MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Project Procedure

TITLE: Semi-Quantitative Macroinvertebrate Stream Bioassessment

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APPLICABILITY: Applies to WQMS personnel who perform community level surveys of aquatic macroinvertebrates in wadeable streams of Missouri.

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1.0 Introduction

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

Minimum qualifications of individuals that perform assessments as described in this procedure should be a Bachelor of Science in a biological field along with at least 1 year of training under a senior aquatic biologist in methods and taxonomy.

2.0 Field Methods

2.1 Habitat Assessment

A stream habitat assessment is completed 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 2003) for further explanation and for blank data forms.

2.2 Length of Stream Reach Sampled

All macroinvertebrate sampling is done in a stream reach approximately twenty times the average width of the stream, measured at the top of the lower bank (Stream Habitat Assessment Project Procedure). 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) according to Hynes (1970).

In Rabeni et al. (1999) multiple reaches of stream, which were twenty times the stream width, were compared to identify 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 efficiently collect organisms from the major 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 primarily found in the Ozark aquatic region of Missouri, but 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 and preserved separately to provide the ability to factor out habitat differences between sites. This will enhance comparisons involving streams where major habitats may be missing. As each habitat sample is collected and processed a label is inserted in the sample jar stating the sampling location, date and habitat. When all samples have been collected, they are transported to the vehicle where they are preserved with 10% formalin. At this time an external sample label (see MDNR 2000) with sample identification number and habitat is placed on the jar lid and a MDNR chain-of-custody form is completed (see MDNR 2001) to accompany the sample back to the laboratory. For a list of suggested field equipment see Appendix B.

2.3.1 Sampling Riffle/Pool Predominant Streams

Flowing water 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, and 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 B). 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 container. After each collection the net is checked for clinging organisms to be added to the composite sample. Field processing of the sample is accomplished by pouring all excess water from the composite through a brine shrimp net. Using a plastic putty knife, the remaining sample can be concentrated into a corner of the container. From here the sample can be scooped into sample jars, making sure that sufficient space remains for preservative. Any small debris left behind is flushed from the container through the brine shrimp net. The brine shrimp net is then inverted and the contents are placed into the sampling jars.

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 are no longer held in suspension. Because water velocities in these areas are not usually discernable with the naked eye, the water is categorized as non-flowing. Six collections from a variety of depositional depths and microhabitats (i.e. backwater, nearshore, forewaters, in channel pools, etc.) are collected with a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix B). Each sample is taken from an approximately one-square meter area of substrate using a traveling kick method. To do this, 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 concentrated and preserved 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 B). 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 concentrated and preserved 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 velocities in these areas are not usually discernable with the naked eye, the water is categorized as non-flowing. Six collections from a variety of depositional depths and microhabitats (i.e. backwater, nearshore, forewaters, in channel pools, etc.) are collected with a bottom aquatic kick net with a 500 x 500 micron mesh bag (see Appendix B). Each sample is taken from approximately one square meter area of substrate using a traveling kick method. To do this, 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 concentrated and preserved as previously described in Section 2.3.1.

Large woody debris substrates are submerged portions of large logs as well as tree branches greater than one inch in diameter. A composite of twelve 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 bag is made by folding a 46 centimeters 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 and 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 twelve 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 sampling container, concentrated, and preserved, 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 B). 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 concentrated and preserved 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 (25 °C) and processed within ninety days.

There have been many discussions and guidance concerning the number of organisms comprising 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).

For flowing water, coarse substrate habitat, 600 organisms (+/- 10%) are sub-sampled. Depositional, woody debris and rootmat habitats consist of 300 organism (+/- 10%) sub-samples. A sub-sampling method from Plafkin et al. (1989), modified from Caton (1991) is used to allow rapid isolation of the target number of organisms. The sample from each habitat is transferred to a 14 inch wide x 20 inch long x 4.5 inch tall stainless steel sieve. The sieve is manufactured from 16-gauge stainless steel with 500 x 500 micron mesh screen cloth in the bottom. The sample is emptied into the sieve and rinsed with water to flush away the formalin preservative. Any large debris is scrubbed, rinsed and removed. The sieve is placed into a 16 x 23 inch or larger plastic container that contains water to a level just above the bottom of the sieve. Keeping the plastic container and sieve level a figure eight stirring method randomly distributes the sample. Once the sample is distributed, it is quickly lifted from the sieve and drained of excess water. For coarse substrate samples, a grid with 1.25 inch x 1.25 inch numbered squares (117 squares) is placed into the sieve. For all other habitats, a grid with 2 inch x 2 inch numbered squares (70 squares) is used to separate material into sub-samples. A random number generator selects squares for sub-sampling. The end of a spatula is used to outline the dimensions of each grid square into the sample after which the grid is removed and the spatula is used to lift out the contents of the outlined squares and place the sub-sample into a marked container.

