the increase in prey organisms.

In summary, the available data on macroinvertebrates indicates that the biological condition at large river sites in the Shenandoah River basin is no worse, and may be better, than it was 40 years ago. The number of taxa and most of the types of dominant taxa collected in 2006 suggest improvement in conditions relative to the 1960s. However, most of the dominant taxa are likely to have high densities because of high nutrient concentrations that stimulate high algae production.

Subwatersheds: Assemblage Structure and Function

General descriptors

We calculated the same eleven non-redundant metrics for macroinvertebrate assemblages collected May 2007 in 26 Shenandoah River tributaries representing different subwatersheds (Table 19). Generally, Total density in tributaries varied widely but did not appear different from large river sites. Taxa richness was only slightly lower at most tributary sites but was very low at a few sites (e.g., Cook's Creek, North River headwaters, Naked Creek – Page Co., Stony Creek headwaters, Linville Creek). Simpson's diversity index is not usually as low as 0.5 or below, but this occurred at some tributary sites (e.g., Linville Creek, Mill Creek – South Fork, Long Glade Creek, Naked Creek – Augusta Co., Stony Creek downstream). The percent modified EPT was at on near zero for several tributaries, mostly the same ones mentioned for the previous metrics. In general, assemblage metrics indicated that the tributary sites represented a wide range of biological conditions, from good to poor, which was the goal of the study design.

Ordination

To assess site groupings and general similarities/differences among the tributary sites we used a combination of Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) ordination techniques. Again we truncated the total taxa list to include only the top 0.2% to eliminate rare taxa that only contribute noise to these types of statistical analyses. In each case tributary sites were arranged according to relative abundances of the top taxa. Then a second matrix (taxa in DCA, environmental
variables in CCA) was used to identify variables that contribute to site separation. Using a second matrix, or data set, ordination allows consideration of parameters that explain separation of data in the first matrix.

We first projected top taxa abundances in a DCA ordination and used a biplot (first and second matrices the same) to show which taxa were most responsible for site separation (Fig. 8). *Prosimulium* were absent at most sites but abundant at Briery Branch and Passage Creek (reference site), which contributed to separation of these sites. Other sites formed two groups based on relative abundances of *Simulium* and *Ephemerella*. These two taxa were 'top taxa', present in nearly all samples, and had varied abundances across tributary sites. A second

reference site, Cedar creek headwaters, was separated from the main cluster of sites along axis two due to a much higher abundance of *Simulium* than other sites. We consider axis two to better explain site differences because axis one was important largely due to *Prosimulium* being present at only five sites. Abundances of *Ephemerella* were especially useful in grouping sites based on density. Some sites had *Ephemerella* densities between 0 and 100 individuals per meter squared, whereas another group of sites ranged from 100 to 2500 individuals per meter squared.

As a follow up ordination we reanalyzed the same data as above but eliminated the outlier site Passage Creek and the outlier taxon *Prosimulium* to better observe differences among the remaining sites (Fig. 9). In this ordination the two axes explain nearly 50% of the variation among sites related to taxa abundance and land use. Several more distinct patterns are apparent with six taxa explaining differences in sites and greater resolution of grouping structure among sites. Removal of outliers exposed patterns among sites whereby key taxa explained major differences among sites distinguishing groups. Planariidae were disproportionately more abundant at Cooks creek and Long Meadow Run which caused separation of these two sites. *Cheumatopsyche* were most abundant at Hawksbill Creek downstream, Holmans creek, and Naked Creek – Page Co. *Chimarra*, *Ephemerella*, and *Maccaffertium/Stenonema* were more abundant at Christians Creek, Smith Creek headwaters, Back Creek, and Smith Creek downstream. Chironomid midges were most abundant at a group of sites including Jennings Creek, Linville Creek, and both Mill Creeks (see lower left quadrat Fig. 9) and explained why these sites were distinct.

CCA ordination of tributary sites, top taxa abundance, and land-use variables shows distinct separation of some sites as well as a clustering of a large number of sites (Fig. 10). Axis 1 explains 36% of the variation among sites, which is a high amount for CCA. Cook's Creek and Smith Creek downstream were the most distant from other sites on the left of axis 1, and the most likely land-use variables separating these sites were high number of animal feeding operations, number of dairy farms, and percent of cropland in the subwatersheds. There was another distinct group of four sites on the right side of axis 1: Passage Creek, Gooney Run, Cedar Creek headwaters, and Back Creek. Three of these sites are reference sites. The positioning of these sites by CCA is the result of low number of animal feeding operations, number of dairy farms, and percent of cropland in the subwatersheds. Thus, these land uses reduce the biological condition of tributary streams.

CCA ordination of tributary sites, top taxa abundance, and sediment chemistry (metals, nitrogen, organic) shows two reference sites separating on the left of axis 1 (Back Creek, Gooney Run) and several non-reference streams on the right of axis 1 (Cooks Creek, Muddy Run, Naked Creek – Augusta Co.; Fig. 11). This separation is produced by high copper, lead, nitrogen, and organic carbon in the sediment at the non-reference sites and *vice versa* for the reference sites. Linville Creek, Hawksbill Creek downstream, Christian's Creek, Stony Creek headwaters, Meadow Creek, Jennings Creek, and Cedar Creek headwaters (a reference site) were separated from remaining sites around the middle of the sediment metals/nutrient gradient. It is possible that assemblages at these sites were responding positively to nutrient enrichment and higher total organic carbon but negatively to sediment copper and lead although it is difficult to determine from CCA alone.

CCA ordination of tributary sites, top taxa abundance, and clam metal concentration also showed a large cluster of sites in the middle surrounded by several outlying sites (Fig. 12). Cedar Creek headwaters and downstream (both reference sites), Passage Creek, Hawksbill Creek downstream, and Meadow creek were separate from other sites. Passage and Hawksbill appeared to separate due to higher clam tissue mercury concentrations, whereas Cedar Creek headwaters and downstream sites separated according to concentrations of clam tissue cadmium and selenium. It is not apparent what influence these heavy metals have on macroinvertebrate assemblages, and these results may not have ecological relevance.

In summary, ordination of tributary sites shows distinct gradients that differentiate sites. Land-use appears to be more important in differentiating sites than specific sediment chemistry variables. All forms of agricultural activity in the subwatersheds appear to influence assemblage structure and function, but these ordinations do not explain the mechanistic causes of these differences or whether specific effluent or sediment runoff is responsible.

Subwatersheds: Relationships Between Macroinvertebrates and Land Use, Environmental Variables

Additional data were collected in tributaries in May 2007 including DEQ nutrients, passive sampler chemistry including 'emerging contaminants', sediment metals and nutrients, clam tissue heavy metal concentration, DEQ fecal coliform and *e. coli* concentrations, and subwatershed land-use measures. Our assessment of assemblage responses to environmental conditions was thus more intensive than in previous analyses. Again we allowed CCA results to prioritize our regression analyses by focusing initially on environmental variables that were important in ordination. Secondarily we also compared macroinvertebrate metrics and top taxa with environmental variables where it was reasonable to hypothesize causal relationships.

Fourteen land-use variables were considered potentially influential to macroinvertebrate assemblage structure and function. We use linear regression to assess relationships between eleven macroinvertebrate metrics and 40 top taxa. There were 49 significant regressions ($p < 0.05$) (Table 20). Often, regressions involving percent Forest showed an inverse relationship with regressions involving percent pasture and hay, percent cropland, and percent developed land. Usually macroinvertebrates responded positively to increased percent forest and negatively to increases in percentage of land area used for different types of agriculture or development. Percent Non-insects, however, responded positively to increases in percentage of land area used for different types of agriculture or development and negatively to increased percent forest. The number of dairy farms, cattle farms, and poultry houses was frequently a predictor of reduced biological condition as quantified by macroinvertebrate assemblage metrics.

The strongest regressions showed that percent collector-filterers were more abundant in forested areas and less abundant in areas characterized by pasture and hayfields. Similarly, the percent modified EPT index was lower in pasture and hayfield areas and higher in forested sites. The highest level of prediction showed that higher numbers of cattle farms, dairy farms, poultry houses, and animal feeding operations led to higher percent non-insects.

In addition to summary assemblage metrics, several individual top taxa responded significantly to subwatershed land use (Table 21). Planariidae responded positively to percent cropland and negatively to percent forest. Similarly, Planariidae responded positively to number of poultry houses, animal feeding operations, and nutrient management plans for poultry waste. *Hydropsyche* also responded positively to what is usually considered detrimental watershed conditions, the number of beef cattle and poultry houses.

A number of other environmental variables were also compared to macroinvertebrate assemblage metrics and top taxa producing 42 significant relationships (Table 22). Several forms of nitrogen and phosphorus predicted metric and top taxa responses including nitrate, nitrite, ammonia, and phosphorus. Typically relatively higher concentrations of these nutrients induced a negative taxonomic response (*i.e.*, taxa abundance decreased). Exceptions were Planariidae responded positively to ammonia, and *Antocha* responded positively to phosphorus. It is unknown whether these organisms prefer enriched conditions or are simply better than other organisms at tolerating high nutrient concentrations. Generally, higher nutrients led to reduced Taxa richness, percent modified EPT, percent sensitive organisms, and percent clingers. We believe nutrients are stimulating primary production on rock surfaces which, in turn, contributes to food and microhabitat availability.

Heavy metals in sediments and clam tissue were also related to macroinvertebrate structure and function as quantified by metrics and top taxa abundance (Table 22). In some cases metal concentrations induced positive responses in macroinvertebrate abundance. In all of these cases the concentration of metal was at or barely above the limit of detection and whether any ecological influence would occur is unknown. Although the direction of relationships was mixed, we expected metals to induce negative invertebrate responses and focus on those results here. The most ecologically and statistically significant results showed that higher clam and sediment lead concentrations predicted negative responses in percent modified EPT, percent sensitive organisms, percent collector-filterers, and percent crawlers.

In summary, regression analysis provided further evidence that the presence of agricultural activity in subwatersheds, various forms of nitrogen and phosphorus, and heavy metals can predict macroinvertebrate structure and function.

Indicator Species

Initially, mayflies, especially members of the family Ephemerellidae, were thought to be good candidates for benthic macroinvertebrates that could be identified to species and might demonstrate differential, predictable responses to environmental stressors in the Shenandoah River. However, there were not many species of ephemerellids, and many early instar nymphs could not be reliably identified. Adult riffle beetles (Elmidae) were common in the benthic samples and could be reliably identified to species. There appeared to be a sufficient number of different species to make this group worthwhile for investigating indicator species. It is possible that increased taxonomic resolution identifies stronger relationships with environmental variables.

In total we collected 14 species of adult elmid beetles in 2007 (Table 23). We conducted similar analyses as we did with assemblage-level information including ordination and regression. In large rivers (2007 data), DCA ordination of elmid species and abundance indicated four distinct groups of sites. Cowpasture and Cedar formed one group, Whitehouse, Mount Jackson, Strasburg, and Woodstock formed a second group, and North River and Cootes Store were separated from all other sites and each other (Fig. 13).

Tributary elmids were also projected in a CCA ordination to examine the influence of land-use in separating sites (Fig. 14). Cooks and Long Meadow Run were separated along axis 1 due to higher numbers of animal feeding operations, poultry houses, and beef cattle per acre. No distinct grouping of other sites was apparent.

Elmid species data were also used in linear regression analyses to detect relationships between land use and environmental variables. Although some relationships were significant, most were weak ($R^2 < 0.3$), and patterns appeared to be caused by outlier points.

Elmid adults were useful in detecting differences among sites but usually just agreed with analyses of the entire assemblage. In general, it does not appear that increased taxonomic resolution of elmids to species provided any more ability to detect differences among sites or to enhance our ability to detect relationships between macroinvertebrate assemblages and environmental variables.

In-Situ Bioassays

Growth

Results from the in-situ tests with the Asian clam (June and August) showed significant differences in growth between the Fish kill and Reference site groups. Asian clams in the Fish kill group of sites consistently showed reduced growth rates relative to Asian clams in the Reference group (Fig. 15). However, based on results from ANOVA, when each site was considered an individual group, Mt. Jackson and Front Royal were the only sites that had significant differences in Asian clam growth in June 2006, and Mt. Jackson and Woodstock were the only sites that had significant differences in Asian clam growth during August 2006 (Fig. 16). During both tests, Asian clams at Mt. Jackson had the highest growth rates. Although growth rates at Woodstock were not detected as significantly different from most sites, it appears that growth at Woodstock was reduced.

Mortality

Mortality responses were very similar to growth responses; there was significantly higher Asian clam mortality in the Fish kill site group than the Reference group during both in-situ tests (Fig. 17). However, ANOVA indicated that significant differences in mortality among sites only occurred in June (Fig. 18). During June, highest mortality occurred at White House. Asian clam mortality at White House was significantly higher than mortality at North River, Harriston, Lynnwood, Woodstock, Berryville, and Cowpasture.

SUMMARY AND CONCLUSIONS

Analyses of benthic macroinvertebrate assemblages have not distinguished any large river sites in the Shenandoah basin with significant reduction in biological condition. The benthic macroinvertebrate assemblages exhibit very high densities and a great deal of taxonomic richness. There are some significant differences among sites, but there are no spatial patterns that correspond with a particular section, such as a fork, or to areas where fish kills have been more prevalent. The benthic macroinvertebrate assemblages at the large river sites fall within the range of what would be expected in similar rivers in the mid-Atlantic region. Comparisons of the benthic macroinvertebrate assemblages at large river sites in the 1960s with those of 2006 show that there are now more taxa and higher densities, thus, biological condition has improved temporally. However, the benthic macroinvertebrate assemblages have become somewhat out of balance, both taxonomically and ecologically. The taxa with exceptionally high densities show strong relationships with various forms of nitrogen and phosphorus that act as nutrients for plant growth. It is likely that high nutrients stimulate algae in the water and on solid substrates. Death and decomposition of algae creates abundant fine detritus along with microbes. Thus, high nutrients provide abundant food for macroinvertebrates that are scrapers, collector-filterers, and collector-gatherers. In addition, heavy growth of plant material on solid stable substrate creates excellent microhabitat for macroinvertebrates. While the present benthic macroinvertebrate assemblages at the large river sites do not indicate much, if any, impairment of biological condition, further increases in nutrient concentrations will eventually cause a decline in biological condition because of over production of algae. This will eventually lead to lower dissolved oxygen, and reduced quantity and quality of food and microhabitat.

Moreover, the results of our benthic macroinvertebrate assemblage analyses at large river sites suggest that macroinvertebrate assemblages are not experiencing any influences similar to fishes. These results lend support to the hypothesis that the fish kills are primarily being caused by a factor specific to fish, probably a biological pathogen. However, results of fish pathology studies seem to indicate a diverse array of fish health problems, including parasites such as trematodes. Some trematodes use snails as intermediate hosts, and snails are one of the taxa whose density has increased greatly and is strongly related to increased nutrients. Other numerically dominant macroinvertebrates may also be involved in the life cycles of trematodes and other parasites that eventually infect fish.

Analyses of tributary streams according to subwatersheds were more informative than the large river studies for elucidating the factors responsible for macroinvertebrate assemblages. Subwatershed tributary analysis facilitated spatially explicit analysis of land-use variables in addition to instream environmental variables. Unlike the large river sites, the biological condition of tributary sites ranged from good to poor. Assemblages in these smaller tributaries were strongly related to agricultural land use, including dairy, beef, poultry, and crops. Biological condition declined in relation to increases in these agricultural land uses, but there did not appear to be a difference in the decline of biological condition according to the particular type of agricultural land use. Nutrients derived from land use were the driving force for determining macroinvertebrate assemblages. There was no clear evidence that toxic contaminants of any kind were a major influence on the benthic macroinvertebrate assemblages. It was obvious in some tributary streams that high nutrients had caused an appreciable decrease

in biological condition. If nutrient concentrations continue to increase in more tributary streams, the impaired biological condition of the tributaries will eventually be manifested in the large river sections of the Shenandoah basin.

