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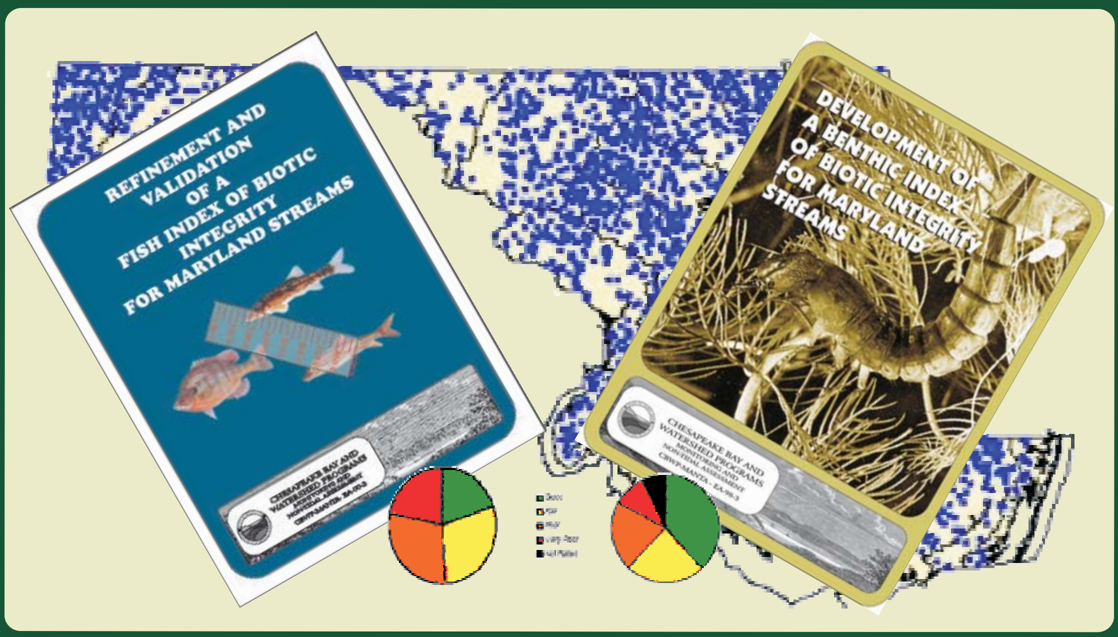

New Biological Indicators To Better Assess The Condition Of Maryland Streams

MARYLAND
DEPARTMENT OF NATURAL RESOURCES

CHESAPEAKE BAY AND WATERSHED PROGRAMS
MONITORING AND NON-TIDAL ASSESSMENT
CBWP-MANTA-EA-05-13

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Secretary

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NEW BIOLOGICAL INDICATORS TO BETTER ASSESS THE CONDITION OF MARYLAND STREAMS

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ABSTRACT

To better assess Maryland streams, Indices of Biotic Integrity (IBIs) that perform better and apply to more stream classes were needed by the Maryland Biological Stream Survey (MBSS). With completion of the second statewide round in 2004**,** the MBSS had collected data from approximately 2500 stream sites, more than doubling the number of sites that were available for the original IBI development. Therefore, development of new fish and benthic macroinvertebrate IBIs was undertaken to achieve the goals of (1) increased confidence that the reference conditions are minimally disturbed; (2) including more natural variation across the geographic regions and stream types of Maryland; and (3) increased sensitivity of IBIs by using more classes (strata), different metric combinations, or alternative scoring methods. New fish IBIs were developed for four geographical and stream type strata: the Coastal Plain, Eastern Piedmont, warmwater Highlands, and coldwater Highlands streams; new benthic macroinvertebrate IBIs were developed for three geographical strata: the Coastal Plain, Eastern Piedmont, and Highlands streams. The addition of one new fish IBI and one new benthic macroinvertebrate IBI reduced the natural variability of these assemblages in each stratum. At the same time, smaller streams (i.e., those draining catchments < 300 acres), which constituted a greater proportion of streams (40%) sampled in Round Two (2000-2004), were included in the reference conditions used to develop the new IBIs. The resulting new IBIs have good-to-excellent classification efficiencies (83% to 96%) and are well balanced between Type I and Type II errors. By scoring coldwater streams, smaller streams, and to some extent blackwater streams higher (i.e., not systematically underscoring them), the new IBIs improve on the original IBIs. Overall, about 20% fewer watersheds in Maryland are designated as degraded using the new IBIs and Maryland's biocriteria framework. The new IBIs remain transparent and understandable, and provide clear thresholds of impairment for both the biointegrity and interim (fishable and swimmable) water quality goals. The consistency between the original and new IBIs allows for joint estimates between MBSS Rounds One and Two, detection of temporal trends in stream condition, and minimal impact on county stream assessment programs.

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1. INTRODUCTION

The Maryland Department of Natural Resources (DNR) has committed to long-term monitoring of its streams under the Maryland Biological Stream Survey (MBSS). The MBSS is a probability-based sampling program that can describe streams at varying spatial scales (Klauda et al. 1998). An objective of the MBSS is to assess the status and trends in biological integrity for all 9400 non-tidal stream miles (on the 1:100,000 map scale) in Maryland. Therefore, it is critical that the MBSS provide estimates of the biological condition of streams using indicators based on references of biological integrity. Karr and Dudley (1981) used reference condition as the basis for their definition of biological integrity, i.e., "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats in the region."

Multi-metric Indices of Biotic Integrity (IBIs), originally developed by Karr et al. (1986), are the most common indicators of stream condition in use today. Most IBIs develop their expectations for the structure and function of biological assemblages from reference sites. Originally, however, the variability in these reference sites was not explicitly modeled nor was the ability of the indicator to distinguish deviation from reference condition directly tested. Currently, it is standard practice to test the performance of IBIs by determining the percentage of reference sites and known degraded sites that are correctly classified. This was first done in Maryland by Weisberg et al. (1997) for the Chesapeake Bay Estuary. More recently, researchers have demonstrated the utility of empirically modeling reference condition from reference sites as exemplified in the Bailey et al. (2004) "reference condition approach." Thus IBI development today involves the careful testing of the performance of individual metrics and their combinations as indicators that work best for the geographic regions and stream types of interest.

The MBSS developed the first fish (Roth et al. 1998) and benthic macroinvertebrate (Stribling et al. 1998) IBIs for Maryland in 1998. Subsequently, Roth et al. (2000) refined the Maryland fish IBI and Southerland et al. (2004) developed a stream salamander IBI for Maryland. To date, salamander sampling to support the stream salamander IBI has not been conducted, though it is being considered for future MBSS sampling. These original Maryland IBIs have performed well, helping Maryland DNR and other agencies better characterize and manage State waters, and have produced dozens of assessments and research findings. At the same time, these IBIs have not adequately captured reference condition for some classes of streams, i.e., some geographic areas, smaller streams, coldwater streams, and blackwater streams. Specifically, either a more general IBI has been applied to two classes of streams (e.g., both Highlands and Piedmont streams) or no IBI has been applied (e.g., streams draining catchments of less than 300 acres).

To better assess Maryland streams, IBIs that accurately characterize stream condition in more stream classes were needed. With completion of the second statewide round in 2004, the MBSS had collected data from approximately 2500 stream sites, more than doubling the number of sites than were available for the original IBI development. Therefore, development of new fish and benthic macroinvertebrate IBIs was undertaken with the following goals:

- Increase confidence that the reference conditions used are minimally disturbed by refining the criteria for selecting reference sites,

- Better capture the full range of natural variation in reference condition in Maryland by including more reference sites from unique geographic regions and stream types,

- Increase the sensitivity of IBIs for distinguishing human disturbance by segregating variation into more classes of reference condition, and

- Evaluate alternative scoring methods that might improve the performance of IBIs.

At the same time, development of the new IBIs had to take into account the following practical constraints:

- Fewer reference sites are available to characterize reference condition when a larger number of geographic or stream type classes are used, and

- IBIs developed for larger geographic or stream type classes may be less sensitive for distinguishing between reference condition and degraded condition.

2. DEVELOPMENT OF NEW IBIS

With these objectives and constraints in mind, we undertook development of new fish and benthic macroinvertebrate IBIs for Maryland following the same steps used to develop the original MBSS IBIs:

- Develop the database,
- Identify reference and degraded sites,
- Determine the appropriate strata,
- Test the candidate metrics, and
- Test and validate the indices.

In addition, we evaluated the effects of alternative metric scoring methods on metric and index performance.

2.1 MBSS DATABASE

It is essential that the data used to develop IBIs (e.g., reference sites) are comparable to the data collected at the sites of concern (test sites). A virtue of the MBSS is that the same biological, chemical, physical habitat, and land use data are collected for all sites used in stream assessment and indicator development. The MBSS is also ideal for the development of IBIs because the sampling protocols are rigorously applied through annual training and quality assurance (Roth et al. 2005a).

MBSS sites are selected using a probability-based design applied to all first- through fourth-order streams in Maryland based on a map scale of 1:100,000 (Roth et al. 2005b). Benthic macroinvertebrates are sampled in the spring and identified to genus or lowest practical taxon in 100-organism subsamples. Fish are sampled in the summer using double-pass electrofishing of 75-m stream segments. Water chemistry and physical habitat data are collected from these same segments. Land use information is extracted from Maryland Office of Planning data for the catchments draining to each segment.

As was done for the original IBIs and was described in Roth et al. (2000), we developed an integrated dataset that included all site and landscape environmental variables linked to the biological data and their derived attributes such as tolerance values and functional groups. The original IBIs were developed with data collected from 1994 to1997 from a maximum of 1098 sites, divided into 732 calibration sites and 366 (33%) validation sites. The dataset for the new IBIs included all samples from 1994 to 2004, totaling 2508 sites with 353 (14%) reserved for validation. Having data from 2508 sites was the primary reason that development of new IBIs was undertaken. We believed this large number of sites provide us with enough reference sites to create reference conditions for additional classes of Maryland stream types. Small numbers of (or no) reference sites in a stream type (e.g., coldwater streams) prevent development of effective IBIs.

2.2 BETTER REFERENCE CONDITIONS

Using reference sites that are minimally disturbed is perhaps the most important component of IBI development. If reference sites are only relatively less degraded than other sites (often referred to as least disturbed), assigning quality levels to IBI scores becomes problematic. Therefore, we reviewed the reference criteria used in the original MBSS IBIs (a site must meet all criteria to be designated as a reference site) to identify changes that would result in greater confidence that the new reference sites were minimally disturbed. We decided to retain the following reference criteria that effectively reflect levels at which individual stressors will probably not result in adverse effects (Roth et al. 2000):

- pH ≥ 6 or blackwater stream (pH < 6 and DOC ≥ 8 mg/l),
- ANC ≥ 50 μeq/l,
- DO ≥ 4 ppm,
- nitrate ≤ 300 μeq/l (4.2 mg/l),
- remoteness rating: optimal or suboptimal,
- aesthetics rating: optimal or suboptimal,
- instream habitat rating: optimal or suboptimal,
- no channelization, and
- no point source discharges.

Because the remoteness variable was replaced with "distance to nearest road" in Round Two and the channel alteration variable was replaced with "channelization," comparable replacement criteria were applied to Round Two sites. Specifically, the surrogate "remoteness" variable was obtained by converting the distance to nearest road value to a 0-20 score using the equation: $= 0.615 + 0.733\sqrt{\text{meters from road}}$ (Paul et al. 2003). A regression of this new remoteness variable on the original variable yielded a reference criterion threshold of 70. For Round Two sites, the reference criterion of no channelization was indicated by a "no" value for the channelization variable.

At the same time, we believed that the land use criteria were not strict enough to eliminate sites with adverse effects. Therefore, we changed the minimum allowable forested land use from ≥ 25% to ≥ 35% of the catchment area and the maximum allowable urban land use from ≤ 20% to ≤ 5% of the catchment area. In addition, studies indicated that wide riparian buffers often ameliorated land use effects, so the minimum allowable riparian buffer width was changed from 15m to 30m.

These changes in land use and riparian width thresholds resulted in a smaller proportion of sites meeting the reference site criteria. Using the original reference site criteria, 152 of the 1098 Round One sites (14%) were designated as reference sites. Using the new criteria, 196 of the total 2508 sites (8%) were designated as reference. Figure 2-1 shows that the cumulative distribution of stream miles with equal or greater benthic macroinvertebrate IBI scores for Round Two sites meeting the new reference criteria is to the right (i.e., higher quality) than sites meeting the original reference criteria. This result is consistent with greater confidence that the sites are minimally disturbed and since the total number of reference sites is greater than that

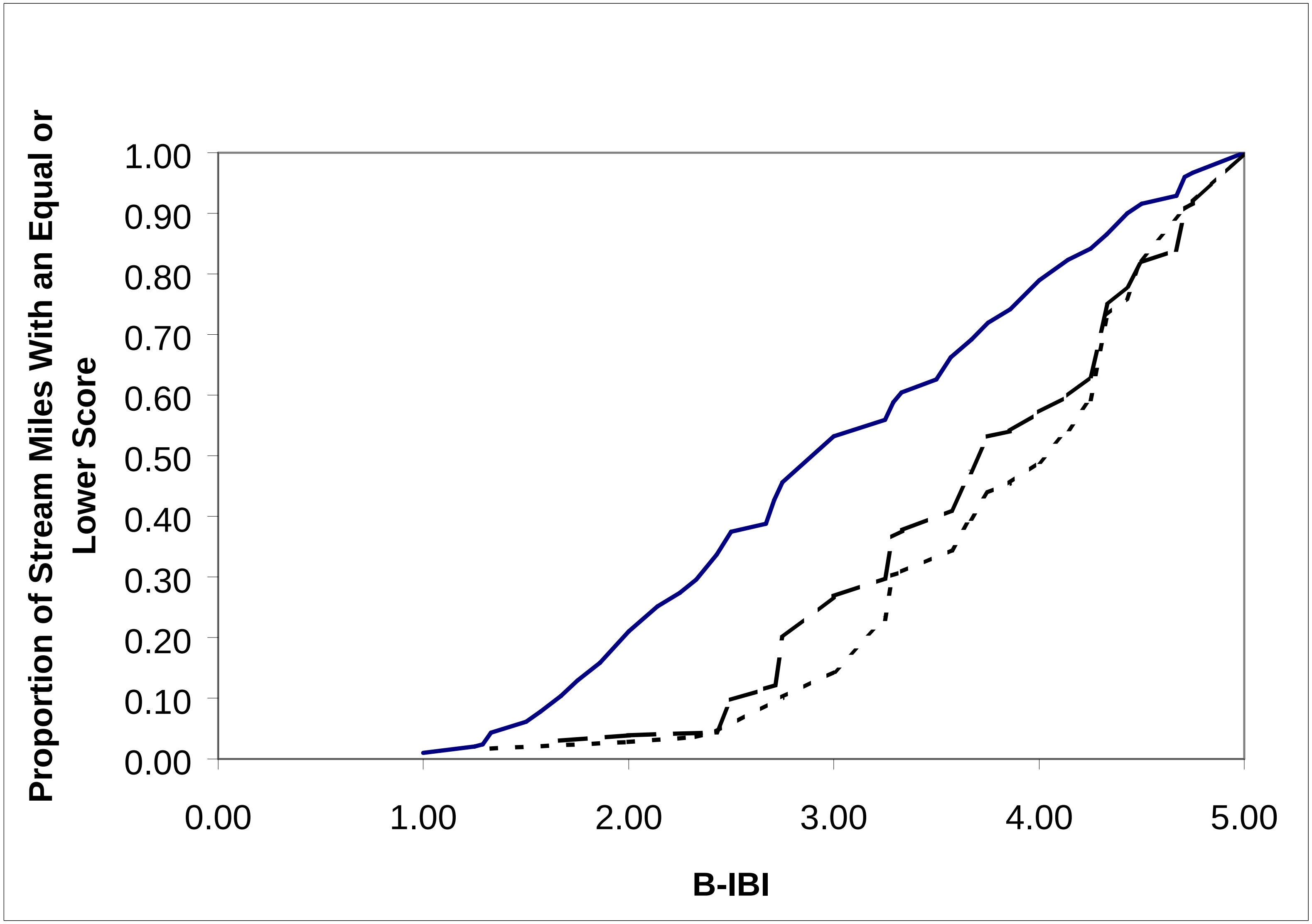

Figure 2-1. Cumulative distribution of stream miles with benthic macroinvertebrate IBI scores for (1) MBSS sites sampled in 2000-2004 (solid line), (2) subset of 2000-2004 sites meeting original reference criteria (dashed line), and (3) subset of 2000-2004 sites meeting new reference criteria (dotted line)

used to develop the original IBIs, the characterization of reference condition should be more robust.

Because it is possible that sites identified as reference are degraded by stressors not captured in the reference criteria, we investigated site records for five reference sites with one original IBI score (fish or benthic) < 2 and the other IBI score < = 3 (or missing). Only one site had any evidence of degradation and this potential degradation was not attributable to a specific source (based on detailed field sheets and recollections of field crews), so these reference sites were retained in the dataset.

