A Macroinvertebrate Bioassessment Index for Headwater Streams of the Eastern Coalfield Region, Kentucky

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Division of Water
Water Quality Branch
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A Macroinvertebrate Bioassessment Index for Headwater Streams of the Eastern Coalfield Region, Kentucky

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10 Executive Summary

Since the promulgation of the Clean Water Act (CWA), as amended in 1987, the Kentucky Division of Water (KDOW) has routinely collected chemical, biological, and habitat information in streams across the Commonwealth. These data have been used primarily for use-support designations in association with 303(d) reports and biennial 305(b) reports to Congress, intensive watershed surveys, and Kentucky Pollutant Discharge Elimination System (KPDES) compliance monitoring. Current KDOW Water Quality Standards are narrative with regard to biological criteria and state that "…Surface waters shall not be aesthetically or otherwise degraded by substances that: (d) Injure, are chronically or acutely toxic to or produce adverse physiological or behavioral responses in humans, animals, fish and other aquatic life; (e) Produce undesirable aquatic life or result in the dominance of nuisance species…" (401 KAR 5:031 Section 2). 401 KAR 5:031 has additional narrative criteria that prevent adverse effects to aquatic communities. In Kentucky, these narrative standards are interpreted using various numeric indices of biological integrity developed from KDOW data. Despite historical biological monitoring, data gaps exist for Kentucky's headwater streams.

Headwater streams, as defined in this document, are 1st or 2nd order streams as depicted on 1:24,000 scale topographic maps and are generally < 3 to 5 mi^2 (~ 8 to 13 km^2) in drainage area. These streams (either intermittent or perennial) and their ephemeral tributaries serve multiple functions often overlooked in environmental planning and landuse decision-making. They are the key interface between the surrounding landscape and larger waterbodies. Healthy headwater streams provide habitat to relatively distinct and diverse invertebrate assemblages, and by assimilating nutrients, organic matter, and sediments, they export high quality water in the form of goods and services (e.g., water supply, recreation, waste assimilation, flood control, and ecological values) (Yoder et al. 2000, Wallace and Meyer 2001). These streams are also closely connected to groundwater resources and provide thermal refuges to many organisms in both winter and summer. Despite possessing these attributes, little is known about the biological potential of small headwater streams in Kentucky.

We sampled macroinvertebrates in the spring index period (mid-February to late-May) from 67 sites (70 sample events) in an effort to calibrate and validate regional expectation criteria for benthic invertebrate communities in small headwater streams (1st–2nd order). Sites were chosen based on topographic maps, aerial photos, and landuse using Arcview GIS software and field reconnaissance. A reference site was determined adequate if it was primarily vegetated with relatively mature native forest, there was little or no residential development, and there were no permitted discharges (coal mining, oil/gas extraction, or sewage treatment plants). Non-reference, or test sites, were chosen to span a range of observed human impacts to the watershed, stream, or individual reach.

In 2000, we sampled 43 sites (25 reference, 18 non-reference, or test) scattered throughout the Kentucky portion of ecoregions 68 (Southwestern Appalachians), 69 (Central Appalachians), and 70

(Western Allegheny Plateau), collectively known as the Eastern Coalfield Region. Another 12 sites (9 reference, 3 test) were sampled in spring 2001, and 10 sites (6 reference, 4 test) were sampled in spring 2002 for validation purposes. Data from three historical sample events (two 1998 and 1999 reference sites) were also used as validation sites.

Landuse within the Eastern Coalfield Region is dominated by silviculture, mining, oil/gas extraction, and residential development. All reference streams were located in highly forested, undisturbed areas, whereas test sites ranged from slightly to severely impacted by regional landuses. Although the selected sites had catchment areas ranging from 50 to 880 ha (0.18 to 3.4 mi^2), reference and test streams did not differ significantly in mean catchment area, riffle substrate size, stream width, elevation, slope, and distance-to-source (Mann-Whitney, $p>0.1$). In contrast, reference and test streams differed significantly in mean riffle embeddedness, riparian width, canopy score, pH, conductivity, and temperature ($p< 0.01$). Both stepwise discriminant function analysis (DFA) and principal components analysis (PCA) showed that conductivity, riparian width, canopy, and embeddedness best separated reference and test sites. In addition, EPA RBP habitat scores successfully distinguished reference from test sites.

Macroinvertebrates were collected with both semi-quantitative (composite of 4-0.25 m^2 kicknets) and multi-habitat qualitative techniques. Approximately 40,000 specimens representing more than 330 taxa from 75 families were collected from all sites combined. Multivariate ordination of reference sites using nonmetric multidimensional scaling (NMDS) showed no evident patterns in taxonomic composition with respect to geographic location. Another multivariate technique (Canonical Correspondence Analysis) clearly separated most test sites from reference sites based on genus-level abundances, indicating that taxonomic structure was considerably modified at test sites, and measures of conductivity, riparian zone width, canopy cover, embeddedness, and RBP habitat scores accounted for this variation.

Thirty-three (33) macroinvertebrate biological attributes (metrics) were calculated and evaluated for discrimination efficiency, sensitivity, redundancy, and variability. Effort was given to include metrics covering a wide scope of ecological attributes (e.g., structure, tolerance, habit, and function). The evaluation process selected seven metrics (taxa richness, EPT richness, mHBI, m%EPT, %Ephemeroptera, %Chironomidae+Oligochaeta, and %Clingers) for use in the Macroinvertebrate Bioassessment Index (MBI).

Three metric scoring methods were also evaluated for discriminatory power and simplicity of calculation: (1) the 25^{th} %ile of the reference distribution, (2) quadrisection of metric values below the 95^{th} %ile for all sites, and (3) percent of standard (95^{th} %ile, 100-point scale) for all sites. All scoring methods were considered to be equally robust. The 100-point percent-of-standard scale was chosen for use in the MBI because of its ease of use and interpretation. Narrative ratings were assigned using the median (Excellent), 10^{th} percentile (Good), and trisection (Fair, Poor, and Very Poor) of the reference distribution below the 10^{th} percentile.

Correlation analysis and linear regression were used to evaluate the response of the MBI to habitat and human disturbance. A moderately strong correlation (r = 0.65, p<0.0001) was seen with the MBI and habitat assessment scores, and an even stronger relationship (r = 0.81, p<0.0001) was found when comparing the MBI to a perceived human disturbance gradient identified by the 1st PCA axis. These analyses showed that the MBI responded negatively to increasing disturbance and was thus useful in distinguishing a range of impairment.

This index will be used to assess headwater streams for point and nonpoint source impacts, 305(b) use assessments, or to identify new high quality streams in need of protection as Exceptional Waters of the Commonwealth (401 KAR 5:030 Section 1 (Implementation of Antidegradation Policy)). In order for the MBI scores to be effective, adherence to sampling procedures and sample index period is important. Recommended time frames for sampling headwater streams ranges from mid-February to June. Samples collected before or after these dates may give inaccurate results and caution should be used when interpreting that data. In some cases (e.g., due to natural or investigator variability), best professional judgement or re-sampling may be warranted if index scores fall close to narrative-rating cutoffs.

A comparative study on the potential use of family-level taxonomy was also done. A 5-metric family-level MBI (F-MBI) was highly correlated (r^2 = 0.93, p<0.0001) with the genus/species MBI. This modified index uses family taxa richness, family EPT richness, family HBI, %Ephemeroptera, and %Chironomidae + %Oligochaeta. Although family-level taxonomy would reduce time, effort and the need for more highly skilled taxonomists, a reduction in sensitivity of the MBI was detected. The use of the F-MBI is therefore recommended in headwater streams as a quick screening tool to delineate obvious impairment from the reference condition and to be used by non-KDOW personnel (e.g., volunteer Watershed Watch participants, private consultants, university students) that may lack adequate taxonomic skills. At this time, the F-MBI is not recommended for monitoring associated with permit compliance, enforcement cases, or to be used in larger, wadeable streams (e.g., 4th or 5th order) where diversity within individual families is much greater.

1.0 Introduction

Determining the ecological health of streams is a major focus of the various aquatic-monitoring programs in the Kentucky Division of Water (KDOW). This effort is mandated by the U.S. Environmental Protection Agency (EPA) under the Clean Water Act (CWA) and integrates the collection of physical, chemical, and biological elements to assess water pollution. Since the turn of the century, aquatic organisms have been used extensively in water quality monitoring and impact assessment (reviewed by Cairns and Pratt 1993), and macroinvertebrate assemblages have proven to be useful in detecting even subtle changes in habitat and water quality. To accurately characterize patterns of stream degradation, impact assessment procedures must be based on sound ecological principles and the ability to feasibly measure the response of a macroinvertebrate community to disturbance.

To address levels of impact to any given stream, a firm understanding of the inherent biological variability and natural potential of streams in a collective region is necessary. This is accomplished using a regional reference approach (Hughes 1995) that is based on the range of conditions found in a population of sites or streams with similar physical characteristics and minimal human impact. Many federal, state, and tribal agencies have used ecoregions (Omernik 1987) as a convenient, stratified means to understand regional differences in biological potential among waterbodies within their jurisdiction. The Reference Reach Program in the KDOW Water Quality Branch was initiated in 1991 to collect and analyze data from least-disturbed streams using an ecoregional framework; however, until now, data collection has focused primarily on larger, wadeable streams.

The reference condition collectively refers to the range of quantifiable ecological elements (i.e., chemistry, habitat, and biology) that are found in natural environments. In many regions of Kentucky, finding reference streams can be a difficult task because few regions are without areas of human disturbance. However, in small forested catchments in the mountainous area of the state, reference sites can be found with a relatively high level of confidence. The application of the reference condition involves its comparison to a stream reach exposed to environmental stress using defined sampling methodology and assessment criteria. Impairment of the test site would be detected if indicator measurements (e.g., species richness, habitat rating, nutrient concentrations) fall outside the range of threshold criteria established by the reference condition.

With this goal in mind, our intent was to numerically define reference conditions and document levels of water quality impairment in small, often intermittent, headwater stream reaches in the Eastern Coalfield Region of Kentucky. Small streams in this region are generally depicted as 1st or 2nd order streams on 7.5 minute USGS topographical maps (1:24,000 scale). With regard to biological integrity, this region has not been thoroughly assessed despite the CWA and regulatory actions associated with the Federal Surface Mining and Reclamation Control Act of 1977, Kentucky's 1998 Forest Conversation Act, and Kentucky's 1994 Agriculture Water Quality Act. Although these intermittent streams often match the species composition of perennial streams (Delucchi 1988, Feminella 1996), they receive little attention with regard to land management and regulatory policy.

Headwater streams, as defined in this document, are 1st or 2nd order streams as depicted on 1:24,000 scale topographic maps and are generally < 3 to 5 mi^2 (~ 8 to 13 km^2) in drainage area. These streams (either intermittent or perennial) and their ephemeral tributaries serve multiple functions often overlooked in environmental planning and landuse decision-making. They are the key interface between the surrounding landscape and larger waterbodies. Healthy headwater streams provide habitat to relatively distinct and diverse invertebrate assemblages, and by assimilating nutrients, organic matter, and sediments, they export high quality water in the form of goods and services (e.g., water supply, recreation, waste assimilation, flood control, and ecological values) (Yoder et al. 2000, Wallace and Meyer 2001). These streams are also closely connected to groundwater resources and provide thermal refuges to many organisms in both winter and summer. Despite possessing these attributes, little is known about the biological potential of small headwater streams in Kentucky.

The objectives of the study were to sample macroinvertebrate assemblages from 1st and 2nd order streams in the Eastern Coalfield Region using a standardized protocol and to develop an index of biotic integrity, the Macroinvertebrate Bioassessment Index (MBI), based on a multimetric approach (Karr et al. 1986, Gerritsen 1995, Barbour et al. 1999). The index would then accurately rank the quality of stream reaches affected by regional stressors such as mining, silviculture, residential and commercial development, or road and bridge construction. It would also identify those high quality or "Exceptional Waters" deserving regulatory protection under Kentucky's anti-degradation regulations (401 KAR 5:030 Section 1).

2.0 Study Area

The study region includes parts of the Central Appalachian (CA), Southwestern Appalachian (SA), and Western Allegheny Plateau (WA) Level III ecoregions (Omernik 1987, USEPA 2000) in Kentucky (Figure 1). These ecoregions lie within the Eastern Coalfield Physiographic Province (Appalachian Plateaus Province) and are characterized by highly dissected terrain with similar forest types, geology, and climate. Bedrock geology is sedimentary and consists of interbedded sandstones, siltstones, shale, and coal. The dominant vegetation is part of the mixed mesophytic forest classification (Braun 1950). Headwater streams in this region typically flow through constrained valleys with relatively high gradients and have boulder-cobble substrates. Precipitation patterns are generally uniform throughout the study region; however in summer 1999, the summer prior to this study, the eastern Kentucky region reached extreme drought status (Drought Mitigation Center 2000). The regional drought of 1999 fell near the 5th %ile for normal annual precipitation with a recurrence interval of >20 yr (Institute for Water Resources 2001).

A series of reference and test sites were selected from six relatively separate geographic areas scattered throughout the Eastern Coalfield region (Figure 1). This was done to document taxonomic

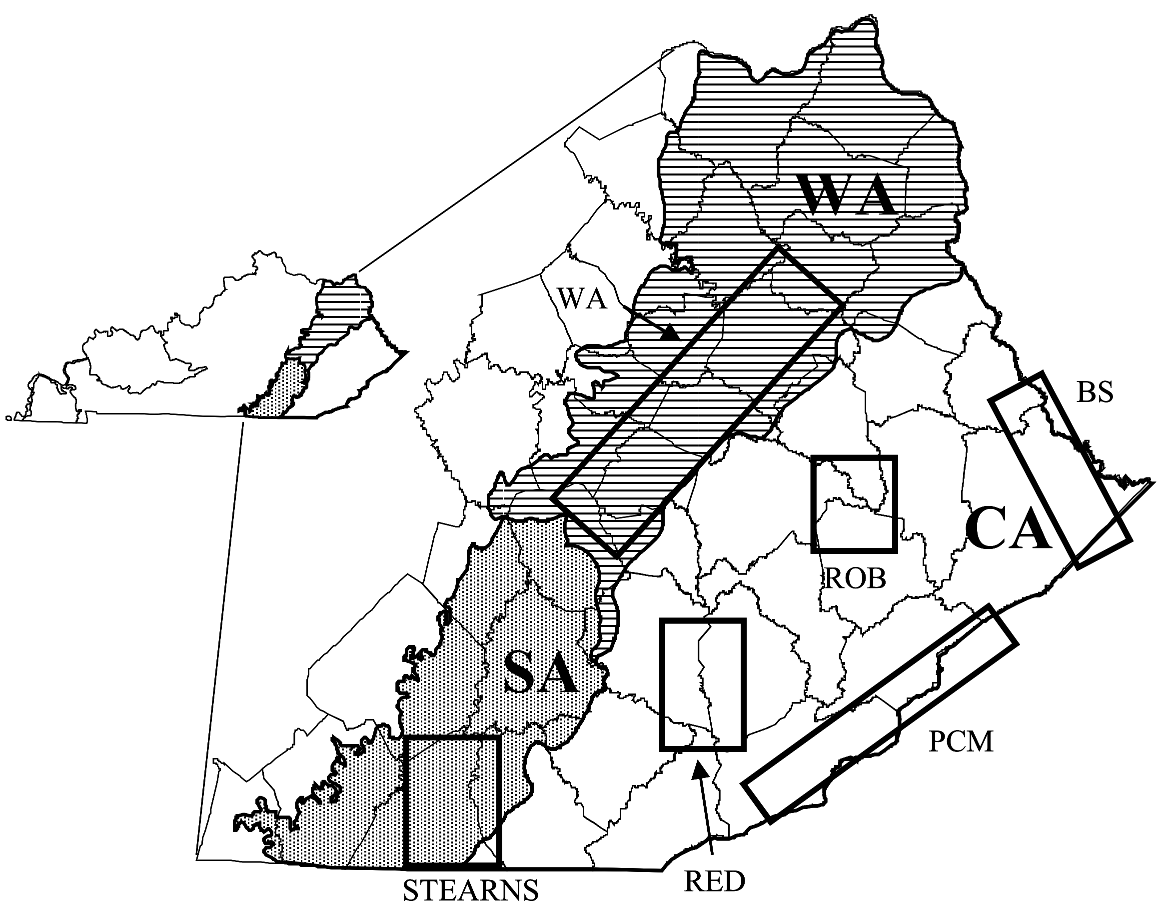


Figure 1. Generalized map of the 6 sampling areas showing ecoregions found within the Eastern Coalfield Region. CA=Central Appalachians, SA=Southwestern Appalachians, WA= Western Allegheny. See text for sampling area descriptions.

similarity, or dissimilarity, across the region. The actual selection of these areas conformed to both the availability of multiple reference sites and an intent to span the major river basins (Upper Cumberland, Kentucky, Licking, Big Sandy, Little Sandy). A previous KDOW study (Pond et al. 2000) showed that this region was taxonomically distinct from other physiographic regions in the Commonwealth and that the CA, SA, and WA ecoregions had the highest among-class similarity compared to other Kentucky ecoregions. For this reason, KDOW considers the Eastern Coalfields a relatively homogeneous region with regard to headwater stream macroinvertebrate communities.

Stream sizes (as drainage area) for all sites ranged from 0.18 to 3.4 mi^2 (Figure 2). Sites were chosen using GIS software (e.g., topographic maps, aerial photos, and landuse) and field reconnaissance. A reference site was determined adequate if it was primarily vegetated with relatively mature native forest, little or no residential development, and there were no permitted discharges (coal mining, oil/gas extraction, or sewage treatment plants). Non-reference, or test sites, were chosen to span a range of observed human impacts to the watershed, stream, or individual reach.

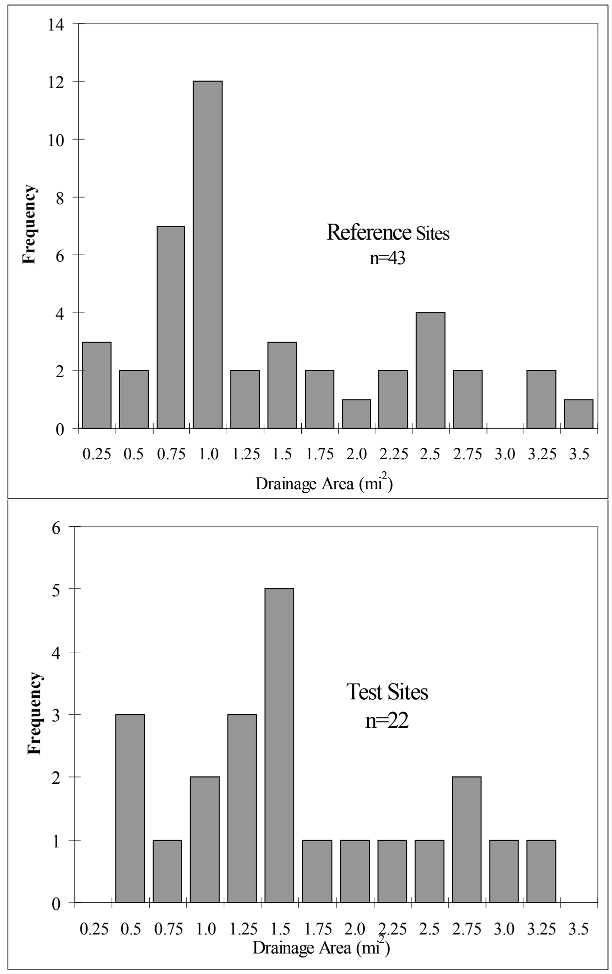


Figure 2. Distribution of reference and test sites by drainage area. Duplicate sample events excluded.

A calibration data set (from CA and SA ecoregions only) was collected in spring (March-April) 2000 from 25 reference streams located in highly forested watersheds with intact physical habitat and channel structure suggestive of least-disturbed conditions. An additional 18 test sites were sampled from streams that had subtle to obvious impacts ranging from channelization, sediment, nutrients, and loss of canopy and riparian vegetation. An independent validation data set was gathered in spring 2001 from 12 sites (9 reference, 3 test) and spring 2002 from 11 sites (7 reference, 4 test) in the CA, SA, and WA ecoregions. Two other sites were taken from 1998 and 1999 independent data sets (CA ecoregion). An attempt was made to include test sites ranging from what appeared to be slightly to heavily impacted. The total number of sites was biased toward reference sites since our primary goal was to document reference conditions.

Many of the sites were situated within the Daniel Boone National Forest (DBNF). The DBNF Stearns Ranger District (STRNS) area was located in the SA ecoregion whereas the DBNF Redbird

Ranger District (RED), Robinson Forest (ROB), Pine/Cumberland Mountain (PCM), Big Sandy (BS) areas, and other more scattered sites, were situated in the CA ecoregion. Additional reference sites in the WA ecoregion were also sampled. Site location information is shown in Appendix A.

3.0 Methods

3.1 *Physical Measurements*

A 100 m study reach was established for each site. At each of five transects spaced 20 m apart, riparian width and canopy cover, estimated bankfull width, and the two dominant substrate types (e.g., cobble-boulder, sand-gravel, bedrock-sand) were recorded. Canopy cover was scored on an ordinal scale (4=full, 2=partial, 0=open) and summed among transects (maximum score=20). We also recorded conductivity, pH, dissolved oxygen, and stream temperature with a portable Hydrolab meter (Hydrolab Corp., Austin, TX). Finally, habitat features were scored with the EPA Rapid Bioassessment Protocol (RBP) Habitat Assessment procedure following Barbour et al. (1999). This latter procedure qualitatively evaluates important habitat components such as epifaunal substrate quantity and quality, embeddedness, velocity/depth regimes, sediment deposition, channel flow status and channel alteration, stream bank stability, bank vegetation protection, and riparian zone width. Within individual benthic samples (see below), we estimated riffle embeddedness (i.e., the mean percent of cobble buried in fine sediment) and substrate size by removing the 5 largest stones found in each quadrat (n=20). An index of substratum size was determined by measuring the diagonal axis of individual stones where diagonal axis > length > width > depth. Substrate size estimation within individual samples was done to establish physical characteristics of the targeted riffle habitat and verify similarity among reference and test sites. Site distance-to-source, elevation, slope, and watershed size were determined from 7.5 minute USGS topographical maps (1:24,000 scale) using Arcview GIS software.

3.2 *Macroinvertebrate Communities*

Benthic invertebrates were collected during the spring index period (mid-February to late-May), as this period offers the highest potential for macroinvertebrate diversity and abundance in these small, headwater streams (Pond 2000, KDOW unpub. data). Moreover, samples collected in this season offer the maximum amount of information for assessment purposes in intermittent streams that may dry up in summer and fall seasons.

