Studies of Benthic Macroinvertebrates in Western Virginia Streams as Related to the

Implementation of Rapid Bioassessment Techniques

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(ABSTRACT)

This study tested two key assumptions in developing regional biocriteria: (1) the accuracy of the ecoregion classification framework and (2) the accuracy of standardized qualitative sampling. Except for the Central Appalachians ecoregion, there was little or no correspondence of benthic macroinvertebrate distribution with the ecoregions or subregions of western Virginia. I found that it was more accurate to rearrange the subregions into three larger regions called bioregions: the forested hills and mountains (subregion 69a), valleys and plateaus (subregions 66c, 67a, and 67b), and the mountains (subregions 66a, 66b, 67c, and 67d). As an alternative to the ecoregion classification scheme, I classified my reference sites in biotic groups and then assessed the effect of several environmental variables on discriminating between the groups. There was a 69.8% correct classification rate using 14 environmental variables. Stepwise multiple discriminant analysis and graphical analysis showed that sampling date, slope, pH, habitat assessment score and distance to source were the best predictors of community structure. These environmental variables correctly classified 52.8% of the reference sites. These classification rates are comparable to rates published in similar studies. My study demonstrated that aggregations of subregions into bioregions and a biotic approach are more accurate classification schemes than ecoregions or subregions for biocriteria based on benthic macroinvertebrates.

A second study evaluated the accuracy of a standardized qualitative sampling approach, commonly used in rapid bioassessments, in assessing the biological condition of lotic systems. I

compared a typical standardized qualitative sampling method with subsampling with a typical quantitative sampling method on a stream with varying degrees of impairment. Although some metrics did respond to differences in sample abundance, overall the two methods made similar estimates of community composition. The two sampling methods made the same assessment an average 89% of the time using multimetric index developed for the Mid-Atlantic Region. I found no pattern showing one method was more accurate in making assessments of biological condition than the other. Given the greater time and costs associated with quantitative sampling methods, I conclude that standardized qualitative methods, are preferable for rapid bioassessment approaches to environmental assessment.

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INTRODUCTION

Early naturalists recognized that water pollution changed the structure of aquatic communities. Later researchers endeavored to characterize the aquatic biota of unimpacted stream conditions as well as describe the response of aquatic communities to various types and levels of pollution. Thresholds (biological criteria) were eventually set to indicate when the aquatic biota of a test site differed enough from the reference condition to be classified as impaired (Cairns and Pratt 1993, Davis 1995, Southerland and Stribling 1995).

In 1981, Karr published a set of assessment thresholds for fish communities in the streams of Illinois and Indiana. This revolutionary biocriterion was called the Index of Biotic Integrity (IBI). The IBI was based on discrete measurements of 12 different attributes (metrics) of a fish community. Each of these metrics described a different aspect of the fish community and responded in a predictable way to increasing impairments (Karr et al. 1986, Karr 1991, Barbour et al. 1995). When integrated, the metrics provided an overall assessment of the condition of the community. To make an assessment, each metric was calculated based on the sample of the fish community collected at a test site. The metric values were assigned a numerical score based on their differences from pre-determined reference conditions. An overall assessment score is calculated by summing the 12 metric scores. The site is designated an integrity class (excellent, good, fair, poor, very poor, no fish) based on the overall assessment score.

Before long, multimetric indices were developed for benthic macroinvertebrates. In 1986, the Ohio Environmental Protection Agency developed the Invertebrate Community Index (ICI) based on 10 benthic macroinvertebrate community metrics (DeShon 1995). For the assessment of streams in Arkansas, Shackleford (1988) created a multimetric biotic index based on 7 metrics. In 1989, the US Environmental Protection Agency (USEPA) published a

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multimetric index using benthic macroinvertebrates and fish called Rapid Bioassessment Protocols (RBPs) for use across the nation (Plafkin et al. 1989). Barbour et al. (1992) later revised the USEPA's index for macroinvertebrates by modifying or replacing the less accurate metrics. Kerans and Karr (1994) developed a Benthic Index of Biotic Integrity (B-IBI) for the rivers of the Tennessee Valley. And most recently, Barbour et al. (1996) developed a multimetric index called the Stream Condition Index (SCI) for assessing streams in Florida.

One by one, states are adopting similar multimetric biocriteria into their assessment programs. Southerland and Stribling (1995) report that currently, in the United States, more than 85% of state water quality agencies use some form of multimetric biocriteria to monitor their aquatic resources. Ninety percent of these programs use benthic macroinvertebrates.

The accuracy of assessments depends on the assumption that, in the absence of impairments, a test site and a reference site have similar benthic macroinvertebrate communities (Barbour et al. 1992). Given the high natural variability among benthic macroinvertebrate communities, ensuring the structural similarity between a reference site and a test site can be a problem. If reference sites are grouped improperly or a test site is not correctly matched with the reference condition, then inaccurate assessments will likely result.

Regulatory and natural resource agencies could set site-specific biological criteria for all waters within their jurisdictions. This site-specific approach would provide accurate assessments of current conditions at each site, but would also involve a substantial and extremely costly assessment effort (Hughes and Larsen 1988). For example, to account for natural variability, each site of concern requires a minimum of three reference sites. Therefore, using site-specific criteria, a state would need to monitor a total of 300 reference sites each season to simply assess 100 sites (Hughes 1995). In many areas, finding even one single unimpacted reference site for an assessment site is difficult.

An alternative approach is to select several minimally-impacted streams as reference sites that represent the best available conditions in that region. Test streams within the same region could be compared to these reference sites. A regional approach to biomonitoring is less expensive to develop especially if the state has numerous water quality problems (Hughes 1986).

The major obstacle against implementing a regional approach is the problem of geographic variability in biological conditions among streams. Research has shown that terrestrial features are a major influence on the structure of aquatic communities (Hynes 1970, Whitton 1975, Likens and Borman 1974). As water flows over and through the land to the stream channel, it acquires and integrates characteristics from the land, especially the soils, topography and vegetation. The combination of these factors helps shape the aquatic biota of the stream. The terrestrial factors that form the basis for the natural variability in streams also tend to be spatially associated. If terrestrial features determine much of the character of a stream, then regions with similar terrestrial features should also have stream communities that are similar ecologically. Regions of similar ecological character are called ecoregions (Crowley 1967).

Barbour et al. (1992) maintain that the accuracy and thus usefulness of multimetric biocriteria rests on the regional extent over which the designated metrics and thresholds reflect reality. Currently, ecoregions are being used as the classification framework for multimetric biocriteria. Several studies have shown fairly good correspondence between ecoregions and single ecosystem components [Larsen et al. 1986 (fish), Hughes et al. 1987 (fish), Rohm et al. 1987 (fish, water quality, physical habitat), Larsen et al. 1988 (fish, macroinvertebrates, periphyton, water quality, physical habitat), Whittier. et al. 1988 (fish, macroinvertebrates, water quality, physical habitat), and Lyons 1989 (fish)]. However, there is growing evidence that there is little uniformity of benthic macroinvertebrate communities within regions and some have suggested that benthic macroinvertebrate communities are controlled more by local conditions

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rather than the regional environment (e.g., Cummins et al. 1989, Corkum 1990, Corkum; 1991, Richards et al. 1993, Sweeney 1993).

Nevertheless, ecoregions continue being used for developing biocriteria (Hughes et al. 1994). Despite some concern about developing community-specific regions for large areas (Hughes et al. 1994), several natural resource agencies are currently producing separate ecoregion maps for one or several ecosystem components (usually fish, macroinvertebrates, and/or habitat) and then developing individual criteria for one or all of these components (Ohio EPA 1987, Kerans and Karr 1994, Hornig et al. 1995, Barbour et al. 1996). However, some researchers have attained greater accuracy in biomonitoring by rearranging ecoregions or subregions into "bioregions." Barbour et al. (1996), in developing a multimetric biotic index for Florida streams, found that an aggregation of nine subregions into three bioregions classified benthic macroinvertebrate communities better than subregions alone.

Another classification approach, advanced by Resh and Unzicker (1975) and developed by Wright et al. (1984) and Moss et al. (1987), is to base a classification on the biota first and then identify the predominant environmental factors responsible for structuring the biota. Test sites can then be matched to reference sites with similar environmental conditions. This approach not only ensures a high level of uniformity among the reference sites within each group, but also expands the possibility of finding better predictive environmental variables. The obvious disadvantage of this approach is that it requires sampling an enormous number of reference sites (Hughes 1985).

Another characteristic of many multimetric biocriteria is the use of a standardized qualitative approach in making assessments. Conventional assessments often rely on quantitative sampling methods that require replicate sample units and detailed statistical analysis. Generally, quantitative bioassessments are very accurate in detecting impairment. However,

processing quantitative samples is slow and tedious work. Such assessments quickly become very expensive and time consuming, especially when a large number of streams need to be monitored.

Standardized qualitative sampling with subsampling, commonly used in rapid bioassessment programs, reduces sample processing time substantially. Lenat and Barbour (1994) estimate that three to five sites can be completely sampled, processed, and analyzed by a single worker in five working days. Standardized qualitative sampling with subsampling enables regulatory and natural resource agencies to assess more streams. However, the approach is primarily a screening tool and was not designed to completely replace traditional quantitative sampling methods. If an impairment is found, the site can then be more thoroughly investigated with quantitative methods (Resh and Jackson 1993).

Until now, studies of rapid bioassessment have evaluated the accuracy of the method either by assessing the accuracy of the approach at sites with known levels of impact (Plafkin et al. 1989, Hannaford and Resh 1995) or by examining the variability of rapid bioassessment's benthic metrics in ascertaining impairment (Barbour et al. 1992, Barton and Metcalfe-Smith 1992, Resh and Jackson 1993, and Resh 1994). Rapid bioassessment was developed, in part, to replace unnecessary quantitative assessments, yet there has not been a direct comparison of rapid bioassessment with any quantitative sampling method.

Therefore, the success of large-scale multimetric biocriteria as a monitoring framework depends on two basic factors: (1) the accuracy of the classification scheme in matching test sites and reference sites; (2) the accuracy of a standardized qualitative approach in ascertaining impairments. I focused my research on these two factors. My study had three objectives:

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1) to determine if the distribution of benthic macroinvertebrates in western Virginia streams corresponds with the aquatic ecoregions and subregions proposed by Omernik (1987).

2) to compare two alternative classification schemes (landscape and biotic approaches) with ecoregions and subregions.

3) to compare the effectiveness of a standardized qualitative approach with quantitative techniques for analyzing the effects of perturbation on benthic macroinvertebrate communities.

I have organized this dissertation into chapters (one chapter for objectives 1 and 2, and one manuscript for objective 3) ready to be submitted for publication. Since the chapters will be submitted as the work of multiple authors, I have used the pronoun "we" consistently throughout each chapter. At the end of the dissertation, I have summarized my findings in a conclusions section.

Literature cited

Barbour, M.T., J. Gerritsen, G.E. Griffith, R. Frydenborg, E. McCarron, J.S. White, M.L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society 15:185-211.

Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves, and R.W. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11:437-449.

Barbour, M.T., J.B. Stribling, and J.R. Karr. 1995. The multimetric approach for establishing biocriteria and measuring biological condition. Pages 63-80 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers Boca Raton, Florida.

Barton, D.R., and J.L. Metcalfe-Smith. 1992. A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Quebec, based on benthic macroinvertebrates. Environmental Monitoring and Assessment 21:225-244.

Cairns, J.,Jr., and J.R. Pratt. 1993. A history of biological monitoring using benthic macroinvertebrates. Pages 10-27 *in* D.M. Rosenberg and V.H. Resh (editors). Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall. New York, New York.

Corkum, L.D. 1990. Intrabiome distributional patterns of lotic macroinvertebrate assemblages. Canadian Journal of Fisheries and Aquatic Sciences 47:2147-2157.

Corkum, L.D. 1991. Spatial patterns of macroinvertebrate distributions along rivers in eastern deciduous forest and grasslands biomes. Journal of the North American Benthological Society 10:358-371.

Crowley, J.M. 1967. Biogeography. Canadian Geographer 11:312-326.

Cummins, K.W., M.A. Wilzbach, D.M. Gates, J.B. Perry, and W.B. Taliaferro. 1989. Shredders and riparian vegetation. BioScience 39:24-30.

Davis, W.S. 1995. Biological assessment and criteria: building on the past. Pages 15-29 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers Boca Raton, Florida.

DeShon, J.E. 1995. Development and application of the Invertebrate Community Index (ICI). Pages 217-243 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Hannaford, M.J. and V.H. Resh. 1995. Variability in macroinvertebrate rapid-bioassessment surveys and habitat assessments in a northern California stream. Journal of the North American Benthological Society 14:430-439.

Hornig, C.E., C.W. Bayer, S.R. Tidwell, J.R. Davis, R.J. Kleinsasser, G.W. Linam, and K.B. Mayes. 1995. Development of regionally based biological criteria in Texas. Pages 145-152 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Hughes, R.M. 1985. Use of watershed characteristics to select control streams for estimating effects of metal mining wastes on extensively disturbed streams. Environmental Management 9:253-262.

Hughes, R.M. 1995. Defining acceptable biological status by comparing with reference conditions. Pages 31-48 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Hughes, R.M., D.P. Larsen, and J.M. Omernik. 1986. Regional reference sites: a method for assessing stream potential. Environmental Management 10:629-635

Hughes, R.M., E. Rexstad, and C.E. Bond. 1987. The relationship of aquatic ecoregions, river basins, and physiographic provinces to the ichthyogeographic regions of Oregon. Copeia 1987:423-432.

Hughes, R.M., and D.P. Larsen. 1988. Ecoregions: an approach to surface water protection. Journal of the Water Pollution Control Federation 60:486-493.

Hughes, R.M., S.A. Heiskary, W.J. Matthews, and C.O. Yoder. 1994. Use of ecoregions in biological monitoring. Pages 125-151 *in* S.L. Loeb and A. Spacie (editors). Biological monitoring of aquatic systems. Lewis Publishers, Boca Raton, Florida.

Hynes, H.B.N. 1970. The ecology of running waters. University of Toronto Press, Toronto.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Illinois Natural History Survey Special Publication 5, Champaign, Illinois.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66-84.

Kerans, B.L. and J.R. Karr. 1994. Development and testing of a benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications 4(4).

Larsen, D.P., J.M. Omernik, R.M. Hughes, C.M. Rohm, T.R. Whittier, A.J. Kinney, A.L. Gallant, and D.R. Dudley. 1986. The correspondence between spatial patterns in fish assemblages in Ohio streams and aquatic ecoregions. Environmental Management 10:815-828.

Lenat, D.R., and M.T. Barbour. 1994. Using benthic macroinvertebrate community structure for rapid, cost effective, water quality monitoring: rapid bioassessment. Pages 187-215 *in* S.L. Loeb and A. Spacie (editors). Biological Monitoring of Aquatic Systems. Lewis Publishers, Ann Arbor, Michigan.

Likens, G.E. and F.H. Borman. 1974. Linkages between terrestrial and aquatic ecosystems. Bioscience 24:447-456.

Lyons, J. 1989. Correspondence between the distribution of fish assemblages in Wisconsin streams and Omernik's ecoregions. American Midland Naturalist 122:163-182.

Moss, D., M.T. Furse, J.F. Wright, and P.D. Armitage. 1987. The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. Freshwater Biology 17:42-52.

Ohio EPA (Environmental Protection Agency). 1987. Biological criteria for the protection of aquatic life. Volumes 1-3. Monitoring and Assessment Program, Surface Water Section, Division of Water Quality, Columbus, Ohio.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118-125.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. US Environmental Protection Agency, Office of Water. Washington, DC. EPA/444/4-89/001.

Resh, V.H. 1994. Variability, accuracy, and taxonomic costs of rapid assessment approaches in benthic biomonitoring. Bollettino di Zoologia 61:375-383.

Resh, V.H., and J.K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. Pages 195-233 *in* D.M. Rosenburg and V.H. Resh (editors). Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.

Resh, V.H. and J.D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. Journal of the Water Pollution Control Federation 49:9-19.

Richards, C., G.E. Host, and J.W. Arthur. 1993. Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshwater Biology 29:285-294.

Rohm, C.M., J.W. Giese, and C.C. Bennett. 1987. Evaluation of an aquatic ecoregion classification of streams in Arkansas. Journal of Freshwater Ecology 4:127-140.

Shackleford, B. 1988. Rapid bioassessment of lotic macroinvertebrate communities: biocriteria development, biomonitoring section. Arkansas Department of Pollution Control and Ecology, Little Rock, Arkansas.

Southerland, M.T. and J.B. Stribling. 1995. Status of biological criteria development and implementation. Pages 81-96 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Sweeney, B.W. 1993. Effects of streamside vegetation on macroinvertebrate communities of White Clay Creek in eastern North America. Proceedings of the Academy of Natural Sciences, Philadelphia 144:291-340.

Whittier, T.R., R.M. Hughes, and D.P. Larsen. 1988. The correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Canadian Journal of Fisheries and Aquatic Sciences 45:1264-1278.

Whitton, B.A. (editor). 1975. River ecology. Studies in ecology. Volume 2. University of California Press, Berkeley, California.

Wright, J.F., D. Moss, P.D. Armitage, and M.T. Furse. 1984. A preliminary classification of running-water in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. Freshwater Biology 14:221-256.