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FIGURES

Fig. 1 Benthic Macroinvertebrate Study Sites

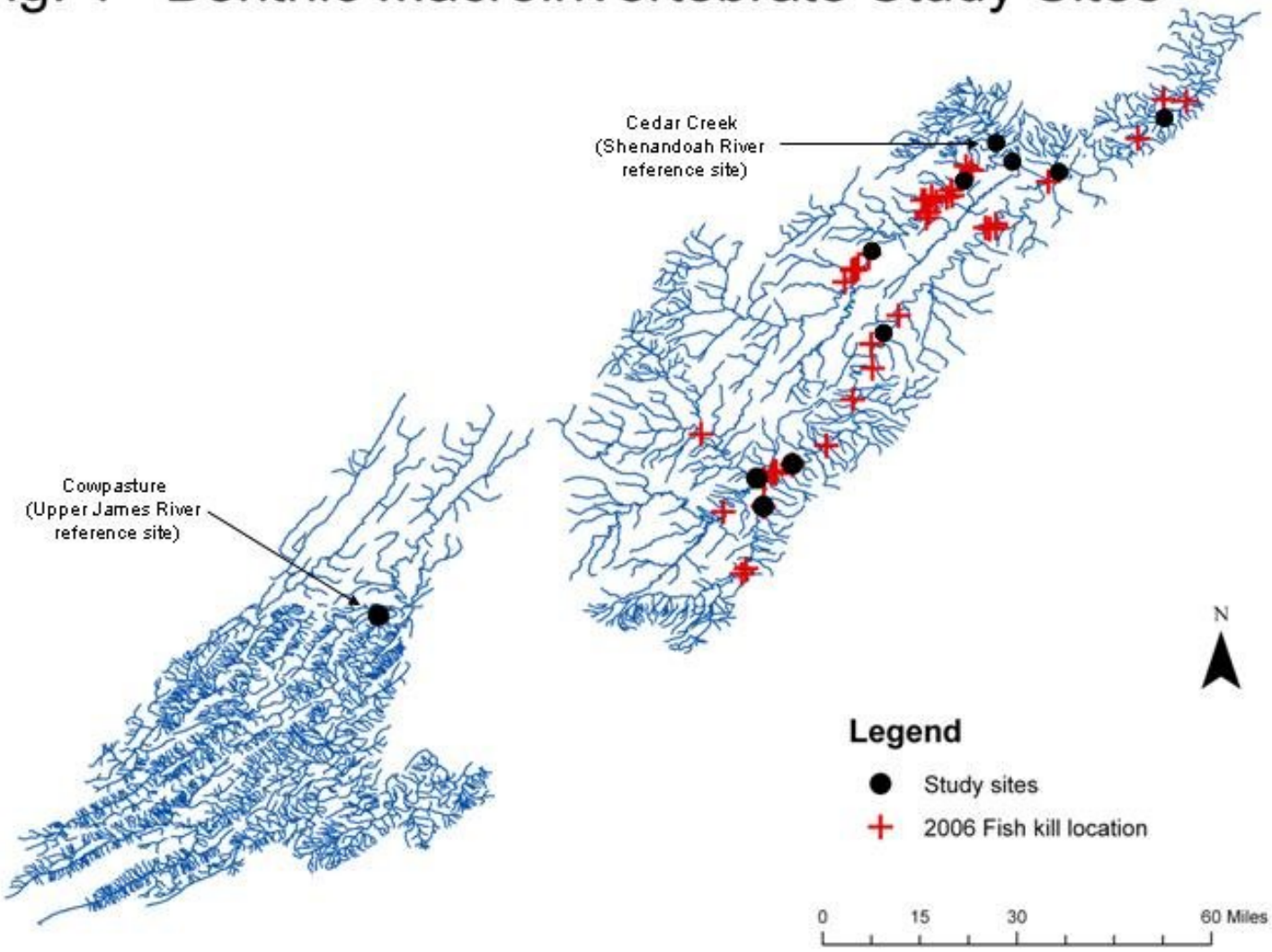
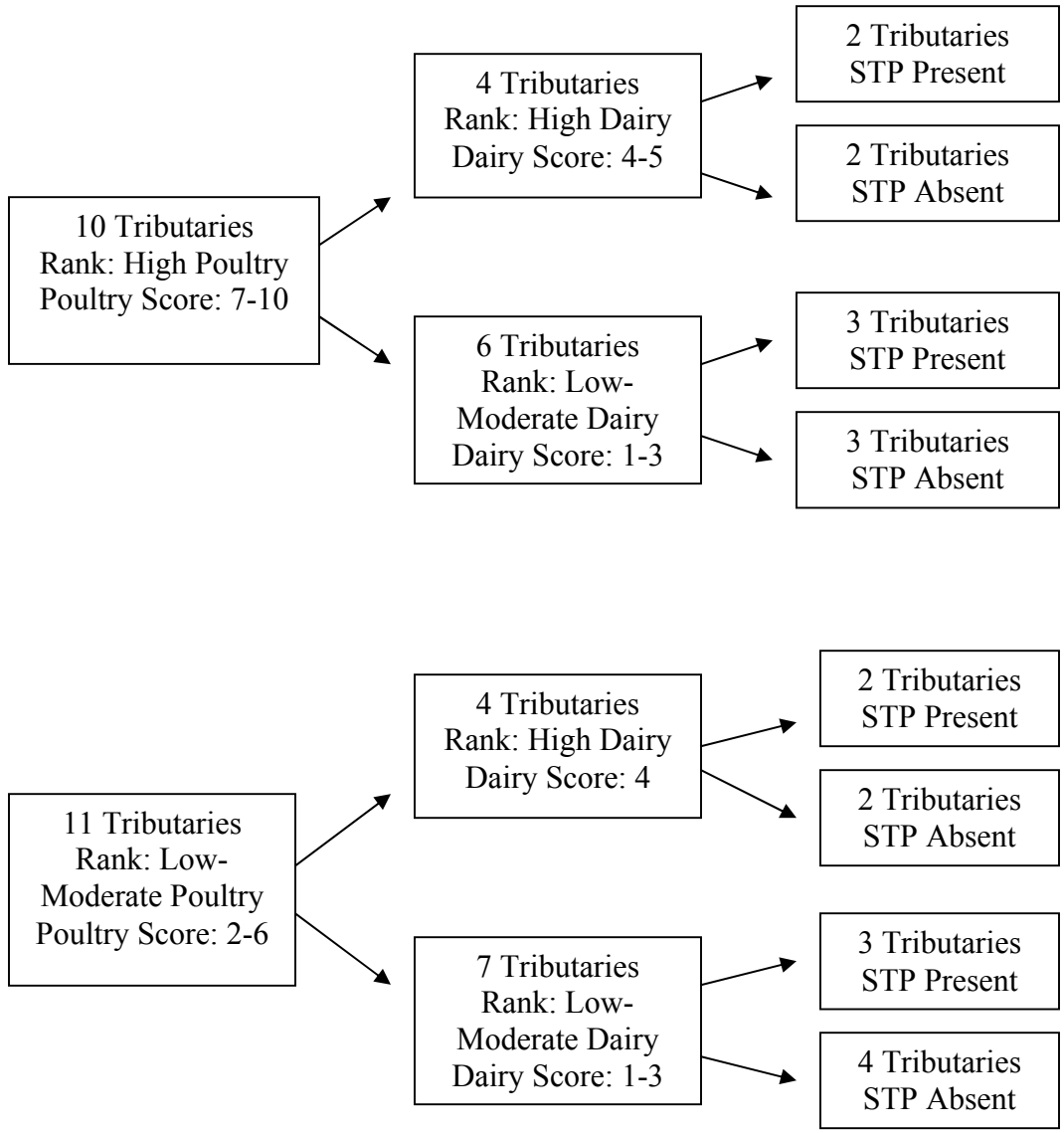


Figure 2. Factorial sampling design for tributary sites within subwatersheds. Boxes illustrate different land-use types used to categorize sites. STP = sewage treatment plant.



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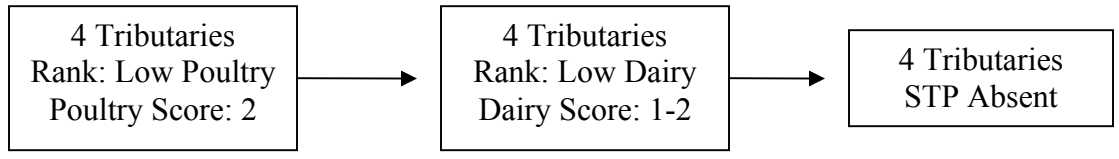


Figure 3. Map of study sites on tributary streams representing subwatersheds of the Shenandoah River basin. Site codes are explained in Table 2.

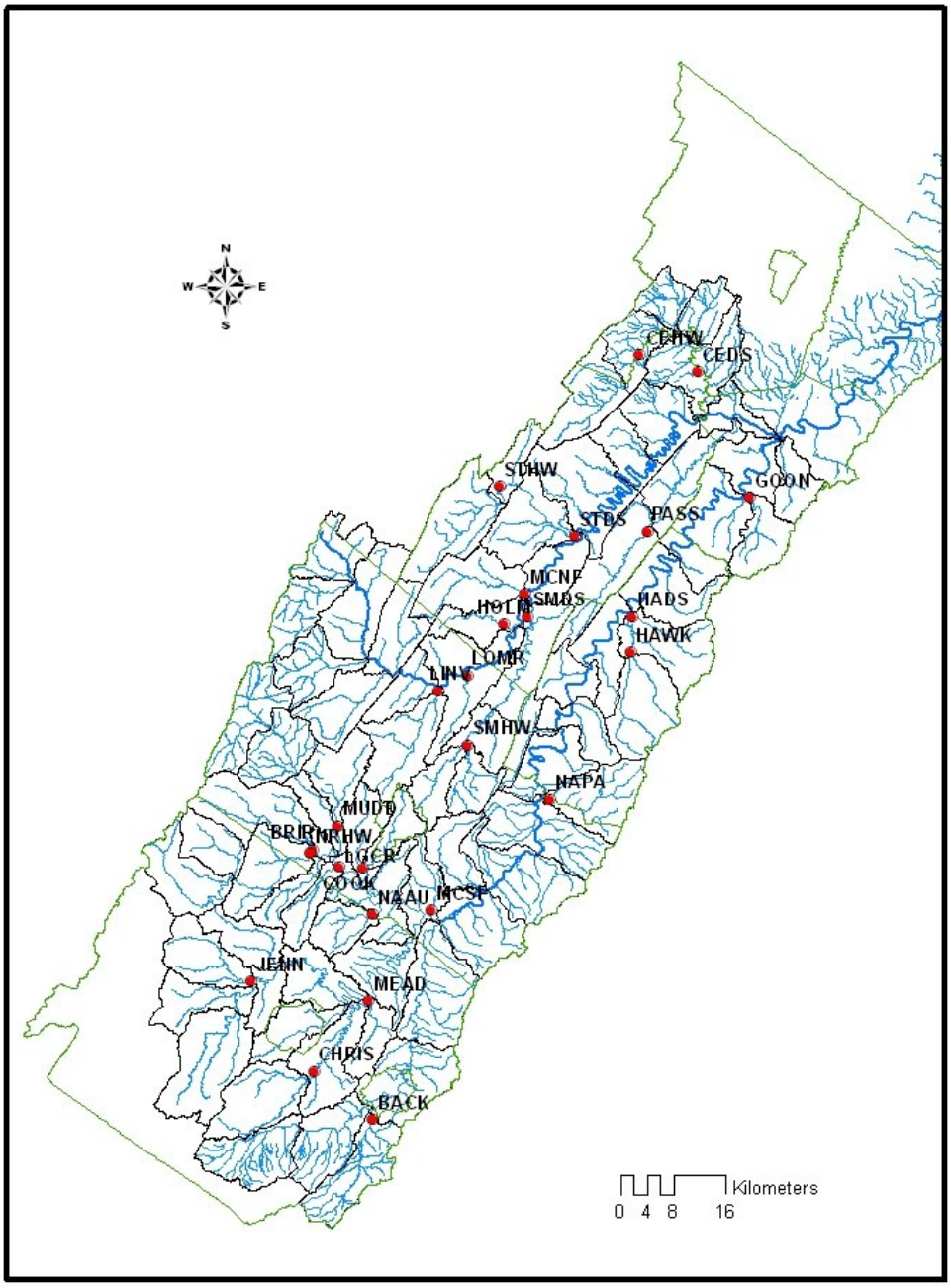


Figure 4. Canonical Correspondence Analysis ordination (CCA) of spring 2006 large river taxa (main matrix) using DEQ nutrient parameters (second matrix) to explain differences in taxa among sites. Axis one explained 18.3% of variation in site separation whereas axis 2 explained 17.2%.. Site codes are explained in Table 1. ORTHP = orthophosphate; TP = total phosphorus. CCA cutoff value for significance R=0.4

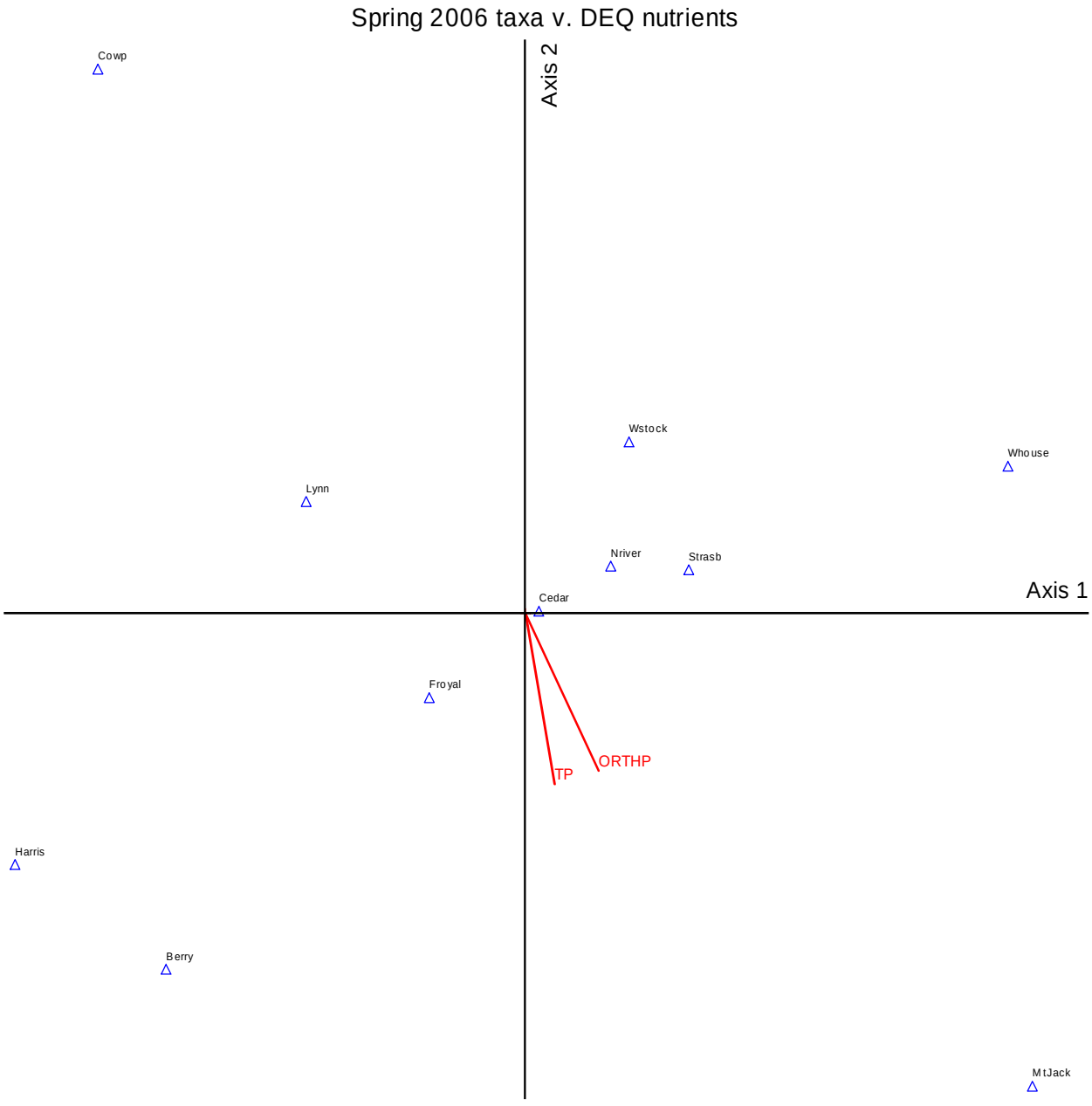


Figure 5. Canonical Correspondence Analysis ordination (CCA) of August 2006 large river taxa (main matrix) using DEQ nutrient parameters (second matrix) to explain differences in taxa among sites. Axis one explained 20% of variation in site separation whereas axis 2 explained 14%. Site codes are explained in Table 1. NN = Nitrate + Nitrite; TP = total phosphorus; AMMON = dissolved ammonia. Cutoff value for significance R=0.5.