We retained the criteria for degraded sites from the original IBIs as follows (a site failing any one of the criteria is designated as a degraded site):

- pH $\leq$ 5 and ANC $\leq$ 0 μeq/l (except for blackwater streams, DOC $\geq$ 8 mg/l) (n=23 sites),
- DO $\leq$ 2 ppm (n=20),
- nitrate > 500 μeq/l (7 mg/l) and DO < 3 ppm (n=0),
- instream habitat rating poor and urban land use > 50% of catchment area (n=15),
- instream habitat rating poor and bank stability rating poor (n=34),
- instream habitat rating poor and channel alteration rating poor (n=69), and
- urban land use > 50% of catchment area and riparian buffer width = 0 m (n=48).

A total of 170 of the 2508 sites (7%) were designated as degraded.

2.3 FULL RANGE OF NATURAL VARIABILITY

While the original benthic macroinvertebrate IBI was applied to all sampled streams, the MBSS recognized that the reference sites did not adequately capture the natural variation of fish assemblages in small streams. This was in part due to the lower abundance of fish and fewer fish species in small streams, but also due to the small number of reference sites in these streams. Therefore, streams draining catchments of less than 300 ac (i.e., where the number of fish and fish species sampled were frequently less than 100 and 5, respectively) were not rated using the original fish IBI (Roth et al. 2000). This resulted in 98 (11%) of streams sampled from 1995 to 1997 being not rated for fish because of their small size (an additional 5% of sites were not rated because they were dry and therefore not sampleable in the summer).

From 2000 to 2004 the MBSS sampled streams from a new 1:100,000-scale map that included a greater proportion of small streams than was sampled from 1995 to 1997. Specifically, while 11% of streams sampled in 1995-1997 drained < 300 ac, 25% of streams sampled in 2000-2004 drained < 300 ac. Only 5% of streams draining < 300 ac had < 100 fish sampled, so data limitation was not a justification for excluding all < 300 ac streams. Therefore, we attempted to include these smaller streams in the development of the new fish IBI. We included all stream sizes in the analyses, creating a more representative but more variable reference condition; subsequently we investigated partitioning this variability into separate small stream or coldwater stream type classes (see next section).

2.4 MORE CLASSES OF REFERENCE CONDITION

As described above, the 196 reference sites meeting the more restrictive reference criteria are representative of the best 8% of streams in Maryland. These reference sites were randomly selected within 84 primary sampling units (i.e., individual and combinations of Maryland 8-digit watersheds) and are distributed across all regions and stream types. This distribution allowed us to identify stream classes using empirical data, which is generally preferable to a priori classification (Hawkins et al. 2000). The goal of the classification step in indicator development is to partition the variability in reference condition into homogeneous regions or stream types that are best addressed with separate IBIs.

The original fish IBIs were developed for three strata: Coastal Plain, Eastern Piedmont (as defined by fish distributions ending at Great Falls), and Highlands (Figure 2-2). The original benthic macroinvertebrate IBIs were developed for the Coastal Plain and non-Coastal Plain (Highlands and Eastern Piedmont combined). For the new IBIs, we performed cluster analyses as described in Roth et al. (2000) to identify groups of sites with similar biological assemblages (as represented by log-transformed percentages of species abundance). To ensure sufficient sample size, sites meeting the original reference criteria (i.e., 261 of the 2500 sites) were used in the analysis. Separate cluster analyses were done for fish and benthic macroinvertebrates. Another approach to identifying useful strata is to apply cluster analysis or other multivariate techniques to the metrics likely to be used in the IBIs (Angermeier et al. 2000).

The cluster analyses in this study indicated that fish assemblages divided into four fairly distinct groups: Coastal Plain, Eastern Piedmont, small streams in Highlands (draining < 3,000-ac catchments), and large streams in Highlands (Figure 2-2). Smaller clusters were also significantly different, but only these larger clusters could be associated with consistent abiotic variables (e.g., geographic boundaries or stream size). The differentiation among benthic macroinvertebrate assemblages was similar but less strong; comparisons of the benthic macroinvertebrate taxa list among the groups also indicated consistent differences.

Distinct blackwater assemblages were not discernable in cluster analyses for either fish or benthic macroinvertebrates. Sites with presumed blackwater fish species (e.g., pirate perch) match well with water chemistry values diagnostic of blackwater (i.e., pH $\leq$ 5 and DOC $\geq$8 mg/l); however, preliminary analysis indicates that benthic macroinvertebrates in blackwater streams are primarily non-insects. In addition, there were not enough blackwater reference sites to create a separate stratum. Nonetheless, other evidence (including analysis of sentinel blackwater sites) indicates that there are differences that may justify not rating blackwater sites with the new fish and benthic macroinvertebrate IBIs. We also investigated whether lower eastern shore streams should be a separate stratum based on the lack of riffles in this region, but decided that it could not be definitively determined that the lack of riffles was a natural condition and not a result of historical degradation.

Using the original fish IBIs, the MBSS determined that most smaller streams, and especially coldwater streams, were scoring lower than larger streams of comparable quality by approximately one-third. These erroneously low scores could lead to designating small streams

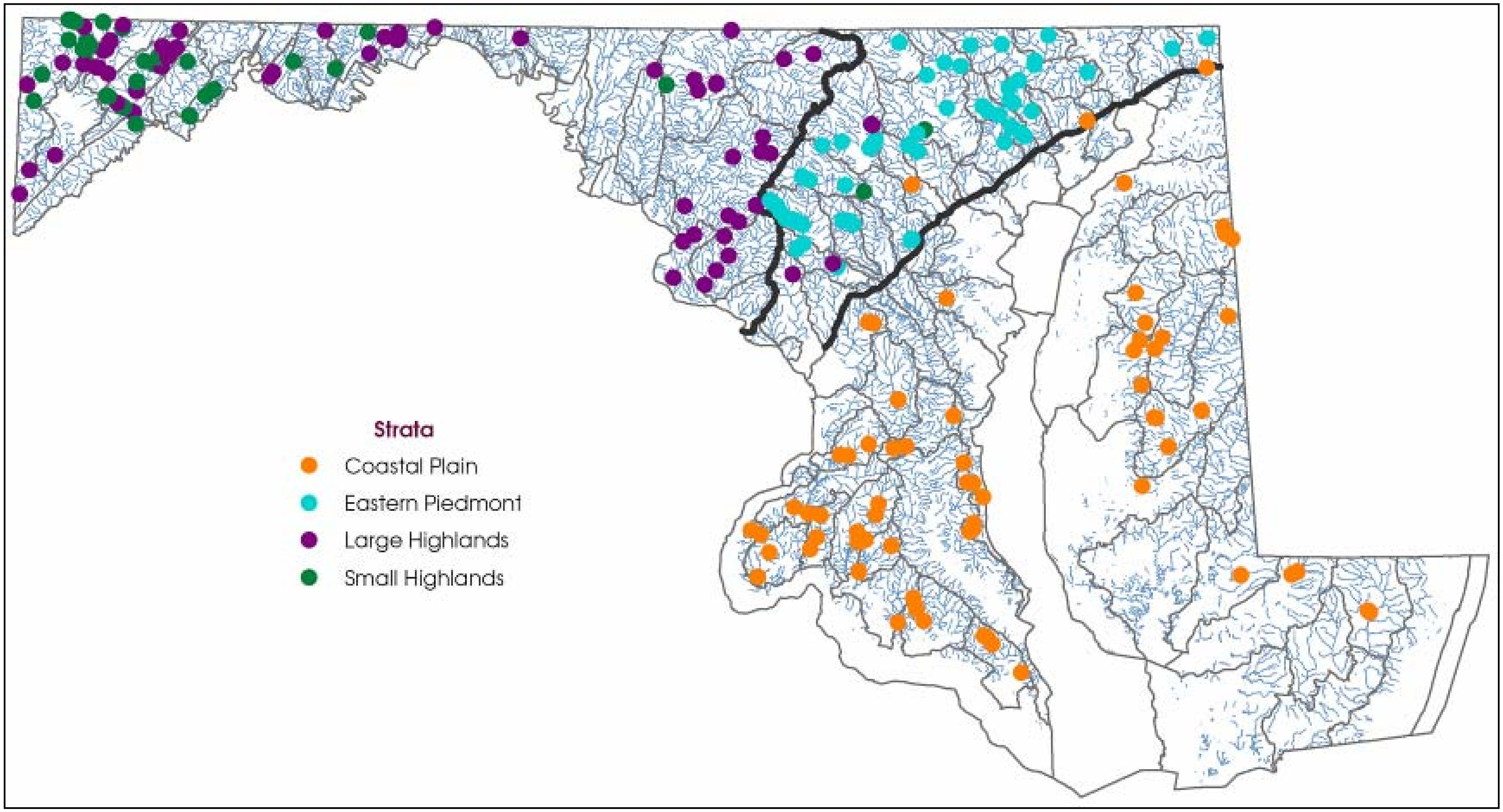

Figure 2-2. Map of ecological strata of Maryland (as designated by the dark lines separating, from the left, Highlands, Eastern Piedmont, and Coastal Plain) and distribution of non-degraded sites with distinctly different fish assemblages. Each stratum represents sites that were found to be similar using cluster analysis.

as impaired when they are not. Therefore, we used both the segregated Highlands stream strata (small and large streams separately) and the combined Highlands stream stratum in subsequent indicator development steps. We also developed a coldwater streams stratum based on current and likely sustainable distributions of brook trout (Matt Kline, University of Maryland-Appalachian Laboratory, personal communication) for use in indicator development. The coldwater stratum included all streams west of Evitts Creek in western MD; isolated brook trout streams in the Catoctin Mountain area, and parts of the Patapsco, Gunpowder, and Susquehanna watersheds were not included in the coldwater streams stratum.

The selection of each of these geographic or stream type strata has a strong ecological basis and potential for improving the performance of IBIs by reducing the variation in reference condition within each stratum. The number of reference sites occurring in each stratum is a practical limitation to IBI development. Bailey et al. (2004) recommends using 5 to 10 reference sites per class (stratum) as a minimum; experience of the MBSS indicates that 40 reference sites in each stratum is effective for developing IBIs. Even though more restrictive reference criteria were used to develop the new IBIs, the large dataset of 2500 sites still provided enough reference sites (approximately 40) for fish IBI development in each of four naturally different stream types: Coastal Plain, Eastern Piedmont, warmwater Highlands, and coldwater Highlands. For the new benthic macroinvertebrate IBI, the coldwater stratum was not used because, unlike fish, benthic macroinvertebrates assemblages are not typically depauperate in minimally disturbed coldwater streams. Table 2-1 shows the number of reference and degraded sites occurring in each geographic or stream type stratum considered in new IBI development.

Table 2-1. Reference and degraded sites occurring in each geographic or stream type stratum*

	Reference	Degraded
Coastal Plain	52	82
Eastern Piedmont	43	40
Warmwater Highlands	53	35
Coldwater Highlands	48	13

* Includes both calibration and validation data

2.5 TESTING CANDIDATE METRICS

In developing the original fish and benthic macroinvertebrate IBIs, the MBSS compiled and tested more than 100 candidate metrics (Roth et al. 2000, Stribling et al. 1998). For the new IBIs, we retained all metrics that showed promise in the original analysis (i.e., all that had significantly different values for reference and degraded sites) and added selected new candidate metrics. The list of candidate metrics for the new fish IBI included 44 original metrics and the following new metrics:

- Pirhalla (2004) habitat tolerance metrics,

- Log-transformed metrics that included sculpins (10 metrics including versions adjusted for catchment area), and

- Observed/Expected (O/E) for fish (Stranko et al. 2005).

The list of candidate metrics for the new benthic IBI included 51 original metrics and the following new metrics calculated based on new benthic macroinvertebrate tolerance values for urban and agriculture calculated from the MBSS dataset (Bressler et al. 2004):

- Number and percentage of intolerants,
- Percentage of tolerants
- Hilsenhoff Biotic Index (HBI), and
- Beck's index.

The log-transformed metrics were included because of analysis indicated that the original fish IBIs may have been overly influenced by sculpin abundance. The other new metrics were not available when the original IBIs were developed. Tables 2-2 and 2-3 describe what each metric means; Tables 4 and 5 present the result of testing these candidate metrics.

As was done for the original fish IBIs, metrics of fish abundance and species richness were tested within each stratum, both as raw values and adjusted for catchment area (Roth et al. 2000). Specifically, equations were developed that regressed the raw sample values against the area of the upstream catchment for each site. The derived values for these adjusted metrics were obtained as the ratio of the raw value over the value expected from the regression equation, with the m (slope) and b (intercept) as follows:

adjusted value = observed value / expected value
where expected value = m * log (catchment area in acres) + b

We tested all candidate metrics by comparing mean values and distributions between reference and degraded sites in each stratum, in combined strata, and statewide. We also looked at including and excluding sites with no fish, sites draining < 300 ac, and sites with < 60 benthic macroinvertebrates to evaluate these effects on the metrics. These different comparisons ensured that the usefulness of each metric for all possible IBIs were considered.

The ability of fish metrics to discriminate between reference and degraded sites (i.e., the number of such sites correctly classified) was similar when sites draining < 300 ac were included. Ann Roseberry-Lincoln (Maryland DNR, personal communication) found no evidence of a bias in benthic IBIs resulting from small stream size. Only three reference sites had < 60 benthic macroinvertebrates, so low count sites were included in the metric testing.

The first step in metric testing was to test for significant differences in (1) the mean values between reference and degraded sites using the Mann-Whitney U test and (2) the distributions of values using the Kolmogorov-Smirnov test. The next step was to score the metrics based on the distribution of values observed at reference sites within each stratum. In developing the original

Table 2-2. Definitions of candidate fish metrics tested and each metric's expected response to anthropogenic stress (direction of change). Percentages are based on the total number of identified individuals.

Metric	Description	Predicted Response
Species Richness and Composition		
Number of Benthic Species	Total number of benthic fish species	Decrease
Number of Benthic Species Adjusted	Adjusted for catchment area	Decrease
Number of Darter, Sculpin, Madtom Species	Number of darter, sculpin, and madtom species (bottom feeders)	Decrease
Number of Darter, Sculpin, Madtom Species Adjusted	Adjusted for catchment area	Decrease
Number of Darter Sculpin Species	Number of darter and sculpin species in the sample	Decrease
Number of Darter Sculpin Species Adjusted	Adjusted for catchment area	Decrease
Number of Darter Species	Number of darter species in the sample	Decrease
Number of Darter Species Adjusted	Adjusted for catchment area	Decrease
Number of Native Species	Number of native fish species in the sample	Decrease
Number of Native Species Adjusted	Adjusted for catchment area	Decrease
Number of Salamanders	Total number of salamanders in the sample	Decrease
Number of Salamanders Adjusted	Adjusted for catchment area	Decrease
Number of Salamanders/Fish	Total number of salamanders and fish in the sample	Decrease
Number of Salamanders/Fish Adjusted	Adjusted for catchment area	Decrease
Number of Species	Total number of fish species in the sample	Decrease
Number of Species Adjusted	Adjusted for catchment area	Decrease
Number of Sucker Species	Number of sucker species in the sample	Decrease
Number of Sucker Species Adjusted	Adjusted for catchment area	Decrease
Number of Sunfish Species	Number of sunfish species in the sample	Decrease
Number of Sunfish Species Adjusted	Adjusted for catchment area	Decrease
% Round-bodied Suckers	Percent of sample* that are round-bodied suckers (intolerant suckers species)	Decrease
% White, Hog Suckers	Percent of sample that are white suckers or hog suckers (tolerant sucker species)	Increase
Indicator Species		
Ln Number of Tolerants	Natural log transformed total number of tolerant fish	Increase
Ln Number of Tolerants Adjusted	Adjusted for catchment area	Increase
Number of Intolerant Species	Total number of intolerant fish species in the sample	Decrease
Number of Intolerant Species Adjusted	Adjusted for catchment area	Decrease
% Abundance of Dominant Species	Percent of fish sample of most dominant species	Increase
% Creek Chub	Percent of sample that are creek chubs (intolerant species)	Increase
% Green Sunfish	Percent of sample that are green sunfish (intolerant species)	Increase

Table 2-2. (Continued)

