

APPENDIX D

METHODOLOGY FOR DETERMINING BIOLOGICAL CONDITION

Determining biological condition involves calculating the MMI for the appropriate site class and comparing the MMI score to a threshold. While this application is relatively straightforward, the collection, preservation and enumeration of a macroinvertebrate sample and the prediction of the biological site class from which that sample was collected is a meticulous process.

The process of using macroinvertebrate data in the application of the MMI first begins with collecting a representative sample from a single or multi-habitat as a means to standardize assessments among waterbodies having similar of habitats. Field sampling is typically followed by preservation, transporting to a laboratory for identification and enumeration analysis, returning data spreadsheets, resolving taxonomic inconsistencies, and lastly electronically uploading the data to a biological database capable of running the MMI.

The process of predicting site class involves extracting three environmental variables (elevation, stream slope and Level III or IV Ecoregion) from maps or ArcMap GIS feature layers for each site. The values are then evaluated against a set of pre-defined classification rules, which determines the site class and the appropriate index to calculate a score. The following sections explain the step-wise procedures necessary to compute a site class and a MMI score from which to assess biological condition.

A. Macroinvertebrate Data Collection

Aquatic benthic macroinvertebrates are animals without backbones that live on submerged rocks, logs, sediment, debris and aquatic plants during some period in their life. They are an important part of the food chain, especially as a food source for fish. The primary advantage of using macroinvertebrates to monitor water quality is that they cannot move around much, so they are less able to evade the deleterious effects of pollutants that diminish water quality. The abundance and species composition of macroinvertebrates can provide water quality scientists with reliable and valuable information on stream and river water quality.

Macroinvertebrate samples are primarily collected using a semi-quantitative method that varies depending on the type of substrate. This sampling method targets riffle and run habitats in rocky bottom streams. These habitats are characteristic of high to intermediate elevation streams in Colorado. However, large portions of eastern Colorado and several regions abutting Utah have streams with sandy or silty substrates. In these low gradient streams a multi-habitat approach is taken. This method targets an abundance of representative habitat types, such as snags, submerged macrophytes and vegetated banks.

The equipment used to collect macroinvertebrate samples, regardless of habitat or stream type, is a modified kick net (or dip net), which includes a long handle, an 18" x 9" rectangular frame and a 500 to 600 μm mesh net that measures no less than 36" long (**Figure 1**).

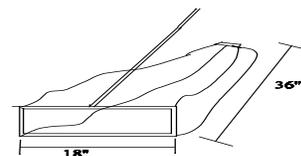


Figure 1. Modified Kick Net

In rocky bottom streams, a riffle or run is selected for sampling. Sampling begins at the downstream portion of the sampling reach and proceeds in an upstream direction. A “kick” is accomplished by positioning the net frame flush against the stream substrate and disturbing one square meter of bottom substrate upstream of the net for a period of one minute. The heel or toe of the samplers’ boot dislodges the upper layer of cobble and organisms are washed into the net. Sampling in low gradient streams, lacking riffle/run habitats, involves systematically disturbing multiple habitat types that result in sampling one square meter of mixed habitats for a period of one minute.

The entire content of the kick net is deposited into a one liter polyurethane jar, which is labeled, 95% ethyl alcohol (EtOH) or ethanol preservative is added and the jar is sealed. If excessive detritus or material prohibits sufficient preservation then the sampler will either split the sample into multiple jars or try to remove some larger rocks and organic matter/litter from the sample to leave enough space to add ethanol preservative.

These methods and equipment have been utilized by Division from 1996 through 2009. Further information related to the Division’s Standard Operating Procedure for the collection of benthic macroinvertebrates, including more details germane to sample processing and preservation may be found in Appendix B of Water Quality Control Commission (“WQCC”) Policy Statement 2010-1.

B. Laboratory Processing and Identification

Preserved samples are delivered to a qualified laboratory, which performs benthic macroinvertebrate sample analysis including taxonomic identification, enumeration and preparation of project reference collections. Taxonomic identification and abundance results are reported to the Division. Further information related to the Standard Operating Procedures for laboratory processing and identification may be found in Appendix C of WQCC Policy Statement 2010-1.

