Guide To The Maryland Biological Stream Survey Benthic Macroinvertebrate and Fish Indices of Biotic Integrity

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Department of Natural Resources
Resource Assessment Service

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Published Guidance

The Maryland Biological Stream Survey (MBSS) was developed with a set of protocols that could be universally applied throughout Maryland to monitor the conditions of wadeable streams. The associated methodologies were developed to be easy-to-learn and easy-to-apply. The MBSS protocols, or components thereof, have been used by DNR, MDE, county-level environmental agencies, academics, citizen-scientists, and others. To ensure that the protocols are applied similarly by the various practitioners and that resulting data are comparable, guidance is provided through written documentation, annual training seminars, and certification. These guidance materials are updated as needed. It is incumbent upon all practitioners of MBSS Protocols to read and understand the written guidance, and to pursue training and certification as necessary.

Guidance for Field Protocols

Guidance for Field Protocols is laid out in the *The Maryland Biological Stream Survey: Field Sampling Manual* (Harbold et al. 2024). This manual has been updated every several years to reflect slight changes in the variables that DNR includes with MBSS sampling. The manual defines and explains all the variables that are investigated as part of the MBSS field sampling protocols and thoroughly describes the associated methods. The manual defines the spring (March 1 - April 30) and summer (June 1- September 30) sampling index periods and provides guidance for each of the different variables. It describes the standard MBSS protocols for assessing physical, chemical, thermal, and biological variables. It also conveys DNR's expertise on safety, planning, quality assurance, and datasheet design.

Guidance for Benthic Laboratory Procedures and Identification

Guidance for subsampling and laboratory protocols is given in *Maryland Biological Stream Survey Laboratory Methods for Benthic Macroinvertebrate Processing and Taxonomy* (Resource Assessment Service 2024). This manual details the standard MBSS methods for preparing a sample, subsampling from a standard-sized MBSS gridded tray, and ensuring a representative subsample consisting of a target number of organisms. It provides guidance for randomly selecting, appropriately counting, and consistently recording the numbers of grids picked. It defines the EXCLUDE designation and describes how it is appropriately applied and recorded. It also provides general guidance on identification procedures and quality assurance. Taxonomists should be aware of Appendix G in the MBSS Laboratory Methods manual (Resource Assessment Service 2024), which lists organisms that should not be picked or counted for the subsample and specifies target hierarchical-levels for organism identification (if not genus).

Guidance for benthic macroinvertebrate identification and a list of recommended taxonomic keys are given in the MBSS Laboratory Methods manual (Resource Assessment Service 2024). Examples of taxonomic keys used by DNR/MBSS staff include *An Introduction to the Aquatic Insects of North America* (Merritt et al. 2019), *Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina: A Guide to the Taxonomy of the Midges of the Southeastern United States, Including Florida* (Epler 2001), and other standard published references. The most recent versions of keys should be referenced, as they may be updated or revised to reflect changes in taxonomy.

The MBSS Benthic Master Taxa List should also be referenced to aid in identification of benthic macroinvertebrates. This is a list of benthic macroinvertebrates that have been verified to exist within non-tidal streams in Maryland. Organisms on the list are represented at the genus-level or less precise classification levels. This list was developed by DNR, is updated on an annual basis, and the most updated version is available through the MDE TMDL Stormwater Implementation Resources - MS4 Monitoring Guidance website (https://mde.maryland.gov/programs/water/TMDL/DataCenter/Pages/TMDLStormwaterImplementation.aspx). New records are added if novel taxa (organisms not previously listed on the Benthic Master Taxa List) are sampled by DNR or reported by an outside entity and validated by DNR. The Master Taxa List may be treated as a list of benthic macroinvertebrates known to be present in Maryland. However, we acknowledge it is never complete and novel taxa may sometimes be found and added to the list. Additions to the Master Taxa List incorporate phylogenetic, tolerance value, and trait information (e.g., functional feeding group, habit) associated with each added taxon.

Guidance for Fish Identification

Guidance for fish identification is given in the *Key to Maryland Fishes* (Kazyak and Raesly 2009). This guide is specific to Maryland, and presents a dichotomous key that includes all of the fishes that are known to have non-tidal resident populations within the state. Other useful guides to fish identification (e.g., Jenkins and Burkhead 1994, Page and Burr 2011, Stauffer et al. 2016) may include descriptions of many of the same fishes that are found in Maryland, and they can be used to confirm distinguishing characteristics and to gain a better understanding of the species. However, any dichotomous keys that are not specifically designed for the unique composition of species known to occur in Maryland should not be used.

Guidance for IBI Calculations

Comprehensive guidance for MBSS Index of Biotic Integrity (IBI) calculations is currently somewhat lacking. The present document aims to address this, by providing detailed instructions for calculating the MBSS Benthic Index of Biotic Integrity (BIBI) and MBSS Fish Index of Biotic Integrity (FIBI), addressing special considerations that may complicate calculations, and presenting appropriate metric thresholds for the indices.

The MBSS Indices of Biotic Integrity for benthic macroinvertebrate and fish sampling data are presented in *New Biological Indicators to Better Assess the Condition of Maryland Streams* (Southerland et al. 2005) and, more succinctly, in *Improving Biological Indicators to Better Assess the Condition of Streams* (Southerland et al. 2007). These documents describe the MBSS IBIs, the processes that were used for metric selection and associated threshold values, and the underpinning knowledge and assumptions. They also provide an overview of how to apply and calculate the MBSS BIBI and MBSS FIBI. The MBSS IBIs described in these documents represent the most recent updates and the currently utilized methodology. However, the aforementioned documents lack certain details and include inconsistencies for some metric thresholds; the present document addresses these issues.

Other documents that describe the background of the MBSS Indices of Biotic Integrity may refer to earlier versions of the MBSS IBIs and should not be used to guide current IBI calculations. These include: *Maryland Biological Stream Survey: Development of a Fish Index of Biotic Integrity* (Roth et al. 1998), and *Refinement and Validation of a Fish Index of Biotic Integrity for Maryland Streams* (Roth et al. 2000).

A biotic index based on family-level identification of Maryland benthic macroinvertebrates was presented in *Development of a Benthic Index of Biotic Integrity for Maryland Streams* (Stribling et al. 1998). The family-level index has not been changed or updated since it was described. It was primarily used to summarize data collected for the Maryland Stream Waders citizen-science program. The present document does not further address the processes for calculating the family-level benthic index.

Training and Certification

Training

Training sessions are held by DNR for MBSS protocols. Training is available for Spring and Summer MBSS Field Protocols, Benthic Macroinvertebrate Sampling, Physical Habitat Assessment, and Fish Sampling. Training for fish identification is also offered annually, in conjunction with summer MBSS field protocol training. Training for laboratory processing and subsampling is also offered, and may be scheduled. All practitioners of MBSS protocols are encouraged to attend relevant training sessions.

Certification Requirements

Certification is offered to document consistency with certain aspects of the state-standard MBSS field sampling and benthic macroinvertebrate laboratory processing protocols. In order to ensure and document adherence to standard MBSS protocols, the following certifications are offered by DNR: (1) Benthic Macroinvertebrate Sample Collection, (2) Physical Habitat Assessment, (3) Laboratory Benthic Sample Processing and Subsampling, (4) Fish Taxonomy, and (5) Fish Sampling Crew Leader. To comply with each MBSS certification process, specific requirements must be met. These requirements, along with the duration of each certification, are described on the MBSS Training & Certification website (https://dnr.maryland.gov/streams/Pages/mbsstraining.aspx).

DNR does not provide certification in benthic macroinvertebrate taxonomy, but instead recognizes certifications issued by the Society for Freshwater Science (SFS). To comply with MBSS standards, the *individual taxonomist* identifying the macroinvertebrates must be certified by SFS in Ephemeroptera, Plecoptera, Trichoptera East (or EPT East Images) and North America Chironomidae (or North America Chironomidae Images). Multiple taxonomists may be involved in identification, but *each* should be appropriately certified for the taxonomic groups that they identify. The SFS certifications are considered valid for five years.

MBSS Master Taxa Lists

Benthic Master Taxa List

The MBSS Benthic Master Taxa List is a genus-level list of benthic macroinvertebrates that have been verified to exist within non-tidal streams in Maryland, accompanied by phylogenetic, tolerance value, and trait information. The Benthic Master Taxa List is primarily designed to provide information that is necessary for calculating MBSS BIBIs. It is also useful for guiding taxonomic identification of benthic macroinvertebrates observed in Maryland. Consistency and comparability of BIBI scores depends on referencing this standardized list.

The MBSS Benthic Master Taxa List was originally developed in the beginning of Round 1 MBSS Sampling (1994-1995), at which point it contained only phylogenetic designations. It was later updated with trophic, habit, and tolerance value (0 - 10) information (presented in Stribling et al. 1998). Initially, trophic information was gathered from Merritt and Cummins (1996; Mid-Atlantic) and U.S. EPA (1999; Mid-Atlantic). Habit information was gathered from Merritt and Cummins (1996; Mid-Atlantic) and Merritt et al. (1996). Tolerance values were gleaned from regional lists (Mid-Atlantic) in U.S. EPA (1999) and are presented in Stribling et al. 1998. Bressler et al. (2004) generated revised tolerance values for taxa in Maryland based upon their response to urban and agricultural stressors, which improved the ability of the Percent Intolerant Taxa metric within BIBIs to detect impairment. Tetra Tech revised the MBSS Benthic Master Taxa List in 2009, to reflect current taxonomic designations, the revised tolerance values (Bressler et al. 2004), and the best available scientific knowledge regarding functional feeding groups and habit. Subsequent updates to the Benthic Master Taxa List have been based off of the 2009 revision, and have only involved the additions of novel taxa, as described in this document. It is reviewed by DNR after all MBSS samples from the most recent sampling year have been processed and identified, and it is updated annually.

Within the MBSS Benthic Master Taxa List, each entry consists of several standard pieces of information. The **TAXON** column is the primary identifier for the organism. Taxonomic designations are presented in separate columns, and the primary identifier corresponds to the lowest-identifiable-level that is indicated; **PHYLUM** is specified for all entries, **CLASS** is specified for all entries except Nematoda and Mollusca, and **ORDER**, **FAMILY**, and **GENUS** is specified for all other entries to the lowest-identifiable taxonomic-level. The **TRIBE** field is used to calculate Percent Tanytarsini (a tribe of midges) for the Highland benthic IBI, but this field is often left blank within the Benthic Master Taxa List. Certain more specific classifications may be presented in the **OTHER_TAXA** field (subphylum, superfamily, subfamily). The functional feeding group (**FFG**), if known, is specified as only one of the following: Collector, Filterer, Piercer, Predator, Scraper, or Shredder. The **Habit**, if known, is specified as one or more of the following: burrowers (bu), climbers (cb), clingers (cn), sk (skaters), sp (sprawlers), sw (swimmers), divers (dv). If an organism conforms to more than one habit, the corresponding habits are listed in no particular order. If **FFG** or **Habit** is unknown for a taxa, the corresponding entry is left blank. **FINALTOLVAL07** references tolerance to stressors, and values less than or equal to 3.0 indicate intolerant taxa. The family level tolerance values (**FAM_TV**) are also included in the Benthic Master Taxa List.

Fish Master Taxa List

The MBSS Fish Master Taxa List is a species-level list of fishes that may be encountered in non-tidal systems in Maryland, accompanied by information about several relevant traits. The Fish Master Taxa List is primarily designed to assist in calculating MBSS FIBIs. Consistency and comparability of FIBI scores depends on referencing this standardized list.

Within the Fish Master Taxa List, each entry consists of several standard pieces of information. **SPECIES** is the primary identifier for the fish and is usually the common species name (except for those records corresponding to genus- or family-level identification). The fields **FAM, GENUS,** and **SP_SCI** indicate taxonomic classification (family, genus, and species, respectively). **APPROX_ID** is used to indicate whether records are at the target species-level. **IN_KEY** indicates whether taxon is in the Key to the Freshwater Fishes of Maryland (Kazyak and Raesly 2009). **TYPE** indicates a general taxonomic grouping that is relevant to several FIBI metrics; fish may be assigned to one of the following: DARTER, LAMPREY, MADTOM, OTHRCYPR, SCULPIN, SHINER, SUCKER, SUNFISH, NOTYPE, [blank]. The tolerance value assigned to species is indicated as **PTOLR** (T = Tolerant, I = Intolerant, NOTYPE/[blank] = Not categorized); this rating of fish tolerance to anthropogenic stress was derived using MBSS data collected prior to 1998 and an analysis that ranked occurrences of individual species at degraded or minimally disturbed sites (Roth et al. 1998). All of the fishes listed in the table are assigned to one, and only one, trophic habit, as determined from literature, and indicated as **TROPHIC**; trophic habit of Maryland fishes are: FF=Filter Feeder, GE=Generalist, IS=Insectivore, IV=Invertivore, OM=Omnivore, TP=Top Predator, AL=Algivore, HE=Herbivore, NOTYPE/[blank]=Not categorized. The **SILT** field indicates whether a Maryland fish species is categorized as a lithophilic spawner, as determined from literature (Yes=lithophilic spawner). **NATIVE** is a column included in the Master Taxa List that is not relevant to the FIBI but still includes useful information related to species nativity to Maryland and is specified as one of the following: native (N), introduced statewide (I), introduced to the Chesapeake Bay watersheds (IC), introduced to Youghiogheny watershed (IY), no type (NOTYPE). . **PIRHALLA**, another column included in the Master Taxa List, is a fish habitat tolerance index developed by Pirhalla (2004) but not relevant to the FIBI.

Master Taxa List Updates

The master taxa lists for both fish and benthic macroinvertebrates are reviewed annually and compared to the taxa encountered by MBSS during the preceding year of sampling by DNR, as well as verified taxa submitted by other agencies. If necessary, taxa lists are updated annually to ensure that all sampled organisms are properly included in the IBI calculations.

Because these master taxa lists are based on extensive sampling throughout the state, the majority of organisms that may be encountered in samples from Maryland are already included. Despite this, novel benthic macroinvertebrates are occasionally encountered and the Benthic Master Taxa List is updated accordingly. The Benthic Master Taxa List may also need to be updated to reflect changes in standard taxonomy (lumping and splitting of taxonomic groups; taxonomic name changes), as laboratory protocols specify that the most up-to-date taxonomy should be used. When a new taxon is added due to a name change, taxonomic split, or taxonomic lump, its reason for addition is indicated in the **COMMENT** column.

To provide continuity and compatibility with historical data, records in the Benthic Master Taxa List (since 2009) are never deleted or edited; the list is only appended. After all the benthic macroinvertebrates that were sampled by MBSS during a year are identified, the resulting list of names of sampled organisms is compared to the most recent Benthic Master Taxa List. Subsequently, records for any organisms that are not on that version of the Benthic Master Taxa List are generated and are added to the Benthic Master Taxa List. Additions to the Benthic Master Taxa List incorporate phylogenetic (e.g., order, family, tribe), trait (e.g., functional feeding group, habit), and tolerance information associated with each additional taxon. Phylogenetic information is gathered from the Integrated Taxonomic Information System (itis.gov). Trait information is gathered from *Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers* (Mid-Atlantic; US EPA 1999) and/or *An Introduction to the Aquatic Insects of North America* (Mid-Atlantic; Merritt et al. 2019). Tolerance values for organisms that are not presented in Bressler et al. (2004) are copied from US EPA (1999) and/or Merritt et al. (2019) from Mid-Atlantic columns only, which are calculated or estimated from literature for their responses to organic pollution.

The ichthyofauna in Maryland has been more thoroughly cataloged, so additions of native species to the Fish Master Taxa List are not expected. However, the Fish Master Taxa List may be updated to include novel non-native species. Additionally, the common names and taxonomic information of fishes may be revised to reflect currently accepted classifications.

If an organism (benthic macroinvertebrate or fish) that does not appear on the MBSS Master Taxa list is sampled by a different agency than DNR, and a physical specimen or photograph of the organism is available, it may be submitted for confirmation and consideration for subsequent inclusion in the appropriate MBSS Master Taxa List. Please notify DNR of any novel benthic macroinvertebrates by communicating with Neal Dziepak (neal.dziepak@maryland.gov). Please notify DNR of any novel fishes by communicating with William Harbold (william.harbold@maryland.gov). Upon confirmation of the specimen or photograph, a record for that organism may be included in the appropriate Master Taxa list with the next update.

