

**Appendix F:**

**MAIS (Macroinvertebrate Aggregated Index for Streams) Manual**

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## MAIS (Macroinvertebrate Aggregated Index for Strams)

### What is it?

The MAIS is a rapid bioassessment protocol designed by researchers in Virginia in the late 1990's (Smith and Voshell 1997). It is used by several agencies in the eastern US, including the state of Virginia and the George Washington and Jefferson National Forest system as their primary rapid screening tool (including post-project monitoring). The Ohio EPA recognizes modified RBP protocols (Barbour et al 1999) and the MAIS as potentially useful Level II bioassessment protocols for ambient biological assessments. However, it should be noted that the MAIS has not been thoroughly tested in Ohio, and it is therefore be regarded as experimental until its performance has been thoroughly evaluated. It is currently recommended that the MAIS be restricted to evaluation of mine impacted streams in the WAP ecoregion until performance evaluation is complete.



MAIS scores are based on macroinvertebrates collected with a prescribed number of kick and dip net sweeps following RBP protocols described by Barbour et al. (1999). Organisms are identified to the family (rather than genus) level. Family level identifications require more training than order level (e.g. Ephemeroptera, Plecoptera, Trichoptera, etc.) but can be performed individuals with an intermediate level of skill. Investigators completing the taxonomic identifications should be trained and certified proficient in Level II stream assessment methods.

Once macroinvertebrates are collected, identified and enumerated, an MAIS index score ranging between 0 and 18 is generated from 9 aggregated macroinvertebrate metrics that describe the diversity and abundance of different groups (listed in table below). In the Mid Atlantic highlands, four narrative categories are assigned based on the scores: 0-7 = very poor, 8-11 = poor, 12-15 = good, 16-18 = very good. It has not been determined whether this calibration is appropriate for the WAP ecoregion; however, in the event that the index is recalibrated for the WAP, the actual numerical values of the scores will not change, so data collected in prior years will still be comparable.

Table 1. The nine biological metrics that comprise the final MAIS index score.

	METRIC	DEFINITION
1	EPT richness	Number of caddisfly, stonefly and mayfly families
2	# Ephemeroptera	Number of mayfly families
3	% Ephemeroptera	% abundance of mayflies
4	% 5 dominant taxa	5 most dominant taxa combined
5	Simpson Diversity Index	Integrates richness and evenness
6	Modified Hilsenhoff Biotic Index	Taxa are weighted by pollution tolerance
7	# intolerant taxa	# of families with tolerance values of 5 or less (very sensitive)
8	% scrapers	Abundance of macros that feed on periphyton
9	% haptobenthos	Abundance of macros that require clean, coarse, firm substrates

## Using the MAIS for long-term monitoring of abandoned mining impacts in southeastern Ohio

Detecting incremental improvements in biological quality requires repeated sampling, either in space or time, to account for normal variation that occurs in natural biological assemblages. Level III bioassessment protocols, which require sampling at least two of the three most common assemblages (fish, macroinvertebrates or periphyton) and taxonomic identifications by highly trained biologists are costly and time-consuming. Performing them on an annual basis is beyond the resources available for most projects.

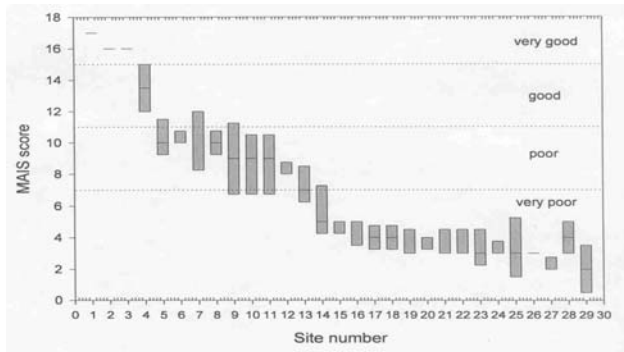


The MAIS is a simpler protocol that requires fewer trips to the site, relatively simple field methods, and an intermediate level of taxonomic training compared to the Level III bioassessments performed by Ohio EPA. It is expected to perform significantly better than Level I volunteer-based protocols like the Isaac Walton league or SQM protocols because the aggregate metrics were derived and tested in a more scientifically robust manner, and because family-level taxonomy provides higher resolution of the biological community than order-level.