After the taxonomic identifications are completed, the data is transferred from the benchsheets into an Excel spreadsheet that is structured specifically for uploading data directly into Colorado’s Ecological Data Application System (“EDAS”). These spreadsheets are known as “Benthic Import Sheets”. After entry is completed, the data sets are printed out and proofed against the bench sheets in order to ensure accurate data entry. Spreadsheets are checked so that all spelling and calculations are correct.

C. Data Management

Electronic copies and printed versions of the Benthic Import Sheets are submitted to the Division. The Benthic Import Sheets are checked against the authenticated final identifications in EDAS to identify potential taxonomic nomenclature inconsistencies and the subsequent actions necessary to remedy those inconsistencies (see Table 1). The authenticated final identifications are those listed under the “FinalID” column in the Benthics Master Taxa table.

Table 1. Common Taxonomic Nomenclature Inconsistencies

Inconsistencies	Action
Taxonomic spelling errors	Check correct spelling in EDAS and fix error on Benthic Import Sheets
Taxonomic suffixes (such as “species”, “adults” and “groups”)	Check correct FinalID in EDAS and remove suffixes on Benthic Import Sheets
Taxonomic authorities	Remove authorities on Benthic Import Sheets
New taxa are identified	Verify taxa and add to EDAS’ Benthics Master Taxa table

Final identifications in EDAS are streamlined so nomenclature is based upon the taxonomic hierarchy system, with few exceptions. Thus, final identifications do not include suffixes nor do they include taxonomic references to “authorities”.

New taxa shall be verified on the Integrated Taxonomic Information System (“ITIS”) website¹. This website shall be considered an authoritative source on taxonomic information. In some instances, an invalid taxonomic name is reported by the laboratory, but the valid taxonomic name exists in EDAS. In these instances, it is appropriate to change the reported taxonomic name to the valid EDAS equivalent on the Benthic Import Sheets.

New taxa are input into EDAS’ Benthics Master Taxa database table. When new taxa are added to this table, ancillary taxonomic information related to that new taxon must be added as well. The generation of metrics and index scores is dependent upon the most updated information available for each new taxon. For instance, many species richness and composition metrics are dependent upon a complete taxonomic hierarchy. Also, many metrics are dependent upon the species tolerance value, functional feeding group, and habit preference type.

Taxonomic hierarchies can be verified on ITIS and added to the Benthics Master Taxa table.

Regional tolerance values, functional feeding groups and habit types shall be verified using the following reliable sources:

- Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. United States Environmental Protection Agency, Office of Water, Washington, D.C.
- Merritt, R. W., and K. W. Cummins (eds.) 1984. An introduction to the aquatic insects of North America. Second edition. Kendall/Hunt, Dubuque, Iowa.

Additional literature sources may include:

¹ <http://www.itis.gov/>

- Bode, R. W., M.A. Novak, L.A. Abele. 1996. Quality assurance work plan for biological stream monitoring in New York State. NYS Department of Environmental Protection; Division of Water; Bureau of Monitoring and Assessment; Stream Biomonitoring Unit; Albany, NY.

Once verified, regional tolerance values, functional feeding groups and habit types, along with their respective sources shall be added to the Benthics Master Taxa table.

At this point, the reported taxonomic names on the Benthic Import Sheets will be in full synchronization with the “FinalID” column in the Benthics Master Taxa table. The Benthic Import Sheets are now ready for importing into EDAS.

D. Generating Predictor Variables

A prerequisite to importing the Benthic Import Sheets into EDAS is the “Predictor Variable Import Sheet”. The function of this spreadsheet is to populate EDAS with a nominal amount of new station information from which to run the multi-metric indices (“MMI”). This spreadsheet includes general site information, such as the station ID, waterbody name, station description and latitude and longitude geographic coordinates in decimal degrees (i.e. 37.3923 and -104.5687). It also includes the set of MMI predictor variables that will determine both the station’s biotype and the MMI to be used in calculating an index score.

