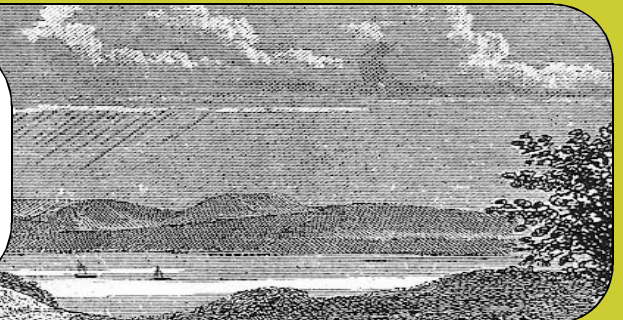


DEVELOPMENT OF A BENTHIC INDEX OF BIOTIC INTEGRITY FOR MARYLAND STREAMS



CHESAPEAKE BAY AND
WATERSHED PROGRAMS
MONITORING AND
NON-TIDAL ASSESSMENT
CBWP-MANTA - EA-98-3



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Development of a Benthic Index of Biotic Integrity for Maryland Streams

Report no. CBWP-EA-98-3

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December 1998

Acknowledgement

This project was funded by Maryland Department of Natural Resources Power Plant Topical Research Program under Contract # CB95-003-002. We are extremely grateful to Dr. Paul Miller (MDNR) for his interest in and management of this project throughout substantial DNR reorganization. Dr. Ron Klauda (MDNR) has been instrumental in advancing the development and use of ecological indicators in Maryland. This research results from ecological indicator development by the Maryland Biological Stream Survey (MBSS), a cooperative effort among state agencies, several counties, academia, and consulting groups. The benthic IBI workgroup consists of the authors, Walt Butler, Niles Primrose, and Scott Stranko (MDNR), Nancy Roth and Mark Southerland (Versar), Ray Morgan, II (University of Maryland, Appalachian Laboratory), Lenwood Hall (University of Maryland, Wye Research Center) and Kieth Van Ness (Montgomery County, Department of Environmental Protection). We thank all of these individuals for their technical discussions and input into this process. Drs. Jeroen Gerritsen and Mike Barbour provided initial discussions on statistical and other procedural concerns. We are grateful to two outside reviewers, Drs. Reese Voshell (Virginia Polytechnic Institute and State University, Blacksburg, VA) and Billie Kerans (Montana State University, Bozeman, Montana), whose careful review and thoughtful comments helped improve this report.

Abstract

To help the state of Maryland assess the impact of acid deposition on its streams and to address the principal Clean Water Act goal of biological integrity, the Department of Natural Resources implemented the Maryland Biological Stream Survey (MBSS) in 1994 to monitor and assess small to medium-sized streams across the state using a probability-based network design. Biological condition is the primary indicator of ecological quality, with physical habitat, water chemistry, and land use characteristics providing indicators of stressors. The biological condition indicator developed through this project is the Index of Biological Integrity (IBI), an approach that 1) aggregates multiple characteristics of a biological assemblage; 2) establishes regional reference conditions, and; 3) allows direct translation of raw data to narrative assessments of site conditions. This workgroup-based effort follows development of a fish IBI, and it has been simultaneous with that for an index of physical habitat quality. Benthic macroinvertebrate data collected in 1994 and 1995 were used to develop the IBI; additional data from 1996 and 1997 were used to test its effectiveness in detecting stream impairment. Using physical, chemical, and land use criteria, reference and degraded sites were identified from among approximately 1,100 sites randomly selected and sampled. Biological metrics were calculated from the data and impairment decision thresholds were determined based on values produced from reference site data. The IBI was found to be most efficient when calibrated separately for 1) low-gradient Coastal Plain streams and for 2) higher gradient non-Coastal Plain streams, with classification efficiencies of 87% and 88%, respectively. Seven metrics were used in the Coastal Plain IBI, total number of taxa, number of EPT taxa, % Ephemeroptera, % Tanytarsini of Chironomidae, Beck's Biotic Index, number of scraper taxa, and % clingers. Nine metrics were used in the non-Coastal Plain; total number of taxa, number of EPT taxa, number of Ephemeroptera taxa, number of Diptera taxa, % Ephemeroptera, % Tanytarsini, number of intolerant taxa, % tolerant individuals, and % collectors.

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I. INTRODUCTION

The Maryland Biological Stream Survey (MBSS) was implemented by the State Department of Natural Resources (MDNR) to provide to the public and natural resource decisionmakers an accounting of the biological status of non-tidal streams and watersheds statewide (Kazyak and Jacobson 1994). In addition to the Department's mandate to assess impacts of acid deposition, their impetus was the Clean Water Act of 1972 and its directive "to protect and restore the chemical, physical, and biological integrity of our Nation's waters" and the recognition that existing stream assessments were not addressing biological integrity. Biological integrity is defined by Frey (1977) and Karr et al. (1986) as the capacity of an ecosystem to support and maintain a biota that is comparable to that found in natural conditions. Development of a biological indicator in this framework required objective definition of reference conditions and of the measures that are used to describe the biota.

A geographically-broad response to this need required an organized, systematic sampling and analysis of indicators of stream quality (Index of Biological Integrity [IBI] for fish and benthic macroinvertebrates) across Maryland, beginning with non-tidal streams. The MBSS developed regionally calibrated indicators following four years of stream surveys collecting physical, chemical, biological, and land use data. A provisional indicator of biological condition using fish assemblage data has been developed (Roth et al. 1997) from those four years of sampling (1994-97). This report presents the process and results of developing an IBI from the MBSS's benthic macroinvertebrate database.

Reference Conditions

Reference conditions, as used here, are numerical descriptions of the variability of biological measurements taken from a composite of multiple reference sites (Gibson et al. 1996, Barbour et al. 1996). Reference sites are generally defined as those sites having minimal exposure to human activities and are representative of the waterbody type and region of interest (Hughes et al. 1986). More specifically, stream reference sites have criteria for in-channel physical and chemical conditions, riparian conditions, and land use that dictate their inclusion within a reference database. These criteria, which can exclude sites from consideration as reference, vary by waterbody type and region and can be developed either *a priori* or *a posteriori* (Gibson et al. 1996). The database of reference sites and the analyses performed in developing and calibrating reference conditions provide an objective, framework for determining ecological impairment of streams. The IBI developed here will be useful in assessing stream ecological conditions across the state in individual streams and small watersheds, and those assessments may be extrapolated to characterize the large river basins.

Biological Measurements and Their Characteristics

Biota are affected by environmental conditions at multiple levels of organization including genes, cells, individuals, species, assemblages, communities, and populations (Karr 1991). Since different stressors can have variable effects on biota, response to changes in

environmental conditions can be reflected at any of these levels and perhaps simultaneously at multiple levels. Because of this complexity, it is desirable to use a method of characterizing components of the community or assemblage that integrates and composites multiple, quantitative descriptors of that assemblage. Karr et al. (1986) developed the multimetric approach (the Index of Biological Integrity or IBI) that combined a series of metrics (biological descriptors) to characterize biological condition with fish assemblage data from streams of the Midwestern U. S. There have been numerous adaptations of the approach using different groups of organisms and calibrated for different geographic areas and waterbody types (Southerland and Stribling 1995, Davis et al. 1996, U.S. EPA 1997). The approach has also been endorsed by the Intergovernmental Task Force on Monitoring Water Quality (ITFM 1995) and the U. S. Environmental Protection Agency (Gibson et al. 1996) as an appropriate means for assessing biological condition, and is used by numerous states in water resource management and regulatory programs (Southerland and Stribling 1995, Davis et al. 1996).

The MBSS sampled approximately 1,100 stream site locations across the state from 1994 to 1997. The database for IBI development consisted of fish and benthic macroinvertebrate assemblage, chemical, physical habitat, and land use data (Roth et al. 1997). The resulting indicators of biological and physical habitat quality are (or will be) based on the database assembled by the MBSS over four years of field sampling and analysis. The purpose of this report is to document the analytical process and the resulting benthic IBI that will be used to assess streams in Maryland.

The benthic macroinvertebrate assemblage has been used as an indicator of stream conditions for many years (Cairns and Pratt 1993). Benthic macroinvertebrates are resident in aquatic ecosystems during some or all periods of their life histories. Benthic macroinvertebrates are useful indicators of stream and ecosystem condition because they respond to short-term episodic events, such as flooding or toxic discharges, and longer-term cumulative effects of climatic or landscape level changes.

Previous efforts at developing benthic macroinvertebrate-based multimetric indices in Maryland (Stribling et al. 1989, 1996, Gerritsen et al. 1995, Van Ness et al. 1997, Maxted et al. 1998) used the same conceptual framework but somewhat different approaches in field sampling methods, selection of reference sites, and development of scoring criteria. The dataset used in this project is the largest of all of these studies. It is statewide, encompassing much of Maryland's geographic range and physiographic variability, has used consistent sampling and analytical methods, and uses sampling sites selected on a probability basis. However, the number of stream site locations within this current database that met reference criteria was fairly small (37) and was not distributed evenly across Maryland's physiographic regions (Figure 1). The implications of a small and clustered distribution of reference sites are that benthic IBI development may not capture all of the variability inherent in the state.

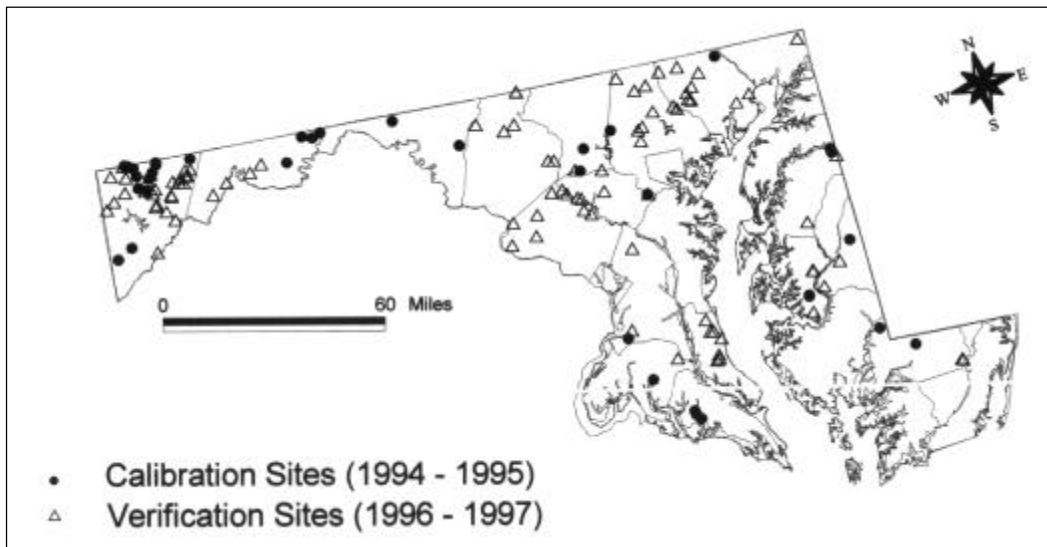


Figure 1. Locations of calibration (1994-1995) and verification (1996-1997) reference sites across the state of Maryland.

II. METHODS

Reference and degraded sites were previously determined following establishment of criteria for these two site groups. For this study, appropriate and relevant strata (or site classes) were determined by examining the geographic variability of the biological data. Candidate metrics were calculated, and their responsiveness to stressors evaluated, using data from reference and degraded sites. Multiple combinations of metrics were tested for their efficiency in correctly categorizing known impaired and nonimpaired sites, and a final index formed. The steps are as follows:

- Step 1. Developing the Data Base
- Step 2. Identifying Reference and Degraded Sites
- Step 3. Determining Appropriate Strata
- Step 4. Compiling Candidate Metrics
- Step 5. Testing Candidate Metrics
- Step 6. Combining Metrics into an Index

This project also tested the effectiveness of the index using an independent data set. The index

was developed and calibrated using sampling results from 1994-95 (181 sites); confirmation relied on 1996-97 results (574 sites). Using the same data (1994-95), we also developed a family-level index, allowing coarse assessments to be performed at a greater cost efficiency. Finally, we discuss the feasibility of using order-level data for performing assessments.

The approach followed in developing this benthic IBI was similar in many respects to those advocated by others, but varies in some details. However, it is identical to that used by the MBSS in development of the IBI using fish assemblage data (Roth et al. 1997).

A. Developing the Database

The database developed previously for water chemistry, physical habitat, and land use (Roth et al. 1997) required no alteration for this research, and only minor formatting changes were necessary for macroinvertebrate sampling and taxonomic data. Appendix A provides the list of all taxa used in these analyses.

1. Site Selection

All sampling sites were selected on a stratified probability basis (randomly within category) (Roth et al. 1997). The most coarse categories were basins within the eastern, central, and western portions of Maryland, making up a total of 18 distinct drainages. The first-level random selection was two basins within each of the geographic regions for each sampling year.

Potential sampling segments comprised all first through third order streams (Strahler 1957), which are about 90% of lotic systems in the state. Selection of stream segments by order, in proportion to their abundance within a basin, make up the second-level of random selection.

2. Benthic Macroinvertebrate Sampling and Processing

Benthic macroinvertebrates were sampled in the spring index period (March 1 to May 1) as outlined in the MBSS Sampling Manual (Kazyak 1995). To summarize, a 600 micron mesh D-net was used to trap organisms dislodged from approximately 20 square feet of multiple habitat types. Riffles and other productive habitat types were sampled preferentially when available in the 75m sampling segment. The composited sample was preserved and subsampled to approximately 100 individual macroinvertebrates. If a sample contained less than 80 organisms, it was not used in metric testing and evaluation. Most organisms were identified to genus, if possible, using stereoscopes. Chironomidae were slide-mounted and identified using compound microscopes. A list of taxa and their abundance within each subsample was generated from laboratory identifications.

3. Water Chemistry

Water samples were collected during the spring index period and analyzed in the laboratory for pH, acid neutralizing capacity (ANC), conductivity, sulfate, nitrate, and dissolved organic carbon (DOC) using standard methods (Roth et al. 1997). During the summer, *in situ* measurements of dissolved oxygen (DO), pH, temperature, and conductivity were made. This combination of variables, in part, describes basic water quality conditions with an emphasis on those that may impact aquatic life.

4. Physical Habitat

Instream and riparian conditions were assessed for fish and benthic macroinvertebrate habitat quality at all sites as specified for the MBSS in Kazyak (1995) and Roth et al. (1997). Within each stream segment, scores were assigned to a set of habitat parameters based on visual field observations. Substrate types, habitat features, bank conditions, riparian vegetation width, remoteness, aesthetic value, and other parameters which describe potential natural and anthropogenic stresses were scored individually. A summary of all physical habitat information is presented in Roth et al. (1997, Appendix F).

5. Land Use

Urban, agricultural, and forested land use in the catchment area of each site has been previously determined (Roth et al. 1997). The catchment of each site was digitized from county topographic maps (1:62,500 scale) and areal percentages of 1990 land uses were determined. This land use information was considered among a set of criteria for helping determine the reference status of the site.

B. Identifying Reference and Degraded Sites

Reference and degraded sites used for selection and calibration of benthic macroinvertebrate metrics are the same as those used for the Maryland Fish IBI (Roth et al. 1997). They were designated as reference or degraded based on chemical and physical criteria that comprise a mixture of laboratory analytical chemistry, field chemistry, visual-based physical habitat and riparian conditions, and land use (Table II-1) as determined by the MBSS Indicators Workgroup. High-end (reference) criteria were determined based on groupings of parameter measurement values that ensured adequate numbers of minimally-impaired sites in each potential site grouping. Low-end (degraded) criteria were selected to ensure that sites clearly exposed to human-induced stressors were used for metric selection and index calibration. The set of stressors is representative of any of three types of degradation, including acidification, eutrophication, or physical habitat alteration. Similar to the reference criteria, these were set to ensure adequate representation by degraded sites in each potential site class. Specific criteria were determined by break points or groupings within scatterplots. Sites were considered as reference *if they met all of the reference site criteria* and degraded *if they met any of the*

degraded site criteria. Thirty-seven sites meeting *all* reference criteria were identified; as were 51 sites meeting *any* of the degraded criteria.