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, MI. Once microscope dimensions and measurements are determined a company that owns a computerized router can manufacture a sorting wheel that will be calibrated to fit each individual stereo microscope. The trough width and depth are changed 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. One vial contains slide-mountable (Chironomidae and Oligochaeta) organisms and the other non-mountable organisms. Once started, a sub-sample square must be completely sorted. Additional squares are randomly selected and sorted until the target number of organisms is reached. If the whole sample is processed and the target number of organisms has not been reached, a notation is made on the Laboratory Sub-

sampling Form (Appendix C). Final counts, percent of sub-sampler sorted, and sorter initials are placed on the Laboratory Sub-sampling Form.

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 have very good overhead illumination. When large and/or rare organisms are found while picking through the debris, they are removed and placed in a separate labeled vial also identified as Large Rare (LR). Therefore there is a potential for three vials per habitat.

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, Taxonomic Levels for Macroinvertebrate Identification, (MDNR-FSS-209) details the identification level and contains the taxonomic references required for macroinvertebrate identification. Representatives of all genus and species determinations are placed in the macroinvertebrate reference collection which is located at the MDNR, ALPD, ESP, Water Quality Monitoring Section, 2710 West Main Street, Jefferson City, Missouri, 65109. Once the non-mountable organisms have been identified under magnification, all individuals are readied for permanent storage. Reference organisms and two internal labels (label one: name of the waterway, county, map coordinates, collection date, habitat and the name of the analyst; and label two: a taxonomic identification) are placed in a sample vial filled with 70% ethyl alcohol and permanently stored within a labeled cabinet. The remaining organisms and one internal label (name of the waterway, county, map coordinates, collection date, habitat and the name of the analyst) are placed in a vial filled with 70% ethyl alcohol and temporarily stored for at least three years.

Chironomidae and Oligochaeta are permanently mounted for identification on microscope slides with CMCP-10 mounting media. All slides are labeled by indelible marker with the stream name, habitat and date at the time of mounting. After a sufficient drying and clearing time, organisms are identified using a compound microscope capable of 1000x magnification. Organisms to be kept for reference are re-labeled with a self-adhesive paper label (name of waterway, county, map coordinates, collection date, habitat, name of analyst and taxonomic identification) and placed into a reference slide box. The remainders of the slides are discarded.

4.2 Data Recording

4.2.1 Laboratory Bench Sheets

For each sampling station a Laboratory Bench Sheet (Appendix A) containing taxonomic identification, date, sample number, location, and organism enumeration is completed. Large/rare taxa are recorded as present with a designation of -99. The number of taxa for each sample habitat type is recorded in the appropriate column on the lab bench sheet. Each taxon is listed only once in the left-

hand column. The lab bench sheet is constructed with a flexible format to enable the analyst to use the composite data or to use data from individual habitats. The bench sheet also contains a column for quality control purposes. The purpose of this column is explained in section 7.2.1.

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. If the investigator feels it is necessary to compare the total values from streams that did not have identical sets of habitats, as is the case with many water chemistry studies, new total values may be calculated from which the discrepant habitat data can be omitted.

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 (EPT)
3. Biotic Index (BI)
4. Shannon Diversity Index (SDI)

In special circumstances additional, or secondary, metrics are required.

