



Biological Assessment Methodology for Non-Tidal Wadeable Streams

**Last Revised on June 4, 2014
Approved with the 2014 Integrated Report**



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I. BACKGROUND

As mandated by the Clean Water Act (CWA), the Maryland Department of the Environment (MDE) is required to describe the methodology used to assess use support and define impaired waters (CWA sections 305b/303d). The assessment methodology should be consistent with the State's WQSs, describe how data and information were used to make attainment determinations, and report changes in the assessment methodology since the last reporting cycle (US EPA 2006). This document describes how biological data is assessed for the purposes of the Integrated [combined 303(d) and 305(b)] Report. The methodology considers all existing and readily available data and information, and explains the analytical approaches used to infer watershed conditions at the 8-digit scale.

All of the State's waters must be of sufficient quality to provide for the protection and propagation of a balanced population of shellfish, fish, and wildlife and allow for recreational activities in and on the water (40 CFR §130.11). Biological criteria (biocriteria) provide a tool with which water quality managers may directly evaluate whether such balanced populations are present. Maryland's biocriteria use two multi-metric indices of biological integrity (IBI); one based on fish communities (F-IBI) and the other on benthic (bottom) macroinvertebrate communities (B-IBI). These indices are developed from reference sites that consider regional differences in biological communities. These indices, as described below, are based on characteristics of fish and benthic communities commonly used to assess the ability of streams to support aquatic life, and can be calculated in a consistent and objective manner. Both indices will be used in Maryland to evaluate biological data for the Clean Water Act requirements.

The Maryland Department of Natural Resources' (DNR) Maryland Biological Stream Survey (MBSS)¹ program, on which these biocriteria methods are based, uses a statewide probability-based design to assess the biological condition of first-, second-, third-, and fourth-order, non-tidal streams (determined based on the solid blue line shown on U.S. Geological Survey 1:100,000-scale maps) within Maryland's 8-digit watersheds (Klauda et al. 1998, Roth et al. 2005). To date, the MBSS has completed three rounds of sampling between 1995 and 2013: the first round of MBSS sampling was designed to assess major drainage basins (i.e., Maryland 6-digit) on 1:250,000-scale maps; and the second and third rounds were designed to assess smaller (i.e., Maryland 8-digit) watersheds on 1:100,000-scale maps. The use of random assignment of sampling locations within the population of first- through fourth-order streams supports the assessment of all of the State's waters.

For the purposes of the Integrated Report (IR), the results of biological sampling will be applied at the Maryland 8-digit watershed level. If a watershed is determined to be impaired, corrective action must be taken. That action may begin with additional monitoring and evaluation to determine the cause of the impairment (i.e., stressor identification). Once the stressor has been identified, it may be appropriate to develop a TMDL for the stressor.

¹ Data produced by the DNR MBSS constitutes the vast majority of data used for this methodology. In this document, the terms "MBSS data" and "State data" will be used interchangeably so as to also allow the use of biological data collected by MDE to serve these same purposes, when appropriate.

II. BRIEF HISTORY OF THE METHODOLOGY AND RATIONALE FOR THE CURRENT APPROACH

The first biological assessment methodology, developed for the 2002 IR, used MBSS data on fish and benthic communities to obtain an average 8-digit watershed IBI score. State assessors used the average watershed IBI scores and their associated confidence limits to determine if a watershed was impaired (Category 5). While this method (i.e. the average IBI score method) provided information on the magnitude of the degradation it did not give an indication of the extent of degradation (e.g., length of stream) found within a watershed, a current EPA requirement for integrated reporting. In addition, this method also utilized a smaller scale assessment process that classified 12-digit watersheds (approximately 10 square miles) as impaired if one low IBI value from one site (i.e., 75 meter sample) was found. This site-level assessment scale negated the advantage of the random monitoring design and the ability to report on the total stream system. Moreover, Southerland et al. (2007) assessed the average variability of the F-IBI and B-IBI scores at different spatial scales, and demonstrated that single site IBI scores were not representative of the 12-digit watershed scale.

Due to the limitations of this first biological assessment methodology, MDE, in coordination with DNR, set out to develop a new methodology to be used in the 2008 IR. This new methodology removed the 12-digit watershed scale assessment and made major changes to how the 8-digit watersheds assessments were conducted. The overarching goals for this new methodology (which has gone relatively unchanged since 2008) were that it:

1. Maintain consistent application at the current water quality management spatial scale (i.e., MD 8-digit watersheds);
2. Maximize the advantages of a probabilistic monitoring design;
3. Include a report on the extent of impact within the stream system (i.e., number of stream miles not supporting the aquatic life designated use);
4. Consider the uncertainty in various components of the assessment approach.

Addressing these four key goals helps to ensure the accuracy of regulatory decisions regarding water quality in Maryland. For goal number one, the advantages of using this assessment scale is that it is (1) consistent with many of the other water quality assessments contained within the Integrated Report; (2) it promotes consistency with subsequent TMDL development; (3) it allows for further spatial refinements during the TMDL development process, where more data may be available; and (4) it supports the use of probabilistically sampled biomonitoring data. Regarding goal number two, states are required by the Clean Water Act to assess all their waters on a regular basis for 303(d)/305(b) purposes. By incorporating a probabilistic monitoring and assessment method, Maryland is able to draw statistical inferences about the quality of all Maryland streams (first- through fourth-order) without the need to conduct census sampling. The MBSS, the State's primary data source for non-tidal biological assessments, helps fulfill this goal due to its stratified random monitoring design which is both meaningful and appropriate for management purposes.

To address the third goal, the biological reporting metric was changed so that now, the extent of degradation in stream miles (or proportion of stream miles) can be applied in assessment, a

metric that was unavailable in the previous biocriteria assessment methodology. Identifying the extent of degraded stream miles within an assessment unit is consistent with EPA Integrated Reporting requirements and meets EPA EMAP reporting recommendations. Using a watershed-based approach and reporting the extent of degraded conditions also allows the converse estimate, i.e., the extent of non-degraded or healthy streams. This allows the inclusion and identification of high quality (Tier II) waters that may be present in assessment units (8-digit watersheds) that are listed as impaired.

The fourth and final major goal for the biological assessment methodology was to account for the uncertainty involved with various aspects of biological sampling. Addressing uncertainty is critical to making accurate water quality management decisions that have significant implications on water quality improvement funding. Therefore, the current biological assessment methodology incorporates methods to account for the uncertainty that results from the temporal and spatial variability in the sampling design. Section III visits this topic in more detail.

This biological assessment methodology has remained largely unchanged since the 2008 IR. However, for the 2014 IR, Maryland began incorporating County-collected biological datasets to help bolster State assessments. This effort added new complexity to the process as county datasets were sampled randomly, but only within county borders. In addition, many counties sampled only benthic macroinvertebrate communities rather than both benthos and fish communities (as done by the MBSS). As a result, MDE added an alternate assessment procedure to be used for those watersheds where counties provided high quality biological data (in addition to State data). These new steps help to account for the sampling differences between the MBSS and the county and allow for a statistically valid 8-digit watershed assessment. In order to address the fact that some counties do not collect fish community data, MDE decided to use two independent assessments: one that assesses only MBSS data (both fish and benthos) and another that assesses only benthic data but uses both MBSS and county data. The results from each of these independent assessments are then compared for agreement (e.g. both meeting standards) to determine the appropriate IR listing category (e.g. Category 2, 3, 5). An important part of the benthic-only assessment is the incorporation of a spatial weighting scheme that weights county data according to the percentage of stream miles within an 8-digit watershed that are also within the county. This helps to ensure that an abundance of county data representing only a small geographic area within a watershed will not bias the entire 8-digit watershed assessment.

