SOP #EH-04
Benthic Macroinvertebrate Sampling & Processing

(*Adapted from ERT/REAC SOP and Great Lakes National Program Office Sampling Method LG406 Revision 07*)

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TECHNICAL STANDARD OPERATING PROCEDURE
BENTHIC MACROINVERTEBRATE SAMPLING & PROCESSING

SYNOPSIS: A standardized method for collecting benthic macroinvertebrates for ecological assessment. This procedure is based on USEPA Rapid Bioassessment Protocol III. Protocols for sample collection and handling are provided.

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for sampling the benthic population at hazardous waste sites. This protocol summarizes the USEPA Rapid Bioassessment Protocol III (RBP III) for benthic macroinvertebrates. RBP III utilizes the systematic field collection and analysis of major benthic taxa, and can detect subtle degrees of impairment at potentially contaminated sites. Discrimination of four levels of impairment should be possible with this assessment. This SOP may be used by employees of USEPA Region 8, or contractors and subcontractors supporting USEPA Region 8 projects and tasks. Deviations from the procedures outlined in this document must be approved by the USEPA Region 8 Remedial Project Manager, Regional Toxicologist or On-Scene Coordinator prior to initiation of the sampling activity.

2.0 RESPONSIBILITIES

The Field Project Leader (FPL) may be an USEPA employee or contractor who is responsible for overseeing the benthic macroinvertebrate sampling activities. The FPL is also responsible for checking all work performed and verifying that the work satisfies the specific tasks outlined by this SOP and the Project Plan. It is the responsibility of the FPL to communicate with the Field Personnel regarding specific collection objectives and anticipated situations that require any deviation from the Project Plan. It is also the responsibility of the FPL to communicate the need for any deviations from the Project Plan with the appropriate USEPA Region 8 personnel (Remedial Project Manager, Regional Toxicologist or On-Scene Coordinator).

Field personnel performing benthic macroinvertebrate sampling are responsible for adhering to the applicable tasks outlined in this procedure while collecting samples.

3.0 EQUIPMENT

- D-frame dip net - 0.3 m^2 "D"-shaped net (500um nytex screen) where the net attaches to a long pole. Net is cone-shaped for capture of organisms.

- Kick-net - 1 m^2 net (500um nytex screen) attached to 2 poles, which functions in a similar manner to a fish kick seine.

- Ponar/Ekman Dredge - for collection of sediment-dwelling invertebrates in non-wadable waterbodies.

- Elutriator Bucket or 500 um Mesh Sieve Bucket - used to remove invertebrates from sediment.

- Collection containers - wide-mouth bottles (500 to 1,000 ml capacity).

- Gloves - for personal protection and to prevent cross-contamination of samples. May be plastic or latex; should be disposable and powderless.

- Field notebook - a bound book used to record progress of sampling effort and record any problems and field observations during sampling.

- Three-ring binder book- to store necessary forms used to record and track samples collected at the site. Binders will contain the Benthic Macroinvertebrate Field Data Sheet, Physical Characterization/Water Quality Field Data Sheet, and sample labels. Example forms are provided in **Attachment 1**.

- Permanent marking pen - used to label samples and to record information in field logbooks and data sheets.

- Sieve Buckets - with 500um mesh. Must have 10 - 12 liter capacity.

- Forceps - to pick organisms from mesh screens and collection nets.

- 95% Ethanol - to preserve samples for analysis.

- Trash Bag - used to dispose of gloves and any other non-hazardous waste generated during sampling.

4.0 METHOD SUMMARY

Benthic macroinvertebrates are collected systematically from all available in-stream habitats by kicking the substrate or jabbing with a D-frame dip net. A total of 20 jabs (or kicks) are taken from all major habitat types in the reach, resulting in sampling approximately 3.1 m^2 of habitat. An organism-based subsample (usually 100, 200, 300, or 500 organisms) is sorted in the laboratory and identified to the lowest practical taxon, generally genus or species.

