BIOLOGICAL MONITORING PROGRAM

QUALITY ASSURANCE PROJECT PLAN FOR

WADEABLE STREAMS AND RIVERS

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Group A: Project Management Elements

A1 – Title and Approval Sheet

Virginia Department of Environmental Quality
Biological Monitoring
Quality Assurance Project Plan for
Wadeable Streams and Rivers

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A2 – Table of Contents

Element/ Section

TABLES

APPENDICES

A3 – Distribution List

Name	Organization
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Darryl Glover	Virginia Department of Environmental Quality
Trisha Johnson	Virginia Department of Environmental Quality
Aimee Genung	Virginia Department of Environmental Quality

Other staff from Virginia Department of Environmental Quality as appropriate.

A4 – Project/ Task Organization

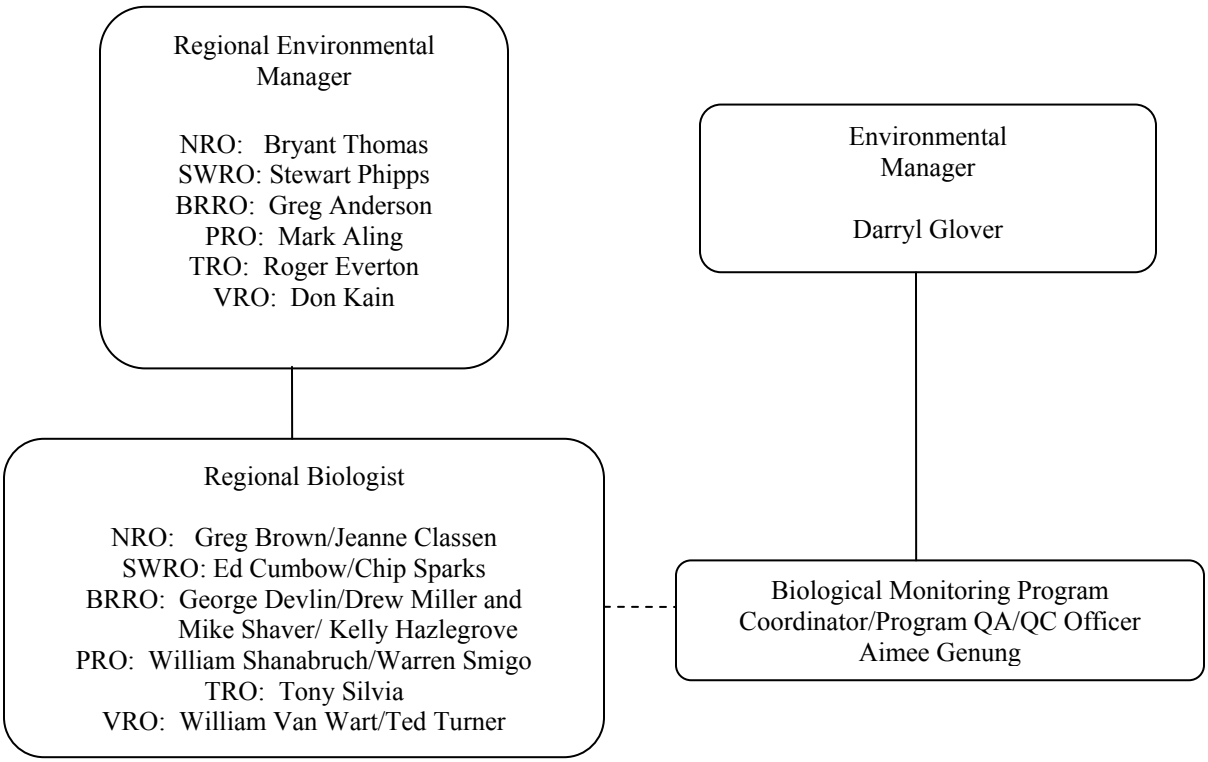

Figure 1: Organizational Chart for VADEQ's Biological Monitoring Program

Virginia Department of Environmental Quality's (VADEQ's) freshwater biological monitoring program is conducted out of six regional offices located throughout Virginia. These offices are located in Abingdon (Southwest Regional Office), Roanoke and Lynchburg (Blue Ridge Regional Office), Harrisonburg (Valley Regional Office), Woodbridge (Northern Regional Office), Glen Allen (Piedmont Regional Office), and Virginia Beach (Tidewater Regional Office). Each regional offices', regional biologists, are under the direction of the regional environmental manager (Figure 1). The Biological Monitoring Program Coordinator in the VADEQ's Central Office in Richmond is responsible for the coordination of the biological monitoring program and also serves as the program QA/QC officer. The program coordinator is under the direction of the environmental manager in the Richmond Central Office.

A5 –Background

Virginia's freshwater biological monitoring program began in the1970's to fulfill requirements of the Federal 106 grant agreement. VADEQ uses benthic macroinvertebrate communities to assess the ecological health of freshwater streams and rivers. Benthic

macroinvertebrates are larger-than-microscopic invertebrate organisms such as insects, crustaceans, snails, mussels, or worms that inhabit stream bottoms.

VADEQ's biological monitoring program examines over 150 stations annually. Reasons for bioassessments include, but are not limited to: targeted monitoring, probabilistic monitoring, tracking local pollution events, follow-up on waters of concern identified through volunteer citizen monitoring, and TMDL monitoring. Data from the biological monitoring program are used in the periodic review and assessment of state waters as required by Section 305(b) of the Clean Water Act. Benthic macroinvertebrate monitoring is used in assessing the designated use of state waters established in 9 VAC 25-260-10 A. that states in part that "All state waters, including wetlands, are designated for the following uses:…the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them…" .

Biological monitoring using benthic macroinvertebrates is an invaluable tool for evaluating the overall, temporally integrated effects of the water and sediment quality in streams and rivers. Benthic macroinvertebrate communities indicate water quality both over time and the effects of different pollution stressors, thus providing a measure of their collective impact, including antagonism and/or synergism among chemical and physical pollutants. Because of their sedentary nature, macroinvertebrates are good indicators of localized conditions. Most species have a complex life cycle of approximately one and, therefore, integrate the effects of fluctuations in water quality over time, which periodic, conventional water quality surveys may miss. In essence, benthic macroinvertebrates are considered to be virtual "living recorders" of water quality conditions over time. The structure and functioning of macroinvertebrate communities are also extremely sensitive, and may exhibit responses to water quality parameters for which specific criteria or standards have not been defined, for which chemical analyses are not normally performed, or for which biological tolerance is below chemical detection limits.

A6 – Project/ Task Description

The VADEQ Data from the biological monitoring program are used in the periodic review and assessment of state waters as required by Section 305(b) of the Clean Water Act. The following are the primary data uses:

1. 305(b) reports: Data are used to provide water quality assessments for the biennial 305(b) reports to the U.S. EPA and Congress.

2. 303(d) listing/delisting: All stream segments assessed as stressed and those where repeated sample data confirm stress are listed on the 303(d) list of waters prioritized for TMDL development and remediation activities. Stream segments assessed as excellent and those where repeated sample data confirm good are delisted from the 303 (d) list of waters prioritized for TMDL development and remediation activities.

3. Virginia Pollutant Discharge Elimination System (VPDES) permits: Some data are used in the permitting process. Biological Assessment Reports may determine if an existing discharge permit is protective of the resident fauna. If the discharge is found to impair the benthic macroinvertebrate community, the permit may be recommended to be reviewed.

4. Probabilistic monitoring (ProbMon): The ProbMon network is a set of randomly chosen stations used to make statistically-based assessments of Virginia's streams.

5. Tracking local pollution events: Biological data may be used to determine the effect of local pollution events in streams and to track the rate of recovery of the benthic communities in these streams.

6. Exceptional State Waters designation: Benthic macroinvertebrate data may be used to determine the exceptional aquatic communities eligibility criterion of Virginia streams and rivers to be classified as "Exceptional State Waters" (9 VAC 25-260-30 (3)).

Coastal Plain Macroinvertebrate Index (CPMI)

The VADEQ uses two bioassessment indices to assess the biotic integrity in non-tidal freshwater streams and rivers in Virginia. In the Coastal Plain, which is characterized by low gradient streams east of the fall line, the Coastal Plain Macroinvertebrate Index (CPMI) is used. This multimetric bioassessment index was developed in 1997 by the Mid-Atlantic Coastal Streams (MACS) workgroup (USEPA 1997 and Maxted et al. 2000). The CPMI was calibrated for low gradient Coastal Plain streams, which exhibit different expected benthic macroinvertebrate communities from non-coastal streams.

Virginia Stream Condition Index

For non-coastal streams, biological assessment of the benthic macroinvertebrate community is based on the methods of the Virginia Stream Condition Index (VSCI). The VSCI was developed for Virginia freshwater non-coastal streams by USEPA's contractor Tetra Tech, Inc., using historical data collected in Virginia at reference and stressed streams in 1994-1998, and was tested against additional data collected in 1999-2002. This review has resulted in the development of the Virginia Stream Condition Index (VSCI) for use in assessing wadeable, non-coastal streams. The VSCI is based upon recent advances in bioassessment methods contained in "*Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers, Second Edition*" (Barbour et al. 1999). The VSCI, a multimetric calculation of benthic integrity converted into a single numerical score, resulted in a single reference condition for the entire non-coastal portion of the Commonwealth against which all future benthic samples will be compared. The development of this index is considered a significant step in the advancement of the biomonitoring program to address a wide range of monitoring and assessment needs. Based on recommendations from public comment and the Academic Advisory Committee (AAC), the VSCI was validated using a spatially diverse (ecoregionally and stream size) data set free of pseudoreplication (http://www.deq.virginia.gov/probmon/). These probabilistic data sets have allowed

VADEQ to narrow data gaps, test the VSCI against many classification variables and confirm with certainty that the VSCI is a good assessment tool for Virginia streams.