III. THE FOUNDATION FOR THE WATERSHED ASSESSMENT

Desirable properties for any assessment methodology are clarity and transparency. While water quality evaluations often deal with complex issues, the priorities for this assessment methodology are that it be objective, transparent, and quantitative. Specifically, the revised biological assessment methodology should: 1) use a scientifically defensible numeric indicator (IBI) based on reference sites, 2) produce unbiased results for the assessment units, 3) follow a clear and logical framework and 4) be robust enough to yield the same results when applied by multiple analysts.

The revised assessment methodology uses the scientifically robust F- and B- IBI developed by the MBSS program and documented in Southerland et al. (2005). To obtain unbiased results, the Department invoked a quantitative component to address temporal variability and sampling uncertainty from the MBSS monitoring design. In this report, variability is the year-to-year change in stream conditions that results from non-anthropogenic variation (e.g., climate, hydrology); and uncertainty is the result of inferring condition from the limited number of sites that can be sampled, given available resources. Finally, the assessment method employs an assessment approach that is transparent and can be understood by a wide audience.

A. Reference Sites and Conditions

Reference sites are the foundation for biological assessment. Using reference sites that are minimally disturbed is critical to IBI development because reference conditions define the scoring criteria applied to the individual metrics (Figure 1). Selection of metrics for inclusion in the IBIs is based on how well they distinguish between reference and degraded sites. In Maryland, reference and degraded sites are identified using lists of abiotic criteria. A complete list of criteria for reference and degraded conditions can be found in Southerland et al. (2005).

Once reference sites have been identified, DNR sequestered them into groups at minimal natural ecological variability by geography and stream type. The MBSS dataset provided enough reference sites (approximately 40) for F-IBI development in each of four naturally different stream types: Coastal Plain, Eastern Piedmont, warmwater Highlands, and coldwater Highlands. For the B-IBI, the Highland stratum was not split by temperature because, unlike fish, benthic macroinvertebrates assemblages are not typically depauperate in minimally disturbed coldwater streams.

The MBSS computes the IBI as the average of individual metric scores for a site (see Southerland et al. 2005). Individual metric scores are based on comparison with the distribution of metric values at reference sites within each geographic stratum (Figure 1). Metrics are scored 1 (if < 10th percentile of reference value), 3 (10th to 50th percentile), or 5 (> 50th percentile). The final IBI scores are calculated as the average of the scores and therefore range from 1 to 5.

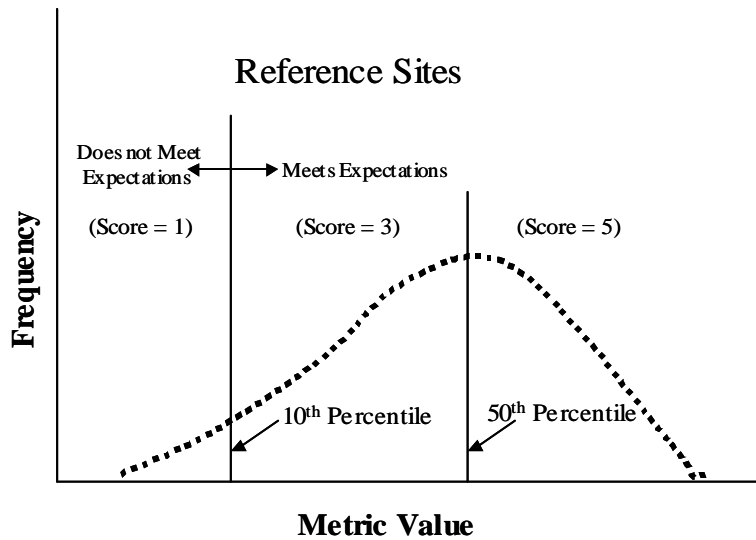


Figure 1: Scoring Criteria based on reference site distribution.

B. Year-to-Year Variability

All streams, regardless of anthropogenic changes, experience natural variability. These changes are a result of variability in precipitation and corresponding flows that result in fluctuation in the physical characteristics of the stream systems (Grossman et al. 1990). MBSS sentinel sites used to evaluate the natural year-to-year variability represent the best (based on physical, chemical and biological data) streams in Maryland. Sentinel sites are present in all regions (Highland, Eastern Piedmont and Coastal Plain) and stream orders (first through third). Most importantly, they are located in catchments that are not likely to experience a change in anthropogenic disturbances over time.

The year-to-year variability of the sentinel sites was examined by comparing the annual IBI values for individual sites over a five-year monitoring period. The coefficient of variation was used to compare site results since this normalizes the site variability to the mean site score. There were a total of 17 sites that had five years of B-IBI scores and 15 sites with five years of F-IBI scores. The average coefficient of variation was approximately 9% for the B-IBI and 13% for the F-IBI. Therefore, it can be expected that over a five-year period the standard deviation of year-to-year IBI scores will vary by 9 – 13% of the mean score.

C. Spatial Uncertainty of Stream Condition

The condition of all streams could in principle be measured through a census (i.e., without the need to resort to inferring condition), but would require visiting every length of stream in the State. The reality is that monitoring cannot be conducted on every foot or even mile of streams in a state due to resource constraints. Also, the sampling of a targeted non-random stream segments does not provide an unbiased estimate on the conditions of streams within a larger assessment unit. Therefore, MDE uses the MBSS dataset, which is a statewide probability-based sample survey, for assessing the biological condition of wadeable, non-tidal streams in Maryland's 8-digit watersheds (Klauda et al. 1998, Roth et al. 2005). MBSS sites are randomly selected from

the 1:100,000-scale stream network and sampled within a 75-m segment of stream length. Individual sampling results are considered representative at the 75-m segment, but because of design the data can be used to estimate unbiased conditions of streams within an assessment unit.

Realizing that randomly selected sampling sites may not always proportionately represent the assessment unit in which they are selected; MDE investigated the relationship between the number of sampling sites and the representation of watershed land use heterogeneity (See Appendix A). Generally, it was found that when approximately 10 sites were sampled within a watershed, that the average percent similarity between the number of sites within each land use were 85% similar to the stream mileage found within those same land uses (within the same watershed). Using this information as a guide, and a precision level of 25%, a minimum sample size of 8 samples was developed so as to capture both spatial heterogeneity and sample uncertainty for the watershed assessments.