Data validation

Benthic Macroinvertebrate Data

Appropriate certification of benthic macroinvertebrate sample collectors, subsamplers, and taxonomists and adherence to relevant protocols should be verified. When identification and count data are returned from the laboratory, it is important for the data user to review and validate the data. It is important that data expectations are met, an acceptable sample-size is reported, that data formatting is conducive to subsequent calculations, and that reported benthic macroinvertebrates can be referenced from the Benthic Master Taxa List for their relevant traits. Data users may need to revise the returned data to make it consistent with the MBSS methodology, can work with the taxonomist to correct issues, and should maintain consistent communication with contracted laboratories to ensure quality control.

Data Expectations

For the purposes of calculating a BIBI score, data that are returned from the laboratory should include, at a minimum: target- and non-target-level identifications, associated counts of individuals, and EXCLUDE designations, and these data should be linked to the appropriate sample name. Grid counts should also be reported for data validation purposes. The data user should review the data for these components and assure that they meet expectations.

The target-level for identification of most organisms used in the MBSS BIBI is genus, but some groups of organisms should be identified to a less precise target-level specified in Appendix G in the MBSS Laboratory Methods manual (Resource Assessment Service 2024). Taxonomists should put effort into achieving target-level identifications, but non-target-level identifications are acceptable for organisms that could not be accurately identified due to immaturity, size, or damage. Non-target-level identification is preferable to potential inaccuracy or omission from the dataset.

Each organism's identity should be accompanied by a quantification of the number within the sample, represented as a positive whole number. Each organism should be represented by a single count that encompasses all encountered forms (sizes, instars, etc.) of the organism. Including such differences could erroneously inflate measures of taxonomic richness.

Any organisms that cannot be definitively identified as different from another taxon in the sample (due to immaturity, size, damage, or other complications) should still be recorded and should be indicated using the EXCLUDE field. The EXCLUDE field should be completed for each record as either "TRUE" or "FALSE". EXCLUDE = "TRUE" only when an organism cannot be identified to the target-level and is recorded at the lowest practical taxonomic grouping and the sample includes at least one taxon from the same taxonomic grouping that has been identified to a more precise taxonomic level. If the taxonomist is able to distinguish the organisms that were identified to a less precise taxonomic grouping as different from those identified to a more precise grouping, the taxonomist should mark EXCLUDE = "FALSE" for the taxon left at the less precise grouping. It is important that this designation is provided by the laboratory, because it is reliant on the best professional judgment of the benthic taxonomist, and this field can not be accurately assigned after identification is completed. Data used for calculating a BIBI requires the EXCLUDE field, and the appropriate designation for each record is essential for correctly calculating taxa richness metrics in the BIBI calculations.

The table below is provided for illustrative purposes. It depicts the identities of organisms identified from an MBSS benthic macroinvertebrate sample and provides examples of correctly-applied EXCLUDE designations along with relevant taxonomist comments. For all taxa that were identified to the target-level (genus), EXCLUDE = “FALSE”. For three taxa that could not be identified to the target level and could not be differentiated from other taxa in the sample that were identified to a more precise classification (Plecoptera, Perlodidae, and Orthocladiinae), EXCLUDE = “TRUE”. For one taxon that could not be identified to the target level but could be differentiated from other taxa in the sample (Nemouridae), EXCLUDE = “FALSE”. For one taxon that could not be identified to the target level but the sample did not contain any other taxa identified to a more precise classification (Ephemerellidae), EXCLUDE = “FALSE”.

TAXON	EXCLUDE	Class	Order	Family	Other_Taxa	Genus	TAXONOMIST COMMENT
PLECOPTERA	TRUE	Insecta	Plecoptera				Unable to identify more precisely than order. Likely the same taxon as one of the Stoneflies listed below.
PERLODIDAE	TRUE	Insecta	Plecoptera	Perlodidae			Unable to definitively identify more precisely than family. Could be either Isoperla (documented below) or Clioperla (not documented in sample).
ISOPERLA	FALSE	Insecta	Plecoptera	Perlodidae		Isoperla	Target-level identification
NEMOURIDAE	FALSE	Insecta	Plecoptera	Nemouridae			Unable to definitively identify more precisely than family. Potentially 1-2 different taxa, but definitely not Amphinemura (documented below).
AMPHINEMURA	FALSE	Insecta	Plecoptera	Nemouridae		Amphinemura	Target-level identification
ORTHOCLADIINAE	TRUE	Insecta	Diptera	Chironomidae	Orthocladiinae		Unable to identify more precisely than the subfamily. Could be either Cricotopus or Orthocladius (both documented below).
CRICOTOPUS	FALSE	Insecta	Diptera	Chironomidae	Orthocladiinae	Cricotopus	Target-level identification
ORTHOCLADIUS	FALSE	Insecta	Diptera	Chironomidae	Orthocladiinae	Orthocladius	Target-level identification
EPHEMERELLIDAE	FALSE	Insecta	Ephemeroptera	Ephemerellidae			Unable to identify more precisely than family. The sample does not contain any other organisms in the Family Ephemerellidae.

Benthic laboratories should also report a log of the number of grids that were picked to obtain subsamples. These values should be represented as positive whole numbers ranging 1 - 100. Further guidance on counting the grids, including instructions for samples split among several trays, is given in the MBSS Laboratory Methods manual (Resource Assessment Service 2024). The reported counts of grids that were picked should be used to ascertain the completeness of the sample and general adherence to MBSS laboratory protocols.

Subsample Size

The protocols for MBSS subsampling specify a procedure that should result in a target number of at least 120 organisms and a final subsample size of at least 100 identified organisms, provided that the sample contained at least that many organisms (Resource Assessment Service 2024). The end user should validate that the data reflect this convention by checking that (1) for a reported subsample containing less than 100 organisms, all of the grids composing the sample should have been picked, and (2) for a reported subsample containing many more than 120 organisms, relatively few grids should have been picked. For the user to validate data in this way, the number of grids picked during subsampling must be recorded and reported by the laboratory. Individuals certified in MBSS Laboratory Benthic Sample Processing and Subsampling are made aware of this requirement during the training process.

MBSS protocols do not specify a minimum or maximum number of sampled benthic macroinvertebrates required to calculate a BIBI. However, it is imperative to ascertain that samples with many more or many fewer organisms than the target number were collected and processed in a manner that conformed to MBSS protocols. It is also important to review the results for any scores that are potentially skewed due to numerical inconsistencies, as certain BIBI metrics (i.e., richness metrics) can be influenced by sample size.

If a reported subsample contains less than 100 organisms, and all grids were picked, it can be presumed that it had a low density of organisms. No adjustments should be made to the data, *post hoc*. In general, low-density assemblages reflect an underlying stream condition that is reflected in the BIBI score. However, in some cases, a low sample size could skew the mathematics for the BIBI and result in an errant score. Data and resulting BIBI scores from such samples should be reviewed, and any misleading results should be flagged. If a reported subsample contains less than 100 organisms and the sample was not picked in its entirety, the sample is incomplete and does not conform to MBSS protocols. This can only be rectified in the laboratory, by spreading the remaining sortate (the remainder of the sample, after the identified bugs have been removed), then picking additional grids until the target number is reached or the sample has been picked in its entirety and identifying those organisms (Resource Assessment Service 2024).

If a reported subsample contains many more than 120 organisms, and relatively few grids were picked, it can be presumed that it had a high density. No adjustments should be made to the data, *post hoc*. Although rarefaction and other procedures could be appropriate in certain research contexts, BIBIs that are consistent with MBSS and used for regulatory purposes must be calculated based on the complete laboratory subsample. A reported subsample containing many more than 120 organisms that were picked from many grids may indicate a deviation from the standard laboratory protocols of evenly spreading the sample across the sorting tray. Conformity to standard protocols should be confirmed, the data and resulting BIBI scores from such samples should be reviewed, and any misleading results should not be used or reported.

Data Formatting

The end-user of the data must also ensure that it is properly formatted. Taxa lists are presented in a long-table format, and the site name and stratum should be consistent among all corresponding records. Each taxon encountered in a sample should be listed one time, accompanied by a number reflecting the total number of individuals classified as that taxon. Each record should also include an appropriate designation (“TRUE” or “FALSE”) in the EXCLUDE field. Data that are specific to each organism (i.e., taxonomic information and traits) should accompany each row of data and reflect the formatting conventions set forth in the Master Taxa List. An example of properly formatted data is given in Appendix 1.

Naming

It is imperative that all of the identified organisms can be assigned the relevant traits that are necessary for the BIBI calculations. The MBSS Benthic Master Taxa List is the standard reference for this information.

It can be easily queried via software, if the names associated with identified organisms match those on the MBSS Benthic Master Taxa List.

The list of names of identified organisms must be compared to the most recent MBSS Benthic Master Taxa List to ascertain that they are present. A portion of the R code provided in Appendix 3 can be useful for recognizing names that are not present on the MBSS Benthic Master Taxa List. Alternatively, the vlookup function may be used in Microsoft Excel, a Find Unmatched Query in Microsoft Access may be executed, or other solutions could be implemented. Any names that do not match should be examined in further detail and should be addressed accordingly:

- If the names do not match due to a misspelling in the list of identified organisms, the misspelled name should simply be corrected.
- If the reported name is synonymous with (or an antiquation of) an organism name that is included in the Master Taxa List, the listed name should be changed to be consistent with the Master Taxa List.
- If the name on the sampled list of organisms appears in Appendix G in the MBSS Laboratory Methods manual (Resource Assessment Service 2024) as an organism that should not have been included in the identified sample, records of this organism should be deleted. Subsequently, the total number of organisms in the valid sample should be ascertained and, if less than 100 and the sample was not picked in its entirety, the original sample (minus the benthic organisms that were already identified) should be subsampled again to achieve a final number of organisms exceeding 100.
- If the name on the sampled list of organisms is more precise than the target-level for the MBSS BIBI (family, or higher, as specified in Appendix G in the MBSS Laboratory Methods manual; Resource Assessment Service 2024), the taxonomy should be ‘rolled back’ to the valid level. If this procedure results in duplicate entries for the same taxon at a site, entries should be combined with the counts summed.
- If the reported name reflects changes in taxonomic keys and the new name is a direct replacement for one that is already in the Master Taxa List, the updated name should be appended to the Master Taxa List using the same traits associated with the antiquated taxonomy. In these cases, please notify DNR, by communicating with Neal Dziepak (neal.dziepak@maryland.gov), so that the Master Taxa List can be appropriately updated.
- If the reported name is indicative of a novel organism (not listed on the Benthic Master Taxa List), that benthic macroinvertebrate may be added to the Benthic Master Taxa List using the procedures described below.

Taxonomic Reconciliation

In order to promote standardization and comparability of BIBI scores collected throughout Maryland, the MBSS Benthic Master Taxa List should be regarded as the central repository for Maryland’s non-tidal benthic macroinvertebrates and the characteristics that are relevant to the MBSS BIBI.

If an organism that does not appear on the MBSS Master Taxa list is sampled by a different agency than DNR, and a physical specimen or photograph of the organism is available, it may be submitted for confirmation and subsequent inclusion in the MBSS Master Taxa List. Please notify DNR of any novel organisms by communicating with Neal Dziepak (neal.dziepak@maryland.gov). Upon confirmation of the specimen or photograph, a record for that organism will be included in the Master Taxa list, facilitating the subsequent calculation of a consistent and comparable BIBI score.

Fish Data

After fish counts, identification, and biomass are recorded in the field, these data should be reviewed. Data pertaining to stream surface area should also be verified and used to calculate an appropriate estimate, and catchment size should be derived. Data expectations should be met and formatting should be conducive to FIBI calculations. It is important to make sure that all fish data can be referenced from the Fish Master Taxa List for relevant fish traits.

Data Expectations

A complete fish sample dataset should include, at a minimum: target and non-target-level identifications, total counts of individuals, total biomass, an estimate of surface area within the site (typically derived from average stream width and length), and upstream catchment acreage. These variables are required for FIBI calculation. It may also be important, for validation purposes, that sampling crew, crew leader, and taxonomist(s) are reported and hold the appropriate certifications.

The reported total counts of individuals and total fish biomasses represent the respective sums across the two electrofishing passes comprising each sample (Harbold et al. 2024).

The target-level for identification of all organisms used in the MBSS FIBI is species. Taxonomists should put effort into achieving this level of identification. Specimens that could not be positively identified in the field should be vouchered (or thoroughly photographed) for identification in the laboratory. Any necessary corrections to the dataset that are based on verified identifications of vouchered specimens should be made prior to FIBI calculation. Hybrid fishes should be included in the dataset, but should be named using the taxa designations that match the Fish Master Taxa List; the traits that are listed for hybrids in the Master Taxa List ensure that they are correctly incorporated into the FIBI calculations. If any fishes could not be definitively identified to species, even after further examination in a laboratory setting, they should be recorded at the lowest-possible taxonomic level using the taxa designations that match the Fish Master Taxa List; the traits that are listed for non-target-level identifications in the Master Taxa List ensure that they are correctly incorporated into the FIBI calculations.

The estimate of stream surface area for the sampled reach is derived from field-collected data. In streams that are wetted and sampleable along the entire 75-m stream segment, the transect measurements that are recorded as part of the standard MBSS habitat inventory are typically used to establish this estimate by multiplying the average width by the total length of wetted segment sampled. In streams with a partially dewatered channel and standing pools, a better estimate of surface area may be obtained by summing individual estimates of surface area within each wetted segment. In such instances, the estimated surface area used in the FIBI calculation will differ from estimates associated with habitat data for the site, as the average width reported for habitat (in MBSS surveys) is always the arithmetic mean of the transect measurements.

Upstream catchment acreage is also required for calculating the MBSS FIBI. This value may be derived using ESRI ArcGIS®, the USGS StreamStats tool (USGS 2019), or similar geographic analysis software. The site midpoint is used for this analysis, and the hydrologic basin upstream of this point is subsequently delineated. The area of the delineated basin, expressed as acreage, represents the upstream catchment acreage.

Sample Size

The FIBI may be calculated using samples containing any number of fish. MBSS samples where no fish were observed are given a default FIBI score of 1.00. Samples from streams with a partially dewatered channel or standing pools can be used to calculate a FIBI score, provided that a valid estimate of the site's surface area is used in the calculation. Because the FIBI incorporates one or zero richness metrics, depending on the geographic stratum, it is generally robust to differences in sample size. However, due to naturally low diversity and abundances of fish in small streams, especially those with catchment areas <300 ac, FIBI scores derived from samples at such sites should be interpreted with some degree of uncertainty (Roth et. al 1998, Roth et al. 2000).

Data Formatting

The end-user of the data must also ensure that it is properly formatted. Taxa lists are presented in a long-table format, and the site name, stratum, total biomass, site surface area, and upstream catchment acreage should be consistent among all corresponding records. Each fish species encountered at a site should be listed one time, and the accompanying count should be represented as a positive whole number reflecting the total number of relevant individuals, regardless of age, condition, or any other non-taxonomic differences. Data that are specific to each species (i.e., taxonomic information and traits) should accompany each row of data and reflect the formatting conventions set forth in the Master Taxa List. An example of properly formatted data is given in Appendix 2.

Naming

It is imperative that all of the names associated with identified fishes can be assigned the relevant traits that are necessary for the FIBI calculations. All fish that are identified to species are referred to using the standard accepted common name, as given in *Common and Scientific Names of Fishes from the United States, Canada, and Mexico, Eighth Edition* (Page et al. 2023), and echoed in the MBSS Sampling Manual (Harbold et al. 2024).

Should the standard common name of a species change, the record on the MBSS Fish Master Taxa List is changed to reflect the most recently accepted common name. This occurred for several species on the MBSS Fish Master Taxa List in 2023 (Page et al. 2023). Fish should be reported using the most recent standardized names, as reflected in the current Fish Master Taxa List. Sampling records referring to antiquated naming conventions should be changed for consistency or indicated in a fashion that is coherent across years.

