

**MISSOURI DEPARTMENT OF NATURAL RESOURCES
 DIVISION OF ENVIRONMENTAL QUALITY
 ENVIRONMENTAL SERVICES PROGRAM
 Project Procedure**

TITLE: Semi-Quantitative Macroinvertebrate Stream Bioassessment

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 personnel who conduct aquatic biological assessments of
 wadeable streams in Missouri using benthic
 macroinvertebrates.

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1.0 Introduction

The purpose of this document is to provide guidance to Water Quality Monitoring Section (WQMS) personnel for the field collection and preservation, laboratory processing, identification and recording, and data analysis of semi-quantitative macroinvertebrate samples used in the biological assessment (bioassessment) of Missouri's wadeable streams and rivers. Any deviation from this procedure should be documented in the final report, explaining the reason for deviation and its possible effects on the data. Whenever possible, technical support documents are cited for consultation.

Minimum qualifications of individuals who perform assessments described in this procedure should be a Bachelor of Science in a biological or related field, along with at least one year of training in methods and taxonomy with a senior aquatic biologist.

2.0 Field Methods

2.1 Stream Habitat Assessment

A stream habitat assessment is conducted at each macroinvertebrate sampling location. The purpose of a habitat assessment is to evaluate the ability of streams to support comparable macroinvertebrate communities. See the *Stream Habitat Assessment Project Procedure* (MDNR 2010c) for further explanation and blank data forms.

2.2 Length of Stream Reach Sampled

All macroinvertebrate sampling is done in a stream reach approximately 20 times the average width of the stream, measured at the top of the lower bank as defined in the Stream Habitat Assessment Project Procedure. According to Hynes (1970), this length of stream will normally encompass approximately two riffle sequences (10 to 14 stream widths) or two meander sequences (14 to 20 stream widths). Rabeni et al. (1999) compared multiple reaches of stream that were 20 times the stream width to judge the adequacy of the sampling reach. In only 6% of the possible cases was the coefficient of variation for any metric reduced by >10% by sampling additional reaches. Results concluded that a single well-chosen reach is adequate for sampling macroinvertebrate communities and, depending on the potential impairment, a single reach can be representative of an entire stream segment. If more accuracy is needed, two comparable reaches within three stream miles of each other will be sampled to characterize the aquatic community.

2.3 Collection and Preservation of Samples

These multi-habitat sampling methods are designed to be used in permanently flowing wadeable streams. Wadeable streams are defined as having an average depth less than 1.5 meters. If necessary, these sampling procedures can be adapted for use in the accessible, shallow portions of larger streams. Sampling should be done only when flow and depth conditions do not impair the ability of the investigator to effectively collect organisms from the targeted habitats or

threaten the safety of the individual. Ideally, sampling efforts should be carried out during periods of stable base flow before peak aquatic insect emergence times. In Missouri the sampling periods are from mid-March through mid-April and from mid-September through mid-October. For the purpose of this document, Missouri has two stream types:

- 1) Streams with riffle/pool predominance are found primarily in the Ozark aquatic region of Missouri, but they are also found in some portions of the Prairie region (Missouri Resource Assessment Partnership 2000). A typical and characteristic feature of a riffle/pool stream type is a repeated and regular frequency of riffles. Riffles typically form every 7-10 stream widths. The three predominant habitats sampled for riffle/pool streams are: a) flowing water over coarse substrate; b) non-flowing water over depositional substrate; and c) rootmat substrate.
- 2) Streams with glide/pool predominance are found in the Prairie and Mississippi Alluvial Plains aquatic regions of Missouri (Missouri Resource Assessment Partnership 2000). Glide/pool stream types generally have a repeated and predictable meander sequence. Pools typically form immediately after a bend. The three predominant habitats sampled for glide/pool streams are: a) non-flowing water over depositional substrate; b) large woody debris substrate; and c) rootmat substrate.

Samples from each major habitat are collected separately to provide the ability to factor out habitat differences between sites. Each habitat sample is collected and placed into one-liter containers or quart-sized jars (see Section 2.3.1). An external sample label includes a sample number, habitat, and number of jars for that habitat (e.g., 1 of 3) and is placed on the lid of each container. A waterproof processed label, including stream name, station number, county, map coordinates, collection date, habitat, and sample number is inserted into the sample jar. At this time, all data are recorded in a field notebook, and an MDNR-ESP-002 chain-of-custody form (MDNR 2010a) is completed to accompany the sample to the laboratory. For a list of suggested field equipment, see Appendix A.

Quality control of collection and preservation of samples is discussed in 7.1.

2.3.1 Sampling Riffle/Pool Predominant Streams

Flowing water over *coarse substrate* samples are taken in riffles and runs, which typically have a coarse bottom substrate mixture of gravel and cobble. Riffles are shallow, turbulent stream segments with higher gradients than pools or runs. Runs are moderately shallow stream channels with laminar flow lacking pronounced turbulence. A total of six collections from a variety of depth, current velocities, and coarse substrate mixtures should be sampled using a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix A). Initially all large pieces of coarse substrate should be brushed off in a manner that allows the current to carry organisms into the net. The remaining substrate is then disturbed to a depth of 15-25 cm by using a foot shuffling action or through the use of a three-pronged hand cultivation tool. Each disturbance area shall be approximately one square meter. A composite of the six collections is made by emptying each kick net into a 16 x 23 inch or larger plastic pan. After each collection, the net is checked for clinging organisms that are added to the composite sample.