Metric	Description	Predicted Response
Indicator Species (Continued)		
% Mudminnows	Percent of sample that are mudminnows (intolerant species)	Increase
% Pioneers	Percent of fish sample that are pioneer species	Increase
% Tolerant	Percent of fish sample considered tolerant of disturbance	Increase
% White Suckers	Percent of sample that are white suckers (intolerant species)	Increase
Pirhalla Average (Highlands)	Average Pirhalla tolerance value of all individuals that are given a tolerance value	Increase
Pirhalla Intolerant (Coastal Plain)	Average tolerance value of all intolerant and moderately tolerant benthic species (darters, sculpins, madtoms, and lampreys)	Increase
Pirhalla Number (Coastal Plain)	Number of intolerant and moderately tolerant species	Decrease
Pirhalla Zero (Coastal Plain)	Average tolerance value of all individuals with tolerance value > 0	Increase
Pirhalla Benthic (Piedmont, All)	Average tolerance value of all benthic individuals (darters, sculpins, madtoms, and lampreys)	Increase
Trophic Composition		
Ln Number of Generalists, Omnivores, Invertivores	Natural log transformed number of fish that are generalist, omnivores, or invertivores	Increase
Ln Number of Generalists, Omnivores, Invertivores Adjusted	Adjusted for catchment area	Increase
Ln Number of Insectivores	Natural log transformed number of fish that are insectivores	Decrease
Ln Number of Insectivores Adjusted	Adjusted for catchment area	Decrease
% Generalists, Omnivores,	Percent of sample that are generalists or omnivores	Increase
% Generalists, Omnivores, Invertivores	Percent of sample that are generalists, omnivores, or invertivores	Increase
% Insectivores	Percent of sample that feed on insects	Decrease
% Invertivores	Percent of sample that feed on invertebrates	Decrease
% Omnivores	Percent of sample that are omnivores	Increase
% Omnivores, Invertivores	Percent of sample that are omnivores or invertivores	Increase
% Top Predators	Percent of fish sample that are top predators	Decrease
Fish Abundance and Condition		
Abundance per square meter	Abundance of fish per square meter	Decrease
Biomass per square meter	Biomass of fish per square meter	Decrease
Ln Abundance of Dominant Species	Natural log transformed abundance of dominant fish species in the sample	Decrease
Ln Abundance of Dominant Species Adjusted	Adjusted for catchment area	Decrease
Number Individuals No Exotics	Number of Individuals, excluding introduced/exotic species	Decrease
Number Individuals No Exotics Adjusted	Adjusted for catchment area	Decrease
Number Individuals No Tolerants	Number of Individuals, excluding tolerant species	Decrease

Table 2-2. (Continued)

Metric	Description	Predicted Response
Fish Abundance and Condition (Continued)		
Number Individuals No Tolerants Adjusted	Adjusted for catchment area	Decrease
Number of Individuals Adjusted	Total number of individual fish	Decrease
Total Biomass	Total biomass of fish	Decrease
Total Biomass Adjusted	Adjusted for catchment area	Decrease
Reproductive Function		
Ln Number of Lithophilic Spawners	Natural log transformed number or lithophilic spawners	Decrease
Ln Number of Lithophilic Spawners Adjusted	Adjusted for catchment area	Decrease
% Lithophilic Spawners	Percent of sample that primarily lives on top of plant or sediment substrates	Decrease

* “Percent of sample” refers to percent of individuals in a sample

Table 2-3. Definitions of candidate benthic macroinvertebrate metrics tested and expected response to anthropogenic stress. Percentages are based on the total number of identified individuals. Tolerance metrics from Bressler, 2004.

Metric	Description	Predicted Response
Taxonomic Richness		
Number of Chironomidae	Number of midge taxa	Decrease
Number of Coleoptera	Number of beetle taxa	Decrease
Number of Crustacea, Mollusca	Sum of the number of calcium dependent taxa	Decrease
Number of Diptera	Number of "true" fly taxa (includes midges)	Decrease
Number of Ephemeroptera	Number of mayfly taxa	Decrease
Number of EPT	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
Number of Orthocladiinae	Number of taxa in the midge subfamily Orthocladiinae	Decrease
Number of Plecoptera	Number of stonefly taxa	Decrease
Number of Tanytarsini	Number of taxa in the midge tribe Tanytarsini	Decrease
Number of Taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
Number of Trichoptera	Number of caddisfly taxa	Decrease
Taxonomic Composition		
% Amphipods	Percent amphipods in the sample	Decrease
% Chironomidae	Percent midge larvae and pupae	Increase
% Coleoptera	Percent beetle larvae and aquatic adults	Decrease
% Corbicula	Percent Asiatic clams	Increase
% Crustacea, Mollusca	Percent Crustacea and Mollusca individuals	Decrease
% Diptera	Percent "true" fly larvae and pupae	Increase
% Ephemeroptera	Percent mayfly nymphs	Decrease
% EPT	Percent mayfly, stonefly, and caddisfly individuals in the sample	Decrease
% Gastropoda	Percent snails and limpets	Decrease
% Isopoda	Percent isopods	Increase
% Non Insects	Percent non-insects	Increase
% Odonata	Percent dragonfly and damselfly nymphs	Decrease
% Oligochaeta	Percent aquatic worms	Increase
% Orthocladiinae of Chironomidae	Percent of Chironomids in the subfamily Orthocladiinae	Increase
% Pelecypoda	Percent bivalves	Decrease
% Plecoptera	Percent stonefly nymphs	Decrease
% Tanytarsini	Percent of Tanytarsini midges to total fauna	Decrease
% Tanytarsini of Chironomidae	Percent of Chironomids in the tribe Tanytarsini	Decrease
% Trichoptera	Percent caddisfly larvae	Decrease

Table 2-3. (Continued)

Metric	Description	Predicted Response
Tolerance/Intolerance		
Beck's Original	Weighted sum of intolerant taxa (= 2* number of Class 1 taxa + number of Class 2 taxa; where Class 1 taxa have tolerance values 0 or 1, Class 2 taxa have values from 2 to 4)	Decrease
Beck's Agriculture	Beck's alternative measure using tolerance values for agriculture	Decrease
Beck's Urban	Beck's alternative measure using tolerance values for urbanization	Decrease
HBI Original	The general tolerance/intolerance of the assemblage; considers the number of individuals in each tolerance class	Increase
HBI Agriculture	HBI alternative using tolerance values for agriculture	Increase
HBI Urban	HBI alternative measure using tolerance values for urbanization	Increase
Number of Intolerant Original	Number of taxa considered to be sensitive to perturbation (Tolerance values 0-3)	Decrease
Number of Intolerant Agriculture	Number of taxa that are intolerant to agriculture	Decrease
Number of Intolerant Urban	Number of taxa that are intolerant to urbanization	Decrease
% Baetidae of Ephemeroptera	Percent pollution tolerant mayflies of all mayflies	Increase
% Hydropsyche and Cheumatopsychye of EPT	Percent pollution tolerant caddisflies of all mayflies, stoneflies and caddisflies	Increase
% Hydropsychidae of Trichoptera	Percent pollution tolerant caddisflies of all caddisflies	Increase
% Intolerant Original	Percent of sample considered intolerant of perturbation (tolerance values 0-3)	Decrease
% Intolerant Agriculture	Percent of sample considered intolerant to agriculture (tolerance values 0-3)	Decrease
% Intolerant Urban	Percent of sample considered intolerant to urbanization (tolerance values 0-3)	Decrease
% Tolerant Original	Percent of sample considered tolerant of perturbation (tolerance values 7-10)	Increase
% Tolerant Agriculture	Percent of sample considered tolerant to agriculture (tolerance values 7-10)	Increase
% Tolerant Urban	Percent of sample considered tolerant to urbanization (tolerance values 7-10)	Increase
Trophic Feeding		
Number of Predators	Number of taxa that capture living food organisms	Decrease
Number of Scrapers	Number of taxa that scrape food from substrate	Decrease
% Collectors	Percent of sample that feed on detrital deposits or loose surface films	Decrease
% Predators	Percent predator individuals	Decrease
% Scrapers	Percent scraper individuals	Decrease
% Shredders	Percent of sample that "shreds" organic litter	Decrease
Habitat		
% Burrowers	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
% Clingers	Percent of sample primarily adapted for inhabiting flowing water, as in riffles	Decrease
% Swimmers	Percent of sample that primarily swims	Decrease

Table 2-4. Results of testing fish metrics for differences among reference and degraded sites by stratum. Classification efficiencies (CEs) are the percentage of reference and degraded sites correctly classified by each metric.

Metric	K-S Test			M-W Test			Classification Efficiencies		
	COASTAL	EASTERN PIEDMONT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS
Abundance per square meter	0.00000	0.00003	0.00000	0.00000	0.00000	0.03154	77.48	70.67	77.69
Biomass per square meter	0.00000	0.00000	0.00000	0.00000	0.00000	0.00014	79.28	84.00	79.34
Ln Abundance of Dominant Species	0.00003	0.00001	0.00000	0.00073	0.00002	0.00014	44.14	46.67	63.64
Ln Abundance of Dominant Species Adjusted	0.00002	0.00002	0.00001	0.00063	0.00002	0.02115	45.95	48.00	66.94
Ln Insectivores	1.00000	0.00001	0.00000	1.00000	0.00000	0.00000	0.00	78.67	81.82
Ln Insectivores Adjusted	1.00000	0.00027	0.00000	1.00000	0.00013	0.00000	0.00	26.67	66.12
Ln Number of Generalist, Omnivores, Invertivores	0.00000	0.00001	0.00001	0.00001	0.00001	0.00522	43.24	48.00	66.12
Ln Number Generalist, Omnivores, Invertivores Adjusted	0.00000	0.00002	0.00001	0.00000	0.00002	0.10092	40.54	46.67	68.60
Ln Number of Lithophilic Spawners	0.57946	0.00000	0.00000	0.09469	0.00000	0.00000	61.26	85.33	79.34
Ln Number of Lithophilic Spawners Adjusted	0.57946	0.00000	0.00000	0.09923	0.00000	0.00007	61.26	86.67	80.17
Ln Number of Tolerants	0.00000	0.00007	0.00273	0.00000	0.00015	0.10038	41.44	49.33	66.94
Ln Number of Tolerants Adjusted	0.00000	0.00007	0.00442	0.00000	0.00016	0.42945	39.64	49.33	70.25
Number Individuals No Exotics	0.00006	0.00059	0.28032	0.00005	0.00004	0.16381	68.47	72.00	84.30
Number Individuals No Exotics Adjusted	0.00006	0.00025	0.28032	0.00006	0.00003	0.28481	72.97	70.67	51.24
Number Individuals No Tolerants	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	69.37	86.67	45.45
Number Individuals No Tolerants Adjusted	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	69.37	86.67	68.60
Number of Benthic Species	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	75.68	92.00	80.17
Number of Benthic Species Adjusted	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	77.48	92.00	80.17
Number of Darter, Sculpin, Madtom Species Adjusted	0.00024	0.00000	0.00000	0.00004	0.00000	0.00000	70.27	92.00	80.17
Number of Darter, Sculpin, Madtom Species	0.00089	0.00000	0.00000	0.00005	0.00000	0.00000	71.17	92.00	80.17
Number of Darter Sculpin Species	0.00118	0.00000	0.00000	0.00022	0.00000	0.00000	70.27	92.00	56.20
Number of Darter Sculpin Species Adjusted	0.00064	0.00000	0.00000	0.00052	0.00000	0.00000	70.27	92.00	80.17
Number of Darter Species	0.00118	0.00000	0.04069	0.00022	0.00000	0.01106	70.27	80.00	85.12
Number of Darter Species Adjusted	0.00064	0.00000	0.04069	0.00052	0.00000	0.02874	70.27	80.00	58.68
Number of Individuals Adjusted	0.00000	0.00000	0.00000	0.00000	0.00000	0.00111	73.87	78.67	79.34
Number of Intolerant Species	0.02602	0.00000	0.00000	0.00145	0.00000	0.00000	67.57	84.00	83.47
Number of Intolerant Species Adjusted	0.02602	0.00000	0.00000	0.00173	0.00000	0.00000	67.57	81.33	81.82
Number of Native Species	0.00001	0.00000	0.00003	0.00000	0.00000	0.00001	65.77	84.00	78.51
Number of Native Species Adjusted	0.00000	0.00000	0.00000	0.00000	0.00000	0.00008	71.17	86.67	78.51
Number of Species	0.00003	0.00000	0.00027	0.00000	0.00000	0.00002	69.37	80.00	76.86
Number of Species Adjusted	0.00000	0.00000	0.00001	0.00000	0.00000	0.00021	69.37	88.00	77.69

Table2- 4. (Continued)

Metric	K-S Test			M-W Test			Classification Efficiencies		
	COASTAL	EASTERN PIEDMONT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS
Number of Sucker Species	0.00162	0.00914	0.06438	0.00011	0.00041	0.00544	69.37	66.67	60.33
Number of Sucker Species Adjusted	0.00089	0.00003	0.06438	0.00004	0.00010	0.06141	69.37	66.67	60.33
Number of Sunfish Species	0.00325	0.30351	0.99541	0.00012	0.03093	0.82214	60.36	61.33	43.80
Number of Sunfish Species Adjusted	0.00144	0.06717	0.57414	0.00024	0.02334	0.59894	58.56	61.33	43.80
% Abundance of Dominant Species	0.00000	0.00000	0.00027	0.00000	0.00000	0.00002	72.07	86.67	80.99
% Creek Chub	0.17988	0.00605	0.00022	0.01585	0.57035	0.00015	52.25	68.00	75.21
% Generalist, Omnivores	0.00505	0.00000	0.00000	0.96393	0.00000	0.00000	58.56	89.33	81.82
% Generalist, Omnivores, Invertivores	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	72.07	86.67	58.68
% Green Sunfish	0.12603	0.24677	0.00001	0.05995	0.21162	0.00000	50.45	60.00	76.03
% Insectivores	1.00000	0.00000	0.00000	0.80441	0.00000	0.00000	0.00	80.00	90.08
% Invertivores	0.01728	0.00000	0.95927	0.89229	0.00000	0.82193	59.46	84.00	77.69
% Lithophilic Spawners	0.40182	0.00000	0.00000	0.14314	0.00000	0.00000	62.16	81.33	81.82
% Mudminnows	0.00047	0.37702	0.00035	0.09643	0.00349	0.00000	63.06	60.00	78.51
% Omnivores	0.05280	0.00000	0.00000	0.64250	0.00013	0.00000	54.05	80.00	85.12
% Omnivores, Invertivores	0.00000	0.00002	0.00000	0.00000	0.00005	0.00000	72.97	73.33	85.95
% Pioneers	0.00156	0.00000	0.00000	0.01428	0.00000	0.00001	63.96	85.33	79.34
% Round-bodied Suckers	0.00000	0.00914	0.59764	0.00000	0.00013	0.03077	78.38	69.33	54.55
% Tolerant	0.00003	0.00000	0.00000	0.00018	0.00000	0.00000	69.37	86.67	84.30
% Top Predators	0.00269	0.00068	0.12783	0.00047	0.00002	0.01284	67.57	73.33	57.85
% White Suckers	0.01421	0.54808	0.00024	0.00020	0.83387	0.00156	59.46	57.33	74.38
% White, Hog Suckers	0.01421	0.54808	0.00035	0.00020	0.67890	0.00268	59.46	57.33	74.38
Pirhalla Average (Highlands)	0.22979	0.00007	0.00000	0.44692	0.00001	0.00000	48.65	70.67	77.69
Total Biomass	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	72.07	74.67	40.50
Total Biomass Adjusted	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	75.68	77.33	80.17
Number of Salamanders	0.02126	0.05526	0.07044	0.00043	0.00083	0.00226	68.47	65.33	62.81
Number of Salamanders Adjusted	0.00000	0.00000	0.00016	0.00000	0.00000	0.00001	58.56	61.33	38.84
Number of Salamanders/Fish	0.02126	0.12199	0.05137	0.00021	0.32424	0.05200	70.27	85.33	77.69
Number of Salamanders/Fish Adjusted	0.00000	0.00000	0.00004	0.00000	0.00000	0.00011	77.48	90.67	76.86
Pirhalla Benthic (Piedmont, All)	0.00101	0.00000	0.00000	0.02050	0.00000	0.00000	37.84	81.33	75.21
Pirhalla Zero (Coastal Plain)	0.00000	0.00019	0.00000	0.00000	0.00001	0.00000	72.97	70.67	77.69
Pirhalla Intolerant (Coastal Plain)	0.98459	0.03808	0.00000	0.01520	0.00043	0.00000	62.16	66.67	75.21
Pirhalla Number (Coastal Plain)	0.98459	0.00002	0.00000	0.01518	0.00000	0.00000	62.16	77.33	79.34

Table 2-5. Results of testing benthic macroinvertebrate metrics for differences among reference and degraded sites by stratum. Classification efficiencies (CEs) are the percentage of reference and degraded sites correctly classified by each metric.