Should scientific advances indicate the presence of a distinct species that was previously named as something else, the record on the MBSS Fish Master Taxa List is changed to reflect the understood and accepted common name based on the most recent scientific understanding. For example, this occurred for records that previously referenced Logperch *Percina caprodes*, when it was determined that all of such records within Maryland's portion of the Chesapeake Bay watershed represented a distinct species, the Chesapeake Logperch *Percina bimaculata* (Near 2008).

If a non-native fish species is suspected to be established or invasive in Maryland, a corresponding record is added to the MBSS Fish Master Taxa List. For example, this occurred when Bluehead Chub was sampled from several sites in Maryland's Potomac River Basin.

Any sampled exotic fishes that are not suspected to be established or invasive in Maryland (i.e., aquarium- or bait bucket-released fishes) should also be included in the documented sample. To retain an accurate and complete record of sampled non-native fishes, these fishes should be identified to the lowest practical taxonomic level and recorded as such. However, for the purpose of the FIBI, the appropriate characteristics are simply assigned from the corresponding genus- or family-level record in the Fish Master Taxa List.

Hybrid fish that are sampled are recorded using the lowest-level taxonomic designation shared by the parents (typically genus) and are designated as 'hybrid.' For example, a Bluegill x Green Sunfish hybrid would be recorded as "Lepomis Hybrid." More specific details regarding the suspected parental species can be listed as notes and photographs may be taken, but neither is required for the FIBI.

Any fish that could not be positively identified in the field or in the laboratory from vouchered specimens or photographs should be recorded at the lowest-possible taxonomic level and the 'sp.' designation. For example, "Notropis sp." would be used to classify individuals from a population Mimic Shiners and Spottail Shiners that do not clearly demonstrate the distinguishing characteristics.

Taxonomic Reconciliation

In order to promote standardization and comparability of FIBI scores collected throughout Maryland, the MBSS Fish Master Taxa List should be regarded as the central repository for Maryland's non-tidal fishes and the characteristics that are relevant to the MBSS FIBI.

Although significant effort should be made to accurately identify all sampled fishes to species-level, the MBSS Fish Master Taxa List has traits that are listed for non-target-level identification. Fish that cannot be reconciled to the species-level should be recorded at the lowest-possible taxonomic level using the taxa designations that match the Fish Master Taxa List. Similarly, exotic fishes that are not suspected to be established or invasive in Maryland should be incorporated into the FIBI using the lowest taxonomic level that matches the Master Taxa List. This ensures that such fishes are correctly and consistently incorporated into the FIBI calculations.

If a fish taxon that is not currently included on the Fish Master Taxa List is encountered at a high enough frequency that it should be included, please notify DNR by communicating with William Harbold (william.harbold@maryland.gov). Upon confirmation of a preserved physical specimen or photographs depicting key physical features, a record for that organism will be included in the Fish Master Taxa list with the next update.

IBI Calculation

Software

The formulae related to calculating the MBSS Indices of Biotic Integrity were originally given in Southerland et al. 2005. These calculations are generally based on simple algebraic equations and subsequent comparisons to predefined thresholds that correspond to ecological conditions. As such, it is feasible to perform such calculations without the aid of a computer. However, hand calculations are prone to error and can be time-consuming, so computer software is recommended.

Most mathematical computer programs can be programmed to generate MBSS IBI scores based on inputted lists of sampled organisms. Spreadsheet-based software can allow for straightforward visualization of the data and transparency in the steps taken to analyze it, but application to many samples may be tedious. Command-based software can fully automate calculations, providing for rapid and accurate calculation of IBIs for many samples, but may be more difficult to program and are not as transparent as spreadsheets. Although no specific software is required to generate valid IBIs, it is important that the program that is used has been thoroughly tested. Recommended procedures for testing a program involve comparing the calculated IBI scores and component metric values and scores at a subset of sites with those resulting from manual calculations and/or a different (reliable) computer algorithm. It is also recommended that all subsequent output values are spot-checked to ensure that the IBI scores reflects the average of the correct component metrics (as determined by the stratum), that component metric scores were appropriately binned, and that the appropriate characteristics were assigned to the organisms.

MBSS Tools R Package

Currently, DNR uses MBSS Tools for calculating IBI scores. MBSS Tools is a computer software package that performs some of the calculations used to analyze data that were collected following the Maryland Biological Stream Survey (MBSS) protocols. MBSS Tools was created by Tetra Tech Inc. in 2017 in collaboration with the Maryland Department of Natural Resources. The MBSS Tools package is run through R, a free software environment for statistical computing and graphics. Thus, R must be installed before using MBSS Tools. Results are only valid for sampling data that were collected using standard MBSS protocols.

MBSS Tools currently has the capability to perform the following calculations:

- Maryland Benthic Macroinvertebrate Index of Biotic Integrity (BIBI)
- Maryland Fish Index of Biotic Integrity (FIBI)
- Maryland Stream Wader benthic macroinvertebrate family-level index
- Stream discharge calculations
- MBSS Physical Habitat Index (PHI; Paul, et al. 2002)[1]
- Ion matrix conductivity contributions and graphing
- Generates standard distribution maps for Maryland

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[1] The utility of the MBSS Physical Habitat Index (PHI) is limited by its narrow scope, the weighting of variables, and a statistical disconnect with other measures of stream quality. Recorded observations for the various MBSS stream habitat variables are generally more informative.

This MBSS Tools package is currently available for download through GitHub (https://github.com/leppott/MBSStools). The current version of the MBSS package can be installed by typing the following code into the R console:

```
if(!require(remotes)){install.packages("remotes")}
install_github("leppott/MBSStools", force=TRUE, build_vignettes = TRUE)  # if errors, try build_vignettes = FALSE
```

If the above code results in errors, more favorable results may be achieved by modifying the preceding code with ‘force=FALSE’ and/or ‘build_vignettes = FALSE’. Once installed, it may then be initiated with the following code in the R Console:

```
library(MBSStools)
```

The vignette, with help files and examples of how to carry out the various calculations, can be referenced by entering the following code:

```
help(package = "MBSStools")
```

Alternatively, the vignette and help files are stored in the MBSS Tools folder under the directory that is returned by the following code:

```
.libPaths()
```

Care must be taken in preparing the data input files for MBSS Tools, and users must follow the formatting specifications outlined in the accompanying help files. Properly formatted MBSS benthic macroinvertebrate and fish data are given in Appendix 1 and Appendix 2 of this document, respectively. This software is sensitive to errors in input files such as spelling, capitalization, formatting. It is also sensitive to missing data fields, even if the field is not applicable to the specific stratum being analyzed. All results must also be carefully examined. USER BEWARE: MBSS Tools may produce results with disregard to some input errors, which result in erroneous outputs.

Examples of properly-formatted input files and code for generating BIBI and FIBI scores using the MBSS Tools package in R are given in Appendices 1 and 2, respectively. Examples of code for calculating BIBI and FIBI scores are given in Appendices 3 and 4, respectively. Users of the MBSS Tools software are urged to refer to these examples and to thoroughly read the vignette, help files, and references provided in the software package.

A MBSS Index of Biotic Integrity calculator with a graphic-user-interface (Shiny App) is also included in the MBSS Tools package. Input files for this calculator must be formatted as outlined in the MBSS Tools help files. The Shiny App calculator can be installed and accessed on computers with R and MBSS Tools by typing the following code into the R console:

```
install.packages("shiny") # installs the shiny package, only needed on first run
library(shiny)
library(MBSStools)
runShiny()
```

Computers that are not installed with R and MBSS Tools can currently access a version of the MBSS Index of Biotic Integrity Shiny App calculator online at: https://tetratech-wtr-wne.shinyapps.io/MBSStools/. Requirements for data formatting are the same as for the algorithms run using R. Screenshots and instructions are presented in Appendix 5.

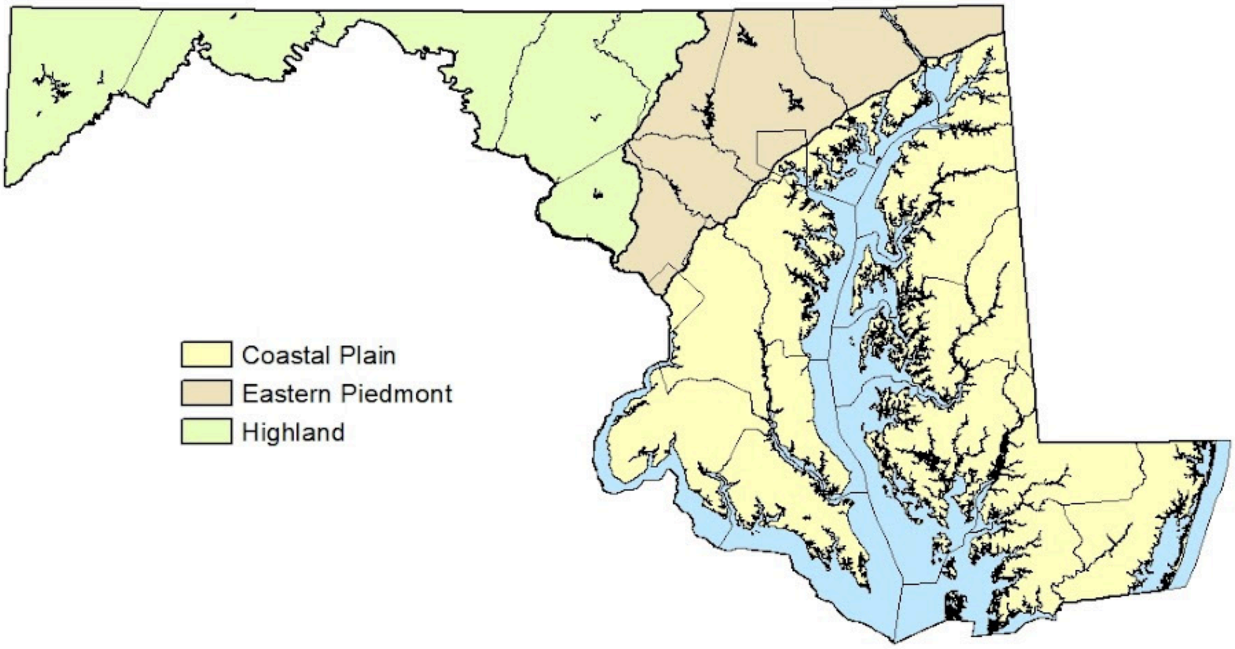
BIBI

The data that are required for calculation of the MBSS BIBI are site or sample designation (**SITE**), the names of sampled taxa (**TAXON)**, the number of individuals that were identified as each taxa in the subsample (**N_TAXA**), and EXCLUDE designations for each record (**EXCLUDE**). Additional taxon-specific information that is necessary for calculation of the MBSS BIBI should be copied from the Master Taxa List from corresponding entries (Step 2, below).

The steps involved in calculating the BIBI for each sample are: (1) determine the physiographic stratum that the sample was taken in and select the appropriate suite of metrics, (2) assign the necessary characteristics to each of the sampled organisms, as determined from the MBSS Master Taxa List, (3) calculate the numerical response to each metric, (4) convert each metric response to a 1, 3, or 5, based on defined thresholds, and (5) calculate the BIBI score by taking the average of component metrics.

STEP 1 - Determine the physiographic stratum

The BIBI is composed of six to eight metrics, depending on the geographic stratum where the sample was collected. The three physiographic strata that are considered for the MBSS BIBI are: Coastal Plain (hereafter referred to as Coastal), Eastern Piedmont (hereafter referred to as Piedmont), and Highland. This strata designation can be determined from the GIS layer (https://data.imap.maryland.gov/datasets/maryland::maryland-biological-stream-survey-ibi-strata/about).



STEP 2 - Assign necessary characteristics to sampled organisms

In order to calculate a BIBI score, certain characteristics must be assigned to the organisms that were sampled. These characteristics can be determined from the MBSS Master Taxa List. If data have been carefully QC'd for consistency with the MBSS Master Taxa List, and are appropriately formatted, this procedure can be carried out using software that joins the table of sampled organisms with the Master Taxa List based on matching the **TAXON** column of the MBSS Master Taxa list with the reported identification name in the dataset.

Some characteristics assigned to each organism reference their taxonomic classifications and are used in BIBI calculations. **ORDER** is important for determining Ephemeroptera, Plecoptera, and Trichoptera taxa, which is used in BIBI calculations in all strata. **ORDER** is also used for determining Diptera, which is applicable in the Highland stratum. **FAMILY** is important for determining Chironomidae, which is applicable in the Piedmont stratum. **TRIBE** is used to determine Tanytarsini (a tribe of midges) in the Highland stratum.

Some characteristics assigned to each organism reference aspects of their life history and are used in BIBI calculations. **FINALTOLVAL07** is important for determining the proportion of organisms that are intolerant, and is used in BIBI calculations in all strata. **FFG** is important for determining scraper taxa, and is applicable in the Coastal and Highland strata. **Habit** is important for determining Climbers in the Coastal stratum, Clingers in the Piedmont stratum, and Swimmers in the Highland stratum. An organism's **Habit** may include several designations, all of which should be considered for IBI calculations. If a taxon does not have any information listed in **Habit, TolVal**, or **FFG**, it is excluded from the numerator (i.e., the number conforming to the metric) but is included in the denominator (i.e. the total number under consideration) for relevant BIBI metric calculations.

STEP 3 - Calculate the numerical response to each metric

Using the list of organisms in the MBSS subsample, their abundances, and the characteristics that were assigned to each record in STEP 2, above, numerical responses may be calculated for each metric. Although it is possible to calculate all of the metrics within each of Maryland's three BIBI strata, it is only necessary to calculate those that pertain to the stratum in which the sampled site was located, as determined in STEP 1, above.

Richness metrics in the BIBI, which are titled 'Number of…', describe the number of taxa (regardless of abundance) that satisfy a given condition. The term "unique taxa", is used in the following descriptions about how to calculate richness metrics . Unique taxa are those that were definitively identified as different from others in the sample, and are indicated by EXCLUDE = "FALSE". Non-unique taxa records, indicated by EXCLUDE = "TRUE", should not be counted toward taxa richness metrics.

Proportional metrics in the BIBI, which are titled 'Percent…', describe the percentage of individuals (regardless of their diversity) that satisfy a given condition as compared to the total number of individual organisms identified. The EXCLUDE designation is irrelevant to proportional metrics.

Throughout the following calculation instructions, the applicable strata for each metric are listed in parentheses, following the underlined metric name. Definitions and calculation for each of the metrics are as follows:

- Number of Taxa (Coastal, Piedmont, and Highland) - Number of macroinvertebrate taxa in the sub-sample. This is calculated as the count of all valid unique records that were included in the representative subsample.
- Number of Ephemeroptera Taxa (Coastal, Piedmont, and Highland) - Number of Ephemeroptera taxa identified. This is calculated as the count of unique records where **ORDER** = Ephemeroptera.
- Number of EPT Taxa (Coastal, Piedmont, and Highland) - Number of Ephemeroptera, Plecoptera, and Trichoptera taxa. This is calculated as the count of unique records where **ORDER** = Ephemeroptera or Plecoptera or Trichoptera.
- Percent Intolerant Urban (Coastal, Piedmont, and Highland) - Percentage of individuals intolerant to urban conditions. This is calculated as the sum of individuals for all records where **FINALTOLVAL07** is less than or equal to 3.0 (i.e., the total number of individuals intolerant to urban conditions) divided by the sum of individuals across all records (i.e., the total number of individual organisms identified), and then multiplied by 100.
- Number of Scraper Taxa (Coastal) - Total number of taxa with a functional feeding group classified as scrapers. This is calculated as the count of unique records where **FFG** = Scraper.
- Percent Climbers (Coastal) - Percentage of individuals classified as climbers. This is calculated as the sum of individuals for all records where **HABIT** includes ‘cb’ (i.e., the total number of individuals classified as climbers) divided by the sum of all records (i.e., the total number of individual organisms identified) and multiplied by 100.
- Percent Ephemeroptera (Coastal) - Percentage of individuals classified as Ephemeroptera. The total number of Ephemeroptera individuals (the sum of individuals for all records where **ORDER** = Ephemeroptera) is divided by the total number of individual organisms identified and multiplied by 100.
- Percent Chironomidae (Piedmont) - Percentage of individuals classified as Chironomidae. The total number of individuals in the family Chironomidae (the sum of individuals for all records where **FAMILY** = Chironomidae) is divided by the total number of individual organisms identified and multiplied by 100.
- Percent Clingers (Piedmont) - Percentage of individuals classified as clingers. This is calculated as the the sum of individuals for all records where **HABIT** includes ‘cn’ (i.e., the total number of individuals classified as clingers) divided by the total number of individual organisms identified and multiplied by 100.
- Percent Diptera (Highland) - Percentage of individuals classified as Diptera. Total number of diptera individuals (the sum of individuals for all records where **ORDER** = Diptera) is divided by the total number of individual organisms identified and multiplied by 100.