Quantitatively, macroinvertebrates were collected from four $0.25m^2$ quadrat kicknet samples (800 x 900 µm mesh) stratified within the thalweg (path of deepest thread of water) of cobble-boulder riffle habitat. Two sample events in the validation data set were collected with a composite of four Surber samples ($0.09\ m^2$ 800 x 900µm) that were stratified in a similar manner (Pond 2000). Riffle habitat was targeted to ensure the highest species richness and abundance of macroinvertebrates (Brown and Brussock 1991, Feminella 1996). The thalweg of a riffle also guarantees the most flow permanence and substrate stability in these often intermittent streams (pers. obs.). To reduce between-riffle variability, two kicknet samples were allocated to each of two distinct riffles separated by at least one pool or run. The four samples were composited into a 600 µm mesh bottom bucket to yield a 1 m^2 quantitative sample. The composited sample was partially field processed using a US #30 sieve (600 µm mesh) and wash bucket. Large stones, leaves, and sticks were individually rinsed and inspected for organisms and then discarded. Small stones and sediment were removed by elutriation using the wash bucket and US #30 sieve. Invertebrates were then picked from the remaining debris until approximately 1 pint or less of debris remained. This material was then preserved in 95% ethyl alcohol.

A **qualitative** composite sample of 3 leafpacks, 3 jabs in sticks/wood, 3 jabs in soft sediments, 3 jabs into undercut banks/submerged roots with an A-frame or D-frame dipnet (800 x 900 µm mesh), and hand-picking of 5 small pool boulders and approximately 2 linear meters of large woody debris was made (modified after Lenat 1988). All qualitative collections were made by the same investigator to reduce inter-observer variability. An effort was made to rinse, inspect, and discard leaves and sticks and sieve fine sediments so that 1 pint or less of material remained which was then preserved in 95% ethyl alcohol. A summary of these techniques is shown in Table 1. In the laboratory, all invertebrates were picked, identified to the lowest practicable taxon (usually genus/species), and enumerated (except qualitative sample). Chironomids were also identified to the genus/species level and oligochaetes to the family level.

Table 1. Summary of sampling methods for headwater, moderate/high gradient streams.

Technique	Sampling Device	Habitat	Replicates
1m^2 Kicknet*	Kick Seine/Mesh Bucket	Riffle	4-0.25m^2
Sweep Sample	Dipnet/Mesh Bucket	All Applicable	
Undercut Banks/Roots	Dipnet/Mesh Bucket		3
Sticks/Wood			3
Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
Silt,Sand, Fine Gravel	Dipnet/Mesh Bucket	Margins	3
Rock Pick	Forceps	Pool	5 sm. boulders
Wood Sample	Forceps/Mesh Bucket	Riffle-Run-Pool	2 linear m

* Sample contents kept separate from other habitats

4.0 Data Analysis

4.1 *Environmental Parameters*

Multivariate statistical procedures were used to identify a subset of environmental parameters that could distinguish *a priori* reference and test sites. This subset would also be used to evaluate MBI performance and offer insight into causes of stream impairment. To assure statistical normality, physical variables were transformed (log, sqrt, or arcsine), where appropriate, prior to entering them into a stepwise discriminant function analysis (DFA) and principal components analysis (PCA) (SYSTAT, Version 7.0, Evanston, Illinois). We also used box-and-whisker plots of all variables to look for discrimination on a more visual level, and tested whether variables were significantly different between reference and test sites using the nonparametric Mann-Whitney U-test.

4.2 *Macroinvertebrate Communities*

To test the hypothesis of regional taxonomic similarity, or homogeneity, we ordinated macroinvertebrate communities at reference sites using the Bray-Curtis dissimilarity index based on $\log_{10}$ abundance at the genus level, and subsequent nonmetric multidimensional scaling (NMDS) (Ludwig and Reynolds 1988). For these analyses, the genus-level of resolution was used to reduce the statistical variability sometimes inherent in species-level data (Maxted et al. 2000). In general, NMDS attempts to arrange objects or communities found at individual sites in a spatial orientation with a particular number of dimensions (two in our study) so as to reproduce the observed statistical distances. This allowed us to graphically identify either regional homogeneity, or geographic separation conforming to the *a priori* geographic designations shown in Figure 1.

We also ordinated all sites in a canonical correspondence analysis (CCA, ter Braak 1986), which combines transformed taxa-site data (correspondence analysis) and environmental-site data (weighted multiple regression) within one algorithm. All physical variables listed in Section 3.1 were used for this analysis. In a two-dimensional plot, taxonomic and site data are produced as points while environmental data are plotted as vectors. Vector length and direction are proportional to the statistical contribution of the variable to the ordination. This technique would provide insight into taxonomic shifts in relation to environmental differences among sites.

4.3 *Metric Selection*

Thirty-three (33) biological attributes, or metrics (Table 2), were evaluated for discrimination efficiency (DE), redundancy, variability, and sensitivity. These metrics spanned a broad range of community ecology including richness, composition, structure, tolerance, habit, and trophic, or functional feeding groups (Barbour et al. 1999). Richness metrics were calculated from both quantitative and qualitative collections combined, whereas all other metrics were calculated using the quantitative riffle samples only. **Discrimination Efficiency** was determined as the percent of the test site metric values (calibration data set) that fell below the 25th %ile or 75th %ile (depending on metric direction) of the reference distribution for a particular metric. Metrics having greater than 50% DE were initially retained for further evaluation (Maxted et al. 2000). **Redundancy** was determined for reference metric values with Pearson correlation coefficients. A high correlation ($r>0.80$, $p<0.05$) between metric pairs would suggest redundancy and be grounds for rejection of one of the metrics. **Variability** of reference metrics was assessed using the interquartile coefficient, which was calculated as the interquartile range (i.e., 75th %ile to 25th %ile) divided by the lower quartile (or upper quartile for negative metrics). This is analogous to the coefficient of variation and a value >1.0 would indicate high variability and thus be unfavorable. For **sensitivity**, or the ability of a metric to discriminate reference and test sites, we used a scoring system based on box-and-whisker plots after Barbour et al. (1996) shown in Figure 3. We considered metrics that scored a 2 or 3 to be sensitive and thus useful for the aggregate index.

Table 2. Candidate metrics, abbreviations, and expected response to disturbance.

METRIC	Abbeviation	Response
No. of Intolerant Taxa[1]	IntolTax	Decrease
No. of Clinger Taxa[2]	ClngTax	Decrease
Rel. Abun. of Clingers	%Clingers	Decrease
Modified Hilsenhoff Biotic Index[3]	mHBI	Increase
TotalTaxa Richness	TR	Decrease
No. of Plecoptera Taxa	PlecoTax	Decrease
No. of Trichoptera Taxa	TrichTax	Decrease
No. of Ephemeroptera Taxa	EphemTax	Decrease
No. of Ephemeroptera+Plecoptera+Trichoptera	EPT	Decrease
Rel. Abun. of Chironomidae	%Chiro	Increase
Rel. Abun. Of Chironomidae+Oligochaeta	%Chir+Olig	Increase
Rel. Abun. Of Ephemeroptera	%Ephem	Decrease
Rel. Abun. Of Tolerants[4]	%Toler	Increase
Proportion of 5 Dominant Taxa	$\%DOM_5$	Increase
Rel. Abun. Of Tanytarsini	%Tany	Decrease
Rel. Abun. Of Hydropsychidae	%Hydro	Increase
Rel. Abun. Of Scrapers[5]	%Scrapers	Decrease
Ratio of EPT/ Chironomidae+Oligochaeta	EPT/C+O	Decrease
Total Individuals	TotInd	Variable
Rel. Abun. Of EPT	%EPT	Decrease
Rel. Abun. Of EPT (minus *Cheumatopsyche*)	m%EPT	Decrease
Rel. Abun. Of Trichoptera	%Trich	Variable
Rel. Abun. Of Diptera	%Dip	Increase
No. of Chironomidae Taxa	ChiroTax	Increase
Rel. Abun. Of Plecotera	%Pleco	Decrease
Rel. Abun. Of Oligochaeta	%Oligo	Increase
Rel. Abun. Of Collector-Gatherers[5]	%Cllct	Variable
Rel. Abun. Of Shredders[5]	%Shred	Decrease
Shannon Diversity	Diversity	Decrease
Rel. Abun. Filter Feeders[5]	%Filtr	Variable
Rel. Abun. Of Dominant Taxon	%1Dom	Decrease
Rel. Abun. Of Baetidae	%Baetid	Increase
No. of Diptera Taxa	DipTax	Variable

[1]Based on tolerance values <3.0
[2]Based on habit designations in Merritt and Cummins (1996)
[3]Based on tolerance values provided in Lenat (1993), Hilsenhoff (1988), and KDOW (unpub. data)
[4]Based on tolerance values >7.0
[5]Based on functional feeding groups designations in Merritt and Cummins (1996)

4.4 *Metric Scoring and Index Development*

Retained metrics were scored using 3 methods (Figure 4): (1) the 25th %ile (or 75th %ile depending on metric direction) of the reference values (Barbour et al. 1996), (2) a quadrisection of all (reference and test) site metric values below the 95th %ile (or 5th %ile) (DeShon 1995), and (3) the percent of standard method (95th or 5th %ile) for all sites. For the first method, we modified the traditional 5, 3, 1 scoring scheme (Karr et al. 1986, Barbour et al. 1996) after Maxted et al. (2000), so that a score of 6 was given to values falling at or above this criterion and was thus considered representative of reference conditions. Below the 25th %ile, metric values were bisected to yield scores of 3 (deviates from reference) and 0 (strongly deviates from reference). The second method quadrisected all calibration site (reference and test) metric values (below or above the 95th or 5th %iles, respectively) using a 6, 4, 2, 0 scoring scheme. All metric scores are then summed to yield the total index value, or MBI. These unitless and weighted scoring methods not only rate metrics by water quality, but also overcome the problem of normalization so that metrics using counts, proportions, and logarithmic functions can be compared uniformly when applied to the aggregate index. Finally, the percent-of-standard method used the entire range of metric values below the 95th %ile, scored them on a continual scale of 0−100 percent, and averaged all metric scores (Gerritsen et al. 2000). If a calculated metric scored over 100 (i.e., a value above the 95th %ile) then it was corrected to the maximum score of 100.

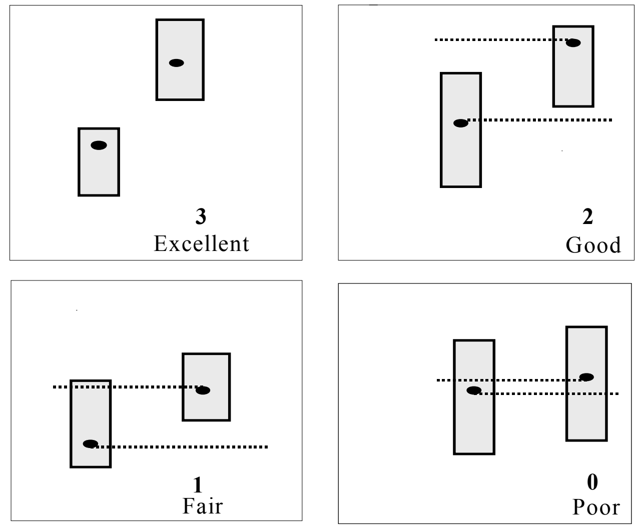


Figure 3. Hypothetical interquartile plots showing sensitivity, or discriminatory power scoring criteria (after Barbour et al 1996).

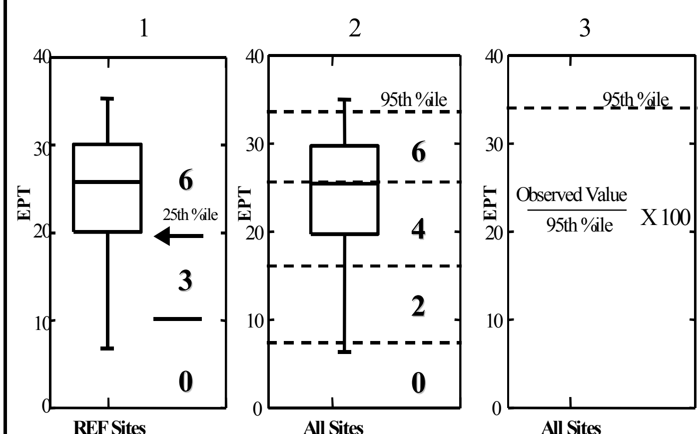
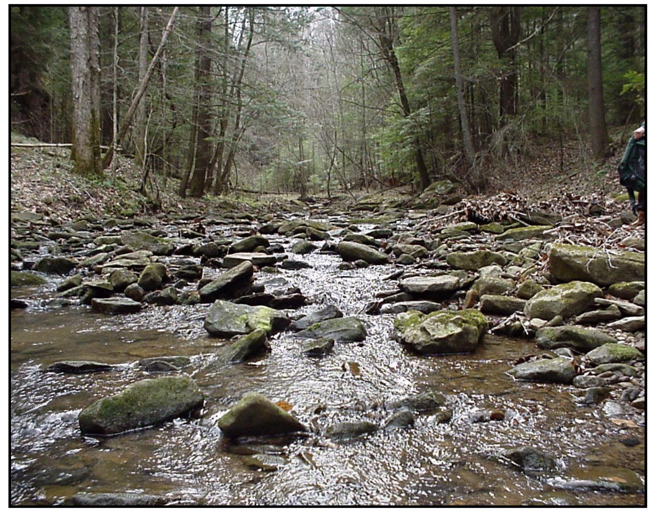


Figure 4. Metric Scoring schemes used to evaluate the MBI. EPT example shown.

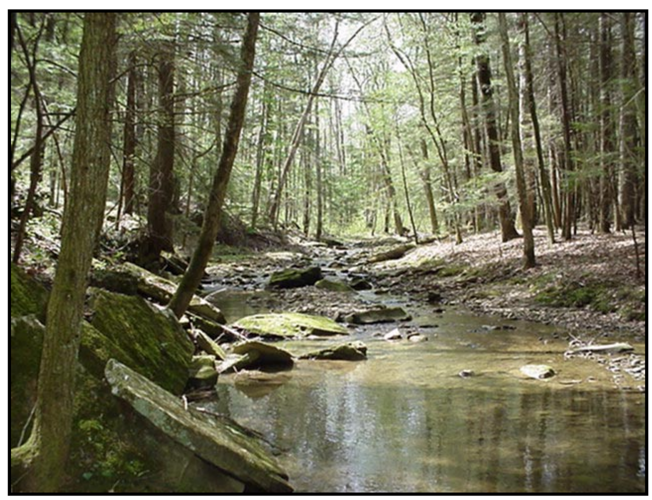
Initially, metric and MBI scoring criteria were established with the calibration dataset. Narrative ratings using the thresholds excellent (median of the reference data), good (10th %ile of reference data), fair (2/3 of the 10th %ile value of reference data), poor (1/3 of the 10th %ile value of reference data), and very poor (below 1/3 of the 10th %ile value of reference data), were also calculated. A check on the discrimination efficiency of each MBI scoring method was done by calculating the percent of the validation reference and test site MBI scores that fell below the 10th %ile of the reference condition.

UT to Big South Fork (Stearns Ranger District)



Right Fork Big Double Creek (Redbird Ranger District)

Falling Rock Branch (UK Robinson Forest)



Steer Fork (Western Allegheny Ecoregion)

Representative headwater reference streams with sampling areas denoted. See Figure 1 for area locations.

5.0 Results

5.1 *Environmental Parameters*

We were interested in which environmental variables could distinguish between reference and test sites. Ideally, one would assume variables like catchment area, elevation, slope, latitude, longitude, and riffle substrate size to be similar between reference and test sites within the study area based on our study design. However, other variables known to change with the degree of impairment were a greater concern. PCA factor 1 (Table 3) accounted for nearly 35% of the total variance of the calibration data set, while axis 2 accounted for 16%. Variables with the highest factor loadings on the 1st axis were conductivity, total habitat score, pH, mean embeddedness, mean riparian width, and canopy cover score. Canopy cover and riparian width were slightly autocorrelated as were pH and conductivity. Factor 2 suggested a less significant stream size gradient and showed that stream width and catchment area were the most important; however, these variables were also highly correlated with one another.

Table 3. Correlations of transformed environmental variables on the first 2 PCA axes.

	Factor1	Factor2
% Variance	35%	16%
logConductivity (mS/cm)	0.903	0.083
logTotal Habitat Score	-0.859	-0.208
pH (S.U.)	0.796	-0.149
arcsine Embeddedness (%)	0.765	0.111
logRiparianWidth (m)	-0.638	-0.387
logCanopy Score*	-0.619	-0.478
Latitude (dec. deg.)	0.449	-0.572
logTemperature (C)	0.422	-0.252
logStream Width (m)	-0.361	0.5703
logDissolved Oxygen (mg/L)	0.356	0.003
Longitude (dec. deg.)	0.329	-0.448
logElevation (m)	-0.306	-0.313
logSubstrate Size (cm)	-0.291	0.569
logCatchment Area (ha)	0.175	0.675

* Canopy score based on 0=open, 2=partial, 4=full per transect

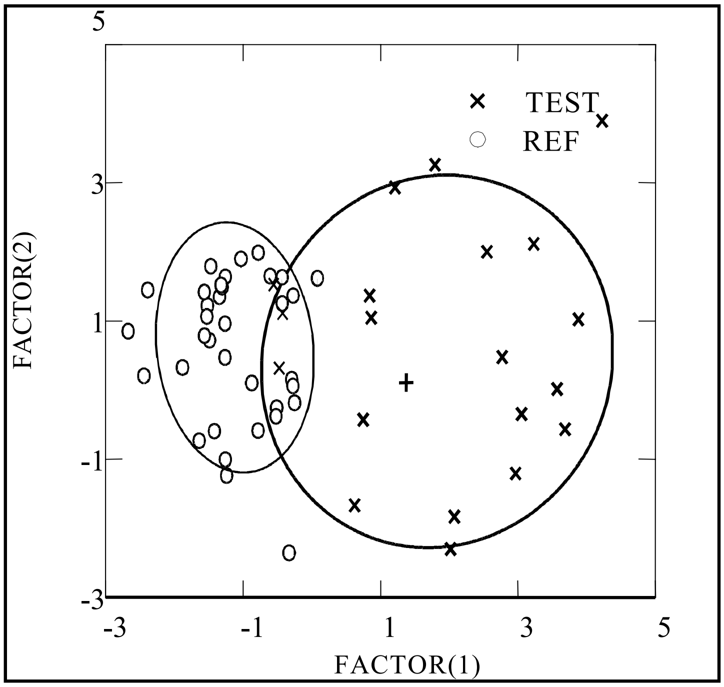


Figure 5. Discriminant root scores using %embeddedness, conductivity, canopy, and total habitat score (2000-2001 data).

The stepwise DFA model chose 4 variables: %embeddedness (*F*=3.47), canopy score (*F*=7.65), conductivity (*F*=12.02), and total habitat score (*F*=1.76) that classified the 43 *a priori* reference and test sites with 98% accuracy (Figure 5). An internal jackknife test of the data also classified the sites with only a 6% misclassification rate. Overall, the 4-variable discriminant model was highly significant (Wilk's λ= 0.256, *F*= 37.05, p<0.0001).

Nonparametric univariate tests (Mann-Whitney U) and box-and-whisker plots showed similar trends in that variables influenced by disturbance were significantly different (p<0.01), whereas physical variables unassociated with disturbance were not (Figure 6). For the 2000 calibration data set, the PCA and DFA identified similar variables that were important to reference and test sites. Using the 4-variable discriminant model, we found that the 22 validation events (2001-2002) were classified with only an 8% misclassification rate. Environmental variables for all sites are listed in Appendix B. Habitat assessment scores (discussed below) are provided in Appendix C.

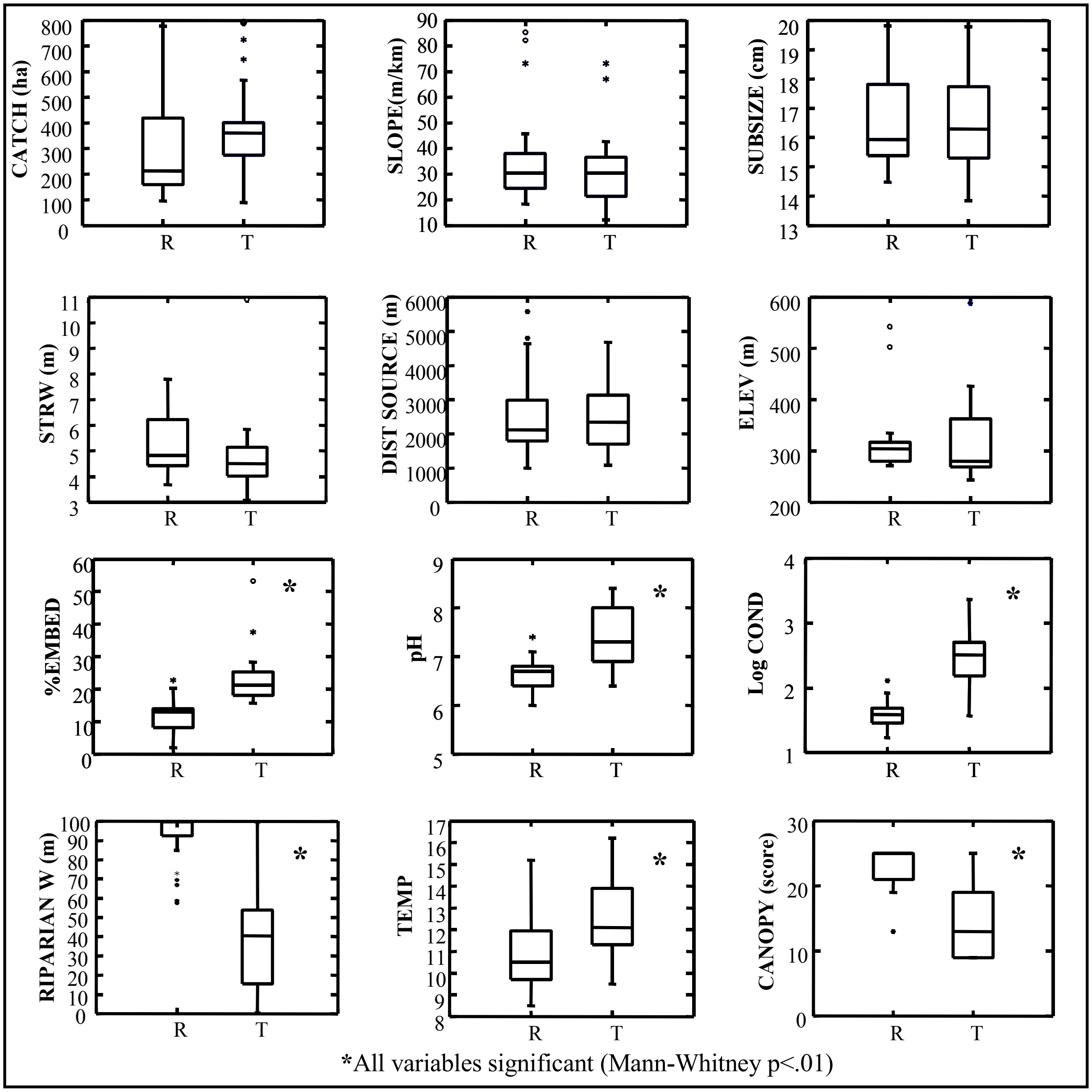


Figure 6. Box plots of selected environmental variables from reference and test sites (2000 calibration data).