Metric	K-S Test			M-W Test			Classification Efficiencies		
	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS
Number of Taxa	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	69.37	81.94	82.61
Number of EPT	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	72.97	90.28	84.78
Number of Ephemeroptera	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	81.08	91.67	89.13
Number of Plecoptera	0.00453	0.00000	0.00001	0.00004	0.00000	0.00000	65.77	83.33	84.78
Number of Trichoptera	0.00139	0.00001	0.00001	0.00001	0.00000	0.00000	62.16	69.44	84.78
Number of Coleoptera	0.03219	0.00522	0.00625	0.00441	0.00007	0.00525	61.26	70.83	56.52
Number of Diptera	0.00003	0.00194	0.01221	0.00001	0.00013	0.00201	50.45	68.06	82.61
Number of Chironomidae	0.00096	0.05388	0.03121	0.00035	0.00394	0.00653	43.24	63.89	82.61
Number of Tanytarsini	0.00123	0.00160	0.00070	0.00002	0.00043	0.00001	69.37	72.22	71.74
Number of Orthocladiinae	0.06774	0.03486	0.11307	0.01227	0.01138	0.04134	45.95	61.11	86.96
Number of Crustaceans, Mollusks	0.51147	0.05581	0.00560	0.18997	0.00941	0.00011	48.65	33.33	28.26
% EPT	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	71.17	83.33	82.61
% Ephemeroptera	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	82.88	90.28	86.96
% Plecoptera	0.00542	0.00000	0.00022	0.00245	0.00000	0.01767	65.77	81.94	80.43
% Trichoptera	0.00198	0.00124	0.01286	0.01287	0.00063	0.02591	62.16	72.22	82.61
% Odonata	0.19257	0.44197	0.99807	0.04880	0.06191	0.23193	63.06	56.94	23.91
% Coleoptera	0.03219	0.00522	0.00473	0.02588	0.00224	0.00205	61.26	70.83	56.52
% Diptera	0.67598	0.00236	0.00007	0.33806	0.00237	0.00006	45.95	69.44	82.61
% Chironomidae	0.05280	0.00037	0.00022	0.02315	0.00009	0.00917	50.45	73.61	84.78
% Orthocladiinae of Chironomidae	0.00773	0.00260	0.00185	0.02519	0.05988	0.00014	48.65	63.89	82.61
% Tanytarsini	0.00035	0.00015	0.00000	0.00007	0.00016	0.00003	69.37	72.22	71.74
% Tanytarsini of Chironomidae	0.00123	0.00160	0.00006	0.00039	0.00108	0.00056	69.37	72.22	71.74
% Non Insects	0.00672	0.02288	0.11529	0.00211	0.68563	0.75839	46.85	36.11	54.35
% Crustaceans, Mollusks	0.47876	0.05581	0.00203	0.69718	0.00338	0.00011	48.65	33.33	28.26
% Gastropods	0.81118	1.00000	0.42450	0.23612	0.85119	0.00181	59.46	47.22	15.22
% Pelecypoda	0.91890	0.99995	0.99059	0.31926	0.78387	0.05118	59.46	48.61	15.22
% Corbicula	1.00000	1.00000	1.00000	0.23298	0.35857	0.21324	60.36	48.61	13.04
% Amphipods	0.81118	0.14357	1.00000	0.60658	0.00672	0.60037	47.75	36.11	26.09
% Isopods	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	59.46	47.22	13.04
% Oligochaetes	0.51147	0.10876	0.36816	0.60530	0.38539	0.53117	45.95	36.11	43.48
Number of Scrapers	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	63.96	79.17	80.43
% Scrapers	0.00034	0.00000	0.00000	0.00004	0.00000	0.00000	64.86	81.94	80.43
Number of Predators	0.00169	0.03486	0.00345	0.00002	0.00068	0.00002	54.95	61.11	89.13
% Predators	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	59.46	47.22	13.04
% Collectors	0.01784	0.84186	0.00222	0.00808	0.81273	0.15068	40.54	56.94	78.26

Table 2-5. (Continued)

Metric	K-S Test			M-W Test			Classification Efficiencies		
	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS	COASTAL	EASTERN PIEDMNT	HIGHLANDS
% Shredders	0.68477	0.20910	0.05144	0.60961	0.92807	0.61375	48.65	63.89	80.43
% Burrowers	0.00016	0.00001	0.00010	0.00109	0.00000	0.00024	58.56	75.00	84.78
% Clingers	0.00169	0.00033	0.00164	0.00012	0.00215	0.00326	63.06	63.89	80.43
% Climbers	0.14574	0.00625	0.02709	0.22460	0.00375	0.02358	54.05	70.83	78.26
% Swimmers	0.00040	0.00000	0.00000	0.00004	0.00000	0.00000	65.77	90.28	84.78
Number of Intolerant Original	0.00012	0.00000	0.00000	0.00000	0.00000	0.00000	67.57	86.11	82.61
Number of Intolerant Urban	0.00004	0.00000	0.00000	0.00001	0.00000	0.00000	63.06	88.89	82.61
Number of Intolerant Agriculture	0.00009	0.00000	0.00000	0.00000	0.00000	0.00000	66.67	90.28	80.43
% Intolerant Original	0.00069	0.00000	0.00000	0.00002	0.00000	0.00000	64.86	91.67	82.61
% Intolerant Urban	0.00279	0.00000	0.00250	0.01602	0.00000	0.03953	63.06	81.94	82.61
% Intolerant Agriculture	0.00011	0.00000	0.00002	0.00005	0.00000	0.00005	66.67	86.11	80.43
% Tolerant Original	0.04450	0.00000	0.00002	0.00898	0.00000	0.00000	50.45	73.61	82.61
% Tolerant Urban	0.00004	0.00000	0.00000	0.00004	0.00001	0.00000	63.06	63.89	84.78
% Tolerant Agriculture	0.01619	0.00044	0.00000	0.02729	0.00006	0.00000	52.25	62.50	84.78
HBI Original	0.00828	0.00000	0.00001	0.00208	0.00000	0.00004	61.26	80.56	82.61
HBI Urban	0.00034	0.00000	0.00473	0.00130	0.00000	0.07124	64.86	77.78	82.61
HBI Agriculture	0.02767	0.00000	0.00008	0.00972	0.00001	0.00052	56.76	66.67	82.61
Beck's Original	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	67.57	86.11	89.13
Beck's Urban	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	63.06	90.28	82.61
Beck's Agriculture	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	58.56	90.28	84.78
% Hydropsychidae of Trichoptera	0.00562	0.00145	0.00000	0.00023	0.00146	0.00000	70.27	72.22	67.39
% Hydro and Cheumatopsyche of EPT	0.99977	0.00035	0.38455	0.24936	0.00000	0.11644	56.76	70.83	52.17
% Baetidae of Ephemeroptera	0.00010	0.00204	0.00000	0.00000	0.00007	0.00000	76.58	70.83	69.57

IBIs we scored each metric as 5, 3, or 1, depending on whether its value at a site approximates, deviates slightly from, or deviates greatly from conditions at reference sites (Karr et al. 1986). In other IBI applications (e.g., Fore et al. 1996, Lyons et al. 1996, Barbour et al. 1996), a number of different methods have been used to establish scoring thresholds, based on varying subdivisions of observed values. For the new IBIs, we retained our discrete scoring approach so that direct comparisons with the original IBIs could be made. Our evaluation of alternative scoring methods is described in Section 2.8.

In our analysis, threshold values for each selected metric were established as approximately the 10^{th} and 50^{th} (median) percentile values for reference sites (see Figure 2-3), and were established separately for each stratum. For each metric expected to decrease with degradation, values below the 10th percentile were scored as 1. Values between the 10th and 50th percentiles were scored as 3, as they fell short of median expected values for reference sites. Values above the 50th percentile were scored as 5. Scoring was reversed for metrics expected to increase with degradation (e.g., values below the 50th percentile were scored as 5, and values above the 90th percentile were scored as 1). In this method, both the upper and lower thresholds are independently derived from the distribution of reference site values. The 10^{th} percentile threshold for designating scores of 1 represents our intent to identify values that are outside the natural expectation for reference sites. This approach is consistent with the likelihood that in Maryland (and most other states), even reference sites have some degree of anthropogenic impact.

To test the discriminatory power of each candidate metric, we evaluated the degree of overlap between metric values at reference and degraded sites by examining the number of sites scoring above and below the lower threshold. A classification efficiency was calculated as the percent of reference sites with values scoring ≥ 3 plus degraded sites scoring < 3, out of the total number of sites evaluated. Reference sites misclassified as degraded (score < 3) and degraded sites misclassified as reference (score ≥ 3) make up the remainder of the sites. A high classification efficiency indicates a small amount of overlap between values for reference and degraded sites. In addition to overall classification efficiencies, classification efficiencies were also reported separately for reference and degraded sites. The term discrimination efficiency is often applied to the percentage of degraded sites alone that are correctly classified (Gerritsen et al. 2000).

Most candidate metrics were significantly different between reference and degraded sites, and many had high classification efficiencies (i.e., exceeding 70%). Certain metrics in some strata exceeded 90%. Classification efficiencies were used as the primary means of selecting metrics for potential inclusion in the IBIs. Among similar metrics (e.g., number of species versus number of native species to describe species richness), the best performing metric (balanced across strata for core metrics) was used.

The classification efficiencies of the fish abundance and richness metrics were very similar for both raw scores and scores adjusted for catchment area. We selected only adjusted metrics for inclusion in IBI testing because they make ecological sense and are consistent with the original MBSS IBIs. The lognormal metrics of sculpin abundance rarely had good classification efficiencies and were not selected; it is possible that sculpin absence at apparent reference sites is actually linked to current (or historical) degradation rather than unaccounted for natural

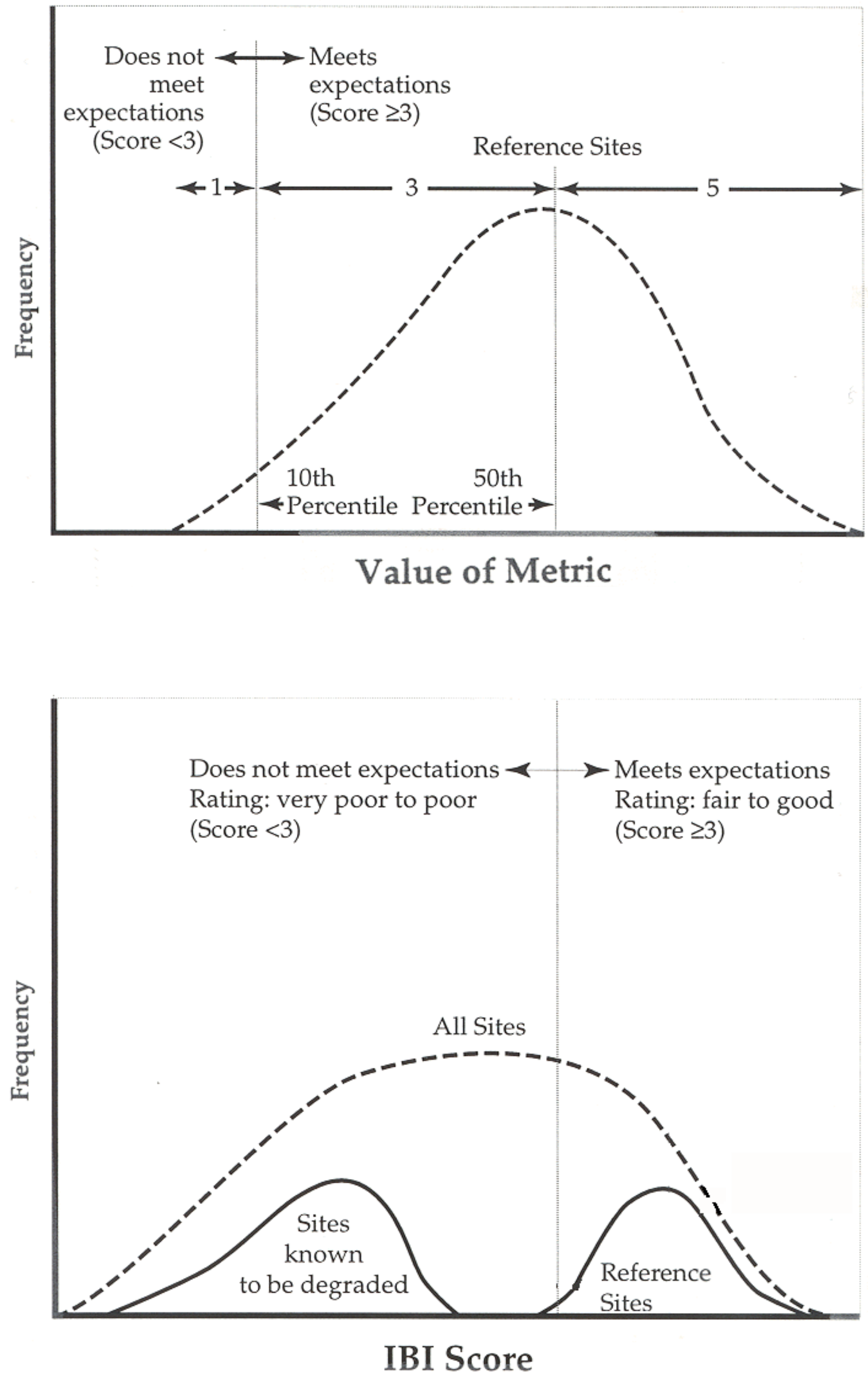

Figure 2-3. Schematic illustration of the process used to derive and interpret scores for the MBSS Indices of Biotic Integrity (IBIs). Scores are based on the distribution of reference sites, as depicted in the top figure. The bottom figure shows hypothetical reference sites in the context of other hypothetical sites, including those with known degradation.

differences. The observed/expected (O/E) metric for fish species could not be calculated for MBSS sites outside the values used to develop the model (i.e., later sample years), so the metric did not perform well overall and was not selected for IBI testing. In the future, refinement of the O/E models with more data may support its use as an independent indicator of stream condition. Some of the Pirhalla metrics performed adequately but were not better than traditional metrics, so they were not selected. The number of salamander species metric had a high classification efficiency in the Coastal Plain and small stream Highlands, but was not selected because salamander sampling is not currently conducted at all MBSS sites. Some metrics with narrow thresholds, i.e., number of benthic species adjusted for catchment area, percent non-tolerant suckers (all suckers except white sucker), percent Tanytarsini, number of Ephemeroptera, and number of Scrapers, are essentially presence/absence metrics (in some cases no scores of 3 were assigned). Three such metrics were included in the original benthic IBIs. We evaluated the effect of eliminating these "presence/absence" metrics (or using the number of benthic species without adjustment) but determined that the original formulations performed better (had higher classification efficiencies).

2.6 IBI COMBINATIONS AND TESTING

As with the original IBIs, we iteratively tested many combinations of metrics to develop the new fish and benthic macroinvertebrate IBIs. For each combination, an index was calculated as the mean of the metrics included, scaled from 1 to 5. Classification efficiencies of different metric combinations (indices) were calculated as above, separately for reference and degraded sites, and overall. Individual IBI combinations were done separately for each of the provisional strata. This is required because each of the metrics is scored independently within each stratum.

At first, the combinations of metrics for IBI testing were selected in a stepwise manner, starting with the best performing metric (i.e., highest classification efficiency). Additional metrics were added as long as they increased the overall classification efficiency of the index. In no stratum did the classification efficiency improve after a second metric was added. This is a result of the very high classification efficiencies achieved by individual metrics in each stratum.

To ensure that the final IBIs were a more complete representation of the fish and benthic assemblages (as recommended by Karr et al. 1986 and done for the original MBSS IBIs), we selected a core of four metrics that performed well and represented different assemblage characteristics for each of the strata (in the coldwater Highlands stratum, only two of these core metrics were used). The core metrics for the new fish IBI were abundance per square meter; number of benthic species (adjusted for catchment area); percentage of tolerant fish; and percentage of generalists, omnivores, and invertivores. Only the abundance per square meter and number of benthic species (adjusted for catchment area) core metrics were included in the coldwater Highlands fish IBI. The core metrics for the new benthic IBI were number of taxa; number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa; number of Ephemeroptera taxa; and percentage of benthic macroinvertebrates intolerant to urban stress (after Bressler et al. 2004). The core fish metrics represent four of the five assemblage components identified by Karr et al. (1986): species richness and composition, indicator species, trophic composition, and fish abundance and condition. The reproductive function component was not represented in the core metrics as no reproductive metrics had high classification efficiencies.

Subsequently, we attempted to improve the performance of the IBIs by adding other metrics to the core suite in the same stepwise fashion. Additional metrics were added until they no longer improved the classification efficiency of the index. The provisional small stream Highlands stratum had the lowest classification efficiency and the large stream Highlands stratum had so few degraded sites that its performance was suspect. At the same time, the coldwater stratum performed well and effectively captured most of the streams draining catchments < 5000 ac, so the small stream Highlands stratum was abandoned and the remaining Highlands streams combined as a separate warmwater Highlands stratum (i.e., the remaining Highlands streams outside the geographic boundaries of the coldwater streams stratum). For the four final strata, two additional metrics were added to each new fish IBI, improving the calibration classification efficiency to at least 83%. For the final three new benthic macroinvertebrate IBIs, two to four additional metrics were added to the core suite, improving the calibration classification efficiency to at least 85%.

2.7 ALTERNATIVE SCORING METHODS

We also looked at alternative IBI scoring approaches that might improve the utility of the new MBSS IBIs. Specifically, we compared (after the approach of Blocksom 2003) the discrete 1-3-5 metric scoring method used by the original MBSS IBIs (as well as the discrete scoring methods of Ohio and Florida) with continuous 0-100 metric scoring methods used by West Virginia (Gerritsen et al. 2000) and proposed by EPA, i.e., MBII and CALU (see Blocksom 2003).