CHAPTER I: CORRESPONDENCE OF ECOREGIONS WITH BENTHIC MACROINVERTEBRATE DISTRIBUTION IN STREAMS OF WESTERN VIRGINIA

Abstract

We evaluated the ecoregion approach to stream classification frequently used in establishing biocriteria. The two objectives were: (1) to determine if the distribution of benthic macroinvertebrates in western Virginia streams corresponds with the proposed ecoregions and subregions, and (2) compare two alternative classification schemes (landscape and biotic) with the ecoregion approach for predicting the structure of benthic macroinvertebrate communities in streams of western Virginia. We compared the benthic macroinvertebrate communities of three ecoregions; Blue Ridge Mountains (66), Central Appalachian Ridges and Valleys (67), Central Appalachians (69), and their component subregions. Regional comparisons were made using individual taxa comprising 1% of the abundance in any region, benthic macroinvertebrate community metrics, and community similiarity indices. We found little or no difference between the the Blue Ridge Mountains ecoregion and the Central Appalachian Ridges and Valleys ecoregion. The Central Appalachians ecoregion had significantly greater abundances of taxa characteristic of headwater streams as well as significantly different benthic macroinvertebrate community structure than the Blue Ridge Mountains ecoregion and Central Appalachian Ridges and Valleys ecoregion. However, given the relatively small number of sites sampled, these results may not be representative of the entire region. Analysis of the benthic macroinvertebrates across subregions showed differences in community structure. We found that it was more accurate to rearrange the subregions into three new regions called bioregions (landscape approach): the forested hills and mountains (subregion 69a), the valleys and plateaus (subregions 66c, 67a, and 67b), and the mountains (subregions 66a, 66b, 67c, and 67d). A multiple discriminant analysis showed that the bioregion classification framework was a better predictor of benthic macroinvertebrate community structure than the original ecoregions or subregions.

The results of this study indicated that, with the possible exception of the Central Appalachians ecoregion, land form and land use (two of the factors originally used to define ecoregions and subregions) play a predominant role in structuring benthic macroinvertebrate communities in western Virginia. In our evaluation of the biotic approach, a hierarchical cluster analysis classified the sites into 7 homogeneous biotic groups. Multiple discriminant analysis using 14 environmental variables correctly classified 69.8% of the reference sites. Stepwise multiple discriminant analysis and graphical analysis showed that sampling date, slope, pH, distance to source and habitat assessment scores were the best predictors of benthic macroinvertebrate community structure. These selected environmental variables were able to correctly classify 52.8% of the reference sites. These classification rates are comparable with classification rates considered acceptable in similar studies. We found that landscape (bioregions) and biotic classification schemes using benthic macroinvertebrates are better for classifying streams than ecoregions. Because of the high sampling cost and rigorous statistical assumptions required for developing a biotic classification scheme, we recommend the landscape approach for developing biocriteria using benthic macroinvertebrates.

Introduction

Much biomonitoring in streams is based on a comparison of the benthic macroinvertebrate community between one or more reference streams, which reflect the best attainable or least impaired conditions, and a test stream, which is suspected of being impaired. The accuracy of the assessment depends on the assumption that, barring impacts, the two sites would have similar benthic macroinvertebrate communities (Barbour et al. 1992). Given the high natural variability among benthic macroinvertebrate communities, ensuring the structural similarity between the benthic macroinvertebrate community at reference sites and a test site can be a problem. If reference sites are grouped improperly or a test site is not correctly matched with the expected reference condition, then inaccurate assessments will likely result.

One popular approach to classifying streams is the use of ecoregions. Omernik's (1987) ecoregion classification system groups reference sites into large regions sharing comparable land surface form, underlying geology, land use, and potential natural vegetation. The ecoregion approach has been used in aquatic environments to help reduce the number of reference sites required for bioassessments and to provide natural resource agencies with a more realistic view of attainable aquatic conditions across their jurisdictions (Hughes et al. 1986, Hughes and Larsen 1988). Ecoregions play a central role in the US Environmental Protection Agency's (USEPA) plan to develop and implement regional biological criteria in every state (Plafkin et al. 1989, USEPA 1991).

Although ecoregions were designed primarily for classifying ecosystems, several studies have shown fairly good correspondence between ecoregions and single ecosystem components [Larsen et al. 1986 (fish); Hughes et al. 1987 (fish); Rohm et al. 1987 (fish, water quality, physical habitat); Whittier et al. 1987 (fish, macroinvertebrates, water quality, physical habitat); Larsen et al. 1988 (fish, macroinvertebrates, periphyton, water quality, physical habitat);

Omernik et al. 1988 (water quality); Whittier. et al. 1988 (fish, macroinvertebrates, water quality, physical habitat); and Lyons 1989 (fish)]. However, there has been only limited testing using benthic macroinvertebrates (Hughes et al. 1987, Larsen et al. 1988; Whittier et al. 1987, Whittier. et al. 1988, and Biggs et al. 1990), and none of the ecoregions in western Virginia have been evaluated. We decided to test the ecoregion concept in western Virginia because biologists in the George Washington and Jefferson National Forest were interested in developing a biomonitoring program with macroinvertebrates for their regulatory and natural resource management responsibilities. The George Washington and Jefferson National Forest comprises much of western Virginia and the vast number of recently undisturbed streams made this an ideal situation for analyzing ecoregions and benthic macroinvertebrate communities.

Classification schemes like the ecoregion approach are generally built on landscape variables first and then stream communities are assessed to see if they match. An alternative approach, proposed by Resh and Unzicker (1975) and further developed by Wright et al. (1984), Moss et al. (1987), and Wright (1995) is first to classify reference sites into groups by their biota and then identify the predominant environmental factors responsible for segregating the biotic groups. Test sites can then be matched to a biotic group by comparing their environmental conditions. One obvious advantage of this biotic approach is that a large number of environmental variables can be tested. However, depending on the number of environmental variables to be tested, this approach requires sampling an enormous number of reference sites (Hughes 1985). Nevertheless, this approach not only ensures a high level of uniformity among the reference sites within each group, but also expands the possibility of finding more predictive environmental variables. Biologists in the United Kingdom have successfully developed a nationwide biological stream assessment program, the River Invertebrate Prediction And Classification System (RIVPACS) using this biotic approach (Wright 1995). They are now able to match a test site from anywhere in the United Kingdom with a set of comparable reference

sites using only 10-12 environmental variables with a reasonable level of accuracy. In their 1990 River Quality Survey, they made biological assessments of almost 9000 streams (Wright 1995).

The present study had two objectives. The first objective was to determine how well the distribution of benthic macroinvertebrates in western Virginia streams corresponds with the ecoregions and subregions proposed by Omernik (1987). We hypothesized that benthic macroinvertebrate communities in the streams of one ecoregion should be more structurally similar to each other than to the benthic macroinvertebrate communities in other ecoregions. Likewise, benthic macroinvertebrate communities within a subregion should be more structurally similar to each other than to benthic communities outside the subregion. Specifically, we evaluated the three mountainous ecoregions in western Virginia and their corresponding subregions (Fig.1).

The second objective of the study was to examine two alternative classification schemes that might explain a greater proportion of the variability among reference sites throughout western Virginia than ecoregions or subregions. First, using the landscape approach, we endeavored to increase the classification accuracy of ecoregions by rearranging ecoregions or subregions into more biologically homogeneous bioregions. We also applied the biotic approach. Second, using the biotic approach, we classified our stream sites into homogeneous groups based on their benthic macroinvertebrate communities and assessed the effect of several physical and chemical characteristics in defining these biotic groups.

Methods

Site selection

To determine the suitability of a site, we examined 1:100,000 scale topographic maps. The USEPA provided us with transparent overlays (personal communication, Omernik 1992) marked with the most recent revisions in the boundaries of ecoregions and subregions for these

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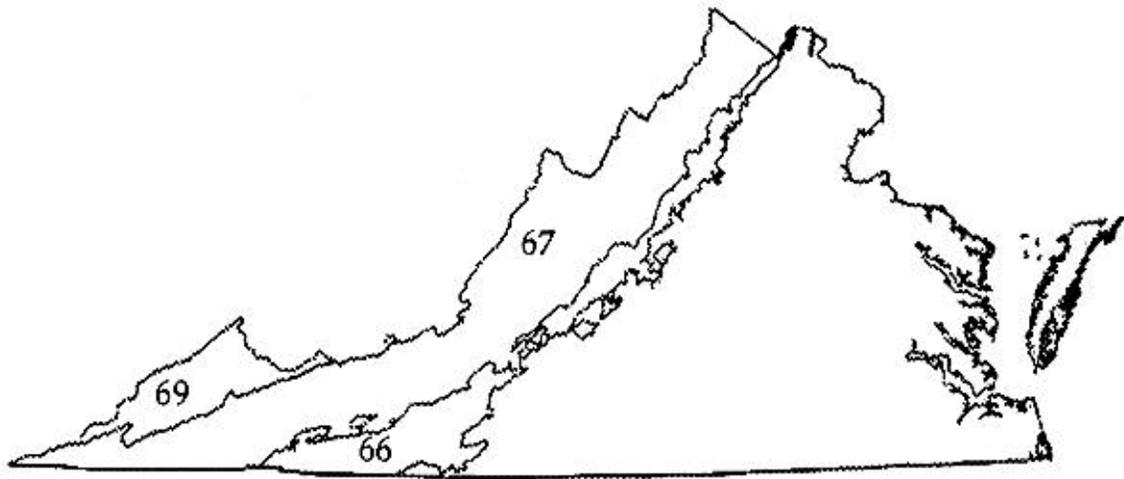
Fig. 1. Map of Virginia showing the three ecoregions of western Virginia (66 = Blue Ridge Mountains, 67 = Central Appalachian Ridges and Valleys, 69 = Central Appalachians).

maps. We selected streams with watersheds that fall entirely within the boundaries of their respective ecoregions and subregions. By using these overlays, we were able to select potential sampling sites representative of each ecoregion and subregion. In addition to the overlays, the USEPA also provided a list of potential reference sites for each ecoregion and subregion. The USEPA selected these sites from maps and from information provided by state biologists. Many of the sites we selected were located on land under the jurisdiction of the US Forest Service (USFS) and personnel in the fisheries biology program assisted with choosing unimpaired sites.

Field methods

We sampled a total of 141 stream sites throughout western Virginia. These sites represented the Blue Ridge Mountains ecoregion, Central Appalachian Ridges and Valleys ecoregion, Central Appalachians ecoregion and their corresponding subregions (Tables 1-2). Twenty one of these streams flow through two or more subregions and were designated as Mixed subregions (Table 1). Most sites ranged from first to third order; only a few fourth order streams were included. Sites were sampled during the months of June through October of 1992, June through September of 1993 and July through August 1994. Each site was sampled only once.

We sampled the benthic macroinvertebrate community at each site with a kick screen that had a 500-μm mesh net. We followed a standardized qualitative method outlined by the US Environmental Protection Agency in its Rapid Bioassessment Protocol III (Plafkin et al. 1989). The protocol recommends taking two 1-m^2 riffle samples with a kick screen and then combining them into a single sample. We assessed the habitat at each site by a standardized qualitative technique that involves making visual observations of specific habitat characteristics in the 300 meters immediately upstream (Barbour and Stribling 1991). The habitat assessment is divided into three sets of habitat parameters. Each set of parameters is weighted progressively less. The primary parameters (worth 20 points each) assess substrate composition, instream cover, flow and riparian canopy cover. The secondary parameters (worth 15 points each) focus on specific

TABLE 1. Number of sites sampled by ecoregion and subregion.

Region	Number of sites/ ecoregion	Number of sites/ subregion
Blue Ridge Mountains (66)	58	
Non Calcareous (66a)		26
Shale Dominated Ridges (66b)		17
Interior Plateau (66c)		5
Mixed		10
Central Appalachian Ridges and Valleys (67)	76	
Limestone Valleys (67a)		10
Shale Valleys (67b)		4
Sandstone Ridges (67c)		37
Shale Ridges (67d)		14
Mixed		11
Central Appalachians (69)	7	
Forested Hills and Mountains (69a)		7
Total	141	141

TABLE 2. Predominant terrestrial characteristics of the three ecoregions of western Virginia (from Omernik 1987).

Ecoregion	Land-Surface Form	Potential Natural Vegetation	Land Use	Soils
Blue Ridge Mountains	Open Low Mountains (elevation 305-914 meters)	Appalachian Oak Forest (*Quercus*) / Northern Hardwoods (*Acer-Betula-Fagus-Tsuga*)	Forest and woodland mostly ungrazed / Woodland and forest with some cropland and forest	Ultisols
Central Appalachian Ridges and Valleys	Plains with Low Mountains / Open Low Mountains (elevation 305-914 meters)	Appalachian Oak Forest (*Quercus*) / Oak-Hickory-Pine Forest (*Quercus-Carya-Pinus*)	Forest and woodland mostly ungrazed / Woodland and forest with some cropland and forest / Cropland with pasture, woodland and forest / Mostly cropland	Ultisols / Alfisols / Inceptisols
Central Appalachians	Low Mountains / High Hills (elevation 152-914 meters)	Mixed Mesophytic Forest (*Acer-Aesculus-Fagus-Liriodendron-Quercus-Tilia*)	Forest and woodland mostly ungrazed / Woodland and forest with some cropland and forest	Inceptisols

characteristics of channel morphology: riffle/pool ratio, scouring and deposition, channelization, and channel capacity. The tertiary parameters (worth 10 points each) examine bank stability and the riparian quality. At a site, each parameter is rated as optimal, sub-optimal, marginal or poor. The ratings are based on written descriptions outlined by Barbour and Stribling (1991). The scores from each habitat parameter are summed to determine an overall habitat assessment score for the site.

We also made several other physical and chemical measurements at each site. We measured temperature with a long stem thermometer and dissolved oxygen by the azide modification of the Winkler method (American Public Health Association 1989). To determine alkalinity (mg/L $CaCO_3$), we collected 250 ml of stream water at each site and titrated the sample with 0.02 N H_2SO_4, in accordance with the EPA's two-endpoint method [(USEPA 1983 (Method 310.1)]. We measured pH with an Orion SA250 pH meter, calibrated with pH 4.0 and 7.0 buffers, measured conductivity with a Yellow Springs Instruments model 33 meter and measured hardness (mg/L $CaCO_3$) with a Hach model HA-71A kit.

Field samples of benthic macroinvertebrates were preserved in 95% ethanol. In the laboratory, benthic macroinvertebrate samples were randomly subsampled to 200 organisms (±10%) by a method developed by Caton (1991). This method involved washing the sample and spreading the contents evenly on a gridded wire screen submerged in a few centimeters of water. The gridded wire screen was removed from the water, and randomly selected squares were sorted until 180 organisms were obtained. If it was necessary to begin another square, all organisms on a square were sorted even if the total number in the subsample exceeded 200 organisms. The 200-organism subsamples were identified to the lowest practical taxonomic level (usually genus) using keys in Wiggins (1977), Brigham et al. (1982), Merritt and Cummins (1984), and Stewart and Stark (1988). Chironomidae were identified only to family. We used Pennak (1989) to identify the non-insect macroinvertebrates.

We determined the UTM coordinates (northing and easting), watershed area, elevation, slope, and distance to source of each sample site using 1:24,000 scale USGS topographic maps. We obtained watershed area (km^2) and elevation data for all 100 of our national forest sites from the George Washington and Jefferson National Forest's Geographic Information System (GIS). To calculate watershed area of the remaining 41 sites outside of the national forest, we used a transparent grid marked out in 1/4 km^2 overlaid on a 1:24,000 scale USGS topographic maps.

Data analysis

Ecoregion approach.- To determine the correspondence of benthic macroinvertebrate communities with the two ecoregions and eight subregions, we tested for differences in the mean abundances of individual taxa, various benthic macroinvertebrate community metrics, and community similarity indices. Since we had unequal sample sizes among ecoregions and subregions, we used an unbalanced ANOVA and a Least-Squares Means test (LSM) for comparing the regions using individual taxa abundance and metrics. We also made orthogonal contrasts between several groupings of subregions: the valley/plateau subregions (66c, 67a and 67b) versus the mountain subregions (66a, 66b, 67c, 67d and 69a) and the montane Blue Ridge Mountains subregions (66a and 66b) versus the montane Central Appalachian Ridges and Valleys subregions (67c and 67d).

Abundance of individual taxa.- One method we used to determine if benthic macroinvertebrate assemblages correspond with aquatic ecoregions was to test for regional differences in abundances of individual taxa (Larsen et al. 1986). In this study, we collected a total of 153 taxa. We limited our analysis to those taxa with mean abundances 1% for any region.

Metrics.- We also tested regional differences by comparing individual metrics across regions (Larsen et al. 1986). Metrics measure different components of benthic macroinvertebrate

community structure, and some are designed to respond in a predictable way to increasing human-influenced disturbances (Karr et al. 1986, Karr 1991, Barbour et al. 1995). We used a total of 12 metrics (Table 3). Metrics are commonly divided into six categories: richness, composition, balance, tolerance, trophic, and habits. Each category describes a different aspect of the benthic macroinvertebrate community. In order to characterize the communities as completely as possible, we selected metrics from five of these categories. We did not select any metrics within the tolerance category because we were primarily interested in ecological differences and not pollution tolerances. We also selected metrics with low coefficients of variation and sizable means.