5.2 *Macroinvertebrate Communities*

More than 330 taxa from 75 families were identified from the combined calibration samples and validation data sets. Approximately 40,000 organisms were enumerated for the entire study, and a synoptic list is shown in Appendix D. Riffle kicknet samples averaged 512 (±92, 95% CI) organisms per site with a range of 66 to 1671. Members of the insect orders Ephemeroptera, Plecoptera, and Trichoptera, or EPT, were most numerous in both numbers of individuals and taxa richness in the calibration samples. Among these sites, EPT richness ranged from 5 to 36, and total taxon richness values ranged from 21 to 68 (genus/species level resolution). Most of the validation sites also fell within these ranges for EPT richness and total taxon richness.

No strong patterns suggesting geographic affinities of the reference assemblages using the six regions were found with NMDS (Figure 7). When examining whether the scatter of sites in the

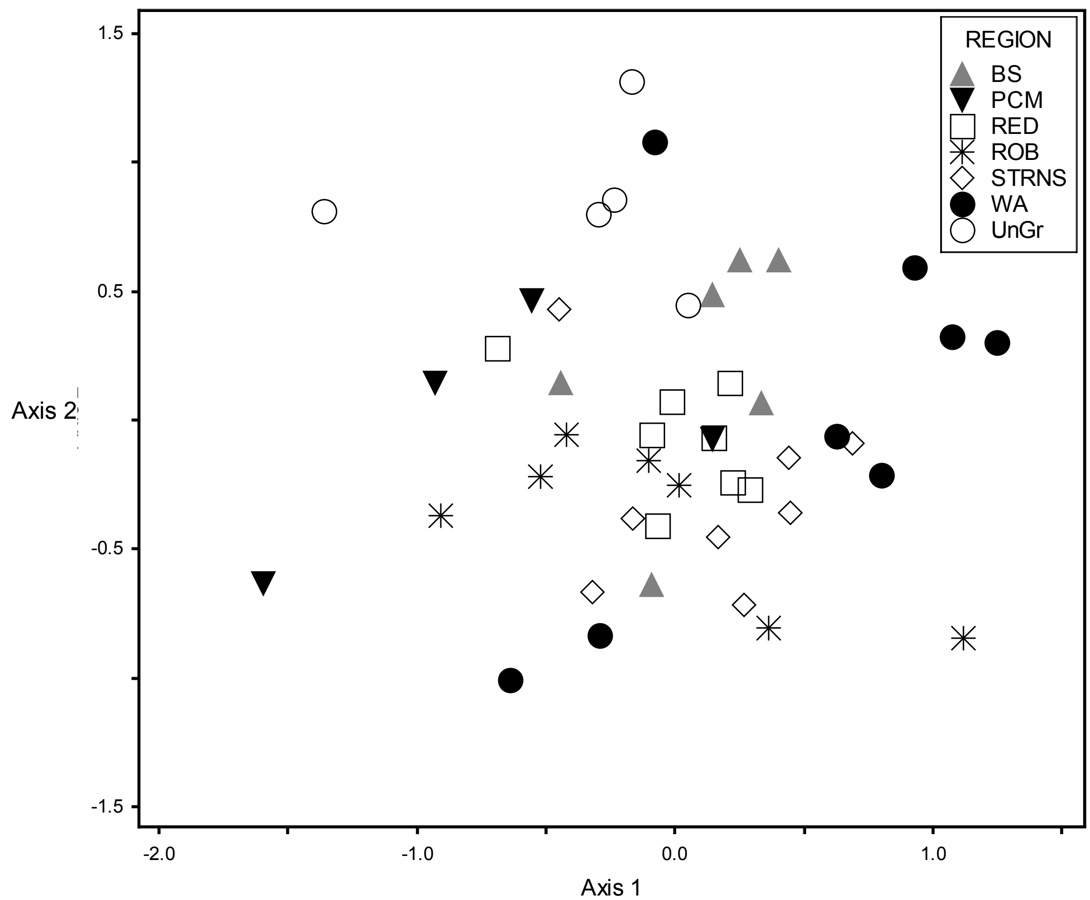


Figure 7. NMDS ordination (genus level) of all reference sites grouped by geographic region. ROB=Robinson Forest, RED=Redbird Ranger District, STRNS=Stearns Ranger District, PCM=Pine/Cumberland Mountains, BS=Big Sandy, WA=Western Allegheny and UnGr=Un- Grouped.

NMDS ordination could be correlated with any measured variable (Pearson correlation coefficient), we found pH and conductivity had the highest significant correlations (p>0.01) with Dimension 1 (r=0.51 and 0.55, respectively). Dimension 2 correlated best with slope and elevation area (r=0.50 and 0.33, respectively). We investigated this further in a separate analysis, where a stepwise DFA of only reference sites in which invertebrate assemblages (genus level) were grouped by UPGMA cluster analysis (flexible β = -0.1) with the Bray-Curtis index. This analysis indicated that elevation and catchment area contributed the most to site groupings (KDOW unpub. data). We chose to ignore this issue since (1) the DFA model's classification efficiency was low (44%, 31% jackknifed) and somewhat counterintuitive with respect to the clusters; (2) NMDS patterns were weak; and (3) because the metric selection and calibration process would likely inhibit any effects caused by slight differences in taxonomic structure of reference sites.

Another multivariate technique (CCA) revealed a disturbance gradient pattern with regard to genus-level taxonomic structure between reference and test sites. The CCA (Figure 8) confirmed the previous multivariate analyses by combining taxonomic and physical relationships into a single plot. The CCA revealed that test sites corresponded positively to environmental vectors along axis 1 (e.g., increasing %embeddedness, pH, and conductivity). An arrow's length is proportional to the variable's importance in the ordination.

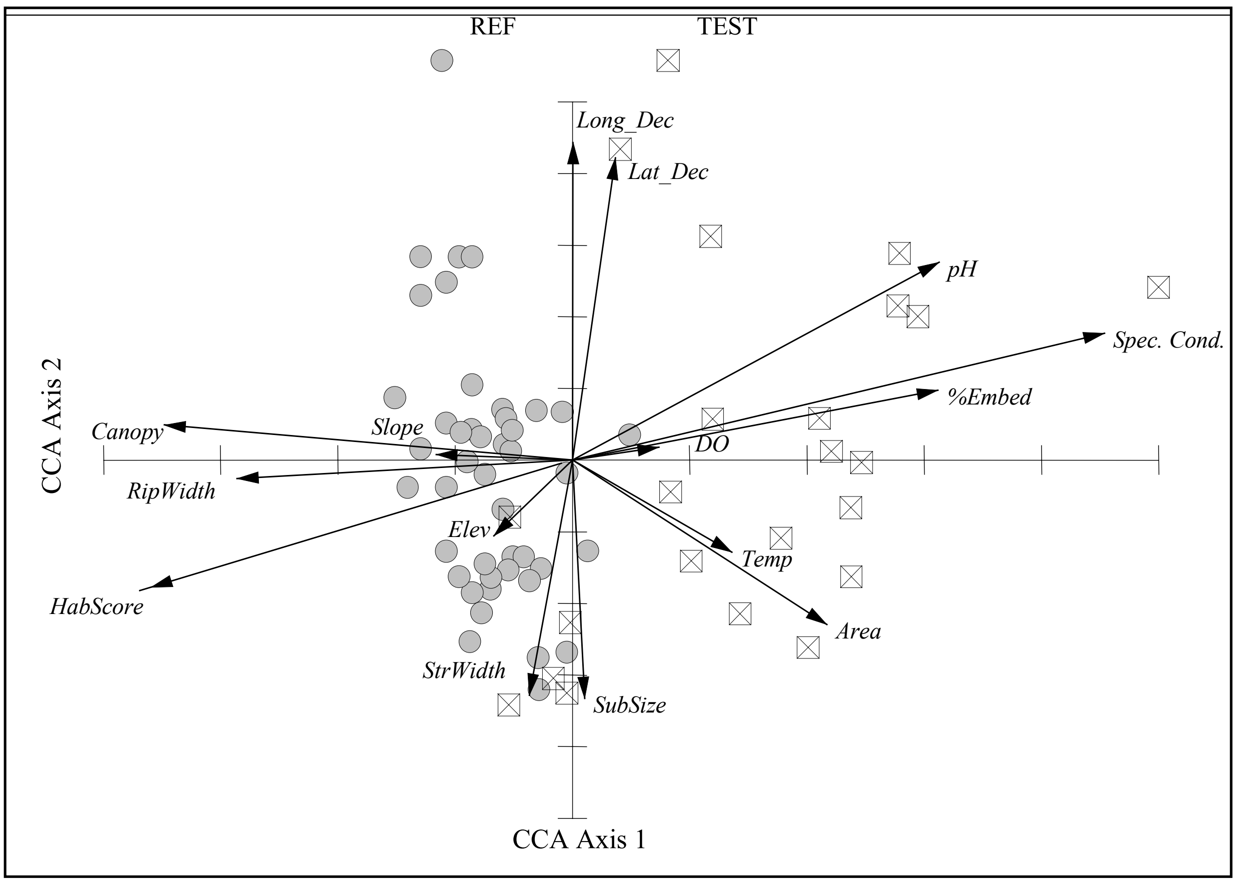


Figure 8. Canonical Correspondence Analysis (CCA) of all reference and test sites.

5.3 *Metric Performance*

Metrics were evaluated for various qualities that, when combined into an index, could distinguish site condition. Table 4 represents the list of metrics calculated from the 2000 calibration data set showing discrimination efficiency (DE), sensitivity (interquartile overlap), interquartile coefficient (variability), and redundancy. The DE ranged from 35%–94%, more than half the metrics had good to excellent sensitivity (score of 2 or 3), only three metrics showed high variability (interquartile coefficient > 1.0) and several metrics showed some redundancy. This process helped identify a suite of metrics that could distinguish site condition. Box-and-whisker plots of particular metrics that were rejected and retained are shown in Figures 9 and 10, respectively. Through a process of elimination and professional judgement, seven core metrics were chosen for the mountain headwater MBI (taxa richness, EPT richness, mHBI, %Ephemeroptera, %Clingers and %Chironomidae +Oligochaeta). Raw metric values for all sites are shown in Appendix E. A Pearson correlation matrix of the seven recommended metrics from reference sites is shown in Table 5. Only TR and EPT richness were calculated from a composite of the quantitative and qualitative samples. The mHBI and the % compositional metrics were calculated from quantitative samples only. The mHBI used the formula:

$$m\,HBI = \frac{\sum n_i\, x\, a_i}{N}$$

where: n_i = number of individuals within a species **(maximum of 25)**,
a_i = tolerance value of the species,
N = total number of organisms in the sample (**adjusted for $n_i \geq 25$**).

Table 4. Metrics evaluated for the MBI showing dicrimination efficiency (%DE), sensitivity score, interquartile coefficient (IQC), and redundancy, based on 2000 calibration dataset.

METRIC	DE	SENSITIVITY	IQC	REDUNDANT (r>0.80) WITH:
IntolTax	94.1	3	0.24	TR, EPT
ClngTax	94.1	3	0.18	TR, EPT, IntolTax
%Clingers	88.2	3	0.33	
HBI	88.2	3	0.15	%Tol
TR	88.2	3	0.24	IntolTax, DipTax, ClngTax, EPT
PlecoTax	88.2	3	0.29	
EPT	88.2	3	0.28	TR
TrichTax	82.4	3	0.22	
%mEPT	76.5	2	0.22	
%Chiro	76.5	2	0.71	%Ch+O
%Chir+Olig	76.5	3	0.71	%Chiro
%Ephem	70.6	2	0.37	
%Toler	70.6	3	0.58	HBI
%DOM5	70.6	2	0.18	Diversity
EphemTax	70.6	3	0.22	
%Hyro	70.6	2	0.75	%Trich
%Scrapers	64.7	3	0.91	
EPT/C+O	64.7	3	1.77	
TotInd	64.7	3	0.55	
%EPT	58.8	2	0.11	
%Trich	58.8	1	1.15	
%Dip	58.8	0	0.50	
ChiroTax	58.8	0	0.40	DipTax
%Pleco	52.9	0	0.90	%Shred
%Oligo	52.9	1	1.00	
%Cllct	52.9	2	0.37	
%Shred	52.9	0	0.77	%Pleco
Diversity	52.9	2	0.17	%DOM5
%Filtr	41.2	0	0.62	%Dip
%1Dom	41.2	0	0.38	
%Baetid	41.2	0	0.89	
DipTax	35.3	1	0.88	TR

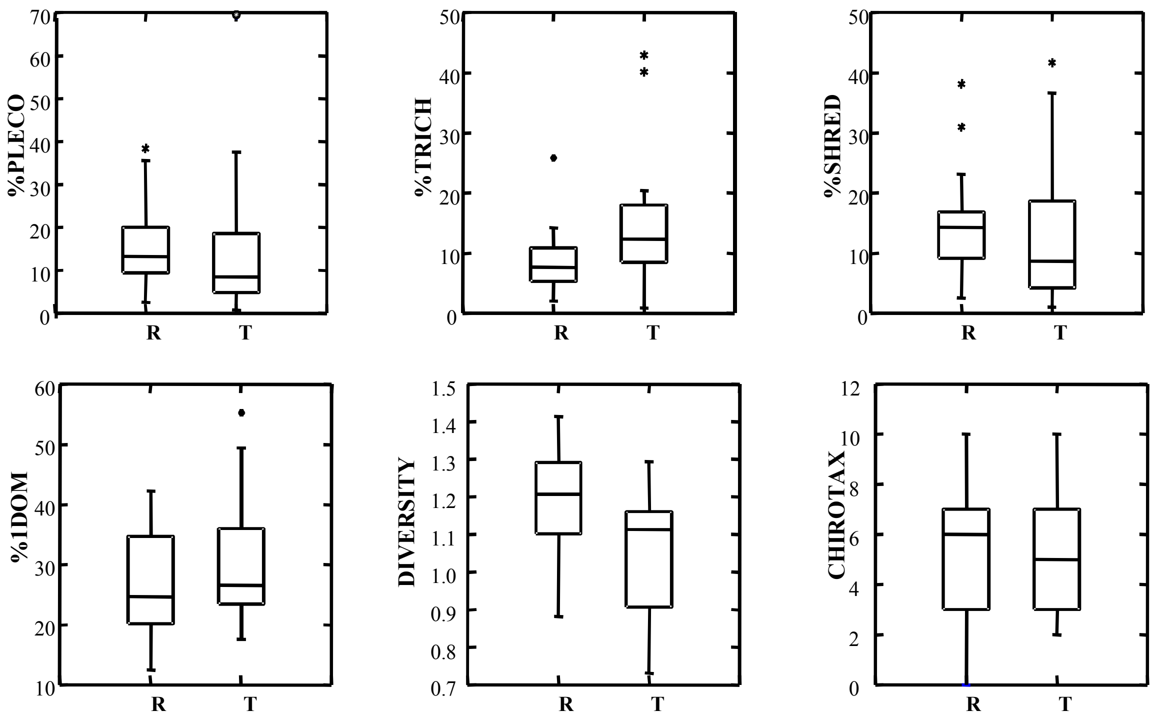


Figure 9. Box-and-whisker plots of selected macroinvertebrate metrics that were rejected for use in the MBI (from calibration data set).

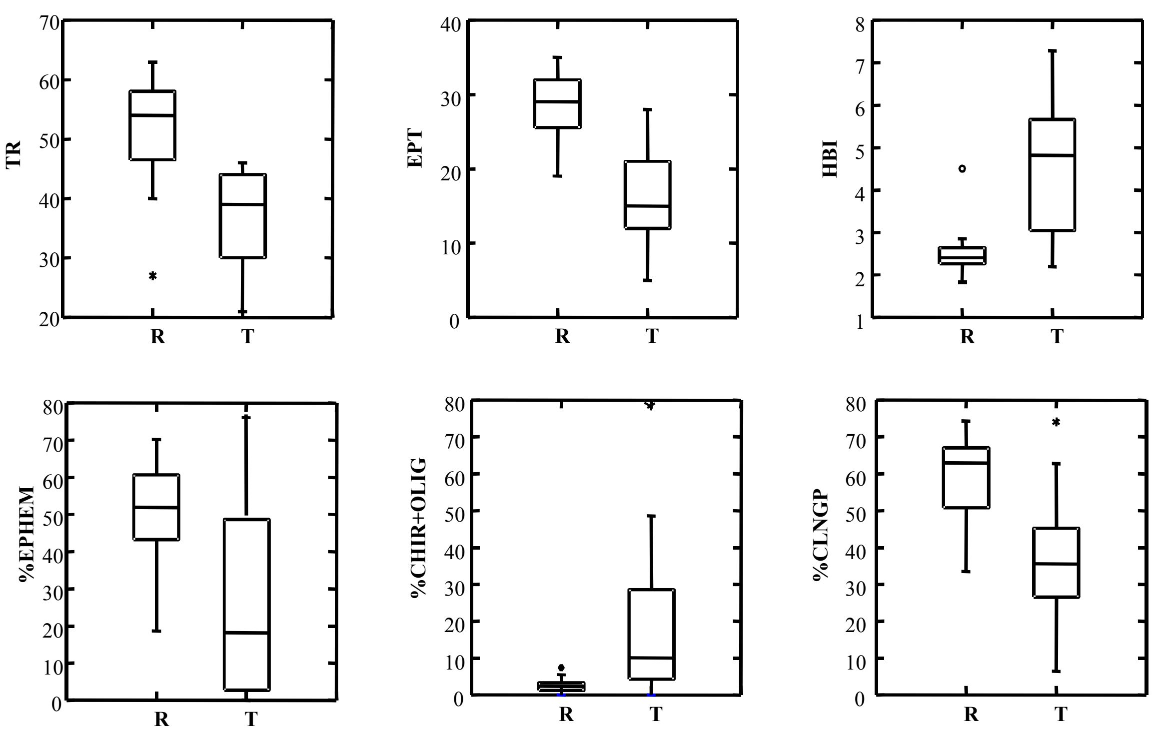


Figure 10. Box-and-whisker plots of selected macroinvertebrate metrics that were retained for use in the MBI (from calibration data set).

Table 5. Pearson correlation matrix for reference metrics used in the MBI.

	TR	***EPT***	***mHBI***	***m%EPT***	***%Ephem***	***%Chir+Olig***	***%Clng***
TR	----						
EPT	0.80	----					
mHBI	-0.22	-0.59	----				
m%EPT	0.12	0.42	-0.78	----			
%Ephem	0.07	0.34	-0.58	0.72	----		
%Chir+Olig	-0.16	-0.42	0.74	-0.74	-0.58	----	
%Clng	0.32	0.53	-0.50	0.34	0.58	-0.48	----

Taxa richness and EPT richness were also evaluated to look at potential influence of drainage area on metric values. A simple linear regression of all reference sites showed no effect of stream size on taxa richness (r^2 = 0.014, p= 0.486) and EPT richness (r^2=0.004, p=0.703). In fact, some of the smallest streams (e.g., <0.4 mi^2) had taxa and EPT richness values roughly equal to or greater than many streams over 2 mi^2 (Appendix E). We recognize that when comparing headwater streams to larger, wadeable systems, stream size can influence richness (KDOW unpub. data) but within the range of sites used in our study (0.18 to 3.38 mi^2), no influence was detected.

5.4 *Scoring Formulae*

The calculated 95th %iles (or 5th %iles) and appropriate scoring formulae for the seven metrics are shown in Table 6. An example MBI calculation is provided for Bear Branch. Figure 12 (Section 5.4) shows narrative rating cutoff points for assigning water quality classifications.

Table 6. Metric scoring formulae and example calculation for the MBI.

Metric	**95th or 5th %ile**	**Formula**	**Example for Bear Branch**	**Metric Score**
Genus TR	63	$\frac{TR}{95th\%ile}X100$	$\frac{42}{63}X100$	66.67
Genus EPT	33	$\frac{EPT}{95th\%ile}X100$	$\frac{17}{33}X100$	51.54
mHBI	2.18	$\frac{10-mHBI}{10-5th\%ile}X100$	$\frac{10-4.12}{10-2.18}X100$	75.19
m%EPT	86.9	$\frac{m\%EPT}{95th\%ile}X100$	$\frac{63.81}{86.9}X100$	73.43
%Ephem	66.5	$\frac{\%Ephem}{95th\%ile}X100$	$\frac{18.09}{66.5}X100$	27.2
%Chir+Olig	0.68	$\frac{100-\%Chir+Olig}{100-5th\%ile}X100$	$\frac{100-9.53}{100-0.68}X100$	91.09
%Clingers	75.5	$\frac{\%Clingers}{95th\%ile}X100$	$\frac{34.82}{75.5}X100$	46.12
			MBI (Average Score) =	**61.60**

5.5 *MBI Performance*

Summed index scores for the three scoring methods were evaluated using box-and-whisker plots of calibration reference and test sites. Results indicated the three MBI scoring methods all have excellent sensitivity (Figure 11). For simplicity of calculation and interpretation, we chose the 100-point, percent of the 95th %ile standard method for use in the MBI. Currently, this scoring method is under development with algal and fish community data at KDOW.

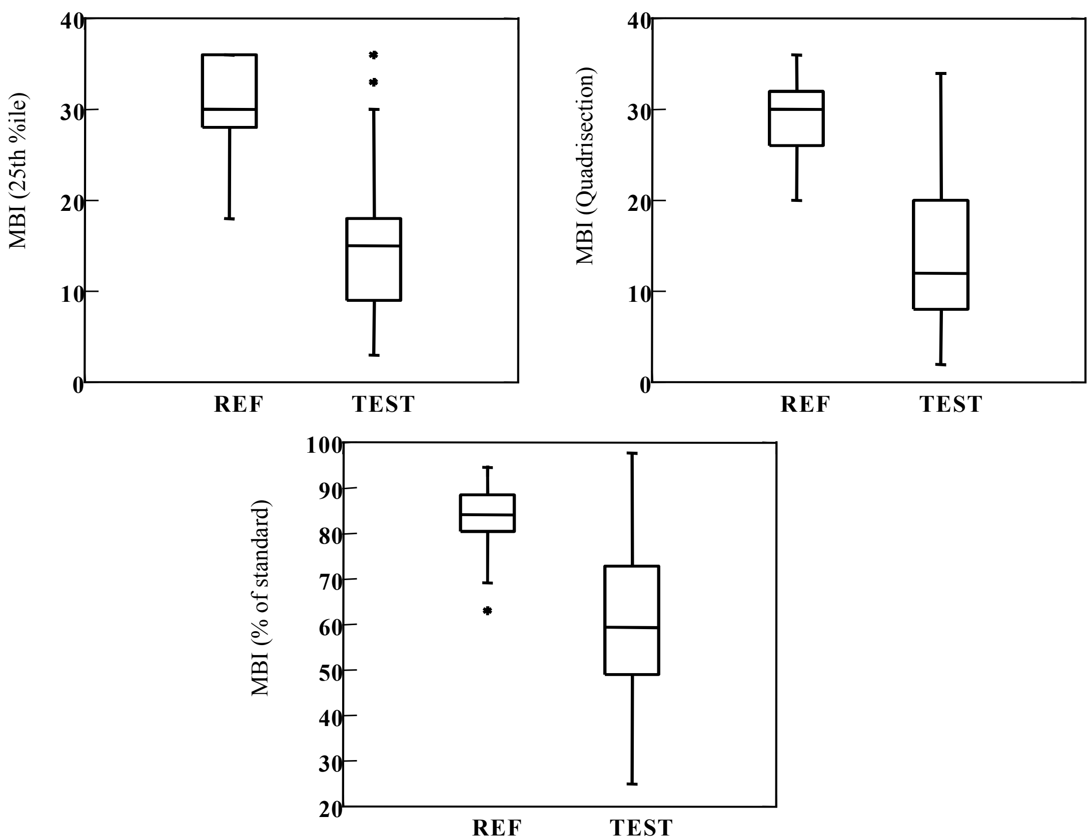


Figure 11. Comparison of MBI Scores from Reference and Test sites: 25%th ile, Quadrisection, 100-point % of Standard.