The 2008 Integrated Report will be assessing the biological data using the VSCI and the CPMI in the 305(b) report. VADEQ finalized this Stream Condition Index in 2006 and this will be the first 305(b) report that uses the VSCI to assess the biological data.

Stream Macroinvertebrate Sampling

VADEQ uses two sampling procedures for benthic macroinvertebrates depending on stream geomorphology and instream characteristics. The single habitat sampling approach is used for streams in which riffles with appropriate substrate (cobble) are available for sampling and are large enough so that at least 1m² of the substrate can be sampled. The single habitat sampling approach is used exclusively in high gradient streams (see Appendix B i). The multihabitat sampling method is used in cases where no riffles are present, the riffles in the reach are too small and/or too few to sample 1m² of substrate. These riffles are, however, candidates for sampling using the multi-habitat method if they represent at least 5% of the available substrate (see Appendix B ii). Multi-habitat sampling is most commonly performed in, but not limited to, low gradient streams.

Seasonality– VADEQ sample index period for spring sampling is March 1 through May 31 and for fall sampling the sample index period is September 1 through November 30. Professional judgment is applied when sample dates fall close to season cutoffs due to temperatures or weather occurrences. VADEQ applies a 2-week buffer between seasons to account for seasonal uncertainties and improve assessment performance. Biological samples should not be collected during periods of excessively high or low flows or within two weeks of a scouring flow event. It is understood that in some cases, sampling outside of these index periods is necessary to assess immediate impacts. Samples collected outside of these index periods may be considered unacceptable.

Habitat Assessment

Habitat assessment is conducted at each bioassessment site. Both in-stream and riparian habitat are important determinants of the composition, structure, and function of macroinvertebrate communities. Habitat quality is often an indicator of water quality stressors in streams. In addition, poor habitat quality can obscure the effects of specific pollutants. A systematic assessment of in-stream and riparian habitat quality is necessary to fully assess water quality conditions in streams and rivers.

Habitat assessment is considered an important tool for the final evaluation of impairment. Habitat parameters that are evaluated are related to the overall aquatic life use and are a potential source of limitation to the aquatic biota. Both the quality and quantity of available habitat can affect the resident biological community structure and composition. The final conclusion of a bioassessment should take into consideration the habitat quality of a water body and whether the health of aquatic biological communities is limited by habitat conditions. Procedures for habitat assessments are located in Appendix B (iii).

Physicochemical Parameters

Physicochemical parameters, including Dissolved Oxygen (DO), pH, specific conductance, and temperature, are collected at each site using several different types of multi-probe meters. These parameters may provide valuable information in determining what water physicochemical characteristics may be limiting to the health of aquatic biological communities.

Reference Site Selection

Due to the rarity of "pristine" waterways, reference sites are considered to be stream reaches that are the "least disturbed," or are considered to be in the best available condition for a certain ecoregion. Ecoregions are defined as being contiguous land forms with similar geology, soils, vegetative cover, and climate and it is hypothesized that biotic communities within ecoregions are likely to be similar. Reference sites are not needed for VSCI or CPMI assessments, but may aid in future revalidation of these indices.

Reference streams are determined in part by using data from land cover, water quality, and habitat surveys. Biologist's best professional judgment (BPJ) may also be used to determine if a stream has any legacy pollution issues that may result in the stream not meeting the reference requirements.

A7 – Data Quality Objectives

High quality data is imperative to the VADEQ's biological monitoring program's ability to accurately assess the condition of Virginia's streams and rivers. The specific data quality objectives, as discussed below, include accuracy and precision, representativeness, and comparability.

Accuracy and Precision

Data quality objectives for this program emphasize accuracy and precision of benthic macroinvertebrate identification at the family level of taxonomy, which will be maintained by following appropriate Standard Operating Procedures (SOPs) and QA/QC procedures (Appendix C i-ii and Appendix D i-ii).

Representativeness

Sampling methods and techniques, sample preservation, and sample handling are interactive factors that directly affect achievement of representativeness of benthic macroinvertebrate sampling. The experimental design for the biological monitoring program is described in section B of this document. Standard Operating Procedures are utilized by the regional biologists that address station selection, sampling techniques, collection, preservation, handling, and processing to maintain standards of representativeness in the surveys.

Comparability

Comparability of biomonitoring data is a summation of quality products at each phase of the data gathering process. It includes representative sampling, sample handling procedures, and procedures for reporting of biological data. Following SOPs based on published

methodology, uniform sampling procedures, and semi-annual training workshops ensure that regional biologists make accurate assessments of water quality statewide.

A8 – Training Requirements/ Certification

All field sampling as well as laboratory sample processing (subsorting of benthic macroinvertebrates) will be performed by, or under the supervision of, a professional regional biologist.

All benthic taxonomic identifications will be performed by a biologist that has obtained a certification from Virginia Commonwealth University or the North American Benthological Society. Certifications are earned by passing a benthic family level taxonomic identification proficiency test established by professional benthic macroinvertebrate taxonomists. All regional biologists will also be trained, but not certified, in the following; EDAS (biological) Database and freshwater and saltwater fish identification.

Agencies and organizations outside of the VADEQ must submit a QAPP to the VADEQ and this QAPP must be approved by the Biological Monitoring Program Coordinator before their biological data will be used for assessment purposes. QAPP requirements for non-DEQ agencies and organizations are provided in the document Guidance Memo No. 06-2010 "Guidelines for DEQ review and approval of biological monitoring QAPPs submitted by non-DEQ sources" (2006).

A9 – Documentation and Records

The QAPP for this project was written by VADEQ staff and will be sent to the appropriate EPA Region 3 contact for review. The most up-to date version of this QAPP will be available through the Biological Monitoring Program Coordinator and will also be available on VADEQ's website.

All field data (habitat assessments, field observations, and water physicochemical measurements) are entered on standardized forms that are completed at the time of sampling (see Appendix D i). Water physicochemical data are later entered into CEDS in the laboratory. Lists of all identified taxa, physicochemical data, and habitat scores are entered and stored by station in VA Ecological Data Application System (EDAS), an ACCESS© database that facilitates the archiving and retrieving by queries, of taxonomic information. The VA EDAS database provides information that is summarized in the Agency's biennial 305(b) Water Quality Assessment Report. Results are also submitted to EPA under VADEQ's Section 106 grant agreement.

Each regional biologist will keep originals of all field data sheets, taxonomic records, quality control records, instrument calibration records, and miscellaneous correspondence

and notes related to the specific sampling stations in the appropriate dedicated storage locations for a period of five years. Final assessment reports will be sent to the appropriate VADEQ staff for each regional office.

Group B: Measurement/ Data Acquisition Elements

B1 – Sampling Process Design

The VADEQ employs two main types of sampling strategies, probabilistic monitoring and targeted monitoring. The probabilistic monitoring network is a set of randomly chosen stations used to make statistically based assessments of Virginia's streams. This approach differs from targeted monitoring by selecting stations randomly rather than with bias for access or specific data needs. Data from randomly selected stations represents an unbiased distribution of statewide conditions and allows a measure of accuracy of these data.

Targeted monitoring is based on choosing stations for specific data needs, such as reviewing VPDES permits, tracking local pollution events, and other rationale described in section A – 6 of this document.

B2 - Sampling Methods

The sampling methods for the biological monitoring program are shown in the SOPs in Appendix B (i & ii). See section A-6 (stream macroinvertebrate sampling) for sample method determination.

B3 – Sample Handling and Custody

Each regional biologist will be responsible for the sample collection, appropriate preservation, labeling, transport, and storage of benthic macroinvertebrate samples. (For details, see respective SOP in Appendix B). No special custody requirements of samples are required in the current program.

B4 – Analytical Methods

The SOP for benthic macroinvertebrate sub-sampling is located in Appendix B (iv).

B5 – Quality Control

Acceptable relative percent difference values and accuracy levels for quality control procedures for field and laboratory techniques for the biological monitoring program are located in Table 1.

Table 1. Quality Control Objectives for the biological monitoring program

Comparability	Accuracy	Sorting Efficiency
The expected degree of agreement between replicate benthic macroinvertebrate samples is ≥ 85%	The expected MQO for taxonomic precision is a PTD value ≤ 10%	The expected sorting efficiency of benthic macroinvertebrate samples is ≥ 90%

Comparability- Replicate samples are taken at 10% of sampling sites. The degree of agreement is based on the percent comparability of the assessment VSCI scores between replicates. If the percent comparability is < 85%, an evaluation of the consistency of field sampling techniques may be warranted.

Accuracy - The VADEQ's Measurement Quality Objective (MQO) for taxonomic precision was suggested by the EPA to be set at a Percent Taxonomic Disagreement (PTD) value of ≤ 10%. PTD is calculated:

$$\mathrm{PTD} = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] \times 100$$

$comp_{pos}$ is the number of agreements and N is the total number of specimens in the larger of the 2 counts

PTDs are calculated for 10% of samples taken annually from each VADEQ regional biologist and other VADEQ staff certified for taxonomic identification annually. Samples are re-identified by an EPA approved independent taxonomist or the Biological Monitoring Program Coordinator. Samples that do not meet the MQO are evaluated for the types of errors involved. Counting and transcribing errors indicate that greater attention to sample processing may need to be practiced. However, consistent MQOs greater than the suggested PTD due to taxonomic mis-identification may warrant the need for increased taxonomic identification training.