### The MAIS as an indicator of Acid Mine Drainage

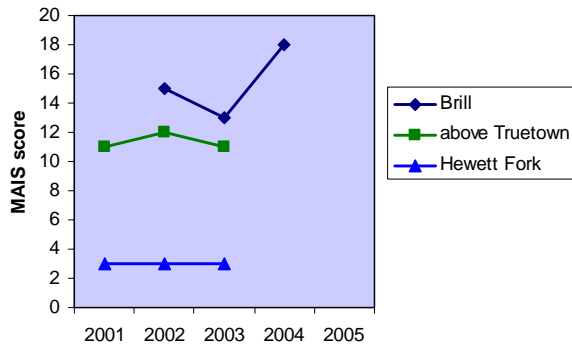
In a study involving 29 sites in three watersheds in southeastern Ohio, the MAIS index correlated well with water column pH ( $r = 0.78$ ), sulfate ( $r = -0.71$ ), conductivity ( $r = -0.40$ ), total Al ( $r = -0.67$ ) and total Mn ( $r = -0.67$ ) (Johnson et al. unpublished).

### The Importance of Repeated Sampling for Assessing Long Term Trends



When sites are sampled repeatedly (e.g. year after year), scores can be expected to vary by one or two points on the 0-18 scale, even when there has been no known change in stream condition. This type of variation is common in biological indices and underscores the importance of establishing a reliable baseline in order to detect change over time.

The figure to the left shows MAIS scores from 26 impacted sites that were sampled in 3 years and 3 unimpacted (very good) sites that were sampled once. Scores were generally similar from year to year, although some sites were clearly more variable than others. Nineteen of the 26 sites that were sampled multiple times were categorized the same each year (73%).

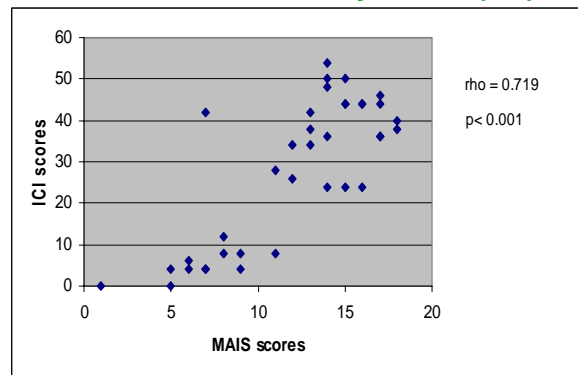


Such year to year variation in scores can be due to annual differences in rainfall and the hydrological regime, random variation in the distribution of organisms and the limitations of the field methods for capturing this variation, worker error (e.g. subtle differences in field sampling, laboratory processing and errors in macroinvertebrate identifications) as well as inherent “noise” in the precision of the metric. In the figure to the left, the decline in quality of Brill Run in 2003 correlated with the stream becoming intermittent due to low rainfall.

Standardizing field and laboratory processing methods helps minimize worker related variation so that real changes in biological quality over time can be more easily detected.

### How Does the MAIS correlate with the Ohio Invertebrate Community Index (ICI)?

In a recent side-by-side comparison at 37 impacted and unimpacted sites in southeastern Ohio, MAIS scores correlated well with Ohio EPA’s Level 3 ICI ( $r = .719$ ), but the correlation is not exact (Kinney, 2006). This is not surprising, given that the two methods involve slightly different methods of collection (direct from natural substrate versus artificial substrate samplers) and thus slightly different communities of organisms are used for index calculation.



The Ohio ICI is particularly responsive to changes in the midge community, whereas the MAIS is more broadly influenced by other taxa inhabiting the natural substrate on the stream bottom. In spite of these differences, the MAIS has comparable ability to detect differences between categories (good vs fair, for example) and is repeatable over time. Thus, it meets the primary goal of long-term monitoring, which is to detect improvement or degradation of the biological community over time (Kinney, 2006). The MAIS and ICI agreed 81% of the time when categorizing sites as meeting Warm Water Habitat criteria or not.