D. Developing a Target Value for Degradation

Using the scoring criteria at reference sites, an $IBI > 3$ indicates the presence of a biological community with attributes (metric values) comparable to those of reference sites, while an $IBI < 3$ means that, on average, metric values fall short of reference expectations. Because a metric score of 3 represents the 10th percentile threshold of reference conditions, IBI values less than 3 represent sites that are suspected to be degraded. In contrast, values greater than or equal to 3 (i.e., fair or good) indicate that most attributes of the community are within the range of those at reference sites. However, Southerland et al. (2005) reported that “good” water quality was found at reference sites with low IBIs and that the distribution of reference and degraded site IBI values overlap, thus sites with a metric below the 10th percentile of reference sites (used for scoring) may have good quality waters. Ideally the State would be able to compute an average site IBI score, based on a minimum of three consecutive years of data, to be compared to the threshold of 3. However, this is rarely possible and therefore, the year-to-year variability will be based on the information from sentinel sites. Given the natural variation of IBI scores in time (observed at sentinel sites), it is expected that a site with an average score of 3 will likely have a distribution of annual values above and below 3 (Figure 2). For these cases the coefficient of variation in combination with an assumed normal distribution is used to determine the minimum detectable difference and the subsequent minimum allowable limit (MAL). The MAL decreases the likelihood of a type I error, classifying a site as degraded when it is actually in good condition, given there is only one sample in time. The following formula is applied to estimate the MAL:

$$MAL = IBI_{avg} - z * IBI_{avg} * CV$$

where

MAL = Minimum Allowable IBI Limit to determine if a site is degraded

IBI_{avg} = Average annual allowable IBI value (3 for B-IBI and F-IBI)

z = Standard normal score (1.28 for 90% one-sided confidence interval)

CV = Coefficient of variation

The minimum allowable limit for the F-IBI is 2.5, assuming a coefficient of variation of 13%, while the minimum allowable limit for the B-IBI is 2.65, assuming a coefficient of variation of 9%.

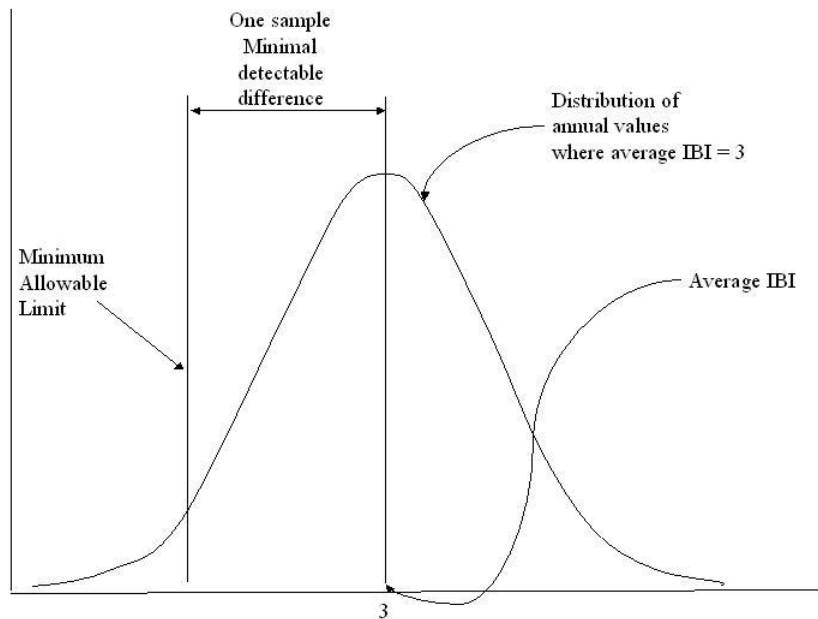


Figure 2: Distribution of annual values at site with average IBI of 3.

E. Watershed Assessment: The Null Hypothesis

The watershed assessment method tests the null hypothesis that the candidate assessment unit (8-digit watershed) does not violate narrative criteria for the support of aquatic life. In the watershed assessment method there is a general sample size provision to ensure that the random monitoring sites generally represent the spatial heterogeneity in the Maryland 8-digit assessment units. This sample size helps control the type II error (false negative - classifying a water body as meeting criteria when it does not) and an alpha level is set to control the type I error (false positive - listing a water body as impaired when it is not).

To test the null hypothesis (i.e., assess a watershed), the exact binomial confidence intervals are calculated using the Pearson-Clopper method and monitoring data in an assessment unit. Calculation of the binomial confidence intervals requires the total number of monitoring sites, the number of sites that are degraded, and the confidence level. The null hypothesis is that the populations of streams in the assessment unit are similar to the population of reference sites, which equates to less than 10% of the streams classified as degraded. A degraded site is defined as a site with either the B-IBI or F-IBI score below the specified threshold of the MAL. With small sample sizes the type II error rate is typically large and can result in accepting the null hypothesis when it is not true (classifying a watershed as meeting criteria when it does not). To reduce the type II error rate, a required precision is specified in the method. The three possible outcomes are as follows:

- Null hypothesis accepted but precision is low: If the lower confidence limit is less than or equal to 10% but half the width of the confidence interval is greater than 25% (low precision), the watershed will be classified as inconclusive and assigned to Category 3 of the Integrated Report and considered for future monitoring.
- Null hypothesis accepted and precision is acceptable: If the lower confidence limit is less than or equal to 10% and half the width of the confidence interval is less than 25% (acceptable precision), the watershed will be classified as pass and assigned to Category 2 on the Integrated Report.
- Null hypothesis rejected: If the lower confidence limit is greater than 10%, the watershed will be classified as failing and assigned to Category 5 on the Integrated Report.

To further reduce possible listing errors, the development of the methodology took into account the spatial distribution of the random monitoring sites as compared to the spatial heterogeneity of landscape features in the watershed. To do so, the Maryland 8-digit watershed landscape heterogeneity was determined using landscape clusters (groups of similar landscape conditions) that incorporate land use, land use change, soil erodibility, slope, precipitation, and population density (US EPA 2007). For all assessment units, the distribution of streams within landscape clusters were compared to the distribution of MBSS round 1 and round 2 monitoring sites. Results indicated that, on average, approximately 85% of the heterogeneity in 8-digit watersheds was captured with ten monitoring stations (see Appendix A).

To ensure clarity and transparency, the assessment method was summarized in a simple lookup table (Table 1) below. The table incorporates (1) testing the null hypothesis that the candidate assessment unit does not violate narrative criteria for the support of aquatic life; (2) applying 90% exact binomial confidence intervals; (3) requiring a precision of 25%; and (4) ensuring that the monitoring sites capture the watershed landscape heterogeneity.

Table 1: Biocriteria Assessment Table

Total Number of Random Sites in Assessment Unit	Maximum Number of Degraded Samples in Assessment Unit to be Classified as Pass (Category 2)	Minimum Number of Degraded Samples in Assessment Unit to be Classified as Fail (Category 5)
≤7	1 (a)	3 (b)
8-11	2	3
12-18	3	4
19-25	4	5
26-32	5	6
33-40	6	7
41-47	7	8
48-55	8	9
56-63	9	10
64-71	10	11
72-79	11	12

Notes:

- a. If $n \leq 7$ and at least 6 samples are not degraded then watershed classified as Pass (Category 2).
- b. If $n \leq 7$ and 3 or more samples are degraded then watershed classified as Fail (Category 5).

IV. THE BIOLOGICAL ASSESSMENT PROCESS

This section describes the current biocriteria assessment approach which was adapted to allow for the incorporation of non-State data² into the 2014 Integrated Report assessments. This process was specifically modified to address two different biological data scenarios: 1) when 8-digit watersheds have only been sampled by the MBSS, and 2) when watersheds have been sampled both by the MBSS and by a non-state government organization (usually county). Figure 3 and Figure 4 illustrate the generalized steps in the assessment process for these two data scenarios. The individual steps (identified alphanumerically in the decision diagrams) are then discussed in greater detail in their corresponding sections (e.g. Step 1, Substep 2b, etc). In general, the assessment methodology has not changed drastically. It still uses the MAL thresholds and the Biocriteria Assessment Table (Table 1) based on confidence intervals to determine the appropriate IR listing categories. The main difference is that for watersheds that have non-state data, the process will involve several more steps that help to account for differences in spatial sampling scale and in indicators³ assessed. This entire assessment process focuses on assessing the condition of 8-digit watersheds with multiple sites by assessing the percentage of sampling sites that are degraded. Use of the percentage of degraded sampling sites allows for State assessors to approximate the number of stream miles degraded in a sampled watershed.