An "Excellent" rating was achieved when a site scored at or above the median of the reference MBI (≥83), while a "Good" rating fell between the median and 10th percentile (72–82). Below the 10th percentile, the reference MBI was further trisected to yield "Fair" (48–71), "Poor" (24–47), and "Very Poor" (<24) conditions. Figure 12 shows sensitivity of the MBI and narrative rating cut-off points. Although these criteria are arbitrary, we considered the cut-off points to be protective since reference sites were located within undisturbed watersheds. By assigning narrative water quality ratings we showed that 84% of the 2001 and 2002 validation sites were properly assigned to *a priori* designations. For both calibration and validation sites, the MBI correctly classified 84% of all test sites as impaired (Fair or Poor), and 91% of all reference sites as Good or Excellent.

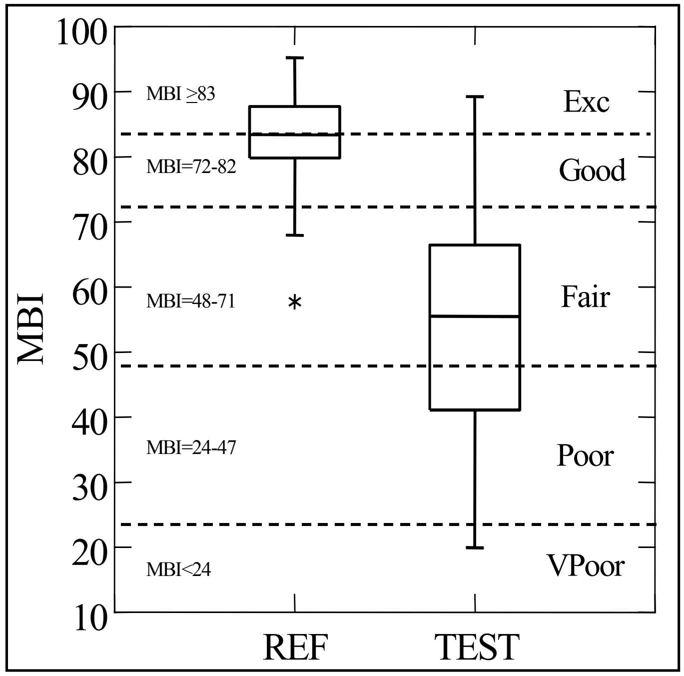


Figure 12. Box plot of MBI scores from reference and test sites (all sites) showing thresholds for narrative ratings.

Habitat assessment scores from all reference and test sites showed the excellent sensitivity of this assessment tool (Figure 13). The RBP habitat assessment scores for all sites were plotted against the MBI and are shown in Figure 14. A moderate relationship was found (r^2=0.53, n=57, p<0.00001) suggesting habitat quality was a good predictor of macroinvertebrate community health. In some cases where habitat was generally good at test sites, excessive conductivity, lack of canopy, or excess nutrients (inferred from excessive algal growth observations) were probable factors leading to low MBI scores. A Pearson correlation matrix of individual metric values and environmental and habitat variables is shown in Appendix F.

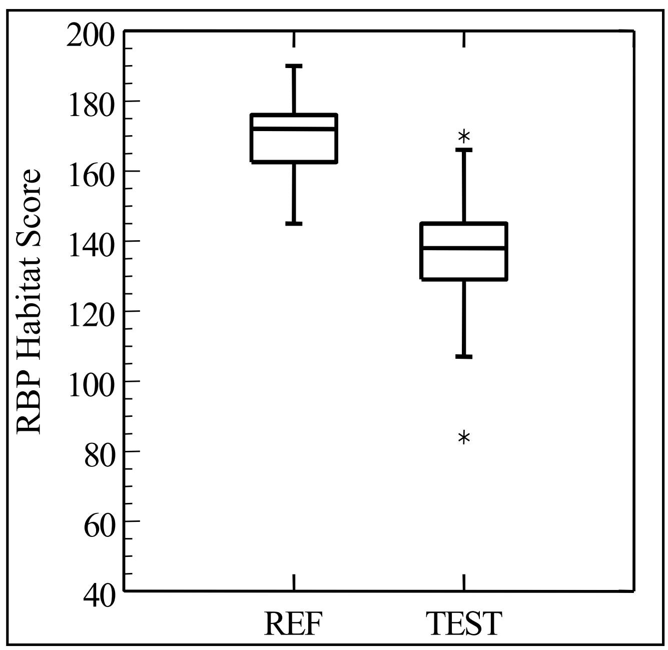


Figure 13. Box plot of RBP habitat scores from reference and test sites (2000-2002).

A further evaluation of MBI scores compared to perceived human disturbance was done using the PCA site scores for the first factor, or axis (Figure 15). This axis was most influenced by conductivity, pH, %embeddedness, total habitat score, riparian width, and canopy score (see Table 2). A strong relationship (r^2=0.65, p<.00001) indicated that the MBI responded negatively to increasing human disturbance.

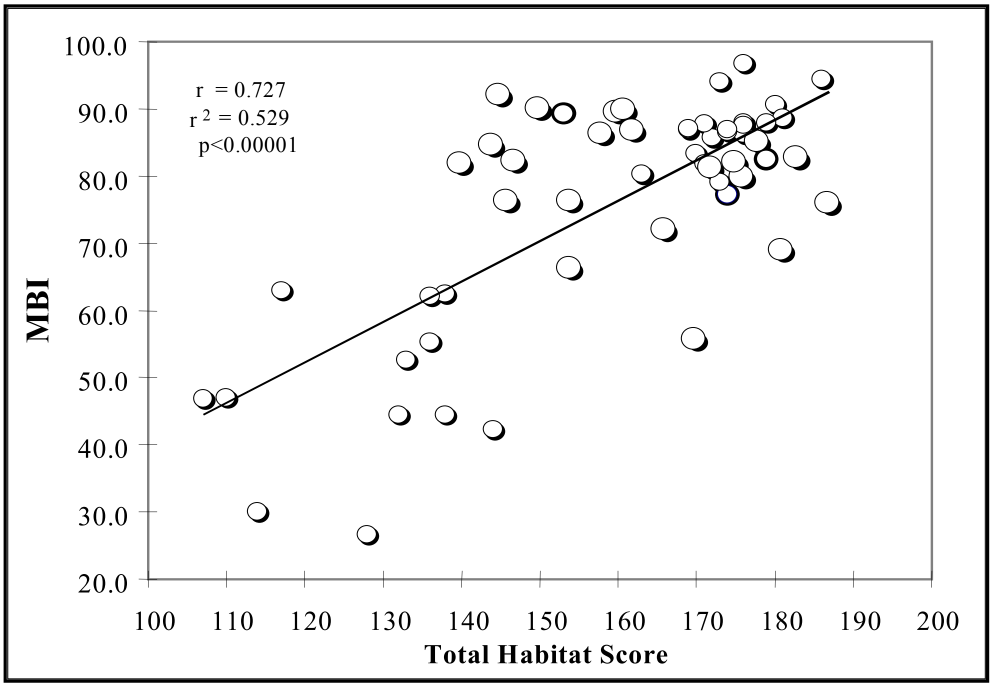


Figure 14. Relationship of MBI to Total Habitat Score (2000-2001 sites).

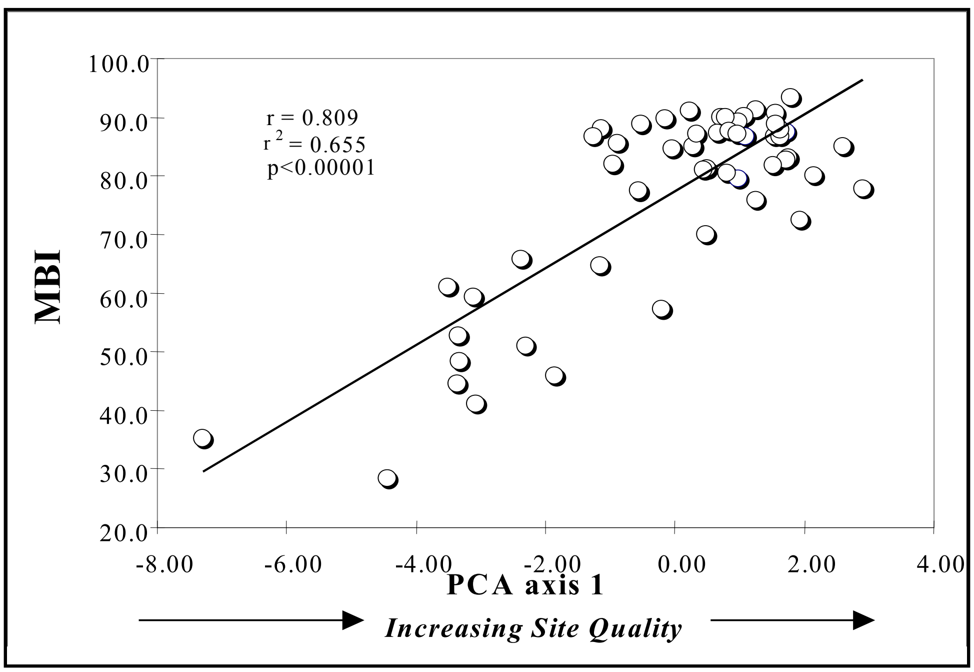


Figure 15. Relationship of the MBI to a perceived disturbance gradient as defined by PCA axis 1 (2000-2001 sites).

5.6 *Geographical Differences in MBI Scores*

While the NMDS taxonomic ordination showed minimal variability among the six *a priori* regions, there were outlier streams within the WA and PCM regions. Similarly, reference MBI scores from those regions were the lowest on average (Figure 16), suggesting differences in biotic potential. None of the reference sites in the PCM and only two in the WA scored in the excellent range. After careful inspection of metrics and environmental factors from the WA and PCM, it was apparent that %Ephem values were markedly reduced compared to other regions. %Ephem values averaged 17.9% and 36% in the PCM and WA, respectively; other regions combined averaged 52%. Mayfly richness did not significantly differ among all regions, but we suspect that relatively lower pH in some PCM and WA streams (5.1 to 6.1 range) was responsible for decreased mayfly densities. Feldman and Conner (1992) and Moeykens and Voshell (2002) also found reduced mayfly abundances in small mountain streams with lower pH. Until more data can be gathered to elucidate this phenomenon, we are confident that the MBI can be used in the entire Eastern Coalfield Region. Users of the index should be cautious when assessing streams in the PCM and WA areas. MBI scores in the BS, RED, ROB, STRNS regions were not significantly different.

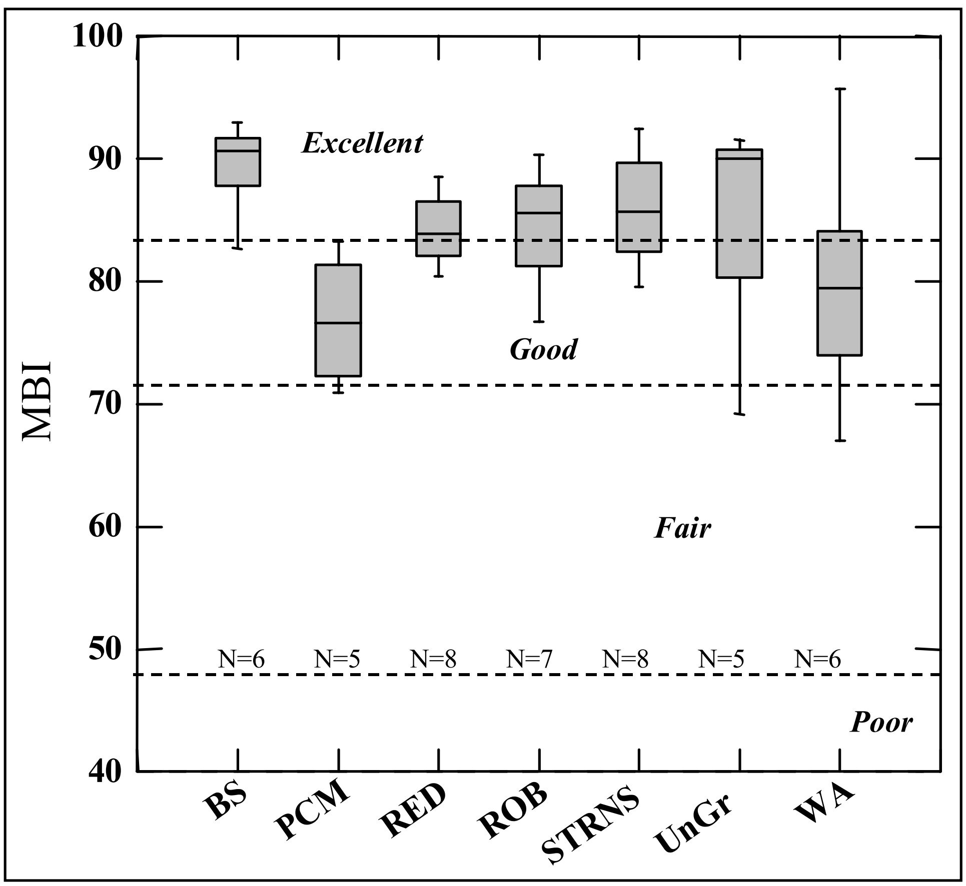


Figure 16. Box plot of reference MBI scores among study regions. Narrative ratings delineated by dashed lines. BS=Big Sandy, PCM=Pine/Cumberland Mountains, RED= Redbird District, ROB=Robinson Forest, STRNS=Stearns District, UnGr=UnGrouped, WA=Western Allegheny.

6.0 Discussion

6.1 *EnvironmentalParameters*

The regional variables modified by disturbance in this study are well-documented elsewhere in the literature (Branson and Batch 1972, Curtis 1973, Talak 1977, Dyer 1982, Sweeney 1993, Green et al. 2000). We were not surprised that conductivity and % embeddedness were the most significant factors in discriminating reference and test sites. Natural stream chemistry in small streams in this region is often low in dissolved ions and has slightly acidic to circumneutral pH. Mean conductivity of all reference sites was 55 µS/cm (range 16–159) compared to 505 µS/cm (range 37–2320) for test sites. Land disturbance and associated erosion typically increase streamwater ionic concentrations and subsequent conductivity (Curtis 1973, Dyer 1982, Dow and Zampella 2000). For example, surface water runoff and groundwater seepage from coal mining operations (particularly mining methods that place overburden into hollow- or valleyfills) contributes to this elevated conductivity and can add high amounts of sediment to receiving streams. In 2000, the Federal Office of Surface Mining estimated that approximately 320 miles of streams have been permanently buried by these mining practices in Kentucky alone (OSM unpub. data). However, this figure only takes into consideration those blue-line streams that are shown on 1:24000 scale topographic maps; hundreds of miles of other headwater streams have likely been filled. Oil and gas drilling also has the potential to elevate stream conductivity through leakage of underground brine water particularly high in chlorides. As of 2001, there were over 30,000 oil or gas wells in the Eastern Coalfield region (Kentucky Geological Survey unpub. data). Based on our study and data found in Green et al. (2000) and Howard et al. (2000), we think that conductivity values >400 µS/cm are excessive for headwater streams in the Eastern Coalfield Region.

Another problem associated with coal mining in this region is acid mine drainage (AMD), where impacted streams are stressed by low pH and high total metals. This condition occurs when coal mining exposes rock that is laden with pyrite that oxidizes into $Fe(OH)_2$ and H_2SO_4. We did not encounter this in the CA, but it is more common in the SA streams where the coal geology is different. In the Stearns region for example, several streams are impacted by AMD. We chose not to sample test sites affected by AMD because the streams are nearly "dead," with only scarce macroinvertebrate populations (e.g., McMurray and Schuster 2001, KDOW unpub. data) and thus not good candidates for testing the MBI. By contrast, many of the streams influenced by mining in our study displayed elevated pH compared to non-mined watersheds. In this case, an alkaline mine drainage phenomenon occurred and was associated with the differing coal geology in the region. A study by Eastern Kentucky University (1975) concluded, “Alkaline pollution caused by surface mining is as real as acid mine drainage pollution.” Dyer (1982) and Green et al. (2000) also documented this occurrence. Relatively low pH at three reference sites (Bad Branch, pH=5.1, Presley House Branch, pH=6.1, Watts Branch, pH=6.0) is consistent with observations in forested streams draining the south side of Pine Mountain and the north side of Cumberland Mountain. Here geological phenomena are suspected, in that streams lack the capacity to buffer acidic rainfall. The patterns of potential acid deposition and acid neutralizing capacity of streams in this area deserve further investigation.

Although % embeddedness used in the DFA and PCA was estimated in thalweg quadrat samples only, we also scored relative embeddedness for the entire riffle with the RBP habitat assessment forms in which the metric showed excellent sensitivity. Sediment pollution from nonpoint sources is a serious problem in Kentucky (KDOW 2000) and elsewhere (Waters 1995). Small streams in the study area that have been exposed to mining and logging are subject to high sediment loading. Moreover, intensified bank erosion caused by hydrologic modification (e.g., impoundments, roads, bridges, and culverts) can substantially increase sedimentation in these streams.

Other factors, such as reduced canopy cover and riparian width, can have direct influences on macroinvertebrate communities that respond to stream temperature, bank habitat and stability, and seasonal changes in the food-energy base (Sweeney 1993). Furthermore, riparian buffers have shown to be critical in reducing the inflow of excessive nutrients, sediments, or contaminants into small streams (Brinson 1993, Sweeney 1993). With regard to canopy cover, our reference sites had the natural compliment of mature forest with dense canopies, albeit mostly second-growth, but this condition was met at very few of the test sites. In intermittent streams, many aquatic insect taxa are adapted to resist desiccation through resting or diapausing eggs, larvae or pupae (Sweeney 1984). We suggest that dense summer canopies may help to regulate high relative humidity and cooler temperatures in the dry streambed sediments, thus helping to assure recruitment of the insect community in subsequent years.

6.2 *Macroinvertebrate Communities*

The Eastern Coalfield region as a whole supports a rich and diverse macroinvertebrate fauna typical of the Appalachian Mountains. Many of these are EPT taxa, and their presence, as in most regions of the country, indicate relatively healthy ecological conditions. The headwater streams in the present study were dominated by EPT, even at many test sites, and EPT made up roughly 45% of the taxa collected overall. This further supports the notion of a large regional pool of EPT species. Our rapid sampling protocol yielded high taxonomic richness in some very small, intermittent streams often considered to have reduced diversity and richness (Harker et al. 1982). Pond (2000) showed results similar to ours and argued that a rich fauna adapted to resist seasonal desiccation can proliferate in these intermittent streams. Moreover, we found high faunal diversity and abundance despite the fact that the calibration communities were essentially recruited from the 1999 populations, those that endured one of the worst droughts on record in the Commonwealth. Finally, Feminella (1996) concluded that, because of the high diversity and faunal similarity to perennial streams, intermittent streams deserve adequate management or regulatory plans to protect species and their habitats.

Strong taxonomic differences in reference communities among the six study regions were not found, suggesting that geographic position and physical variation in these regions are not strikingly influencing macroinvertebrate composition and that higher physical (e.g., geology, topography) and zoogeographical (e.g., speciation, dispersal) factors drive these compositional and structural patterns on a larger spatial scale. Despite the identification of several outlier sites, the NMDS analysis gave us confidence that our reference sites could be used to develop the index for the entire Eastern Coalfield region. The CCA ordination clearly separated reference and test sites, demonstrating that taxonomic composition was indeed altered in streams with varying degrees of impairment. Absence of key indicator taxa (particularly EPT) was frequently observed at test sites. For example, in modified watersheds with elevated stream conductivity (e.g., conductivity >400 µS/cm), Ephemeroptera (mayflies) were markedly reduced or absent. Other workers (Green et al. 2000; H. Howard, US EPA, Athens, GA, pers. comm.; KDOW unpub. data) have seen this phenomenon, and we speculate that many mayfly species are susceptible to high ionic strength that interferes with gill function. Another pattern observed at disturbed sites was an increase in the relative abundance of chironomids and oligochaetes. This pattern is also well documented by others in the region (Arnwine and Denton 2001, Gerritsen et al. 2000, Yoder and Rankin 1995) and signifies that these groups, in general, are tolerant of disturbance.

While the use of indicator species in bioassessment has drawn much criticism in the past (Cairns 1974, Roback 1974), the concept of indicator assemblages provide insight into taxonomic shifts between reference and impaired sites. We think it is also beneficial to look at the presence or absence of taxa frequently associated with reference sites as supplemental information for describing the reference condition. Table 7 reveals the 25 most common taxa found among the 45 reference sample events used in this study. Eighteen of these top 25 taxa were EPT. The mayflies *Ephemerella*, *Epeorus* and *Ameletus*, the stoneflies *Amphinemura* and *Leuctra*, and the caddisflies *Neophylax* and *Rhyacophila* made up the top seven taxa overall with the highest importance values (relative frequency + mean relative abundance). Several notable taxa with high frequencies but low abundances (i.e., <1%) were *Cambarus, Eurylophella, Pycnopsyche,* and *Tipula.*

Table 7. Top 25 taxa collected from all reference sites based on relative frequency + mean relative abundance (= relative importance value).

Family	Genus	Rel. Freq.	Rel. Abun.	Rel. Import.
Ephemerellidae	*Ephemerella*	95.2	13.8	109.0
Heptageniidae	*Epeorus*	97.6	9.2	106.8
Ameletidae	*Ameletus*	95.2	8.3	103.6
Nemouridae	*Amphinemura*	95.2	7.8	103.1
Uenoidae	*Neophylax*	97.6	2.0	99.7
Leuctridae	*Leuctra*	97.6	2.0	99.6
Rhyacophilidae	*Rhyacophila*	97.6	1.4	99.1
Cambaridae	*Cambarus*	97.6	0.7	98.3
Ephemerellidae	*Eurylophella*	97.6	0.5	98.1
Limnephilidae	*Pycnopsyche*	97.6	0.3	97.9
Tipulidae	*Tipula*	95.2	1.0	96.2
Hydropsychidae	*Diplectrona*	92.9	3.0	95.9
Tipulidae	*Hexatoma*	88.1	1.3	89.4
Perlodidae	*Isoperla*	85.7	1.5	87.2
Perlidae	*Acroneuria*	85.7	1.2	86.9
Psephenidae	*Ectopria*	83.3	1.5	84.9
Heptageniidae	*Stenacron*	83.3	0.3	83.7
Simuliidae	*Prosimulium*	78.6	3.8	82.4
Heptageniidae	*Cinygmula*	73.8	7.5	81.3
Lepidostomatidae	*Lepidostoma*	78.6	0.7	79.3
Simuliidae	*Simulium*	76.2	1.4	77.6
Dryopidae	*Helichus*	76.2	1.1	77.3
Polycentropodidae	*Polycentropus*	76.2	0.4	76.6
Leptophlebiidae	*Paraleptophlebia*	73.8	2.6	76.4
Philopotamidae	*Wormaldia*	73.8	0.7	74.5

6.3 *Metric Evaluation*

The metrics chosen for inclusion in the MBI (Genus-TR, Genus-EPT, mHBI, %Ephem, %Chir+Olig, and %Clingers) have also been accepted as good indicators of ecological health in many regions of the U.S. (Plafkin et al. 1989, Resh and Jackson 1993, Kerans and Karr 1994, Barbour et al. 1999, Karr and Chu 1999, Gerritsen et al. 2000, Arnwine and Denton 2001), but contradictory statements on the use of metrics have been offered by Norris (1995) and Reynoldson et al. (1997). By testing metric sensitivity and calibrating scoring criteria, we were able to set regional expectations for macroinvertebrate communities of 1st and 2nd order streams typically between 0.15 to 5 mi^2 (0.5 to 13 km^2) in catchment area.