Finally, although the MAIS has been used in the Mid-Atlantic Highlands, it has not been fully *calibrated* for our ecoregion (Western Allegheny Plateau). This means that the exact numerical cutoffs for “very good” versus “good” designation may be a little different for this ecoregion compared to the Mid Atlantic Highlands. We are in the process of calibrating the MAIS against a set of minimally impacted sites previously designated by the Ohio EPA as representative of the reference condition in the eastern part of Ohio. In the event that the cutoffs for the four categories are adjusted, note that the actual numerical score would not be calculated any differently; thus, MAIS scores collected in 2006 can still be directly compared to those collected in 2005 or earlier years.

## FIELD METHODS

### Site Selection

Long term monitoring sites are generally selected based on their proximity to key confluences or projects, and ease of access. A few other considerations will help ensure a good biological sample:

1) Scout sites to make sure that there are **at least three riffles** present in a 150 meter stretch. Kick samples performed in riffle habitat produce the greatest diversity and abundance of macroinvertebrates. If the channel is deep and water slow-moving it will be difficult to take a kick sample at all. If runs (but not riffles) are present, the kick net can be used as long as the water velocity is high enough to carry organisms into the net, but the diversity and abundance of organisms will be low. If MAIS samples are collected from the same reach year after year, scores will still be comparable, but may not show improvement after remediation of AMD impacts because organisms may remain habitat-limited.



2) **Avoid sampling near bridges** (e.g. within 50 feet) because of unnatural alterations of channel morphology and flow.

3) At sites where the streambed is wide and flat, the water course may meander and carve new sub-channels during high flow events. **Rain events can produce temporary shallow riffles** that quickly dry out when flow drops again. Although these can house transient organisms, the fauna will not be as rich as in a permanent riffle. If sampling after rain events, make sure you locate the kick net in riffles associated with the deepest channels, as they are most likely more permanent.

### When to sample - seasonal index period

July is the ideal month for collecting, although anytime during the index period (June 15 – Sept 15) is acceptable. Spring and fall macroinvertebrate communities differ in their composition and may not be comparable. In our area, flows in some streams become so reduced by late August that sampling by kick net is difficult.

### Kick net and Dip net Sampling Procedures

Macroinvertebrates are collected natural substrate via two methods:

- 1) Kick net (3 riffles per site)
- 2) D-ring dip net (20 “jabs” in all available habitats)

The kick net sample is the most quantitative, and provides information on both diversity and relative abundance of organisms in riffle habitat. The dip samples represent many different habitats (edges, root wads, woody debris, leaf packs, and pool bottoms) and yield a different assemblage of organisms than the kick.

### **Labeling jars**

Although information from kick and dip samples are combined to calculate the final biological index, you should keep the samples separate during collection, sorting, enumeration and identification stages. Thus, from each site you will have one jar of specimens from the three kicks, and one jar of specimens from the 20 jabs.

Jars should be carefully labeled with the date, site number/name, collecting method and initials of the team leader before you begin sampling. 70% ethanol is used to preserve organisms. Remember that water loosens tape and 70% alcohol can wash off some ink when sloshed out of jar. Sharpies work well. Label the body of the jar, not the lid, as lids can easily get mixed up.

### **Filling out field sheets**

A new field sheet should be filled out each year, even though at many of the long-term monitoring sites, physical habitat may have already been recorded (e.g. QHEI). However, high flow events in spring can shift substrate, and beaver dams, dumping of garbage, or changes in riparian characteristics can significantly alter flow from year to year. A quick sketch of the stream channel and notes on substrate and flow should be recorded on the **field sheet** for each site (Copy of field sheet in Appendix B). For groups returning to the site in later years, it is especially helpful to note how far apart riffles are, and whether riffles are permanent or made of woody debris. Woody debris riffles should only be sampled if no other hard substrate riffles are available, as they are temporary and may not be present in subsequent years.