Table II-1. Criteria used for designating reference and degraded stream sites (adapted from Roth et al. 1997).

Reference (all criteria must be met)	Degraded (any criterion must be met)
pH ≥ 6 (if blackwater stream, pH < 6 and DOC ≥ 8 mg/l) ANC ≥ 50 $\mu\text{eq/l}$ Dissolved O ₂ ≥ 4 ppm Nitrate-N ≤ 4.2 mg/l Urban land use $\leq 20\%$ of catchment area Forested land use $\geq 25\%$ of catchment area Remoteness rating optimal or sub-optimal Aesthetics rating optimal or sub-optimal Instream habitat optimal or sub-optimal Riparian buffer width ≥ 15 m No channelization No point source discharges	pH ≤ 5 (except for blackwater streams) <i>and</i> ANC ≤ 0 $\mu\text{eq/l}$ Dissolved O ₂ ≤ 2 ppm Nitrate-N ≥ 7 mg/l <i>and</i> dissolved O ₂ ≤ 3 ppm Urban land use $> 50\%$ of area <i>and</i> instream habitat poor Instream habitat poor <i>and</i> bank stability poor Channel alteration rating poor <i>and</i> instream habitat poor

C. Determining Appropriate Strata

Detection of anthropogenic stresses on the benthic macroinvertebrate assemblage must occur independently of inherent differences due to natural factors. Natural variability in community composition across the state was explored using two analytical techniques: cluster analysis and nonmetric multi-dimensional scaling (NMDS). Both techniques were used to compare measures of similarity within and among groups of reference and other minimally-impaired sites. For this portion of the analysis, a total of 130 sites were used, including the 37 reference sites and an additional 93 considered not substantially degraded. These sites met criteria slightly less stringent than those used for reference sites. Physical and geographic variables examined included stream order, catchment area, gradient, conductivity, ANC, DOC, major river basin, Level IV subcoregion, and physiographic region to determine their appropriateness as natural strata.

Site Similarity Indices. Clustering and ordination require some measure of similarity (e.g., dissimilarity matrix) using relative abundance of taxa as the input data (Ludwig and Reynolds 1988). For cluster analysis, the Jaccard coefficient (C) was selected to create a dissimilarity matrix. It is one of the most widely-accepted measures used to examine similarity of pairs of sites in terms of taxa presence and absence (Magurran 1988) and is expressed as the percentage of taxa shared by the following:

$$C_j = j / (a + b - j)$$

where:

j = the number of taxa common to both samples

a = the number of taxa in sample A

b = the number of taxa in sample B

Ordination consisted of non-metric multidimensional scaling (NMDS) using the Bray-Curtis index. The Bray-Curtis index or coefficient (BC, also known as percentage dissimilarity) is commonly used in ecology and was selected for creating the input matrix (Ludwig and Reynolds 1988, Boesch 1977):

$$BC_{jk} = \frac{1}{2} \sum_{i=1}^s |p_{ij} - p_{ik}|$$

p_{ij} = percentage of i taxon from j th sample

p_{ik} = percentage of i taxon from k th sample

Following calculation, each similarity matrix was imported into Statistica® (StatSoft 1997).

Cluster Analysis. Cluster analysis is a multivariate process for putting information into meaningful groups in order to classify sites (van Tongeren 1987). Clusters produced by the Jaccard-based dissimilarity matrix that align with physical attributes are interpreted as reflecting the natural variability of benthic macroinvertebrate assemblages within a stratum, and provide supporting evidence for consideration as a geographic stratum. Cluster analysis was performed on the Jaccard matrix using unweighted pair group averaging (UPGMA, Ludwig and Reynolds 1988). Using tree clustering, sites were partitioned into discrete clusters.

Non Metric Multidimensional Scaling. NMDS arranges sites along axes so points close together correspond to sites with similar taxonomic composition and points farthest apart are most dissimilar (Jongman et al. 1987). This approach is more robust in producing separation of classes than other ordination methods (e.g., Kenkel and Orloci 1986, Reynoldson et al. 1995). The most widely used technique is based on an ordination algorithm developed by Kruskal

(Kenkel and Orloci 1986, ter Braak 1987). Each dimension explains variation in the data, with the first explaining the most, continuing with the second in descending amounts of explained variation. Dimension values are plotted as two- or three-dimensional graphs depending on the view or perspective of the dimensions that best illustrate site classes or similarity groupings. For this analysis, the Bray-Curtis percent dissimilarity matrix was used.

D. Compiling and Calculating Candidate Metrics

Candidate metrics for testing and potential inclusion in the IBI, selected primarily from previous or parallel studies or guidance documents (Barbour et al. 1996, Gibson et al. 1996, U.S. EPA 1997a), are grouped into five categories: richness, composition, tolerance/intolerance, feeding behavior or trophic structure, and habit tendencies. A total of 57 metrics within these five categories were considered as potential or candidate index components (Table II-2).

Taxonomic Richness. Metrics in this category are counts of the distinct number of taxa within selected taxonomic groups. “Total taxa” and “EPT taxa” are broadly used metrics that provide information on overall taxonomic diversity (at specified hierarchies), with the latter based on three insect orders generally known to be sensitive to disturbance (Ephemeroptera [mayflies], Plecoptera [stoneflies], and Trichoptera [caddisflies]). Other candidate metrics of this category are focused upon different orders (e.g., beetles), or subfamilies and tribes of the family Chironomidae.

Taxonomic Composition. These metrics are based on the proportion of individuals in a sample belonging to a specified taxonomic group. Two exceptions are “% Orthocladiinae of Chironomidae” and “% Tanytarsini of Chironomidae”, each of which are the proportion of midges in a sample that are of this subfamily and tribe, respectively.

Tolerance/Intolerance. Tolerance of a taxon is based on its ability to survive short- and long-term exposure to physicochemical stressors that result from chemical pollution, hydrologic alteration, or habitat degradation. Following the basic framework established by Hilsenhoff (Hilsenhoff 1982), tolerance values were assigned to individual taxa on a scale of 0-10, with 0 identifying those taxa with greatest sensitivity (least tolerance) to stressors, and 10, those taxa with the least sensitivity (most tolerance) to stressors. Tolerance values (Appendix A) were found using primarily a compilation of regional lists (U. S. EPA 1990 [draft]). If more than one tolerance value was listed for a genus, typically the one that was higher was chosen. In cases where tolerance values were listed for all species in a genus, but not the genus itself, the value that occurred most frequently among the species was used. If no value at all was listed, value assignment was based on best professional judgement.

Trophic/Feeding. All of these metrics are based on mode of feeding. Designations for each taxon were taken primarily from Merritt and Cummins (1996) and U. S. EPA (1990 [draft]). When a taxon was not listed in either of these sources, it was assigned the feeding type of its most closely-related taxon, an approach in agreement with other researchers (Merritt et al. 1996). If more than one feeding designation was listed for a genus, and it was known that they were separated by larvae and adults, that for the appropriate life stage was selected.

Table II-2. Definitions of candidate benthic metrics and expected response to increasing stressors.

Metric	Definition	Expected response
Taxonomic Richness		
Total taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
EPT taxa	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
Ephemeroptera taxa	Number of mayfly taxa	Decrease
Plecoptera taxa	Number of stonefly taxa	Decrease
Trichoptera taxa	Number of caddisfly taxa	Decrease
Coleoptera taxa	Number of beetle taxa	Decrease
Diptera taxa	Number of “true” fly taxa (includes midges)	Decrease
Chironomidae taxa	Number of midge taxa	Decrease
Orthocladiinae taxa	Number of taxa in the midge subfamily Orthocladiinae	Decrease
Tanytarsini taxa	Number of taxa in the midge tribe Tanytarsini	Decrease
Crustacea or Mollusca	Sum of the number of calcium dependent taxa	Decrease
Taxonomic Composition		
% EPT	Percent mayfly, stonefly, and caddisfly individuals in the sample	Decrease
% Ephemeroptera	Percent mayfly nymphs	Decrease
% Plecoptera	Percent stonefly nymphs	Decrease
% Trichoptera	Percent caddisfly larvae	Decrease
% Odonata	Percent dragonfly and damselfly nymphs	Decrease
% Coleoptera	Percent beetle larvae and aquatic adults	Decrease
% Diptera	Percent “true” fly larvae and pupae	Increase
% Chironomidae	Percent midge larvae and pupae	Increase
% Orthocladiinae of Chiron.	Percent of chironomids in the subfamily Orthocladiinae	Increase
% Tanytarsini of Chiron.	Percent of chironomids in the tribe Tanytarsini	Decrease
% Tanytarsini	Percent of Tanytarsini midges to total fauna	Decrease
% non-insects	Percent non-insects	Increase
% Crustacea & Mollusca	Percent Crustacea and Mollusca individuals	Decrease
% Gastropoda	Percent snails and limpets	Decrease
% Pelecypoda	Percent bivalves	Decrease
% <i>Corbicula</i>	Percent asiatic clams	Increase
% Amphipoda	Percent Amphipods	Decrease
% Isopoda	Percent Isopods	Increase
Shannon-Wiener Index ^a	A measure of general richness and composition (diversity and evenness)	Decrease
% Oligochaeta	Percent aquatic worms	Increase

Table II-2. Definitions of candidate benthic metrics and expected response to increasing stressors (continued).

Metric	Definition	Expected response
Tolerance/Intolerance		
Intolerant taxa	Number of taxa considered to be sensitive to perturbation (Hilsenhoff values 0 - 3)	Decrease
% tolerant	Percent of sample considered tolerant of perturbation (tolerance values 7 - 10)	Increase
% intolerant	Percent of sample considered intolerant of perturbation (tolerance values 0 - 3)	Decrease
% dominant taxon	Percent of the most abundant taxon	Increase
Hilsenhoff Biotic Index ^b	The general tolerance/intolerance of the assemblage; considers the number of individuals in each tolerance class	Increase
Beck's Biotic Index	Weighted sum of intolerant taxa (= 2* number of Class 1 taxa + number of Class 2 taxa; where Class 1 taxa have tolerance values 0 and 1, Class 2 taxa have values from 2 to 4)	Decrease
% Hydropsychidae of Trich.	Percent pollution tolerant caddisflies of all caddisflies	Increase
% <i>Hydropsyche</i> & <i>Cheumatopsyche</i> of EPT	Percent pollution tolerant caddisflies of all mayflies, stoneflies and caddisflies	Increase
% Baetidae of Ephemeroptera	Percent pollution tolerant mayflies of all mayflies	Increase
Trophic feeding		
Scraper taxa	Number of taxa that scrape food from substrate	Decrease
Predator taxa	Number of taxa that capture living food organisms	Decrease
% scrapers	Percent scraper individuals	Decrease
% predators	Percent predator individuals	Decrease
% collectors	Percent of sample that feeds on detrital deposits or loose surface films	Decrease
% filterers	Percent of sample that feeds on suspended detritus	Variable
% shredders	Percent of sample that "shreds" organic litter	Decrease
Habit		
% burrowers	Percent of sample that is primarily infauna	Increase
% burrowers (general)	Percent of sample that is primarily infauna or burrows as a secondary habit	Increase
% climbers	Percent of sample that primarily lives on stem type surfaces	Decrease
% climbers (general)	Percent of sample that lives on stem type surfaces as a primary or secondary habit	Decrease
% clingers	Percent of sample primarily adapted for inhabiting flowing water, as in riffles	Decrease
% clingers (general)	Percent of sample adapted for inhabiting flowing water as a primary or secondary habit	Decrease
% sprawlers	Percent of sample that primarily lives on top of plant or sediment substrates	Decrease

Table II-2. Definitions of candidate benthic metrics and expected response to increasing stressors (continued).

Metric	Definition	Expected response
% sprawlers (general)	Percent of sample that lives on top of substrates or sprawls as a secondary habit	Decrease

Table II-2. Definitions of candidate benthic metrics and expected response to increasing stressors (continued).

Metric	Definition	Expected response
Habit (continued)		
% swimmers	Percent of sample that primarily swims	Decrease
% swimmers (general)	Percent of sample that swims as a primary or secondary habit	Decrease
a Shannon-Wiener Index = $\sum -((n/N)*\text{Log}(n/N))/\text{Log}(2)$; where n is the number of individuals in a taxon and N is the number of individuals in the sample, summed for all taxa in the sample.		
b Hilsenhoff Biotic Index = $\sum (n)*(tolerance\ value)/N$; where n is the number of individuals in a taxon and N is the number of individuals in the sample that have known tolerance values; summed for all taxa in the sample.		

The functional feeding group designation for an organism reflects the dominant mode of feeding, not the specific nutritional source or benefits (Cummins and Klug 1979, Anderson and Cargill 1987, Merritt and Cummins 1996, Wallace and Webster 1996). Designations for each taxon are listed in Appendix A and include the following functional feeding groups: scrapers, predators, collectors, filterers, and shredders. Scrapers are those organisms that remove periphyton or other algal material and the associated microbes from mineral or vegetable substrates. Predators

engulf or actively capture living animal tissue or prey. Collectors feed on organic materials that are deposited or trapped within epibenthic layers of fine sediments or detritus. Filterers trap, engulf, or strain suspended particulates from the water column that may be plant or animal in origin. Shredders chew and break up woody materials, coarse organic particulates, or living macrophyte tissue.

Habit. Organisms were assigned habit classifications of burrower, climber, clinger, sprawler, or swimmer, according to their locomotion or behavior in relation to their habitat (Merritt and Cummins 1996, Merritt et al. 1996). Burrowers are those animals that live in the fine sediments of stream bottoms, particularly depositional areas. However, some burrow into plant materials. Organisms that live on the surfaces of living plant materials (leaves, stems, roots) or decaying organic detritus are called climbers. Some aquatic invertebrates have morphological or behavioral adaptations that allow them to avoid or withstand the hydraulic forces of stream riffles or other fast-flowing zones. These animals are known as clingers and may have ventral suckers, dorsoventral flattening, well-developed tarsal claws, or fixed constructed retreats. Sprawlers live on the surfaces of leaves or on top of fine sediments, and have the ability to maintain silt-free respiratory capacity. Swimmers are adapted for locomotion in the open water column using fish-like movements. Habit designations were assigned to taxa using primarily Merritt and Cummins (1996), and are listed in Appendix A. Approaches for selecting a genus-level habit when there were either multiple designations or none are similar to those discussed above for tolerance values and feeding designations.

E. Testing Candidate Metrics

Metrics that are strongly correlated with drainage area require transformation before applying scoring criteria. Each candidate metric was tested against log transformed drainage area using Pearson's product-moment correlation (Ludwig and Reynolds 1988).

The distribution of metric values at reference and degraded sites were compared using two non-parametric statistical tests. Differences of the medians were tested using Mann-Whitney U and differences in distribution characteristics (such as variance) were detected using the Kolmogorov-Smirnov test (Steel and Torrie 1980). The tests were applied separately within each of the two strata (Coastal Plain and non-Coastal Plain, see Results, Section A). Metrics whose values differed between reference and degraded sites ($p < 0.05$) by both criteria were retained for further analysis, whereas those metrics having similar medians and distributions in reference and degraded sites were not considered in subsequent analyses.

Calculated metric values were converted (normalized) to metric scores of 5, 3 or 1 depending on their proximity to optimal values. To investigate the most appropriate metric value thresholds for the data, optimal and sub-optimal value ranges were established using four scoring criteria. The four criteria were based on percentiles of the data as follows:

- 1) the 50th percentile (or median) and the 10th percentile,
- 2) the 50th percentile and a bisection to the extreme minimum value,
- 3) the 25th percentile and a bisection to the extreme minimum value, and
- 4) a trisection of the 95th percentile.

All scoring criteria considered only reference site data, except for #4, which included metric values from reference and degraded sites.

In the first method, metric values above the 50th percentile were scored as 5, metric values between and including the 10th and 50th percentiles were scored as 3, and all metric values below the 10th percentile were scored as 1 (Figure 2). Those metrics that increase in response to perturbation (reverse metrics) were scored such that values below the median received a score of 5, values between and including the 50th and 90th percentiles were scored as 3, and values above the 90th percentile were scored as 1.