Blocksom (2003) evaluated the precision of index scores sampled at the same site in the same or subsequent years as the signal-to-noise ratio of condition "signal" to temporal variability "noise." Using the MBSS data from 57 pairs of field duplicate benthic samples (collected at selected sites on the same day), we calculated the signal-to-noise ratio of each of the six metric scoring methods. The three continuous metric scoring methods and the discrete MBSS method had high signal-to-noise ratios ranging from 10.23 to 12.21**,** indicating good precision. The Ohio and Florida discrete methods had substantially lower ratios of 6.93 and 6.82, respectively.

Blocksom (2003) determined the number of condition classes that could be distinguished (based on the minimum detectable difference) under each metric scoring method by conducting a power analysis of the mean difference in index scores between both two and three site visits. We did not conduct a comparable analysis for minimum detectable difference, because the MBSS data do not include multiple revisits to sites, except for sentinel sites. Sentinel sites (i.e., high quality sites usually located on protected land, Prochaska 2004) represent only a single condition class. Instead, we calculated the coefficient of variation for the sentinel sites that were sampled each year for up to four years, using the different metric scoring methods. The lower the CV, the higher the power of the index. The MBSS method has the lowest CV (highest power) at 0.13, with the other methods ranging from 0.18 to 0.24.

In addition, we looked at variability in index scores at the scale they are used as biological criteria to designate Maryland watersheds (primary sampling units) as impaired under water quality standards (MDE 2005). The method for designation involves the mean (and 90%

confidence intervals) of IBI scores obtained at 10 or more sites randomly selected from all stream segments in the watershed; no sites are revisited.

We calculated the mean index scores for each of 84 Maryland watersheds using the MBSS, WV, and MBII metric scoring methods (Table 2-6). The relative standard errors (RSEs) for each watershed (based on 10 or more sites) averaged for each method indicated that the MBSS metric scoring method is less variable than the other methods. Higher variability in watershed mean index scores will require more sites to designate impairment with the same confidence. For example, the best performing continuous metric scoring method, WV, would require sampling 65% more sites to make watershed impairment determinations with the same confidence.

Table 2-6. Mean relative standard errors (RSEs) for index scores from multiple sites in Maryland watersheds, using different metric scoring methods (see Blocksom 2003).

Method	**Mean RSE**
MBSS	0.07
Florida	0.08
WV	0.09
MBII	0.11
CALU	0.11
Ohio	0.14

As mentioned above, the variability in mean index scores for watersheds has implications for designating impairment under Maryland water quality standards. Therefore, we calculated confidence intervals for each watershed mean and applied the biocriteria rule to determine if each watershed would be designated as impaired. We did this for the MBSS discrete and WV continuous metric scoring methods. In all but five watersheds, the impairment designations (pass, indeterminate, or fail biocriteria) were the same. In three cases a watershed that failed MBSS biocriteria was ruled indeterminate by the WV method, while in two cases a watershed that passed MBSS biocriteria was ruled indeterminate by the WV method. The weighted Kappa statistic for this comparison showed a 99% concordance between the two metric scoring methods for designating watershed impairment.

We conclude that differences in metric scoring methods, both continuous versus discrete scoring and wide versus compressed scoring ranges, have measurable but small effects on index performance. While some methods perform better in some ways, other methods may perform better in other ways. Most important for the application of indices to Maryland water resource management is the responsiveness to stream condition (good in MBSS and other methods) and the variability of mean IBI scores by watershed. Based on our analyses, the current discrete MBSS scoring method is better than all other methods in reducing the variability of mean watershed IBI scores and the number of sites required to designate impairment with the same confidence.

Based on these results using the original MBSS IBIs, we decided to apply the best performing continuous, 0-100 metric scoring method to the candidate metrics and new MBSS IBIs to evaluate their performance. Specifically, we used the West Virginia method of scoring all fish and benthic metrics on 100-point scale based on the range of all sites from 0 to 95th percentile (or 5th percentile for inverse metrics). The classification efficiencies for each metric (when the 10th percentile of reference was applied) were similar but not exactly the same as for the 1-3-5 scoring. The classification efficiencies for the 4-core-metric fish IBIs and benthic IBIs using the 100-point scaled metrics (with the 10th percentile of reference as the degradation threshold) were consistently lower than for IBIs developed with 1-3-5 metric scoring (with the 3 threshold of reference). In addition, the classification efficiencies for the best possible fish IBIs using the 100-point metric scoring were 5 to 10% lower (except in the Eastern Piedmont where the classification efficiency was similar).

These differences in classification efficiencies resulted from the wider range of values inherent in 100-point scale, e.g., not all metric values assigned a 3 in the discrete scoring would be above the degradation threshold in the continuous scoring. Specifically, the continuous 100-point metric scale allows extreme values to affect the IBI more. The discrete 1-3-5 metric scoring standardizes metric values against the reference condition and therefore should be less variable over time (and with different datasets) than are values from all sites. While fewer IBI values are possible with discrete metric scoring, these values may be more ecologically relevant (i.e., extreme values in additional metrics can dilute performance of key metrics). Because the 100-point metric scoring method was not demonstrably better (either theoretically or empirically), we decided to retain the 1-3-5 metric and IBI scoring methods. This has the advantage of continuity with the original MBSS IBI method and 100-point metric scores can still be calculated for any metric or IBI as needed for comparison with other assessment programs.

2.8 IBI VALIDATION

As described above, we reserved 353 of all sites sampled from 1994 to 2004 for validation of the new MBSS IBIs. This number of sites is comparable to the number of validation sites used to develop the original MBSS IBIs, but still includes less than 5 degraded sites in three strata by chance.

For the fish IBIs in the Highlands and Coastal Plain, the overall classification efficiencies of the validation sites were even higher than the calibration classification efficiencies (88%). The validation classification efficiency for the fish IBI in the Eastern Piedmont was lower at 71%, but this validation is less reliable because only 7 reference and degraded sites were in the validation dataset by chance.

The validation classification efficiencies for the benthic IBI in the Coastal Plain was higher than for calibration at 96%, somewhat lower in the Eastern Piedmont at 86% (but again there were only 7 validation sites in this stratum), and comparable in the Highlands at 88%.

These high classification efficiencies using only validation sites indicate that the performance of the IBIs was not derived from overfitting to the calibration dataset. Therefore, the IBIs are likely to be robust when applied to new data.

3. COMPARISON OF ORIGINAL AND NEW IBIS

Using the indicator development process described above, we created new MBSS fish and benthic macroinvertebrate IBIs as shown in Tables 3-1 and 3-2. The new fish IBIs differ from the original IBIs in that they divide the original Highlands stratum into two strata, one for coldwater Highlands streams and one for the remaining warmwater Highlands streams. In addition, smaller streams (i.e., those draining < 300 ac catchments) that were not included in the original fish IBI development have been included in the new IBIs; therefore the new IBIs can be applied to these smaller streams (25% of stream miles in 2000-2004). The new benthic IBIs differ from the original IBIs in that they divide the original non-Coastal Plain stratum into new Highlands and Eastern Piedmont strata. As with the original benthic and new fish IBIs, smaller (< 300 ac) streams are included in the new benthic IBIs.

Table 3-1. The new fish IBI metrics by stratum and their threshold values (metrics adjusted for catchment size are indicated by *)

Fish IBIs (metrics)	Thresholds		
	5	3	1
Coastal Plain			
Abundance per square meter	≥ 0.72	0.45 – 0.71	< 0.45
Number of Benthic species *	≥ 0.22	0.01 – 0.21	0
% Tolerant	≤ 68	69 - 97	> 97
% Generalist, Omnivores, Invertivores	≤ 92	93 - 99	100
% Round-bodied Suckers	≥ 2	1	0
% Abundance Dominant Taxa	≤ 40	41 - 69	> 69
Eastern Piedmont			
Abundance per square meter	≥ 1.25	0.25 – 1.24	< 0.25
Number of Benthic species *	≥ 0.26	0.09 – 0.25	< 0.09
% Tolerant	≤ 45	46 – 68	> 68
% Generalist, Omnivores, Invertivores	≤ 80	81 - 99	100
Biomass per square meter	≥ 8.6	4.0 – 8.5	< 4.0
% Lithophilic Spawners	≥ 61	32 - 60	< 32
Warmwater Highlands			
Abundance per square meter	≥ 0.65	0.31 – 0.64	< 0.31
Number of Benthic species *	≥ 0.25	0.11 – 0.24	< 0.11
% Tolerant	≤ 39	40 – 80	> 80
% Generalist, Omnivores, Invertivores	≤ 61	62 – 96	> 96
% Insectivores	≥ 33	1 – 32	< 1
% Abundance of Dominant Taxa	≤ 38	39 - 89	> 89
Coldwater Highlands			
Abundance per square meter	≤ 0.88	0.89 – 2.24	> 2.24
% Tolerant	≤ 0.22	0.23 – 0.81	> 0.81
% Brook Trout	≥ 0.14	0.01 – 0.13	< 0
% Sculpins	≥ 0.44	0.01 – 0.43	< 0

* Slope and intercept values, based on linear regression relationships between metric and log (watershed area) in acres, m * log (catchment area in acres) + b are:

Stratum	m (slope)	b (intercept)
Coastal Plain	1.69	-3.33
Eastern Piedmont	1.25	-2.36
Warmwater Highland	1.23	-2.35

Table 3-2. The new benthic macroinvertebrate IBI metrics by strata and their threshold values. Note that the new benthic macroinvertebrate IBI is intended for use with subsample sizes ranging from 80 to 120 individuals.

Benthic IBIs (metrics)	Thresholds		
	5	**3**	**1**
Coastal Plain			
Number of Taxa	≥ 22	14 – 21	< 14
Number of EPT	≥ 5	2 – 4	< 2
Number of Ephemeroptera	≥ 2	1 - 1	< 1
% Intolerant Urban	≥ 28	10 – 27	< 10
% Ephemeroptera	≥ 11	0.8 – 10.9	< 0.8
Number of Scrapers	≥ 2	1 – 1	< 1
% Climbers	≥ 8	0.9 – 7.9	< 0.9
Piedmont			
Number of Taxa	≥ 25	15 – 24	< 15
Number of EPT	≥ 11	5 – 10	< 5
Number of Ephemeroptera	≥ 4	2 – 3	< 2
% Intolerant Urban	≥ 51	12 – 50	< 12
% Chironomidae	≤ 4.6	4.7 – 63	> 63
% Clingers	≥ 74	31 – 73	< 31
Combined Highlands			
Number of Taxa	≥ 24	15 – 23	< 15
Number of EPT	≥ 14	8 – 13	< 8
Number of Ephemeroptera	≥ 5	3 – 4	< 3
% Intolerant Urban	≥ 80	38 – 79	< 38
% Tanytarsini	≥ 4	0.1 – 3.9	< 0.1
% Scrapers	≥ 13	3 – 12	< 3
% Swimmers	≥ 18	3 – 17	< 3
% Diptera	≤ 26	27-49	> 50

The number and composition of metrics differ between the new and original IBIs. The following metrics from the original fish IBIs are included in the new fish IBIs for the same strata: number of benthic species (adjusted for catchment area); percent tolerants; and percent generalists, omnivores, and invertivores. The abundance per square meter metric that appeared in the original Coastal Plain and Eastern Piedmont fish IBIs is now in all four new fish IBIs. The new Coastal

Plain fish IBI has only six metrics compared to the eight metrics in the original IBI, and the only new metric is the percent non-tolerant suckers (i.e., all suckers except white sucker). The new Eastern Piedmont fish IBI has six metrics compared to the nine metrics in the original IBI and includes no new metrics. The new warmwater Highlands fish IBI has six metrics compared to the seven metrics in the original Highlands IBI, while the new coldwater Highlands fish IBI has only four metrics, including two new metrics appropriate to its stream type: percent brook trout and percent sculpins.
The following metrics from the original benthic IBIs are included in the new benthic IBIs for the same strata: number of taxa and number of EPT. The new Coastal Plain IBI also includes the percent Ephemeroptera and number of scrapers metric from the original IBI, plus three new metrics: number of Ephemeroptera, percent intolerant to urban stressors, and percent climbers. The new benthic IBI for Eastern Piedmont includes number of Ephemeroptera, and the new benthic IBI for Highlands includes number of Ephemeroptera and percent Tanytarsini, both of which were included in the original non-Coastal Plain IBI. The Eastern Piedmont benthic IBI has six metrics and the Highlands IBI has eight metrics compared to the nine metrics in the original non-Coastal Plain IBI. The new Eastern Piedmont benthic IBI includes three new metrics and the Highlands IBI four new metrics.

In addition to including different combinations of metrics, the new IBIs have different scoring thresholds. Because a new set of reference sites were used to develop the new fish and benthic IBIs, the metric values at the 10^{th} and 50^{th} percentiles of reference were different. Different scoring thresholds for the same metrics also vary among strata as they did in the original IBIs (because reference conditions differ). For example, the degradation threshold (above which a score of 3 is given) for the abundance per square meter metric changed from 0.42 (old) to 0.45 (new) in the Coastal Plain fish IBIs and from 0.56 to 0.25 in the Eastern Piedmont fish IBIs. The percent tolerants metric threshold changed from 93 to 97 in the Coastal Plain, from 65 to 68 in the Eastern Piedmont, and from 71 (old combined Highlands) to 80 in the warmwater Highlands and 81 in the coldwater Highlands.

For the Coastal Plain benthic macroinvertebrate IBIs, the degradation threshold for number of taxa changed from 11 to 14 and the threshold for the number of EPT changed from 3 to 2. In the non-Coastal Plain, the threshold for number of taxa changed from 16 (old) to 15 in both the Eastern Piedmont and Highlands. The threshold for the number of EPT changed from 5 (old) to 8 in the Highlands but stayed the same in the Eastern Piedmont. Larger changes occurred for some metrics and are attributable to changes in the reference condition resulting from stricter criteria, more small streams, and chance.

3.1 COMPARISON OF HOW ORIGINAL AND NEW IBIS SCORE REFERENCE CONDITION

As described above, the new MBSS IBIs were developed using a more restrictive set of reference sites (8% of all sites versus 14% of all sites for the original IBIs). Because stricter thresholds for land use and riparian disturbance were applied, we are more confident that the new IBI reference sites are minimally disturbed. At the same time, the reference sites for the new fish IBIs included smaller streams draining < 300 ac that were not included in the original fish IBI. In addition, the sampling design for 2000-2004 on the 1:100,000-scale stream network resulted in more small streams being sampled. The distribution of the new reference sites included 38% that were < 1,000 ac, compared to 19% of the original reference sites. Including more small streams in the new reference condition ensures that more natural variability is included in the new IBIs.

The mean score for all new reference sites was 3.7 using the original fish IBIs and 4.0 using the new fish IBIs. Mean reference sites scores were 3.6 for the original benthic IBIs and 3.9 using the new benthic IBIs. For the 16 reference sites < 300 ac, the mean benthic BIBI was 4.4 and the fish IBI was 3.0; reference sites >300 ac were 4.0 for both IBIs. For reference sites < 1000 ac, the mean benthic IBI was 3.7 compared to 3.6 for larger streams and the fish IBI was 3.3 compared to 3.9. This indicates that smaller streams still scored somewhat lower using the new fish IBI.

3.2 COMPARISONS OF HOW ALL STREAMS SCORE WITH ORIGINAL AND NEW IBIS

We conducted a direct comparison of the original and new MBSS IBIs (both fish and benthic macroinvertebrate) by applying them to the 2000-2004 MBSS dataset of 1367 sites. The statewide mean for the new fish IBIs was virtually unchanged with an original IBI of 2.91 and a new IBI of 2.93. The statewide mean of the new benthic IBIs was only 3% higher, increasing from 2.96 to 3.07.

On a regional basis, the greatest difference in mean scores between original and new IBIs was an increase of 0.64 (16%) for the Coastal Plain benthic IBI and 0.32 (8%) for the Highlands fish IBI. In the other regions, the mean benthic IBIs decreased 9% in the Highlands and 2% in the Eastern Piedmont, using the new IBIs. The mean fish IBIs decreased 5% in the Coastal Plain while staying the same in the Eastern Piedmont.

On a county basis, 17 (one-third) of the 48 possible original and new IBI pairs (24 counties times both fish and benthic IBIs) changed by 0.5 units or more. The greatest increase was 1.14 for the benthic IBI in Caroline County and the greatest decrease was 0.58 for benthic IBI in Frederick County. Most of these changes were for the benthic IBIs in the non-Coastal Plain, which was separated into Highlands and Eastern Piedmont strata in the new benthic IBIs.

The distributions of stream miles among the four MBSS condition classes (good, fair, poor, very poor) were also somewhat different between the original and new IBIs (Figure 3-1). For both the new fish and benthic IBIs**,** the proportion of stream miles statewide changed by less than 10% in each condition class. Overall, the distribution of stream miles in each condition class was more even with the new IBIs than with the original IBIs. Using the new benthic IBI, there were fewer fair and very poor streams, but more good and poor streams. Using the new fish IBI, there were also fewer fair streams but more good streams.