Field processing the sample is accomplished by removing the material from the large plastic pan, placing it into the sample containers, and then preserving the material in the sample containers. A plastic putty knife may be used initially to remove large amounts of material from the large plastic pan, and to place that material into the sample containers. The material remaining in the large plastic pan is poured through a brine shrimp net and placed into the sample containers. Water may be added to the large plastic pan to allow all small debris to be flushed through the brine shrimp net. Make sure that sufficient space remains in the sample containers for preservative. All samples are transported to the vehicle where the sample containers are filled with a 10% formalin solution buffered with calcium carbonate.

Non-flowing water depositional substrate samples are taken from depositional areas, usually formed when water current drops to low velocities. This reduced velocity results in substrate deposits of fine sediment and particulate organic matter that are no longer held in suspension. Because water movement in these areas is not visually discernible, the water is categorized as non-flowing. A composite sample of six collections from a variety of depositional depths and microhabitats (e.g., backwater, near shore, fore waters, in channel pools, etc.) are collected with a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix A). Each sample is taken from an approximately one square meter area of substrate using a traveling kick method. To accomplish this method, the substrate is disturbed by the collector's feet to a depth of 15-25 cm while sweeping the net back and forth immediately over the substrate to collect organisms that are suspended in the water column. A composite of the six collections is made by emptying each kick net into a 16 x 23 inch or larger plastic pan. After each collection the net is checked for clinging organisms, which are added to the composite sample. Water is added to the composite sample, and all large pieces of debris are vigorously washed, inspected for clinging organisms, and discarded. The remaining sample is processed as previously described in Section 2.3.1.

Rootmat substrate samples are submerged roots from terrestrial vegetation, which are important habitat and sources of refuge for aquatic organisms. Rootmat is best defined as the immersed portion of fine fibrous roots of woody vegetation that are found along the bank. Collections are made from six distinctly different areas along the sampling reach. Each collection is made from approximately one meter of shoreline exhibiting good quality rootmat. Sampling is accomplished by using a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix A). If current is present, the net is placed so that the substrate can be disturbed by a kicking action, which causes the organisms to be swept into the net. If no current is present, the net is placed under the substrate and shaken vigorously, causing any clinging organisms to fall into the net. A composite of the six collections is made by emptying each kick net into a 16 x 23 inch or larger plastic container. Water is added to the composite sample, and any large debris is vigorously washed, checked for clinging organisms, and discarded. The remaining sample is processed as previously described in Section 2.3.1.

2.3.2 Sampling Glide/Pool Predominant Streams

Non-flowing water depositional substrate samples are taken from depositional areas formed when water current drops to low velocities, resulting in deposits of sediment and particulate organic matter that is no longer held in suspension. Because water movement in these areas is not visually discernible, the water is categorized as non-flowing. Six collections from a variety of depositional depths and microhabitats (e.g., backwater, near shore, fore waters, in channel pools, etc.) are collected with a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix A). Each sample is taken from approximately one square meter area of substrate using a traveling kick method. To accomplish this method, the substrate is disturbed by the collector's feet to a depth of 15-25 cm while sweeping the net back and forth immediately over the substrate to collect organisms that are suspended in the water column. A composite of the six collections is made by emptying each kick net into a 16 x 23 inch or larger plastic container. After each collection, the net is checked for clinging organisms, which are added to the composite sample. Water is added to the composite sample, and all large pieces of debris are vigorously washed, inspected for clinging organisms, and discarded. The remaining sample is processed as previously described in Section 2.3.1.

Large woody debris (also known as “snag”) substrates are submerged portions of large logs as well as tree branches greater than one inch in diameter. A composite sample of 12 collections is made from different pieces of woody debris. The pieces of woody debris selected should represent a variety of conditioned wood types, sizes, water depths, and velocities. The sampling area on each piece of woody debris is an area of approximately 400-600 square centimeters. Organisms associated with the large woody debris and associated growths of periphyton or moss are collected by using a hand scrub brush and a nitex bag with dimensions of 44 centimeters wide by 50 centimeters deep. The snag bag is made by folding a 46 centimeter wide by 102 centimeter long piece of 500 x 500 micron mesh nitex cloth in half. The sides are folded over 10 centimeters and sewn together. Each edge at the top is also folded and sewn for extra strength.

The sampling of woody debris usually requires two people. When possible, large woody debris is gently lifted off the stream bottom and slid into the bag by one individual while the other individual holds the bag open. The wood and bag can then be tilted to vertical after which the first individual holds and brushes the wood while the second individual continues to hold the bag open. Woody debris too large to lift can be sampled using different strategies depending upon water velocity. Both strategies require one individual to hold the bag opening open while molding one side of the bag to fit the contour of the wood. If water current is present, the bag is placed immediately downstream from the sampling area such that the current carries organisms into the bag. When there is no natural current available, an artificial current can be created by repeatedly sweeping the brush along the log only in the direction of the bag opening. When the 12 collections have been made, the sample is processed by concentrating the material into one corner of the bag by splashing the outside of the bag with water. The corner of the bag and concentrated material can then be grasped and inverted into a sample jar. Any material remaining on the bag is rinsed in the plastic pan as previously described in Section 2.3.1.