- Percent Scrapers (Highland) - Percentage of individuals classified as scrapers. Total number of individuals with a functional feeding group classified as scrapers (the sum of individuals for all records where **FFG** = Scraper) is divided by the total number of individual organisms identified and multiplied by 100.
- Percent Swimmers (Highland) - Percentage of individuals classified as swimmers. The total number of individuals classified as swimmers (the sum of individuals for all records where **HABIT** includes ‘sw’) is divided by the total number of individual organisms identified and multiplied by 100.
- Percent Tanytarsini (Highland) - Percentage of individuals classified as Tanytarsini. Total number of individuals in the tribe Tanytarsini (the sum of individuals for all records where **TRIBE** = Tanytarsini) is divided by the total number of individual organisms identified and multiplied by 100.

STEP 4 - Compare numerical responses to scoring thresholds

The metric values obtained in STEP 3, above, are compared to standard thresholds, given in the table below. The thresholds were initially given in *New Biological Indicators to Better Assess the Condition of Maryland Streams* (Southerland et al. 2005), but the table given in the present document is refined to include all potential metric values. As each metric response is compared to the thresholds, the corresponding numerical score (5, 3, or 1) should be recorded for each component metric.

Benthic IBI metrics	**Thresholds**		
	5	**3**	**1**
Coastal (aka Coastal Plain)			
Number of Taxa	≥ 22	< 22 to 14	< 14
Number of EPT Taxa	≥ 5	4 to 2	< 2
Number of Ephemeroptera Taxa	≥ 2	1	< 1
Percent Intolerant Urban	≥ 28	< 28 to 10	< 10
Percent Ephemeroptera	≥ 11	< 11 to 0.8	< 0.8
Number of Scraper Taxa	≥ 2	1	< 1
Percent Climbers	≥ 8	< 8 to 0.9	< 0.9
Eastern Piedmont (aka Piedmont)			
Number of Taxa	≥ 25	24 to 15	< 15
Number of EPT Taxa	≥ 11	5 to 10	< 5
Number of Ephemeroptera Taxa	≥ 4	3 to 2	< 2
Percent Intolerant Urban	≥ 51	< 51 to 12	< 12
Percent Chironomidae	≤ 24	> 24 to 63	> 63
Percent Clingers	≥ 74	< 74 to 31	< 31
Highland (aka Combined Highlands)			
Number of Taxa	≥ 24	> 24 to 15	< 15
Number of EPT Taxa	≥ 14	< 14 to 8	< 8
Number of Ephemeroptera Taxa	≥ 5	< 5 to 3	< 3
Percent Intolerant Urban	≥ 80	< 80 to 38	< 38
Percent Tanytarsini	≥ 4	< 4 to 0.1	< 0.1
Percent Scrapers	≥ 13	< 13 to 3	< 3
Percent Swimmers	≥ 18	< 18 to 3	< 3
Percent Diptera	≤ 26	> 26 to 50	> 50

STEP 5 - Calculate the average of metric scores

To generate a BIBI score for the sample, calculate the average of all component metric scores as the sum of component metric scores divided by the number of metrics corresponding to the stratum.

Because they are calculated based on the average of component metrics, each of which may be assigned only 3 discrete scores, resulting BIBI scores are somewhat ordinal. BIBI scores for all of the strata range from 1.00 - 5.00. In the Coastal stratum, scores vary by increments of 0.28571428571429 for 15 potential scores. In the Piedmont stratum, scores vary by increments of 0.33333333333333 for 13 potential scores. In the Highland stratum, scores vary by increments of 0.25000000000000 for 17 potential scores.

The gradient of BIBI scores that may be observed in any of the strata was designed to correspond to the spectrum of stream conditions in Maryland (Southerland et al. 2007). In general, minimally degraded sites tend to have benthic macroinvertebrate assemblages that score relatively high, whereas heavily degraded sites tend to have benthic macroinvertebrate assemblages that score relatively low. Scores ranging 4.0 – 5.0 are considered “good” and are comparable to reference streams considered to be minimally impacted by anthropogenic disturbance. Scores 3.0 – < 4.0 are considered “fair” and are comparable to reference conditions, but some aspects of biological integrity may not resemble the qualities of minimally-impacted streams. Scores 2.0 – < 3.0 are considered “poor” and indicate significant deviation from reference conditions, with many aspects of biological integrity not resembling the qualities of minimally-impacted streams. Scores 1.0 – < 2.0 are considered “very poor” and indicate strong deviation from reference conditions, with most aspects of biological integrity not resembling the qualities of minimally-impacted streams (Boward et al. 1999).

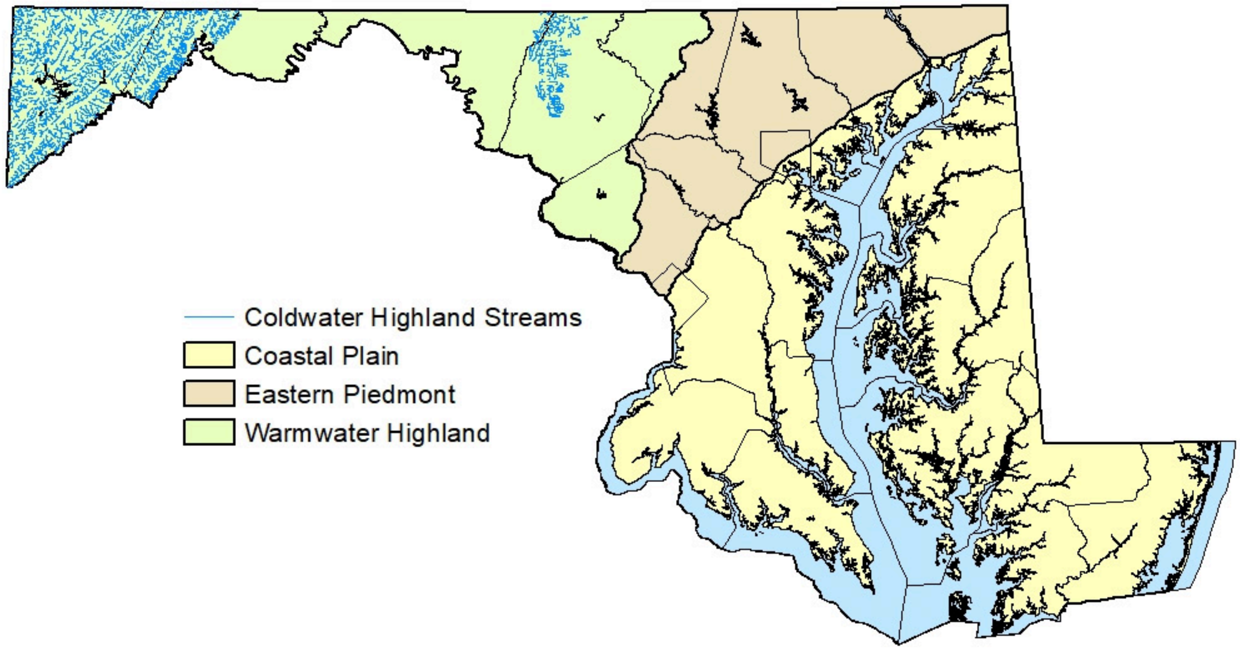
FIBI

The data that are required for calculation of the MBSS FIBI are site or sample designation (**SITE**), the names of sampled fishes (**SPECIES)**, the total numbers of each species in the two-pass electrofishing sample (**TOTAL**), and the total fish biomass in the two-pass electrofishing sample (**TOTBIOM**). Calculations also require estimates of sampled stream surface area, derived from field-measurements, and the upstream catchment acreage, derived from geographic analysis software. Additional species-specific information that is necessary for calculation of the MBSS FIBI should be copied from the Master Taxa List from corresponding entries (Step 2, below).

Like calculating the BIBI, the steps involved in calculating the FIBI for each sample are: (1) determine the physiographic stratum that the sample was taken in and select the appropriate suite of metrics, (2) assign the necessary characteristics to each of the sampled organisms, as determined from the MBSS Master Taxa List, (3) calculate the numerical response to each metric, (4) convert each metric response to a 1, 3, or 5, based on defined thresholds, and (5) calculate the BIBI score by taking the average of component metrics.

STEP 1 - Determine the physiographic stratum

The FIBI is composed of four to six metrics, depending on the geographic stratum where the sample was collected. The four physiographic strata that are considered for the MBSS FIBI are: Coastal Plain (hereafter referred to as Coastal), Eastern Piedmont (hereafter referred to as Piedmont), Warmwater Highland, and Coldwater Highland. This strata designation can be determined from this GIS layer (https://data.imap.maryland.gov/datasets/maryland::maryland-biological-stream-survey-ibi-strata/about) and the "MBSS Coldwater Stratum" file for differentiating between Warmwater and Coldwater Highland streams.



STEP 2 - Assign Necessary Characteristics to Sampled Organisms

Characteristics assigned to organisms in a sample required to calculate a FIBI score can be determined from the MBSS master taxa list. Provided that the data are formatted correctly, this can be carried out using software to join the table of organisms with the Master Taxa List by matching the **SPECIES** column in the formatted dataset with that of the Master Taxa List.

Some characteristics assigned to each organism reference their taxonomic classifications and are used in FIBI calculations. **SPECIES** is used in determining brook trout in the Coldwater Highland stratum and dominant species in the Coastal and Warmwater Highland strata. **TYPE** is important in determining benthic species (darters, madtoms, sculpins, and lamprey) in the Coastal, Piedmont, and Warmwater Highland strata; non-tolerant suckers in the Coastal stratum; and sculpin in the Coldwater Highland stratum.

Some characteristics assigned to each organism reference aspects of their life history and are used in FIBI calculations. **PTOLR** (tolerance) is used in determining tolerance percentages in all four strata and non-tolerant suckers in the Coastal stratum. **TROPHIC** (trophic level) is used in determining generalists, omnivores, and invertivores in the Coastal, Piedmont, and Warmwater Highland strata, as well as insectivores in the Warmwater Highland stratum. **SILT** is used in determining lithophilic spawners in the Piedmont stratum.

STEP 3 - Calculate the numerical response to each metric

Using the list of organisms in a sample, their abundances, and the characteristics that were assigned to each record in STEP 2, above, numerical responses may be calculated for each metric. Although it is possible to calculate all of the metrics within each of Maryland's four FIBI strata, it is only necessary to calculate those that pertain to the stratum in which the site was located, as determined in STEP 1, above. Throughout the following calculation instructions, the applicable strata for each metric are listed in parentheses, following the underlined metric name. Definitions and calculations for each of the metrics are as follows:

- Abundance per square meter (Coastal, Piedmont, Warmwater Highland, Coldwater Highland) - Number of fish observed per square meter in a site. This is calculated by dividing the total number of fish observed at a site by the estimated surface area (in square meters) of sampled stream.
- Number of benthic species, adjusted (Coastal, Piedmont, Warmwater Highland) - The number of benthic species in a sample is the count of species that are classified (**TYPE**) as darters, madtoms, sculpins, or lamprey. This value is normalized according to the upstream catchment size (in Acres), by dividing it by an adjustment factor that is appropriate for the geographic stratum:

  Coastal:
  # of Benthic Species Adjusted = (# of Benthic Species)/(1.69 × log(Catchment Size)– 3.33)
  Piedmont:
  # of Benthic Species Adjusted = (# of Benthic Species)/(1.25 × log(Catchment Size)– 2.36)
  Warmwater Highland:
  # of Benthic Species Adjusted= (# of Benthic Species)/(1.23 × log(Catchment Size)– 2.35)

*Benthic fishes in Maryland (**TYPE** = "DARTER", "SCULPIN", "MADTOM", or "LAMPREY"):* Darters: Banded, Blackside, Fantail, Glassy, Greenside, Johnny, Maryland, Rainbow, Shield, Stripeback, Striped, Swamp, Tessellated, and Variegate Darters, and Chesapeake Logperch. Sculpins: Blue Ridge, Checkered, Mottled, Potomac, and Slimy Sculpins. Madtoms: Margined Madtom, Tadpole Madtom, and Stonecat. Lamprey: American Brook, Least Brook, and Sea Lampreys.

- Percent tolerant (Coastal, Piedmont, Warmwater Highland, Coldwater Highland) - The percentage of individuals in a sample that are tolerant to anthropogenic stressors. This is calculated by dividing the total number of tolerant individuals by the total number of sampled fish and multiplying the quotient by 100.

*Tolerant fishes in Maryland (**PTOLR** = "T"):* Blacknose Dace, Bluegill, Bluntnose Minnow, Brown Bullhead, Creek Chub, Eastern Mudminnow, Golden Shiner, Green Sunfish, Largemouth Bass, Pirate Perch, Pumpkinseed, Redfin Pickerel, Tessellated Darter, White Sucker.

- Percent generalists, omnivores, invertivores (Coastal, Piedmont, Warmwater Highland) - The percentage of the number of individuals within a sample that are classified as generalists, omnivores, or invertivores. This is calculated by dividing the total number of generalists, omnivores, and invertivores by the total number of sampled fish and multiplying the quotient by 100.

*Generalist, omnivorous, and invertivorous fishes in Maryland (**TROPHIC** = "GE", "OM", or "IV"):* Generalist: American Eel, Black Crappie, Brook Trout, Creek Chub, Fallfish, Green Sunfish, Redbreast Sunfish, Rock Bass, Tiger Trout, Warmouth, White Crappie, Yellow Perch. Omnivore: Blacknose Dace, Blue Catfish, Bluehead Chub, Bluntnose Minnow, Brown Bullhead, Channel Catfish, Common Carp, Common Shiner, Fathead Minnow, Flathead Catfish, Golden Redhorse, Golden Shiner, Goldfish, Longnose Dace, River Chub, Shorthead Redhorse, Silverjaw Minnow, Spottail Shiner, Striped Shiner, White Catfish, White Sucker, Yellow Bullhead. Invertivore: Banded Killifish, Banded Sunfish, Blueback Herring, Bluegill, Bluespotted Sunfish, Chesapeake Logperch, Comely Shiner, Creek Chubsucker, Cutlip Minnow, Eastern Mosquitofish, Eastern Mudminnow, Flier, Johnny Darter, Longear Sunfish, Margined Madtom, Mimic Shiner, Mud Sunfish, Mummichog, Northern Hogsucker, Pearl Dace, Pirate Perch, Pumpkinseed, Rainwater Killifish, Rosyface Shiner, Rosyside Dace, Satinfin Shiner, Spot, Spotfin Shiner, Stonecat, Stripeback Darter, Striped Darter, Striped Killifish, Swallowtail Shiner, Swamp Darter, Tadpole Madtom, Tessellated Darter, White Perch.

- Percent non-tolerant suckers (round-bodied suckers) (Coastal) - The percentage of non-tolerant suckers (all suckers except for white sucker) in a sample. This is calculated by taking the sum of non-tolerant suckers in a sample, dividing that total by the total number of fish sampled, then multiplying the quotient by 100.

Round-bodied sucker species in Maryland: Black Redhorse, Creek Chubsucker, Golden Redhorse, Longnose Sucker, Northern Hogsucker, and Shorthead Redhorse (**NOT** White Sucker).

- Percent abundance of dominant species (Coastal, Warmwater Highland) - The percentage of a sample that is composed of the most abundant fish species in the sample. This is calculated by dividing the total number of the most abundant fish species in the sample by the total number of fish in the sample and multiplying the quotient by 100.
- Biomass per square meter (Piedmont) - The total fish biomass per square meter in the site. This is calculated by dividing the total biomass by the estimated surface area (in meters) of sampled stream.
- Percent lithophilic spawners (Piedmont) - The percentage of individuals classified as lithophilic spawners. This is calculated by dividing the number of lithophilic spawners by the total number of fish in the sample and multiplying the quotient by 100.