Sorting Efficiency- VADEQ staff involved in laboratory sub-sampling of samples must first demonstrate the ability to remove ≥ 90% of the specimens per grid. For detailed sub-sampling procedures and QA/QC, (see Appendix B iv).

The QA/QC officer/Biological Monitoring Coordinator will be responsible for conducting program audits to ensure appropriate SOPs are being followed in the field and lab.

B6 – Instrument / Equipment Testing, Inspecting, and Maintenance Requirements

Detailed information on testing, inspection, and maintenance requirements of all multi-probe meters for measurement of stream physicochemical parameters can be found in Section IV of the "Standard Operating Procedures Manual for the Department of Environmental Quality Office of Water Quality Monitoring and Assessment" located at www.deq.virginia.gov/watermonitoring/pdf/wqmsop.pdf.

B7 – Instrument Calibration and Frequency

Detailed descriptions of frequency and calibration procedures can be found in Section IV of the "Standard Operating Procedures Manual for the Department of Environmental Quality Office of Water Quality Monitoring and Assessment" located at www.deq.virginia.us/watermonitoring/pdf/wqmsop.pdf

B8 – Inspection/ Acceptance Requirements for Supplies and Consumables

Supplies and consumables used by the biological monitoring program are purchased through various sources. Inspections are made before each sampling event on the D-frame dip net to ensure that there are no tears in the mesh. Sample containers are also to be inspected for damage before use.

B9 –Non-direct Measurements

GIS data may be used in the determination of appropriate reference stations and to facilitate interpretation of sampling results based on watershed characteristics.

B10 – Data Management

Refer to Section A9.

Group C: Assessment/ Oversight Elements

C1 – Assessment and Response Actions

As mentioned in section A5, the VADEQ uses two bioassessment indices to assess the biotic integrity in non-tidal freshwater streams and rivers in Virginia.

For non-coastal streams, biological assessment of the benthic macroinvertebrate community is based on the Virginia Stream Condition Index (VSCI). The individual metrics, metric calculations, and assessment categories used for VSCI assessments are presented in Appendix C (i).

The CPMI is a multimetric bioassessment index which was calibrated for low gradient Coastal Plain streams which exhibit different expected benthic macroinvertebrate communities from non-coastal streams and developed by the MACS workgroup in 1997. The CPMI consists of five metrics: Taxonomic Richness, EPT Richness, % Dominant Taxon, Hilsenhoff Biotic Index, and Percent Clingers. The scores for each metric and assessment category are summarized in Appendix C (ii).

For both the VSCI and CPMI indices, a bioassessment categorized as "excellent" or "good" results in the designation of the stream reach as "fully supporting" for Aquatic Life Use Support (ALUS). A bioassessment categorized as "stressed" or "severely stressed", results in the designation of the stream reach as "impaired needing a TMDL" unless a documented justification for not assessing as impaired is provided. (For detailed assessment determination, see the Water Quality Assessment Guidance Manual for Y2006 located at www.deq.virginia.gov/waterguidance/pdf/052017.pdf).

For the CPMI, values obtained may sometimes be intermediate to established ranges and require some subjective judgment as to the assessment of biological condition. In these instances, habitat assessment and water quality data may aid in the assessment process.

Each regional biologist is required to document any problems encountered during data collection, sample processing, or data analysis, and to take remedial action where required. Such action may include resampling or eliminating data from further consideration.

C2 – Reports to Management

Biomonitoring program staff will discuss QA/QC issues at regularly scheduled meetings or as the need arises. Yearly reports will be developed by the program QA/QC officer and distributed to the regional environmental managers and biologists. A summary of QA/QC activities , including any conditions or situations affecting data completeness or quality, corrective actions, and outcomes of corrective actions will be prepared as part of the final report.

Group D: Data Validation and Usability

D1 – Data Review, Validation, and Verification Requirements

All field and laboratory data will be reviewed, verified, and validated to ensure they conform to program specifications. Regional biologists will confer with one another in the field while collecting physical habitat data and collection of macroinvertebrates to ensure quality data is collected. It will be the responsibility of each regional biologist whether to accept or reject physical habitat data. Taxonomic identification of macroinvertebrates will have a QA/QC of 10% of samples collected per region, per year.

D2 – Validation and Verification Methods

Data review, verification, and validation will be performed using self-assessment and peer and management review. Data will initially be validated by the regional biologist when returning from the field and further validated during entry into the EDAS database. Any errors detected will be rectified by editing incorrect database entries, resampling, or excluding questionable data. Sorting efficiency of sub-sampling macroinvertebrates are QA/QC'd by experienced personnel who will check all sorted quadrates from the first three samples processed by a sorter to ensure that all organisms were removed once per year. This will not only apply to inexperienced sorters, but also to those deemed "experienced." Qualification will only occur when sorters are consistent in achieving $\geq$ 90% sorting efficiency after at least three samples have been checked. The program coordinator will QA/QC 10% of macroinvertebrate samples identified to family level collected in one year's time. Biological data approved by the regional environmental managers will be given to the appropriate waterbody assessment personnel.

D3 – Reconciliation with Data Quality Objectives

All data collected by the biological monitoring program will be reviewed on an ongoing basis for accuracy, precision, and completeness. If data quality does not meet the appropriate specifications, data will be discarded and resampling may occur. If there are problem taxa, from the 10% QA/QC'd by the program coordinator, the regional biologist will be informed and must review the past years samples and re-identify those samples containing problem taxa. Once those taxa are re-identified the program coordinator will QA/QC 10% of the samples for verification.

Group E: Program Assurance

E1 – Audit Verification

The Program and Performance Audits verify that procedures specified in this Project Plan are being utilized. These audits insure the integrity of the reported data. For this program, audits are divided into two major topic areas:

- Field Sampling
- Laboratory

E2 - Field Audits

The internal audits used to evaluate field sampling will examine:

- Sampling Sites
- Sample Collection Procedures
- Assessment of Site

E3 - Laboratory Audits

The internal audits used to evaluate the laboratory will examine:

- SubSampling Procedures
- QA/QC Efficiency
- Taxonomic Skill
- Equipment check

References

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002.

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Stribling, J.B., S.R. Moulton, G.T. Lester. 2003. Determining the quality of taxonomic data. J. N. Am. Benthol. Soc., 22(4):621-631.

Appendix A

List of Acronyms

AAC .....Academic Advisory Committee
ALUS .....Aquatic Life Use Support
BPJ……..Best Professional Judgment
BRRO……..Blue Ridge Regional Office
CEDS .....Comprehensive Environmental Data System
CO .....Central Office
CPMI.....Coastal Plain Macroinvertebrate Index
EDAS .....Ecological Data Application System
GIS .....Geographical Information Systems
MACS .....Mid-Atlantic Coastal Streams
MQO .....Measurement Quality Objective
NRO .....Northern Regional Office
PTD .....Percent Taxonomic Disagreement
PRO.....Piedmont Regional Office
QA.....Quality Assurance
QAPP .....Quality Assurance Project Plan
QC .....Quality Control
RBP II .....Rapid Bioassessment Protocols (II)
SOP/SOPs .....Standard Operating Procedure(s)
SWRO .....South West Regional Office
TMDL .....Total Maximum Daily Load
TRO.....Tidewater Regional Office
EPA .....United States Environmental Protection Agency
VADEQ.....Virginia Department of Environmental Quality
VPDES.....Virginia Pollutant Discharge Elimination System
VRO .....Valley Regional Office
VSCI .....Virginia Stream Condition Index

Appendix B (i)

SOP Title: Methods for Benthic Macroinvertebrate Collections in Cobble Substrate (single habitat)

Date of Last Revision: 07/17/2008

Equipment/Materials:

Standard aquatic dip net
(0.3 meter width (~1 foot))
Wash bucket
Sample containers
Field notebook
First aid kit
D-frame (500-µm mesh openings)
Sieve bucket (500-µm mesh openings)
70-99% isopropyl
Forceps
Pencils

References:

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002.

Procedures:

Habitat:	Riffles, Runs
Area:	2m² total; i.e. 6 kicks of 1/3 m² or 12 kicks of 1/6 m²
Mesh Size	500-µm mesh openings
Index Period	Regional consideration or sample reference sites during same period, decisions based on project/program objectives

1. The sample reach (considered to be a station) should extend to a 100-meter instream segment of habitat having no major tributaries in the assessment area. Sampling should be conducted at least 100-meters upstream of any road or bridge crossing to minimize the affects on stream velocity, depth, and overall habitat.

2. Starting at the downstream end of the reach and moving upstream, all riffles and runs are candidates for sampling throughout the reach. Sampling is conducted holding the dipnet on the bottom of the stream and kicking the cobble substrate (i.e., riffles and runs) to agitate and dislodge organisms. A single kick consists of disturbing the substrate upstream of the net by kicking with the feet and/or by using the hands to dislodge the cobble/boulder for **30 seconds – 1 ½ minutes**. For example six kicks disturbing a 1/3 of a m² above the dip net or 12 kicks disturbing a 1/6 of a m² of above dip net should be used to sample a total of **2m²**, at **30 seconds – 1 ½ minutes** per kick net sample.

3. *Riffles/Runs* – Shallow part of the stream where water flows swiftly over completely or partially submerged pebble to boulder sized rocks to produce surface agitation. Sample by holding the bottom rim of the dip net against the substrate downstream of the riffle

and perpendicular to the flow while disturbing the substrate just upstream of the net with feet and hands to dislodge organisms.

4. The collected sample is washed by running clean stream water through the net 2-3 times. The sample is then transferred to the sieve bucket if needed. Do not let the net become so clogged with debris that it results in the diversion of water around the net rather than through the net. If clogging occurs, discard the sample that is in the net and redo that portion of the sample in a different location.