### **Kick net procedure**

First, survey the stream reach and identify three riffles to sample. Ideally, you will be sampling about 150 meters of reach, but sometimes you may have to walk upstream/downstream in search of riffles. When using the kick net, one person holds the net while another disturbs the substrate in a one meter square area. It helps to mentally delineate the sides and corner of the area before you begin kicking to make sure you cover it all. Slide your feet from side to side and disrupt the entire stream bed. Be thorough in covering the entire area, although you don't have to go deep (a few cm will suffice). Large boulders can be lifted by hand and shaken. It may take both people to lift the net carefully so that material does not slip out.

**Tip:** its fine to include small plant mats and leaf packs (in fact it's recommended), but avoid large ones, as they will add considerably to the time required for picking/sorting.



**Three options for picking/processing the collected organisms:**

Different groups will be under different constraints in terms of field time and number of available personnel. An ideal arrangement is for two people to dedicate a full day to field and lab work; in our experience the team can comfortably sample, sort and preserve specimens from 4-6 sites per day, depending on driving distance. Options 1 (“field pick”) and 2 (“live pick at the lab”) are faster and easier than the Option 3 (preserve all and pick later).

**1) On site picking**

Field-pick all organisms directly out of nets. Place specimens in a small jar of 70% ethanol.

**2) “Live” pick in lab later the same day**

Wash collected material (rocks, leaves, debris) into a tub, sieve it, and store in 1 qt containers of stream water. Store large containers in ice cooler until they can be “live picked” at lab later in the day.

**3) Preserve in ethanol and pick weeks later**

Preserve all material (leaves, rocks, debris) in 70% ethanol after sieving. Material can be sorted weeks or months later.

**Option 1) On-site picking** – This method works well if there are 2 or 3 people on the field team, or if sites are impacted and organisms are not abundant. A piece of plastic (an old shower curtain works well) should be laid on the ground under the kick net to capture any macroinvertebrates that wiggle through the mesh. Spend up to 20 minutes studying the net, removing stones and organic debris as you go. Check each piece for cryptic taxa. Riffle beetles, damselfly nymphs, mollusks and some small diving beetles, in particular, are often overlooked because they move slowly. Collect everything that is visible to the naked eye (midge larvae, oligochaetes, freshwater shrimp, mollusks, true worms, crayfish etc.).

Since all the crayfish in our area belong to one family (Cambaridae) and don’t differ in their tolerance to most types of impairment, you may elect to collect 2 or 3 representative specimens, and release the rest unharmed. Just be sure to record the number released!

Note: At high quality sites, the kick net may contain up to 300 organisms and take up to an hour to pick thoroughly! Under these conditions, you may want to consider picking only ½ of the net each time. Choose the left or right half before you begin picking, and pick one side thoroughly. Be sure to sample all three riffles...do not just sample one riffle and call it quits! Be sure to note the manner of subsampling on the field sheet.

**Option 2) Live-picking at the lab later in the day** – This method is ideal if you have limited field time or helpers, or during inclement or buggy weather, because you can visit sites in the morning and spend afternoons picking and sorting. Instead of picking organisms off the net, they are washed with a pail into a large tub. We use a 20 gallon Tupperware tub. Remove larger debris (sticks, large rocks, leaves) by hand (checking for attached macroinvertebrates), then concentrate the catch by pouring the tub contents through a 600 µm sieve. Material can

be placed in large containers (one quart size usually works), covered with stream water and kept in an ice cooler until transported back to the lab for live picking later in the day.

It is recommended that samples be picked **the same afternoon or evening**, as predators remain active (even when refrigerated) and can eat other specimens.

The identity of the picker(s) back at the lab should be recorded on the field sheet. A subsample (10% of the samples) should be double-checked by a supervisor for QA/QC.

**Option 3) Preserve material in ethanol for later sorting (e.g. weeks later)** – This method is the most time-consuming, since organisms are more difficult to see when preserved in alcohol than when live and wiggling. However, if enough time is devoted, fewer small organisms (such as midges) are overlooked. It requires more ethanol and a place to store jars, and be aware that picking should be done in a well-ventilated area to minimize inhaling fumes from the ethanol.