*Lithophilic spawning fishes in Maryland (**SILT** = "Y"):* Banded Darter, Blue Ridge Sculpin, Bluehead Chub, Brook Trout, Brown Trout, Central Stoneroller, Checkered Sculpin, Chesapeake Logperch, Comely Shiner, Common Shiner, Creek Chub, Cutlip Minnow, Fallfish, Fantail Darter, Glassy Darter, Golden Redhorse, Ironcolor Shiner, Longear Sunfish, Mottled Sculpin, Northern Hogsucker, Pearl Dace, Potomac Sculpin, Rainbow Darter, Rainbow Trout, River Chub, Rock Bass, Rosyface Shiner, Rosyside Dace, Shield Darter, Shorthead Redhorse, Silverjaw Minnow, Spottail Shiner, Striped Shiner, Swallowtail Shiner, Tiger Trout, White Sucker.

- Percent insectivores (Warmwater Highland) - The percentage of individuals classified as insectivores. This is calculated by dividing the total number of insectivores by the total number of fish in the sample and multiplying the quotient by 100.

*Insectivorous fishes in Maryland (**TROPHIC** = "IS")*: Banded Darter, Blue Ridge Sculpin, Checkered Sculpin, Fantail Darter, Glassy Darter, Greenside Darter, Ironcolor Shiner, Mottled Sculpin, Potomac Sculpin, Rainbow Darter, Shield Darter.

- Percent brook trout (Coldwater Highland) - The percentage of individuals in the sample that are brook trout. This is calculated by dividing the total number of brook trout by the total number of fish in a sample and multiplying the quotient by 100.
- Percent sculpins (Coldwater Highland) - The percentage of individuals in the sample that are sculpins. This is calculated by dividing the total number of sculpins by the total number of fish in a sample and multiplying the quotient by 100.

STEP 4 - Compare numerical responses to scoring thresholds

The metric values obtained in STEP 3, above, are compared to standard thresholds, given in the table below. The thresholds were initially given in *New Biological Indicators to Better Assess the Condition of Maryland Streams* (Southerland et al. 2005), but the table given in the present document is refined to include all potential metric values. As each metric response is compared to the thresholds, the corresponding numerical score (5, 3, or 1) should be recorded for each component metric.

Fish IBI metrics	**Thresholds**		
	5	**3**	**1**
Coastal Plain (aka Coastal)			
Abundance per square meter	≥ 0.72	0.45 to < 0.72	< 0.45
Number of benthic species adjusted	≥ 0.22	> 0 to < 0.22	0
Percent tolerant	≤ 68	97 to > 68	> 97
Percent generalists, omnivores, invertivores	≤ 92	< 100 to > 92	100
Percent non-tolerant suckers (all except white suckers)	≥ 2	> 0 to < 2	0
Percent abundance of dominant species	≤ 40	≤ 69 to > 40	> 69
Eastern Piedmont (aka Piedmont)			
Abundance per square meter	≥ 1.25	≥ 0.25 to < 1.25	< 0.25
Number of benthic species adjusted	≥ 0.26	≥ 0.09 to < 0.26	< 0.09
Percent tolerant	≤ 45	≤ 68 to > 45	> 68
Percent generalists, omnivores, invertivores	≤ 80	< 100 to > 80	100
Biomass per square meter	≥ 8.6	≥ 4.0 to < 8.6	< 4.0
Percent lithophilic spawners	≥ 61	≥ 32 to < 61	< 32
Warmwater Highland			
Abundance per square meter	≥ 0.65	≥ 0.31 to < 0.65	< 0.31
Number of benthic species adjusted	≥ 0.25	≥ 0.11 to < 0.25	< 0.11
Percent tolerants	≤ 39	≤ 80 to > 39	> 80
Percent generalists, omnivores, invertivores	≤ 61	≤ 96 to > 61	> 96
Percent insectivores	≥ 33	≥ 1 to < 33	< 1
Percent abundance of dominant species	≤ 38	≤ 89 to > 38	> 89
Coldwater Highland			
Abundance per square meter	≤ 0.88	< 2.24 to > 0.88	≥ 2.24
Percent tolerant	≤ 0.22	< 0.81 to > 0.22	≥ 0.81
Percent brook trout	≥ 0.14	> 0 to < 0.14	0
Percent sculpins	≥ 0.44	< 0 to < 0.44	0

STEP 5 - Calculate the average of metric scores

To generate a FIBI score for the sample, calculate the average of all component metric scores as the sum of component metric scores divided by the number of metrics corresponding to the stratum.

Because they are calculated based on the average of component metrics, each of which may be assigned only 3 discrete scores, resulting FIBI scores are somewhat ordinal. FIBI scores for all of the strata range from 1.00 - 5.00. In the Coastal, Piedmont, and Warmwater Highland strata, scores vary by increments of 0.33333333333333 for 13 potential scores. In the Coldwater Highland stratum, scores vary by increments of 0.25 for 9 potential scores.

MBSS samples where no fish were observed are given a default FIBI score of 1.00, provided that the entire wetted portion of the 75-m reach was sampled using standard MBSS protocols.

The gradient of FIBI scores that may be observed in any of the strata was designed to correspond to the spectrum of stream conditions in Maryland (Southerland et al. 2007). In general, minimally degraded sites tend to have fish assemblages that score relatively high, whereas heavily degraded sites tend to have fish assemblages that score relatively low. Scores ranging 4.0 – 5.0 are considered "good" and are comparable to reference streams considered to be minimally impacted by anthropogenic disturbance. Scores 3.0 – < 4.0 are considered "fair" and are comparable to reference conditions, but some aspects of biological integrity may not resemble the qualities of minimally-impacted streams. Scores 2.0 – < 3.0 are considered "poor" and indicate significant deviation from reference conditions, with many aspects of biological integrity not resembling the qualities of minimally-impacted streams. Scores 1.0 – < 2.0 are considered "very poor" and indicate strong deviation from reference conditions, with most aspects of biological integrity not resembling the qualities of minimally-impacted streams (Boward et al. 1999).

Generating BIBI Scores with Unreconciled Taxa

If a physical specimen or photograph of an organism that does not appear on the MBSS Master Taxa List is submitted for confirmation and inclusion in the MBSS Master Taxa List, the resulting record should subsequently be used in the BIBI calculations. However, if a physical specimen or photograph of the organism is not available for confirmation, it may or may not be possible to generate a valid BIBI score for any samples that included the unreconciled taxon. In such cases, the identity of the organism should be treated with skepticism, and its impact on the final IBI score should be tested. The following steps should be followed to determine if a reliable and comparable BIBI score can be generated for a sample containing unreconciled taxa:

STEP 1 - Research known geographic distributions and life-history traits (e.g., lentic, lotic, marine) of the unreconciled taxon (which should be a target-level genus entry). Geographic distributions and life-history traits are described in Merritt et al. 2019, but are not included for each individual genus. If the taxon can be reasonably found in a non-tidal Maryland stream, or geographic distributions and life-history traits are unknown, consider it *potentially valid* and proceed to step 2.1. If the published information about the taxon indicates that it is restricted to disparate geographic regions or habitats, consider it *invalid* and proceed to step 3.1. These steps outlined below should be conducted for each unreconciled taxon individually and within each sample in which they are included.

STEP 2.1 - Assign phylogenetic information from itis.gov, and assign trait information from Merritt et al. 2019 (Mid-Atlantic) and/or US EPA 1999 (Mid-Atlantic) for the unreconciled taxon. Add this information to a provisional version of the Benthic Master Taxa File for use in these BIBI calculations. Calculate BIBI scores for all samples containing the unreconciled taxon. Keep note of generated BIBI scores. Proceed to step 3.1.

STEP 3.1 - 'Roll back' the name of the unreconciled taxon to the lowest taxonomic level that is included in the Benthic Master Taxa List (e.g., Family Tipulidae). After 'rolling back', investigate whether the naming conversion is already present within each sample containing the unreconciled taxon. If the naming conversion is not present within a given sample, proceed to step 3.2. If the naming conversion is present within a given sample, proceed to step 3.3.

STEP 3.2 - If the naming conversion is not present within a given sample, these should be treated as separate distinct entries and the EXCLUDE designation can be applied unambiguously as "FALSE" to the converted taxon name. This will ensure that the converted taxon name is counted for richness metrics. Calculate BIBI scores for all samples containing the unreconciled taxon. Keep note of generated BIBI scores. Proceed to step 4.1.

STEP 3.3 - If the naming conversion is already present within a given sample, it is uncertain whether the novel organism is indeed unique. Calculate BIBI scores for all samples containing the unreconciled taxon, assuming EXCLUDE = "FALSE." Under this scenario, the two records would be combined and the total number of individuals would reflect the sum of the counts for the unreconciled taxon and the other lower-taxonomic-level taxon. Keep note of generated BIBI scores. Proceed to step 3.4.

STEP 3.4 - Calculate BIBI scores for all samples containing the unreconciled taxon, assuming EXCLUDE = "TRUE." Keep note of generated BIBI scores. Proceed to step 4.1.

STEP 4.1 - Compare each of the BIBI scores that were generated in the steps above. If the final composite BIBI scores are the same, that score is comparable to MBSS. However, if the resulting scores do not match, it is not possible to determine a score that is comparable with MBSS.

IBI Applicability

BIBI

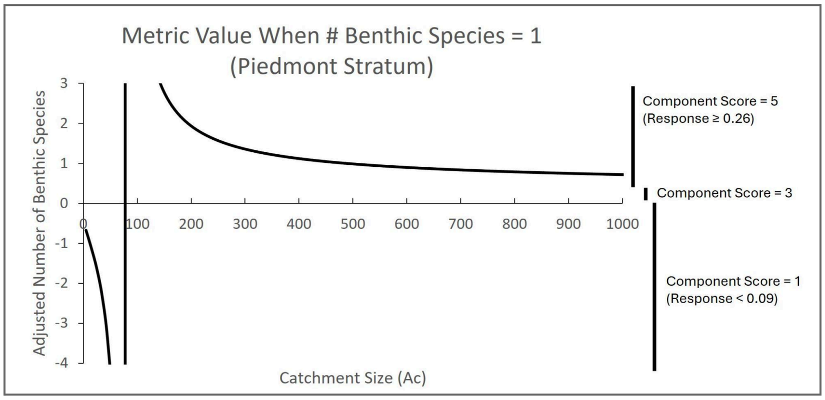
The BIBI was developed based on a dataset that included approximately 2,500 first- through fourth-order streams in Maryland, based on a map scale of 1:100,000, including coldwater streams, small streams (catchment areas <300 ac), and blackwater streams (Southerland et al. 2005). Although the BIBI generally characterizes stream health adequately, certain metrics may not be calibrated for unique conditions or stream types that occur in Maryland. Examples may include limestone spring creeks or exceedingly small, intermittent, or ephemeral streams. DNR is currently examining component metric response to stream size, flow duration, and other variables to evaluate the Maryland BIBI's ability to assess underlying ecological conditions in these unique situations.

In addition to the site conditions mentioned above, taxonomic changes could impact BIBI results and influence how accurately they represent stream health. Taxonomic changes have been due to advances, over time, in the scientific understanding of benthic macroinvertebrate taxonomy and subsequent improvements to classification keys. BIBI scoring results can be influenced by such changes to taxonomic classification. Simple changes to the naming or grouping of taxa are accommodated through updates to the Master Taxa List, and do not impact the BIBI, provided their ecological information is transcribed across records. These taxonomic changes also have variously resulted in the lumping of some taxa that were previously considered distinct and, more frequently, the splitting of some taxa that were previously considered uniform (Merritt et al. 2019). Such changes can directly influence richness metrics; lumping could potentially reduce richness, whereas splitting could potentially inflate richness. Such taxonomic changes could also affect any metric that depends on ecological characteristics of the organism, as both lumping and splitting could affect the understanding of a taxon's tolerance, habit, and/or functional feeding group. It is important to consider taxonomic changes if comparing benthic macroinvertebrate assemblages and BIBI results over time.

FIBI

The FIBI was developed based on a similar dataset to the BIBI, that included first- through fourth-order streams in Maryland and was based on a map scale of 1:100,000. The uncertainty of applying the FIBI to sites with catchment areas <300 ac has been reiterated (Roth et. al 1998, Roth et al. 2000). Small streams in Maryland may naturally lack fish diversity, which reduces the responsiveness of the index. Furthermore, fish populations in small streams may comprise relatively few individuals, and low sample sizes may increase variability of estimates.

Due to the mathematical equation, the metric Adjusted Number of Benthic Species exhibits erratic responses if any benthic fish species are sampled in very small catchments. This metric is incorporated into the MBSS FIBI for all strata except the Coldwater Highland stratum. If the catchment size is less than a critical value (93.4 ac in the Coastal stratum, 77.3 ac in the Piedmont stratum, and 81.4 ac in the Warmwater Highland stratum), the metric response is mathematically negative. This does produce some counterintuitive results where small streams cannot score higher than a 1 on this metric, even if several benthic species are present. If the catchment size is greater than the previously described critical value but less than an upper critical value (45,800 ac in the Coastal stratum, 92,300 ac in the Piedmont stratum, and 145,500 ac in the Warmwater Highland stratum) the presence of any benthic species in the sample



will result in a component metric score of 5.0. A component metric score of 3.0 is only possible if the catchment size is greater than the upper critical value. Thus, for the vast majority of Maryland's wadeable streams (those with catchment sizes between the two critical values), this metric is dichotomous, where sampling one or more benthic species results in a component score of 5 but failing to detect a benthic species in the sample results in a component score of 1. In regard to the FIBI, detecting a benthic species in such a stream results in a $0.\overline{66}$ increase in final score in the Coastal, Piedmont, and Warmwater Highland strata.

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Appendix 1. Properly formatted input data for calculating a BIBI using MBSS Tools in program R.

Column headings and included data demonstrate expected spelling, capitalization, and key words. The variables **SITE**, **TAXON**, **N_TAXA**, and **EXCLUDE** are the data that were collected. The physiographic variable **STRATA_R** must be entered on each line of data for a site. The variable **Index.Name** is required for the R code, and should be “MBSS.2005.Bugs” to invoke the MBSS BIBI; this field is created within the R code in Appendix 3. The other variables (taxonomy and relevant characteristics) are listed in the MBSS Master Taxa List and can be referenced using software that joins the tables based on matching the **TAXON** column of the MBSS Master Taxa list with the reported identification name in the dataset; this join can be accomplished within the R code in Appendix 3. Other fields may be included in the input data, provided that they are named differently than required column headings.

SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	PHYLUM	CLASS	ORDER	FAMILY	GENUS	OTHER_TAXA	TRIBE	FFG	HABIT	FINALTOLVAL07	Index.Name
Site1	ANTOCHA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Tipulidae	Antocha			Collector	cn	8	MBSS.2005.Bugs
Site1	CHEUMATOPSYCHE	1	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche			Filterer	cn	6.5	MBSS.2005.Bugs
Site1	CLINOCERA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Empididae	Clinocera			Predator	cn	7.4	MBSS.2005.Bugs
Site1	CRICOTOPUS	10	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	Orthocladiinae		Shredder	cn, bu	9.6	MBSS.2005.Bugs
Site1	DIAMESA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Diamesa	Diamesinae	Diamesini	Collector	sp	8.5	MBSS.2005.Bugs
Site1	DICROTENDIPES	2	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Dicrotendipes	Chironominae	Chironomini	Collector	bu	9	MBSS.2005.Bugs
Site1	HEMERODROMIA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Empididae	Hemerodromia	Hemerodromiini	Hemerodromiini	Predator	sp, bu	7.9	MBSS.2005.Bugs
Site1	HYDROBAENUS	2	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Hydrobaenus	Orthocladiinae		Scraper	sp	7.2	MBSS.2005.Bugs
Site1	HYDROPSYCHE	10	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche			Filterer	cn	7.5	MBSS.2005.Bugs
Site1	MICROPSECTRA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Micropsectra	Chironominae	Tanytarsini	Collector	cb, sp	2.1	MBSS.2005.Bugs
Site1	ORTHOCLADIINAE	3	TRUE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae		Orthocladiinae		Collector	bu	7.6	MBSS.2005.Bugs
Site1	ORTHOCLADIUS	25	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius	Orthocladiinae		Collector	sp, bu	9.2	MBSS.2005.Bugs
Site1	PARAKIEFFERIELLA	4	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Parakiefferiella	Orthocladiinae		Collector	sp	2.1	MBSS.2005.Bugs
Site1	PARAMETRIOCNEMUS	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Parametriocnemus	Orthocladiinae		Collector	sp	4.6	MBSS.2005.Bugs
Site1	PARATANYTARSUS	2	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus	Chironominae	Tanytarsini	Collector	sp	7.7	MBSS.2005.Bugs
Site1	PHAENOPSECTRA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Phaenopsectra	Chironominae	Chironomini	Collector	cn	8.7	MBSS.2005.Bugs
Site1	POLYPEDILUM	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Chironominae	Chironomini	Shredder	cb, cn	6.3	MBSS.2005.Bugs
Site1	PROSTOMA	1	FALSE	HIGHLAND	Nemertea	Enopla	Hoplonemertea	Tetrastemmatidae	Prostoma			Predator		7.3	MBSS.2005.Bugs
Site1	RHEOTANYTARSUS	6	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Rheotanytarsus	Chironominae	Tanytarsini	Filterer	cn	7.2	MBSS.2005.Bugs
Site1	STENELMIS	3	FALSE	HIGHLAND	Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis			Scraper	cn	7.1	MBSS.2005.Bugs
Site1	SUBLETTEA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Sublettea	Chironominae	Tanytarsini	Collector		10	MBSS.2005.Bugs
Site1	SYMPOTTHASTIA	3	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Sympotthastia	Diamesinae	Diamesini	Collector	sp	8.2	MBSS.2005.Bugs
Site1	TANYTARSUS	30	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	Chironominae	Tanytarsini	Filterer	cb, cn	4.9	MBSS.2005.Bugs
Site1	TVETENIA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Tvetenia	Orthocladiinae		Collector	sp	5.1	MBSS.2005.Bugs
Site2	ANTOCHA	3	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Tipulidae	Antocha			Collector	cn	8	MBSS.2005.Bugs
Site2	CHEUMATOPSYCHE	5	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche			Filterer	cn	6.5	MBSS.2005.Bugs
Site2	CHIMARRA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Philopotamidae	Chimarra			Filterer	cn	4.4	MBSS.2005.Bugs
Site2	CRICOTOPUS	6	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	Orthocladiinae		Shredder	cn, bu	9.6	MBSS.2005.Bugs

Site2	CRYPTOCHIRONOMUS	3	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Cryptochironomus	Chironominae	Chironomini	Predator	sp, bu	7.6	MBSS.2005.Bugs
Site2	DIAMESA	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Diamesa	Diamesinae	Diamesini	Collector	sp	8.5	MBSS.2005.Bugs
Site2	DIAMESINAE	1	TRUE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae		Diamesinae	Diamesini	Collector		7.1	MBSS.2005.Bugs
Site2	DICROTENDIPES	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Dicrotendipes	Chironominae	Chironomini	Collector	bu	9	MBSS.2005.Bugs
Site2	HEMERODROMIA	2	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Empididae	Hemerodromia	Hemerodromiini	Hemerodromiini	Predator	sp, bu	7.9	MBSS.2005.Bugs
Site2	HYDROBAENUS	6	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Hydrobaenus	Orthocladiinae		Scraper	sp	7.2	MBSS.2005.Bugs
Site2	HYDROPSYCHE	8	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche			Filterer	cn	7.5	MBSS.2005.Bugs
Site2	LUMBRICULIDAE	2	FALSE	HIGHLAND	Annelida	Oligochaeta	Lumbriculida	Lumbriculidae				Collector	bu	6.6	MBSS.2005.Bugs
Site2	LYPE	1	FALSE	HIGHLAND	Arthropoda	Insecta	Trichoptera	Psychomyiidae	Lype			Scraper	cn	4.7	MBSS.2005.Bugs
Site2	MICROTENDIPES	4	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Microtendipes	Chironominae	Chironomini	Filterer	cn	4.9	MBSS.2005.Bugs
Site2	NAIDIDAE	1	FALSE	HIGHLAND	Annelida	Oligochaeta	Haplotaxida	Naididae				Collector	bu	8.5	MBSS.2005.Bugs
Site2	ORTHOCLADIINAE	3	TRUE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae		Orthocladiinae		Collector	bu	7.6	MBSS.2005.Bugs
Site2	ORTHOCLADIUS	22	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius	Orthocladiinae		Collector	sp, bu	9.2	MBSS.2005.Bugs
Site2	PARAKIEFFERIELLA	2	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Parakiefferiella	Orthocladiinae		Collector	sp	2.1	MBSS.2005.Bugs
Site2	PARAMETRIOCNEMUS	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Parametriocnemus	Orthocladiinae		Collector	sp	4.6	MBSS.2005.Bugs
Site2	PARATANYTARSUS	7	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus	Chironominae	Tanytarsini	Collector	sp	7.7	MBSS.2005.Bugs
Site2	RHEOCRICOTOPUS	1	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Rheocricotopus	Orthocladiinae		Collector	sp	6.2	MBSS.2005.Bugs
Site2	RHEOTANYTARSUS	3	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Rheotanytarsus	Chironominae	Tanytarsini	Filterer	cn	7.2	MBSS.2005.Bugs
Site2	STENELMIS	2	FALSE	HIGHLAND	Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis			Scraper	cn	7.1	MBSS.2005.Bugs
Site2	SYMPOTTHASTIA	4	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Sympotthastia	Diamesinae	Diamesini	Collector	sp	8.2	MBSS.2005.Bugs
Site2	TANYTARSINI	2	TRUE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae		Chironominae	Tanytarsini	Collector		3.5	MBSS.2005.Bugs
Site2	TANYTARSUS	18	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	Chironominae	Tanytarsini	Filterer	cb, cn	4.9	MBSS.2005.Bugs
Site2	THIENEMANNIMYIA GROUP	4	FALSE	HIGHLAND	Arthropoda	Insecta	Diptera	Chironomidae	Thienemannimyia group	Orthocladiinae		Predator	sp	8.2	MBSS.2005.Bugs

Appendix 2. Properly formatted input data for calculating a FIBI using MBSS Tools in program R.

Column headings and included data demonstrate expected spelling, capitalization, and key words. The variables **SITE**, **SPECIES**, **TOTAL,** and **TOTBIOM** are the data that were collected. The physiographic variable **FIBISTRATA**, the catchment size **ACREAGE**, and site dimensions (**LEN_SAMP** and **AVWID**; for determining wetted area) must be entered on each line of data for a site. The variable **Index.Name** is required for the R code, and should be "MBSS.2005.Fish" to invoke the MBSS FIBI; this field is created within the R code in Appendix 4. The other variables (relevant trait characteristics) are listed in the MBSS Master Taxa List and can be referenced using software that joins the tables based on matching the **SPECIES** column of the MBSS Master Taxa list with the reported identification name in the dataset; this join can be accomplished within the R code in Appendix 4. Other fields may be included in the input data, provided that they are named differently than required column headings. All column headings are required for MBSS Tools, but the corresponding cells may be left blank if not relevant to the stratum's metrics.

SITE	SPECIES	TOTAL	TOTBIOM	FIBISTRATA	ACREAGE	LEN_SAMP	AVWID	TYPE	PTOLR	TROPHIC_MBSS	NATIVE_MBSS	SILT	Index.Name
Site1	BLACKNOSE DACE	52	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	T	OM	N	N	MBSS.2005.Fish
Site1	CENTRAL STONEROLLER	31	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	I	AL	N	Y	MBSS.2005.Fish
Site1	CREEK CHUB	22	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	T	GE	N	Y	MBSS.2005.Fish
Site1	CUTLIP MINNOW	34	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site1	LARGEMOUTH BASS	3	1791	EPIEDMONT	4327.75	75	6	NOTYPE	T	TP	IC	N	MBSS.2005.Fish
Site1	LONGNOSE DACE	20	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	NOTYPE	OM	N	N	MBSS.2005.Fish
Site1	NORTHERN HOGSUCKER	6	1791	EPIEDMONT	4327.75	75	6	SUCKER	I	IV	N	Y	MBSS.2005.Fish
Site1	ROSYSIDE DACE	118	1791	EPIEDMONT	4327.75	75	6	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site1	TESSELLATED DARTER	8	1791	EPIEDMONT	4327.75	75	6	DARTER	T	IV	N	N	MBSS.2005.Fish
Site1	WHITE SUCKER	13	1791	EPIEDMONT	4327.75	75	6	SUCKER	T	OM	N	Y	MBSS.2005.Fish
Site2	BLACKNOSE DACE	92	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	T	OM	N	N	MBSS.2005.Fish
Site2	BLUE RIDGE SCULPIN	244	1223	EPIEDMONT	663.261	75	3.575	SCULPIN	I	IS	N	Y	MBSS.2005.Fish
Site2	BLUNTNOSE MINNOW	1	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	T	OM	N	N	MBSS.2005.Fish
Site2	CENTRAL STONEROLLER	1	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	I	AL	N	Y	MBSS.2005.Fish
Site2	CREEK CHUB	49	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	T	GE	N	Y	MBSS.2005.Fish
Site2	CUTLIP MINNOW	1	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site2	LONGNOSE DACE	26	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	NOTYPE	OM	N	N	MBSS.2005.Fish
Site2	ROSYSIDE DACE	17	1223	EPIEDMONT	663.261	75	3.575	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site2	TESSELLATED DARTER	3	1223	EPIEDMONT	663.261	75	3.575	DARTER	T	IV	N	N	MBSS.2005.Fish
Site3	BLACKNOSE DACE	78	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	T	OM	N	N	MBSS.2005.Fish
Site3	BLUE RIDGE SCULPIN	268	4068	EPIEDMONT	1753.88	75	4.375	SCULPIN	I	IS	N	Y	MBSS.2005.Fish
Site3	BLUEGILL	9	4068	EPIEDMONT	1753.88	75	4.375	SUNFISH	T	IV	IC	N	MBSS.2005.Fish
Site3	BLUNTNOSE MINNOW	1	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	T	OM	N	N	MBSS.2005.Fish
Site3	BROWN TROUT	3	4068	EPIEDMONT	1753.88	75	4.375	NOTYPE	NOTYPE	TP	I	Y	MBSS.2005.Fish
Site3	CENTRAL STONEROLLER	16	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	I	AL	N	Y	MBSS.2005.Fish
Site3	COMMON SHINER	2	4068	EPIEDMONT	1753.88	75	4.375	SHINER	I	OM	N	Y	MBSS.2005.Fish
Site3	CREEK CHUB	90	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	T	GE	N	Y	MBSS.2005.Fish
Site3	CUTLIP MINNOW	9	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site3	GREEN SUNFISH	1	4068	EPIEDMONT	1753.88	75	4.375	SUNFISH	T	GE	IC	N	MBSS.2005.Fish
Site3	LARGEMOUTH BASS	2	4068	EPIEDMONT	1753.88	75	4.375	NOTYPE	T	TP	IC	N	MBSS.2005.Fish
Site3	LONGNOSE DACE	17	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	NOTYPE	OM	N	N	MBSS.2005.Fish
Site3	NORTHERN HOGSUCKER	1	4068	EPIEDMONT	1753.88	75	4.375	SUCKER	I	IV	N	Y	MBSS.2005.Fish
Site3	ROSYSIDE DACE	94	4068	EPIEDMONT	1753.88	75	4.375	OTHRCYPR	NOTYPE	IV	N	Y	MBSS.2005.Fish
Site3	TESSELLATED DARTER	10	4068	EPIEDMONT	1753.88	75	4.375	DARTER	T	IV	N	N	MBSS.2005.Fish
Site3	WHITE SUCKER	45	4068	EPIEDMONT	1753.88	75	4.375	SUCKER	T	OM	N	Y	MBSS.2005.Fish

Appendix 3. Example R script for generating BIBI scores using the MBSS Tools package.

This script assumes that the file ‘BenthicSampleData.csv’ is formatted as in Appendix 1, and contains column headings and data for **SITE**, **TAXON**, **N_TAXA**, **EXCLUDE**, and **STRATA_R**. Other variables (taxonomy and relevant characteristics) may be appended prior to reading the data into the R program (as it is in Appendix 1). Alternatively, these fields may be appended using the code below, provided that “MBSS Benthic Master Taxa List.csv” is saved in the working directory, and can be joined based on matching the **TAXON** column of the MBSS Master Taxa list with the reported identification names.

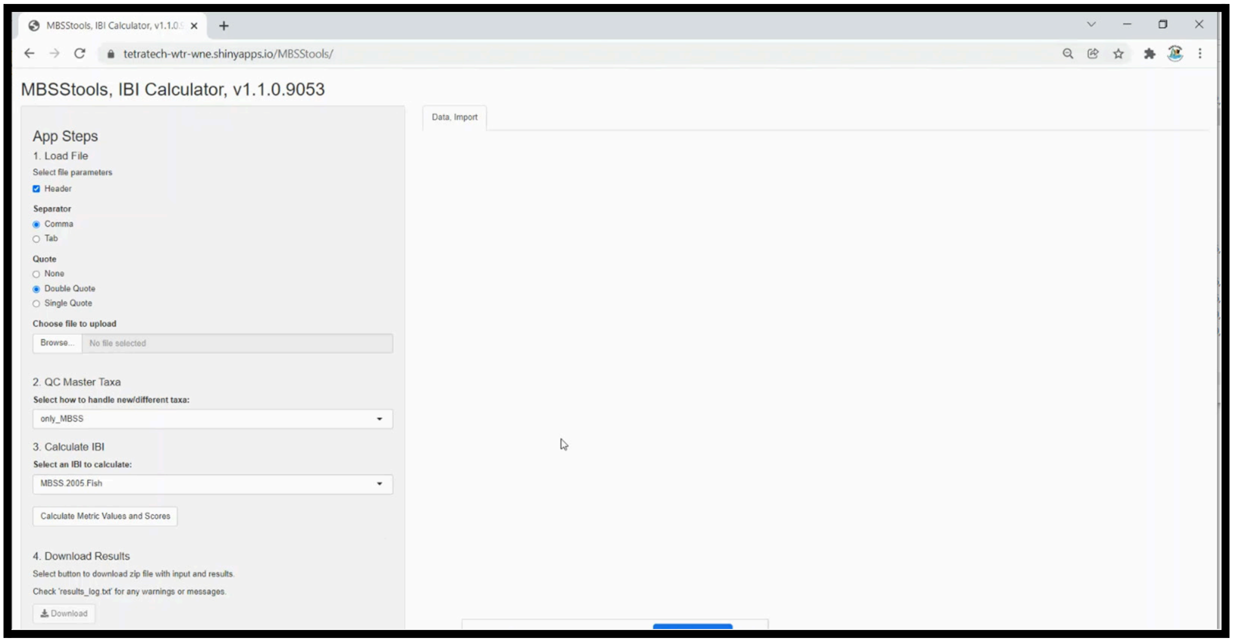
```
## Load required libraries ##
library(MBSStools)
library(dplyr)

## Specify working directory ##
setwd("C:/Working Directory")

## Read data ##
taxa_benthic <- read.csv("BenthicSampleData.csv", stringsAsFactors=FALSE)

##Minor formatting##
taxa_benthic$TAXON<-toupper(taxa_benthic$TAXON)
taxa_benthic$Index.Name = "MBSS.2005.Bugs"

## Read and join the Master Taxa List table.  (Only necessary if phylogenetic and trait information not included in input data) ##
char15 <- read.csv("MBSS Benthic Master Taxa List.csv") #Read table as csv file
taxa_benthic<- merge.data.frame(taxa_benthic,char15, by="TAXON", all.x = TRUE) ## Join the Master Taxa List to data

## Check if there are any reported taxa that can not be matched to the Master Taxa List ##
UnMatchedTaxa <-  taxa_benthic[is.na(Taxa_and_Char$PHYLUM),]
UnMatchedTaxa  #Provides a list of any taxa that could not be appended with an entry from the Master Taxa List

## Format EXCLUDE column for MBSS Tools; must be formatted as TRUE and FALSE ##
taxa_benthic$EXCLUDE[taxa_benthic$EXCLUDE %in% NA] <- "FALSE"
taxa_benthic$EXCLUDE[taxa_benthic$EXCLUDE %in% ""] <- "FALSE"
taxa_benthic$EXCLUDE[taxa_benthic$EXCLUDE %in% "N"] <- "FALSE"
taxa_benthic$EXCLUDE[taxa_benthic$EXCLUDE %in% "Y"] <- "TRUE"

## Write a CSV file with final data used for the BIBI ##
write.csv(taxa_benthic,"taxa_benthic.csv")

## Standard MBSS Tools code below ##
myIndex <- "MBSS.2005.Bugs"
thresh <- metrics_scoring
myMetrics.Bugs.MBSS <- as.character(unique(thresh[thresh[,"Index.Name"]==myIndex,"MetricName.Other"]))
myMetric.Values.Bugs.MBSS <- metric.values(taxa_benthic,"bugs", myMetrics.Bugs.MBSS)
Metrics.Bugs.Scores.MBSS <- metric.scores(myMetric.Values.Bugs.MBSS, myMetrics.Bugs.MBSS, "INDEX.NAME",
    "STRATA_R", thresh)

## Output and write a CSV file with BIBI metric response values, metric scores, and BIBI score ##
Metrics.Bugs.Scores.MBSS
write.csv(Metrics.Bugs.Scores.MBSS,"BIBI_output.csv")
```

Appendix 4. Example R script for generating FIBI scores using the MBSS Tools package.