Rootmat substrate samples are submerged roots from terrestrial vegetation, which are important habitat and sources of refuge for aquatic organisms. Rootmat is best defined as the immersed portion of fine fibrous roots of woody vegetation that are found along the bank. Collections are made from six distinctly different areas along the sampling reach. Each collection is made from approximately one meter of shoreline exhibiting good quality rootmat. Sampling is accomplished by using a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix A). If current is present, the net is placed so that the substrate can be disturbed by a kicking action, which causes the organisms to be swept into the net. If no current is present, the net is placed under the substrate and shaken vigorously, causing any clinging organisms to fall into the net. A composite of the six collections is made by emptying each kick net into a 16 x 23 inch or larger plastic container. Water is added to the composite sample, and any large debris is vigorously washed, checked for clinging organisms, and discarded. The remaining sample is then processed as previously described in Section 2.3.1.

3.0 Laboratory Processing of Samples

Once samples are collected and preserved, they are stored at room temperature (approximately 25°C) and processed within 90 days. Laboratory processing includes subsampling macroinvertebrates to a designated number of organisms based on habitat type, and sorting these organisms into vials for later identification.

Past studies have provided guidance to determine the most suitable number of organisms to compose a representative sample. A pilot study in North Carolina compared 100-organism versus 300-organism sub-samples (Plafkin et al. 1989). It was determined that 100 organisms were adequate for making a good evaluation of water quality at the family level of identification. A 100-organism sub-sample has also proven adequate in numerous other studies for impact detection (Hilsenhoff 1982, 1987; Nuzzo 1986; Bode 1988). Because of these publications, the earliest draft versions (1994) of this procedure recommended a 100-organism sub-sample. Later analyses of MDNR data have found results to be more consistent with greater numbers of organisms in the sub-sample, independently arriving at fixed count numbers similar to recommendations found in publications such as Vinson and Hawkins (1996), Barbour and Gerritsen (1996), and Larsen and Herlihy (1998).

The designated target number of organisms to be sub-sampled is based on habitat type. For flowing water over coarse substrate habitat, 600 organisms (+/- 10%) are sub-sampled. Depositional, woody debris, and rootmat habitat subsamples consist of 300 organisms (+/- 10%). A sub-sampling method from Plafkin et al. (1989), modified by Caton (1991), is used to allow rapid isolation of the target number of organisms.

The sub-sampling method includes using a rectangular shaped sieve (Appendix B). The sides are high enough to retain the sample. The bottom of a sieve must have a 500 x 500 micron mesh screen cloth to retain the macroinvertebrates and evenly drain the sample. The sample sieve is subdivided into equal size squares, and the squares are numbered.

The sample is transferred from the sample jars into the sieve and rinsed with water to flush away the formalin preservative. Any large debris is scrubbed, rinsed, and removed. The sieve and sample are placed into a larger plastic pan filled with water to a level just above the sample. Keeping the plastic container and sieve level, a figure eight stirring method randomly, yet evenly, distributes the sample. Once the sample is evenly distributed within the sieve, it is quickly lifted from the larger pan and drained of excess water.

A random number generator is used when selecting a numbered square to be sub-sampled. The ends of two one-inch wide flat spatulas, or putty knives, are used to outline the dimensions of each grid square and to remove the sub-sample contents from the outlined square. The sub-sample is placed into a marked container, such as a plastic cup labeled "sample," prior to sorting.

To sort organisms from debris, place small amounts of the sample into a counting wheel similar to a Wards Zooplankton Counting Wheel sold by Wildco, 301 Cass Street, Saginaw, Michigan. A sorting wheel can be made from cultured marble countertop material that is available from local manufacturers. The wheel can be made by the manufacturer or a local contractor using a computerized router. The trough diameter and width can be calibrated to maximize visibility for each individual dissecting microscope at 10x magnification. The depth of the trough is designed to accommodate the maximum amount of sample that will be visible under 10x magnification. As organisms are separated from the debris, entries are made on a counter, and specimens are sorted into one of two vials filled with 80% alcohol. Each vial contains a processed label (as used in the sample jars) that includes stream name, station number, county, map coordinates, collection date, habitat, and sample number. One vial contains slide-mountable organisms (Chironomidae and Oligochaeta) and is labeled as such; the second vial contains the remaining organisms and is considered the non-mountable organisms vial. Additional squares are randomly selected and sorted until the target number of organisms is reached (+/- 10%). Once sorting of a square is started, the sub-sample square must be completely sorted even if the number of organisms surpasses the target number. If half of the sample is processed, and the target number of organisms has not been reached, sorting can be terminated. The number of organisms, percent sub-sampled, and sorter's initials are recorded on the Laboratory Sub-sampling Form (Appendix C).

When sub-sampling has been completed, any remaining debris is searched for large and/or rare taxa. Large and/or rare taxa are any readily visible organisms (excluding Chironomidae) not found during the sub-sampling process. They are best located by placing one handful of debris at a time in a large white pan with enough water to cover the debris. The pan should be very well illuminated. When large and/or rare organisms are found while picking through the debris, they are removed and placed in a separate labeled vial identified as Large/Rare (L/R). Therefore, there is a potential for three vials per habitat.

Quality control of laboratory-processed samples is discussed in Section 7.2.1.

4.0 Identification and Recording of Organisms

4.1 Identification

Identifications are made to the lowest possible taxonomic level (usually genus or species). The Standard Operating Procedure MDNR-ESP-209 *Taxonomic Levels for Macroinvertebrate Identification* (MDNR 2010d) details the identification level and contains the taxonomic references required for macroinvertebrate identification. A reference collection (described in 7.2.2) may be used to verify the identification of most stream-dwelling aquatic organisms found in Missouri.