Community similarity.- We computed a community similarity matrix comparing each site to the other sites with the Bray-Curtis Coefficient (Bray and Curtis 1957) and the Index of Biotic Similarity (Pinkham and Pearson 1974). We calculated the Bray- Curtis Coefficient as percent similarity (PS) (Ludwig and Reynolds 1988) of community a and community b (PS_{ab}) as follows:

$$PS_{ab} = \frac{2W}{A + B} \ (100)$$

where

$$W = \sum_{i}^{k} \left[\min\left(X_{ia}, X_{ib}\right)\right]$$

$$A = \sum_{i}^{k} X_{ia}$$

$$B = \sum_{i}^{k} X_{ib}$$

X_{ia} = abundance of the ith species in community a

X_{ib} = abundance of the ith species in community b

k = number of taxa compared

TABLE 3. Definitions of benthic macroinvertebrate community metrics used in western Virginia ecoregion/subregion comparison.

Category	Metric	Definition
Richness measures	Taxa richness	Number of total taxa. Measures the collective variety of the assemblage.
	No. of families	Total number of macroinvertebrate families. Measures the collective variety of the assemblage.
	EPT index	Number of taxa in the insect orders Ephemeroptera, Plecoptera, and Trichoptera. These orders are generally considered to be sensitive to a wide variety of impairments.
Composition measures	% EPT	Percent abundance of the insect orders Ephemeroptera, Plecoptera, and Trichoptera. These orders are generally considered to be sensitive to a wide variety of impairments.
	% EPT(−Hydropsychidae)	Percent abundance of the insect orders Ephemeroptera, Plecoptera, and Trichoptera minus the pollution-tolerant caddisfly family Hydropsychidae.
Balance measures	% 5 Most Dominant Taxa	Measures the dominance of the 5 most abundant taxa. The greater the percentage, the greater the redundancy of taxa in the assemblage.
	Hydropsychidae/Trichoptera	Percentage of the moderately pollution-tolerant caddisfly family Hydropsychidae to total Trichoptera.
	Simpson's Index of Diversity	Integrates taxa richness and evenness into a measure of general diversity.
Trophic Status measures	% Collector-Gatherers	Percent abundance of collector gatherer functional feeding group.

	% Collector-Filterers	Percent abundance of collect-filterer functional feeding group.
	% Scrapers	Percent abundance of scraper functional feeding group.
Habit measures	% Haptobenthos	Percent abundance of taxa requiring clean coarse substrate.

PS_{ab} ranges from 0 to 100 with 0 for communities with no taxa in common and 100 for identical communities. One shortcoming of the Bray-Curtis Coefficient is that the index is weighted toward dominant taxa and may neglect rare taxa. Since rare taxa might be ecologically important in assessing regional differences, we also calculated the Index of Biotic Similarity, *B*, (Pinkham and Pearson 1974). This index is more sensitive to rare taxa than the Bray-Curtis Coefficient because it weighs all the taxa equally. The Index of Biotic Similarity ranges from 0 to 1, with 0 for entirely different communities and 1 for identical communities. The index is calculated as follows:

$$B = \frac{1}{k} * \sum_{i}^{k} \frac{\min(X_{ia}, X_{ib})}{\max(X_{ia}, X_{ib})}$$

where

X_{ia} = abundance of the ith species in community *a*

X_{ib} = abundance of the ith species in community *b*

k = number of taxa compared

We used a randomization or permutation procedure (Pitman 1937) to compare similarities between benthic macroinvertebrate communities within and among ecoregions and subregions (Smith et al. 1990). We first calculated the mean similarities within (*W*) and between (*B*) each ecoregion and subregion. Since regional differences can be summarized by the ratio of *B/W*, we used *L* as our measure of similarity (Good 1982).

$$L = B / W$$

The observed data (L_{data}) were then permutated by randomly switching some of the data between the regions and then computing a new mean similarity ($L_{permute}$). This process was repeated 1000 times. We then compared the L_{data} value with the 1000 $L_{permute}$ values. Under the null hypothesis of no differences between the regions, $L_{permute}$ should be approximately equal to the L_{data}. If, however, $L_{permute}$ is significantly larger relative to the L_{data}, then the null hypothesis is rejected. The *P*-value is estimated by $(t+1)/(N+1)$ where *N* is the number of permutations sampled and *t* the number of values greater than the L_{data}.

Biotic approach

Biotic groups.-The 141 stream sites were classified into groups based on their faunal composition. We made a pairwise comparison of every site using the Bray-Curtis Coefficient (Bray and Curtis 1957). Since the Bray-Curtis Coefficient is sensitive to abundance, we standardized the similarity index by converting the absolute numbers to proportions in addition to using 200-organism subsamples (Krebs 1989).

To identify biotic groups, we used Number Cruncher Statistical Systems (NCSS) 6.0.21 (Number Cruncher Statistical Systems 1996) to calculate a hierarchical cluster analysis based on Bray-Curtis Coefficient distances. The cluster analysis was based on Unweighted Pair-Groups using Arithmetic Averages (UPGMA) linkage. Sites were placed in the same group if they had a similarity greater than 40.0. We set this threshold based on the range of similarity we found comparing the benthic macroinvertebrate communities from equally-sized streams within the same watershed (Fig. 2). Groups with less than 5 sites were assigned to their closest neighboring group.

Graphical analysis.- To determine if there was any separation between groups based on the individual factors, we graphed the environmental variables separately against the biotic groups as well as the variables against each other. Since biotic groups are independent of each other, the level of separation between groups shows the influence of an environmental variable. To make the graphs easier to interpret, we plotted the means of each environmental variable and 95% confidence interval for each group.
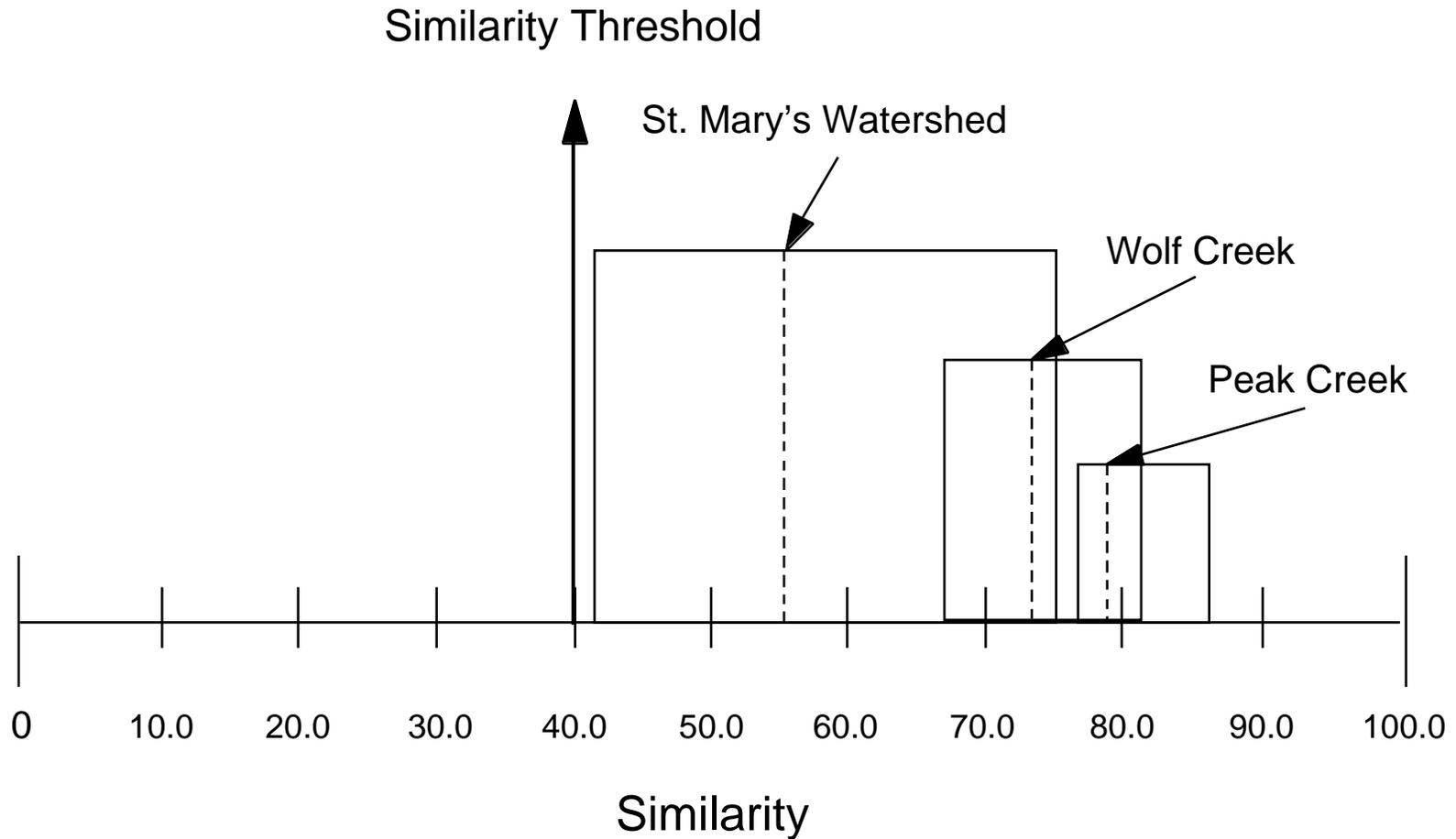
Fig 2. Comparison of 40.0 Bray-Curtis Coefficient similarity threshold with means (dashed lines) and ranges (rectangles) of samples within the same riffle compared to themselves (Wolf Creek and Peak Creek) and equal-sized streams within the same watershed compared to themselves (St. Mary's watershed).

Discriminant analysis.- Discriminant analysis was used to determine the predictive ability of our combined environmental variables. Stepwise discriminant analysis was also used to determine which variables were best in discriminating among the biotic groups. For the stepwise discriminant analysis, we set the significance level at 0.05 for each environmental variable to enter and stay within the model. Discriminant analysis is sensitive to two main assumptions: multivariate normality of the independent variables within classification groups and equality of covariance matrices among classification groups. Although ecological studies tend to violate these assumptions (Williams 1983), we tried to meet them as best we could. We checked the normality of the environmental variables within each biotic group (D'Agostino et al. 1990). We used Bartlett's univariate homogeneity of variance test (Bartlett 1937a, 1937b; based on a principle of Neyman and Pearson 1931) and Box's M test (Box 1949) to test for homogeneity of covariance matrices between and among the biotic groups. We used a log transformation on northing, easting, elevation, slope, distance to source, watershed area, temperature, dissolved oxygen, alkalinity, hardness and conductivity (Sokal and Rohlf 1995). Each habitat assessment score was first converted into a proportion of the highest possible habitat assessment score, 180, and then transformed using an arcsine transformation (Sokal and Rohlf 1995). Likewise, sampling date was converted into a proportion of the total number of days in a year and then transformed using an arcsine transformation (Sokal and Rohlf 1995). Since pH is already in log scale, it was not transformed. All the statistics were calculated using NCCS 6.0.21 (Number Cruncher Statistical Systems 1996).

Results

Ecoregion approach

Abundance of individual taxa.- The mean abundances of only a few taxa were significantly different between the ecoregions (Table 4). There were significantly greater mean abundances of taxa characteristic of headwater streams in the Blue Ridge Mountains ecoregion (66) and in the Central Appalachians ecoregion (69) (e.g., *Diplectrona modesta* and *Ectopria*)

TABLE 4. Mean (± 95% confidence interval) benthic macroinvertebrate taxa abundance by ecoregion. Only taxa with individual mean abundances greater than 1% for any ecoregion are listed. Ecoregions with the same letter are not significantly different (P 0.05; least-squares means).

Taxon	Blue Ridge Mountains	Central Appalachian Ridges and Valleys	Central Appalachians
Lirceus	0.00 (0.00)a	2.53 (3.57)a	0.00 (0.00)a
Gammarus	0.00 (0.00)a	7.64 (14.26)a	0.00 (0.00)a
Ephemerellidae*	4.50 (1.82)a	3.96 (1.50)a	2.14 (2.02)a
Leptophlebiidae	6.91 (2.48)a	5.43 (1.69)a	3.86 (3.88)a
Baetidae	18.17 (5.31)a	22.64 (7.60)a	29.71 (36.56)a
Heptageniidae*	17.44 (3.11)a	15.16 (3.68)a	10.43 (7.76)a
Stenonema	8.53 (2.32)a	6.99 (2.54)a	8.86 (7.29)a
Epeorus	5.79 (1.87)a	5.66 (2.76)a	1.29 (2.21)a
Isonychia	2.83 (1.36)a	4.00 (1.96)a	0.29 (0.56)a
Peltoperlidae	7.40 (3.15)a	5.55 (2.75)a	4.71 (4.27)a
Perlidae*	7.06 (1.38)a	5.28 (1.38)a	4.86 (3.71)a
Acroneuria	3.10 (0.91)a	1.45 (0.56)a	2.43 (4.12)a
Perlodidae	1.22 (0.64)b	1.14 (0.68)b	4.86 (6.64)a
Chloroperlidae	3.52 (1.19)a	0.89 (0.33)b	2.86 (2.16)a
Leuctra	15.60 (4.29)a	18.04 (5.58)a	20.29 (21.86)a
Hydropsyche	20.53 (6.60)a	13.84 (3.71)a	16.57 (12.60)a
Cheumatopsyche	10.64 (5.39)a	7.33 (3.45)a	7.71 (12.10)a
Diplectrona modesta	6.09 (2.91)b	4.08 (2.22)b	18.14 (17.39)a

Rhyacophila	4.41 (1.16)a	2.66 (0.92)b	4.57 (3.52)a
Chimarra	0.14 (0.21)a	2.30 (2.09)a	0.00 (0.00)a
Dolophilodes distinctus	7.83 (2.29)a	11.99 (3.25)a	12.71 (8.07)a
Psephenus herricki	3.60 (1.52)a	3.55 (2.62)a	0.71 (1.12)a
Stenelmis	0.14 (0.15)a	4.25 (3.93)a	0.00 (0.00)a
Optioservus	2.76 (1.12)a	5.87 (3.21)a	1.86 (2.44)a
Oulimnius latiusculus	6.19 (2.09)a	4.66 (1.68)a	6.43 (4.17)a
Ectopria	0.36 (0.11)b	0.47 (0.15)b	2.43 (0.84)a
Simulium	17.28 (7.50)a	16.45 (5.12)a	22.43 (22.07)a
Chironomidae	32.71 (4.90)a	41.12 (7.26)a	40.29 (18.48)a

* represents individuals identified to genera within family plus individuals identified only to family.

than the Central Appalachians Ridges and Valleys ecoregion (Wiggins 1977, Unzicker and McCaskill 1982, Unzicker et al. 1982, S.W. Hiner, Virginia Polytechnic Institute and State University, personal communication).

Among the subregions, however, we found many more differences in taxa abundance (Table 5). The Limestone Valleys subregion (67a) was the most distinctive subregion in our study. The mean abundances of individual taxa reflected environmental conditions characteristic of Limestone Valleys streams: hard water (*Lirceus* and *Gammarus*), medium size (*Serratella*), warm water (*Chimarra*), open canopy (*Stenonema*, *Stenelmis* and *Optioservus*), abundance of fine sediments (*Caenis*), and organic enrichment (Oligochaeta, Baetidae, *Hydropsyche*, *Cheumatopsyche*, *Simulium*, and Chironomidae)[Mingo 1979, Seagle and Hendricks 1979, Brigham 1982, Unzicker and Carlson 1982, Covich and Thorpe 1991, Merritt and Cummins 1996]. In the mountain subregions (66a, 66b, 67c and 67d), we found significantly greater mean abundances of taxa that typically occur in cool mountain streams (*Drunella*, *Epeorus*, Perlidae, Chloroperlidae, *Rhyacophila* and *Dolophilodes distinctus*), closed canopies (*Pteronarcys*, Peltoperlidae, *Amphinemura* and *Leuctra*) and cobble/pebble substrates (*Oulimnius latiusculus*)[Unzicker and Carlson 1982, Unzicker and McCaskill 1982, Unzicker et al. 1982, Merritt and Cummins 1996]. The taxa of the Forested Hills and Mountains subregion (69a) were very similar to the other mountain subregions (66a, 66b, 67c and 67d). In addition, the Forested Hills and Mountains subregion (69a) also had greater mean abundances of taxa typical of headwater streams (*Diplectrona modesta* and *Ectopria*)[Wiggins 1977, S.W. Hiner, Virginia Polytechnic Institute and State University, personal communication]. Individual taxa abundances indicated that the Interior Plateau subregion (66c) and Shale Valleys subregion (67b) differed from the mountain subregions (66a, 66b, 67c and 67d) in taxa commonly found in warm water streams (*Chimarra*) with open canopies (Heptageniidae, *Psephenus herricki*, *Stenelmis* and Ancylidae)[Mingo 1979, Seagle and Hendricks 1979, Merritt and Cummins 1996]. Nevertheless, these subregions also had significant mean abundances of taxa characteristic of the

TABLE 5. Mean (± 95% confidence interval) benthic macroinvertebrate taxa abundance by subregion. Only taxa with individual mean abundances greater than 1% for any subregion are listed. Subregions with the same letter are not significantly different (P 0.05; least squares means).