The other methods of establishing scoring thresholds were similarly based on the upper and lower percentile and bisection or trisection. In the case of the 95th percentile and trisection, all metric values above two-thirds of the 95th percentile were scored as 5, values between and including one-third and two-thirds of the 95th were scored as 3 and all values below one third of the 95th received a score of 1. Metrics whose expectations were reversed were scored conversely.

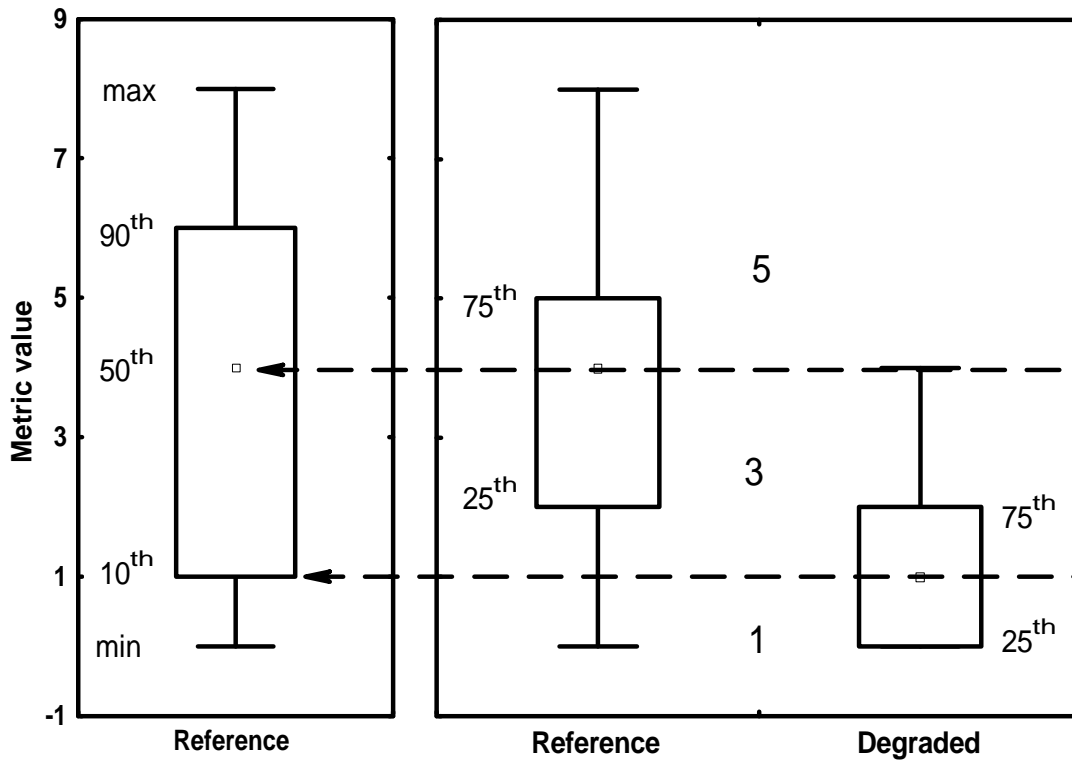


Figure 2. Percentile distribution of metric values as a mechanism for establishing scoring criteria.

The responsiveness of each metric to stressors was determined by the agreement of metric scoring with site status (reference or degraded). Criteria correctly classified sites previously determined as “reference” (Table II-1) if they produced metric scores of 3 and 5. Likewise, a metric score of 1 indicated impaired biological conditions and correctly classified sites previously determined as “degraded”, again using criteria in Table II-1. Incorrect classifications occurred when a reference site had a metric score of 1 and when an impaired site had a metric score of 3 or 5. Although increasing the chances of false positives (that is, of saying there is impairment when, in fact, none exists), scoring and evaluating metrics in this manner decreases the likelihood of false negatives (or, of concluding no impairment exists, when, in fact, it does). The classification efficiencies (CEs) of each metric in both strata and all scoring methods were calculated as the percentage of correct site classifications. The final scoring method was determined by examining overall performance of the metrics across both strata using both reference and degraded sites (combined and separate). The initial goal was to retain metrics for consideration if their CE $\geq 70\%$. However, the universality of a metric (i.e., how widespread geographically is it used and understood), and whether it was useful in multiple strata, were considered during the metric evaluation process.

F. Combining Metrics into an Index

The process by which metrics were chosen for the index required iterative testing of the classification efficiencies of several metric combinations. At least one metric from each category (richness, composition, tolerance/intolerance, functional feeding group, and habit) was included

in every index combination considered. This was to ensure representation in the final index of a diversity of community characteristics and thus, the sensitivity of the index to a broader range of stressors (Karr and Chu 1997). The CE of the index was calculated as above, with scores ≥ 3.0 in the reference sites and 3.0 in the impaired sites considered correct and where the efficiency equals the percentage of correctly classified sites. Metrics with the highest CE per category per stratum were used in the preliminary combinations. The basic set of the best metrics from each metric category was augmented in a stepwise manner until the highest index CE attainable was determined. When more than one metric in a category shared the top rank in efficiency, trials were run in a stepwise manner, with one, with the other, and with both (or all) until the highest index CE was attained.

Testing of metric combinations for the index continued in a stepwise manner to include the second highest ranking metric in each category, and the third if appropriate. Upon building a large metric set, trials continued, which selectively deleted metrics if deletion did not reduce classification efficiency. Exceptions to this general format were the trials run without the habit metrics and those with “total number of taxa” in the Coastal Plain, which had a low classification efficiency, but contains essential information about the assemblage. The selected metric scores were averaged to yield a single index value for each site.

If several combinations yielded the same high classification efficiency, the final index was determined on the basis of the ecological importance of the metrics within the combination. Redundancy among metrics within each stratum was calculated using Pearson’s product moment correlation (Ludwig and Reynolds 1988). Redundant metrics ($r \geq 0.75$) were used in index combinations if inclusion increased the index classification efficiency. The metrics selected for the final index included those which yielded the highest overall classification efficiency and which contained the most appropriate ecological information.

G. Testing the Index Using an Independent Data Set

After developing the genus level index using 1994 and 1995 data, the index was tested using 1996 and 1997 data. This verification dataset consisted of sampling and taxonomic results from 687 sites selected randomly, of which 584 produced samples with ≥ 80 organisms. New reference ($n = 92$) and degraded ($n = 23$) sites were identified among the 1996 and 1997 sites using the previously developed criteria (Table II-1). The index metrics were calculated in the Coastal Plain and non-Coastal Plain and metrics were scored using established scoring criteria. Metric scores were combined into an index and the percentage of correct classifications (CE) determined as before.

H. Developing Indices for Use with Higher Level Taxonomic Identifications

Family Level. A family level index was developed using much the same procedure as described above. Metrics that were already part of the genus level index and could be calculated with family-level data were used. Tolerance values were assigned to each family in the samples. Functional feeding group and habit preference metrics were not used in the family level index, because the technical literature did not support assignment of families into feeding group or habit categories. Metric scoring criteria were determined using the 50th and 10th percentile thresholds. Various sets of metrics were tested starting with only those metrics applied within the respective regions at the genus level. Metrics were subsequently added and deleted from the trial sets, using all metrics in both regions, regardless of regional specificity at the genus-level. The set of metrics chosen for the final index yielded the best CE in both physiographic strata, i.e., only one set of metrics for the family level is proposed for both the CP and NCP regions, to simplify application of the index.

Order level. Of the metrics used at the family level, five could be applied using order level data: “number of taxa” (orders), “number of EPT orders”, “% Ephemeroptera”, “number of Ephemeroptera taxa and number of Diptera taxa”. Except for “% Ephemeroptera”, all of these metrics have limited ranges of values, e. g., the number of Ephemeroptera orders would range from 0-1 (that is, present or absent). Such a limited array of metrics and metric values may yield imprecise assessment results. Other uses of higher level taxonomy in water resource assessments tend to mix taxonomic levels according to ease of identifications for novice or volunteer monitors (U.S. EPA 1997b, IWLA 1992).

III. RESULTS

A. Determination of Strata

Both cluster analysis and NMDS suggested that the Coastal Plain (CP) and non-Coastal Plain (NCP) had different benthic macroinvertebrate assemblages. To a varying extent, site groupings are best reflected by physiographic regions. This can be seen with both the dendrogram and the NMDS ordination plots (Figures 3 and 4). Vertical lines drawn on Figure 3 demonstrate reasonable site groupings; small clusters to the left of the numbered clusters are comprised of relatively heterogeneous sites and are not numbered. Based on the clusters, numbers of sites, and their distribution, the most sensible strata are NCP (clusters 1 and 3) and CP (cluster 2 and the heterogeneous sites). Three clusters were identified at the linkage level of approximately 0.83. Cluster 1 consists mostly of sites from Washington and Allegheny counties (22 out of 25); cluster 2 is comprised of 15 sites from southern Maryland and all are in the CP; and cluster 3 is spread out geographically with 57 of the 61 sites occurring in counties that are within the NCP. To the left of cluster 1 are heterogeneous sites based on taxonomic composition. Relative to sites within clusters 1, 2, and 3 they have a large percentage of taxa not shared with other sites. Of these, 23 out of 29 sites occur in counties that are completely in the CP or have small areas that are transitional to the Piedmont.

Tree Diagram for 130 Sites
Unweighted pair-group average
 Dissimilarities from Jaccard matrix (Taxa >= 5 occ.)

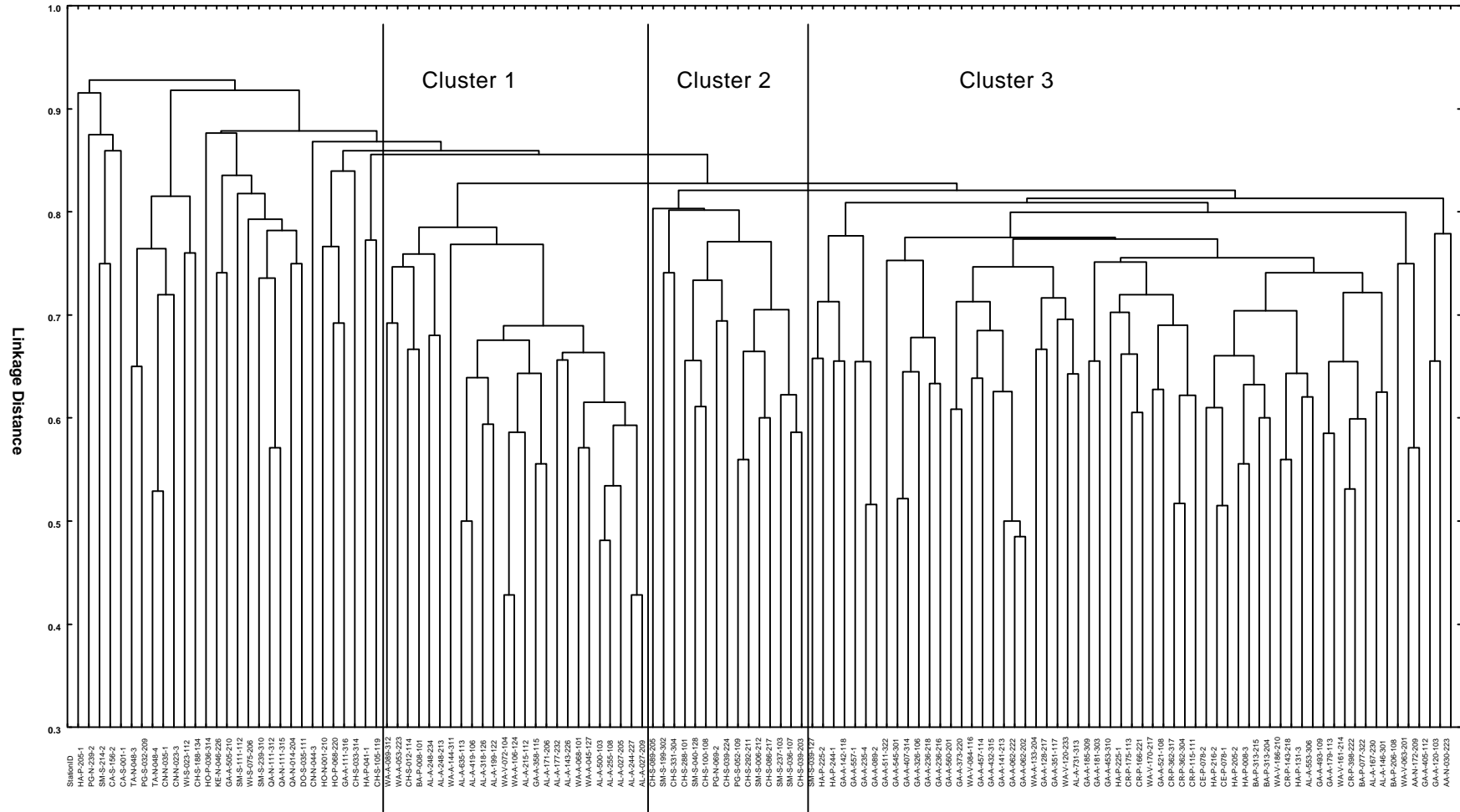


Figure 3. Dendrogram results of cluster analysis produced through unweighted pair-group averaging (UPGMA) of biological dissimilarity distances (benthic macroinvertebrates). Sites (n=130) are from 1994-95 MBSS sampling, and represent those identified as least impaired. Station identification codes include county designations in the first two letters and physiographic region in the third letter. The Coastal Plain (CP) sites are denoted with N or S. Non-Coastal Plain (NCP) sites are denoted with A, P or V.

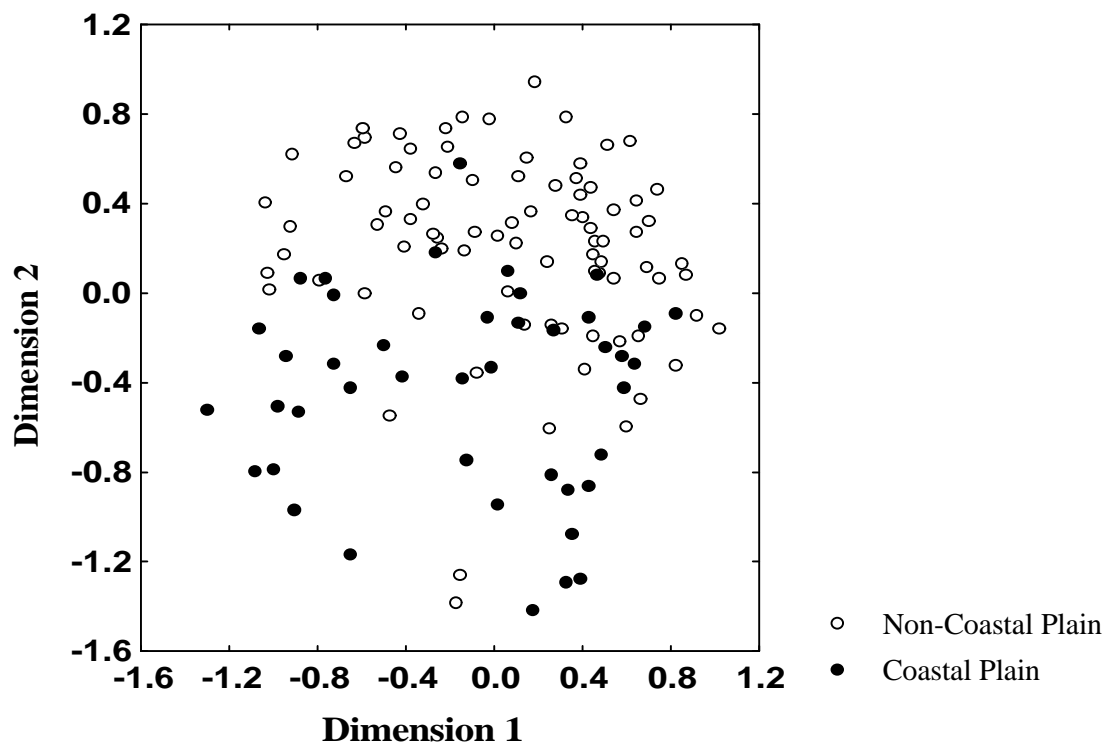


Figure 4. Results of multivariate ordination using nonmetric multidimensional scaling (NMDS) of Bray-Curtis dissimilarity coefficients (benthic macroinvertebrates). Sites (n=130) are from 1994-95 MBSS sampling, and represent those identified as least impaired.