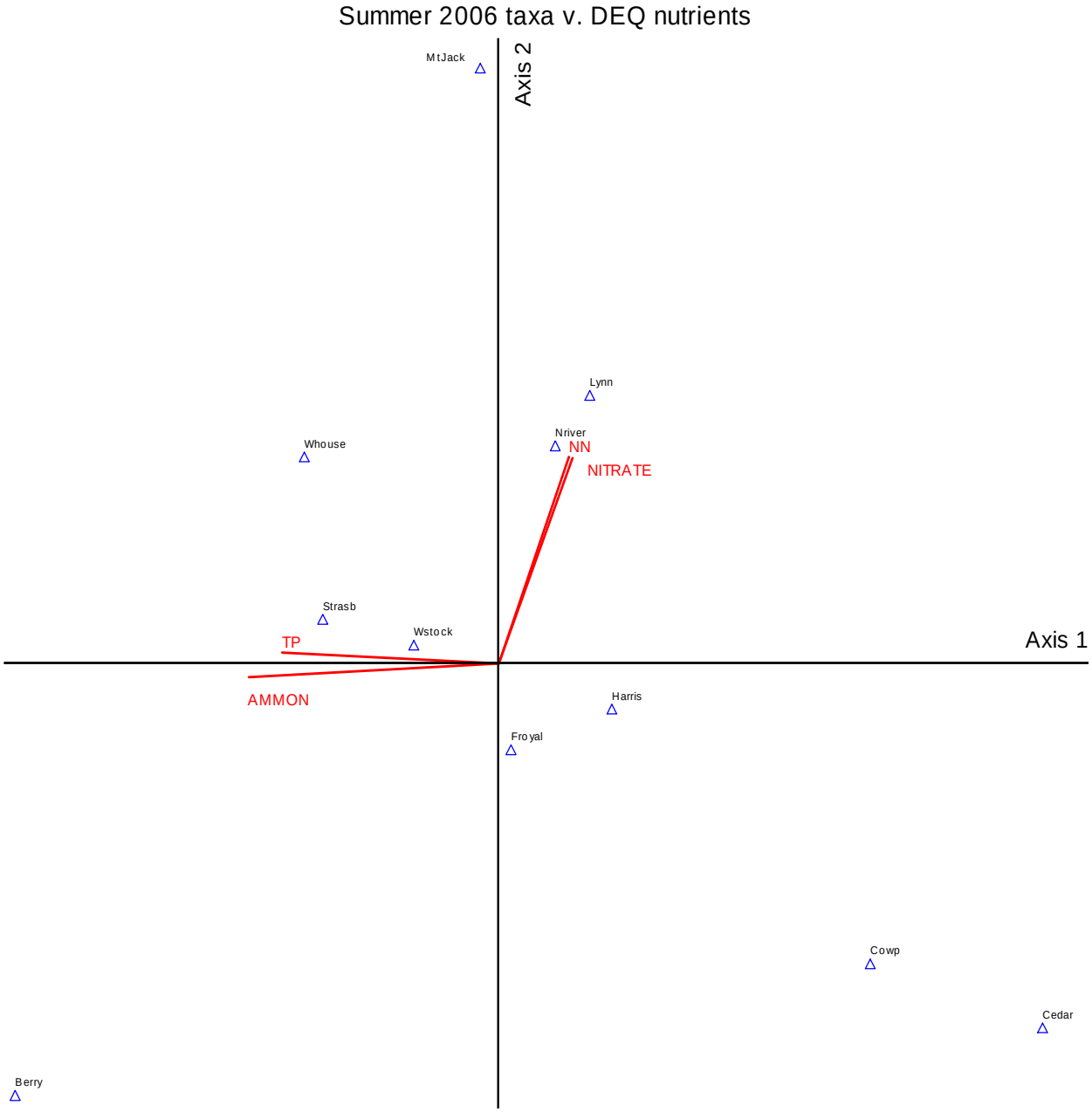


Figure 6. Canonical Correspondence Analysis ordination (CCA) of August 2006 large river taxa (main matrix) using substrate parameters (second matrix) to explain differences in taxa among sites. Axis one explained 21.2% of variation in site separation whereas axis 2 explained 18.6%. Site codes are explained in Table 1. grav = percent gravel; depsed = % deposited sediments; cob = percent cobble; AFDM = ash-free-dry-mass of substrate epilithon. Cutoff value for significance R=0.2.

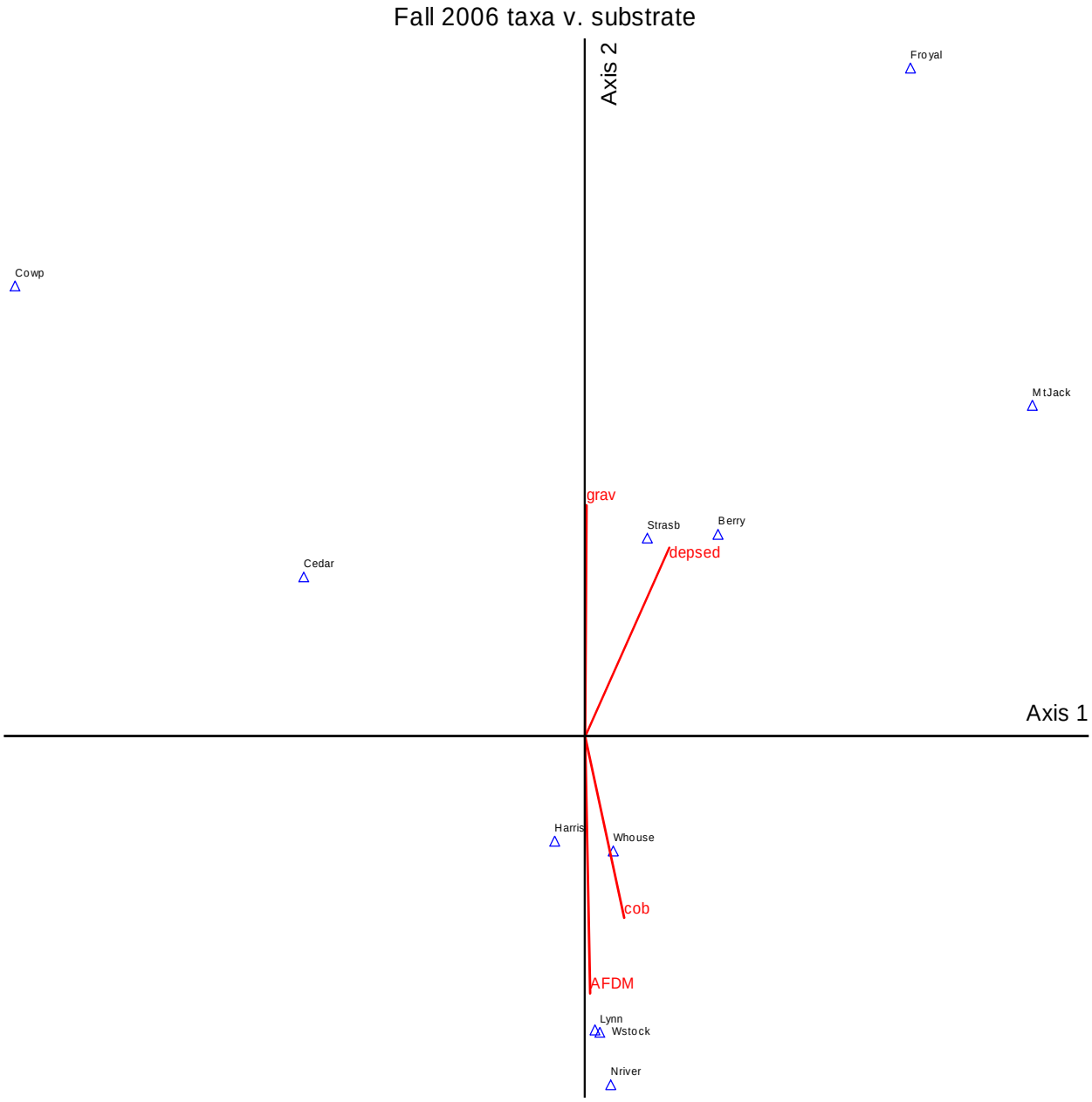


Figure 7. Virginia Stream Condition Index (SCI) by site for large rivers. Black circles are May 2006 data; open circles are August 2006 data. The dashed line indicates that cutoff between impaired and non-impaired. Site codes along x-axis are explained in Table 1.

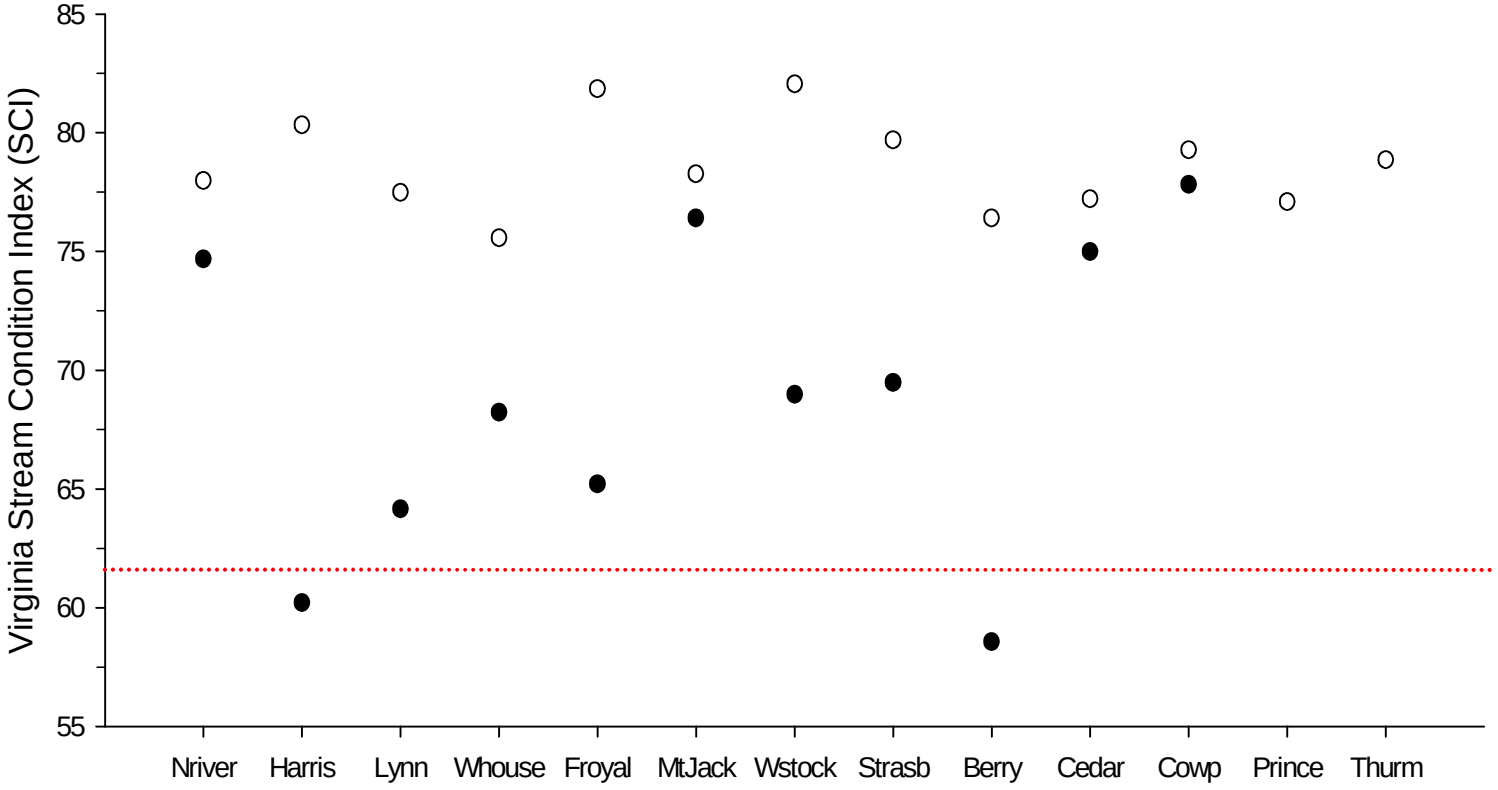


Figure 8. Detrended Correspondence Analysis ordination (DCA) biplot of 2007 tributary top taxa abundance showing taxa most important in site separation. Vector arrows are directly on axes and are difficult to see. Site codes are explained in Table 2. Prosimul = *Prosimulium*; Ephemere = *Ephemerella*.

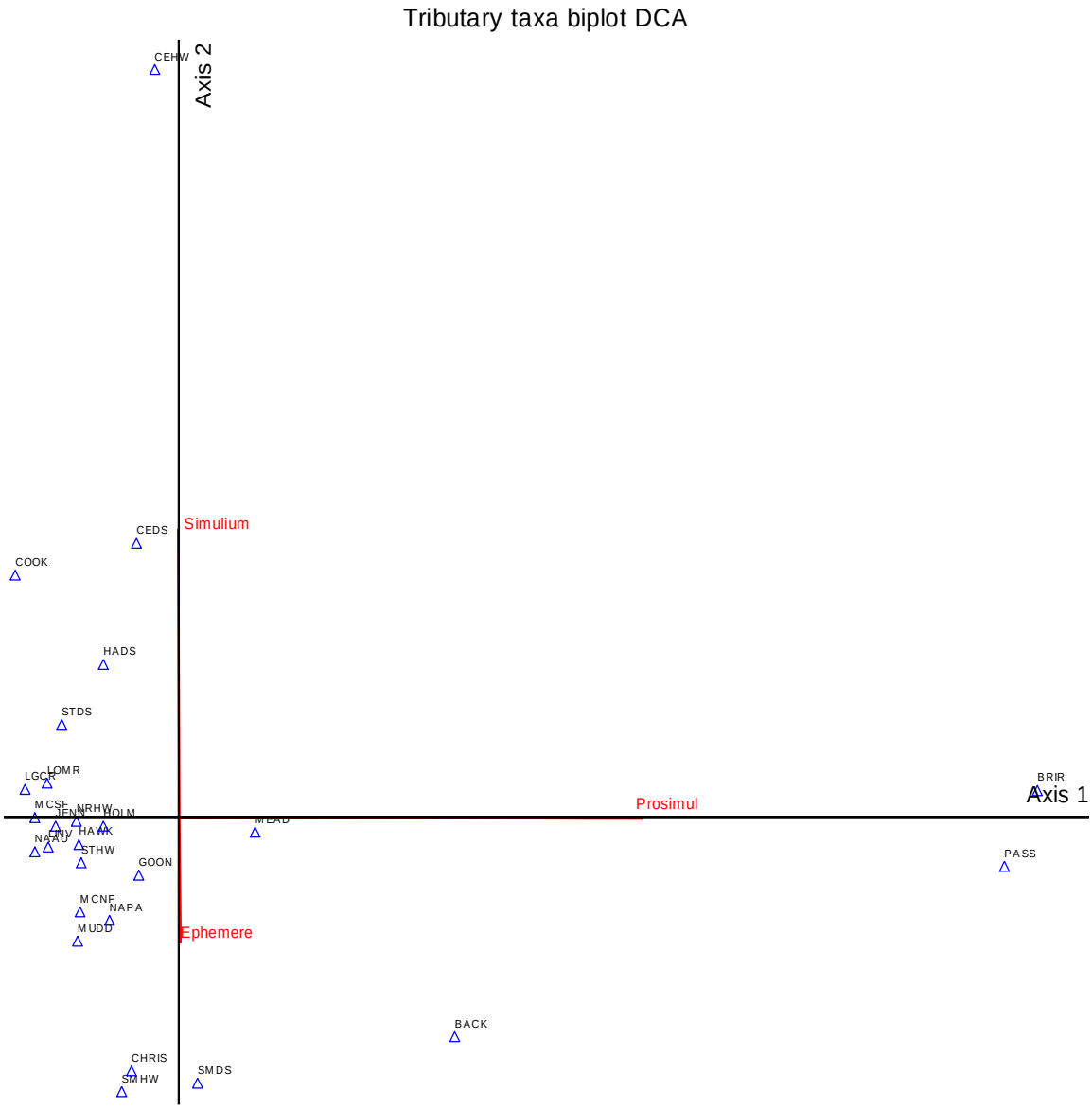


Figure 9. Detrended Correspondence Analysis ordination (DCA) biplot of 2007 tributary top taxa with Passage Creek and *Prosimulium* omitted showing taxa most important in site separation. Site codes are explained in Table 2. Cheumato = *Cheumatopsyche*; Chironom = Chironomidae; Planarii = Planariidae; Ephemere = *Ephemerella*; Maccafe = *Maccaffertium/Stenonema*.