The selected metrics all had high discrimination efficiency, good to excellent sensitivity, low variability, and an acceptable level of redundancy. The highest correlation ($r = 0.80$, $p<0.05$) was found between EPT and TR. In our study area, EPT dominated the reference communities and accounted for this high redundancy. However, species richness is an important indicator used, by many opinion makers, to describe biodiversity in the current public debate about the importance of biodiversity in maintaining healthy ecosystems, and thus gave us impetus to include both metrics. Moreover, we think habitat diversity and niche partitioning in small streams can be better inferred with the TR metric. All other metric combinations had low to moderate correlations (range ±0.12–0.74, see Table 5) indicating that each metric contributed different information about the community.

Other metrics included in the MBI showed various responses to stream conditions. The mHBI, which is considered most sensitive to organic pollution (Hilsenhoff 1988, Lenat 1993), showed excellent utility in our study. Because assigned tolerance values indirectly integrate a wide variety of species responses to stress, the mHBI responded to impacts ranging from chronic sedimentation, elevated conductivity, to habitat degradation. The %Ephem metric showed the most sensitivity to coal mining and oil brine impacted streams, and was inversely related to %Chir+Olig. Chironomids and oligochaetes are generally tolerant of various forms of stream degradation including sediment, nutrients and organic wastes. In streams impacted by residential landuse with improper onsite sewage treatment, these organisms were frequently more abundant. The %Clinger metric responds primarily to siltation, as these organisms are adapted to "cling" to hard, stable substrates with minimal silt cover. In addition, they are reduced or absent from shifting sand or fine gravel habitat associated with sediment-impacted streams. This metric has recently been adopted for use in a multimetric index by Tennessee (Arnwine and Denton 2001), that shares similar ecoregions with Kentucky.

6.4 *MBI Performance and Application*

Overall, we conclude the aggregate index provides relevant information to characterize various landuse impacts inherent to the region. Moreover, the MBI responded to stressors associated with the burial and elimination of upstream tributaries from mining or construction practices (e.g., increased conductivity and embeddedness). The final 100-point scale MBI utilized all available reference metric data from this study (calibration and validation data sets).

Using the 10th%ile of the reference MBI as the threshold for separating impaired from unimpaired, we found that the MBI correctly classified 83% of *a priori* designated test sites as being impaired. Because we chose test sites ranging from slightly to heavily impacted, the MBI shows excellent promise for detecting a range of impairment. It should be emphasized that in some cases (e.g., due to natural or investigator variability, or minimal disturbance effects), best professional judgement or re-sampling may be warranted if index scores fall close to narrative-rating cutoffs. In the four test streams that rated as unimpaired, conductivity values were <325 µS/cm (mean = 177.2 µS/cm) and had RBP habitat scores >140 (mean = 145), values considered to not greatly deviate from the reference condition. Faunistically, these streams had taxa comparable to those found at most reference sites. The majority of the test streams in our study were derived from largely forested catchments; therefore, near- and in-stream disturbances adjacent to or upstream of the site likely influenced overall impairment ratings.

Regional stressors arising from mining and residential development are some of the most influential regarding headwater stream biointegrity in the Appalachian coal region. In mining regions of West Virginia, Green et al. (2000) found that biological conditions in mined and mined/residential watersheds were substantially more impaired than unmined watersheds. Our data showed that mined and mined/residential watersheds had the lowest MBI scores, and unmined sites were the ones likely to score Excellent or Good (Figure 17).

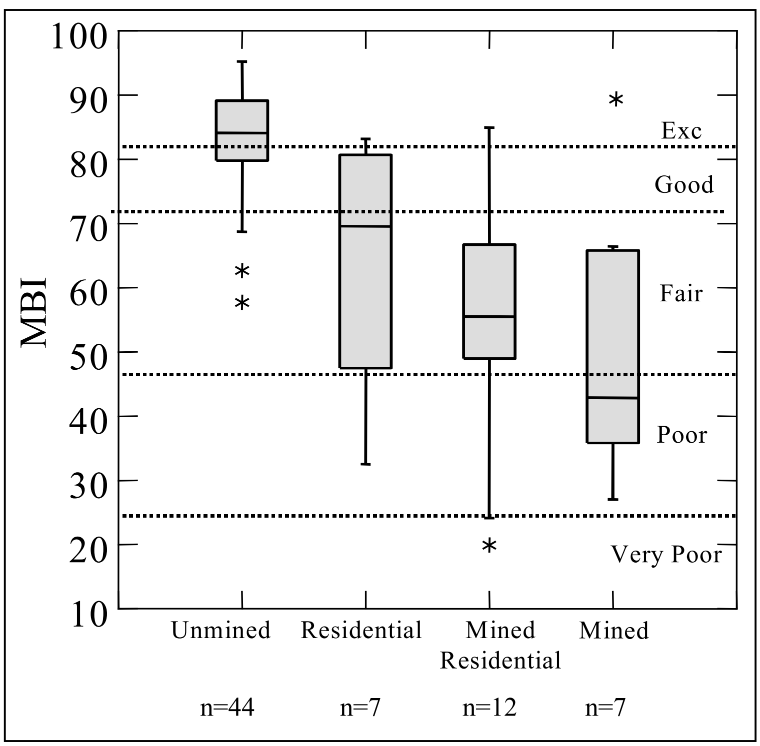


Figure 17. Boxplot of MBI scores among landuse types.

When used together with the EPA RBP Habitat Assessment scores and simple measurements such as canopy cover and conductivity, we feel the MBI can be both a powerful and practical tool for point and nonpoint source impact studies, 305(b) reporting, or to identify new high quality streams in need of protection as Exceptional Waters of the Commonwealth. Sample methodology and seasonality should be adhered to for the MBI to be effective, and we suggest sampling in the spring index period in this region, which ranges from mid-February to June.

It is anticipated that as new data are collected, metric scoring criteria may be refined during subsequent triennial reviews of water quality standards. Future needs include expanding the geographic area so that the quality of reference and disturbed sites in other parts of the Eastern Coalfield region can be assessed. Additional testing on the effects of annual variability on MBI scores, and to provide a check on the precision of the MBI, is also needed. Although very few sample events were replicated in this study, a preliminary analysis on one reference site showed promising results (Table 8). Here, MBI scores had very low variability despite two metrics (mHBI and %Chir+Olig) and total number of individuals having coefficient of variations over 20%.

Table 8. Variability in the MBI and metrics from revisit and duplicate samples at Lower Pigeon Branch (REF). SD=Standard Deviation, C.I.=Confidence Interval; CV=Coefficient of Variation.

Stream Name	CollDate	Narrative	MBI	TR	EPT	mHBI	m%EPT	%Ephem	%Chir+Olig	%Clng
Lower Pigeon Br	4/12/01	Exc	83.43	53	29	2.55	66.86	42.20	5.65	61.37
Lower Pigeon Br	5/15/02	Exc	87.74	45	30	1.68	91.71	54.15	1.95	54.15
Lower Pigeon Br	5/16/02	Exc	85.51	49	27	2.22	85.94	46.68	1.86	53.58
Mean			85.6	49.0	28.7	2.1	81.5	47.7	3.2	56.4
SD			2.2	4.0	1.5	0.4	13.0	6.0	2.2	4.3
95% C.I.			2.4	4.5	1.7	0.5	14.7	6.8	2.4	4.9
C.V. (%)			2.5	8.2	5.3	20.3	16.0	12.7	68.6	7.7

7.0 Application of Family-level Taxonomic Resolution

The level of taxonomic effort (i.e., family vs. genus/species) was compared to determine applicability and sensitivity of the MBI in headwater streams in the Eastern Coalfield Region. While it is well-accepted that a finer level of taxonomic resolution provides more detailed and defensible information than a coarser one (Hawkins et al. 2000, Guerold 2000, Lenat and Resh 2001), there are a number of studies that show the utility of family-level taxonomy in bioassessments (Bailey et al. 2001, Gerritsen et al. 2000, Green et al. 2000). In headwater mountain streams in Kentucky, we have observed reduced species diversity within individual invertebrate families (i.e., low genus/species: family ratios). Exceptions to this are, for example, the families Chironomidae, Heptageniidae, Hydropsychidae, Elmidae, and Perlodidae.

We modified the headwater stream MBI to use 5 metrics: family taxa richness, family EPT, family biotic index, % Ephem, and % Chir+Olig. The % Ephem and % Chir+Olig metric scoring criteria remained the same as in the original MBI since these metrics are derived at lower levels of taxonomic resolution. The %Clinger metric cannot be used since genus-level resolution is needed to designate many of the taxa's habit. The 95th or 5th %iles were recalculated for the remaining metrics and scored on the 100-point scale as described in Section 4.3. The family biotic index (FBI) is analogous to the mHBI, but it uses family-level tolerance values, which were based on the calculated mean tolerance value of all genus/species within a particular family. Metric scoring formulae and an example calculation for the family-level MBI (F-MBI) is provided in Table 9.

Table 9. Metric scoring formulae and example calculation for the F-MBI.

Metric	95th or 5th %ile	Formula	Example for Bear Branch	Metric Score
Family-TR	35.7	$\frac{FamTR}{95th\%ile} X100$	$\frac{26}{35.7} X100$	72.8
Family-EPT	21	$\frac{FamEPT}{95th\%ile} X100$	$\frac{11}{21} X100$	52.4
FBI	3.10	$\frac{10 - FBI}{10 - 5th\%ile} X100$	$\frac{10 - 4.84}{10 - 3.10} X100$	74.8
% Ephem	66.5	$\frac{\%Ephem}{95th\%ile} X100$	$\frac{18.1}{66.3} X100$	27.2
% Chir+Olig	0.68	$\frac{100 - \%Chir + Olig}{100 - 5th\%ile} X100$	$\frac{100-9.53}{100-0.68} X100$	91.08
			Final F-MBI	63.65

A strong relationship (r^2=0.93) was found between the F-MBI and the original index (Figure 18). This, in conjunction with the strong discriminatory power of the F-MBI (Figure 19), suggested that the F-MBI could be used for bioassessment of headwater streams in this region. Narrative ratings were established with modified percentile thresholds found in Section 4.4. These values correspond to water quality ratings of Unimpaired (>75), Partially Impaired (50-74), and Impaired (<50).
Use of these three use-based water quality classifications acknowledges a conservative viewpoint because of the decreased sensitivity inherent in family level taxonomy. Using the 10^{th}%ile of the

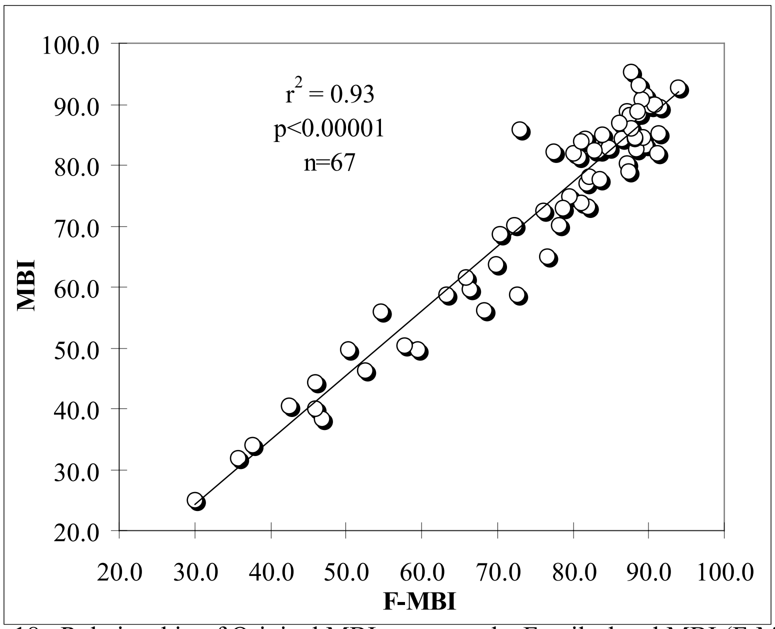


Figure 18. Relationship of Original MBI scores to the Family-level MBI (F-MBI)

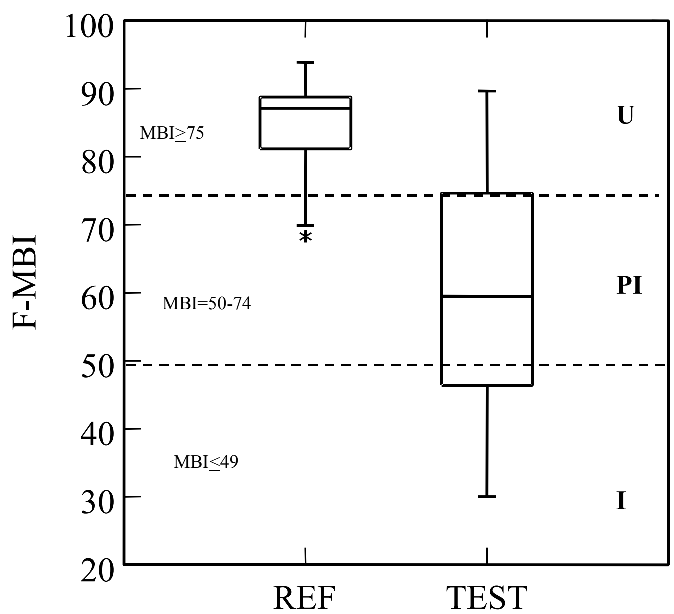


Figure 19. Box plot of F-MBI scores from reference and test sites (all sites) showing thresholds for use-support designations. U=Unimpaired, PI=Partially Impaired, I=Impaired.

reference F-MBI as the threshold for separating impaired from unimpaired, the MBI correctly classified 74% of *a priori* designated test sites (calibration and validation) as being impaired. Thus, a difference of 9% discrimination efficiency was noted when comparing the MBI to the F-MBI. In addition, there was a narrower central tendency in test site F-MBI assessments, whereas test streams assessed with the MBI showed a broader range of impairment. This also indicated a slight decrease in sensitivity of the F-MBI. Furthermore, MBI interquartiles showed better discrimination than with the F-MBI (Figure 20).

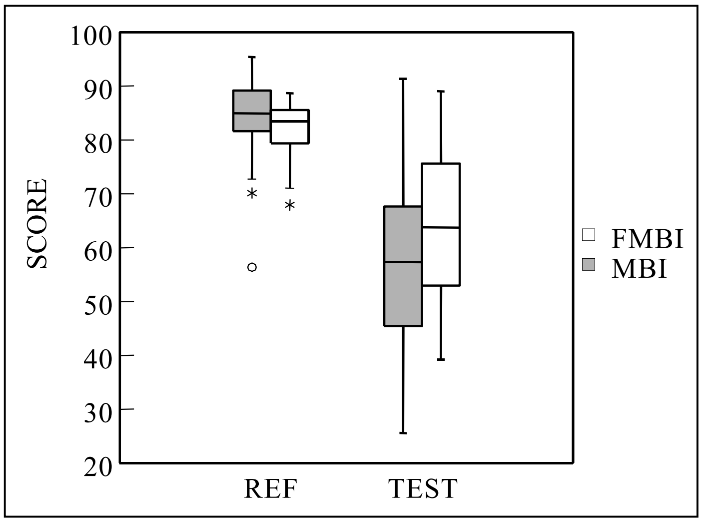


Figure 20. Box plot of MBI and Family-level MBI (F-MBI).

Despite a loss of discriminatory power, the F-MBI is recommended for use in mountain headwater streams as (1) a quick screening tool to delineate obviously impaired streams from the reference condition, (2) an assessment protocol for non-KDOW personnel (e.g., volunteer Watershed Watch, private consultants, university students) that may lack taxonomic expertise, and (3) a means for KDOW to assess a large number of headwater streams in a very short time with fewer personnel. Finally, KDOW asserts that genus/species level taxonomy should be made when the goal of biomonitoring is to show incremental improvements in water quality for permit compliance and enforcement or for other pollution abatement activities.

Toms Branch (Big Sandy Basin)

Puncheoncamp Branch (Stearns District)

Presley House Branch (Pine/Cumberland Mountain Region)

John Carpenter Branch (UK Robinson Forest)

Representative headwater reference streams with sampling areas denoted. See Figure 1 for area locations.

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Appendix A. Site locations for reference and test sites. Groups refer to geographic location: BS=Big Sandy, WA=Western Allegheny, PCM=Pine/Cumberland Mountains, ROB=Robinson Forest, RED, Redbird District, STRNS= Stearns, UNG=Ungrouped. Ecoregion abbreviations are CA=Central Appalachians, SW=Southwestern Appalachians, WA=Western Allegheny Plateau.

SiteID	Condition	StreamName	Group	RM	Order	Area (mi^2)	Basin	Ecoregion	County	Lat_Dec	Long_Dec	TOPO
1007005	REF	HOBBS FORK	BS	0	2	1.15	BIG SANDY	CA	MARTIN	37.69166	-82.40413	VARNEY
1007006	REF	UT HOBBS FORK	BS	0.1	1	0.18	BIG SANDY	CA	MARTIN	37.6829	-82.40664	VARNEY
1017001	TEST	LONG BR	BS	2	1	0.36	BIG SANDY	CA	FLOYD	37.73322	-82.69648	LANCER
1022008	TEST	CALEB FORK	BS	0.35	2	1.78	BIG SANDY	CA	FLOYD	37.32683	-82.6878	WHEELWRIGHT
1032001	REF	TOMS BR	BS	0.35	1	0.95	BIG SANDY	CA	PIKE	37.25827	-82.44763	HELLIER
1032002	REF	LOWER PIGEON BR	BS	0.65	1	0.89	BIG SANDY	CA	PIKE	37.24081	-82.4871	CLINTWOOD
1032003	TEST	UPPER PIGEON BR	BS	0.15	2	2.01	BIG SANDY	CA	PIKE	37.24141	-82.51691	JENKINS EAST
2006027	TEST	HATCHELL BR	STRNS	0.1	1	0.35	UPPER	SW	MCCREARY	36.86816	-84.3669	CUMBERLAND FALLS
2006030	REF	JACKIE BR	STRNS	0.1	2	1.14	UPPER	SW	WHITLEY	36.90527	-84.2791	SAWYER
2006031	REF	CANE CR	STRNS	0.3	1	0.65	UPPER	SW	WHITLEY	36.76649	-84.30595	CUMBERLAND FALLS
2008017	REF	ROCK CR1	STRNS	0.2	1	0.82	UPPER	SW	MCCREARY	36.64218	-84.70962	BELL FARM
2008018	REF	WATTS BR	STRNS	0.1	2	2.2	UPPER	SW	MCCREARY	36.65685	-84.65647	BELL FARM
2008019	REF	PUNCHEONCAMP BR	STRNS	0.1	2	1.7	UPPER	SW	MCCREARY	36.65766	-84.64091	BELL FARM
2008020	REF	ROCK CR2	STRNS	0.1	2	0.63	UPPER	SW	MCCREARY	36.66325	-84.62916	BELL FARM
2008021	REF	ROCK CR3	STRNS	0.1	1	0.37	UPPER	SW	MCCREARY	36.66859	-84.62849	BELL FARM
2008022	REF	UT BS FK CUMBERLAND	STRNS	0.1	2	0.89	UPPER	SW	MCCREARY	36.7124	-84.5526	BARTHELL
2008023	TEST	COFFEY BR	STRNS	0.1	2	1.25	UPPER	SW	MCCREARY	36.69082	-84.51865	BARTHELL
2014004	TEST	JENNEYS BR	STRNS	0.3	1	0.66	UPPER	SW	MCCREARY	36.7366	-84.45815	WHITLEY CITY
2023004	REF	DRY FORK	UNG	1.7	2	2.05	UPPER	SW	JACKSON	37.39283	-84.13898	JOHNETTA
2041003	REF	BROWNIES CR	PCM	14.1	2	2.3	UPPER	CA	HARLAN	36.6981	-83.44046	EWING
2041004	TEST	BROWNIES CR2	PCM	0.1	1	0.31	UPPER	CA	HARLAN	36.69928	-83.4399	EWING
2042002	TEST	EWING CR	PCM	0.2	2	3.06	UPPER	CA	HARLAN	36.8389	-83.37168	HARLAN
2042003	REF	WATTS CR	PCM	2.65	2	0.85	UPPER	CA	HARLAN	36.86211	-83.37577	WALLINS CREEK
2046002	REF	BAD BR	PCM	0.25	2	2.6	UPPER	CA	LETCHER	37.0711	-82.77193	WHITESBURG
2046004	REF	PRESLEY HOUSE BR	PCM	0.2	2	0.9	UPPER	CA	LETCHER	37.06656	-82.7916	WHITESBURG
2046005	TEST	FRANKS CR	PCM	3.0	2	1.36	UPPER	CA	LETCHER	37.03002	-82.8015	WHITESBURG
4036017	REF	STEER FORK	WA	0.2	2	3	KENTUCKY	WA	JACKSON	37.45352	-83.93016	MCKEE
4042003	REF	CHESTER CR	WA	0.15	2	2.65	KENTUCKY	WA	WOLFE	37.72722	-83.66139	ZACHARIAH
4042016	TEST	MF RED RIVER	WA	13	2	1.8	KENTUCKY	WA	WOLFE	37.72497	-83.65929	ZACHARIAH
4050002	REF	CLEMONS FORK	ROB	3.0	2	2.0	KENTUCKY	CA	BREATHITT	37.4806	-83.1356	NOBLE
4050007	TEST	FUGATE FORK	ROB	0.2	2	2.6	KENTUCKY	CA	BREATHITT	37.46033	-83.2353	NOBLE
4050008	TEST	JENNY FORK	ROB	0.1	1	0.45	KENTUCKY	CA	BREATHITT	37.45763	-83.19653	NOBLE
4050009	TEST	BEAR BR	ROB	0.7	2	1.54	KENTUCKY	CA	BREATHITT	37.45706	-83.19544	NOBLE
4050010	REF	CLEMONS FORK	ROB	3.9	2	0.8	KENTUCKY	CA	BREATHITT	37.48593	-83.13222	NOBLE
4050011	REF	FALLING ROCK BR	ROB	0.1	1	0.41	KENTUCKY	CA	BREATHITT	37.47624	-83.1388	NOBLE
4050012	REF	JOHN CARPENTER FORK	ROB	0.2	1	0.58	KENTUCKY	CA	BREATHITT	37.48239	-83.12843	NOBLE
4050013	REF	SHELLY ROCK FORK	ROB	0.1	1	0.55	KENTUCKY	CA	BREATHITT	37.48165	-83.15128	NOBLE
4050014	REF	MILLSEAT BR	ROB	0.7	2	0.58	KENTUCKY	CA	BREATHITT	37.48242	-83.15023	NOBLE
4050015	REF	LITTLE MILLSEAT BR	ROB	0.1	2	0.82	KENTUCKY	CA	BREATHITT	37.47224	-83.1466	NOBLE
4050016	TEST	LICK BR	ROB	0.4	2	2.81	KENTUCKY	CA	PERRY	37.39266	-83.13835	NOBLE
4050017	TEST	WILLIAMS BR	ROB	0.6	2	1.08	KENTUCKY	CA	PERRY	37.39329	-83.15638	NOBLE
4050018	TEST	CANEY CR	ROB	0.75	2	2.5	KENTUCKY	CA	BREATHITT	37.44875	-83.2611	HADDIX
4052017	REF	LITTLE DOUBLE CR	RED	0.7	2	1.5	KENTUCKY	CA	CLAY	37.1312	-83.5983	BIG CREEK
4052018	REF	RF BIG DOUBLE CR2	RED	0.7	2	1.46	KENTUCKY	CA	CLAY	37.08907	-83.6184	CREEKVILLE
4052019	REF	LF BIG DOUBLE CR	RED	0.5	2	0.6	KENTUCKY	CA	CLAY	37.08321	-83.60373	CREEKVILLE
4052020	REF	RF ELISHA CR	RED	2.1	2	2.35	KENTUCKY	CA	LESLIE	37.07628	-83.51512	CREEKVILLE
4052021	REF	BIG MF ELISHA CR	RED	0.2	1	0.82	KENTUCKY	CA	CLAY	37.0815	-83.51472	CREEKVILLE
4052022	REF	LF ELISHA CR	RED	0.6	2	2.47	KENTUCKY	CA	LESLIE	37.09225	-83.52559	CREEKVILLE
4052023	REF	RF BIG DOUBLE CR	RED	0.2	2	1.53	KENTUCKY	CA	CLAY	37.09037	-83.60673	CREEKVILLE
4052024	TEST	RED BIRD CR	RED	86.05	2	1.4	KENTUCKY	CA	BELL	36.91241	-83.54094	BEVERLY
4052025	TEST	MUD LICK BR	RED	0.2	1	1.1	KENTUCKY	CA	BELL	36.91261	-83.53675	BEVERLY
4052026	TEST	LAWSON CR	RED	1.3	2	1.48	KENTUCKY	CA	BELL	36.92718	-83.55332	BEVERLY
4052027	TEST	SPRUCE BR	RED	0.1	2	0.95	KENTUCKY	CA	CLAY	36.95668	-83.53017	BEVERLY
4052028	TEST	GILBERTS LITTLE CR	RED	0.2	2	1.47	KENTUCKY	CA	CLAY	37.09083	-83.56353	CREEKVILLE
4052029	TEST	ARNETTS FORK	RED	0.9	2	1.42	KENTUCKY	CA	CLAY	37.11115	-83.59735	CREEKVILLE
4052030	REF	SUGAR CR	RED	2.1	2	3.05	KENTUCKY	CA	LESLIE	37.12376	-83.5243	CREEKVILLE
4054005	REF	CAWOOD BR	RED	0.1	1	0.8	KENTUCKY	CA	LESLIE	36.93714	-83.37177	BLEDSOE
4054007	TEST	LF CAMP CR	UNG	0.1	1	0.93	KENTUCKY	CA	LESLIE	37.113	-83.34741	CUTSHIN
4054008	TEST	CAMP CR	UNG	1.3	2	2.7	KENTUCKY	CA	LESLIE	37.10556	-83.34114	CUTSHIN
4054009	REF	BILL BR	UNG	0.2	2	2.3	KENTUCKY	CA	LESLIE	36.93219	-83.30634	BLEDSOE
4054010	REF	HONEY BR	UNG	0.1	2	0.82	KENTUCKY	CA	LESLIE	37.01735	-83.35649	CUTSHIN
4055002	REF	UT LINE FORK	UNG	0.2	1	0.22	KENTUCKY	CA	LETCHER	37.07736	-82.99397	ROXANA
5037002	REF	BOTTS FORK	WA	0.2	2	3.38	LICKING	WA	MENIFEE	37.94811	-83.50826	SCRANTON
5037004	REF	WELCH FORK	WA	0.1	2	1.5	LICKING	WA	MENIFEE	37.94361	-83.50491	SCRANTON
6012003	REF	NICHOLS FORK	WA	0.2	2	0.65	LITTLE SANDY	WA	ELLIOTT	38.08026	-83.00607	ISONVILLE
6012004	REF	MEADOW BR	WA	0.3	2	0.93	LITTLE SANDY	WA	ELLIOTT	38.07261	-82.99451	MAZIE
6013014	REF	NEWCOMBE CR UT	WA	0.1	1	0.25	LITTLE SANDY	WA	ELLIOTT	38.10296	-83.06426	ISONVILLE