Follow procedure for picking in the lab later, but place sieved material from kick net into quart jars with 70% ethanol instead of stream water. Note that if there is a lot of organic debris (which retains water), the ethanol may become diluted. It will then need to be decanted and replaced with new 70% at the lab within a day or so to prevent decay.

The identity of the picker/sorter(s) should be recorded on the field sheet. A subsample (usually 10%) of the samples should be double-checked by a supervisor for QA/QC.



### Dip Net Procedure

The dip net samples are considered a type of multihabitat sampling. The goal is to effectively **sample all the different habitats you see in roughly the proportion that they occur.**

Dip netting is a little more difficult for beginners because it requires some knowledge of the different types of microhabitats that might be present (pools, woody debris, leaf packs, root overhangs, grassy edges, etc) and which taxa typically inhabit them. Not all microhabitats are present at all sites, or in the same proportions, so collectors rely on individual judgment more than with kick net sampling.

A good approach is to walk slowly upstream or downstream, taking a “jab” every 6 to 10 feet as you encounter new types of microhabitats, until you have completed 20 jabs. You can examine and pick the contents of the net yourself after each jab if you carry a jar of ethanol in your pocket and forceps in a pocket or on a string around your neck. Or, empty the contents of each jab into a white pan and let an assistant on the bank pick organisms out. If field time is limited, the same options for picking later in the day, or preserving in 70% ethanol for picking weeks later could be used. A thorough dip net sample typically takes at least 45 minutes to collect.

A “jab” might consist of a jiggly two foot sweep of the surface of a sandy reach, a 10” pass through a leaf pack, or a 10 second scraping of root wads on the bank. Skim the surface (top 3 cm) of the substrate; don’t use the net like a shovel!



*Some typical stream microhabitats to look for :*

Leaf packs in riffles	Woody debris (knock and scrape)
Leaf packs in pools	Root wads hanging from bank
Sandy/coarse substrate runs	Mounds of grass/grassy margins
Silty areas/pool bottoms	Moss or other vegetation
Sides of large rocks	

### A word about ‘non-benthic’ macroinvertebrates

Occasionally questions arise regarding whether to include taxa that are aquatic but not benthic (such as whirligig beetles, water striders, some diving beetles, etc) or flying adults that have a benthic stage (like adult midges, dragonflies, or adult mayflies seen on the surface of the water). Our recommendation is to include all aquatic insects, whether or not they are technically benthic, even though they don’t contribute a lot to the MAIS score (most have a ‘neutral’ weighting in the index). However, flying adults should not be collected, since they could have easily emigrated from other sites.

## LABORATORY METHODS

Once samples are transported back to the lab, they should be double-checked to make sure that the jar labels are legible and contain the necessary information. Field sheets should be checked for completion. If additional picking or sorting is conducted in the lab, the identity of the picker should be recorded on the field sheet and 10% of the samples double-checked by a supervisor for QA/QC.

### Identification of Macroinvertebrates

The MAIS score is derived from family level identifications, which are easier for “non-experts” to master than genus/species level identifications, which can take years of training. Family level identifications certainly take longer than a day to master, but a dedicated person can become fluent at the family-level after 6-8 hours of instruction and a week or two of practice with a good lab manual.

A stereo microscope and some reliable taxonomic references are needed for family-level identifications. Note that the Ohio EPA offers periodic training and certification for Level II bioassessment protocols.



### Laboratory data sheets

In addition to the field sheet for each site, we recommend two other data sheets be associated with each site. The **Macroinvertebrate Identification sheet** contains handwritten notes made while identifying and enumerating taxa and the **Site Data Summary sheet** contains the final list

of taxa and their abundances in the Kick and Dip samples that will be entered into a spreadsheet later.

### 1) Macroinvertebrate Identification Sheet

Even experienced taxonomists often make notes on the characters of more problematic taxa to leave a clear record of how they arrived at the final identification. It is especially important that beginners leave this type of record, because it makes it easier to keep up with specimens that are separated on the basis of 3 or 4 characters, not just one, or specimens that will need a second opinion to verify.