Taxon	Non Calcareous	Shale Dominated Ridges	Interior Plateau	Limestone Valleys	Shale Valleys	Sandstone Ridges	Shale Ridges	Forested Hills and Mountains
Oligochaeta	1.27 (0.67)c	0.71 (0.84)c	1.00 (1.23)bc	3.70 (5.42)ab	0.25 (0.49)bc	1.08 (0.84)c	0.07 (0.14)c	1.14 (1.08)bc
Lirceus	0.00 (0.00)b	0.00 (0.00)b	0.00 (0.00)b	19.20 (25.97)a	0.00 (0.00)b	0.00 (0.00)b	0.00 (0.00)b	0.00 (0.00)b
Gammarus	0.00 (0.00)c	0.00 (0.00)c	0.00 (0.00)c	55.40 (108.37)ab	0.00 (0.00)bc	0.68 (1.27)c	0.14 (0.28)c	0.00 (0.00)c
Caenis	0.04 (0.07)b	0.12 (0.16)b	0.00 (0.00)b	5.00 (4.07)a	1.25 (1.47)b	0.59 (0.96)b	0.00 (0.00)b	0.00 (0.00)b
Ephemerellidae*	6.88 (3.58)a	2.18 (1.80)b	2.80 (4.57)ab	9.20 (6.50)a	3.00 (4.60)ab	2.65 (1.31)b	6.07 (4.99)ab	2.14 (2.02)ab
Serratella	0.27 (0.14)b	0.00 (0.00)b	0.40 (0.40)b	4.60 (2.68)a	0.00 (0.00)b	0.24 (0.15)b	0.00 (0.00)b	0.00 (0.00)b
Drunella	3.77 (2.55)a	0.12 (0.16)b	1.00 (1.96)ab	0.20 (0.39)b	0.25 (0.49)b	1.46 (0.93)b	1.36 (1.63)ab	0.00 (0.00)b
Ephemerella	1.00 (0.71)bc	1.41 (1.47)abc	1.40 (2.74)abc	4.40 (5.75)ab	2.50 (4.27)abc	0.65 (0.68)c	4.71 (5.11)a	0.71 (1.40)abc
Leptophlebiidae	9.73 (4.48)a	2.88 (1.81)b	3.00 (4.47)ab	0.80 (1.16)b	6.50 (9.75)ab	4.86 (1.75)b	10.43 (6.27)a	3.86 (3.88)ab
Baetidae	18.46 (6.84)b	11.65 (11.75)b	25.80 (7.97)ab	47.00 (48.65)a	34.25 (22.41)ab	17.81 (6.81)b	23.64 (7.07)ab	29.71 (36.56)ab
Heptageniidae*	19.38 (4.16)b	10.94 (3.69)c	13.00 (4.99)bc	14.50 (11.51)bc	35.00 (33.79)a	12.51 (4.16)c	13.21 (7.55)bc	10.43 (7.76)bc
Stenonema	8.04 (3.51)ab	6.76 (3.45)abc	12.20 (5.49)ab	12.80 (11.14)a	8.25 (9.34)abc	5.32 (2.35)bc	0.86 (0.71)c	8.86 (7.29)abc
Epeorus	6.58 (1.78)cd	3.71 (2.93)cd	0.60 (0.78)cd	1.00 (1.20)d	21.25 (37.79)a	4.38 (2.64)cd	10.43 (7.52)abc	1.29 (2.21)d
Isonychia	4.19 (2.64)bc	0.71 (0.84)c	3.60 (3.75)bc	6.70 (5.82)b	19.00 (23.76)a	2.22 (1.54)bc	2.71 (3.75)bc	0.29 (0.56)bc
Pteronarcys	2.31 (1.24)a	0.35 (0.69)b	0.20 (0.39)ab	0.00 (0.00)b	0.25 (0.49)ab	1.54 (1.20)ab	2.64 (2.16)a	0.43 (0.58)ab
Peltoperlidae	8.31 (5.08)ab	10.47 (7.04)a	0.40 (0.48)ab	0.10 (0.19)b	1.00 (1.96)ab	9.22 (5.12)a	3.36 (3.68)ab	4.71 (4.27)ab
Amphinemura	0.23 (0.38)c	3.35 (3.80)a	0.00 (0.00)abc	0.00 (0.00)bc	2.00 (3.92)abc	1.84 (1.47)abc	1.50 (1.83)abc	0.00 (0.00)abc
Perlidae*	6.69 (1.83)a	8.12 (3.20)a	9.40 (5.49)a	0.80 (0.70)b	6.25 (10.38)ab	5.62 (2.02)a	6.79 (3.58)a	4.86 (3.71)ab
Paragnetina	0.88 (0.68)b	0.00 (0.00)d	2.60 (2.11)a	0.10 (0.20)cd	0.50 (0.98)cd	0.05 (0.11)bcd	0.21 (0.42)bcd	0.00 (0.00)cd
Acroneuria	2.92 (1.37)ab	3.29 (1.58)ab	4.80 (5.20)a	0.10 (0.20)c	0.75 (1.47)abc	1.92 (0.93)a	1.43 (0.79)b	2.43 (4.12)ab
Perlodidae	1.15 (0.72)bc	1.59 (1.78)bc	0.00 (0.00)bc	0.10 (0.20)c	2.50 (4.90)abc	0.73 (0.39)c	3.36 (3.09)ab	4.86 (6.64)a

Chloroperlidae	3.77 (1.79)a	3.18 (2.43)ab	0.80 (1.14)abc	0.00 (0.00)c	0.00 (0.00)bc	1.38 (0.57)bc	0.71 (0.60)c	2.86 (2.16)abc
Leuctra	13.65 (5.00)bcd	25.65 (10.71)ab	2.60 (3.70)cd	1.20 (2.14)d	2.00 (1.79)bcd	22.22 (9.43)bc	21.50 (11.02)bc	20.29 (21.86)bcd
Nigronia	1.38 (0.84)b	2.06 (0.91)ab	4.00 (5.58)a	1.20 (1.33)b	0.00 (0.00)b	1.70 (0.79)ab	0.93 (0.59)b	1.43 (2.80)ab
Hydropsyche	23.65 (11.59)a	12.76 (7.51)ab	19.20 (10.04)ab	20.40 (8.13)ab	33.75 (35.09)ab	13.16 (5.26)b	6.43 (4.46)ab	19.33 (12.60)ab
Cheumatopsyche	7.54 (4.62)ab	17.06 (15.49)a	6.20 (4.18)ab	13.10 (11.46)ab	7.50 (14.70)ab	6.08 (5.47)b	6.21 (5.94)ab	7.71 (12.10)ab
Diplectrona modesta	7.38 (4.02)b	5.06 (5.15)bc	0.00 (0.00)bc	0.00 (0.00)c	0.25 (0.49)bc	5.05 (2.75)bc	1.14 (1.65)bc	18.14 (17.39)a
Rhyacophila	4.81 (1.92)a	5.06 (2.17)a	2.00 (2.23)ab	0.90 (0.80)b	2.25 (2.58)ab	3.32 (1.38)ab	1.50 (1.33)b	4.57 (3.52)ab
Chimarra	0.00 (0.00)c	0.47 (0.71)c	0.00 (0.00)bc	6.20 (9.19)b	15.50 (29.09)a	0.95 (1.34)c	0.00 (0.00)c	0.00 (0.00)c
Dolophilodes distinctus	9.53 (2.99)bc	7.82 (4.99)bc	3.20 (2.58)bc	0.50 (0.44)c	6.00 (8.00)abc	13.38 (5.13)ab	18.93 (7.31)a	12.71 (8.07)ab
Psephenus herricki	4.04 (2.35)d	1.35 (1.21)cd	2.80 (1.90)cd	3.00 (4.35)cd	10.75 (18.50)a	1.46 (0.80)bc	0.79 (1.03)cd	0.71 (1.11)cd
Stenelmis	0.04 (0.07)c	0.12 (0.16)c	0.80 (1.57)c	14.80 (12.68)b	34.50 (65.03)a	0.46 (0.47)c	0.07 (0.14)c	0.00 (0.00)c
Optioservus	3.81 (1.98)b	0.76 (0.748)b	7.00 (4.92)b	19.90 (16.15)a	7.75 (11.38)b	4.65 (3.99)b	0.29 (0.25)b	1.86 (2.44)b
Promoresia	0.77 (0.86)b	0.12 (0.16)b	0.00 (0.00)ab	0.90 (1.14)ab	0.00 (0.00)ab	2.68 (1.90)a	0.07 (0.14)b	0.14 (0.28)ab
Oulimnius latiusculus	8.73 (3.55)a	4.65 (3.72)abc	0.40 (0.78)bc	0.00 (0.00)c	0.50 (0.57)bc	7.11 (2.97)ab	0.93 (1.26)c	6.43 (4.17)bc
Ectopria	0.38 (0.34)b	0.47 (0.42)b	0.00 (0.00)b	0.10 (0.20)b	0.00 (0.00)b	0.84 (0.56)b	0.07 (0.14)b	2.43 (1.65)a
Hexatoma	1.77 (1.16)ab	0.65 (0.53)b	3.20 (5.79)a	0.30 (0.30)b	0.25 (0.49)ab	1.14 (0.58)ab	0.86 (0.40)b	1.57 (1.47)ab
Simulium	8.77 (4.50)b	22.41 (15.51)ab	33.60 (16.53)a	18.90 (17.26)ab	8.00 (6.84)ab	17.11 (7.57)ab	26.00 (13.04)a	22.43 (22.07)ab
Chironomidae	25.08 (5.96)b	44.06 (8.36)a	42.00 (18.02)ab	42.30 (28.41)ab	64.50 (74.24)a	39.16 (8.54)a	36.50 (12.89)ab	40.29 (18.48)ab
Atherix	0.88 (1.14)ab	2.76 (5.42)ab	1.20 (1.90)ab	4.30 (4.95)a	1.00 (1.96)ab	0.38 (0.37)b	0.21 (0.42)ab	2.29 (4.16)ab
Ancylidae	0.00 (0.00)b	0.06 (0.11)b	2.20 (1.69)a	0.00 (0.00)b	0.00 (0.00)b	0.11 (0.17)b	0.00 (0.00)b	0.00 (0.00)b

* represents individuals identified to genera within family plus individuals identified only to family.

mountain subregions (66a, 66b, 67c and 67d): *Drunella*, *Epeorus*, *Pteronarcys*, *Amphinemura*, Perlidae, *Rhyacophila*, and *Dolophilodes distinctus*)[Unzicker and Carlson 1982, Unzicker et al. 1982, Merritt and Cummins 1996].

In the valley/plateau subregions (66c, 67a and 67b), we found greater mean abundances of taxa characteristic of streams with hardwater (*Lirceus*), fine sediments (*Caenis*), open canopies (Heptageniidae, *Stenonema*, *Psephenus herricki*, *Stenelmis* and *Optioservus*), and warmer waters (*Chimarra*)[Brigham 1982, Unzicker and Carlson 1982, Unzicker et al. 1982, Covich and Thorp 1991, Merritt and Cummins 1996](Table 6). In contrast, the mountain subregions (66a, 66b, 67c, 67d and 69a) were characterized by greater mean abundances of taxa typical of small, cool headwater streams (*Diplectrona modesta* and *Dolophilodes distinctus*) with closed canopies (*Leuctra*) and cobble/pebble substrate (*Oulimnius latiusculus*) [Wiggins 1977, Unzicker et al 1982] (Table 6).

A few regional differences in individual taxa abundance were found to exist between the two mountain regions (Table 7). The mountain subregions of the Blue Ridge Mountains ecoregion (66a and 66b) had significantly greater abundances of *Stenonema*, *Acroneuria*, *Chloroperlidae*, and *Rhyacophila* than the ridges of the Central Appalachian Ridges and Valleys ecoregion (67c and 67d). Only *Dolophilodes distinctus* was significantly more abundant in the mountain subregions of the Central Appalachian Ridges and Valleys ecoregion (67c and 67d). The greater abundances of these aquatic insects in either region probably do not indicate any ecologically significant differences between the mountain subregions.

Metrics.- The Central Appalachians Ridges and Valleys ecoregion (67) was significantly less taxonomically rich than the Blue Ridge Mountains ecoregion (66) and the Central Appalachians ecoregion (69) and significantly less diverse than the Blue Ridge Mountains ecoregion (66) (Table 8). Among subregions (Table 9), the Interior Plateau subregion (66c) and

TABLE 6.   Mean (±95% confidence interval) individual taxa abundance of the valley/plain subregions (66c, 67a and 67b) and mountain subregions (66a, 66b, 67c, 67d and 69a).   Only taxa with individual mean abundances greater than 1% for any region are listed. Regions with the same letter are not significantly different (P 0.05; orthogonal contrasts; valley/plateau (n=19), mountain (n=101)).

| | Region | |
Taxon	Valley/Plateau	Mountain
Lirceus	10.11 (14.04) a	0.00 (0.00) b
Gammarus	29.16 (57.04) a	0.27 (0.47) a
Caenis	2.89 (2.35) a	0.25 (0.35) b
Ephemerellidae*	6.21 (3.90)a	4.10 (1.33)a
Ephemerella	3.21 (3.19) a	1.44 (0.84) a
Leptophlebiidae	2.58 (2.43)a	6.49 (1.70)a
Baetidae	38.74 (25.74)a	18.57 (4.48)a
Heptageniidae*	18.42 (9.60)a	13.97 (2.34)b
Stenonema	11.68 (6.18) a	5.89 (1.51) b
Epeorus	5.16 (8.08) a	5.46 (1.62) a
Isonychia	8.47 (6.02) a	2.41 (1.05) b
Peltoperlidae	0.37 (0.43)a	8.07 (2.64)a
Perlidae*	4.21 (2.95)a	6.43 (1.17)a
Acroneuria	1.47 (1.59) a	2.38 (0.63) a
Perlodidae	0.58 (1.03)a	1.63 (0.74)a
Chloroperlidae	0.21 (0.32)b	2.31 (0.70)a
Leuctra	1.74 (1.48) b	20.36 (4.61) a
Hydropsyche	22.89 (8.56) a	15.10 (4.01) a
Cheumatopsyche	10.11 (6.73) a	8.44 (3.69) a

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Diplectrona modesta	0.05 (0.10) b	6.02 (2.15) a
Rhyacophila	1.47 (0.88) a	3.83 (0.87) a
Chimarra	6.53 (7.61) a	0.43 (0.51) b
Dolophilodes distinctus	2.37 (1.93) b	12.13 (2.53) a
Psephenus herricki	4.58 (4.40) a	1.96 (0.75) b
Stenelmis	15.26 (14.81) a	0.21 (0.18) b
Optioservus	13.95 (9.11) a	2.98 (1.59) b
Oulimnius latiusculus	0.21 (0.24) b	6.21 (1.64) a
Simulium	20.47 (10.63) a	17.46 (4.67) a
Chironomidae	46.89 (21.04) a	36.07 (4.47) a

* represents individuals identified to genera within family plus individuals identified only to family.

TABLE 7.   Mean (± 95% confidence interval) individual taxa abundance of the mountainous subregions of the Blue Ridge (66a and 66b) and the ridges of the Central Appalachian Ridges and Valleys ecoregion (67c and 67d).   Only taxa with individual mean abundances greater than 1% for any region are listed.   Regions with the same letter are not significantly different (*P*   0.05; orthogonal contrast; Blue Ridge (*n*=43), Central Appalachian Ridges and Valleys (*n*=51)).

Taxon	Region	
	Blue Ridge Mountains	Central Appalachian Ridges and Valleys
Ephemerellidae*	5.02 (2.36)a	3.59 (1.69)a
Drunella	2.33 (1.62)	1.43 (0.80)
Leptophlebiidae	7.02 (2.96)a	6.39 (2.21)a
Baetidae	15.77 (6.22)a	19.41 (5.32)a
Heptageniidae*	16.05 (3.14)a	12.71 (3.63)a
Stenonema	7.53 (2.50) a	4.10 (1.80) b
Epeorus	5.44 (1.62) a	6.04 (2.87) a
Isonychia	2.81 (1.70) a	2.35 (1.50) a
Peltoperlidae	9.16 (4.11)a	7.61 (3.89)a
Perlidae*	7.26 (1.67)a	5.94 (1.76)a
Acroneuria	3.07 (1.03) a	1.78 (0.71) b
Chloroperlidae	3.53 (1.43)a	1.20 (0.45)b
Leuctra	18.40 (5.43) a	22.02 (7.43) a
Hydropsyche	19.35 (7.71) a	11.31 (4.07) a
Cheumatopsyche	11.30 (6.77) a	6.12 (4.26) a
Diplectrona modesta	6.47 (3.15) a	3.98 (2.09) a
Rhyacophila	4.91 (1.43) a	2.82 (1.08) b

Dolophilodes distinctus	8.74 (2.65) b	14.90 (4.25) a
Psephenus herricki	2.98 (1.54) a	1.27 (0.64) a
Optioservus	2.60 (1.30) a	3.45 (2.94) a
Oulimnius latiusculus	7.12 (2.65) a	5.41 (2.30) a
Ectopria	0.42 (0.26)	0.63 (0.42)
Simulium	14.16 (6.90) a	19.55 (6.58) a
Chironomidae	32.58 (5.59) a	38.43 (7.08) a

* represents individuals identified to genera within family plus individuals identified only to family.