The NMDS plot of all 130 sites further supports the two somewhat distinct strata (Figure 4). The majority of NCP sites are in the top half of the graph, above 0.0 on the Y axis, while the majority of CP sites are below 0.0 on the Y axis. After separation of CP from NCP sites, further subdivision using both clustering and NMDS suggested site groups by physiographic province, ecoregion, and subecoregion (Omernik 1987, Woods et al. 1996, White 1997). The most distinct ecoregions or subecoregions were Inner and Outer Coastal Plain, Piedmont, Shale Ridges, and Limestone Valleys. However, due to insufficient number of sites in many of these geographic areas, their distinctness cannot be fully evaluated. None of the other physical, chemical, or geographic variables including basin, stream order, and water chemistry showed as strong a correspondence as geographic regions.

B. Metric Evaluation

Of the 57 metrics evaluated, 16 in the CP (Table III-1a) and 31 in the NCP (Table III-1b) passed both tests of significance (Mann-Whitney U and Kolmogorov-Smirnov) at $p < 0.05$. Of the 16 and 31 metrics, respectively, 12 were common to both regions. In the CP, the richness category and the trophic/feeding category had few metrics that met these criteria for inclusion in the index. They were “total taxa” and “EPT taxa” in the richness category and “scraper taxa” in the trophic/feeding category. In the NCP the habit category included only “% climbers (general)” and “% swimmers (general)” as passing metrics.

Table III-1a. Evaluation of individual candidate benthic metrics for Coastal Plain (* indicates p<0.05).

COASTAL PLAIN	Mann-Whitney (p)	Kolmogorov-Smirnov (p)	Classification		Reason for including or excluding the metric in the final index	Correlation to log drainage area: R values
			Efficiency 50th & 10th%ile	Used in final index		
Richness Metrics						
Total taxa	0.002 *	p < .025 *	47	x	Universally applied and understood metric	0.01
EPT taxa	0.003 *	p < .05 *	55	x	Universally applied and understood metric	0.02
Ephemeroptera taxa	0.004 *	p < .10			Did not pass both significance tests	0.05
Plecoptera taxa	0.082	p > .10			Did not pass both significance tests	-0.06
Trichoptera taxa	0.011 *	p > .10			Did not pass both significance tests	0.06
Coleoptera taxa	0.018 *	p < .10			Did not pass both significance tests	0.29
Diptera taxa	0.047 *	p < .10			Did not pass both significance tests	-0.22
Chironomidae taxa	0.186	p > .10			Did not pass both significance tests	-0.04
Orthoclaadiinae taxa	0.162	p > .10			Did not pass both significance tests	-0.02
Tanytarsini taxa	0.011 *	p < .10			Did not pass both significance tests	0.03
Crustacea or Mollusca	0.508	p > .10			Did not pass both significance tests	0.21
Composition Metrics						
% EPT	0.191	p > .10			Did not pass both significance tests	0.09
% Ephemeroptera	0.012 *	p < .01 *	74	x	High classification efficiency	0.12
% Plecoptera	0.633	p > .10			Did not pass both significance tests	-0.12
% Trichoptera	0.001 *	p < .01 *	71		Other composition metrics used	0.15
% Odonata	0.032 *	p < .10			Did not pass both significance tests	0.1
% Coleoptera	0.053	p < .10			Did not pass both significance tests	0.16
% Diptera	0.794	p > .10			Did not pass both significance tests	-0.02
% Chironomidae	0.878	p > .10			Did not pass both significance tests	-0.14
% Orthoclaadiinae of Chiro	0.549	p > .10			Did not pass both significance tests	0.04
% Tanytarsini of Chiro	0.010 *	p < .01 *	74	x	High classification efficiency	-0.03
% Tanytarsini	0.072	p < .05 *			Did not pass both significance tests	0.13
% non-insects	0.056	p > .10			Did not pass both significance tests	-0.15
% Crustacea & Mollusca	0.061	p > .10			Did not pass both significance tests	-0.15
% Gastropoda	0.782	p > .10			Did not pass both significance tests	0.06
% Pelecypoda	0.364	p > .10			Did not pass both significance tests	0.09
% Corbicula	0.701	p > .10			Did not pass both significance tests	0.24
% Amphipoda	0.340	p > .10			Did not pass both significance tests	-0.19
% Isopoda	0.024 *	p > .10			Did not pass both significance tests	-0.03
Shannon-Wiener	0.005 *	p < .05 *	53		Low efficiency in this category	-0.06
% Oligochaeta	0.498	p > .10			Did not pass both significance tests	0.22
Tolerance/intolerance Metrics						
Intolerant taxa	0.002 *	p < .05 *	55		Low efficiency in this category	-0.09
% tolerant	0.029 *	p < .10			Did not pass both significance tests	0.05
% intolerant	0.020 *	p < .10			Did not pass both significance tests	-0.05
% dominant taxon	0.058	p > .10			Did not pass both significance tests	0.13
Hilsenhoff Biotic Index	0.017 *	p < .05 *	66		Other tolerance metric used	0.03
Beck's Biotic Index	0.000 *	p < .01 *	68	x	High classification efficiency	-0.04
% Hydropsychidae of Trich	0.001 *	p < .005 *	82		Uncertain significance of 0 metric value	0.29
% Hydro & Cheum of EPT	0.002 *	p < .005 *	84		Uncertain significance of 0 metric value	0.3
% Baetidae of Ephem	0.162	p > .10			Did not pass both significance tests	-0.06
Trophic/Feeding Metrics						
Scraper taxa	0.002 *	p < .05 *	55	x	High efficiency in this category	0.3
Predator taxa	0.011 *	p > .10			Did not pass both significance tests	-0.06
% scrapers	0.032 *	p < .10			Did not pass both significance tests	0.16
% predators	0.077	p > .10			Did not pass both significance tests	-0.14
% collectors	0.723	p > .10			Did not pass both significance tests	-0.18
% filterers	0.249	p > .10			Did not pass both significance tests	0.26
% shredders	0.255	p > .10			Did not pass both significance tests	-0.19
Habit Metrics						
% burrowers	0.381	p < .10			Did not pass both significance tests	-0.12
% burrowers (gen)	0.332	p > .10			Did not pass both significance tests	-0.13
% climbers	0.878	p > .10			Did not pass both significance tests	0.01
% climbers (gen)	0.397	p > .10			Did not pass both significance tests	-0.04
% clingers	0.001 *	p < .005 *	74	x	High classification efficiency	0.31 *

Table III-1a. Evaluation of individual candidate benthic metrics for Coastal Plain (* indicates p<0.05) (continued).

COASTAL PLAIN	Mann-Whitney (p)	Kolmogorov-Smirnov (p)	Classification		Reason for including or excluding the metric in the final index	Correlation to log drainage area: R values
			Efficiency 50th & 10th%ile	Used in final index		
% clingers (gen)	0.031 *	p < .01 *	74		Other habit metric used	0.32 *
% sprawlers	0.010 *	p < .05 *	68		Other habit metric used	-0.3
% sprawlers (gen)	0.010 *	p < .025 *	63		Other habit metric used	-0.24
% swimmers	0.498	p > .10			Did not pass both significance tests	0.06
% swimmers (gen)	0.185	p > .10			Did not pass both significance tests	0.03

Table III-1b. Evaluation of individual candidate benthic metrics for Non-Coastal Plain (* indicates p<0.05).

NON-COASTAL PLAIN	Mann-Whitney (p)	Kolmogorov-Smirnov (p)	Classification		Reason for including or excluding the metric in the final index	Correlation to log drainage area: R values
			Efficiency 50th& 10 th %ile	Used in final index		
Richness Metrics						
Total taxa	0.000 *	p < .001 *	80	X	High classification efficiency in this category	0.15
EPT taxa	0.000 *	p < .001 *	74	X	Universally applied and understood metric	0.06
Ephemeroptera taxa	0.000 *	p < .001 *	82	X	High classification efficiency in this category	0.15
Plecoptera taxa	0.002 *	p < .05 *	58		Other richness metrics used	-0.01
Trichoptera taxa	0.000 *	p < .005 *	56		Other richness metrics used	-0.01
Coleoptera taxa	0.200	p > .10			Did not pass both significance tests	0.01
Diptera taxa	0.008 *	p < .025 *	72	X	Increased overall index efficiency	0.14
Chironomidae taxa	0.116	p > .10			Did not pass both significance tests	0.23 0
Orthoclaadiinae taxa	0.137	p > .10			Did not pass both significance tests	0.08
Tanytarsini taxa	0.071	p > .10			Did not pass both significance tests	0.05
Crustacea or Mollusca	0.003 *	p < .025 *	no range		Range is indiscernable at 50th and 10th %iles	-0.04
Composition Metrics						
% EPT	0.028 *	p < .05 *	60		Other composition metrics used	0.06
% Ephemeroptera	0.000 *	p < .001 *	82	X	High classification efficiency in this category	0.11
% Plecoptera	0.655	p > .10			Did not pass both significance tests	-0.13
% Trichoptera	0.004 *	p < .005 *	68		Other composition metrics used	0.12
% Odonata	0.449	p > .10			Did not pass both significance tests	0.12
% Coleoptera	0.193	p > .10			Did not pass both significance tests	-0.03
% Diptera	0.168	p > .10			Did not pass both significance tests	-0.03
% Chironomidae	0.332	p > .10			Did not pass both significance tests	0.12
% Orthoclaadiinae of Chiro	0.892	p > .10			Did not pass both significance tests	-0.04
% Tanytarsini of Chiro	0.040 *	p < .025 *	66		Other composition metrics used	-0.04
% Tanytarsini	0.007 *	p < .025 *	72	X	Increased overall index efficiency	0.14
% non-insects	0.002 *	p < .025 *	70		Other composition metrics used	-0.14
% Crustacea & Mollusca	0.001 *	p < .005 *	no range		Range is indiscernable at 50th and 10th %iles	-0.22 0
% Gastropoda	0.162	p > .10			Did not pass both significance tests	0.07
% Pelecypoda	0.485	p > .10			Did not pass both significance tests	-0.02
% Corbicula	1.000	p > .10			Did not pass both significance tests	0.09
% Amphipoda	0.356	p > .10			Did not pass both significance tests	0.06
% Isopoda	0.148	p > .10			Did not pass both significance tests	-0.26 0
Shannon-Wiener	0.000 *	p < .001 *	80		Other composition metric used	-0.04
% Oligochaeta	0.763	p > .10			Did not pass both significance tests	0.28 0
Tolerance/Intolerance Metrics						
Intolerant taxa	0.000 *	p < .001 *	74	X	High classification efficiency in this category	-0.13
% tolerant	0.000 *	p < .005 *	74	X	High classification efficiency in this category	-0.25 0
% intolerant	0.007 *	p < .01 *	68		Other tolerance metrics used	-0.13
% dominant taxon	0.000 *	p < .001 *	68		Other tolerance metrics used	0.1
Hilsenhoff Biotic Index	0.001 *	p < .001 *	74		Other tolerance metrics used	0.05
Beck's Biotic Index	0.000 *	p < .001 *	74		Other tolerance metrics used	-0.05
% Hydropsychidae of Trich	0.005 *	p < .005 *	74		Uncertain significance of 0 metric value	0.11

Table III-1b. Evaluation of individual candidate benthic metrics for Non-Coastal Plain (* indicates p<0.05) (continued).

NON-COASTAL PLAIN	Mann-Whitney (p)	Kolmogorov-Smirnov (p)	Classification		Reason for including or excluding the metric in the final index	Correlation to log drainage area: R values
			Efficiency 50th& 10 th ile	Used in final index		
% Hydro & Cheum of EPT	0.071 *	p < .05 *	68		Uncertain significance of 0 metric value	0.09
% Baetidae of Ephem	0.009 *	p < .01 *	74		Uncertain significance of 0 metric value	0.08
Trophic/Feeding Metrics						
Scraper taxa	0.000 *	p < .005 *	66		Other feeding metric used	0.15
Predator taxa	0.000 *	p < .01 *	58		Other feeding metric used	-0.06
% scrapers	0.002 *	p < .025 *	66		Other feeding metric used	0.09
% predators	0.011 *	p < .01 *	66		Other feeding metric used	-0.05
% collectors	0.017 *	p < .025 *	72	X	High classification efficiency in this category	0.09
% filterers	0.503	p > .10			Did not pass both significance tests	-0.02
% shredders	0.236	p > .10			Did not pass both significance tests	-0.13
Habit Metrics						
% burrowers	0.793	p > .10			Did not pass both significance tests	0.14
% burrowers (gen)	0.676	p > .10			Did not pass both significance tests	0.23 0
% climbers	0.031 *	p < .10			Did not pass both significance tests	0.04
% climbers (gen)	0.011 *	p < .025 *	58		Did not increase overall efficiency	-0.12
% clingers	0.268	p > .10			Did not pass both significance tests	0.21 0
% clingers (gen)	0.907	p > .10			Did not pass both significance tests	0.11
% sprawlers	0.116	p > .10			Did not pass both significance tests	-0.13
% sprawlers (gen)	0.303	p > .10			Did not pass both significance tests	-0.09
% swimmers	0.010 *	p < .10			Did not pass both significance tests	-0.21
% swimmers (gen)	0.000 *	p < .001 *	76		Did not increase overall efficiency	0.07

The scoring criteria that provided the best overall CE was the 50th and 10th percentile. Of the other scoring techniques, the 50th percentile and bisection yielded similar metric CEs, while the other techniques (25th and bisection and 95th and trisection) proved less efficient (Table III-2). It should be noted that the 50th and 10th percentiles as threshold criteria were used in development of the MBSS fish IBI (Roth et al. 1997). Only those metrics scored with the 50th and 10th percentile criteria were carried further in the analysis.

Two metrics in the CP and seven metrics in the NCP were significantly correlated (p<0.05) with the log of the drainage area of their sites, though r values were all less than 0.35 (Table III-1a, b). In the CP, the strongest correlation (r = 0.32) was found in the metric “% clingers (general)”. In the NCP, “% Oligochaeta” was most strongly correlated (r = 0.28) of all metrics and “% tolerant individuals” was mostly strongly correlated (r = -0.25) of those metrics which had statistical significance. These r values were considered insufficient to warrant metric value adjustments for drainage area.

Of all the metrics which passed both significance tests, CEs ranged from 47 to 84%. In the Coastal Plain, 7 metrics had efficiencies greater than 70%; however, the two highest (“% Hydropsychidae of Trichoptera” and “% Hydropsyche and Cheumatopsyche of EPT”) were excluded from the index groupings because a metric value of zero had uncertain meaning (Table III-1a). A zero percentage of tolerant individuals within a generally intolerant group (e.g., “Hydropsychidae of Trichoptera”) could signify either that no tolerant individuals are present (a potential indicator of reference conditions) or that no tolerant *or* intolerant individuals are present (a potential indicator of stress). And, in the latter

case, rather than counting the metric value as a zero, it would have to be treated as “missing data” (cannot calculate fractions with a denominator of zero), or automatically assigned a score of “1”. Since it would be very unusual to have even the most-degraded sites produce a sample with zero Chironomidae, the metric “% Tanytarsini of Chironomidae” was retained for the CP index.

Table III-2. Average performance of metric scoring criteria.

Strata	Classification efficiency (average of significant metrics)			
	50 th and 10 th	50 th and bisect	25 th and bisect	95 th and trisect
Coastal Plain	66	65	61	62
Non-Coastal Plain	70	68	67	63

In the NCP, 16 metrics had CEs greater than 70%, though two (“% Hydropsychidae of Trichoptera” and “% Baetidae of Ephemeroptera”) were excluded from index combinations (Table III-1b) for the reason mentioned above. Classification efficiencies of two metrics showing statistical significance (“number of Crustacea or Mollusca taxa” and “% Crustacea and Mollusca”) could not be calculated because the range of values between the 50th and 10th percentiles was insufficient (both percentiles were zero).