Appendix B. Mean physical and chemical variables from all sites.

StationID	StreamName	Condition	Date	Area (Mi^2)	DO	pH	Spec. Cond.	Temp	%Embed	RipWidth (m)	StrWidth (m)	Canopy Score	SubSize (cm)	Elevation (m)	Slope (m/km)
01007005	HOBBS FORK	REF	4/11/01	1.15	9.14	7.2	62	18.5	17.25	54.1	4.2	16	11.21	256	15.2
01007005	UT HOBBS FORK2	REF	4/11/01	0.18	9	6.88	43	17	15.00	>100.0	2.2	18	12.80	292	48.8
01017001	LONG BR	TEST	4/23/02	0.36	10.1	8.6	702	15.6	ND	5.0	1.0	ND	ND	231	36.5
1022008	CALEB FORK	TEST	5/1/02	1.78	8.9	7.8	347.1	16.3	ND	51.0	3.1	ND	ND	341.3	24.4
01032001	TOMS BRANCH	REF	4/12/01	0.95	10.1	8.12	159	14.1	14.00	75.0	5.0	18	14.16	360	54.8
01032002	LOWER PIGEON BRANCH	REF	4/12/01	0.89	9.95	7.88	119	13.3	14.00	55.5	5.1	18	ND	402.3	60.9
01032003	LOWER PIGEON BRANCH	REF	5/15/02	0.89	ND	7.54	108	12.2	16.00	55.5	5.0	18	ND	402.3	60.9
01032003	LOWER PIGEON BRANCH	REF	5/16/02	0.89	ND	7.62	109	12.5	19.00	55.5	5.0	18	ND	402.3	60.9
01032003	UPPER PIGEON BR	TEST	5/16/02	2.01	ND	7.94	1410	14.7	35.00	50.7	4.9	12	ND	390.1	24.4
02006027	HATCHELL BRANCH	TEST	4/19/00	0.35	9.3	6.4	37.2	14	28.00	>100.0	3.59	20	16.48	280.4	42.7
02006030	JACKIE BRANCH	REF	4/20/00	1.14	9.7	6.6	22.1	11.3	2.00	>100.0	7.55	20	18.38	274.3	30.5
02006031	CANE CREEK	REF	4/25/00	0.65	8.5	6.2	19.5	12	15.00	>100.0	4.26	20	18.48	292.6	27.4
02008017	UT ROCK CREEK1	REF	4/17/00	0.82	9.5	6.3	38.6	11.9	13.00	>100.0	4.53	18	14.48	304.8	39.6
02008018	WATTS BRANCH	REF	4/17/00	2.2	9.1	6.2	27	12.4	17.30	57.5	4.57	20	15.43	280.4	30.5
02008019	PUNCHEONCAMP BRANCH	REF	4/18/00	1.7	10.7	6.7	26.2	10.4	13.30	>100.0	7.80	20	15.34	280.4	39.6
02008020	UT ROCK CREEK3	REF	4/18/00	0.63	10.6	6.7	38.9	10.45	22.80	>100.0	3.74	18	15.27	274.3	82.3
02008021	UT ROCK CREEK2	REF	4/18/00	0.37	10.1	6.9	30.9	10.5	6.50	>100.0	3.97	16	18.12	271.3	73.2
02008022	UT BS FK CUMBERLAND	REF	4/18/00	0.89	9.7	7	41.8	11	12.30	>100.0	4.98	20	15.93	231.6	54.9
02008023	COFFEY BRANCH	TEST	4/19/00	1.25	10.1	6.8	66.9	10.7	18.00	21.5	5.7	10	16.29	274.3	36.6
02014004	JENNEYS BRANCH	TEST	4/19/00	0.66	9.7	7.3	188.7	12.9	37.50	50.0	4.4	4	14.00	356.6	30.5
02023004	DRY FORK	REF	4/19/01	2.05	11.27	8.02	68	12.2	6.25	50.9	3.37	12	ND	386	30.5
02041004	BROWNIES CREEK	REF	4/26/00	2.3	10.2	6.8	60.2	10.2	5.50	85.0	6.66	20	17.59	493.8	22.3
02041005	BROWNIES CREEK2	TEST	4/26/00	0.31	8.7	6.7	95	13.1	9.30	>100.0	3.56	16	16.01	502.9	64.1
02042002	EWING CREEK	TEST	4/26/00	3.06	8	7.4	485	15.5	16.50	59.0	10.9	4	16.16	353.6	25.3
02042003	WATTS CREEK	REF	3/29/01	0.85	11.7	6.03	20	5.64	5.50	>100.0	5.4	14	16.35	402.3	30.5
02046002	BAD BRANCH	REF	4/27/00	2.6	8.7	5.1	16.7	7.5	8.00	>100.0	6.6	20	19.97	548.6	79.2
02046004	PRESLEY HOUSE BRANCH	REF	4/27/00	0.9	10.2	6.1	17.1	8.6	2.50	>100.0	4.8	20	18.03	542.5	85.3
02046005	FRANKS CREEK	TEST	4/27/00	1.36	9.1	7.1	324.4	11.7	15.75	2.6	5.8	16	15.30	588.2	73.1
04036017	STEER FORK	REF	4/18/01	3.01	12	7.55	44	8.75	16.00	>100.0	9.4	20	ND	289.5	36.6
04042703	CHESTER CREEK	REF	4/10/02	2.65	ND	6.8	69	9.98	ND	>100.0	6.1	18	ND	304.8	18.3
04042016	MF RED RIVER	TEST	4/10/02	1.8	ND	7.5	132	14.3	ND	4.0	4.9	8	ND	277.4	24.4
04050002	CLEMONS FORK	REF	5/19/98	2.0	ND	7.2	77	ND	11.00	>100.0	ND	ND	ND	299.5	21.3
04050007	FUGATE FORK	TEST	4/10/00	2.6	10.94	8.1	609.8	14.9	22.80	1.6	5.4	8	19.87	243.8	18.29
04050008	JENNY FORK	TEST	4/10/00	0.45	11.3	7.6	635.1	13.3	19.50	>100.0	5.1	16	14.64	268.2	36.58
04050009	BEAR BRANCH	TEST	4/10/00	1.54	11.6	8	431.2	16.2	20.00	30.5	3.1	4	18.73	268.2	27.43
04050010	CLEMONS FORK	REF	4/10/00	0.8	13	6.8	83.4	12.70	7.3	>100.0	7	20	15.81	316.9	18.3
04050011	FALLING ROCK BRANCH	REF	4/11/00	0.41	13.3	6.7	41.4	8.9	13.30	>100.0	4.46	20	19.34	292.6	45.7
04050012	JOHN CARPENTER FORK	REF	4/11/00	0.58	12.9	6.8	38.8	9.1	8.50	>100.0	4.59	20	15.84	316.9	24.4
04050013	SHELLY ROCK FORK	REF	4/11/00	0.55	12.2	7.1	39.4	10.1	12.30	>100.0	4.62	20	15.08	304.8	36.6
04050014	MILLSEAT BRANCH	REF	4/11/00	0.58	12.7	7.4	130.3	10.9	9.50	>100.0	4.36	20	15.94	304.8	24.4
04050015	LITTLE MILLSEAT BRANCH	REF	4/12/00	0.82	10.9	7.1	40.2	10.4	11.80	>100.0	4.1	20	14.70	280.4	24.4
04050016	LICK BRANCH	TEST	4/12/00	2.81	13.4	8.3	2320	10.9	53.30	14.1	3.3	14	13.84	268.2	18.3
04050017	WILLIAMS BRANCH	TEST	4/12/00	1.08	15.7	8.4	1228	10.5	23.80	30.5	3.1	10	14.61	268.2	18.3
04050018	CANEY CREEK	TEST	4/12/00	2.5	13.5	8.1	152.9	11.6	28.30	40.5	4.0	6	16.07	243.8	12.2
04052017	LITTLE DOUBLE CREEK	REF	3/29/00	1.5	11.34	6.9	60	9.1	13.40	67.0	5.15	14	15.80	280.4	30.5
04052018	RIGHT FORK BIG DOUBLE CREEK2	REF	3/29/00	1.46	11	6.4	38.3	9.4	7.80	73.0	6.0	8	16.54	329.2	36.6
04052019	LEFT FORK BIG DOUBLE CREEK	REF	3/29/00	0.6	11.2	6.4	48.4	10	18.60	>100.0	3.7	14	16.26	329.2	30.5
04052020	RIGHT FORK ELISHA CREEK	REF	3/30/00	2.35	11.5	6.8	49.2	11	13.10	>100.0	6.7	16	16.59	316.9	18.3
04052021	BIG MIDDLE FORK ELISHA CREEK	REF	3/30/00	0.82	10.3	6.5	54.3	13.1	14.10	>100.0	5.7	14	15.12	316.9	36.6
04052022	LEFT FORK ELISHA CREEK	REF	3/30/00	2.47	9.8	6.4	45	15.2	10.60	58.8	6.1	20	15.72	329.2	18.3
04052023	RIGHT FORK BIG DOUBLE CREEK	REF	4/ 5/00	1.53	12.4	6.4	35	8.5	20.30	69.5	6.0	18	18.12	316.9	24.4
04052024	RED BIRD CREEK	TEST	4/ 5/00	1.4	13.7	6.9	505	11.9	25.30	0.4	4.4	6	17.62	420.6	30.5
04052025	MUD LICK BRANCH	TEST	4/ 5/00	1.1	11.1	6.5	156.5	12.1	19.40	53.9	4.5	10	16.03	414.5	30.5
04052026	LAWSON CREEK	TEST	4/ 5/00	1.48	10.6	7	436	12.9	18.10	15.5	4.7	4	16.87	426.7	30.5
04052027	SPRUCE BRANCH	TEST	9/ 6/00	0.95	12.3	7.3	161	9.5	21.30	77.0	4.7	14	18.26	362.7	67.1
04052028	GILBERTS LITTLE CREEK	TEST	4/ 6/00	1.47	11.4	6.97	63	11.3	24.10	52.2	4.5	4	17.74	280.4	36.6
04052029	ARNETTS FORK	TEST	4/ 6/00	1.42	10.6	6.7	56	13.9	16.30	52.5	4.98	8	18.30	292.6	21.3
04052030	SUGAR CREEK	REF	4/ 6/00	3.05	10.9	6	26.3	12.5	13.10	>100.0	6.3	16	19.80	316.9	21.3
04054005	CAWOOD BRANCH	REF	3/28/01	0.8	11.94	6.63	29	3.55	10.25	>100.0	5.5	14	18.67	420.56	91.4
04054007	LEFT FORK CAMP CREEK	TEST	3/27/01	0.93	10.7	7.99	505	9.35	18.25	>100.0	4.7	16	16.29	292	73.1
04054008	CAMP CREEK	TEST	3/27/01	2.7	11.5	8.25	926	8.35	23.75	10.5	5.5	12	15.94	298.1	60.9
04054009	BILL BRANCH	REF	3/28/01	2.3	11.66	6.55	23	5.04	13.75	>100.0	8.5	14	16.67	457.2	73.1
04054010	HONEY BRANCH	REF	3/28/01	0.82	11.05	6.83	42	8.64	11.50	52.1	4.9	14	17.05	347.5	48.8
04055002	UT LINE FORK	REF	4/16/98	0.22	12.1	7.57	52	12.9	ND	>100.0	3.7	18	ND	350.5	91.4
05037002	BOTTS FORK	REF	4/18/02	3.38	ND	7.58	132	15.85	ND	>100.0	7.6	18	ND	246.9	12.2
05037004	WELCH FORK	REF	4/1802	1.5	ND	7.5	94	18.2	ND	>100.0	4.87	18	ND	249.9	18.4
06012003	NICHOLS FORK	REF	4/29/02	0.65	ND	6.65	47	12.02	ND	>100.0	4.6	20	ND	259.1	12.2
06012004	MEADOW BRANCH	REF	4/29/02	0.93	ND	6.3	46	11.99	ND	>100	4.8	18	ND	252.9	9.2
06013014	UT NEWCOMBE CREEK	REF	3/14/02	0.25	ND	7.9	89	11.05	ND	>100	ND	18	ND	213.36	36.6

Appendix C. RBP Habitat Assessment Scores from all sites.

StationID	StreamName	Condition	CollDate	Total HabScore	BankSta-LB	BankSta-RB	Bank Stabilty	BankVegP-LB	BankVegP-RB	BankVeg	ChaFlowS	ChanAlter	Embeddedness	EpiFauSub	FreqOfRiffles	RipVegZW-LB	RipVegZW-RB	RipScore	SedDep	Vel/Dep Regime
1007005	HOBBS FORK	REF	4/11/01	145	6	8	14	7	9	16	15	18	15	14	11	10	10	20	8	14
1007006	UT HOBBS FORK	REF	4/11/01	153	9	9	18	8	9	17	15	19	16	16	11	10	10	20	10	11
1017001	LONG BR	TEST	4/23/02	84	9	9	18	2	3	5	19	1	11	1	4	2	2	4	11	10
1022008	CALEB FORK	TEST	5/1/02	136	8	7	15	9	4	13	16	10	15	16	16	10	2	12	11	16
1032001	TOMS BRANCH	REF	4/12/01	173	8	10	18	9	10	19	15	20	16	17	19	10	10	20	10	19
1032002	LOWER PIGEON BRANCH	REF	4/12/01	172	10	7	17	9	9	18	15	19	17	19	19	7	8	15	15	18
1032003	LOWER PIGEON BRANCH	REF	5/15/02	167	9	8	17	9	10	19	15	18	16	17	18	8	10	18	14	15
1032003	UPPER PIGEON BR	TEST	5/16/02	138	10	8	18	9	4	13	15	14	12	13	19	10	2	12	8	14
2006027	HATCHELL BRANCH	TEST	4/19/00	154	8	8	16	9	9	18	15	18	11	16	18	10	10	20	7	15
2006030	JACKIE BRANCH	REF	4/20/00	179	9	9	18	10	10	20	15	20	19	18	17	10	10	20	17	15
2006031	CANE CREEK	REF	4/25/00	163	7	10	17	9	10	19	15	19	14	16	15	10	10	20	13	15
2008017	UT ROCK CREEK1	REF	4/17/00	176	8	8	16	9	9	18	15	20	17	19	18	10	10	20	14	19
2008018	WATTS BRANCH	REF	4/17/00	174	9	9	18	9	9	18	15	17	16	18	18	8	10	18	18	18
2008019	PUNCHEONCAMP BRANCH	REF	4/17/00	186	9	9	18	9	9	18	15	18	19	20	19	10	10	20	19	20
2008020	UT ROCK CREEK3	REF	4/18/00	176	9	9	18	9	9	18	15	19	16	19	19	10	10	20	17	15
2008021	UT ROCK CREEK2	REF	4/18/00	170	8	8	16	9	9	18	15	19	14	18	18	10	10	20	17	15
2008022	UT BS FK CUMBERLAND	REF	4/18/00	183	8	8	16	9	9	18	15	20	18	19	19	10	10	20	19	19
2008023	COFFEY BRANCH	TEST	4/19/00	146	7	7	14	5	5	10	15	16	13	16	17	5	7	12	15	18
2014004	UT JENNEYS BRANCH	TEST	4/19/00	110	7	8	15	3	8	11	15	11	8	10	11	0	9	9	9	11
2023004	DRY FORK	REF	4/19/01	162	8	9	17	8	9	17	15	15	19	16	17	2	10	12	19	15
2041003	BROWNIES CREEK	REF	4/26/00	176	8	8	16	8	8	16	15	18	18	19	19	10	8	18	18	19
2041004	BROWNIES CREEK2	TEST	4/26/00	166	6	7	13	8	8	16	15	17	18	19	19	10	10	20	14	15
2042002	EWING CREEK	TEST	4/26/00	107	2	2	4	2	2	4	15	14	14	7	15	3	10	13	5	16
2042003	WATTS CREEK	REF	3/29/01	178	9	8	17	10	10	20	15	20	19	17	17	10	10	20	18	15
2046002	BAD BRANCH	REF	4/27/00	190	10	10	20	10	10	20	15	20	18	19	20	10	10	20	19	19
2046004	PRESLEY HOUSE BRANCH	REF	4/27/00	187	10	10	20	10	10	20	15	20	19	18	17	10	10	20	19	19
2046005	FRANKS CREEK	TEST	4/27/00	140	7	7	14	7	7	14	15	10	17	18	18	1	1	2	14	18
4036017	STEER FORK	REF	4/18/01	176	8	10	18	9	9	18	15	20	17	19	19	6	10	16	16	18
4042703	CHESTER CREEK	REF	4/10/02	172	8	8	16	7	9	16	16	17	19	18	17	10	10	20	16	17
4042016	MF RED RIVER	TEST	4/10/02	130	6	6	12	5	3	8	16	14	13	15	18	5	1	6	12	16
4050002	CLEMONS FORK	REF	5/19/98	176	9	9	18	9	9	18	18	16	20	18	18	10	4	14	18	18
4050007	FUGATE FORK	TEST	4/10/00	136	7	8	15	7	9	16	15	14	13	16	19	1	1	2	10	16
4050008	JENNY FORK	TEST	4/10/00	138	7	7	14	7	8	15	15	15	12	12	16	9	9	18	6	15
4050009	BEAR BRANCH	TEST	4/10/00	117	7	6	13	7	5	12	15	12	9	17	18	2	0	2	8	11
4050010	CLEMONS FORK	REF	4/10/00	180	10	8	18	9	9	18	15	20	19	19	19	10	10	20	16	16
4050011	FALLING ROCK BRANCH	REF	4/11/00	160	4	8	12	5	9	14	15	19	18	18	18	10	10	20	11	15
4050012	JOHN CARPENTER FORK	REF	4/11/00	174	7	9	16	8	9	17	15	18	17	19	19	10	10	20	15	18
4050013	SHELLY ROCK FORK	REF	4/11/00	171	9	9	18	9	9	18	15	19	17	16	17	10	10	20	15	16
4050014	MILLSEAT BRANCH	REF	4/11/00	175	8	8	16	9	9	18	15	19	17	19	19	10	10	20	15	17
4050015	LITTLE MILLSEAT BRANCH	REF	4/12/00	169	9	6	15	8	8	16	15	17	17	18	18	10	10	20	15	18
4050016	LICK BRANCH	TEST	4/12/00	114	9	9	18	6	6	12	15	16	3	9	17	2	6	8	6	10
4050017	WILLIAMS BRANCH	TEST	4/12/00	128	8	6	14	5	7	12	15	15	10	14	18	7	1	8	11	11
4050018	CANEY CREEK	TEST	4/12/00	144	8	8	16	7	8	15	15	14	13	15	16	9	1	10	15	15
4052017	LITTLE DOUBLE CREEK	REF	3/29/00	173	8	8	16	7	7	14	15	20	18	19	20	10	9	19	17	15
4052018	RIGHT FORK BIG DOUBLE CREEK2	REF	3/29/00	172	9	9	18	9	9	18	15	20	15	18	19	10	9	19	15	15
4052019	LEFT FORK BIG DOUBLE CREEK	REF	3/29/00	162	7	7	14	7	7	14	15	18	14	18	20	10	10	20	14	15
4052020	RIGHT FORK ELISHA CREEK	REF	3/30/00	174	9	9	18	9	9	18	15	16	15	19	19	10	10	20	15	19
4052021	BIG MIDDLE FORK ELISHA CREEK	REF	3/30/00	161	7	7	14	8	8	16	15	17	16	17	19	10	10	20	12	15
4052022	LEFT FORK ELISHA CREEK	REF	3/30/00	171	8	8	16	8	8	16	15	18	16	19	19	8	10	18	16	18
4052023	RIGHT FORK BIG DOUBLE CREEK	REF	4/ 5/00	147	2	3	5	3	3	6	15	15	15	19	20	10	10	20	14	18
4052024	RED BIRD CREEK	TEST	4/ 5/00	133	8	6	14	8	6	14	15	13	13	13	17	1	0	1	14	19
4052025	MUD LICK BRANCH	TEST	4/ 5/00	144	9	9	18	8	8	16	15	11	13	16	16	6	7	13	10	16
4052026	LAWSON CREEK	TEST	4/ 5/00	136	9	9	18	7	7	14	15	11	14	11	15	9	6	15	17	6
4052027	SPRUCE BRANCH	TEST	4/ 6/00	150	6	6	12	6	8	14	15	16	11	17	19	10	10	20	7	19
4052028	GILBERTS LITTLE CREEK	TEST	4/ 6/00	132	8	8	16	8	9	17	15	14	13	11	16	0	8	8	11	11
4052029	ARNETTS FORK	TEST	4/ 6/00	154	9	8	17	7	8	15	15	15	16	17	18	1	9	10	15	16
4052030	SUGAR CREEK	REF	4/ 6/00	181	9	9	18	9	9	18	15	19	17	19	19	10	10	20	17	19
4054005	CAWOOD BRANCH	REF	3/28/01	181	10	10	20	10	10	20	15	19	19	18	19	10	10	20	14	17
4054007	LEFT FORK CAMP CREEK	TEST	3/27/01	170	10	10	20	7	9	16	15	19	17	17	19	10	10	20	10	17
4054008	CAMP CREEK	TEST	3/27/01	138	8	6	14	5	9	14	15	14	13	17	18	0	9	9	6	18
4054009	BILL BRANCH	REF	3/28/01	179	10	10	20	10	10	20	15	20	17	19	19	9	10	19	15	15
4054010	HONEY BRANCH	REF	3/28/01	158	9	9	18	10	10	20	15	16	18	18	18	10	3	13	7	15
4055002	UT LINE FORK	REF	4/16/98	169	9	8	17	9	8	17	15	18	19	16	16	10	10	20	16	15
5037002	BOTTS FORK	REF	4/18/02	161	4	6	10	5	8	13	15	18	17	19	17	10	10	20	14	18
5037004	WELCH FORK	REF	4/18/02	168	7	8	15	7	8	15	15	19	17	18	18	10	10	20	15	16
6012003	NICHOLS FORK	REF	4/29/02	156	6	6	12	7	7	14	17	18	16	16	16	10	10	20	10	17
6012004	MEADOW BRANCH	REF	4/30/02	148	5	5	10	6	6	12	16	20	15	15	16	10	10	20	9	15
6013014	UT NEWCOMBE CREEK	REF	3/14/02	171	10	10	20	10	10	20	15	16	16	17	19	9	9	20	14	16