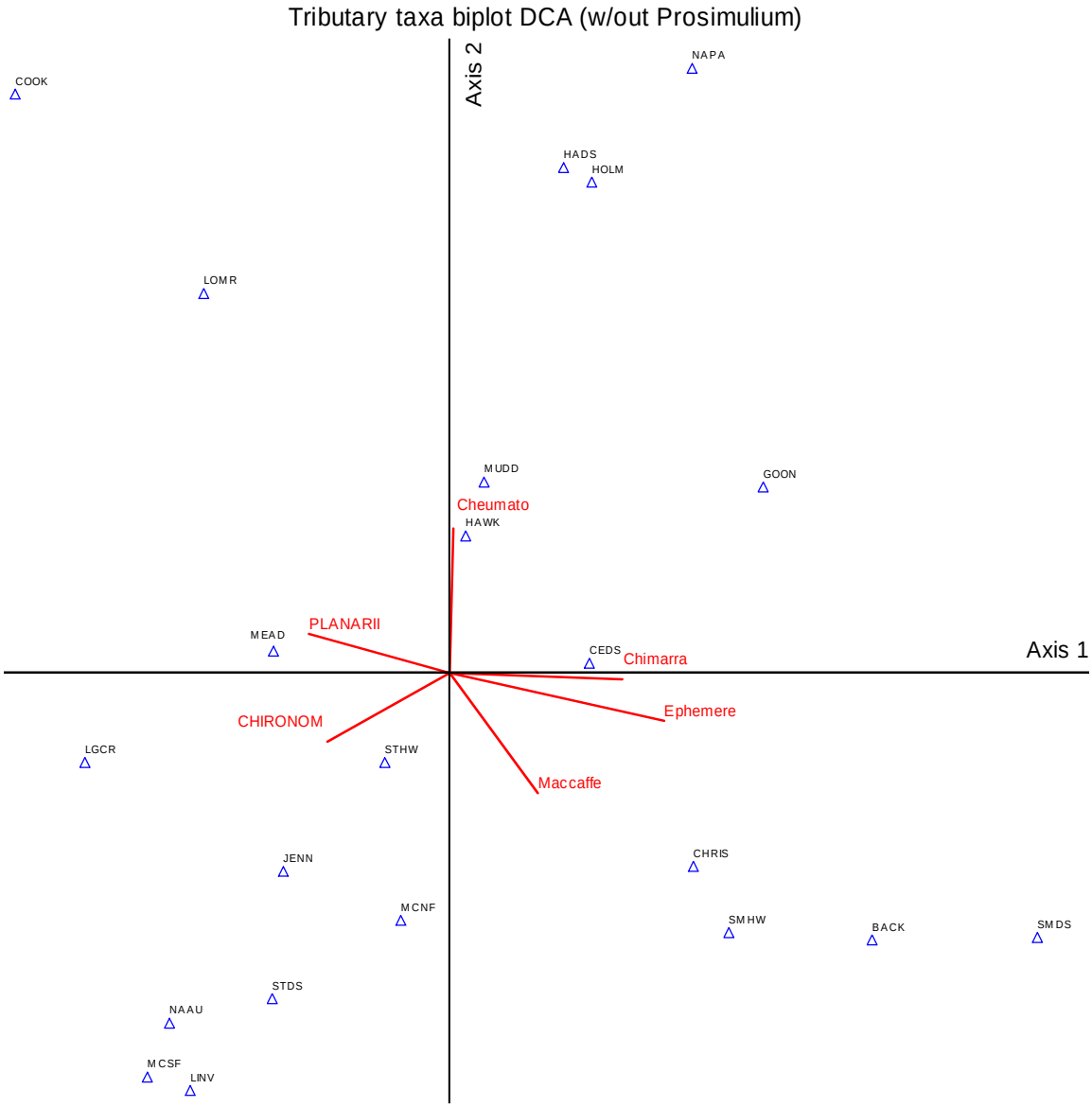


Figure 10. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using land-use (second matrix) to explain differences in taxa among sites. Axis one explained 16% of variation in site separation whereas axis 2 explained 6%. Site codes are explained in Table 2. #AFO/A = number of animal feeding operations per 1000 acres; %_CROP = percent cropland in watershed; # Dai = number of dairy farms; #_AFO = absolute number of animal feeding operations in watershed. Cutoff value for significance R=0.6.

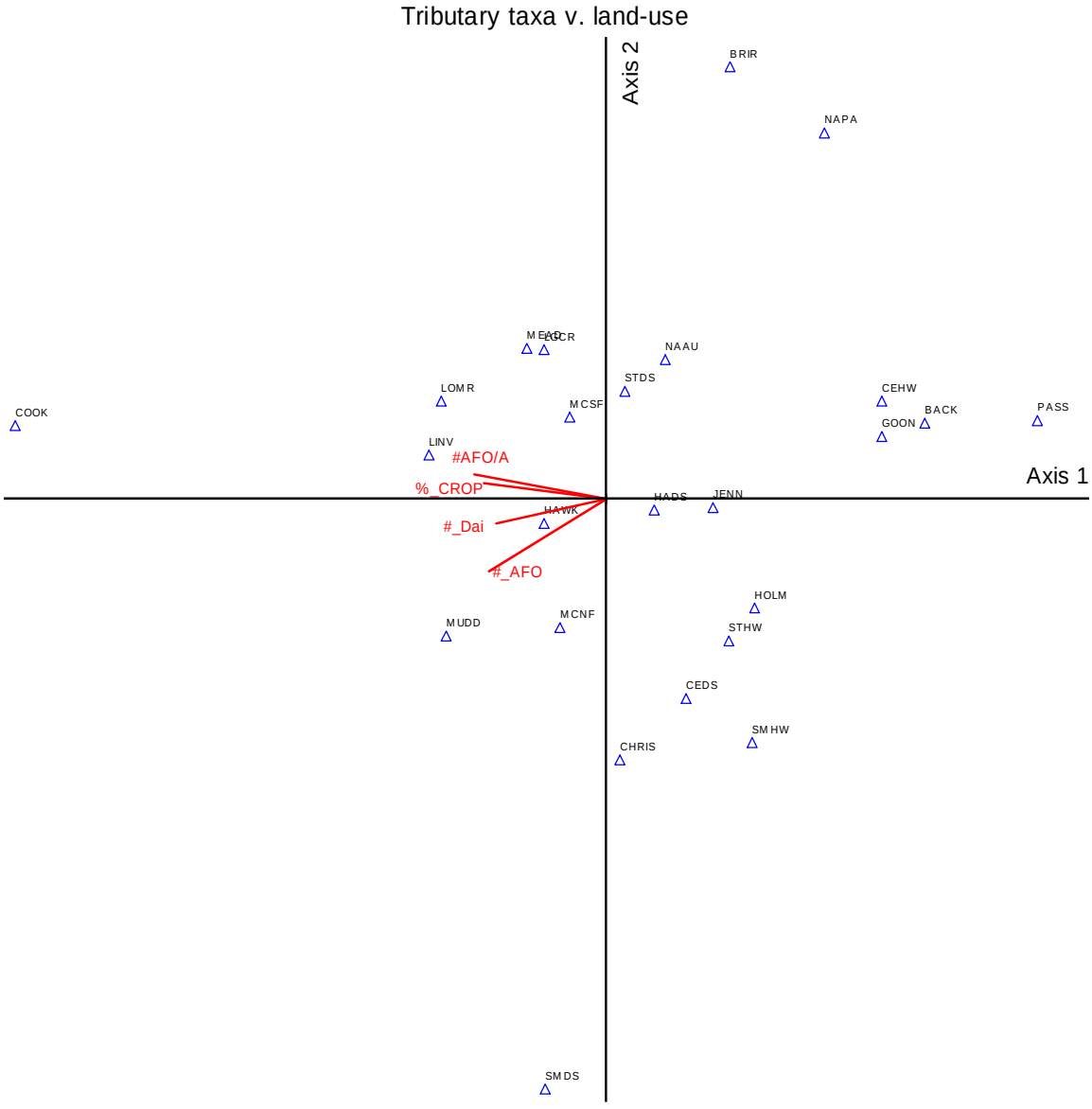


Figure 11. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using sediment metal, nutrient, and carbon variables (second matrix) to explain differences in taxa among sites. Axis one explained 24% of variation in site separation whereas axis 2 explained 15%. Site codes are explained in Table 2. sed_Cu = sediment copper; TN_% = percent total nitrogen; TOC_% = percent total organic carbon; Sed_Pb = sediment lead. Cutoff value for significance R=0.4.

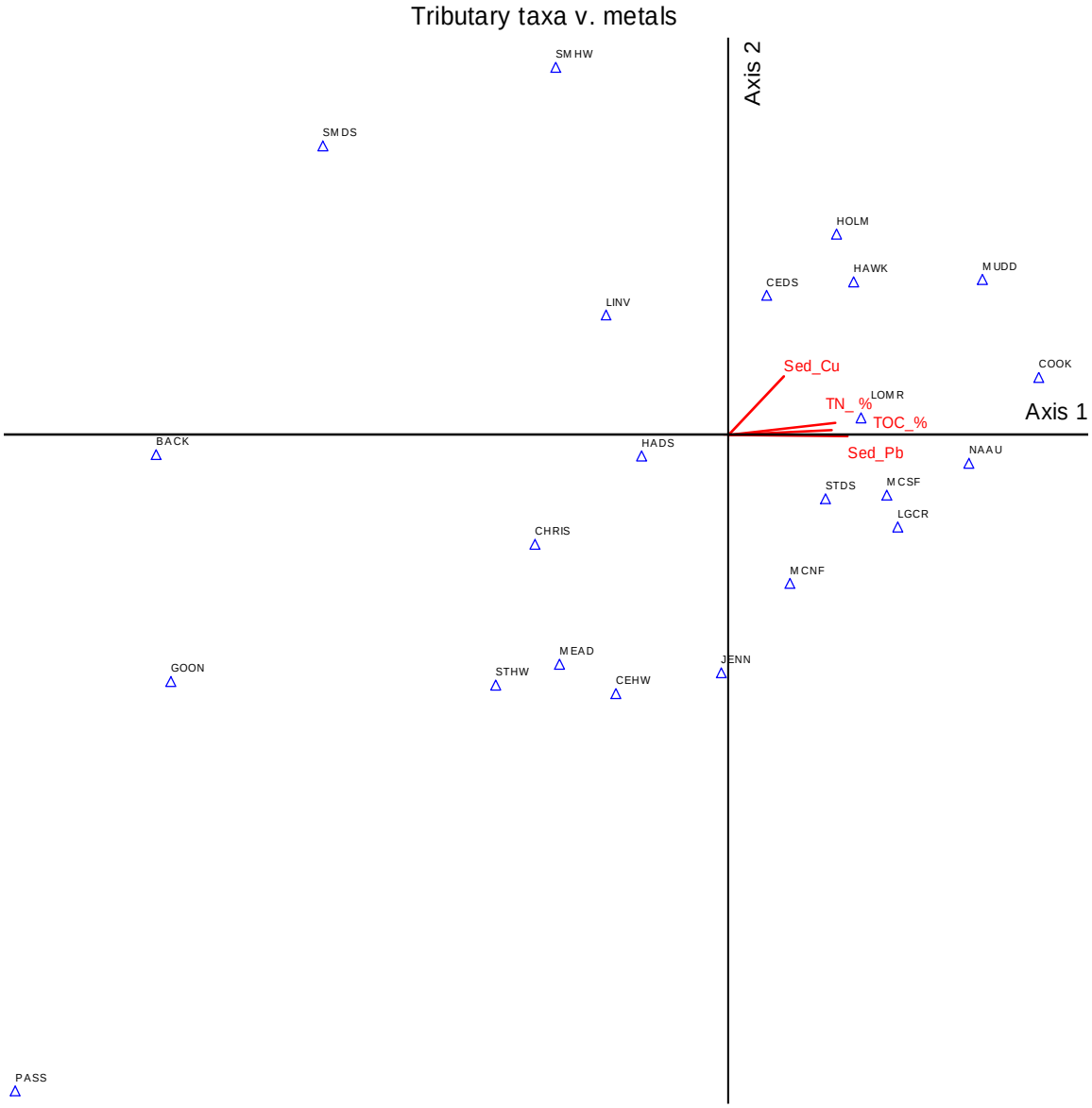


Figure 12. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using clam tissue metal concentration (second matrix) to explain differences in taxa among sites. Axis one explained 23.4% of variation in site separation whereas axis 2 explained 10.5%. Site codes are explained in Table 2. Clam_Se = clam selenium; Cd = cadmium; Hg = mercury. Cutoff value for significance R=0.4.

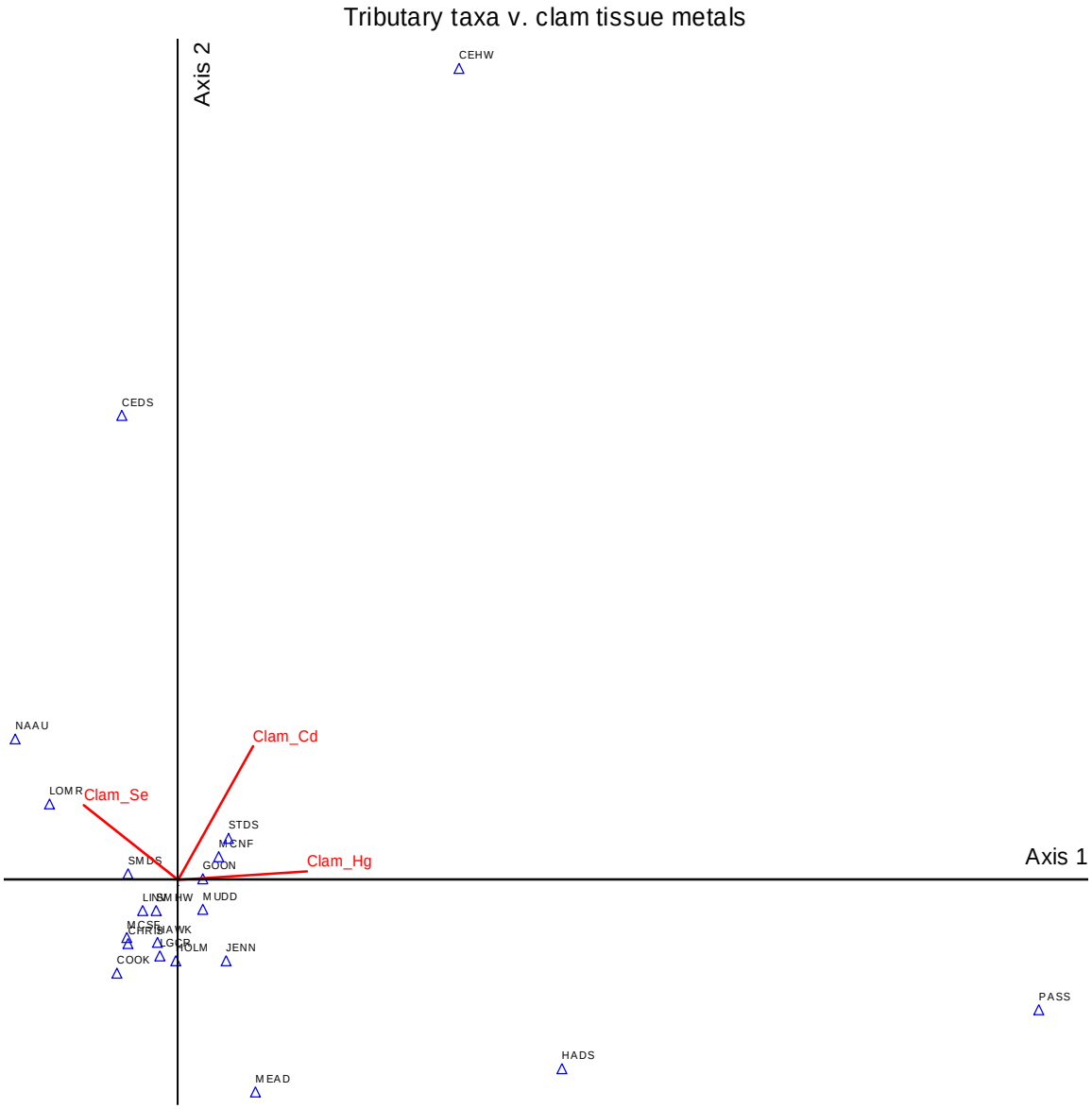


Figure 13. Detrended Correspondence Analysis ordination (DCA) 2007 large river elmid species abundance. Site codes are explained in Table 1.