C. Combination of Metrics into an Index

The metrics selected for the final indices included those that contained the most appropriate ecological information and which, as a group, yielded the highest overall CE (Table III-3). Three metrics that were included are common to the indices of both strata: “total number of taxa”, “number of EPT taxa”, and “% Ephemeroptera”. Statistics and scoring criteria of the metrics included in the final indices are shown in Table III-4.

The basic set of metrics in the preliminary index for the CP, composed of one metric (of high CE) from each category, included the following five metrics; “number of EPT taxa”, “% Ephemeroptera”, “the Beck’s Biotic Index”, “number of scraper taxa”, and “% clingers”. This combination correctly classified 82% of the sites as reference or impaired. With two additional metrics (“total number of taxa” and “% Tanytarsini of Chironomidae”), the final index correctly classified 87% of the sites, performing best when using only degraded sites (Table III-3). Of these seven metrics, only two showed potential redundancy. The “number of EPT taxa” and “Beck’s Biotic Index” were highly correlated ($r = 0.90$) but were not excluded from the index metric set because of their importances as a universally applied richness metric and a tolerance/ intolerance metric with high classification efficiency.

Table III-3. Classification efficiencies for various combinations of metrics. An “x” signifies inclusion of the metric in the index.

	Coastal Plain		Non-Coastal Plain	
	Preliminary	Final	Preliminary	Final
Overall classification efficiency	84	87	82	88
Efficiency in reference sites	85	77	92	92
Efficiency in degraded sites	84	92	73	85
Taxonomic Richness Metrics				
Total number of taxa		x		x
Number of EPT taxa	x	x		x
Number of Ephemeroptera taxa			x	x
Number of Diptera taxa				x
Taxonomic Composition Metrics				
% Ephemeroptera	x	x	x	x
% Tanytar. of Chiron.		x		
% Tanytarsini				x
Tolerance/Intolerance Metrics				
Number of intolerant taxa			x	x
% tolerant individuals				x
Beck’s Biotic Index	x	x		
Feeding Metrics				
Number of scraper taxa	x	x		
% collectors			x	x
Habit Metrics				
% clingers	x	x		
% swimmers (general)			x	

Table III-4. Descriptive statistics of reference sites and scoring criteria for the final genus-level index metrics in the Coastal Plain and Non-Coastal Plain regions.

Coastal Plain (n = 13)

Metric	Statistic					Score		
	min	10 th	50 th	90 th	max	5	3	1
Total number of taxa	8	11	24	32	36	>24	11 - 24	<11
Number of EPT taxa	2	3	6	11	13	>6	3 - 6	<3
% Ephemeroptera	0.8	2.0	11.4	46.2	47.7	>11.4	2.0 - 11.4	<2.0
% Tanytarsini of Chiron.	0.0	0.0	13.0	46.2	100.0	>13.0	>0.0 - 13.0	0.0
Beck’s Biotic Index	2	4	12	16	18	>12	4 - 12	<4
Number of scraper taxa	0	1	4	6	8	>4	1 - 4	<1
% clingers	20.0	38.7	62.1	86.1	99.2	>62.1	38.7 - 62.1	<38.7

Table III-4. Descriptive statistics of reference sites and scoring criteria for the final genus-level index metrics in the Coastal Plain and Non-Coastal Plain regions (continued).

Non-Coastal Plain (n = 24)

Metric	Statistic					Score		
	min	10 th	50 th	90 th	max	5	3	1
Total number of taxa	14	16	22	30	36	>22	16 - 22	<16
Number of EPT taxa	3	5	12	16	19	>12	5 - 12	<5
Number of Ephemeroptera taxa	1	2	4	6	7	>4	2 - 4	<2
Number of Diptera taxa	2	6	9	11	16	>9	6 - 9	<6
% Ephemeroptera	2.1	5.7	20.3	60.2	78.0	>20.3	5.7 - 20.3	<5.7
% Tanytarsini	0.0	0.0	4.8	21.1	37.6	>4.8	>0.0 - 4.8	0.0
Number of intolerant taxa	2	3	8	12	13	>8	3 - 8	<3
% tolerant	0.9	1.1	11.8	48.0	69.9	<11.8	11.8 - 48.0	>48.0
% collectors	7.4	13.5	31.0	73.1	82.8	>31.0	13.5 - 31.0	<13.5

In the NCP, the preliminary index (basic set of metrics) had a CE of 82% and was composed of the following metrics: “number of Ephemeroptera taxa”, “% Ephemeroptera”, “number of intolerant taxa”, “% collectors”, and “% swimmers”. The final index included five additional metrics (“total number of taxa”, “number of EPT taxa”, “number of Diptera taxa”, “% Tanytarsini”, and “% tolerant individuals”) and deleted the habit metric (“% swimmers”). This final index had a CE of 88% overall and performed better within the reference sites (Table III-3). The “number of Ephemeroptera taxa” was correlated to “total number of taxa” ($r = 0.82$), “number of EPT taxa” ($r = 0.88$), and “number of intolerant taxa” ($r = 0.85$). The “number of EPT taxa” was also correlated to “number of intolerant taxa” ($r=0.92$). These metrics were included in the index despite this potential redundancy because of their values as metrics with universal applicability or high classification efficiency within their metric categories.

Raw index scores for the Coastal and non-Coastal Plain indices ranged from 7 to 35 and 9 to 45, respectively. To facilitate statewide comparisons and to be consistent with the MBSS fish IBI, these scores were adjusted to a common scale ranging from 1 to 5. Index score ranges and their respective narrative ratings are shown in Table III-5. The relative separation of reference and degraded sites by total index score is shown in Figure 5. Metric values and final index scores by site (for 88 reference and degraded sites sampled in 1994-1995) are included in Appendices B and C, respectively.

Table III-5. IBI score ranges and corresponding narrative ratings.

IBI Score Range	Narrative Rating
4.0 - 5.0	Good
3.0 - 3.9	Fair
2.0 - 2.9	Poor

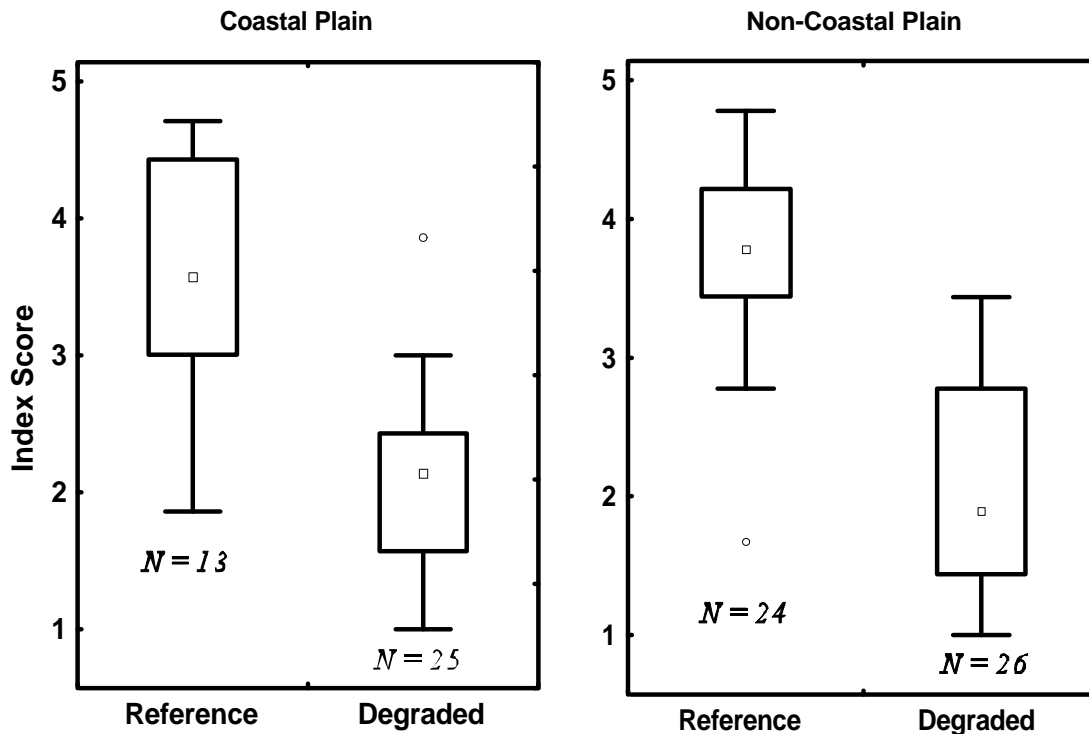


Figure 5. Comparison of overall genus-level index scores between reference and degraded sites using 1994 - 95 data.

1.0 - 1.9

Very Poor

D. Results of Index Testing with Independent Data Set

Classification efficiencies attained with the 1996-97 dataset were high (Table III-6). In the CP, correct classification of reference and degraded sites occurred 72% of the time. When calculated on degraded sites only, the CE was 94%. For the NCP, these statistics were 82 and 100%, respectively.

Table III-6. Result of index testing using MBSS 96-97 data set for verification.

	Coastal Plain		Non-Coastal Plain	
	Class. Efficiency.	<i>N</i>	Class. Efficiency	<i>N</i>
Reference and Degraded	72%	39	82%	76
Degraded sites	94%	16	100%	7
Reference sites	57%	23	78%	69

E. Indices of Biological Integrity for Higher Taxonomic Levels

Several different combinations of family level metrics were tested for their effectiveness in correctly classifying reference and degraded sites (Table III-7). Initially, only those metrics which were used in the indices developed at the genus level and which had significance at the family level were tested within each region (CP and NCP). The addition of metrics which were used in either genus-level regional index improved the overall efficiency of the family level index in both regions. The final index formulation has the same metrics for both CP and NCP: “total number of families”, “number of EPT families”, “number of Ephemeroptera families”, “number of Diptera families”, “% Ephemeroptera”, and “Beck’s Biotic Index”. The overall CE for the CP (71%) was not quite as high as it was for the genus-level index (87%) (Table III-3); however, for the NCP it was identical (88%). The CP family level index performed best among reference sites and the NCP family index performed equally well between reference and degraded sites. Metric scoring criteria were developed using the 50th and 10th percentiles (Table III-8), as described for the genus-level data.

Table III-7. Family level index classification efficiencies for different metric suites.

Coastal Plain Overall Efficiency	71% ^a	66%	71%	71%
Efficiency in reference sites	85%	77%	85%	85%
Efficiency in degraded sites	64%	60%	64%	64%
Non-Coastal Plain Overall Efficiency	82% ^b	86%	88%	86%
Efficiency in reference sites	83%	83%	88%	83%
Efficiency in degraded sites	81%	88%	88%	88%
Number of families	x	x	x	x
Number of EPT families	x	x	x	x
Number Ephemeroptera families		x	x	x
Number Diptera families		x	x	
% Ephemeroptera	x	x	x	x
Number intolerant families		x	x	x
% tolerant		x		x
Beck’s Biotic Index	x		x	x

a Only metrics from genus-level CP index usable with family-level data.

b Only metrics from genus-level NCP index usable with family-level data.

Five metrics were evaluated at the taxonomic level of order: “total taxa”, “EPT taxa”, “% Ephemeroptera”, “number of Ephemeroptera taxa”, and “number of Diptera taxa”. The resulting ranges of metric and threshold values were narrow, but metric scores were generated using the same rules as used for the family- and genus-level analyses. In the CP, three metrics were combined into an index (“total taxa”, “EPT taxa”, and “% Ephemeroptera”); in the NCP, these plus the “number of Ephemeroptera taxa” were used.

Table III-8. Statistics from reference sites and scoring criteria based on the 50th and 10th percentiles for the family level index.

<i>Coastal Plain (n = 13)</i>	Statistic					Score		
	min	10th	50th	90th	max	5	3	1
Metric								
Number of families	6	8	16	20	27	>16	8 - 16	<8
EPT families	2	3	6	10	10	>6	3 - 6	<3
Ephemeroptera families	1	1	2	3	5	>2	1 - 2	<1
Diptera families	1	2	3	4	5	>3	2 - 3	<2
% Ephemeroptera	0.8	2.0	11.4	46.2	47.7	>11.4	2.0 - 11.4	<2.0
Intolerant families	1	2	5	9	9	>5	2 - 5	<2
Beck's Biotic Index	2	4	9	14	15	>9	4 - 9	<4
<i>Non-Coastal Plain (n = 24)</i>	Statistic					Score		
Metric	min	10th	50th	90th	max	5	3	1
Number of families	7	9	14	21	23	>14	9 - 14	<9
EPT families	3	4	10	13	15	>10	4 - 10	<4
Ephemeroptera families	1	2	3	4	4	>3	2 - 3	<2
Diptera families	2	2	3	4	5	>3	2 - 3	<2
% Ephemeroptera	2.1	5.7	20.3	60.2	78.0	>20.3	5.7 - 20.3	<5.7
Intolerant families	2	4	8	12	15	>8	4 - 8	<4
Beck's Biotic Index	3	8	12.5	19	24	>12.5	8 - 12.5	<8

The index had a CE of 76% in the Coastal Plain (85% among reference and 72% among degraded sites). In the Non-Coastal Plain, the index correctly classified 60% of the sites (16% among reference and 100% among degraded sites). Other metric combinations with and without “number of Ephemeroptera taxa” in both CP and NCP resulted in no differences in CEs. The metric “number of Diptera taxa” was not included in indices because the score was always 3 for all sites.

Even with relatively high classification efficiencies, use of order level indices is not recommended, although it may be somewhat useful for highly qualitative assessments. There is limited information and a diminished capacity for additional interpretation of results: pollution tolerance values and functional feeding group and habit designations have little meaning. Since there are only narrow ranges of calculated metric values, the sensitivity of a metric or an index for detecting impairment would be decreased over those with broader ranges.

IV. DISCUSSION

Maryland DNR developed biological indices for both fish and benthic macroinvertebrates because these indicators respond when exposed to both physical and chemical stressors. Also, a community- or assemblage-level measure (such as the multimetric Index of Biotic Integrity) that integrates multiple types of responses (i.e., at different levels of biological organization) is more likely to reflect those responses than any individual metric (Karr et al. 1986). Following Karr, there were several attempts at adapting the multimetric approach to different areas of the country or to different assemblages. Those

efforts met with variable success because guidance documents, such as Plafkin et al. (1989), were often taken “off the shelf” and methods were applied without modification.

There has been increasing recognition that regional calibration of biological indices is necessary for improving their accuracy and sensitivity (Fore et al. 1996, Kerans et al. 1992, Barbour et al. 1995, 1996, Maxted et al. 1998, Roth et al. 1997). The process used here is a direct effort to formulate the Maryland benthic IBI according to specific regional conditions. The indices proposed by the MBSS are intended as indicators of stream and watershed ecological condition. Because of this, it is important that an identical set of stream locations (reference, degraded, and in-between) are used for their development. It would be without ecological rationale, for example, to develop a fish IBI using data from sites producing “good” fish samples, and a benthic IBI from sites producing “good” benthic samples. Definition of reference and degraded conditions were based on physical, chemical, and land use characteristics. Biological indicators (the metrics and indices selected and calibrated in this project) represent a measurement of the biota that are able to survive and reproduce in those conditions. All data used in this project came from randomly-selected sites, avoiding some of the potential biases such as mentioned above, as well as allowing aggregation to watershed-level assessments.

The majority of metrics selected for the two IBIs regionalized for Maryland Coastal Plain and non-Coastal Plain streams are supported by several demonstrations of their ability to discriminate between reference and degraded conditions. Two methods used for testing the discriminatory power of candidate metrics were statistical tests with subjectively selected significance levels (p-values). Additionally, the effectiveness of the individual metrics and aggregated indices in correctly classifying reference and degraded sites was documented by classification efficiency, which directly defines their ability to detect degradation. The MBSS quantitatively determined reference and degraded sites using criteria for physical habitat quality, water chemistry, and land use data; individual metrics and indices are *known* to reflect those conditions. The importance of this approach and the results is that metrics and index formulations are reflecting the occurrence of multiple, site-specific stressors which accumulate from landscape-level sources.