5.0 SAMPLING PROCEDURES

A 100m reach that is representative of the characteristics of the stream should be selected. Whenever possible, the area should be at least 100m upstream from any road or bridge crossing to minimize its effect on stream velocity, depth and overall habitat quality. There should be no major tributaries discharging to the stream in the study area. If a 100m reach is not available for sampling, a standard number of stream widths can be used to measure the stream distance. For example, the EPA's Environmental Monitoring and Assessment Program (EMAP) uses a standard of 40 stream widths for sampling. This approach allows variation in the length of the reach, based on the size of the stream.

Before sampling, complete the physical/chemical field sheet to document site description, weather conditions, and land use. Example forms are provided in **Attachment 1**. After sampling, review this information for accuracy and completeness.

Draw a map of the sampling reach or stream widths on the Field Data Sheet. This map should include in-stream attributes (e.g., riffles, falls, fallen trees, pools, bends, etc.) and important structures, plants, and attributes of the bank and near stream areas. Use an arrow to indicate the direction of flow. Indicate the areas that were sampled for macroinvertebrates, with each sample identification number on the map. Use a hand-held GPS for latitude and longitude determination of the furthest upstream and downstream points of the sampling reach.

5.1 Habitat Types

The following major stream habitat types are colonized by macroinvertebrates and generally support macroinvertebrate diversity in stream ecosystems. Some combination of these habitats will be sampled using this multi-habitat approach to benthic sampling.

Cobble (hard substrate) - In many high-gradient streams, this habitat type will be dominant. Sample shallow areas with coarse (mixed gravel, cobble or larger) substrates by holding the bottom of the dip net against the substrate and dislodging organisms by kicking the substrate for 0.5 m upstream of the net.

Snags - Snags and other woody debris that have been submerged for a relatively long period (not recent deadfall) provide excellent colonization habitat. Sample submerged woody debris by jabbing in medium-sized snag material (sticks and branches). The snag habitat may be kicked first to help dislodge organisms, but only after placing the net downstream of the snag. Accumulated woody material in pool areas are considered snag habitat. Large logs should be avoided because they are generally difficult to sample adequately.

Vegetated banks - When lower banks are submerged and have roots and emergent plants associated with them, they are sampled in a fashion similar to snags. Submerged areas of undercut banks are good habitats to sample. Sample banks with protruding roots and plants by jabbing into the habitat. Bank habitat can be kicked first to help dislodge organisms, but only after placing the net downstream.

Submerged macrophytes - Submerged macrophytes are seasonal in their occurrence and may not be a common feature of many streams, particularly those that are high-gradient. Sample aquatic plants that are rooted on the bottom of the stream in deep water by drawing the net through the vegetation from the bottom to the surface of the water (maximum of 0.5m each jab). In shallow water, sample by bumping or jabbing the net along the bottom in the rooted area, avoiding sediments where possible.

Sand (and other fine sediment) - Usually the least productive macroinvertebrate habitat in streams, this habitat may be the most prevalent in some streams. Sample banks of unvegetated or soft soil by bumping the net along the surface of the substrate rather than dragging the net through soft substrates; this reduces the amount of debris in the sample.

5.2 Sample Collection of Community Samples

5.2.1 General Information

Record the percentage of each habitat type in the reach. Note the sampling gear used, and comment on conditions of the sampling, e.g., high flows, treacherous rocks, difficult access to stream, or anything that would indicate adverse sampling conditions.

Document observations of aquatic flora and fauna. Make qualitative estimates of macroinvertebrate composition and relative abundance as a cursory estimate of ecosystem health and to check adequacy of sampling.

5.2.2 Non-Wadable Waterbodies

Non-wadable waterbodies should use a Ponar or Ekman dredge to collect sediment-dwelling benthic macroinvertebrates. Sediment collection procedures are similar to the collection techniques discussed in the Sediment Sampling SOP (#EH-03). Multiple sediment grab samples will be composited and placed in an elutriator bucket or 500um mesh sieve bucket. Add water to the sample and hand-mix gently to break up lumps of sediment. Pour the sample slurry from the tub through the elutriator or sieve bucket which is placed over a second tub to catch the rinse water. Wash the sediment through the mesh with water at very low pressure. Excessive pressure will result in damage to organisms, in particular oligochaetes, and could compromise taxonomic

analysis of the sample. Gently agitate the sieve bucket to aid in rinsing the fine sediment out of the sample. It may be necessary to sieve the slurry in small portions to prevent clogging of the mesh. Continue to rinse the composite with surface water until all sediments have been removed, leaving behind any sediment-dwelling invertebrates.