After non-mountable organisms are identified, they are placed in a vial filled with 80% ethyl alcohol and one internal label (stream name, county, map coordinates, collection date, habitat, and sample number) and temporarily stored for at least three years. Mountable Chironomidae and Oligochaeta are permanently mounted for identification on microscope slides with CMCP-10 or comparable mounting media. All slides are labeled using an indelible marker with the stream name, sample number, and habitat. After a sufficient drying and clearing time, organisms are identified using a compound microscope capable of 1000x magnification. Slides not retained for the reference collection (Section 7.2.2) are discarded.

4.2 Data Recording

4.2.1 Laboratory Bench Sheets

For each sampling station, a Laboratory Bench Sheet (Appendix D) containing taxonomic identification, date, analyst, sample number, station number, location, and columns for organism enumeration in four habitats (covers riffle/pool or glide pool requirements; see Section 2.3) is completed. Large/rare taxa are recorded as present with a designation of -99. Each taxon is listed only once in the left-hand column. The number of each taxon for each sample habitat type is recorded in the appropriate column on the lab bench sheet. The lab bench sheet is constructed with a flexible format to enable the analyst to use the composite data or to examine data from individual habitats. The bench sheet also contains a column for quality control for each habitat. Quality control is explained in Section 7.2.2.

4.2.2 Electronic Data Recording

The raw data from the lab bench sheets are entered into an electronic database. The data are organized by column and row headers. Each column header is a label for both a single habitat and stream location. In addition, each stream has a column included for the total of the three potential habitats. Each row header is a label for a distinct macroinvertebrate taxon. Metric values are calculated through the functions present in the software. It is important to note that large/rare taxa will be used only in the calculation of Taxa Richness and the Ephemeroptera/Plecoptera/Trichoptera (EPT) Taxa Index. There is a distinct advantage to the flexibility provided by keeping each habitat distinct and separate from all others. The investigator may examine metric values within specific habitats to identify areas of impairment.

Quality Control (QC) procedures for identification and recording of data are discussed in Section 7.2.2.

5.0 Data Analysis

There are several benefits of multi-habitat sampling over single habitat sampling. Lenat (1988) reported that more taxa were collected using the multi-habitat method, as would be expected from a greater number of samples from a variety of habitats. More importantly, taxa richness data produced by the multi-habitat method were less variable than taxa richness data from single habitat samples, and between site differences were more significant. Greater difference between sites improves the ability to discriminate degrees of impairment.

In the Environmental Protection Agency's Rapid Bioassessment Protocol (Plafkin et al. 1989), eight metrics were proposed for macroinvertebrate community analysis. Barbour et al. (1992) evaluated these eight metrics and others for redundancy and variability. Results from this evaluation suggest that the most reliable metrics are Taxa Richness, EPT Taxa, and Biotic Indices. Metric research done within Missouri by Rabeni et al. (1997) independently confirmed Taxa Richness, EPT Taxa, and the Biotic Index as most reliable, in addition to the Shannon Diversity Index. These four constitute the primary metrics calculated at each sampling station to derive scoring criteria.

Primary Metrics:

1. Taxa Richness (TR)
2. Ephemeroptera/Plecoptera/Trichoptera Taxa Index (EPTT)
3. Biotic Index (BI)
4. Shannon Diversity Index (SDI)

Secondary metrics include, but are not limited to:

1. Quantitative Similarity Index for Taxa (QSI-T)
2. Pinkham and Pearson Similarity Index (PPSI)
3. Dominant Macroinvertebrate Taxa (DMT)
4. Dominant Macroinvertebrate Families (DMF)
5. Percent Contribution of Dominant Taxon (PDT)
6. Dominants In Common (DIC)
7. Percent Sensitive Taxa (PST)
8. Functional Feeding Groups and Functional Habit Groups (FFG/ FHG)
9. Percent Scrapers (PS)
10. Quantitative Similarity Index for Functional Feeding Groups (QSI-FFG)

5.1 Primary Metrics

5.1.1. Taxa Richness (TR)

Taxa Richness reflects the health of the community through a measurement of the number of taxa present. In general, the total number of taxa increases with improving water quality, habitat

diversity, and/or habitat suitability. However, some pristine headwater streams may be less productive, whereas mild organic enrichment may actually increase the number of taxa (Plafkin et al. 1989). Taxa Richness is calculated by counting all taxa, including large rare taxa, from the sub-sampling effort.

5.1.2 Ephemeroptera/Plecoptera/Trichoptera Taxa Index (EPTT)

The EPTT index is the total number of distinct taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera Taxa (see MDNR 2010d). The EPTT index generally increases with increasing water quality. This value summarizes taxa richness within the insect orders that are generally considered to be pollution sensitive. Headwater streams that are naturally unproductive may experience an increase in taxa, including EPT taxa, in response to mild organic enrichment. The EPTT Index is calculated by counting EPT taxa, including large rare taxa, from the sub-sampling effort.

5.1.3 Biotic Index (BI)

The biotic index was first developed by Chutter in 1972 and then modified for Wisconsin by Hilsenhoff in 1977. Hilsenhoff reported evaluations and further modifications in 1982 and 1987. The Hilsenhoff Biotic Index was developed as a means of detecting organic pollution in communities inhabiting rock or gravel riffles of Wisconsin streams. The BI tolerance value for each taxon used by MNDR can be found in MDNR-ESP-209 *Taxonomic Levels for Macroinvertebrate Identifications* (MDNR 2010d). Tolerance values for each taxon (T_i) range from 0 to 10 with the higher values indicating increased tolerance to organic pollution. The calculated BI for the macroinvertebrate community is also expressed as a single value between 0 and 10. Temporary tolerance values for the BI used in this procedure are based on North Carolina (Lenat 1993), Wisconsin (Hilsenhoff 1987), New York (Bode et al. 1988), Kentucky (KDOW 2009), and Kansas (Huggins and Moffett 1988).