Ecological relevance of selected metrics

The initial compilation of candidate metrics and the process of metric selection and testing was in part driven by a goal of representing different categories of ecological information. Effort was made not only to maximize the effectiveness of detecting degradation, but also to communicate meaningful ecological information. The following provides a description of the ecological relevance of metrics that were selected and what changes in their values may mean.

Total number of taxa. The richness of the community in terms of number of genera indicates biodiversity of ecosystems and is commonly used as a quantitative measure of stream water and habitat quality. Taxa richness generally decreases as a stream ecosystem degrades (Resh and Grodhaus 1983) and may be a factor of habitat elimination, competitive displacement by opportunistic taxa following disturbance, and/or local extirpation of relatively intolerant taxa. Some stream systems often naturally support fewer taxa, such as high-gradient, cold-water streams. This

metric can also reflect temporarily higher numbers of taxa (relative to reference conditions) due to nutrient enrichment.

Number of EPT taxa. The richness of the generally intolerant insect orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) can indicate stream condition, since these taxa tend to become more scarce with increasing levels of disturbance (Lenat 1988). Some EPT taxa are less sensitive to pollutants or disturbance, so low taxa counts are usually represented by more tolerant taxa.

Number of Ephemeroptera taxa. The richness of mayfly taxa indicates the ability of a stream to support this generally intolerant insect order. Mayflies have medium to high oxygen requirements and some taxa need clean gravel substrate. Organic enrichment and excess fine sediment, indicators of anthropogenic stress, will often reduce the diversity of mayflies.

Number of Diptera taxa. Diptera as an order are relatively diverse and Dipterans are variable in their tolerance to stress. Many taxa, especially Chironomidae, have cosmopolitan distributions and may occur even in highly-polluted streams. However, a high diversity of Diptera taxa generally suggests good water and habitat quality.

Percent Ephemeroptera. The degree to which mayflies dominate the community can indicate the relative success of these generally pollution intolerant individuals in sustaining reproduction. The presence of stresses will reduce the abundance of mayflies relative to other, more tolerant individuals; although, some mayfly groups, such several genera of the family Baetidae, are known to increase in numbers in cases of nutrient enrichment.

Percent Tanytarsini of Chironomidae. The tribe Tanytarsini is a relatively intolerant group of midges. The degree to which they represent the total number of midges indicates the general sensitivity of the midge assemblage. A high percentage of Tanytarsini among the midges may indicate lower levels of anthropogenic stress. This metric increases with high numbers of Tanytarsini (among all Chironomidae) and decreases with high numbers of non-tanytarsine Chironomidae.

Percent Tanytarsini. Tanytarsini as a percentage of the entire sample has a significance similar to the percent Tanytarsini of Chironomidae, except that other midges do not affect the metric value.

Number of intolerant taxa. Intolerant taxa are the first to be eliminated by perturbations. Often, intolerant taxa are specialists and perturbations can disturb or eliminate specialized habitat or water quality requirements. Taxa with tolerance ratings from 0 to 3 on the 0 to 10 scale were considered intolerant.

Percent tolerant. As perturbation increases, tolerant individuals (tolerance values 7 - 10) tend to predominate in the sample. Intolerant individuals become less abundant as stress increases, leading to more individuals in tolerant, opportunistic taxa.

Beck's Biotic Index. The weighted enumeration of intolerant individuals in the community expresses the relative abundance of individuals in the most intolerant and second most intolerant classes. Since the most intolerant taxa are weighted more heavily, their abundance in the assemblage is more important to this metric. The metric increases with better water and habitat quality.

Number of scraper taxa. High diversity of the herbivorous scraper fauna can indicate a lack of stressors. This metric illustrates a food web effect; these genera feed on periphyton and associated microfauna which may themselves be more abundant under conditions of minimal perturbation.

Percent collectors. Abundance of detritivores, which feed on fine particulate organic matter in deposits, typically decreases with increased disturbance. This ecological response may be a food web effect, where organic material becomes scarce or unsuitable with increased perturbation, or membership within this feeding group may be highly represented by intolerant taxa.

Percent clingers. The taxa which cling to surfaces in fast moving water by means of morphological adaptations or construction of fixed retreats increase in abundance in the absence of stressors. The stressors which most adversely affect this metric are those that directly disturb or eliminate high quality habitat, such as clean gravel riffles.

The final suite of metrics in the MBSS CP IBI contained four of the five metrics used by Maxted et al. (1998) in the Mid-Atlantic Coastal Plain. These include "total number of taxa", "number of EPT taxa", "% Ephemeroptera", and "% clingers". In that study, these metrics had mean CE's of 44%, 83%, 63%, and 65%, respectively. The largest difference in performance is with the metric "number of EPT taxa", which produced a CE of only 55% in the MBSS CP. Differences in metric-specific and index CEs can be attributed to a different set of reference sites and index period. For the latter, the Mid-Atlantic Coastal Streams Workgroup samples during the fall (October 1 - December 1), whereas the MBSS index period is spring. Seasonal differences in sampling can cause differences in metric effectiveness. The "HBI" metric that completes the index developed by Maxted et al. was replaced in this study by "Beck's Biotic Index", which slightly outperforms the "HBI" in correctly identifying degradation.

Of the nine metrics selected for the NCP index, four were also used by Smith and Voshell (1997) in a 10-metric index developed for the Mid-Appalachian Highlands. They were "number of EPT taxa", "number of Ephemeroptera taxa", "% Ephemeroptera", and "number of intolerant taxa". Three metrics used by Smith and Voshell ("% EPT", "HBI", and "% scrapers") were not selected for the MBSS index because other metrics in the same categories either matched their CE or outperformed them. Habit metrics were not used for the MBSS NCP index, while Smith and Voshell used a broader category of habit (haptobenthos, or inhabiting clean substrate) to develop a useable metric.

The indices in the CP and NCP include three metrics in-common: "total number of taxa", "number of EPT taxa" and "% Ephemeroptera". In the CP, "total number of taxa" and "number of EPT taxa" were the only metrics in the richness category that significantly distinguished reference and degraded sites according to the Kolmogorov-Smirnov test, and then their classification efficiencies were low (47 and 55%, respectively). In the NCP, 6 richness metrics significantly discriminated, and those mostly had

higher CEs (56 to 82%).

Other differences in the two physiographic regions became apparent during the index development process. Notably, roughly twice as many candidate metrics significantly discriminated reference and impaired sites in the NCP. In the CP, this may be an artifact of a smaller number of sites, or it may indicate either that benthic macroinvertebrate assemblages are less responsive to environmental perturbation in this region or that reference site selection criteria were somehow inappropriate. Stressors seem to have a more profound ecological impact in the NCP.

Some metrics performed in a qualitatively different manner between the two regions as well. “Percent collectors” was significant in the NCP and decreased in response to perturbation. In the CP, this metric was not only non-discriminating, but on examination of value distributions, it appeared to slightly increase in response to stresses. The initial suite of metrics selected as the best performers in their metric categories had high discriminatory power (84 and 82% in the CP and NCP, respectively). This was also recognized during development of the fish IBI (Roth et al. 1997). As well as increasing the power, additional metrics in the index serve to broaden the applicability of the index by capturing ecological information that may be less common to all sites.

Although the CP and NCP division between the two indices may have been an intuitive result, the exercises of performing cluster and ordination analyses were useful in investigating other potential site classes. They also helped illuminate the need for additional sampling, since several finer regions were under-represented by sites. Several of the sub-ecoregional site groupings (Inner and Outer Coastal Plain [White 1997], Piedmont, Shale Ridges, Limestone Valleys [Omernik 1987, Woods et al. 1996]) as well as black water streams may, when represented by additional data from future sampling events, warrant consideration as separate site classes.

Though substantial confidence was placed in the ability of each metric and the overall index to discriminate degraded sites from nondegraded ones, other factors were also considered in the process of determining whether metrics should be included. The metrics “total number of taxa” and “number of EPT taxa” did not have extremely high discriminatory power from our tests; however, their near universal recognition in benthic assessment efforts and value in communication balances that concern. The goal of having metrics representative of the five categories necessitated inclusion of some metrics with relatively low classification efficiencies. For example, in the CP, the metric “number of scraper taxa” had a CE of only 55%, but it was also the only metric to pass other evaluation tests within the category of trophic/feeding metrics. Similarly, the metric “% collectors” is the only trophic/feeding metric included in the NCP index. Even with lower individual CEs, these metrics either helped improve those of the overall indices, or did not lower them. Their inclusion will help with the interpretive power of assessments.

Management and Policy Implications

The benthic Index of Biotic Integrity (IBI) described in this report has several applications that could enhance surface water protection and regulatory programs in Maryland. Whether numeric or narrative, this verified index should be an integral component of water quality biocriteria in Maryland. Integration with fish assemblage and physical habitat indices, as well as water chemistry data, will provide a comprehensive and defensible approach for assessing cumulative impacts to the state's streams. These biocriteria could provide a valuable tool for updating the state's list of impaired waters (the 303[d] list), evaluating the effectiveness of TMDL actions, and could ultimately be incorporated into Maryland's surface water use classes.

The benthic IBI provides many opportunities for coordination and cooperation among agencies monitoring stream benthos in Maryland. For example, four Maryland counties (Baltimore, Montgomery, Prince George's, and Anne Arundel) and Baltimore City currently monitor benthic assemblages in streams and three (Carroll, Howard, and Harford) are developing benthic monitoring programs. For existing programs, methods and assessment approaches could be compared as well as the feasibility of data integration. For programs under development, the approach outlined in this report could provide a template for program design. Meshing monitoring and assessment approaches could ultimately lead to increased data sharing and integration in reporting (e.g., 305[b], Maryland Tributary Strategies reports, county reports, municipal NPDES permits) as well as substantial cost savings. Other state agencies, such as Maryland Department of the Environment and Maryland State Highways Administration, could use this approach for evaluating point source and road construction impacts.

The IBI has already proven to be valuable for Maryland's developing Watershed Restoration Priorities through its Clean Water Action Plan. Because the IBI directly determines the quality of streams by measuring degradation of a biological resource (i.e., the benthic assemblage), the IBI should continue to be an integral component of the state's Watershed Restoration Action Strategies. Once restoration programs are implemented, the IBI will provide information on the degree of effectiveness of such programs.

The indices developed for higher level taxonomy (i.e., family and order) described in this report may provide a valuable tool for volunteer groups and school-based educational programs. With appropriate training, citizens and students could use these indices to obtain qualitative information on their local streams. Pending acceptance of Quality Assurance Project Plans, Maryland DNR and other resource agencies could then incorporate these citizen-based assessments into state water quality reports.

The benthic IBIs reported here were developed and verified using field data from non-tidal, first through third order streams. Their use for assessment of other waterbody types, e. g., larger streams, wetlands, or tidally-influenced waters, would not be appropriate. Though a similar approach for those waterbody types would produce effective indices, sufficient data have not yet been collected.

The MBSS is intended to be a long-term ecological monitoring program. Although it has often been suggested that a greater level of detail in data collection from a larger number of sites (such as species level taxonomy and more intensive geographic stratification), substantial increases in the costs of such a

program need to be considered. It is likely that such changes in the monitoring strategy of the MBSS would be cost-prohibitive and result in diminished data utility.

Integration of reference site and degraded site data from other programs (such as Montgomery County and Delaware) would increase the sample size of the dataset and perhaps the sensitivity of the biological indices. Use and integration of assessments from the previous indices with those from this development effort should be subjected to a performance-based comparison to define their level of comparability. The MBSS plans to update these indices with additional sites and sampling results in future years, in essence, recalibrating the IBI with new information. It is recognized that new reference sites may cause upward, or downward, adjustment of decision thresholds; they may also cause re-evaluation of site classification or strata. This dynamic nature of reference conditions should not be seen as a shortcoming to this process, rather, it should be seen as a means of improving this ecological indicator's ability to recognize degraded conditions.

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Appendices

Appendix A. Master Taxa List with designated tolerance value (TolVal), functional feeding group (FFG), and habit. Abbreviations of habits are as follow: bu - burrower, cn - clinger, cb - climber, sp - sprawler, dv - diver, and sk - skater. Notes are keyed to comments at end of table

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
Nematomorpha						bu	1
Enopla	Hoplonemertea	Tetrastemmatidae			Predator		
			Prostoma		Predator		
Turbellaria				4	Predator	sp	
	Tricladida	Planariidae		1	Predator	sp	
			Cura			sp	
			Dugesia	7	Predator	sp	
Oligochaeta				10	Collector	bu	
	Lumbriculida	Lumbriculidae		10	Collector	bu	
	Tubificida	Enchytraeidae		10	Collector	bu	2
		Naididae		10	Collector	bu	2
		Tubificidae		10	Collector	cn	2
			Limnodrilus	10	Collector	cn	
			Spirosperma	10	Collector	cn	
Hirudinea					Predator	sp	
	Pharyngobdellida	Erpobdellidae		10	Predator	sp	
			Mooreobdella	8	Predator	sp	
	Rhynchobdellida	Glossiphoniidae			Predator	sp	
			Helobdella		Predator	sp	
		Piscicolidae	Piscicola		Predator	sp	
Gastropoda	Basommatophora	Ancylidae			Scraper	cb	
			Ferrissia	7	Scraper	cb	
		Lymnaeidae		6	Scraper	cb	
			Fossaria	8	Scraper	cb	
			Lymnaea	7	Scraper	cb	
			Pseudosuccinea	6	Collector	cb	
			Radix	6	Collector	cb	
			Stagnicola	7	Scraper	cb	
		Physidae		8	Scraper	cb	
			Physella	8	Scraper	cb	
		Planorbidae		7	Scraper	cb	
			Gyraulus	8	Scraper	cb	
			Helisoma	6	Scraper	cb	
			Menetus	8	Scraper	cb	
			Planorbella	7	Scraper	cb	
			Promenetus	7	Scraper	cb	
	Mesogastropoda	Bithyniidae	Bithynia		Scraper	cb	
		Hydrobiidae		8	Scraper	cb	
			Amnicola	8	Scraper	cb	
			Hydrobia		Scraper	cb	

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
		Pleuroceridae	Goniobasis		Scraper	cb	
			Leptoxis		Scraper	cb	
		Valvatidae	Valvata				
		Viviparidae	Campeloma	6	Scraper	cb	
			Viviparus	1	Scraper	cb	
Pelecypoda	Unionoida	Unionidae			Filterer	bu	3
	Veneroida	Corbiculidae	Corbicula	6	Filterer	bu	
		Sphaeriidae			Filterer	bu	
			Pisidium	8	Filterer	bu	
			Sphaerium	8	Filterer	bu	
Malacostraca	Amphipoda					sp	
		Crangonyctidae		6	Collector	sp	
			Crangonyx	4	Collector	sp	
		Gammaridae	Gammarus	6	Shredder	sp	
			Stygonectes	6	Shredder	sp	
		Hyalellidae	Hyalella	6	Shredder	sp	
	Decapoda	Cambaridae		6	Shredder	sp	
			Cambarus	6	Collector	sp	
			Orconectes	6	Shredder	sp	
		Palaemonidae	Palaemonetes	7		sp	
	Isopoda			8	Collector		
		Asellidae	Caecidotea	8	Collector	sp	
			Lirceus	8	Collector	sp	
Insecta	Collembola						
		Isotomidae	Isotomurus				
	Ephemeroptera				Collector		
		Ameletidae					
			Ameletus	0	Collector	sw, cb	
		Baetidae			Collector	sw, cn	
			Acentrella	4	Collector	sw, cn	
			Acerpenna	4	Collector	sw, cn	
			Baetis	6	Collector	sw, cb, cn	
			Barbaetis	10	Collector		
			Callibaetis	9	Collector	sw, cn	
			Centroptilum	2	Collector	sw, cn	
			Dipheter		Collector	sw, cn	
			Procloeon	4	Collector		
		Baetiscidae	Baetisca	4	Collector	sp	
		Caenidae	Caenis	7	Collector	sp	
		Ephemerellidae				cn, sp, sw	