Secondary Metrics:
1. Quantitative Similarity Index for Taxa (QSI-T)
2. Pinkham and Pearson Similarity Index (PPSI)

3. % Dominant Taxa (%DT)
4. Dominants In Common (DIC)
5. Percent Scrapers (PS)
6. 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. Some pristine headwater streams may be less productive than the middle reaches and support a limited number of taxa. In these streams, mild organic enrichment may actually result in an increase in 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 Index (EPT)

The EPT index is the total number of distinct taxa within the orders Ephemeroptera, Plecoptera and Trichoptera (see MDNR 2001). The EPT 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 EPT 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. Biotic Index values to be used for the calculation of this metric can be found in the Standard Operating Procedure MDNR-FSS-209 (MDNR 2001). Tolerance values for each taxon range from 0 to 10, higher values indicating increased tolerance. The overall pollution tolerance of the macroinvertebrate community is expressed as a single value between 0 and 10. Temporary tolerance values for the Biotic Index used in this procedure are based upon North Carolina (Lenat 1993), Wisconsin (Hilsenhoff 1987), New York (Bode et al. 1988) and Kansas (Huggins and Moffett 1988). Tolerance values specific to Missouri are under development.

The formula for calculating the Biotic Index is:

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

Where: X_i = number of individuals within each species
T_i = tolerance value of that species

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:

$$H' = -\sum_{i=1}^{n} (p_i)(\log_e p_1)$$

Where: H' = Information content of sample (= index of diversity)
S = Number of species
p_i = Proportion of total sample belonging to ith species

5.2 Secondary Metrics

5.2.1 Quantitative Similarity Index for Taxa (QSIT)

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 taxa 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%. Identical communities have a value of 100% and totally different communities have a value of 0%. In general, values less than 65% indicate environmental stress whereas values greater than 65% occur as expected natural variation between duplicate samples of the same communities (Shackleford 1988).

This metric has been found useful by MDNR for quality control purposes. In Arkansas an average of 75.0 and a range of 60.0 to 85.0 were obtained with duplicate bioassessment samples. See Section 7, Quality Control, for values from duplicate samples using this procedure.

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:

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

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

5.2.3 Percent Contribution of Dominant Taxon (%DT)

Percent Contribution of Dominant Taxon is a simple measure of redundancy and evenness and 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 is used (Barbour et al. 1992).

5.2.4 Dominants In Common (DIC)

Shackleford (1988) modified the Percent Dominant Taxa to reflect "dominants in common". This metric utilizes the dominant five taxa at the stations of comparison. The Dominants In Common approach will best provide a measure of replacement or substitution between the reference community and a downstream 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.

5.2.5 Percent Scrapers (PS)

The Percent Scrapers is a measure of relative abundance of herbivores that graze 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 being scrapers are found in the functional feeding group column of the Standard Operating Procedure MDNR-FSS-209 (MDNR 2001).

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

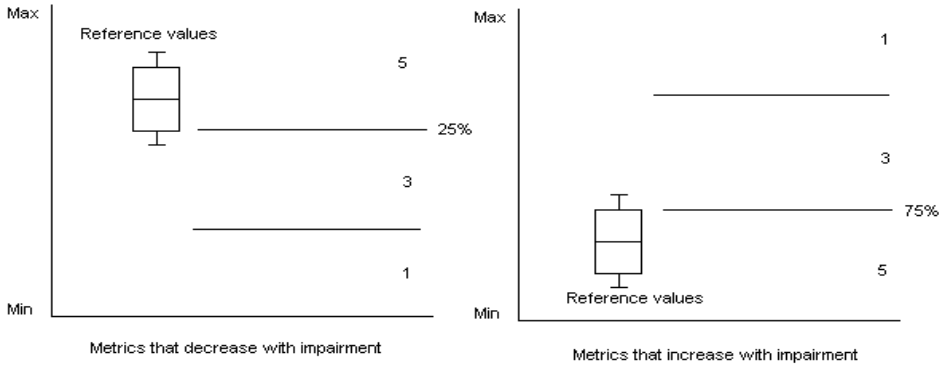
The Quantitative Similarity Index for Functional Feeding Groups is a useful method of comparing the composition of two communities. The equation not only functionally compares two communities in terms of presence or absence (qualitative), but also takes relative abundance (semi-quantitative) into account (Shackleford 1988). This index is calculated in the same manner as QSIT. Functional Feeding Group designations may be found in MDNR-FSS-209 (MDNR 2001).