The same volume of sediment should be collected and the same number of rinses should be performed at each sampling location to ensure representativeness between locations. The number of grabs collected and rinses performed should be recorded on the Benthic Macroinvertebrate Field Data Sheet (example provided in **Attachment 1**).

Transfer the sample from the bucket to sample container(s) and preserve in enough 95% ethanol to cover the sample. Forceps may be needed to remove organisms from the bucket. Place a sample identification label that includes date, stream name, sampling location, and collector name into the sample container. The outside of the container should include the same information and the words "preservative: 95% ethanol". If more that one container is needed for a sample, each container label should contain all the information for the sample and should be numbered (e.g., 1 of 2, 2 of 2, etc.). This information will be recorded in the "Sample Log" at the biological laboratory.

Complete the top portion of the Benthic Macroinvertebrate Field Data Sheet (**Attachment 1**).

5.2.3 Wadable Waterbodies

Wadable waterbodies (eg: streams, rivers) can be sampled using a kick-net or dip net. Begin sampling at the downstream end of the reach and proceed upstream. A total of 20 jabs or kicks will be taken over the length of the reach; a single jab consists of forcefully thrusting the net into a productive habitat for a linear distance of 0.5m. A kick is a stationary sampling accomplished by positioning the net and disturbing the substrate for a distance of 0.5m upstream of the net.

Different types of habitat are to be sampled in approximate proportion to their representation of surface area of the total macroinvertebrate habitat in the reach. For example, if snags comprise 50% of the habitat in a reach and riffles comprise 20%, then 10 jabs should be taken in snag material and 4 jabs should be take in riffle areas. The remainder of the jabs (6) would be taken in any remaining habitat type. Habitat types contributing less than 5% of the stable habitat in the stream reach should not be sampled. In this case, allocate the remaining jabs proportionately among the predominant substrates. The number of jabs taken in each habitat type should be recorded on the Benthic Macroinvertebrate Field Data Sheet (example provided in **Attachment 1**).

The jabs or kicks collected from the multiple habitats will be composited to obtain a single homogeneous sample. Every 3 jabs (more often if necessary) wash the collected material by running clean stream water through the net two to three times, being careful to retain the sample inside the net. If clogging does occur, discard the material in the net and redo that portion of the sample in the same habitat type but in a different location. Remove large debris after rinsing and inspecting it for organisms; place any organisms found into the sample container. Do not spend time inspecting small debris in the field.

Transfer the sample from the net to sample container(s) and preserve in enough 95% ethanol to cover the sample. Forceps may be needed to remove organisms from the dip net. Place a sample identification label that includes date, stream name, sampling location, and collector name into the sample container. The outside of the container should include the same information and the words "preservative: 95% ethanol". If more that one container is needed for a sample, each container label should contain all the information for the sample and should be numbered (e.g., 1 of 2, 2 of 2, etc.). This information will be recorded in the "Sample Log" at the biological laboratory.

Complete the top portion of the Benthic Macroinvertebrate Field Data Sheet (**Attachment 1**).

5.3 Sample Collection for Analytical Measurements

Sample collection techniques are identical to those utilized to collect benthic community samples. The jabs/kicks or dredge samples collected from multiple habitats will be composited to obtain a single homogeneous sample. Remove any large debris manually and use forceps or elutriation buckets to extract any organisms from the sample; place any organisms found into the appropriate sample container as specified in the QAPP. Continue to collect and composite organisms until the mass requirements for the analytical method are met. Benthic macroinvertebrate samples that will be analyzed for contaminants should be kept on dry ice.

5.4 Habitat Assessment

Perform habitat assessment after sampling has been completed, and record all observations on the Habitat Assessment Field Data Sheet (**Attachment 1**). Having sampled the various microhabitats and walked the reach helps ensure a more accurate assessment. Conduct the habitat assessment with another team member, if possible.