5. As the sample is added to the sieve bucket (when needed), it should be further washed to remove fines. While sieving, remove large debris from the sample after rinsing and inspecting for organisms, and place any organisms back into the sieve bucket. Do not attempt to inspect small debris.

6. Transfer the sample from the kick net or sieve bucket to a prelabeled sample container(s) and preserve in 70-99. percent isopropyl alcohol. Forceps may be needed to remove organisms from the screen and dipnet.

7. Complete the Benthic Macroinvertebrate Field Data Sheet including comments on weather and wildlife observations, etc. Notes on the stable habitats sampled should be recorded. (i.e., the proportion of snags, vegetation, etc. sampled, the type of substrate, and the condition of the habitats).

Quality Control (QC)

1. Field sampling QC involves the collection of replicate samples at various reaches to verify the repeatability of the results obtained by a single set of field investigators. Each investigation team should conduct replicate sampling at 10 percent of the sampling reaches. Replicate sampling is conducted either on an adjacent reach upstream of the initial sampling area or within the initial sampling area in close proximity, (not in the same locations as the first set of samples). The replicated sample should be similar to the initial site in respect to habitat, stressors, point source pollution, etc. Replicate samples are preserved, subsampled, and the organisms are identified using SOPs. Results are recorded in a sampling QC log book.

2. Sample labels should include the following information: station ID, date, habitat sampled, sampler's name, and 1of 2, 2 of 2, etc.

Appendix B (ii).

SOP Title: Methods for Multi-habitat Benthic Macroinvertebrate Collections

Date of Last Revision: 07/17/2008

Equipment/Materials:

Standard aquatic dip net
(0.3 meter width (~1 foot))
Wash bucket
Sample containers
Field notebook
First aid kit
D-frame (500-µm mesh openings)
Sieve bucket (500-µm mesh openings)
70-99% isopropyl
Forceps
Pencils

References:

United States Environmental Protection Agency. 1997. Field and laboratory methods for macroinvertebrate and habitat assessment of low-gradient nontidal streams. Mid-Atlantic Coastal Streams Workgroup, Environmental Services Division, Region 3, Wheeling, W.V

Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002.

Procedures:

Habitat:	Snags, Vegetation, Banks, Riffles
Area:	20 jabs, each 1-m in length
Mesh size:	500-µm mesh openings
Index Period	Regional consideration or sample reference sites during same Period decisions based on project/program objectives

1. The sample reach (considered to be a station) should extend to a 100-meter instream segment of habitat having no major tributaries in the assessment area. Sampling should be conducted at least 100-meters upstream of any road or bridge crossing to minimize the affects on stream velocity, depth and overall habitat.

2. Sampling is conducted from downstream to upstream by jabbing the D-frame net into productive and stable habitats 20 times. A single jab consists of forcefully thrusting the net into a productive habitat for a linear distance of 1-meter, followed by 2-3 sweeps of the same area to collect dislodged organisms for **20 seconds – 1 ½ minutes / jab, sweep, or kick**.

3. Different types of habitat should be sampled in rough proportion to their frequency within the reach. Unique habitat types (i.e., those consisting of less than 5 percent of stable habitat within the sampling reach) should not be sampled.

4. Identify proportional representation of habitat types. Characterize the bottom and shore-zones according to features present at the time the sample is collected. Do not base characterizations on anticipated oscillations of flow regime or substrate compositions.

    a) Bottom-zone (within channel substrate)
        - Riffles have relatively fast velocity, shallow steam depth, steep surface gradient, and a straight to convex channel profile. Riffles are usually topographic high areas produced by the accumulation of coarse materials.
        - Non-riffle encompasses all other forms (i.e., pools, runs, and slack areas) and generally possesses intermediate to fine particle substrate.
        - Vegetation, such as submerged macrophytes, serve as habitat for macroinvertebrates and may constitute large areas of the available substrate.

    b) Shore-zone (allochthonous material)
        - Overhanging vegetation includes terrestrial shore-zone plant material that is living, submerged, and provides in-stream cover for fish and macroinvertebrates.
        - Submerged tree roots include living root material from shoreline or overhanging vegetation that is submerged and provides in-stream cover for fish and macroinvertebrates.
        - Woody debris includes submerged snags and/or other woody material that has been microbially conditioned. Woody debris in the channel is considered part of the shoreline for estimating allocation of sampling.

5. Proportionally allocate sampling effort (20 jabs/sweeps/kicks) to shore-zone and bottom-zone, 2**0 seconds – 1 ½ minutes/jab, sweep, or kick**.

6. The collected sample is washed by running clean stream water through the net 2-3 times The sample is then transferred to the sieve bucket (if needed). Samples should be cleaned and transferred to the sieve bucket at least every five jabs, more often if necessary. Do not let the net become so clogged with debris that it results in the diversion of water around the net rather than through the net. If clogging occurs, discard the sample that is in the net and redo that portion of the sample in a different location.

7. As the sample is added to the sieve bucket (when needed), it should be further washed to remove fines. While sieving, remove large debris from the sample after rinsing and inspecting of organisms, and place any organisms back into the sieve bucket. Do not attempt to inspect small debris.

8. Transfer the sample from the kick net or sieve bucket to a pre-labeled sample container(s) and preserve in 70-99 percent isopropyl alcohol. Forceps may be needed to remove organisms from the sieve screen and dipnet.

Following are specific sampling techniques for different productive and stable habitats:

Riffles/Runs – Shallow part of the stream where water flows swiftly over completely or partially submerged pebble to boulder sized rocks to produce surface agitation. Sample by holding the bottom rim of the dip net against the substrate downstream of the riffle and perpendicular to the flow while disturbing the substrate just upstream of the net with feet and hands to dislodge organisms.

Snags- Submerged woody debris, sampled by jabbing in medium-sized snag material (sticks and branches). The 1-meter section of this habitat is estimated. The snag habitat may be kicked first to help dislodge organisms, but do so only after placing net in water downstream of the snag. Accumulated woody material in pool areas can also be considered as snag habitat.

Vegetation – Aquatic plants that are rooted on the bottom of the stream. They are sampled in deep water by drawing the net through the vegetation from the bottom to the surface of the water. In shallow water, they are sampled by bumping the net along the bottom in the rooted area.

Banks – When banks have roots, plants, and snags associated with them, they are sampled in a fashion similar to snags. When the banks are of unvegetated or soft soil, they are sampled by bumping the net along the substrate rather than dragging the net through soft substrates. This will reduce the amount of detritus (defined as sticks, leaves, and/or pieces of bark) through which you would have to pick. Also, the bank habitat can be kicked first in order to help dislodge organisms.

9. Complete the Benthic Macroinvertebrate Field Data Sheet including comments on weather and wildlife observations etc. Notes on the stable habitats sampled should be recorded. (i.e., the proportion of snags, vegetation, etc. sampled, the type of substrate, and the condition of the habitats).

Quality Control (QC)

1. Field sampling QC involves the collection of replicate samples at various reaches to verify the repeatability of the results obtained by a single set of field investigators. Each investigation team should conduct replicate sampling at 10 percent of the sampling reaches. Replicate sampling is conducted on an adjacent reach upstream of the initial sampling. The adjacent reach should be similar to the initial site in respect to habitat, stressors, point source pollution, etc. Replicate samples are preserved, sub-sampled, and the organisms are identified using SOPs. Results are recorded in a sampling QC log book.

2. Sample labels should include the following information; station ID, date, habitat sampled, sampler's name, and 1 of 2, 2 of 2, etc.

Appendix B (iii)

SOP Title: Methods for Habitat Assessment for Streams

Date of Last Revision: 12/28/2007

Equipment/Materials:
Habitat Assessment Field Sheets for (1) High Gradient Streams
(2) Low Gradient Streams
Pencils
Field notebook

References:
Barbour, M.T., J. Gerritsen, and B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers; periphyton, benthic macroinvertebrates, and fish 2nd edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-b-99-002.

Procedures:

1. Select the reaches for conducting the habitat assessment and complete the sections on general characteristics and land use.

2. The habitat assessment will be focused on evaluating the physical habitat structure of a 100-meter section of the stream and upper reaches in the catchment for the large-scale parameters.

    a) Identify the downstream point of the reach that was sampled for macroinvertebrates. Measure a 100-meter section, upstream, that is consistent with the biological sampling reach to assess large-scale parameters.

    b) Complete the identifying information on the field data sheets for the habitat assessment.

Physical Habitat Structure:

Conduct the habitat assessment. Refer to the descriptors described here and the decision criteria on the habitat assessment field data sheet.

<u>High Gradient Streams</u>

The first 5 parameters are assessed directly in the entire 100-meter reach that was used for the macroinvertebrate sampling.

1. **Epifaunal substrate/available cover** includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs and branches, cobble and large rocks,

and undercut banks that are available to fish and macroinvertebrates for refugia, spawning/nursery activities, and/or feeding. A wide variety of submerged structures in the stream provide aquatic organisms with many living spaces; the more living spaces in a stream, the more types of organisms the stream can support.

2. **Embeddedness** refers to the extent to which rocks (gravel, cobble, and boulders) are surrounded by, covered, or sunken into the silt, sand, or mud of the stream bottom. Generally, as rocks become embedded, fewer living spaces are available to macroinvertebrates and fish for shelter, spawning, and egg incubation. This parameter is assessed primarily in the riffles, if present. To estimate the percent of embeddedness, observe the amount of silt or finer sediments surrounding the rocks. If kicking does not dislodge the rocks or cobbles, they may be greatly embedded. It may be useful to lift a few rocks and observe how much of the rock (e.g., ½, ⅓) is darker due to anoxic reaction on the inorganic surface.