The general site information is gathered while in the field and recorded to field sheets. Station coordinates are determined in the field with a hand-held GPS unit and verified using desktop tools such as Google Earth or Google Maps to ensure an accurate location. This information is appended to the Predictor Variable Import Sheet.

The verified station coordinates are converted to “point” layers on ArcMap GIS. From these points, the MMI predictor variables are extracted from feature or raster layers. The MMI predictor variables and brief descriptions are listed below:

- **Elevation** - a National Elevation Dataset raster layer from the U.S. Geological Survey. The unit is meters or a resolution of 1 arc-second (about 30 meters).
- **Slope** - a feature stream layer developed by Utah State University. Slope was calculated by identifying segments of channel that were between upstream and downstream tributaries. The range of elevation values, extracted from a 30-meter Digital Elevation Model, was divided by the length of the segment to obtain the slope of the segment. The slope value was then applied to the entire segment.
- **Level IV Ecological Subregions** - a feature layer that was developed through a collaborative effort between the U.S. Environmental Protection Agency, National Health and Environmental Effects Laboratory, U.S. Forest Service, Natural Resources Conservation Service and various other state and federal agencies. The subregions

denote areas of general similarity in ecosystems and in the type, quality, and quantity of environmental resources.

The MMI predictor variables are appended to the Predictor Variable Import Sheet. It is not necessary to include any of the River Invertebrate Prediction and Classification System (“RIVPACS”) Observed over Expected (“O/E”) predictor variables to the Predictor Variable Import Sheet, since they are not relevant to the generation of MMI scores. The Predictor Variable Import Sheet will upload correctly without the O/E predictor variables.

The Predictor Variable Import Sheet can be uploaded into EDAS by selecting the “Multimetric and Predictive Models” button from the EDAS home page and opening the “Model Analysis” form. The user clicks the “Import/Update Predictor Variables for MMI and Predictive Model” box. A new window opens. The user navigates to the Predictor Variable Import Sheet location and selects the file to upload. After uploading, check the “TTImportPredictors” table to confirm that all the data was imported into EDAS. The “TTImportPredictors” table can be accessed from the home page of EDAS by clicking the “Access Database Structure” button. If the “TTImportPredictors” table is empty the import was successful. This table temporarily stores stations and predictor variable data that EDAS is not programmed to recognize and therefore did not properly upload to their assigned tables. Errors in the import process are often due to incorrect spreadsheet formatting. For instance, the date format is something other than “short date” or a Station ID is formatted as a number rather than generally unformatted. These errors can be remedied within the Predictor Variable Import Sheet. First, clear the contents of the “TTImportPredictors” table. Then re-attempt to import the Predictor Variable Import Sheet as described above. Repeat until the upload results in an empty “TTImportPredictors” table.

If the Predictor Variable Import Sheet import was successful, proceed to upload the Benthic Import Sheet through the Model Analysis form. The Model Analysis form can be accessed by selecting the “Multimetric and Predictive Models” button from the home page of EDAS. The user clicks the “Import Benthic Data” box. A new window opens and the user selects the file to upload. After the upload is completed, check the “TTImport” table to check for taxonomic identifications that were not imported into EDAS. The “TTImport” table can be accessed from the home page of EDAS by clicking the “Access Database Structure” button. If the “TTImport” table is empty the import was successful. If not, re-check those taxonomic identifications and apply these changes to the Benthic Import Sheet. Clear the contents of the “TTImport” table. It is not necessary to remove the taxonomic data that did successfully imported on the first attempt. Re-attempt to import the entire Benthic Import Sheet. Repeat until the upload results in an empty “TTImport” table.

EDAS is programmed to allow transfer of data from the two aforementioned import sheets into a series of relational database tables. Station metadata and predictor variables are stored in the “Stations” table in EDAS. EDAS automatically predicts a “biotype” for each new station based upon a sequence of classification rules using the three predictor variables. The predicted “biotype” is stored within the “Stations” table and becomes the prompt for which MMI will be used to generate an index score.