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Drunella	1	Scraper	cn, sp	
			Ephemerella	2	Collector	cn, sw	
			Eurylophella	4	Scraper	cn, sp	
			Serratella	2	Collector	cn	
			Timpanoga	2	Collector	sp	
		Ephemeridae	Ephemera	3	Collector	bu	
			Hexagenia	6	Collector	bu	
		Heptageniidae			Scraper	cn	
			Cinygmula		Scraper	cn	
			Epeorus	0	Scraper	cn	
			Heptagenia	4	Scraper	cn, sw	
			Leucrocuta	1	Scraper	cn	
			Nixe	2	Scraper	cn	
			Stenacron	4	Collector	cn	
			Stenonema	4	Scraper	cn	
		Isonychiidae	Isonychia	2	Filterer	sw, cn	
		Leptophlebiidae			Collector	sw, cn	
			Habrophlebia		Collector	sw, cn, sp	
			Leptophlebia	4	Collector	sw, cn, sp	
			Paraleptophlebia	2	Collector	sw, cn, sp	
		Metretopodidae	Siphloplectron	2	Predator	sw, cn	
		Potamanthidae	Anthopotamus				
		Siphonuridae			Collector	sw, cb	
			Siphonurus	7	Collector	sw, cb	
	Odonata				Predator		
		Aeshnidae			Predator	cb	
			Basiaeschna	6	Predator	cb, sp, cn	
			Boyeria	2	Predator	cb, sp	
		Calopterygidae	Calopteryx	6	Predator	cb	
		Coenagrionidae			Predator	cb	
			Argia	8	Predator	cn, cb, sp	
			Enallagma	8	Predator	cb	
			Ischnura	9	Predator	cb	
			Nehalennia		Predator	cb	
		Cordulegastridae	Cordulegaster	3	Predator	bu	
		Corduliidae			Predator	sp, cb	
			Macromia	3	Predator	sp	
			Somatochlora	1	Predator	sp	
		Gomphidae			Predator	bu	
			Arigomphus		Predator	bu	

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Dromogomphus	4	Predator	bu	
			Erpetogomphus		Predator	bu	
			Gomphus	5	Predator	bu	
			Hagenius	1	Predator	sp	
			Lanthus	6	Predator	bu	
			Progomphus	5	Predator	bu	
			Stylogomphus		Predator	bu	
		Libellulidae		9	Predator		
			Leucorrhinia		Predator	cb	
			Libellula		Predator	sp	
	Plecoptera	Capniidae			Shredder	sp, cn	
			Allocapnia	3	Shredder	cn	
			Capnia	1	Shredder	sp, cn	
			Paracapnia	1	Shredder		
		Chloroperlidae			Predator	cn	
			Alloperla		Predator	cn	
			Haploperla		Predator	cn	
			Perlinella		Predator	cn	
			Sweltsa		Predator	cn	
		Leuctridae			Shredder	sp, cn	
			Leuctra	0	Shredder	cn	
			Paraleuctra		Shredder	sp, cn	
		Nemouridae			Shredder	sp, cn	
			Amphinemura	3	Shredder	sp, cn	
			Nemoura	1	Shredder	sp, cn	
			Ostrocerca		Shredder	sp, cn	
			Prostoia		Shredder	sp, cn	
			Shipsa		Shredder	sp, cn	
			Soyedina		Shredder	sp, cn	
		Peltoperlidae			Shredder	cn, sp	
			Peltoperla		Shredder	cn, sp	
			Tallaperla		Shredder	cn, sp	
		Perlidae			Predator	cn	
			Acroneuria	0	Predator	cn	
			Eccoptura		Predator	cn	
			Neoperla	3	Predator	cn	
			Paragnetina	1	Predator	cn	
			Perlesta	4	Predator	cn	4
			Phasganophora		Predator	cn	5
		Perlodidae			Predator	cn	

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Clioperla	1	Predator	cn	
			Cultus		Predator	cn	
			Diploperla		Predator	cn	
			Isoperla	2	Predator	cn, sp	
			Malirekus		Predator	cn	
		Pteronarcyidae	Pteronarcys	2	Shredder	cn, sp	
		Taeniopterygidae		2	Shredder		
			Oemopteryx		Shredder	sp, cn	
			Strophopteryx		Shredder	sp, cn	
			Taeniopteryx	2	Shredder	sp, cn	
	Hemiptera	Belostomatidae	Belostoma	10	Predator	cb, sw	6
		Corixidae			Predator	sw	
			Palmacorixa		Predator		
			Trichocorixa	5	Predator	sw, cb	
		Gerridae	Gerris		Predator	sk	
			Trepobates		Predator	sk	
		Notonectidae	Notonecta	10	Predator	sw, cb	
		Veliidae	Microvelia	6	Predator	sk	
	Megaloptera	Corydalidae	Chauliodes	4	Predator	cn, cb	
			Corydalus	5	Predator	cn, cb	
			Nigronia	0	Predator	cn, cb	
		Sialidae		4	Predator	bu, cb, cn	
			Sialis	4	Predator	bu, cb, cn	
	Neuroptera	Sisyridae	Climacia		Predator	cb	7
	Trichoptera	Brachycentridae		1	Filterer		
			Brachycentrus	1	Filterer	cn	
			Micrasema	2	Shredder	cn, sp	
		Calamoceratidae	Heteroplectron	3	Shredder	sp	
		Dipseudopsidae	Phylocentropus	5	Collector	bu	8
		Glossosomatidae			Scraper	cn	
			Agapetus	2	Scraper	cn	
			Glossosoma	0	Scraper	cn	
		Hydropsychidae			Filterer	cn	
			Cheumatopsyche	5	Filterer	cn	
			Dipletrona	2	Filterer	cn	
			Homoplectra		Filterer	cn	
			Hydropsyche	6	Filterer	cn	
			Parapsyche	1	Filterer	cn	
		Hydroptilidae		4			

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Hydroptila	6	Scraper	cn	
			Leucotrichia		Scraper	cn	
			Ochrotrichia	4	Scraper	cn	
			Oxyethira	3	Collector	cb	
		Lepidostomatidae	Lepidostoma	3	Shredder	cb, sp, cn	
		Leptoceridae		4	Collector		
			Ceraclea	3	Collector	sp, cb	
			Mystacides	4	Collector	sp, cb	
			Nectopsyche	3	Shredder	cb, sw	
			Oecetis	8	Predator	cn, sp, cb	
			Trienodes	6	Shredder	sw, cb	
		Limnephilidae			Shredder	cb, sp, cn	
			Goera		Scraper	cn	
			Hydatophylax	2	Shredder	sp, cb	
			Ironoquia	3	Shredder	sp	
			Limnephilus	3	Shredder	cb, sp, cn	
			Platycentropus	4	Shredder	cb	
			Pycnopsyche	4	Shredder	sp, cb, cn	
		Molannidae	Molanna	6	Scraper	sp, cn	
		Odontoceridae	Psilotreta	0	Scraper	sp	
		Philopotamidae			Filterer	cn	
			Chimarra	4	Filterer	cn	
			Dolophilodes	0	Filterer	cn	
			Wormaldia		Filterer	cn	
		Phryganeidae	Ptilostomis	5	Shredder	cb	
		Polycentropodidae				cn	
			Neureclipsis	7	Filterer	cn	
			Nyctiophylax	5	Filterer	cn	
			Polycentropus	5	Filterer	cn	
		Psychomyiidae	Lype	2	Scraper	cn	
			Psychomyia	2	Collector	cn	
		Rhyacophilidae	Rhyacophila	1	Predator	cn	
		Sericostomatidae	Agarodes	3	Shredder	sp	
		Uenoidae				cn	
			Neophylax	3	Scraper	cn	9
	Lepidoptera			6			
		Cosmopterygidae	Pyroderces		Shredder	bu	
		Pyralidae			Shredder	cb	
		Tortricidae			Shredder	bu, cb	
Coleoptera		Curculionidae					

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Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
		Dryopidae	Helichus	5	Scraper	cn	
		Dytiscidae		5	Predator	sw, dv	
			Agabus	5	Predator	sw, dv	
			Cybister	5	Predator	sw, dv	
			Deronectes	5	Predator	sw	
			Derovatellus		Predator	sw, dv	
			Hydroporus	5	Predator	sw, cb	
			Uvarus	5	Predator	sw, cb	
		Elmidae		5	Collector	cn	
			Ancyronyx	2	Scraper	cn, sp	
			Dubiraphia	6	Scraper	cn, cb	
			Macronychus	4	Scraper	cn	
			Optioservus	4	Scraper	cn	
			Oulimnius	2	Scraper	cn	
			Promoesia	2	Scraper	cn	
			Stenelmis	6	Scraper	cn	
		Gyrinidae	Dineutus	4	Predator	sw, dv	
			Gyrinus	4	Predator	sw, dv	
		Halipidae	Halipus	5	Shredder	cb	
			Peltodytes	5	Shredder	cb, cn	
		Hydrophilidae	Berosus	5	Collector	sw, dv, cb	
			Cymbiodyta	5	Collector	bu	
			Enochrus	5	Collector	bu, sp	
			Hydrobius	5	Collector	cb, cn, sp	
			Hydrochus		Shredder	cb	
			Hydrophilus	5	Collector	sw, dv, cb	
			Sperchopsis	5	Collector	cn	
			Tropisternus	10	Collector	cb	
		Psephenidae	Ectopria	5	Scraper	cn	
			Psephenus	4	Scraper	cn	
		Ptilodactylidae	Anchytarsus	4	Shredder	cn	
		Scirtidae		4	Collector	cb, sp	
			Cyphon	7	Scraper	cb	
<hr/>							
	Diptera						
		Athericidae	Atherix	2	Predator	sp, bu	
		Blephariceridae	Blepharicera		Scraper	cn	
		Ceratopogonidae			Predator	sp, bu	
			Alluaudomyia		Predator	bu	
			Bezzia	6	Predator	bu	
			Ceratopogon	6	Predator	sp, bu	

Appendix A. Master Taxa List with designated tolerance value (ToIVal), functional feeding group (FFG), and habit. Abbreviations of habits are as follow: bu - burrower, cn - clinger, cb - climber, sp - sprawler, dv - diver, and sk - skater. Notes are keyed to comments at end of table (continued).

Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Culicoides	10	Predator	bu	
			Helius	4	Predator	sp, bu	
			Mallochohelea		Predator	bu	
			Probezzia	6	Predator	bu	
			Sphaeromias		Predator	bu	
		Chaoboridae	Chaoborus		Predator	sp, sw	
		Chironomidae					
			Ablabesmyia	8	Predator	sp	Tanp
			Apsectrotanypus	5	Predator	bu, sp	Tanp
			Brillia	5	Shredder	bu, sp	Orth
			Brundiniella	5	Predator	bu, sp	Tanp
			Cardiocladius	6	Predator	bu, cn	Orth
			Chaetocladius	6	Collector	sp	Orth
			Chironomini	6			Chir
			Chironomus	10	Collector	bu	Chir
			Cladopelma	7	Collector	bu	Chir
			Cladotanytarsus	7	Filterer		Tant
			Clinotanypus	8	Predator	bu	Tanp
			Conchapelopia	6	Predator	sp	Tanp
			Corynoneura	7	Collector	sp	Orth
			Cricotopus	7	Shredder	cn, bu	Orth
			Cricotopus/Orthocladius		Shredder		Orth
			Cryptochironomus	8	Predator	sp, bu	Chir
			Cryptotendipes	8	Collector	sp	Chir
			Diamesa	5	Collector	sp	Diam
			Dicrotendipes	10	Collector	bu	Chir
			Diplocladius	7	Collector	sp	Orth
			Endochironomus	10	Shredder	cn	Chir
			Eukiefferiella	8	Collector	sp	Orth
			Glyptotendipes	10	Filterer	bu, cn	Chir
			Heleniella		Predator	sp	Orth
			Heterotrissocladius		Collector	sp, bu	Orth
			Hydrobaenus	8	Scraper	sp	Orth
			Kiefferulus	10	Collector	bu	Chir
			Krenopelopia		Predator	sp	Tanp
			Labrundinia	7	Predator	sp	Tanp
			Larsia	6	Predator	sp	Tanp
			Limnophyes		Collector	sp	Orth
			Lopescladius		Collector	sp	Orth
			Macropelopia	7	Predator	sp	Tanp

Appendix A. Master Taxa List with designated tolerance value (ToIVal), functional feeding group (FFG), and habit. Abbreviations of habits are as follow: bu - burrower, cn - clinger, cb - climber, sp - sprawler, dv - diver, and sk - skater. Notes are keyed to comments at end of table (continued).

Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Meropelopia	7			Tanp
			Micropsectra	7	Collector	cb, sp	Tant
			Microtendipes	6	Filterer	cn	Chir
			Nanocladius	3	Collector	sp	Orth
			Natarsia	8	Predator	sp	Tanp
			Nilotanypus	6	Predator	sp	Tanp
			Odontomesa	4	Collector	sp	Prod
			Omisus				Chir
			Orthocladius	6	Collector	sp, bu	Orth
			Pagastia	1	Collector		Diam
			Parachaetocladius	2	Collector	sp	Orth
			Parachironomus	10	Predator	sp	Chir
			Paracladopelma	7	Collector	sp	Chir
			Parakiefferiella	4	Collector	sp	Orth
			Paralauterborniella	8	Collector	cn	Chir
			Paramerina	4	Predator	sp	Tanp
			Parametriocnemus	5	Collector	sp	Orth
			Paraphaenocladius	4	Collector	sp	Orth
			Paratanytarsus	6	Collector	sp	Tant
			Paratendipes	8	Collector	bu	Chir
			Paratrichocladius		Collector	sp	Orth
			Pentaneura	6	Predator	sp	Tanp
			Phaenopsectra	7	Collector	cn	Chir
			Polypedilum	6	Shredder	cb, cn	Chir
			Potthastia	2	Collector	sp	Diam
			Procladius	9	Predator	sp	Tanp
			Prodiamesa	3	Collector	bu, sp	Prod
			Psectrocladius	8	Shredder	sp, bu	Orth
			Pseudorthocladius	0	Collector	sp	Orth
			Psilometriocnemus		Collector	sp	Orth
			Rheocricotopus	6	Collector	sp	Orth
			Rheopelopia	4	Predator	sp	Tanp
			Rheosmittia				Orth
			Rheotanytarsus	6	Filterer	cn	Tant
			Saetheria	4	Collector	bu	Chir
			Stempellinella	4	Collector	cb, sp, cn	Tant
			Stenochironomus	5	Shredder	bu	Chir
			Stictochironomus	9	Collector	bu	Chir
			Sublettea		Collector		Tant
			Symposiocladius		Predator	sp	Orth

Appendix A. Master Taxa List with designated tolerance value (ToIVal), functional feeding group (FFG), and habit. Abbreviations of habits are as follow: bu - burrower, cn - clinger, cb - climber, sp - sprawler, dv - diver, and sk - skater. Notes are keyed to comments at end of table (continued).

Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Sympothastia	2	Collector	sp	Diam
			Syndiamesa			sp	Diam
			Tanypus	10	Predator		Tanp
			Tanytarsus	6	Filterer	cb, cn	Tant
			Thienemanniella	6	Collector	sp	Orth
			Thienemannimyia		Predator	sp	Tanp
			Tribelos	5	Collector	bu	Chir
			Trissopelopia		Predator	sp	Tanp
			Tvetenia	5	Collector	sp	Orth
			Unniella		Collector		Orth
			Xylotopus	2	Shredder	bu	Orth
			Zavrelia	4	Collector	cb, sp, cn	Tant
			Zavrelimyia	8	Predator	sp	Tanp
		Culicidae	Aedes	8	Filterer	sw	
		Dixidae	Dixa	4	Predator	sw, cb	
		Dolichopodidae			Predator	sp, bu	
		Empididae			Predator	sp, bu	
			Chelifera		Predator	sp, bu	
			Clinocera		Predator	cn	
			Hemerodromia	6	Predator	sp, bu	
		Ephydriidae			Collector	bu, sp	
		Muscidae		7	Predator	sp	
			Limnophora		Predator	bu	
		Psychodidae	Pericoma	4	Collector		
		Ptychopteridae	Bittacomorpha		Collector	bu	
		Simuliidae		7	Filterer	cn	
			Cnephia	4	Filterer	cn	
			Prosimulium	7	Filterer	cn	
			Simulium	7	Filterer	cn	
			Stegopterna	7	Filterer	cn	
		Stratiomyidae	Stratiomys		Collector	sp, bu	
		Syrphidae	Chrysogaster		Collector	bu	
		Tabanidae		8	Predator		
			Chrysops	7	Predator	sp, bu	
			Tabanus	5	Predator	sp, bu	
		Tipulidae			Predator	bu, sp	
			Antocha	5	Collector	cn	
			Cryptolabis			bu	
			Dicranota	4	Predator	sp, bu	
			Erioptera	7	Collector	bu	

Appendix A. Master Taxa List with designated tolerance value (ToIVal), functional feeding group (FFG), and habit. Abbreviations of habits are as follow: bu - burrower, cn - clinger, cb - climber, sp - sprawler, dv - diver, and sk - skater. Notes are keyed to comments at end of table (continued).