The formula for calculating the Biotic Index is:

$$BI = \frac{\sum_{i=1}^n X_i T_i}{n}$$

Where: X_i = number of individuals within each species
 T_i = tolerance value of that taxon
 n = total number of organisms in the sample with tolerance values

5.1.4 Shannon Diversity Index (SDI)

The Shannon Diversity Index (Shannon and Weaver 1949) is a measure of community composition, which takes into account both richness and evenness. It is assumed that a more diverse community is a more healthy community; diversity increases as the number of taxa increase, and as the distribution of individuals among those taxa is more evenly distributed.

The formula for calculating the Shannon Diversity Index is:

$$H' = -\sum_{i=1}^s (p_i)(\log_e p_i)$$

Where: H' = Information content of sample (= index of diversity)
 S = Number of species
 p_i = Proportion of total sample belonging to i^{th} species

5.2 Secondary Metrics

5.2.1 Quantitative Similarity Index for Taxa (QSI-T)

The Quantitative Similarity Index for Taxa compares two aquatic communities in terms of presence or absence of taxa, also taking relative abundance (percent composition) of each taxon into account.

The formula for calculating the Quantitative Similarity Index for Taxa is:

$$QSIT_{ab} = \sum \min(P_{ia}, P_{ib})$$

Where: P_{ia} = the relative abundance of species i at Station a
 P_{ib} = the relative abundance of species i at Station b
 $\min(P_{ia}, P_{ib})$ = the minimum relative abundance of species i at Station a or b

Values for this index range from 0-100% with zero being totally different and 100% absolutely identical. It has been used for bracketing potential stressors in streams by comparing samples upstream of a pollutant source (control) with samples collected downstream (test). Shackleford (1988) suggested that a QSI-T value less than 65% can indicate environmental stress, and values greater than 65% are more typical of natural variation between duplicate samples of same communities. In Arkansas, a range of 60.0 to 85.0 and an average of 75.0 were obtained when the QSI-T was used between duplicate bioassessment samples. WQMS uses the QSI-T for quality control by calculating percent similarity between duplicate macroinvertebrate samples. This similarity gauges the level of success between two sample collectors and limits potential bias in samples. See Section 7.1 for suggested acceptable values using the QSI-T metric for quality control purposes.

5.2.2 Pinkham and Pearson Similarity Index (PPSI)

Community similarity indices are used in situations where reference communities exist. The reference community can be derived through sampling or prediction for a region through the use of a reference database. The Pinkham and Pearson Similarity Index measures the degree of similarity in taxonomic composition in terms of taxa abundance, and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant species. See Pinkham and Pearson (1976) and U.S. EPA (1983) for more detail.

The formula for calculating the Pinkham and Pearson Similarity Index is:

$$PPSI_{ab} = \sum \frac{\min(X_{ia}, X_{ib})}{\max(X_{ia}, X_{ib})} \left[\left(\frac{X_{ia}}{X_a} \right) \left(\frac{X_{ib}}{X_b} \right) / 2 \right]$$

Where: X_{ia}, X_{ib} = number of individuals in the i^{th} species in Sample a or b.

5.2.3 Dominant Macroinvertebrate Taxa (DMT)

The Dominant Macroinvertebrate Taxa metric is a measure of the prevalence of each taxon within a station. Each taxon occupies a percentage of a sample, based on the total number of individuals, and the percentage is ranked from high to low. The DMT ranks and percentages may be compared among test stations, as well as with the combined reference stations of their corresponding Ecological Drainage Unit (EDU). This metric may identify changes in community structure, and differences from what is expected in high quality streams. This metric also may highlight responses by individual taxa known to be sensitive to specific stressor(s), as well as taxa generally regarded as sensitive or tolerant. The level of identification used to develop this metric is described in MDNR-ESP-209 *Taxonomic Levels for Macroinvertebrate Identifications*. The DMT metric is the percentage based on the total number of individuals, and is calculated as the number of individuals within each taxon, divided by the total number of individuals in a station, and multiplied by 100. Each taxon is ranked based on dominance.

5.2.4 Dominant Macroinvertebrate Families (DMF)

The Dominant Macroinvertebrate Families metric is a measure of the prevalence of each taxonomic family within a station. It is a percentage based on the total number of individuals within each family, and is calculated as the number of individuals within each family, divided by total number of individuals in the sample, and multiplied by 100. Each family occupies a percentage of a sample, based on the total number of individuals in a sample, and the percentage is ranked from high to low. Usually, the top seven most abundant families are listed for each station for comparison. The rank and percentage of test stations may be compared to individual reference or control stations. Changes in the dominance of certain families may assist in the identification of the type and potential source(s) of impairment.

5.2.5 Percent Contribution of Dominant Taxon (PDT)

Percent Contribution of Dominant Taxon is a simple measure of redundancy and evenness, and it assumes that a highly redundant community (major abundance contributed by a single taxon) reflects an impaired community. This index may be redundant if the Pinkham and Pearson Similarity Index or DMT is used (Barbour et al. 1992). The PDT calculates the percentage of each taxon within a sample.