The new Highlands benthic IBI resulted in a greater proportion of poor and very poor streams; the new Eastern Piedmont benthic IBI more good and very poor streams; and the new Coastal Plain benthic IBI more good and fair streams. The new Highlands fish IBI resulted in 16% more good streams and fewer fair and very poor streams. The increase in proportion of good streams is likely attributable to the appropriately higher scores for coldwater streams which have their own stratum in the new fish IBIs. The new Eastern Piedmont fish IBI resulted in more good streams and fewer fair streams as well; the new Coastal Plain fish IBI resulted in more very poor streams and fewer fair streams.

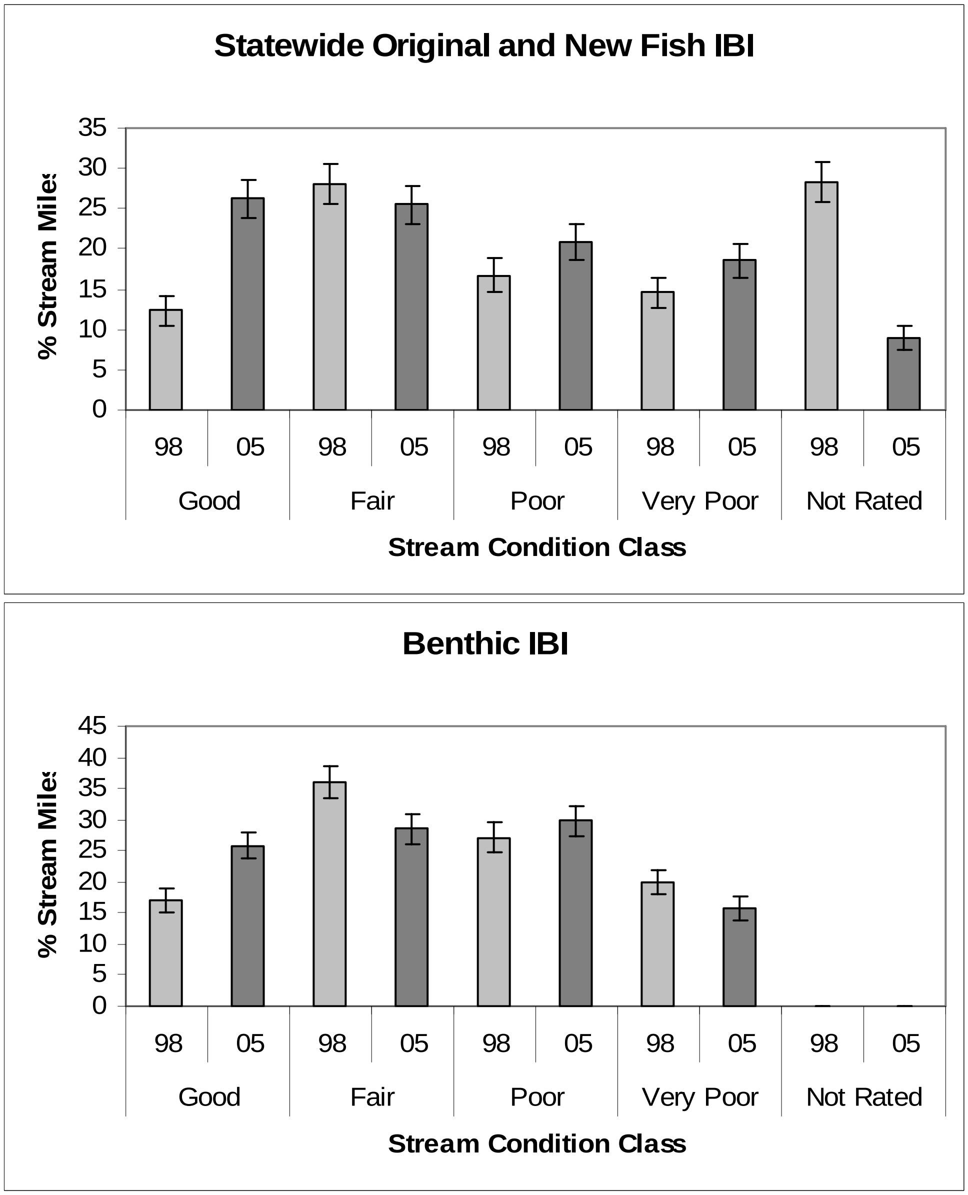

Figure 3-1. Percentage of stream miles in each condition class statewide for 2000-2004 sites scored with original (98) and new (05) MBSS IBIs. Fish and benthic IBIs are shown separately.

Among sites draining catchments of < 300 ac only, the statewide mean new fish IBI increased 0.58 (15%), while the statewide mean new benthic IBI was virtually the same, differing by only 1%. The differences between the original and new mean benthic IBIs were variable among the regions: decreasing 10% in the Highlands and increasing 11% in the Coastal Plain. In contrast, the differences between the original and new mean fish IBIs were consistently high in all three regions by 13% to 16%.

The influence of the new coldwater Highlands fish IBI is also evident when comparing it to the original Highlands fish IBI. The mean score on coldwater streams was 3.56 for the new fish IBI and 2.75 for the original fish IBI, an increase of 0.81 (20%).

Although a separate blackwater stratum was not developed, the new Coastal Plain fish IBI rates blackwater streams 0.34 (8%) higher and the new Coastal Plain benthic IBI rates them 0.74 (18%) higher. This is likely due to the greater number of blackwater reference sites sampled in 2000-2004 and used to develop the new IBIs; only 24 blackwater reference sites were used to develop the original IBIs, while 64 were used for the new IBIs. Even though the blackwater stream type may still be scored lower than other types, the new IBIs better represent the expectation for natural blackwater streams.

The original IBIs were developed with and applied to 1000 sites selected randomly on a 1:250,000-scale stream network. Smaller streams draining catchments < 300 ac were 11% of this stream network. The additional 1500 sites sampled from 2000 to 2004 were selected from a 1:100,000-scale stream network that included 25% of sites draining catchments < 300 ac. Therefore, applying the original fish IBI to the 2000-2004 sites would increase the proportion of non-rated sites (i.e., those < 300 ac) from 11% to 25%. By including these smaller streams in our reference condition (16 of 250 reference sites) we developed a fish IBI that could be applied to smaller streams and eliminate these non-rated streams. Note that some streams are dry (or otherwise unsampleable) in the summer and therefore will continue to be not rated; the proportion of not-rated streams using the new fish IBI is 5%.

The different IBI scores that result from using the new IBIs rather than the old IBIs would also affect the designations of watersheds as impaired according to Maryland's biological criteria (MDE 2005). These biological criteria are applied to Maryland 8-digit watersheds (or combined watershed Primary Sampling Units, PSUs) with 10 or more MBSS sample sites. Mean IBIs and one-sided 90% confidence interval values are calculated to give one of three ratings:

- Does not meet criteria (Fails): If the mean and upper bound of the one-sided 90% confidence interval (upper) of either index (FIBI or BIBI) is less than 3.0, the 8-digit watershed (or PSU) is listed as failing to meet the proposed criteria.
- Meets criteria (Passes): If the mean and lower bound of the one-sided 90% confidence interval (lower) of both indices (FIBI and BIBI) are greater than or equal than 3.0, the 8-digit watershed (or PSU) is listed as meeting the proposed criteria.

- Inconclusive: All other cases are inconclusive.

Applying the original MBSS IBIs to 2000-2004 data, 40 watersheds fail, 37 are inconclusive, and 7 pass biological criteria; using the new MBSS IBIs, 31 watersheds fail, 41 are inconclusive, and 12 pass. Overall, 22% fewer watersheds fail biological criteria with the new IBIs. The most frequent changes in the designation of individual watersheds are the 17 watersheds that failed with the original IBIs, but that are inconclusive with the new IBIs. In addition, among the 37 watersheds that were inconclusive with the original IBIs, 24 (65%) remain inconclusive with the new IBIs, while 5 pass and 8 fail.

4. DISCUSSION

As stated at the outset, the development of new fish and benthic macroinvertebrate IBIs was undertaken to achieve the goals of (1) increased confidence that the reference conditions are minimally disturbed, (2) including more natural variation across the geographic regions and stream types of Maryland, and (3) increased sensitivity of IBIs with more classes (strata), different metric combinations, or alternative scoring methods. The large number of sites in the 1994-2004 MBSS dataset reduced but did not eliminate the constraint of small numbers of reference sites when more geographic or stream type classes are used.
It was also important that the new MBSS IBIs be as consistent as possible with the old IBIs. In particular, they should remain transparent and understandable, and provide clear thresholds of impairment for of both biointegrity and interim (fishable and swimmable) water quality goals. Consistency between the original and new IBIs is needed to calculate joint estimates between sampling rounds, detect trends in stream condition, and minimize the impact of the change on county programs and MDE listings of impaired waters.

4.1 MINIMALLY DISTURBED REFERENCE CONDITION

The reference conditions used to develop the new MBSS IBIs represented only 8% of all sites in Maryland. They did not include original reference sites that were most likely to be affected by land use changes. For these reasons, we are more confident that the new IBIs are based on minimally disturbed reference conditions for Maryland streams.

4.2 IBI THAT BETTER PREDICT DEGRADATION

Given minimally disturbed reference conditions, the ability of IBIs to distinguish deviation from those reference conditions is based on how predictably IBI scores change with disturbance. This ability to predict deviation comes from (1) choosing metrics that vary predictably and precisely with disturbance and (2) combining these metrics into an index that consistently changes with disturbance across the natural variation gradients encountered. We reduced the natural variation that each new IBI had to address by increasing the number of geographic or stream type classes, i.e., the number of new MBSS IBIs. In the new version, we have four rather than three fish IBIs and three rather than two benthic IBIs. In the case of the new fish IBIs, we increased the natural variation of reference condition by adding smaller streams < 300 ac, but this did not adversely affect the performance of the new IBIs given the four strata.

Within each stratum (i.e., new IBI), the combination of metrics changes from the old IBIs and in every case the ability of the IBI to distinguish reference from degraded sites (i.e., the classification efficiency) increased (Table 9). By convention, classification efficiencies above 80% are good and above 90% are excellent.

In addition to these good-to-excellent overall IBI classification efficiencies, each new IBI was effective at correctly classifying both reference and degraded sites (Table 4-1). Misclassification of reference sites (saying they are degraded) is essentially a false negative or Type I error. Among the new fish IBIs, the classification efficiencies for reference sites ranged from 80% to

95%; among new benthic IBIs, these classification efficiencies ranged from 89% to 94%. Misclassification of degraded sites (saying they are not degraded) is essentially a false positive or Type II error. Among the new fish IBIs, the classification efficiencies for degraded sites ranged from 78% to 97%; among new benthic IBIs, these classification efficiencies ranged from 83% to 92%. Low classification rates for both reference and degraded sites indicates that the new MBSS IBIs are a good balance of both types of error, i.e., not many degraded streams will be missed, nor will we be "crying wolf" about streams that are actually not degraded.

Table 4-1. Comparison of classification efficiencies (CEs) between original and new MBSS IBIs. CEs are the percentage of reference and degraded sites that are correctly classified by each IBI.

	Stratum	Original IBI Calibration (Validation) CE	New IBI Calibration (Validation) CE	Reference CE	Degraded CE
Fish IBI	Coastal Plain	74 (72)	85 (88)	89%	80%
	Eastern Piedmont	90 (94)	96 (71)	95%	97%
	Highlands	86 (75)			
	Warmwater Highlands		83 (88)	85%	78%
	Coldwater Highlands		85		
Benthic IBI					
	Coastal Plain	87 (72)	87 (96)	89%	83%
	Non-Coastal Plain	88 (82)			
	Eastern Piedmont		93 (86)	94%	92%
	Highlands		91 (88)	93%	88%

4.3 APPLYING THE NEW MBSS IBIS

The MBSS IBIs are central to water resource management in Maryland and have special implications for the designation of watersheds as impaired under Section 303d of the Clean Water Act. Therefore, it is critical that stream condition ratings be founded in ecological knowledge and solid science. The MBSS recognizes that there are no pristine streams in Maryland; most have a history of human disturbance and all are affected by atmospheric deposition. Nonetheless**,** there are high quality streams in Maryland that can be called minimally disturbed and equated with Biological Condition Gradient (BCG) level 2, "minimal changes in structure and function" (EPA 2005). Adoption of the new IBIs will provides us with more confidence that the reference conditions we are using to create IBIs and rate stream condition reflect BCG level 2, rather than BCG level 3, "evident changes in structure and minimal changes in function."

In addition to indicating when stream condition deviates from reference condition (i.e., is degraded), IBIs provide a means of determining the degree to which streams deviate or the "severity of failing" to meet the criterion (Bailey et al. 2004). The original MBSS IBIs used four "bands" of IBI scores to designate stream condition: 1.0 to 1.9 very poor, 2.0 to 2.9 poor, 3.0 to 3.9 fair, and 4.0 to 5.0 good. This convention was retained for the new IBIs. Given the new reference conditions, these bands can be more confidently assigned to the biointegrity goal of

CWA (good) and the interim goal of CWA (fair). The two additional bands (i.e., the poor and very poor classes of stream condition) are consistent with variability in stream condition relative to reference condition.

Creation of more bands is not justified by the precision of the IBIs. The limits on IBI precision are to be expected, as IBIs balance sensitivity to degradation and incorporation of natural variability. While IBIs are founded in the concept of biological integrity, they are only a rough approximation of the ecological structure and function of stream resources. We argue that protection of biological diversity in its most expansive definition (CEQ 1993, Noss and Cooperrider 1994) cannot be achieved solely through the use of IBIs. Augmented or separate monitoring and assessment focused on rare species and habitats is needed to fully protect stream ecosystem (see Kazyak et al. 2005).

4.4 CONTINUITY ACROSS THE ORIGINAL AND NEW IBIS

We determined that the improvements in the performance of the new IBIs, especially the more accurate coldwater Highlands and Coastal Plain fish IBIs, and the ability to rate the more abundant small streams with the fish IBI warranted adoption of the new IBIs. At the same time, the final construction of the new fish and benthic IBIs for the MBSS is very similar to the original MBSS IBIs. The basis in reference condition, the discrete 1-3-5 scoring, and the four bands of stream condition were retained. More elaborate modeling of reference condition (e.g., independent of geographic or stream type classification) was not incorporated. While new IBIs need to be calculated (using new metric combinations and thresholds), the IBI application process is unchanged.

As needed, the new MBSS IBIs can be calculated for past sites to maintain continuity of the long-term MBSS dataset. It is also possible to convert IBI results between different sampling periods by using regressions between the original and new IBIs. In general the regression R^2 are about 0.75; lower R^2 occurs for the Non-Coastal Plain benthic IBI where two new strata have been created and for original Highlands fish IBI when compared to the new coldwater fish IBI.

Five of the metrics in the new MBSS benthic IBIs are shared by the benthic indices (Stream Condition Indices) of Virginia and West Virginia. The metric combinations in these indices performed adequately in Maryland but with lower classification efficiencies. Similarly, the new MBSS IBIs also share metrics with the Montgomery County IBIs. Comparability studies (Vølstad et al. 2003) indicate that the indices for all these programs can be readily integrated.