Class	Order	Family	Genus	ToIVal	FFG	Habit	Note
			Hexatoma	4	Predator	bu, sp	
			Limnophila	4	Predator	bu	
			Limonia	6	Shredder	bu, sp	
			Molophilus			bu	
			Ormosia		Collector	bu	
			Pilaria	7	Predator	bu	
			Pseudolimnophila	2	Predator	bu	
			Rhabdomastix			bu	
			Tipula	4	Shredder	bu	

1. Nematomorpha is a phylum level identification. No class level identification was made.
 2. Brinkhurst (1986). ITIS (1998) places the family in the order Haplotaxida.
 3. Margulis and Schwartz (1988). ITIS (1998) uses the class name Bivalvia.
 4. Merritt and Cummins (1996). ITIS (1998) places *Perlesta* in the family Chloroperlidae.
 5. Merritt and Cummins (1996). ITIS (1998) uses the genus name *Agetina*.
 6. Merritt and Cummins (1996). ITIS (1998) uses the order name Heteroptera.
 7. Merritt and Cummins (1996). ITIS (1998) places Sisyridae in the order Megaloptera.
 8. Merritt and Cummins (1996). ITIS (1998) places *Phylocentropus* in the family Psychomyiidae.
 9. Merritt and Cummins (1996). ITIS (1998) places *Neophylax* in the family Limnephilidae.
- Tanp Subfamily Tanypodinae
 Orth Subfamily Orthocladiinae
 Chir Tribe Chironomini
 Tant Tribe Tanytarsini
 Diam Subfamily Diamesinae
 Prod Subfamily Prodiamesinae

Appendix B. Metric values used for analysis in this project and their corresponding reference and degraded status.

COASTAL PLAIN		Index Metrics						
Site ID	Ref Status	Total taxa	EPT taxa	% Ephem	% Tany/ of Chir	Beck's Biotic Index	Scraper taxa	% clinger
CH-S-012-114	Ref	25	9	21.8	21.4	14	4	62.1
CN-N-044-3	Ref	8	5	0.8	0.0	7	2	99.2
KE-N-046-226	Ref	18	5	9.7	18.2	4	3	80.6
PG-N-069-2	Ref	24	13	10.2	100.0	16	6	83.0
QA-N-111-312	Ref	26	9	13.7	13.0	12	5	55.8
QA-N-112-315	Ref	36	9	14.3	7.7	14	8	60.2
SM-S-039-127	Ref	31	11	21.7	14.3	18	2	65.8
SM-S-199-302	Ref	24	11	46.2	21.4	13	4	38.7
SM-S-239-310	Ref	17	6	47.7	0.0	7	5	79.4
TA-N-048-3	Ref	20	5	2.0	0.0	7	1	52.0
TA-N-048-4	Ref	14	3	5.6	0.0	4	0	20.0
WI-S-023-112	Ref	32	5	11.4	8.6	12	6	41.9
WI-S-075-206	Ref	11	2	4.3	46.2	2	2	86.1
AA-N-164-1	Deg	7	3	0.0	0.0	3	0	0.9
AA-N-161-2	Deg	6	3	0.0	0.0	2	0	3.3
AA-N-180-130	Deg	13	5	0.0	0.0	6	3	75.0
CA-S-088-2	Deg	17	6	4.3	0.0	7	2	7.4
CA-S-108-3	Deg	13	6	1.0	0.0	7	1	4.0
CA-S-108-7	Deg	10	3	3.2	0.0	4	1	16.1
CA-S-209-2	Deg	11	5	42.4	0.0	2	0	2.2
CH-S-080-222	Deg	13	6	55.3	0.0	3	2	14.9
CH-S-177-129	Deg	16	1	8.3	8.9	4	1	40.7
CH-S-213-120	Deg	8	1	0.0	0.0	2	0	35.4
CH-S-293-136	Deg	18	7	0.0	64.3	11	0	31.3
CH-S-331-304	Deg	19	7	82.2	0.0	7	3	25.9
KE-N-096-102	Deg	24	1	0.0	0.0	2	3	0.0
PG-N-087-2	Deg	11	3	2.0	0.0	3	0	77.5
PG-N-205-2	Deg	14	5	0.9	3.0	6	0	45.3
PG-N-271-9	Deg	7	2	0.0	0.0	2	0	52.6
QA-N-030-128	Deg	14	3	23.0	0.0	2	2	21.8
QA-N-031-202	Deg	10	1	1.0	0.0	1	4	13.6
QA-N-041-109	Deg	19	1	1.8	6.3	1	1	16.7
QA-N-041-113	Deg	16	1	0.0	0.0	0	3	7.4
QA-N-086-118	Deg	23	9	18.0	35.0	7	4	43.8
QA-N-086-126	Deg	11	2	0.0	0.0	1	1	83.7
SM-S-104-126	Deg	11	3	0.0	0.0	5	0	89.4
SM-S-209-105	Deg	11	4	37.9	0.0	5	1	2.9
SO-S-005-109	Deg	19	2	0.0	6.5	3	1	22.4

Appendix B. Metric values of sites status reference (ref) and, degraded (deg) used for analysis in this project (Continued).

NON-COASTAL PLAIN		Index Metrics								
Site ID	Ref Status	Total taxa	EPT taxa	Ephem taxa	Dip taxa	% Ephem	% Tany-tarsini	% tolerant	Intol taxa	% collectors
AL-A-199-122	Ref	26	13	5	8	30.7	2.6	22.8	12	30.1
AL-A-215-112	Ref	14	7	3	7	29.7	33.7	11.4	7	14.9
AL-A-318-126	Ref	17	10	2	7	21.6	6.4	42.4	6	26.4
BA-P-206-108	Ref	16	11	5	2	78.0	0.0	1.7	9	78.9
CR-P-175-113	Ref	28	16	6	7	31.3	3.6	1.1	10	28.6
GA-A-062-202	Ref	25	15	6	9	63.5	2.9	2.1	12	40.9
GA-A-062-222	Ref	30	17	5	9	53.9	2.2	4.8	13	45.2
GA-A-111-316	Ref	18	5	4	11	14.1	18.5	16.3	3	62.0
GA-A-120-103	Ref	18	8	3	9	12.5	5.4	48.0	5	19.6
GA-A-141-213	Ref	33	19	6	9	40.7	5.1	1.0	13	39.0
GA-A-185-309	Ref	28	9	4	16	7.5	37.6	19.3	6	31.6
GA-A-236-216	Ref	22	12	4	10	12.6	21.1	22.4	8	30.5
GA-A-236-218	Ref	26	12	4	11	7.8	8.9	5.1	10	13.5
GA-A-407-314	Ref	21	14	5	6	29.0	0.0	0.9	7	31.5
GA-A-457-114	Ref	25	13	4	8	19.7	5.1	12.1	11	28.2
GA-A-511-322	Ref	18	13	5	4	4.1	0.0	1.8	7	7.4
GA-A-521-108	Ref	36	16	7	11	10.4	15.6	31.3	12	51.0
GA-A-545-301	Ref	19	10	3	7	12.5	4.8	6.4	5	22.5
HA-P-008-3	Ref	21	11	5	7	60.2	2.0	9.3	10	49.0
HO-P-036-314	Ref	17	3	3	11	5.7	9.8	3.4	3	82.8
HO-P-068-220	Ref	18	5	2	11	32.3	1.1	20.0	3	73.1
WA-A-053-223	Ref	14	4	1	10	2.1	3.5	69.9	2	8.5
WA-A-089-312	Ref	23	15	4	8	19.8	0.8	52.0	6	24.0
WA-V-161-214	Ref	27	12	4	13	20.8	4.9	14.6	10	52.1
AL-A-020-228	Deg	14	9	3	5	6.1	5.1	83.1	5	15.2
BA-P-107-123	Deg	18	6	2	8	9.0	2.2	48.1	4	56.2
GA-A-001-105	Deg	13	8	2	4	6.7	0.7	0.8	6	32.1
GA-A-010-205	Deg	26	7	3	17	7.6	5.0	60.6	4	25.2
GA-A-021-1	Deg	7	3	0	4	0.0	0.0	86.8	2	3.0
GA-A-021-2	Deg	7	4	0	2	0.0	0.9	34.8	1	0.9
GA-A-089-1	Deg	17	2	0	11	0.0	1.0	60.5	2	19.6
GA-A-143-1	Deg	6	2	0	2	0.0	0.0	71.6	2	0.0
GA-A-152-5	Deg	11	8	0	2	0.0	0.0	17.9	7	5.0
GA-A-235-215	Deg	13	8	4	5	29.1	3.5	2.6	6	31.4
GA-A-347-1	Deg	10	5	0	4	0.0	2.7	5.0	2	1.8
GA-A-520-1	Deg	11	6	0	2	0.0	0.9	19.2	3	7.9
GA-A-520-2	Deg	19	7	1	9	0.9	2.6	50.8	4	8.8
GA-A-553-1	Deg	4	2	0	1	0.0	0.0	97.6	1	0.0
GA-A-557-1	Deg	17	1	0	12	0.0	0.0	64.5	2	10.6
MO-P-022-3	Deg	14	7	1	6	2.1	0.0	71.0	6	8.2
MO-P-053-2	Deg	20	8	2	7	1.9	0.0	31.3	4	12.6
MO-P-265-5	Deg	11	2	0	5	0.0	0.0	91.0	2	69.6
MO-P-501-1	Deg	9	1	0	5	0.0	0.0	82.6	0	11.9
MO-P-501-3	Deg	8	2	0	3	0.0	0.0	77.6	1	10.8
WA-A-101-219	Deg	12	4	2	7	3.0	24.8	28.1	4	36.6
WA-V-003-123	Deg	6	0	0	4	0.0	0.0	6.2	0	8.2
WA-V-075-220	Deg	20	3	1	12	1.8	5.3	51.8	4	54.4
WA-V-157-111	Deg	18	4	2	10	11.1	25.2	53.8	1	40.0
WA-V-192-115	Deg	12	0	0	9	0.0	0.0	20.5	0	82.9
WA-V-193-110	Deg	14	3	1	7	1.8	35.7	43.8	0	70.5

Appendix C. Index scores and ratings of reference (Ref) and degraded (deg) sites from the 1994-95 data set. Raw scores range from 7 to 35 in the Coastal Plain and from 9 to 45 in the Non-Coastal Plain. IBI scores range from 1.0 to 5.0. Ratings are good (G), fair (F), poor (p) and very poor (vp).

Station ID code	Status	Raw Score	IBI Score	Rating	Station ID code	Status	Raw Score	IBI Score	Rating
COASTAL					NON-COASTAL				
CH-S-012-114	Ref	31	4.4	G	AL-A-199-122	Ref	37	4.1	G
CN-N-044-3	Ref	17	2.4	p	AL-A-215-112	Ref	31	3.4	F
KE-N-046-226	Ref	23	3.3	F	AL-A-318-126	Ref	31	3.4	F
PG-N-069-2	Ref	31	4.4	G	BA-P-206-108	Ref	33	3.7	F
QA-N-111-312	Ref	31	4.4	G	CR-P-175-113	Ref	39	4.3	G
QA-N-112-315	Ref	31	4.4	G	GA-A-062-202	Ref	41	4.6	G
SM-S-039-127	Ref	33	4.7	G	GA-A-062-222	Ref	41	4.6	G
SM-S-199-302	Ref	27	3.9	F	GA-A-111-316	Ref	33	3.7	F
SM-S-239-310	Ref	25	3.6	F	GA-A-120-103	Ref	29	3.2	F
TA-N-048-3	Ref	19	2.7	p	GA-A-141-213	Ref	43	4.8	G
TA-N-048-4	Ref	13	1.9	vp	GA-A-185-309	Ref	35	3.9	F
WI-S-023-112	Ref	25	3.6	F	GA-A-236-216	Ref	31	3.4	F
WI-S-075-206	Ref	21	3.0	F	GA-A-236-218	Ref	37	4.1	G
AA-N-164-1	Deg	9	1.3	vp	GA-A-407-314	Ref	35	3.9	F
AA-N-161-2	Deg	9	1.3	vp	GA-A-457-114	Ref	35	3.9	F
AA-N-180-130	Deg	19	2.7	p	GA-A-511-322	Ref	25	2.8	p
CA-S-088-2	Deg	17	2.4	p	GA-A-521-108	Ref	41	4.6	G
CA-S-108-3	Deg	15	2.1	p	GA-A-545-301	Ref	29	3.2	F
CA-S-108-7	Deg	13	1.9	vp	HA-P-008-3	Ref	37	4.1	G
CA-S-209-2	Deg	15	2.1	p	HO-P-036-314	Ref	31	3.4	F
CH-S-080-222	Deg	17	2.4	p	HO-P-068-220	Ref	33	3.7	F
CH-S-177-129	Deg	17	2.4	p	WA-A-053-223	Ref	15	1.7	vp
CH-S-213-120	Deg	7	1.0	vp	WA-A-089-312	Ref	27	3.0	F
CH-S-293-136	Deg	19	2.7	p	WA-V-161-214	Ref	39	4.3	G
CH-S-331-304	Deg	21	3.0	F	AL-A-020-228	Deg	23	2.6	p
KE-N-096-102	Deg	11	1.6	vp	BA-P-107-123	Deg	27	3.0	F
PG-N-087-2	Deg	15	2.1	p	GA-A-001-105	Deg	25	2.8	p
PG-N-205-2	Deg	17	2.4	p	GA-A-010-205	Deg	31	3.4	F
PG-N-271-9	Deg	9	1.3	vp	GA-A-021-1	Deg	9	1.0	vp
QA-N-030-128	Deg	17	2.4	p	GA-A-021-2	Deg	11	1.2	vp
QA-N-031-202	Deg	9	1.3	vp	GA-A-089-1	Deg	17	1.9	vp
QA-N-041-109	Deg	13	1.9	vp	GA-A-143-1	Deg	9	1.0	vp
QA-N-041-113	Deg	11	1.6	vp	GA-A-152-5	Deg	15	1.7	vp
QA-N-086-118	Deg	27	3.9	F	GA-A-235-215	Deg	29	3.2	F
QA-N-086-126	Deg	15	2.1	p	GA-A-347-1	Deg	17	1.9	vp
SM-S-104-126	Deg	17	2.4	p	GA-A-520-1	Deg	15	1.7	vp
SM-S-209-105	Deg	19	2.7	p	GA-A-520-2	Deg	19	2.1	p
SO-S-005-109	Deg	13	1.9	vp	GA-A-553-1	Deg	9	1.0	vp
					GA-A-557-1	Deg	15	1.7	vp
					MO-P-022-3	Deg	15	1.7	vp
					MO-P-053-2	Deg	21	2.3	p
					MO-P-265-5	Deg	13	1.4	vp
					MO-P-501-1	Deg	9	1.0	vp
					MO-P-501-3	Deg	9	1.0	vp
					WA-A-101-219	Deg	25	2.8	p
					WA-V-003-123	Deg	13	1.4	vp
					WA-V-075-220	Deg	25	2.8	p
					WA-V-157-111	Deg	27	3.0	F
					WA-V-192-115	Deg	17	1.9	vp
					WA-V-193-110	Deg	21	2.3	p