A. Assessment Process for Watersheds with MBSS Data Only

The following assessment process outlined in Figure 3 is for use for those 8-digit watersheds that have only been sampled randomly by the MBSS (no county data is available). This biological assessment process has remained unchanged from the previous (2012) version. A detailed description of each step shown in Figure 3 is provided in the corresponding numbered sections below.

² The use of non-state data is currently limited to those datasets which use the MBSS IBI framework. This helps to ensure comparability with the state-established reference conditions.

³ Most county biological sampling programs sample for benthic macroinvertebrates and do not include sampling for fish communities.

Biological Assessment Process for Watersheds where only State Data is Available

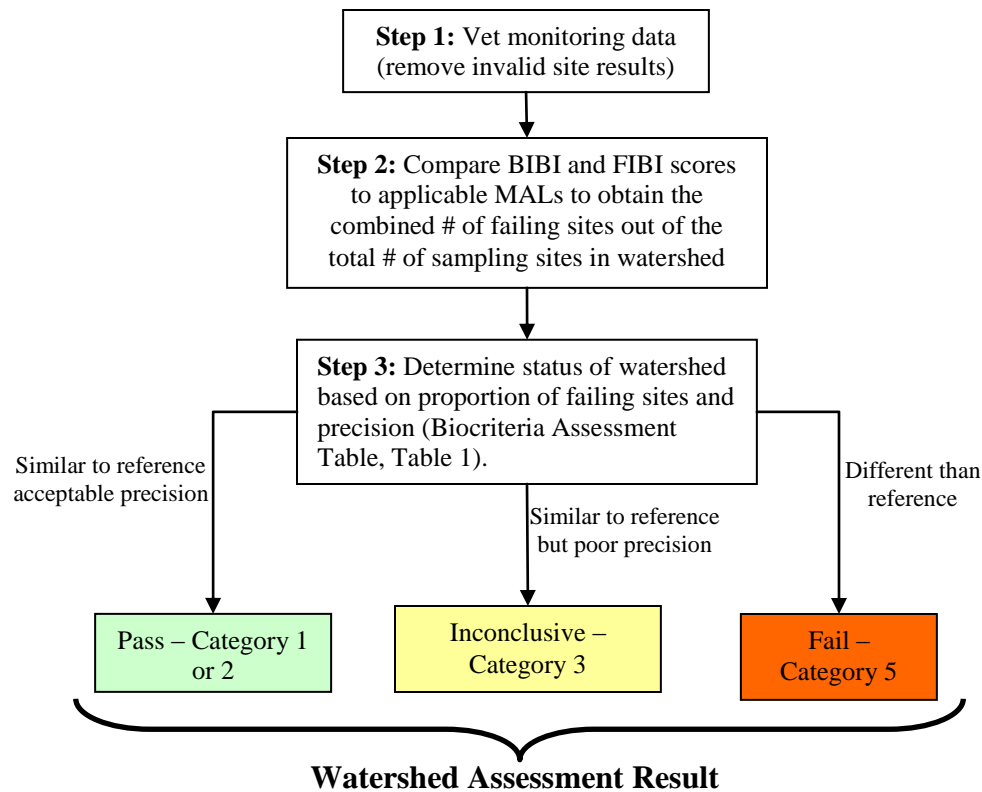


Figure 3: Watershed assessment procedure for watersheds having only State-collected data.

Step 1: Vetting Monitoring Data

In all cases, State biologists may use professional judgment in evaluating biological results. However, to aid in the data review, a set of rules is used to guide the data vetting process. These rules evaluate specific data parameters such as flow, catchment size, and buffer width to determine if the IBIs are reliable indicators of current watershed conditions. As a specific example, if there was a temporary or significant natural stressor such as drought or flood, sample results were evaluated to determine whether IBI scores resulted from anthropogenic influences or natural conditions. The final master database contains all biological sites considered valid for use in the assessment process. The following rules for eliminating site results were developed by MDE with help from DNR to address situations when the IBIs are not representative of stream condition.

- (a) Sampling locations with less than a 300-acre catchment or watershed often have limited fish habitat and naturally low fish diversity. As a result, the F-IBI will not be used for assessment decisions at these sites unless the score is significantly greater than 3.
- (b) Due to the unique chemistry of blackwater streams and the lack of defined blackwater reference conditions, the IBIs tend to underrate this stream type. For this reason, all blackwater sites (dissolved organic carbon > 8 mg/l and either pH <5 or acid neutralizing capacity (ANC) <200 µeq/L) with either the B-IBI or F-IBI indeterminate or significantly less than 3 will not be used. If the B-IBI and the F-IBI are significantly greater than 3, the stream will be rated as meeting the aquatic life designated use.
- (c) If the number of organisms in a benthic sample is less than 60, that sample will not be used unless the B-IBI is significantly greater than 3 or supporting data (e.g., habitat rating, water quality data) indicate impairment (presence of anthropogenic stressors) and there is no evidence of sampling error or unusual natural phenomena.
- (d) Heavy rain and other runoff events (e.g., sudden heavy snowmelt) can scour the streambed and transport fish and/or benthics out of a stream segment. As such, samples taken within two weeks of such events may be considered invalid in the best professional judgment of State biologists and not used for evaluation of stream condition.
- (e) The IBI scores of stream sampling sites that are tidally influenced will not be used to determine designated use attainment.
- (f) The IBI scores of streams affected by excessive drought or intermittent conditions will not be used in assessment decisions. Other sampling sites influenced by low flow conditions may also not be used.
- (g) The IBI scores of sampling sites that are dominated by wetland-like conditions (e.g., no flowing water, shallow, abundant organic matter) may be considered invalid in the best professional judgment of State biologists.
- (h) The IBI scores of streams impounded by beaver dams may be considered invalid. For example, a site within a natural impoundment that was created by beaver activity between the spring benthic macroinvertebrate sampling and the summer fish sampling. Man-made alterations to selected stream segments (e.g., channelization, dredging) should be noted, but they do not invalidate the IBIs.
- (i) Sampling sites where the results may be skewed due to sampling error will not be used for assessment purposes.

In addition to these cases, State biologists may use best professional judgment to evaluate any streams sampled under conditions that are not characterized by reference stations.

Step 2: Comparing IBI Scores to the MALs

In step 2, State assessors compare the F-IBI and B-IBI score from each sampling location to the applicable minimum allowable limit (MAL), which for F-IBIs equals 2.5 and for B-IBIs equals 2.65. For any sampling location that has either a F-IBI and/or a B-IBI below the MAL, that site will be classified as a failing site. Next, the total number of failing sites is summed for each 8-digit watershed. Note: Some sites may have both a failing F-IBI and a failing B-IBI. Regardless, such a site will only count once toward the total number of failing sites within an 8-digit watershed.