3. **Velocity/Depth regime** is important to the maintenance of healthy aquatic communities. Fast water increases the amount of dissolved oxygen in the water, keeps pools from being filled with sediment, and helps food items like leaves, twigs, and algae move more quickly through the aquatic system. Slow water provides spawning areas for fish and shelters macroinvertebrates that might be washed downstream in higher stream velocities. Similarly, shallow water tends to be more easily aerated (i.e., hold more oxygen), but deeper water stays cooler longer. Thus, the best stream habitat will include all of the following velocity/depth combinations and can maintain a wide variety of organisms.

    a) Slow (<0.3 m/sec), Shallow (<0.5 m)
    b) Fast (>0.3 m/sec), Deep (>0.5 m)
    c) Fast, Shallow
    d) Slow, Deep

4. **Sediment deposition** is a measure of the amount of sediment that has been deposited in the stream channel and of the changes to the stream bottom that have occurred as a result of the deposition. Excessive levels of sediment deposition create an unstable and continually changing environment that is unsuitable for many aquatic organisms. Sediments are naturally deposited in areas where flow is obstructed. These deposits can lead to the formation of islands, shoals, or point bars (sediment that builds up in the stream, usually at the beginning of a meander) and can result in the complete filling in of pools. To determine whether or not these sediment deposits are new, look for vegetation growing on them: new sediments will not yet have been colonized by vegetation.

5. **Channel flow status** determines the percentage of the channel that is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, less living area is available for aquatic organisms. Assess the wetted width of the stream in relation to the location of the lower bank.

The next 2 parameters should be assessed along a length of stream that includes the sampling reach plus 1 or 2 reaches upstream.

6. **Channel alteration** is basically a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened (e.g. dredged), or diverted into concrete channels, often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present when the stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when combined sewer overflow (CSO) pipes are present; when the stream is of uniform depth due to dredging; and when other such changes have occurred. Signs that indicate the occurrence of dredging include straightened, deepened, and otherwise uniform stream channels, and the removal of streamside vegetation to provide dredging equipment access to the stream.

7. **Frequency of riffles (or bends)** is a way to measure the heterogeneity occurring in a stream. Because riffles are a good source of high-quality habitat and faunal diversity, an increase in the frequency of riffles provides for greater diversity of the stream community. In streams where riffles are uncommon, a measure of the frequency of bends can be used as a measure of meandering or sinuosity, which also provides for a diverse habitat and fauna. Additionally, streams with a high degree of sinuosity are better suited to handle storm surges through absorption of energy by bends as well as providing refugia for fauna during storm events.

For the last 3 parameters, visually evaluate the condition of the right and left stream banks, separately. Face downstream to determine left from right. Assess these parameters along the stream margins for the sampling reach as well as 1 or 2 adjacent reaches, up or down stream, also facing downstream.

8. **Bank stability** measures erosion potential and whether or not the stream banks are eroded. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks and are, therefore, considered to have high erosion potential. Signs of erosion include crumbling; unvegetated banks, exposed tree roots, and exposed soil.

9. **Bank vegetative protection** measures the amount of the stream bank that is covered by natural (i.e., growing wild and not obviously planted) vegetation. The root systems of plants growing on stream banks help hold soil in place, reducing erosion. Vegetation on banks provides shade for fish and macroinvertebrates and serves as a food source by dropping leaves and other organic matter into the stream. Ideally, a variety of vegetation should be present, including trees, shrubs, and grasses. Vegetative disruption may occur when the grasses and plants on the streambanks are mowed or grazed upon, or the trees and shrubs are cut back or cleared.

10. **Riparian vegetative zone width** is defined here as the width of natural vegetation from the edge of the stream bank. The riparian vegetative zone is a buffer zone to pollutants

entering a stream from runoff. It also controls erosion and provides stream habitat and nutrient input into the stream. A wide, relatively undisturbed riparian vegetative zone reflects a healthy stream system. Narrow, far less useful riparian zones occur when roads, parking lots, fields, lawns and other artificially cultivated areas, bare soil, rocks, or buildings are near the stream bank. The presence of "old fields" (i.e., previously developed agricultural fields allowed to convert to natural conditions) should rate higher than fields in continuous or periodic use.

Low Gradient Streams

11. **Epifaunal substrate/available cover** includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs and branches, cobble and large rocks, and undercut banks, that are available to fish and macroinvertebrates for refugia, spawning/nursery activities, and/or feeding. A wide variety of submerged structures in the stream provide aquatic organisms with many living spaces. The more living spaces in a stream, the more types of organisms the stream can support.

12. **Pool substrate characterization** refers to the type and condition of bottom substrates found in pool sediment types (e.g., gravel, sand) and rooted aquatic plants that support a wider array of organisms than pools dominated by mud or bedrock and with little or no plants. Additionally, streams with a variety of substrate types will support far more types of organisms than streams with uniform pool substrates.

13. **Pool variability** rates the overall mixture of pool types found in streams according to size and depth. Streams with many pool types support a wider variety of organisms than streams with fewer pool types. Thus, the best stream habitat will include all of the following pool types and can maintain a wider variety of aquatic species.

    a) Large (>half cross-section of stream), Shallow (<1.0 m)
    b) Small (<half cross-section of stream), Deep (>1.0 m)
    c) Large, Deep
    d) Small, Shallow

14. **Sediment deposition** is a measure of the amount of sediment that has been deposited in the stream channel and of the changes to the stream bottom that have occurred as a result of the deposition. Excessive levels of sediment deposition create an unstable and continually changing environment that is unsuitable for many aquatic organisms. Sediments are naturally deposited in areas where the stream flow is reduced, such as pools and bends, or where flow is obstructed. These deposits can lead to the formation of islands, shoals, or point bars (sediments that build up in the stream, usually at the beginning of a meander) or can result in the complete filling in of pools. To determine whether or not these sediment deposits are new, look for vegetation growing on them: new sediments will not yet have been colonized by vegetation.

15. **Channel flow status** determines the percent of the channel that is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams

and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, less living area is available for aquatic organisms. Assess the wetted width of the stream in relation to the location of the lower bank.

The next 2 parameters should be assessed along a length of stream that includes the sampling reach plus one or two reaches upstream.

16. **Channel alteration** is basically a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened (e.g., dredged), or diverted into concrete channels, often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present when the stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams, bridges, and flow-altering structures, such as combined sewer overflow (CSO) pipes are present; when the stream is of uniform depth due to dredging; and when other such changes have occurred. Signs that indicate the occurrence of dredging include straightened, deepened, and otherwise uniform stream channels, and the removal of streamside vegetation to provide dredging equipment access to the stream.

17. **Channel sinuosity** is a way to measure the meandering or sinuosity occurring in a stream. A stream with a high degree of sinuosity provides for a more diverse habitat and fauna than a stream with a low degree of sinuosity. Additionally; streams with a high degree of sinuosity are better suited to handle storm surges through absorption of energy by bends as well as providing refugia for fauna during storm events.

For the last 3 parameters, visually evaluate the condition of the right and left stream banks, separately. Face downstream to determine left from right. Assess these parameters along the stream margins for the sampling reach as well as 1 or 2 adjacent reaches.

18. **Bank stability** measures erosion potential and whether or not the stream banks are eroded. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks and are therefore considered to have a high erosion potential. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.

19. **Bank vegetative protection** measures the amount of the stream bank that is covered by natural vegetation (i.e., growing on stream banks) which helps hold soil in place, reducing erosion. Vegetation on banks provides shade for fish and macroinvertebrates and serves as a food source by dropping leaves and other organic matter into the stream. Ideally, a variety of vegetation should be present, including trees, shrubs, and grasses. Vegetative disruption may occur when the grasses and plants on the streambanks are mowed or grazed upon, or the trees and shrubs are cut back or cleared.

20. **Riparian vegetative zone width** is defined here as the width of natural vegetation from the edge of the stream bank. The riparian vegetative zone is a buffer zone to pollutants

entering a stream from runoff. It also controls erosion and provides stream habitat and nutrient input into the stream. A wide, relatively undisturbed riparian vegetative zone reflects a healthy stream system. Narrow, far les useful riparian zones occur when roads, parking lots, fields, lawns and other artificially cultivated areas, bare soil, rocks, or buildings are near the stream bank. The presence of "old fields" (i.e., previously developed agricultural fields allowed to convert to natural conditions) should rate higher than fields in continuous or periodic use.

21. Perform QC on the datasheets. Habitat assessment sheets and any field data sheets should be filled out as accurately and completely as possible. All field data sheets should be properly labeled and filled out.

Habitat assessments are subjective evaluations and are potentially subject to variability among investigators. Minimize variability by proper training, discuss habitat parameters, and conduct evaluations as a team. See Barbour et al. (1999) for more specific guidance.