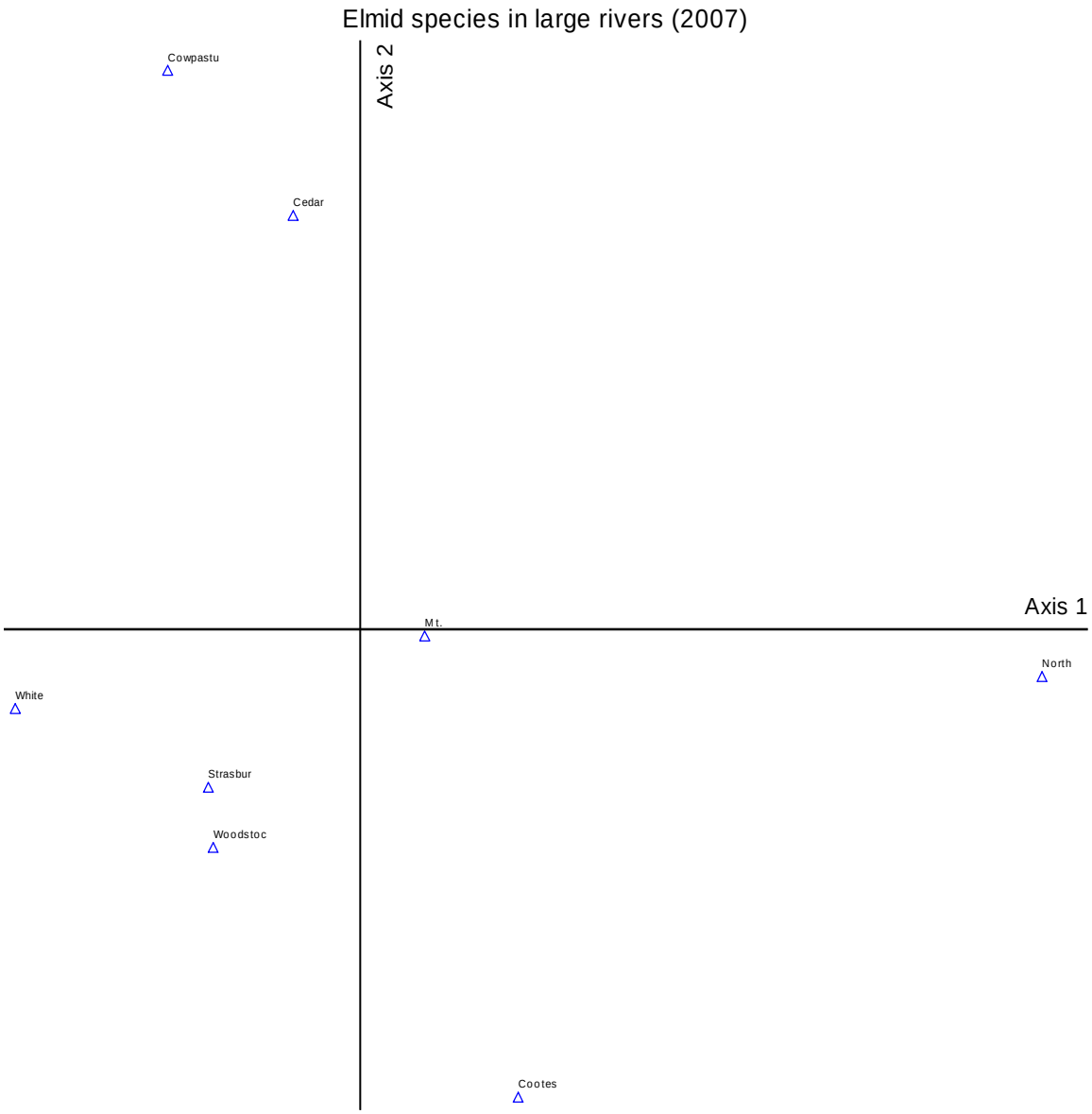


Figure 14. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary elmid species abundance (main matrix) using land-use (second matrix) to explain differences in taxa among sites. Axis one explained 30% of variation in site separation whereas axis 2 explained 17.4%. Site codes are explained in Table 2. #AFO/A = number of animal feeding operations per 1000 acres; #PH/A = number of poultry houses per 1000 acres; #Beef/A = number of beef operations per 1000 acres. Cutoff value for significance R=0.5.

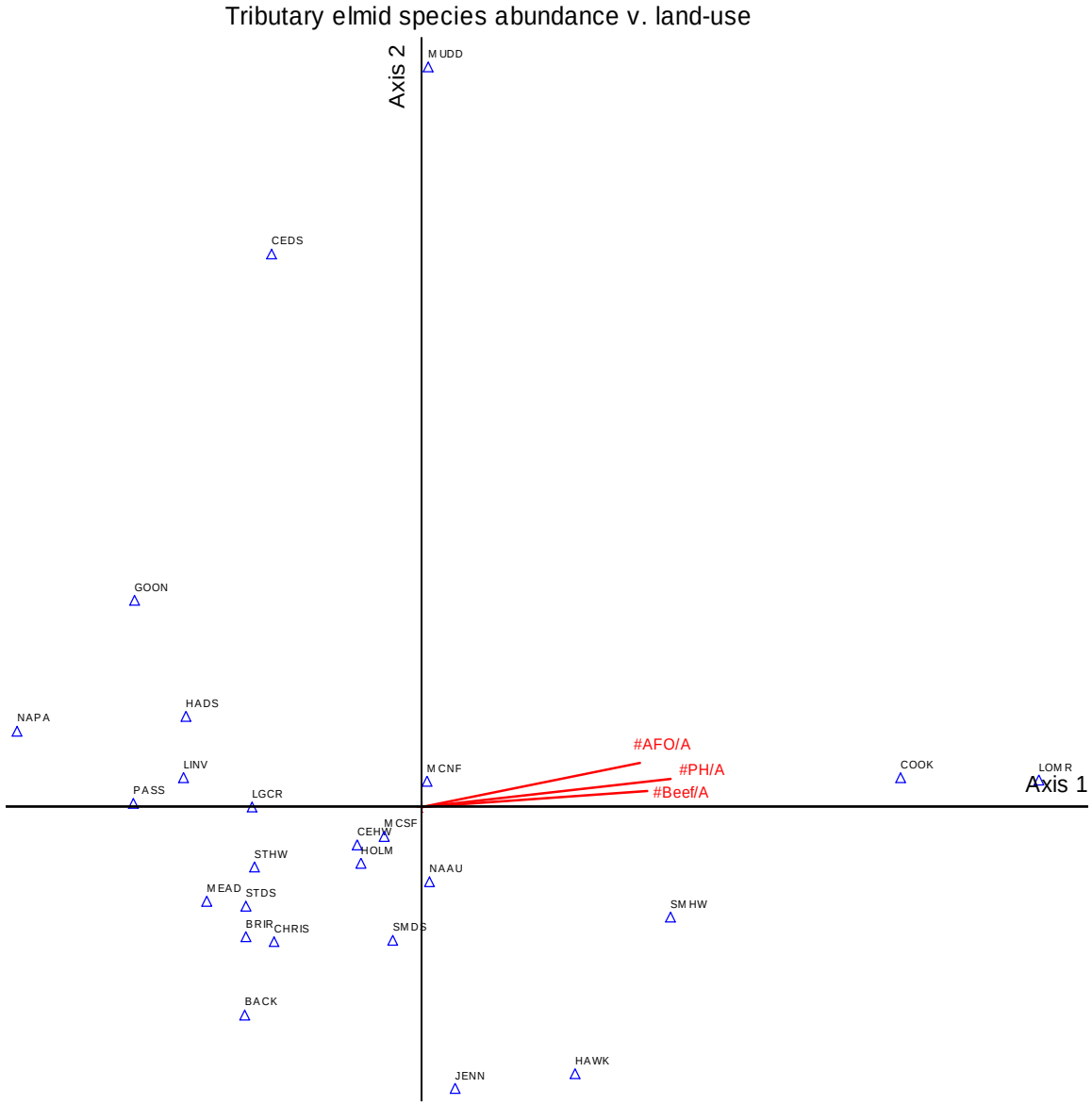


Figure 15. In-situ bioassay results for 2006 *Corbicula* growth (mm/day) between fish kill and reference large river site groups. ANOVA indicated that the site groups were significantly different in June and August.

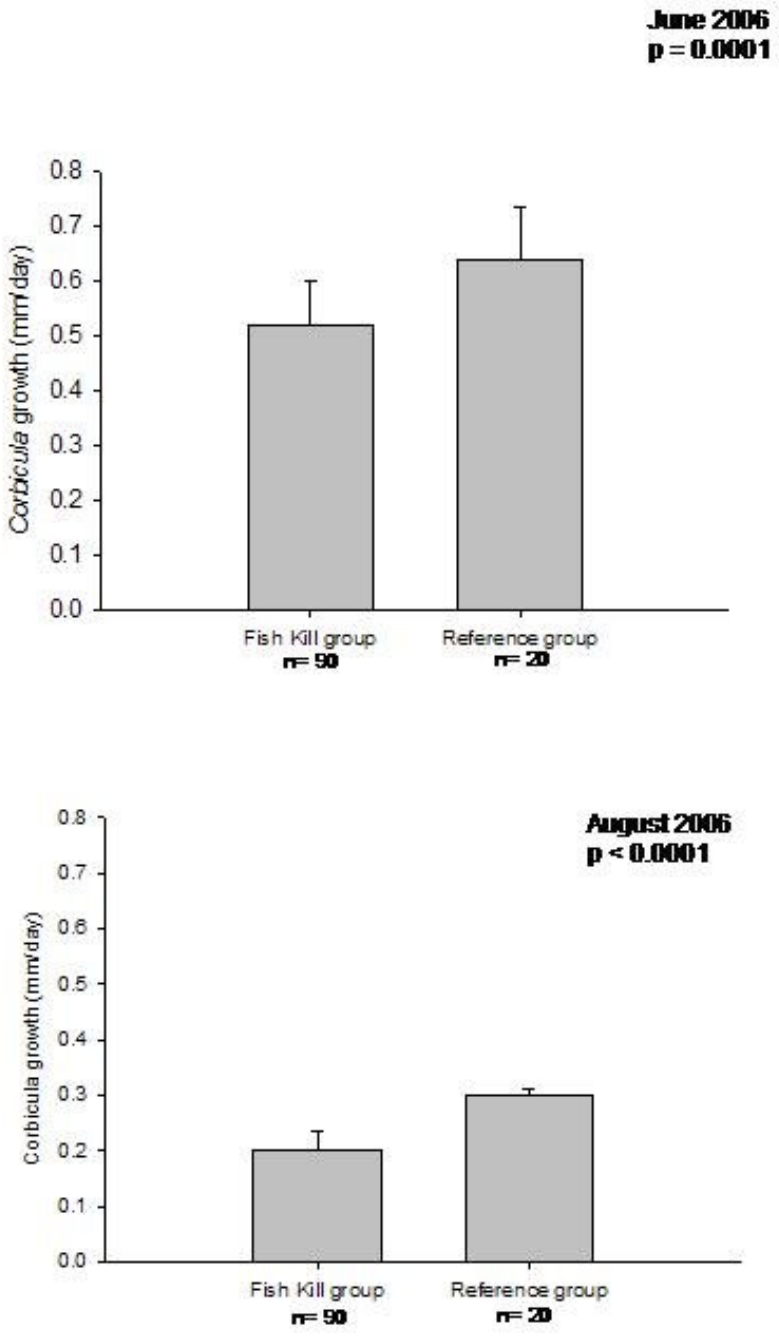


Figure 16. In-situ bioassay results for 2006 *Corbicula* growth (mm/day) among all individual large river sites without any grouping. ANOVA indicated that only Front Royal and Mt Jackson were significantly different in June and only Mt Jackson and Woodstock were significantly different in August.

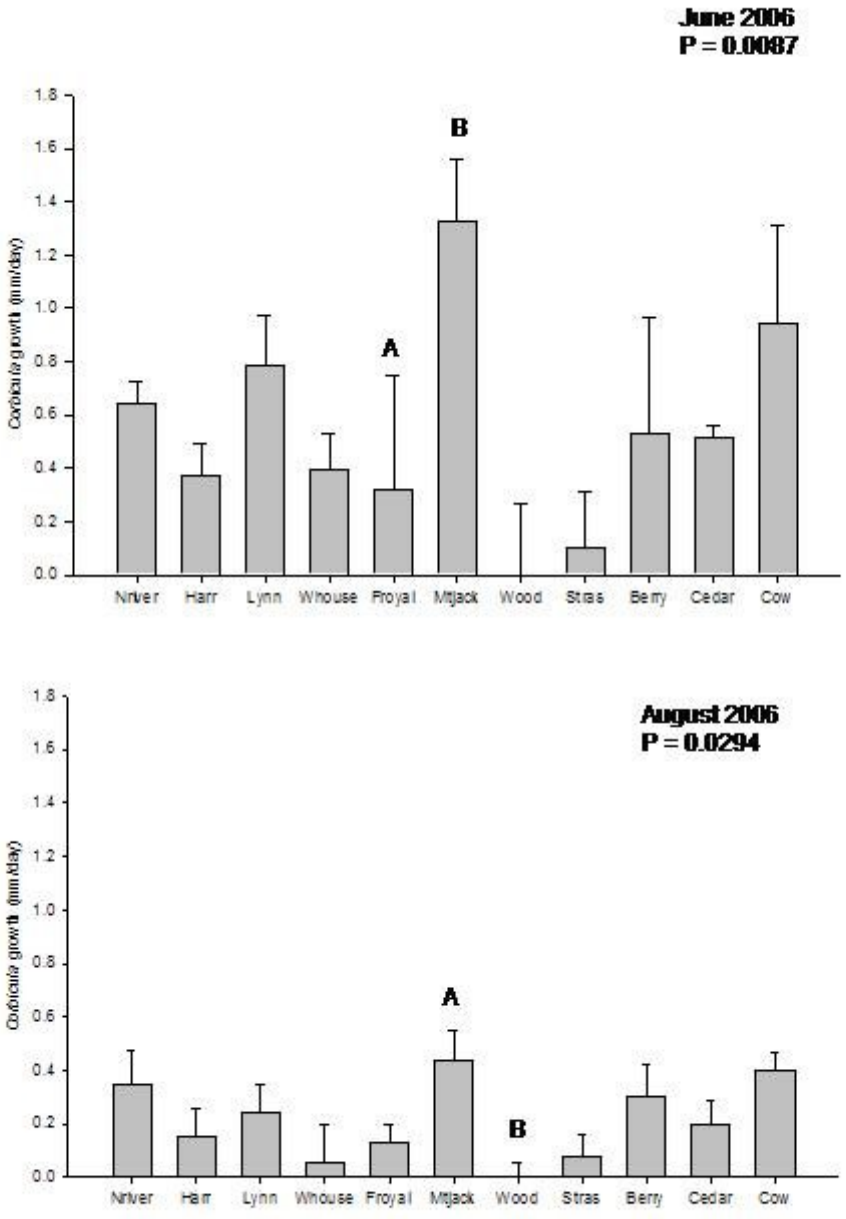


Figure 17. In-situ bioassay results for2006 *Corbicula* mortality between fish kill and reference large river site groups. ANOVA indicated that the site groups were significantly different in June and August.

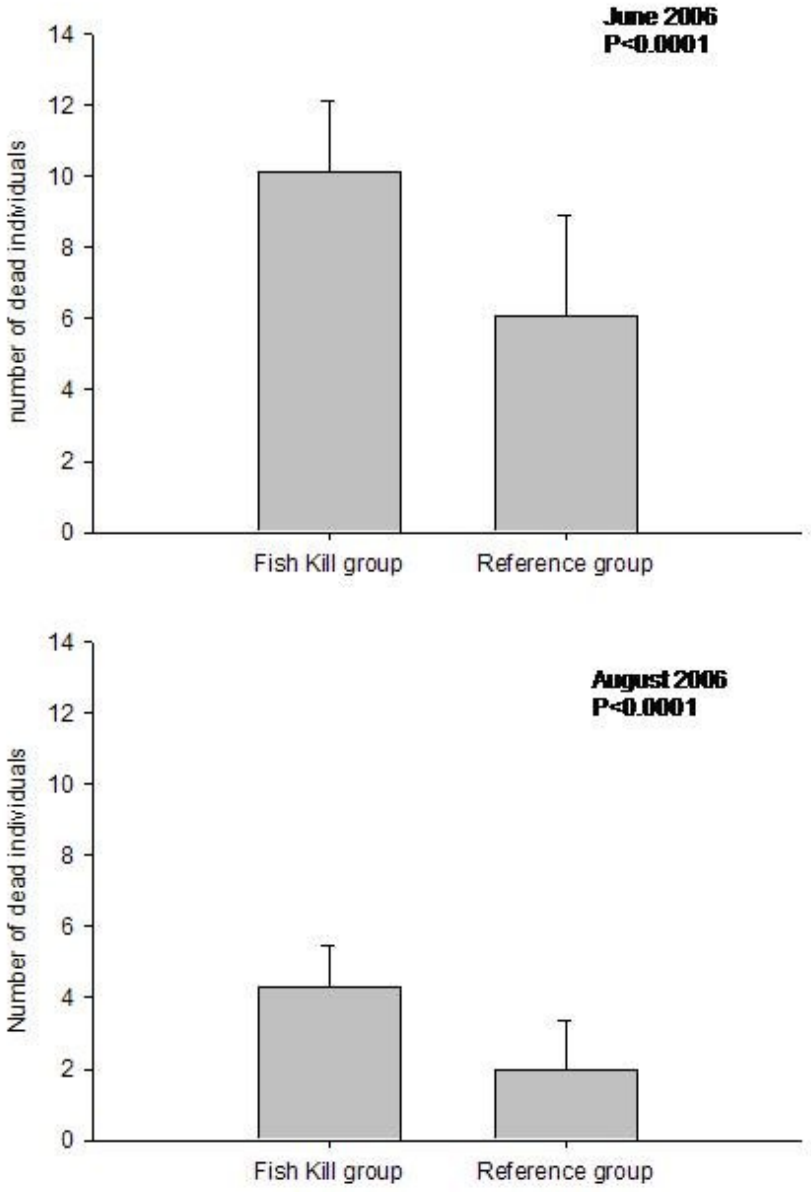
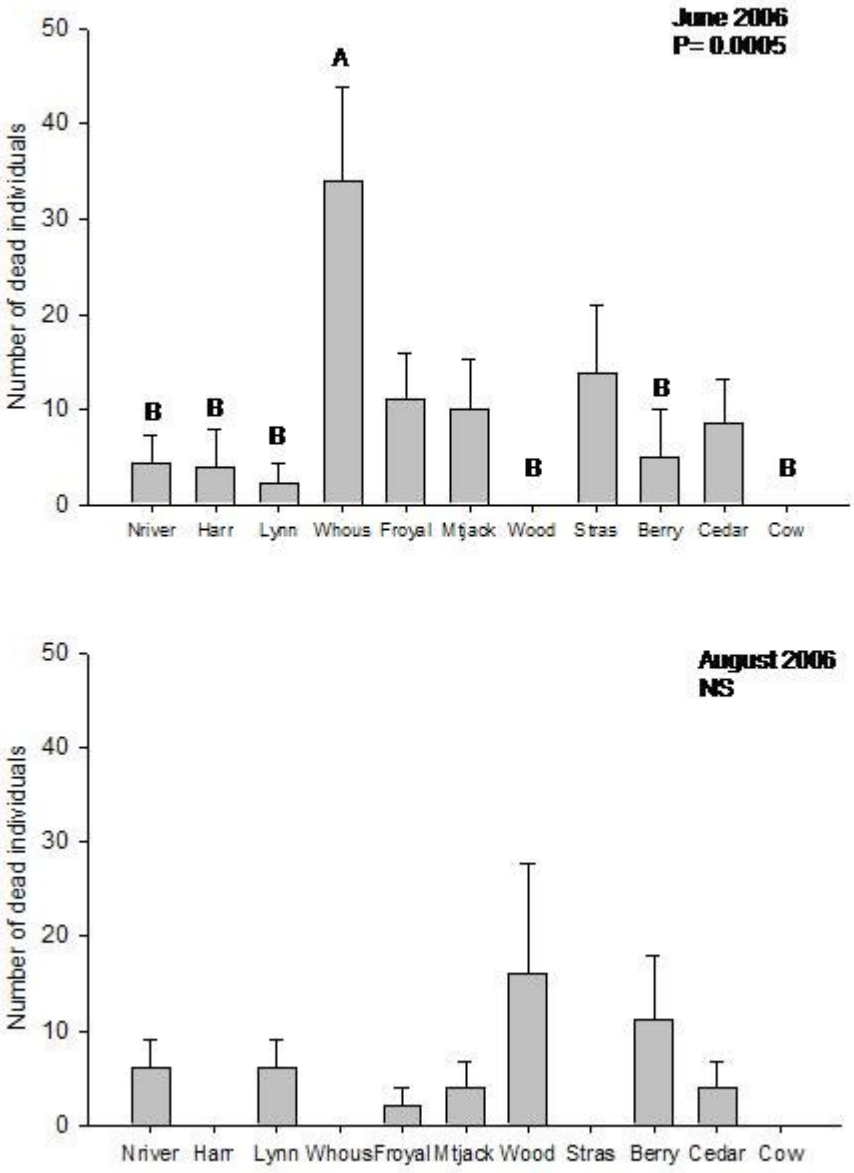