Step 3: Determining Status Based on Proportion of Failing Sites

Using the number of the failing sites in a watershed and the total number of sites within that watershed, State assessors use 90% confidence intervals and precision to determine watershed status. This is equivalent to using Table 1 above. This lookup table was developed as a simple way to test watersheds for similarity with reference watersheds. The minimum sample size incorporated into this table accounts for spatial variability by requiring an acceptable level of precision. A watershed that is significantly different than the reference condition is classified as impaired and listed on Category 5 in the Integrated Report. If a watershed is not determined to be significantly different from reference conditions, the assessment must have an acceptable precision (half the width of the confidence interval is <25%) before the watershed is listed as attaining the water quality criterion (Category 1 or 2). If the precision is not acceptable, the watershed is listed as inconclusive and placed in Category 3.

Minimum Sample Size

Considering the watershed/monitoring site similarity analysis results and the required statistical precision for a definitive classification, a watershed can be reasonably assessed if it has at least eight random monitoring sites. However, if less than eight sites are within an 8-digit watershed and three of them are classified as degraded, the watershed will be classified as not supporting aquatic life and placed on Category 5 of the Integrated Report. The rationale is that if five more samples were collected (to total eight) then the watershed would be listed on Category 5 regardless of the results at the new sites. Likewise, if there are less than eight monitoring sites but at least six sites are not degraded then the watershed will be classified as supporting aquatic life and placed on Category 2. Similarly, the rationale is that if two more sites were added to the monitoring design, the watershed would be listed on Category 2 regardless of the new site results. However, in the future, it is recommended that biological monitoring designs have at least eight sites per 8-digit watershed.

B. Alternate Assessment Process for Watersheds with State and County Data

Prior to the 2014 IR, State assessors depended solely upon MBSS data for assessing entire 8-digit watersheds. Starting with the 2014 IR, the State began integrating county-collected biological data with MBSS data so as to increase watershed sample sizes and provide more up-to-date information. In order to do this, however, State assessors had to address two major differences between the MBSS and county data. For one, county data is typically only sampled in that portion of a watershed that is within county borders. As a result, using this data to assess an entire 8-digit watershed (that crosses into another jurisdiction) has the potential to bias the watershed assessment. Secondly, many county biological sampling programs collect only benthic data instead of both benthic and fish information (as in the MBSS). This too can bias the result as

each county site has only one chance (benthos) to be classified as failing instead of two chances (benthos and fish). In order to account for these differences in biological sampling, it was necessary for the State to develop an alternate assessment process that weights IBI scores according to the spatial scale sampled and the number of indicators used. Please note: This assessment process, shown in Figure 4, is only applied for those 8-digit watersheds where both MBSS and county data is available. All other watersheds will be assessed using the process described in Section IV.A.

This new assessment process includes two separate analyses: one that makes use of both B-IBI and F-IBI scores (Step 1) and another that only uses B-IBI scores (Step 2). Having these two independent analyses ensures that F-IBI scores from the MBSS still play a role in the final assessment result but also ensures that the lack of a F-IBI score in the county datasets does not significantly bias the overall assessment. In the second analysis (Step 2), weights are applied to county B-IBI results to account for the portion of stream miles in the watershed that the county data assessed. These weights help to nullify any spatial scale bias that might occur from using the county data. Figure 4 outlines the assessment process used for those 8-digit watersheds that have been sampled both by the MBSS and by a county. A detailed description of each step shown in Figure 4 is provided in the corresponding sections below.

Alternate Biological Assessment Process for Watersheds with both State and County Data

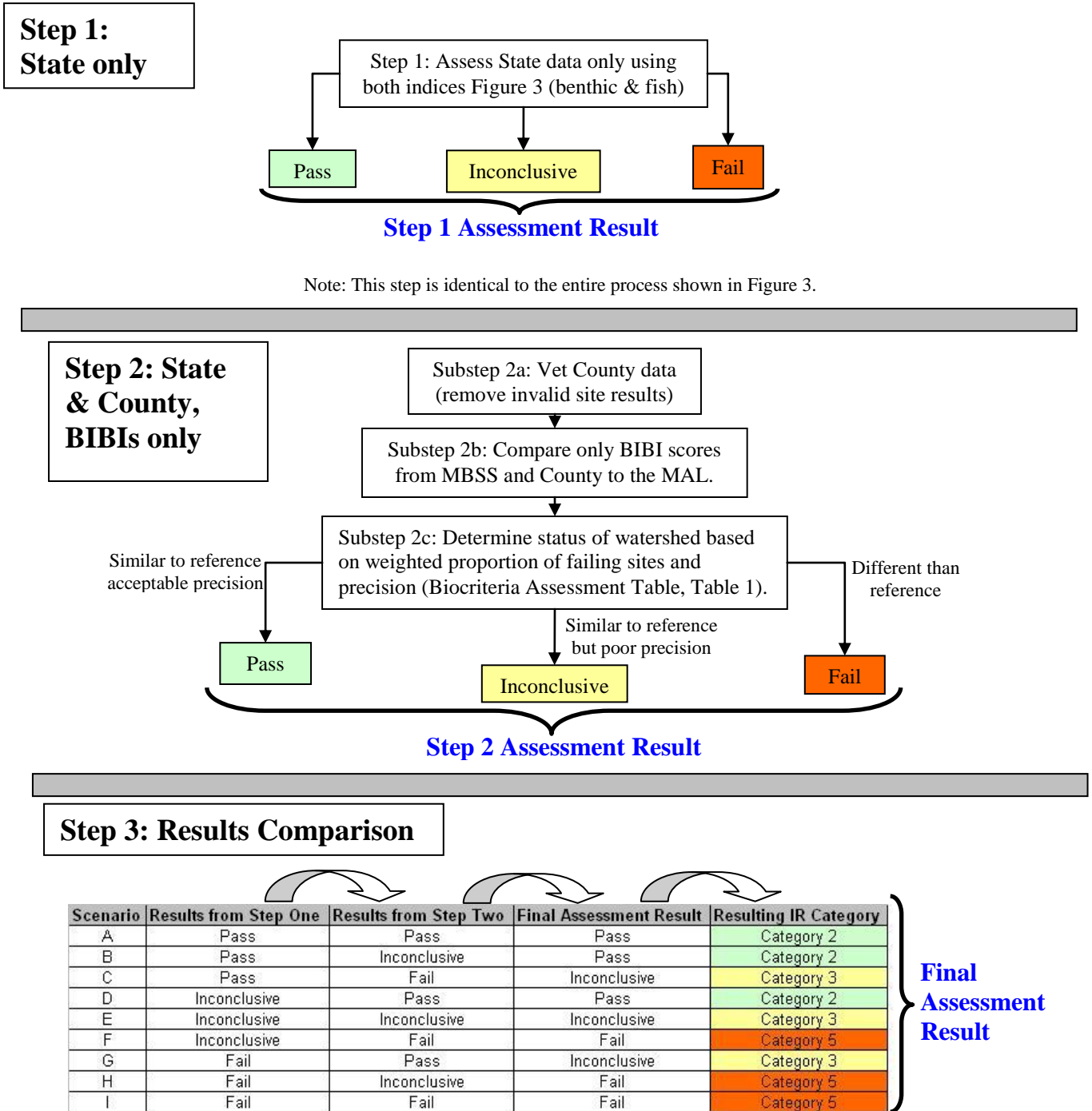


Figure 4: Watershed assessment procedure for watersheds that have both State and County data.