Return samples to the laboratory and complete the log-in forms (example provided in **Attachment 2**).

6.0 SAMPLE CONTAINERS AND LABELING

Sample labels must be properly completed, including the sample identification code, date, stream name, sampling location, and collector's name and placed into the sample container. The outside of the container should be labeled with the same information. Chain-of-custody forms must include the same information as the sample container labels.

7.0 LABORATORY PROCESSING FOR COMMUNITY SAMPLES

7.1 Laboratory Equipment/Supplies

- log-in sheet for samples (example provided in **Attachment 2**)
- standardized gridded pan (30cm x 36cm) with approximately 30 grids (6cm x 6cm)
- 500 micron sieve
- forceps
- white plastic or enamel pan (15cm x 23cm) for sorting
- specimen vials with caps or stoppers
- sample labels
- benthic macroinvertebrate laboratory bench sheet (example provided in **Attachment 2**)
- dissecting microscope for organism identification
- fiber optics light source
- compound microscope with phase contrast for identification of mounted organisms (e.g., midges)
- 70% ethanol for storage of specimens
- appropriate taxonomic keys

Macroinvertebrate samples should be processed in the laboratory under controlled conditions. Aspects of laboratory processing include subsampling, sorting, and identification of organisms.

All samples should be dated and recorded in the "Sample Log" notebook, which is a three-ring binder book used to store Sample Log forms (**Attachment 2**) upon receipt by laboratory personnel. All information from the sample container label must be included on the sample log sheet. If more than one container was used, the number of containers should be indicated as well. All samples should be sorted in a single laboratory to enhance quality control.

7.2 Subsampling and Sorting

The Rapid Bioassessment Protocol III uses a fixed-count approach to subsampling and sorting the organisms from the sample matrix of detritus, sand, and mud. The following protocol is based on a 200-organism subsample, but it could be used for any subsample size (100, 300, 500,

etc.). The subsample is sorted and preserved separately from the remaining sample for quality control checks.

Prior to processing any samples in a lot (i.e., samples within a collection date, specific watershed, or project), complete the sample log-in sheet to verify that all samples have arrived at the laboratory, and are in proper condition for processing.

Thoroughly rinse sample in a 500um mesh sieve to remove preservative and fine sediment. Large organic material (whole leaves, twigs, algal or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected, and discarded. If the samples have been preserved in alcohol, it will be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms, which will prevent them from floating on the water surface during sorting. If the sample was stored in more than one container, the contents of all containers for a given sample should be combined at this time. Gently mix the sample by hand while rinsing to make homogeneous.

After washing, spread the sample evenly across a pan marked with grids approximately 6cm x 6cm. On the laboratory bench sheet, note the presence of large or obviously abundant organisms; do not remove them from the pan.

Use a random numbers table to select 4 numbers corresponding to squares (grids) within the gridded pan. Remove all material (organisms and debris) from the four grid squares, and place the material into a shallow white pan and add a small amount of water to facilitate sorting. If there appear (through a cursory count or observation) to be 200 organisms ± 20% (cumulative of 4 grids), then subsampling is complete.

Any organism that is lying over a line separating two grids is considered to be on the grid containing its head. In those instances where it may not be possible to determine the location of the head (worms for instance), the organism is considered to be in the grid containing most of its body.

If the density of organisms is high enough that many more than 200 organisms are contained in the 4 grids, transfer the contents of the 4 grids to a second gridded pan. Randomly select grids for this second level of sorting as was done for the first, sorting grids one at a time until 200 organisms ± 20% are found. If picking through the entire next grid is likely to result in a subsample of greater than 240 organisms, then that grid may be subsampled in the same manner as before to decrease the likelihood of exceeding 240 organisms. That is, spread the contents of the last grid into another gridded pan. Pick grids one at a time until the desired number is reached. The total number of grids for each subsorting level should be noted on the laboratory bench sheet.

7.3 Identification of Macroinvertebrates

Taxonomy can be at any level, but should be done consistently among samples. Genus/species provides more accurate information on ecological/ environmental relationships and sensitivity to impairment. Family level provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. In either case, only those taxonomic keys that have been peer-reviewed and are available to other taxonomists should be used.