6.0 Stream Condition Index

The Stream Condition Index (SCI) was developed by Rabeni et al. (1997) and is used by MDNR as an aquatic biological integrity measure. It is a multi-metric approach where four primary metrics, and under special circumstances some of the secondary metrics, are aggregated into a single value. At this time the SCI is calculated for individual season and year and is established by collecting data from at least ten reference streams in the same ecoregion as the study stream. The lower quartile of the distribution of each metric is used as the minimum value representative of reference conditions. To make the metrics comparable and have equal importance in the SCI, all values are normalized to unitless values. The range of each metric is divided into one of three possible scores of 5, 3, or 1 (see Figure 2). For those metrics whose values decrease with impairment (TR, EPT, and SDI) any value above the lower quartile (25%) of the reference distribution receives the highest score (5). The range of the values from 0 through the minimum value designated for reference conditions are then bisected. The lowest half designates a score of 1 and the upper half a score of 3. For the BI whose values increase with impairment, any value below the upper quartile (75%) of the reference distribution receives the highest score (5). The range of the values from 10 through the value established by the upper quartile (75%) are then bisected. The upper half designates a score of 1 and the lower half a score of 3.

Each study stream is evaluated by calculating the metrics, scoring them using this scale determined in the SCI, and totaling the scores into a single value. If the study stream scores 100%-80% of the reference biological criteria it is considered fully supporting, 70%-50% partially supporting, and 40%-20% non-supporting. See the Missouri Water Quality Standards, 10 CSR 20-7 (MDNR 1996), for identification of reference streams and narrative criteria.

Figure 2. An illustration of metric scoring procedure (after Barbour et al. 1992).



7.0 Quality Control

7.1 Field Samples

The goal of the sampling protocol is to collect a majority of the taxa with consistent relative abundance from each of three major habitats in a reach of stream. Within the sampling reach, duplicate samples will be collected and analyzed for QSIT (Section 5.2.1) at 10% of the sites. 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 Sub-sampling

Ten percent (10%) of all habitats sub-sampled will be searched by another biologist for organisms missed. When finished the biologist will initial the Laboratory Sub-sampling Form (Appendix C) under the QC column. All organisms found during the QC process will be identified in the far left-hand column and enumerated in the appropriate habitat QC column of the Laboratory Bench Sheet.

7.2.2 Organism Identification

Newly hired biologists involved in taxonomic identifications will keep a taxonomic list of organisms that have been confirmed by at least two senior in-house biologists. Confirmation of identifications for each taxon will be required until three successful and independent identifications have been made. An overall reference collection will be kept at the MDNR, ALPD, ESP, WQMS laboratory. Reference specimens shall be kept for each taxon. All reference specimens shall be confirmed by an expert in the specific taxonomic field and then deposited in the reference collection.

7.3 Data Entry

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.

8.0 Literature Cited

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Appendix A
Laboratory Bench Sheet

Laboratory Bench Sheet
MDNR Semi-Quantitative Macroinvertebrate Stream Bioassessment Procedure

Date:	Analyst:	Sample #: Station #:	Location:

Taxa Name	Flow / Coarse	QC	Non-flow / Depositional	QC	Large Woody Debris	QC	Rootmat	QC

Taxa Name	Flow / Coarse	QC	Non-flow / Depositional	QC	Large Woody Debris	QC	Rootmat	QC

Appendix B

Field Equipment List

Field Equipment List
MDNR Semi-Quantitative Macroinvertebrate Stream Bioassessment Procedure

2) 18" x 8" Bottom Aquatic Kick Nets with 500 x 500 micron mesh bags* (Catalog #425-A50)
500 x 500 micron mesh bag for sampling large woody debris
Three prong garden tool
Large woody debris scrub brush
3" plastic scrapers
Brine shrimp nets
Plastic containers for contents of kick nets
Wash bucket with 500 x 500 micron mesh bottom screen*
Replacement bag for kick-net* (Catalog # 425-J51-500)
Wet suit or trapper's gloves
Chest waders with suspenders & belt
Knee pads
Carboy full of 10% formalin
Extra two-liter containers with full strength formaldehyde
Sample jars (1-quart wide mouth canning jars and lids)
Forceps
Vials filled with 80% ethanol
Camera and film
Silicone
Duct tape
Maps
Clip board
Data sheets
Internal location labels for jars
Self-adhesive sample labels
Chain of custody

* Available from:
Wildco (Wildlife Supply Co.)
301 Cass St.
Saginaw, MI 48602
(517) 799-8100

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				
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				
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				
Depositional				
Woody				
Rootmat				