VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY WATER MONITORING AND ASSESSMENT PROGRAM

BIOLOGICAL MONITORING QUALITY ASSURANCE PROJECT PLAN FOR WADEABLE STREAMS AND RIVERS

Effective Date: October 24, 2024
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1. PROGRAM MANAGEMENT AND INFORMATION/DATA QUALITY OBJECTIVES

1.1 Approval Page

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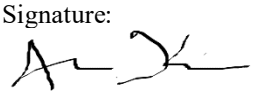
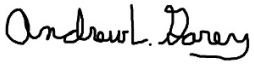
Name: Andrew Kirk Title: Biological Monitoring Coordinator	Signature:  Date: 10/18/2024
Name: Andrew Garey Title: Water Monitoring Team Lead	Signature:  Date: 10/18/2024

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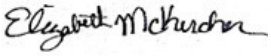
Name: Elizabeth McKercher Title: Director, Water Planning Division	 Signature: Date: 10/22/2024

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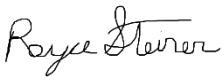
Name: Royce Steiner Title: WQM Quality Assurance Coordinator	Signature:  Date: 10/15/2024

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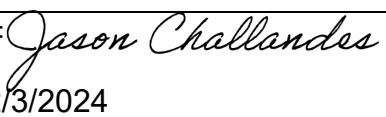
Name: Jason Challandes Title: R3 Designated Project Manager Organization: EPA R3 WD SWPS	Signature:  Date: 12/3/2024

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1.2 Document Format, Document Control, Table of Contents

Document Format

This Quality Assurance Project Plan (QAPP) was developed in accordance with the U.S. EPA Quality Assurance Project Plan Standard. The order of the elements in this QAPP follows the Standard, as seen in the Table of Contents. The QAPP is also in accordance with the U.S. EPA Region 3 Quality Management Plan, DCN R3QMP001-20200601.

Document Control

This table shows changes to this controlled document over time. The most recent version is presented in the top row of the table. Previous versions of the document are maintained by Quality Manager.

Table 1.2-1 QAPP Revisions

EPA Document Control #	DEQ Revision #	History/ Changes	Effective Date
240327.1	03	Updated in accordance with EPA QAPP standard template.	October 24, 2024
NA	02	Updated enumeration and taxonomy protocols: specimens identified primarily to genus under laboratory dissecting microscope.	August 13, 2008

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1.3 Purpose and Background

Virginia's freshwater biological monitoring program began in the1970's to fulfill requirements of the Federal 106 grant agreement. DEQ uses benthic macroinvertebrate communities to assess the ecological health of freshwater streams and rivers. Benthic macroinvertebrates are larger-than-microscopic invertebrate organisms and include animals such as insects, crustaceans, snails, mussels, and worms that inhabit stream bottoms.

DEQ's biological monitoring program examines over 150 stations annually. The program focuses on wadeable, non-tidal, streams and rivers. Estuarine biomonitoring is conducted as part of the Estuarine Probabilistic Monitoring Program (not reviewed in this document), and the agency has not developed biomonitoring methods for other freshwater systems (e.g., swamps and impoundments). Reasons for bioassessments include, but are not limited to conventional monitoring, probabilistic monitoring, tracking local pollution events, follow-up on waters of concern identified through volunteer citizen monitoring, and monitoring to support Total Maximum Daily Load (TMDL) or watershed implementation studies. Data from the biological monitoring program are used in the periodic review and assessment of state waters as required by the Virginia Water Quality Monitoring, Information and Restoration Act (WQMIRA) and section 305(b) of the Clean Water Act. Benthic macroinvertebrate monitoring is used in assessing the designated use of state waters established in 9VAC25-260-10A. that states in part: "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. Because macroinvertebrates do not generally travel great distances during their lifetime, they are good indicators of localized conditions. Most species have relatively long-life cycles and, therefore, integrate the effects of fluctuations in water quality over longer time periods than water quality surveys, which focus on physical and chemical measurements. The structure and functioning of macroinvertebrate communities are also sensitive to water quality parameters for which specific criteria have not been defined, for which chemical analyses are not normally performed, or for which biological tolerance is below chemical detection limits. In essence, benthic macroinvertebrates function as living recorders of water quality conditions over time.

1.4 Project/ Task Description

Stream Macroinvertebrate Sampling

Benthic macroinvertebrate samples are collected following the agency's Standard Operating Procedures (Appendix B-iii). Briefly, multiple grab samples are collected using a D-frame dipnet, from areas along the stream bottom where the highest biodiversity is expected to occur.

DEQ uses two sampling procedures--single and multihabitat sampling methods--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 $2m^2$ 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, or the riffles in the reach are too small and/or too few to obtain a complete sample. 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.

DEQ collects macroinvertebrate samples during both spring and fall of the same year for most stations monitored. The 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. Depending on weather patterns for a given season, samples may be taken up to two weeks before, or two weeks after, each seasonal window. Biological samples are not collected during periods of excessively high or low flows. 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 for use in water quality assessments.

In the laboratory, each sample is sorted, using a randomized procedure such that at least 210 organisms are selected (i.e., subsampled) from each sample. If the sample contains less than 210 individuals, the entire sample is used. Except for some true flies, insects in this subsample are typically identified to genus. Non-insects and some true flies (Order: Diptera) in the subsample are typically identified to Family or a higher taxonomic level whereas other insect taxa are typically identified to genus.

The data from the biological monitoring program are used in the periodic review and assessment of state waters as required by the Virginia Water Quality Monitoring, Information and Restoration Act (WQMIRA) and section 305(b) of the Clean Water Act. The following are the primary data uses:

1. 305(b)/303(d) Water Quality Assessment Integrated Report (IR): Data are used to provide water quality assessments for the biennial IR to the U.S. EPA and Congress. Waters assessed as impaired are listed on the 303(d) impaired waters list and subsequently scheduled for development of a Total Maximum Daily Load (TMDL) or other remediation activities.

2. 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.

3. Probabilistic monitoring (ProbMon): The ProbMon network is a set of randomly selected stations used to make statistically based inferences of Virginia's waters. The program spans fresh and estuarine waterway however, the methodologies in this QAPP are only relevant in freshwater streams.

4. 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.

5. Exceptional State Waters designation: Benthic macroinvertebrate data may be used to determine the exceptional aquatic community's eligibility criterion of Virginia streams and rivers to be classified as "Exceptional State Waters" (9VAC25-260-30(3)).

6. Public Information: DEQ provide and explain the data to interested residents to inform their understanding of Virginia's waters.

Macroinvertebrate Indices and future development

Based on the data produced from the laboratory identification, DEQ uses one of three bioassessment indices (depending on the geographic region sample) to assess attainment of the aquatic life use at biological monitoring sites. There are two multimetric indices for the coastal plain region and one multimetric index for non-coastal streams. The specific index used depends on the geographic region sampled, as defined by physiographic province and ecoregion. For definition and designation of ecoregions see Woods et al. 2012.

Coastal Plain Macroinvertebrate Indices

DEQ uses two multimetric indices to assess the biotic integrity in non-tidal freshwater streams in the Coastal Plain of Virginia. Both are adaptations of the Coastal Plain Macroinvertebrate Index (CPMI) which was used for assessment of macroinvertebrate data through 2012. The original CPMI was developed in 1997 by the Mid-Atlantic Coastal Streams (MACS) workgroup (Maxted et al. 2000) and utilized a relatively small number of Virginia streams (15 unique biomonitoring stations). By 2012, DEQ had accumulated a robust data set for Virginia Coastal Plain streams which enabled development of more refined bioassessment tools for these low gradient streams based on 55 unique biomonitoring stations (Dail et al. 2013). The new Virginia Coastal Plain Macroinvertebrate Index VCPMI (65- Chowan) is applied to Virginia streams in the Southeastern Plains (Ecoregion 65) except for waters located in the Chowan River basin. Streams in the Chowan basin tend to be very low gradient, blackwater systems with different macroinvertebrate communities than other streams in Ecoregion 65. The VCPMI (63 + Chowan) is used for bioassessment of Virginia streams located in the Middle Atlantic Coastal Plain (Ecoregion 63) or coastal plains of the Chowan basin.