Most organisms are identified to the lowest practical level (generally genus or species) by a qualified taxonomist using a dissecting microscope. Midges (Diptera: Chironomidae) are mounted on slides in an appropriate medium and identified using a compound microscope. Each taxon found in a sample is recorded and enumerated in a laboratory bench notebook and then transcribed to the laboratory bench sheet for subsequent reports. Any difficulties encountered during identification (e.g., missing gills) are noted on these sheets.

Labels with specific taxa names (and the taxonomist's initials) are added to the vials of specimens by the taxonomist. (Note that individual specimens may be extracted from the sample to be included in a reference collection or to be verified by a second taxonomist.) Slides are initialed by the identifying taxonomist. A separate label may be added to slides to include the taxon (taxa) name(s) for use in a voucher or reference collection.

Record the identity and number of organisms on the Laboratory Bench Sheet (**Attachment 2**). Either a tally counter or "slash" marks on the bench sheet can be used to keep track of the cumulative count. Also, record the life stage of the organisms, the taxonomist's initials and the Taxonomic Certainty Rating (TCR) as a measure of confidence.

In the spaces provided on the bench sheet, explain certain TCR ratings or condition of organisms. Other comments can be included to provide additional insights for data interpretation. If QC was performed, record on the back of the bench sheet.

For archiving samples, specimen vials, (grouped by station and date), are placed in jars with a small amount of denatured 70% ethanol and tightly capped. The ethanol level in these jars must be examined periodically and replenished as needed, before ethanol loss from the specimen vials takes place. A stick-on label is placed on the outside of the jar indicating sample identifier, date, and preservative (denatured 70% ethanol).

All samples should be stored on wet ice (4^{o}C) in a secured cooler. Ship samples under chain-of-custody, protected with suitable resilient packing material to reduce shock, vibration, and disturbance.

8.0 SUBSAMPLE PROCEDURE MODIFICATIONS

As an improvement to the mechanics of the technique, a sorting tray was designed that consists of two parts, a rectangular plastic or plexiglass pan (36cm x 30cm) with a rectangular sieve insert. The sample is placed on the sieve, in the pan and dispersed evenly.

When a random grid(s) is selected, the sieve is lifted to temporarily drain the water. A "cookie-cutter" like metal frame 6cm x 6cm is used to clearly define the selected grid; debris overhanging the grid may be cut with scissors. A 6cm flat scoop is used to remove all debris and organisms from the grid. The contents are then transferred to a separate sorting pan with water for removal of macroinvertebrates.

These modifications have allowed for rapid isolation of organisms within the selected grids and easy removal of all organisms and debris within a grid while eliminating investigator bias. Save the sorted debris residue in a separate container. Add a label that includes the words "sorted residue" in addition to all prior sample label information and preserve in 95% ethanol. Save the remaining unsorted sample debris residue in a separate container labeled "sample residue"; this container should include the original sample label. Length of storage and archival is determined by the laboratory or benthic section supervisor.

Place the sorted 200-organism (± 20%) subsample into glass vials, and preserve in 70% ethanol. Label the vials inside with the sample identifier or lot number, date, stream name, sampling location and taxonomic group. If more than one vial is needed, each should be labeled separately and numbered (e.g., 1 of 2, 2 of 2). For convenience in reading the labels inside the vials, insert the labels left-edge first. If identification is to occur immediately after sorting, a petri dish or watch glass can be used instead of vials.

Midge (Chironomidae) larvae and pupae should be mounted on slides in an appropriate medium (e.g., Euperal, CMC-9); slides should be labeled with the site identifier, date collected, and the first initial and last name of the collector. As with midges, worms (Oligochaeta) must also be mounted on slides and should be appropriately labeled.

Fill out header information on Laboratory Bench Sheet (see **Attachment 2**). Also check subsample target number. Complete back of sheet for subsampling/sorting information. Note number of grids picked, time expenditure, and number of organisms. If QC check was performed on a particular sample, person conducting QC should note findings on the back of the Laboratory Bench Sheet. Calculate sorting efficiency to determine whether sorting effort passes or fails.