Appendix D. Complete list of taxa from REF and TEST sites indicating the number of sites where taxa were collected. TV= Tolerance Value, Clinger habit denoted by "X".

Order	Family	FinalID	TV	Clinger	REF	TEST
Tricladida	Planariidae	Unidentified Planariid	5.0		3	1
Hoplonemertea	Prostomidae	Prostoma sp	6.1	X	0	2
Lymnophila	Ancylidae	Ferrissia rivularis	6.6		3	0
Lymnophila	Lymnaeidae	Fossaria sp	7.0		0	1
Lymnophila	Lymnaeidae	Lymnaea sp	7.0		0	7
Lymnophila	Lymnaeidae	Stagnicola sp	8.2		0	1
Lymnophila	Lymnaeidae	Unidentified Lymnaeid	7.0		0	1
Basommatophora	Physidae	Physella sp	8.8		0	8
Heterodonta	Sphaeriidae	Pisidium sp	6.5		3	0
Heterodonta	Sphaeriidae	Sphaerium sp	7.6		8	3
Lumbriculida	Lumbriculidae	Eclipidrilus sp	7.3		1	1
Lumbriculida	Lumbriculidae	Unidentified Lumbriculid	7.3		31	19
Haplotaxida	Naididae	Nais sp	8.9		1	0
Haplotaxida	Naididae	Unidentified Naidid	9.1		6	1
Haplotaxida	Tubificidae	Unidentified Tubificidae	9.0		1	0
Ephemeroptera	Leptophlebiidae	Choroterpes sp	2.3	X	2	0
Ephemeroptera	Leptophlebiidae	Habrophlebia vibrans	0.5		6	0
Ephemeroptera	Leptophlebiidae	Leptophlebia sp	6.2		1	0
Ephemeroptera	Leptophlebiidae	Paraleptophlebia sp	0.9		28	8
Ephemeroptera	Isonychiidae	Isonychia sp	3.5		3	7
Ephemeroptera	Heptageniidae	Cinygmula subequalis	0.0	X	32	4
Ephemeroptera	Heptageniidae	Epeorus sp	1.3	X	38	8
Ephemeroptera	Heptageniidae	Leucrocuta aphrodite	2.4	X	2	0
Ephemeroptera	Heptageniidae	Leucrocuta juno	2.8	X	2	0
Ephemeroptera	Heptageniidae	Leucrocuta sp	2.4	X	2	0
Ephemeroptera	Heptageniidae	Stenacron carolina	1.1	X	2	0
Ephemeroptera	Heptageniidae	Stenacron gildersleevi	2.5	X	9	0
Ephemeroptera	Heptageniidae	Stenacron interpunctatum	6.9	X	8	2
Ephemeroptera	Heptageniidae	Stenacron minnetonka	4.0	X	6	1
Ephemeroptera	Heptageniidae	Stenacron pallidum	2.7	X	12	1
Ephemeroptera	Heptageniidae	Stenacron sp	4.0	X	7	1
Ephemeroptera	Heptageniidae	Stenonema femoratum	7.2	X	4	3
Ephemeroptera	Heptageniidae	Stenonema ithaca	3.6	X	1	0
Ephemeroptera	Heptageniidae	Stenonema meririvulanum	0.1	X	11	0
Ephemeroptera	Heptageniidae	Stenonema modestum	5.5	X	2	2
Ephemeroptera	Heptageniidae	Stenonema pudicum	2.0	X	0	1
Ephemeroptera	Heptageniidae	Stenonema vicarium	1.3	X	19	5
Ephemeroptera	Siphlonuridae	Siphlonurus sp	5.8		1	0
Ephemeroptera	Ameletidae	Ameletus sp	2.4		37	15
Ephemeroptera	Tricorythidae	Tricorythodes sp	5.1		0	1
Ephemeroptera	Ephemeridae	Ephemera guttulata	0.0		11	1
Ephemeroptera	Ephemeridae	Ephemera simulans	2.2		6	2
Ephemeroptera	Ephemeridae	Ephemera sp	1.1		3	2
Ephemeroptera	Ephemerellidae	Attenella attenuata	1.6	X	1	0
Ephemeroptera	Ephemerellidae	Drunella cornutella	0.0	X	2	0
Ephemeroptera	Ephemerellidae	Drunella sp	0.7	X	21	6
Ephemeroptera	Ephemerellidae	Ephemerella dorothea	1.7	X	1	0
Ephemeroptera	Ephemerellidae	Ephemerella sp	2.0	X	36	16
Ephemeroptera	Ephemerellidae	Eurylophella funeralis	2.1	X	4	0
Ephemeroptera	Ephemerellidae	Eurylophella macdunnoughi	1.5	X	1	0
Ephemeroptera	Ephemerellidae	Eurylophella sp	4.3	X	35	17
Ephemeroptera	Ephemerellidae	Serratella sp	2.7	X	3	0
Ephemeroptera	Ephemerellidae	Timpanoga lita	0.0	X	1	0
Ephemeroptera	Caenidae	Caenis latipennis	7.4		0	1
Ephemeroptera	Caenidae	Caenis sp	7.4		1	6
Ephemeroptera	Baetidae	Acentrella ampla	3.6		17	8
Ephemeroptera	Baetidae	Acentrella sp	3.6		8	0
Ephemeroptera	Baetidae	Acentrella turbida	3.6		9	7
Ephemeroptera	Baetidae	Acerpenna macdunnoughi	5.4		2	1
Ephemeroptera	Baetidae	Baetis brunneicolor	5.4		1	0
Ephemeroptera	Baetidae	Baetis flavistriga	6.6		10	0
Ephemeroptera	Baetidae	Baetis intercalaris	5.0		1	2
Ephemeroptera	Baetidae	Baetis sp	5.4		8	5
Ephemeroptera	Baetidae	Baetis sp #1	5.4		1	0
Ephemeroptera	Baetidae	Baetis tricaudatus	1.6		5	3
Ephemeroptera	Baetidae	Callibaetis sp	9.8		1	0
Ephemeroptera	Baetidae	Centroptilum sp	6.6		5	4

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Order	Family	FinalID	TV	Clinger	REF	TEST
Ephemeroptera	Baetidae	Diphetor hageni	1.6		10	1
Ephemeroptera	Baetidae	Plauditus dubius	5.4		1	1
Ephemeroptera	Baetidae	Plauditus sp	5.4		6	4
Ephemeroptera	Baetidae	Procloeon sp	5.0		4	0
Ephemeroptera	Baetidae	Unidentified Baetid	5.0		1	0
Ephemeroptera	Baetiscidae	Baetisca lacustris	1.0		1	0
Ephemeroptera	Baetiscidae	Baetisca sp	2.1		1	1
Odonata	Coenagrionidae	Argia moesta	8.2		0	1
Odonata	Coenagrionidae	Argia sedula	8.5		0	1
Odonata	Coenagrionidae	Argia sp	8.2		0	3
Odonata	Coenagrionidae	Argia tibialis	8.2		0	1
Odonata	Coenagrionidae	Enallagma sp	8.9		0	1
Odonata	Coenagrionidae	Ischnura sp	9.5		0	1
Odonata	Calopterygidae	Calopteryx maculata	7.8		13	14
Odonata	Aeshnidae	Boyeria grafiana	6.1		14	5
Odonata	Aeshnidae	Boyeria vinosa	5.9		1	5
Odonata	Gomphidae	Gomphus sp	5.8		1	2
Odonata	Gomphidae	Lanthus parvulus	1.8		1	0
Odonata	Gomphidae	Lanthus sp	1.8		10	1
Odonata	Gomphidae	Progomphus obscurus	8.2		0	1
Odonata	Gomphidae	Stylogomphus albistylus	4.7		20	11
Odonata	Corduliidae	Unidentified Corduliid	6.6		0	1
Odonata	Libellulidae	Libellula sp	9.6		3	1
Odonata	Cordulegastridae	Cordulegaster erronea	5.7		1	1
Odonata	Cordulegastridae	Cordulegaster maculata	5.7		0	2
Odonata	Cordulegastridae	Cordulegaster sp	5.7		24	13
Plecoptera	Pteronarcyidae	Pteronarcys proteus	1.7	X	4	0
Plecoptera	Perlodidae	Clioperla clio	4.7	X	14	2
Plecoptera	Perlodidae	Diploperla robusta	2.7	X	18	9
Plecoptera	Perlodidae	Isoperla bilineata	5.4	X	1	0
Plecoptera	Perlodidae	Isoperla holochlora	0.0	X	24	6
Plecoptera	Perlodidae	Isoperla sp	1.8	X	29	14
Plecoptera	Perlodidae	Malirekus hastatus	1.2	X	8	4
Plecoptera	Perlodidae	Remenus bilobatus	0.3	X	12	1
Plecoptera	Perlodidae	Yugus sp	0.0	X	18	1
Plecoptera	Capniidae	Allocapnia sp	2.5		0	1
Plecoptera	Capniidae	Paracapnia angulata	0.1		1	0
Plecoptera	Peltoperlidae	Peltoperla arcuata	1.0	X	19	4
Plecoptera	Peltoperlidae	Tallaperla sp	1.2	X	1	0
Plecoptera	Nemouridae	Amphinemura delosa	3.3		2	0
Plecoptera	Nemouridae	Amphinemura nigritta	3.3		0	2
Plecoptera	Nemouridae	Amphinemura sp	3.3		36	20
Plecoptera	Nemouridae	Amphinemura wui	3.3		2	0
Plecoptera	Nemouridae	Ostrocerca sp	2.5		11	6
Plecoptera	Nemouridae	Paranemura perfecta	2.0		2	1
Plecotera	Nemouridae	Soyedina vallicularia	0.0		2	0
Plecoptera	Leuctridae	Leuctra ferruginea	0.7		1	0
Plecoptera	Leuctridae	Leuctra sibleyi	0.7		2	0
Plecoptera	Leuctridae	Leuctra sp	0.7		36	13
Plecoptera	Leuctridae	Paraleuctra sp	2.8		1	0
Plecoptera	Taeniopterygidae	Strophopteryx sp	2.7		1	1
Plecoptera	Taeniopterygidae	Taenionema atlanticum	5.0		3	1
Plecoptera	Taeniopterygidae	Taeniopteryx sp	5.4		0	1
Plecoptera	Perlidae	Acroneuria abnormis	2.1	X	14	2
Plecoptera	Perlidae	Acroneuria carolinensis	0.0	X	23	6
Plecoptera	Perlidae	Acroneuria sp	1.4	X	0	1
Plecoptera	Perlidae	Eccoptura xanthenes	3.7	X	12	4
Plecoptera	Perlidae	Perlesta sp	4.7	X	1	0
Plecoptera	Chloroperlidae	Alloperla sp	1.2	X	6	1
Plecoptera	Chloroperlidae	Haploperla brevis	1.0	X	26	3
Plecoptera	Chloroperlidae	Sweltsa sp	0.0	X	23	6
Hemiptera	Corixidae	Unidentified Corixid	9.0		1	1
Hemiptera	Veliidae	Microvelia sp	9.0		1	0
Hemiptera	Notonectidae	Notonecta sp	8.7		0	1
Megaloptera	Corydalidae	Corydalus cornutus	5.2	X	0	11
Megaloptera	Corydalidae	Nigronia fasciatus	5.6	X	19	5
Megaloptera	Corydalidae	Nigronia serricornis	5.0	X	9	9
Megaloptera	Sialidae	Sialis sp	7.2		6	6
Trichoptera	Limnephilidae	Goera sp	0.1		14	0

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Order	Family	FinalID	TV	Clinger	REF	TEST
Trichoptera	Limnephilidae	Ironoquia sp	7.7		5	5
Trichoptera	Limnephilidae	Pycnopsyche gentilis	0.6		6	1
Trichoptera	Limnephilidae	Pycnopsyche sp	2.5		13	3
Trichoptera	Limnephilidae	Pycnopsyche sp1	2.5		28	7
Trichoptera	Limnephilidae	Pycnopsyche sp2	2.5		12	1
Trichoptera	Molannidae	Molanna blenda	2.0		4	0
Trichoptera	Hydroptilidae	Hydroptila sp	6.2	X	5	2
Trichoptera	Hydroptilidae	Ochrotrichia sp	4.0	X	1	0
Trichoptera	Hydroptilidae	Stactobiella sp	1.3	X	1	0
Trichoptera	Lepidostomatidae	Lepidostoma sp	0.9		33	5
Trichoptera	Lepidostomatidae	Theliopsyche sp			1	0
Trichoptera	Leptoceridae	Nectopsyche exquisita	4.1		1	0
Trichoptera	Leptoceridae	Oecetis sp	4.7		0	1
Trichoptera	Leptoceridae	Triaenodes marginatus	4.5		0	1
Trichoptera	Leptoceridae	Triaenodes sp	4.5		2	0
Trichoptera	Calamoceratidae	Anisocentropus pyraloides	0.9		5	0
Trichoptera	Calamoceratidae	Heteroplectron americanum	3.2		3	0
Trichoptera	Rhyacophilidae	Rhyacophila carolina	1.0	X	25	8
Trichoptera	Rhyacophilidae	Rhyacophila fuscula	1.9	X	3	1
Trichoptera	Rhyacophilidae	Rhyacophila glaberrima	0.8	X	3	0
Trichoptera	Rhyacophilidae	Rhyacophila invaria gp	0.0	X	29	8
Trichoptera	Rhyacophilidae	Rhyacophila ledra/fenestra	3.9	X	9	1
Trichoptera	Rhyacophilidae	Rhyacophila lobifera	2.5	X	2	0
Trichoptera	Rhyacophilidae	Rhyacophila minor	0.0	X	9	0
Trichoptera	Rhyacophilidae	Rhyacophila nigrita	0.0	X	1	0
Trichoptera	Rhyacophilidae	Rhyacophila sp	0.8	X	4	1
Trichoptera	Rhyacophilidae	Rhyacophila torva	1.6	X	2	0
Trichoptera	Helicopsychidae	Helicopsyche borealis	5.0	X	2	0
Trichoptera	Uenoidae	Neophylax sp	2.2	X	39	10
Trichoptera	Glossosomatidae	Agapetus sp	0.0	X	10	2
Trichoptera	Glossosomatidae	Glossosoma intermedium	1.6	X	1	0
Trichoptera	Glossosomatidae	Glossosoma sp	1.6	X	2	0
Trichoptera	Hydropsychidae	Ceratopsyche bronta	2.7	X	1	5
Trichoptera	Hydropsychidae	Ceratopsyche cheilonis	1.4	X	0	1
Trichoptera	Hydropsychidae	Ceratopsyche slossonae	0.0	X	1	1
Trichoptera	Hydropsychidae	Ceratopsyche sp	1.4	X	1	0
Trichoptera	Hydropsychidae	Ceratopsyche sparna	3.2	X	1	3
Trichoptera	Hydropsychidae	Ceratopsyche ventura	0.0	X	5	0
Trichoptera	Hydropsychidae	Cheumatopsyche sp	6.2	X	25	17
Trichoptera	Hydropsychidae	Diplectrona metaqui	2.0	X	1	0
Trichoptera	Hydropsychidae	Diplectrona modesta	2.2	X	37	15
Trichoptera	Hydropsychidae	Homoplectra doringa	3.0	X	2	1
Trichoptera	Hydropsychidae	Hydropsyche betteni	7.8	X	13	15
Trichoptera	Hydropsychidae	Hydropsyche sp	4.0	X	1	1
Trichoptera	Psychomyiidae	Lype diversa	4.1	X	7	1
Trichoptera	Psychomyiidae	Psychomyia flavida	2.9	X	2	0
Trichoptera	Odontoceridae	Psilotreta sp	0.0	X	1	0
Trichoptera	Philopotamidae	Chimarra aterrima	2.0	X	3	4
Trichoptera	Philopotamidae	Chimarra sp	2.8	X	3	8
Trichoptera	Philopotamidae	Dolophilodes distinctus	0.8	X	10	2
Trichoptera	Philopotamidae	Wormaldia moesta	0.7	X	1	0
Trichoptera	Philopotamidae	Wormaldia sp	0.7	X	30	1
Trichoptera	Polycentropodidae	Nyctiophylax sp	0.9	X	7	2
Trichoptera	Polycentropodidae	Phylocentropus carolinus	5.6		2	0
Trichoptera	Polycentropodidae	Polycentropus sp	3.5	X	30	9
Coleoptera	Dryopidae	Helichus basalis	4.6	X	23	7
Coleoptera	Dryopidae	Helichus fastigiatus	4.6	X	15	10
Coleoptera	Dryopidae	Helichus lithophilus	4.6	X	3	1
Coleoptera	Psephenidae	Ectopria sp larva	4.2	X	33	5
Coleoptera	Psephenidae	Psephenus herricki	2.4	X	27	15
Coleoptera	Ptilodactylidae	Anchytarsus bicolor	3.6	X	13	1
Coleoptera	Hydrophilidae	Cymbiodyta sp	8.3		1	0
Coleoptera	Hydrophilidae	Hydrobius tumidus	8.3		0	1
Coleoptera	Hydrophilidae	Tropisternus sp (larvae)	9.7		0	3
Coleoptera	Hydrophilidae	Unidentified Hydrophilid	6.3		0	1
Coleoptera	Gyrinidae	Dineutus assimilis	5.5		0	1
Coleoptera	Dytiscidae	Agabus punctatus	8.9		1	0
Coleoptera	Dytiscidae	Hydroporus sp	8.6		2	0
Coleoptera	Dytiscidae	Neoporus sp	8.9		1	1

Appendix D. Complete list of taxa from REF and TEST sites indicating the number of sites where taxa were collected. TV= Tolerance Value, Clinger habit denoted by "X".