Table 8. Means (± 95% confidence interval) of 12 benthic macroinvertebrate community metrics by ecoregion. Numbers in the same row followed by the same letter are not significantly different ($P < 0.05$; least squares means).

Metric	Blue Ridge Mountains	Central Appalachian Ridges and Valleys	Central Appalachians
Taxa richness	25.17 (1.34) a	22.66 (1.42) b	26.57 (5.29) a
No. of families	19.78 (0.96) a	17.87 (0.97) b	20.29 (3.49) a
EPT index	16.31 (0.96) a	13.97 (1.03) b	14.86 (3.27) a
% EPT	65.09 (3.42) a	59.33 (3.52) b	60.30 (14.4) a
% EPT (−Hydropsychidae)	42.93 (4.13) a	40.08 (3.60) a	40.91 (12.3) a
% 5 most dominant taxa	55.00 (1.89) b	58.91 (1.85) a	57.71 (3.78) ab
Hydropsychidae/Trichoptera	0.64 (0.06) a	0.54 (0.06) a	0.63 (0.19) a
Simpson's index of diversity	0.87 (0.01) a	0.83 (0.02) b	0.84 (0.04) ab
% Collector-gatherers	34.17 (1.73) a	37.20 (2.53) a	35.03 (5.68) a
% Collector-filterers	32.17 (2.43) a	30.80 (2.22) a	37.12 (8.13) a
% Scrapers	20.82 (2.27) a	20.02 (2.48) a	17.36 (3.29) a
% Haptobenthos	66.12 (1.94) a	63.78 (1.99) a	63.44 (5.09) a

TABLE 9. Means (± 95% confidence interval) of 12 benthic macroinvertebrate community metrics by subregions. Numbers in the same row followed by the same letter are not significantly different (P 0.05; least squares means).

Metric	Non Calcarous	Shale Dominated Ridges	Interior Plateau	Limestone Valleys	Shale Valleys	Sandstone Ridges	Shale Ridges	Forested Hills and Mountains
Taxa richness	27.81 (1.93) a	21.47 (2.34) bc	23.00 (1.86) abc	19.10 (4.49) c	24.50 (8.71) abc	23.67 (2.11) b	20.78 (2.15) bc	26.57 (5.29) ab
No. of families	21.27 (1.46) a	17.29 (1.62) b	18.60 (2.37) bc	15.40 (3.15) c	18.00 (4.31) ab	18.76 (1.45) b	16.64 (1.26) b	20.28 (3.49) ab
EPT index	18.19 (1.30) a	13.76 (1.69) bc	13.80 (0.73) cd	9.90 (3.17) d	14.75 (6.01) abc	14.78 (1.56) bc	14.71 (1.26) ab	14.86 (3.27) abc
% EPT	69.73 (4.33) a	60.68 (6.62) ab	47.96 (4.02) bc	44.71 (12.4) c	64.61 (17.9) a	60.29 (4.54) ab	65.68 (5.64) a	60.30 (14.4) ab
% EPT (- Hydropsychidae)	46.60 (4.47) a	44.24 (8.94) a	21.65 (7.11) bc	17.48 (7.98) c	35.80 (18.0) ab	42.74 (4.67) a	47.75 (5.78) a	40.91 (12.3) a
% 5 most dominant taxa	51.87 (2.29) d	59.94 (3.80) abc	55.32 (5.10) c	64.26 (5.95) a	62.83 (5.62) abc	57.56 (2.82) bc	58.94 (3.46) abc	57.71 (3.78) abcd
Hydropsychidae/ Trichoptera	0.63 (0.10) bc	0.56 (0.13) bc	0.82 (0.09) ab	0.83 (0.07) a	0.58 (0.38) abcd	0.53 (0.08) c	0.31 (0.14) d	0.63 (0.18) abc
Simpson's index of diversity	0.89 (0.02) a	0.85 (0.02) ab	0.87 (0.03) ab	0.77 (0.08) c	0.83 (0.04) abc	0.84 (0.03) b	0.85 (0.03) ab	0.84 (0.04) ab
% Collector- gatherers	33.75 (2.26) d	32.90 (2.68) d	38.41 (8.22) bcd	43.38 (10.29) ab	42.52 (12.48) bcd	34.68 (3.21) cd	40.36 (5.97) abc	35.03 (5.68) bcd
% Collector-filterers	31.44 (3.31) a	31.13 (5.19) a	35.48 (6.25) a	30.29 (8.77) a	32.70 (10.98) a	31.04 (2.72) a	31.95 (5.90) a	37.12 (8.13) a
% Scrapers	23.83 (3.03) a	14.69 (4.14) bc	21.98 (3.98) ab	24.53 (9.56) a	19.56 (16.07) abc	20.34 (2.85) a	10.18 (3.08) c	17.37 (3.29) abc
% Haptobenthos	68.92 (2.82) a	61.60 (2.94) b	61.67 (6.17) ab	62.36 (5.80) b	65.46 (7.36) ab	64.15 (3.01) b	65.31 (4.40) ab	63.44 (5.09) ab

the Limestone Valleys subregion (67a) were considerably different from the other subregions. These two low-gradient subregions had significantly lower numbers and abundances of sensitive EPT taxa. Notably, the metrics indicated that benthic macroinvertebrate communities of the Shale Valleys subregion (67b) were more similar to the benthic macroinvertebrate communities of the mountain subregions (66a, 66b, 67c, 67d and 69a) than to the communities of the Interior Plateau subregion (66c) or Limestone Valleys subregion (67a).

The mountain subregions (66a, 66b, 67c, 67d and 69a) had significantly greater numbers and abundances of pollution-sensitive EPT taxa than the valley/plateau subregions (66c, 67a and 67b) (Table 10). The valley/plateau subregions (66c, 67a and 67b) had a significantly greater ratio of Hydropsychidae/Trichoptera as well as significantly greater % collector-gatherers and % scrapers than the mountain subregions (66a, 66b, 67c, 67d and 69a). Therefore, the metrics also reflect environmental conditions (i.e., organic enrichment, open canopies) typically found in the streams of the valley/plateau subregions.

The montane subregions of the Blue Ridge Mountains ecoregion (66a and 66b) were significantly more diverse than the mountainous ridges of the Central Appalachian Ridges and Valleys (67c and 67d) (Table 11). However, the Blue Ridge Mountains montane subregions also had significantly greater ratio of pollution-tolerant Hydropsychidae to total Trichoptera than the montane subregions of the Central Appalachian Ridges and Valleys (67c and 67d). The mountainous Central Appalachian Ridges and Valleys subregions (67c and 67d) had significantly greater % collector- gatherer abundance than the montane subregions of the Blue Ridge Mountains ecoregion (67c and 67d).

Community similarity.- The randomization test using the Bray-Curtis Coefficient showed a significant difference between the three ecoregions (*P*   0.001). A matrix of the lambda (*L*) values for pairwise comparisons indicated that there was little difference between the Blue Ridge

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TABLE 10. Mean (± 95% confidence interval) values of 12 benthic macroinvertebrate community metrics of the valley/plateau subregions (66c, 67a and 67b) with mountainous subregions (66a, 66b, 66c, 67c, 67d and 69a). Regions with the same letter are not significantly different (P 0.05; orthogonal contrast; valley/plateau (n=19), mountain (n=101))

Metric	Region	
	Valley/Plateau	Mountain
Taxa richness	21.26 (3.06) a	24.17 (1.20) a
No. of families	16.79 (2.01) a	18.97 (0.82) a
EPT index	11.95 (2.23) b	15.49 (0.83) a
% EPT	49.76 (8.12) b	63.53 (2.68) a
% EPT (−Hydropsychidae)	22.44 (6.48) b	44.55 (2.78) a
% 5 most dominant taxa	61.61 (3.88) a	56.70 (1.55) a
Hydropsychidae/Trichoptera	0.77 (0.09) a	0.54 (0.05) b
Simpson diversity index	0.81 (0.05) a	0.85 (0.01) a
% Collector-gatherers	41.89 (6.18) a	34.95 (1.69) b
% Collector-filterers	32.16 (5.27) a	31.70 (1.84) a
% Scrapers	22.81 (5.91) a	18.67 (1.77) b
% Haptobenthos	62.83 (3.66) a	65.06 (1.62) a

TABLE 11. Mean (± 95% confidence interval) values of 12 benthic macroinvertebrate community metrics of the mountainous subregions of the Blue Ridge Mountains Ecoregion (66a and 66b) and the ridges of the Central Appalachian Ridges and Valleys Ecoregion (67c and 67d). Regions with the same letter are not significantly different (P 0.05; orthogonal contrast; mountains (Blue Ridge) (n=43), ridges (Central Appalachian Ridges and Valleys) (n=51)).

Metric	Region	
	Mountains	Ridges
Taxa richness	25.30 (1.74) a	22.88 (1.67) a
No. of families	19.70 (1.23) a	18.18 (1.13) a
EPT index	16.44 (1.21) a	14.76 (1.18) a
% EPT	66.15 (3.89) a	61.77 (3.67) a
% EPT (−Hydropsychidae)	45.67 (4.40) a	44.11 (3.76) a
% 5 most dominant taxa	55.06 (2.34) a	57.94 (2.24) a
Hydropsychidae/Trichoptera	0.60 (0.08) a	0.47 (0.08) b
Simpson's index of diversity	0.87 (0.02) a	0.84 (0.02) b
% Collector-gatherers	33.41 (1.71) b	36.23 (2.90) a
% Collector-filterers	31.32 (2.83) a	31.29 (2.52) a
% Scrapers	20.21 (2.78) a	17.55 (2.55) a
% Haptobenthos	66.03 (2.31) a	64.47 (2.48) a

Mountains ecoregion (66) and the Central Appalachians Ridges and Valleys ecoregion (67). However, the Central Appalachians ecoregion (69) was significantly different from the Blue Ridge Mountains ecoregion and the Central Appalachian Ridges and Valleys ecoregion (Table 12). The randomization test using the Index of Biotic Similarity showed no significant difference between the three ecoregions (P 0.067). Like the Bray-Curtis Coefficient, the lambda (L) values for pairwise comparisons indicated that there was little difference between the Blue Ridge Mountains ecoregion (66) and the Central Appalachians Ridges and Valleys ecoregion (67). The Central Appalachians ecoregion (69) was substantially different from the other two ecoregions (Table 12). Specifically, the Central Appalachians ecoregion (69) was more similar to the Central Appalachian Ridges and Valleys ecoregion (67) than the Blue Ridge Mountains ecoregion (66).

Randomization tests using the Bray-Curtis Coefficient and the Index of Biotic Similarity showed significant differences between the subregions (P 0.001). The matrices of the lambda (L) values for pairwise comparisons indicated that there was little difference between the Non Calcareous subregion (66a), Shale Dominated Ridges subregion (66b), Interior Plateau subregion (66c), Sandstone Ridges subregion (67c) and Shale Ridges subregion (67d). The Interior Plateau subregion (66c) was similar to the Limestone Valleys subregion (67a) but was substantially different from the Shale Valleys subregion (67b) and the Forested Hills and Mountains subregion (69a). The Limestone Valleys subregion (67a) was similar to the Shale Valleys subregion (67b). The Forested Hills and Mountains subregion (69a) was substantially different from all the other subregions (Tables 13-14).

Alternative classification schemes

Landscape approach.- Our results suggest that the subregions can be rearranged more accurately into several large regions that are different from the original ecoregions. Barbour et al. (1996) also found this to be true in Florida streams and termed the redefined areas

TABLE 12. Matrix of Lambda values for pairwise comparisons (ratios of the mean between similarities to the mean within similarities) of the Blue Ridge Mountains Ecoregion (66), the Central Appalachians Ridges and Valleys Ecoregion (67) and the Central Appalachians Ecoregion (69) using the Index of Biotic Similarity and Bray-Curtis Coefficient. Values near 1 indicate little difference between the ecoregions.

Ecoregion	67	69
66		
Index of Biotic Similarity	1.0083	0.6971
Bray-Curtis Coefficient	1.0012	0.6412
67		
Index of Biotic Similarity	1.0000	0.6825
Bray-Curtis Coefficient	1.0000	0.8559

TABLE 13.   Matrix of Lambda values for pairwise comparisons (ratios of the mean between similarities to the mean within similarities) of the Non Calcareous subregion (66a), the Shale Dominated Ridges subregion (66b), the Interior Plateau subregion (66c), the Limestone Valleys subregion (67a), the Shale Valleys subregion (67b), the Sandstone Ridges subregion (67c), the Shale Ridges subregion (67d) and the Forested Hills and Mountains Subregion (69a) using the Bray-Curtis Coefficient.   Values near 1 indicate little difference between the subregions.

Subregion	66b	66c	67a	67b	67c	67d	69a
66a	0.9086	0.9906	0.6763	0.6979	0.9723	0.9011	0.5985
66b	1.0000	0.9648	0.6563	0.6523	1.0143	0.9093	0.5947
66c		1.0000	1.1015	0.7760	1.0726	0.9338	0.7122
67a			1.0000	0.9564	0.6896	0.6312	0.6543
67b				1.0000	0.7136	0.6156	0.4638
67c					1.0000	1.0220	0.6257
67d						1.0000	0.5879

TABLE 14. Matrix of Lambda values for pairwise comparisons (ratios of the mean between similarities to the mean within similarities) of the Non Calcareous subregion (66a), the Shale Dominated Ridges subregion (66b), the Interior Plateau subregion (66c), the Limestone Valley subregion (67a), the Shale Valley subregion (67b), the Sandstone Ridges subregion (67c), the Shale Ridges subregion (67d) and the Forested Hills and Mountains subregion (69a) using the Index of Biotic Similarity. Values near 1 indicate little difference between the subregions.

Subregion	66b	66c	67a	67b	67c	67d	69a
66a	0.8612	0.8455	0.5849	0.6901	1.0144	0.8123	0.6357
66b	1.0000	0.8943	0.6557	0.7819	0.9739	0.8928	0.7059
66c		1.0000	1.0543	0.8711	0.9465	0.8896	0.5788
67a			1.0000	0.9888	0.6510	0.6467	0.6029
67b				1.0000	0.7950	0.7537	0.5450
67c					1.0000	0.9303	0.7723
67d						1.0000	0.6461

"bioregions." In our study, there were three bioregions: the forested hills and mountains (subregion 69a), the valleys and plateaus (subregions (66c, 67a and 67b), and the mountain subregions (66a, 66b, 67c and 67d). Next, we compared the predictive accuracy of the original ecoregion and subregion framework with a bioregion classification using the benthic macroinvertebrate community metrics. A multiple discriminant analysis showed that the bioregion classification framework was a better predictor of benthic community structure than the original ecoregions or subregions (Number Cruncher Statistical Systems 1996). Bioregions classified 73.0% of the sites correctly (Table 15). Ecoregions classified 60.3% of the sites correctly (Table 16), and subregions correctly classified only 55.0% of the sites (Table 17). Stepwise discriminant analysis showed that the most influential metric in discriminating bioregions was % EPT-Hydropsychidae. Taxa richness, EPT index, Hydropsychidae/Trichoptera and % scrapers were the most influential in discriminating ecoregions. The most influential metrics in discriminating subregions were taxa richness, % EPT-Hydropsychidae, % 5 most dominant taxa and % scrapers.

Biotic Approach

Graphical Analysis.- Using a similarity threshold of 40.0 for the Bray-Curtis Coefficient, the 141 sample sites were divided into 7 biotic groups (Fig. 3). After graphing each environmental variable against the biotic groups, we found little or no separation between biotic groups for most of the environmental variables. However, sampling date and slope each separated the biotic groups into 3 independent clusters (Figs. 4-5). The two-dimensional scatter plots indicated that slope, pH, habitat assessment score and especially sampling date were the most predictive variables (Figs. 6-9).

Discriminant analysis.- In discriminant analysis, one missing variable at a site automatically removes the site from the analysis. Due to missing on-site environmental variables at some of our sites, only 106 sites were used in the analysis. We found that 69.8% of the sites

TABLE 15. Prediction of bioregions using multiple discriminant analysis on benthic macroinvertebrate community metrics (*n*=141 sites).

| Bioregion | Predicted group membership | | | | Percentage of sites correctly predicted |
	No. of sites	Mountain	Valley/ Plateau	Forested Hills and Mountains	
Mountain	115	83	12	20	72.2
Valley/Plateau	19	3	14	2	73.7
Forested Hills and Mountains	7	1		6	85.7
Overall	141				73.0

Percentage classification accuracy greater than chance = 59.6%

TABLE 16. Prediction of ecoregions using multiple discriminant analysis on benthic macroinvertebrate community metrics (*n*=141 sites).

| Ecoregion | Predicted group membership | | | | Percentage of sites correctly predicted |
	No. of sites	Blue Ridge Mountains	Central Appalachian Ridges and Valleys	Central Appalachians	
Blue Ridge Mountains	58	41	10	7	70.7
Central Appalachian Ridges and Valleys	76	24	38	14	50.0
Central Appalachians	7		1	6	85.7
Overall	141				60.3

Percentage classification accuracy greater than chance = 40.4%

TABLE 17. Prediction of subregions using multiple discriminant analysis on benthic macroinvertebrate community metrics (n=120 sites).