5.2.6 Dominants in Common (DIC)

Shackelford (1988) modified the Percent Contribution of Dominant Taxa to reflect "Dominants In Common." This metric utilizes the dominant five taxa at the stations of comparison. The DIC approach will best provide a measure of replacement or substitution between the reference community and a test station. An examination of community dominants can provide insight into community structure because dominants specialize on prevailing environmental conditions. Benthic studies have shown that tolerant species are present in nearly all streams, but dominate only in polluted systems (Nuzzo 1986; Lenat 1988; Bode 1988). For this metric, the dominants are the five most abundant taxa. Each of these taxa usually has a relative abundance that is greater than four percent of the total. This metric is redundant if used with DMT. The DIC is a rank of the PDT.

5.2.7 Percent Sensitive Taxa (PST)

The Percent Sensitive Taxa calculates the distribution of taxa within a community based on their BI values. The BI value enumerates the sensitivity of taxa to organic pollution or disturbance in a range from 0 (intolerant) to 10 (tolerant). The PST metric calculates the abundance of all taxa that occur within the following ranges of BI percentiles: >90th; 90th-75th; 75th-50th; 50th-25th; and <25th. This metric allows for comparison between test stations and the aggregate of reference samples in the EDU.

5.2.8 Functional Feeding Groups and Functional Habit Groups (FFG/FHG)

The relative abundance of taxa within Functional Feeding Groups and Functional Habit Groups may be related to the quality of food or habitat resources. For example, Rabeni et al. (2005) identified responses of particular feeding and habit groups to increased benthic fine sediment. The percentage within each group may be compared between test stations and controls, as well as with combined references of each EDU. The percentage within each group is based on the primary feeding and habit group for the total number of individuals in the sample. Groups are identified in Merritt, Cummins, and Berg (2008) and are listed below:

Functional Feeding Groups –

- Filterer Collectors
- Filterer Gatherers
- Parasites
- Piercers
- Predators
- Scrapers
- Shredders

Functional Habit Groups –

- Burrowers
- Clingers
- Climbers
- Divers
- Skaters
- Sprawlers
- Swimmers

5.2.9 Percent Scrapers (PS)

The Percent Scrapers is a measure of relative abundance of herbivores that graze on attached algae and associated material from mineral and organic surfaces (Cummins 1973, Cummins and

Wilzbach 1985). The proportion of scrapers may indicate complex responses of invertebrate assemblages to water quality, nutrient enrichment and physical differences among streams. Those organisms considered to be scrapers are found in the functional feeding group column of the Standard Operating Procedure MDNR-ESP-209 (MDNR 2010d).

5.2.10 Quantitative Similarity Index for Functional Feeding Groups (QSI-FFG)

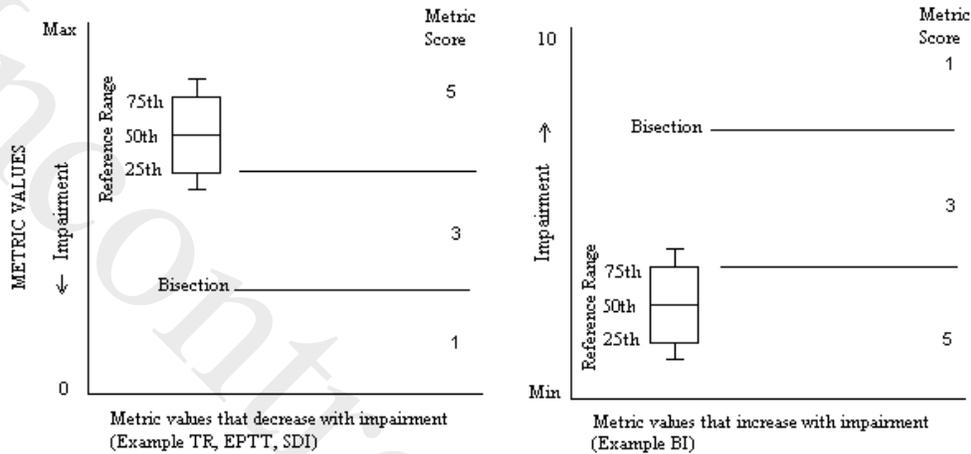
The Quantitative Similarity Index for Functional Feeding Groups is a useful method of comparing the composition of two communities. The equation not only functionally compares two communities in terms of presence or absence (qualitative), but also takes relative abundance (semi-quantitative) into account (Shackleford 1988). This index is calculated in the same manner as QSI-T. Functional Feeding Group designations may be found in MDNR-ESP-209 (MDNR 2010d).

6.0 Macroinvertebrate Stream Condition Index (MSCI)

The Stream Condition Index (SCI) was developed by Rabeni et al. (1997) as a measure of aquatic biological integrity. The SCI was further refined for reference streams within each EDU into the *Biological Criteria for Perennial/Wadeable Streams* (MDNR 2002). This multi-metric approach utilizes a comparison of four primary metric (i.e., TR, EPTT, BI, SDI) values with biological criteria (biocriteria) scoring ranges for each metric that are developed by compiling data from at least five reference streams/stations for each season. Each test station's individual metric value is compared with the corresponding reference scoring range and that metric receives a score. The sum of all four individual metric scores is what is now known as the Macroinvertebrate Stream Condition Index (MSCI) score.