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APPENDIX
MASTER TAXA LIST

Appendix Table 1. Ecological characteristics of fish species for use in IBI metrics. Tolerance: I = intolerant, T = tolerant; Native/introduced status: N = native statewide, IC = introduced to Chesapeake drainage, IY = introduced to Youghiogheny, I = introduced statewide; Trophic groups: FF = filter feeder, TP = top predator, GE = generalist, IV = invertivore, IS = insectivore, OM = omnivore, AL = algivore, HE = herbivore; NOTYPE = no category assigned; Composition: B=Benthic, R-Round-Bodied Sucker

Common Name	Tolerance (Based on Data)	Native or Introduced	Trophic Status	Lithophilic Spawner	Composition
AMERICAN BROOK LAMPREY	NOTYPE	N	FF	N	B
LAMPREY SP.	NOTYPE	N	FF	N	B
LEAST BROOK LAMPREY	NOTYPE	N	FF	N	B
SEA LAMPREY	I	N	FF	N	
LONGNOSE GAR	NOTYPE	N	TP	N	
AMERICAN EEL	NOTYPE	N	GE	N	
ALEWIFE	NOTYPE	N	FF	N	
AMERICAN SHAD	NOTYPE	N	FF	N	
BLUEBACK HERRING	NOTYPE	N	IV	N	
GIZZARD SHAD	NOTYPE	N	FF	N	
BLACKNOSE DACE	T	N	OM	N	
BLUNTNOSE MINNOW	T	N	OM	N	
BRIDLE SHINER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
CENTRAL STONEROLLER	I	N	AL	Y	
CHEAT MINNOW	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
COMELY SHINER	I	N	IV	Y	
COMMON CARP	NOTYPE	I	OM	N	
COMMON SHINER	I	N	OM	Y	
CREEK CHUB	T	N	GE	Y	
CUTLIPS MINNOW	NOTYPE	N	IV	Y	
CYPRINELLA SP.	I	N	IV	N	
CYPRINID HYBRID	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
CYPRINID SP.	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
EASTERN SILVERY MINNOW	NOTYPE	N	AL	N	
EMERALD SHINER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
FALLFISH	I	N	GE	Y	
FATHEAD MINNOW	NOTYPE	I	OM	N	
GOLDEN SHINER	T	N	OM	N	
GOLDFISH	NOTYPE	I	OM	N	
GRASS CARP	NOTYPE	I	HE	N	
IRONCOLOR SHINER	I	N	IS	Y	
LONGNOSE DACE	NOTYPE	N	OM	N	
LUXILUS SP.	NOTYPE	N	OM	Y	

Common Name	Tolerance (Based on Data)	Native or Introduced	Trophic Status	Lithophilic Spawner	Composition
NOTROPIS SP.	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
PEARL DACE	NOTYPE	N	IV	Y	
REDSIDE DACE	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
RIVER CHUB	I	N	OM	Y	
ROSYFACE SHINER	NOTYPE	N	IV	Y	
ROSYSIDE DACE	NOTYPE	N	IV	Y	
SATINFIN SHINER	I	N	IV	N	
SILVERJAW MINNOW	NOTYPE	N	OM	Y	
SPOTFIN SHINER	I	N	IV	N	
SPOTTAIL SHINER	I	N	OM	Y	
STRIPED SHINER	I	N	OM	Y	
SWALLOWTAIL SHINER	NOTYPE	N	IV	Y	
TENCH	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
BLACK REDHORSE	NOTYPE	NOTYPE	NOTYPE	NOTYPE	R
CREEK CHUBSUCKER	NOTYPE	N	IV	N	R
GOLDEN REDHORSE	NOTYPE	N	OM	Y	R
LONGNOSE SUCKER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	R
NORTHERN HOGSUCKER	I	N	IV	Y	R
QUILLBACK	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
SHORTHEAD REDHORSE	NOTYPE	N	OM	Y	R
WHITE SUCKER	T	N	OM	Y	
BROWN BULLHEAD	T	N	OM	N	
BULLHEAD (UNKNOWN)	NOTYPE	N	OM	N	
CHANNEL CATFISH	NOTYPE	IC	OM	N	
MARGINED MADTOM	I	IY	IV	N	B
STONECAT	NOTYPE	N	IV	N	B
TADPOLE MADTOM	NOTYPE	N	IV	N	B
WHITE CATFISH	NOTYPE	IY	OM	N	
YELLOW BULLHEAD	NOTYPE	N	OM	N	
CHAIN PICKEREL	NOTYPE	IY	TP	N	
NORTHERN PIKE	NOTYPE	IC	TP	N	
REDFIN PICKEREL	T	IY	TP	N	
EASTERN MUDMINNOW	T	N	IV	N	
BROOK TROUT	I	N	GE	Y	
BROWN TROUT	NOTYPE	I	TP	Y	
CUTTHROAT TROUT	NOTYPE	I	TP	Y	
RAINBOW TROUT	NOTYPE	I	TP	Y	
PIRATE PERCH	T	N	IV	N	
INLAND SILVERSIDE	NOTYPE	N	FF	N	
BANDED KILLIFISH	NOTYPE	N	IV	N	

Common Name	Tolerance (Based on Data)	Native or Introduced	Trophic Status	Lithophilic Spawner	Composition
MUMMICHOG	NOTYPE	N	IV	N	
RAINWATER KILLIFISH	NOTYPE	N	IV	N	
STRIPED KILLIFISH	NOTYPE	N	IV	N	
MOSQUITOFISH	NOTYPE	N	IV	N	
BLUE RIDGE SCULPIN	I	N	IS	Y	B
CHECKERED SCULPIN	NOTYPE	N	IS	Y	B
MOTTLED SCULPIN	I	N	IS	Y	B
POTOMAC SCULPIN	NOTYPE	N	IS	Y	B
SCULPIN SP.	NOTYPE	N	IS	Y	
SLIMY SCULPIN	NOTYPE	NOTYPE	NOTYPE	NOTYPE	B
STRIPED BASS	NOTYPE	N	TP	N	
WHITE PERCH	NOTYPE	N	IV	N	
BANDED SUNFISH	NOTYPE	N	IV	N	
BLACK CRAPPIE	NOTYPE	IC	GE	N	
BLACKBANDED SUNFISH	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
BLUEGILL	T	IC	IV	N	
BLUESPOTTED SUNFISH	NOTYPE	N	IV	N	
FLIER	I	N	IV	N	
GREEN SUNFISH	T	IC	GE	N	
LARGEMOUTH BASS	T	IC	TP	N	
LONGEAR SUNFISH	NOTYPE	IC	IV	Y	
MUD SUNFISH	NOTYPE	N	IV	N	
PUMPKINSEED	T	IY	IV	N	
REDBREAST SUNFISH	NOTYPE	IY	GE	N	
REDEAR SUNFISH	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
ROCK BASS	NOTYPE	IC	GE	Y	
SMALLMOUTH BASS	NOTYPE	IC	TP	N	
SUNFISH SP.	NOTYPE	NOTYPE	NOTYPE	NOTYPE	
WARMOUTH	NOTYPE	N	GE	N	
WHITE CRAPPIE	NOTYPE	IC	GE	N	
BANDED DARTER	I	I	IS	Y	B
BLACKSIDE DARTER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	B
DARTER SP.	NOTYPE	N	NOTYPE	Y	
FANTAIL DARTER	NOTYPE	N	IS	Y	B
GLASSY DARTER	NOTYPE	N	IS	Y	B
GREENSIDE DARTER	NOTYPE	N	IS	N	B
JOHNNY DARTER	NOTYPE	N	IV	N	B
LOGPERCH	NOTYPE	N	IV	Y	B
MARYLAND DARTER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	B
RAINBOW DARTER	NOTYPE	N	IS	Y	B

Common Name	Tolerance (Based on Data)	Native or Introduced	Trophic Status	Lithophilic Spawner	Composition
SHIELD DARTER	I	N	IS	Y	B
STRIPEBACK DARTER	NOTYPE	N	IV	N	B
SWAMP DARTER	I	N	IV	N	B
TESSELLATED DARTER	T	N	IV	N	B
VARIEGATE DARTER	NOTYPE	NOTYPE	NOTYPE	NOTYPE	B
YELLOW PERCH	NOTYPE	IY	GE	N	B
SPOT	NOTYPE	N	IV	N	

Appendix Table 2. Master Taxa List with designated tolerance value (Tol/Val), functional feeding group (FFG), and habitat. 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		7.3	Predator		
			Prostoma	7.3	Predator		
Turbellaria				4	Predator	sp	
	Tricladida	Planariidae		8.4	Predator	sp	
			Cura	6.5		sp	
			Dugesia	9.3	Predator	sp	
			Phagocata	8.4	Predator		
Oligochaeta				10	Collector	bu	
	Haplotaxida	Enchytraeidae		9.1	Collector	bu	
		Naididae		8.5	Collector	bu	2
	Lumbricina				Collector	bu	
	Lumbriculida	Lumbriculidae		6.6	Collector	bu	2
	Tubificida	Haplotaxidae					
		Naididae	*Chaetogaster*				
		Tubificidae		8.4	Collector	cn	2
			Branchiura				
			Limnodrilus	8.6	Collector	cn	
			Spirosperma	6.6	Collector	cn	
Hirudinea		Hirudinea			Predator	sp	
	Pharyngobdellida	Erpobdellidae		10	Predator	sp	
			Mooreobdella	8	Predator	sp	
	Rhynchobdellida	Glossiphoniidae		6	Predator	sp	
			Alboglossiphonia	6	Predator		
			Batracobdella	6	Predator		
			Helobdella	6	Predator	sp	
			Placobdella	6	Predator		
		Piscicolidae					
Gastropoda							
	Basommatophora	Ancylidae		7	Scraper	cb	
		Lymnaeidae		6.9	Scraper	cb	
			Fossaria	6.9	Scraper	cb	
			Lymnaea	6.9	Scraper	cb	
			Pseudosuccinea	6.3	Collector	cb	
			Radix	6.9	Collector	cb	
			Stagnicola	7.8	Scraper	cb	
		Physidae		7	Scraper	cb	
			Physella	7	Scraper	cb	
		Planorbidae		7.6	Scraper	cb	
			Gyraulus	7.6	Scraper	cb	
			Helisoma	7.6	Scraper	cb	
			Menetus	7.6	Scraper	cb	
			Planorbella	7.6	Scraper	cb	
			Promenetus	7.6	Scraper	cb	
	Bivalvia	ORDER					
	Limnophila	Ancylidae	*Ferrissia*	7	Scraper	cb	
	Mesogastropoda	Bithyniidae					

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
		Hydrobiidae		8	Scraper	cb	
			Amnicola	8	Scraper	cb	
			Hydrobia	8	Scraper	cb	
		Pleuroceridae					
			Goniobasis	10	Scraper	cb	
			Leptoxis	10	Scraper	cb	
		Valvatidae					
			Valvata	9			
		Viviparidae					
			Campeloma	6	Scraper	cb	
			Viviparus	1	Scraper	cb	
Pelecypoda	Unionoida	Unionidae		6	Filterer	bu	3
	Veneroida	Corbiculidae			Filterer		
			Corbicula	6	Filterer	bu	
		Sphaeriidae		6.5	Filterer	bu	
			Pisidium	5.7	Filterer	bu	
			Sphaerium	5.5	Filterer	bu	
		Piscicolidae	*Piscicola*		Predator	sp	
Malacostraca	Amphipoda			6		sp	
		Crangonyctidae		6.5	Collector	sp	
			Crangonyx	6.7	Collector	sp	
		Gammaridae					
			Gammarus	6.7	Shredder	sp	
			Stygonectes	9.3	Shredder	sp	
		Hyalellidae			Shredder		
			Hyalella	4.2	Shredder	sp	
		Taltridae					
	Cladocera	ORDER					
	Decapoda	Cambaridae		2.8	Shredder	sp	
			Cambarus	0.4	Collector	sp	
			Orconectes	2.8	Shredder	sp	
			Procambarus	2.8	Collector		
		Palaemonidae					
			Palaemonetes	7		sp	
	Isopoda			3.3	Collector		
		Asellidae		3.3			
			Caecidotea	2.6	Collector	sp	
			Lirceus	3.3	Collector	sp	
	Gordioidea	GORDIIDAE		6.8	Predator		
Insecta	Amphipoda	Crangonyctidae	*Synurella*	0.4			
	Coleoptera			4.1			
		Carabidae					
			Chlaenius			-	
		Chrysomelidae			Shredder	cn	
		Curculionidae			Shredder	cn	
		Dryopidae					
			Helichus	6.4	Scraper	cn	
		Dytiscidae		5.4	Predator	sw, dv	10
			Acilius	5.4			
			Agabetes	5.4	Predator		
			Agabus	5.4	Predator	sw, dv	10
			Copelatus		Predator	sw	
			Coptotomus				
			Cybister	5.4	Predator	sw, dv	10

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Deronectes	5.4	Predator	sw	
			Derovatellus	5.4	Predator	sw, dv	10
			Helocombus	4.1			
			Hydaticus		Predator	sw	
			Hydroporus	4.6	Predator	sw, cb	10
			Laccophilus	5.4	Predator	sw, dv	10
			Laccornis	5.4		sw	
			Matus	5.4			
			Rhantus	5.4	Predator	sw	
			Uvarus	5.4	Predator	sw, cb	10
		Elmidae		4.8	Collector	cn	
			Ancyronyx	7.8	Scraper	cn, sp	10
			Dubiraphia	5.7	Scraper	cn, cb	10
			Macronychus	6.8	Scraper	cn	
			Microcylloepus	4.8	Collector		
			Optioservus	5.4	Scraper	cn	
			Oulimnius	2.7	Scraper	cn	
			Promoresia	0	Scraper	cn	
			Stenelmis	7.1	Scraper	cn	
		Gyrinidae			Predator		
			Dineutus	4	Predator	sw, dv	10
			Gyrinus	4	Predator	sw, dv	10
		Haliplidae					
			Haliplus	9	Shredder	cb	
			Peltodytes	8.9	Shredder	cb, cn	10
		Helophoridae			Shredder	cl	
			Helophorus	4.1	Shredder		
		Hydrochidae					
		Hydrophilidae	*Berosus*	4.1	Collector	sw, dv, cb	10
			Cymbiodyta	4.1	Collector	bu	
			Enochrus	4.1	Collector	bu, sp	10
			Helochares				
			Hydrobius	4.1	Collector	cb, cn, sp	10
			Hydrochara	4.1			
			Hydrochus	4.1	Shredder	cb	
			Hydrophilus	4.1	Collector	sw, dv, cb	10
			Sperchopsis	4.1	Collector	cn	
			Tropisternus	4.1	Collector	cb	
		Psephenidae					
			Ectopria	2.2	Scraper	cn	
			Psephenus	4.4	Scraper	cn	
		Ptilodactylidae					
			Anchytarsus	3.1	Shredder	cn	
		Scirtidae		4	Collector	cb, sp	10
			Cyphon	7	Scraper	cb	
	Collembola			6			
		Isotomidae		4.8			
			Isotomurus	4.8			
		Sminthuridae					
	Diptera			6			
		Athericidae				sp, bu	10
			Atherix	2	Predator	sp, bu	10
		Blephariceridae					
			Blepharicera	4	Scraper	cn	

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
		Ceratopogonidae		3.6	Predator	sp, bu	10
			Alluaudomyia	3.6	Predator	bu	
			Atrichopogon	3.6	Predator		
			Bezzia	3.3	Predator	bu	
			Ceratopogon	2.7	Predator	sp, bu	10
			Culicoides	5.9	Predator	bu	
			Dasyhelea	3.6	Collector	sp	
			Helius	3.6	Predator	sp, bu	10
			Mallochohelea	3.6	Predator	bu	
			Probezzia	3	Predator	bu	
			Sphaeromias	3.6	Predator	bu	
			Stilobezzia	3.6	Predator	sp	
		Chaoboridae					
			Chaoborus	4	Predator	sp, sw	10
		Chironomidae		6.6			
			SF Chironominae	6.6	Collector		
			TR Diamesini	7.1	Collector		
			SF Orthocladiinae	7.6	Collector		
			SF Tanypodinae	7.5	Predator		
			TR Tanytarsini	3.5	Collector		
			TR Chironomini	5.9			
			Ablabesmyia	8.1	Predator	sp	
			Alotanypus	6.6			
			Apsectrotanypus	6.6	Predator	bu, sp	10
			Brillia	7.4	Shredder	bu, sp	10
			Brundiniella	6.6	Predator	bu, sp	10
			Cardiocladius	10	Predator	bu, cn	10
			Chaetocladius	7	Collector	sp	
			Chironomus	4.6	Collector	bu	
			Cladopelma	6.6	Collector	bu	
			Cladotanytarsus	6.6	Filterer	-	
			Clinotanypus	6.6	Predator	bu	
			Conchapelopia	6.1	Predator	sp	
			Constempellina	6.6	Collector		
			Corynoneura	4.1	Collector	sp	
			Cricotopus	9.6	Shredder	cn, bu	10
			Cricotopus/Orthocladius	7.7	Shredder		
			Cryptochironomus	7.6	Predator	sp, bu	10
			Cryptotendipes	6.6	Collector	sp	
			Diamesa	8.5	Collector	sp	
			Dicrotendipes	9	Collector	bu	
			Diplocladius	5.9	Collector	sp	
			Einfeldia	6.6	Collector		
			Endochironomus	6.2	Shredder	cn	
			Eukiefferiella	6.1	Collector	sp	
			Georthocladius			sp	
			Glyptotendipes	6.6	Filterer	bu, cn	10
			Guttipelopia	6.6	Predator		
			Heleniella	0.9	Predator	sp	
			Heterotrissocladius	2	Collector	sp, bu	10
			Hydrobaenus	7.2	Scraper	sp	
			Kiefferulus	6.6	Collector	bu	
			Krenopelopia	6.6	Predator	sp	
			Labrundinia	6.6	Predator	sp	
			Larsia	8.5	Predator	sp	