Subregion	No. of sites	Predicted group membership								Percentage of sites correctly predicted
		Non Calcareous	Shale Dominated Ridges	Interior Plateau	Limestone Valleys	Shale Valleys	Sandstone Ridges	Shale Ridges	Forested Hills and Mountains	
Non Calcareous	26	17	1	1		1	2	2	2	65.4
Shale Dominated Ridges	17		8	2			2	3	2	47.1
Interior Plateau	5			5						100.0
Limestone Valleys	10	1			7	2				70.0
Shale Valleys	4					3		1		75.0
Sandstone Ridges	37	9	6	3	2	1	10	3	3	27.0
Shale Ridges	14		2			2		10		71.4
Forested Hills and Mountains	7		1						6	85.7
Overall	120									55.0

Percentage classification accuracy greater than chance = 48.6%
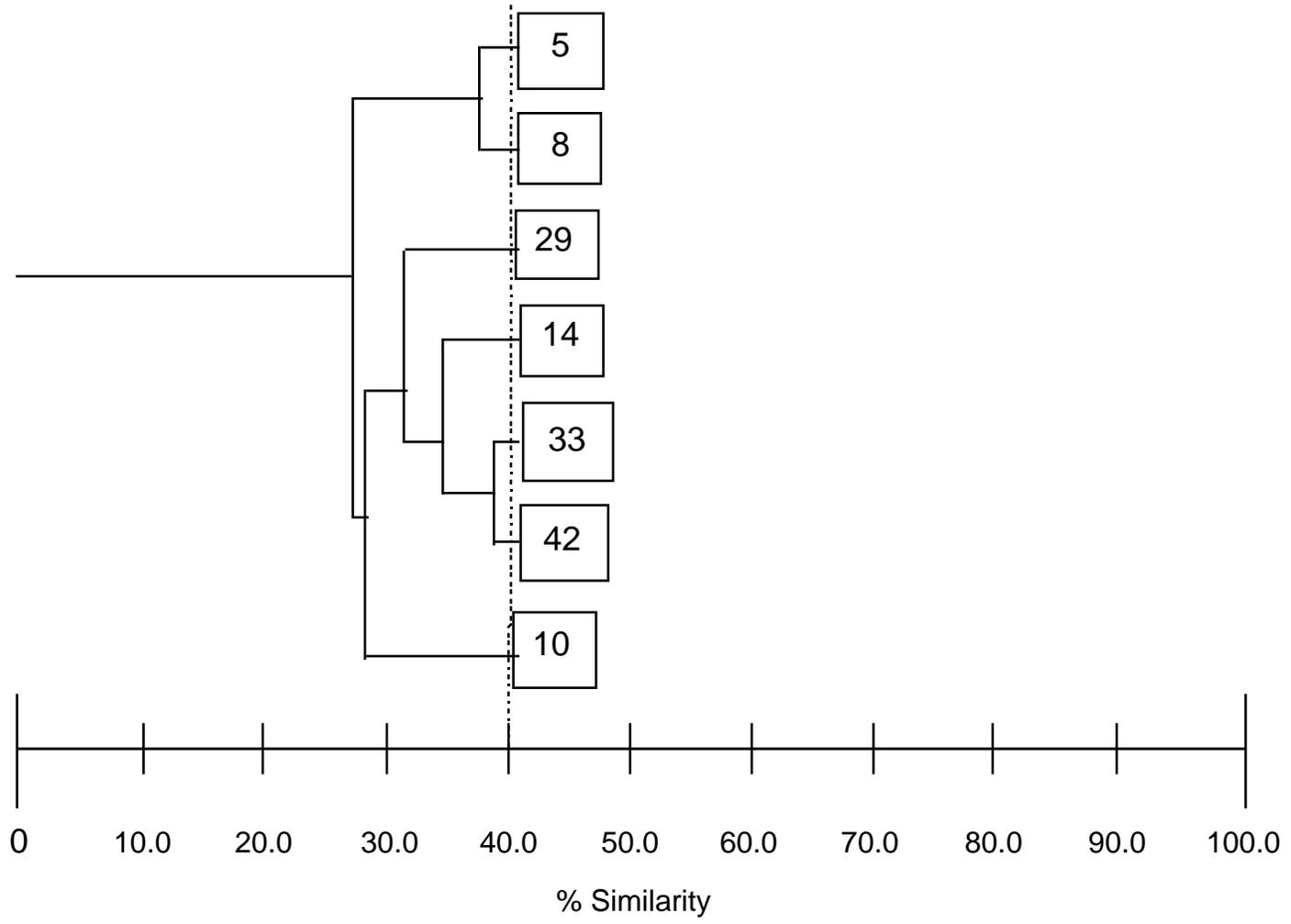
FIG. 3.   Dendrogram of UPGMA cluster analysis of 7 biotic groups based on Bray-Curtis distances between benthic macroinvertebrate communities.
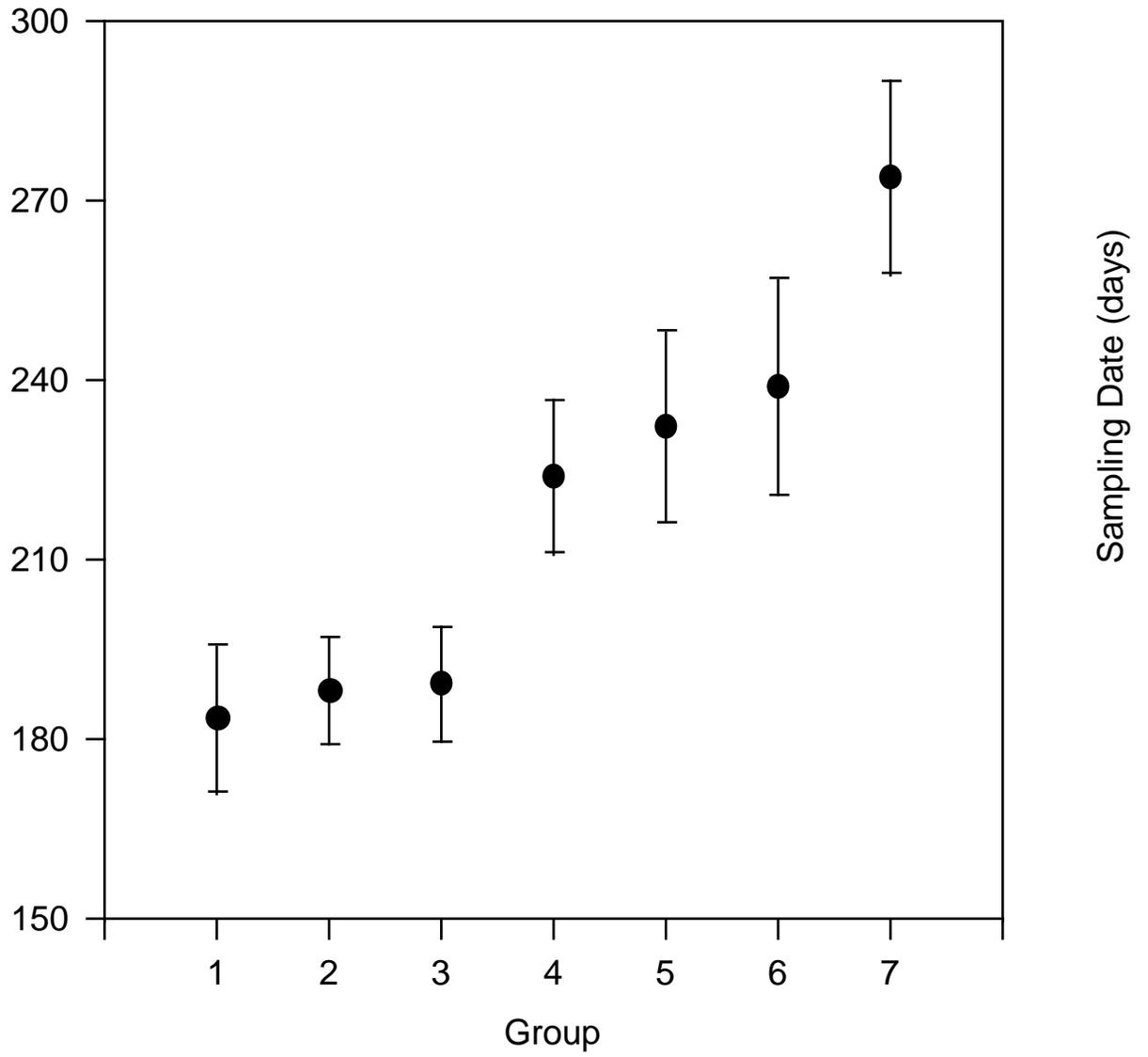
FIG. 4. Mean sampling date (days) and 95% confidence interval for each biotic group.
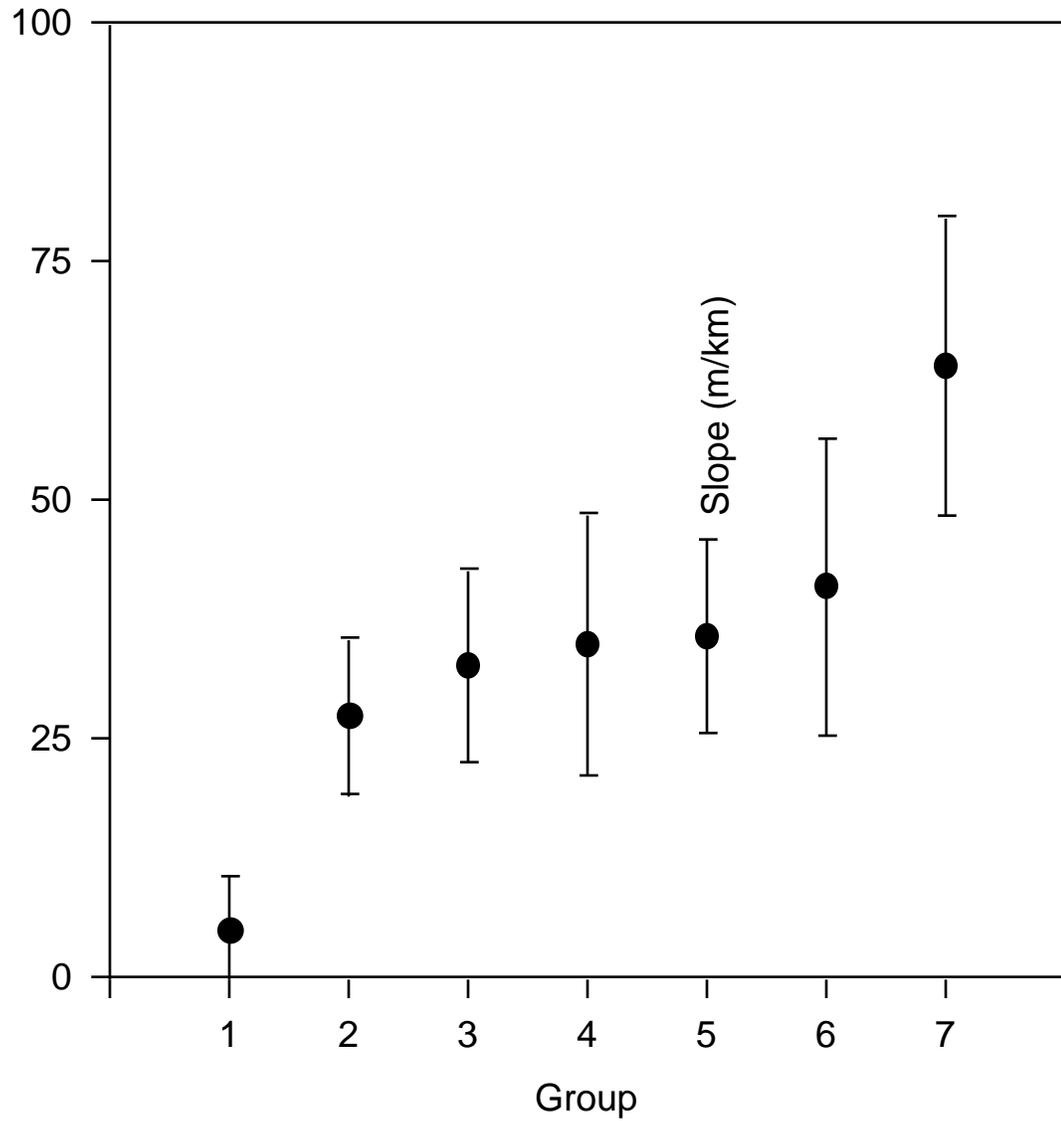
FIG. 5. Mean slope (m/km) and 95% confidence interval for each biotic group.
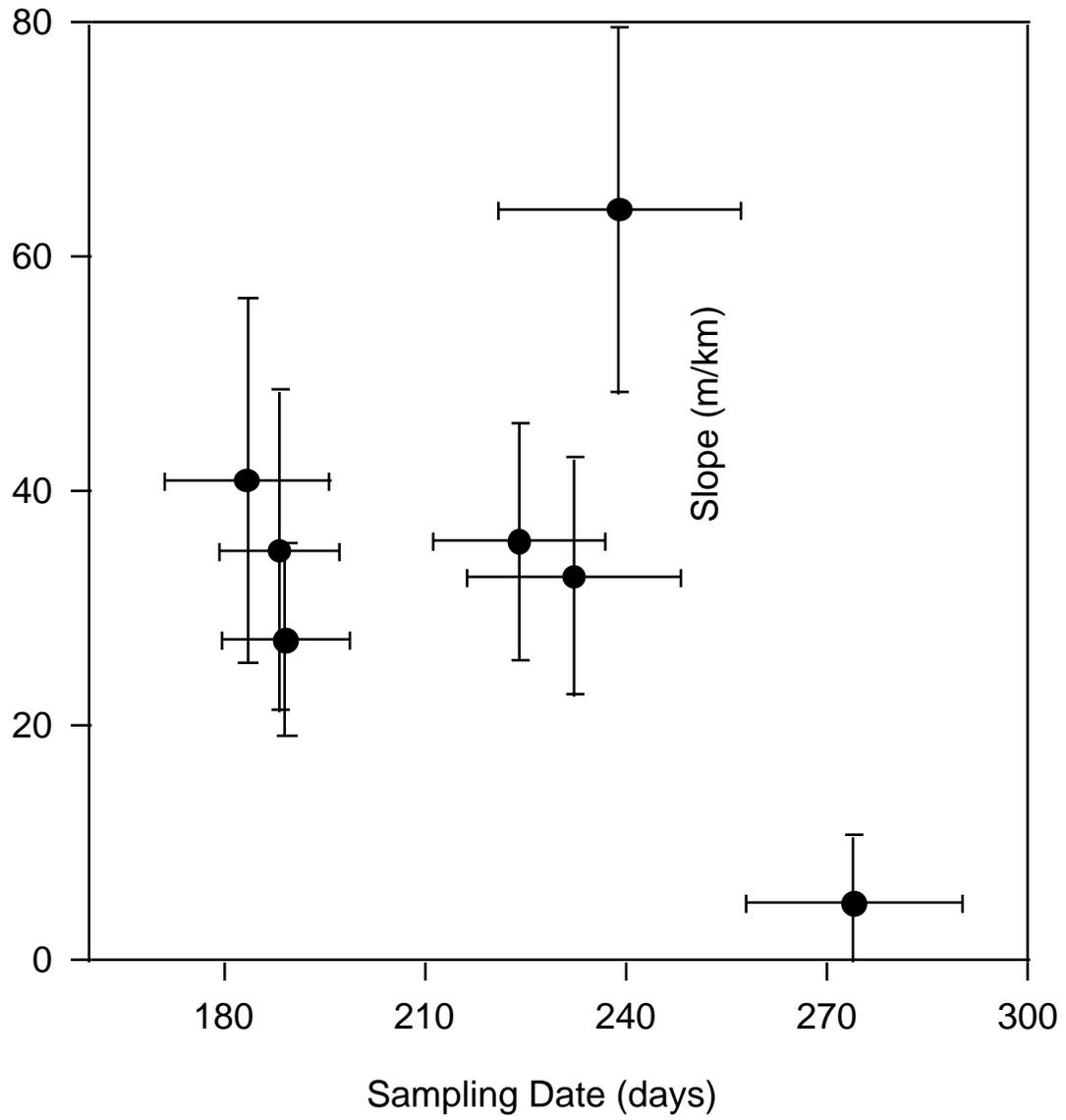
FIG. 6. Relationship of sampling date (days) and slope (m/km) for 7 biotic groups (means and 95% confidence intervals).
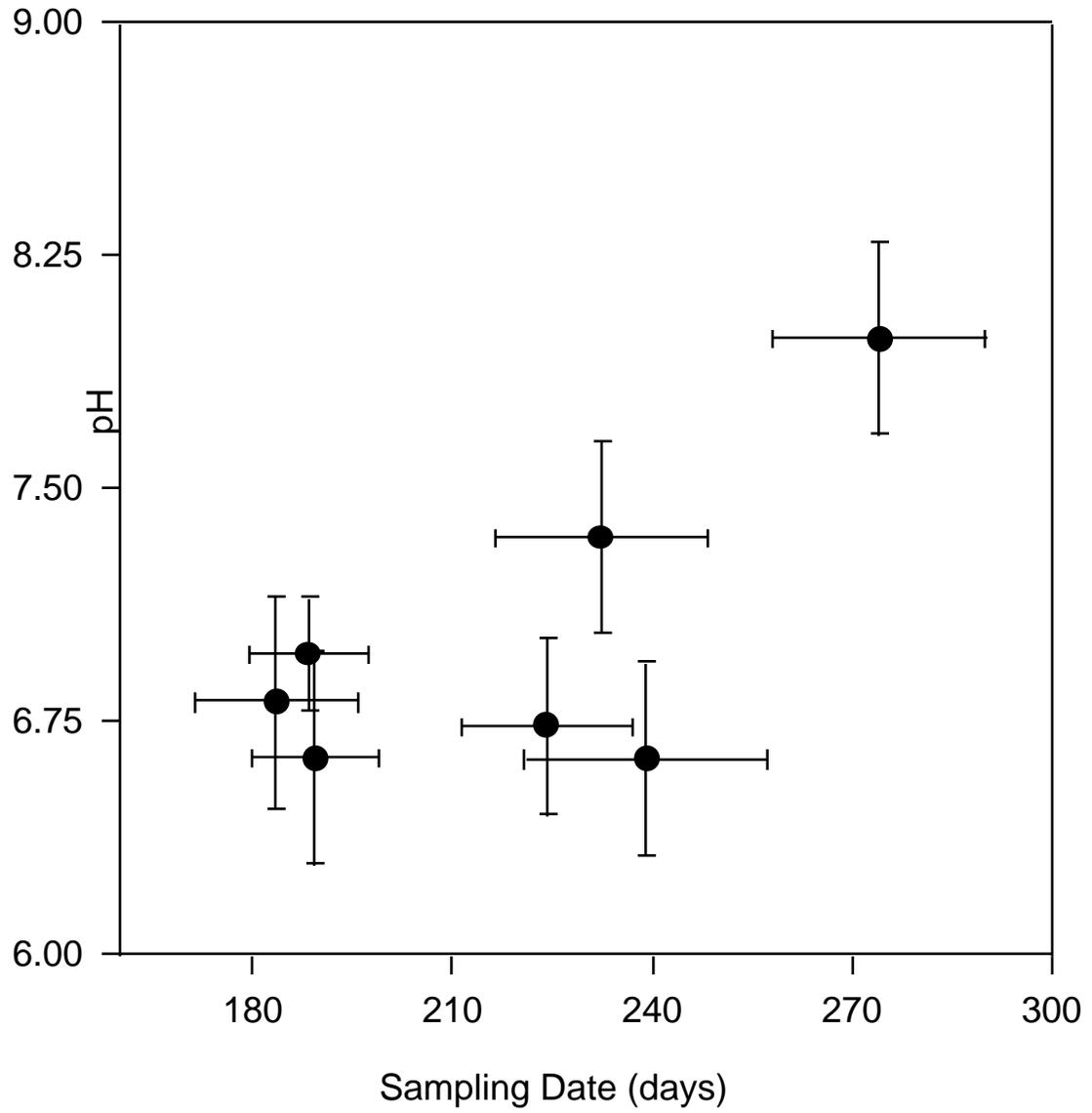
FIG. 7. Relationship of sampling date (days) and pH for 7 biotic groups (means and 95% confidence intervals).
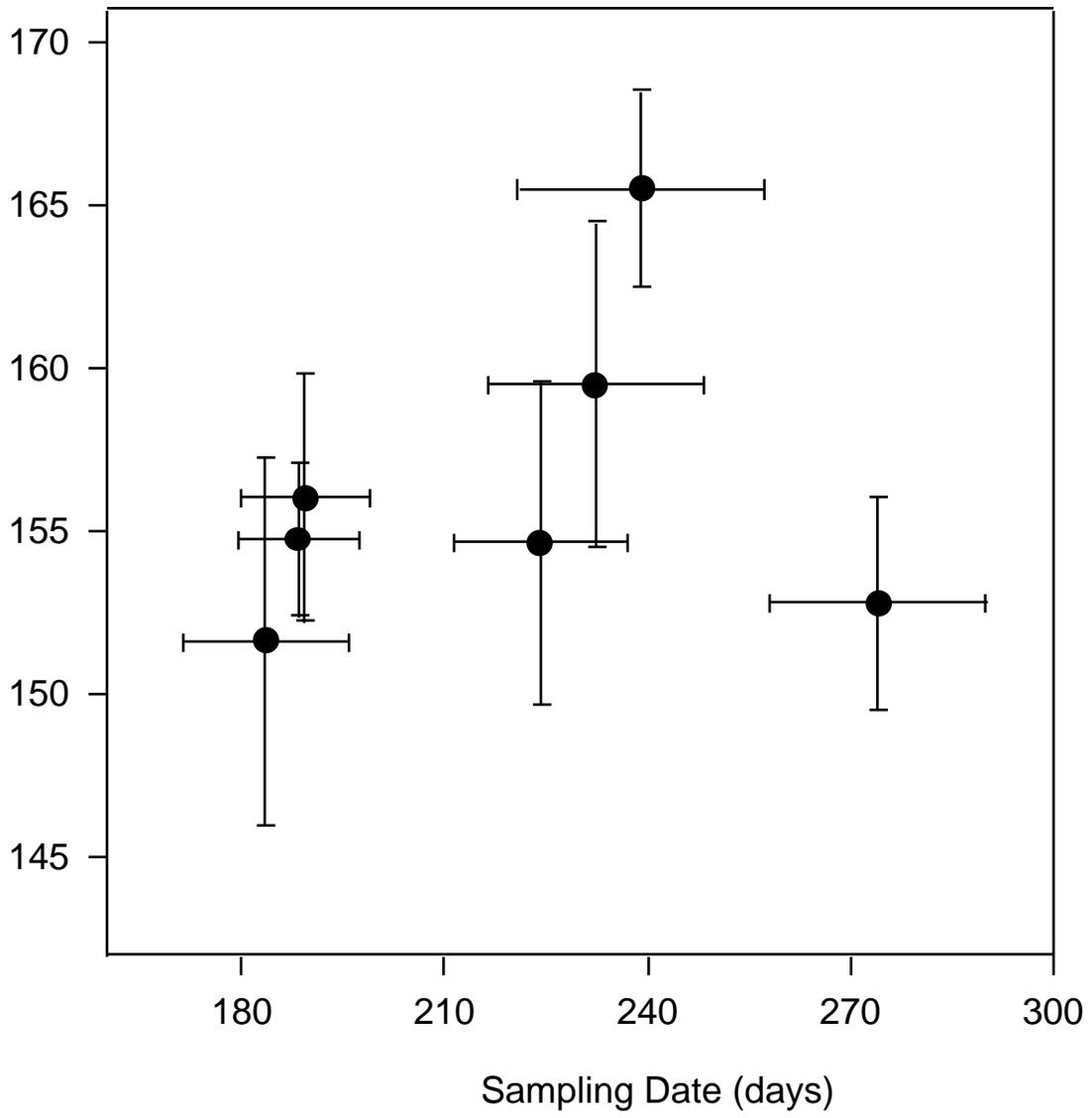
FIG. 8. Relationship of sampling date (days) and habitat assessment score for 7 biotic groups (means and 95% confidence intervals).
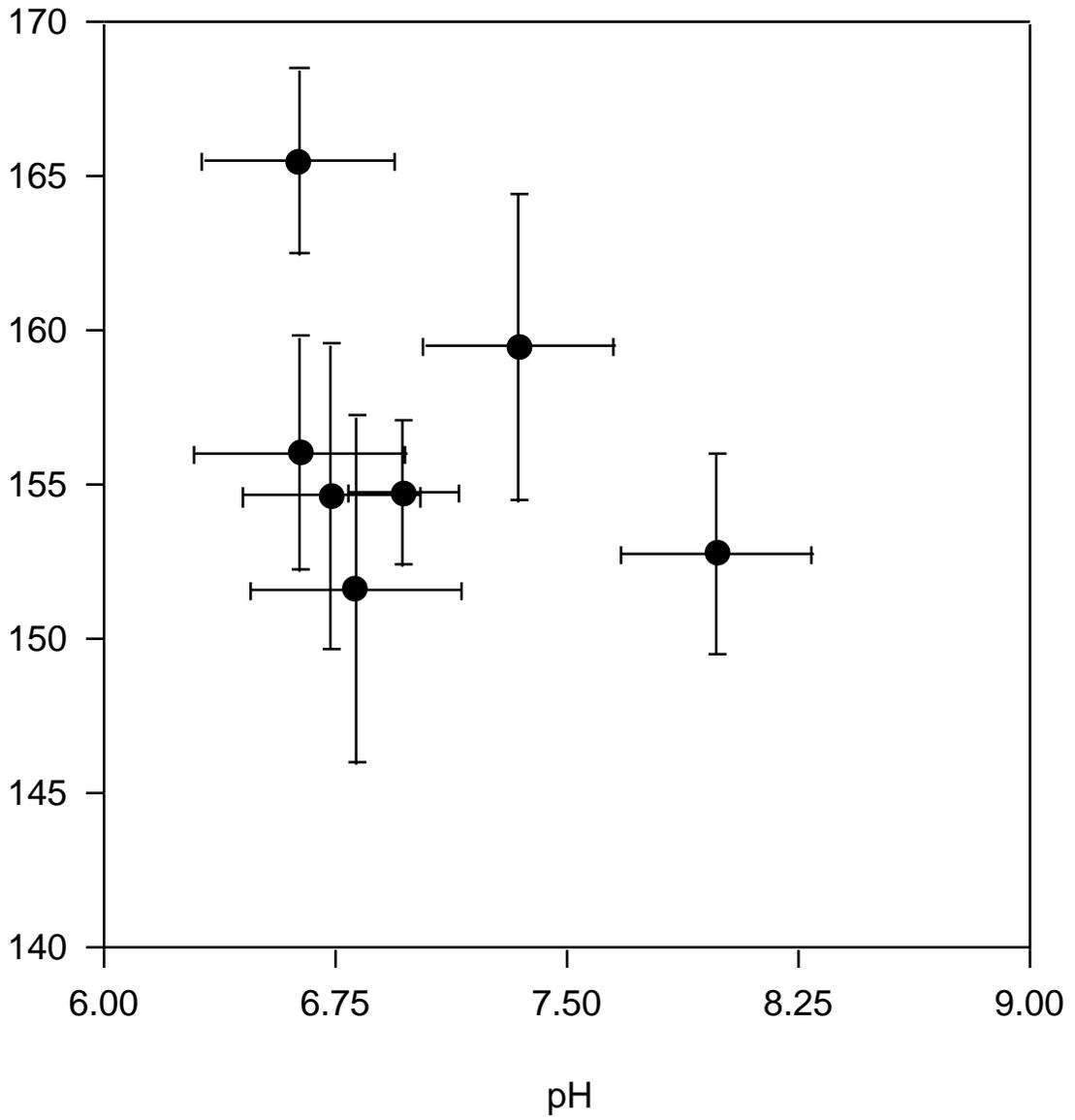
FIG. 9. Relationship of pH and habitat assessment score for 7 biotic groups (means and 95% confidence intervals).