To make the metrics comparable and have equal importance in the MSCI, all metric values are normalized to unitless scores. Each metric value receives a score of 5, 3, or 1 (see Figure 1). For metric values that decrease with impairment (TR, EPTT, and SDI), any value above the lower quartile (25%) of the reference range receives the highest score of 5. Between the 25% of the reference range and 0 is bisected to determine the next two possible metric scores. A metric value located below 25% and above the bisection receives a score of 3, whereas a metric value below the bisection receives a score of 1. For metric values that increase with impairment (BI), any metric value below the upper quartile (75%) of the reference range receives the highest score of 5. Between the 75% and the highest possible BI score of 10 is bisected to determine the next two possible metric scores. Metric values located above the 75% and below the bisection receive a score of 3, whereas metrics above the bisection receive a score of 1.

Figure 1: An illustration of the individual metric scoring procedure (after Barbour et al.1992).



Therefore, an MSCI score of 20 is possible when using the four primary metrics. If the study stream has an MSCI score that is 100%-80% of the reference biological criteria, or 20 – 16, it is considered *fully supporting*; 70%-50%, or 14 – 10, is considered *partially supporting*; and 40%-20%, or 8 – 4 is considered *non-supporting* of the beneficial use designation for the protection of aquatic life. See the Missouri Water Quality Standards, 10 CSR 20-7 (MDNR 2014) for a listing of reference streams and an explanation of narrative criteria.

7.0 Quality Control (QC)

7.1 Collection and Preservation

The goal of quality control (QC) in sample collection is to ensure a level of consistency between sample collectors. Each sample team will select one station that will be used for the collection of duplicate macroinvertebrate samples. These duplicate samples then will be analyzed for QSI-T (Section 5.2.1). Duplicate samples are expected to have a 70% or greater taxa similarity, as determined in Rabeni et al. (1999).

7.2 Laboratory

7.2.1 Processing

Each biologist must undergo QC of their processing abilities at least once per processing season. A sub-sample of processed material is saved for QC of laboratory processing (see Section 3.0). Another biologist (QC biologist) will re-sort the material to find organisms that were missed in the initial processing. All taxonomically appropriate macroinvertebrates (according to MDNR-ESP-209) found in the sub-sample cup are placed into a vial for later identification. The vial is labeled with the stream name, sample number, as was done with sample bottles, and are

identified as a QC vial. The QC biologist records the total number of individuals missed in the QC column and initials the Laboratory Sub-sampling Form (Appendix C). The biologist's QC examination is considered successful if they missed fewer than 10% of the total number picked in sorting. The biologist fails the processing QC if more than 10% is missed. If the QC examination is failed, the biologist must continue the QC process until they are successful in three consecutive sample attempts.

7.2.2 Organism Identification and Recording

Organisms will be identified by trained biologists to taxonomic levels designated in Standard Operating Procedure MDNR-ESP-209 *Taxonomic Levels for Macroinvertebrate Identifications* (MDNR 2010d). All organisms found during the laboratory process (see Section 7.2.1) will be identified and enumerated in the appropriate habitat column of the Laboratory Bench Sheet (Appendix D; see Section 4.2.1) and included in the final taxa list.

Newly hired biologists must have independent confirmation of their taxonomic identifications. An in-house senior biologist will confirm all of their identifications. The newly hired biologist will keep a list of accurately identified taxa. The newly hired biologist may independently identify taxa that they have accurately identified three times.

An overall reference collection will be kept in the Biology/Toxicology Laboratory of ESP located at the Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program, Water Quality Monitoring Section, 2710 West Main Street, Jefferson City, Missouri, 65109. All reference specimens shall be confirmed by a second biologist or expert in the specific taxonomic field and then deposited in the reference collection. Reference specimens should be kept for each taxon.

Once the non-mountable reference organisms have been identified under magnification, all individuals are readied for permanent storage. Reference organisms and two internal labels (label one: stream name, county, map coordinates, collection date, habitat, sample number, and the name of the analyst; label two: a taxonomic identification) are placed in a sample vial filled with 80% ethyl alcohol and permanently stored within a labeled cabinet.

Slides used for mountable Chironomidae and Oligochaeta specimens kept for reference are re-labeled with a self-adhesive paper label (stream name, county, map coordinates, collection date, habitat, sample number, name of analyst, and taxonomic identification) and placed in a reference slide box.

7.3 Data Recording

As discussed in Section 4.2.2, the raw data from the laboratory bench sheet are recorded in electronic data files. All data entries will be checked by another biologist before being approved and imported by the entry biologist or a third biologist.

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Appendix A
Field Equipment List

Field Equipment List

MDNR Semi-Quantitative Macroinvertebrate Stream Bioassessment Procedure

□ Waders and Equipment Bag

Macroinvertebrate gear

- 2) 18" X 8" bottom aquatic kick nets w/500 micron mesh bags
- Replacement bag for kick net
- 500 micron snag sampling bags
- Snag scrub brush
- Large flat plastic pans
- Wash bucket w/500 micron mesh bottom screen
- Regular wash bucket
- 3" plastic scrapers
- Brine shrimp nets
- Forceps
- Sample jars
- Internal labels
- External labels
- Carboy w/10% formalin
- Extra two-liter containers w/full strength formaldehyde
- Preservative buffer
- Three-pronged hand cultivation tool

Water quality gear

- Sample bottles
- Labels (numbered and blank)
- Preservatives
- Vacuum Pump
- Filters
- DI Carboy
- DI Bottle & water
- Turbidity vials & Baggies
- pH meter
- Conductivity meter
- Dissolved oxygen meter
- Thermometer
- Flow meter
- Adjustable wading rod
- Iron posts (rebar)
- Tape measures (100' and 25')
- Hammer
- Clipboard
- Sample Cooler
- Ice
- Latex/Nitrile Gloves