E. Automatically Generating Index Scores

EDAS allows users to automatically generate MMI index scores.

From EDAS' home page, click on the "Multimetric and Predictive Models" box to open the "Model Analysis" form. On the Model Analysis form any number of stations can be selected upon which to run the MMI tool. If new macroinvertebrate samples were recently uploaded into EDAS, it is necessary to click the "Verify 300 organism subsample" box. This runs a Visual Basic macro that subsamples any sample count greater than 360 individuals down to a 300 count. Samples with counts less than 360 individuals remain as reported by the laboratory. The 300 count subsample is stored in a table and cannot be sub-sampled again. All metric calculations and MMI scores are based upon this one and only subsample.

Once sub-sampling is completed, click the "Calculate MMIs" box to open the "Multimetric Index Model (MMI) Calculation and Reporting" form. The user is prompted to select the output of the MMI calculations. Selecting "View MMI results report" opens a reporting form within EDAS. Selecting "Export MMI results as 'Excel'" opens a save file window and allows the user to save the output spreadsheet to a file location of their choice.

Depending on the number of stations selected on the Model Analysis form, the MMI calculation can take anywhere between 30 seconds and 3 minutes. When the calculations are finished, the Excel spreadsheet will open and MMI scores can be viewed immediately.

F. Manually Generating Index Scores

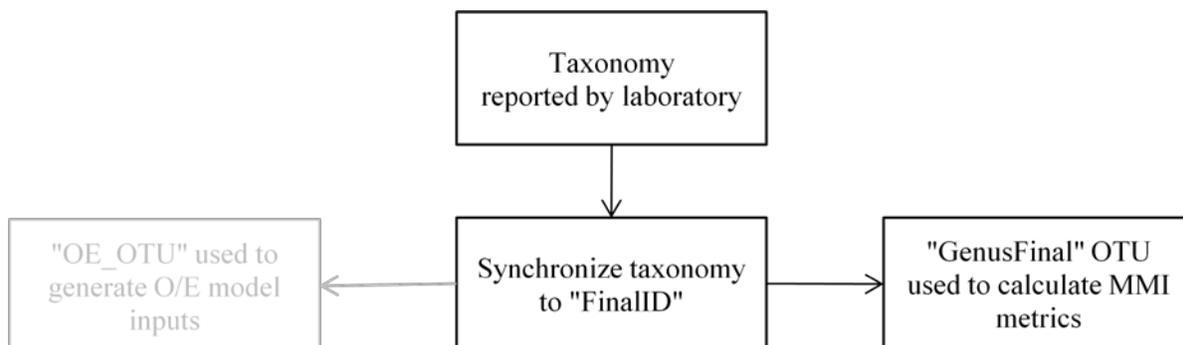
Manually deriving MMI scores, independent of EDAS, is best handled through formulaic calculations in an Excel spreadsheet. However, there are many steps that must occur between the receipt of laboratory reported taxonomic data and setting up the MMI calculation spreadsheet. These steps are organized in sequence and explained below:

Taxonomic Resolution

Biological assessment tools require that a consistent taxonomy be applied across all samples because ambiguous taxa amongst samples can be confusing. This issue was first encountered during the first version of the O/E model completed in 2005. To counteract this problem, consistent taxonomy was identified and referred to as operational taxonomic units or OTU's (Hawkins, 2009). A comparable taxonomic structure was developed for the MMI. The MMI equivalent to the O/E OTU was given the name of "GenusFinal". Thus, the MMI utilizes high-resolution taxonomy that includes identifying midges to genus and other taxa treatments that improve the indices' sensitivity to stress.

Accordingly, the Division strongly encourages that any identified and enumerated data first be synchronized with the consistent taxonomy provided in the "FinalID" column in the Benthics Master Taxa table in EDAS. Each taxon with a "FinalID" will have an equivalent OTU designation associated with each bioassessment tool. Figure 2 illustrates this intention.