were correctly classified using all 14 environmental variables (Table 18). The classification accuracy of the discriminant functions was 64.8% greater than expected if the sites had been randomly classified. The stepwise discriminant analysis showed that sampling date, slope, pH, habitat assessment score, and distance to source were the best predictors of benthic macroinvertebrate community structure out of the environmental variables we tested. The overall Wilk's lambda (analogous to 1-R^2 in multiple regression) for the model was 0.233 (Table 19). We found that 52.8% of the sites were correctly classified using the 7 environmental variables previously screened by the stepwise discriminant analysis (Table 20). The classification accuracy of the discriminant functions was 45.0% greater than expected if the sites were randomly classified.

Discussion

Ecoregion approach

We found no difference in benthic macroinvertebrate community structure between the Blue Ridge Mountains ecoregion (66) and the Central Appalachian Ridges and Valleys ecoregion (67). However, the benthic macroinvertebrate community structure of the Central Appalachians ecoregion (69) was fairly different from the Blue Ridge Mountains ecoregion (66) and the Central Appalachian Ridges and Valleys ecoregion (67). Nonetheless, given the relatively small number of sites sampled, the difference may not be truly representative of the entire region.

Analysis of the benthic macroinvertebrate community across subregions showed differences in community structure. We found that the subregions were roughly divided into three larger regions: the Forested Hills and Mountains subregion (69a), the valley/plateau subregions (66c, 67a and 67b), and the mountain subregions (66a, 66b, 67c and 67d). Our pairwise comparison of the communities showed that the Forested Hills and Mountains

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TABLE 18. Prediction of biotic groups using multiple discriminant analysis on 14 selected environmental variables (n=106 sites).

Group	No. of sites	Predicted Group Membership							Percentage of sites correctly predicted
		1	2	3	4	5	6	7	
1	8	6	1	1					75.0
2	34	3	25	2	1	2		1	73.5
3	24	4	1	16	2	1			66.7
4	10			1	7		2		70.0
5	20		3	2	2	12	1		60.0
6	8			1	1		6		75.0
7	2							2	100.0
Overall	106								69.8

Percentage classification accuracy greater than chance = 64.8%

TABLE 19. Components of the final stepwise discrimination model for assessing the influence of environmental variables in structuring benthic macroinvertebrate communities. ($n = 106$ sites; Overall Wilks' Lambda = 0.233)

Environmental Variable	Percent Change in Lambda	F-value	P-value
Sampling date	35.07	8.91	< 0.0001
Slope	27.14	6.08	< 0.0001
pH	27.01	5.98	< 0.0001
Distance to source	18.68	3.64	0.0027
Habitat Assessment Score	16.85	3.24	0.006

TABLE 20. Prediction of biotic groups using multiple discriminant analysis on selected environmental variables (*n*=106 sites).

Group	No. of sites	Predicted Group Membership							Percentage of sites correctly predicted
		1	2	3	4	5	6	7	
1	8	5	2	1					62.5
2	34	11	14	6		2	1		41.2
3	24	3	3	15	2	1			62.5
4	10			1	7	1	1		70.0
5	20	2	3	2	2	8	2	1	40.0
6	8		1		2		5		62.5
7	2							2	100.0
Overall	106								52.8

Percentage classification accuracy greater than chance = 45.0%

subregion (69a) was most unlike any of the other subregions. The valley/plateau subregions (66c, 67a and 67b) generally had higher percentages of taxa tolerant of sediment and pollution than the mountain subregions and larger mean abundances of benthic macroinvertebrates typical of valley streams. The Interior Plateau subregion (66c), though aligned closely with the valley subregions, demonstrated some similarity in community structure and certain individual taxa with the mountain subregions (66a, 66b, 67c and 67d). The Shale Valleys subregion (67b) also showed metric values and mean abundances of certain individual taxa more similar to the mountain subregions (66a, 66b, 67c and 67d). Our comparison of the montane regions of the Blue Ridge with the ridges of the Central Appalachian Ridges and Valleys showed little difference in the mean abundance of individual taxa, metrics and community similarity. Therefore, even without the influence of valley/plateau subregions, there was no appreciable difference between the benthic macroinvertebrate communities in the mountain ranges.

The results of this study indicated that there are regional differences in benthic macroinvertebrate communities. However, except for possibly the Central Appalachians ecoregion (69), these differences fail to correlate with the western Virginia ecoregions and subregions proposed by Omernik (1987). There are several factors that may explain why ecoregions failed to match the benthic macroinvertebrate communities in western Virginia. Many of the previous studies that found correspondences between ecoregions and a particular target community focused mainly on the distribution of fish [Larsen et al. 1986 (fish); Hughes et al. 1987 (fish); Rohm et al. 1987 (fish, water quality, physical habitat); Whittier et al. 1987 (fish, macroinvertebrates, water quality, physical habitat); Larsen et al. 1988 (fish, macroinvertebrates, periphyton, water quality, physical habitat); Whittier. et al. 1988 (fish, macroinvertebrates, water quality, physical habitat); and Lyons 1989 (fish)]. Benthic macroinvertebrates should show a greater correspondence to ecoregions than fish. Since fish are strictly limited to current and historical bodies of water, they may not be able to fully exploit the environmental conditions available within a given ecoregion. Whereas most macroinvertebrates are able to fly as adults

and may disperse overland. Nevertheless, regional differences may be easier to detect using fish assemblages because fish are considerably easier to identify to species. In our study, most benthic macroinvertebrates were identified to the genus level. Differences in ecoregions using benthic macroinvertebrates as a target community may only become apparent at the species level.

In addition, the growing trend of environmental management has been to focus on ecosystems as a whole. Natural resource managers are expected to know the attainable conditions of ecosystems and recognize the effects of human activities on all ecosystem components. Since ecoregions were developed for assessing the conditions of entire ecosystems, this classification approach may be less accurate in predicting the macrodistribution of individual target communities than classifications developed for specific communities (Omernik 1995).