Step 1: Assess State Data Only (Benthos and Fish)

This step simply follows the assessment process described in Section IV.A. and shown in Figure 3. Here the assessment result will be used only as an endpoint for Step 1 in the alternate assessment process rather than as the final watershed assessment result as in Section IV.A.

Step 2: Assess State and County Data, Benthic Data Only

In Step 2 of the alternate assessment process, State and county benthic monitoring data is integrated to fill in data gaps and increase the overall sample size. The purpose of introducing the separate second step was to allow for a means to include samples with B-IBI scores only, and to account for non-spatially coincident watershed-county boundaries. Here, the process incorporates weighting to account for the difference in spatial scale represented by the county data. The specifics of this are described in more detail in the following substeps.

Substep 2a: Vetting Monitoring Data

This substep involves vetting the county monitoring data according to the same procedures used for State data and described in Section IV.A. - Step 1 of this document. This step helps to eliminate invalid data and to ensure that county-submitted data is subjected to the same standards of review as State data.

Substep 2b: Compare B-IBI Scores to MAL

Next, the B-IBI scores from both State and county datasets are compared to the MAL for B-IBIs which equals 2.65. For any site that has a B-IBI below the MAL, that site will be classified as failing. In this step it is important to maintain the distinction between the number of failing county sites and the number of failing State sites. These two values will be assigned different weights in Substep c.

Substep 2c: Weighting Procedure and Determination of Step 2 Status

For this part of the assessment, the assessor must determine the percentage of stream miles of an 8-digit watershed that is within the county's borders (for watersheds that cross county boundaries). Since counties typically only sample within their borders, their sampling sites will be limited to this portion of any given 8-digit watershed. To avoid biasing the assessment for the entire 8-digit watershed the assessor must weight the county sampling sites by this percentage. These weighted county values are then combined with the State values to obtain an overall weighted proportion of failing sites. The equation below describes this procedure.

$$\frac{D_w}{T_w} = \frac{\sum_{i=1}^n (B_i W_i)}{\sum_{i=1}^n W_i}$$

where

- D_w = Weighted number of sites with degraded B-IBI scores in a watershed
- T_w = Weighted total number of sites with B-IBI scores in a watershed
- n = Number of sites with B-IBI scores in a watershed
- i = Counter for each site with a B-IBI score in a watershed
- B_i = Binary B-IBI score for each site: 1 for degraded, 0 for not degraded
- W_i = Weight. For State MBSS sites, 1; for county sites, the proportion of a watershed's stream miles within each county's borders

Note: An alternate equation, with corresponding explanation, that yields identical results is provided in Appendix B.

These proportions are then used calculate 90% confidence intervals, which are the basis for the Biocriteria Assessment Table (Table 1). The output is a pass/fail/inconclusive designation for Step 2. Similar to Step 1 from Section IV.B., the assessor uses the assessment result from this step (Step 2) for use in Step 3, following.

Step 3: Results Comparison

The final step of the alternate assessment process involves a simple comparison of the results from Step 1 and Step 2. The purpose of this is to mute any bias that may be introduced as a result of using county data while at the same time, taking advantage of the increased sample size. The following table (Table 2) shows the possible assessment scenarios that can result from this process. As noted, any time agreement exists between Step 1 and Step 2 (Scenarios A, E, and I), the shared result will stand. Any time the results of one step are inconclusive and the other step is not, the conclusive result will be used for IR listing (Scenarios D and F). Finally, when the results from the two steps disagree (Scenarios C and G), it will generally result in a Category 3 listing as more data is needed to confirm watershed status. In certain cases though, State assessors may use professional judgment and default to an impaired status (Category 5) so as to be more conservative with the overall assessment.

Table 2: List of assessment scenarios that can result from the alternate bioassessment process.

Scenario	Results from Step One	Results from Step Two	Final Assessment Result	Resulting IR Category
A	Pass	Pass	Pass	Category 2 – meets standards
B	Pass	Inconclusive	Pass	Category 2– meets standards
C	Pass	Fail	Inconclusive	Category 3 – insufficient info
D	Inconclusive	Pass	Pass	Category 2– meets standards
E	Inconclusive	Inconclusive	Inconclusive	Category 3– insufficient info
F	Inconclusive	Fail	Fail	Category 5 - impaired
G	Fail	Pass	Inconclusive	Category 3– insufficient info
H	Fail	Inconclusive	Fail	Category 5- impaired
I	Fail	Fail	Fail	Category 5- impaired

C. Data Limitations

Previous versions of the Biological Assessment Methodology discussed the State's preference to use only the most recent 10 years of biological data for IR assessments. However, since there is an insufficient sample size for some 8-digit watersheds as of the 2014, it makes using older data (e.g. Round One and Two of the MBSS) necessary. As a result, for the 2014 IR, MDE will continue to use all three rounds of data from the MBSS to probabilistically assess 8-digit watersheds. MDE will review this matter in future assessments to determine if and when older data should be omitted from State assessments.

As the MBSS Program continues to collect more data around the State, they may continue to refine and enhance the respective benthic and fish IBIs in order to better discriminate between healthy and degraded stream conditions. In doing so, the IBI scores from an older site may change depending on what metrics are used and how the IBI is calculated. To keep assessments transparent and repeatable for regulatory purposes, MDE may choose to continue using the 2005 IBIs and corresponding metrics. Specific data scenarios may arise in the future that cannot be predicted. At all times, it is MDE's goal to maintain the scientific defensibility of these assessments and others that depend on the use of biological data.

V. USE OF NON-STATE DATA

Given that a key use of these procedures is for the Integrated Report and that the State is required to consider all readily available data, MDE recognizes the need to incorporate local biological data into the assessment process. Counties or other water monitoring programs that intend to submit their data to support decisions made using the biological framework should carefully follow the general guidelines below. Additional detail is also provided in the document named "Biological Data Quality Guidelines" and can be found on MDE's website at:

<http://mde.maryland.gov/programs/water/TMDL/Integrated303dReports/Documents/AssessmentMethodologies/BiologicalDataQualityGuidelines4172017.pdf>.

- Data collected using MBSS (field, laboratory and IBI protocols) or comparable methodology must be:
 - Documented to be of good quality;
 - Fully integratable with MBSS data;
 - Provided in a format readily available for merging into the MBSS database;
 - Contain the additional habitat, physical, and chemical information that the MBSS provides that allow for vetting.

- If MBSS methodology is not used but data are documented to be of good quality, in accordance with guidance and technical direction from the State, the data may still be used to supplement fully integrated MBSS and local data.

Data not meeting the requirements stated above may be helpful for non-regulatory purposes (e.g., targeting, education). Such data will be stored and documented for these uses. State biologists

may refer submitters to information sources that will help them to improve the quality of their monitoring data.