An important piece of information to record for **each** specimen is the **identity of the person who identified the organism**. There may be instances where some identifications were made by a beginner and the more difficult taxa identified by someone more experienced. There should be a clear record of this. Systematic misidentifications sometimes occur, even with experienced taxonomists, and keeping records will help locate misidentified specimens later should the need arise. A sample Identification Sheet is included in Appendix C.

### 2) Site Data Summary sheet

This sheet summarizes the taxa found at each site in the kick and dip samples, and their abundances. It contains the raw data that will be entered into the MAIS EXCEL sheet to calculate the final index score. A sample Site Summary sheet is included in the Appendix D.

## DATA ENTRY AND CALCULATION OF MAIS SCORES

Once the families in the sample have been counted and identified on the Site Summary sheet, the data is typed into an EXCEL worksheet template that will calculate the MAIS score for that site.

Step 1. Open a blank master EXCEL worksheet. There are three sheets in the xls file:

- 1) **Data worksheet** –enter raw abundances
- 2) **Metrics** – where metrics and final score appear
- 3) **Scrap** – used to calculate one metric (% 5 dominant)

Step 2. On the **Data worksheet**, enter the abundance of each taxa (total collected in kick and dip net samples) in the third column. Taxa names, tolerance values, functional feeding group information are already entered into the spreadsheet.

Step 3. You must manually calculate one of the metrics, % 5 dominant, using the **Scrap** worksheet. To do this, return to the **Data Worksheet** and copy column C. Paste column C into the first column of the **Scrap** sheet and use the data sort option to sort the abundances in descending order. The **Scrap** sheet will automatically calculate % 5 dominant and insert the value on the **Metrics** sheet.

Step 4. Return to the Metrics sheet, where the MAIS metrics, final MAIS score and condition category are now calculated.

## REFERENCES

Barbour, M. T., J. Gerritsen, B. D. Snyder and J. B. Stribling. 1999. Rapid Bioassessment Protocols for use in streams and wadeable rivers: periphyton, macroinvertebrates and fish. 2<sup>nd</sup> edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D. C.

Kinney, C. J. 2006. A comparison of two methods of bioassessment in streams. Masters thesis, Environmental Studies Program, Ohio University.

Smith, E. P. and J. R. Voshell. 1997. Studies of benthic macroinvertebrates and fish in streams within EPA Region 3 for development of Biological Indicators of Ecological Condition. Part 1, Benthic Macroinvertebrates. Report to U. S. Environmental Protection Agency. Cooperative Agreement CF821462010. EPA, Washington, D.C.

## APPENDIX A.

### Field Equipment Checklist:

- map and/or directions to site
- Field notes sheets
- kick net
- dip net
- featherweight forceps (extra pair always a good idea)
- waterproof markers and tape
- waders
- insect repellent, sunscreen, water, etc.

### Option 1 (on site picking)

- two 4 – 8 oz jars per site
- piece of plastic or shower curtain
- white pan for sorting dip samples
- 70% ethanol (1/2 to 1 gallon)

### Option 2 (live-picking back at lab)

- large tupper ware tub (20 gal)
- extra pail for washing net
- brass sieve
- large containers (1 qt to 1 gallon, one per site)
- small jars (4 – 8 oz, one per site)
- ice cooler
- white pan for sorting dip samples (optional)
- 70 % ethanol (1/2 to 1 gallon)

### Option 3 (preserving material for later picking)

- large containers (1 qt to 1 gallon, one per site)

APPENDIX B. Field Note Sheet

## APPENDIX C. Macroinvertebrate Identification Sheet

Site number/code \_\_\_\_\_ Date sampled \_\_\_\_\_

Watershed \_\_\_\_\_ Date identifications made \_\_\_\_\_

Page \_\_\_\_\_ of \_\_\_\_\_

Specimen or taxa description	Notes, identification and identifier's initials



## APPENDIX E. List of taxa (families) commonly collected in Western Allegheny Plateau streams