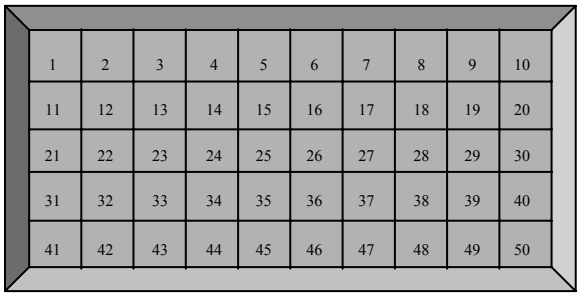
Appendix B (iv)

Title: Methods for Laboratory Sorting and Subsampling of Benthic Macroinvertebrate Samples

Date of Last Revision: 07/17/2008

Equipment/Materials:

Forceps
Standardized gridded tray (500 µm screen, 50 quadrants, each 25 cm^2)
Gridded subsample tray (25 quadrants, each 1 m^2)
Small putty knife
Quadrant-sized square metal "cookie cutter"
White plastic or enamel pan for sorting
70% isopropyl alcohol
Specimen vials, caps, or stoppers
Sample labels
Scissors
Dissecting microscope for organism identification (10-40x)
Macroinvertebrate Log Book
Benthic Macroinvertebrate Subsampling bench sheet



Subsampling Tray

References:

Caton, L. W. 1991. Improved sub-sampling methods for the EPA "Rapid Bioassessment" Benthic protocols. Bulletin for the North American Benthological Society 8(3):317-319.

Barbour, M.T., J. Gerritsen, and B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and rivers: periphyton, benthic macroinvertebrates, and fish, 2nd Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA841-B-00-002.

General:

The sorting and subsampling of the macroinvertebrate samples in the laboratory facilities include processing and identification of organisms collected in wadeable streams. A randomized 110-organism sub-sample is sorted and preserved using a special Caton gridded tray and screen, designed by Larry Caton, Oregon Department of Environmental Quality

(Caton, 1991). Documentation for the level of effort, or proportion of sample processed, is recorded on the Benthic Macroinvertebrate Laboratory Bench Sheet.

Internal Label Information Required for each Vial of Sorted Material and Vial of Identified Macroinvertebrates:

- Station ID
- Stream Name
- Sampling Date
- Sorter's Initials
- "1 of 2" "2 of 2" if necessary
- Habitat sampled

Procedures:

1. Log each sample (as it is received) on the Benthic Sample Log-in sheet (located in the Benthic Log Book) until ready for processing.

2. Remove the lid from the sample container or open the sample and pull out the internal sample label (save the sample label – it will need to be transferred into the sample vial of macroinvertebrates, or prepare a new label). Record sample collection information on the Benthic Macroinvertebrate Laboratory Bench Sheet. Header information required includes: station ID, stream name, date the sample was collected, sampling method, person subsorting, # of grids subsorted, person identifying the insects, total # of subsorted insects, date identified, and sorting date.

3. Transfer the homogenized sample material to the gridded Caton tray. Wash the sample thoroughly by running tap water over it to remove any fine material.

4. Place the gridded tray into a larger container or sink. Add enough water to spread the sample evenly throughout the Caton grid, use BPJ. Spread the sample material over the bottom of the pan as evenly as possible. Move the sample into the corners of the pan using forceps, a spoon, or by hand. Vibrate or shake the pan gently to help spread the sample.

5. Slowly lift the screen out of the larger tray or sink to drain.

6. Use a random number generator to select a grid to process. Remove all the material from that grid and place the removed material into a separate holding container, such as a white, plastic or enamel pan or petri dish. The material is removed as follows:

    a. Place the metal dividing frame or "cookie cutter" over the sample at the approximate location of the grid selected for processing (based on the numbers marked on the sides of the gridded tray). Use a pair of rulers or other

straight edges to facilitate lining up the cookie cutter at the intersection, if necessary.

b. Remove the material within the "cookie cutter" using a putty knife, a teaspoon, or forceps. Depending on the consistency of what is in the sample, it might be necessary to cut the material along the outside of the "cookie cutter" with scissors or putty knife so that only one grid's worth of sample material is used. Inspect the screen for any remaining organisms. An organism is considered to be in the grid containing most of its body that is if more than 50% of an organism is in a grid it belongs to that grid.

c. Place the material from the selected grid(s) into a separate white plastic or enamel pan or petri dish. Add the necessary amount of water to the pan/dish to facilitate sorting.

7. Completely remove all macroinvertebrates from the selected (**First**) grid by examining the material beneath a dissecting microscope or place the selected grid in a tray and place under a magnifying glass to remove organisms (**all organisms should NOT be removed with the naked eye only**) and store organisms in an internally-labeled vial (or larger container, if necessary) containing **70%** isopropyl alcohol as a preservative. If more than 30-45 organisms are selected from the first grid, use your **best professional judgment**, with regards to whether or not you should subsample. If subsampling skip to step 8, if not continue with step 7a;

   a. Keep a count of the number of organisms removed and enter the number of organisms found in each grid under the correct column on the Sub-Sample and Sample Reduction Sheet (Appendix D ii).

   b. Continue selecting and processing randomly selected quadrates until 110 organisms +/- 10% (99-121) are counted. **Each grid begun must be picked to completion; that is, even if the target is reached halfway through a grid, finish the entire grid. A minimum of 4 grids must be picked.** Record the number of quadrates in the subsample on the Benthic Macroinvertebrate Laboratory Bench Sheet (use multipliers from the table for high density samples).

   c. **Do not remove or count empty snail or bivalve shells, pupae, or incidentally-collected terrestrial taxa**. Also do not count fragments such as legs, heads, antennae, gills, or wings, which do not include the head. For Oligochaeta, attempt to remove and count only whole organisms and fragments that include the head.

   d. If the last grid being processed results in more than 121 organisms (i.e., 10% above target number), evenly redistribute all of the organisms (without detritus) in a 25 grid tray. Use a random numbers table and counting backwards, from your total count, remove organisms from

selected grid (s) (remember to remove ALL organisms in selected grid) until you are left with your target count of 110 organisms within 10% (99-121) remaining in the tray. The organisms that are removed may be discarded and the organisms that are remaining in your tray are your benthic sample to be identified.

e. Identify all the organisms in the sample to lowest identifiable taxonomic level **(retain identified sample in vial for up to FIVE years)**, record the number of organisms on the Benthic Macroinvertebrate Bench Sheet, and enter the data into EDAS.

8. Processing of high density samples

a. Discard all of the organisms picked from the first grid.

b. Using a random numbers table, take the number of grids designated by the table below **all at once, these removed grids will** depend upon the number of organisms found in the first grid. The removed grids will now be your sample to re-subsample. An **example** of removal would be the following; when removing 15, 20, or 25 grids you should be able to remove 3, 4, or 5 columns from the box. For example if you are to remove 15 grids, choose 3 random numbers (i.e. 3, 28, 55) and remove **columns** 3, 8, and 5. If you are to remove 10 grids, choose 5 numbers (i.e. 2, 45, 77, 66, and 91) and remove grids next to one another. For example, grids 2 and 3 as well as 5 and 6 that are located in column 4, and grids 7 and 8 that are located in column 7, etc…. Place the selected removed grids in the sorting tray and set aside. Discard the remaining sample in the subsampling box.

c. Completely mix the selected grids in the tray. If the first grid has more than 30 organisms, use your ***best professional judgment***, with regards to whether or not you should re-subsample, and then go back to step 7 a-f.

Organisms per grid in original sample	Remove and keep following number of grids	Predicted number of organisms per grid	Predicted number of grids to reach 110	Multiplier for recording total number of grids picked
30-45	25	15 - 25.5	5 – 7	0.5
46-55	20	18.4 - 22	5 – 6	0.4
56-75	15	16.8 – 22.5	7 – 5	0.3
76-110	10	15.2 - 22	5 – 7	0.2
111-230	5	11.1 - 22	5 – 10	0.1
231-315	4	18.48 – 25.2	6 – 4	0.08

*4 quadrates must be removed. If removal leads to over 121 organisms, subsampling will continue as described in step 6d.

Documentation:

1. Complete a Benthic Macroinvertebrate Laboratory Bench Sheet for each sample as it is processed.

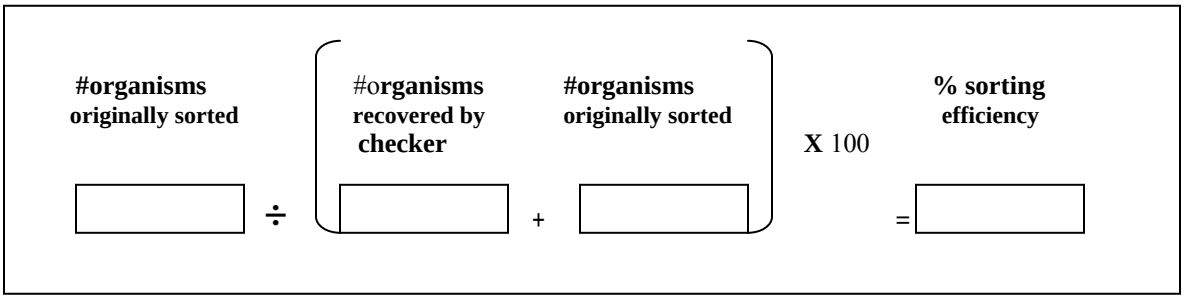
QA/QC

Because it can be difficult to detect the organisms in stream samples (due to inexperience, detritus, etc.), only persons who have received instruction by a regional biologist familiar with processing benthic samples can perform a quality control (QC) check. Regional biologists must perform QC checks anytime samples are processed by an inexperienced individual. These QC checks must be performed immediately following sorting of each grid. All sorters, whether inexperienced or experienced, will be checked on their first three samples **once per year** by a regional biologist or an experienced QC checker.

1. Initially, a regional biologist will check all sorted quadrates from the first three samples processed by a sorter to ensure that all organisms were removed from the detritus. This will not only apply to inexperienced sorters, but also other regional biologists. Qualification will only occur when sorters are consistent in achieving ≥ 90% sorting efficiency after at least three samples have been checked.