VI. BIOLOGICAL STRESSOR IDENTIFICATION

If a watershed is determined to be impaired (Category 5) based on biological data, it is MDE’s goal to identify the impairing pollutant(s) so as to facilitate TMDL development and/or to direct water quality restoration. To support this effort, the MDE Science Services Administration has developed a biological stressor identification (BSID) analysis that uses a case-control, risk-based approach to systematically and objectively determine the likely cause(s) of reduced biological conditions. In effect, the BSID process links potential causes/stressors identified by the analysis with general causal scenarios and concludes with a review for ecological plausibility by State scientists. Once the BSID process is completed, one or several stressors (i.e., pollutants) may be identified as probable causes of the poor biological conditions within the Maryland 8-digit watershed.⁴

MDE will use identified stressor(s) (e.g., sediment, chlorides, and nutrients) to support current pollutant listings, add new pollutant listings, and/or change the category assessment for a pollutant on the Integrated Report. As a result, when stressor(s)/pollutant(s) are identified for a biologically-impaired 8-digit watershed, the biological listing will be removed from Category 5 and will be replaced by the appropriate pollutant listing(s) (in Category 4c or 5, as appropriate). An example of this is illustrated below in Tables 3 and 4.

Table 3: Example of a Category 5 Biological Listing

AU-ID	Basin Name	Category	Cause	Indicator
MD-02130906	Patapsco Lower North Branch	5	Cause Unknown	Fish and Benthic IBIs

⁴ These probable causes each have an associated ‘percent attributable risk’ value which is an estimate of the excess prevalence of the specified stressor at impaired sites beyond stressor prevalence at unimpaired sites.

Table 4: Example of changes to the IR Listings that result from the BSID Analysis. These three listings essentially take the place of the previous biological listing (combination benthic/fish bioassessment) for watershed MD-02130906.

Cycle First Listed	Assessment Unit-ID	Basin Name	Category	Cause	Indicator	Notes
1996	MD-02130906	Patapsco Lower North Branch	5	Total Suspended Solids (TSS)	Fish and Benthic IBIs	This pollutant listing existed previous to the BSID analysis. The BSID confirmed that this pollutant was impairing the watershed.
2010	MD-02130906	Patapsco Lower North Branch	5	Chlorides	Fish and Benthic IBIs	Newly identified stressor/cause
2010	MD-02130906	Patapsco Lower North Branch	5	Sulfates	Fish and Benthic IBIs	Newly identified stressor/cause

As shown in Table 4, the impairment ‘cause’ field was changed to reflect the actual cause/pollutant impairing the watershed. Those watersheds that do not have the stressor identification process completed will remain as “Cause Unknown” until stressors are identified. In some cases, more biological, chemical, or physical data may need to be collected in order to inform the BSID analyses. The BSID analysis and process can be reviewed in more detail by visiting MDE’s webpage at: http://mde.maryland.gov/programs/water/TMDL/Pages/bsid_studies.aspx. This page includes a link to the report titled *Maryland Biological Stressor Identification Process which provides the background on the analysis methods. Please note that this report will soon be updated in late 2014.*

VII. USING BIOLOGICAL DATA FOR TIER II DESIGNATION

As specified in COMAR [26.08.02.04-1] biological assessment data will be used for the purpose of identifying Tier II waters to be protected under the Department’s Anti-degradation Policy Implementation Procedures. According to these regulations, when biological assessment data indicates that water quality is within 20% of the maximum attainable value of the index of biological integrity, those waters will be assigned a Tier II designation. For data sampled and scored according to MBSS protocols, this equates to having both a fish and benthic IBI score of 4.00 or greater at a single site. Using these two pieces of biological information sampled during different seasons of the year helps to independently validate the high quality status of a segment.

Tier II segments can exist in watersheds that are listed as impaired (Category 5) by the methodology spelled out in this document, despite Section 26.08.02.04-1D(2) of the Anti-degradation Procedures. This section states, “Water bodies included in the List of Impaired Waters (303(d) List) are not Tier II waters for the impairing substance.” The biological assessment methodology only assesses the biological condition of streams at the 8-digit

watershed scale (approximately 90 square miles) and calculates the percentage of sites impaired within this larger scale. As a result, it is possible for smaller stream segments located within 'impaired' (Category 5) 8-digit watersheds to be of Tier II quality due to local variation in stressors and land use. Since local water quality conditions are better characterized through site-specific monitoring, individual stations are used to identify and designate Tier II segments regardless of the watershed assessment result. For more information on Maryland's Tier II high quality waters please visit:

<http://mde.maryland.gov/programs/water/TMDL/WaterQualityStandards/Pages/AntidegradationPolicy.aspx>. To see what waters are currently designated as Tier II please refer to <http://mde.maryland.gov/programs/water/TMDL/WaterQualityStandards/Pages/HighQualityWatersMap.aspx>.

VIII. FUTURE MONITORING PRIORITIES

Monitoring prioritization will focus on the watersheds determined to be inconclusive in the final assessment (Category 3) and will be based on the following specific factors. First, the watersheds with the largest percentage of perennial non-tidal 1st through 4th order stream miles/drainage area will receive preference over basins with a large percentage of tidal stream miles/drainage area. Secondly, the available data for each watershed will be evaluated and best professional judgment applied to determine whether obvious causes of low IBI scores exist due to natural conditions (i.e., a high percentage of intermittent or blackwater streams in the watershed) and/or anthropogenic influences. In some cases, watersheds will be addressed by a Water Quality Analysis or referred for further stressor identification. To allow for the most efficient use of resources, consideration will be given to the number of stations monitored by DNR and the counties so as to limit redundant sampling efforts.

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Appendix A. Evaluating the Spatial Representation of the Monitoring Data

An analysis of MBSS data representation of each 8-digit watershed determines if stream monitoring stations adequately capture watershed landscape heterogeneity and can thus be used to support a biological assessment. Watershed landscape heterogeneity is assessed using the distribution of landscape clusters (groups of similar landscape conditions) that incorporate land use, land use change, soil erodibility, slope, precipitation, and population density (US EPA 2007). Nine distinct cluster types were identified and are presented in Figure A-1.

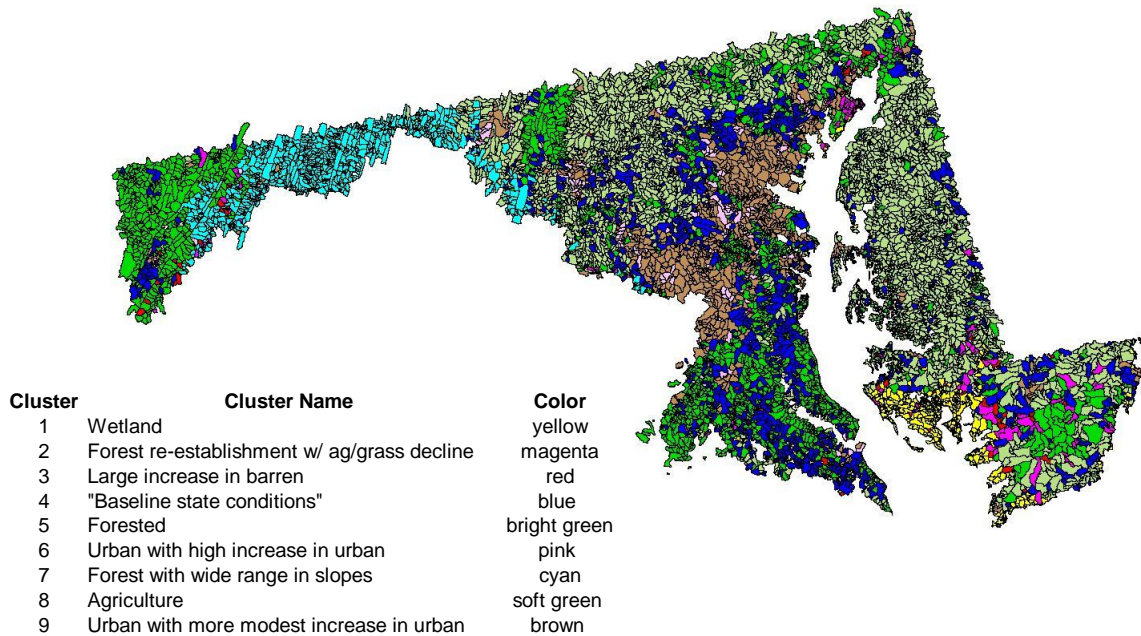


Figure A-1. Landscape similarity in Maryland.