Figure 18. In-situ bioassay results for 2006 *Corbicula* mortality among all individual large river sites without any grouping. ANOVA indicated that only Mt Jackson was significantly different in June, and no sites were significantly different in August.



TABLES

Table 1. Sampling and location information for large river study sites. All are in the Shenandoah River basin, except Cowpasture River, which is in the James River basin. The locations of these sites are shown on a map in Fig. 1.

Site Name	Code	Dates Sampled			Location (latitude/ longitude)	Watershed Area (ha)	Elevation (ft)
		May 2006	Aug 2006	May 2007			
Main Stem							
Berryville	Berry	X	X		39°07'16"N 77°53'40"W	746,877	380
North Fork							
Coote's Store				X			
Mount Jackson	Mtjack	X	X	X	38°44'41"N 78°38'22"W	131,411	840
Woodstock	Wood	X	X	X	38°54'08"N 78°28'50"W	183,652	690
Strasburg	Stras	X	X	X	38°58'21"N 78°21'02"W	199,563	520
South Fork Tribs							
Harriston	Harr	X	X		38°13'06"N 78°50'13"W	53,127	1160
North River	Nriver	X	X	X	38°16'55"N 78°51'05"W	197,059	1060
South Fork							
Lynnwood	Lynn	X	X		38°18'49"N 78°46'18"W	270,134	1040
White House	Whouse	X	X	X	38°38'50"N 78°32'18"W	347,031	740
Front Royal	Froyal	X	X		38°54'53"N 78°12'40"W	418,911	460
Reference							
Cedar Creek	Cedar	X	X	X	39°00'01"N 78°20'00"W	40,756	520
Cowpasture River	Cow	X	X	X	37°58'31"N 78°41'46"W	82,361	1220

Table 2. Tributary sites with codes. Locations of these sites are shown on a map in Fig. 3.

Site Names	Code
Cooks Creek	COOK
Briery Branch	BRIR
Linville Creek	LINV
Muddy Creek	MUDD
Long Meadow Run	LOMR
Mill Creek - N. Fork	MCNF
Naked Creek (Augusta Co.)	NAAU
Hawksbill Creek	HAWK
Hawksbill Creek Downstream	HADS
Holmans Creek	HOLM
Long Glade Creek	LGCR
Mill Creek - S. Fork (Rockingham Co.)	MCSF
Christians Creek	CHRIS
Meadow Run	MEAD
Naked Creek (Page Co.)	NAPA
Jennings Branch	JENN
Smith Creek Headwaters	SMHW
Smith Creek Downstream	SMDS
Stony Creek Headwaters	STHW
Stony Creek Downstream	STDS
Back Creek	BACK
Gooney Run - Ref.	GOON
Passage Creek - Ref.	PASS
Cedar Creek Headwaters - Ref.	CEHW
Cedar Creek Downstream - Ref.	CEDS

Table 3. Land use categories that were quantified for the subwatersheds in the tributary study with codes used in statistical analyses.

Land use categories	Code
# Acres in subwatershed(s)	Area_Acres
% Area as wetland	%_WETL
% Area as forest	%_FOR
% Area as pasture/hay	%_PASTHAY
% Area as crops	%_CROP
% Area as developed land (all types)	%_DEVEL
% Area as barren land	%_BARR
# Dairies	#_Dairy
# Beef operations	#_Beef
# Poultry houses	#_PoultryH
# Acres in a nutrient mgmt plan for poultry litter	SumA_NMP
# Animal feeding operations (total)	#_AFO
# Dairies/1000 acres	#Dairy/1000A
# Beef operations/1000 acres	#Beef/1000A
# Poultry houses/1000 acres	#PH/1000A
# Animal feeding operations/1000 acres	#AFO/1000A
% Acres in a nutrient mgmt plan for poultry litter	%AcresNMP
# Municipal STPs	#MUNSTP
Total flow (MGD) of municipal STPs	MUNFLOW

Table 4. Benthic macroinvertebrate assemblage metrics: definitions and usually expected responses to environmental stressors. The + or – symbols indicate an expected increase or decrease, respectively, in the metric's numerical value.

Metrics (within categories)	Definition	Expected response to environmental stressors
Density		
Total density	Number of all individuals per m^2	+ or -
Richness, Diversity, Evenness		
Total richness	Number of all taxa	-
Simpson's diversity index	Combines richness and abundance with the equation: $\frac{\sum n(n-1)}{N(N-1)}$ Where: n= total number of individuals of a taxon, N=total number of individuals	+ or -
Composition		
% Modified EPT	Proportion of total density consisting of organisms within the orders Ephemeroptera (excluding Baetidae), Plecoptera, and Trichoptera (excluding Hydropsychidae)	-
% Non-insects	Proportion of total density consisting of organisms that are not insects	+
Tolerance		
% Sensitive	Proportion of total density consisting of organisms with pollution tolerance values of 0, 1, or 2	-
Trophic		
% Scrapers	Proportion of total density consisting of organisms that shear the	+ or -

	layer of material growing on firm substrates	
% Collector-gatherers	Proportion of total density consisting of organisms that acquire and ingest fine particles of detritus lying on the bottom	+
% Collector-filterers	Proportion of total density consisting of organisms that acquire and ingest fine particles of detritus suspended in the water	
Habits		
% Crawlers	Proportion of total density consisting of organisms that move around slowly in small spaces within mineral or plant substrate	-
% Modified Clingers	Proportion of total density consisting of organisms that maintain a fixed position on mineral or plant substrate in current (excluding Hydropsychidae)	

Table 5. List of all taxa collected from large river study sites in 2006. (M) indicates the taxon was present in May; (A) indicates the taxon was present in August ; blank cells indicate that a taxon was not collected.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
NON- INSECT TAXA											
NEMATODA	A	M A	M A	M	M A	M		M	M		
ANNELIDA											
HIRUDINEA	M						M	M			M
PLANARIIDAE	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
NEMERTEAN	A	M A	M A	M A		M	M A	M A	M A	M A	A
MOLLUSCA											
CORBICULIDAE											
Corbicula fluminea	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
SPHAERIIDAE	M	M A	M A			A		M A	M	M A	M A
unknown gastropod							M A				
PLEUROCERIDAE											
Leptoxis carinata	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
ANCYLIDAE			M A	M A		M A			M A	M A	
PHYSIDAE											
Physa	M A						M A	M A		M A	
PLANORBIDAE				M A			A	M A			
CRUSTACEA											
CAMBARIDAE										M A	
ASELLIDAE											
Caecidotea		M									
CRANGONYCTIDAE											
Crangonyx	A		M								
GAMMARIDAE											
Gammarus							M			M	

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
ACARI (HYDRACARINA)								M		M	MA
INSECT TAXA											
EPHEMEROPTERA											
BAETIDAE											
Baetis (complex)	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
CAENIDAE											
Caenis	A	M A	M A	A	A	M A	M A	M A	M A	M A	M A
EPHEMERELLIDAE											
Drunella lata		M				M				M	M
Drunella tuberculata	M	M		M		M	M	M		M	M
Ephemerella	M	M	M	M	M A	M	M A	M	M	M A	M A
Eurylophella											M A
Serratella	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M A
EPHEMERIDAE										M	
HEPTAGENIIDAE											
Leucrocuta	M A	M A		M A		M A	M A	M A		M A	
Maccaffertium/Stenonema	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Stenacron	M	M A	M		A	M A	M A	A	M A	M	
Rhithrogena								M			M
ISONYCHIIDAE											
Isonychia	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M A
LEPTOPHLEBIIDAE		M A			A					M A	
LEPTOHYPHIDAE											
Tricorythodes	A	M	M A	A	A	M A	A	A	M A	M A	A
POTAMANTHIDAE											
Anthopotamus	M A	M			A	M			A	M	M A
PLECOPTERA											
CHLOROPERLIDAE					M						

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
LEUCTRIDAE											
Leuctra							M			M	M
NEMOURIDAE											
Amphinemura		M									
PERLIDAE		M A				M A				M A	M A
Acroneuria	A	M A			A				A	M A	M A
Agnetina	M A	M A	M A	M A	M A	M		A	M A		M A
Neoperla											M A
Paragnetina											A
Perlesta placida (group)	M	M	M A	M A		M	M	M	M	M	M A
PERLODIDAE											
Isoperla				M							
PTERONARCYIDAE											
Pteronarcys										M A	A
ODONATA											
CALOPTERYGIDAE											
Calopteryx							M				
COENAGRIONIDAE											
Argia	M A	M A	M A	M A	A	M A	M A	M A	M	M A	M
GOMPHIDAE											
Gomphus							M			M	
Lanthus		M					A	A		M A	M A
Stylogomphus		A	M						M	A	A
NEUROPTERA											
SISYRIDAE											
Climacia		A								M A	M A
MEGALOPTERA											
CORYDALIDAE											
Corydalus cornutus	M A	A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Nigronia fasciatus		A									M

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
SIALIDAE											
Sialis						M A					
TRICHOPTERA											
BRACHYCENTRIDAE											
Brachycentrus	M A	M A	M A	M A	A	M	M	M	M	M A	A
Micrasema	M					M	M		A	M A	M A
GLOSSOSOMATIDAE											
Glossosoma nigrior										M	M
Protoptila	M A	M A	M A	M A	M A	M	M A	M A	M A	M A	M A
HELICOPSYCHIIDAE											
Helicopsyche borealis	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
HYDROPSHYCHIDAE											
Diplectrona							M	M			
Cheumatopsyche	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Hydropsyche	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Macrostemum											MA
Parasyche		A									
HYDROPTILIDAE											
Agraylea	M A		M A	M A	M A	M A	M A	M A	M A	M A	M A
Hydroptila	M A	M A	M A	M A	A	M A	A	A	M A	M A	
Ochrotrichia	M A										
LEPTOCERIDAE											
Ceraclea		A	M			M		M A	M A	M	M A
Nectopsyche								M			
Oecetis	M		M	M	MA	M	M	M A	A	M	A
Triaenodes	M		M				M	M			
LEPIDOSTOMATIDAE											
Lepidostoma	M A	A	M A		M			A	M A	A	M A

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
LIMNEPHILIDAE											
Pycnopsyche				A							
UENOIDAE											
Neophylax			M		A	M			M A		
PHILOPOTAMIDAE											
Chimarra	M A	A	M A	M A	M	M A	M A	M A	M A	M A	M A
Dolophilodes	M									M	M
POLYCENTROPODIDAE											
Polycentropus				A							
Neureclipsis	A										
Nyctiophylax						M	M				
PSYCHOMYIIDAE											
Lype diversa	A	M A	M A		M				M	M	
Psychomyia	M									M	
RHYACOPHILIDAE											
Rhyacophila			M			M	M		M	M	M
LEPIDOPTERA											
PYRALIDAE											
Petrophila	A		A	A	A		A	A	A		M A
COLEOPTERA											
ELMIDAE											
Ancronyx				M							
Dubiraphia minima	M A	M A	M A	M A	M	M	M A	M A	M A	M A	M A
Macronychus			A	A				A		M	
Microcylloepus pusillus	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Optioservus trivittatus	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Oulimnius latiusculus		M A	A								
Promoresia elegans	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Stenelmis crenata	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
Stenelmis mera	A		M A	M A	A	M A	M A	M A	M A	M A	M A
Stenelmis sandersoni										M A	M A
Stenelmis musgravei			A		M		A		M	A	A
Stenelmis markeli					A						
Stenelmis lateralis											M A
GYRINIDAE											
Dineutus									M		
HYDROPHILIDAE											
Berosus	M	M	M	M	M	M	M	M	M	M	
LUTROCHIDAE											
Lutrochus										M A	
PSEPHENIDAE											
Ectopria			M		M			M	M	M A	A
Psephenus herricki	M A	M A	M A	M A		M A	M A	M	M A	M A	M A
SCIRTIDAE											
Scirtes								M			
DIPTERA											
ATHERICIDAE											
Atherix	A		A							M A	A
BLEPHARICERIDAE											
Blepharicera											M
CERATOPOGONIDAE		M				M	M A	M		M	M A
CHIRONOMIDAE	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
EMPIDIDAE											
Clinocera						M				M	
Hemerodromia	M A		M A	M A	M A	M A	M A	M A	M	M	M
SIMULIIDAE											
Simulium	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
TABANIDAE										M	

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
TANYDERIDAE											
Protoplasa fitchii											M
TIPULIDAE											
Antocha	M A	M A	M A	M	M A	M	M	M	M A	M A	M A
Hexatoma									M		
Tipula		M	M	M				M A	M		
Total taxa	55	56	54	48	44	54	55	58	56	72	67

Table 6. 'Top taxa', selected as comprising > 0.2 % of total abundance at a site collected during three sample periods listed in order of descending overall abundance.

Spring 2006	August 2006	2007 Large river
Chironomidae	*Baetis* (complex)	*Stenelmis*
Stenelmis	*Stenelmis*	*Baetis (*complex)
Leptoxis carinata	*Leptoxis carinata*	Chironomidae
Optioservus	*Macaffertium/Stenonema*	*Leptoxis carinata*
Baetis (complex)	*Optioservus*	Empididae
Planariidae	*Isonychia*	Macaffertium/Stenonema
Cheumatopsyche	*Cheumatopsyche*	Ephemerella
Corbicula fluminea	Chironomidae	Hydropsyche
Hydropsyche	*Hydropsyche*	Simulium
Serratella	Planariidae	Optioservus
Macaffertium/Stenonema	*Serratella*	Planariidae
Simuliium	*Tricorythodes*	*Hydropsyche*
Ephemerella	*Leuctra*	*Caenis*
Microsema	*Chimarra*	*Cheumatopsyche*
Psephenus	*Simulium*	*Anthopotamus*
Caenis	*Micrasema*	*Psephenus herricki*
Chimarra	*Corbicula fluminea*	*Microcylloepus*
Helicopsyche	*Agraylea*	*Isonychia*
Perlesta	*Corydalus*	*Stenacron*
Berosus	*Promoresia*	*Eurylophella*
Dubiraphia	*Psephenus*	*Chimarra*
Isonychia	*Helicopsyche*	Dryopidae
Brachycentrus	*Agnetina*	Tabanidae
Protoptila	*Caenis*	*Dubiraphia*
Leuctra	*Protoptila*	*Serratella*
Ceratopogonidae	*Hydroptila*	*Leptophlebia*
Anthopotamus	*Heptagenia*	*Corbicula fluminea*
Promoresia	*Lepidostoma*	Ceratopogonidae
Hemerodromia		Taeniopterygidae
Antocha		*Agnetina*
Drunella tuberculata		*Isoperla*
Diplectrona		*Macrostemum*
Corydalus		*Promoresia*
Rithrogena		*Leucrocuta*
Stenacron		*Acroneuria*
Nemertean		Leptophyidae
		Scirtidae
		Helicopsyche borealis
		Oligochaeta
		Antocha
		Nematoda

Table 7. Spring 2006 macroinvertebrate metrics calculated for eleven large river sites and two New River sites (*). mod = modified; CG = collector-gatherer; CF = collector-filterer.

Site	Total abundance	Taxa richness	Simpson's Diversity Index	%mod EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawler	Mod % Clinger
North river	714	30	0.884	26	18	24	47	45	15	10	65
Harriston	445	23	0.820	18	9	14	25	72	21	4	53
Lynnwood	566	25	0.819	18	22	19	33	64	11	7	44
Whitehouse	846	28	0.846	12	38	21	43	47	8	6	52
Front Royal	554	25	0.783	18	17	9	33	62	21	5	51
Mount Jackson	441	28	0.870	38	13	24	45	43	4	13	59
Woodstock	637	31	0.846	16	20	19	47	48	11	5	62
Strasburg	598	28	0.797	12	28	23	64	32	10	3	73
Berryville	726	24	0.739	18	4	2	32	63	12	4	52
Cedar	664	35	0.891	27	21	29	51	41	5	12	61
Cowpasture	498	27	0.881	35	11	24	55	29	10	12	72
Prince*	218	19	0.764	36	31	39	40	49	14	8	73
Thurmond*	164	17	0.857	43	17	30	37	54	28	8	77

Table 8. Summer 2006 macroinvertebrate metrics calculated for eleven large river sites and two New River sites. mod = modified; CG = collector-gatherer; CF = collector-filterer.

Site	Total abundance	Taxa richness	Simpson's Diversity Index	%mod EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawler	Mod % Clinger
North river	929	29	0.868	49	17	21	30	56	14	76	7
Harriston	713	25	0.873	58	8	24	16	66	34	84	6
Lynnwood	913	29	0.875	36	24	25	41	52	17	81	4
Whitehouse	840	25	0.852	29	15	19	49	43	20	84	6
Front Royal	533	25	0.858	69	6	13	30	60	10	67	4
Mount Jackson	188	19	0.818	63	10	22	32	56	12	89	5
Woodstock	691	22	0.863	57	14	31	40	51	16	88	5
Strasburg	393	24	0.861	51	13	15	45	47	5	70	3
Berryville	807	27	0.875	42	5	18	35	52	28	79	8
Cedar	512	27	0.903	31	17	31	56	29	15	86	9
Cowpasture	529	27	0.895	42	6	18	36	51	23	71	9

Table 9. 2007 macroinvertebrate metrics calculated for seven large river sites. mod = modified; CG = collector-gatherer; CF = collector-filterer.

	Total abundance	Taxa richness	Simpson's Diversity Index	% modified EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawlers	Modified % clingers
Whitehouse	577	20	0.936	9	12	10	71	55	14	3	74
Cedar	533	22	0.956	10	13	12	69	54	15	4	71
North River	412	21	0.946	12	13	14	59	58	15	5	68
Woodstock	343	24	0.949	13	15	16	59	55	12	6	67
Cowpasture	296	23	0.938	13	18	19	68	48	9	7	71
Mount Jackson	334	24	0.945	17	15	18	58	54	12	8	68
Strasburg	289	26	0.948	18	14	20	51	55	13	9	66

Table 10. Virginia Stream Condition Index (VSCI) by site calculated from August 2006 data. (*) indicates data from the New River collected during late summer 1988 and 1989 (Thurm = Thurmond). Site codes are explained in Table 1. E = Ephemeroptera; P = Plecoptera; T = Trichoptera; HBI = Hilsinhoff Biotic Index.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp	Prince*	Thurm*
Taxa Richness	34	29	32	28	30	23	27	31	29	31	32	24	21
EPT Index	17	15	15	14	16	10	11	13	16	15	17	12	10
% P+T - Hydropsychidae	8.84	7.02	3.95	2.54	5.59	0.89	0.31	1.78	3.70	4.76	17.05	3.60	8.22
HBI (modified family)	4.48	4.34	4.31	4.35	4.42	4.24	4.09	4.35	4.34	3.77	3.96	3.62	4.00
% Top 2 Dominant Family	42.68	32.25	39.70	49.31	41.84	52.88	45.08	49.11	49.22	50.68	39.65	48.05	36.24
% Chironomidae	5.58	4.19	4.45	2.46	2.53	2.13	0.75	1.31	3.91	1.30	15.47	10.42	3.76
% Scraper	36.24	31.78	44.75	53.55	37.66	40.91	46.94	52.50	42.25	63.17	39.84	52.49	42.34
% Ephemeroptera	39.87	48.58	31.05	26.31	62.34	61.93	56.44	46.23	37.26	25.78	26.60	29.04	35.13
SCI	**77.97**	**80.32**	**77.49**	**75.57**	**81.84**	**78.27**	**82.04**	**79.96**	**76.41**	**77.21**	**79.28**	**77.09**	**78.85**
	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired

Table 11. Virginia Stream Condition Index (VCI) by site calculated from May 2006 data. Site codes are explained in Table 1. E = Ephemeroptera; P = Plecoptera; T = Trichoptera; HBI = Hilsinhoff Biotic Index.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp
Taxa Richness	34	34	30	27	30	33	35	32	36	46	36
EPT Index	16	17	16	13	15	16	15	12	18	20	17
% P+T - Hydropsychidae	2.68	2.15	3.67	1.98	5.36	4.19	1.25	4.44	2.27	6.01	14.39
HBI (modified family)	4.26	5.10	4.86	5.06	4.99	4.08	4.63	4.34	5.17	4.05	3.64
% Top 2 Dominant	44.87	50.45	50.01	45.92	52.35	45.31	54.61	57.78	67.73	43.62	53.91
% Chironomidae	8.93	36.37	33.68	14.13	27.90	14.09	19.62	12.74	40.78	18.39	4.42
% Scraper	52.43	21.06	32.60	43.84	34.43	54.19	50.27	61.61	29.52	54.64	62.20
% Ephemeroptera	21.20	15.68	13.82	9.18	13.40	28.61	14.40	7.45	15.22	19.80	15.76
SCI	**74.68**	**60.21**	**64.16**	**68.22**	**65.21**	**76.40**	**68.98**	**69.47**	**58.57**	**74.99**	**77.82**
	Not Impaired	Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Impaired	Not Impaired	Not Impaired

Table 12. Macroinvertebrate mean biomass calculated from large river riffle areas at each study site in spring 2006. Only taxa that dominate the non-mollusk biomass are shown. Values are mean biomass (mg dry mass per m^2) of 6 replicate samples. Site codes are explained in Table 1.

TAXON	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp
Stenelmis	610	103	243	700	325	284	605	541	453	113	371
Chironomidae	91	216	301		265	97	230	53	547	188	
Corydalus	141			2133		370	424	43	702	282	117
Isonychia	126			508	94	125	144	90		61	
Maccaffertium/Stenonema	75	200				210	62	57		69	
Agnetina	124		250								96
Hdyropsyche	111	47	151								
Cheumatopsyche					64	97			192		
Ephemerella	278		90			148					
Serratella	100	82								80	
Baetis			87		66		69				
Optioservus										52	82
Leucrocuta						140	111				
Psephenus		61								70	
Chimarra											154
Simulium		47									
Tipula			108								
Berosus				239							
Helicopsyche					44						
Nigronia										52	
Dubiraphia										50	
Acroneuria											154
Rithrogena											96
Number of dominant taxa	9	7	7	4	6	8	7	5	4	10	7

Total biomass (no mollusks)	2225	1018	1597	4728	1094	1850	2136	984	2519	1365	1434
Total biomass	7746	2914	6743	16960	4408	4513	8535	5139	3038	6469	3429

Table 13. Linear regression results showing spring 2006 metrics and top taxa versus DEQ water quality environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". EPT = Ephemeroptera, Plecoptera, and Trichoptera.

Response/Metric	Independent variable	p-value	R^2	Direction
Modified EPT	**Total phosphorus**	**0.029**	**0.428**	+
Simpson's Diversity Index	**Ammonia**	**0.038**	**0.397**	-
	Kjeldahl nitrogen	**0.031**	**0.420**	-
	Total phosphorus	**0.039**	**0.395**	-
Percent Crawler taxa	**Ammonia**	**0.05**	**0.362**	-
Optioservus	**Maximum Nitrate + Nitrite (N-N)**	**0.003**	**0.636***	-

Table 14. Linear regression results showing August 2006 metrics versus DEQ water quality, epilithic, and substrate environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". AFDM = ash-free-dry-mass of epilithon.

Response/Metric	Independent Variable	p-value	R^2	Direction
Total abundance	**Gravel**	**0.019**	**0.477**	**-**
	AFDM epilithon	**0.005**	**0.594***	**+**
	Ammonia max	**0.040**	**0.427**	+
	Kjeldahl max	**0.022**	**0.500**	+
Taxa richness	**Phosphorus**	**0.049**	**0.403**	**-**
Simpson's Diversity Index	**Nitrate**	**0.033**	**0.452**	**-**
	N-N	**0.033**	**0.453**	**-**
	Phosphorus	**0.011**	**0.577***	**-**
	Total phosphorus	**0.016**	**0.538**	**-**
	Nitrate max	**0.018**	**0.524**	**-**
	N-N max	**0.018**	**0.526**	**-**
	Phosphorus max	**0.015**	**0.545**	**-**
	Orthophosphate max	**0.010**	**0.587***	**-**
Percent non-insect	**AFDM**	**0.010**	**0.536**	**+**
Percent sensitive taxa	**Chlorophyll-a**	**0.007**	**0.576***	**+**
	Kjeldahl nitrogen	**0.035**	**0.444**	**-**
Percent collector taxa	**Orthophosphate**	**0.050**	**0.399**	+
Percent CR taxa	**Phosphorus**	**0.034**	**0.447**	**-**
Modified % clingers	**Nitrate**	**0.018**	**0.524**	**+**
	Nitrite	**0.034**	**0.449**	+
	Nitrate + Nitrite	**0.017**	**0.527**	**+**
	Nitrate max	**0.020**	**0.510**	**+**
	Nitrate + Nitrite max	**0.020**	**0.511**	**+**
	Phosphorus max	**0.011**	**0.573***	**+**
	Orthophosphate max	**0.010**	**0.586***	**+**

Table 15. Linear regression results showing 2007 large river 'top taxa' versus DEQ water quality variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". AFDM = ash-free-dry-mass of epilithon.

Response/Metric	Independent Variable	p-value	R^2	Direction
Leptoxis caranita	**Nitrite**	**0.041**	**0.689**	+
	Nitrate + Nitrite	**0.026**	**0.747**	**+**
	Ammonia	**0.014**	**0.812***	**+**
Stenelmis	**Nitrite**	**0.017**	**0.797**	**+**
	Nitrate + Nitrite	**0.008**	**0.856***	**+**
	Ammonia	**0.031**	**0.725**	**+**
Baetis	**Kjeldahl nitrogen**	**0.021**	**0.774**	**+**
	Phosphorus	**0.034**	**0.716**	**+**
	Orthophosphate	**0.041**	**0.689**	+
Macaffertium	**Phosphorus**	**0.007**	**0.866***	**+**
	Orthophosphate	**0.010**	**0.845***	**+**

Table 16. Comparison of taxa present in the Shenandoah River, Virginia (Berryville, Strasburg, Front Royal, 2006) and two other similar rivers in the mid-Atlantic region (New River, West Virginia, 1988-89; Susquehanna River, Pennsylvania, 1989).

	Shenandoah River	New River	Susquehanna River
NON- INSECT TAXA			
NEMATODA	X		X
HIRUDINEA	X		
OLIGOCHAETA	X		X
PLANARIIDAE	X	X	X
MOLLUSCA			
Bivalve	X	X	X
GASTROPODA			
PLEUROCERIDAE	X	X	
ANCYLIDAE	X		X
PHYSIDAE			
Physa	X		
PLANORBIDAE	X		
CRUSTACEA			
CAMBARIDAE	X	X	
ACARI (HYDRACARINA)	X		X
INSECT TAXA			
EPHEMEROPTERA			
BAETIDAE			
Baetis (complex)	X	X	X
Centroptilum			X
Heterocloeon			X
Pseudocloeon			X
Unidentified Baetidae			X
CAENIDAE			
Caenis	X	X	X
EPHEMERELLIDAE			
Drunella tuberculata	X		
Ephemerella	X		
Serratella	X	X	X
EPHEMERIDAE			
Hexagenia			X
HEPTAGENIIDAE			
Epeorus		X	
Heptagenia		X	X
Leucrocuta	X		X
Maccaffertium/Stenonema	X	X	X
Stenacron	X	X	X
Rhithrogena	X		X

	Shenandoah	New	Susquehanna
Unidentified Heptageniidae			X
ISONYCHIIDAE			
Isonychia	X	X	X
LEPTOPHLEBIIDAE	X		
Choroterpes			X
LEPTOHYPHIDAE			
Tricorythodes	X	X	X
POLYMITARCYIDAE			
Ephoron		X	X
POTAMANTHIDAE			
Anthopotamus	X		X
PLECOPTERA			
CHLOROPERLIDAE	X		
LEUCTRIDAE			
Leuctra	X		
PERLIDAE		X	X
Acroneuria	X	X	
Agnetina	X		
Neoperla		X	
Perlesta placida (group)	X		
ODONATA			
COENAGRIONIDAE			
Argia	X	X	
GOMPHIDAE			
Lanthus	X	X	
Stylogomphus	X		
MEGALOPTERA			
CORYDALIDAE			
Corydalus cornutus	X	X	
Nigronia fasciatus		X	
SIALIDAE			
Sialis			X
NEUROPTERA			
SISYRIDAE			
Clinacia	X		
TRICHOPTERA			
BRACHYCENTRIDAE			
Brachycentrus	X		
Micrasema	X	X	
GLOSSOSOMATIDAE			
Protoptila	X		X
HELICOPSYCHIIDAE			
Helicopsyche borealis	X		
HYDROPSHYCHIDAE			
Diplectrona	X		
Cheumatopsyche	X	X	X
Hydropsyche	X	X	X
Macrostemum			X

	Shenandoah	New	Susquehanna
HYDROPTILIDAE			
Agraylea	X		
Hydroptila	X	X	X
LEPTOCERIDAE			
Ceraclea	X	X	X
Nectopsyche	X		
Oecetis	X		
Triaenodes	X		
LEPIDOSTOMATIDAE			
Lepidostoma	X		
UENOIDAE			
Neophylax	X		
PHILOPOTAMIDAE			
Chimarra	X	X	X
PSYCHOMYIIDAE			
Lype diversa	X		
RHYACOPHILIDAE			
Rhyacophila	X		
LEPIDOPTERA			
PYRALIDAE			
Petrophila	X		X
COLEOPTERA			
ELMIDAE			
Dubiraphia	X		X
Microcylloepus	X	X	
Optioservus	X	X	X
Promoresia	X		
Stenelmis	X	X	X
HYDROPHILIDAE			
Berosus	X		X
PSEPHENIDAE			
Ectopria	X		
Psephenus herricki	X	X	X
SCIRTIDAE			
Scirtes	X		
DIPTERA			
CERATOPOGONIDAE	X		X
CHIRONOMIDAE	X	X	X
EMPIDIDAE			
Hemerodromia	X	X	
SIMULIIDAE			
Simulium	X	X	X
TIPULIDAE			
Antocha	X	X	
Tipula	X		
Total number of taxa	68	35	43

Table 17. Mean metric values for benthic macroinvertebrates assemblage in samples taken from Shenandoah large river sites by Virginia Tech in May 2006 compared to samples taken by Eugene Surber in the 1960s. Site codes are explained in Table 1.

	Surber 1960s							VT 2006						
Metrics	MtJack	Wood	Stras	Lynn	White	Froyal	Berry	MtJack	Wood	Stras	Lynn	White	Froyal	Berry
Total Density	702	704	221	489	581	365	352	441	637	598	566	846	554	726
Total Richness	24	25	20	21	19	22	15	28	31	28	25	28	25	24
Simpsons Diversity Index	0.849	0.852	0.844	0.822	0.774	0.837	0.754	0.870	0.846	0.797	0.819	0.846	0.783	0.739
% Modified EPT	11	26	41	22	9	39	3	38	16	12	18	12	18	18
% Non-insects	34	33	19	6	8	7	7	13	20	28	22	38	17	4
% Sensitive	31	29	16	6	2	7	1	24	19	23	19	21	9	2
% Scrapers	41	28	45	13	8	21	13	45	47	64	33	43	33	32
% Collector-gatherers	56	70	48	76	86	76	83	43	48	32	64	47	62	63
% Collector-filterers	38	28	4	25	56	42	35	4	11	10	11	8	21	12
% Crawlers	12	36	38	18	9	25	9	13	5	3	7	6	5	4
% Modified Clingers	9	22	29	8	6	17	4	59	62	73	44	52	51	52

Table 18. List of taxa and mean density (numbers of individuals per m^2) collected by Eugene Surber in the 1960's and in May 2006 by Virginia Tech in the current study. All samples were collected at the same seven large river sites and in the same season (see text).

	Surber (52 taxa)	**VT** (65 taxa)
NON- INSECT TAXA		
NEMATODA	100	22
OLIGOCHAETA	1578	
HIRUDINEA	89	
PLANARIIDAE	1522	16811
NEMERTEAN		589
MOLLUSCA		
CORBICULIDAE		
Corbicula fluminea		562
SPHAERIIDAE	2100	222
PLEUROCERIDAE		
Leptoxis carinata	6956	38511
ANCYLIDAE	556	156
PHYSIDAE		
Physa	211	189
PLANORBIDAE	44	344
UNIONIDAE	11	
CRUSTACEA		
CAMBARIDAE	11	22
CRANGONYCTIDAE		
Crangonyx		322
TALITRIDAE		
Hyalella azteca	500	
ACARI (HYDRACARINA)		22
INSECT TAXA		
EPHEMEROPTERA		
BAETIDAE		
Baetis (complex)	2178	80333
CAENIDAE		
Caenis	322	1467
EPHEMERELLIDAE	6400	14400
HEPTAGENIIDAE		
Maccaffertium/Stenonema	2578	33600
Stenacron		311
Epeorus	11	88
ISONYCHIIDAE		
Isonychia	689	28667
LEPTOPHLEBIIDAE		1
LEPTOHYPHIDAE		
Tricorythodes	144	14922
POTAMANTHIDAE		
Anthopotamus	144	189
POLYMITARCYIDAE		
Ephoron	667	
PLECOPTERA		
LEUCTRIDAE		
Leuctra		13867

PERLIDAE		
Acroneuria	178	656
Agnetina		2011
Neoperla		67
Paragnetina		22
Perlesta placida (group)		167
PERLODIDAE	22	
PTERONARCYIDAE		
Pteronarcys		33
ODONATA		
CALOPTERYGIDAE		
Calopteryx	33	
COENAGRIONIDAE		
Argia	89	855
GOMPHIDAE		
Lanthus		178
Stylogomphus		67
Progomphus	11	
MACROMIIDAE		
Micromia	11	
LEPIDOPTERA		
PYRALIDAE		
Petrophila		522
NEUROPTERA		
SISYRIDAE		
Climacia		178
MEGALOPTERA		
CORYDALIDAE		
Corydalus cornutus	111	5078
Nigronia fasciatus		11
SIALIDAE		
Sialis	122	22
TRICHOPTERA		
BRACHYCENTRIDAE		
Brachycentrus		522
Micrasema		11
GLOSSOSOMATIDAE		
Glossosoma nigrior	244	
Protoptila		1467
HELICOPSYCHIIDAE		
Helicopsyche borealis	222	2156
HYDROPSHYCHIDAE		
Cheumatopsyche	11044	25211
Hydropsyche	8978	16578
Macrostemum	111	33
Parasyche		167
HYDROPTILIDAE	244	7011
LEPTOCERIDAE		
Ceraclea		156
Oecetis		89
LEPIDOSTOMATIDAE		
Lepidostoma		1122
LIMNEPHILIDAE	44	
Pycnopsyche		11
UENOIDAE		
Neophylax		11

PHILOPOTAMIDAE	44	
Chimarra		9411
POLYCENTROPODIDAE		
Polycentropus		11
Neureclipsis	211	11
PSYCHOMYIIDAE		
Lype diversa		56
RHYACOPHILIDAE		
Rhyacophila	156	
COLEOPTERA		
ELMIDAE	6200	113211
DRYOPIDAE		
Helichus	122	
HYDROPHILIDAE		
Berosus	433	
PSEPHENIDAE		
Ectopria		44
Psephenus herricki	1322	3022
HALIPLIDAE	44	
DIPTERA	1622	
ATHERICIDAE		
Atherix	156	444
CERATOPOGONIDAE		144
CHIRONOMIDAE	12756	19422
EMPIDIDAE		
Hemerodromia	78	200
SIMULIIDAE	380	
Simulium		7567
TIPULIDAE		
Antocha	33	611
Hexatoma	122	
Tipula	33	11

Table 19. Eleven metrics summarizing assemblage structure and function at 26 Shenandoah river tributary sites in 2007. EPT = Ephemeroptera, Trichoptera, Plecoptera; CG = collector-gatherer; CF = collector-filterer.

	Total abundance	Taxa richness	Simpson's Diversity Index	% modified EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawlers	Modified % clingers
HAWK	211	13	0.779	24	4	1	1	23	24	22	18
COOK	231	6	0.502	0	28	0	0	27	8	1	7
LOMR	1157	15	0.673	1	22	7	21	16	10	6	24
SMHW	970	18	0.712	25	2	3	10	36	10	24	26
JENN	290	15	0.530	18	1	10	3	14	13	14	10
LGCR	841	19	0.424	2	8	0	2	8	8	7	5
MUDD	335	14	0.583	13	3	3	6	20	15	10	17
BRIR	228	15	0.750	32	5	21	19	25	25	20	49
GOON	193	15	0.726	34	1	13	8	27	25	27	27
CEHW	288	20	0.728	22	4	15	13	12	46	13	59
STHW	127	11	0.661	27	2	8	11	20	12	23	18
LINV	231	11	0.331	3	2	1	13	4	2	2	15
PASS	184	15	0.664	32	2	6	14	21	29	27	39
HOLM	307	15	0.754	19	2	0	6	22	33	17	21
MCNF	370	15	0.511	15	1	2	10	15	7	12	15
MEAD	303	15	0.531	9	6	7	23	5	6	6	27
BACK	173	12	0.703	53	0	9	7	41	16	47	15
CHRIS	315	18	0.727	28	3	6	22	31	7	28	27
CEDS	91	12	0.805	16	8	9	26	24	25	7	67
NRHW	142	10	0.537	21	1	6	7	16	10	15	14
STDS	906	19	0.471	8	4	8	8	10	11	6	20
HADS	216	13	0.832	20	4	5	7	26	33	15	37
SMDS	771	21	0.755	54	5	17	17	43	16	46	28
NAAU	472	14	0.441	7	5	2	11	10	11	9	14
NAPA	150	10	0.726	34	1	3	7	39	19	26	29
MCSF	1940	23	0.394	6	5	5	9	8	3	6	11

Table 20. Linear regression results showing eleven 2007 tributary metrics versus land-cover variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". Beef = beef operations; dairy = dairy operations; PH = poultry houses; AFO = animal feeding operations; NMP = nutrient management plan for poultry litter.

Response/Metric	Independent Variable	p-value	R^2	Direction
Total abundance	% forest	0.025	0.200	-
	% pasture/hay	0.012	0.242	+
	# beef/1000acres	0.022	0.208	+
Taxa richness	# dairy/1000acres	0.048	0.159	-
Simpson's Diversity Index	**% forest**	**0.005**	**0.300**	**+**
	% pasture/hay	0.006	0.288	-
	% crop	0.033	0.183	-
% modified EPT	**% forest**	**<0.001**	**0.427**	**+**
	% pasture/hay	**<0.001**	**0.428**	**-**
	% crop	0.007	0.278	-
	# dairy/1000acres	0.037	0.175	-
	# beef/1000acres	0.019	0.217	-
	# PH/1000acres	0.004	0.302	-
	# AFO/1000acres	0.007	0.273	-
	# acresNMP	0.013	0.240	-
% non-insect taxa	% forest	0.013	0.240	-
	% pasture/hay	0.032	0.185	+
	% crop	**<0.001**	**0.462**	**+**
	% development	0.034	0.180	+
	# dairy/1000acres	**<0.001**	**0.540***	**+**
	# beef/1000acres	**<0.001**	**0.382**	**+**
	# PH/1000acres	**<0.001**	**0.596***	**+**
	# AFO/1000acres	**<0.001**	**0.670***	**+**
	% acres NMP	0.005	0.293	+
% sensitive taxa	**% forest**	**0.004**	**0.305**	**+**
	% pasture/hay	0.005	0.296	-
	% crop	0.025	0.199	-
	% acresNMP	0.010	0.256	-
% collector-filterer	**% forest**	**0.002**	**0.351**	**+**
	% pasture/hay	**0.004**	**0.314**	**-**
	% crop	0.013	0.242	-
	% development	0.012	0.247	-
	# beef/1000acres	0.013	0.240	-
% crawler taxa	% forest	0.010	0.257	+
	% pasture/hay	0.011	0.252	-
	% crop	0.034	0.181	-
	# PH/1000acres	0.024	0.203	-
	# AFO/1000acres	0.025	0.200	-
	% acres NMP	0.041	0.170	-

% modified clingers	Watershed area	0.012	0.246	+
	% forest	**0.005**	**0.301**	**+**
	% pasture/hay	0.008	0.268	-
	% crop	0.012	0.244	-
	% development	0.031	0.187	-
	# beef/1000acres	0.035	0.179	-
	# AFO/1000acres	0.043	0.167	-
	% acres NMP	0.042	0.167	-

Table 21. Linear regression results showing 2007 tributary 'top taxa' versus land-cover variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". PH = poultry houses; beef = beef operations; AFO = animal feeding operations; NMP = nutrient management plan for poultry litter; MUNSTP = municipal sewage treatment plant.

Response/eMtric	Independent Variable	p-value	R^2	Direction
Chironomidae	% forest	0.014	0.233	+
	% pasture/hay	0.009	0.262	-
Ephemerella	# beef/1000acres	0.007	0.273	+
	# MUNSTP	**<0.001**	**0.424**	**+**
Hydropsyche	% pasthay	0.019	0.216	+
	# beef/1000acres	**<0.001**	**0.416**	**+**
	# PH/1000acres	**0.002**	**0.336**	**+**
Stenelmis	% forest	0.012	0.246	-
	% pasture/hay	0.007	0.274	+
	# beef/1000acres	0.021	0.211	+
Cheumatopsyche	# PH/1000acres	0.031	0.187	+
Leptoxis carinata	# beef/1000acres	0.017	0.222	+
	# MUNSTP	0.038	0.172	+
Planariidae	**% forest**	**0.002**	**0.334**	**-**
	% crop	**<0.001**	**0.521**	**+**
	# AFO	0.029	0.190	+
	# dairy/1000acres	**<0.001**	**0.391**	+
	# PH/1000acres	**<0.001**	**0.614***	**+**
	# AFO	**<0.001**	**0.588***	**+**
	# acresNMP	**<0.001**	**0.364**	**+**
Oligochaeta	% forest	0.022	0.208	-
	# beef/1000acres	0.035	0.180	+
	# PH/1000acres	0.05	0.157	+
Hemerodromia	% forest	0.017	0.222	-
	% pasture/hay	0.006	0.286	+
	# beef/1000acres	0.042	0.167	+
Macaffertium	Watershed area	0.022	0.209	+
	# AFO/1000acres	0.027	0.195	-
	# PH/1000acres	0.021	0.210	-
	# MUNSTP	0.008	0.270	+

Table 22. Linear regression results showing eleven 2007 tributary macroinvertebrate metrics and 'top taxa' versus other environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". Mod = modified

Response/eMtric	Independent Variable	p-value	R^2	Direction
Taxa richness	**Phosphorus**	**0.041**	**0.696***	**-**
% mod EPT	**Nitrate**	**0.04**	**0.475**	**-**
	Nitrogen	**0.033**	**0.499**	**-**
% sensitive taxa	**Nitrate**	**0.009**	**0.345**	**-**
	Nitrogen	**0.011**	**0.628***	**-**
mod% clingers	**Phosphorus**	**0.043**	**0.995***	**-**
Hydropsyche	**Phosphorus**	**0.022**	**0.999***	**-**
Antocha	**Phosphorus**	**0.013**	**0.741***	**+**
Leptoxus caranita	**Total phosphorus**	**0.033**	**0.997***	**-**
Planaria	**Ammonia**	**0.029**	**0.579**	**+**
Taxa richness	Clam arsenic	0.048	0.190	+
Simpson's Diversity	Clam mercury	0.047	0.192	+
	Clam lead	0.022	0.227	-
% mod EPT	Clam arsenic	0.049	0.172	-
	Clam lead	**<0.001**	**0.471**	**-**
% non-insect	Sediment chromium	0.030	0.206	+
	Sediment lead	0.034	0.198	+
% sensitive taxa	Clam cadmium	0.043	0.199	+
	Sediment lead	**0.006**	**0.306**	**-**
	Total Organic Carbon	**0.003**	**0.360**	**-**
% CG	Sediment lead	0.036	0.193	-
% CF	Clam arsenic	0.039	0.205	-
	Clam cadmium	**0.002**	**0.407**	**+**
	Clam mercury	**0.005**	**0.349**	**+**
	Sediment lead	**0.007**	**0.301**	**-**
% crawlers	**Sediment lead**	**0.002**	**0.364**	**-**
Mod % clingers	**Clam cadmium**	**<0.001**	**0.519***	**+**
	Clam mercury	0.011	0.297	+
	Sediment lead	0.038	0.189	-
	Total Organic Carbon	0.015	0.260	-
Stenelmis	Clam arsenic	0.025	0.236	+
	Sediment manganese	0.04	0.185	+
Simuliidae	**Clam cadmium**	**<0.001**	**0.533***	**+**
	Sediment cobalt	0.021	0.227	-
	Clam mercury	0.015	0.345	+
Oligochaeta	Sediment arsenic	0.044	0.179	+
	Sediment chromium	0.023	0.222	+
Baetis	Clam chromium	0.026	0.235	+

Hemerodromia	Index of Estrogenic Activity (E2Eq)	0.037	0.200	+

Table 23. Abundance of elmid adults collected at each tributary sites. Where genus abbreviation is used it is the same as the preceding column reading left to right.

Site Code	*Ancronyx verigata*	*Macronychus glabratus*	*Microcylloepus pusillis*	*Optioservus trivittatus*	*O. ovalis*	*Oulimnius latiusculus*	*Promoresia tardella*	*P. elegans*	*Stenelmis crenata*	*S. mera*	*S .sandersoni*	*S. musgravei*	*Stenelmis markeli*	*Stenelmis lateralis*
HAWK	0	0	1	1	0	0	0	0	0	0	0	0	0	0
COOK	0	0	0	0	0	0	0	0	0	1.3	0	0	0	0
LOMR	0	23.8	0	0	0	0	0	0	2	20.3	0	0	0	0
SMHW	0	1	0	0	2.3	1.5	0	0	0	10.8	0	0	0	0
JENN	3.5	0	1	0	1	0	0	0	0	0	1	0	0	0
LGCR	3.5	0	5.6	0	2.2	1	0	0	0	1	10.2	0	0	0
MUDD	3.5	0	1	0	0	1.5	0	0	0	0	3.7	0	0	0
BRIR	3.5	0	0	0	0	0	0	0	0	0	0	0	0	0
GOON	3.5	0	1	0	0	2	1	0	0	1	1	0	0	0
CEHW	3.5	0	2	0	0	1	0	1	0	0	2	1.5	0	0
STHW	3.5	0	0	0	0	0	0	2	0	0	4	0	0	0
LINV	3.5	0	0	0	0	1	0	0	0	0	2.3	1	0	0
PASS	3.5	0	0	0	1	5	0	1	1	0	0	3	0	0
HOLM	3.5	0	0	0	0	0	4	0	0	0	5.8	0	0	0
MCNF	3.5	0	1	0	0	1	0	0	0	1	2.5	0	0	0
MEAD	3.5	0	1.3	0	0	1.3	0	0	0	0	9.5	0	0	0
BACK	3.5	0	0	0	0	0	0	0	0	0	1	0	0	0
CHRIS	3.5	0	2.5	1	1	2.3	2	0	0	2.5	15.5	0	0	0
CEDS	3.5	0	0	0	0	2	0	0	0	0	0	4	1	0
NRHW	3.5	0	0	0	0	0	0	0	0	0	0	0	0	0
STDS	3.5	0	1	0	0	1	0	0	0	0	2.3	0	0	0
HADS	3.5	0	0	0	0	1	0	0	0	0	4.3	0	0	0
SMDS	3.5	0	1	0	1	3.5	0	0	0	3.7	5.8	0	0	1
NAAU	3.5	0	3	0	0	0	0	0	0	3	3.2	0	0	0
NAPA	3.5	0	0	0	0	1	0	0	0	0	0	0	0	0
MCSF	3.5	1.25	2.2	1	1.7	1.7	1	0	0	4.3	11	1	0	0