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Limnophyes	8.6	Collector	sp	
			Lopescladius	6.6	Collector	sp	
			Macropelopia	6.6	Predator	sp	
			Meropelopia	6.8			
			Mesocricotopus	6.6			
			Mesosmittia	6.6		sp	
			Metriocnemus				
			Micropsectra	2.1	Collector	cb, sp	10
			Microtendipes	4.9	Filterer	cn	
			Nanocladius	7.6	Collector	sp	
			Natarsia	6.6	Predator	sp	
			Nilotanypus	6.6	Predator	sp	
			Nilothauma	6.6		lotic	
			Odontomesa	6.6	Collector	sp	
			Omisus	6.6			
			Orthocladiinae A	8.4	Collector		
			Orthocladiinae B	6.6	Collector		
			Orthocladius	9.2	Collector	sp, bu	10
			Pagastia	6.6	Collector	-	
			Pagastiella		Collector	sp	
			Parachaetocladius	3.3	Collector	sp	
			Parachironomus	6.6	Predator	sp	
			Paracladopelma	6.6	Collector	sp	
			Parakiefferiella	2.1	Collector	sp	
			Paralauterborniella	6.6	Collector	cn	
			Paramerina	6.6	Predator	sp	
			Parametriocnemus	4.6	Collector	sp	
			Paraphaenocladius	4	Collector	sp	
			Parasmittia	6.6			
			Paratanytarsus	7.7	Collector	sp	
			Paratendipes	6.6	Collector	bu	
			Paratrichocladius	6.6	Collector	sp	
			Pentaneura	6.6	Predator	sp	
			Phaenopsectra	8.7	Collector	cn	
			Platysmittia	6.6			
			Polypedilum	6.3	Shredder	cb, cn	10
			Potthastia	0	Collector	sp	
			Procladius	1.2	Predator	sp	
			Prodiamesa	6.6	Collector	bu, sp	10
			Psectrocladius	6.6	Shredder	sp, bu	10
			Psectrotanypus	6.6	Predator	bu	
			Pseudochironomus	6.6	Collector		
			Pseudorthocladius	6	Collector	sp	
			Pseudosmittia	6.6	Collector	sp	
			Psilometriocnemus	6.6	Collector	sp	
			Rheocricotopus	6.2	Collector	sp	
			Rheopelopia	6.6	Predator	sp	
			Rheosmittia	6.6			
			Rheotanytarsus	7.2	Filterer	cn	
			Robackia		Collector		
			Saetheria	6.6	Collector	bu	
			Smittia	6.6	Collector	lentic	
			Stempellina	6.6	Collector	cb	
			Stempellinella	4.2	Collector	cb, sp, cn	10
			Stenochironomus	7.9	Shredder	bu	

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Stictochironomus	9.2	Collector	bu	
			Stilocladius	6.6	Collector	sp	
			Sublettea	10	Collector	-	
			Symposiocladius	4.8	Predator	sp	
			Sympotthastia	8.2	Collector	sp	
			Syndiamesa	6.6		sp	
			Synorthocladius	6.6	Collector		
			Tanypus	6.6	Predator		
			Tanytarsus	4.9	Filterer	cb, cn	10
			Thienemanniella	5.1	Collector	sp	
			Thienemannimyia	6.7	Predator	sp	
			Thienemannimyia group	8.2	Predator	sp	
			Tribelos	7	Collector	bu	
			Trissopelopia	4.1	Predator	sp	
			Tvetenia	5.1	Collector	sp	
			Unniella	6.6	Collector	-	
			Xenochironomus				
			Xylotopus	6.6	Shredder	bu	
			Zalutschia	6.6	Shredder		
			Zavrelia	6.6	Collector	cb, sp, cn	10
			Zavreliella			bu	
			Zavrelimyia	5.3	Predator	sp	
			Demicryptochironomus				
		Culicidae			Collector	sw	
				8			
			Aedes	8	Filterer	sw	
			Culex		Collector	sw	
		Dixidae		5.8			
			Dixa	5.8	Predator	sw, cb	10
			Dixella	5.8	Collector		
		Dolichopodidae		7.5	Predator	sp, bu	10
		Empididae		7.5	Predator	sp, bu	10
			Chelifera	7.1	Predator	sp, bu	10
			Clinocera	7.4	Predator	cn	
			Hemerodromia	7.9	Predator	sp, bu	10
		Ephydridae			Collector	bu, sp	10
		Muscidae		7	Predator	sp	
			Limnophora	7	Predator	bu	
		Pelechorhynchidae			Predator		
		Psychodidae		4			
			Pericoma	4	Collector		
			Psychoda	4	Collector	bu	
		Ptychopteridae					
			Bittacomorpha	4	Collector	bu	
			Ptychoptera	4	Collector		
		Sarcophagidae					
		Sciomyzidae		6	Predator	bu	
		Simuliidae		3.2	Filterer	cn	
			Cnephia	3.2	Filterer	cn	
			Greniera	3.2	Filterer		
			Prosimulium	2.4	Filterer	cn	
			Simulium	5.7	Filterer	cn	
			Stegopterna	2.4	Filterer	cn	
		Stratiomyidae			Collector		
			Stratiomys	2.8	Collector	sp, bu	10

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
		Syrphidae			Collector		
			Chrysogaster		Collector	bu	
		Tabanidae		2.8	Predator		
			Chrysops	2.9	Predator	sp, bu	10
			Tabanus	2.8	Predator	sp, bu	10
		Tanyderidae	*Protoplasa*		Collector		
		Tipulidae		4.8	Predator	bu, sp	10
			Antocha	8	Collector	cn	
			Cryptolabis	4.8		bu	
			Dicranota	1.1	Predator	sp, bu	10
			Erioptera	4.8	Collector	bu	
			Hexatoma	1.5	Predator	bu, sp	10
			Limnophila	4.8	Predator	bu	
			Limonia	4.8	Shredder	bu, sp	10
			Liogma	4.8			
			Molophilus	4.8		bu	
			Ormosia	6.3	Collector	bu	
			Pedicia	4.8	Predator	bu	
			Pilaria	4.8	Predator	bu	
			Pseudolimnophila	2.8	Predator	bu	
			Rhabdomastix	4.8		bu	
			Tipula	6.7	Shredder	bu	
	Ephemeroptera			2.9	Collector		
		Ameletidae		2.6			
			Ameletus	2.6	Collector	sw, cb	10
		Baetidae		2.3	Collector	sw, cn	10
			Acentrella	4.9	Collector	sw, cn	10
			Acerpenna	2.6	Collector	sw, cn	10
			Baetis	3.9	Collector	sw, cb, cn	10
			Barbaetis	2.3	Collector		
			Callibaetis	2.3	Collector	sw, cn	10
			Centroptilum	2.3	Collector	sw, cn	10
			Cloeon				
			Diphetor	2.3	Collector	sw, cn	10
			Fallceon	2.3			
			Procloeon	2.3	Collector		
		Baetiscidae				sp	
			Baetisca	4	Collector	sp	
		Caenidae					
			Caenis	2.1	Collector	sp	
		Ephemerellidae		2.6		cn, sp, sw	10
			Attenella	2.6	Collector		
			Drunella	1.9	Scraper	cn, sp	10
			Ephemerella	2.3	Collector	cn, sw	10
			Eurylophella	4.5	Scraper	cn, sp	10
			Serratella	2.8	Collector	cn	
			Timpanoga	2.6	Collector	sp	
		Ephemeridae					
			Ephemera	3	Collector	bu	
			Hexagenia	6	Collector	bu	
			Litobrancha				
			Pentagenia	3	Collector		
		Heptageniidae		2.6	Scraper	cn	
			Cinygmula	1.6	Scraper	cn	

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Epeorus	1.7	Scraper	cn	
			Heptagenia	2.6	Scraper	cn, sw	10
			Leucrocuta	1.8	Scraper	cn	
			Nixe	2.6	Scraper	cn	
			Stenacron	2	Collector	cn	
			Stenonema	4.6	Scraper	cn	
		Isonychiidae					
			Isonychia	2.5	Filterer	sw, cn	10
		Leptophlebiidae		1.7	Collector	sw, cn	10
			Habrophlebia	1.7	Collector	sw, cn, sp	10
			Leptophlebia	1.8	Collector	sw, cn, sp	10
			Paraleptophlebia	2	Collector	sw, cn, sp	10
		Metretopodidae					
			Siphloplecton	2	Predator	sw, cn	10
		Polymitarcyidae			Collector	bu	
		Potamanthidae					
			Anthopotamus	3			
		Siphlonuridae		7	Collector	sw, cb	10
			Siphlonurus	7	Collector	sw, cb	10
		Tricorythidae			Collector	cn, sp	10
	HYMENOPTERA	ORDER					
	Hemiptera	Belostomatidae					
			Belostoma	10	Predator	cb, sw	10
		Corixidae		5.6	Predator	sw	
			Hesperocorixa	5.6	Piercer	sw	
			Palmacorixa	5.6	Predator	-	
			Trichocorixa	5.6	Predator	sw, cb	10
		Gerridae					
			Aquarius				
			Gerris	6	Predator	skater	
			Limnoporus	6	Predator	skater	
			Metrobates		Predator	skater	
			Trepobates	6	Predator	skater	
		Mesoveliidae			Predator	cn	
		Naucoridae			Predator	cb, sw	10
		Nepidae			Predator		
			Ranatra	5.6	Predator		
		Noteridae	*Hydrocanthus*				
		Notonectidae					
			Bueno	5.6			
			Notonecta	10	Predator	sw, cb	1
		SALDIDAE		6	Predator		0
		Veliidae					
			Microvelia	6	Predator	skater	
			Rhagovelia		Predator	skater	
	Hymenoptera	BRACONIDAE			Parasite		
	Lepidoptera			6.7			
		Cosmopterygidae			Shredder		
			Pyroderces	6.7	Shredder	bu	
		Noctuidae		6.7	Shredder	bu	
		Pyralidae		6.7	Shredder	cb	
			Crambus	5			
		Tortricidae		6.7	Shredder	bu, cb	10
	Megaloptera	Corydalidae		1.4	Predator		

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Chauliodes	1.4	Predator	cn, cb	10
			Corydalus	1.4	Predator	cn, cb	10
			Nigronia	1.4	Predator	cn, cb	10
		ORDER					
		Sialidae		1.9	Predator	bu, cb, cn	10
			Sialis	1.9	Predator	bu, cb, cn	10
	Neuroptera	Sisyridae					
			Climacia		Predator	cb	7
	Odonata			6.6	Predator		
		Aeshnidae		6.2	Predator	cb	
			Aeshna				
			Anax		Predator		
			Basiaeschna	6.2	Predator	cb, sp, cn	10
			Boyeria	6.3	Predator	cb, sp	10
			Nasiaeschna				
		Calopterygidae			Predator		
			Calopteryx	8.3	Predator	cb	
		Coenagrionidae		9	Predator	cb	
			Argia	9.3	Predator	cn, cb, sp	10
			Enallagma	9	Predator	cb	
			Ischnura	9	Predator	cb	
			Nehalennia	9	Predator	cb	
		Cordulegastridae			Predator		
			Cordulegaster	2.4	Predator	bu	
		Corduliidae		2	Predator	sp, cb	10
			Helocordulia				
			Macromia	3	Predator	sp	
			Somatochlora	1	Predator	sp	
		Gomphidae		2.2	Predator	bu	
			Arigomphus	2.2	Predator	bu	
			Dromogomphus	2.2	Predator	bu	
			Erpetogomphus	2.2	Predator	bu	
			Gomphus	2.2	Predator	bu	
			Hagenius	2.2	Predator	sp	
			Lanthus	1.1	Predator	bu	
			Progomphus	2.2	Predator	bu	
			Stylogomphus	2.2	Predator	bu	
		Lestidae			Predator		
			Lestes	9	Predator	cb	
		Libellulidae		9	Predator		
			Erythemis	7	Predator	sp	
			Leucorrhinia	7	Predator	cb	
			Libellula	7	Predator	sp	
			Pachydiplax	8	Predator		
			Plathemis	3	Predator		
	Plecoptera			2.4			
		Capniidae		3.7	Shredder	sp, cn	10
			Allocapnia	4.2	Shredder	cn	
			Capnia	3.7	Shredder	sp, cn	10
			Paracapnia	2.8	Shredder	-	
		Chloroperlidae		1.6	Predator	cn	
			Alloperla	1.6	Predator	cn	
			Haploperla	1.6	Predator	cn	
			Perlinella	1.6	Predator	cn	

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Sweltsa	1.9	Predator	cn	
		Leuctridae		0.8	Shredder	sp, cn	10
			Leuctra	0.4	Shredder	cn	
			Paraleuctra	0.8	Shredder	sp, cn	10
		Nemouridae		2.9	Shredder	sp, cn	10
			Amphinemura	3	Shredder	sp, cn	10
			Nemoura	2.9	Shredder	sp, cn	10
			Ostrocerca	1.7	Shredder	sp, cn	10
			Paranemoura	2.9			
			Prostoia	4.5	Shredder	sp, cn	10
			Shipsa	2.9	Shredder	sp, cn	10
			Soyedina	2.9	Shredder	sp, cn	10
		Peltoperlidae		1.3	Shredder	cn, sp	10
			Peltoperla	1.1	Shredder	cn, sp	10
			Tallaperla	1.5	Shredder	cn, sp	10
		Perlidae		2.2	Predator	cn	
			Acroneuria	2.5	Predator	cn	
			Eccoptura	0.6	Predator	cn	
			Neoperla	2.2	Predator	cn	
			Paragnetina	2.2	Predator	cn	
			Perlesta	1.6	Predator	cn	4
			Phasganophora	2.2	Predator	cn	5
		Perlodidae		2.2	Predator	cn	
			Clioperla	1.7	Predator	cn	
			Cultus	2.2	Predator	cn	
			Diploperla	2.2	Predator	cn	
			Helopicus				
			Isoperla	2.4	Predator	cn, sp	10
			Malirekus	2.2	Predator	cn	
			Yugus		Predator	cn	
		Pteronarcyidae					
			Pteronarcys	1.1	Shredder	cn, sp	10
		Taeniopterygidae		3.1	Shredder		
			Oemopteryx	1.8	Shredder	sp, cn	10
			Strophopteryx	3.3	Shredder	sp, cn	10
			Taeniopteryx	4.8	Shredder	sp, cn	10
	TRICHOPTERA			4.6			
	Trichoptera	Brachycentridae		2.3	Filterer		
			Brachycentrus	2.3	Filterer	cn	
			Micrasema	2.3	Shredder	cn, sp	10
		Calamoceratidae					
			Anisocentropus				
			Heteroplectron	3	Shredder	sp	
		Dipseudopsidae					
			Phylocentropus	5	Collector	bu	8
		Glossosomatidae		1	Scraper	cn	
			Agapetus	2	Scraper	cn	
			Glossosoma	0	Scraper	cn	
		Goeridae			Scraper	cn	
			Goera	3.4	Scraper	cn	
		Helicopsychidae	*Helicopsyche*		Scraper	cn	
		Hydropsychidae		5.7	Filterer	cn	
			Cheumatopsyche	6.5	Filterer	cn	
			Diplectrona	2.7	Filterer	cn	

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
			Homoplectra	5.7	Filterer	cn	
			Hydropsyche	7.5	Filterer	cn	
			Parapsyche	5.7	Filterer	cn	
			Potamyia	5.7	Filterer	cn	
		Hydroptilidae		4			
			Hydroptila	6	Scraper	cn	
		Hydroptilidae	*Leucotrichia*	5	Scraper	cn	
			Ochrotrichia	4	Scraper	cn	
			Orthotrichia	5	Piercer		
			Oxyethira	3	Collector	cb	
		Lepidostomatidae					
			Lepidostoma	0	Shredder	cb, sp, cn	10
		Leptoceridae		4.1	Collector		
			Ceraclea	4.1	Collector	sp, cb	10
			Mystacides	4.1	Collector	sp, cb	10
			Nectopsyche	4.1	Shredder	cb, sw	10
			Oecetis	4.7	Predator	cn, sp, cb	10
			Triaenodes	5	Shredder	sw, cb	10
		Limnephilidae		3.4	Shredder	cb, sp, cn	10
			Hydatophylax	3.4	Shredder	sp, cb	10
			Ironoquia	4.9	Shredder	sp	
			Limnephilus	3.4	Shredder	cb, sp, cn	10
			Limnophilus				
			Platycentropus	3.4	Shredder	cb	
			Pycnopsyche	3.1	Shredder	sp, cb, cn	10
		Molannidae					
			Molanna	6	Scraper	sp, cn	10
			Molannodes	6			
		Odontoceridae					
			Psilotreta	0.9	Scraper	sp	
		Philopotamidae		2.6	Filterer	cn	
			Chimarra	4.4	Filterer	cn	
			Dolophilodes	1.7	Filterer	cn	
			Wormaldia	1.8	Filterer	cn	
		Phryganeidae		4.3	Shredder		
			Oligostomis	2			
			Ptilostomis	4.3	Shredder	cb	
		Polycentropodidae		0.2		cn	
			Neureclipsis	0.2	Filterer	cn	
			Nyctiophylax	0.2	Filterer	cn	
			Polycentropus	1.1	Filterer	cn	
		Psychomyiidae		4.9			
			Lype	4.7	Scraper	cn	
			Psychomyia	4.9	Collector	cn	
		Rhyacophilidae					
			Rhyacophila	2.1	Predator	cn	
		Sericostomatidae					
			Agarodes	3	Shredder	sp	
		Uenoidae		2.7		cn	
			Neophylax	2.7	Scraper	cn	9
BRANCHIOBDELLIDA							
Crustacea	Bivalvia	Sphaeriidae	*Musculium*	5.5	Filterer		
	Cladocera			8	Filterer		
	Copepoda			8	Collector		

Appendix Table 2. (Continued)

Class	Order	Family	Genus	TolVal	FFG	Habit	Note
	Ostracoda			8	Collector		

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 *Agnetina*.
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.
10. Use the leftmost Habit when calculating the % Climbers, % Clingers and % Swimmers metrics.

SF Subfamily
TR Tribe