The nine cluster groups can be described as follows: Cluster 1 watersheds are dominated by wetlands and concentrated in the southwest corner of the Delmarva Peninsula. Cluster 2 watersheds are characterized by forest re-growth mainly at the expense of agriculture. Cluster 3 watersheds are characterized by large increases in barren land. They are mainly scattered around the margins of the Chesapeake Bay with another concentration in the westernmost portion of the panhandle. Cluster 4 is perhaps best labeled as “baseline state condition,” since all cluster means are close to the average. Cluster 4 watersheds are scattered throughout the State. Cluster 5 and 7 watersheds are dominated by forest with the main difference being that cluster 7 watersheds have a broader range of slopes. Clusters 6 and 9 are dominated by urban land use, with cluster 6 having a much higher rate of urban increase. Cluster 8 watersheds are dominated by agriculture.

Representation of watershed heterogeneity is assessed by determining if the distribution of sample stations within cluster groups is proportional to the distribution of stream length within cluster groups. A Percent Similarity Index (PSI), also called the Renkonen Index (Krebs 1989), is calculated using proportions of 1st through 4th order streams within

clusters and proportions of monitoring stations within clusters. Despite the simplicity of this measure, it is a robust quantitative similarity coefficient and is commonly used in ecological research when comparing communities using species proportions. The PSI ranges from 0% (no similarity) to 100% (complete similarity). The index is calculated as

$$PSI = \sum_{i=1}^S \text{minimum}(p_i^{streams}, p_i^{stations})$$

where $p_i^{streams}$ is the percentage of 1st – 4th order streams in cluster i
 $p_i^{stations}$ is the percentage of monitoring stations in cluster i
 i is a cluster type
 S is the number of cluster types occurring in a watershed (sum of proportions must equal 100% within a watershed)

A plot of the similarity between the watershed landscape clusters and the number of MBSS round 1 and round 2 monitoring sites in an 8-digit watershed is presented in Figure A-2. It is evident that a greater number of sites results in a higher watershed Percent Similarity Index. Also, Figure A-2 illustrates that PSI has a large range for watersheds with less than ten sites but begins to reach an average of about 85% approximately when the number of sites is greater than eight.

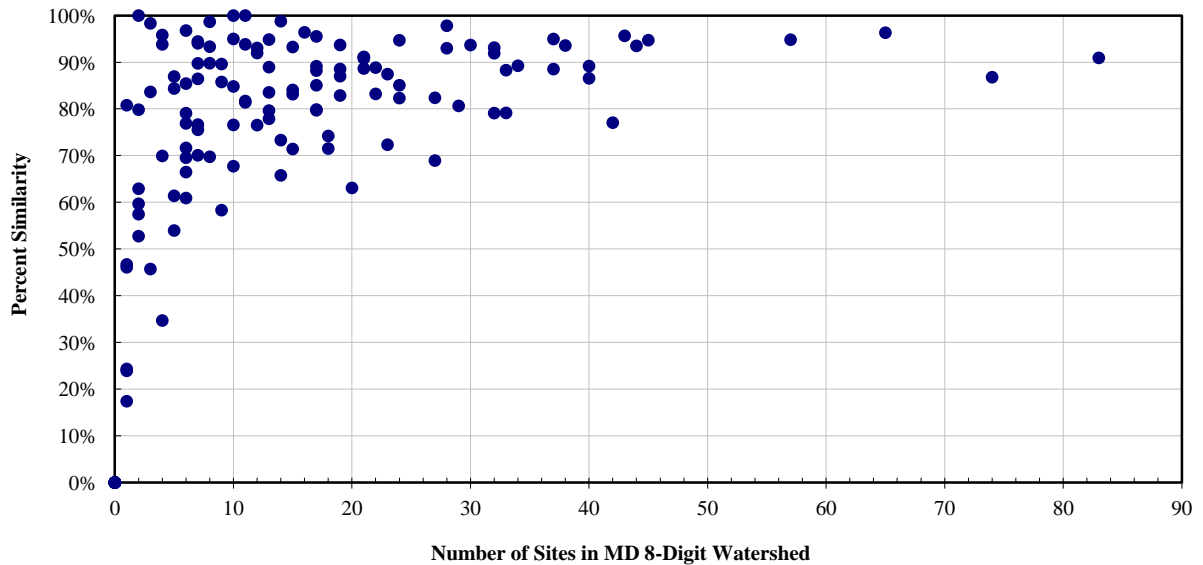


Figure A-2. Watershed Percent similarity index vs. number of sites in a Maryland 8-digit watershed.

Appendix B. Alternate Weighting Procedure for Assessing State and Non-State Data

To enable better understanding of the weighting procedure used for incorporating non-state biological data into the 8-digit watershed assessments (Substep 2c), Maryland provides the following alternate weighting equation. Please note that watershed results produced by this equation are identical to those yielded by the equation in the main portion of this methodology document (specifically Section IV.B.). The strength of this equation is that it lends itself to doing the calculations by hand whereas the previously mentioned equation is more suited to automated calculation by statistical software programming.

$$\frac{D_w}{T_w} = \frac{D_s + D_c W}{T_s + T_c W}$$

where

- D_w = Weighted number of sites with degraded B-IBI scores in a watershed
- T_w = Weighted total number of sites with B-IBI scores in a watershed
- D_s = Number of sites with degraded B-IBI scores from State MBSS data in a watershed
- T_s = Total number of sites with B-IBI scores from State MBSS data in a watershed
- D_c = Number of sites with degraded B-IBI scores from county data in a watershed
- T_c = Total number of sites with B-IBI scores from county data in a watershed
- W = Weight: proportion of a watershed's stream miles falling within county borders

This equation can also be adapted for watersheds that are split by county borders and sampled by more than one county. In those cases, the equation would be modified to include the additional county's failing scores, total scores and weights. An example is provided below for illustration.

$$\frac{D_w}{T_w} = \frac{D_s + D_{c1} W_{c1} + D_{c2} W_{c2} \dots}{T_s + T_{c1} W_{c1} + D_{c2} W_{c2} \dots}$$

where the altered terms

- D_{c1} = Number of sites with degraded B-IBI scores from one county in the watershed
- T_{c1} = Total number of sites with B-IBI scores from that same county in the watershed
- W_{c1} = Weight: proportion of a watershed's stream miles within that one county's borders

D_{c2} = Number of sites with degraded B-IBI scores from the second county in the watershed

T_{c2} = Total number of sites with B-IBI scores from the second county in the watershed

W_{c2} = Weight: proportion of a watershed's stream miles within that second county's borders