Location gear

- GPS unit
- Maps
- Cell phone

Forms and records

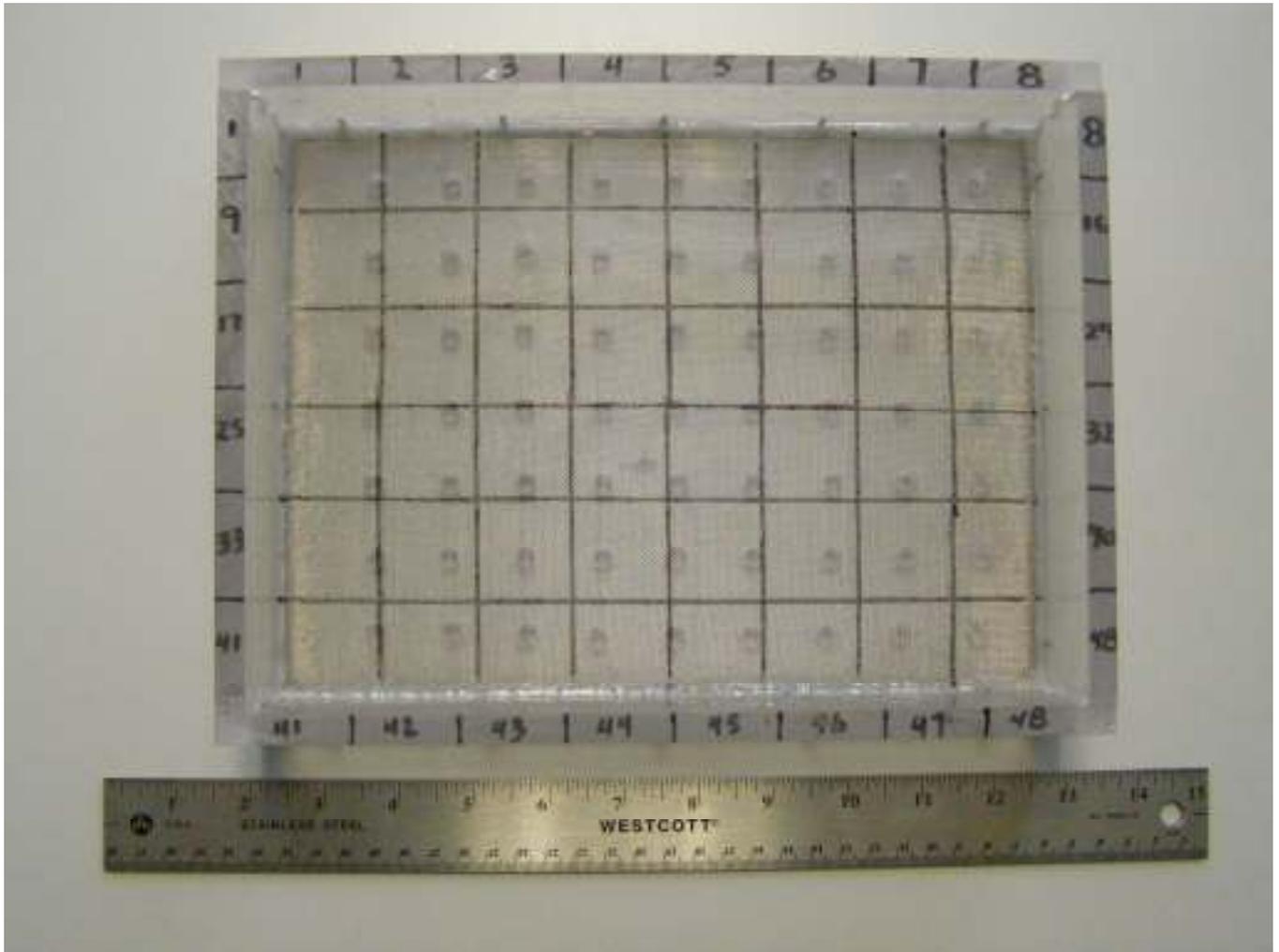
- Mechanical pencils
- Write in the rain pen
- Sharpie (fine)
- Field notebook
- Chains of Custody
- Flow
- Habitat assessment
- Channel measurements

Extras

- Duct tape
- Wader repair kit
- Cotton gloves
- Trapper's gloves
- Silicone
- Insect repellent
- Hand cleaner
- Paper towels
- Machete/pruners
- Rain gear

Appendix B

Photograph of Sub-Sample Sieve



Appendix C

Laboratory Sub-sampling Form

Laboratory Sub-sampling Form
MDNR Semi-Quantitative Macroinvertebrate Stream Bioassessment Procedure

Name				
Sample #				
Date				
	# org	%sub	by	QC
Coarse				
Depositional				
Woody				
Rootmat				

Name				
Sample #				
Date				
	# org	%sub	by	QC
Coarse				
Depositional				
Woody				
Rootmat				

Name				
Sample #				
Date				
	# org	%sub	by	QC
Coarse				
Depositional				
Woody				
Rootmat				

Name				
Sample #				
Date				
	# org	%sub	by	QC
Coarse				
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	# org	%sub	by	QC
Coarse				
Depositional				
Woody				
Rootmat				

Name				
Sample #				
Date				
	# org	%sub	by	QC
Coarse				
Depositional				
Woody				
Rootmat				

Appendix D
Laboratory Bench Sheet