Despite previous evidence showing a link between benthic macroinvertebrate distribution and landscape formations (Ross 1963, Woodall and Wallace 1972, Pennak 1977, Molles 1982, Minshall et al. 1985, Whittier et al. 1988 and Corkum 1989), several researchers have found that local conditions often overwhelm the influence of the regional environment on the structure of benthic macroinvertebrate communities (Cummins et al. 1989, Corkum 1990, Richards et al. 1993 and Sweeney 1993). Our study confirms these findings. We discovered that, with the possible exception of the Central Appalachians ecoregion, land form and land use (two of the factors originally used to define ecoregions and subregions) appear to play a predominant role in structuring benthic macroinvertebrate communities in western Virginia. Considering the ecological requirements of benthic macroinvertebrates, we found it unusual that regions with such widely contrasting land forms, soils and land use (e.g., Limestone Valleys subregion (67a) and Sandstone Ridges subregion (67c)) were expected to have similar benthic macroinvertebrate communities. Resh et al. (1995) argue that the ecoregions defined by Omernik (1987) are too large for making accurate water resource assessments using benthic macroinvertebrates.

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Alternative classification schemes

Landscape approach.- Some researchers have attained greater accuracy in biomonitoring by rearranging ecoregions or subregions into "bioregions." Barbour et al. (1996), in developing a multimetric biotic index for Florida streams, found that an aggregation of 9 subregions into 3 bioregions classified benthic macroinvertebrate communities better than subregions alone. Since our data strongly indicate regional differences between mountain, valley/plateau and Central Appalachian streams, we consider classifications based on bioregions especially promising in assuring assessment accuracy.

Biotic approach.- Wright et al. (1984), Moss et al. (1987), Wright (1995) and Norris (1996) have demonstrated that a small number of environmental variables can be used to predict the benthic macroinvertebrate community expected at a site that is free from any major environmental impact. Wright et al. (1984) in developing a general classification scheme for streams throughout the United Kingdom, correctly classified 76.1% of their sites using 28 environmental variables. In our study, we found a 69.8% correct classification rate using 14 environmental variables.

Moss et al. (1987), starting with the 28 environmental variables tested by Wright et al. (1984), examined how reducing the number of environmental variables affected the accuracy of their classification. They found a 65.7% correct classification rate with 11 variables and a 52.2% correct classification rate with five variables. Currently, UK biologists are using 12 environmental variables in their classification scheme (Wright 1995). In classifying 51 reference sites around Canberra, Australia, Norris (1996) found that four variables correctly classified 66% of the reference sites at the family level. In our study, stepwise discriminant analysis showed that sampling date, slope, pH, habitat assessment score and distance from source were the most predictive environmental variables out of the 14 we tested. These five factors correctly classified 52.8% of the sites. If we ignore sampling date, these environmental variables compare favorably

with similar factors found to correspond with benthic macroinvertebrate distribution patterns in similar studies (Wright et al. 1984, Corkum and Currie 1987, Moss et al. 1987, Corkum and Ciborowski 1988, Corkum 1989, Corkum 1990, Wright 1995 and Norris 1996).

Wright et al. (1984), Moss et al. (1987) and Wright (1995) based their classification on species-level presence/absence data. Norris (1996) based his classification on a subset of families that occurred at 10 percent or more of the 51 reference sites. In some ways, our classification was more rigorous. We based our classification on genus-level taxonomy and abundances of all taxa, not just presence/absence or a subset of the most dominant families. In addition, these other studies incorporated more quantifiable estimations of important on-site environmental variables such as mean substrate size, discharge, stream width and stream depth. Unfortunately, we were often limited to visual-based estimations of on-site physical variables. Sampling date was among the most influential variables in our study. The predictive ability of sampling date demonstrates significant changes in benthic communities throughout the summer and early fall. Greater accuracy may have resulted if our sampling season was limited to a shorter window of time. Since classification rates of 66% were considered acceptable in similar studies (Moss et al. 1987, Wright 1995, and Norris 1996), we feel that, in light of some of the unique features of our study, our classification rate using five variables is comparable to these rates.

Our study demonstrates that in western Virginia, benthic macroinvertebrate distribution does not correspond with the proposed ecoregions and subregions. We found that reliable biocriteria can be developed using a landscape approach (aggregations of subregions into bioregions) or a biotic approach. Because of the high cost of developing a biotic classification scheme, we recommend the landscape approach for developing biocriteria using benthic macroinvertebrates.

Literature Cited

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater, 17th edition. American Public Health Association, Washington, DC.

Barbour, M.T., J. Gerritsen, G.E. Griffith, R. Frydenborg, E. McCarron, J.S. White, M.L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society 15:185-211

Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves, and R.M. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11:437-449.

Barbour, M.T., and J.B. Stribling. 1991. Use of habitat assessment in evaluating the biological integrity of stream communities. Pages 25-38 *in* Biological criteria: research and regulation, 1991,. EPA-440/5-91-005. US EPA, Office of Water, Washington, DC

Barbour, M.T., J.B. Stribling and J.R. Karr. 1995. The multimetric approach for establishing biocriteria and measuring biological condition. Pages 63-80 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Bartlett, M.S. 1937a. Some examples of statistical methods of research in agriculture and applied biology. Journal of the Royal Statistical Society Supplement 3:68-78.

Bartlett, M.S. 1937b. Properties of sufficiency and statistical tests. Proceedings of the Royal Statistical Society. Series A, 160:268-282.

Biggs, B.J., M.J. Duncan, I.G. Jowett, J.M. Quinn, C.W. Hickey, R.J. Davis-Colley, and M.E. Close. 1990. Ecological characterization, classification, and modeling of New Zealand rivers: an introduction and synthesis. New Zealand Journal of Marine and Freshwater Research 24:277-304.

Box, G.E.P. 1949. A general distribution theory for a class of likelihood criteria. Biometrika 36:317-346.

Bray, J.R. and J.T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349.

Brigham, A.R., W.U. Brigham, and A. Gnilke. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, Illinois.

Brigham, W.U. 1982. Aquatic coleoptera. Pages 10.1-10.136 *in* Brigham, A.R., W.U. Brigham, and A. Gnilke (editors). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, Illinois.

Caton, L.W. 1991. Improved subsampling methods for the EPA "Rapid Bioassessment Benthic Protocols." Bulletin North American Benthological Society 8: 317-319

Corkum, L.D. 1989. Patterns of benthic invertebrate assemblages in rivers of northwestern North America. Freshwater Biology 21:191-205.

Corkum, L.D. 1990. Intrabiome distributional patterns of lotic macroinvertebrate assemblages. Canadian Journal of Fisheries and Aquatic Sciences 47:2147-2157.

Corkum, L.D. and D.C. Currie. 1987. Distributional patterns of immature Simuliidae (Diptera) in northwestern North America. Freshwater Biology 17:201-221.

Corkum, L.D. and J.J.H. Ciborowski. 1988. Use of alternative classifications in studying broad-scale distributional patterns of lotic invertebrates. Journal of the North American Benthological Society 7:167-179.

Covich, A.L. and J.H. Thorp. 1991. Crustacea: introduction and Peracarida. Pages 665-689 *in* A.L. Covich and J.H. Thorp (editors). Ecology and classification of North American freshwater invertebrates. Academic Press, Inc. San Diego, California.

D'Agostino, R.B., A. Belanger, and R.B. D'Agostino, Jr. 1990. A suggestion for using powerful and informative tests of normality. American Statistician 44:316-321.

Good, I.J. 1982. An index of separateness of clusters and a permutation test for its significance. Journal of Statistical Computation and Simulation 15:81-84.

Hornig, C.E., C.W. Bayer, S.R. Tidwell, J.R. Davis, R.J. Kleinsasser, G.W. Linam, and K.B. Mayes. 1995. Development of regionally based biological criteria in Texas. Pages 145-152 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers Boca Raton, Florida.

Hughes, R.M. 1985. Use of watershed characteristics to select control streams for estimating effects of metal mining wastes on extensively disturbed streams. Environmental Management 9:253-262.

Hughes, R.M., S.A. Heiskary, W.J. Matthews, and C.O. Yoder. 1994. Use of ecoregions in biological monitoring. Pages 125-151 *in* S.L. Loeb and A. Spacie (editors). Biological monitoring of aquatic systems. Lewis Publishers, Boca Raton, Florida.

Hughes, R.M. and D.P. Larsen. 1988. Ecoregions: an approach to surface water protection. Journal of the Water Pollution Control Federation 60:486-493.

Hughes, R.M., D.P. Larsen, and J.M. Omernik. 1986. Regional reference sites: a method for assessing stream potentials. Environmental Management 10:629-635.

Hughes, R.M., E. Rexstad, and C.E. Bond. 1987. The relationship of aquatic ecoregions, river basins, and physiographic provinces to the ichthyogeographic regions of Oregon. Copeia 1987:423-432.

Hughes, R.M., and J.M. Omernik. 1983. An alternative for characterizing stream size. Pages 87-101 *in* T.D. Fontaine III and S.M. Bartell (editors), Dynamics of lotic ecosystems. Ann Arbor Science, Ann Arbor, Michigan.

Huryn, A.D. and J.B. Wallace. 1987. The exopterygote insect community of a mountain stream in North Carolina, USA: life histories, production and functional structure. Aquatic Insects 9:229-251.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66-84.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Special Publication 5. Illinois Natural History Survey, Urbana, Illinois.

Kerans, B.L. and J.R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications 4:768-785.

Krebs, C.J. 1987. Ecological methodology. Harper and Row, Publishers. New York, New York.

Larsen, D.P., J.M. Omernik, R.M. Hughes, C.M. Rohm, T.R. Whittier, A.J. Kinney, A.L. Gallant and D.R. Dudley. 1986. Correspondence between spatial patterns in fish assemblages in Ohio streams and aquatic ecoregions. Environmental Management 10:815-828.

Larsen, D.P., D.R. Dudley and R.M. Hughes. 1988. A regional approach for assessing attainable surface water quality: Ohio as a case study. Journal of Soil and Water Conservation 40:55-61.

Ludwig, J.A., and J.F. Reynolds. 1988. Statistical ecology: a primer on methods and computing. Wiley Interscience Publication, John Wiley and Sons. New York, New York.

Lyons, J. 1989. Correspondence between the distribution of fish assemblages in Wisconsin streams and Omernik's ecoregions. American Midland Naturalist 122:163-182.

Merritt, R.W., and K.W. Cummins (eds.). 1984. An Introduction to the aquatic insects of North America. 2nd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Merritt, R.W., and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Mingo, T.M. 1979. Distribution of aquatic Dryopoidea (Coleoptera) in Maine. Entomological News 90:177-185.

Minshall, G.W., K.W. Cummins, R.C. Petersen, C.F. Cushing, D.A. Bruns, J.R. Sedell, and R.L. Vannote. 1985. Developments in stream ecosystem theory. Canadian Journal of Fisheries and Aquatic Sciences 42:1045-1055.

Molles, M.C., Jr. 1982. Trichopteran communities of streams associated with aspen and conifer forests: long-term structural change. Ecology 63:1-6.

Moss, D., M.T. Furse, J.F. Wright, and P.D. Armitage. 1987. The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. Freshwater Biology 17:42-52.

Neyman, J. and E.S. Pearson. 1931. On the problem of k samples. Bulletin. Academie Polonaise des Sciences. Serie. A 3:460-481.

Norris, R.H. 1996. Predicting water quality using reference conditions and associated communities. Pages 32-52 *in* R.C. Bailey, R.H. Norris and T.B. Reynoldson (editors). Study design and data analysis in benthic macroinvertebrate assessments of freshwater ecosystems using a reference site approach. Technical Information Workshop, North American Benthological Society, 44th Annual Meeting, Kalispell, Montana.

Number Cruncher Statistical Systems 1996. Number Cruncher Statistical Systems User's Guide:Vols. I-III. Version 6.0.21. Number Cruncher Statistical Systems., Kaysville, Utah.

Ohio EPA (Environmental Protection Agency). 1987. Biological criteria for the protection of aquatic life. Volumes 1-3. Monitoring and Assessment Program, Surface Water Section, Division of Water Quality, Columbus, Ohio.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118-125.

Omernik, J.M. 1995. Ecoregions: a spatial framework for environmental management. Pages 49-62 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers Boca Raton, Florida.

Omernik, J.M., C.M. Rohm, S.E. Clarke, and D.P. Larsen. 1988. Summer total phosphorus in lakes: a map of Minnesota, Wisconsin and Michigan. Environmental Management 12:815-825.

Pennak, R.W. 1977. Trophic variables in Rocky Mountain trout streams. Archiv fur Hydrobiologie 80:253-285.

Pennak, R.W. 1989. Freshwater invertebrates of the United States: Protozoa to Mollusca. 3rd edition. Wiley Interscience, John Wiley and Sons, New York, New York.

Pinkham, C.F.A. and J.G. Pearson. 1974. A new measure of biotic similarity between samples and its applications with a cluster analysis program. Edgewood Arsenal Technical Report. EB-TR-74062. Department of the Army. Headquarters, Edgewood Arsenal, Aberdeen Proving Ground, Maryland.

Pitman, J. 1937. Significance tests which may be applied to samples from any population. Journal of the Royal Statistical Society, Series B 4:119-130.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-001. US Environmental Protection Agency, Office of Water, Washington, DC

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1997. Revisions to Rapid Bioassessment protocols for use in streams and rivers: periphyton, benthic macroinvertebrates and fish. http://www.epa.gov/OWOW/monitoring/AWPD/RBP

Resh, V.H., R.H. Norris, and M.T. Barbour. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Australian Journal of Ecology 20:108-121.

Resh, V.H. and J.D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. Journal of the Water Pollution Control Federation 47:9-19.

Richards, C., G.E. Host and J.W. Arthur. 1993. Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshwater Biology 29:285-294.

Rohm, C.M., J.W. Giese, and C.C. Bennett. 1987. Test of an aquatic ecoregion classification of streams in Arkansas. Journal of Freshwater Ecology 4:127-140.

Ross, H.H. 1963. Stream communities and terrestrial biomes. Archiv fur Hydrobiologie 59:235-242.

Seagle, H.H., and A.C. Hendricks. 1979. Dryopid beetles of the south fork of the Shenandoah river basin, Virginia, with some biological notes. The Coleopterists Bulletin 33:205-208.

Smith, E.P., K.W. Pontasch and J.C. Cairns, Jr. 1990. Community similarity and the analysis of multispecies environmental data: a unified statistical approach. Water Research 24:507-514.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry: The principles and practice of statistics in biological research. 3rd ed. W.H. Freeman and Company. New York, New York.

Stewart, K.W. and B.P. Stark. 1988. Nymphs of the North American stonefly genera (Plecoptera). Thomas Say Foundation. The Entomological Society of America.

Sweeney, B.W. 1993. Effects of streamside vegetation on macroinvertebrate communities of White Clay Creek in eastern North America. Proceedings of the Academy of Natural Sciences, Philidelphia 144:291-340.

Unzicker, J.D., and P.H. Carlson. 1982. Ephemeroptera. Pages 3.1-3.97 *in* Brigham, A.R., W.U. Brigham, and A. Gnilke (editors). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, Illinois.

Unzicker, J.D., and V.H. McCaskill. 1982. Plecoptera. Pages 5.1-5.50 *in* Brigham, A.R., W.U. Brigham, and A. Gnilke (editors). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, Illinois.

Unzicker, J.D., V.H, Resh and J.C. Morse. 1982. Trichoptera. Pages 9.1-9.138 *in* Brigham, A.R., W.U. Brigham, and A. Gnilke (editors). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, Illinois.

USEPA (US Environmental Protection Agency). 1983. Methods for chemical analysis of water and wastes. EPA-600/4-79-020 Environmental Monitoring and Support Laboratory, Office of Research and Development. US Environmental Protection Agency, Cincinnati, Ohio, 45268

USEPA (US Environmental Protection Agency). 1991. Policy on the use of biological assessments and criteria in the water quality program. US Environmental Protection Agency, Office of Science and Technology, Washington, DC

Whittier, T.R., D.P. Larsen, R.M. Hughes, C.M. Rohm, A.L. Gallant and J.M. Omernik. 1987. The Ohio stream regionalization project: a compendium of results. US Environmental Protection Agency Publication EPA/600/3-87/025, Corvallis, Oregon.

Whittier, T.R., R.M. Hughes, and D.P. Larsen. 1988. The correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Canadian Journal of Fisheries and Aquatic Sciences 45:1264-1278.

Wiggins, G.B. 1977. Larvae of the North American Caddisfly Genera (Trichoptera). University of Toronto Press. Toronto, Canada and Buffalo, New York.

Williams, B.K. 1983. Some observations on the use of discriminant analysis in ecology. Ecology 64:1283-1291.

Woodall, W.R., and J.B. Wallace. 1972. The benthic fauna in four small southern Appalachian streams. American Midland Naturalist 88:393-407.

Wright, J.F. 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. Australian Journal of Ecology 20:181-197.

Wright, J.F., D. Moss, P.D. Armitage, and M.T. Furse. 1984. A preliminary classification of running-water in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. Freshwater Biology 14:221-256.

Yoder, C.O., and E.T. Rankin. 1995. Biological criteria program development and implementation in Ohio. Pages 109-144 *in* W.S. Davis and T.P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.