Figure 2. Organized Taxonomic Unit Assignments for each Bioassessment Tool



Sample Standardization

Because the MMI is based on estimates of the number of taxa present in a sample, MMI scores can be affected by any modification to sampling technique, such as increasing or decreasing sampling area or timed effort. For instance, increasing sampling area or time generally results in increased individual counts, which in turn increases richness. Conversely, decreasing sampling area or time may result in low individual counts, which in turn under-estimates richness. For this reason, samples shall be standardized on area and count.

The Division recommends that raw sample counts greater than 360 individuals be randomly resampled by computer to a fixed 300 count. A free executable program (Matrify.exe) is available for download at Utah State University's The Western Center for Monitoring and Assessment of Freshwater Ecosystems. Additionally, a Visual Basic macro can be scripted to perform this function. Raw sample counts less than 360 individuals need not be resampled and can be used in further analysis. However, raw sample counts less than 150 individuals shall be flagged as not conforming to the data treatments ensconced in the MMI development.

Metric Prerequisites

Taxonomic hierarchy, regional tolerance values, functional feeding designations and habit/behavior assignments are requirements for computing accurate metrics. The best method to compute metrics would be to set up another Excel spreadsheet. Include columns for taxonomic identifications (harmonized beforehand), individual counts (subsamped beforehand) and columns for the following information:

Taxonomic Hierarchy

Several richness and composition metrics require information pertaining to an organism's taxonomic hierarchy. This will become evident when metrics are calculated further in this process. For instance, in Biotype 2 one of the composition metrics is % Ephemeroptera. For those organisms with an OTU designation of sub-family (rare), tribe or genus, the taxonomic hierarchy is vital to those affected richness and composition

metrics. The taxonomic hierarchy shall include phylum, class, order, family, tribe, genus and species.

Regional Tolerance Values

Several tolerance metrics are components of all three MMI's. It will be necessary to research information pertaining to each organism's regional tolerance value. Tolerance values do not need to be specifically from the Southern Rocky Mountain region. It is acceptable to use tolerance values from other regions in the United States as long as they are cited from valid sources. Valid tolerance values can be found in the literature sources cited under Subsection C.

Functional Feeding Group Designations

The predator/shredder taxa metric is a component of all three MMI's. Therefore, it will be necessary to research information pertaining to each organism's FFG. Functional feeding group designations can be found in the literature sources cited under Subsection C. Functional feeding designations include parasite, predator, omnivore, scraper, gatherer/collector, filter/collector, shredder, and piercer.

Habit/Behavior Assignments

Several habit metrics are components of all three MMI's. It will be necessary to research information pertaining to each organism's habit preferences. Habit designations can be found in the literature sources cited under Subsection C. Habit and behavior designations include clinger, climber, sprawler, burrower, swimmer, diver, and skater.

Metric Calculations

Computing individual metrics will involve setting up Excel spreadsheet formulas or equations whereby the breadth of taxonomic information is transformed into measureable statistics. Guidance on building these formulae will not be provided within this document.

MMI Calculation

The same Excel spreadsheet can be used to compute the MMI scores. Computing the MMI score involves applying a specific scoring formula to each metric. Scoring formulas for all metrics inclusive to each MMI are provided on pages 20-22 of the MMI report located in Appendix A of WQCC Policy Statement 2010-1. This results in an individual metric score. The sum of these individual metric scores is divided by the number of metrics that comprise that particular MMI. For instance, if MMI #1 is comprised of metrics A, B, C, D and E, then the scores for metrics A, B, C, D, and E are summed and divided by five. The output is the MMI score. The range of MMI scores is 0-100.

Of note, individual metric scores greater than 100 or less than zero will occur. It is necessary to modify those occurrences by adjusting scores greater than 100 down to 100 and scores less than

zero up to zero. This will have to be done manually within the spreadsheet cell. If this data treatment is ignored, there is a chance that an MMI score can exceed 100 or fall below zero.