This script assumes that the file ‘FishSampleData.csv’ is formatted as in Appendix 2, containing column headings and data for **SITE**, **SPECIES**, **TOTAL**, **TOTBIOM**, **FIBISTRATA**, **ACREAGE**, **LEN_SAMP**, and **AVWID.** Relevant fish trait characteristics (i.e., **TYPE**, **PTOLR**, **TROPHIC_MBSS**, and **SILT**) may be appended prior to reading the data into the R program (as it is in Appendix 2). Alternatively, these fields may be appended using the code below, provided that“MBSS Fish Master Taxa List.csv” is saved in the working directory, and can be joined based on matching the **SPECIES** column of the MBSS Master Taxa list with the reported identification names.

```
## Load required libraries ##
library(MBSStools)
library(dplyr)

## Specify working directory ##
setwd("C:/Working Directory")

## Read data ##
taxa_fish <- read.csv("FishSampleData.csv", stringsAsFactors=FALSE)
taxa_fish$SPECIES<-toupper(taxa_fish$SPECIES)
taxa_fish$Index.Name = "MBSS.2005.Fish"

## Read and append Fish Master Taxa List to the data.  (Only necessary if  relevant trait information not included in input data)##
char <- read.csv("MBSS Fish Master Taxa List.csv")
taxa_fish<- merge.data.frame(taxa_fish,char, by="SPECIES", all.x=TRUE)

## Write a CSV file with final data used for the FIBI ##
write.csv(taxa_fish,"taxa_fish.csv")

## Standard MBSS Tools code below ##
myIndex <- "MBSS.2005.Fish"
thresh <- metrics_scoring
taxa_fish$NATIVE_MBSS = "."
myMetrics.Fish <- as.character(unique(thresh[thresh[,"Index.Name"]==myIndex,"MetricName.Other"]))
myMetric.Values.Fish <- metric.values(taxa_fish, "fish", myMetrics.Fish, TRUE)
Metrics.Fish.Scores <- metric.scores(myMetric.Values.Fish, myMetrics.Fish, "Index.Name", "FIBISTRATA", thresh)

## Output and write a CSV file with FIBI metric response values, metric scores, and FIBI score ##
Metrics.Fish.Scores
write.csv(Metrics.Fish.Scores,"FIBI_output.csv")
```

Appendix 5. Using the MBSS Index of Biotic Integrity Shiny App calculator



MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma

○ Tab

Quote

○ None

● Double Quote

○ Single Quote

Choose file to upload

Browse... No file selected

2. QC Master Taxa

Select how to handle new/different taxa: only_MBSS

3. Calculate IBI

Select an IBI to calculate: MBSS.2005.Fish

Calculate Metric Values and Scores

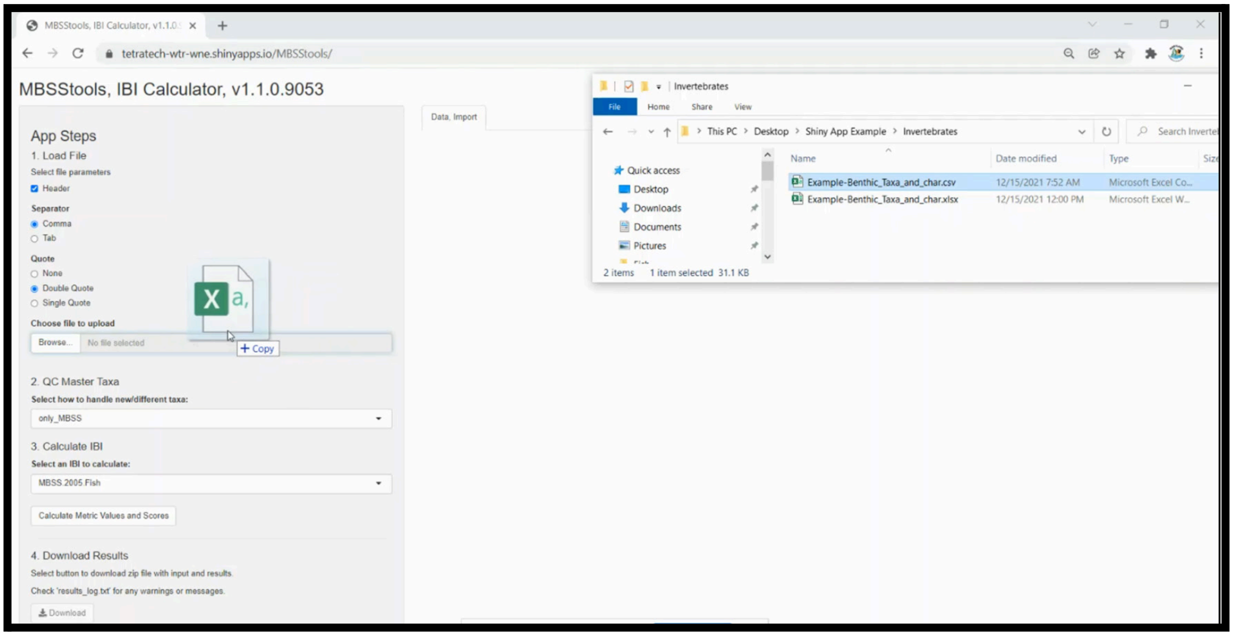
4. Download Results

Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

Download

STEP 1 - Open the MBSSTool IBI Calculator SHINY app in a web browser using the URL https://tetratech-wtr-wne.shinyapps.io/MBSStools/ or through the R console.



MBSStools, IBI Calculator, v1.1.0.9053

Name	Date modified	Type
Example-Benthic_Taxa_and_char.csv	12/15/2021 7:52 AM	Microsoft Excel Co...
Example-Benthic_Taxa_and_char.xlsx	12/15/2021 12:00 PM	Microsoft Excel W...

2 items   1 item selected 31.1 KB

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma

○ Tab

Quote

○ None

● Double Quote

○ Single Quote

Choose file to upload

Browse... No file selected

2. QC Master Taxa

Select how to handle new/different taxa: only_MBSS

3. Calculate IBI

Select an IBI to calculate: MBSS.2005.Fish

Calculate Metric Values and Scores

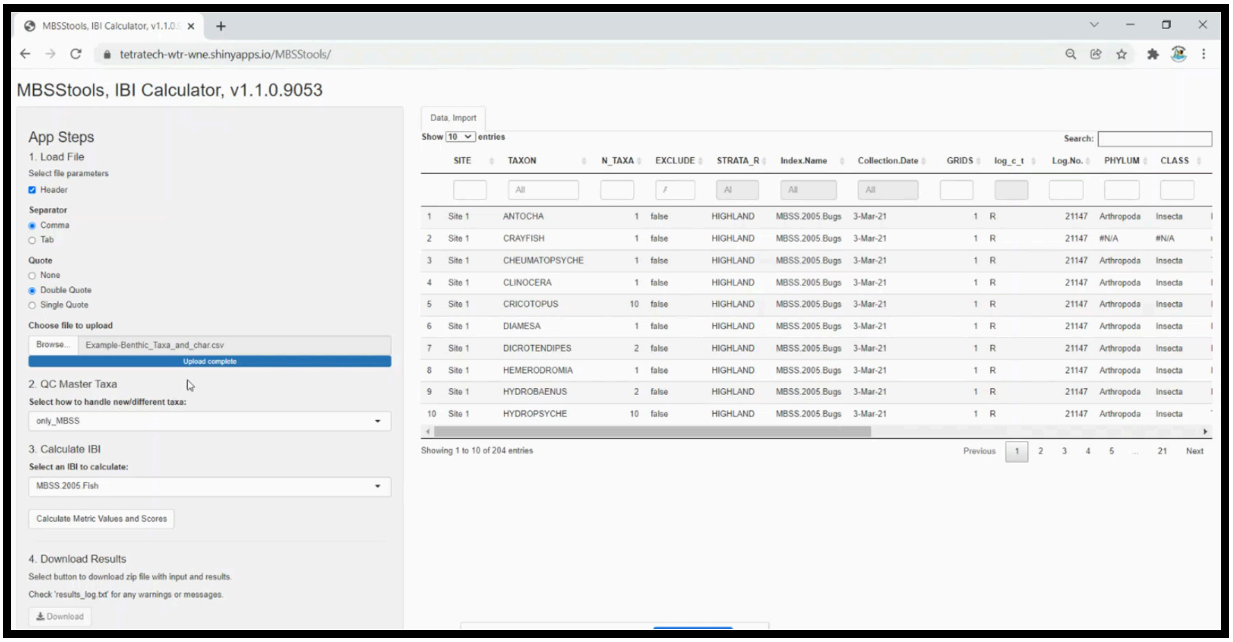
4. Download Results

Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

Download

STEP 2- Load the properly formatted data file by dragging-and-dropping (pictured above) or by browsing to the appropriate file. The uploaded file should include a Header row containing ALL of the fields presented in Appendix 1 or Appendix 2 (with the same spelling, capitalization, and formatting as depicted), and the spreadsheet should be fully-populated. The user must specify that the header row is entered, must specify comma-separated values for a *.csv file or tab-separated values for a *.tsv file, and whether quotes (if present) are single or double.



MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma
○ Tab

Quote

○ None
● Double Quote
○ Single Quote

Choose file to upload

Browse... Example-Benthic_Taxa_and_char.csv

Upload complete

2. QC Master Taxa

Select how to handle new/different taxa:

only_MBSS

3. Calculate IBI

Select an IBI to calculate:

MBSS.2005.Fish

4. Download Results

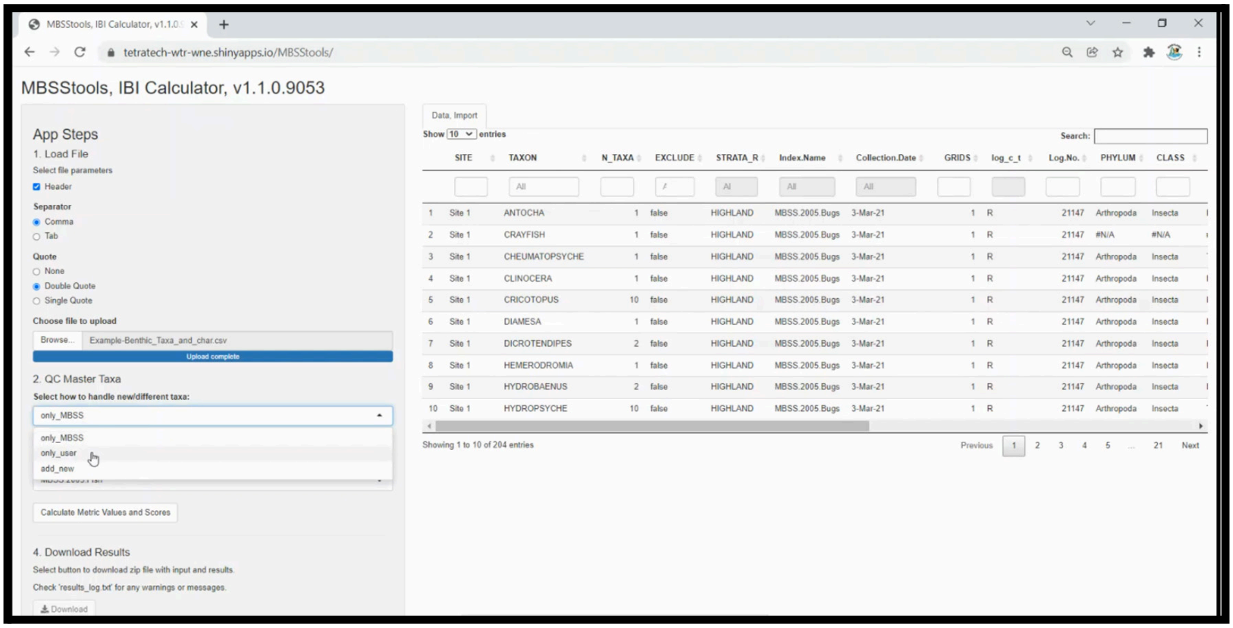
Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

	SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	Index.Name	Collection.Date	GRIDS	log_c_t	Log.No.	PHYLUM	CLASS
		All			Al	All	All					
1	Site 1	ANTOCHA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
2	Site 1	CRAYFISH	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	#N/A	#N/A
3	Site 1	CHEUMATOPSYCHE	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
4	Site 1	CLINOCERA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
5	Site 1	CRICOTOPUS	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
6	Site 1	DIAMESA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
7	Site 1	DICROTENDIPES	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
8	Site 1	HEMERODROMIA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
9	Site 1	HYDROBAENUS	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
10	Site 1	HYDROPSYCHE	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta

Showing 1 to 10 of 204 entries

<u>STEP 3</u>- Use the data preview pane (on the right side of the screen) to confirm that the data were properly uploaded and imported. Column headings should appear across the top of the preview as formatted in the uploaded file, the proper data should align with each column, and all rows of data should appear in the preview.



MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma
○ Tab

Quote

○ None
● Double Quote
○ Single Quote

Choose file to upload

Browse... Example-Benthic_Taxa_and_char.csv

Upload complete

2. QC Master Taxa

Select how to handle new/different taxa:

only_MBSS

4. Download Results

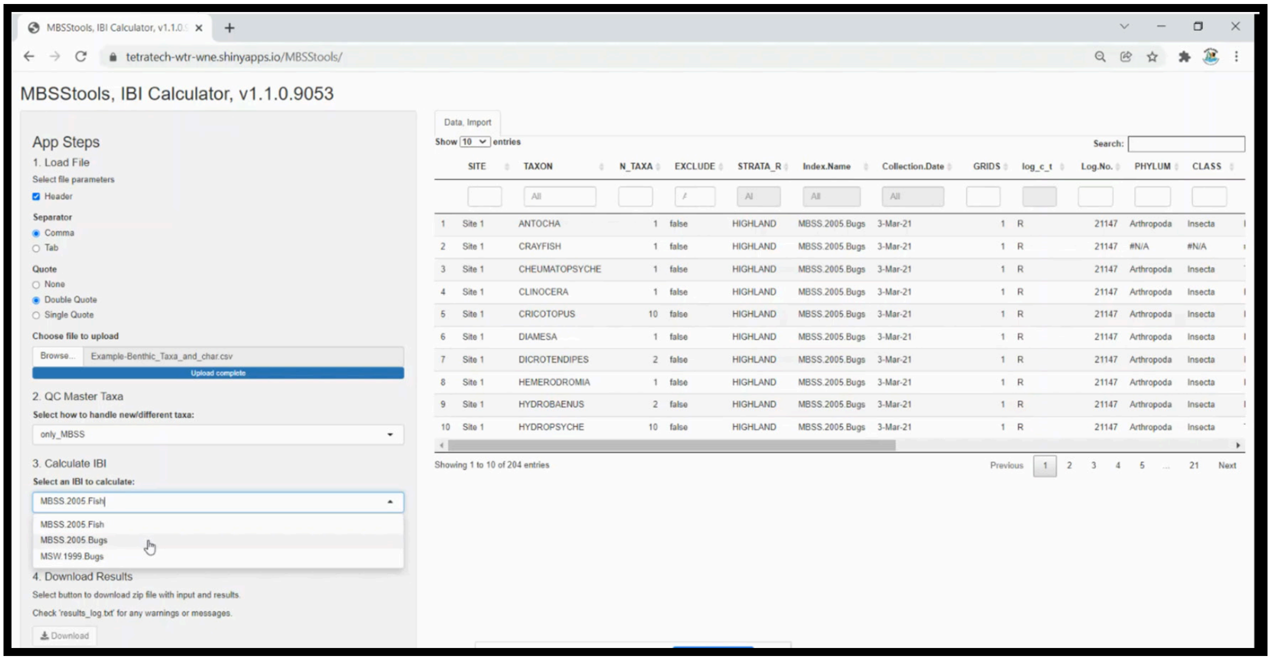
Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

	SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	Index.Name	Collection.Date	GRIDS	log_c_t	Log.No.	PHYLUM	CLASS
		All			Al	All	All					
1	Site 1	ANTOCHA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
2	Site 1	CRAYFISH	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	#N/A	#N/A
3	Site 1	CHEUMATOPSYCHE	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
4	Site 1	CLINOCERA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
5	Site 1	CRICOTOPUS	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
6	Site 1	DIAMESA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
7	Site 1	DICROTENDIPES	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
8	Site 1	HEMERODROMIA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
9	Site 1	HYDROBAENUS	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
10	Site 1	HYDROPSYCHE	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta

Showing 1 to 10 of 204 entries

<u>STEP 4</u>- The IBI Calculator will cross-reference the taxa designations to ensure that they are on the MBSS Master Taxa List (which is loaded into the MBSS Tools IBI Calculator) or a user-defined list, and will include the results of this cross-check in the output files. The user should select the appropriate cross-reference from the dropdown. This option does not affect the IBI calculation.



MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma
○ Tab

Quote

○ None
● Double Quote
○ Single Quote

Choose file to upload

Browse... Example-Benthic_Taxa_and_char.csv

Upload complete

2. QC Master Taxa

Select how to handle new/different taxa: only_MBSS

3. Calculate IBI

Select an IBI to calculate: MBSS.2005.Fish

- MBSS.2005.Fish
- MBSS.2005.Bugs
- MSW.1999.Bugs

4. Download Results

Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

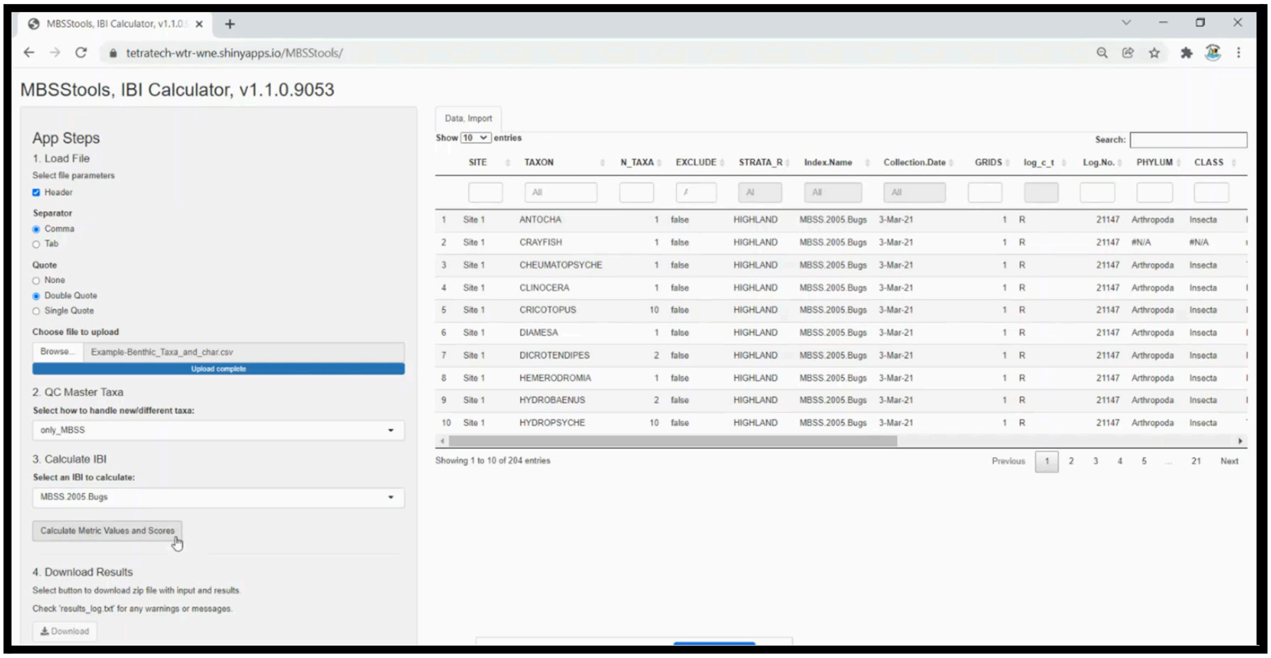
Data, Import

Show 10 entries

	SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	Index.Name	Collection.Date	GRIDS	log_c_t	Log.No.	PHYLUM	CLASS
1	Site 1	ANTOCHA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
2	Site 1	CRAYFISH	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	#N/A	#N/A
3	Site 1	CHEUMATOPSYCHE	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
4	Site 1	CLINOCERA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
5	Site 1	CRICOTOPUS	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
6	Site 1	DIAMESA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
7	Site 1	DICROTENDIPES	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
8	Site 1	HEMERODROMIA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
9	Site 1	HYDROBAENUS	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
10	Site 1	HYDROPSYCHE	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta

Showing 1 to 10 of 204 entries

STEP 5- The user must select whether to calculate the MBSS FIBI (MBSS.2005.Fish), MBSS BIBI (MBSS.2005.Bugs), or the family-level benthic index (MSW.1999.Bugs).



MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma
○ Tab

Quote

○ None
● Double Quote
○ Single Quote

Choose file to upload

Browse... Example-Benthic_Taxa_and_char.csv

Upload complete

2. QC Master Taxa

Select how to handle new/different taxa: only_MBSS

3. Calculate IBI

Select an IBI to calculate: MBSS.2005.Bugs

4. Download Results

Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

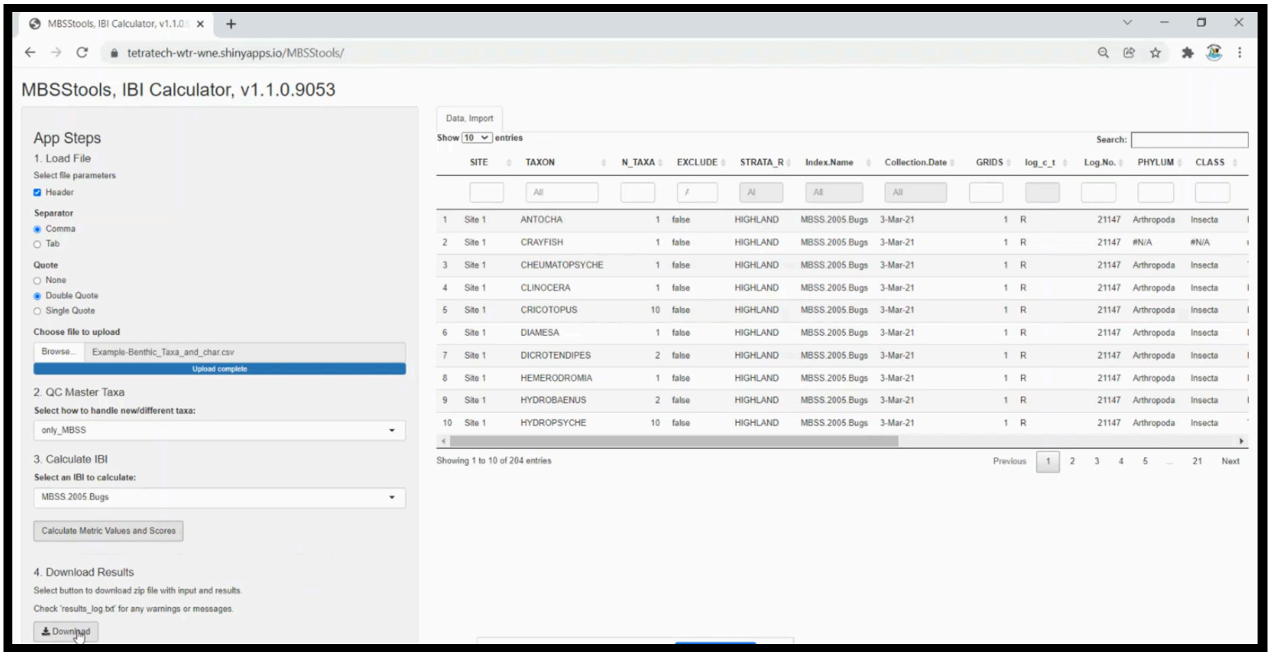
Data, Import

Show 10 entries

	SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	Index.Name	Collection.Date	GRIDS	log_c_t	Log.No.	PHYLUM	CLASS
1	Site 1	ANTOCHA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
2	Site 1	CRAYFISH	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	#N/A	#N/A
3	Site 1	CHEUMATOPSYCHE	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
4	Site 1	CLINOCERA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
5	Site 1	CRICOTOPUS	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
6	Site 1	DIAMESA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
7	Site 1	DICROTENDIPES	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
8	Site 1	HEMERODROMIA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
9	Site 1	HYDROBAENUS	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
10	Site 1	HYDROPSYCHE	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta

Showing 1 to 10 of 204 entries

STEP 6- Click "Calculate Metric Values and Scores." The software will then work through the appropriate algorithms. If the software crashes or becomes unresponsive at this step, it is likely due to an issue with formatting of the input file (e.g., missing or misnamed column headings).


MBSStools, IBI Calculator, v1.1.0.9053

App Steps

1. Load File

Select file parameters

☑ Header

Separator

● Comma
○ Tab

Quote

○ None
● Double Quote
○ Single Quote

Choose file to upload

Browse... Example-Benthic_Taxa_and_char.csv

Upload complete

2. QC Master Taxa

Select how to handle new/different taxa: only_MBSS

3. Calculate IBI

Select an IBI to calculate: MBSS.2005.Bugs

Calculate Metric Values and Scores

4. Download Results

Select button to download zip file with input and results.

Check 'results_log.txt' for any warnings or messages.

Download

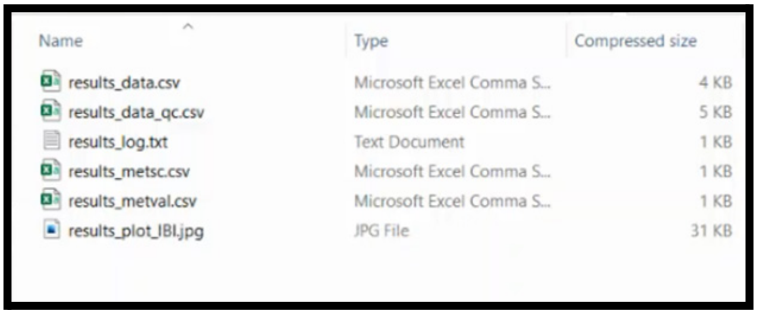
Data, Import

Show 10 entries

	SITE	TAXON	N_TAXA	EXCLUDE	STRATA_R	Index.Name	Collection.Date	GRIDS	log_c_t	Log.No.	PHYLUM	CLASS
1	Site 1	ANTOCHA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
2	Site 1	CRAYFISH	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	#N/A	#N/A
3	Site 1	CHEUMATOPSYCHE	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
4	Site 1	CLINOCERA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
5	Site 1	CRICOTOPUS	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
6	Site 1	DIAMESA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
7	Site 1	DICROTENDIPES	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
8	Site 1	HEMERODROMIA	1	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
9	Site 1	HYDROBAENUS	2	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta
10	Site 1	HYDROPSYCHE	10	false	HIGHLAND	MBSS.2005.Bugs	3-Mar-21	1	R	21147	Arthropoda	Insecta

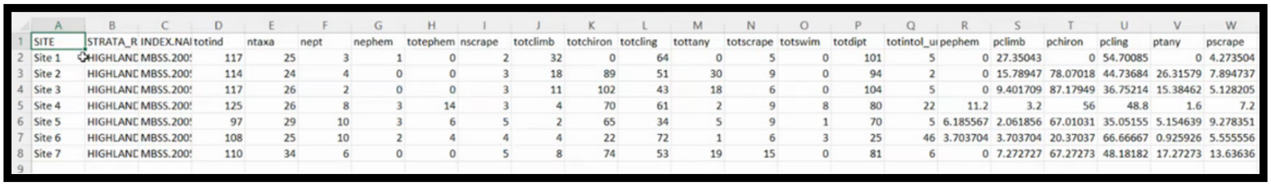
Showing 1 to 10 of 204 entries

STEP 7- Upon completion of the algorithm for calculating metric values and scores, the "Download" button will turn black. Click the "Download" button icon, and direct the software to the appropriate local directory for saving results.



Name	Type	Compressed size
results_data.csv	Microsoft Excel Comma S...	4 KB
results_data_qc.csv	Microsoft Excel Comma S...	5 KB
results_log.txt	Text Document	1 KB
results_metsc.csv	Microsoft Excel Comma S...	1 KB
results_metval.csv	Microsoft Excel Comma S...	1 KB
results_plot_IBI.jpg	JPG File	31 KB

STEP 8- Open or extract the downloaded *.zip file to view the files. Downloaded files include a copy of the data that were uploaded (results_data.csv) and the cross-reference (results_data_qc.csv), a summary of several basic checks on the data (results_log.txt), and the metric values (results_metval.csv) and scores (results_metsc.csv), as well as a plot summarizing the frequencies of IBI scores for samples in the dataset.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	SITE	STRATA_R	INDEX.NA	totind	ntaxa	nept	nephem	totephem	nscrape	totclimb	totchiron	totcling	tottany	totscrape	totswim	totdipt	totintol_u	pephem	pclimb	pchiron	pcling	ptany	pscrape
2	Site 1	HIGHLAN	MBSS.200	117	25	3	1	0	2	32	0	64	0	5	0	101	5	0	27.35043	0	54.70085	0	4.273504
3	Site 2	HIGHLAN	MBSS.200	114	24	4	0	0	3	18	89	51	30	9	0	94	2	0	15.78947	78.07018	44.73684	26.31579	7.894737
4	Site 3	HIGHLAN	MBSS.200	117	26	2	0	0	3	11	102	43	18	6	0	104	5	0	9.401709	87.17949	36.75214	15.38462	5.128205
5	Site 4	HIGHLAN	MBSS.200	125	26	8	3	14	3	4	70	61	2	9	8	80	22	11.2	3.2	56	48.8	1.6	7.2
6	Site 5	HIGHLAN	MBSS.200	97	29	10	3	6	5	2	65	34	5	9	1	70	5	6.185567	2.061856	67.01031	35.05155	5.154639	9.278351
7	Site 6	HIGHLAN	MBSS.200	108	25	10	2	4	4	4	22	72	1	6	3	25	46	3.703704	3.703704	20.37037	66.66667	0.925926	5.555556
8	Site 7	HIGHLAN	MBSS.200	110	34	6	0	0	5	8	74	53	19	15	0	81	6	0	7.272727	67.27273	48.18182	17.27273	13.63636
9																							

<u>STEP 9</u>- Review the downloaded files to ascertain completeness and accuracy. It is recommended that a subset of output values and scores are compared to manual calculations and/or a different computer algorithm.