Order	Family	FinalID	TV	Clinger	REF	TEST
Coleoptera	Elmidae	Dubiraphia vittata	4.1	X	0	5
Coleoptera	Elmidae	Macronychus glabratus	4.6	X	0	3
Coleoptera	Elmidae	Microcylloepus pusillus	2.1	X	1	0
Coleoptera	Elmidae	Optioservus ovalis	2.4	X	7	9
Coleoptera	Elmidae	Optioservus sp	2.4	X	2	0
Coleoptera	Elmidae	Optioservus sp(larvae)	2.4	X	26	12
Coleoptera	Elmidae	Optioservus trivittatus	2.4	X	2	0
Coleoptera	Elmidae	Oulimnius latiusculus	1.8	X	14	0
Coleoptera	Elmidae	Promoresia elegans	2.2	X	2	0
Coleoptera	Elmidae	Promoresia sp (larvae)	2.4	X	1	0
Coleoptera	Elmidae	Promoresia tardella	0.0	X	1	1
Coleoptera	Elmidae	Stenelmis crenata	5.1	X	25	3
Coleoptera	Elmidae	Stenelmis sp(larvae)	5.1	X	10	7
Diptera	Muscidae	Limnophora sp	8.4		0	1
Diptera	Chaoboridae	Chaoborus sp	8.5		0	1
Diptera	Tipulidae	Antocha sp	4.3	X	11	7
Diptera	Tipulidae	Dicranota sp	0.0		10	2
Diptera	Tipulidae	Dolichopeza sp	5.5		1	0
Diptera	Tipulidae	Hexatoma sp	4.3		35	7
Diptera	Tipulidae	Limnophila sp	4.9		3	1
Diptera	Tipulidae	Limonia sp	9.6		2	3
Diptera	Tipulidae	Ormosia sp	4.9		5	1
Diptera	Tipulidae	Pedicia sp	4.9		1	0
Diptera	Tipulidae	Pseudolimnophila sp	7.2		21	3
Diptera	Tipulidae	Tipula sp	7.3		37	20
Diptera	Tipulidae	Tipula sp1	7.3		3	2
Diptera	Tipulidae	Unidentified Tipulid	5.0		9	3
Diptera	Empididae	Chelifera sp	8.1		3	0
Diptera	Empididae	Clinocera sp	8.1	X	8	3
Diptera	Empididae	Hemerodromia sp	8.1		4	14
Diptera	Empididae	Unidentified Empidid	8.1		2	2
Diptera	Athericidae	Atherix sp	2.1		2	1
Diptera	Dixidae	Dixa sp	2.6		5	0
Diptera	Dixidae	Dixella sp	5.0		4	1
Diptera	Chironomidae	Ablabesmyia mallochi gr	7.2		2	1
Diptera	Chironomidae	Ablabesmyia sp	7.2		1	0
Diptera	Chironomidae	Brillia flavifrons	5.2		1	0
Diptera	Chironomidae	Cardiocladius sp	5.9	X	0	1
Diptera	Chironomidae	Chaetocladius sp			1	0
Diptera	Chironomidae	Chironomus sp	9.6		0	2
Diptera	Chironomidae	Corynoneura sp	6.0		0	1
Diptera	Chironomidae	Cricotopus annulator	7.0		1	0
Diptera	Chironomidae	Cricotopus bicinctus gr	8.5		0	3
Diptera	Chironomidae	Cricotopus sp	7.0		4	4
Diptera	Chironomidae	Cricotopus trifascia gr	2.8		1	0
Diptera	Chironomidae	Cricotopus/Orthocladius gr	7.1		11	17
Diptera	Chironomidae	Cryptochironomus sp	6.4		2	0
Diptera	Chironomidae	Diamesa sp	8.1		10	15
Diptera	Chironomidae	Dicrotendipes sp	8.1		1	1
Diptera	Chironomidae	Diplocladius sp	7.0		1	0
Diptera	Chironomidae	Endochironomus sp	7.8		1	0
Diptera	Chironomidae	Epoicocladius sp	2.0		2	0
Diptera	Chironomidae	Eukiefferiella sp	3.4		14	7
Diptera	Chironomidae	Euryhapsis sp			0	1
Diptera	Chironomidae	Heleniella sp	0.0		2	0
Diptera	Chironomidae	Heterotrissocladius marcidus gr	5.4		0	1
Diptera	Chironomidae	Larsia sp	9.3		2	1
Diptera	Chironomidae	Lopescladius sp	1.7		2	0
Diptera	Chironomidae	Mesosmittia sp			0	1
Diptera	Chironomidae	Microspectra sp	1.5		15	3
Diptera	Chironomidae	Microtendipes pedellus gr	5.5		4	0
Diptera	Chironomidae	Microtendipes rydalensis gp	5.5		2	0
Diptera	Chironomidae	Microtendipes sp	5.5		18	5
Diptera	Chironomidae	Nanocladius sp	7.1		1	0
Diptera	Chironomidae	Natarsia sp	10.0		1	1
Diptera	Chironomidae	Nilotanypus sp	3.9		1	0
Diptera	Chironomidae	Orthocladius sp	7.3		2	1
Diptera	Chironomidae	Parachaetocladius sp	0.0		8	1
Diptera	Chironomidae	Parametriocnemus lundbecki	3.7		30	18

Appendix D. Complete list of taxa from REF and TEST sites indicating the number of sites where taxa were collected. TV= Tolerance Value, Clinger habit denoted by "X".

Order	Family	FinalID	TV	Clinger	REF	TEST
Diptera	Chironomidae	Parametriocnemus sp	3.7		2	1
Diptera	Chironomidae	Paratendipes albimanus	9.2	X	0	1
Diptera	Chironomidae	Phaenopsectra sp	6.5		4	0
Diptera	Chironomidae	Phaenopsectra/Tribelos sp	6.8		0	1
Diptera	Chironomidae	Polypedilum aviceps	3.7		4	1
Diptera	Chironomidae	Polypedilum fallax	6.4		2	1
Diptera	Chironomidae	Polypedilum flavum	5.3		12	4
Diptera	Chironomidae	Polypedilum illinoense	9.0		0	1
Diptera	Chironomidae	Polypedilum scalaenum gr	8.4		0	2
Diptera	Chironomidae	Polypedilum sp	6.8		3	1
Diptera	Chironomidae	Polypedilum tritum	6.8		1	1
Diptera	Chironomidae	Potthastia longimanus	6.5		1	0
Diptera	Chironomidae	Potthastia sp	6.4		3	1
Diptera	Chironomidae	Rheocricotopus sp	7.3		5	5
Diptera	Chironomidae	Rheotanytarsus sp	6.4	X	8	2
Diptera	Chironomidae	Stempellina sp	0.0		9	0
Diptera	Chironomidae	Stenochironomus sp	6.5		0	1
Diptera	Chironomidae	Stilocladius sp	5.0		1	0
Diptera	Chironomidae	Symposiocladius sp	5.4		1	1
Diptera	Chironomidae	Sympotthastia spinifera	5.7		0	1
Diptera	Chironomidae	Tanytarsus sp	6.7		23	10
Diptera	Chironomidae	Thienemanniella sp	5.9		1	3
Diptera	Chironomidae	Thienemannimyia gr	5.9		34	15
Diptera	Chironomidae	Tvetenia bavarica gr	3.7		2	0
Diptera	Chironomidae	Tvetenia discoloripes gr	3.6		0	1
Diptera	Chironomidae	Tvetenia sp	3.6		11	3
Diptera	Chironomidae	Unidentified Chironomid	5.0		4	2
Diptera	Chironomidae	Unidentified Larvae	5.0		1	0
Diptera	Chironomidae	Unidentified Podonominae			1	0
Diptera	Chironomidae	Unidentified Pupae	7.0		0	1
Diptera	Ephydridae	Unidentified Ephydrid	9.0		1	2
Diptera	Dolichopodidae	Unidentified Dolichopodid	5.0		1	0
Diptera	Psychodidae	Pericoma sp	10.0		1	0
Diptera	Psychodidae	Psychoda alternata	9.9		1	2
Diptera	Ptychopteridae	Ptychoptera sp	7.0		1	0
Diptera	Simuliidae	Prosimulium magmun	2.6	X	1	0
Diptera	Simuliidae	Prosimulium sp	4.0	X	30	9
Diptera	Simuliidae	Simulium sp	4.4	X	33	13
Diptera	Stratiomyidae	Myxosargus sp	10.0		0	1
Diptera	Stratiomyidae	Odontomyia sp	10.0		0	2
Diptera	Stratiomyidae	Stratiomys sp	8.1		0	1
Diptera	Tabanidae	Chrysops sp	6.7		2	1
Diptera	Tabanidae	Tabanus sp	9.2		1	1
Diptera	Tabanidae	Unidentified tabanid	8.6		6	0
Diptera	Ceratopogonidae	Bezzia/Palpomyia gr	6.9		10	2
Diptera	Ceratopogonidae	Culicoides sp	7.7		0	1
Diptera	Ceratopogonidae	Dasyhelea sp	6.7		2	0
Diptera	Ceratopogonidae	Monohelea sp	6.8		1	1
Diptera	Ceratopogonidae	Probezzia sp	6.9		2	2
Diptera	Sciaridae	Unidentified Sciarid	5.0		13	3
Hydracarina	Hydrachnidae	Unidentified Hydracarina (mite)	5.5		1	0
Amphipoda	Crangonyctidae	Crangonyx sp	8.0		3	1
Amphipoda	Gammaridae	Gammarus sp	8.0		2	0
Amphipoda	Talitridae	Hyalella azteca	7.8		1	1
Isopoda	Asellidae	Caecidotea sp	9.1		9	2
Isopoda	Asellidae	Lirceus fontinalis	7.9		7	3
Decapoda	Cambaridae	Cambarus bartonii cavatus	4.6		17	5
Decapoda	Cambaridae	Cambarus buntingi	4.9		2	1
Decapoda	Cambaridae	Cambarus cumberlandensis	4.1		3	2
Decapoda	Cambaridae	Cambarus distans	3.9		21	9
Decapoda	Cambaridae	Cambarus parvoculus	3.2		9	2
Decapoda	Cambaridae	Cambarus robustus	4.9		13	7
Decapoda	Cambaridae	Cambarus rusticiformis	4.0		3	1
Decapoda	Cambaridae	Cambarus sciotensis	6.4		0	2
Decapoda	Cambaridae	Cambarus sp	4.9		15	4
Decapoda	Cambaridae	Cambarus sphenoides	4.9		2	0
Decapoda	Cambaridae	Cambarus striatus	4.9		0	1
Decapoda	Cambaridae	Orconectes cristavarius	5.5		3	11
Decapoda	Cambaridae	Orconectes sp	5.5		1	6

Appendix E. MBI scores and metric values for all sites.

StationID	Program	StreamName	CollDate	Area (Mi2)	MBI	Genus TR	Genus EPT	mHBI	m%EPT	%Ephem	%Chir+Olig	%Clng	Family TR	Family EPT	FBI
1007005	REF	HOBBS FORK	4/11/01	1.15	91.7	56	31	2.77	78.95	56.43	2.05	69.59	27	17	3.53
1007006	REF	HOBBS FORK2	4/11/01	0.18	90.6	48	29	2.18	87.07	54.96	0.86	66.38	30	17	3.10
1017001	TEST	LONG BRANCH	4/23/02	0.36	41.1	41	9	5.61	44.21	0.00	26.45	10.74	18	8	5.36
1022008	TEST	CALEB FORK	5/ 1/02	1.78	19.9	21	4	6.70	0.88	0.88	54.39	2.63	14	3	7.07
1032001	REF	TOMS BRANCH	4/12/01	0.95	93.4	58	32	2.62	82.53	59.34	3.46	67.30	30	17	3.84
1032002	REF	LOWER PIGEON BRANCH	4/12/01	0.89	83.4	53	29	2.55	66.86	42.20	5.65	61.37	28	19	3.96
1032003	REF	LOWER PIGEON BRANCH	5/15/02	0.89	87.7	45	30	1.68	91.71	54.15	1.95	54.15	27	19	3.33
1032003	REF	LOWER PIGEON BRANCH	5/16/02	0.89	85.5	49	27	2.22	85.94	46.68	1.86	53.58	27	18	3.51
1032003	TEST	UPPER PIGEON BRANCH	5/16/02	2.01	28.4	35	8	6.33	9.13	6.25	69.23	15.87	20	6	6.27
2006027	TEST	HATCHELL BRANCH	4/19/00	0.35	57.5	29	18	3.69	46.25	30.63	0.30	16.52	16	14	3.92
2006030	REF	JACKIE BRANCH	4/20/00	1.14	82.2	53	25	2.94	62.53	43.40	4.85	69.81	29	18	3.90
2006031	REF	CANE CREEK	4/24/00	0.65	79.6	52	26	2.66	77.95	32.29	3.56	50.11	29	17	3.21
2008017	REF	UT ROCK CREEK1	4/12/00	0.82	85.5	57	30	3.25	62.02	40.87	2.56	75.48	29	20	3.90
2008018	REF	WATTS BRANCH	4/17/00	2.2	90.2	46	25	3.14	84.97	66.67	1.78	74.32	26	16	3.82
2008019	REF	PUNCHEONCAMP BRANCH	4/18/00	1.7	92.4	55	30	2.89	82.29	70.19	2.68	64.20	27	19	3.68
2008020	REF	UT ROCK CREEK3	4/18/00	0.63	89.2	56	26	2.68	74.92	52.10	1.95	76.88	30	19	3.79
2008021	REF	UT ROCK CREEK2	4/18/00	0.37	85.9	39	19	2.47	81.82	70.74	0.85	69.03	24	14	3.53
2008022	REF	UT BS FK CUMBERLAND	4/18/00	0.89	82.5	37	21	2.98	86.52	73.35	3.05	51.52	22	15	3.51
2008023	TEST	COFFEY BRANCH	4/19/00	1.25	75.5	41	21	3.24	78.65	48.54	11.24	45.62	25	17	3.80
2014004	TEST	JENNEYS BRANCH	4/19/00	0.66	48.2	37	13	5.71	27.74	6.19	28.54	53.49	17	9	5.63
2023004	REF	DRY FORK	4/19/01	2.05	65.6	34	18	3.67	34.49	25.93	0.54	68.78	23	14	4.15
2041003	REF	BROWNIES CREEK	4/26/00	2.3	70.9	52	31	2.93	50.10	18.38	2.22	34.55	31	22	3.85
2041004	TEST	BROWNIES CREEK2	4/26/00	0.31	61.4	39	24	2.53	36.32	18.25	0.71	23.21	24	18	3.74
2042002	TEST	EWING CREEK	4/26/00	3.06	42.7	25	11	4.88	32.20	20.34	33.90	18.64	16	9	5.49
2042003	REF	WATTS CREEK	3/29/01	0.85	83.2	61	34	2.19	68.11	17.27	6.71	66.19	35	20	3.33
2046002	TEST	BAD BRANCH	4/27/00	2.6	60.8	38	18	3.02	79.61	7.54	4.47	17.04	28	20	3.35
2046004	REF	PRESLEY HOUSE BRANCH	4/27/00	0.9	73.7	46	24	2.64	72.14	26.01	2.79	42.41	24	14	3.62
2046005	TEST	FRANKS CREEK	4/27/00	1.36	81.1	42	25	3.41	80.24	56.99	5.32	50.91	27	16	3.71
4036017	REF	STEER FORK	4/18/01	3	95.7	59	36	3.03	84.80	62.12	4.70	76.72	26	19	4.27
4042016	TEST	MF RED RIVER	4/10/02	1.80	69.3	57	28	4.14	50.44	37.24	29.91	38.12	26	18	3.79
4042703	REF	CHESTER CREEK	4/10/02	2.65	84.1	58	30	2.42	68.67	32.53	10.24	68.37	27	16	4.72
4050002	REF	CLEMONS FORK	5/14/99	2.0	80.5	66	32	3.12	59.80	35.78	12.99	51.72	25	18	3.57
4050007	TEST	FUGATE FORK	4/10/00	2.6	55.7	43	13	3.87	45.65	1.85	16.89	49.08	27	20	4.08
4050008	TEST	JENNY FORK	4/10/00	0.45	65.8	42	19	3.05	84.05	2.37	9.70	42.46	19	9	4.91
4050009	TEST	BEAR BRANCH	4/10/00	1.54	61.6	42	17	4.12	63.81	18.09	9.53	34.82	24	14	4.14
4050010	REF	CLEMONS FORK	4/10/00	0.8	90.3	59	30	2.55	74.12	51.97	2.69	68.74	19	11	4.84
4050011	REF	FALLING ROCK BRANCH	4/11/00	0.41	88.9	57	32	2.79	71.69	46.86	2.37	68.76	30	19	3.46
4050012	REF	JOHN CARPENTER FORK	4/12/00	0.58	76.7	40	22	2.98	59.94	42.98	0.88	63.16	30	21	3.46
4050013	REF	SHELLY ROCK FORK	4/11/00	0.55	85.6	38	20	2.41	78.84	62.09	0.70	73.26	23	17	3.57
4050014	REF	MILLSEAT BRANCH	4/11/00	0.58	82.0	53	31	2.45	75.42	24.92	7.41	61.95	23	15	3.10
4050015	REF	LITTLE MILLSEAT BRANCH	4/12/00	0.82	86.8	44	28	2.61	79.69	57.59	0.45	60.71	25	20	3.57
4050016	TEST	LICK BRANCH	4/12/00	2.81	34.9	21	8	6.87	35.77	0.00	48.54	40.51	26	18	3.15
4050017	TEST	WILLIAMS BRANCH	4/12/00	1.08	21.7	25	5	5.82	1.74	0.00	75.96	12.89	11	6	5.83
4050018	TEST	CANEY CREEK	4/12/00	2.5	37.0	36	10	5.42	9.62	5.13	44.23	28.85	13	4	6.10
4052017	REF	LITTLE DOUBLE CREEK	3/29/00	1.5	80.4	27	19	2.16	94.26	64.09	0.00	49.93	16	7	6.03
4052018	REF	RF BIG DOUBLE CREEK2	3/29/00	1.46	81.1	46	22	2.39	68.77	46.53	3.00	63.25	16	13	3.08
4052019	REF	LF BIG DOUBLE CREEK	3/29/00	0.6	87.4	52	25	2.55	74.42	54.09	1.53	69.69	23	15	3.59
4052020	REF	RF ELISHA CREEK	3/30/00	2.35	83.1	48	31	2.63	72.03	47.97	4.49	50.00	27	17	3.59
4052021	REF	BM FORK ELISHA CREEK	3/30/00	0.82	83.2	57	28	2.82	74.35	55.90	5.54	38.01	28	21	3.70
4052022	REF	LF ELISHA CREEK	3/30/00	2.47	85.7	42	25	2.52	81.80	69.32	0.52	50.95	33	18	3.81
4052023	REF	RF BIG DOUBLE CREEK	4/ 5/00	1.53	84.6	40	22	2.45	82.23	59.31	4.71	64.67	21	16	3.39
4052024	TEST	RED BIRD CREEK	4/ 5/00	1.4	49.7	28	13	4.66	42.14	13.21	10.06	27.67	24	15	3.36
4052025	TEST	MUD LICK BRANCH	4/ 5/00	1.1	85.2	42	24	2.49	75.52	60.00	0.90	63.58	19	11	4.88
4052026	TEST	LAWSON CREEK	4/ 5/00	1.48	59.3	33	15	4.65	49.65	31.91	4.26	35.46	24	15	3.48
4052027	TEST	SPRUCE BRANCH	4/ 6/00	0.95	91.8	43	26	2.39	88.17	76.10	1.16	74.25	20	10	4.77
4052028	TEST	GILBERTS LITTLE CREEK	4/ 6/00	1.47	33.6	32	11	5.33	5.94	2.74	28.31	6.39	23	16	3.48
4052029	TEST	ARNETTS FORK	4/ 6/00	1.42	74.6	27	20	2.09	97.06	51.47	0.00	30.15	16	9	5.90
4052030	REF	SUGAR CREEK	4/ 6/00	3.05	88.5	54	29	2.79	73.04	52.07	2.30	70.28	16	13	2.99
4054005	REF	CAWOOD BRANCH	3/28/01	0.8	69.2	38	20	2.95	58.12	21.57	3.81	58.12	28	18	3.73
4054007	TEST	LF CAMP CREEK	3/27/01	0.93	53.9	36	18	4.29	67.74	5.99	19.59	18.43	22	15	3.58
4054008	TEST	CAMP CREEK	3/27/01	2.7	41.4	29	15	4.36	22.94	5.29	51.76	32.94	20	12	4.82
4054009	REF	BILL BRANCH	3/28/01	2.3	91.9	43	28	1.99	91.16	59.18	2.04	82.99	16	9	5.05
4054010	REF	HONEY BRANCH	3/28/01	0.82	90.0	40	26	2.83	86.42	65.34	2.34	81.73	22	19	2.85
4055002	REF	UT LINE FORK	2/ 9/99	0.22	90.7	60	31	1.92	81.94	50.29	10.49	64.85	22	17	3.60
4055002	REF	UT LINE FORK	4/16/98	0.22	80.3	57	26	2.84	79.44	55.36	11.60	28.34	30	18	4.13
05037002	REF	BOTTS FORK	4/18/02	3.38	80.4	55	31	3.31	63.86	37.28	13.90	60.16	27	21	3.51
05037004	REF	WELCH FORK	4/18/02	1.5	83.1	62	36	2.62	67.47	28.80	8.00	56.80	26	19	4.18
06012003	REF	NICHOLS BRANCH	4/29/02	0.65	75.8	49	25	2.95	73.83	31.46	4.05	43.93	28	21	3.53
06012004	REF	MEADOW BRANCH	4/30/02	0.93	74.0	53	24	3.10	73.65	29.64	5.09	36.23	27	18	3.44
06013014	REF	UT NEWCOMBE CREEK	3/14/02	0.25	67.0	41	17	3.71	77.38	28.31	2.31	31.69	29	17	3.52

Appendix F. Pearson correlation coefficients for biological attributes and environmental/habitat variables for all sites. Values in italics are statistically significant ($p<0.01$).

	MBI	Genus TR	Genus EPT	mHBI	m%EPT	%Ephem	%Chir+Olig	%Clng
Area (mi^2)	-0.26	-0.19	-0.19	*0.34*	0.12	-0.17	0.32	-0.18
DO	-0.23	-0.15	-0.22	0.25	0.02	-0.19	0.41	0.00
pH	*-0.43*	-0.24	*-0.35*	*0.51*	*-0.39*	-0.33	0.62	-0.21
Spec. Cond.	*-0.66*	*-0.51*	*-0.59*	*0.71*	*-0.50*	*-0.53*	*0.73*	*-0.36*
Temp	-0.06	0.04	-0.06	0.14	0.09	0.02	0.00	-0.20
%Embed	*-0.59*	*-0.51*	*-0.60*	*0.75*	*-0.44*	*-0.39*	*0.58*	*-0.37*
RipWidth (m)	*0.55*	*0.53*	*0.59*	*-0.61*	*0.49*	0.31	-0.50	0.36
StrWidth (m)	0.17	0.14	0.21	-0.12	0.11	0.15	-0.09	0.14
Canopy Score	*0.60*	*0.51*	*0.65*	*-0.59*	*0.39*	*0.43*	*-0.46*	*0.44*
SubSize (cm)	-0.11	-0.14	-0.16	-0.09	-0.19	-0.15	-0.16	-0.18
Tot HabScore	*0.76*	*0.56*	*0.67*	*-0.73*	*0.52*	*0.46*	*-0.60*	*0.44*
Bank Stabilty Score	0.20	0.22	0.23	-0.18	0.09	0.05	-0.22	0.16
BankVeg Score	*0.44*	*0.46*	*0.49*	*-0.46*	*0.32*	0.20	*-0.44*	*0.32*
ChanAlter Score	*0.51*	*0.45*	*0.52*	*-0.56*	*0.40*	0.32	*-0.39*	*0.41*
Embeddedness Score	*0.61*	*0.55*	*0.66*	*-0.69*	*0.47*	*0.40*	*-0.55*	*0.38*
EpiFauSub Score	*0.65*	*0.50*	*0.64*	*-0.73*	*0.56*	*0.51*	*-0.56*	*0.42*
FreqOfRiffles Score	0.19	0.07	0.16	-0.30	0.10	0.19	-0.20	0.07
RipScore Score	*0.62*	*0.46*	*0.59*	*-0.66*	*0.52*	*0.46*	*-0.53*	*0.45*
SedDep Score	*0.40*	*0.37*	*0.37*	*-0.43*	*0.40*	*0.31*	*-0.41*	*0.24*
Vel/Dep Regime Score	*0.41*	0.30	*0.44*	*-0.45*	*0.36*	0.32	*-0.34*	0.24