Record date of sorting and slide monitoring, if applicable, on Log-In Sheet as documentation of progress and status of completion of sample lot.

9.0 DECONTAMINATION

After sampling has been completed at a given site, all nets, pans, etc. that have come in contact with the sample should be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to use at the next sampling site.

Excess sediment and substrate material not included in the sample should be washed into the stream, pond, lake, or surface impoundment where it came from. All marker flags (if reused) should be decontaminated by wiping off with towels and/or baby wipes before re-use.

Throw all used wipes and gloves into the trash bags and take with you to dispose of at the field office.

10.0 RECORD KEEPING AND QUALITY CONTROL

Each field crew will carry a three-ring binder book that contains the Benthic Macroinvertebrate Field Data Sheet, Physical Characterization/Water Quality Field Data Sheet, and sample labels. In addition, a field notebook should be maintained by each individual or team that is collecting samples, as described in the Project Plan. Each sampling location must be recorded on the site diagram. Each sample should have an ID number affixed to the outside of the wide-mouth bottle, and the duplicate label must be affixed to the sample data sheet. Deviations from this sampling plan should be noted in the field notebook, as necessary.

10.1 Required Information

For each location, the notebook information must include:

a. date
b. time
c. personnel
d. weather conditions
e. sample identification numbers that were used
f. descriptions of any deviations to the Project Plan and the reason for the deviation

Samples taken from waters with visible color abnormalities, foaming, unusual odor, iridescent film, or other indications of non-homogeneous conditions should also be noted. Field personnel

will collect the proper type and quantity of quality control samples as prescribed in the QAPP.

10.2 Field Quality Control Samples

The type of quality control samples, and the frequency of collection, are specified in the QAPP. The following quality control samples will be collected.

> Field Duplicate: Field duplicate samples are collected at the same time as the primary sample, and are used to evaluate precision and reproducibility of the analysis and sampling technique or collection team. In this case, the field duplicate sample is a second sample of benthic macroinvertebrates collected from the same reach or widths of a stream.

11.0 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, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

USEPA. 2003. Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes; Chapter 4 - Biological Parameters; LG406 - Standard Operating Procedure for Benthic Invertebrate Field Sampling Procedure, Revision 07. EPA 905-R-03-002. March 2003.

ATTACHMENT 1

Benthic Macroinvertebrate Field Data Sheet

BENTHIC MACROINVERTEBRATE FIELD DATA SHEET

STREAM NAME	LOCATION	
STATION #_____RIVERMILE_____	STREAM CLASS	
LAT _____LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		LOT NUMBER
FORM COMPLETED BY	DATE _____ TIME____ AM PM	REASON FOR SURVEY

HABITAT TYPES	**Indicate the percentage of each habitat type present** ❑ Cobble_____% ❑ Snags_____% ❑ Vegetated Banks_____% ❑ Sand_____% ❑ Submerged Macrophytes_____% ❑ Other ( )_____%
SAMPLE COLLECTION	**Gear used** ❑ D-frame ❑ kick-net ❑ Other _____ **How were the samples collected?** ❑ wading ❑ from bank ❑ from boat **Indicate the number of jabs/kicks taken in each habitat type.** ❑ Cobble_____ ❑ Snags_____ ❑ Vegetated Banks_____ ❑ Sand_____ ❑ Submerged Macrophytes_____ ❑ Other ( )_____
GENERAL COMMENTS	

QUALITATIVE LISTING OF AQUATIC BIOTA
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare, 2 = Common, 3= Abundant, 4 = Dominant

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

FIELD OBSERVATIONS OF MACROBENTHOS
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare (1-3 organisms), 2 = Common (3-9 organisms), 3= Abundant (>10 organisms), 4 = Dominant (>50 organisms)

Porifera	0	1	2	3	4	Anisoptera	0	1	2	3	4	Chironomidae	0	1	2	3	4
Hydrozoa	0	1	2	3	4	Zygoptera	0	1	2	3	4	Ephemeroptera	0	1	2	3	4
Platyhelminthes	0	1	2	3	4	Hemiptera	0	1	2	3	4	Trichoptera	0	1	2	3	4
Turbellaria	0	1	2	3	4	Coleoptera	0	1	2	3	4	Other	0	1	2	3	4
Hirudinea	0	1	2	3	4	Lepidoptera	0	1	2	3	4						
Oligochaeta	0	1	2	3	4	Sialidae	0	1	2	3	4						
Isopoda	0	1	2	3	4	Corydalidae	0	1	2	3	4						
Amphipoda	0	1	2	3	4	Tipulidae	0	1	2	3	4						
Decapoda	0	1	2	3	4	Empididae	0	1	2	3	4						
Gastropoda	0	1	2	3	4	Simuliidae	0	1	2	3	4						
Bivalvia	0	1	2	3	4	Tabinidae	0	1	2	3	4						
						Culcidae	0	1	2	3	4						

Physical Characterization/Water Quality Field Data Sheet

PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET
(Pg. 1)

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

WEATHER CONDITIONS

Now		**Past 24 hours**
❑	storm (heavy rain)	❑
❑	rain (steady rain)	❑
❑	showers (intermittent)	❑
____%❑	%cloud cover	____%
❑	clear/sunny	❑
		❑

Has there been a heavy rain in the last 7 days?

❑ Yes ❑ No

Air Temperature_____^{0} C

Other_____

SITE LOCATION AND MAP

Draw a map of the site and indicate the areas sampled (or attach a photograph)

STREAM CHARACTERIZATION

Stream Subsystem
❑ Perennial ❑ Intermittent ❑ Tidal

Stream Origin
❑ Glacial
❑ Non-glacial montane
❑ Swamp and bog
❑ Spring-fed
❑ Mixture of origins
❑ Other_____

Stream Type
❑ Coldwater ❑ Warmwater

Catchment Area_____km^2

PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET (Pg. 2)

WATERSHED FEATURES	**Predominant Surrounding Landuse** ❑ Forest ❑ Commercial ❑ Field/Pasture ❑ Industrial ❑ Agricultural ❑ Other _____ ❑ Residential **Local Watershed NPS Pollution** ❑ No evidence ❑ Some potential sources ❑ Obvious sources **Local Watershed Erosion** ❑ None ❑ Moderate ❑ Heavy
RIPARIAN VEGETATION (18 meter buffer)	**Indicate the dominant type and record the dominant species present** ❑ Trees ❑ Shrubs ❑ Grasses ❑ Herbaceous **dominant species present** _____
INSTREAM FEATURES	**Estimated Reach Length** _____m **Estimated Stream Width** _____m **Sampling Reach Area** _____m^2 **Area in km^2 (m^2x1000)** _____km^2 **Estimated Stream Depth** _____m **Surface Velocity (at thalweg)** _____m/sec **Canopy Cover** ❑ Partly open ❑ Partly shaded ❑ Shaded **High Water Mark** _____m **Proportion of Reach Represented by Stream Morphology Types** ❑ Riffle_____% ❑ Run_____% ❑ Pool_____% **Channelized** ❑ Yes ❑ No **Dam Present** ❑ Yes ❑ No
LARGE WOODY DEBRIS	**LWD** _____m^2 **Density of LWD** _____m^2/km^2 **(LWD/ reach area)**
AQUATIC VEGETATION	**Indicate the dominant type and record the dominant species present** ❑ Rooted emergent ❑ Rooted submergent ❑ Rooted floating ❑ Free floating ❑ Floating Algae ❑ Attached Algae **dominant species present** _____ **Portion of the reach with aquatic vegetation** _____%
WATER QUALITY	**Temperature**_____ ^{0} C **Specific Conductance**_____ **Dissolved Oxygen** _____ **pH** _____ **Turbidity** _____ **WQ Instrument Used** _____ **Water Odors** ❑ Normal/None ❑ Sewage ❑ Petroleum ❑ Chemical ❑ Fishy ❑ Other_____ **Water Surface Oils** ❑ Slick ❑ Sheen ❑ Globs ❑ Flecks ❑ None ❑ Other_____ **Turbidity (if not measured)** ❑ Clear ❑ Slightly turbid ❑ Turbid ❑ Opaque ❑ Stained ❑ Other_____
SEDIMENT/ SUBSTRATE	**Odors** ❑ Normal ❑ Sewage ❑ Petroleum ❑ Chemical ❑ Anaerobic ❑ None ❑Other_____ **Oils** ❑ Absent ❑ Slight ❑ Moderate ❑ Profuse **Deposits** ❑ Sludge ❑ Sawdust ❑ Paper fiber ❑ Sand ❑ Relict shells ❑ Other_____ **Looking at stones which are not deeply embedded, are the undersides black in color?** ❑ Yes ❑ No

PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET
(Pg. 3)

INORGANIC SUBSTRATE COMPONENTS (should add up to 100%)			ORGANIC SUBSTRATE COMPONENTS (does not necessarily add up to 100%)		
Substrate Type	**Diameter**	**% Composition in Sampling Reach**	**Substrate Type**	**Characteristic**	**% Composition in Sampling Area**
Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"-10")		Muck-Mud	black, very fine organic (FPOM)	
Gravel	2-64 mm (0.1"-2.5")				
Sand	0.06-2mm (gritty)		Marl	grey, shell fragments	
Silt	0.004-0.06 mm				
Clay	< 0.004 mm (slick)				

Habitat Assessment Field Data Sheet

HABITAT ASSESSMENT FIELD DATA SHEET - LOW GRADIENT STREAMS

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Parameters to be evaluated broader than sampling reach	Habitat Parameter	Condition Category			
		Optimal	**Suboptimal**	**Marginal**	**Poor**
	6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
	SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	9. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
	SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
	SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score _____

ATTACHMENT 2

Sample Log-in Sheet

BENTHIC MACROINVERTEBRATE SAMPLE LOG-IN SHEET

Date Collected	Collected By	Number of Containers	Preservation	Station #	Stream Name and Location	Date Received by Lab	Lot Number	Date of Completion		
								sorting	mounting	identification

Benthic Macroinvertebrate Laboratory Bench Sheet

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (Pg. 1)

page _____ of _____

STREAM NAME	LOCATION
STATION #_____ RIVERMILE_____	STREAM CLASS
LAT _____ LONG _____	RIVER BASIN
STORET #	AGENCY
COLLECTED BY DATE_____	LOT #
TAXONOMIST DATE_____	SUBSAMPLE TARGET ❑ 100 ❑ 200 ❑ 300 ❑ Other ____

Enter Family and/or Genus and Species name on blank line.

Organisms		No.	LS	TI	TCR	Organisms		No.	LS	TI	TCR
Oligochaeta						Megaloptera					
Hirudinea						Coleoptera					
Isopoda											
Amphipoda						Diptera					
Decapoda											
Ephemeroptera											
						Gastropoda					
						Pelecypoda					
Plecoptera											
						Other					
Trichoptera											
Hemiptera											

Taxonomic certainty rating (TCR) 1-5:1=most certain, 5=least certain. If rating is 3-5, give reason (e.g., missing gills).

LS= life stage: I = immature; P = pupa; A = adult TI = Taxonomists initials

Total No. Organisms _____ **Total No. Taxa** _____

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (Pg. 2)

SUBSAMPLING/SORTING INFORMATION

Sorter _____

Date _____

Number of grids picked: _____

Time expenditure _____ No. of organisms _____

Indicate the presence of large or obviously abundant organisms:

QC: ❑ YES ❑ NO **QC Checker**

_____

$$\frac{\text{\# organisms originally sorted}}{\text{\# organisms recovered by checker} + \text{\# organisms originally sorted}} = \text{\% sorting efficiency}$$

≥90%, sample passes _____

<90%, sample fails, action taken _____

_____

TAXONOMY

ID _____

Date _____

Explain TCR ratings of 3-5:

Other Comments (e.g. condition of specimens):

QC: ❑ YES ❑ NO **QC Checker**

_____

Organism recognition ❑ pass ❑ fail

Verification complete ❑ YES ❑ NO