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 resulted in the development of the Virginia Stream Condition Index (VSCI) for use in assessing wadeable, non-coastal streams (Burton & Gerritsen 2003). 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 benthic samples are compared. The development of this index was 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. These probabilistic data sets have allowed DEQ 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 (Dail et al. 2006).

Future Index Development

The agency is currently working in partnership with EPA Region 3 and the Office of Research and Development on new methodology for conducting benthic assessments. The result of this work will likely be a set of indices based on genus-level taxonomy and 220-individual subsamples, whereas the current indices incorporate family level taxonomy and 110-individual subsamples. The number of indices developed, and the regions of the state over which they are applied, are to-be-determined, and are dependent on analysis results. This QAPP will be revised to include additional details on the genus-level indices when they are implemented for assessment.

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 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 in Appendix B-iii.

Physicochemical Parameters

Physicochemical parameters, including dissolved oxygen (DO), pH, specific conductance, and temperature, are collected at each site using 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. Collection of physiochemical data over multiple seasons provides additional utility for determining what factors may affect aquatic life.

Reference Site Selection

The concept of reference conditions, that is, the expected biological conditions in the absence of substantial human disturbance, is critical to biomonitoring. The selection of sites that reflect such conditions, which are commonly referred to as reference sites, is a key component of index development. The benthic macroinvertebrate data collected at reference sites in Virginia are the foundation of the biological assessment indices used by the agency.

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 landforms 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 directly for stream assessments but are a critical element in future refinement of the multimetric indices used for bioassessment, and the continued validation of the agency's current biological assessment processes. Therefore, reference

site selection is ongoing in order to strengthen the biological monitoring dataset and increase the accuracy of assessment outcomes based on those data.

Reference streams are determined in part by using data on abiotic factors such as land cover, water quality, and habitat survey. Biologist's best professional judgment (BPJ) may also be used to determine if a stream should be excluded as a reference site due to anthropogenic impacts that are apparent on site, but not reflected in the data used for reference site selection.

1.5 Quality Objectives and Criteria for Data

Data Quality Objectives

Data Quality Objectives (DQOs) are qualitative and quantitative statements that specify the quality of data required to support specific WMA decisions. DQOs also specify the level of uncertainty that a decision maker is willing to accept in results derived from monitoring data that are used for a regulatory or programmatic decision, such as establishing analytical method requirements, establishing sampling protocols or the revision or development of industry standards.

The WMA program, using existing performance information on the methods and procedures contained in this document, developed the DQOs defined in this section. Because DQOs are established through an iterative process, these values may be adjusted by the WMA QA Coordinator based on continual evaluation of performance data generated by this program.

The main objective of this document is to provide biological monitoring data of known and documented quality for the purpose of:

1. Performing water quality assessments in the biennial water quality assessment integrated report (IR) to EPA.
2. Stream segment rankings (303(d) listing).
3. Evaluating of the effectiveness of implementation of best management practices (BMPs).
4. Providing data and guidance to managers and modelers for restoration programs.

The DQOs for this program are provided in Table 1.5-1.

Table 1.5-1. Data Quality Objectives for the biological monitoring program

Comparability	Accuracy	Sorting Efficiency	Completeness
The expected degree of agreement between field replicate benthic macroinvertebrate samples is ≥ 85%	The expected Measurement Quality Objective for taxonomic precision is a PTD value ≤ 15%	The expected sorting efficiency of benthic macroinvertebrate samples is ≥ 90%	The expected data completeness is 90% to generate a meaningful dataset for its intended purpose.

Action Limits/Levels

High quality data are imperative for accurately assessing the condition of Virginia's streams and rivers. The quality of data generated by the biological monitoring activities can be expressed in terms of accuracy, precision, representativeness, and comparability.

Precision and Bias

The precision and bias of data are influenced by the procedures used by the field staff during the collection and analysis of a sample. Data quality objectives for this program emphasize accuracy and precision of benthic macroinvertebrate identification at the genus level of taxonomy, which will be maintained by following appropriate Standard Operating Procedures (SOPs) and QA/QC procedures (Appendices B i-ii and D).

Representativeness

The representativeness of the benthic macroinvertebrate data is mainly dependent on establishment of sampling locations and sampling procedures that produce results representative of the true conditions at the time of sampling. 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 refers to the extent to which the data generated by this program are comparable to other studies conducted in the past or from other areas. 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. To ensure comparability, DEQ requires the use of standardized sampling methods as defined in the SOP, standard laboratory methods for macroinvertebrate identification, uniform sampling procedures, standardized site selection procedures, and annual training workshops to ensure accurate assessments of water quality statewide.

Completeness

The completeness of data identifies how many data are required to meet Quality Objectives, e.g., the % of data needed to evaluate results for your purpose. Ideally, 100% of the data should be available for its intended use. However, there is always the possibility of data loss due to laboratory or equipment error, insufficient sample volume, or samples broken during transport. In addition, unexpected situations may arise where field conditions do not allow for 100% data completeness. Due to these unforeseen possibilities, DEQ considerers 90% data completeness sufficient to evaluate results for the intended purpose.

1.6 Distribution List

The following individuals in Table 1.6-1 will receive a copy of this QAPP and any subsequent revisions. A complete copy of the original version and all revisions of the QAPP shall be

maintained in the organization's files by the Project Manager and made available to approval authorities upon request. The project roles listed in Table 1.6-1 are detailed in Section 1.7.

Table 1.6-1. QAPP Distribution List and Project Roles

Name	Project Role	Organization
Jason Challandes	EPA R3 Designated Project Manager Senior Manager	EPA Region 3
Central: Sandra Mueller Blue Ridge: Jason Hill Northern: Jeff Talbott Piedmont: Heather Deihls Southwest Willard Keene Tidewater: Cory Routh Valley: Tara Wyrick	Senior Managers - Water Monitoring and Assessment Program Manager and Regional Managers	DEQ Central Office DEQ Regional Offices
Andrew Garey Andrew Kirk	Project Managers – DEQ Monitoring Team Lead and DEQ Biological Monitoring Coordinator	DEQ Central Office
DEQ Regional Biological Monitoring Staff	Scientists	DEQ Regional Offices
Royce Steiner	Quality Assurance Manager (QAM)	DEQ Central Office
DEQ Assessment Staff, Regional Program Planners and Monitoring Staff	Principal Data Users	DEQ Central Office DEQ Regional Offices
Cindy Johnson	DEQ Laboratory Liaison	DEQ Central Office

1.7 Project Organization

The Virginia Department of Environmental Quality's (DEQ) freshwater biological monitoring program is conducted out of six regional offices located throughout Virginia. These offices are in Abingdon (Southwest Regional Office), Salem (Blue Ridge Regional Office), Harrisonburg (Valley Regional Office), Woodbridge (Northern Regional Office), Glen Allen (Piedmont Regional Office), and Virginia Beach (Tidewater Regional Office). Regional Biologists in each office are under the direction of the regional water monitoring and assessment manager (Figure 1.9-1). The associated responsibilities for program and project personnel are described below. The Biological Monitoring Program Coordinator in DEQ's Central Office in Richmond is responsible for the coordination of the biological monitoring program. The program coordinator is under the direction of the Water Quality Team Lead in the Central Office located in Richmond, Virginia. Each staff member is individually and ultimately responsible for understanding and adhering to the quality and operation procedures they perform, and for the quality of the data they collect or produce. The responsibilities of personnel involved in project implementation are enumerated below.

The Delegated Approving Official (DAO) has the responsibility to:

- Be knowledgeable of EPA requirements for QAPPs and other equivalent QA documents.

- Follow the quality document review process outlined in the US EPA Region 3 Quality Management Plan (QMP)Acquire DAO certification as outlined in the QMP.
- Not approve QA documents they authored or prepared, or for projects/programs they manage.

The Senior Managers, who have leadership authority for the project, will be responsible for the following activities:

- Oversee resource allocation.
- Review and internally approve the QAPP and any other relevant documentation (e.g. SOP)

The Project Managers will:

- Conduct outreach with potential participants, data users, and stakeholders.
- Ensure all project personnel are properly trained and/or have the skills to fulfill assigned project tasks.
- Conduct a readiness review prior to any data collection step, including acquiring collection permits or other permissions as applicable, and ensuring all equipment and supplies are sufficient.
- Oversee participation, data collection, and data analysis tasks, ensuring all protocols and this QAPP are followed during sampling and other operations.
- Authorize all changes or deviations in the operation of the project, including management and implementation of any corrective actions.
- Issue reports as applicable, including preparing a summary of any data quality issues.
- Retain project records according to applicable Agency policy.
- Review and approve QAPP and any other relevant documentation.
- Distribute final QAPP and any subsequent revisions.
- Maintain and amend this QAPP as necessary and notify QAM.

The Scientists will be responsible for:

- Reading and being very familiar with this QAPP and the related standard operating procedure(s) (SOPs) or methods for any operation they perform.
- Ensuring they are properly trained and/or have the skills to fulfill assigned task.
- Identifying and reporting to the Project Manager any emerging/unanticipated problems, data anomalies, or other project/data issues.
- Annotating the related SOPs for any activity they perform if necessary and permanent changes arise or authoring new SOPs if a gap exists.
- Recording, entering, verifying, and validating data as outlined in this QAPP.
- Maintaining data and retaining project records in conjunction with the project manager and in accordance with applicable Agency policy.

The Quality Assurance Manager (QAM) will be responsible for the following activities:

- Reviewing QAPP
- Assessing effectiveness of the QAPP.
- Discussing any corrective actions or other quality issues with Project Manager and any relevant staff as applicable.
- As necessary, discussing quality-related issues with their organization's senior manager, even if outside of their direct supervisory chain.

The Principal Data User will need to:

- Communicate early in the project with Senior or Project management about any specific needs and objectives.
- Read reports or other documentation to understand any quality concerns, e.g., any limitations to data use, flags on lab data, etc., before using information/data.

DEQ Laboratory Liaison will, as needed:

- Coordinate program activities between the Regional Office staff and the state lab (DCLS) including sample collection scheduling based on laboratory capabilities.

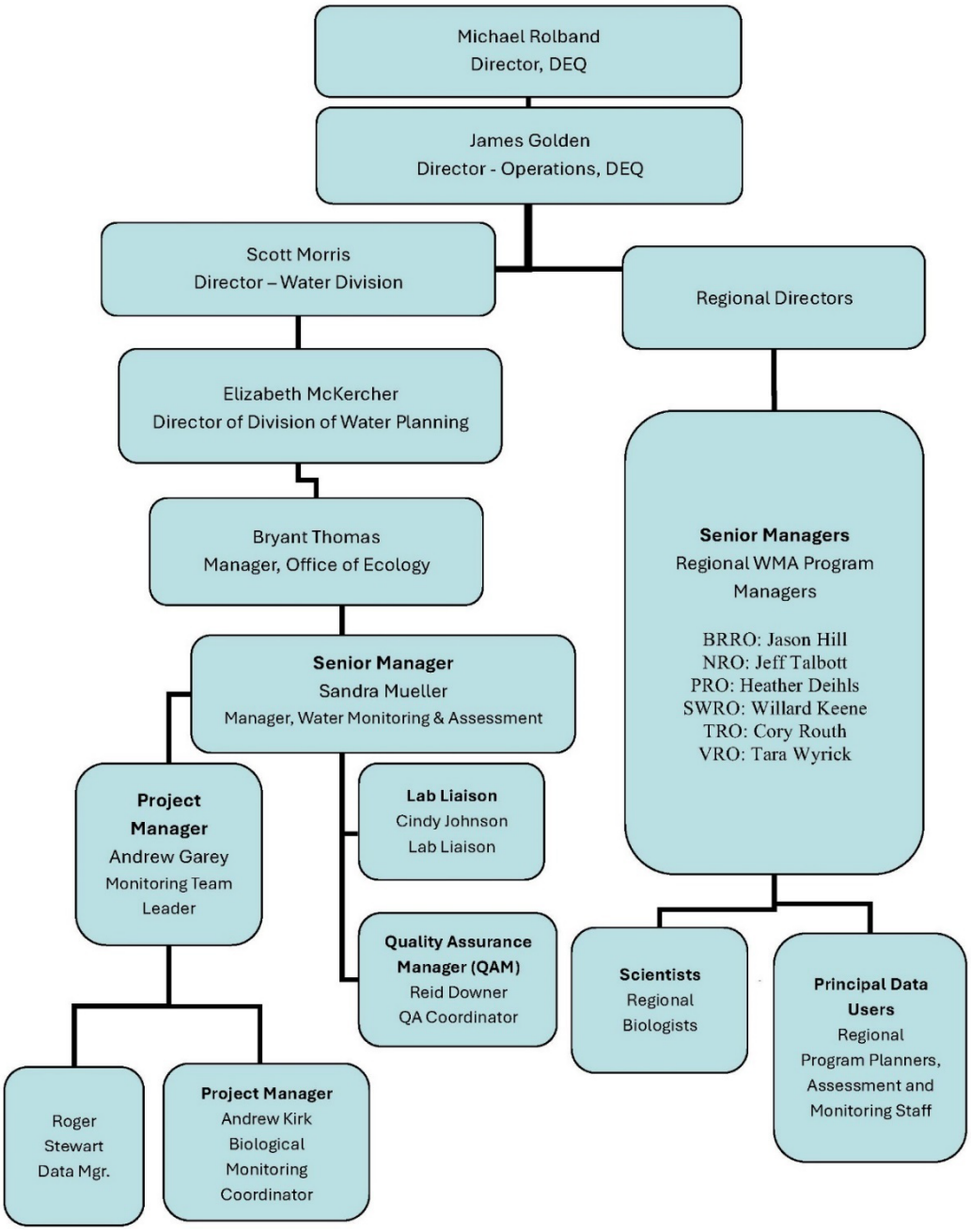
1.8 Project Quality Assurance Manager (QAM) Independence

The Project QAM is independent of environmental information operations. This independence is ensured by the QAM not participating in any environmental information collection activities outside of their role of quality oversight, e.g., the QAM does not collect data but can conduct assessments in the field. The Project QAM is not required to be independent of senior management who are nominally, but not functionally, involved in operations.

1.9 Project Organization Chart and Communications Project Organization Chart

Figure 1.9-1 depicts the organizational structures of the Virginia Department of Environmental Quality (DEQ) for the Water Monitoring and Assessment (WMA) program Lines of authority are shown in the organizational chart. Project roles are bolded.

FIGURE 1.9-1: DEQ ORGANIZATIONAL CHART FOR WMA PROGRAM



Communication

Project communication is detailed in Table 1.9-1.

Table 1.9-1 Communication Pathways

Communication Driver	Responsible Entity	Procedure (timing, pathway, etc.)
Manage field project phase	Project Manager	The Project Manager will inform the scientists about all field activities and any other pertinent project information.
Issues encountered in field or with data analysis	Scientists	Scientists will communicate any issues or deviations from the QAPP to the Project Manager within one business day of that activity.
Corrective actions for field activities and data analysis	Project Manager and QAM	The Project Manager, in coordination with the QAM, will determine the need for corrective action for field and analytical issues and communicate those to affected staff as soon as possible.
Amending the QAPP	Project Manager with notification to QAM	The Project Manager, in coordination with the QAM, will maintain and amend this QAPP as necessary. The Project Manager will ensure the distribution of the final QAPP and any subsequent revisions.
Authority to issue stop work orders	Project Managers, QAM, and Senior Managers	The Project Manager, QAM and Senior Managers will determine the need to issue stop work orders in the event of an environmental emergency, due to potential risk to human health, or for other reasons. They will communicate stop work orders to affected staff immediately.

The Project Manager and/or the Regional Senior Managers will be the communications champions for any communications outside of the project team. Landowners will be contacted by phone or in person to seek approval to access their property.

1.10 Training Requirements/ Certification

The Project Manager is responsible for ensuring that all scientists involved with data generation have the necessary training to successfully complete their tasks and functions. The Project Manager will document attendance at any training or certification sessions. All project members must be familiar with the protocols. Any unknown procedures will be reviewed in the office and/or field prior to sampling.

Field Personnel Training

Proper training of field personnel represents a critical aspect of quality control. Field biologists are trained to conduct biological monitoring and a wide variety of ancillary monitoring activities using standardized protocols to ensure comparability in data collection among field teams and across geographic regions. These standardized protocols are outlined in the Standard Operating Procedures in Appendix B: Single Habitat Benthic Macroinvertebrate Collections, Multi-habitat Benthic Macroinvertebrate Collections, Habitat Assessment for Streams, and Lab Sorting and Subsampling of Macroinvertebrate Samples.

Each field team member receives training to enable compliance with all applicable Occupational Safety and Health Administration or equivalent state or local regulation requirements including proper handling and disposal of routinely used chemicals.

All biological monitoring and related field sampling activities as well as laboratory sample processing (e.g., subsampling 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 genus-level EPT (Eastern) certification from Society for Freshwater Science. Certifications are earned by correctly identifying 95% or more of the specimens and must be renewed every five years.

Continued Proficiency of Field Personnel

To ensure continued proficiency in Quality Assurance/Quality Control procedures, the agency Quality Assurance Coordinator, or designee, performs a field audit of staff collecting samples. All field staff undergoes an audit at least once every two years. Staff performing monitoring for more than one type of program using significantly different protocols (example: riverine ambient and lake monitoring) may be audited more frequently.

1.11 Documentation and Records

QA Project Plan Distribution

This QAPP will be distributed to each Regional Office and responsible for the collection of samples and generation of biological macroinvertebrate data. The Biological Monitoring Coordinator will be responsible for ensuring that any necessary changes required to keep the QAPP up to date with actual practices are documented and implemented. The Biological Monitoring Coordinator will ensure that a distribution list of QAPP recipients is maintained, such that revisions and updates can be distributed. The document control format used in this QAPP will identify the QAPP revision number and revision data. A QAPP revision history will be maintained that identifies each revision and changes to the program throughout its implementation.

The QAPP shall be reviewed at least annually to ensure that the project will achieve all intended purposes. In addition, it is expected that from time to time ongoing and perhaps unexpected changes will need to be made to the project. The Biological Monitoring Coordinator and the Project Quality Assurance Manager shall authorize all changes or deviations in the operation of the project. Changes will be noted in the project file. Significant changes (i.e. those judged by the

Quality Assurance Manager to have potential effects on the quality or meaning of the resulting data) will be incorporated into an amended QAPP which will be submitted to EPA for review prior to implementing those changes in monitoring. The Biological Monitoring Coordinator will document the effective date of all changes made in the QAPP and will distribute new revisions to all applicable personnel whenever a substantial change is made.

Field Data Documentation

All field data (habitat assessments, field observations, and water physicochemical measurements) and benthic macroinvertebrate data are entered on standardized forms that are completed at the time of data collection (see Appendix C-i). These data are then entered and stored in the agency's Comprehensive Environmental Data System (CEDS). Results are also submitted to EPA under DEQ'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 DEQ staff for each regional office for inclusion in the Integrated Report.

2. IMPLEMENTING ENVIRONMENTAL OPERATIONS

2.1 Site Locations and Sampling Design

Regional Offices submit biological monitoring site selections annually to the Water Quality Monitoring data scientist for the upcoming year in mid to late December via the Yearly Run Schedule of DEQ's Comprehensive Environmental Data System (CEDS). Regions select sites based on programmatic needs: assessment, TMDL development, impairment listing/delisting follow-up, special studies and follow up on citizen nominations. Once all the information is received from the regional offices, the sites are downloaded by individual programs along with the parameters to be monitored. The information is then reviewed by the Water Monitoring and Assessment Program Manager and their team to ensure site selection is appropriate for the agency's needs and fits the agency's budget constraints. If adjustments are needed, changes are coordinated with the Regional Water Quality Monitoring Managers to produce the final list of sites in January. The final monitoring plan is subsequently made available to the public on DEQ's website.

DEQ employs two main types of sampling strategies: probabilistic monitoring and conventional monitoring.

Probabilistic monitoring design

The DEQ Biological Monitoring Program initiated the Freshwater Probabilistic Monitoring module—a network of randomly chosen stations—in the spring of 2001. Since that time, the agency has been sampling 40 to 50 new wadeable probabilistic freshwater sites each year. In the freshwater resource class, the distribution of site selection among stream sizes has been chosen to ensure approximately equal representation among five sampling strata: streams of the 1st, 2nd, 3rd, 4th, and ≥5th Strahler Orders. In wadeable streams, DEQ collects biological samples and measures

conventional field parameters (temperature, dissolved oxygen, pH and conductivity) during separate spring and fall visits to each site. This provides for the evaluation of seasonal variations at the site and ensures that field parameters and biological information are collected during two critical periods for the principal groups of water quality parameters of interest. For sites in this resource class, spring and fall sampling coincides with high- and low-flow periods, respectively, providing evaluations of worst-case and best-case NPS scenarios, as well as two different phases of benthic organism life cycles. In non-wadeable streams, the randomly selected sites are visited in the spring and the fall, but larger boatable river sites are only sampled in the fall.

In addition to providing unbiased physical, chemical, and biological characterizations with well-defined statistical confidence intervals for Virginia's free-running, freshwater streams and rivers, the randomly selected biological monitoring sites provide valuable insight for the subsequent definition of regional "best attainable" and "reference" biological communities. The accumulation of such information, over time, will also provide additional data for the refinement of the VSCI and CPMI. This will assure the continued improvement of DEQ's Biological Monitoring Program.

Conventional biological monitoring design

For conventional biological monitoring, site selection ensures the collection of data to meet specific purposes. As described in section 1.6, benthic data are used for providing biological water quality assessments of the aquatic life designated use in wadeable free-flowing streams, supporting the VPDES permitting program, tracking local pollution events, rendering exceptional state water designations, and providing information to the public on the biological condition of waters of interest.

Site selection is determined so data collection meets the defined objectives. Conventional site locations are determined based on-site accessibility, wadeable condition (generally defined as average depth less than 0.75m), free-flowing, and best available habitat suitable for collection using biological monitoring protocols defined in the SOPs for Single Habitat Benthic Macroinvertebrate Collections and Multi-habitat Benthic Macroinvertebrate Collections (Appendix B).

2.2 Sampling Methods

Biological monitoring sampling methods are defined in the SOPs and are found in Appendix B-i B-ii. Section 1.4 (Stream Macroinvertebrate Sampling) includes more information on biological monitoring sample method determination.

2.3 Sample Handling and Custody

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

2.4 Analytical Methods

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

2.5 Quality Control

Data Quality Objectives (DQOs) are qualitative and quantitative statements that specify the quality of data required to support specific WMA decisions. DQOs also specify the level of uncertainty that a decision maker is willing to accept in results derived from monitoring data that are used for a regulatory or programmatic decision, such as establishing analytical method requirements, establishing sampling protocols or the revision or development of industry standards.

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 2.5-1.

Table 2.5-1. Quality Control Objectives for the biological monitoring program

Comparability	Accuracy	Sorting Efficiency	Completeness
The expected degree of agreement between field replicate benthic macroinvertebrate samples is ≥ 85%	The expected Measurement Quality Objective for taxonomic precision is a PTD	The expected sorting efficiency of benthic macroinvertebrate samples is ≥ 90%	The expected data completeness is 90% to generate a meaningful dataset for its intended purpose.

Comparability - Field replicate pairs are collected, subsampled, and identified. The degree of agreement is based on the percent comparability of the multimetric index scores between replicates. If the percent comparability is < 85%, an evaluation of the consistency of field sampling techniques may be warranted.

Accuracy - The DEQ's Measurement Quality Objective (MQO) for taxonomic precision was suggested by the EPA to be set at a Percent Taxonomic Disagreement (PTD) value of ≤ 15% 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 DEQ regional biologist and other DEQ staff certified for taxonomic identification. Samples are re-identified by another certified biologist from a different regional office. The Biological Monitoring Coordinator assigns samples to biologists for re-identification and removes all identifiable sample information from each sample before placing a sample tag in the container with a unique sample ID, the major river basin and season on it. In this way, the samples are distributed anonymously with only the Biological Monitoring Coordinator knowing the identity of the two taxonomists. Once the secondary taxonomist identifies and submits their bench sheets to the Biological Monitoring Coordinator, the coordinator provides the necessary sample information for them to enter their data into CEDS. The two biologists for a given sample then discuss where their identifications or enumerations differed and provide an updated PTD for each sample. A subset of these QA samples (approximately 25%) are again identified by an independent certified taxonomist that is not employed by VADEQ. The DEQ biologist and the independent taxonomist discuss where their identifications or enumerations differed and provide an updated PTD for each sample. 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 whereas consistent MQOs greater than the suggested PTD due to taxonomic misidentification may warrant the need for increased taxonomic identification training.

Sorting Efficiency - DEQ staff involved in laboratory subsampling must demonstrate the ability to remove ≥ 90% of the specimens present. For detailed subsampling procedures and QA/QC, see Appendix B-iv and Appendix D.

Completeness - The completeness of data identifies how many data are required to meet Quality Objectives, e.g., the % of data needed to evaluate results for your purpose. Ideally, 100% of the data should be available for its intended use. However, there is always the possibility of data loss due to laboratory or equipment error, insufficient sample volume, or samples broken during transport. In addition, unexpected situations may arise where field conditions do not allow for 100% data completeness. Due to these unforeseen possibilities, DEQ considerers 90% data completeness sufficient to evaluate results for the intended purpose.

Audits - The QA/QC Coordinator/Biological Monitoring Coordinator will be responsible for conducting program audits to ensure appropriate SOPs are being followed in the field and lab (see Section 3.1).

2.6 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 Chapter 3 of DEQ's Water Quality Monitoring Standard Operating Procedures Manual located on the DEQ website: https://www.deq.virginia.gov/our-programs/water/water-quality/monitoring.

2.7 Instrument Calibration and Frequency

Detailed descriptions of frequency and calibration procedures can be found in Chapter 3 of DEQ's Water Quality Monitoring Standard Operating Procedures Manual located on the DEQ website: https://www.deq.virginia.gov/our-programs/water/water-quality/monitoring.

2.8 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 inspected for damage before use. Supplies and consumables procured centrally, for statewide use, will be inspected and accepted by a project manager or by the project quality assurance manager. Those procured by an individual region will be inspected and accepted by staff assigned by the regional WMA manager, with the final responsibility for acceptance resting with that manager.

2.9 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. When GIS data are used, the specific sources of the data will be cited, along with all approved QAPPs and other quality

assurance and quality control information affecting the data. The Biological Monitoring Coordinator and the Project Quality Assurance Manager must approve all data sources used in significant decision-making in the monitoring and assessment processes. The decisions made, and the data upon which they were made, will be documented in the project file. Whenever possible, the data themselves will be either publicly available, or stored by DEQ so that they may be provided upon request.

2.10 Data Acquisition Requirements

Data will primarily be generated through DEQ field activities and consequent laboratory analyses of benthic macroinvertebrate samples.

2.11 Data Management

All field data (habitat assessments, field observations, and water physicochemical measurements) and benthic macroinvertebrate data are entered on standardized forms that are completed at the time of data collection (see Appendix C-i). These data are then entered and stored in the agency's Comprehensive Environmental Data System (CEDS). Results are also submitted to EPA under DEQ'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 ten years, per Library of Virginia's agency-specific retention schedule. Final assessment reports will be sent to the appropriate DEQ staff for each regional office for inclusion in the Integrated Report.

3. ASSESSMENT, RESPONSE ACTIONS, AND OVERSIGHT

3.1 Assessments and Response Actions

Performance and system audits of both field and laboratory activities are routinely conducted to verify that sampling and analysis are performed in accordance with the procedures established in the SOP and QAPP.

Audits of Data Quality

Field duplicate data will be reviewed in order to assess the quality of sampling activities. Analytical and measurement data should be reviewed in order to assess the quality of measurement and analytical activities, respectively. Metadata should be reviewed in order to assess precision and accuracy. The QAM has the ultimate responsibility to accept or reject data.

Technical Systems Audits

Field Sampling Audits

Field sampling audits evaluate field operations in comparison to the written procedures outlined in SOPs and other requirements established in the project plan and WQM SOP. The QAM, Project Manager, or designated staff member, will conduct field sampling audits at each Regional Office at

least once a year. Additional audits will be scheduled if warranted by the initial audit observations and findings. The primary audit elements for the program are:

- Availability, appropriateness and use of field SOPs.
- Sampling methodology
- Sample handling procedures
- QA procedures
- Field instrument operation logbook
- Field maintenance logbooks
- Field documentation
- Field data quality, quantity and timeliness
- Follow-up on previous corrective action and recommendations

The QAM and Project Manager will prepare, or review and approve the audit report prepared by the designated staff member, which discusses deficiencies found during the on-site evaluation with recommendations for corrective action. The report will be forwarded to the regional water quality monitoring program managers.

Laboratory Audits

The QAM performs laboratory audits annually for each regional office. They are responsible for all QA/QC functions in the laboratory. During these audits, one or more components of a lab are reviewed to determine if that part is functioning in compliance with the relevant quality assurance project plans, the approved standard operating procedures and approved methodology. An audit report includes a list of deficiencies that must be addressed in order to correct or improve the lab operations.

System components to be audited during the internal audit include, but are not limited to:

- Use of established approved procedures as outlined in the relevant QAPP and SOPs
- Personnel training records
- Proper execution of established procedures
- Follow-up to corrective actions from previous audits
- Sample and data handling activities: all sample login, routing and disposal; sample preparations; method calibrations; sample analyses; data reduction, validation and reporting; preventative maintenance and repair procedures; standard and reagent preparation, documentation and storage; sample and waste disposal; container and lab ware decontamination; QC management practices and assessment of analytical precision, accuracy and sensitivity

Deficiency lists and associated corrective action orders are formally communicated to responsible staff.

Performance Audits

The laboratory is involved in external performance audits conducted through the analysis of performance evaluation samples provided by the regional biologists and the Project Manager. These audits consist of performance sample audits and blind sample audits, respectively.

Performance Sample Audits

Performance sample audits are conducted periodically by regional biologists through the collection and identification of duplicate samples to assess comparability. The results of these audits are

documented and reported to managers so that any necessary adjustments can be made.

Blind Sample Peer Review
Blind sample peer reviews are performed for each biologist annually. Each biologist randomly selects 10% of their samples to send to the Project Manager for redistribution and reidentification by another biologist from a different regional office. A subset of the samples is reviewed by a third-party certified taxonomist (see Section 2.5 for more detailed information regarding the process).

Corrective Action

The first level of responsibility for identifying the need for corrective action is with field and laboratory technical staff during routine sampling and analysis activities. Each regional biologist is required to document any potential problems encountered during data collection and sample processing and to address potential data quality issues as needed. Such action may include resampling or eliminating data from further consideration. The second level of responsibility is with any person observing deviations during field audits, while reviewing field documentation, or while reviewing laboratory results.

Each time the need for corrective action is identified; the problem and steps taken to resolve it are documented on the corrective action request and tracking form used by DEQ, or similar variant. This form documents the problem, the recommended corrective action, mechanism of implementing the corrective action and responsible personnel.

Field Corrective Action

Corrective actions will be initiated if the field team is not adhering to the prescribed sampling or documented procedures or if laboratory analyses are experiencing interference or systematic contamination due to field sampling procedures or sample handling protocol. Corrective actions begin with identifying the source of the problem. Corrective action responses may include more intensive staff training, modification of field procedures, or removal of the source of systematic contamination. Once resolved, the corrective action procedure will be fully documented.

Laboratory Corrective Action
Problems should be resolved at lowest level possible. When quality assurance data exceed a threshold of acceptable limits corrective action should be taken immediately and all actions documented. Laboratory staff notifies supervisors when unsure of the appropriate corrective action. The group manager, QA Coordinator and Project Manager review all corrective actions. The QA Coordinator provide recommendations and continue to monitor to ensure detected problems are resolved. If the initial corrective action fails to resolve the problem or a trend is established, the QA Coordinator may make additional recommendations or establish an action team to seek a resolution. The goal of the laboratory is to detect problems early, implement changes to improve services, and monitor for effect.

3.2 Reports to Management

Biomonitoring program staff will discuss QA/QC issues and solutions at regularly scheduled quarterly meetings or as the need arises.

Project Managers and the QAM will prepare QA reports to DEQ management and regional program managers on a quarterly basis. Each report will address the following topic areas:

- Results of performance and system and field audits.
- Evaluation of compliance with QA project plan.
- Evaluation of data quality measurement trends.
- Identification of QA problems, program needs and recommendations for solutions.

Project Managers and the QAM will prepare an annual Quality Assurance report. The quality assurance report will summarize the results of QA/QC assessments and evaluations, including precision, accuracy, comparability, representativeness and completeness of the monitoring data; will provide a summary of the field split and equipment blank analyses and will provide a summary of any lab and/or field performance audits that were conducted. The annual report will be distributed to the program managers, agency management, and EPA personnel.

4. ENVIRONMENTAL INFORMATION REVIEW AND USEABILITY DETERMINATION

4.1 Data Review, Validation, and Verification Requirements

The field, laboratory and data management activities described in this QAPP will be reviewed to assess whether these activities were performed in a manner that is appropriate for accomplishing the program objectives. This assessment will include electronic verification of the data and data validation. Data verification is confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. Data verification concerns the process of examining a result of a given activity to determine conformance to the stated requirements for that activity. Data validation is confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. Data validation concerns the process of examining a product or result to determine conformance to the user needs.

Regional biologists will confer with one another in the field while collecting physical habitat data and 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 biologist, per year.

4.2 Data 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 CEDS database. Any errors detected will be rectified by editing incorrect database entries, resampling, or excluding questionable data. Sorting efficiency of macroinvertebrates will be evaluated by experienced personnel who will check all sorted quadrates from the first three samples processed by a sorter to ensure that >90% of organisms are removed. This will not only apply to inexperienced sorters, but also to those deemed "experienced." Qualification will only occur when sorters are consistent in achieving ≥ 90% sorting efficiency after at least three samples have been checked. For evaluation of completeness, if the specific completeness percentage drops below 90%, agency staff will remedy this by either

resampling sites or adding new sites to the annual monitoring plan. It may be necessary to add monitoring efforts to a future monitoring plan if this percentage falls below 90% for a given year. Regardless of the timing of such changes, the overall goal will be to achieve 90% completeness for the dataset used for each Integrated Report.

4.3 Reconciliation with Data Quality Objectives

Once all the data have been reviewed, the Project Manager and QAM will make an overall assessment concerning the final usability of the data (and any limitations on its use) in meeting programmatic needs.

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 QA/QC results for taxonomic identification reveal systematic errors for certain taxa the regional biologist (scientists) will be informed and may be required to review past samples and re-identify those samples containing problem taxa. Once those taxa are re-identified, 10% of the samples will be submitted to an independent certified taxonomist for verification.

5. PROGRAM ASSURANCE

5.1 Audit Verification

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

5.2 Field Audits

The internal audits used to evaluate field sampling will examine:

- Sampling Site Selection
- Sample Collection Procedures
- Habitat Evaluation

5.3 Laboratory Audits

The internal audits used to evaluate the laboratory will examine:

- Subsampling Procedures
- Accuracy of specimen identification
- Equipment maintenance

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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 .....Southwest Regional Office
TMDL .....Total Maximum Daily Load
TRO.....Tidewater Regional Office
EPA.....United States Environmental Protection Agency
DEQ .....Virginia Department of Environmental Quality
VCPMI……Virginia Coastal Plain Macroinvertebrate Index
VPDES.....Virginia Pollutant Discharge Elimination System
VRO .....Valley Regional Office
VSCI .....Virginia Stream Condition Index

Appendix B-i: SOP, Single Habitat Benthic Macroinvertebrate Collections

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

Date of Last Revision: 03/01/2024

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)
70-95% Ethanol
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:	Snags, Vegetation, Banks, Riffles
Area:	$2m^2$ of stream bottom
Mesh size:	500-µm mesh openings
Sampling Period:	Biannual, generally spring: March 1 through May 31 and fall: September 1 through November 30. See additional considerations based on project/program objectives and weather variability; section A6 of the DEQ Biomonitoring QAPP.

1. The sample reach (considered to be a station) should encompass enough habitat to sample $2m^2$ of stream bottom, as defined below. Sampling should generally be conducted 100 meters or more, upstream of road or bridge crossings to minimize the effects of these structures 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 cobbles and boulders. The total area sampled should encompass approximately $2m^2$ of stream bottom. For example, six kicks, each disturbing 1/3 m^2 above the dip net or 12 kicks disturbing a 1/6 m^2 each could be used.

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 either by pouring or by partially submerging the net (with care taken not to submerge past metal frame of net) with 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. 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 95% ethanol. Forceps may be needed to remove organisms from the screen and dipnet.

7. Complete the Benthic Macroinvertebrate Field Data Sheet including habitat assessment and comments on weather, wildlife, and other observations.

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. 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.

2. Sample labels should include the following information: station ID, date, sampling method, sampler's name, and container number and total number of containers (e.g., 1 of 2).

Appendix B-ii: SOP, Multi-habitat Benthic Macroinvertebrate Collections

SOP Title: Methods for Multi-habitat Benthic Macroinvertebrate Collections

Date of Last Revision: 03/01/2024

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)
70-95% ethanol
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 or kicks, 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 encompass enough habitat to collect 20 kicks or jabs (total of both) as defined below. Sampling should be conducted at least 100-meters upstream of any road or bridge crossing to minimize the effects 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 habitat for a linear distance of 1 m, followed by 2-3 sweeps of the same area to collect dislodged organisms. Kicks may also be used on appropriate bottom substrate, if present (see Appendix B-i).

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. 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.

4. Proportionally allocate sampling effort (20 jabs/sweeps/kicks) to the habitat types observed over the reach.

5. 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.

6. As the sample is added to the sieve bucket (when needed), it should be further washed to remove fines. Rinse and remove organisms from large debris in the sieve bucket, Place any organisms back into the sieve bucket. Do not attempt to inspect small debris.

7. Transfer the sample from the kick net or sieve bucket to a pre-labeled sample container(s) and preserve in 95 percent ethanol. Forceps may be needed to remove organisms from the sieve screen and dipnet.

9. Complete the Benthic Macroinvertebrate Field Data Sheet including habitat assessment with comments on weather and wildlife observations etc. Notes on the stable habitats

sampled should be recorded (e.g., 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. 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.

2. Sample labels should include the following information; station ID, date collected, habitat sampled, collector ID, and container number and total number of containers (e.g., 1 of 2).

Appendix B-iii: SOP, Habitat Assessment for Streams

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 sample reach for conducting the habitat assessment and complete the sections on general characteristics and land use. The habitat reach should include the stream channel section over which the sample was collected and the immediately adjacent and upstream riparian zone.
2. The habitat assessment will be focused on evaluating the physical habitat structure of the sampled reach of 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. All habitat parameters within the sampled reach of stream will be evaluated.

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 listed 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 reach used for 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., 1/2, 1/3) 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 reach lengths 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 reach lengths, 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 pollutant 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

The first 5 parameters are assessed directly in the reach used for macroinvertebrate sampling.

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 reach lengths 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 reach lengths.

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 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.

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. Periodic review and "calibration" of habitat parameters among regional biologists and field personnel will aid with the consistent assessment of instream habitat quality. See Barbour et al. (1999) for more specific guidance.

Appendix B-iv: Lab Sorting and Subsampling of Macroinvertebrate Samples

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

Date of Last Revision: 07/17/2008

Equipment/Materials:

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

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50

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 200-organism sub-sample is sorted and preserved using a special Caton gridded tray and screen (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
- Unique lab number (if necessary)

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. Assigning a unique lab number for each sample aids with organization

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 subsampling, # of grids subsampled, person identifying the insects, total # of subsampled insects, date identified, and sorting date.

3. Transfer the homogenized sample material to the gridded Caton tray. Wash the sample thoroughly by gently 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. Ensure materials of differing densities (leaves versus sand) are spread evenly across the tray. 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. The addition of a small amount of ethanol to the sorting tray may reduce the "stirring" of the sample caused by alcohol covered forceps being introduced to the water within the sorting tray.

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 (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% ethyl alcohol as a preservative. If more than 51 organisms (see table below) 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).
    b. Continue selecting and processing randomly selected quadrates until 200 organisms +/- 10% (180-220) 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. An organism must have a head attached to be counted.
    d. If the last grid being processed results in more than 220 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 to select which of the 25 grids will be excluded from the sample. Remove ALL organisms from the selected grid(s)

and subtract the number of organisms removed from the original total count. Select grids and remove organisms from the sample until the target count of 180-220 organisms remains in the tray. The organisms removed may be discarded and the organisms remaining in the tray are the benthic sample to be identified.

e. Identify all the organisms in the sample to lowest identifiable taxonomic level record the number of organisms on the Benthic Macroinvertebrate Bench Sheet, and enter the data into CEDS. Retain identified sample in vial for a minimum of five years, at which time the sample may be discarded.

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 (e.g., 3, 28, 55) and remove columns 3, 8, and 5. If you are to remove 10 grids, choose 5 numbers (e.g., 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 51 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
51-80	25	25.5-40	5 -8	0.5
81-110	20	–32.4-44	5-6	0.4
111-150	15	33.3-45	4-6	0.3
151-220	10	–30.2-44	5-7	0.2
221-460	5	–22.1-46	4-9	0.1
461-630	4	36.8-50.4	4-5	0.08

*4 quadrates must be removed. If removal leads to over 220 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 (as mentioned in section B5)

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.

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.

1. 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 = %; see Appendix D). 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.

Appendix C-i: Field Data Sheets

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BENTHIC MACROINVERTEBRATE FIELD DATA

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

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

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

Date ___/___/___ **Latitude** _____ **Longitude** _____

Stream Physicochemical Measurement

Instrument ID Number _____ **pH** _____ **Average Width (m)** _____

Temperature _____ °C **Conductivity** _____ µS/cm **Reach Length (m)** _____

Dissolved Oxygen _____ mg/L Did instrument pass all post calibration checks? Y / N

If NO- which parameter(s) failed and action taken _____ _____

Benthic Macroinvertebrate Collection

Methods 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**

 # jabs _____ _____ _____ _____ **Area Sample (sq. m):** _____

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

NOTES: _____

_____

HIGH GRADIENT HABITAT DATA

	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/Available Cover	Greater than 70% 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).	40-70% mix 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 colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate 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 0
2. Embeddedness (observed in the upstream and central portions of riffle and cobble areas)	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 particles are more than 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 0
3. Velocity/Depth Regine	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). Slow is <0.3 m/s, deep is >0.5 m/s.	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 velocity/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 0
4. Sediment Deposition	Optimal	Suboptimal	Marginal	Poor

(observed in pools)	**Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.**	**Some new increase in bar formation, mostly from gravel, sand or fine sediment. 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.**	**Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of bottom affected; moderate deposition in pools prevalent.**	**Heavy deposits of fine material, increased bar 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 0**
5. Channel Flow Status	**Optimal** **Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.**	**Suboptimal** **Water fills >75% of the available channel; or 25% of channel substrate is exposed.**	**Marginal** **Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.**	**Poor** **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**
6. Channel Alteration	**Optimal** **Channelization or dredging absent or minimal; stream with normal pattern.**	**Suboptimal** **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.**	**Marginal** **Channelization may be extensive; embankments or shoring structures present on both banks; and 40 - 80% of stream reach channelized and disrupted.**	**Poor** **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. Frequency of Riffles (or bends)	**Optimal** **Occurrence of riffles relatively frequent; ratio of distance btw. riffle divided by width of the stream <7:1(*generally 5-7); variety of habitats is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.**	**Suboptimal** **Occurrence of riffles infrequent; distance btw. riffles divided by the width of the stream is btw. 7 to 15.**	**Marginal** **Occasional riffle or bend; bottom contours provide some habitat; distance btw. riffles divided by the width of the stream is btw. 15 to 25.**	**Poor** **Generally all flat water or shallow riffles; poor habitat; distance btw. riffles divided by the width of the stream is a ratio of >25.**
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 (right and left facing downstream)	**Optimal** **Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. < 5% of bank affected.**	**Suboptimal** **Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.**	**Marginal** **Moderately unstable, 30-60% of bank in reach has areas of erosion; high erosion potential during floods.**	**Poor** **Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; 60-100% of bank has erosional scars.**
SCORE	**Left Bank 10 9** **Right Bank 10 9**	**8 7 6** **8 7 6**	**5 4 3** **5 4 3**	**2 1 0** **2 1 0**
9. Bank Vegetative Protection (right and left facing downstream)	**Optimal** **More than 90% of the streambank surfaces and immediate riparian zones 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.**	**Suboptimal** **70-90% of 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.**	**Marginal** **50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less then one-half of the potential plant stubble height remaining.**	**Poor** **Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 cm or less in average stubble height.**
SCORE	**Left Bank 10 9** **Right Bank 10 9**	**8 7 6** **8 7 6**	**5 4 3** **5 4 3**	**2 1 0** **2 1 0**
10. Riparian Vegetative Zone Width (right and left facing downstream)	**Optimal** **Width of riparian zone >18 m; human activities (i.e. parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.**	**Suboptimal** **Width of riparian zone 12-18 m; human activities have impacted zone only minimally.**	**Marginal** **Width of riparian zone 6-12 m: human activities have impacted zone a great deal.**	**Poor** **Width of riparian zone <6 m; little or no riparian vegetation due to human activities.**
SCORE	**Left Bank 10 9** **Right Bank 10 9**	**8 7 6** **8 7 6**	**5 4 3** **5 4 3**	**2 1 0** **2 1 0**

TOTAL SCORE _____

LANDUSE CHECKLIST	(Blank= Not observed, L= Low, M= Moderate, H=Heavy)	(circle all that apply within reach)		
Residential	Recreational	Agricultural	Industrial	Stream Management

L M H Residences	L M H Hiking Trails	L M H Crops	L M H Industrial	L M H Liming
L M H Lawns	L M H Parks, Camps	L M H Pasture	L M H Mines/Quarries	L M H Chemical
L M H Construction	L M H Primitive Parks	L M H Livestock on pasture	L M H Oil/Gas	L M H Angling
L M H Pipes. Drains	L M H Trash/Litter	L M H Animal feeding operation	L M H Power Plants	L M H Dredging
L M H Roads	L M H Surface Films	L M H Orchards	L M H Logging	L M H Channelization
L M H Dumping		L M H Irrigation	L M H Fire	L M H Flow Alterations
L M H Bridge Culverts			L M H Odor	L M H Fish Stocking
L M H STPs			L M H Commercial	L M H Dams

BENTHIC MACROINVERTEBRATE FIELD DATA

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

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

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

Date ___/___/___ **Latitude** _____ **Longitude** _____

Stream Physicochemical Measurement

Instrument ID Number _____ **pH** _____ **Average Width (m)** _____

Temperature _____ °C **Conductivity** _____ µS/cm **Reach Length (m)** _____

Dissolved Oxygen _____ mg/L Did instrument pass all post calibration checks? Y / N

If NO- which parameter(s) failed and action taken _____ _____

Benthic Macroinvertebrate Collection

Methods 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**

 # jabs _____ _____ _____ _____ **Area Sample (sq. m):** _____

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

NOTES: _____

_____

LOW GRADIENT HABITAT DATA

	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/Available Cover	Greater than 70% 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).	40-70% mix 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 colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate 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 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 submerged 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 (large = > 1/2 cross section of stream; deep = > 1 m)	**Optimal** Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	**Suboptimal** Majority of pools large-deep; very few shallow.	**Marginal** Shallow pools much more prevalent than deep pools.	**Poor** 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	**Optimal** Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	**Suboptimal** Some new increase in bar formation, mostly from gravel, sand or fine sediment. 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	**Marginal** Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of bottom affected; moderate deposition in pools prevalent.	**Poor** Heavy deposits of fine material, increased bar 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 0
5. Channel Flow Status	**Optimal** Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	**Suboptimal** Water fills >75% of the available channel; or 25% of channel substrate is exposed.	**Marginal** Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	**Poor** 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
6. Channel Alteration	**Optimal** Channelization or dredging absent or minimal; stream with normal pattern.	**Suboptimal** 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.	**Marginal** Channelization may be extensive; embankments or shoring structures present on both banks; and 40 - 80% of stream reach channelized and disrupted.	**Poor** 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	**Optimal** 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).	**Suboptimal** The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	**Marginal** The bends in the stream increase the stream length 1 to 2 times longer then if it was in a straight line.	**Poor** 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 (right and left facing downstream)	**Optimal** Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. < 5% of bank affected.	**Suboptimal** Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	**Marginal** Moderately unstable, 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	**Poor** Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; 60-100% of bank has erosional scars.
SCORE	Left Bank 10 9 Right Bank 10 9	8 7 6 8 7 6	5 4 3 5 4 3	2 1 0 2 1 0
9. Bank Vegetative Protection (right and left facing downstream)	**Optimal** More than 90% of the streambank surfaces and immediate riparian zones 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.	**Suboptimal** 70-90% of 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.	**Marginal** 50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less then one-half of the potential plant stubble height remaining.	**Poor** Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 cm or less in average stubble height.
SCORE	Left Bank 10 9 Right Bank 10 9	8 7 6 8 7 6	5 4 3 5 4 3	2 1 0 2 1 0
10. Riparian Vegetative Zone Width (right and left facing downstream)	**Optimal** Width of riparian zone >18 m; human activities (i.e. parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	**Suboptimal** Width of riparian zone 12-18 m; human activities have impacted zone only minimally.	**Marginal** Width of riparian zone 6-12 m: human activities have impacted zone a great deal.	**Poor** Width of riparian zone <6 m; little or no riparian vegetation due to human activities.

	Left Bank	**10**	**9**		**8**	**7**	**6**		**5**	**4**	**3**		**2**	**1**	**0**
SCORE	**Right Bank**	**10**	**9**		**8**	**7**	**6**		**5**	**4**	**3**		**2**	**1**	**0**

TOTAL SCORE _____

LANDUSE CHECKLIST	(Blank= Not observed, L= Low, M= Moderate, H=Heavy)		(circle all that apply within reach)	
Residential	Recreational	Agricultural	Industrial	Stream Management
L M H Residences	L M H Hiking Trails	L M H Crops	L M H Industrial	L M H Liming
L M H Lawns	L M H Parks, Camps	L M H Pasture	L M H Mines/Quarries	L M H Chemical
L M H Construction	L M H Primitive Parks	L M H Livestock on pasture	L M H Oil/Gas	L M H Angling
L M H Pipes. Drains	L M H Trash/Litter	L M H Animal feeding operation	L M H Power Plants	L M H Dredging
L M H Roads	L M H Surface Films	L M H Orchards	L M H Logging	L M H Channelization
L M H Dumping		L M H Irrigation	L M H Fire	L M H Flow Alterations
L M H Bridge Culverts			L M H Odor	L M H Fish Stocking
L M H STPs			L M H Commercial	L M H Dams

Appendix C-ii: Laboratory Bench Sheets

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Benthic Macroinvertebrate Laboratory Bench Sheet

Station ID:_____ Sample Subsorted by: _____ Date Subsorted: __/__/__
Stream Name: _____ # of Grids subsorted: _____
Date Sampled: __/__/__ Total # of subsorted organisms: _____
Sampling Method: _____ Sample Identified by: _____ Date Identified: __/__/__

	Taxon Excluded	Final ID	Tally	Total # of organism	# to ref. coll.	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
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16						
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26						
27						
28						
29						
30						
31						
32						
33						
34						
35						
36						
37						
38						
39						

40						
				Totals:		

Subsample and Sample Reduction

(per SOP)

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

If <50 organisms found, continue to table below.
If >50 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 ID #	# of Organisms	Grid ID #	# of Organisms	Grid ID #	# of Organisms	Grid ID #	# of Organisms

Total organisms = _____ Total grids = _____

For sample reduction: _____ x _____ = _____

(# of grids after reduction) (correction multiplier) (corrected # of grids from original sample) {A}

IF after picking, there are >220 organisms, return the picked sample to a 15-30 grid tray and remove grids (per SOP) to reduce sample to 220 organisms or less. Record data below.

Total # of organisms retained = _____

Grids removed to reduce sample to 220 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 D: QA/QC Sorting Efficiency Sheet

QC Initials		**SORTER Initials**			**Pass or Fail** (circle one)
________		__________			
#organisms originally sorted		[#organisms recovered by checker	#organisms originally sorted]		% sorting efficiency
☐	÷	[☐	+ ☐]	X 100 =	☐

QC Initials		**SORTER Initials**			**Pass or Fail** (circle one)
________		__________			
#organisms originally sorted		[#organisms recovered by checker	#organisms originally sorted]		% sorting efficiency
☐	÷	[☐	+ ☐]	X 100 =	☐

QC Initials		**SORTER Initials**			**Pass or Fail** (circle one)
________		__________			
#organisms originally sorted		[#organisms recovered by checker	#organisms originally sorted]		% sorting efficiency
☐	÷	[☐	+ ☐]	X 100 =	☐

Appendix E: Biomonitoring Audit Summary

Station ID:_____ **Region:**_____ **QAQC Observer:**_____

Field Personnel: _____**Laboratory Personnel:** _____

Date: (Field)_____**(Lab)**_____ **Collection Procedure:** ☐ **Single Habitat** ☐ **Multi-Habitat**

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 m^2) or 12 (1/6 of a m^2) sampling sites.	☐	☐
3.	Kicks were timed 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 95% ethanol.	☐	☐
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 timed 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.
5. Collected sample was sieved and transferred to sample container according to SOP.
6. Collected sample was correctly preserved in a minimum 95% ethanol.

	Yes	No
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 51 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 ± 10% (99-121).	☐	☐
	☐	☐
	☐	☐
	☐	☐

9. If number 8 resulted in NO, then SOP was followed to result in 200 organisms ± 10% (180-220).
10. Only aquatic organisms were removed from sample according to SOP.

11. QA/QC sorting efficiency is up to date.

NOTES: _____

_____