2. The QC checker will calculate sorting efficiency for each sample (number of organisms/sample found by the initial sorter ÷ total number of organisms/sample found by QC Officers × 100 = %), If sorting efficiency for each of these three consecutive samples is ≥ 90% for a particular individual, this individual is considered "experienced" and can serve as a QC checker. In the event that an individual fails to achieve ≥ 90% sorting efficiency, they will be required to sort an additional three samples in order to continue to monitor their sorting efficiency. However, if they show marked improvement in their sorting efficiency prior to completion of the next three samples, whereby they acquire the ≥ 90% sorting efficiency, the QC checker may, at his/her discretion, consider this individual to be "experienced." Sorting

efficiency should not be calculated for samples processed by more than one individual.



Audits on sample collection practices will be conducted at each regional office at least every two years. Audits for laboratory identification and equipment maintenance will be conducted at least every two years on a separate occasion from the field audits. See Appendix E.

Appendix C (i)

Virginia Stream Condition Index (VSCI): Metric scoring criteria, assessment catergories, and metric definitions

Metrics
1. Total Taxa (a)
2. EPT Taxa (a)
3. % Ephemeroptera (a)
4. % Plecoptera + Trichoptera less Hydropsychidae (a)
5. % Scrapers (a)
6. % Chironomidae (b)
7. % Top 2 Dominant (b)
8. HBI (family) (c)

a. Score is the total possible score * the (metric value / by the standard best value X_{95}).

b. Score is the total possible score * the (total possible score - the metric value/the total possible score - the standard best value X_5).

c. Score is the total possible score * the (total possible score - the metric value/the total possible score - the standard best value X_5).

Total Possible Score = 100

Assessment Category	Score Range
Excellent	≥73
Good	60-72
Stress	59-43
Severe Stress	≤42

Metric	Definition	Responses to Increased pertubation
1. Total Taxa	Measures total number of taxa observed.	Decrease
2. EPT Taxa	Measures total number of pollution sensitive Ephemeroptera, Plecoptera, and Trichoptera observed.	Decrease
3. % Ephemeroptera	Measures % Ephemeroptera taxa present in sample.	Decrease
4. % Plecoptera + Trichoptera less Hydropsychidae	Measures % Plecoptera + Trichoptera, subtracting pollution tolerant Hydropsychidae	Decrease
5. % Scrapers	Measures % scraper functional feeding group present in sample.	Decrease
6. % Chironomidae	Measures % pollution tolerant Chironomidae present in sample.	Increase
7. % Top 2 Dominant Taxa	Measures % dominance of the 2 most abundant taxa.	Increase
8. HBI (family)	Hilsenhoff Biotic Index.	Increase

The Coastal Plain Macroinvertebrate Index (CPMI): Metric scoring criteria, assessment categories, and metric definitions

	Metric Scoring Criteria			
Metric	6	4	2	0
1. Total Taxa	>17	12-17	6-11	<6
2. EPT Taxa	>6	5-6	3-4	<3
3. % Ephemeroptera	>24%	16-24%	8-15%	<8%
4. HBI	<5.7	5.7-6.4	6.5-7.2	>7.2
5. % Clingers	>26%	18-26%	9-17%	<9%

Total Possible Score = 30

Assessment Category	**Score Range**
Excellent	24 - 30
Good	16 - 22
Stress	6 - 14
Severe Stress	0 - 4

Metric	**Definition**	**Response to increased perturbation**
Total Taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
EPT Taxa	Number of taxa in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
% Ephemeroptera	Percent of mayfly nymphs	Decrease
Hilsenhoff Biotic Index (HBI)	Uses tolerance values to weight abundance in an estimate of overall pollution	Increase
% Clingers	Percent of insects having fixed retreats or adaptations for attachment to surfaces in flowing water	Decrease

Benthic Macroinvertebrate Field Data Sheet (front)

Station ID: _____ **Ecoregion:** _____ **Land Use** _____

Field Team: _____ **Survey Reason:** _____ **Start Time:** __:__

Stream Name: _____ **Location:** _____ **Finish Time:** __:__

Date: __/__/__ **Latitude:** _____ **Longitude:** _____

Stream Physicochemical

Instrument ID number: _____ **pH:** _____

Temperature: _____Co **Conductivity:** _____uS/cm

Dissolved Oxygen: _____mg/l Did instrument pass all post-calibration checks? Y / N

If NO - which parameter(s) failed and action_____

Benthic Macroinvertebrate Collection

Method used (circle one) **Single Habitat** (Riffle) **Multi Habitat** (Logs, plants, etc)

Riffle Quality (circle one) **Good** **Marginal** **Poor** **None**

Habitats sampled (circle one) **Riffle** **Snags** **Banks** **Vegetation** **Area Sampled** (sq. m.):

jabs _____ _____ _____ _____ _____

Weather Observations

Current Weather (circle one) **Cloudy** **Clear** **Rain/Snow** **Foggy**

Recent precipitation (circle one) **Clear** **Showers** **Rain** **Storms** **Other** _____

Stream flow (circle one) **Low** **Normal** **Above Normal** **Flood**

Biological Observations

0 1 2 3	Periphyton	0 1 2 3	Salamanders	0 1 2 3	Other....
0 1 2 3	Filamentous algae	0 1 2 3	Warmwater Fish	0 1 2 3	_____
0 1 2 3	Submerged Macrophytes	0 1 2 3	Coldwater Fish	0 = Not Observed	
0 1 2 3	Emergent Macrophytes	0 1 2 3	Beavers	1 = Sparse	
0 1 2 3	Crayfish	0 1 2 3	Muskrats	2 = Common to Abundant	
0 1 2 3	Corbicula	0 1 2 3	Ducks/Geese	3 = Dominant -	
0 1 2 3	Unionidae	0 1 2 3	Snakes	abnormally high density where other taxa are insignificant	
0 1 2 3	Operculate Snails	0 1 2 3	Turtles	in relation to the dominant taxa. There can be situations	
0 1 2 3	Non-operculate Snails	0 1 2 3	Frogs/Tadpoles	where mulitiple taxa are dominant such as algae and snails.	

NOTES:

_____

_____

HighGradient Habitat Data Sheet

	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/Available Cover	Greater then 70% of substrate favorable for epifauna 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 tranisent).r	40-70% mis of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colinzation (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; sbustrate frequently disturbed or removed.	Less than 20% 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
2. Embeddedness	Optimal	Suboptimal	Marginal	Poor
	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder partilces are more thatn 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
3. Velocity/Depth Regime	Optimal	Suboptimal	Marginal	Poor
	CoverAll four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). Slow is <0.3 m/s, deep is >0.5	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 veolcity/depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
4. Sediment Deposition	Optimal	Suboptimal	Marginal	Poor
	Little or no enlargement of islands or point bars	Some new increase in bar formation, mostly	Moderate deposition of new gravel, sand or fine	Heavy deposits of fine material, increased bar

	and less than 5% (<20% for l;ow-gradient streams) of the bottom affected by sediment deposition.	from gravel, sand or fine sediment. 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	sediment on old and new bars; 30-50% (50-80% for low-gradient) of	development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
	m/s.			
	Optimal	Suboptimal	Marginal	Poor
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
6. Channel Alteration	Optimal	Suboptimal	Marginal	Poor
	Channelization or dredging absent or minimal; stream with normal patter.	Some channelization present, usually in areas of bridge abutements; 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 - 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
7. Frequency of Riffles (or bends)	Optimal	Suboptimal	Marginal	Poor
	Occurrence of riffles relatively frequent ratio of distance btw. riffled divided by width of the stream <7:1 *generally 5 to 7): variety of habitats if key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance btw. riffles divided by the width of the stream is btw. 7 to 15.	Occasional riffle or bend; bottom contours provide saome habitat; distance btw. riffles divided by the width of the stream is btw. 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance btw. riffles dividied by the width of the stream is a ration of >25%.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
8. Bank Stability (score each bank)	Optimal	Suboptimal	Marginal	Poor
	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
SCORE RB	10 9	8 7 6	5 4 3	2 1 0
SCORE LB	10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	Optimal	Suboptimal	Marginal	Poor
	More than 90% of the stream bank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or non-woody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of stream bank 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 stream bank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less then one-half of the potential plant stuibble height remaining.	Less than 50% of the stream bank surfaces covered by vegetation; disruption of stream bank vegetation is very high; vegetation has been removed to 5 cm or less in average stubble height.
SCORE RB	10 9	8 7 6	5 4 3	2 1 0
SCORE LB	10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank)	Optimal	Suboptimal	Marginal	Poor
	Width of riparian zone >18 m; human activities (i.e. parking lots, roadbaeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 m; human activites have impacted zone only minimally.	Width of riparian zone 6-12 m: human activiteid have impacted zone a great deal.	Width if riparian zone <6 m; little or no riparian vegetation due to human activities.
SCORE RB	10 9	8 7 6	5 4 3	2 1 0
SCORE LB	10 9	8 7 6	5 4 3	2 1 0

SCORE _____

Appendix D (ii)

Biological Monitoring
ver. 1
August 2008

Benthic Macroinvertebrate Laboratory Bench Sheet

Station ID: _____ Sample subsorted by: _____ Date: _____

Stream Name: _____ # of Grids subsorted: _____

Date Sampled: _____ Total # of subsorted insects: _____

Sampling Method: _____ Sample Identified by: _____ Date: _____

Taxa Collected:

Porifera			Metretopodidae_____		Limnephilidae_____
	Spongillidae_____		Neoephemeridae_____		Molannidae_____
Flatworms			Oligoneuridae_____		Odontoceridae_____
	Planariidae_____		Psuedironidae_____		Philopotamidae_____
Gastropoda	Unknown_____		Polymitarcyidae_____		Phryganeidae_____
Limpets	Ancylidae_____		Potamanthidae_____		Polycentropodidae_____
Snails			Siphlonuridae_____		Psychomyiidae_____
	Lymnaeidae_____	Zygoptera	Tricorythidae_____		Rhyacophilidae_____
	Physidae_____		Early Instar/Damaged		Sericostomatidae_____
	Planorbidae_____		Calopterygidae_____		Uenoidae_____
	Hydrobiidae_____		Coenagrionidae_____	Lepidoptera	Unknown_____
	Pleuroceridae_____		Lestidae_____		Pyralidae_____
	Viviparidae_____		Protoneuridae_____	Coleoptera	Early Instar/Damaged
Unionida	Immature_____	Anisoptera	Early Instar/Damaged		Chrysomelidae_____
	Corbiculidae_____		Aeshnidae_____		Curculionidae_____
	Sphaeriidae_____		Cordulegastridae_____		Dryopidae_____
	Unionidae_____		Corduliidae_____		Dytiscidae_____
Oligochaeta	Unknown_____		Gomphidae_____		Elmidae_____
Lumbriculida			Libellulidae_____		Gyrinidae_____
	Lumbriculidae_____		Macromiidae_____		Haliplidae_____
Tubificida			Petaluridae_____		Helodidae_____
	Enchytraeidae_____		Cordullidae/Libelluidae_____		Helophoridae_____
	Naididae_____	Plecoptera	Early Instar/Damaged		Hydraenidae_____
	Tubificidae_____		Capniidae_____		Hydrochidae_____
Haplotaxida			Chloroperlidae_____		Hydrophilidae_____
	Haplotaxidae_____		Leuctridae_____		Limnichidae_____
Leeches	Hirudinea _____		Nemouridae_____		Noteridae_____
	Erpobdellidae_____		Peltoperlidae_____		Psephenidae_____
	Glossiphoniidae_____		Perlidae_____		Ptilodactylidae_____
	Hirudinidae_____		Perlodidae_____		Scirtidae_____
	Pisciolidae_____		Pteronarcyidae_____	Diptera	Early Instar/Damaged
Branchiobdellida			Taeniopterygidae_____		Athericidae_____
	Branchiobdellidae_____	Hemiptera	Early Instar/Damaged		Blephariceridae_____
Decapoda	Cambaridae_____		Belostomatidae_____		Canaceidae_____
	Portunidae_____		Corixidae_____		Ceratopogonidae_____
Shrimp			Gelastocoridae_____		Chaoboridae_____
	Palaemonidae_____		Gerridae_____		Chironomidae (A)_____
Isopoda			Hebridae_____		Chironomidae (B)_____
	Asellidae_____		Hydrometridae_____		Culicidae_____
Amphipoda			Mesoveliidae_____		Dixidae_____
	Crangonyctidae_____		Naucoridae_____		Dolichopodidae_____
	Gammaridae_____		Nepidae_____		Empididae_____
	Talitridae_____		Notonectidae_____		Ephydridae_____
Water Mites			Veliidae_____		Muscidae_____
	Hydracarina_____		Pleidae_____		Nymphomyiidae_____
Ephemeroptera	Early Instar/Damaged	Neuroptera			Pelecorhynchidae_____
	Acanthometropodidae_____		Sisyridae_____		Psychodidae_____
	Ameletidae_____	Megaloptera			Ptychopteridae_____
	Baetidae_____		Corydalidae_____		Sciomyzidae_____
	Baetiscidae_____		Sialidae_____		Simuliidae_____
	Behningiidae_____	Trichoptera	Early Instar/Damaged		Stratiomyidae_____
	Caenidae_____		Brachycentridae_____		Syrphidae_____
	Ephemerellidae_____		Calamoceratidae_____		Tabanidae_____
	Ephemeridae_____		Glossosomatidae_____		Tanyderidae_____
	Heptageniidae_____		Helicopsychidae_____		Thaumaleidae_____
	Isonychiidae_____		Hydropsychidae_____		Tipulidae_____
	Leptophlebiidae_____		Hydroptilidae_____		
			Lepidostomatidae_____		
TOTAL:			Leptoceridae_____		

Use back of sheet for subsampling information

Sub-sample and Sample Reduction Sheet

Organisms found in first grid = _____ (Grid #_____)

If <30 organisms found, continue to table below.
If >30 organisms found, discard 1st grid, enter # of grids for sample reduction and continue to table below.

Sample Reduction? Y___ N ____ Number of Grids selected for reduction =_____

Grid I.D. #	# of Organisms	Grid I.D. #	# of Organisms	Grid I.D. #	# of Organisms	Grid I.D. #	# of Organisms

Total organisms = _____ Total grids = _____

For sample reduction: _____ x _____ = _____
(# of grids after reduction) (correction multiplier) (corrected # of grids from orig. sample) {A}

IF after picking, there are >121 organisms, then return picked sample to 15-30 grid tray and remove grids (per SOP) to reduce sample to 121 organisms or less. Record data below.

Total # of organisms retained = _____
Grids removed to reduce sample to 121 organisms or fewer = _____
Percentage of grids retained for sample (to total grids) = _____

_____ x _____ = _____
(# of grids from original sample {A}) (% of grids retained) (final corrected # of grids from original sample)

Appendix E (i)

QA/QC Sorting Efficiency Sheet

QC Initials ________ **SORTERS Initials** ____________ **Pass or Fail** (Circle)

$$\frac{\#\text{organisms originally sorted } [\quad]}{\#\text{organisms recovered by checker } [\quad] + \#\text{organisms originally sorted } [\quad]} \times 100 = \%\text{ sorting efficiency } [\quad]$$

QC Initials ________ **SORTERS Initials** ____________ **Pass or Fail** (Circle)

$$\frac{\#\text{organisms originally sorted } [\quad]}{\#\text{organisms recovered by checker } [\quad] + \#\text{organisms originally sorted } [\quad]} \times 100 = \%\text{ sorting efficiency } [\quad]$$

QC Initials ________ **SORTERS Initials** ____________ **Pass or Fail** (Circle)

$$\frac{\#\text{organisms originally sorted } [\quad]}{\#\text{organisms recovered by checker } [\quad] + \#\text{organisms originally sorted } [\quad]} \times 100 = \%\text{ sorting efficiency } [\quad]$$

QC Initials ________ **SORTERS Initials** ____________ **Pass or Fail** (Circle)

$$\frac{\#\text{organisms originally sorted } [\quad]}{\#\text{organisms recovered by checker } [\quad] + \#\text{organisms originally sorted } [\quad]} \times 100 = \%\text{ sorting efficiency } [\quad]$$

Appendix E (ii)

Biomonitoring Audit Summary

Date:

Field personnel:

River:

Region:

Site visit by:

A. Collection Procedures for Single Habitat:

	Yes	No
1. Reach is at least 100-meters upstream of any road or bridge crossing.	☐	☐
2. Kick sampling consisted of 6 (1/3 of a m²) or 12 (1/6 of a m²) sampling sites.	☐	☐
3. Kicks were times according to SOP.	☐	☐
4. Sample was collected in adequate sampling area i.e. riffle/run.	☐	☐
5. Collected sample was sieved and transferred to sample container according to SOP.	☐	☐
6. Collected sample was correctly preserved in a minimum 70% isopropyl alcohol.	☐	☐
7. Benthic Macroinvertebrate Field Data Sheet was filled out appropriately.	☐	☐
8. Benthic Sample replicate (if required at site) followed SOP protocol.	☐	☐
9. Sample labels written according to SOP.	☐	☐

NOTES:

B. Collection Procedures for Multi Habitat:

	Yes	No	
1. Reach is at least 100-meters upstream of any road or bridge crossing.	☐	☐	
2. Sampling consisted of 20 jabs, each 1 m in length, followed by 2-3 sweeps.	☐	☐	
3. Kicks were times according to SOP.	☐	☐	
4. Sample was collected in adequate sampling area according to SOP, i.e. different types of habitat should represent proportion of their frequency.	☐	☐	Percent Habitat _____
5. Collected sample was sieved and transferred to sample container according to SOP.	☐	☐	

6. Collected sample was correctly preserved in a minimum 70% isopropyl alcohol. ☐ ☐
7. Benthic Macroinvertebrate Field Data Sheet was filled out appropriately. ☐ ☐
8. Benthic Sample replicate (if required at site) followed SOP protocol. ☐ ☐
9. Sample labels written according to SOP. ☐ ☐

NOTES:

C. Habitat Assessment Procedures:

	Yes	No
1. Was assessment sheet filled out according to high or low (circle one) gradient systems.	☐	☐
2. Habitat assessment was scored according to SOP.	☐	☐

NOTES:

D. Laboratory Sorting and Subsampling Procedures:

	Yes	No
1. Sample information was recorded in Log-In book according to SOP.	☐	☐
2. Sample was washed and spread evenly in Caton Grid Tray according to SOP.	☐	☐
3. Random number was used to select first grid.	☐	☐
4. Material from grid was removed according to SOP.	☐	☐
5. **ALL** macroinvertebrates were removed from grid material according to SOP.	☐	☐
6. If more than 30 organisms in first grid, SOP was followed to continue sub-sampling.	☐	☐
7. A minimum of 4 grids were picked.	☐	☐
8. The processed sample resulted in 110 organisms $\pm$ 10% (99-121).	☐	☐
9. If number 8 resulted in NO, then SOP was followed to result in 110 organisms $\pm$ 10% (99-121).	☐	☐
10. Only **aquatic** organisms were removed from sample according to SOP.	☐	☐
11. QA/QC sorting efficiency is up to date.	☐	☐

NOTES:
