Quality Assurance Project Plan for

Anne Arundel County Biological Monitoring and Assessment Program

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May 2011
Revision 2

This quality assurance project plan (QAPP) has been prepared according to guidance provided in the following documents: (1) *Generic Quality Assurance Project Plan Guidance for Programs Using Community Level Biological Assessment in Wadeable Streams and Rivers* (EPA 841-B-95-004, U.S. Environmental Protection Agency, Office of Water, Washington, DC, July 1995) and (2) *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R-5, U.S. Environmental Protection Agency, Quality Staff, Washington, DC, Interim Final, November 1995) to ensure that environmental and related data collected, compiled, and/or generated for this project are complete, accurate, and of the type, quantity, and quality required for their intended use.

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LIST OF ACRONYMS

B-IBI	Benthic-Index of Biotic Integrity
BMP	Best Management Practice
COC	Chain-of-Custody
CP	Coastal Plain
CV	Coefficient of Variability
DNR	Department of Natural Resources
DNREC	Department of Natural Resources and Environmental Conservation
DO	Dissolved Oxygen
DQO	Data Quality Objectives
EPA	Environmental Protection Agency
EPT	Ephemertoptera, Plecoptera, Trichoptera
GPS	Global Positioning System
MBSS	Maryland Biological Stream Survey
NIST	National Institute for Standards and Technology
NPDES	National Pollutant Discharge Elimination System
NPS	Nonpoint Source
ODNR	Ohio Department of Natural Resources
PM	Project Manager
PS	Point Source
PDE	Percent Difference in Enumeration
PSE	Percent Sorting Efficiency
PTD	Percent Taxonomic Disagreement
QA	Quality Assurance
QC	Quality Control
QHEI	Qualitative Habitat Assessment Index
RMSE	Root Mean Square Error
RPD	Relative Percent Difference
PSE	Percent Sorting Efficiency
SOP	Standard Operating Procedure
WERS	Watershed, Ecosystem, and Restoration Services
WRD	Watershed Restoration Division

DISTRIBUTION LIST

This document has been distributed to the following Anne Arundel County, KCI Technologies, Inc., Maryland Department of Natural Resources (DNR) Watershed Restoration Division (WRD), and Maryland Biological Stream Survey (MBSS) staff who are involved in this project.

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1.0 PROJECT/TASK ORGANIZATION

The purpose of this document is to present the Quality Assurance (QA) Project Plan for collecting, analyzing, and integrating environmental data from streams and watersheds to support a long-term biological monitoring program for the Anne Arundel County, Maryland, Department of Public Works, Watershed, Ecosystem, and Restoration Services (WERS). This revised QA Project Plan has been updated to reflect changes following completion of Round One (2004-2008), which will be implemented during Round Two (2009-2013), currently underway. The QA Project Plan provides general descriptions of the work to be performed to collect and analyze the data, and the procedures used to ensure the data are scientifically valid and defensible and that uncertainty has been reduced to a known and practical minimum. The County utilizes a professional consulting firm under contract with Anne Arundel County and any required specialty firms as subconsultants (referred to here as Contractor and Subcontractor respectively) to complete sample collection, provide taxonomic identifications, compile the data, calculate metrics for the Maryland Biological Stream Survey (MBSS) Coastal Plain (CP) Benthic-Index of Biological Integrity (B-IBI), and prepare final site assessments and summary reports.

The organizational aspects of a program provide the framework for conducting the required tasks. The organizational structure and function also can facilitate project performance and adherence to quality control (QC) procedures and QA requirements. Key project roles are filled by those persons responsible for ensuring the collection of valid data and the routine assessment of the data for calculation of precision and accuracy, as well as the data users and the persons responsible for approving and accepting final products and deliverables. The project organizational chart is presented in Figure 1; it reflects the relationships and lines of communication among all participants and data users. The responsibilities of these persons are described below.

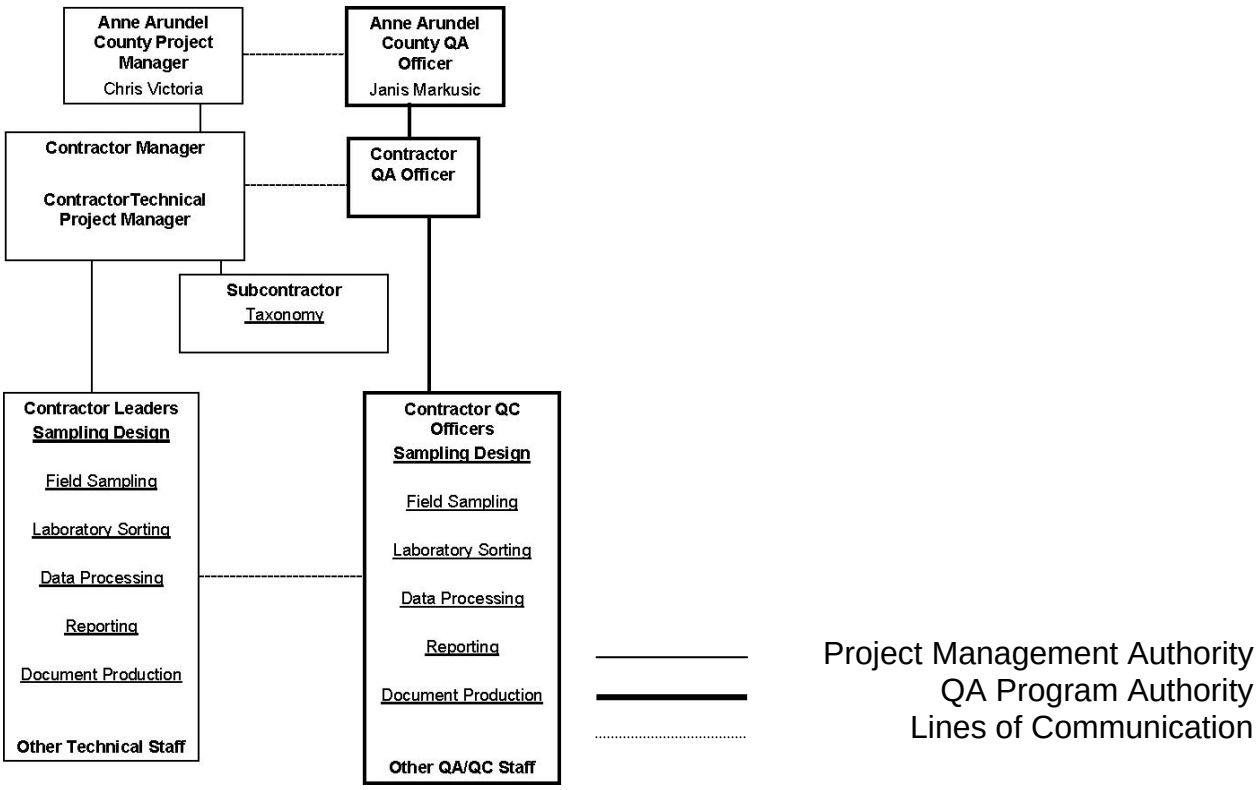

Figure 1. Project Organization

Project funding and general oversight will be provided by Anne Arundel County, WERS. Chris Victoria, Environmental Scientist, and Janis Markusic, NPDES Coordination/Ecosystem Assessment Program Manager. The County's management staff will review and approve the QA Project Plan and ensure that necessary County resources are provided for completion of the project.

The contractor will provide project oversight for this study as the Contractor Project Manager (PM). This individual will supervise the assigned project personnel to ensure their efficient utilization. Other specific responsibilities include the following:

- Preparing the scope of work for pertinent task orders.
- Reviewing and revising the QA Project Plan.
- Coordinating any subcontractors and project assignments in establishing priorities and scheduling.
- Ensuring completion of tasks within established budgets and time schedules.
- Providing guidance, technical advice, and performance evaluations to those assigned to the project.
- Performing field audits.

- Ensuring laboratory procedures are followed and conducting laboratory audits.
- Implementing corrective actions and providing professional advice to staff.
- Preparing and/or reviewing preparation of project deliverables and other materials developed to support the project.
- Providing support to Anne Arundel County in interacting with the project team, technical reviewers, and others to ensure technical quality requirements are met in accordance with study design objectives.

The primary responsibilities of the Contractor QA Officer include the following:

- Providing support to the PM in the preparation and distribution of the QA Project Plan.
- Reviewing and approving the QA Project Plan.
- Reviewing and evaluating field and laboratory procedures.
- Monitoring QC activities to determine conformance, if requested.
- Conducting performance and system audits of the procedures during the project, if requested.

Contractor Task Leaders will be responsible for managing specific tasks during the project (i.e., reviewing and potentially modifying the sampling design, performing field sampling to obtain biological, chemical water quality, physical habitat, and geomorphological data, processing samples in the laboratory, processing data [data entry and analysis], reporting, and producing documents). Task Leaders will supervise the technical staff participating in their group and their activities, implement the QC program, complete assigned work on schedule with strict adherence to established procedures, and complete required documentation.

The Field Sampling Task Leader will direct the work of the sampling team, including taking measurements, collecting samples, and completing field records. The field team will include scientific staff with specialization and technical competence in their particular field sampling activities to effectively and efficiently perform the required work. The Field Sampling Task Leader is responsible for:

- Ensuring that the team adheres to the project scope of work and QA Project Plan.
- Understanding all sampling operations, the standard operating procedures (SOPs), and the working order, readiness and completeness of all sampling gear, equipment, and supplies.
- Ensuring that acceptable progress is made in acquiring field samples that meet or exceed the specified standards for quality and data.
- Completing and signing field records, following custody procedures to ensure the integrity of the samples with respect to prevention of loss or contamination, maintenance of proper

sample identification during handling, and verifying the completeness and accuracy of chain-of-custody documentation.

- Controlling and monitoring access to samples while in their custody.

Additional oversight will be provided by the Contractor QC Officers for the task teams, who are responsible for performing evaluations to ensure that QC is maintained throughout the measuring and sampling processes in the field and subsequent analyses. The QC evaluations will include double-checking work as it is completed and providing written documentation of these reviews to ensure that the standards set forth in the QA Project Plan are met or exceeded. Other QA/QC Staff, such as technical reviewers and technical editors selected as needed, will provide peer review oversight on the content of work products and ensure that work products comply with Anne Arundel County specifications, respectively.

For this project, sample processing and taxonomic identifications will be provided by a subcontractor. The Contractor's PM will oversee work performed by the subcontractor to verify that all work assigned to the subcontractor is completed in accordance with the County's QA/QC requirements.

2.0 PROBLEM DEFINITION/BACKGROUND

Biological monitoring and assessment provide a direct measure of the ecological health of a stream. Stream organisms are continuous monitors of both short- and long-term water quality and other environmental factors and provide direct indicators of the quality of a stream. Aquatic communities have been used for more than 150 years to assess the conditions of stream ecosystems. Advantages of using benthic macroinvertebrates include their generally restricted mobility and often multi-year life cycles, allowing them to integrate the effects of both chemical and physical perturbations over time. When hydrologic regimes of streams are altered, the physical nature of the habitat changes due to accelerated erosion and deposition of channel soils and other materials. This changes the capacity of a stream to support a healthy biota. Changes in the quality of the water resource are reflected as changes in the structural and functional attributes of the macroinvertebrate assemblage. Biological monitoring and assessment results can be used to detect impairment of the biological community and to assess the severity of impacts from both point source (PS) and nonpoint source (NPS) pollution. When coupled with information on chemical and physical stressors, these types of exposure-and-effect data can be used to improve water quality assessments. Over the past several decades, biological monitoring and assessment of aquatic communities along with characterization of their chemical and physical habitats have increased with application of these data to watershed management policies and practices.

The primary goal of the Clean Water Act of 1972 is the protection and restoration of the chemical, physical, and biological integrity of the water resources of the United States. This goal provides the stimulus by which state and county agencies have begun to manage their water resources. Historically, many municipalities have been hampered in their ability to recommend and implement pollution control and remediation efforts because the chemical, physical, and biological condition of most of their water resources have not been adequately characterized. To expand its monitoring program, Anne Arundel County developed a stream monitoring program consisting of chemical, physical, and biological assessment techniques to document and track changes in the condition of stream resources County-wide. Problems resulting from chemical contamination and physical

habitat alteration are reflected by changes in the aquatic biota. Therefore, inclusion of a biological monitoring component is providing Anne Arundel County with the relevant indicators for assessing the condition of, and managing, its water resources.

The biological monitoring program was initiated with Round 1 (2004-2008) and is continuing with Round 2 (2009-2013). The primary goals of the program are to assess the current status of biological stream resources, establish a baseline for comparison with current and future assessments, and to relate them to specific programmatic activities. Example activities include best management practice (BMP) installation, storm water permitting, watershed assessment and management, and guidelines for future development. The County currently uses a combination of chemical sampling, geomorphic assessment, storm water sampling, and biological sampling to assist in its environmental management decision-making process. The continuation of the comprehensive biological monitoring program described in this document is a significant contribution to the needs of Anne Arundel County to evaluate and manage streams in the county. By sampling biology and stream habitat at approximately 240 sites over a 5-year period and integrating the biological sampling program results with chemical, hydrologic, physical habitat, geomorphological, and land use data, Anne Arundel County will be able to characterize stream condition.

Questions regarding the County's streams can be asked in three scales: county-wide, watershed-wide, and stream-specific. The design of this program is intended to allow the County to address questions at all three levels. It should be noted, however, that the use of the word 'watershed' in this document is meant to reflect a functional sampling unit, or primary sampling unit (PSU) as described in Hill and Stribling (2004), which in some cases is a discrete watershed unit (e.g., West River, Rhode River) and others a component of a larger watershed (e.g., Lower Severn, Upper Severn), and thus are used interchangeably herein. Some of the specific questions the program will be able to address with the monitoring data and results are:

Status:

Countywide: What percentage of streams in the County are impaired based on evaluation of the monitoring data.

Watershed wide: What is the overall ecological condition of an individual watershed? What is the status of streams in an individual watershed? Where are the most stressed streams (prioritized in order of severity)?

Stream specific: What is the ecological condition of individual streams located downstream from known or unidentified disturbances?

Trends:

Countywide: How has the percentage of impaired streams in the County changed from the conditions documented in Round 1 to those in Round 2?

Watershed wide: How has the percentage of impaired streams in an individual watershed changed from one monitoring year to another, for example, from monitoring year one to monitoring year six?

Stream specific: How have individual sites changed in condition over time? Are previously degraded streams showing improvement?

Problem identification/prioritization:

All streams: What are the locations of the impaired streams that were assessed? Of the streams and watersheds assessed, what are the locations of those that are most degraded? Conversely, what streams and watersheds are of high quality and require prioritization for protection.

Cause-and-effect relationships:

Metrics, bioassessment scores: What is the predictable response of individual metrics or biological attributes from exposure to specific human-induced stressors?

Evaluation of environmental management activities:

Countywide: Have the environmental protection policies of Anne Arundel County been adequate to maintain a healthy condition in the County's streams? Are the activities cost-effective for the County and industry?

Watershed wide: Have the environmental protection policies of Anne Arundel County been adequate to maintain a healthy condition in the County's watersheds?

Stream specific: Did the restoration of a specific stream lead to an improvement in biological condition? Did the implementation of restoration and protection measures lead to improvement in a particular stream segment?

Data generated by biological monitoring will allow the County to address questions regarding the quality of its targeted streams on a county-wide basis. These data will allow the County to document and monitor the biological status of the targeted streams and determine trends in their condition. The County will be able to integrate the biological and physical data to create a more comprehensive assessment of the targeted streams and aid the development and support of comprehensive watershed management practices. Anne Arundel County has identified the following specific objectives for their monitoring program:

- Document the ecological status of Anne Arundel County watersheds,
- Contribute to understanding dominant stressors and stressor sources affecting stream and watershed ecology,
- Track ecological health trends in the County's watersheds over time, and
- Have monitoring data be an integral part of resource management in the County.

3.0 PROJECT/TASK DESCRIPTION

Major activities for the development of the biological monitoring program for Anne Arundel County include developing a sampling design, coordinating field and laboratory activities, analyzing data generated, and creating formal reports. Each of these activities has inherent QC requirements and requires oversight by a trained staff person. They can also be divided into a number of tasks, each requiring management and QC by qualified personnel.

Task 1: Identify Sites

This task has already been completed for Round One (2004-2008) and Round Two (2009-2013); however, it is included here to outline the site-selection process for future monitoring rounds.

(a) Develop a list of the population of potential sites (i.e., sampling segments) segregated into 24-subwatershed primary sampling units (PSUs). This requires manipulation of the National Hydrography Dataset (NHD), 1:100,000 scale, to segregate and account for each segment within each subwatershed, and to designate them as 1st, 2nd, or 3rd order streams.

(b) Perform two-stage selection of sampling segments. The first-stage selection is to pick from among the 24 subwatersheds, at random, those to be done in each of five years in a monitoring round. The result of the first stage selection is a list of those subwatersheds to be sampled and assessed during each sampling year (years 1 through 5) of each round. The second-stage selection consists of randomly selecting a certain percentage of the segments within each stream order as a proportion of total stream miles within each subwatershed. A total of ten primary and ten alternate sites are selected for each PSU. This stage results in a final list of sampling segments, segregated by subwatershed and year.

Task 2: Obtain Sampling Equipment and Supplies

The Contractor will obtain all necessary sampling equipment and supplies for successfully conducting the sampling at each site. The Contractor will comply with all safety requirements and will make all logistical arrangements to have equipment, supplies, and appropriate personnel at the sites in accordance with the schedule established by Anne Arundel County and the Contractor PM.

Task 3: Conduct Sampling

The Contractor will conduct all sampling in accordance with MBSS methods guidance and the approved QA Project Plan (this document). Sampling will occur during March–April of each sampling year. The sampling team will consist of two to three persons, one of which will be designated as the Field Sampling Task Leader and another designated as the QC Officer. The Contractor will typically perform sampling and analysis at 50 stream sites and 5 duplicate QC sites throughout five primary sampling units (PSUs) each year, with the potential for an additional 20 targeted sites. Sampling will include acquiring latitude and longitude via portable global positioning system (GPS) units; taking site photographs; measuring pH, dissolved oxygen (DO), water temperature, specific conductance and turbidity; collecting benthic macroinvertebrates; assessing physical habitat; and measuring geomorphic characteristics of the survey reach. All

macroinvertebrate samples will be preserved in 80-90% ethanol, and transported to the subcontractor's laboratory for processing and identification.

Task 4: Conduct Laboratory Processing

The subcontractor laboratory will randomly subsample the benthic macroinvertebrate samples to obtain between 100 and 120 organisms from each site, and identify each (primarily to genus level), resulting in a final count of 100 ± 20 identified organisms. At least 10 percent of these subsamples will be sent to an independent taxonomic laboratory for re-identification and enumeration of the organisms.

Task 5: Perform Data Management

All physical, chemical, and biological data, including field and laboratory results produced by the Contractor will be entered into an ESRI Personal GeoDatabase. Data will be provided in a format suitable for inclusion in the Watershed Management Tool.

Task 6: Analyze Data

The MBSS B-IBI will be calculated using the most up to date version of the Coastal Plain B-IBI, and site assessments performed. Physical habitat scores will also be calculated, using the Rapid Bioassessment Protocols (RBPs) for Low Gradient streams and MBSS's Physical Habitat Index (PHI). Geomorphic data will be analyzed within Ohio Department of Natural Resources (ODNR) Reference Reach Survey 4.3L spreadsheets.

Task 7: Prepare Final Reports

The Contractor will provide a comprehensive annual summary report report for each year sampling is completed. The report will present all physical, chemical, and biological data and assessments, aggregated to subwatershed scales, with conclusions and recommendations based on evaluation of the results from all subwatersheds. Individual site assessment summaries will also be provided as an Appendix to the report.

Implementation of the monitoring program during each year will proceed with several milestones as presented in Table 1. They include selection of sampling sites, initiation of sampling, fieldwork, laboratory and data analyses, and annual reports. Sites have been selected for Round Two and initial sampling will begin with the 2009 spring index period (March 1- April 30). If tasks are added to address the monitoring program during additional years, addenda to this QA Project Plan (e.g., sampling and analysis plans) will be prepared and distributed to participating staff.

Table 1. Annual Timeline for Anne Arundel County Biological Monitoring and Assessment Program Activity

ACTIVITIES & MILESTONES	J	F	M	A	M	J	J	A	S	O	N	D
Review/Finalize QA Project Plan (January)												
Logistical Arrangements & Scheduling for Spring (January – February)												
Benthic Sampling (March 1 – April 30)												
Laboratory Processing & Sample Taxonomy (March 15 – June 15)												
Draft Report (September)												
Final Report (November)												

The monitoring program will coordinate, to the extent possible, with other ongoing monitoring programs so increased benefits can be derived from data sharing, the use of joint reference sites and reference conditions, the ability to produce ecological assessments that are more regional in scope, and the potential for increased cost- and time-efficiencies. Comparability of methods and results will provide a stronger link to monitoring activities in adjacent counties (or other agencies or universities), the District of Columbia, state monitoring and reporting activities, and national monitoring efforts. Contacts for some of these different monitoring programs and groups are provided in Table 2.

Table 2. Contacts for Biological Monitoring Programs Run by Adjacent Counties (or Other Agencies), State Monitoring and Reporting Activities, and National/Regional Monitoring Efforts. See Distribution List for detailed contact information.

Contact/Agency	Program/Area of Coverage/Activities
Ron Klauda Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division	Division Director, Resource Assessment Service, Monitoring & Nontidal Assessment Division
Dan Boward Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division	Ecological Assessment, Maryland Biological Stream Survey (MBSS), (Benthos)
Scott Stranko Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division	Biologist, QA/QC officer, Maryland Biological Stream Survey (MBSS)
Keith Van Ness Montgomery County Department of Environmental Protection, Water Resources Division	Program Director, Chief Biologist, Montgomery County Biological Monitoring Program
Howard Saltzman Howard County Department of Public Works	Program Director, Stormwater Management Division
Angela Morales Howard County Department of Public Works	Environmental Planner, Stormwater Management Division

4.0 QUALITY OBJECTIVES AND CRITERIA

Data quality objectives (DQOs) are qualitative and quantitative statements that clarify the intended use of the data, define the type of data needed to support the decision, identify the conditions under which the data should be collected, and specify tolerable limits on the probability of making a decision error due to uncertainty in the data (if applicable). DQOs are developed by data users to specify the data quality needed to support specific decisions.

4.1 Project Quality Objectives

The quality of an environmental monitoring program can be evaluated in three steps: (1) establishing scientific assessment quality objectives, (2) evaluating program design for whether the objectives can be met, and (3) establishing assessment and measurement quality objectives that can be used to evaluate the appropriateness of the methods being used in the program. The process of establishing DQOs involves identifying the allowable uncertainty of a data set which may lead to two types of error: *false positives* (Type I error: a problem is found to exist when in fact it does not) and *false negatives* (Type II error: a problem is not found when in fact it does exist). The acceptance probabilities of those errors as established by the data users are the DQOs. The DQO process entails establishing action-triggering values and selecting rates of false positives and false negatives that are acceptable to the data user (decision maker). The quality of a particular data set is some measure of the types and amount of error associated with the data.

Sources of error or uncertainty associated with variables and indicators include the following:

- Sampling (or random) error: The difference between sample values and *in situ* "true" values from unknown biases due to sampling design. Sampling error includes natural variability (spatial heterogeneity and temporal variability in population abundance and distribution) not specifically accounted for in a design (for design-based inference), and variability associated with model parameters or incorrect model specification (for model-based inference).

- Measurement (or systematic) error: The difference between sample values and *in situ* "true" values associated with the measurement process. Measurement error includes bias and imprecision associated with sampling methodology, specification of the sampling unit, sample handling, storage, preservation, identification, instrumentation, etc.

The data requirements for this project encompass aspects of field, laboratory analysis, and database management to reduce sources of errors and uncertainty in the use of the data. Data needed for the project are listed in Table 3.

This study is being undertaken to identify the condition of streams to characterize the watersheds of Anne Arundel County. This information will be used to develop a framework of sites to be sampled that will represent the various stream classes in the County. Ultimately, the County will use these data to explore whether decreasing or eliminating pollutant/stressor loadings might reduce risks to the overall ecological condition of its streams and watersheds. Substantial stress to the instream invertebrate assemblage, for example, can occur if chemical contaminants exceed certain thresholds or risk-based criteria, or, if physical habitat becomes degraded beyond the point of the stream's capacity to support a vigorous biota (= loss of complexity). However, some habitat features can modify the bioavailability of contaminants such that invertebrate populations are not adversely affected. By using a biological indicator to assess the ecological condition of streams sampled in this project, a series of quantitative, numeric benchmarks can be developed to identify sites (based on physical, biological, and chemical data) with little or no impairment to the benthic macroinvertebrate assemblage ("reference" or "subreference") for comparison to sites with similar conditions and habitat features. This will be accomplished by calculating the B-IBI for each site based on multiple metrics and by comparing the B-IBIs with those determined for reference (unimpaired) vs. impaired conditions for aquatic life in wadeable (1st to 3rd order), non-tidal streams across the County. The

biological index to be used was developed by the MBSS (Southerland et al., 2005) and is specifically calibrated to the physiographic regions of Maryland. Anne Arundel County will use the Coastal Plain version of the B-IBI to classify its streams.

Table 3. Types of environmental data to be collected for this project.

DATA TYPE	MEASUREMENT ENDPOINT(S) OR UNITS
Site Information Parameters	
GPS (global positioning system)	latitude and longitude (decimal degrees)
Photodocumentation	visual record of sampling sites
Drainage area and land use	area in acres
Ancillary observations	standard units used for parameter of interest or specific descriptive codes or description
Chemical Parameters	
Dissolved oxygen	milligrams per liter [mg/L]
pH	range from 0 to 14 standard units [SU]
Temperature	degrees Celsius [EC]
Specific conductance	microSiemans per centimeter [µS/cm] at 25ºC
Turbidity	nephelometric turbidity units (NTU).
Biological Parameters	
Benthic macroinvertebrates	number of each taxon
Ancillary observations	standard units used for parameter of interest or specific descriptive codes or description
Physical and Geomorphological Parameters	
Physical habitat assessment	rating of multiple parameters typically on scale of 0-20, 20 is highest possible score, but also as a percentage
Stream cross sectional measurement, water surface slope, and reach sinuosity	survey of channel dimensions, water depth, and valley distance recorded in feet and tenths
Modified Wolman pebble count	number in each size class, measured in mm
Additional Information as Appropriate (Nondirect Measurements)	
Historical data on watershed and stream conditions: aerial photographs, past IBI listings, stream channel cross-section dimensions, channel pattern, channel elevation, etc.	standard units used for parameter of interest or specific descriptive codes

The principal study questions for this project are:

- What is the status of a particular stream site (based on B-IBI score)?
- What is the status of a particular watershed (based on the mean B-IBI score, n = 10 sites)?

The index score obtained for each site will be compared with threshold levels developed by MBSS that correspond to different levels of impairment or reference conditions. Uncertainty in the data due to sampling and measurement errors or errors introduced during data manipulation, could result in identifying an effect on a macroinvertebrate assemblage when one does not exist, or in not identifying an effect when one does exist. By examining available benthic macroinvertebrate B-IBI data (via the MBSS database) from Anne Arundel and nearby counties (Prince George's, Calvert, Charles, Caroline, Dorchester, Kent, Queen Anne's, Somerset, St. Mary's, Talbot, Wicomico, and Worcester), a power analysis was conducted to determine the sample size required to meet the County's management goals:

- Detect a 30% change in the biological condition (IBI), 80% of the time, with 95% confidence.

The procedures used and results of the power analysis are explained in more detail in Appendix A. The County was divided into 24 total watersheds; four to five watersheds will be sampled during each year of the project using a rotating basin design (Hill and Stribling 2003).

The null hypothesis to be tested for Maryland's B-IBI nonimpaired thresholds (i.e., B-IBI = "good" or "fair") represents a baseline condition that is presumed to be true in the absence of strong evidence to the contrary. A decision error occurs when the null hypothesis is rejected when it is true (Type I–false positive decision error), or the null hypothesis is rejected when it is false (Type II–false negative decision error). The reason this might occur is because the measurement data on which the analyses are based can only estimate the true state of an environmental variable, such as the concentration of a nutrient or the number of benthic macroinvertebrate taxa in a waterbody. The true value cannot be known because (1) sampling must be limited and limits the capture of the complete extent of natural variability that exists in the true state of the environment (known as sampling design error), and (2) analytical methods and instruments are never absolutely perfect and can only estimate the true value of an environmental sample (known as measurement error). These errors, in addition to the uncertainties introduced in the biological index because relationships among variables must be limited to reduce complexity or might be imperfectly developed, means that basing decisions on the measurement data used in the metric selection could lead to decision errors. If the IBI for a site indicates that it is nonimpaired and the true value of the B-IBI score is above the lowest range value for the "good" condition, then consequences of accepting this value are negligible (i.e., the decision is correct). If the decision maker accepts this value and the true value of the B-IBI is below the impairment threshold, then failing to implement actions to improve the stream condition based on acceptance of this value could have severe consequences for the stream. The severity of potential consequences should also be considered to establish which decision error has more severe consequences near the cutoff. The ranges for each category and the tolerable limit on the decision error might be changed based on this evaluation.

While such errors cannot be eliminated, they can be controlled, for example, by collecting a large number of samples to control sampling design error and analyzing individual samples several times

or by using more precise laboratory methods to control measurement error. Verification and validation activities undertaken during the process of index development will help to control errors in the ranges established for each condition category. Limits to controlling errors will depend on available resources.

Responses of metrics and indices to water quality, habitat, and watershed perturbations can be examined through the data set analysis. The power analysis showed that investigation of a specific impact type would require approximately 10 sites affected by the impact, to detect a 30% change in index value with 80% probability. Many impacts co-occur, for example, sediment loading and hydrological "flashiness" are both common in watersheds with ongoing suburban construction (land cover alteration). It might not be possible to examine responses to individual stressors, only the responses to suites of stressors that occur simultaneously. For example, it is known that uncontrolled urban stormwater causes scouring and sedimentation of the stream bottom, increased instability of stream banks, and can often be coupled with removal of riparian vegetation and water temperature increases. This suite of stressors will cause the benthic macroinvertebrate community to be impaired, but it may be difficult to determine which of the stress components is having the greatest impact. The relative severity of the cumulative stressors can be determined through biological assessment; thus, sites with different suites of stress components can be ranked relative to one another.

The efficacy of management and restoration activities is evaluated by annual monitoring of known targeted problem sites to detect trends. Restored streams are monitored to determine if biological conditions improve. Bioassessment results from the restored streams are then compared to similarly impaired streams that were not restored, as well as to the reference condition. Finding trends of biological improvement in restored streams, and no trends in the other streams, would be strong evidence that restoration has been at least in part, effective.

Methods and procedures described in this document are intended to reduce the magnitude of measurement error sources and frequency of occurrence. The relevant measurement quality objectives for this project are related to sample handling, as well as making measurements of certain parameters onsite. General activities intended to help allow attainment of project quality objectives include the following:

- Use of standardized, repeatable data and sample collection procedures,
- Use of trained personnel to perform the data and sample collection and analyses, and
- Use of GPS coordinates and photographs to record the actual sampling locations for future reference purposes and for ensuring that the correct locations were sampled for all parameters.

Reducing data uncertainty is of highest priority. Since these data may also be used for water resources management and regulatory purposes, it is important to reduce uncertainty using appropriate QC protocols. Project quality objectives for chemical, physical/geomorphic and biological data are detailed below. Discussion of conventional data quality indicators, i.e., precision, accuracy, completeness, representativeness, and comparability, follows this section.

Chemical Parameters

Several parameters will be measured using an *in situ* multiprobe, while turbidity will be measured using a portable turbidimeter. Dissolved oxygen will be monitored because it is an important measure of the quantity of oxygen that is available to aquatic organisms. Without sufficient oxygen, aquatic organisms cannot survive and reproduce. Another parameter that will be measured is pH. This measures the acidity (hydrogen/hydroxide ion concentration) in effluents. Most aquatic organisms have a preferred range of pH between 6 and 9. Water temperature will be measured for use in taking temperature-dependent measurements such as pH and specific conductance. Specific conductance is an indirect measure of the dissolved ion concentrations in water (i.e., conductivity), corrected to a standard temperature of 25 degrees Celsius. Changes in ion concentrations from runoff and other sources can cause stress to aquatic organisms. Turbidity is a measure of water clarity which is influenced by suspended particles and organisms such as clay or silt sediments and phytoplankton. Aquatic organisms are particularly susceptible to the effects of increased sediments and turbidity. Project quality objectives for analyses of chemical parameters needed to determine the appropriateness of identifying the categories of impaired/nonimpaired wadeable, non-tidal streams in Anne Arundel County are:

- *In situ* measurements of water quality will follow approved methods.
- Calibration of the *in situ* measurement device and turbidimeter will be within 10% of known standards as per manufacturer's specifications.

Biological Parameters

The list of candidate metrics describing the benthic macroinvertebrate communities to be used to calculate the B-IBI will be those used by MBSS for the CP physiographic province. They were selected through evaluation of measurement parameters (metrics) relative to stated selection criteria (Table 4) which ensures linkage of the data analysis and resultant interpretation to project quality objectives. Samples are assessed by calculating the metrics on each and comparing them to the reference conditions; results for each location are translated into a narrative stream quality assessment of "good," "fair," "poor," and "very poor."

The sources of error or uncertainty yield measurable variability in the multimetric index as represented by the range of individual metric values or scores (Figure 2), or from aggregated metric scores from multiple reference sites (= population variance). This variability may be due to sampling error (natural variability among similar sites) or measurement error (method variability). Sampling a population of ecological reference sites, during the same index period as proposed in this program, yields quantitative estimates of the combined sources of error. For example, the interquartile range of values for the metric "Percent Contribution of Dominant Taxon" from a set of 12 reference sites may be 17–26; the interquartile range of the total bioassessment score from twelve reference sites might be 48–56. These ranges would represent the expected variability associated with the individual metric as well as the variability associated with sampling error or natural variability for the total bioassessment score. For this biological monitoring and assessment program, these values were calculated from 30-40 reference sites that will likely be located within the candidate reference watersheds that have already been identified. Reference sites are discussed in more detail in Section 7.0.

Table 4. Summary of indicator selection criteria.

CRITERIA/QUALITY	DEFINITION(S)
Scientific Validity (Technical Considerations)	
Measurable/ Quantitative	Feature of environment measurable over time; has defined numerical scale and can be quantified simply.
Sensitivity	Responds to broad range of conditions or perturbations within an appropriate time frame and geographic scale; sensitive to potential impacts being evaluated.
Resolution/ Discriminatory Power	Ability to discriminate meaningful differences in environmental condition with a high degree of resolution (high signal:noise ratio).
Integrates Effects/ Exposure	Integrates effects or exposure over time and space.
Validity/Accuracy	Parameter is true measure of some environmental condition within constraints of existing science.
	Related or linked unambiguously to an endpoint in an assessment process.
Reproducible	Reproducible within defined and acceptable limits for data collection over time and space.
Representative	Changes in parameters indicate trends in other parameters they are selected to represent.
Scope/Applicability	Responds to environmental changes on a geographic and temporal scale appropriate to the goal or issue.
Reference Value	Has reference condition or benchmark against which to measure progress.
Data Comparability	Can be compared to existing datasets/past conditions.
Anticipatory	Provides an early warning of changes.
Practical Considerations	
Cost-effectiveness	Information is available or can be obtained with reasonable cost/effort.
	High information return per cost.
Level of Difficulty	Ability to obtain expertise to monitor.
	Ability to find, identify, and interpret chemical parameters, biological taxa, or habitat parameter.
	Easily detected.
	Generally accepted method available.
	Sampling produces minimal environmental impact.
Programmatic Considerations	
Relevance	Relevant to desired goal, issue, or agency mission (e.g., fish fillets for consumption advisories; species of recreational or commercial value).
Program Coverage	Program uses suite of indicators that encompass major components of the ecosystem over the range of environmental conditions that can be expected.
Understandable	Indicator is or can be transformed into a format that target audience can understand (e.g., nontechnical for public).

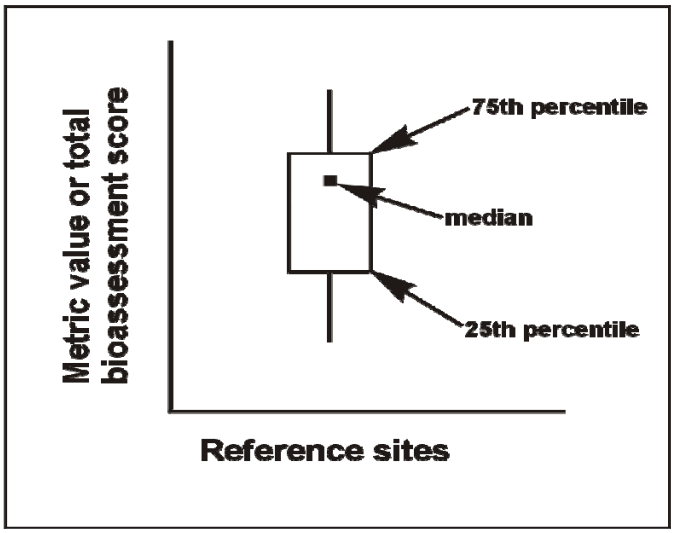

Figure 2. Box-and-Whisker plot showing the upper and lower percentiles and the median of individual metric value distribution among reference sites.

The project quality objectives for the biological component are:

- No more than a 10% sorting error will occur using trained laboratory technicians (sorting efficiency ≥ 90%).
- Accuracy of data entry will be ensured by 100% hand-checking of all values.

Physical and Geomorphological Parameters

Physical habitat assessment will be performed at each sampling location using U.S. EPA's RPB approach and the MPHI approach used by MBSS, both of which are visually-based assessment methods. Complex in-channel characteristics of streams function to provide (1) dissipation of erosive flow energy and (2) suitable habitat for support of a "healthy" aquatic biota. As streams degrade through physical and hydrologic processes, the physical habitat structure becomes less complex, and thus has a diminished capacity to support biota and withstand the erosiveness of storm flows. Physical habitat assessment will rate streams based on their qualitative position within a continuum of structural complexity. It is based on field observation of a series of instream, channel shape, and riparian characteristics, each of which is placed within the continuum for that site. The scores are summed for an overall physical habitat quality score. Stream channel cross sections, physical habitat assessments, and pebble counts will be made at each of the sampling locations to describe the physical characteristics of each stream. Pebble counts characterize the particle size distribution in stream and river beds. Shifts to fine materials (<0.25 mm diameter) in streams can negatively impact aquatic communities. An increase in fine-sized sediments can alter the biochemical conditions of the stream, and reduce food sources, change respiratory diffusion gradients, and decrease habitat space for macroinvertebrates and other organisms by decreasing the interstitial space between gravel and cobble particles. Also, fine materials can transport contaminants from roadways and soils through runoff and erosion processes. Physical characteristics indicate relative stream channel stability as well as the estimated biological potential of the stream. This information can also provide guidance on what types of macroinvertebrates might be present, because different species are generally adapted to different stream bed particle size, steam bed shape and area, and water flow (fast, moderate, or slow moving). In addition, the physical characteristics can serve as a snapshot of current conditions to which past and future measurements can be compared. The project quality objectives for these data are:

- Physical habitat assessments, measurement of stream channel dimensions, and pebble counts will follow approved methods.

4.2 Data Quality Objectives & Measurement Quality Objectives (DQOs/MQOs)

Field-based stream assessments are a series of steps combined into application as a protocol (MDEQ QAPP 2003). The measurement quality objectives (MQOs) for any protocol are most appropriately established for each step within the protocol. MQOs are used as an indicator of potential method problems. Data are not always discarded simply because MQOs are not met. Instead, this is a signal to further investigate and correct problems. Once the problem(s) are rectified, the data can still be utilized, as long as the correction is satisfactory. When individual performance characteristics are not applicable to some aspect of the assessment process, it is indicated as such. The steps for the biological assessments in the Anne Arundel County program include:

- Field Sampling
- Laboratory Sorting & Subsampling
- Taxonomy
- Enumeration
- Data Entry
- Metric Calculation
- Final Index & Site Assessment

Several performance characteristics are also provided for physical and geomorphic parameters.

4.2.1 Precision

4.2.1.1 Biological Assessments

a. Field Sampling

The replicate samples (QCs) are combined and used to calculate several measures of precision for individual metrics and the final index (B-IBI). Three measures will be calculated, relative percent difference (RPD), root mean square error (RMSE), and coefficient of variability (CV), which are described in detail below. Results from Round One monitoring demonstrated that there are varying levels of consistency for individual metrics, and, accordingly, metric-specific MQOs have been established (Hill and Pieper, 2010; Table 5). Values exceeding these should be investigated for potential error, and corrective actions or adjustments may be made as required.

Relative Percent Difference

Relative percent difference (RPD) represents the proportional difference between two measures and is calculated as:

$$RPD = \left(\frac{|A - B|}{A + B} \times 2 \right) \times 100$$

where, A is the metric or index value of the first sample and B is the metric or index value of the second sample **(**Berger et al. 1996**)**.

Root Mean Square Error

Root mean square error (RMSE) (or standard error of estimate), is a pooled standard error for a set of *k* group means, typically associated with a one-way ANOVA and is calculated by:

$$RMSE = \sqrt{\frac{\sum_{j=1}^{k}\sum_{i=1}^{n_j}(y_{ij}-\overline{y_j})^2}{\sum df_{1\ldots k}}}$$

Where y_{ij} is the i^{th} individual observation in group j.$j = 1\ldots k$ (Zar 1999). It is important to note that the denominator in this operation is the sum of degrees of freedom (df) for each group of replicated samples. Similar to RPD, RMSE decreases as precision increases. However, unlike RPD, RMSE is scale-dependent; therefore, metric and index RMSE values that are on different scales cannot be directly compared. Unlike RPD values, however, RMSE values are not distorted by metric values of zero.

Coefficient of Variation

To standardize the scale-dependent RMSE values, the coefficient of variation (CV) was calculated for the individual metric and B-IBI scores. CV is calculated from RMSE by:

$$CV = \frac{RMSE}{\overline{Y}} \times 100$$

where $\overline{Y}$ is the mean of the dependent variable (e.g., metric, index; Zar 1999). The CV allows direct comparison of the standard deviations among metrics and indices.

Table 5. Measurement quality objectives (MQO) for evaluating field sampling precision of benthic macroinvertebrate sampling represented by median relative percent difference, root mean square error (RMSE), and coefficient of variation (CV).

Metric or Index	Median RPD	RMSE	CV
Total Number of Taxa	20	4.3	20
Number of EPT Taxa	30	1.7	50
% Ephemeroptera	30	2.8	100
% Intolerant to Urban	80	15.9	80
Number of Ephemeroptera Taxa	30	0.5	100
Number of Scraper Taxa	30	0.9	100
% Climbers	30	6.9	70
B-IBI	20	0.6	22

b. Laboratory Sorting and Subsampling

Not applicable.

c. Taxonomy

Ten percent of the benthic macroinvertebrate samples will be randomly selected for re-identification by an independent laboratory. Comparison of the results will provide an estimate of taxonomic precision, or Percent Taxonomic Disagreement. This is calculated by the formula:

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

where $comp_{pos}$ is the number of agreements, and $comp_{tot}$ is the total number of taxonomic comparisons (which is equivalent to the total number of identifications in the sample). The lower the PTD value, the more similar are sample taxonomic results, and the greater is the overall taxonomic precision. The MQO for taxonomy is 15%. Individual sample PTD should be evaluated for determining the reasons for disagreement on certain identifications, but corrective actions are unnecessary if mean PTD for the dataset is ≤15%.

d. Enumeration

Final specimen counts for samples are dependent on the taxonomic identifications, not the rough counts obtained during the initial sorting activity. Comparison of counts is quantified by calculation of Percent Difference in Enumeration (PDE), where

$$PDE = \left(\frac{|Lab1 - Lab2|}{Lab1 + Lab2}\right) \times 100$$

The MQO for specimen enumeration is 5%, where samples having greater than 5% count difference are examined for sample integrity and reasons for the differences. The MQO for the dataset overall is a mean percent difference of ≤ 5%.

e. Data Entry

Not applicable.

f. Metric Calculation (i. e., Data Reduction)

Not applicable.

g. Final Index and Site Assessment

The replicate samples (QCs) are combined and used to calculate several measures of precision for the final index (B-IBI). Four measures will be calculated, relative percent difference (RPD), root mean

square error (RMSE), and coefficient of variability (CV), which are described above under subsection a. Field Sampling.

The MQOs for *a sampling event (field season, watershed, or other strata) are the B-IBI mRPD should be <15 and/or the coefficient of variability (CV) should be <22%,* where values in excess will be examined for potential error that may have arisen during the assessment process.

4.2.1.2 Physical and Geomorphological Parameters

Repeat physical habitat assessments at quality control sites will provide data to calculate RPD and will be based on the overall aggregated score (i. e., not individual parameters). The MQO for overall habitat scores is RPD ≤ 30%. Values exceeding these should be investigated for potential error, and corrective actions or adjustments may be made as required.

4.2.2 Accuracy

4.2.2.1 Biological Assessments

a. Field Sampling

Not applicable.

b. Laboratory Sorting and Subsampling

Not applicable.

c. Taxonomy

Definition of accuracy requires specification of an analytical truth (Taylor 1988, Clark and Whitfield 1994). For taxonomy that could be 1) the most up-to-date technical literature/keys, 2) an identified reference collection verified by specialists in different taxonomic groups, or 3) specimen by specimen comparison with museum-based type material (specimens). All taxonomy in this project will be completed using up to date technical literature. Option 3 is not feasible, nor considered necessary, for this project.

d. Enumeration

Not applicable.

e. Data Entry

The accuracy of data entry will be checked by direct comparison of original datasheets (handwritten in the field or laboratory) with spreadsheets prior to analysis and upload to the database. An individual *other than* the primary data entry technician will check all data entries (100%). Similar projects have shown that high error rates are generally associated with specific problem areas, such as how the information is written on a data sheet, the format of the entry sheet, or even a possible

problem with a specific data entry technician. All data entry errors will be corrected prior to any analyses taking place, thus, all data will be correctly entered.

f. Metric Calculation (i.e., Data Reduction)

A subset of metric values will be hand-calculated using only the taxonomic and enumeration data, and then compared to those that resulted from the programmed spreadsheets. This QC check procedure ensures that the interaction between metric calculation formulas and raw data is performing as expected. Thus, the analytical truth is the understanding of the technical, mathematical, and scientific logic behind each metric. The pattern to be used to select values for recalculation will be a combination of systematic and random characteristics, and should result approximately 5-10% of the metric values being recalculated by hand. If differences are found, each value will be checked for error in the calculation process (hand calculator vs. computer algorithm), and corrections made. Upon "re-running" of the metrics, and additional hand re-calculations, 100% of the computer generated metric values will be correct.

g. Final Index and Site Assessment

The analytical truth for final B-IBI scores was the number of sites designated as "degraded" using physical and chemical indicators of degradation. The percentage of degraded sites correctly identified as biologically impaired by the B-IBI is the classification efficiency (CE) (Stribling et al. 1998, MDEQ 2003). The accuracy of the Coastal Plain B-IBI is a CE of 96 (Southerland et al. 2005).

Bias can be a problem in interpretation of CEs if only a small number of quantitatively determined degraded sites is available (see discussion of bias).

4.2.2.2 Physical and Geomorphological Parameters

Not applicable.

4.2.3 Bias

4.2.3.1 Biological Assessments

a. Field Sampling

The MBSS sampling protocols intentionally focus sampling effort toward stable, productive habitat. Across the state these tend to be riffle and/or cobble habitat. However in the Coastal Plain, also designated as Low Gradient streams, those habitats are sparse. Therefore, other stream habitat is concentrated on, in the following order of preference: rootwads, rootmats and woody debris and associated snag habitat; leaf packs; submerged macrophytes and associated substrate; and undercut banks (DNR 2010). Other less preferred habitats include gravel, broken peat, clay lumps and detrital or sand areas in runs; however, of the aforementioned habitat types, those that are located within moving water are preferred over those in still water (DNR 2010). This format allows the sampler to obtain the maximum number of individual organisms while still sample each available stream habitat.

b. Laboratory Sorting and Subsampling

Percent sorting efficiency (PSE) is used to evaluate the effectiveness of a laboratory sorter in finding and removing specimens from a particular sample. It is calculated not only for an individual sorter, but for the overall project or "lot" of samples. Percent sorting efficiency is calculated as:

$$\frac{A}{A+B} \times 100$$

where, A is the number of organisms found by the original sorter, and B is the number of missed organisms recovered by the QC laboratory sort checker. The laboratory sorting/subsampling measurement quality objective (MQO) for this project is to have a database *where ≤10% of the samples overall have a sort efficiency of <90%*. Individual sorters will consistently attain a 90% or greater sort efficiency. Metrics and a final biological index are not calculated if the entire sample (all 30 grids) are sorted and less than 60 organisms are recovered.

c. Taxonomy

This type of error in taxonomy would be problematic if there were consistent misinterpretation of technical keys, misunderstanding of morphological features, poor processing of samples (including slide mounts of Chironomidae and Oligochaeta). Occasional problems with poor slide mounts have been noted in previous comparisons, but the extent to which these affected error in the taxonomic analysis was not evaluated. It is assumed that good taxonomic precision (low PTD) also somewhat reflects a minimum of bias in identifications.

d. Enumeration

Not applicable.

e. Data Entry

Not applicable.

f. Metric Calculation (i.e., Data Reduction)

Not applicable.

g. Final Index and Site Assessment

An artifact of calculating CE is that high values (e. g., between 95-100%) can be associated with low numbers of stressor sites. That is, if a dataset has a high number of stressor sites, and also a high CE, confidence can be placed in the result. Conversely, if a high CE is obtained with a low number of sites, the result should be accepted only with lower confidence. Thus, CE can be biased by low numbers to give potentially artificially high values.

4.2.3.2 Physical and Geomorphological Parameters

The level of bias with these methods can be substantial if the operator is undertrained or has a minimum of experience. Each field team will have one member experienced in the methods (with at least one year of prior field team member experience); and all field personnel will receive MBSS training and/or County orientation prior to sampling. Additionally, at least one team member will have Rosgen Level I training, or have at least one year of prior field experience performing Rosgen classifications.

4.2.4 Representativeness

4.2.4.1 Biological Assessments

a. Field Sampling

Representativeness of the sampling approach is inherent in its design. The method targets multiple sub-habitats in order of most stable, productive habitat (cobble/riffles, rootwads, rootmats and woody debris and associated snag habitat, leaf packs, , submerged macrophytes and associated substrate, undercut banks, gravel/broken peat and/or clay lumps and detrital or sand areas in runs.) and allocates a fixed sampling effort (20 ft^2) among the habitats in rough proportion to their occurrence through the 75m reach. This sampling approach is designed to produce a multi-taxon sample that reflects the benthic macroinvertebrate assemblage that the stream physical habitat has the capacity to support.

b. Laboratory Sorting and Subsampling

Two aspects of the sample handling and laboratory processing method, in part, ensure representativeness. First, the initial laboratory handling of the sample, specifically the effort to evenly spread the entire sample across the Caton tray, and, second, the randomization process for original selection of grids for sorting. Four grids are initially selected for sorting. If after an initial sort of the first grid reveals that there is a potential to find well over 100 organisms out of the four original grids, those four grids are re-spread into another Caton tray. Then, four new grids are randomly chosen to sort. An important aspect of subsampling representativeness would be those samples where the 100 organism level was attained in a low number of grids (e.g., 4 or 5). If the sample was not well mixed prior to spreading, it is possible that the selected grid(s) are not characteristic of the sample overall.

c. Taxonomy

Not applicable.

d. Enumeration

Not applicable.

e. Data Entry

Not applicable.

f. Metric Calculation

Not applicable.

g. Final Index and Site Assessment

Sites for this project are randomly selected. This allows them to be considered representative of the conditions for the individual targeted streams, as well as complete watersheds, and at the end of the basin rotation, the County overall.

4.2.4.2 Physical and Geomorphological Parameters

The habitat assessment approach used in this project is intended to simultaneously represent the structural complexity of the stream channel morphology, its capacity to dissipate erosive flow energies, and its overall relative value as habitat for the stream biota.

4.2.5 Completeness

The MQO for all sample types and assessments is that 95% of the planned data points will be obtained. Percent completeness is calculated as:

$$\%C = \frac{V}{T} X 100$$

where V = number of measurements/samples judged valid, and T = total number of planned measurements/samples.

4.2.6 Comparability

Two data sets are considered to be comparable when there is confidence that they are equivalent with respect to the measurement of a specific variable or group of variables. For this project, data will be considered comparable if they meet the performance criteria, or MQOs, for each step of the sampling and analysis process. Measurement data collected in this project will follow procedures established by MBSS. Comparability is dependent on the proper design of the sampling program, and on adherence to sampling techniques and SOPs. All sampling will be conducted during March and April.

5.0 SPECIAL TRAINING REQUIREMENTS/CERTIFICATION

This QA Project Plan and other supporting materials will be distributed to all people listed in the distribution list (p. vii). All field, laboratory, and data analytical personnel have training and/or experience in performing all duties for which they are responsible.

One week prior to initiation of field work, all field team members and alternates will attend an orientation session to review the QA Project Plan and other materials; check that all equipment and sampling gear are ready; receive supplemental or refresher training in all field methods (biological,

physical, chemical monitoring); and review documentation requirements, QC procedures, and health and safety gear and procedures. Each sampling team will consist of the Field Sampling Task Leader, or qualified designee, who will direct the measurement and/or sampling effort, and a QC Officer, who will ensure strict adherence to project protocols. One person on any field team will also attend the MBSS spring index period training, usually held at the end of February.

Sample handling, sorting/subsampling, and primary taxonomic identifications will be performed by personnel with extensive experience or who are currently in training. All samples picked by sorters-in-training will undergo a QC check until the sorter-in-training consistently passes the 90% sorting efficiency criterion.

6.0 DOCUMENTATION AND RECORDS

Thorough documentation of all field activities related to sample collection is necessary for proper sample processing in the laboratory and, ultimately, for the interpretation of study results. Each type of field measurement, sample collection, and sample handling will be documented for each site sampled using either standard forms or electronic data forms. Specific information requirements for the various parameters are detailed in the following subsections.

To ensure completeness and accuracy of the data recorded in the field directly into the geodatabase, the digital data forms have been designed with numerous data entry safeguards in place. Such safeguards include setting all required data fields to 'mandatory' status so that the user will be prompted to enter the required information into a field before the form can be closed and saved. Additionally, upper and lower limits are placed on numeric fields to ensure that data are within acceptable ranges. For example, most RBP and PHI parameter fields have limits of 0 – 20, while water chemistry parameters such as pH have appropriate ranges of 0 – 14, since values outside of this range would be erroneous.

Chemical Parameters

Field water quality measurements will be logged within the sampling device (YSI), with the exception of turbidity, and recorded on the appropriate electronic data form on the field computer. Turbidity will only be recorded on the appropriate electronic data form. Additionally, a Calibration Log Book will be used for recording multiprobe calibrations.

Physical/Geomorphic Parameters

The following forms will be used to record stream channel cross sections, habitat evaluations, and pebble counts:

- ODNR Reference Reach Spreadsheet 4.3L (electronic)
- Habitat Assessment Field Data Form (electronic)

Biological Parameters

Collection and processing of benthic macroinvertebrate samples will be documented in writing using the following forms and labels:

- A sample identification label to accompany each sample, one on the outside of the container and one placed inside with the preserved benthic macroinvertebrate sample.
- A Chain-of-Custody Record.
- Benthic sample log-in sheet for logging in samples.
- Benthic Macroinvertebrate Laboratory Bench Sheet.
- Laboratory taxonomy bench sheets (provided by subcontracted taxonomist).

The original handwritten laboratory bench sheets and sample sorting efficiency forms (Benthic Macroinvertebrate Laboratory Bench Sheets) will document laboratory activities and will be submitted to the PM. Voucher samples will be assembled and maintained in the subcontractor laboratory. The laboratory manager will have primary responsibility for the voucher collection.

The PM will maintain files, as appropriate, as repositories for information and data used in the preparation of any reports and documents during the project and will supervise the use of materials in the project files. The following information will be included:

- Any reports and documents prepared.
- Contract and work assignment information.
- QA Project Plan.
- Results of technical reviews, data quality assessments, and audits.
- Communications (memoranda; internal notes; telephone conversation records; letters; meeting minutes; and all written correspondence among the project team personnel, subcontractors, suppliers, or others).
- Maps, photographs, and drawings.
- Studies, reports, documents, and newspaper articles pertaining to the project.
- Special data compilations.
- Spreadsheet data files: Records of physical habitat, taxonomy, and metric calculations (hard copy and electronically).
- GIS files (shapefiles and personal geodatabases).

Original, handwritten field and laboratory data sheets, chain-of-custody forms, and hard copies of data spreadsheets will be maintained in the Contractor's files. Formal reports generated from data collection (electronic and hard copy) will also be maintained with the Contractor's project files and copies will be forwarded to Anne Arundel County Project Manager. The data reports will include a summary of the types of data collected, sampling dates, their values, and any problems or anomalies observed during sample collection.

If any change(s) in this QA Project Plan is required during the study, a memo will be sent to each person on the distribution list describing the change(s), following approval by the appropriate persons. The memos will be attached to the QA Project Plan.

All written records relevant to the sampling and processing of samples will be maintained by the Contractor's PM with copies submitted to the Anne Arundel County PM. Unless other arrangements are made with Anne Arundel County, records will be maintained with the Contractor for a maximum of 2 years following project completion.

7.0 SAMPLING DESIGN

This section describes the strategy and procedures to be used to collect site information and chemical, biological, and physical data for Anne Arundel County. More details are presented in Appendix A. A single benthic macroinvertebrate sample will be collected during the spring index period from each of the approximately 240 stream locations in this project during five years. An index period is a restricted timeframe within which sampling can occur to limit seasonal variability in benthic macroinvertebrate community composition. Anne Arundel County will use the early spring index period from March 1 - April 30, which corresponds to the period used by the MBSS.

GPS coordinates will be followed to and recorded at each site, physical habitat and geomorphological assessments will be performed at all sites, as well *in situ* measurements of pH, dissolved oxygen, temperature, specific conductance, and turbidity. The biological and physical data are considered critical; the geomorphological and field chemistry data are considered noncritical.

Three types of sites will be monitored in this program to address Anne Arundel County's goals: probability, QC, and reference. Table 6 presents several design elements relative to sampling and site assessment.

Table 6. Sampling design elements for Anne Arundel County's Biological Monitoring and Assessment Program.

Data Quality Objectives	
To be able to detect a 30% change in biological condition, 80% of the time, with 95% confidence.	
Site Types	**Number of Sites**
1. Probability	~ 50 per year
2. QC sites	10 % of sampled sites
3. Reference	MBSS CP Reference Condition (13)
Sampling Frequency (Index Period)	
Spring: March 1 – April 30, coincides with MBSS	
Sampling Method	
Benthic macroinvertebrates: 20 ft² sample, multihabitat, randomized 100-organism subsamples, 595-600 µm mesh.	
Benthic Macroinvertebrate Taxonomic Level	
Genus for most taxa, unless immature or damaged, then next higher classification level	

Benthic Macroinvertebrate Metrics

1. Total Taxa
2. EPT Taxa (Ephemeroptera, Plecoptera, Trichoptera)
3. % Ephemeroptera
4. % Intolerant to Urban
5. Number of Ephemeroptera Taxa
6. Number of Scraper Taxa
7. % Climbers

Probability sites are those sites randomly selected each year for sampling. Neither targeted nor reference sites alone yield information that can be used to estimate status of stream resources in the County, nor in single watersheds. Conclusions such as "20% of stream segments in the County are impaired" require a representative sample of stream segments, which is best selected with a probability-based design. A probability-based design usually includes some form of random selection of sites, such that each site has a finite probability of being selected for sampling. This ensures the representativeness of the sample, in that a concerted effort is made to eliminate bias in site selection. In addition, design of a sampling program inevitably requires compromises to be able to answer the intended questions in a reasonable time at a reasonable cost. Assumptions were made on annual sampling effort and on defining the population of interest. Prior knowledge was applied to stratification of watersheds and sites. As described in the Design of the Biological Monitoring and Assessment Program for Anne Arundel County, Maryland (Appendix A), 24 watersheds have been delineated in the county. In the first stage of site selection, watersheds were prioritized for County needs, and in the second stage, stream segments within the selected watersheds were chosen at random for sampling. In each year of the monitoring program, a set of four to five watersheds will be selected, and approximately 40 to 50 stream segments will be sampled. After five years, all of the watersheds in Anne Arundel County are sampled, completing one round.

Quality control sites are duplicate reaches that are sampled at 10% of the total sites (i.e, one per sampling unit) to provide data for calculating sampling and method precision as relative percent difference (RPD), root mean square error (RMSE), coefficient of variability (CV), and confidence interval (CI). They will constitute 75m reaches that are immediately upstream of probability sites. To ensure that no additional stressor sources are present and that physical habitat appears similar to the original reach, the locations of these reaches will be selected following the procedure described in KCI-SOP-BI-010 "Selecting a QC Site for Duplicate Sampling" (Appendix S). Prior to sampling, site maps displaying the most recent orthophotography will be reviewed to determine which sites may be good candidates for a quality control site (i.e., absence of road crossings or tributaries, absence of pipe outfalls or other point source discharges, consistent buffers from adjacent land use). In general, sites where there is an increased potential for additional stressor sources in the upstream reach should be avoided. Of the remaining sites, the first one encountered in each sampling unit where the physical habitat and geomorphic conditions are consistent and no obvious additional stressor sources are present for 75 meters above the randomly selected site will become a quality control site and have a duplicate reach sampled.

Reference sites are used in biological assessment to compare data from assessment or test sites. The reference sites are used to establish a reference condition as an objective standard of comparison (Gibson et al., 1996). Reference sites represent least or minimally disturbed stream conditions in the region. Criteria used to select reference sites include an abundance of natural vegetation in the watershed, especially riparian vegetation near the stream channel; the absence of known pollution discharges and stream alterations; a minimum of roads, residential areas, and other human alterations

(Gerritsen et al., 1993; Hughes et al., 1986, 1994). The reference conditions for assessing Maryland CP streams (which includes Anne Arundel County) were developed by the MBSS (Stribling et al., 1998) using quantitative physical, chemical, and land use criteria. Scoring criteria were developed using metric value distributions from multiple CP sites (Table 7).

See Gibson et al. (1996), for a more complete description of reference condition development; Barbour et al. (1995), for national guidance; and Stribling et al. (1998) and Southerland et al. (2005), for development and documentation of stream reference conditions in the Mid-Atlantic Coastal Plain.

Table 7. Bioassessment scoring criteria for benthic macroinvertebrate metrics.

Core Metrics	5	3	1
Total Taxa	≥ 22	14 - 21	< 14
Number of EPT Taxa	≥ 5	2 – 4	<2
Number Ephemeroptera Taxa	≥ 2	1	< 1
% Ephemeroptera	≥ 11	0.8 – 10.9	<0.8
% Intolerant Urban	≥ 28	10 – 27	< 10
Number of scraper taxa	≥ 2	1	< 1
% climbers	≥ 8.0	0.9 – 7.9	< 0.9

(Southerland et al 2005)

Assessment of individual streams and subwatersheds is possible within each index period, for all randomly-selected sites. However, aggregation of stream and watershed assessment to a Countywide estimate will not be done until the end of each round of sampling. It should be noted that targeted sites will not be used for Countywide or subwatershed scale assessment.

8.0 SAMPLING METHODS

Specific sampling methods are detailed in the SOPs in each Appendix.

Site Information Parameters

Site establishment is detailed in KCI-SOP-BI-005 "Establishing and Marking a Random Site (Appendix F). GPS coordinates will be obtained in accordance with KCI-SOP-TE-001, "Use of GPS (Trimble Pathfinder ProXT)" (Appendix C).

Chemical Parameters

The in-stream chemical data (pH, temperature, DO, specific conductance) will be collected by the field sampling team using a YSI multiprobe in accordance with the KCI-SOP-WQ-001 "Operation of the Professional Plus Instrument" (Appendix D). Turbidity will also be measured in the field in accordance with KCI-SOP-WQ-002 "Operation of the Hach Model 2100P Turbidimenter" (Appendix E).

Biological Parameters

Benthic macroinvertebrate sampling will be conducted by the field sampling team following the methods outlined in the MBSS Sampling Manual (DNR, 2010). Samples will be logged in the field on the Chain-of-Custody Record, according to KCI-SOP-BI-006 "Completing Benthic Sample Log-

in Forms" (Appendix N). A list of equipment and expendable supplies needed in the field is provided in Table 8 for sampling benthic macroinvertebrates.

Table 8. Equipment and Supply List for Benthic Macroinvertebrate Sampling.

General	**Sampling Gear**
Maps, of site location and access routes	YSI and Turbidimeter with buffers/standards
GPS receiver & field computer	Standard aquatic dipnet, D-frame net (595-600 µm openings)
Pencils, pens	Additional D-frame net as backup (with extra clips)
Alcohol pens, Sharpies	Sieve bucket, standard no. 30 mesh (595-600 µm openings)
Calibration log	Sample containers, two to three 1-quart, plastic, opaque, straight-sided (per station)
Field data sheets	Scissors, one pair
Labels (internal, external, as specified)	Forceps
Clipboard	Wash bottle, 1-liter capacity
Hip or chest waders, one per crew member (plus backup or repair kit)	95% ethanol, 0.5 gallon per station (permit pouring into sample containers w/minimum spillage)
Gloves	Box or cooler for sample transport

Physical and Geomorphological Parameters

Evaluation of physical habitat quality is accomplished using two procedures. The first was developed for use with Rapid Bioassessment Protocols (RBPs; Barbour and Stribling 1994; Barbour et al., 1999). The approach is visual-based and consists of scoring a continuum of conditions for each parameter into one of four categories represented as optimal, suboptimal, marginal, and poor (Figure 3). Included is a 20-point scale for each parameter with 0 being poor and 20 optimal. Habitat quality is scored by visually assessing (i.e., scoring or rating) parameters along an approximate 75-meter segment of stream, including about 25 m upstream of the sampling reach.

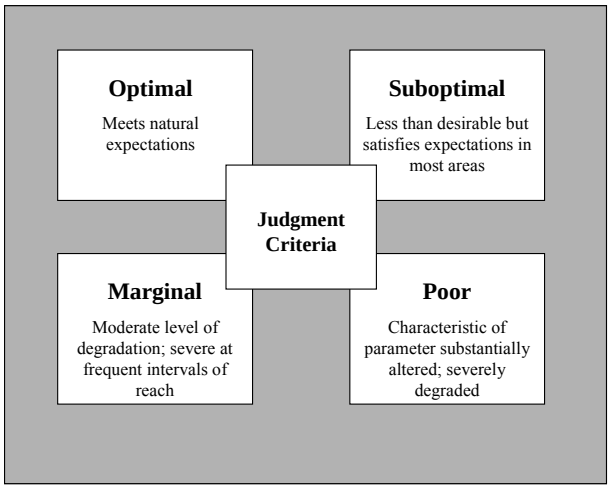

Figure 3. Narrative attributes for judging the quality of habitat parameters along a continuum of conditions.

The second is the approach used by the MBSS, and is a combination of the RBP-type of assessment with that of Ohio EPA's Qualitative Habitat Assessment Index (QHEI). This approach is detailed in the most current MBSS sampling manual (DNR, 2010). The Contractor will conduct the habitat assessments following the procedures in KCI-SOP-BI-008 "Physical Habitat Assessment (MBSS Methods)" (Appendix K) and KCI-SOP-BI-009 "Physical Habitat Assessment for Low Gradient Streams" (Appendix L).

After thorough visual assessment of the channel characteristics is made, a representative section, based on the best professional judgment of the crew leader, is selected for analysis as the assessment reach. This section should be representative of the channel condition and form within the reach of interest. One cross section is measured within the assessment reach and bankfull channel, slope, sinuosity, and other features measured or calculated. Cross sectional measurements will be performed following the procedures in KCI-SOP-GE-004 "Stream Cross Sectional Measurement "

(Appendix I). Channel cross section data will be used to perform Rosgen Level II classifications for each survey reach following procedures described in Rosgen (1996).

In addition to the cross sectional measurements, an abbreviated longitudinal profile will also be performed following the procedures in KCI-SOP-GE-005 "Abbreviated Stream Longitudinal Profile Measurement" (Appendix J). Distance and elevation measurements are taken as close as possible to the end and the beginning of the sampling reach (0 and 75 meters). The resulting points are coupled with the measurements taken during the cross section survey and are used to characterize the slope of the sampling reach.

In addition, pebble counts will be performed following the procedures in KCI-SOP-GE-003 "Modified Wolman Pebble Count" (Appendix M). An estimate of the distribution of channel features (i.e., riffles, pools, run, steps, etc.) in the assessment reach will be made based upon the total percentage made up by a particular feature. A total of 10 transects will be proportionally distributed through the assessment reach spanning the estimated bankfull width of the channel. Within each transect, a total of 10 particles, selected at equally spaced intervals, will be measured with a ruler along the intermediate axis or compared to a sand gauge depending on the size of the particle. A total of 100 particles are counted for the entire reach unless the substrate is entirely comprised of sand (or finer) particles, in which case only 20 particles will be measured.

9.0 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

The benthic macroinvertebrate samples are preserved in the field using 95% ethanol with proper internal and external labeling. A Benthic Sample Log-in Record (Appendix N) will be completed at the time of sample collection to accompany the samples to the Contractor's laboratory for storage prior to shipping to the subcontractor's laboratory for processing. Proper chain-of-custody procedures are necessary for tracking sample possession from field to laboratory. The form will document the sampling date, sampler's initials, sampling site location/description, and sample description.

The appropriate sample identification label (to be placed on or in the sample bottle/container) will be completed to accompany each sample throughout the chain of custody. The label will document the project name, sampling personnel names, sample type, sampling site location, preservative, and the sample number. All entries will coincide with specimen and sample information on the Benthic Sample Log-in Record.

Samples will be logged in when received by the Contractor's lab, following KCI-SOP-BI-006, "Completing Benthic Sample Log-In Procedure" (Appendix N). Prior to shipping the samples to the subcontractor's laboratory for processing, samples will be logged onto a Chain-of-Custody Record following KCI-SOP-BI-007, "Completing Biological Sample Chain-of-Custody Record" (Appendix H).

Methods of benthic macroinvertebrate sample processing are based on U.S.EPA's rapid bioassessment protocols (Barbour et al. 1999). Biological sample laboratory processing falls into two divisions. The initial or primary sample processing includes sorting, subsampling, and re-sorting checks. In biomonitoring programs subsampling is recommended as a cost-effective and valid procedure for (1) selecting a representative estimate of the total sample collected and (2)

standardizing the level of effort expended on each sample. Anne Arundel County will use a randomized 100-organism subsample obtained from a gridded screen (Caton, 1991) to increase subsampling efficiency in accordance with KCI-SOP-BI-004 "Benthic Macroinvertebrate Sample Processing – 100 Organism Subsample" (Appendix O). Quality control of the benthic macroinvertebrate sample processing will be performed following INVERT009.01 "Macroinvertebrate Sorting Quality Control" (Appendix P). Secondary or final phase processing includes taxonomic identification and verification procedures, tabulation, enumeration, and measurements.

10.0 ANALYTICAL METHODS REQUIREMENTS

This section presents a description of the taxonomic identification guidelines and the basic data analysis procedure. In the latter, the process for calculation of metrics, and selection and development of an aggregated, multimetric index is presented.

Benthic Macroinvertebrate Taxonomy

Training, experience, and possession of proper laboratory equipment and taxonomic literature are crucial factors affecting the quality of identification activities. For Anne Arundel County, specimens generally will be identified to the genus level using the most current literature available. However, some organisms (early instars or those with damaged or missing parts) may be left at a higher taxonomic level, such as family or order. Taxonomic identifications will be performed in accordance with SOP INVERT005.02 "Macroinvertebrate Identification" (Appendix Q). Quality control of the taxonomic identifications will be ensured through adherence to SOP INVERT010.00 "Macroinvertebrate QA/QC" (Appendix R).

A level of uncertainty is identified for all taxonomic identifications. A scale of uncertainty is applied for each identification, where 1 is most certain and 5 is least certain. Reasons for any uncertain identification (e.g., missing gills, headless specimen, etc.) will be documented on the laboratory bench sheet. The taxonomic uncertainty ratings are used as documentation in quality control checks (calculation of PTD), and can be used in seeking confirmation of specimen identifications. When identifications are uncertain, an average tolerance value for the next higher taxonomic level will be assigned. For example, if the generic level of identification is questionable, an average tolerance value for the family level will be used.

Verification will be accomplished by comparison with a pre-established reference collection. A reference collection is defined as a set of biological specimens, each representing some taxonomic level and not necessarily limited to specific projects or activities. Specimens whose identification is uncertain may be sent to taxonomic experts familiar with the group in question for confirmation. A reference collection of benthic macroinvertebrates is currently being developed based on the list of taxa accumulated from several local and regional studies in and around the Mid-Atlantic. The voucher samples from those studies (Cummins and Stribling 1992, Stribling et al., 1990, Stribling and Thaler 1991) are being curated to build the reference collection. Reference specimens will represent taxa collected in the Anne Arundel County area from several watersheds including tributaries of the Severn, Patapsco, Patuxent, and Magothy River watersheds. The majority of the specimens are identified to the generic level; Oligochaeta and Hirudinea are identified to class.

The true data of a project are the actual specimens collected in a survey for that project. Following identification and enumeration, these specimens will be maintained in a voucher collection for at least five years or as prearranged by contract agreement. Voucher collections may sometimes serve as reference collections but not vice-versa. This is primarily because reference collections are arranged/curated based on taxonomic and/or phylogenetic order and are not usually associated with particular projects or specific waterbodies (although that information will be included with label data). If there are ever questions regarding the accuracy of taxonomic identifications that have been used in metric calculation and reporting, referral to the voucher collection should be an initial step taken in resolution. Selected basic taxonomic literature is provided in Table 9.

Table 9. General taxonomic and functional feeding group literature for benthic macroinvertebrates.

Borror, D.J., C.A. Triplehorn, and N.F. Johnson. 1989. *An Introduction to the Study of Insects*. 6th edition. Sauners College Publishers, Philadelphia, PA.

Cummins, K. W. and M. A. Wilzbach. 1985. *Field Procedures for Analysis of Functional Feeding Groups of Stream Macroinvertebrates.* University of Maryland, Frostburg, MD.

McCafferty, W. P. 1981. *Aquatic Entomology: The Fishermen's and Ecologists' Illustrated Guide to Insects and Their Relatives.* Science Books International, Boston, MS.

Merritt, R. W. and K. W. Cummins, eds. 2007. *An Introduction to the Aquatic Insects of North America.* 4th. ed. Kendall/Hunt Publishing Company, Dubuque, IA.

Needham, J. G. and P. R. Needham. 1989. *A Guide to the Study of Freshwater Biology.* 5th ed. Holden-Day, Inc., San Francisco, CA.

Peckarsky, B. L., et al., 1990. *Freshwater Macroinvertebrates of Northeastern North America.* Cornell University Press, Ithaca, NY.

Pennak, R. W. 1989. *Fresh-Water Invertebrates of the United States: Protoza to Mollusca.* 3rd. ed. John Wiley and Sons, New York, NY.

Stehr, Frederick W. 1991. *Immature Insects*. Vol. 2. Kendall/Hunt Publ. Co., Dubuque, IA.

Thorp, J.H. and A.P. Covich, eds. 2009. *Ecology and Classification of North American Freshwater Invertebrates.* 3rd. ed. Academic Press, New York, NY.

Data Analysis and Site Assessment

The process for analyzing biological data is patterned after the multimetric approach advocated by U.S.EPA in their technical guidance for developing biocriteria (Gibson et al., 1996, Barbour et al., 1995). Metric values calculated from all test sites are compared to the reference condition (= metric scoring criteria) developed for the MBSS (Stribling et al., 1998, Southerland et al., 2005). Each of the seven metrics is assigned the appropriate bioassessment rating score according to the reference condition and its metric scoring criteria (see Table 7). The seven rating scores for the metrics are averaged to an overall bioassessment score for each site. The averaged score for a site is converted to narrative assessments by comparing to the following:

4.0 - 5.0	good
3.0 - 3.9	fair
2.0 - 2.9	poor
1.0 - 1.9	very poor

The scoring of IBI metrics was based on Maryland DNR's MBSS approach (Stribling et al., 1998, Southerland et al., 2005). The IBI approach involves scoring each metric as 5, 3, or 1, depending on whether its value at a site approximates, deviates slightly from, or deviates greatly from conditions at the best reference sites. According to MBSS methods, threshold values for each selected metric are established as approximately the 10^{th} and 50^{th} (median) percentile values for reference sites. For each positive metric, values below the 10^{th} percentile are scored as 1; values between the 10^{th} and 50^{th} percentiles are scored as 3; and values above the 50^{th} percentile are scored as 5. Scoring for negative metrics is reversed (e.g. values below the 50^{th} percentile are scored as 5; values above the 90^{th} percentile are scored as 1). To develop an overall index, a mean of all metric scores is calculated, resulting in an index scaled from 1 to 5.

Integration of biological assessment results, physical habitat quality, stream stability (cross sections), pebble count, land cover characteristics, and selected water chemistry characteristics will provide the Anne Arundel County WERS with a mechanism for rating the overall health of a given watershed and to monitor trends within County watersheds as regular data collection continues.

11.0 QUALITY CONTROL

Data quality is addressed, in part, by consistent performance of valid procedures documented in the SOPs. It is enhanced by the training and experience of project staff (Section 5.0) and documentation of project activities (Section 6.0). This QAPP and other supporting materials will be distributed to all project personnel. An orientation and methods refresher sessions will be held prior to commencement of sampling. A QC Officer will ensure that samples are taken according to the established protocols and that all forms, check lists, and measurements are recorded and completed correctly during the sampling episode. Staff performance will be reviewed during the sampling and analysis phases to ensure adherence to project protocols.

QC samples for macroinvertebrate laboratory analyses will be collected at 10% of total sample locations (Section 4.1). Duplicate samples will be collected to verify the precision and repeatability of the results obtained by a single set of field investigators. An overall report will describe all QC activities and analyses, as explained in Sections 4 and 7 of this QAPP. Summary statistics will include:

- Precision (consistency) of field sampling using intra-team site duplication
  - relative percent difference (RPD)
  - root mean square error (RMSE)
  - coefficient of variability (CV)
  - 90% confidence interval (CI)
- Precision of laboratory sample sorting
  - percent sorting efficiency
  - percent taxonomic disagreement (PTD)
  - percent difference in enumeration (PDE)
- Accuracy of data entry
  - number of errors/corrective actions
- Completeness
  - number of valid data points obtained as a proportion of those planned

Wherever applicable, spreadsheets will be proofread using the original handwritten field and/or laboratory data sheets. This review will be done by someone other than the data entry specialist. A minimum of 10% of randomly selected metric values will be recalculated by hand to verify the computer-generated values.

The above QC statistics will be calculated as described in Section 4.2 Data Quality Objectives & Measurement Quality Objectives (DQOs/MQOs), with a general description below.

Precision

Precision is a measure of the nearness of two values and can be used as an indicator of method consistency. It is demonstrated by the degree of mutual agreement between individual measurements or enumerated values of the same property of a sample, usually under demonstrated similar conditions. Precision of sampling methods is estimated by taking duplicate samples at the same or immediately adjacent sampling site, typically at about 10% of the sites.

For this project, duplicate collection of water quality parameters (pH, DO, temperature, specific conductance, turbidity), benthic macroinvertebrate sampling, and habitat assessment will be performed at approximately 10% (randomly chosen) of the sampling sites by the same sampling team. To measure the precision of laboratory sorting (i.e., measurement error due to analytical error), QC personnel or a qualified coworker rechecks the sorted samples, missed specimens are removed and counted, and sorting efficiency is calculated.

Accuracy

Accuracy is defined as the degree of agreement between an observed value and an accepted reference or true value. Accuracy is a combination of random error (precision) and systematic error (bias), which are due to sampling and analytical operations. Bias is the systematic distortion of a measurement process that causes errors in one direction so that the expected sample measurement is always greater (or lesser to the same degree) than the sample's true value.

Since accuracy is the measurement of a parameter and comparison with a "truth," and the true values of environmental physicochemical characteristics cannot be known with confidence, use of a surrogate is required. Accuracy of field measurements will be assumed to be determined through use of precision. Field equipment for the measurement of dissolved oxygen (DO), pH, specific conductance, and turbidity will be calibrated for accuracy according to manufacturer's specifications. Instruments used and procedures for determining accuracy include the following:

> **DO sensors** (YSI): The accuracy of DO sensors and methods used in this project will be determined at 100 percent solubility. The solubility concentration is determined in water that has been saturated with air. The actual concentration of DO at 100 percent solubility is determined internally by the unit from measurements of temperature and barometric pressure.
>
> **pH sensors** (YSI): The accuracy of pH sensors used in this project will be checked using certified pH 4, pH 7, and pH 10 buffer solutions traceable to National Institute for Standards and Technology (NIST) Standard Reference Material.

Conductivity sensors (YSI): The accuracy of conductivity sensors used in this project will be checked using a standard solution of 1413 µS/cm and by making the appropriate corrections for nonstandard specific conductance measurement.

Turbidimeter (Hach 2100P): The accuracy of the nepholmetric turbidimeter used in this project will be checked using StableCal Standards ranging from <0.1 NTU, 20 NTU, 100 NTU, and 800 NTU, and periodically checked with Gelex Secondary standards.

Representativeness

Data representativeness is defined as the degree to which data accurately and precisely illustrate a characteristic of a population or community and, therefore, addresses the natural variability or the spatial and temporal heterogeneity of a site. The Anne Arundel County sampling program is designed to ensure representative sample collection of the habitat or population being sampled and adequate sample replication. In the relatively low gradient streams of the Maryland CP, benthic sampling focuses on the snags, rootwads, and vegetated banks, which are considered to be the most biologically productive habitat in such streams. However, if riffles are present in a reach, they will be considered the best available habitat.

Comparability

Comparability is a description of the confidence with which one data set can correspond to another. For this biological monitoring program, comparability of data is ensured by similarity in geographic, seasonal, and method characteristics and by the consistent training and experience of field sampling and laboratory personnel. All field teams have a leader, who has **at least** one year of sampling experience using the methods.

- Samples collected in Anne Arundel County will be compared only with reference conditions developed from Coastal Plain streams of the same type, that is, of a similar size or order.

- Benthic macroinvertebrate sampling will be conducted within one index period: spring (March 1 - April 30). The spring index period ensures seasonal comparability with Howard, Montgomery, and Prince George's Counties (MD), the Maryland Biological Stream Survey (MBSS), and the State of Delaware, Department of Natural Resources and Environmental Conservation (DNREC), which all sample during the same time period.

- In Anne Arundel County, all benthic macroinvertebrate samples will be taken with the 20 "jab" multihabitat method (DNR 2010, Barbour et al. 1999; Appendix A), one that has been shown to have good consistency that translates directly to comparability. This method is comparable with that used by the MBSS, Howard, and Montgomery Counties, and the Mid-Atlantic Highlands Assessment (EMAP/REMAP; U. S. Environmental Protection Agency).

- All field personnel conducting sampling and habitat assessment will have adequate training and appropriate experience (Section 5.0).

- Field audits will be performed by the PM and by the MBSS to ensure comparability of methods and methods application.

Completeness

Completeness is defined as the percentage of measurements made that are judged to be valid according to specific criteria and entered into the data management system. To achieve this objective, every effort is made to avoid sample and/or data loss through accidents or inadvertence. Accidents during sample transport or lab activities that cause the loss of the original samples will result in irreparable loss of data. Samples will be stored and transported in unbreakable (plastic) containers. All sample processing (subsampling, sorting, identification, and enumeration) will occur in a controlled environment within the subcontractor laboratory. The assignment of a set of continuous (serial) laboratory numbers to a batch of samples that have undergone chain of custody inspection makes it less likely for the technician or taxonomist to overlook samples when preparing them for processing and identification. The laboratory serial (or log) numbers also make it easy during the data compilation stage to recognize if some samples have not been analyzed. With a sampling program in part based upon a randomized site selection process, it is anticipated that some of the selected segments will not be able to be sampled because of, for example:

- Denial of access to stream over private land, or

- The randomly selected site is intermittent (however, the fact that the stream is dry is valuable information on that stream).

Sites that are not sampled based on such circumstances will be treated "unsampleable" and **will** be replaced with alternate sites to ensure that the required number of samples is obtained within each sampling unit.

12.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

Preventive maintenance of field and laboratory equipment is an ongoing task. Field personnel routinely inspect equipment for defects, wear and tear, and proper calibration. Dip nets and sieve buckets are inspected for holes or tears prior to each field event as well as throughout the sampling period. Back-up dip nets are kept on hand in the field equipment box. If small tears are found, they may be sewn; larger holes require replacing the net. The physicochemical measurement equipment will be cleaned and calibrated according to the manufacturer's instructions prior to the sampling event. Calibration will be performed daily during the sampling period. Laser levels will be checked for accuracy and/or calibrated on an annual basis. Critical spare parts (e.g., dipnet) and backup equipment will always available in the event equipment needs to be returned to the manufacturer for repair. One field person will be designated to gather and inspect all equipment in the equipment supply list (see Table 8) the week prior to the sampling event to ensure proper working order or to obtain replacement gear, if necessary.

13.0 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

The YSI will be calibrated with standard buffers for pH (4.00, 7.00 and 10.00) and conductivity (standard 1413 µS/cm). The DO calibration will be performed using percent air saturation. Field

calibrations will be conducted daily and recorded in the calibration log book; calibration must be within 90% of standards or replacement of sensors, repair, or substitution of the multiprobe will be required. The Hach Turbidimeter will be calibrated with StablCal primary standards (<0.1 NTU, 20 NTU, 100 NTU, and 800 NTU) prior to initiation of sampling. Accuracy of the readings will be checked once per week using the Gelex secondary standards. If the reading is not within 5% of the previously established value, the instrument will be recalibrated.

14.0 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Supplies and consumables are those items necessary to support the sampling and analytical operation, including but not limited to bottles, calibration solutions, decontamination supplies, preservatives, and various types of water (potable, deionized, organic-free, etc.). Upon delivery of supplies, the Contractor PM will ensure that types and quantities of supplies received are consistent with what was ordered, and with what is indicated on the packing list and invoice for the material. The supplier will be contacted immediately if any discrepancies are found. Field gear (dipnets, boots, buckets, sieves, etc.), sample containers, buffers, and standards will be inspected by the Biological Field Sampling Leader the week prior to a sampling event. Sample containers will be inspected for holes and tight fit of the caps, as well as the presence of wet or dry material indicating contamination. Other materials must also meet specific requirements as indicated by the appropriate manufacturer; for example, only certified standard solutions will be used for the multiprobe calibration. Buffers and standards will be checked for expiration dates and appearance (correct color). Any supplies or consumables not meeting basic requirements will be discarded and replaced by new materials.

15.0 NON-DIRECT MEASUREMENTS

Non-direct measurement data will include taxonomic bench sheets and electronic files, metric calculation database, regional tolerance values and functional feeding group designations. All data entered from taxonomic benchsheets will be confirmed by a staff member other than the data entry technician. Corrections will be entered as needed. Metric calculations will be confirmed by hand calculating data values from randomly-selected sites. Regional tolerance values will be reviewed by the PM. Data compiled from the sampling event will not be suitable for use prior to the above QC checks.

Comparison of data collected during this field effort with historical data will be used for qualitative assessment only. Chemical, biological , and physical/geomorphological data will be incorporated into quantitative assessment where appropriate, noting collection and analysis methods, and will be subject to the same standards as data collected under this QAPP.

16.0 DATA MANAGEMENT

Digital data will be backed-up regularly on external storage drives and periodically uploaded to an ftp site for processing. Upon completion of sampling, data files will be transferred to the project directory for QC checks and processing. Samples will be transported to the subcontractor laboratory and logged in using the COC Record (Appendix F). Samples will be stored appropriately until processing begins. Sample processing (sorting and subsampling) records will be recorded in the "sample processing" notebook in the subcontractor laboratory. Information includes project name

and number, sample identification and sampling date, sorting date and sorter initials, time required for processing the sample and notes on the general organic matter type contained in the sample. Subsampling and sorting efficiency sheets (Appendix J) will be completed to record the number of grids sorted in order to record the level of effort and quality control.

Taxonomic benchsheets provided by the laboratory subconsultant will be archived in the Contractor's project folder. These data will also be provided as an Excel file in the Anne Arundel County folder which will be stored in the central network file for Anne Arundel County. All files pertaining to the data, calculations, figures, and text for data reporting are stored in this central file location.

Data manipulation will be conducted primarily using Microsoft Excel, after all QC checks have been conducted and approved. All computer files associated with the project will be stored in a project subdirectory by the Contractor (subject to regular system backups) and will be copied to disk for archive for the 2 years subsequent to project completion (unless otherwise directed by the Anne Arundel County PM). Data will be maintained in an ESRI Personal Geodatabase, however, manipulations and statistical analyses will be performed in Excel after all QC checks are completed.

Cross section, pebble count, and longitudinal profile data will be recorded and managed using Excel spreadsheets developed by ODNR, which can be found at the following URL as of March 2010: http://www.dnr.state.oh.us/soilandwater/water/streammorphology/default/tabid/9188/Default.aspx

17.0 ASSESSMENT AND RESPONSE ACTIONS

The QA program under which this project will operate includes performance and system audits with independent checks of the data obtained from sampling, analysis, and data gathering activities. The Contractor will follow established QA protocols specified in this QA Project Plan. The QA programs followed by subcontractors and consultants will be reviewed by The Contractor, to ensure that similar levels of QA/QC are attained.

The essential steps in the QA program for any organization participating in this project are as follows:

- Identify and define the problem,
- Assign responsibility for investigating the problem,
- Investigate and determine the cause of the problem,
- Assign and accept responsibility for implementing appropriate corrective action,
- Establish effectiveness of and implement the corrective action, and
- Verify that the corrective action has eliminated the problem.

Many of the technical problems that might occur can be solved on the spot by the staff members involved; for example, by modifying the technical approach, repairing instrumentation that is not

working properly, or correcting errors or deficiencies in documentation. Immediate corrective actions form part of normal operating procedures and are noted in records for the project. For example, field audits are conducted close to the beginning of sampling to ensure proper technique and method application. If the auditor witnesses any problems, they are recorded and corrected on site. All corrective actions are recorded in the Field Audit Report. During laboratory sorting, sorting efficiency is checked for 10% of the samples by examining the sorted remains for any missed organisms. If the calculated sorting efficiency is <89%, remaining grids will be checked until the sorter consistently passes. Any missed organisms will be shown to the sorter so that they become aware of the type(s) of organisms they are missing. Sorting efficiency forms will be attached to the bench sheet for that site. If field and laboratory QC reviews result in repeated corrective actions, the Field Sampling Task Leader or Laboratory Task Leader must report personnel to the appropriate QC officer for retraining or reassignment of duties.

Problems not solved this way require more formalized, long-term corrective action. In the event quality problems that require attention are identified, the appropriate PM (Anne Arundel County or the Contractor) will determine whether attainment of acceptable quality requires either short- or long-term actions. If a failure in an analytical system occurs (e.g., performance requirements are not met), the appropriate QC Officer or QA Officer will be responsible for corrective action and will immediately inform either the PM or QA Officer, as appropriate. The Contractor PM is senior-level staff having primary responsibility for monitoring the activities of this project and identifying/confirming any quality problems. These problems will also be brought to the attention of the Contractor QA Officer, who will initiate the corrective action system described above, documenting the nature of the problem and ensuring that the recommended corrective action is carried out. The Contractor QA Officer is also senior-level staff and has been granted authority to stop work on the project if problems affecting data quality and requiring extensive effort to resolve are identified. The Contractor PM will be notified of major corrective actions and stop work orders.

Corrective actions may include the following:

- Reemphasizing to staff the project objectives, limitations in scope, the need to adhere to the agreed-upon schedule, and the need to document QC and QA activities.
- Securing additional commitment of staff time to devote to the project.
- Retaining outside consultants to review problems in specialized technical areas.
- Changing procedures.

The Contractor PM may exercise their authority to replace a staff member, subcontractor, or consultant, as appropriate, if it is in the best interest of the project. Performance audits are quantitative checks on different segments of project activities; they are most appropriate for sampling, analysis, and data processing activities. The Task QC Officers are responsible for overseeing work as it is performed and periodically conducting checks during the data entry and analysis phases of the project. The Contractor PM and QA Officer or designee will conduct one field audit of the field sampling team, reviewing sampling operations and conformance with SOPs and other guidance. An audit report will be prepared and submitted to the Anne Arundel County PM and QA Officer at the completion of this activity. As data entries, calculations, or other activities are checked, the person performing the check will sign and date a hard copy of the material or complete

a review form, as appropriate, and provide this to the Contractor PM for inclusion in the project files. Laboratory performance audits are beyond the scope of this QA Project Plan. The Directors and QA Officer of the subcontract laboratory are responsible for ensuring the quality of the data produced by the organization and conducting internal audits as appropriate.

System audits are qualitative reviews of project activity to check that the overall quality program is functioning and that the appropriate QC measures identified in the QA Project Plan are being implemented. A system audit will not be conducted during the Anne Arundel County project unless additional funds are received by the Contractor for this task.

18.0 REPORTS TO MANAGEMENT

The project's status will be reported to the County's PM quarterly. The results of the field audit, QC activities, data quality assessments, and performance evaluations will be incorporated into the final report. The report will be reviewed by the Contractor PM.

The Anne Arundel County PM will review the reports and discuss any concerns with the Contractor PM for immediate resolution, as discussed in Section 17.0.

The final project reports will provide data and narratives explaining the results of the sampling and analyses as described in Section 3.0.

19.0 DATA REVIEW, VALIDATION, AND VERIFICATION

Data review, validation, and verification provide methods for determining the usability and limitations of data, and provide a standardized data quality assessment. The Contractor will be responsible for reviewing field and laboratory data sheets, data entries, transmittals, and analyses for completeness and adherence to QC requirements. Data quality will be assessed by comparing entered data to original data or by comparing analytical results with the performance criteria summarized in Table 5 and Section 11.0 to determine whether to accept, reject, or qualify the data. Additional evaluations will be performed to verify and validate the data and metric calculations.

20.0 VALIDATION AND VERIFICATION METHODS

Verification confirms that specified requirements have been fulfilled. Field measurement data will be reviewed by a qualified person who did not participate in the collection of the data or the analysis of the samples. The data will be evaluated for (1) data representativeness, (2) data comparability, and (3) data completeness. In addition, the distribution of data for measurement parameters will be plotted and assessed for normality. Data points that exceed two standard deviations of the mean (95% of the observations) will be subject to strenuous review and rejected from the data set if determined to be due to measurement error or some other problem. All field data sheets, Meter Calibration Logs, and Chain-of-Custody Records will be reviewed by the Contractor PM (assisted by the QA Officer, as needed) for completeness and correctness. Biological data provided by the taxonomist will be reviewed for completeness and certainty (e.g., number of individuals, taxonomic certainty ratings).

The Contractor will be responsible for reviewing data entries and transmittals for completeness and correctness based on the original data sheet or manual recalculations. Data quality will be assessed by comparing entered data with original data or by comparing results with the measurement performance criteria.

Validation confirms that the particular requirements for a specific intended use are fulfilled. Statistical analyses will be reviewed and examined for errors or nonsensical results by the Data Processing QC Officer. Note that data qualifier flags will not be used in this process. A narrative discussion will be prepared describing the appropriate use of the data based on the findings of the evaluation and the level of confidence associated with the data. Data that do not meet the requirements of Table 5 will be identified and uncontrolled sampling error investigated.

Results of the verification and validation processes will be reported to the Anne Arundel County PM. The Contractor and County PMs will make the final determination to reject data and remove the unusable data from the ESRI Personal Geodatabase. If fewer than 100% of the data are judged valid (completeness requirement), statistical procedures and best professional judgment will be applied to verify whether the remaining data will make it possible to draw the correct conclusions for the project. Limitations in the data set will be communicated to the end user (Anne Arundel County) in the final report prepared for the project.

21.0 RECONCILIATION WITH USER REQUIREMENTS

Biological/habitat sampling and stream stability measures for this project are scheduled to begin on March 1 of each monitoring year. Following completion of fieldwork, all completeness measures will be calculated. If values indicate a need for additional sampling, samples will be collected within the index period time constraints. Taxonomic bench sheets and Chain-of-Custody Records will be reviewed by the Contractor PM. Any discrepancies in the records will be reconciled with the appropriate associated field personnel and will be reported to the Anne Arundel County PM.

The chemical, biological, and physical/geomorphological parameters measured and results of calculations performed for this project (i.e., metrics, B-IBI for each site) will be evaluated qualitatively and quantitatively to determine whether the data are of the type, quality, and quantity to support the decisions to be made (site or subwatershed status). Precision, accuracy, and completeness measures will be assessed by the Contractor and compared with the criteria discussed in Section 4.2. This will represent the final determination of whether the data collected are of the correct type, quantity, and quality to support their intended use for this project. Any problems encountered in meeting the performance criteria (or uncertainties and limitations in the use of the data) will be discussed with the Anne Arundel County PM and QA Officer and will be reconciled, if possible. Reconciliation might involve reanalyzing a benthic macroinvertebrate sample or reviewing the performance criteria to determine whether different criteria (for example, 90% completeness) are capable of meeting project objectives. Noncompliant data that cannot be reconciled will be rejected.

GLOSSARY

Accuracy: a measure of how close repeated trails are to the desired target.

Assemblage: the set of related organisms that represent a portion of a biological community (e.g. benthic macroinvertebrates).

Benthic: pertaining to the bottom (bed) of a waterbody.

Biological Assessment: an evaluation of the biological condition of a waterbody that uses biological surveys and other direct measurements of resident biota in surface waters.

Biological Integrity: the condition of the aquatic community inhabiting unimpaired water bodies of a specified habitat as measured by community structure and function.

Biological Indicators: plant or animal species of communities with a narrow range of ecological tolerance *that may* be selected for emphasis and monitored because their presence and relative abundance serve a s a barometer of ecological conditions within a management unit.

Biological Survey (biosurvey): the process of collecting, processing, and analyzing representative portions of a resident aquatic community to determine the community structure and function.

Community: the whole of the plant and animal population inhabititing a given area.

Community Structure: number and kinds of species in an aquatic community.

Ecoregion: geographic areas that are distinguished from others by ecological characteristics such as climate, soils, geology, and vegetation.

Habitat: a place where the physical and biological elements of ecosystems provide a suitable environment and the food, cover, and space resources needed for plant and animal livelihood.

Impairment: degradation.

Land Uses: activities that take place on land, such as construction, farming, or tree clearing.

Macroinvertebrate: organisms that lack a backbone and can be seen with the naked eye.

Multiple metric or multimetric approaches: analysis techniques using several measurable characteristics of the biological assemblage.

Pool: deeper portion of a stream where water flows slower than in neighboring, shallow portions.

Reference Condition: the chemical, physical, or biological quality condition exhibited at either a single site or an aggregation of sites that represent the least impacted *or* reasonably attainable condition at the least impacted reference sites.

Riffle: shallow area in a stream where water flows swiftly over gravel and rock.

Riparian: of or pertaining to the banks of a body of water.

Riparian Zone: the vegetative area on each bank of a body of water.

Run/Glide: section of a stream with a low velocity and with little or no turbulence on the surface of the water.

Spatial Heterogeneity: variation in a biological parameter due to different ecological conditions among sites.

Taxon (plural taxa): a level of classification within a scientific system that categorizes living organisms based on their physical characteristics.

Taxonomic key: a quick reference guide used to identify organisms. They are available in varying degrees of complexity and detail.

Temporal variability: variation in biological parameter due to fluctuations over time in ecological condition such as changing water chemistry or sunlight (e.g., diurnal and seasonal variations).

Tolerance: the ability to withstand a particular condition (e.g., pollution tolerant indicates the ability to live in polluted waters).

Tributaries: a body of water that drains into another, typically larger body of water.

Watershed: the area of land drained by a particular river or stream ecosystem. In this document it reflects a functional sampling unit, which may be either a discrete watershed, or a component of a larger watershed.

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Appendix A: Monitoring Program Design Document

Design of the Biological Monitoring and Assessment Program for Anne Arundel County, Maryland

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January 6, 2004

ACKNOWLEDGEMENTS

As part of the development of this document, a Technical Advisory Group was formed to provide input into the process. The authors would like to thank the members of this group for their invaluable assistance in the development of this program:

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A suggested citation for this document is provided below:

Hill, C. and J.B. Stribling. 2004. Design of the Biological Monitoring and Assessment Program for Anne Arundel County, Maryland. Prepared by Tetra Tech, Inc., Owings Mills, Maryland, for the Anne Arundel County Office of Environmental & Cultural Resources, Annapolis, Maryland.

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I. INTRODUCTION

Biological assessments are a highly effective approach to understanding the overall health and quality of streams. Changes in the resident biota (e.g., benthic macroinvertebrates, fish, herpetofauna, etc.) are ultimately caused by changes in their surrounding environment. By comparing the structure and function of biological assemblages in streams of interest to those of a known reference condition, we are able to detect a change from natural conditions (i.e., impairment). The greater the difference between conditions measured in a stream of interest and the reference condition, the greater the extent of impairment, and vice versa. Therefore, biological responses are very useful for indicating changes in overall stream ecosystem health. In other words, by observing shifts in biological assemblages from their natural conditions we can detect impairment in stream ecosystems.

As part of a comprehensive biological assessment program, physical habitat quality is assessed to supplement biological data. Alterations in stream and watershed hydrology can potentially lead to accelerated stream channel erosion, which, in turn, leads to habitat degradation and reduces the capacity of the stream to support a "healthy" biota. While not directly identifying specific cause-effect relationships, combining the results of both biological and physical habitat assessments can provide insight into the types of stressors and stressor sources impacting watersheds of interest, allowing for prioritized implementation of more detailed, diagnostic investigations based on the severity of observed biological responses.

The purpose of this program document is to:

- outline the overall management and technical questions Anne Arundel County is interested in addressing through biological monitoring;
- briefly describe the sampling methods and quality control activities for the program;
- document the technical framework for the design of the sampling network and provide the actual sampling sites for the program.

The primary goals of this biological monitoring program are to assess the current status of the biological stream resources of Anne Arundel County and to establish a baseline for comparing future assessments; to assess the status and trends of the biological stream resources, and to relate them to specific programmatic activities (Table 1).

Table 1. Goals of the Anne Arundel County monitoring and assessment program

1.	Document the ecological status of Anne Arundel County watersheds
2.	Contribute to understanding dominant stressors and stressor sources affecting stream and watershed ecology

3.	Track ecological health trends in the County's watersheds over time
4.	Have monitoring data be an integral part of resource management in the County.

Based upon these goals, the initial program will enable the County to address questions at three different geographic scales: stream-specific, watershed wide; and, after the five-year sampling rotation is complete, countywide. Some of the specific questions the program will be able to address with the monitoring data and results are:

Status:

Countywide: What percentage of streams in the County are impaired based on evaluation of the structure and function of the benthic macroinvertebrate assemblage?

Watershed wide: What is the overall ecological condition of an individual watershed? What is the status of streams in an individual watershed? Where are the most stressed streams (prioritized in order of severity)?

Stream specific: What is the ecological condition of individual streams located downstream from known or unidentified disturbances?

Trends:

Countywide: How has the percentage of impaired streams in the County changed from the end of year five to the end of year ten (two sampling rotations)?

Watershed wide: How has the percentage of impaired streams in an individual watershed changed from one monitoring year to another, for example, from monitoring year one to monitoring year six?

Stream specific: How have individual sites changed in condition over time? Are previously degraded streams showing improvement?

Problem identification/prioritization:

All streams: What are the locations of the impaired streams that were assessed? Of the streams and watersheds assessed, what are the locations of those that are most degraded?

Cause-and-effect relationships:

Metrics, bioassessment scores: What is the predictable response of individual metrics or biological attributes from exposure to specific human-induced stressors?

Evaluation of environmental management activities:

Countywide: Have the environmental protection policies of Anne Arundel County been adequate to maintain a healthy condition in the County's streams?

Watershed wide: Have the environmental protection policies of Anne Arundel County been adequate to maintain a healthy condition in the County's watersheds?

Stream specific: Did the restoration of a specific stream lead to an improvement in biological condition? Did the implementation of restoration & protection BMPs lead to improvement in a particular stream or watershed?

This report describes the sampling design and biological field sampling methods developed for the Anne Arundel County monitoring program, which will ultimately enable them to answer these questions.

II. BACKGROUND

In 1972, the United States Environmental Protection Agency first administered the Clean Water Act to protect and restore the chemical, physical, and biological integrity of the Nation's water resources. Regulating point and nonpoint source pollution, the Act is the foundation by which federal and state agencies manage water resources. To implement the regulations for the control of nonpoint source runoff under this act, the Maryland Department of the Environment requires jurisdictions of certain sizes to obtain a National Pollution Discharge Elimination System (NPDES) permit regulating the nonpoint source discharges from municipal separate storm sewer systems. One aspect of permit compliance requires the Anne Arundel County to prioritize watersheds for restoration activities and to demonstrate the success of these activities, which means that the County must determine which areas are most in need of restoration.

The County has initiated a variety of watershed studies and developed some regular water quality assessment programs to better understand watershed conditions. For example, some programs (e.g., Town Center Monitoring Program, Church Creek water quality monitoring) have been implemented to monitor the chemical and physical conditions in selected County streams. In addition, watershed studies have been completed or are underway in the South and Severn River watersheds. Finally, the Maryland Department of Natural Resources'(DNR) Maryland Biological Stream Survey (MBSS) sampled 85 sites in the County between 1995-2002 and 35 sites were sampled in 2002 for DNR's Upper Patuxent Watershed Restoration Action Strategy (WRAS) initiative.

Despite these efforts, the County currently lacks the information necessary to adequately characterize the biological condition of its major watersheds countywide and to satisfy the needs and goals of the County that are described above. In addition, because the MBSS is a statewide monitoring program, the density of sampling points is not sufficient to answer questions about stream-specific conditions within major watershed units of the County.

Because of the limitations of current programs to characterize watershed health, the County has recognized the need to develop its own comprehensive stream monitoring program consisting of physical, chemical, and biological data collection techniques to document and track changes in the condition of the stream resources. Since aquatic biota responds to a broad array of stressors in their environment, both physical and chemical stressors can be detected by changes in the biology. For that reason, the inclusion of a biological monitoring program will provide the County with the relevant data to assess the condition of its streams and watersheds and to better manage its water resources.

Both the field sampling and data analysis methods are intended to be directly comparable to the DNR's MBSS, and complementary to those currently in place in Prince George's, Montgomery, and Howard Counties. Additional consideration in development of the program methods and sampling site locations include the County's watershed management tool (WMT) and the data requirements associated with the Watershed Improvements through Statistical Evaluations (WISE) model currently in development for the Severn River watershed.

III. METHODS

Benthic Macroinvertebrate Sampling and Processing

In Coastal Plain ("low" gradient) streams, benthic macroinvertebrates are collected using a D-frame net (U.S. Standard No. 30 595-600-μm mesh) following the 20 "jab" multiple habitat method (Kazyak 2001, Barbour et. al 1999). Samples are collected from 75-meter reaches by making 20 one-foot linear sweeps (jabs) through different habitat types (e.g., cobbles, undercut banks, snags). Sampling will focus on allocating "jabs" to the most stable and productive habitats (e.g., riffles, snags, undercut banks) and to the least productive habitats (e.g. mud, sand) only when productive habitats are either absent or not large enough to allocate 20 ft^2. All sampled material is composited in a 600-micron sieve bucket, placed in one or more 1-liter sample containers and preserved in 95% ethanol. Internal and external sample labels are completed for each container. Using a Caton gridded screen in the lab, the composited samples are randomly subsampled to 100-organisms and identified to genus level.

Physical Habitat

Physical habitat quality is evaluated using a visual-based assessment method for low-gradient streams developed for use with Rapid Bioassessment Protocols (RBPs) (Barbour and Stribling 1991, 1994; Barbour et al. 1999). A total of 10 parameters describing physical habitat, instream and planform morphology, riparian zones, and stream banks are visually assessed and ranked as optimal, sub-optimal, marginal, or poor. Each parameter is scored on a 20-point scale, where a score of 20 is optimal (best) and 0 is poor (worst), and then summed for a total habitat score.

Water Chemistry

Water chemistry is measured *in situ* using a YSI 600 QS multi-parameter water quality monitor and 650 MDS display and data-logging system. The unit measures dissolved

oxygen, conductivity, temperature, and pH, which are collected to document physico-chemical characteristics of the stream.

Index Period

The Spring Index Period (March 1-April 15) was chosen for Anne Arundel County benthic macroinvertebrate sampling, which corresponds with MBSS, Prince George's, Montgomery, and Howard Counties.

Quality Assurance/Quality Control

Quality Assurance/Quality Control (QA/QC) activities are designed to ensure data quality and document data characteristics. The Quality Assurance Program plan (QAPP) describes, in detail, the procedures that are used for data collection, the technical rationale behind the procedures, and the series of activities and reporting procedures that will be used to document and communicate data quality.

Standard Operating Procedures (SOPs)

The SOPs and procedures for field sampling, laboratory processing, and completing chain-of-custody forms are documented in the Anne Arundel County QAPP. Chain-of-custody and sample log sheets are maintained to track the inventory and processing status of all samples. Sample documentation forms are kept in three-ring binders in Tetra Tech's Biological Research Facility (BRF).

Field Training

Prior to each sampling year, a field orientation session is held as a "refresher" for experienced samplers and as an introduction for new samplers. All two-person field teams are divided into Team Leader and Crew Member. Team Leaders are required to have completed at least one field season as a Crew Member. Crew Members will have completed either the introductory or "refresher" field orientation. At least one person from each field crew will have also attended an MBSS training session conducted by DNR staff.

Field Audits

The field crew will be visited on-site and observed by an experienced field ecologist who is not involved in the project's fieldwork. Field team procedures are observed for adherence to SOPs and consistency in completion of all data collection requirements including, field data sheets, sample preservation, and photo documentation.

Duplicate Sampling

Duplicate biological and physical habitat samples are taken at 10% of the total sites sampled in each subwatershed to estimate sampling precision. Comparisons of the differences between the results from these sites provide estimates of the precision of the biological assessments and the consistency of sampling activity. Relative percent difference (RPD) provides an estimate of the difference between sample pairs. Duplicate samples will be taken from adjacent 75 meter reaches where no additional stressor sources are observed, and physical habitat appears similar to the original reach.

Laboratory Subsampling and Sorting

Individuals in the Tetra Tech BRF perform all sorting and subsampling of samples. The quality assurance officer checks each individual's samples to ensure that there are no missed specimens in removed grid debris until each individual passes (i.e., removes ≥90% of specimens) ten samples consecutively. Once a 90% sorting efficiency is attained, random checks are performed on 10% of samples sorted by each individual.

Taxonomic Verification

There are two principal sources of error that can cause uncertainty in some taxonomic identifications. One is that the specimens in question are of very early instars (juvenile) and lack morphological structures necessary for positive identification. Another is that any specimen can have damaged or missing morphological features (gills, antennae, legs, caudal filaments) rendering final, positive identification problematic. In addition, for midges, inadequate mounting medium can make genus level identification nearly impossible. Taxonomic data quality will be documented as Percent Taxonomic Disagreement (PTD), which quantifies the error rate identification through comparison of results from re-identified samples.

Reference Collection and Voucher Samples

A taxonomic reference collection for benthic macroinvertebrates collected in the Anne Arundel County will be established. One or more specimens removed from samples are kept as representative of the taxonomist's concept of that taxon. As sampling progresses from year to year, the reference collection will be updated with any new example specimens. Voucher samples (stored in ~ 75% ethanol) will be kept from all sampling in the County for at least three years in the Tetra Tech BRF.

Data Entry and Management System

All biological, physical habitat, chemical, and ancillary data are entered directly from field data sheets or Excel spreadsheets into Ecological Data Applications System (EDAS). The data and analytical results from future index periods will be managed in this system. One hundred percent of the data set, once entered, is checked by hand against the original, hand-written field sheets. If discrepancies are encountered, they will be corrected in EDAS.

Documentation of Performance Characteristics

The documentation of performance characteristics for all methods is known as the performance-based method system (PBMS – see ITFM 1995), which is essentially a system that permits the use of any method of sampling and analysis that meets established requirements for DQOs (Diamond et al. 1996, NWQMC 2001). The basic elements of a PBMS approach include method precision (repeatability of measurements), bias (skewness of measurements), sensitivity (detection limit), and accuracy (proximity to the analytical truth). Calculating the performance characteristics is essential to understanding the robustness of the method for reliably determining the condition of the aquatic ecosystem.

Stressor Identification
Stressors are identified using methods similar to those described in Suter et al. (2002), Norton et al. (2002), and Cormier et al. (2002), as well as in the U.S. Environmental Protection Agency's Stressor Identification Guidance Document (USEPA 2000). The following series of logical steps, adapted from Suter et al. (2002), are used to deduce the most likely causes of biological impairment in streams: 1) Identify impairment, 2) List of candidate causes, 3) Analysis of evidence, and 4) Characterize causes. A potential future use of these assessments will be in diagnostic analyses to more specifically define stressor associations.

IV. MONITORING NETWORK DESIGN

POWER ANALYSIS

According the County's management goals, analyses should be able to detect a 30% change (sensitivity), 80% of the time (power), with a 95% confidence level (confidence). The target population consists of 1^{st} – 3^{rd} order streams (excluding tidal-influenced reaches) of Anne Arundel County, as defined by 1:100,000 map scale. To estimate typical statistics (i.e., mean and standard deviation) that will be expected in the proposed sampling program, summary statistics were computed from data available from MBSS, which included 955 sites collected between 1995-1997. The following rationale was used to screen data from the MBSS to develop a meaningful data set for the analysis:

1. Only coastal MBSS data were used since all Anne Arundel County streams are located on the Coastal Plain.
2. For reference conditions, only those samples where GB CLASS was equal to G (good, reference) were used (criteria for reference sites were taken from Roth et al. 1997 and Stribling et al. 1998, Table 2).
3. For test conditions, only those samples where GB CLASS was equal to N (not bad, sub-reference) were used.
4. Only samples in counties that are entirely on the Coastal Plain were considered (i.e., samples with sample IDs that started with AA (Anne Arundel), CA (Calvert), CH (Charles), CN (Caroline), DO (Dorchester), KE (Kent), PG (Prince George's), QA (Queen Anne's), SO (Somerset), SM (St. Mary's), TA (Talbot), WI (Wicomico), and WO (Worcester)).

Table 2. Criteria used for designating GB CLASS – G (Roth et al. 1997, Stribling et al. 1998).

Good/Reference Criteria (all must be met)
pH ≥ 6
ANC ≥ 50μeq/l
Dissolved O_2 ≥ 4 ppm
Nitrate-N ≤ 4.2 mg/l
Urban land use ≤ 20% of catchment area
Forested land use ≥ 25% of catchment area

Remoteness rating optimal or suboptimal
Aesthetics rating optimal or suboptimal
Instream habitat rating optimal or suboptimal
Riparian buffer width ≥ 15 m
No channelization
No point source discharges

This process resulted in 37 samples to characterize reference conditions and 81 samples to characterize test conditions. The Index of Biotic Integrity (IBI) mean and standard deviation for samples used to characterize reference conditions is 3.25 and 0.88, respectively. The IBI standard deviation for samples used to characterize test conditions is 0.91.

Using data from MBSS sites in the Coastal Plain region of Maryland, a power analysis (one-sided t-test) was conducted following methods described in Steel and Torrie (1980). Figure 1 presents a summary of sample size calculations using the above "best estimate" for reference and test site statistics. It also provides a simplification of assuming the reference and test sample have the same standard deviation of 0.91. From the results of the power analysis, it was determined that approximately 13 sites in each sampling section would be more than sufficient to meet the management goals. There are several caveats that should be mentioned concerning this conservative estimate of sample size. First, the data used for this calculation was from a relatively large geographical distribution, and the variability within this population may be larger that what would be found within Anne Arundel County alone. We originally attempted to use the Coastal Plain data only from counties on the western shore of the Chesapeake Bay (i.e., Anne Arundel, Calvert, Charles, Prince George's, and St. Mary's), however, with the relatively small amount of data available we actually observed a greater level of variance. Therefore, we decided to use a larger data set to minimize the influence of outliers on our population estimates. Secondly, is not known what degree of this variability can be attributed to the sampling personnel. Because the data we used were collected during the first few years of MBSS's sampling program, it is possible that there could be a good deal of variability attributed to differences between sampling personnel. Without a sufficient data set for Anne Arundel County, it is not possible to precisely quantify the amount sampling variability we would expect to encounter within the County. Based on the amount of variability observed in other neighboring counties, we believe that 10 sites per sampling unit should be sufficient meet the data quality objectives (DQO's). However, if it is determined after the first year of sampling that 10 sites is too many or too few to achieve the DQO's, the number of sites per unit can then be adjusted for future sampling efforts.

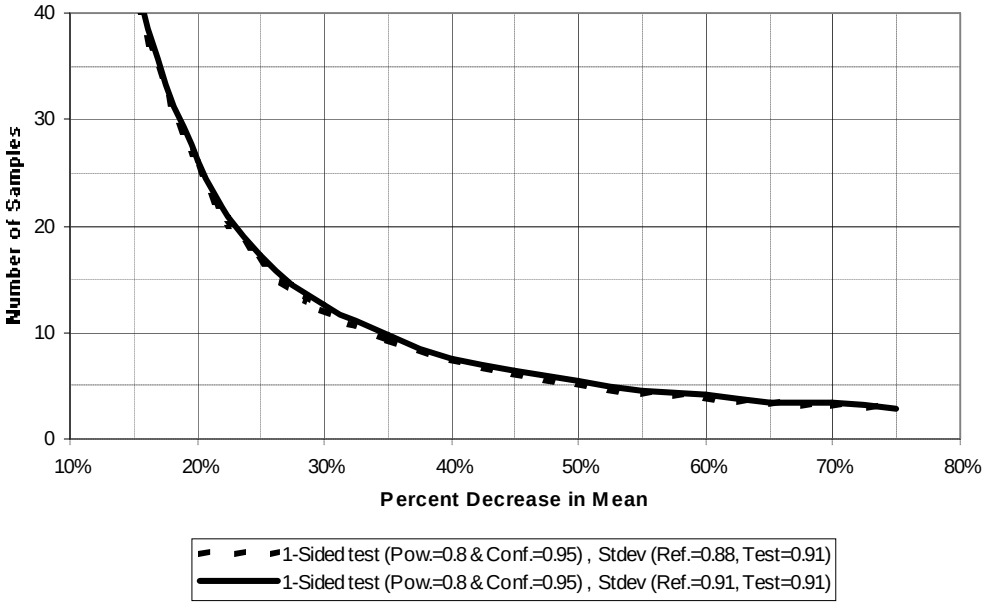

Figure 1. Sample size calculations for reference and test statistics.

V. WATERSHED DIVISIONS AND SITE SELECTION

Maps

The following map breaks out the County's 24 subwatershed primary sampling units (PSUs) and the larger Maryland 8- digit watersheds using the hydrologic unit code (HUC) boundaries defined by MDE (Figure 2). The County decided that the 8-digit watershed coverage was too broad to allow them to answer questions on a smaller scale. DNR's HUA 14-digit watershed coverage was examined to determine a suitable subwatershed coverage for the County. It was determined that many of the 14-digit watersheds in this coverage were not complete subwatersheds. Therefore, several of the 14-digit watersheds were often combined to make up a single functional subwatershed PSU. In the end, we were able to subdivide the County into 24 functional PSUs, which generally conform or fit within the boundaries of the larger 8-digit watersheds, while some of the smaller 8-digit watersheds are retained as a functional subwatershed PSU.

Scale is an important factor to consider when determining the smallest stream order that should be included in the County's biological monitoring program. MBSS currently uses 1:100,000 scale maps to detect and delineate streams for its sampling program. The 1:100,000 scale is the same map scale used to develop USEPA's reach file version number 3 (RF3) data. One of the main goals of Anne Arundel County is to ensure that biological monitoring data collected by Anne Arundel County can be compared, and to some extent merged, with the MBSS data. To avoid potential compatibility problems that could result from combining data collected at different scales (e.g., sampling

ephemeral vs. perennial streams), we recommend that the map scale used by the MBSS program, the 1:100,000 scale, also be used for the Anne Arundel County program.

Site Selection (Random and Targeted)

All of Anne Arundel County will be sampled in a 5-year period, using a rotating basin design. In a rotating basin design, a subset of watersheds is assessed each year, which rotate annually until all of the watersheds in the county are sampled. The county was divided according to watersheds into 24 total PSUs (Figure 2), and approximately 5 will be sampled each year of the program (Table 3).

Within each PSU, there are a total of 10 primary sampling locations (= 75 m sampling segments), and 10 alternate sampling locations. Spatial allocation of the sampling segments is based on random selection within Strahler (1957) stream orders (Figure 3). The number of sampling locations within each of the first, second, and third order channel distances (m) is proportional to their total lengths (See Appendix A). Thus, selection and placement of sampling segments are simple random within each ordinal category. In addition to the randomly selected sites, five least impacted sentinel streams were chosen in and around Anne Arundel County to be sampled every year to measure temporal variability among sampling years (Table 4). Finally, the County may also choose to select a number of targeted sites to be added within each PSU. These extra sites will be added as needed to ensure that areas requiring assessment are characterized. At this time, no targeted sites have been incorporated into the design plan.

To address issues of measurement error (i.e., systematic error), duplicate samples will be collected at 10% of the total number of sites each year. Before sampling begins, the sites where duplicate sampling is to occur will be randomly selected. Where it is not possible to locate an adjacent upstream 75 m sampling segment (due to beaver dams, piping or other physical or hydrologic alteration discovered while in the field), an alternate site for repeat sampling will be selected from an alternate site list. Similarly, an alternate will be selected for a primary site in the event the sampling team cannot obtain site access

Sampling error (i.e., random error) will be addressed using multiple sites that are randomly selected and happen to fall in close proximity (< 1000 m) to other sampling locations.

Table 3. Anne Arundel sampling schedule by subwatershed. The subwatershed groupings can be changed depending on the budget for each year.

Year	Primary Sampling Unit
1 (2004)	9 Severn Run (10 sites)
	10 Severn River (10 sites)
	3 Lower Patapsco (10 sites)
	18 Middle Patuxent (10 sites)
	21 Ferry Branch (10 sites)
2 (2005)	15 Herring Bay (10 sites)
	12 Lower North River (10 sites)
	19 Stocketts Run (10 sites)
	11 Upper North River (10 sites)
	22 Lyons Creek (10 sites)
3 (2006)	7 Upper Magothy River (10 sites)
	24 Hall Creek (10 sites)
	6 Bodkin Creek (10 sites)
	5 Marley Creek (10 sites)
4 (2007)	16 Upper Patuxent (10 sites)
	1 Piney Run (10 sites)
	2 Stony Run (10 sites)
	23 Cabin Branch (10 sites)
	17 Little Patuxent (10 sites)
5 (2008)	14 West River (10 sites)
	8 Lower Magothy River (10 sites)
	20 Rock Branch (10 sites)
	4 Sawmill Creek (10 sites)
	13 Rhode River (10 sites)

Table 4. Least impacted streams where sentinel sites will be located.

County	Stream	Stream order
Anne Arundel	Dorsey Run	2nd
Anne Arundel	Tarnans Branch	1st
Calvert	Battle Creek	2nd
Calvert	Fishing Creek	3rd
Charles	Piney Branch	2nd

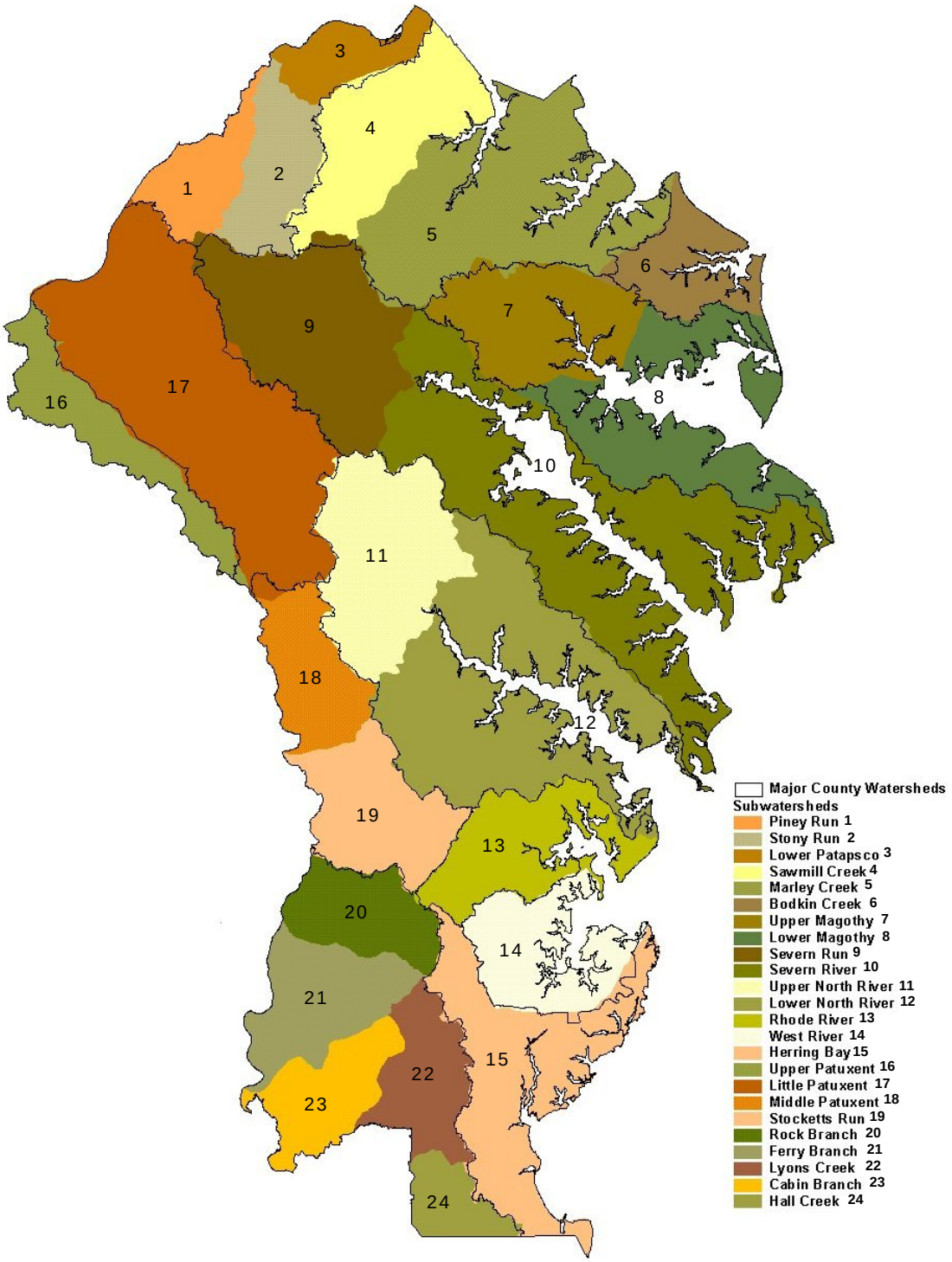

Figure 2. Map of the 24 subwatershed primary sampling units.

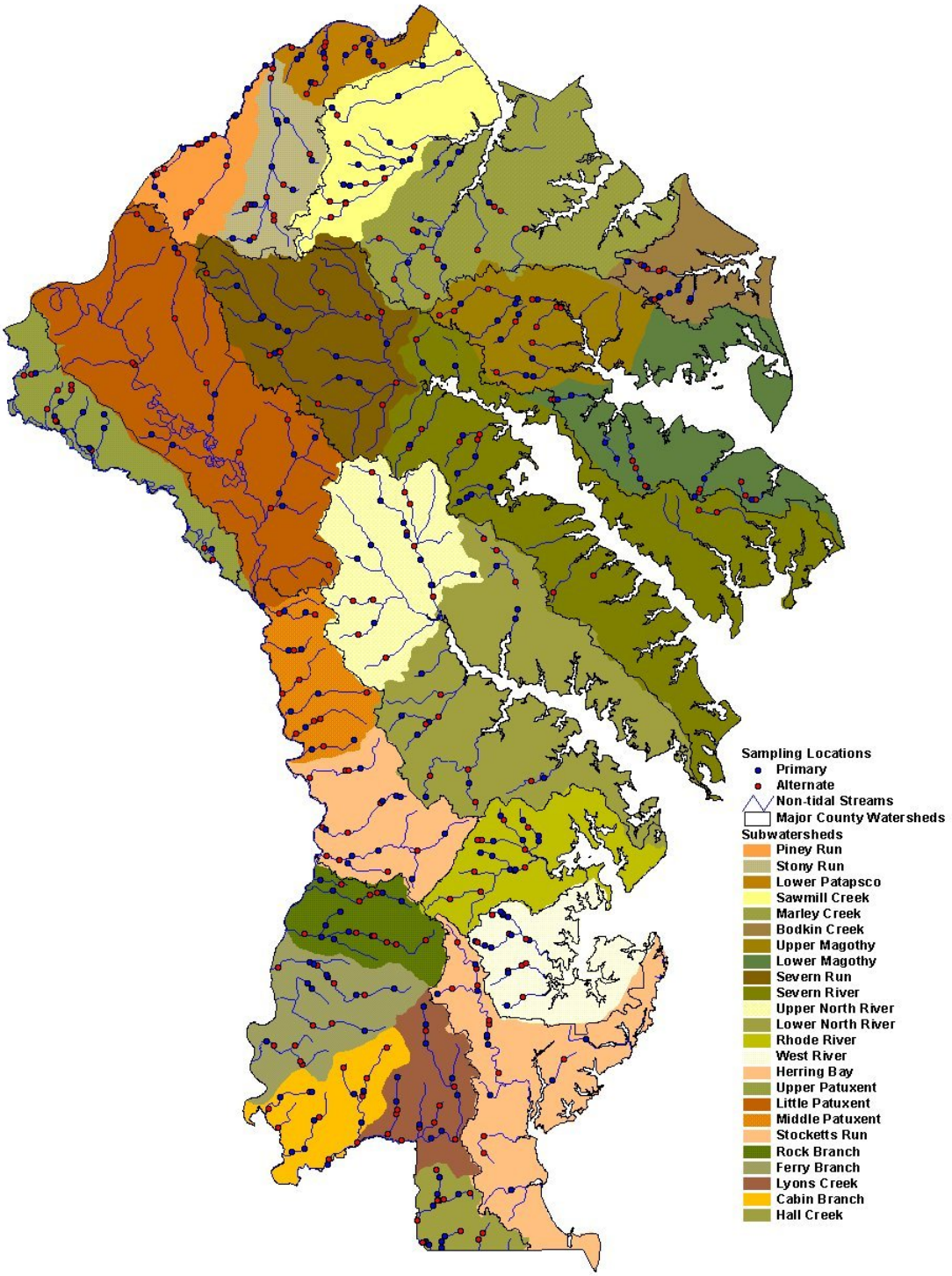

Figure 3. Map of randomly selected sampling locations in Anne Arundel County.

VI. FUTURE DIRECTIONS

The following are future initiatives that the County will consider undertaking in this program as it matures, dependant upon available funding and/or staffing levels:

Tidal Streams

The program as currently designed is not suitable for assessing tidal waters. The County will pursue the development of a tidal stream assessment program as funds permit. The Department of Natural Resources has offered to assist the County in finding grant funds to implement tidal stream biological assessments.

Countywide Water Quality Monitoring Program

While some information exists for a small number of water quality parameters and how they impact stream biological communities, additional work is necessary to better understand these relationships. By collecting biological and water chemistry data at selected sites, particularly at sites where upstream land uses are not well characterized with regard to pollutant loading, it will be possible to gain more understanding into pollutants largely behind degradation of stream communities. A future design document will be developed for this initiative if it is pursued, which will be considered as funds permit.

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APPENDIX A. RANDOM SITE SELECTION METHODOLOGY

Determine number of sites that correspond with stream order percentages in subwatersheds

1. Intersect stream lines with Anne Arundel county shapefile (GIS).
2. Determine percent of each stream order in the Anne Arundel subwatersheds (using stream orders already in shapefile) (Export GIS summary of stream length to Excel).
3. Assign 10 primary sites to subwatersheds in amounts corresponding to percentages.
    a. If > 0.5%, then count as a site.
    b. Distribute sites in percentage allocation to match stream order allocation.
4. Add 10 alternate sites by doubling the allocation values of primary sites.

Sampling point creation

1. Divide streams into 1m points (GIS script).
2. Select points inside Anne Arundel County (GIS).
3. Attach attributes for streams and subwatersheds to points (GIS).
4. Assign Lat/Lon in decimal degrees (GIS).

Random Selection

1. Assign random number from 1 to 1,000,000 to all points in Anne Arundel county (GIS).
2. Sort ascending random numbers to choose sites for each subwatershed (transfer 50,000 ascending random numbers to Excel spreadsheet).
    a. If 10 first order sites for subwatershed A, then take first 10 ascending random number sites with first order attributes.
    b. If three second order sites for subwatershed A, then take first 3 ascending random number points with second order attribute etc.
3. Join selected primary and alternate point identifiers to shapefile and export subset of points.

APPENDIX B. SITE LOCATION INFORMATION

Table A-1. Sampling location coordinates

PRIMARY SAMPLING UNIT	PRIORITY	STREAM ORDER	ID	LATITUDE	LONGITUDE
1 Piney Run	Primary	1	01-01	39.1495797308	-76.7428325353
	Primary	1	01-02	39.1668433129	-76.7598469843
	Primary	1	01-03	39.1585400198	-76.7558073291
	Primary	1	01-04	39.1747017756	-76.7206887573
	Primary	1	01-05	39.1620570367	-76.7593783012
	Primary	2	01-06	39.1781065293	-76.7442528883
	Primary	3	01-07	39.1818864529	-76.7322795741
	Primary	3	01-08	39.2121300010	-76.7004621149
	Primary	3	01-09	39.2025916792	-76.7097961203
	Primary	3	01-10	39.1916800158	-76.7162701868
	Alternate	1	01-11A	39.1514746031	-76.7407166836
	Alternate	1	01-12A	39.1708584181	-76.7211768635
	Alternate	1	01-13A	39.1509046218	-76.7425788846
	Alternate	1	01-14A	39.1673597998	-76.7585022452
	Alternate	1	01-15A	39.1560908893	-76.7351200211
	Alternate	2	01-16A	39.1698367831	-76.7545440416
	Alternate	3	01-17A	39.1801726151	-76.7347508438
	Alternate	3	01-18A	39.1837606523	-76.7282471922
	Alternate	3	01-19A	39.1915368353	-76.7167780502
	Alternate	3	01-20A	39.1797418408	-76.7363668552
2 Stony Run	Primary	1	02-01	39.1546596104	-76.7063655442
	Primary	1	02-02	39.1729998535	-76.6758482289
	Primary	1	02-03	39.1406117461	-76.7180429657
	Primary	1	02-04	39.1400253207	-76.7094235443
	Primary	1	02-05	39.1547858715	-76.7077253873
	Primary	1	02-06	39.1605819146	-76.6860358055
	Primary	1	02-07	39.1894025871	-76.6890497968
	Primary	2	02-08	39.1701909630	-76.6973236109
	Primary	2	02-09	39.1880969988	-76.6938809283
	Primary	2	02-10	39.1893753504	-76.6944484081
	Alternate	1	02-11A	39.1546362356	-76.7096255228
	Alternate	1	02-12A	39.1630371507	-76.6911960054
	Alternate	1	02-13A	39.1502745504	-76.6963030196
	Alternate	1	02-14A	39.1529985160	-76.7115249781
	Alternate	1	02-15A	39.1754822226	-76.6771168204
	Alternate	1	02-16A	39.1481018648	-76.6963300580
	Alternate	1	02-17A	39.1409516839	-76.7070968931
	Alternate	2	02-18A	39.2071126546	-76.6975117605
	Alternate	2	02-19A	39.1575309525	-76.6999571629
	Alternate	2	02-20A	39.2109352588	-76.6962862273
3 Lower Patapsco	Primary	1	03-01	39.2207333918	-76.6446681257

3 Lower Patapsco	Primary	1	03-02	39.2051168596	-76.6760331412
	Primary	1	03-03	39.2164288454	-76.6578482928
	Primary	1	03-04	39.2112924325	-76.6682545242
	Primary	1	03-05	39.2165729685	-76.6814327761
	Primary	1	03-06	39.2176412172	-76.6698129303
	Primary	1	03-07	39.2149266364	-76.6836245037
	Primary	1	03-08	39.2232373789	-76.6482387269
	Primary	1	03-09	39.2177462353	-76.6452587562
	Primary	1	03-10	39.2138619749	-76.6428317758
	Alternate	1	03-11A	39.2197220507	-76.6526113931
	Alternate	1	03-12A	39.2165427422	-76.6451663683
	Alternate	1	03-13A	39.2202667139	-76.6687063321
	Alternate	1	03-14A	39.2151841618	-76.6688650381
	Alternate	1	03-15A	39.2196904636	-76.6866115173
	Alternate	1	03-16A	39.2049108954	-76.6740792648
	Alternate	1	03-17A	39.2217210452	-76.6685329534
	Alternate	1	03-18A	39.2006015688	-76.6786167843
	Alternate	1	03-19A	39.2050657215	-76.6762430100
	Alternate	1	03-20A	39.2122128278	-76.6380038607
4 Sawmill Creek	Primary	1	04-01	39.1623709017	-76.6558064598
	Primary	1	04-02	39.1712418987	-76.6515006977
	Primary	1	04-03	39.2119538127	-76.6166416068
	Primary	1	04-04	39.1698468445	-76.6382901940
	Primary	1	04-05	39.1627830389	-76.6592768600
	Primary	1	04-06	39.1996079279	-76.6299587192
	Primary	1	04-07	39.1945776440	-76.6652264996
	Primary	1	04-08	39.1802806451	-76.6345359831
	Primary	2	04-09	39.1723118174	-76.6277216288
	Primary	3	04-10	39.1732452264	-76.6239195947
	Alternate	1	04-11A	39.1799675764	-76.6570257319
	Alternate	1	04-12A	39.1550434014	-76.6581212839
	Alternate	1	04-13A	39.1633800906	-76.6474739396
	Alternate	1	04-14A	39.1558145361	-76.6662253437
	Alternate	1	04-15A	39.1553963322	-76.6581823499
	Alternate	1	04-16A	39.1916488148	-76.6622319943
	Alternate	1	04-17A	39.1495235332	-76.6658265313
	Alternate	1	04-18A	39.2171518480	-76.5975919417
	Alternate	2	04-19A	39.1654003481	-76.6369502880
	Alternate	3	04-20A	39.1784355016	-76.6212864317
5 Marley Creek	Primary	1	05-01	39.1432230842	-76.6199072545
	Primary	1	05-02	39.1711817969	-76.6021879574
	Primary	1	05-03	39.1760861477	-76.5981223657
	Primary	1	05-04	39.1287707012	-76.6061820939
	Primary	1	05-05	39.1483861889	-76.6181860564
	Primary	1	05-06	39.1550111616	-76.5823869394
	Primary	1	05-07	39.1719133579	-76.6096171124
	Primary	1	05-08	39.1441018955	-76.5633505772

5 Marley Creek	Primary	1	05-09	39.1302522585	-76.6253256370
	Primary	2	05-10	39.1420743915	-76.6086745506
	Alternate	1	05-11A	39.1533748590	-76.5795336371
	Alternate	1	05-12A	39.1406735164	-76.6403213942
	Alternate	1	05-13A	39.1234720767	-76.6321993253
	Alternate	1	05-14A	39.1356338454	-76.5887291324
	Alternate	1	05-15A	39.1441600443	-76.5625589875
	Alternate	1	05-16A	39.1517058518	-76.5760307999
	Alternate	1	05-17A	39.1316844584	-76.6094712055
	Alternate	1	05-18A	39.1441219276	-76.6220321629
	Alternate	1	05-19A	39.1165435039	-76.6149749900
	Alternate	2	05-20A	39.1371126765	-76.6172385223
6 Bodkin Creek	Primary	1	06-01	39.1287408334	-76.5007214345
	Primary	1	06-02	39.1184263027	-76.4822911556
	Primary	1	06-03	39.1284879549	-76.4999442695
	Primary	1	06-04	39.1157807187	-76.4915994627
	Primary	1	06-05	39.1200322718	-76.4813970742
	Primary	1	06-06	39.1147328914	-76.4963566572
	Primary	1	06-07	39.1302183505	-76.5090960570
	Primary	1	06-08	39.1148269500	-76.4753752451
	Primary	1	06-09	39.1265805068	-76.4917463232
	Primary	1	06-10	39.1172425494	-76.4863389486
	Alternate	1	06-11A	39.1271116369	-76.4981216541
	Alternate	1	06-12A	39.1138175924	-76.4758375004
	Alternate	1	06-13A	39.1271701247	-76.4892434700
	Alternate	1	06-14A	39.1153732716	-76.4922320107
	Alternate	1	06-15A	39.1147178302	-76.4942176700
	Alternate	1	06-16A	39.1289525340	-76.5014715094
	Alternate	1	06-17A	39.1285874241	-76.5001779048
	Alternate	1	06-18A	39.1264677468	-76.4921365676
	Alternate	1	06-19A	39.1143943684	-76.4755733561
	Alternate	1	06-20A	39.1306189032	-76.5084055454
7 Upper Magothy	Primary	1	07-01	39.1088611255	-76.5670294052
	Primary	1	07-02	39.0828613043	-76.5592609502
	Primary	1	07-03	39.0902367472	-76.5595317419
	Primary	1	07-04	39.1131090605	-76.5973009435
	Primary	1	07-05	39.1131111831	-76.5683313374
	Primary	1	07-06	39.1063583754	-76.5781091217
	Primary	1	07-07	39.0982640427	-76.5764130707
	Primary	1	07-08	39.0833837449	-76.5633519421
	Primary	1	07-09	39.1153039017	-76.5674474734
	Primary	2	07-10	39.1150526518	-76.5566367665
	Alternate	1	07-11A	39.0971843132	-76.5598601756
	Alternate	1	07-12A	39.1058652851	-76.5684150163
	Alternate	1	07-13A	39.1086155055	-76.6080921300
	Alternate	1	07-14A	39.1112394566	-76.5442161879
	Alternate	1	07-15A	39.1037207349	-76.5574991368

7 Upper Magothy	Alternate	1	07-16A	39.1102481147	-76.6002176813
	Alternate	1	07-17A	39.1194722161	-76.5830059316
	Alternate	1	07-18A	39.1117650931	-76.5468487514
	Alternate	1	07-19A	39.0849221908	-76.5790560317
	Alternate	2	07-20A	39.1146826494	-76.5592800563
8 Lower Magothy	Primary	1	08-01	39.0488366931	-76.5150438513
	Primary	1	08-02	39.0315212798	-76.4432570777
	Primary	1	08-03	39.0750656966	-76.5392611818
	Primary	1	08-04	39.0405285268	-76.5027908084
	Primary	1	08-05	39.0552062042	-76.5212532170
	Primary	1	08-06	39.0367691440	-76.4989126762
	Primary	1	08-07	39.0537585505	-76.5074686389
	Primary	1	08-08	39.0329727118	-76.4739495268
	Primary	1	08-09	39.0402638915	-76.4687660928
	Primary	1	08-10	39.0735074756	-76.5464693846
	Alternate	1	08-11A	39.0476274189	-76.5065378726
	Alternate	1	08-12A	39.0388952825	-76.4491716150
	Alternate	1	08-13A	39.0333380468	-76.4469173770
	Alternate	1	08-14A	39.0734300459	-76.5480702293
	Alternate	1	08-15A	39.0314520413	-76.4412635280
	Alternate	1	08-16A	39.0733815711	-76.5495361863
	Alternate	1	08-17A	39.0346756915	-76.4716297653
	Alternate	1	08-18A	39.0449674595	-76.5049244872
	Alternate	1	08-20A	39.0358483890	-76.4707270571
	Alternate	1	08-19A	39.0373113319	-76.4998346784
9 Severn Run	Primary	1	09-01	39.0807758349	-76.6510920414
	Primary	1	09-02	39.1076714931	-76.7033254526
	Primary	1	09-03	39.0931421878	-76.6957811538
	Primary	1	09-04	39.0914521870	-76.6634938172
	Primary	1	09-05	39.1210754344	-76.7160508730
	Primary	1	09-06	39.1081590731	-76.7021097027
	Primary	1	09-07	39.0828091448	-76.6618302863
	Primary	2	09-08	39.1039590853	-76.6942588150
	Primary	2	09-09	39.1021259586	-76.6888761141
	Primary	3	09-10	39.0966305820	-76.6365499265
	Alternate	1	09-11A	39.0921792107	-76.6984739603
	Alternate	1	09-12A	39.1184513870	-76.6708795002
	Alternate	1	09-13A	39.0712599724	-76.6387707179
	Alternate	1	09-14A	39.0933968743	-76.6931554721
	Alternate	1	09-15A	39.0938656010	-76.6939066880
	Alternate	1	09-16A	39.0718666609	-76.6729554882
	Alternate	1	09-17A	39.1261710435	-76.7321039601
	Alternate	2	09-18A	39.1097813799	-76.6390046102
	Alternate	2	09-19A	39.0806140598	-76.6316980699
	Alternate	3	09-20A	39.1076375762	-76.6467424018
10 Severn River	Primary	1	10-01	39.0311856564	-76.5981147920
	Primary	1	10-02	39.0373391229	-76.5826464036

10 Severn River	Primary	1	10-03	39.0342326586	-76.5925227410
	Primary	1	10-04	39.0497808030	-76.5903628035
	Primary	1	10-05	39.0548768491	-76.6241133436
	Primary	1	10-06	39.0429230748	-76.5992338219
	Primary	1	10-07	39.0585163185	-76.5961716876
	Primary	1	10-08	39.0887246515	-76.6173648257
	Primary	1	10-09	39.0327933704	-76.5948028192
	Primary	1	10-10	39.0874226761	-76.6001340630
	Alternate	1	10-11A	39.0591094127	-76.5879863936
	Alternate	1	10-12A	39.0559203662	-76.5978643714
	Alternate	1	10-13A	39.0537160048	-76.6246753220
	Alternate	1	10-14A	39.0260372146	-76.4620448499
	Alternate	1	10-15A	38.9935589106	-76.5489668625
	Alternate	1	10-16A	39.0612689990	-76.6181297053
	Alternate	1	10-17A	39.0567697674	-76.5891782043
	Alternate	1	10-18A	39.0273560077	-76.4717948733
	Alternate	1	10-19A	39.0985504570	-76.6206623563
	Alternate	1	10-20A	39.0003361066	-76.5278175454
11 Upper North River	Primary	1	11-01	39.0319149118	-76.6403701494
	Primary	1	11-02	39.0010903085	-76.5913716176
	Primary	1	11-03	39.0173588232	-76.6261590705
	Primary	1	11-04	39.0133577509	-76.6453635200
	Primary	1	11-05	38.9816181778	-76.6438134941
	Primary	1	11-06	38.9965400744	-76.6127781106
	Primary	1	11-07	39.0077572214	-76.6527464909
	Primary	1	11-08	39.0226698384	-76.6276941402
	Primary	2	11-09	38.9964698436	-76.6132145403
	Primary	2	11-10	39.0072548446	-76.6187294378
	Alternate	1	11-11A	38.9765510813	-76.6522869087
	Alternate	1	11-12A	38.9907449917	-76.6440765058
	Alternate	1	11-13A	39.0304068602	-76.6242855293
	Alternate	1	11-14A	39.0433998098	-76.6443884611
	Alternate	1	11-15A	39.0352626380	-76.6274941728
	Alternate	1	11-16A	38.9670497041	-76.6378285403
	Alternate	1	11-17A	38.9902846828	-76.6551480370
	Alternate	1	11-18A	39.0185650698	-76.6260685934
	Alternate	2	11-19A	38.9917327330	-76.6125315038
	Alternate	2	11-20A	39.0130555094	-76.6221227146
12 Lower North River	Primary	1	12-01	39.0045736950	-76.5795110103
	Primary	1	12-02	38.9171272410	-76.5543624951
	Primary	1	12-03	38.9350600718	-76.6211279061
	Primary	1	12-04	38.9402723161	-76.6151015074
	Primary	1	12-05	38.9429154887	-76.6293026026
	Primary	1	12-06	38.9093556454	-76.6167874599
	Primary	1	12-07	38.9603784742	-76.6187679434
	Primary	1	12-08	38.9148730985	-76.5943161708
	Primary	2	12-09	38.9865486149	-76.5682151070

12 Lower North River	Primary	2	12-10	38.9823754916	-76.5694525261
	Alternate	1	12-11A	39.0158873793	-76.5858800265
	Alternate	1	12-12A	38.9158435381	-76.5611888194
	Alternate	1	12-13A	38.9393960719	-76.6164454452
	Alternate	1	12-14A	38.9514570697	-76.6085578191
	Alternate	1	12-15A	38.9181387724	-76.6144299994
	Alternate	1	12-16A	38.9069617321	-76.5909379676
	Alternate	1	12-17A	39.0111172624	-76.5791212499
	Alternate	1	12-18A	38.9206255675	-76.5949757808
	Alternate	2	12-19A	38.9977790541	-76.5690411272
	Alternate	2	12-20A	38.9426700735	-76.6099120486
13 Rhode River	Primary	1	13-01	38.8861333590	-76.5881382583
	Primary	1	13-02	38.8848712652	-76.5821657320
	Primary	1	13-03	38.8928578486	-76.5576182921
	Primary	1	13-04	38.8911657774	-76.5657997374
	Primary	1	13-05	38.8909047406	-76.5813234996
	Primary	1	13-06	38.8996138292	-76.5658932780
	Primary	1	13-07	38.8908236402	-76.5575742497
	Primary	1	13-08	38.9011321692	-76.5775773502
	Primary	2	13-09	38.8789742758	-76.5727016662
	Primary	2	13-10	38.8791394082	-76.5698283979
	Alternate	1	13-11A	38.8872292861	-76.5646970513
	Alternate	1	13-12A	38.8669966155	-76.6043423581
	Alternate	1	13-13A	38.8993392181	-76.5760532559
	Alternate	1	13-14A	38.8910716800	-76.5877489663
	Alternate	1	13-15A	38.8781899808	-76.5919800196
	Alternate	1	13-16A	38.8913710995	-76.5901861271
	Alternate	1	13-17A	38.8697447859	-76.5884874795
	Alternate	1	13-18A	38.8964763733	-76.5632849446
	Alternate	2	13-19A	38.8798564724	-76.5647150262
	Alternate	2	13-20A	38.8787053816	-76.5706175421
14 West River	Primary	1	14-01	38.8616464739	-76.5778482469
	Primary	1	14-02	38.8520781006	-76.5720662279
	Primary	1	14-03	38.8608359971	-76.5769761348
	Primary	1	14-04	38.8465622590	-76.5831759519
	Primary	1	14-05	38.8363189652	-76.5742963612
	Primary	1	14-06	38.8475665408	-76.5865814846
	Primary	1	14-07	38.8591780673	-76.5740257695
	Primary	1	14-08	38.8494311999	-76.5911621401
	Primary	1	14-09	38.8225434139	-76.5746507932
	Primary	2	14-10	38.8496554015	-76.5628911929
	Alternate	1	14-11A	38.8260677947	-76.5664477374
	Alternate	1	14-12A	38.8521758897	-76.5736922748
	Alternate	1	14-13A	38.8367361359	-76.5741448710
	Alternate	1	14-14A	38.8392018263	-76.5669169829
	Alternate	1	14-15A	38.8368736081	-76.5739316080
	Alternate	1	14-16A	38.8602250111	-76.5766077852

14 West River	Alternate	1	14-17A	38.8402481095	-76.5644347055
	Alternate	1	14-18A	38.8600716955	-76.5824770832
	Alternate	1	14-19A	38.8489800538	-76.5901933783
	Alternate	2	14-20A	38.8500360872	-76.5646958072
15 Herring Bay	Primary	1	15-01	38.7460193763	-76.5731318368
	Primary	1	15-02	38.8085373959	-76.5327788702
	Primary	1	15-03	38.7855172151	-76.5841341745
	Primary	1	15-04	38.8274813357	-76.6109381796
	Primary	1	15-05	38.8396600852	-76.5914047713
	Primary	1	15-06	38.7917128989	-76.5508325482
	Primary	1	15-07	38.8287819472	-76.5904824984
	Primary	2	15-08	38.8094815357	-76.5851639220
	Primary	2	15-09	38.8102545088	-76.5852133825
	Primary	2	15-10	38.8063207840	-76.5851397732
	Alternate	1	15-11A	38.8487885380	-76.6005394423
	Alternate	1	15-12A	38.8311505835	-76.5904179849
	Alternate	1	15-13A	38.7616313602	-76.5871032942
	Alternate	1	15-14A	38.8001489038	-76.5448162556
	Alternate	1	15-15A	38.8379664167	-76.5910318974
	Alternate	1	15-16A	38.8298413541	-76.6031687562
	Alternate	1	15-17A	38.7683596618	-76.5871921589
	Alternate	2	15-18A	38.7945125385	-76.5795107758
	Alternate	2	15-19A	38.8149478235	-76.5841362572
	Alternate	2	15-20A	38.8164640530	-76.5842622357
16 Upper Patuxent	Primary	1	16-01	39.0620300592	-76.7869976299
	Primary	1	16-02	39.0732454598	-76.8095383189
	Primary	1	16-03	39.0849137404	-76.8233559278
	Primary	1	16-04	39.0678685955	-76.7866418128
	Primary	1	16-05	39.0688323780	-76.7979351428
	Primary	1	16-06	39.0611128706	-76.8086487309
	Primary	1	16-07	39.0670263612	-76.8129527727
	Primary	1	16-08	39.0647092353	-76.8122064947
	Primary	1	16-09	39.0118778117	-76.7298419135
	Primary	2	16-10	39.0539950588	-76.7949454850
	Alternate	1	16-11A	39.0801670546	-76.8040992397
	Alternate	1	16-12A	39.0847205696	-76.8251667223
	Alternate	1	16-13A	39.0651305463	-76.8125620648
	Alternate	1	16-14A	39.0777781280	-76.8040601760
	Alternate	1	16-15A	39.0073329912	-76.7291313068
	Alternate	1	16-16A	39.0778393475	-76.8116301389
	Alternate	1	16-17A	39.0842476033	-76.8292909416
	Alternate	1	16-18A	39.0124300674	-76.7339384893
	Alternate	1	16-19A	39.0701859337	-76.8172883821
	Alternate	2	16-20A	39.0529953990	-76.7938076450
17 Little Patuxent	Primary	1	17-01	39.0298608416	-76.6921581073
	Primary	1	17-02	39.0551271531	-76.7504658883
	Primary	1	17-03	39.0580860861	-76.6745881461

17 Little Patuxent	Primary	1	17-04	39.0509917885	-76.6858832978
	Primary	1	17-05	39.1448332892	-76.7611400451
	Primary	1	17-06	39.0761554729	-76.7279866104
	Primary	1	17-07	39.0594985596	-76.7617874385
	Primary	1	17-08	39.1367307606	-76.7487786848
	Primary	2	17-09	39.0351251813	-76.6939716589
	Primary	2	17-10	39.0662693117	-76.7300998970
	Alternate	1	17-11A	39.0654901613	-76.6895705667
	Alternate	1	17-12A	39.0051156011	-76.6678000454
	Alternate	1	17-13A	39.0885446580	-76.7703829836
	Alternate	1	17-14A	39.1510134636	-76.7692679429
	Alternate	1	17-15A	39.0522262394	-76.7150616499
	Alternate	1	17-16A	39.0893612074	-76.7717788909
	Alternate	1	17-17A	39.1346648518	-76.7474308658
	Alternate	1	17-18A	39.1074884110	-76.7493522559
	Alternate	2	17-19A	39.0287232913	-76.6985337236
	Alternate	2	17-20A	39.0810950725	-76.7323437131
18 Middle Patuxent	Primary	1	18-01	38.9705602346	-76.6822602542
	Primary	1	18-02	38.9859109676	-76.6915150659
	Primary	1	18-03	38.9293210513	-76.6769243515
	Primary	1	18-04	38.9449844810	-76.6879015934
	Primary	1	18-05	38.9330292214	-76.6543692911
	Primary	1	18-06	38.9382897067	-76.6804230873
	Primary	1	18-07	38.9854840847	-76.6803579502
	Primary	1	18-08	38.9703033712	-76.6892540906
	Primary	1	18-09	38.9523831269	-76.6738253507
	Primary	2	18-10	38.9880824637	-76.7027950558
	Alternate	1	18-11A	38.9581573474	-76.6835861398
	Alternate	1	18-12A	38.9407307612	-76.6757385341
	Alternate	1	18-13A	38.9528682789	-76.6478830269
	Alternate	1	18-14A	38.9702981114	-76.6864698316
	Alternate	1	18-15A	38.9357323615	-76.6855486269
	Alternate	1	18-16A	38.9525096277	-76.6925585235
	Alternate	1	18-17A	38.9417759326	-76.6726822624
	Alternate	1	18-18A	38.9844798943	-76.6749670797
	Alternate	1	18-19A	38.9303120756	-76.6709848965
	Alternate	2	18-20A	38.9853158243	-76.6940523263
19 Stocketts Run	Primary	1	19-01	38.8959829885	-76.6701542591
	Primary	1	19-02	38.9077148047	-76.6399171708
	Primary	1	19-03	38.9099618742	-76.6323961275
	Primary	1	19-04	38.8938336129	-76.6065036414
	Primary	1	19-05	38.9095284013	-76.6300412140
	Primary	1	19-06	38.8820142205	-76.6581799935
	Primary	1	19-07	38.8750686920	-76.6241456880
	Primary	1	19-08	38.9210671720	-76.6515744264
	Primary	2	19-09	38.8851284900	-76.6557242789
	Primary	2	19-10	38.8872514793	-76.6420582853

19 Stocketts Run	Alternate	1	19-11A	38.8944120973	-76.6580205115
	Alternate	1	19-12A	38.8967740691	-76.6717972547
	Alternate	1	19-13A	38.9067841172	-76.6407970792
	Alternate	1	19-14A	38.8781734723	-76.6371700195
	Alternate	1	19-15A	38.9204409849	-76.6590764122
	Alternate	1	19-16A	38.8868617643	-76.6213741076
	Alternate	1	19-17A	38.9173960316	-76.6780135354
	Alternate	1	19-18A	38.9204186339	-76.6574223530
	Alternate	2	19-19A	38.8850931633	-76.6693460734
	Alternate	2	19-20A	38.8831828497	-76.6626929377
20 Rock Branch	Primary	1	20-01	38.8516828055	-76.6456636978
	Primary	1	20-02	38.8705215845	-76.6661212049
	Primary	1	20-03	38.8533420832	-76.6575619613
	Primary	1	20-04	38.8620300381	-76.6625042074
	Primary	1	20-05	38.8695733097	-76.6396047339
	Primary	1	20-06	38.8562648363	-76.6699920680
	Primary	1	20-07	38.8659167984	-76.6300831930
	Primary	1	20-08	38.8505553710	-76.6657713849
	Primary	1	20-09	38.8751139656	-76.6726107727
	Primary	2	20-10	38.8514257055	-76.6726048414
	Alternate	1	20-11A	38.8480501967	-76.6325044853
	Alternate	1	20-12A	38.8497024524	-76.6169244524
	Alternate	1	20-13A	38.8695037302	-76.6426004793
	Alternate	1	20-14A	38.8532518908	-76.6540875942
	Alternate	1	20-15A	38.8515088412	-76.6445394725
	Alternate	1	20-16A	38.8688624649	-76.6463197755
	Alternate	1	20-17A	38.8663618997	-76.6520958007
	Alternate	1	20-18A	38.8491913059	-76.6374801044
	Alternate	1	20-19A	38.8733919873	-76.6618378150
	Alternate	2	20-20A	38.8528502609	-76.6813594628
21 Ferry Branch	Primary	1	21-01	38.8124703927	-76.6498151355
	Primary	1	21-02	38.8322217304	-76.6795719634
	Primary	1	21-03	38.8356929602	-76.6695635617
	Primary	1	21-04	38.8296313019	-76.6341282849
	Primary	1	21-05	38.8300924045	-76.6774351743
	Primary	1	21-06	38.8270759822	-76.6542796958
	Primary	1	21-07	38.8348529392	-76.6688934677
	Primary	1	21-08	38.8401560771	-76.6760588859
	Primary	2	21-09	38.8411030917	-76.6784967046
	Primary	2	21-10	38.8081874745	-76.7015406003
	Alternate	1	21-11A	38.7996943813	-76.6838616212
	Alternate	1	21-12A	38.8146125890	-76.6770467948
	Alternate	1	21-13A	38.7984144411	-76.6825413938
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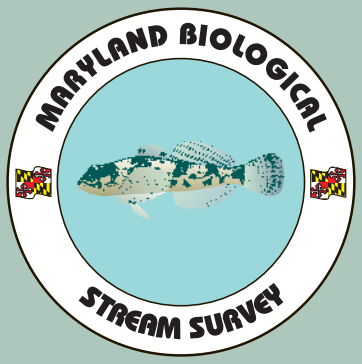
Appendix B: MBSS Sampling Manual

MARYLAND BIOLOGICAL STREAM SURVEY



Sampling Manual : Field Protocols

Rev. Jan. 2010

MARYLAND DEPARTMENT OF NATURAL RESOURCES

CHESAPEAKE BAY AND WATERSHED PROGRAMS
MONITORING AND NON-TIDAL ASSESSMENT
CBW P-MANTA-EA-07-01

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Publication # 12-2162007-190

Published January 2010

PRINTED ON RECYCLED PAPER

Maryland Biological Stream Survey, Round Three Field Sampling Manual

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January 2007

Revised January 2010

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Foreword

This document titled "The Maryland Biological Stream Survey Sampling Manual" was prepared by the Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division. The purpose of this document is to provide written standard operating procedures for all aspects of the Round Three Maryland Biological Stream Survey (MBSS) sampling. Nine distinct manuals are prepared or in preparation that correspond to different aspects of the MBSS; each one is integral to the completion of Round Three. The nine manuals are Survey Design and Site Selection, Landowner Permission, Field Sampling Protocols, Benthic Macroinvertebrate Laboratory Procedures, Water Chemistry Laboratory Procedures, Data Entry and Management, Index Calculations, Generating Results and Reporting, and Applying Results to Management Decisions. In addition to describing the procedures used to conduct each aspect, the quality assurance and quality control measures that accompany each aspect are provided in each manual.

For other manuals or more information on this manual you can consult the MBSS portion of the DNR web site at: ww.dnr.state.md.us/streams/publications.html.

Purpose of Manual

This document was prepared to support the Round Three MBSS. It is imperative that the protocols used for every aspect of the MBSS be provided to guide progress throughout Round Three and to ensure that the goals and objectives of the round are met. These written protocols also provide information to anyone attempting to duplicate procedures used by the MBSS or to ensure comparability of data and results generated by the MBSS. All persons working on the MBSS or generating reports using MBSS protocols should be familiar with the information provided herein.

Maryland Biological Stream Survey Goal and Objectives

The goal and objectives of the Round Three MBSS are the same as those of the first two rounds. The goal is to provide the best possible information for ensuring the protection and restoration of Maryland's stream ecological resources. There are four objectives of the MBSS used to attain this goal. 1) Assess, with known confidence, the current condition of ecological resources in Maryland's streams and rivers; 2) Identify causes of adverse effects (stressors) to ecological resources; 3) Provide an inventory of biodiversity in Maryland's streams; and 4) Document changes (improvements and degradation) over time in Maryland's stream ecological conditions and biodiversity status. Round One provided Maryland's first statewide assessment of ecological conditions (Objective 1). The information from Rounds One and Two was also useful in identifying many of the most pervasive stressors (Objective 2) and providing a preliminary inventory of Maryland's stream biodiversity (Objective 3). Although changes in ecological conditions (Objective 4) between the first two rounds were examined, information available from only two statewide rounds is not sufficient to conclude if any observed changes reflect actual trends. The Round Three MBSS will again provide information on all four objectives. However, a portion of the sampling effort for Round Three has shifted away from assessing statewide conditions to identifying stressors and providing biodiversity inventories. Although the condition of Maryland's individual watersheds will not be provided from Round Three, a statewide assessment of stream ecological conditions will be available and can be compared to results from Rounds One and Two.

SECTION 3
Field Sampling Protocols

3.0 Introduction

Data and results generated by the Maryland Biological Stream Survey have been widely used for making management decisions. Examples include Maryland's 305b report to Congress and the list of impaired waters (303d list), as well as identification of Tiered Aquatic Life Uses and aquatic biodiversity priority areas (See Section 8, Application to Management Decisions for details). To ensure comparability with these data and results, it was imperative that any changes to field sampling protocols for Round Three be minimal. However, the collection of crayfish, more detailed freshwater mussel information, focused stream salamander searches, and vernal pool location and habitat information have been added to Round Three to provide more thorough inventories of aquatic biodiversity than have been available previously. Improvements were also made to the stream physical habitat assessment procedures and to the design of several data sheets to improve the efficiency of field data collection while minimizing loss of comparability.

This section of the MBSS manual is patterned after previous versions of the Maryland Biological Stream Survey Sampling Manual (Kazyak 1994; Kazyak 2000; Kazyak 2001) used during Rounds One and Two.

3.1 Personnel and Crew Qualifications

Persons responsible for field collection of MBSS data fit into one of three positions, Crew Supervisor, Crew Leader, and Crew member. Each position is responsible for different aspects of field data collection. The specific responsibilities of these positions as they apply to each aspect of MBSS field data collection are described along with the description of each aspect. Specific qualifications for each MBSS position are as follows. The Crew Supervisor must be familiar with all aspects of MBSS sampling and have a minimum of five years of experience leading field data collection efforts and the logistics involved with planning and implementing field data collection. The Crew Leader must be intimately familiar with every aspect of MBSS sampling and have at least three years of experience with MBSS sampling or with another comparable ecological field sampling effort. Crew Member qualifications are minimal; however persons in this position must be physically fit for strenuous activity and must follow all safety, data collection, and quality control procedures.

Along with having qualified persons in each of these positions, all MBSS field crew members and the Crew Leader must have received training in MBSS protocols. Additionally, the field crew must be made up of persons who collectively passed all MBSS taxonomy tests for any taxonomic groups on which the crew plans to collect field data (e.g. the fish taxonomy test must be passed to identify fishes). Since benthic macroinvertebrates are identified in the laboratory, no one on the field crew is required to pass the benthic macroinvertebrate taxonomy test to collect benthic macroinvertebrates.

To ensure comparability among data collected by different sampling crews, all crews must also commit to regular field audits (see chapter 3.5, Quality Assurance). Typically audits are performed at a minimum of two sites sampled by each crew. However, additional audits may be required depending on the experience of the crew, performance on previous audits, and intended use of collected data. Audits can only be performed by a qualified MBSS QC officer. A qualified MBSS QC officer has had extensive MBSS crew leader experience, has extensive experience in conducting MBSS training, and is familiar with the intended use of MBSS data by the crew being audited. The QC officer should also be familiar with aspects of the MBSS other than field data collection (e.g. laboratory protocols, IBI calculation, data

management).

3.2 Health and Safety

The purpose of this chapter is to provide recommendations for health and safety aspects to persons involved in MBSS field collections. Suggested training and qualifications are described, along with general safety procedures, sampling hazards, provision of first aid, and emergency situations. The recommendations in this chapter are non-binding; the ultimate responsibility for health and safety of field crews lies with the parent organization for each field crew.

3.2.1 Training and Qualifications

To minimize any potential health and safety risks related to field sampling conducted as part of the MBSS, survey personnel need to be physically able to conduct fieldwork under demanding conditions and be well prepared to handle contingencies or emergencies. The following are suggested requirements for all field survey personnel:

- Recent (within 1 year) physician's approval to conduct rigorous physical work
- Recent (within 1 year) CPR certification
- Recent (within 1 year) Red Cross First Aid Training
- Complete a satisfactory interview about health and safety aspects of the MBSS with the Field Crew Supervisor, including routine safety precautions and a discussion of actions to be taken in an emergency.

In addition to the recommendations identified for all survey personnel, Crew Leaders should have adequate field sampling experience under rigorous conditions.

3.2.2 Duties and Responsibilities

This section outlines the health and safety responsibilities of persons involved with MBSS field activities.

3.2.2.1 Field Crew Supervisor

The Field Crew Supervisor for each organization involved in sampling has overall responsibility for health and safety aspects of the portion of the MBSS for which that organization is responsible.

3.2.2.2 Crew Leader

Field Crew Leaders are responsible for ensuring that day-to-day activities of the field crew are conducted in a safe manner. Recommended health and safety responsibilities of the Crew Leader include:

- instruction and supervision of the survey team such that sampling and travel at a given site are done in a manner which minimizes health and safety risks;
- reporting to the Field Crew Supervisor or his/her designee any unusual health and safety conditions, emergencies, or accidents encountered during the deployment of the crew. In the case of accidents or emergencies, the Crew Leader should, as soon as the situation permits, notify the Field Crew Supervisor or his/her designee by direct phone contact;

- ensuring that vehicles and sampling equipment are in safe operating condition prior to and during field deployments;

- ensuring that all members of the survey team are fully aware of any potentially hazardous materials used as part of sampling. Examples include preservatives for biological and chemical samples;

- determining whether sampling conditions are safe and appropriate;

- informing the survey team of any situation-specific dangers involved at a given site;

- ensuring that vehicles are operated in a safe manner; and

- ensuring that samples and sampling equipment are safely stored prior to vehicle operations.

3.2.2.3 Field Crew Members

All personnel involved in field sampling or field observations (e.g., QA/QC inspections) should be aware of the risks involved with the routine aspects of MBSS. When unsafe or hazardous conditions are observed, crew members should inform the Crew Leader at the earliest opportunity. In addition, crew members should notify the Crew Leader if, for any reason, they cannot perform an assigned task in a safe manner. Examples include sickness, physical limitations, or uncertainty about proper operation of the sampling equipment.

3.2.3 Sampling Hazards and Procedures for Minimizing Risk

There are a number of potential health and safety considerations specific to the MBSS. A number of these hazards are common to all sampling sites, while others may be site- or region-specific. This section lists a number of hazards likely to be encountered during the MBSS as well as measures to minimize the health and safety risks associated with them.

1. **Vehicle Accident.** As with nearly all other field sampling programs, there is a risk of a vehicular accident. To minimize this risk, the following measures should be taken:

    - an inspection of the sampling vehicle should be performed by the Crew Leader or a designee prior to sampling departure. This inspection should include tire condition and operability of wipers, defroster, etc.;

    - during sampling activities, any potentially unsafe vehicle condition should be reported to the Field Crew Supervisor and corrected as soon as is practical;

    - if, in the judgment of the Crew Leader, the sampling vehicle is not safe to operate, the vehicle should not be operated until the condition is rectified;

    - vehicles should not be operated by crew members who are incapable of safely operating them. No sampling vehicle should be operated by a person not holding a valid drivers license.

2. **Electric Shock.** Failure to observe appropriate safety precautions when using backpack electrofishing gear could result in electric shock. Under worst case conditions, this shock could result in cardiac arrest and loss of life. To minimize risks associated with electrofishing during

the MBSS, the following measures shall be taken:

- only personnel designated by Field Crew Leaders should operate the backpack electrofishing unit;

- to minimize the amount of body surface area potentially exposed to electric shock, normal wading gear for the MBSS should be chest waders. Only non-leaking wading gear should be used during electrofishing-- if a leak is discovered, wading gear should be changed and the leaking gear repaired or replaced prior to the next use;

- bare wire portion of the cathode (rattail) or the anode should never be touched while the unit is in operation;

- electrofishing should only be conducted when a minimum of three persons are present at a site. In the event of electric shock, this provides for one person to administer CPR while another seeks medical assistance. Use of a portable phone is also recommended as an effective means to summon emergency medical care if necessary;
- if the Crew Leader determines that stream conditions at the time of the site visit present an abnormal risk of electric shock, he or she will determine that the site is not sampleable and sampling will be conducted at an alternate site or canceled in that reach;

- prior to each use, electrofishing gear should be verified to be in safe working condition by the Crew Leader. This verification should include an examination of external wiring and electrical connections; and

- in cases where two electrofishing units are used or barge shocking is employed at a site, extra care should be taken to ensure that unit operators maintain an awareness of all personnel in the water. In addition, unit operators should maintain adequate spacing between units to minimize the risks of shock from both electric fields in the event a crew member slips or falls into the water, or the discharge of one anode completing the switch circuit for another unit.

3. **Hazardous Terrain.** A routine part of sampling during the MBSS is traveling over rough terrain to access the sample site. One of the risks arising from this aspect of the MBSS is the possibility of injury from falling. To minimize this risk, the following preventive actions are recommended:

- when necessary, the Crew Leader will make a determination that access to the sampling site is not possible and the site will be deemed unsampleable;

- when traveling over any extensive distance, appropriate footwear should be worn instead of waders or hip boots;

- equipment should be distributed equitably among crew members for transport from the vehicle to the site; if determined to be necessary by the Crew Leader, more than one trip to transport equipment should be made;

- to the greatest extent possible, travel between the vehicle and the sample site should occur during daylight hours; and

- only in unusual circumstances (as determined by the Crew Leader) should a crew member travel alone over hazardous terrain.

4. **Fast or Deep Water.** During the MBSS, some sampling sites may be visited which have fast and/or deep water in them. Sampling in locations which are too deep or too fast for wading could result in injury or drowning. It should be noted that sampling fast and/or deep waters also increases the likelihood of electrical shock; thus a high degree of caution is imperative for safe operations. To minimize health and safety risks associated with sampling in fast and/or deep waters, the following steps should be taken:

   - prior to sampling, the Crew Leader should ensure that all crew members who are to enter the stream are physically fit to do so and are aware of any specific sampling risks at the site;

   - prior to sampling, the Crew Leader should make a determination as to whether the site can be sampled by wading without undue risks. If a negative determination is reached, the site should be revisited at another time or not sampled;

   - field crew members should wear chest waders outfitted with waist belts and sticky rubber soled wading boots and/or cleats should be used in rocky areas. Felt soled boots should not be worn to avoid the transmission of harmful aquatic organisms.

5. **Slippery Substrate.** During the MBSS, sampling at some sites will be hazardous due to slippery substrate. Examples of stream types which may have treacherous substrates include those affected by acid mine drainage and streams with high silt loads. To minimize the risks associated with slippery substrates, the following measures are recommended:

   - the Crew Leader should factor the slipperiness of the substrate into decisions as to whether a site can be sampled and any extra precautions to be taken by the field crew; all wading gear should have sticky rubber soled wading boots and/or cleats.

6. **Dangerous Animals or Plants.** Sampling at some sites during the MBSS will include risks associated with dangerous animals and/or plants. Poison ivy is likely to be common along many travel routes used by the sampling crew, as well as in riparian vegetation. Poison ivy roots on tree trunks offer particular risks since they are often unnoticed. Another plant which occurs in boggy areas and should be avoided is poison sumac. Contact with bees, wasps, and certain caterpillars can cause allergic reactions and should also be avoided. A number of other animals also present serious risks including: northern copperheads, timber rattlesnakes, free-ranging domestic dogs, rabid animals of any species, and ticks. To minimize the risks associated with dangerous animals and plants during the MBSS, the following measures are recommended:

   - all field survey personnel should receive training in field identification, avoidance of, and first aid for dangerous plants and animals which may be encountered during the MBSS;

   - crew members should inform their Crew Leader of any known allergies and keep appropriate medical relief in the first aid kit (at a minimum, each crew should keep an emergency supply of benadryl – gel caps or liquid are preferred because they enter the bloodstream more quickly than tablet form);

   - the Crew Leader should make all crew members aware of site- or situation-specific dangers. Similarly, field crew members should inform the Crew Leader as soon as they are discovered;

   - All crew members should be informed of the risks of lyme disease and should check themselves after conducting field work for ticks that may have become attached to the body.

7. **High Bacterial Levels.** When sampling in areas downstream of sewage or other organic waste sources, potentially dangerous bacterial levels may exist. In urban areas, the presence of such inputs may be clearly evident by smell, observation of solids and floatables, and/or the presence of sewage fungus on bottom substrates. However, in some areas, potentially dangerous bacterial levels could be present in a stream without any obvious evidence. To minimize the health risks associated with high bacterial levels in streams, the following measures should be incorporated into field surveys:

   - during development of the itinerary, the Crew Leader should examine the list of NPDES discharge permits and investigate through MDE any known pollution problems in the watershed being sampled. Using this information, a determination should be made as to whether special safety precautions are necessary;

   - prior to entering the stream, the Crew Leader should make note of any evidence of high bacterial levels and inform the field crew;

   - the use of gloves should be maximized during the sampling process;

   - open wounds should not be exposed to contact with stream water; and

   - after exposure to stream water, all crew members should wash their hands in isopropyl alcohol and clean water prior to consuming any food or drink.

8. **Hazardous Waste.** Because of historical disposal practices, hazardous wastes may be present at an unknown number of sites to be sampled during the MBSS. Risks of relatively brief exposure (such as sampling during the MBSS) to hazardous wastes are likely to be low, but precautions still need to be taken to minimize exposure probabilities. These include:

   - prior to commencement of field sampling, existing information (through MDE and EPA) about known or probable hazardous waste sites in Maryland in relation to MBSS sample sites should be reviewed. After review of available hazardous site information, the crew should be informed of any hazardous waste sites in areas designated to be sampled. Any such areas identified will be sampled by a crew that has received OSHA hazardous waste safety training (as specified in 29 CFR 1910.120);

   - all sampling at hazardous waste sites will be conducted in accordance with site health and safety plans and only after proper advance notice has been given to authorities on site;

   - if sampling is to be conducted in an area where hazardous waste is known to be present, MBSS personnel who participate in sampling should participate in a Medical Monitoring Plan established by the Contractor for the hazardous site sampling crew. Medical Monitoring should include baseline, yearly, and exit examinations;

   - after sampling at or in the vicinity of hazardous waste sites, all exposed equipment should be thoroughly rinsed, including waders and any exposed personal equipment and;

   - no food should be consumed at known hazardous waste sites and following sampling, food will only be consumed after thorough hand washing.

9. **Hypothermia.** Many of the sites sampled during the MBSS will be in remote locations. At

these locations, the potential for stranding and prolonged exposure to extreme weather conditions is of concern, especially when sampling is conducted during cold weather. There is also a potential for prolonged exposure to cold water in the case of accidents, emergencies or other unusual conditions. Recommended precautions to reduce the possibility of hypothermia or related illnesses include:

- each field crew should carry several space blankets at all times when in the field during the Spring Index Period;
- Crew Leaders should be responsible for monitoring weather conditions and adjusting or postponing sampling plans as appropriate; and
- prior to leaving the vehicle for a sampling site, the Crew Leader should ensure that crew members are properly clothed and that emergency supplies are taken to the site.

10. **Lightning Strike.** As sampling during the MBSS will occur over relatively long periods of time in spring and summer, exposure of field crews to electrical storms is likely. To minimize risks associated with a lightning strike the following measures should be taken:

    - Crew Leaders should be responsible for monitoring weather conditions, adjusting sampling schedules as appropriate to minimize the chance of a field crew being exposed to an electrical storm while in a remote location; and
    - in the event of an electrical storm while sampling, sampling activities should be halted and the Crew Leader should determine whether to return to the vehicle or seek local shelter.

11. **Dehydration and Hyperthermia.** The most prevalent risk to MBSS sampling crews is the risk of dehydration. Freshwater should be kept with sampling crews at all times and crew members should be encouraged to drink plenty of water. In the event that a crew member suffers from dehydration or heat related illness, all possible attempts should be made to cool and hydrate the person. Make sure to have plenty of fresh drinking water readily available.

3.2.4 First Aid

During any field sampling activity such as the MBSS, there is a possibility that first aid will need to be administered. To meet this need, all personnel should be trained in first aid. In addition, each field crew should maintain a stocked first aid kit in both field sampling equipment and in the sampling vehicle.

3.2.5 Emergencies

In the event of a medical or other emergency, the Crew Leader or qualified crew member should take all appropriate immediate actions and should send for appropriate assistance using the fastest available means. In the event the emergency occurs at a remote location, all necessary information to guide assistance personnel should be provided, including map coordinates if known and appropriate.

3.2.6 Precautions for Minimizing Ecological Risk

An increasing potential exists for transferring non-native and invasive organisms (including those that cause serious diseases to native stream dwelling fauna) from one stream to another while conducting monitoring. Whirling disease (a protist, *Myxobolus cerebralis*), Didymo (an alga , *Didymospenia*

geminata), and amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) are examples of such organisms. In addition, avian influenza can be transferred among farms simply by walking in the chicken litter that came from infected individuals and then walking in another area with chickens. It is important to properly clean all footware or other equipment that may have contacted disease-containing litter.

The risks described above require that field crews conducting MBSS sampling take precautions to minimize, to the greatest extent possible, the transfer of any disease organisms from one place to another. Beginning in June 2007, all MBSS field crews will be required to disinfect all field equipment and waders that come in contact with stream or wetland (e.g. vernal pool) water following sampling at each stream site. This procedure should also be applied to all equipment that comes in contact with chicken litter.

The disinfection procedure consists of soaking or rinsing all equipment that has come in contact with water (or chicken litter) in a 10% bleach solution for at least one minute. Equipment with a smooth surface (e.g. buckets, sides – but not soles - of waders) can be scrubbed with a scrub brush using a 10% bleach solution. After soaking and scrubbing have been completed, all equipment must be rinsed with freshwater to remove the bleach solution. Avoid skin and eye contact with bleach solution as it can be severely irritating. Thoroughly rinsing all equipment with freshwater also minimizes risk of skin and eye irritation.

3.3 Quality Assurance

The purpose of this chapter is to outline QA/QC activities that are part of MBSS field activities. The chapter includes descriptions of documentation procedures, responsibility and accountability of project personnel, training requirements, facilities, and equipment. To achieve the objectives of the MBSS, it is imperative that all project personnel follow the procedures and guidance provided in this chapter.

3.3.1 Introduction

Quality assurance and quality control (QA/QC) are integral parts of data collection and management activities of the MBSS. The field QA program for the MBSS was designed to: 1) ensure comparability of data collected by disparate sampling crews and to data collected previously by MBSS, 2) ensure that data are of known and sufficient quality to meet the project objectives, and 3) provide estimates of various sources of variance associated with the individual variables being measured.

To be effective, the QA program must continually monitor the accuracy, precision, completeness, and comparability of the data during all phases of the program. Components of the MBSS field QA program include:

- thorough training of all persons involved with data collection;
- development of and adherence to strict project protocols and guidelines;
- comprehensive field and laboratory data documentation and management;
- verification of data reproducibility; and
- proper calibration of all equipment used for data collection.

3.3.2 Population of Interest

The current population of interest for the MBSS includes all non-tidal, 4th order and smaller stream reaches of the State of Maryland, with the exception of reservoir-like impoundments which substantially alter the lotic nature of the reach.

3.3.3 Comparability and Completeness

Comparability of data between field crews is maximized by providing standardized training in MBSS techniques prior to sampling. Training requirements are included in the Scope of Work for each organization involved in field sampling. Training is mandatory for all persons involved with data collection.

To utilize data from a given site during analyses, all data included on the MBSS data sheets, which pertains to the analysis being conducted, must be validated along with appropriate site location information.

3.3.4 Documentation

To ensure scientific credibility, study repeatability and cost effectiveness, all field sampling activities of the MBSS need to be adequately documented. These activities include adherence to sampling protocols, equipment calibration, data sheet review, field notes, information management, and data quality assessment. To minimize the possibility that needed documentation or data are not recorded, standardized forms and on-site verification of form completions by supervisory personnel are included as part of the MBSS. Each of the activities listed above is described in other sections of this manual, including documentation procedures and requirements.

3.3.5 Field Audits

For the field data collection component of the MBSS, the QC officer is primarily responsible for conducting field audits. At least two sites sampled by each crew during each year should be subject to audit. However, additional audits may be required depending on the experience of the crew, performance on previous audits, and intended use of collected data. Field audits consist of checking for consistency and accuracy in taxonomic identification, site confirmation, calibration and maintenance of equipment, adherence to established protocols, record keeping, and prompt identification of necessary remedial or corrective actions.

For taxonomic identification, the QC officer may designate someone who is an expert in particular taxa to verify accurate taxonomic identification.

To ensure consistency in data collection, the QC officer is required to fill out an extra set of MBSS data sheets at sites sampled during QC visits. These data sheets are to be filled out independently from the data sheets filled out by the crew. Any decisions regarding safety, sampleability, number of persons involved with sampling at the site, use of equipment, or anything that may affect data quality, comparability, or completeness should be recorded on the extra data sheets or in a QC log book. The data recorded by the QC officer will be compared to the data recorded by each crew. Assuming the QC officer makes decisions and records data consistently, and since the QC officer visits all sampling crews, this provides a measure of comparability of data collection among sampling crews. In addition to field audits, the QC Officer will visit with each crew prior to the Summer Index Period to verify competency prior to initiating sampling. This visit typically consists of protocol review in the field while hypothetical sampling is conducted.

3.3.6 Training Requirements

An important aspect of the MBSS QA program is the training program for field personnel, which will be conducted prior to sampling. Training ensures consistent implementation of required procedures and attainment by each person of a minimum level of technical competency. **All participants in MBSS field sampling must receive MBSS training.** Additionally, the field crew must be made up of persons who collectively passed all MBSS taxonomy tests for any taxonomic groups on which the crew plans to collect field data (e.g. the fish taxonomy test must be passed to collect MBSS fish data). Since benthic macroinvertebrates are identified in the laboratory, no one on the field crew is required to pass the benthic macroinvertebrate taxonomy test to collect benthic macroinvertebrates.

3.3.7 Equipment Maintenance and Calibration

Preventive maintenance and calibration must be performed on all sampling equipment used as part of the MBSS. Maintenance and calibration procedures should be implemented as per manufacturer instructions. Unless otherwise specified, calibration must be performed daily prior to equipment use and anytime equipment problems are suspected. Preventative maintenance must be performed at intervals not to exceed the frequency recommended by the manufacturer. All equipment malfunctions must be fully corrected prior to next use. For weighing scales, weekly checks must be conducted during field sampling using NIST standards or other accepted standards to demonstrate that instrument error is within limits specified by the manufacturer.

For each piece of equipment used as part of the MBSS, a bound logbook for calibration and maintenance must be maintained. Entries in the log must be made for all calibration and maintenance activities. Documentation includes detailed descriptions of all calibrations, adjustments, and replacement of parts, and each entry must be signed and dated.

To ensure that MBSS equipment is operated within QA/QC requirements, the QC Officer should conduct periodic site equipment audits.

3.3.8 Field Information Management

Each MBSS site is assigned a unique identification code. The code is recorded at the top of all MBSS data sheets. The unique code is made up of four parts. 1) Watershed code. The appropriate four letter code indicating the eight digit watershed containing the site (watershed codes are found on page 43). 2) Segment. Three numbers are used to designate the segment. These three letters begin with the stream order and the next two letters refer to the order in which the site was selected. For random sites, the order in which the sites were collected can be important as sites lower in order being sampled indicate less probability of bias (i.e. in being representative of watershed conditions) compared to having many sites with higher order sampled. 3) Type. A one letter code is used to designate the site type. Site type codes that were used during the Round Two MBSS and are likely to be used during the Round Three MBSS include “R” for random sites, “S” for sentinel sites, “X” for special study sites and “T” for targeted sites. 4) Year. The last four digits in the site identification are the calendar year during which sampling occurred.

To facilitate data recording during inclement weather, data sheets should be printed on waterproof paper. Backup copies of all field data sheets must be made. Digital photographs should be labeled appropriately with site identification and backed up.

To ensure that all field data for the MBSS are collected and recorded in a usable manner, all data should

be printed in the units specified on the MBSS data sheets. No writing over is permitted on data sheets. The incorrect entry should be lined out and the correct entry written in an obvious location next to the line out. Data sheets for a given site must be consecutively labeled so that the total number of data sheets generated for each site is known. Recorded data must be reviewed at the point of entry and the Crew Leader and one other member of the crew must review and initial all data sheets prior to departure from the site.

Each sample collected as part of the MBSS will be assigned a sample number. The sample number will contain several unique identifiers to minimize the possibility of misidentification. In addition, chain-of-custody forms (pages 50-52) should be maintained for all water sample, benthic macroinvertebrate, herpetofauna, crayfish, mussel, and fish collections.

3.3.9 Data Quality Assessment

Assessment of data quality against established data quality objectives will be conducted to determine the overall performance of the QA program, identify potential limitations to use and interpretation of the field collected data, and to provide information for other data users regarding usability of the data for other purposes.

The quality of MBSS data will be evaluated in several ways. Precision and bias associated with important elements of the sampling and measurement process for each variable measured will be evaluated using results from replicate sampling and performance evaluation studies. Information about precision, bias, and completeness will be used to determine the comparability of data acquired during each sampling year.

Inherent differences in data collected at independent sites are potentially confounded by differences in sampling efficiency, experience, knowledge of protocols, or sampling effort. Such crew differences can adversely affect data quality and interpretation of regional patterns, but logistics constrain the degree to which these potential limitations can be evaluated and/or corrected. In general, field crews will be assigned sampling sites within discrete geographic regions, and it is likely that sampling efficiency will not be uniform from the beginning to the end of the index period or between years. To minimize this effect, retaining consistent personnel should be a priority.

3.3.10 Duplicate Samples

To aid evaluation of precision and bias, 5% of all MBSS sites will have replicate benthic macroinvertebrate and water chemistry samples collected. For water chemistry samples, one QC sample from each crew will be a blank (filled with deionized water); the remainder of the 5% will be duplicates. These samples are in addition to other duplicate and blank samples analyzed as part of in-laboratory QA/QC protocols. An annual summary of QA/QC results for benthic macroinvertebrate and analytical chemistry sampling will be prepared and maintained on file.

3.3.11 Taxonomic Identification

The MBSS is recognized as providing the highest quality biological data. This is due primarily to the QC requirements for taxonomic identification. The following taxa are identified to species (or sub-species in some cases) in the field: fishes, reptiles, amphibians, crayfishes, freshwater mussels, and select invasive plants. The crew conducting MBSS sampling must consist of members who, collectively, have passed tests for all of these taxonomic groups. Only the person(s) on each crew that has passed the test for the taxonomic group should conduct identification in the field.

During the Round Three MBSS, (with the exception of shells from dead mussels) photographic vouchers

will be accepted in lieu of preserved specimens. Photographs of at least five specimens of each fish, herpetofauna, and crayfish species encountered (as long as five were collected) should be photographed. In addition, any rare, threatened, or endangered species encountered should be photographed, as long as the photograph can be taken without causing any harm to the specimen. Photographs must clearly show the appropriate features necessary for identifying the species. The Maryland Department of Natural Resources Monitoring and Non-Tidal Assessment Division will keep a voucher library of all photographs taken during Round Three MBSS sampling. With the exception of rare, threatened, or endangered species, specimens that are too small to provide photographs that can be used to verify identifications should be preserved for verification. Photographs will be reviewed by an expert in taxonomy for each taxonomic group and results will be kept on record.

Taxonomic experts (or a designee assigned by the taxonomic expert) will also audit field identification of organisms. Field audits will be conducted by taxonomic experts (or designee) at a minimum of two sites per crew.

3.3.12 Legibility

To ensure accurate transfer of information from hard copy data sheets to the MBSS data-base, data must be recorded on data sheets legibly. If the hand writing of certain individuals is deemed illegible by the crew leader, then those individuals should not record data on data sheets.

3.4 Preparation for Sampling

The purpose of this chapter is to outline procedures and provide guidance for pre-deployment activities to be completed prior to each field sampling trip.

3.4.1 Equipment

Prior to each field sampling trip, the Crew Leader should ensure that all necessary sampling equipment is prepared for sampling. A list of equipment for sampling during the Spring Index Period is shown on page 41, and equipment for the Summer Index Period is listed on page 42.

The Crew Leader will be responsible for ensuring that all necessary equipment and supplies are loaded into the vehicle. The crew will depart for sampling only after the Crew Leader has verified the equipment inventory.

At the end of each sampling day, the Crew Leader will ensure that all sampling equipment is properly stored and that gear, data sheets, preservatives, sample bottles, etc., needed for the next day are identified. When conducting water quality sampling, the Crew Leader should ensure that water quality instruments are in working order and calibrated prior to use.

To provide access to unimproved roads and thereby reduce travel time to numerous sample sites, four-wheel drive vehicles should be used when possible for MBSS sampling. Prior to use each day, the Crew Leader will visually inspect the sampling vehicle for any evidence of safety or mechanical problems.

3.4.2 Schedule

Before sampling, Crew Leaders should develop a generalized sampling itinerary. In cases where major exceptions or changes to the generalized schedule must be made due to equipment failure, inclement weather, or other problems, the Crew Leader should keep detailed records justifying changes

Prior to sampling, the Crew Leader should provide the DNR Natural Resources Police and regional fisheries managers with notification of the tentative dates and locations in which sampling is scheduled. Phone numbers for each area are provided in Tables 4-1 and 4-2.

Table 4-1. DNR Freshwater Fisheries Regional Managers

Region	Counties	Biologist	Telephone Number
Western	Garrett, Allegany	Alan Klotz	(301) 334-8218
Central	Montgomery, Howard, Baltimore, Harford, Washington, Frederick, Carroll	Charlie Gougeon	(410) 442-2080
Southern	Anne Arundel, Prince Georges, Charles, Calvert, St. Marys	Mary Groves	(301) 888-2423
Eastern	Cecil, Kent, Queen Anne's, Talbot, Caroline, Dorchester, Wicomico, Somerset, Worcester	Rick Shaffer	(410) 275-9921

Table 4-2. DNR Natural Resources Police

Region	Counties	Telephone Number
Headquarters	N/A	1-800-628-9940 410-260-8880 410-260-8888
Central	Baltimore, Howard, Montgomery, Harford, Carroll	410-356-7060 410-356-7061
Western Region	Frederick, Washington, Allegany, Garrett	301-777-7771 301-777-7645
Southern Region	Anne Arundel, Prince Georges, Charles, Calvert	301-888-1601
Upper Eastern Shore	Queen Anne's, Kent, Cecil, Talbot, Caroline	410-758-2890
Lower Eastern Shore	Dorchester, Wicomico, Worcester, Somerset	410-548-7070

3.5 Sample Collection

3.5.1 Introduction

The purpose of this chapter is to describe, in detail, the specific procedures that must be followed during sampling for the Round Three MBSS, including water quality, benthic macroinvertebrate, fish, reptile, amphibian, crayfish, mussel, invasive plant, and physical habitat sampling. Sections on site location, sampleability determination, photodocumentation, and temperature logger deployment and retrieval are also included. Strict adherence to all of these protocols is imperative. Of particular importance is diligence in completing and verifying the complete and accurate recording of data sheet information while still in the field and completing sampling during the appropriate Index Period.

3.5.2 Index Periods

To provide a synoptic view of the current ecological status of Maryland streams, MBSS sampling takes place during two index periods, spring and summer. The Spring Index Period extends from 1 March to 30 April, and the Summer Index Period extends from 1 June to 30 September each year. Four primary activities are conducted during the Spring Index Period: benthic macroinvertebrate, water chemistry for laboratory analysis, select physical habitat variable sampling, and vernal pool searches. During the Summer Index Period, seven primary activities are conducted: fish, reptile and amphibian, mussel, crayfish, invasive plant, in situ water chemistry, and select physical habitat variable sampling. It is imperative that sampling for these variables be performed during the appropriate index period. Although focused sampling for reptiles and amphibians, crayfishes, and mussels are conducted during the summer index period, incidental observations of any of these taxa should be recorded during any visit to the site, during any time of the year. If no specific place for recording the incidental observation of a particular species is available on data sheets, it should be recorded in the comments section of an available data sheet.

The time period for the Spring Index Period is based on sample degree-day accumulations of mean air temperatures above 4.5ºC. This time period was chosen because studies in Maryland have demonstrated that sampling in spring can estimate the degree of acidification in a stream, within acceptable limits, and also provide benthic macroinvertebrate data most suited for identifying anthropogenic stressors at a site.

Based on the results of benthic macroinvertebrate studies, degree day accumulations above certain thresholds (440ºC for Coastal Plain and 1050ºC for the rest of Maryland) were used as a basis for determining when MBSS Spring Index Period sampling should be completed. Since degree day accumulations rarely approached these thresholds during March and April, the Spring Index Period for Round Three includes all days within these two months. Degree days do not need to be taken into consideration.

The MBSS Summer Index Period was selected to occur during the low flow period, which is most limiting to fishes. Sampling during this period is also advantageous because spawning effects are minimized, temperatures are conducive to wading and water contact, and capture efficiency using electrofishing is typically best when streams are relatively low and warm. The other taxa, which MBSS summer sampling documents, are most active and/or most easily observed/captured (crayfishes, mussels, stream salamanders, invasive plants) during this time period.

Since water levels are typically at their lowest in Maryland streams during the summer, the Summer Index Period is also the time during which physical habitat is most limiting to many stream dwelling organisms (including fishes, mussels, stream salamanders, and crayfishes). Physical habitat quality and quantity measurements taken during this time; therefore, they represent limiting conditions for these organisms.

3.5.3 Site Location and Length

As with Rounds One and Two, all MBSS sites are located only on non-tidal 1st – 4th order (Strahler) streams based on a 1:100,000 scale stream reach file. The site consists of the watered portion of the stream and an area 50 meters perpendicular to the stream. Each site is 75 m in length. Round Three MBSS will consist of sampling sites with locations selected at random (random sites) and sites with pre-determined locations specifically chosen to answer an important management or research question (targeted sites). All MBSS sites (random or targeted) must have geographical coordinates provided with them. The coordinates represent the mid-point of the 75 m long site (37.5 m from the downstream end of the site). Permission to use any landowner's property for access to or sampling of any MBSS site is a requirement for MBSS sampling.

1. **Random Sites.** Geographic coordinates for random sites are provided to the crew leader prior to visiting the site. The sampling crew must locate random sites in the field. Reach file, road, and tax maps showing the locations of random sites are used to get close to the exact location of random sites. The exact location is reached using a Global Positioning System (GPS) unit to avoid bias in deciding where the site should be sampled. When the GPS unit indicates that the site location has been reached and the location is not on a stream, the mid-point of the site should be designated as the point that is reached using the shortest distance to the stream from the location indicated by the GPS. If the stream is more than 30 m from the location identified by the GPS, a new set of geographical coordinates should be provided for the site. A space is provided for the new coordinates on the Spring Habitat data sheet. A copy of the MBSS reach file should be consulted following the identification of the site location to be sure that (based on the reach file) the correct stream is being sampled. Maps showing landowner properties (usually tax maps) should also be consulted to ensure that the site is located on a property or properties where landowner permission has been acquired. In extreme cases, where landowner permission or other sampleability issues prohibit sampling a site in the exact location where the site was chosen, the site may be moved up to one site distance (75 m) from the original location. However, new coordinates must be provided for the site and substantial documentation must be provided to justify the location change. This option should be used only after all other options have been exhausted based on Crew Leader judgment.

2. **Targeted Sites.** Geographic coordinates should be provided for all non-random sites at the mid-point of the site (37.5 m from either end). A hard copy map showing the location of the site must also be included with data sheets as well as proper landowner permission information.

3.5.4 Determination of Sampleability

To ensure that a site can be safely and effectively sampled, the Crew Leader will examine the stream prior to the initiation of any sampling. General criteria for determining sampleability include: safety, landowner permission, ability to electrofish effectively, and non-tidal status. No sampling should take place under dangerous conditions. If the site has non-wadeable areas that can be safely sampled using a combination of long-handled anodes and/or dipnets, the site should be considered sampleable. Examples of conditions which could deem a site unsampleable include: a dry stream bed, obvious tidal influence, and unsafe velocities/depths. The determination of sampleability for benthic macroinvertebrates, spring physical habitat assessment, vernal pools, and spring water chemistry should be noted on the Spring Index Period Data Sheet. Sampleability for electrofishing, summer physical habitat assessment, water chemistry, herpetofauna, mussels, and crayfishes should be noted on the Summer Index Period Data Sheet. A description of how to determine sampleability for each of these is included with the description of sampling methods for each. A list of codes for sampleability is provided on page 43.

1. **Culverts.** It should be noted that some sites may still be sampleable even though they include underpasses, beaver dams, large culverts, and dry sections. In the case of small culverts which can not be electrofished, the length of the culvert should be measured and recorded on the data sheet and the length added to the original 75 m site. If the culvert occurs in the first half of the site, the additional distance should be added to the downstream end of the site. Similarly, the additional distance should be added to the upstream end, if the culvert is within the upper half of the original site. If the culvert can be sampled completely, no change should be made to the original 75 m site.

2. **Moving Sites.** The location of a site (even a randomly selected site) can be changed to ensure that a sample is collected as close as possible to the location originally chosen for sampling. This may be important to avoid any bias that may come from sampling large numbers of replacement sites. However, the maximum distance that a site should be moved is 75 meters. It is imperative that a randomly selected site be moved as little as possible. Sites (especially randomly selected sites) should only be moved after every attempt has been made to sample the site in its originally chosen location.

3.5.5 Marking Sites

The 75 m that make up an MBSS site are measured beginning with the 0 m mark at the downstream end of the site and ending with the 75 m mark at the upstream end. At a minimum the extent of the 75 m site (0 m and 75 m locations) should be clearly marked while sampling is being conducted. During MBSS Rounds One and Two, orange spray paint and flagging were used to mark these locations and flagging was used to mark the 25 m and 50 m locations. The 25 m and 50 m locations need to be identified along with the 75 m and 0 m locations to complete Summer Index Period habitat sampling. Marking of all four locations (0 m, 25 m, 50 m, and 75 m) during the spring index period is recommended. In some rare cases, marking with conspicuous markings, like orange spray paint, may not be allowed or appreciated by landowners. All effort should be made to adequately mark the site in the spring so it can be found again during the summer. To the full extent possible, all flagging or other material used for marking sites should be removed from the site following the last visit to the site. If necessitated by landowner concerns, the orange mark can also be painted over in brown or grey during the summer visit.

3.5.6 Photographic Documentation

All MBSS sites require at least one photograph be taken of the stream being sampled. Typically, at least two photographs are taken from the mid-point of the site, one looking upstream and one looking downstream. These photographs are typically taken during the Spring Index Period and are used to depict the general appearance and conditions of the stream. Any unusual or unique conditions that exist at the site should be documented with a photograph. Examples of unusual or unique conditions include severely eroded stream banks or trash dumping, pipes or other point source discharges, unusual water coloration, abundant flocculent, large silt or sediment deposition, and riparian tree cutting. Many conditions may warrant taking a photograph to document observations. Crew Leader judgment should be used when deciding what conditions should be photographed. However, when in doubt, take a picture.

A unique number should be used to label each digital photograph on the camera. This number, along with a descriptive title, should be entered in the appropriate portion of the Spring or Summer Index Period data sheet, depending on when the photograph(s) was taken. Digital photograph files should be stored with file names that include (at a minimum) the site identification and the unique photograph number. All files should be appropriately backed up.

3.5.7 Water Chemistry for Laboratory Analysis

Selected water quality variables (pH, acid neutralizing capacity, sulfate, nitrite, nitrate, ammonia, total nitrogen (dissolved and particulate), ortho-phosphate, total phosphorous (dissolved and particulate), chloride, conductivity, and dissolved organic carbon) are measured based on grab samples taken during the Spring Index Period (1 March to 30 April). These analytes provide information about the state of acidification and degree of organic loading in the reaches being sampled. Approximately 1.5 L of water and at least 50 mL in a closed syringe are needed to provide data for all of the MBSS laboratory water chemistry parameters.

The basic protocols used to collect samples in spring follow those used in other DNR-sponsored acid deposition studies, including the Western Maryland Stream Survey (Morgan et al., 1991) and the Western Maryland Watershed Mitigation Study (Morgan et al. 1993). All bottles for water sampling should have been leached in deionized water for at least 24 hours prior to field use, and syringes should be new and unopened. All sampling equipment should be carefully packed to eliminate potential contamination. If any contamination is suspected, spare sample bottles or syringes should be used.

Water samples for MBSS laboratory analyses should be collected without regard to stream stage and the amount of precipitation or the time since the last precipitation. The only criterion that must be met is that a water sample can be collected safely. However, sampling during turbid conditions or just after heavy rains should be avoided to ensure that benthic habitat can be properly evaluated.

Water must be collected prior to, or upstream of, any disturbance to the stream caused by site sampling or access. Stepping in the stream upstream of the location where water is being collected should be avoided until after all of the water has been collected. Collecting water at the upstream end (75 m) of the site can ensure that other sampling can occur coincident with the collection of water samples. When possible, the area from which water is taken should be near the center of the stream channel, in flowing water, and where adequate depth is present to completely submerge the water sampling bottles.

Each 1 L and 0.5 L sample bottle and syringe must be labeled. The label should include: "MBSS", the date, and site identification as recorded on the top of the Spring Index Period Data Sheet. Each syringe and sample bottle label must be verified by a member of the field crew for accuracy, with verification indicated on the Spring Index Period data sheet. All labels on samples for laboratory analysis should be covered with clear plastic tape to ensure the labels are not smudged or lost. Labels for QC samples below should use letter characters in place of numbers in the segment portion of the label (e.g, 1=A; 2=B, 3=C, etc., and 0=J).

Using care to avoid potential sample contamination from handling, fill the pre-leached 0.5 and 1 liter sample bottles to half-full, rinse, and discard. Repeat the process twice. Then fill the sample containers such that no or a minimum of air space exists in the neck of the bottle. Check to ensure that the seals on both sample bottles are tight.

Place a Luer Lock valve on the end of the syringe. Fill the syringe three times, expelling the water each time. Fill the syringe a fourth time to approximately the 60 ml mark. Hold the syringe in a vertical position and gently tap it until all bubbles are released. After all air is expelled from the syringe, use the plunger to release 5 to 10 ml of sample. When the volume in the syringe is 50 to 55 ml, and while still discharging water, carefully close the Luer Lock valve. Syringes should not contain more than 55 ml of sample to minimize the possibility of plunger dislodgement during shipping or less than 50 ml to provide sufficient water to determine the pH effectively.

Place samples on ice to maintain samples at 4°C until laboratory analysis is performed.

If a blank sample is to be taken at the site being sampled, that sample should be taken before collecting a routine sample at the randomly selected stream reach. Blanks should be collected following collection procedures outlined above, except that water from the deionized water container should be substituted for stream water. The letter B indicating blank should be entered on the QC label portion of the data sheet. The label for the QC, blank sample should be the same as the original sample, Except that letters should be substituted for numbers in the segment portion of the label (e.g, 1=A; 2=B, 3=C, etc., and 0=J).

If a duplicate sample is to be taken, that duplicate sample should be collected immediately after the routine sample using the same methods described for stream sampling above. The letter D indicating duplicate should be entered on the QC label portion of the data sheet. As with the blank sample, the label for the QC, blank sample should be the same as the original sample, except that letters should be substituted for numbers in the segment portion of the label (e.g, 1=A; 2=B, 3=C, etc., and 0=J).

After sample collections are completed, the field data and chain-of-custody forms (see ATTACHMENT 8) should be completed and checked by the field crew for completeness and accuracy.

Sample bottles must be shipped to the analytical laboratory via overnight mail within 48 hours of collection.

Special attention should be given to packing samples in such a way that they are unlikely to leak or break during transport. During the packing process, re-verify that data sheets, labels on samples, and chain-of-custody sheets are consistent, and that a complete sample has been taken.

NOTE: Because of practical and cost constraints, samples are generally shipped to the lab every other day. This results in an exceedance of filtering time limits for some analytes and some samples, but lab experience has shown that this practice has a negligible influence on results.

3.5.8 In Situ Water Chemistry

In addition to laboratory water chemistry sampling during spring, in situ measurements of dissolved oxygen, pH, and specific conductance will be made during the Summer Index Period (1 June to 30 September). Although technically not a chemistry parameter, temperature is also taken (typically using the same instrument used to measure the other parameters listed above) during the Summer Index Period. Turbidity is another parameter that is technically not a chemical parameter, but is included in this section.

In general, manufacturer's instructions should be followed for using equipment to collect summer water chemistry measurements. Prior to conducting in situ water chemistry sampling, all equipment needed for water chemistry measurements must be calibrated and in working order. The summer index period data sheet includes a section that is to be signed by the person who calibrated the instrument. An extra set of in situ water quality instruments should be on hand (in the field sampling vehicle) during the Summer Index Period as a backup in case of a malfunction to the primary instrument.

As with water collection for laboratory analysis, water must be collected prior to, or upstream of, any disturbance to the stream caused by site sampling or access. Stepping in the stream upstream of the location where in situ chemistry measurements are being made should be avoided until after all measurements have been completed. Collecting water at the upstream end (75 m) of the site can ensure that other sampling can occur coincident with the collection of in situ water chemistry data.

Being careful to avoid direct contact of probes with bottom substrates, instrument probes should be

deployed at a representative location at or near mid-stream. If necessary to protect the probes, one crew member should hold the unit off of the bottom while another person records data. The units should be turned on and allowed to equilibrate according to manufacturers specifications. An instrument that is unstable or that did not pass calibration should not be used.

Turbidity vials should be free of scratches and should be handled with kim wipes, or other clean materials to avoid scratching the glass of the vials. Vials should be rinsed three times prior to filling for the turbidity reading. Condensation often forms on the outside of the vials. This moisture can interfere with turbidity readings and should be wiped off of the vial (with a clean, scratch free material) prior to taking a reading.

After readings have stabilized, temperature, dissolved oxygen, pH, specific conductance, and turbidity data should be recorded on the Summer Index Period Data Sheet.

After in situ measurements have been completed, necessary caps for probes should be replaced and the instruments carefully disassembled and stored for transport.

3.5.9 Physical Habitat

Physical habitat assessments conducted by MBSS are intended to represent the habitat conditions available to the organisms living in the streams and to report on the extent to which certain anthropogenic factors may be affecting Maryland's streams. MBSS Habitat assessment protocols are based on a combination of metrics modified and adapted from USEPA's Rapid Bioassessment Protocols (RBP) and Ohio EPA's Qualitative Habitat Evaluation Index (QHEI). Although EPA's RBP habitat assessment protocols differentiate between riffle-run and pool-glide stream types, all metrics selected for the MBSS are scored at all MBSS sample sites to allow direct comparisons across physiographic regions and summaries of conditions on a statewide basis.

Certain MBSS physical habitat variables are recorded based on counts, measurements, or estimates made in the field. These variables include distance from nearest road to site, width of riparian buffer, stream gradient, width, depth, velocity, culvert width and length, extent and height of eroded bank, numbers of woody debris and root wads, extent of channelization, percent embeddedness, and percent shading. The quality of five habitat assessment metric variables along with the severity of bank erosion, buffer breaks, and bar formation are rated using standardized MBSS rating methods. The collection of data on certain other habitat variables are based on the observation (or not) of certain conditions such as buffer breaks, land use types, and evidence of channelization. Based on observations at sites, the absence, presence or extensive presence of stream character and bar substrate is recorded. The type and relative size of riparian vegetation and the type of land cover adjacent to the buffer are reported using standard MBSS codes. The method used for collecting data in the field for each variable differs based on the expected use of each variable as well as optimizing the time required to collect useable information.

Data sheet entries for all physical habitat variables are based on observations within or from the 75 m site only, unless otherwise stated below.

In all cases where it is necessary to differentiate the left bank of the stream from the right bank, the left and right are determined while facing upstream.

Only persons who have attended MBSS training and have demonstrated proficiency with performing MBSS physical habitat assessments should conduct MBSS physical habitat assessments.

Most MBSS physical habitat assessment information is collected during the Summer Index Period.

However, a number of important measures are rated during the Spring Index Period. Detailed descriptions of how data are to be recorded for each variable follow. The physical habitat data collected during the Spring Index Period are described first followed by those collected during the Summer Index Period.

3.5.9.1 Spring Index Period Physical Habitat Assessment

The physical habitat assessment variables recorded during the Spring Index Period can be found on the MBSS Spring Habitat Data Sheet and should be recorded on this sheet. The methods used to determine exactly what should be recorded for each variable are described, by variable, below. Data sheet entries for all Spring Index Period physical habitat variables are based on observations within or from the 75 m site only.

If the stream cannot be sampled for spring physical habitat assessment, this should be noted on the Spring Index Period Data Sheet. Codes designating reasons that a stream could not be sampled are provided on page 43.

1. **Trash Rating.** The trash rating is scored on a 0-20 scale based on criteria found on the Stream Habitat Assessment Guidance Sheet (page 44).

2. **Distance of Nearest Road to Site.** This variable should be measured when practical with a tape measure or GPS to the nearest meter. If it is not practical to measure this distance, it can be estimated to the nearest 10 m.

3. **Riparian Buffer Width.** The riparian buffer width should be measured to the nearest meter on each side of the stream. The left and right banks of the stream are determined while facing upstream. The average width of the buffer should be recorded. Buffer breaks should not be considered when estimating the average buffer width as buffer breaks are recorded in a different portion of the data sheet (see number 6 below). If the average buffer width is greater than or equal to 50 meters, enter 50 for the buffer width.

4. **Adjacent Land Cover.** Using the codes for adjacent land cover types (page 43), the type of land cover immediately adjacent to the stream buffer should be recorded. If the buffer is 50 m or more, then the same code that was recorded for the buffer should be recorded for the adjacent land cover.

5. **Riparian Vegetation.** Using the codes for vegetation types (page 43) the dominant vegetation types present within the 50m buffer of the 75 m site should be recorded. As many as four types can be recorded. The vegetation types are recorded in order of their dominance within the buffer, with the most dominant recorded first (in the left most box under the bank where the buffer is being recorded). Stem density and canopy density should both be taken into consideration for determining density. However, stem density should take precedence over canopy density.

6. **Buffer Breaks**. Both banks of the stream for the entire 75 m site should be examined for buffer breaks. For each bank of the stream, if any buffer breaks are observed, then a "Y" should be placed in the box on the MBSS Spring Habitat Data Sheet next to the words "Buffer Breaks (Y/N)". If no buffer breaks are observed, write an "N" in the box.

7. **Buffer Break Types.** If a buffer break is observed while examining the stream banks, the severity of the buffer break should be noted and recorded as M (minor) or S (severe) in the box

alongside the most appropriate buffer break type listed on the Spring Habitat Data Sheet.

8. **Channelization.** The site should be inspected for any evidence of channel straightening or dredging. If evidence of channel straightening or dredging are observed anywhere within the 75 m site, the linear extent of the channelization should be measured to the nearest meter. Channelization along each bank and the stream bottom should be measured separately and recorded in the appropriate portion of the Spring Habitat Data Sheet, where the type of channelization is listed. If channelization is observed at a site with a braided stream channel, the total extent of stream channel that is channelized should be recorded. It is possible (when multiple channels are present), using this method, for the total extent of left bank, right bank, or stream bottom channelized to be more than 75 m. Since the objective of this measure is to determine the total length of stream channel that is channelized, this is acceptable.

9. **Land Use.** While at the site, a survey of the surrounding area for land use types is conducted. For each land use type listed on the Spring Habitat Data Sheet mark a "Y" or "N" indicating whether or not the land use type is present near the site. Any land use that can be observed while in or alongside the stream at the site should receive a "Y" and any that cannot be observed should receive an "N".

10. **Stream Gradient.** The intent of this is to measure the slope of the stream over 75 m. This is achieved by recording the difference in water surface height from the 0 m to the 75 m locations of the MBSS site as compared to a level plane. A levelometer was used during the Round Two MBSS to measure stream gradient. Laser levels and other techniques may provide similar results, sometimes with increased precision. Any technique used to measure stream gradient should provide data accurate to at least the nearest 5 centimeters to be comparable to data collected during the Round Two MBSS.

    The calibration and proper functioning of the instrument that is used for determining stream gradient must be verified at least every two weeks while sampling is being conducted and documentation showing verification must be kept with the instrument.

    Measurements of height should be taken from the water's surface and NOT to the stream bottom or any bank locations. Measurements can be taken at a number of locations if the 0 m and 75 m locations cannot be seen at the same time, from the same location. However, if the level must be relocated, height measurements must be taken again from the next closest location where a measurement was already taken.

    If a culvert is present within the MBSS site and the stream level drops below the culvert due to the presence of the culvert, then the stream gradient should be measured without considering the unnatural drop caused by the culvert. This requires two separate sets of height measurements, one downstream from the culvert and one upstream of the culvert. The height difference over the span of the culvert should not be measured in this case.

    Record the height differences that will be used to calculate stream gradient on the Spring Habitat Data Sheet.

11. **Road Culvert.** If a road culvert is present within the 75 m site, an assessment of whether or not the culvert will be sampleable for fish is conducted. The width and length of the culvert should also be measured and recorded on the Spring Habitat Data Sheet.

3.5.9.2 Summer Index Period Physical Habitat Assessment

The physical habitat assessment variables recorded during the Summer Index Period can be found on the MBSS Summer Habitat Data Sheet and should be recorded on this sheet. The methods used to determine exactly what should be recorded for each variable are described, by variable, below. Data sheet entries for all Summer Index Period physical habitat variables are based on observations within or from the 75 m site only, unless otherwise specified.

In all cases where it is necessary to differentiate the left bank of the stream from the right bank, the left and right are determined while facing upstream.

Many of the summer physical habitat assessment measures require sufficiently clear water to observe the stream bottom throughout the majority of the 75 m site. If conditions do not allow sufficient visibility to see all of the features that must be observed, or if conditions are unsafe for wading, the site should be considered unsampleable for physical habitat. In many cases, the stream may be sampleable during a return visit when the water level is lower. However, if the stream cannot be sampled for summer physical habitat assessment, this should be noted on the Summer Index Period Data Sheet. Codes designating reasons that a stream could not be sampled are provided on page 43.

1. **Habitat Assessment Metrics.** Five metrics: instream habitat, epifaunal substrate, pool quality, riffle quality, and velocity depth diversity are rated on a scale of 0-20 using criteria provided on the Habitat Assessment Guidance Sheet (pages 44 and 45). The scores for each of these metrics are meant to characterize a distinct aspect of stream habitat. The instream habitat metric primarily addresses habitat for fishes and epifaunal substrate is meant to rate the suitability of habitat for benthic macroinvertebrates. The general quality of riffle and pool habitats are rated based primarily on the prevalence of sufficient depth and extent of these habitats. Velocity/depth/diversity provides a measure of the how well fast, slow, deep, and shallow areas are represented in the stream.

2. **Embeddedness.** The percent of riffle substrates surrounded by fine substrates, such as sand and silt, is recorded based on visual observation. Riffle substrates that are examined should include the area with the fastest flow within riffle or run habitats. If no riffle is present within the 75 m site, embeddedness can be rated based on the closest available riffle located in the same reach as the site (but should not be more than 75 m away from the upstream or downstream end of the site). Several substrates should be examined within the riffle to determine the approximate average condition within the fast part of the riffle. Substrates should be examined for embeddedness prior to disturbances (such as walking or netting) that are likely to dislodge fine materials from around larger substrate.

3. **Shading.** The percent of the wetted area of the 75 m site that is shaded by overhanging vegetation or other structures is approximated based on a visual assessment. If clearing of vegetation was conducted to facilitate electrofishing, or for any other reason, shading should be rated based on the condition prior to clearing.

4. **Woody Debris.** For the MBSS, large woody debris are defined as any natural woody structures (e.g. logs, snags, dead tree trunks), with the exception of live trees that are at least 10 cm in diameter and more than 1.5 m long. The number of large woody debris, located in the wetted portion of the 75 m stream site (instream woody debris), is counted. The number of large woody debris in the stream channel or immediate riparian area, but not in the wetted portion of the stream (dewatered woody debris) are counted separately from instream woody

debris. Only those dewatered woody debris from the immediate riparian area that (in the opinion of the evaluator) are likely to become wetted during high flows, or fall into the stream channel should be counted.

5. **Root Wads.** For the MBSS, root wads that are on live trees with a chest high trunk diameter (DBH) of at least 15 cm should be counted. These should be counted along both banks of the stream within the 75 m site. Those root wads that are in the water (instream) are counted separately from those not in the stream (dewatered). However, only those dewatered root wads that provide stability to the stream bank or that are likely to become wetted during high flows should be counted.

6. **Stream Character.** The Stream Character portion of the MBSS Summer Habitat Data Sheet lists 15 stream features. For each feature, an A, P, or E should be recorded in the box next to the feature indicating whether the feature is absent, present, or extensive respectively in the 75 m stream site.

7. **Maximum Depth.** The maximum depth of the MBSS site is considered the deepest area found anywhere within the 75 m. Maximum depth is recorded to the nearest cm.

8. **Wetted Width, Thalweg Depth, and Thalweg Velocity.** The wetted width, thalweg depth and thalweg velocity are measured at four transects within the 75 m MBSS site. The four transects are located at the 0 m, 25 m, 50 m, and 75 m portions of the MBSS site (beginning with 0m at the downstream-most end of the site). Wetted width is measured from bank to bank (perpendicular to the direction of the stream flow) to the nearest 0.1 m and includes only the wetted portion of the stream. Islands or other large features in the stream that would not be covered by water during higher base-flow should not be included in the measurement of wetted width. Features that would be covered by water (during higher base-flow should be included in the wetted width measurement. Thalweg depth is the depth (in cm) of the deepest part of the stream at each transect. Thalweg velocity is the stream current velocity (in m/sec) in the deepest part of the stream at each transect.

9. **Flow.** Measurements that can be used to calculate flow (often referred to as discharge) are recorded on the MBSS Summer Habitat Data Sheet. A transect that is suitable for taking these measurements should be located. A suitable transect approximates a "U" shaped channel to the extent possible. The most useful measurements are acquired by avoiding transects with boulders or other irregularities that create backflows and cross flows. The stream channel can be modified to more closely approximate a "U" shaped channel and provide laminar flow with adequate depth for taking velocity measurements. Unless the stream is very small (less than 0.5 m wide), a minimum of 10 measurements should be taken. As many as 25 measurements can be recorded on the MBSS Summer Habitat Data Sheet. In general, more measurements are required in larger streams. The measurements consist of depth (to the nearest 0.5 cm) and velocity (to the nearest 0.001 m/sec) and should be recorded at regular intervals. Velocity measurements should be taken at 0.6 of the distance from the water surface to the bottom (measured from the surface), making sure to orient the sensor to face upstream and taking care to stand well downstream to avoid deflection of flows. Depth and velocity measurements should be taken at the exact same locations. The Lat Loc on the MBSS Summer Habitat Data Sheet refers to the distance from one stream bank (either left or right) where each depth and velocity measurement is taken.

10. **Alternative Flow**. If flows are so low that they can not be measured with a flow meter,

the stream should be constricted as much as possible in a 1 meter section of uniform width and depth. The speed of a floated object should be recorded three times as a substitute for velocity measured with the flow meter. Record on the data sheet the depth, width, and time (3 trials) for the floated object.

11. **Bank Erosion.** The length and average height of erosion on both banks of the stream, within the 75 m site should be recorded along with the severity of erosion, on the MBSS Summer Habitat Data Sheet. In braided streams it is possible to have the total extent of eroded bank add up to more than 75 m. Since the objective of this measure is to determine the total area of erosion present at the site, this is acceptable.

12. **Bar Formation and Substrate**. Boxes in this portion of the MBSS Summer Habitat Data Sheet should be filled in completely to indicate if the bar formation is absent (fill in the box next to "None"), minor, moderate, or extensive; and the dominant substrate type(s) that make up the bars in the site. More than one substrate can be selected. However substrates comprising only a minor part of the substrate should not be selected.

3.5.10 Stream Blockages

Barriers to migration (such as stream blockages) often restrict the movements of resident, as well as diadromous, fishes. The Department of Natural Resources Fisheries Service keeps track of all known barriers to fish migration. The MBSS has provided the locations of many man-made barriers to fish migration to Fisheries Service to aid in documenting their locations so that the most effective possible plans to provide passage can be implemented.

To continue to provide this useful information, any man-made stream blockages either at the MBSS site or en route to the MBSS site, should have the height (to the nearest 0.1 m) and location (latitude and longitude in decimal degrees) recorded on the MBSS Spring Habitat Data Sheet. The type of blockages should also be recorded. Codes for blockage types are provided on page 43. Well known and obvious blockages such as dams on major rivers need not be recorded, but if there is any doubt about whether or not to record a blockage, recording the blockage is recommended.

3.5.11 Temperature Loggers

Temperature loggers should be deployed at all MBSS sites and should be programmed to record temperatures from 1 June to at least 15 August. Each logger should be set to record the highest temperature during an interval not to exceed 20 minutes in duration (shorter durations can be achieved depending on the memory capacity of the logger). Temperature loggers should be deployed within the limits of the sample site, preferably along a bank. The serial number of the temperature logger deployed at each site should be recorded on the MBSS Spring Index Period Data Sheet along with a description of the location where the logger was deployed. Loggers should be secured to a well anchored tree root, gabion, or other stable structure. Care should be taken when selecting the deployment location to ensure that the temperature logger is not in an area with fast current and that it is placed at a depth to ensure that it will remain submerged until time of retrieval. When each temperature logger is retrieved, the time and date of retrieval should be recorded. Verifying that the serial number for the logger that was retrieved matches the serial number entered on the Spring Index Period Data Sheet is recommended. It is often useful (and recommended) to attach a flag or piece of tape to the logger with the site identification, date, and time of retrieval.

3.5.12 Vernal Pools

1. **Definition.** A vernal pool is a small, temporary body of water that provides vitally important habitat for many amphibians and aquatic invertebrates. Despite their importance, very limited information is currently available on Maryland's vernal pools. During Round Three, the MBSS has added the collection of information on the location and size of potential vernal pool habitats encountered, as well as a list of herpetofauna associated with the pool and minimal physical habitat information. Habitats that qualify as potential vernal pools are less than one acre (4,000 m^2), can be very small (less than one square meter), and are not directly connected to a flowing stream.

2. **Index Period.** Vernal pool sampling should take place during the Spring Index Period.

3. **Vernal Pool Searches.** A search for vernal pools should be conducted within the area adjacent to MBSS sites to 50 m perpendicular to each side of the stream and for the entire 75 m length of the site. If an area cannot be searched, the appropriate code is recorded on the data sheet. Examples of conditions that would prohibit or limit searches include areas without permission on one side of the stream and extensive multiflora rose along the stream. If a portion of the area cannot be searched, the approximate unsearchable area should be recorded on the comments section of the data sheet and the appropriate unsampleability code recorded. Vernal pool sampling is only deemed unsampleable if the entire 50 m area on both sides of the stream being searched is unsampleable. If less than the entire 50 m area is sampleable, the approximate area that cannot be searched should be recorded (with a description of the reason it could not be searched) in the comments section of the data sheet. Any vernal pool found in the 50 m area should be recorded on the Vernal Pool Data Sheet and a "Y" should be recorded in the box on the Vernal Pool Data Sheet next to the "Within Transect Y/N" section.

4. **Incidental Findings.** Any vernal pools encountered outside of the transect (the area within 50 m perpendicular to the 75 m site on both sides of the stream) should be recorded on a Vernal Pool Data sheet and an "N" recorded in the "Within Transect Y/N" section.

5. **Vernal Pool Data.** Geographic coordinates (decimal degrees) should be recorded on the MBSS Vernal Pool Data Sheet for pools that are not within the 50 m transect. No coordinates should be recorded for vernal pools within the 50 m area searched for pools adjacent to the stream site. At least one digital photograph of each vernal pool should be taken and the number of the photograph recorded. If a large number of pools are found in close proximity to one another, one photograph that shows this is sufficient. A large group of pools within sight of one another can also be recorded on one section of the vernal pool data sheet as a vernal pool complex. The approximate dimensions of the potential vernal pool, or pool complex (length, width, and depth) should also be estimated and recorded, along with whether or not the pool is in the floodplain of a stream or not (upland pool). The position of the pools (floodplain or upland is recorded on the data sheet. The land cover adjacent to the potential vernal pool or pool complex should be recorded using the codes provided on page 43. Up to three codes can be recorded. The codes should be entered in order, from left to right on the data sheet, starting with the closest land cover to the pool and ending with the land cover that is furthest away from the pool. The land cover types that are recorded should be the dominant types that are in the area that can be seen while standing next to the pool. The presence of fishes or fairy shrimp (order Anostraca) (observed while standing near the pool) should be recorded using a "Y" for presence and an "N" for not observed. It is not necessary to sample for fishes or other aquatic organisms in potential vernal pools. Any amphibians observed in or immediately adjacent to the potential vernal pool should also be recorded along with the life stage of the organism (egg, larval, juvenile, adult). Frogs that are heard calling from within or immediately adjacent to the potential vernal pool, but that are not

observed should also be recorded, with the appropriate box indicating that the species was heard, but not seen, checked next to the species name. Only persons who have passed the MBSS amphibian taxonomic identification test should identify amphibians that are observed and only persons who have passed the frog call test should identify frogs based on calls that are heard.

6. **Wading in Vernal Pools.** Wading in vernal pools with waders that have been in other water bodies can spread diseases that may be highly deleterious to amphibians that use these habitats. In most cases, collecting all of the information described above can be conducted without wading into the water. Wading in potential vernal pools should be avoided.

3.5.13 Biological Sampling

Biological sampling has always been the primary focus of the MBSS. During Rounds One and Two, MBSS focused primarily on fish and benthic macroinvertebrates. Indices of Biological Integrity (IBIs) for these groups were developed using Round One and Round Two MBSS data. These IBIs are now the basis of biocriteria in Maryland and have been extensively used to represent the ecological condition of streams and rivers. A stream salamander IBI was also developed using MBSS sampling data along with supplemental sampling. In addition to providing IBI scores, the MBSS is well known for providing the best possible information on fish, benthic macroinvertebrates and stream salamanders available in Maryland. New distributional records for many species (including rare, threatened, and endangered taxa) have been documented by MBSS. MBSS species specific data have also been used to determine biodiversity priority areas so that effective conservation measures can be implemented. Threats and stressors to biota have also been determined from MBSS data and can be used to implement restoration and protection.

The key to the successes that the MBSS has had with biological data has been the consistency that comes from strict adherence to established sampling protocols and the quality control program which ensures (and documents) that those personnel collecting biological data in the field and laboratory are proficient with taxonomic identification.

Nationally, freshwater mussels and crayfishes are the most imperiled animal groups. A great deal of information is needed on these two groups in Maryland. To help meet this need, crayfish and mussel information will be collected during Round Three. The sampling of stream salamanders has also been expanded to allow more rigorous examinations of salamander information collected at MBSS sites. The information collected on these taxa will provide a great deal of information that will supplement our knowledge of Maryland's biological integrity and biodiversity and will continue to provide much needed information to plan and implement effective restoration and protection measures for aquatic biota in our state.

This section describes the protocols used during the Round Three MBSS for the collection of biological variables.

3.5.13.1 Benthic Macroinvertebrates

Benthic macroinvertebrate sampling is conducted within the same 75 m site used for other MBSS sampling. MBSS benthic macroinvertebrate sampling must occur during the Spring Index Period (1 March to 30 April). The intent of benthic sampling is to provide a representative sample of the community composition and relative abundance in favorable habitat (habitats supporting the greatest benthic diversity) within the site. In addition to representing the diversity at an MBSS site, benthic macroinvertebrate data collected by MBSS are used to calculate the MBSS benthic macroinvertebrate IBI. A D-net (540µ mesh), sieve bucket (540µ mesh sieve), and sample bucket are needed to collect an MBSS benthic macroinvertebrate sample.

1. **Sampleability**. Before sampling benthic macroinvertebrates, the crew leader (with input from other crew members) must determine if the site can be sampled safely and effectively. Sampling can only be conducted safely if the site being sampled is wadeable. If the depth or current velocity precludes safe wading, the site should be considered unsafe and not sampleable.

   Effective MBSS benthic macroinvertebrate sampling requires inspection of suitable habitats. Although turbidity or darkly stained water should not prohibit benthic macroinvertebrate sampling, streams that are so turbid that benthic macroinvertebrate habitat cannot be seen at all should not be sampled. Exceptions are sites with persistent and excessive turbidity problems (based on many return visits none of the stream bottom is ever visible). In these cases a note describing the turbidity problem should be made in the comments section of the spring data sheet. Other situations that may preclude sampling include dry streams, marshes, impoundments such as those produced by beaver dams that are too deep to sample, tidally influenced streams, and areas where permission is denied. The appropriate code for unsampleability (page 43) should be recorded on the Spring Index Period Data Sheet.

2. **Habitats to be Sampled.** Sampling should include a combination of habitats that support the most diverse macroinvertebrate community within a sample site. These habitats often include riffles when one is present. Other habitats, in order of preference, are root wads, root mats and woody debris and associated snag habitat; leaf packs; submerged macrophytes and associated substrate; and undercut banks. Other less preferred habitats include gravel, broken peat, clay lumps and detrital or sand areas in runs. Note that, among all the habitats listed above, those in moving water are preferred to those in still water.

3. **Benthic Macroinvertebrate Sampling Protocols.** Benthic sample buckets must be labeled twice - on the external wall of the bucket and on the inside. The following information must be included on the label: date, time, and site identification code from the Spring Index Period Data Sheet. Verify the information on each label and indicate so on the Spring Index Period Data Sheet. The external label should be covered with clear plastic tape to prevent smudging and/or label loss. Internal labels must be printed on waterproof paper. Both labels should be filled in with pencil. Benthic sample Chain-of-Custody forms (pages 50-52) should also be filled out with the name of the sampler, date, time, and sample site number.

   Immediately before sampling for macroinvertebrates at each site, ensure that there are no holes or remnants of prior samples in the D-net. Holes must be repaired before sampling is continued.

   Survey the site to locate the most productive benthic macroinvertebrate habitats as listed above. Twenty square feet of habitat should be sampled at each site and material collected for this 20 ft^2 sample is pooled into one sample bucket. Conveniently, the standard D-net used for MBSS sampling is about one foot wide, allowing for easy approximation of the necessary 20 ft^2 sample.

   The most productive habitats should be sampled in proportion to the availability of each habitat type in the site, while ensuring that all potentially productive habitats are represented in the sample. Surveying the site before sampling will allow the sampler to develop a plan that meets the objective of appropriately representing habitat types in the sample. Sampling procedures that should be used in each habitat type are described below.

   In a riffle, start at the downstream edge and place the net firmly in the substrate. Aggressively disturb the substrate with hand and/or foot. Sampling typically disturbs riffle habitat about 5 to 8

cm below the substrate surface. Rub by hand any large sticks and/or stones from within the disturbed area to dislodge any organisms that may be clinging to these substrates. Repeat this process near the upstream edge of the riffle. Repeat as necessary until the desired number of square feet has been sampled. Samples should be taken from the range of substrate types and velocities found within the riffle to best represent the community of benthic macroinvertebrates living within the riffle.

Log and snag substrates should be rubbed by hand or with a small brush. The D-net should be positioned with the stream current flowing into the net as the logs or snag substrates are rubbed.

The D-net should be used in a jabbing or sweeping motion to dislodge organisms from root mats, submerged macrophytes, or other habitats. Kicking the habitat prior to jabbing may also be done as needed to dislodge organisms. In soft substrates the net motion should be more gentle to minimize the collection of detritus. In all cases the D-net should be placed downstream of the sampled substrate following jabbing and sweeping to make sure that dislodged organisms are carried into the net.

In some rare cases, (e.g. some large 3rd-4th order streams) a sufficient amount of potentially productive habitat may not be present within the 75 m site to collect a 20 ft^2 sample. If this is the case, moving out of the sample site in an upstream direction to find habitat that can be sampled using a D-net is permissible. This should only be done if it is not possible to collect a sufficient sample within the 75 m site. If sampling is conducted upstream of the 75 m site, a description of the habitats sampled and distance from the upstream end of the 75 m must be recorded in the comments section of the Spring Index Period Data Sheet.

When a complete 20 ft^2 sample has been obtained, or when the D-net becomes filled to the point that water does not pass easily through it, the net should be washed into a sieve bucket that is partially submerged and in a shallow portion of a run or pool. While the sample is in the sieve bucket, all large stones (i.e., those greater than 3 cm in diameter), debris, leaves, etc., should be carefully washed, inspected for organisms, and discarded. If necessary, use forceps to remove any animals remaining on the net. All vertebrates (e.g., herpetofauna and fish) should be removed from the sieve bucket at this time. To remove fine sediments from the sample, the sieve bucket may be gently "slapped" against the stream water surface and very slowly rotated while the bottom of the bucket is submerged. Do not rotate the sieve bucket quickly during this process, as this action may damage many soft-bodied macroinvertebrates potentially rendering them unidentifiable. After processing the sample in the sieve bucket, the benthic net should be rinsed carefully in stream water to make sure that no benthic macroinvertebrates remain that may be transported to the next sample site.

4. **Preservation.** The processed composite sample should be transferred from the sieve bucket to an externally labeled sample bucket and preserved in 95% ethanol. Place the internal label atop the sample material and ensure that the lid to the sample bucket is tight. Gently mix the sample material and preservative and ready the sample for transport.

5. **Delivery to Laboratory.** A Benthic Macroinvertebrate Chain-of-Custody Sheet (page 50-52) must accompany all samples taken to the benthic macroinvertebrate identification laboratory, which includes the sample identification codes for all samples being delivered, sampler name, date, and a signature from a laboratory representative upon transfer of samples to the laboratory.

During the spring visit, record in the comments section of the spring data sheet any herpetofauna (positive identifications only) observed or heard at the site, including those released from the

sieve bucket during benthic macroinvertebrate processing. Maintain as vouchers any species not previously collected from the basin being sampled.

6. **Archiving.** MBSS benthic macroinvertebrate sample sortates are kept for five years. After this time, the sample material is discarded. Benthic macroinvertebrate subsamples are kept as archives in perpetuity.

3.5.13.2 Fish Sampling

The objectives of fish sampling for the MBSS are to assess the ecological integrity, fishability, and biodiversity in the non-tidal, flowing waters of Maryland. Double-pass electrofishing of 75 m stream sites is used to collect the information needed to meet these objectives. MBSS electrofishing occurs only during the Summer Index Period (June-September). This time period was chosen to characterize fish communities during the low flow period. Sampling during this period is also advantageous because spawning effects are minimized, temperatures are conducive to wading and water contact, and capture efficiency using electrofishing is typically best when streams are relatively low and warm.

1. **Electrofishing Safety.** All persons conducting electrofishing should be familiar with chapter 3.2.6, in which hazards and procedures for minimizing risk for electric shock, prior to conducting electrofishing are described.

2. **Sampleability.** Prior to conducting electrofishing, the crew leader (with input from other crew members) must determine if the site can be sampled safely and effectively. Electrofishing can only be conducted safely if the site being sampled can be waded. If the depth or current velocity precludes safe wading, then the site should be considered unsafe for electrofishing. However, where the margins of deep areas can be safely waded and fish can be effectively captured (e.g. using long handled dip nets and anodes), as long as all other sampleability considerations are met, sampling should occur. The most predominant effective sampleability consideration is water visibility. Effective MBSS electrofishing cannot occur in water that is turbid. All areas of the stream bottom must be visible. The only exception to the visibility consideration is a stream that is stained dark from natural organic sources (tannins leached from leaves; blackwater streams). Although sampling can occur in blackwater streams when visibility is relatively limited due to a natural cause, sampling should not occur in a blackwater stream that is also turbid. Whether or not the entire stream bottom is clearly visible in all portions of the site is recorded on the MBSS Fish Data Sheet.

   In addition to turbidity and tannic water, overhanging vegetation (especially multiflora rose) may prohibit clear visibility of (and often access to) the stream and habitats that are to be sampled. Provided proper authorization from the landowner has been acquired, vegetation that substantially limits electrofishing should be cleared prior to electrofishing. Block nets should be put in place prior to commencing clearing (or as early as possible during the clearing process) so that fishes are not chased from the site during clearing. Note that when rating shading on the MBSS Summer Habitat Assessment Data Sheet shading that was present before clearing should be recorded.

   Other situations that may preclude sampling include dry streams, marshes with no defined channel, impoundments or beaver dams that are too deep to sample, tidally influenced streams, and areas where permission is denied. If a stream is unsampleable (typically due to depth, velocity, or turbidity) during the early part of the Summer Index Period or following rain, the stream should be visited later in the Index Period or during a dryer period to re-assess sampleability. If (upon return visits) the stream is found to be continuously too deep, fast, or turbid to sample, then the appropriate code for unsampleability (found on the MBSS Summer

Index Period Data Sheet) should be recorded on the Summer Index Period Data Sheet.

3. **Sampling Considerations.** The width of the stream, number of anodes needed to effectively electrofish, and any other fish sampling considerations should have been recorded on the Spring Index Period Data Sheet during spring sampling. In cases where spring sampling is not being conducted, site reconnaissance is recommended prior to the electrofishing visit to determine the number of anodes and length of block nets needed, as well as any other fish sampling considerations.

4. **Number of Anodes.** The appropriate number of anodes to cover the entire width of the MBSS site must be used. In all cases this number of anodes is at least one for every three meters of stream width. More than this number may be necessary depending on the amount of habitat available within the stream site, deep areas, or other reasons to be determined by the Crew Leader. All anodes used by MBSS sampling crews are outfitted with ¼" mesh netting to facilitate fish capture. The netting on the anodes should not have any holes or tears greater than ¼". As more than one anode can be used for each electrofishing unit, the number of anodes/unit is recorded on the MBSS Fish Data Sheet.

5. **Dip Nets.** At least one person with a dip net should accompany each person using an anode, even if the person with the anode also carries a dip net. Dip nets used by MBSS sampling crews have ¼" mesh and should not have any holes or tears greater than ¼". Fishes must be transferred from dip nets to buckets, live cars, or other appropriate storage containers immediately upon capture to limit, as much as possible, stress to each individual fish that is captured.

6. **Barge.** In large, deep, streams it may be deemed necessary by the crew leader to use a floating barge shocker to ensure effective capture of fishes.

7. **Block Nets.** MBSS sampling of fishes requires the use of block nets. Block nets for MBSS sampling should have ¼" or smaller mesh, be completely free of holes or tears larger than ¼" and be long enough to block the entire width of the stream perpendicular to the flow and be high enough to reach from the bottom to above the surface of the stream. Block nets should be placed at the 0 m and 75 m ends of the MBSS site, so as to effectively prohibit the escape of fishes from within the site and to prohibit entry of additional fishes from outside the site. Any tributaries or seeps entering the site that will not be sampled must also be blocked with block nets to prohibit the movement of fish in or out of the site. In braided streams, all braids should be blocked at the 0 and 75 m locations and all braids should be sampled.

   If the MBSS site includes a culvert that is too small to sample through, block nets should be used to isolate the culvert from the site. The length of the culvert (not the width of the pipe) should then be added to the upstream or downstream end of the site so that the sampled section of stream is a total of 75 m long.

   Although block nets are typically outfitted with small lead weights on the bottom end, these weights are typically insufficient to keep fishes from swimming under the net (especially eels and small benthic species). Therefore, it is necessary to use rocks, stakes, or other objects to anchor the bottom of the net to the stream bottom. Like the lead weights on the bottom of the block net, the top of the block net is also typically outfitted with floats. These floats, however, are typically not sufficient to keep the entire top of the block net above the water's surface, which may permit the escape of small fishes or fishes that can readily jump out of the water. To prohibit the escape of these fishes, it is necessary to lift the top of the block net out of the water and prop it with sticks, rods, or other devices. In most streams, ropes will be needed to anchor the sides of the

block nets so that they are not dislodged by the stream current or by floating debris during electrofishing.

8. **Fish Movement.** Prior to and during the installation of block nets care must be taken to ensure that fishes are not chased out of or into the MBSS site. Any observed movement of fishes in or out of the site should be noted on the MBSS Fish Data Sheet. Disturbing the area within and upstream of the site should also be avoided, to the extent possible, prior to electrofishing so that visibility is not affected by resulting turbidity.

9. **Appropriate Voltage.** The output voltage of the electrofishing unit should be adjusted to ensure that fishes are being recruited effectively. Proper adjustments of electricity output will vary according to the varying conductivity of the water in different streams. The conductivity should be used as a guide to determine the approximate voltage and frequency to be used. In addition most electrofishers are equipped with a signal that can be used to guide the adjustment of these settings. Regardless of the conductivity and any signals that the electrofisher provides, testing of the electrofisher's effectiveness downstream of the MBSS site, prior to use in the site, should be conducted, as this is the best way to definitively be sure that the electrofisher is being effective. Effective electrofishing effectively stuns small and large fishes without causing mortality.

10. **Crew Requirements.** All persons participating in electrofishing must wear watertight chest waders. In rocky bottom streams sticky rubber soles, boot chains, or other appropriate devices must be used to limit slipping on potentially slick substrates. Polarized sunglasses should also be worn to reduce glare and thereby improve capture efficiency. Under cloudy or lower light conditions, amber-lensed glasses should be worn, while green-lensed or brown-lensed glasses are appropriate under sunny conditions. The use of rubber gloves is highly recommended due to the danger of electric shock that could occur from contact with water being sampled.

11. **Time.** The seconds of electrofishing for each unit being used for sampling should be monitored and recorded for each of the two electrofishing passes. On the MBSS Fish Data Sheet the time in seconds is recorded as the time each unit reads at the beginning of the first electrofishing pass, at the beginning of the second electrofishing pass, and at the end of the second pass.

12. **Fish Sampling.** MBSS electrofishing begins at the downstream block net. The entire site is thoroughly electrofished, bank to bank, including backwater areas, sloughs, and shallows, making an equal attempt to capture every fish observed. An exception is that fish too small to be retained by dip nets (body length less than 30 mm) need not be collected. When necessary to ensure capture of fish, the operator of the electrofishing unit should use the net on the anode ring. For the MBSS, continuous rather than intermittent electrofishing is used to avoid bias introduced by selective placement of the electrode and reduce sampling mortality.

    All captured fish are placed into buckets, live cars, or other appropriate storage containers immediately upon capture to limit, as much as possible, stress to each individual fish that is captured. Using flow through live cars and bubblers will substantially increase survival of collected fishes compared to using closed systems without bubblers. Care should be taken to avoid electrofishing near any flow through containers as the fishes in these containers will be affected by the electricity.

    In fast water or where visibility is reduced dip netters should place nets on the stream bottom to increase the probability of capturing bottom dwelling individuals that may be difficult to see.

Particular attention should be given to small benthic fishes (darters, sculpins, and madtoms).

13. **Block Net Check.** Upon completion of each electrofishing pass, the entire downstream block net must be examined for fishes. It is important to make sure that the downstream block net still effectively blocks the movement of fishes after checking it following first pass electrofishing. This may require the removal of debris that has accumulated during the first pass.

14. **Delaying Second Pass.** If water clarity in the site is reduced because of substrate disturbance during the first pass, second pass electrofishing must be delayed up to one hour until visibility improves to the point that visibility is similar to what it was during the first pass. If 2nd pass visibility is poorer that 1st pass visibility, it should be noted on the Fish Data Sheet.

15. **Equal Effort.** To ensure consistency among MBSS sampling crews, it is important to use the same sampling effort on the second pass as was expended for the first pass. This requires that all of the same habitat that was sampled during the first pass be sampled on the second pass. Therefore, the entire site should be electrofished on the second pass. The number of units, netters, and anodes should also be the same during both electrofishing passes.

16. **Biomass.** Fishes are weighed in aggregate to the nearest 10 grams separately for the first and second electrofishing pass. Only fishes should be weighed. Other organisms, rocks, sticks, leaves and other debris must be removed prior to weighing. Aggregate fish biomass is recorded at the bottom of the MBSS fish Data Sheet.

17. **Counting Fishes.** All fishes captured are identified to species and enumerated. The numbers of fish by species are recorded separately for the first and second pass. A "Fish Crib Sheet" is provided on page 46 to aid in counting by species.

    All individuals not clearly identifiable to species should be retained for later inspection in the laboratory. The number of individuals retained should be indicated on the MBSS Fish Data Sheet. **Retention of all specimens which cannot be positively identified is mandatory.** Specimens for preservation should be promptly placed into plastic jars filled with a 10% buffered formalin solution. After a minimum of five days, but no more than a month in formalin the specimens should be soaked for 24-48 hours in water, after which they can be transferred to 70% ethanol solution. Individuals >160 mm should be slit on the lower abdomen of the RIGHT side prior to preservation in formalin. All specimen jars should be labeled with inside labels specifying the date, site number, and name of collector. An example of the MBSS Voucher Specimen Label is included on page 63. The MBSS fish key should be used as needed for positive verification during field identifications. Only persons who have passed the MBSS fish identification test should identify fishes to species in the field.

18. **Unusual Anomalies.** For each species, if any unusual occurrences of anomalies are observed it should be recorded with a Y on the "Unusual Anomalies" section of the MBSS Fish Data Sheet. If unusual anomalies are not observed an N should be entered in this section of the data sheet. Unusual anomalies can include, but are not limited to, excessive black spot or black spot on an atypical species, multiple skeletal deformities, fin erosion, lesions, tumors, fungus, discoloration, excessive external parasites, or other unusual appearance. Any other comments, by species, that may be important in fish data analysis or interpretation can also be entered on the MBSS Fish Data Sheet.

19. **Voucher Specimens**. For the Round Three MBSS, DNR field crews will be required to maintain voucher collections. However, photographic vouchers will be acceptable in lieu of

preserved specimens, provided the features that need to be seen to correctly identify the specimen are clearly visible in the photograph. Photographs of at least five specimens of each fish, reptile, and amphibian, and crayfish species encountered during round three (as long as five were collected) should be photographed. In addition, any rare, threatened, or endangered species encountered should be photographed, as long as the photograph can be taken without causing any harm to the specimen. The Maryland Department of Natural Resources Monitoring and Non-Tidal Assessment Division will keep a voucher library of all photographs taken during MBSS sampling. With the exception of rare, threatened, or endangered species, specimens for which photographs cannot be used to verify identifications should be preserved for verification. Photographs will be reviewed by an expert in taxonomy for each taxonomic group and results will be kept on record. Each species photograph should include a label with the date and site identification.

Taxonomic experts (or a designee assigned by the taxonomic expert) will also audit field identification of organisms. Field audits will be conducted by taxonomic experts (or designee) at a minimum of two sites per crew.

Release individuals not retained as voucher specimens or for laboratory examination. Extreme care should be taken while holding fish prior to release to reduce stress from handling and crowding. Plenty of oxygenated water should also be supplied by holding fishes in flow through live cars and/or using battery-operated bubblers.

20. **Gamefish.** During counting and identification of the fish sample for each pass, gamefish species should be measured to the nearest mm (total length) and recorded on the Gamefish Length Data Sheet. Gamefish species for the MBSS include all bass (*Micropterus spp*.), striped bass (*Morone saxatilis*), trout (Salmonidae), walleye (*Sander vitreum*), and pikes and pickerels (Esocidae) with the exception of redfin pickerel (*Esox americana*). If visual observations suggest that some individuals may be stocked fish (based on fin wear, fin size, etc.), indicate so in the comments section for that species on the Fish Data Sheet.

3.5.13.3 Reptile and Amphibian Sampling

Reptile and amphibian sampling has been an integral part of the Maryland Biological Stream Survey since 1994. Reptiles and amphibians, and particularly stream salamanders, have been shown to be excellent indicators of MBSS site conditions. During Round Two, however, only incidental observations of herpetofauna were recorded for MBSS sites, with no focused search conducted. During Round Three, particular emphasis will be made on searching for stream salamanders and a minimum of fifteen minutes will be spent searching available habitat for herpetofauna. Incidental observations will also continue to be recorded. The life-history stage of reptiles and amphibians should be recorded (egg, larvae, or adult).

1. **Incidental Collection.** The full common name of any reptile or amphibian species that is encountered while sampling or accessing MBSS sites, during either spring or summer, should be recorded on the MBSS Spring Index Period Data Sheet or Summer Index Period Data Sheet, respectively.

2. **General Herpetofauna Search.** Approximately fifteen minutes should be spent searching the best available habitat within the riparian area of the stream during the Summer Index Period for herpetofauna. In rare cases, a site may not have any suitable habitat. In these cases, less than fifteen minutes may be spent searching for herpetofauna. Searches should consist primarily of flipping cover objects such as rocks and logs, or even human refuse. The full common name of all reptile and amphibian species encountered should be recorded on the MBSS

Summer Fauna Data Sheet.

3. **Stream Salamanders.** Stream salamanders include the following species in the family Plethodontidae; the northern red salamander, eastern mud salamander, northern spring salamander, northern two-lined salamander, long-tailed salamander, northern dusky salamander, Appalachian mountain dusky salamander, and seal salamander. Searches focused on finding these species will be conducted during the Summer Index Period, along a 25 by one meter transect paralleling the stream and can be immediately downstream of the 75 m site (but can also be upstream) so as not to interfere with electrofishing. The 25 m transect should be contiguous with, but not within, the 75 m MBSS site and be located so that it follows the wetted edge of the stream. During the search, all available cover objects (including cobbles, small boulders, logs, or other objects) within the transect should be carefully flipped over and then returned as closely as possible to the original position. Only stream salamanders found on one side of the stream should be recorded on the stream salamander portion of the data sheet to ensure comparability with all sites. The side that is searched should be selected randomly (a coin flip is acceptable). If substantial salamander habitat is present on the other side of the stream that bank can also be searched. However, any salamanders found on the other bank should be recorded in the general herptofauna search portion of the data sheet.

   All stream salamanders found incidentally, during the general herpetofauna search, or during the stream salamander search should be counted by species (up to 100 individuals of each species). Numbers by species should be recorded on the MBSS Summer Fauna Data Sheet. In rare cases where more than 100 individuals of a single species are encountered, "> 100" can be recorded. An estimate of the actual number can be written in the comments section of the data sheet.

4. **Sampleability.** General herpetofauna searches should be conducted at any MBSS site that can be safely accessed, even if electrofishing cannot be conducted for a number of reasons (e.g. due to dry stream bed, excessive depth, or beaver pond). The ability to perform stream salamander searches, however, may be precluded by certain stream conditions. As with other aspects of MBSS sampling, the sampleability codes provided on page 43 should be used to indicate sampleability for stream salamanders.

5. **Photographic Vouchers.** Photographs should be taken of any rare, threatened, or endangered species (provided the photograph can be taken without harming the specimen). In addition, voucher photographs of at least five individuals (provided at least 5 individuals are encountered) of each species encountered should be taken to verify proper identification in the field. Photographs should show the anatomical features that are necessary for proper taxonomic identification and files for digital photographs should include the MBSS site identification. The best photographs have the site identification in the photograph, with the specimen. The Department of Natural Resources, Monitoring and Non-Tidal Assessment Division will keep a photographic voucher collection for reptiles and amphibians. It is not necessary to preserve any reptile or amphibian specimens during the Round Three MBSS.

6. **Taxonomic Identification.** Only those members of the field sampling crew who have passed the reptile and amphibian taxonomy test should perform reptile and amphibian identification for the crew. The Key to the Reptiles and Amphibians of Maryland can be consulted to help with identifications.

7. **Frog Calls.** A frog call test will be administered during the Spring Index Period training. Field crew members who pass the frog call test can identify frogs as present in the vicinity of MBSS sites based on hearing the frog call. Frogs that are heard calling can be recorded on the

Spring Index Period Data Sheet during spring sampling and on the Summer Index Period Data Sheet during summer sampling.

8. **Handling Care and precautions.** Live specimens that will be released should be handled as little as possible, while still ensuring sufficient observation to obtain accurate taxonomic identification. Animals should be released as closely as possible to where they were captured. If an animal was found under a cover object, the object should be returned to its original position and the animal should be placed next to the object and allowed to return to underneath the object on its own.

3.5.13.4 Crayfish Sampling

Sampleability. If the site can be safely accessed, and with proper permission, sampling for crayfishes during the summer index period should be conducted. This includes sampling in dry streams. During the spring and summer index period incidental findings should also be recorded.

1. **Stream Crayfishes.** An attempt should be made to capture all crayfishes encountered during each electrofishing pass. Most stream-dwelling crayfishes are primarily nocturnal and reside in shallow burrows under stream substrate (e.g. cobbles, boulders, woody debris) during the day. Effort should be made during each electrofishing pass to overturn or disturb these habitats to optimize the number crayfishes captured. All captured crayfishes are placed into buckets, live cars, or other appropriate storage containers immediately upon capture to limit, as much as possible, stress to each individual crayfish that is captured. Upon the completion of each pass, the downstream blocknet is checked for crayfishes. Identify and enumerate all adult (>15 mm carapace length) crayfishes caught during the first and second electrofishing pass. The full scientific (Latin) name of each species and the number collected during each pass are recorded in the crayfish section of the Summer Index Period Data Sheet.

2. **Burrows.** The presence of crayfish burrows along stream banks or within the floodplain adjacent to the MBSS site is recorded on the datasheet. The abundance of burrows is recorded as (P): Present, (A): Absent, or (E): Extensive. If time allows, an attempt should be made to excavate crayfish burrows to identify the burrowing species.

3. **Taxonomic Identification.** Only those members of the field sampling crew who have passed the crayfish taxonomy test should perform crayfish identification for the crew. The Key to the Crayfishes of Maryland can be consulted to help with identifications.

4. **Crayfish Vouchers.** All specimens that could not be positively identified in the field should be retained for further inspection by a regional crayfish expert. Retention of all specimens which cannot be positively identified is mandatory. For Round Three MBSS, one to five individuals of each species collected in each of the major river basins (6-digit) should be retained so that taxonomic identifications can be verified. For the major river basin collections, the preservation of Form I males is preferred. However, females and small males should be vouchered in the absence of Form I males. Specimens for preservation should be promptly placed into plastic jars filled with 70% ethanol solution. Label all specimen jars with an inside label specifying the date, site name, and name of collector. Release all crayfishes not retained for vouchers.

3.5.13.5 Mussel Sampling

Any freshwater mussel (Family Unionidae), Dreissenidae, or Asiatic clams (Corbicula) that are

observed while sampling MBSS sites should be identified to species with their sceintific names recorded. During the Summer Index Period, suitable bivalve habitats within the sampling segment should be searched, with part of this effort focused on searching the stream bank for shells and animal middens. This can be conducted in conjunction with the herpetofauna search, but should consist of at least 15 minutes of effort. Live specimens that are encountered should be identified in the field, and then immediately returned as closely as possible to where they were collected. The mussel should be gently placed partway into the substrate with the anterior end pointing down. The species encountered and whether they were live (L), or dead (D) should be noted on the Summer Fauna Data Sheet in the appropriate area. A check box is provided to record the apparent absence of mussels from the site. If no Corbicula are encountered, it should be noted on the data sheet as none "N" in the section of the data sheet designated for recording information about Corbicula.

Any unionid mussel or Corbicula incidentally encountered during the Spring Index Period should be recorded on the Spring Faunal Data Sheet. If live mussels are collected in the D-net during benthic macroinvertebrate sampling, they should be place as closely as possible to where they were collected, or into the appropriate habitat if unsure where the specimen was collected. The mussel should be gently placed partway into the substrate with the anterior end pointing down.

No live freshwater mussels should be vouchered. Digital pictures should be taken of live specimens, for which the identification is uncertain provided that the photographs clearly show characters necessary to confirm the identification. At a minimum, photodocumentation will clearly show a lateral and a dorsal aspect of each specimen. Additional characters that may prove beneficial to identification include umbo/beak sculpture and posterior slope. Placing the specimen against a light-colored background for the picture may help produce a clear photograph. Pictures should be forwarded to a mussel expert for confirmation. Valves from as many dead specimens as practical for which the identification is uncertain should be retained. Valves collected from a single site can be placed in one zip-lock bag with a voucher label containing site name, date, and collector. Voucher shells should be cleaned of all debris with a soft brush (e.g. toothbrush) in water before sending to a taxonomic expert for verification.

While it is rare to find evidence of mussels in streams that are dry when visited during the Summer Index Period, they have the ability to withstand short periods of drought. Therefore, mussel sampling can be conducted in streams with standing pools or streams that have become dry.

3.5.13.6 Invasive Plants

The full common name of invasive plants observed at each MBSS site is recorded during the Summer Index Period. The common names of any invasive plant species observed within view of the MBSS site should be recorded. However, the riparian area within five meters of the stream on each bank should be thoroughly searched. The abundance of each invasive plant found is recorded as present (P) or Extensive (E). Only those members of the field sampling crew who have passed the exotic plant taxonomy test should perform invasive plant identification for the crew.

Spring Index Period Equipment List. **This table lists the equipment needed to complete sampling for all variables for which MBSS field sampling is conducted during the Spring Index Period.**

MBSS Sampling Manual	G.P.S. unit
Road maps and itinerary	Compass
Site list/maps	Ice
Spring Habitat Data Sheets	Bubble wrap, packing material, packing tape
Spring Index Period Data Sheets	Clear label tape
Vernal Pool Data Sheets	Deionized water for blanks
Permanent markers	Water quality sample bottles- 1 liter
Pencils	Water quality sample bottles- 500 mL
Taxonomic Keys (reptiles and amphibians, crayfish, freshwater mussels)	Syringes and valves
Machete or other clearing tools	Tripod, level, and stadia
Digital camera	Pre-printed adhesive outside labels and inside labels
First aid kit	Spare batteries
Spray paint	Chain-of-custody forms
Flagging	Ziplock bags
100 m measuring tape	
540 micron mesh D-net	
Spare net bag for D-net	
EtOH (2 liters per site)	
Foul weather gear	
Backpack	
Small cooler for transporting water samples from site to vehicle	
Large cooler for keeping samples cool after collection and for shipping to laboratory	
Temperature loggers	
Chest waders	
Wader repair kit	

Summer Index Period Equipment List. **This table lists the equipment needed to complete sampling for all variables for which MBSS field sampling is conducted during the Summer Index Period.**

MBSS sampling manual	Flowmeter and staff gauge
Road maps	Spring or electronic scale
Site list and site maps	Calibration weights
Summer Index Period Data Sheets	Calibration standard solutions for pH and conductivity
Fish Data Sheets	Backpack electrofishing Unit(s)
Summer Habitat Data Sheets	Anode ring probe(s) (fitted with 3/16" mesh netting);
Gamefish Length Data Sheets	Electrofishing batteries
Habitat Guidance Sheet	Spare netting/cable ties for anode ring nets
Clipboards	25 liter buckets
Pencils	Dip nets
Sample jars	Block nets
Pre-printed voucher labels	Live cars
Taxonomic keys (reptiles and amphibians; fish, freshwater mussels, crayfishes)	DO, temp.,pH, conductivity meter (Hydrolab or equivalent)
Voucher lists	Calibration log
Preservatives (alcohol and formalin)	Waders and wading boots
100 m measuring tape	Cellular phone
Flagging	Backpacks
Digital camera	Measuring board
G.P.S. unit	Meter sticks
Compass	Tool box
Turbidimeter	Kim Wipes
Turbidity standards	Polarized Glasses
Disinfectant lotion	Decontamination solution – 10% bleach or Virkon
Drinking water	
First aid kit	
Foul weather gear	
Wader repair kit	
Pruning tool	
Machete	

MBSS Drainage Basin Codes

YG = Youghiogheny River
NO = North Branch Potomac River
UP = Upper Potomac River
MP = Middle Potomac River
CO = Conawago Creek
PW = Potomac Washington Metro
LP = Lower Potomac River
PX = Patuxent River
WC = West Chesapeake
PP = Patapsco River
BU = Bush River
GU = Gunpowder River
SQ = Lower Susquehanna River
EL = Elk River
CR = Chester River
CK = Choptank River
NW = Nanticoke-Wicomico Rivers
PC = Pocomoke River
OC = Ocean Coastal

Watershed	Abbreviation
Aberdeen Proving Ground	ABPG
Anacostia River	ANAC
Antietam Creek	ANTI
Assawoman Bay	ASSA
Atkisson Reservoir	ATKI
Atlantic Ocean	ATLA
Back River	BACK
Back Creek	BACR
Baltimore Harbor	BALT
Big Annemessex River	BANN
Big Elk Creek	BELK
Bird River	BIRD
Bodkin Creek	BODK
Bohemia River	BOHE
Breton Bay	BRET
Brighton Dam	BRIG
Broad Creek	BROA
Bush River	BUSH
Bynum Run	BYNU
Cabin John Creek	CABJ
Casselman River	CASS
Catoctin Creek	CATO
Conowingo Dam Susquehanna R	CDAM
Chincoteague Bay	CHIN
Christina River	CHRI
Conewago Creek	COCR
Conococheague	CONO
Corsica River	CORS
Deep Creek Lake	DCRL
Deer Creek	DEER
Dividing Creek	DIVI
Double Pipe Creek	DOUB
Eastern Bay	EAST
Evitts Creek	EVIT
Fifteen Mile Creek	FIMI
Fishing Bay	FISH
Furnace Bay	FURN
Georges Creek	GEOR
Gilbert Swamp	GILB
Gunpowder River	GUNP
Gwynns Falls	GWYN
Honga River	HONG
Isle of Wight Bay	ISLE
Jones Falls	JONE
Kent Island Bay	KEIS
Kent Narrows	KENA
Langford Creek	LANG
Little Conococheague	LCON
Liberty Reservoir	LIBE
Little Choptank	LICK
Little Elk Creek	LIEL
Little Gunpowder Falls	LIGU
Licking Creek	LIKG
Lower Monocacy River	LMON
Loch Raven Reservoir	LOCH
Lower Choptank	LOCK
Lower Chester River	LOCR
Lower Elk River	LOEL
Lower Gunpowder Falls	LOGU
Lower Pocomoke River	LOPC
Lower Wicomico	LOWI
Little Patuxent River	LPAX
Lower Susquehanna	LSUS
Little Tonoloway	LTON
Lower Chesapeake Bay	LWCH
Lower Winters Run	LWINT
Little Youghiogheny	LYOU
Magothy River	MAGO
Manokin River	MANO
Marsh Run	MARS
Marshyhope Creek	MACK
Mattawoman Creek	MATT
Middle Chesapeake Bay	MDCH
Middle Chester River	MICR
Middle River-Browns	MIDD
Miles River	MILE
Monie Bay	MONI
Middle Patuxent Rier	MPAX
Nanjemoy Creek	NANJ
Nanticoke River	NANT
Nassawango Creek	NASS
Northeast River	NEAS
Newport Bay	NEWP
Octoraro Creek	OCTO
Oxon Creek	OXON
Patapsco River Lower North Br	PATL
Patuxent River Lower	PAXL
Patuxent River Middle	PAXM
Patuxent River Upper	PAXU
Pocomoke Sound	PCSO
Piscataway Creek	PISC
Potomac AL Co	PRAL
Prettyboy Reservoir	PRET
Potomac River FR Co	PRFR
Potomac River Lower North Br	PRLN
Potomac Lower Tidal	PRLT
Potomac River MO Co	PRMO
Potomac River Middle Tidal	PRMT
Potomac River Upper North Br	PRUN
Potomac Upper Tidal	PRUT
Potomac WA Co	PRWA
Port Tobacco River	PTOB
Rocky Gorge Dam	RKGR
Rock Creek	ROCK
Sassafras River	SASS
Savage River	SAVA
South Branch Patapsco	SBPA
Southeast Creek	SEAS
Seneca Creek	SENE
Severn River	SEVE
Sideling Hill Creek	SIDE
Sinepuxent Bay	SINE
South River	SOUT
St. Clement Bay	STCL
Stillpond-Fairlee	STILL
St. Mary's River	STMA
Swan Creek	SWAN
Tangier Sound	TANG
Tonoloway	TONO
Town Creek	TOWN
Transquaking River	TRAN
Tuckahoe Creek	TUCK
Upper Elk River	UELK
Upper Monocacy River	UMON
Upper Chesapeake Bay	UPCH
Upper Choptank	UPCK
Upper Chester River	UPCR
Upper Pocomoke River	UPPC
West Chesapeake Bay	WCHE
Western Branch	WEBR
West River	WEST
Wicomico River	WICO
Wicomico Creek	WICR
Wills Creek	WILL
Wicomico River Head	WIRH
Wye River	WYER
Youghiogheny River	YOUG
Zekiah Swamp	ZEKI

VEGETATION TYPES

G= Grasses /Forbes
R= Regen Deciduous /Shrubs (<4"DBH)
Y= Young Deciduous (4-12" DBH)
M= Mature Deciduous (12-24" DBH)
O= Old Deciduous (>24" DBH)
A= Regen Coniferous (<4" DBH)
B= Young Coniferous (4-12" DBH)
C= Mature Coniferous (12-24" DBH)
D= Old Coniferous (>24" DBH)
L= Lawn

Riparian Buffer Zone / Adjacent Land Cover Types

FR = Forest
OF = Old Field
EM = Emergent Vegetation
LN = Mowed Lawn
TG = Tall Grass
LO = Logged Area
SL = Bare Soil
RR = Railroad
PV = Paved Road
PK = Parking Lot/ Industrial/ Commercial
GR = Gravel Road
DI = Dirt Road
PA = Pasture
OR = Orchard
CP = Cropland
HO = Housing

INSTREAM BLOCKAGE CODES

DM = Dam
PC = Pipe Culvert
F = Fishway
GW =Gaging Station Weir
G = Gabion
PX = Pipeline Crossing
AC = Arch Culvert
BC = Box Culvert
TG = Tide Gate
(Note: Height is measured in meters from stream surface to water surface above structure)

Sampleability Codes

S = Sampleable
1 = Dry Stream bed
2 = Too Deep
3 = Marsh, no defined channel
4 = Excessive Vegetation
5 = Impoundment
6 = Tidally Influenced
7 = Permission Denied
8 = Unsafe (Describe in Comments)
9 = Beaver
10 = Other

MBSS Stream Habitat Assessment Guidance Sheet

Habitat Parameter	Optimal 16-20	Sub-Optimal 11-15	Marginal 6-10	Poor 0-5
1. Instream Habitat[a]	Greater than 50% of a variety of cobble, boulder, submerged logs, undercut banks, snags, root wads, aquatic plants, or other stable habitat	30-50% of stable habitat. Adequate habitat	10-30% mix of stable habitat. Habitat availability less than desirable	Less than 10% stable habitat. Lack of habitat is obvious
2. Epifaunal Substrate[b]	Preferred substrate abundant, stable, and at full colonization potential (riffles well developed and dominated by cobble; and/or woody debris prevalent, not new, and not transient)	Abund. of cobble with gravel &/or boulders common; or woody debris, aquatic veg., undercut banks, or other productive surfaces common but not prevalent /suited for full colonization	Large boulders and/or bedrock prevalent; cobble, woody debris, or other preferred surfaces uncommon	Stable substrate lacking; or particles are over 75% surrounded by fine sediment or flocculent material
3. Velocity/Depth Diversity[c]	Slow (<0.3 m/s), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m/s), deep; fast, shallow habitats all present	Only 3 of the 4 habitat categories present	Only 2 of the 4 habitat categories present	Dominated by 1 velocity/depth category (usually pools)
4. Pool/Glide/Eddy Quality[d]	Complex cover/&/or depth > 1.5 m; both deep (> .5 m)/shallows (< .2 m) present	Deep (>0.5 m) areas present; but only moderate cover	Shallows (<0.2 m) prevalent in pool/glide/eddy habitat; little cover	Max depth <0.2 m in pool/glide/eddy habitat; or absent completely
5. Riffle/Run Quality[e]	Riffle/run depth generally >10 cm, with maximum depth greater than 50 cm (maximum score); substrate stable (e.g. cobble, boulder) & variety of current velocities	Riffle/run depth generally 5-10 cm, variety of current velocities	Riffle/run depth generally 1-5 cm; primarily a single current velocity	Riffle/run depth < 1 cm; or riffle/run substrates concreted
6. Embeddedness[f]	Percentage that gravel, cobble, and boulder particles are surrounded by line sediment or flocculent material.			
7. Shading[g]	Percentage of segment that is shaded (duration is considered in scoring). 0% = fully exposed to sunlight all day in summer; 100% = fully and densely shaded all day in summer			
8. Trash Rating[h]	Little or no human refuse visible from stream channel or riparian zone	Refuse present in minor amounts	Refuse present in moderate amounts	Refuse abundant and unsightly

a) Instream Habitat Rated based on perceived value of habitat to the fish community. Within each category, higher scores should be assigned to sites with a variety of habitat types and particle sizes. In addition, higher scores should be assigned to sites with a high degree of hypsographic complexity (uneven bottom). In streams where ferric hydroxide is present, instream habitat scores are not lowered unless the precipitate has changed the gross physical nature of the substrate. In streams where substrate types are favorable but flows are so low that fish are essentially precluded from using the habitat, low scores are assigned. If none of the habitat within a segment is useable by fish, a score of zero is assigned.

b) Epifaunal Substrate Rated based on the amount and variety of hard, stable substrates usable by benthic macroinvertebrates. Because they inhibit colonization, floculent materials or fine sediments surrounding otherwise good substrates are assigned low scores. Scores are also reduced when substrates are less stable.

c) Velocity/Depth Diversity Rated based on the variety of velocity/depth regimes present at a site (slow-shallow, slow-deep, fast-shallow, and fast-deep). As with embeddedness, this metric may result in lower scores in low-gradient streams but will provide a statewide information on the physical habitat found in Maryland streams.

d) Pool/Glide/Eddy Quality Rated based on the variety and spatial complexity of slow- or still-water habitat within the sample segment. It should be noted that even in high-gradient segments, functionally important slow-water habitat may exist in the form of larger eddies. Within a category, higher scores are assigned to segments which have undercut banks, woody debris or other types of cover for fish.

e) Riffle/Run Quality Rated based on the depth, complexity, and functional importance of riffle/run habitat in the segment, with

highest scores assigned to segments dominated by deeper riffle/run areas, stable substrates, and a variety of current velocities.

f) Embeddedness Rated as a percentage based on the fraction of surface area of larger particles that is surrounded by fine sediments on the stream bottom. In low gradient streams with substantial natural deposition, the correlation between embeddedness and fishability or ecological health may be weak or non-existent, but this metric is rated in all streams to provide similar information from all sites statewide.

g) Shading Rated based on estimates of the degree and duration of shading at a site during summer, including any effects of shading caused by landforms.

h) Trash Rating The scoring of this metric is based on the amount of human refuse in the stream and along the banks of the sample segment.

v. 2009

CRIB SHEET

Page ☐ Of ☐

SITE

Watershed Code	Segment	Type	Year

____ PASS			____ PASS		
Species	Tally	Anomalies	Species	Tally	Anomalies

COMMON NAMES OF MARYLAND FISHES

LAMPREYS:
American brook lamprey (T)
Least brook lamprey
Sea lamprey

HERRINGS:
Alewife
American shad
Atlantic menhaden
Blueback herring
Gizzard shad
Hickory shad
Threadfin shad

CATFISHES:
Blue catfish
Brown bullhead
Channel catfish
Margined madtom
Stonecat (E)
Tadpole madtom
White catfish
Yellow bullhead

SUCKERS:
Black redhorse*
Creek chubsucker
Golden redhorse
Longnose sucker* (X)
Northern hogsucker
Shorthead redhorse
Quillback
White sucker

KILLIFISH:
Banded killifish
Mummichog
Rainwater killifish
Striped killifish

STICKLEBACKS:
Fourspine stickleback
Threespine stickleback

MINNOWS:
Eastern blacknose dace
Western blacknose dace
Bluntnose minnow
Bridle shiner (E)
Bullhead minnow*
Central stoneroller
Cheat minnow* (X)
Comely shiner (T)
Common carp
Common shiner
Creek chub
Cutlip minnow
Eastern silvery minnow
Emerald shiner*
Fallfish
Fathead minnow
Goldfish
Golden shiner
Grass carp
Ironcolor shiner (E)
Longnose dace
Mimic shiner*
Pearl dace (T)
Redside dace
River chub
Rosyface shiner
Rosyside dace
Satinfin shiner
Sand shiner*
Silver shiner*
Silverjaw minnow
Spotfin shiner
Spottail shiner
Striped shiner (I)
Swallowtail shiner
Tench

SCULPINS:
Blue Ridge sculpin
Checkered sculpin
Mottled sculpin
Potomac sculpin

PERCHES:
Banded darter
Chesapeake Logperch (T)
Fantail darter
Glassy darter (T)
Greenside darter
Johnny darter
Logperch* (T)
Maryland darter (E)
Rainbow darter
Shield darter
Stripeback darter (E)
Swamp darter (I)
Tessellated darter
Yellow perch
Walleye

SUNFISHES:
Banded sunfish
Black crappie
Blackbanded sunfish (E)
Bluespotted sunfish
Bluegill
Flier (T)
Green sunfish
Largemouth bass
Longear sunfish
Mud sunfish (I)
Pumpkinseed
Redbreast sunfish
Redear sunfish
Rock bass
Smallmouth bass
Warmouth
White crappie

TEMPERATE BASSES:
Striped Bass
White Perch

TROUTS:
Brook trout
Brown trout
Cutthroat trout
Lake trout
Rainbow trout

PIKES:
Chain pickerel
Muskellunge
Northern pike
Redfin pickerel

MISCELLANEOUS:
American eel
Bowfin
Eastern mudminnow
Inland silverside
Longnose gar
Mosquitofish
Northern snakehead
Oriental weatherfish
Pirate perch
Rainbow smelt
Sheepshead minnow
Spot
Trout-perch (X)

***Historically from and potentially occurring in the Youghiogheny River basin**

COMMON NAMES OF MARYLAND REPTILES AND AMPHIBIANS

SALAMANDERS
Allegheny Mountain Dusky Salamander
Common Mudpuppy (X)
Eastern Hellbender (E)
Eastern Mud Salamander
Eastern Red-backed Salamander
Eastern Tiger Salamander (E)
Four-toed Salamander
Green Salamander (E)
Jefferson Salamander
Long-tailed Salamander
Marbled Salamander
Northern Dusky Salamander
Northern Red Salamander
Northern Slimy Salamander
Northern Spring Salamander
Northern Two-lined Salamander
Red-spotted Newt
Seal Salamander
Spotted Salamander
Valley and Ridge Salamander
Wehrle's Salamander (I)

FROGS
American Bullfrog
Barking Treefrog
Carpenter Frog
Cope's Gray Treefrog
Eastern American Toad
Eastern Cricket Frog
Eastern Narrow-mouthed Toad (E)
Eastern Spadefoot
Fowler's Toad
Gray Treefrog
Green Treefrog
Mountain Chorus Frog (E)
New Jersey Chorus Frog
Northern Green Frog
Northern Leopard Frog
Northern Spring Peeper
Pickerel Frog
Southern Leopard Frog
Upland Chorus Frog
Wood Frog

TURTLES
Bog Turtle (T)
Eastern Box Turtle
Eastern Mud Turtle
Eastern Painted Turtle
Eastern River Cooter
Eastern Snapping Turtle
Eastern Spiny Softshell (I)
Midland Painted Turtle
Northern Diamond-backed Terrapin
Northern Map Turtle (E)
Northern Red-bellied Cooter
Red-eared Slider
Spotted Turtle
Stinkpot
Wood Turtle

SNAKES
Coastal Plain Milk Snake
Common Rainbow Snake (E)
Common Ribbonsnake
Eastern Gartersnake
Eastern Hog-nosed Snake
Eastern Kingsnake
Eastern Milk Snake
Eastern Ratsnake
Eastern Smooth Earthsnake
Eastern Wormsnake
Mole Kingsnake
Mountain Earthsnake (E)
Northern Black Racer
Northern Brownsnake
Northern Copperhead
Northern Pinesnake
Northern Red-bellied Snake
Northern Ring-necked Snake
Northern Rough Greensnake
Northern Scarletsnake
Northern Water Snake
Red Cornsnake
Red-bellied Water Snake
Queen Snake
Scarlet Kingsnake
Smooth Greensnake
Southern Copperhead
Southern Ring-necked Snake
Timber Rattlesnake

LIZARDS
Broad-headed Skink
Common Five-lined Skink
Eastern Fence Lizard
Eastern Six-lined Racerunner
Little Brown Skink
Northern Coal Skink (E)
Southeastern Five-lined Skink

SCIENTIFIC NAMES OF MARYLAND CRAYFISHES

Cambarus acuminatus
Cambarus bartonii bartonii
Cambarus carinirostris
Cambarus diogenes
Cambarus dubius
Cambarus thomaii
Cambarus monongalensis
Fallicambarus fodiens
Orconectes obscurus
Orconectes limosus
Orconectes virilis
Orconectes rusticus
Procambarus acutus acutus
Procambarus clarkii
Procambarus zonangulus
Procambarus alleni
Procambarus sp.

SCIENTIFIC NAMES OF MARYLAND FRESHWATER BIVALVES

Alasmidonta heterodon (E)
Alasmidonta undulata (E)
Alasmidonta varicosa (E)
Anodonta implicata
Corbicula spp.
Dreissena polymorpha
Dreissena bugensis
Elliptio complanata
Elliptio fisheriana
Elliptio lanceolata
Elliptio producta (I)
Lampsilis cardium
Lampsilis cariosa
Lampsilis radiata
Lasmigona subviridis (E)
Leptodea ochracea
Ligumia nasuta
Pyganodon cataracta
Strophitus undulatus (I)
Utterbackia imbecillis

COMMON NAMES OF INVASIVE PLANT SPECIES

Autumn Olive
Bamboo sp.
Bull Thistle
Burning Bush
Callery/Bradford Pear
Canada Thistle
Cogon Grass
Daylily (Common)
English Ivy
Garlic Mustard
Giant Hogweed
Japanese Barberry
Japanese Honeysuckle
Japanese Hops
Japanese Knotweed
Japanese Spiraea
Japanese Stilt Grass
Kudzu
Lesser Celandine (Fig Buttercup)
Maiden Grass
Mile-a-Minute
Mimosa
Multiflora Rose
Norway Maple
Oriental Bittersweet
Paulownia (Empress tree)
Phragmites (Common Reed)
Porcelainberry
Privet
Purple Loosestrife
Shrub Honeysuckle
Tree of Heaven
Vinca Vine
Wavyleaf Basketgrass
White Mulberry
Wineberry
Wintercreeper
Wisteria

MBSS Water Quality Chain of Custody Sheet

UMCES - Appalachian Laboratory
301 Braddock Road
Frostburg, MD 21532

Chain of Custody Record
Maryland Biological Stream Survey
Spring Index Period

Date of Shipment

YYMMDD

_____

Cooler Temperature on Receipt

_____ °C

Analyze For:

1-L Grab: DOC, TDP, TDN, Cl, NO_2, NO_3, PO_4, SO_4, NH3, PP, PN
0.5-L Grab: Specific conductance, ANC
Syringe: closed pH

Sample Identification

Site ID	Date YYMMDD	Time Military	Site ID	Date YYMMDD	Time Military
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

Field Comments:

Cooler Contents
Total Number of:

Syringes _____
1-L Bottles _____
0.5-L Bottles _____

Lab Comments:

Cooler relinquished by: _____
(print name)

_____
(signature)

Cooler received by: _____
(print name)

_____
(signature)

Date and Time of Receipt at Laboratory:

YYMMDD Time (24hr)

_____ _____

Page ____ of ____

Guidance for MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

General

This sheet provides a means of tracking the transfer of benthic macroinvertebrate samples between field collecting crews and DNR field office personnel responsible for processing the samples. If multiple sample containers are delivered for a single site, enter each container on a separate row. If entries are repeated down a row, it is not necessary to enter the information in each cell. Simply use an arrow or quote marks to indicate the information is repeated down the row. Please write as legibly as possible following the guidelines below. The entry of a printed name indicates responsibility of the individual for relinquishing or receiving each sample.

1. ***Site ID***	Enter the site ID just as it appears on the field data form.
2. ***Collector (print)***	Print the name of the person who collected the benthic sample.
3. ***Collection Date***	Enter the date the sample was collected (using DD/MM/YY format) just as it appears on the field data form.
4. ***Date Delivered to Field Office***	Enter the date the sample was delivered to the field office using DD/MM/YY format.
5. ***Relinquished By (print)***	Enter the printed name of the person relinquishing the sample to the appropriate field office staff member.
7. ***Received By (print)***	Enter the printed name of the person receiving the sample at the field office.
8. ***Field Office Log-In Number***	(Done by field office personnel) Enter the Benthic Sample Log-in number.
9. ***Comments***	Place any pertinent comments regarding the delivered samples, including unusual circumstances, here. Examples include “label for sample from site HA-P-056-312 fell off - see label in container” or “some of sample for site HA-P-056-312 spilled while in transport”.

If you have questions regarding the use of this sheet or the benthic sample chain-of-custody procedure, call Dan Boward at 410-974-3767.

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

Site ID	Collector (print)	Collection Date (DD/MM/YY)	Date Delivered to Field Office (DD/MM/YY)	Relinquished By (print)	Received by (print)	Field Office Log Number

Comments

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MBSS SPRING INDEX PERIOD DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

DATE Year ____ Month ____ Day ____

CREW: _____

STREAM: _____

TIME ____ (Military)

LOCALITY: _____

SAMPLEABILITY

- ____ Benthos
- ____ Habitat Assessment
- ____ Water Quality
- ____ Vernal Pool ____ (PRESENT/ABSENT)

OTHER (SPECIFY) _____

SITE ACCESS ROUTE

_____

_____

_____

_____

_____

SAMPLE LABELS

Verified by: _____

QC LABEL

Watershed Code ____ Segment ____ (Letters only) Type ____ Year ____

Dup. (D) or Blank (B): ____ Verified by: _____

TEMP. LOGGER

	(Y/N)	(TIME - Military)		
WATER	____	____	#	____
AIR	____	____	#	____

LOCATION _____

_____

_____

_____

PHOTODOCUMENTATION

Number	Title	Voucher (Y/N)
____	_____	____
____	_____	____
____	_____	____
____	_____	____
____	_____	____
____	_____	____
____	_____	____
____	_____	____

BENTHIC HABITAT SAMPLED

- ____ Riffle
- ____ Rootwad/Woody Debris
- ____ Leaf Pack
- ____ Macrophytes
- ____ Undercut Banks
- ____ Other _____

SAMPLING CONSID.: (____ NUM. ANODES) _____

_____

_____

STREAM WIDTH (m) ____ 0 m ____ 75 m

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MBSS SPRING HABITAT DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

DATE Year ____ Month ____ Day ____

____ Dist. from Nearest Road to Site (m)

____ Trash Rating 0 - 20

LANDUSE (Y/N)

- ☐ Old Field
- ☐ Deciduous Forest
- ☐ Coniferous Forest
- ☐ Wetland
- ☐ Surface Mine
- ☐ Landfill
- ☐ Residential
- ☐ Commercial/Industrial
- ☐ Cropland
- ☐ Pasture
- ☐ Orchard/Vineyard/Nursery
- ☐ Golf Course

ROAD CULVERT

Present in Segment (Y/N) ☐

Sampleable? (Y/N) ☐

Width of Culvert (m) ____

Length of Culvert (m) ____

STREAM GRADIENT

	Location (m)	Height (m)
	____	____
1	____	____
	____	____
2	____	____
	____	____
3	____	____

RIPARIAN VEGETATION
(facing upstream)

	LEFT BANK	RIGHT BANK
Width (50m max)	____	____
Adjacent Land Cover	____	____
Vegetation Type	____	____
Buffer Breaks (Y/N)	☐	☐

	LEFT BANK	RIGHT BANK
Storm Drain	☐	☐
Tile Drain	☐	☐
Impervious Drainage	☐	☐
Gully	☐	☐
Orchard	☐	☐
Crop	☐	☐
Pasture	☐	☐
New Construction	☐	☐
Dirt Road	☐	☐
Gravel Road	☐	☐
Raw Sewage	☐	☐
Railroad	☐	☐

Buffer Break Types
(M = minor; S = severe)

CHANNELIZATION

☐ Evidence of Channel Straightening or Dredging (Y/N)

TYPE	EXTENT (m)		
	LEFT BANK	BOTTOM	RIGHT BANK
Concrete	____	____	____
Gabion	____	____	____
Rip-Rap	____	____	____
Earthen Berm	____	N/A	____
Dredge Spoil Off Channel	____	N/A	____
Pipe Culvert	____	____	____

Actual Coordinates
(If >30m distance between original coordinates and stream)

Lat ____

Lon ____

Stream Block Ht. (m) ____

Stream Block Type ____

Lat ____

Lon ____

v. 2009

MBSS VERNAL POOL DATA SHEET

Page ____ of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

Within transect? (Y/N): ____ Lat ____ Long ____

Vernal Pool ID: V P ____

Dimensions: ____ m X ____ m

Max Depth: ____ cm

Landscape Setting: ____ Upland or Floodplain

Predominant Surrounding Landuse: ____ ____ ____ Dist. From Pool

Fish Observed (Y/N): ____

Anostraca Observed (Y/N): ____

HERPETOFAUNA — None Observed ____

	Lifestage				Seen	Heard	Num.	Num. Photos
	Adult	Juv.	Larval	Egg	(Y/N)	(Y/N)	Ret.	Taken
_____								
_____								
_____								
_____								
_____								
_____								

PHOTODOCUMENTATION

Number	Title	Voucher (Y/N)
____	_____	____
____	_____	____
____	_____	____

COMMENTS: _____

_____

_____

Within transect? (Y/N): ____ Lat ____ Long ____

Vernal Pool ID: V P ____

Dimensions: ____ m X ____ m

Max Depth: ____ cm

Landscape Setting: ____ Upland or Floodplain

Predominant Surrounding Landuse: ____ ____ ____ Dist. From Pool

Fish Observed (Y/N): ____

Anostraca Observed (Y/N): ____

HERPETOFAUNA — None Observed ____

	Lifestage				Seen	Heard	Num.	Num. Photos
	Adult	Juv.	Larval	Egg	(Y/N)	(Y/N)	Ret.	Taken
_____								
_____								
_____								
_____								
_____								
_____								

PHOTODOCUMENTATION

Number	Title	Voucher (Y/N)
____	_____	____
____	_____	____
____	_____	____

COMMENTS: _____

_____

_____

v. 2009

MBSS SPRING FAUNA DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

HERPETOFAUNA

☐ None Observed

Species	Lifestage (Adult, Juv., Larval, Egg)	SEEN (Y/N)	HEARD (Y/N)	NUMBER RETAINED	NUMBER PHOTOS TAKEN
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐
	☐ ☐ ☐ ☐	☐	☐	☐	☐

MUSSELS

☐ None Observed

Species	LIVE	DEAD	NUMBER RETAINED	NUMBER PHOTOS TAKEN
	☐	☐	☐☐	☐
	☐	☐	☐☐	☐
	☐	☐	☐☐	☐
	☐	☐	☐☐	☐
	☐	☐	☐☐	☐

Corbicula LIVE ☐ DEAD ☐ NONE ☐

CRAYFISHES

☐ None Observed

Species	NUMBER RETAINED
	☐☐
	☐☐
	☐☐
	☐☐
	☐☐

Crayfish Burrows ☐
(Absent, Present, Extensive)

COMMENTS

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MBSS SUMMER INDEX PERIOD DATA SHEET

Page ____ Of ____

SITE — Watershed Code | Segment | Type | Year

DATE — Year | Month | Day

TIME (Military)

Reviewer: First / Second

CREW: _____

COMMENTS: _____

SAMPLEABILITY

s = Sampleable
1 = Dry Streambed
2 = Too Deep
3 = Marsh, no defined channel
4 = Excessive Riparian Vegetation
5 = Impoundment
6 = Tidally Influenced
7 = Permission Denied
8 = Unsafe (describe in comments)
9 = Beaver
10 = Other _____

- ☐ Electrofishing
- ☐ Habitat
- ☐ Water Quality
- ☐ Herpetofauna
- ☐ Salamanders
- ☐ Crayfishes
- ☐ Mussels
- ☐ Aquatic Plants
- ☐ Exotic Plants

EXOTIC PLANTS

☐ None Observed

Species	Relative Abundance (P or E)
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐
_____	☐

WATER QUALITY

Temp (C) | pH | Turbidity (NTU)

DO (ppm) | Cond (mS/cm)

Meter Calibrations by: _____

Meter ID : _____

AQUATIC VEGETATION

(A, P, or E)

- Submerged Aquatic Vegetation ☐
- Emergent Aquatic Vegetation ☐
- Floating Aquatic Vegetation _____ (A, P, or E)

Didymo ☐ Voucher (Y/N) ☐

PHOTODOCUMENTATION

Number		Voucher (Y/N)
☐☐☐	_____	☐
☐☐☐	_____	☐
☐☐☐	_____	☐
☐☐☐	_____	☐

TEMPERATURE LOGGERS

Air Logger Present? (Y/N) ☐

Water Logger De-watered? (Y/N) ☐

COMMENTS: _____

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MBSS SUMMER FAUNA DATA SHEET

Page ____ Of ____

SITE Watershed Code | Segment | Type | Year

Reviewer: First / Second

STREAM SALAMANDERS

☐ None Observed

Species	Electrofishing Catch (Total)	General Catch (Total)	Transect Catch (Total)	Number Retained	Number Photos Taken

Habitat Types Sampled in 25 m Transect

- ☐ Mud
- ☐ Cobble
- ☐ Boulder
- ☐ Gravel
- ☐ Vegetation
- ☐ Leaf Litter
- ☐ Woody Debris
- ☐ Human Refuse
- ☐ Other _____

OTHER HERPETOFAUNA

☐ None Observed

Species	Lifestage: Adult	Juv.	Larval	Egg	Number Retained	Number Photos Taken

MUSSELS

☐ None Observed

Species	Live	Dead	Number Retained	Number Photos Taken

Corbicula: Live ☐ Dead ☐ None ☐

CRAYFISHES

☐ None Observed

Species	1st Pass Catch (Total)	2nd Pass Catch (Total)	Number Retained

Crayfish Burrows ☐ (Absent, Present, Extensive)

Incidental Catch:

Species

COMMENTS

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MBSS SUMMER HABITAT DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

BANK EROSION

	Left Bank	Right Bank
Extent (m)		
Severity (0=none 1=min 2=mod 3=severe)		
Average Height (m)		

BAR FORMATION & SUBSTRATE

Severity (0=none 1=min 2=mod 3=extensive) ____

- Cobble
- Gravel
- Sand
- Silt/Clay

HABITAT ASSESSMENT

1. Instream Habitat (0-20) ____
2. Epifaunal Substrate (0-20) ____
3. Velocity/Depth Diversity (0-20) ____
4. Pool/Glide/Eddy Quality (0-20) ____
   - Extent (m) ____
5. Riffle/Run Quality (0-20) ____
   - Extent (m) ____
6. Embeddedness (%) ____
7. Shading (%) ____

FLOW

Lat Loc (m)	Depth (cm)	Velocity (m/s)
0 0	0 0	0 0
0 0	0	0

STREAM CHARACTER

- Braided
- Riffle
- Run/Glide
- Deep Pool(>= .5m)
- Shallow Pool(< .5m)
- Gravel
- Sand
- Silt/Clay
- Cobble
- Bedrock
- Boulder >2m
- Boulder <2m
- Beaver Pond
- Overhead Cover
- Undercut Bank

A = Absent P = Present E = Extensive

- No. of Instream Woody Debris ____
- No. of Dewatered Woody Debris ____
- No. of Instream Rootwads ____
- No. of Dewatered Rootwads ____

Maximum Depth (cm) ____

	Wetted Width (m)	Thalweg Depth (cm)	Thalweg Velocity (m/s)
0 m			
25 m			
50 m			
75 m			

Alternative Flow Measurements

- Distance (cm) ____
- Depth (cm) ____
- Width (cm) ____
- Time (sec) 1. ____ 2. ____ 3. ____

COMMENTS ____

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MBSS FISH DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

	(Y/N)
Fish Move. During Net Installation?	
Bottom Visible in all Areas of Seg.?	
Same Water Clarity - 2nd Pass?	
Length of Seg. Sampled (m)	
Fish Captured? (Y/N)	Gamefish? (Y/N)

Time (sec)	Unit___	Unit___	Unit___	Unit___	Unit___
Anodes/Unit					
Begin 1st p.					
Begin 2nd p.					
End 2nd p.					
Volt.					

SPECIES	Number Retained?	1st Pass Catch (Total)	2nd Pass Catch (Total)	Anomalies (Y/N)	Comments

Aggregate Fish Biomass ____ ____ (g)

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MBSS GAME FISH LENGTH DATA SHEET

Page ____ Of ____

SITE Watershed Code ____ Segment ____ Type ____ Year ____

Reviewer: First ____ / Second ____

SPECIES	LENGTH (TL; mm)	SPECIES	LENGTH (TL; mm)
1.		31.	
2.		32.	
3.		33.	
4.		34.	
5.		35.	
6.		36.	
7.		37.	
8.		38.	
9.		39.	
10.		40.	
11.		41.	
12.		42.	
13.		43.	
14.		44.	
15.		45.	
16.		46.	
17.		47.	
18.		48.	
19.		49.	
20.		50.	
21.		51.	
22.		52.	
23.		53.	
24.		54.	
25.		55.	
26.		56.	
27.		57.	
28.		58.	
29.		59.	
30.		60.	

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MBSS GAME FISH LENGTH DATA SHEET (continued)

Page ____ Of ____

SITE Watershed Code | Segment | Type | Year

Reviewer: First / Second

SPECIES	LENGTH (TL; mm)	SPECIES	LENGTH (TL; mm)

MBSS VOUCHER SPECIMEN LABELS

Maryland Biological Stream Survey
SITE ID_____
Cat. No._____ Family:_____
Species:_____
Basin: _____ Date: _____
State: _____ County:_____
Locality: _____
Lat: _____ Lon:_____
Col. By: _____
Det. By: _____No.Specimens:_____

Appendix C: GPS Unit Operation

Standard Operating Procedure KCI-SOP-TE-001

Use of GPS Device (Trimble Pathfinder ProXT Receiver)

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	2/15/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/10/10

Scope and Applicability: This procedure is to be used to operate a Trimble Global Positioning System (GPS) receiver and field computer equipped with ESRI ArcPad software and the Trimble GPScorrect extension.

The Global Positioning System (GPS) is a satellite-based positioning system operated by the U.S. Department of Defense (DoD). A constellation of operational NAVSTAR satellites orbit the earth every 12 hours, providing worldwide, all-weather, 24-hour time and position information.

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in the operation of a Trimble ProXT GPS receiver and field computer running ESRI ArcPad.

References:

Trimble GPS Pathfinder Pro Series User Guide. 2005. Version 1.00, Revision A, May 2005. Trimble Navigation Limited, Westminster, CO.

ESRI ArcPad 7.1 Quick Reference Guide. 2007. Last modified December 14, 2007. http://downloads2.esri.com/support/documentation/pad_/ArcPad7.1_QuickRef_dec2007.pdf

Equipment/Materials:
Trimble Pathfinder ProXT GPS receiver
Lithium-ion battery pack
Field computer with ERSI ArcPad software and GPScorrect extension
Screwthread adaptor
Range pole, backpack with pole, or belt clip
External antenna (optional)

Procedure:

Operation

1. Turn on the receiver and the Bluetooth radio.
    a. Press the green power button briefly to turn on the unit and enable the Bluetooth wireless device. On startup, the three LEDs on the front panel should light up briefly. If they do not light up, check that the battery is installed correctly and is charged.
2. Mount the receiver. The receiver can be mounted either on a screwthread adapter for use on a backpack, range pole, or vehicle magnetic mount or on a belt clip.
    a. To mount the receiver on top of a backpack, range pole or magnetic mount attach the screw thread adaptor to the pole on a backpack or range pole, then slide the receiver onto the screwthread adaptor.
3. Connect the receiver to a field computer.
    a. Turn on the field computer and start the ArcPad software. In the ArcPad menu, select *GPS Active* to activate the GPS controller. If the device fails to connect ensure that the Bluetooth radio is enabled on both the computer and the receiver.
    b. When the GPS is active and receiving data, a red compass-like icon will appear on your map with a yellow cross in the center. If you see an icon of a red circle with a diagonally line running through the center, the GPS is active but not receiving adequate satellite data. Make sure the receiver is in the horizontal position and dense foliage is not blocking its view of the sky. You may need to move around until you acquire enough satellites to obtain a positional fix.
4. Capture GPS data.
    a. Select the appropriate layer in the editor drop down menu and make the layer editable.
    b. Select *Point* from the Point drop-down menu.
    c. Tap the Capture Point Using GPS button and wait for the specified number of GPS positions to be captured and averaged.
    d. Complete the attribute entry form (e.g., Site ID, comments).
    e. Tap the OK button. The point is now saved in the selected shapefile.

Pertinent QA and QC Procedures:

1. A second person should check the map file to see that a new point has been collected and that the attributes are filled out and correct.

Appendix D: YSI ProPlus Operation

Standard Operating Procedure KCI-SOP-WQ-001

Operation of the YSI Professional Plus Instrument

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	5/26/11
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	5/27/11

Scope and Applicability: This method is to be used to calibrate and deploy the YSI Professional Plus (Pro Plus) instrument. This electronic instrument is used to measure dissolved oxygen (mg/L), pH (standard pH units), temperature (°C), and conductivity (µS/cm measured as specific conductance) *in situ* using specialized sensors, or probes. Proper training in its operation is required before use, and the procedure should be reviewed before each deployment.

Measurement of water quality parameters must precede any field activities that disrupt the water column or bottom sediments.

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in calibrating and deploying the YSI Pro Plus instrument. One of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below.

- Provides oversight of daily operations, monitors QC activities to determine conformance, and conducts performance and system audits of the procedures.
- Verifies the completeness of the YSI Calibration Log and Water Quality Field Data Sheet (or electronic data form) to ensure that these forms are filled out as accurately and completely as possible.
- Ensures that all field data sheets (or electronic data forms) are filed properly.

References:

YSI Professional Plus User Manual. 2007. Item #605596, Revision A, August 2007. YSI Incorporated, Yellow Springs, Ohio.

Equipment/Materials:

YSI Pro Plus instrument
C-cell alkaline batteries
Weighted sensor guard
Calibration & storage cup
Calibration Log
ProPlus User Manual
Deionized water
Specific conductance standard (1000 µS/cm)
pH buffer standards (pH 4.0, 7.0 & 10.0)
Dissolved oxygen sensor membranes
Dissolved oxygen sensor electrolyte solution
Philips head screwdriver

Procedure:

Calibration

1. Calibrate the dissolved oxygen (DO) sensor using the following steps:
    a. Verify that the DO membrane is without wrinkles and the DO electrolyte solution does not contain bubbles. Should either of these conditions exist, replace the membrane and electrolyte solution according to the manufacturer's instructions (YSI, 2007), prior to calibration.
    b. Turn the display unit on and press the Calibrate button. Highlight 'DO' and press Enter.
    c. Highlight *DO %* and press Enter to confirm.
    d. Pour a small amount of water (approximately 1/8 inch) into the bottom of the plastic storage cup and screw it onto the probe cable assembly. Disengage a thread or two to ensure atmospheric venting, and make sure that there are no water droplets on the DO membrane. After checking the sensor for water droplets, wait approximately 5 to 10 minutes for the storage container to become completely saturated and allow the sensor to stabilize.
    e. Wait for the temperature and DO% values under "Actual Readings" to stabilize, then highlight *Accept Calibration*. Press Enter to calibrate and the message line at the bottom of the screen will display "Calibration Successful". Press Esc to cancel the calibration and "Calibration Aborted" will display in the message line.
    f. Record both the value under 'Actual Readings' before calibration and the value displayed by the unit after calibration in the Calibration Log.

2. Calibrate the conductivity sensor using the following steps:
    a. Turn the display unit on and press the Calibrate button. Highlight 'Conductivity' and press Enter.
    b. Select *Sp. Conductance* and press Enter. Specific conductance is a measure of conductivity reported as a temperature compensated value using a reference temperature of 25°C.
    c. Place the sensor into a fresh, traceable conductivity calibration solution. The solution must cover the holes for the conductivity sensor near the cable. Ensure the entire conductivity sensor is submerged in the solution or you will get approximately half the expected value.
    d. Choose the units in SPC-us/cm and press Enter.
    e. Highlight *Accept Calibration* to accept the actual reading or scroll to *Calibration Value* to enter the numeric entry screen to manually change the reading and press Enter. Once you enter *Accept Calibration* the message line will display "Calibration Successful". Press Esc to cancel the calibration and "Calibration Aborted" will be displayed in the message line.
    f. You may receive a message indicating that the cell constant is out of range. If this occurs you must choose whether to accept or decline the calibration. Before accepting the out of range value, ensure that the calibration solution is clean, that the correct calibration value was entered if it was entered manually, and that you have cleaned the sensor using the conductivity sensor cleaning brush.

g. Record both the value under 'Actual Readings' before calibration and the value displayed by the unit after calibration in the Calibration Log. If the unit was calibrated out of range, this should be clearly noted in the Calibration Log, and the unit should be replaced as soon as possible if subsequent attempts to calibrate are not successful.

3. Calibrate the pH sensor using the following steps:
    a. Turn the display unit on and press the Calibrate button. Highlight 'ISE1 (pH)' and press Enter. The message line will show the instrument is "Ready for the 1st point" calibration value.
    b. Place the sensor into the first buffer solution and once the reading is stable, highlight *Accept Calibration* and press Enter to accept the displayed calibration value or highlight *Calibration value* and press Enter to input a new calibration value. Once this value is accepted, the message line will display "Ready for 2nd Point."
    c. Rinse the pH probe with water and place the sensor in the second buffer solution, and allow the reading to stabilize. The message line shows the instrument is "Ready for 2^{nd} point" calibration value. Once the readings are stable and the instrument has determined the buffer value, verify *Accept Calibration* is highlighted and press Enter to confirm the second calibration point.
    d. If performing a 3-point calibration, rinse the pH probe, place the sensor in the third buffer solution, and allow the reading to stabilize. The message line shows the instrument is "Ready for 3^{rd} point" calibration value. Once the readings are stable and the instrument has determined the buffer value, verify *Accept Calibration* is highlighted and press Enter to confirm the third calibration point.
    e. After completing the final calibration point you must press *Cal* to finalize the calibration. Pressing *Cal* allows the instrument to accept the calibration information and adjust as needed based on those calibration values. The Actual Readings on this display will NOT reflect the updated calibration information. The values will not change until *Cal* has been pressed and "Calibration Successful!" is displayed in the message line. Do not press *Cal* if you wish to move on to calibrate another point.
    f. In general a 3-point calibration should be performed using buffers 4, 7, and 10. If a 2-point calibration is performed, it should be performed using buffers 7 and 4 as that is the typical pH range for Anne Arundel County streams.
    g. Record both the values under 'Actual Readings' before calibration and the values displayed by the unit after calibration in the Calibration Log.
4. The temperature sensor does not require calibration because it has been factory-calibrated, and the accuracy and precision do not vary over time.

Deployment and Operation

1. The Pro Plus must be calibrated and working properly prior to operation/deployment.
2. Deployment of the Pro Plus must occur prior to any field activities that disrupt the water column or bottom sediments.
3. Press the green Power button to turn the unit on.
4. Remove the storage cup from the end of the Quatro cable assembly and attach the weighted guard. Gently lower the sonde into the water, weighted-end first, and make sure

that all sensors are completely submerged. If placing the sonde into a stream or fast flowing waters it is best to place it perpendicular to the flow and NOT facing into the flow.

5. Monitor the water parameter reading via the LCD data display while allowing the unit to stabilize. If *Auto Stable* is enabled, wait for the 'AS' symbol to stop blinking before recording each parameter. When each parameter has stabilized, record the measurement values on the appropriate data sheet or electronic data form or log the sample on the device.
6. When all measurements have been completed, unscrew the weighted guard and replace the storage cup, ensuring that the sponge is moist or a small amount of water is present when the unit is not in use.

Sample Logging

1. With the sonde deployed, select *Log One Sample* and hit the Enter button.
2. If folders or sites have been pre-entered or uploaded to the device, use the key pad to select the appropriate site from the list. If you are logging samples at a new site, select *Sites*, then in the *Sites List* menu select *Add new* and press the Enter key. Key in the site ID and press Enter.
3. Before logging a sample, ensure that the appropriate site and/or folder is displayed in the *Log One* menu. Highlight the *Log Now!* option and press Enter. An audible beep will occur when the sample has been logged and the message 'Sample Logged' will appear at the bottom of the display screen.

Pertinent QA and QC Procedures:

1. Calibration of pH and DO probes will be performed once daily, prior to initial deployment, using the standard solutions. Calibration of the conductivity probe will occur at least once per week, prior to initial deployment, using a standard solution. Calibrations will be recorded on the Calibration Sheet or in the Calibration Log.
2. Be sure that all buffers and standard solutions used for calibration have not expired. Also, be sure to replace buffers and standards solutions that have an unusual color or odor.
3. If the instrument cannot be calibrated by following this procedure and the information in the instruction manual, making adjustments or replacing sensors if necessary, the unit should be sent to YSI for repair and another unit should be used for the project until repairs are completed.
4. The Field QC Officer must perform QC checks on data sheets/data forms. All field data forms should be filled out as accurately and completely as possible. If a measurement cannot be made or is questionable, comments as to the reason should be recorded.

Appendix E: Hach 2100P Turbidimeter Operation

Standard Operating Procedure KCI-SOP-WQ-002

Operation of the Hach 2100P Portable Turbidimeter

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	2/16/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist / Assistant Division Chief NRM
	Signature:	Date:	3/16/10

Scope and Applicability: This method is to be used to calibrate and operate the Hach Model 2100P Portable Turbidimeter. This electronic instrument is used to measure turbidity using the nephelometric principle of turbidity measurement. Proper training in its operation is required before use, and the procedure should be reviewed before each deployment.

Measurement of turbidity must precede any field activities that disrupt the water column or bottom sediments.

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in calibrating and operation of the Hach 2100P Turbidimeter. One of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below.

- Provides oversight of daily operations, monitors QC activities to determine conformance, and conducts performance and system audits of the procedures.
- Verifies the completeness of the Hach 2100P Turbidimeter Calibration Log and Water Quality Field Data Sheet (or electronic data form) to ensure that these forms are filled out as accurately and completely as possible.
- Ensures that all field data sheets (or electronic data forms) are filed properly.

References:

Hach Model 2100P Portable Turbidimeter Instrument and Procedure Manual. 2003. Cat. No. 46500-88. Hach Company, Loveland, Colorado.

Equipment/Materials:

Model 2100P Portable Turbidimeter
AA-cell alkaline batteries
Carrying case
Silicone oil, 15-mL dropping bottle
Calibration Log
Set of StablCal primary standards
Gelex secondary standards
Glass sample cells
Clean, lint-free cloth

Procedure:

Calibration

1. Prepare the StablCal Stabilized Standards:
   Note: These instructions do not apply to the <0.1 NTU StablCal Standards; <0.1 NTU StablCal Standards should NOT be shaken or inverted.
   a. Shake the standard vigorously for 2-3 minutes to resuspend any particles.
   b. Allow the standard to stand undisturbed for 5 minutes.
   c. Gently invert the vial of StablCal 5 to 7 times.
   d. Prepare the vial for measurement using traditional preparation techniques. This usually consists of oiling the vial (see Hach, 2003) and marking the vial to maintain the same orientation in the sample cell compartment (see Hach, 2003).
   e. Let the vial stand for one minute. The standard is now ready for use in calibration.

2. Calibrate the Turbidimeter using StablCal Stabilized Standards using the following steps:
   a. Insert the StablCal<0.1 NTU standard into the cell compartment by aligning the orientation mark on the cell with the mark on the front of the cell compartment. Close the lid and press the POWER key.
   b. Press the CAL key. The 'CAL' and 'S0' icons will be displayed (the '0' will flash). The 4-digit display will show the value of the S0 standard for the previous calibration.
   c. Press the READ key. The instrument will count from 60 to 0, (67 to 0 if signal average is on), read the blank and use it to calculate a correction factor for the 20 NTU standard measurement. The display will automatically increment to the next standard. Remove the sample cell from the cell compartment.
   d. The display will show the S1 (with the 1 flashing) and '20 NTU' or the value of the S1 standard for the previous calibration. If the value is incorrect, edit the value by pressing the → key until the number that needs editing flashes. Use the ↑ key to scroll to the correct number. After editing, insert the 20 NTU StablCal Standard into the cell compartment by aligning the orientation mark on the cell with the mark on the front of the cell compartment. Close the lid.
   e. Press the READ key. The instrument will count from 60 to 0 (67 to 0 if signal average is on), measure the turbidity and store the value. The display will automatically increment to the next standard. Remove the sample cell from the cell compartment.
   f. The display will show the S2 (with the 2 flashing) and '100 NTU' or the value of the S2 standard for the previous calibration. If the value is incorrect, edit the value by pressing the → key until the number that needs editing flashes. Use the ↑ key to scroll to the correct number. After editing, insert the 100 NTU StablCal Standard into the cell compartment by aligning the orientation mark on the cell with the mark on the front of the cell compartment. Close the lid.
   g. Press the READ key. The instrument will count from 60 to 0 (67 to 0 if signal average is on), measure the turbidity and store the value. The display will automatically increment to the next standard. Remove the sample cell from the cell compartment.
   h. The display will show the S3 (with the 3 flashing) and '800 NTU' or the value of the S3 standard for the previous calibration. If the value is incorrect, edit the value by pressing the → key until the number that needs editing flashes. Use the ↑ key to

scroll to the correct number. After editing, insert the 800 NTU StablCal Standard into the cell compartment by aligning the orientation mark on the cell with the mark on the front of the cell compartment. Close the lid.

i. Press the READ key. The instrument will count from 60 to 0 (67 to 0 if signal average is on), measure the turbidity and store the value. The display will increment back to the S0 display. Remove the sample cell from the cell compartment and press the CAL key to accept the calibration.
j. If 'E 1' or 'E 2' are displayed, an error occurred during calibration. Check the standard preparation and review the calibration; repeat the calibration if necessary. Press the DIAG key to cancel the error message (E 1 or E 2). To continue without repeating the calibration, press the POWER key twice to restore the previous calibration. If 'CAL?' is displayed, an error may have occurred during calibration. The previous calibration may not be restored. Either recalibrate or use the calibration as is.

3. Assign values to Gelex Secondary Standards using the following steps:
    a. Select automatic range mode using the RANGE key.
    b. Thoroughly clean the outside of the Gelex vials and apply a thin coating of silicone oil.
    c. Place the 0-10 NTU Gelex standard in the cell compartment so the diamond on the vial aligns with the orientation mark on the instrument. Close the sample lid.
    d. Press the READ key. Record the displayed value, remove the vial from the instrument and mark the value on the band near the top of the vial.
    e. Repeat step b through step d for the other Gelex standards, being careful to orient the cells properly.

Turbidity Measurement Procedure

1. Select a clear sample cell, fill to the line (about 15 mL), and screw on cap. Always use clean sample cells in good condition as dirty, scratched or damaged cells can cause inaccurate readings. Make sure that cold samples do not "fog" the sample cell.
2. Wipe the cell with a soft, lint-free cloth to remove water spots and fingerprints.
3. Apply a thin, even film of silicone oil to the cell using the oil cloth, as needed.
4. Place the instrument on a flat, sturdy surface and press the POWER button to turn the unit on.
5. Insert the sample cell into the instrument cell compartment so that the diamond or orientation mark aligns with the raised orientation mark in front of the cell compartment, and close the lid
6. If 'AUTO RNG' is not displayed on the LCD screen, press the RANGE key. The display will show 'AUTO RNG' when the instrument is in automatic range selection mode.
7. If 'SIG AVG' is not displayed on the LCD screen, press the SIGNAL AVERAGE key. The display will show 'SIG AVG' when the instrument is in signal averaging mode.
8. Press the READ key. The display will show '----' and a lamp symbol will appear at the bottom left hand corner of the display while the unit is measuring. Record the turbidity value after the lamp symbol turns off and 'SIG AVG' stops blinking.

Sample Collection Procedure

1. Identify a location where the flow is representative of the majority of the reach (e.g., non-backwater if mostly fast-flowing stream) and a representative sample can be collected. Avoid locations where there may be excessive turbulence (i.e., below a cascade or headcut) to minimize excessive gas bubbles within the sample. If the sampler is standing in the flowing water and not on the stream bank, the sample should ALWAYS be collected upstream of where the collector is standing.
2. Using either an empty sample cell or sample collection bottle, submerge the container under the water surface, being careful not to disturb the bottom sediments. Discard the water and repeat two more times to ensure that the container has been rinsed with sample water for a total of three times prior to collection of the sample to be analyzed. If the entire surface is fouled with scum, sheen, or other film, care should be taken to sample the water column below the surface without contaminating the sample. One such technique would be to leave the sample container's cap on while inserting the bottle, cap-side first, into the water column, then inverting the bottle and removing the cap once below the surface and allowing it to fill completely before bringing back up to the surface and out of the water.

Pertinent QA and QC Procedures:

1. Calibration of the turbidimeter should occur once every three months (per manufacturers recommendation), or more often as experience dictates. Periodically, check the instrument calibration using the appropriate Gelex Secondary Standard. If the reading is not within 5% of the previously established value, recalibrate the instrument. Calibrations will be recorded on the in the Calibration Log.
2. Be sure that StablCal Stabilized Standards have not expired and that the vials containing the standards are free of scratches.
3. If the instrument cannot be calibrated by following this procedure and the information in the instruction manual, the unit should be sent to Hach for repair.
4. The Field QC Officer must perform QC checks on data sheets/data forms. All field data forms should be filled out as accurately and completely as possible. If a measurement cannot be made or is questionable, comments as to the reason should be recorded.

Appendix F: Establishing and Marking a Random Site

Standard Operating Procedure KCI-SOP-BI-005

Establishing and Marking a Random Site

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	2/19/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/16/10

Scope and Applicability: This procedure is provided to detail the steps involved in locating, establishing, and marking for return visit, randomly selected biological sampling sites in Anne Arundel County.

Responsibility and Personnel Qualifications: Personnel need to be trained or experienced in the use of global positioning systems (GPS) and field maps or mapping software (e.g., ArcPad) to properly locate and mark sampling sites for evaluation.

References: DNR. 2010. Maryland Biological Stream Survey Sampling Manual: Field Protocols. Revised January 2010. CBWP-MANTA-EA-07-01. Published by the Maryland Department of Natural Resources, Annapolis, MD. Publication # 12-2162007-190.

McCandless, T. 2003. Maryland Stream Survey: Bankfull Discharge and Channel Characteristics of Streams in the Coastal Plain Hydrologic Region. Produced by the U.S. Fish and Wildlife Service, CBFO in cooperation with the Maryland State Highway Administration and the U.S. Geological Survey. Annapolis, MD. 29 pp., plus Appendixes.

Equipment/Materials:

GPS Receiver
Field computer and appropriate GIS software and data layers
300 ft tape measure
Survey pins
Pin flags
Foil tree tags
All weather field notebook
Surveyor's flagging
Hammer
Aluminum nails
Permanent marker

Procedure:

General

Permission to access private property should always be obtained prior to attempting to access the stream and sampling location. The County will provide a list of sites where landowners have granted

permission and/or have made specific requests prior to field crews accessing their property. Field crews should always carry a copy of this list in the field vehicles and consult this list prior to attempting access. Wherever possible, field crews should avoid parking on private property unless specific permission has been obtained. It is advised that field crews make an attempt to talk to landowners if they will be accessing or sampling a site on that person's property and within view of their home. If no one is home, a copy of the County provided permission letter should be left at the front door, wherever feasible. If access is denied, this information should be recorded and an alternate site should be chosen, even if you had prior approval.

Stream Verification

1. Use the GPS device and corresponding field computer with mobile GIS software such as ArcPad to navigate to the pre-determined site. The mobile GIS software should have all necessary data layers installed to assist with selection of the appropriate site location (e.g., orthophotography, County stream layer, NHD stream layer).
2. Verify that the pre-determined site falls on, or in the direct vicinity of, a stream channel.
    a. If the site falls directly on a stream channel and there are no other channels within view (or on the mapping software) procede to step 3.
    b. If the site falls near the confluence of (or between) two stream channels, identify the channel which represents the NHD streamline used to generate the sites. Typically, this will be the larger (i.e., higher order) stream carrying the most flow. It is also advised to check the stream order and drainage area assigned to the site and choose the channel which is most representative of those characteristics. For example, if the target site is on a 2nd order stream with 10 square miles of drainage, you should choose the site with a bankfull width of close to 20 feet, as opposed to only 5 feet. Additionally, regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to validate that the correct site is chosen based on the pre-determined drainage area (McCandless, 2003).
    c. If the site falls 10 or more meters from a stream channel and there are no other channels within view, review the mapping software to verify that the NHD line does in fact represent the nearby stream channel. Navigate to the "intended" channel while choosing a location that approximates the stream features and drainage area that the NHD layer represents. Once you reach the "intended" location, procede to step 3.
3. Determine sampleability of the site. Examples of conditions that could deem a site unsampleable include: obvious tidal influence, ponding (i.e., lentic conditions) caused by beaver dams or other impoundments, lack of a defined channel (e.g., wetlands), unsafe velocities/depths, or sites that overlap another site. Sites rejected as unsampleable are noted as such and the reason for this classification is recorded in the field book.
    a. The crew leader must determine if the site can be samples safely and effectively. Sampling can only be conducted safely if the site is considered wadeable. If the depth or current velocity precludes safe wading, the site should be considered unsafe and not sampleable and an alternate site should be selected.

   b. Sites lacking safe access (e.g., contained within barbed-wire topped chain link fence or within a deep, steep-walled gully) should also be considered unsafe and deemed unsampleable and an alternate site should be sampled in its place.
   c. Streams exhibiting obvious tidal influence or which are no longer contained within a defined channel (i.e, due to impoundment or wetland) are deemed unsampleable and an alternate site should be sampled in its place. However, braided or multi-thread channels in wetland areas are considered sampleable. See the SOP for Cross Section measurement (KCI-SOP-GE-004) for details.
   d. Streams that are completely dry (i.e., no water present) are technically unsampleable for macroinvertebrates, and the site should be replaced with an alternate site. However, sites with minimal flow or standing water in pools are considered sampleable if they have sufficient water to facilitate sampling 20 ft^2 of habitat/substrate and should NOT be replaced with an alternate site. A replacement site can be chosen for partially dry sites ONLY if there is not enough water present to collect the full 20 1-square foot jabs required by the MBSS benthic macroinvertebrate sampling protocol.

4. If only a portion of the sampling reach is unsampleable due to the presence of a culvert, impoundment, etc., the location of a site can be modified to ensure that a sample is collected as close as possible to the location originally chosen for sampling.
   a. In the case of small culverts which cannot be sampled, the length of the culvert should be measured and that distance should be added to the sampling reach. If the culvert occurs in the first half of the site, the additional distance should be added to the downstream end of the site. Similarly, the additional distance should be added to the upstream end, if the culvert is within the upper half of the original site. If the culvert is large enough such that it can be safely sampled completely, no change should be made to the original 75 m site.
   b. In extreme cases, where landowner permission or other sampleability issues prohibit sampling a site in the exact location where the site was chosen, the site may be moved up to one site distance (75 m) from the original location. New coordinates must be provided for the site and substantial documentation must be provided to justify the location change. This option should be used only after all other options have been exhausted based on Crew Leader judgment and landowner permissions will be required if not already obtained.
5. Once it is verified that you have reached the target stream channel and the stream is deemed sampleable, capture the mid-point of the 75 meter reach via GPS following the procedure described in the SOP for GPS Operation and lay out the sampling reach as follows:
   a. Following the sinuosity of the stream channel, measure a distance of 123 feet (37.5 meters) downstream from the mid-point. Note: If the stream has multiple threads, follow the thread carrying the most flow. This becomes the downstream extent of the sampling reach, or 0 meter mark. If a tree is present within five meters of the 0 meter mark, attach a tree tag using a hammer and nail. Survey flagging can also be added to assist with the reach survey, but is not required if a tree tag is present. If a tree is not present with five meters of the 0 mark, or has a diameter breast height (dbh) of less than 2 inches, a tree tag should not be used. If possible, locate the

nearest tree or shrub and attach surveyor's flagging. Tree tags and flagging should be labeled with the site ID, year, and station (e.g., R2-04-02 2010 0m or R2-04-02 2010 75m). If no trees are present a pin flag will be used.

b. Using a survey pin, secure the end of the 300-foot measuring tape at the 0 m mark. Following the thalweg thread of the channel, measure off 246 feet (75 meters). This becomes the upstream extent of the sampling reach. Follow the aforementioned procedures for attaching tree tags and/or flagging at the upstream end (75 m mark) of the reach.

Pertinent QA and QC Procedures:

1. Site assessments should always be completed in teams of at least two people.
2. Professional judgment and caution should be used when entering a stream with high, cloudy, or fast-flowing water. In general sites will not be sampled if water is too elevated to be safe and too elevated and turbid to see channel/habitat features. However, sites that appear to be turbid/elevated due to recent rain should be inspected at a later date to see if it is sampleable.

Appendix G: Aquatic Macroinvertebrate Sampling Method

Standard Operating Procedure KCI-SOP-BI-002

Aquatic Macroinvertebrate Sampling (Freshwater Streams) – Probabilistic Assessment

Prepared by:	Name: Colin Hill	Title:	Environmental Scientist
	Signature:	Date:	2/26/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/10/10

Scope and Applicability: This procedure is for use performing multi-habitat aquatic macroinvertebrate sampling in freshwater streams using Maryland Biological Stream Survey (MBSS) methods.

Responsibility and Personnel Qualifications: Individuals performing aquatic macroinvertebrate sampling should have recently attended the MBSS Spring Index Period training session and should have hands-on training from senior staff experienced in performing this work.

References:

DNR. 2010. Maryland Biological Stream Survey Sampling Manual: Field Protocols. Revised January 2010. CBWP-MANTA-EA-07-01. Published by the Maryland Department of Natural Resources, Annapolis, MD. Publication # 12-2162007-190.

General Notes:

- The sampling net and sieve bucket should be inspected prior to use. Any holes in the net or gaps around the frame repaired prior to sampling.
- If in-stream water quality measurements are to be collected, be sure to do so in undisturbed water and prior to collecting the macroinvertebrate sample.

Equipment/Materials:

Standard aquatic dip net (D-Frame) with 595 µm mesh
Sieve bucket with 595 µm mesh
2 wash buckets
Ethanol (95%)
Sample containers and lids (allow at least 2 containers per sample to be collected)
Field computer (optional)
Waders
Biological Assessment Forms or electronic forms
Internal/External bucket labels
Pencils
First Aid Kit
300 ft tape

Procedure:

1. Navigate to the pre-selected sampling site and verify the sampleability of the stream following the procedures described in the Site Verification and Evaluation SOP (KCI-SOP-BI-004). Following the procedures described in KCI-SOP-BI-005, measure off the sampling reach and mark the upstream and downstream extent of the site.

1. Productive habitat types (riffles, logs, snags, submerged macrophytes, root mats) should be sampled in proportion to their frequency along the assessment reach, with the most productive habitats receiving priority. The person performing the sampling should conduct a brief visual inspection of the reach prior to sampling to determine the proportions of the various productive habitats.

2. A total of 20 square feet of habitat should be sampled throughout the 75-meter assessment reach. The frame of the D-net is approximately one square foot. Therefore, 20 jabs/kicks across productive habitats results in approximately 20 square feet of sample area.

3. Sampling is conducted moving from the downstream extent of the reach to the upstream extent. In general, the D-frame net should be positioned such that the stream flow will flow into the open net, moving sampled material into the net. Sampling is conducted by jabbing the D-frame net into productive habitats or placing the flat end of the net frame along the bottom and kicking to disturb the sampled area (substrate, root mat, etc.).

    1 Riffles – When sampling a riffle place the net firmly in the substrate. Forcefully disturb a one-square-foot patch of substrate on the upstream side of the net allowing the organisms to flow into the net. Any large rocks or sticks should be rubbed by hand to dislodge any organisms clinging to the object.
    2 Logs or Snags – When sampling submerged woody debris the one-foot of sampling area is approximated. Place the net in the water downstream of the log/snag and kick or rub it to dislodge organisms.

3 Leaf Packs – Collect a handful (approximately one square foot) of well-conditioned leaf matter. Positioning your net downstream of the material and vigorously shake the leaves in front of the opening of the net so that dislodged organisms are carried into the net and are captured.

4 Submerged macrophytes and root mats – For these habitats the D-net should be used in a jabbing motion to dislodge organisms. The one-square foot of sampling area is approximated.

5 Sand or fine sediment – Sandy substrates are not considered to be highly productive habitat, but may be the dominant habitat in some stream systems. To sample in a sandy substrate, similar to riffles, hold the net downstream of the area to be sampled and disturb the substrate. The sediment will not need to be disturbed as vigorously as in a riffle. Use the net to sweep through the disturbed area to collect any organisms suspended in the water. Alternatively, the D-net can be bumped along the substrate. Care should be taken not to collect too much sediment when sampling these habitats.

4. Samples should be transferred to the sieve bucket every four or five jabs or more, if necessary. The net should not be allowed to fill with debris as this will disturb the flow of water into the net and organisms may be lost.

5. As the sample is added to the sieve bucket it should be rinsed to remove fine sediments. The D-net should also be rinsed over the sieve bucket allowing any organisms to rinse into the bucket. The net should also be visually inspected to ensure that there are no organisms clinging to the net. The sample in the sieve bucket should be gently mixed by hand while rinsing and large debris should be removed after careful rinsing over the sieve bucket and visual inspection for any remaining organisms (which should be placed back into the sieve bucket).

1. Once the sample has been cleaned, transfer it from the sieve bucket to sample containers. Fill sample containers no more than 75% full to allow for a sufficient volume of ethanol preservative. Be sure to check the sieve bucket for any remaining organisms. A label should be completed in pencil and affixed to the outside of the container (top and bottom). An additional label (also in pencil) should be placed inside the sample container on top of the sample. Sample labels should include the project name, the sample date, site ID and initials of the sampler. Additionally, each label should provide information as to whether there are additional buckets in the sample (i.e., ‘1 of 1’ or ‘1 of 2’, etc.)
   The sample should be preserved with 95% ethanol, with a sufficient volume to fully submerge all detrital material. Samples with large amounts of organic material will require greater amounts of ethanol.

6. Complete the appropriate field data sheets or electronic data forms. Be sure to note the habitats sampled and proportions sampled. Log the sample on the Benthic Log-in form according the procedures described in KCI-SOP-BI-006 (Completing Benthic Sample Log-In SOP).

Pertinent QA and QC Procedures:

1. Field sampling QC involves collecting a replicate sample at 10 percent of sampling reaches to verify the repeatability of the field sampler. The replicate sample should be conducted on a reach immediately upstream of the initial sampling reach. The replicate sample reach should be similar to the initial site in terms of habitat and should have no point source inputs or major tributaries within the sampling reach. The replicate sample is collected in the same manner as the initial sample.

2. Upon entering the data digitally a Project Checklist is completed to track entry and QC of the data.

3. Follow procedures outlined in KCI's current Quality Management System Manual section 3.7 Reviews and Checking Policy.

Example of Project Checklist:

Project: _____
Client: _____

SiteID	Field Work Completed (Date)	Collected By (Initials)	Number of Containers	Benthic Samples Logged-in (Date)	Benthic Samples Received by Lab (Date)	Benthic Data Received by KCI (Date)	Date of Completion + Initials								
							Benthic Data Entry	Benthic Data Entry QC	Reference Reach File Completed	Reference Reach File Checked	GPS Point Post-processed	Drainage Area Delineated	Drainage Area Delineation QC	Land Cover/ Impervious Assess	Land Cover/ Impervious QC

Example of Sample Bucket Label:

Project:_____

Site ID:_____

Sample Date:_____

Samplers:_____

Bucket Number _____ of_____

Appendix H: Chain-of-Custody Completion

Standard Operating Procedure KCI-SOP-BI-007

Completing Biological Sample Chain-of-Custody Record

Prepared by:	Name: Megan Crunkleton	Title:	Environmental Scientist
	Signature:	Date:	02/15/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/16/10

Scope and Applicability: The primary objective of the chain-of-custody procedure is to create a written record that can be used to verify that biological samples were transported from KCI to Environmental Services and Consulting (ES&C) for processing and received by laboratory personnel.

Responsibility and Personnel Qualifications: KCI staff and laboratory personnel must follow written chain-of-custody procedures for transporting/receiving samples.

References:

USEPA. 1995. *Generic Quality Assurance Project Plan Guidance for Programs Using Community Level Biological Assessment in Wadeable Streams and Rivers*. EPA 841-B-95-004. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Equipment/Materials:

Biological Sample Chain-of-Custody (COC)
Plastic resealable bag
Black ballpoint pen

Procedure:

1. After benthic samples have been received, inspected, and recorded in the Benthic Sample Log-In COC (please see the Benthic Sample Log-In SOP KCI-SOP-BI-006 for more detail), KCI laboratory personnel will then document benthic samples before they are delivered to the ES&C laboratory for sorting and identification.
2. KCI office information is filled into the upper left portion of the ES&C COC along with the Project Name and Number and Date the samples are packed for delivery to ES&C (DD/MM/YY).
3. ES&C office information is filled into the upper right portion of the COC (Note: It is not necessary to provide information for the “Sampler” line).

4. For the shipment of sorted samples, 'UPS Ground' will be recorded for the "Shipped Via" line along with the date of delivery. For the delivery of unsorted samples, the initials of the individual who will be transporting the samples to ES&C will be recorded for the "Shipped Via" line along with the date of delivery.
5. Check the appropriate box for "Turn Around".
6. Each sample is recorded under the "Field Sample ID/Description" column. In the "Comment" column record the container number (i.e. 1 of 1; 1 of 2; 2 of 2, etc.).
7. For samples requiring taxonomic identification, 'MBSS Genus' is recorded for "Analysis Requested" and the "Preservation Code" used for all samples is "8" (Ethanol). For samples requiring sorting, the type of sorting protocol and target number should be identified in the comment column.
8. KCI laboratory personnel will sign under "Relinquished by" and record the date (DD/MM/YY) and time (0000) the samples are relinquished from the KCI laboratory.
9. The ES&C COC will then be placed in a plastic resealable bag and will be delivered with the benthic samples to the ES&C laboratory.
10. Once the samples are delivered to ES&C, ES&C laboratory personnel will sign under "Received by" and record the date (DD/MM/YY) and time (0000) the samples were delivered by KCI. A copy of the signed form should then be photocopied and a copy retained for KCI's records.
11. ES&C laboratory personnel will inspect each sample container for damage, leakage, or any other problem and will note the condition of the samples on the COC form. If any problems are found with the samples, laboratory personnel will contact the Project Manager and document what steps were taken to remediate the issue.

Pertinent QA and QC Procedures:

1. A second member of KCI's laboratory staff should check the log-in records to be sure they are complete and correct.

Example of Biological Sample Chain-of-Custody Record:

Environmental Services & Consulting LLC.
516 Roanoke Street • Christiansburg, VA 24073
TEL: (540)552-0144 • FAX: (540) 552-1528 • EMAIL: Info@es-and-c.com

Chain of Custody

Client: _____ Lab Used: _____
Attention: _____ Contact Person: _____
Street: _____ Telephone/Fax: _____
City/State/Zip: _____ Sampler: _____

Project No: _____ Date: _____ Shipped Via: _____ Date: _____

Sample Log and Analysis Requested

MATRIX CODE
DW DRINKING WATER
GW GROUND WATER
WW WASTE WATER
S SOIL
H SOLID
L SLUDGE
O OIL
A AIR
M MISC

PRESERVATION CODES
0 NO PRESERVATIVE
1 HYDROCHLORIC ACID
2 NITRIC ACID
3 SULFURIC ACID
4 SODIUM THIOSULFATE
5 SODIUM HYDROXIDE
6 ZINC ACETATE
7 EDTA
8 ETHANOL

Analysis Requested

Turn Around
☐ Rush
☐ Standard

Lab ID	Field Sample ID/Description	Matrix	Sample Start		Sample Stop		Container		Preservation Code	Comment
			Date	Time	Date	Time	Type	No.		

Relinquished by:	Date	Time	Received by:	Date	Time	CONDITION OF SAMPLES

Appendix I: Stream Cross Sectional Measurement

Standard Operating Procedure KCI-SOP-GE-004

Stream Cross-sectional Measurement

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	5/03/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	5/12/10

Scope and Applicability: This method is to be used to determine the cross-sectional measurement of a stream to be used for performing Rosgen stream type classifications.

Responsibility and Personnel Qualifications: Any person who has received training in performing stream cross-sectional measurements may use this procedure. One of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below:

- Provides oversight of daily operations, monitors QC activities to determine conformance, and conducts performance and systems audits of the procedures.
- Checks the measurement data as they are recorded on the Stream Survey Data Forms or Reference Reach Spreadsheets (Attachment).
- Verifies the completeness of every Stream Survey Data Form or Reference Reach Spreadsheet to ensure that they are filled out as accurately and completely as possible.
- Ensures that all field data sheets are filed or archived properly.
- Ensures that all survey equipment is properly calibrated and is in good working order.

References:

McCandless, T.L. 2003. *Maryland Stream Survey: Bankfull Discharge and Channel Characteristics of Streams in the Coastal Plain Hydrologic Region.* Prepared by the U. S. Fish and Wildlife Service, Chesapeake Bay Field Office, Annapolis, MD, in cooperation with the Maryland State Highway Administration and the U.S. Geological Survey. 29 pp. plus Appendices.

AADPW. 2002. Cypress Creek Tributary Assessment and Findings Report. Prepared by Bayland Consultants and Designers, Inc., and Clear Creek Consulting. 32 pp., plus Appendices.

Rosgen, D.L. 1994. *Applied River Morphology*. Published by Wildland Hydrology, Pagosa Springs, CO.

U.S. Department of Agriculture Forest Service. 1994. General Technical Report, RM-245, *Stream Channel Reference Sites: An Illustrated Guide to Field Technique*. Rocky Mountain Research Station.

Precautions and Notes:

1. Ensure that survey equipment is in good working order and that field crews are trained in survey techniques.

2. All language referring to bank locations within the reach are oriented in the downstream direction. For example, if a step is called for involving the left bank, *then the location of the left bank is determined by facing downstream.*

3. Data should be collected and reported in English units (feet and tenths of feet).

Equipment/Materials:
Stream Survey Data Forms or Reference Reach Spreadsheet
Field logbook
Field computer (optional)
Pencils
Laser level
Top-setting survey rod (25-foot or 4 meter)
Survey pins
½ diameter iron reinforcement bar (2-4 ft length)
2-pound sledgehammer
Survey caps
Flagging
Hammer and aluminum nails
GPS Unit and field computer (optional)
100 meter or 300 foot measuring tape
20 meter or 50 foot measuring tape
Bright-colored (Day-Glo) spray paint (optional)
Compass
Aluminum forestry tags

Procedure:

1. If sampling as part of biological assessment work following Maryland Biological Stream Survey (MBSS) protocols, measure a 75-meter section that is consistent with the desired sampling area, flagging the upstream and downstream 75-meter locations.

2. Determine a representative section of the reach for the cross-sectional measurements. If part of biological assessment work, the cross section should be placed within the sampling reach, subject to the following guidance:

- For low gradient, riffle-pool systems, the section should be located within a riffle section, which is typically located within the straight reach between two meander bends. It is quite possible that a textbook type gravel riffle will not be found within the reach. The important thing is to place it between two meanders in a relatively straight reach. DO NOT install the section on a meander. Avoid pools.

- For higher gradient, step-pool systems, the section should be located just downstream of a step within the run area before the beginning of the pool just upstream of the next step. DO NOT install the section on a step or within the pool upstream of it.

- For streams with a braided or anastamosed plan form, the section should be representative of the stream system and incorporate multiple threads if they are present within the sampling reach. If three or less threads are present, all should be included within the section. If more than three are present, only the three largest threads should be incorporated into the section.

Additionally, the section should be located in an area free from direct anthropogenic alterations that is reflective of local geology such that the stream is able to adjust its banks under its current flow regime. For example, when working in a sand bed stream system, don't put your section in an area where riprap has been installed.

- For streams with direct anthropogenic alterations within the sampling reach (e.g., channel stabilization or straightening), it is advised to conduct the cross sectional survey either a short distance upstream or downstream of the reach, but not more than 100 feet outside of the sampling reach, if the morphology outside of the reach is more representative of a natural channel. See Rosgen (1994) for additional guidance.

4. Once the cross section location has been determined, two permanent monuments (iron rebar) to define the cross section are installed. Install the left bank monument first. The monument should be located either one channel width away from the left top of bank or 15 feet away, whichever is greater. Hammer the rebar into the ground, leaving approximately 6 to 8 centimeters (2 to 3 inches) exposed.

Loosely attach a tape measure to the left monument, or use survey pins, and stretch the tape across the channel to the desired location of the right bank monument. Next, adjust the location of the right bank monument up or down stream such that the flow in the stream is approximately perpendicular to the cross section. Then install the right bank monument.

Spray paint the exposed end of each monument with a bright color (e.g., hunter orange) or wrap with engineering tape. Finish the installation by topping the rebar monuments with survey caps. These caps must be installed *before* any survey data are collected.

5. Record GPS coordinates of each permanent monument.

6. Whenever possible, establish at least one benchmark and collect its relative elevation during the

survey. The benchmark is something prominent and long lasting in the area of each cross-sectional site that can be used to find the site at a later date (e.g., manhole cover, boulder, tree, building, culvert, etc.). Note the compass direction and/or distance to the most convenient monument on the Stream Survey Data Form or spreadsheet. If the benchmark is a tree, label and affix an aluminum forestry tag to the tree using a nail. Be sure and describe any relevant circumstances about the benchmark location, as necessary (e.g., bottom or top of culvert, center of manhole cover, etc.)

7. *After installing zero on the tape measure at the left bank monument*, begin to collect survey data. For each survey point, collect the distance from the left monument and a relative elevation. Data should be collected in feet (tenths).

As the main intent of this task is to characterize the dimensions of the bankfull channel and adjacent floodplain, collect enough survey points from left to right to accomplish this task. Typically, 15 to 20 points are necessary. However, the following minimum elevation points shall be collected:

- Left monument at the ground and on the survey cap.
- Floodplain shots sufficient to characterize topography.
- Left top of bank.
- Left bankfull indicator.
- Other significant depositional features or bank slope breaks on the left side, if present.
- Left edge of water.
- Thalweg.
- Right edge of water.
- Right bankfull indicator.
- Other significant depositional features or bank slope breaks on the right side, if present.
- Right top of bank.
- Floodplain shots sufficient to characterize topography.
- Right monument at the ground and on the survey cap.
- Floodprone width. See Rosgen (1994) for details.

When surveying multi-thread channels, only the main channel will require a detailed survey as described above. Additional channels can be captured in less detail, including elevations of the following points:

- Left top of bank
- Left bank toe
- Thalweg
- Right bank toe,
- Right top of bank
- Right or left bankfull
- Water surface (if necessary)

Additionally, all the threads across the floodplain that were not surveyed should be counted and their locations on the tag line note if they are included between the monuments. Threads located

outside the survey limits established by the monuments only need to be noted. All of the unsurveyed threads should be photodocumented.

When you have entrenched channels in disturbed landscapes, deciding on the bankfull indicator can be challenging. When in doubt, multiple calls for bankfull can be made on the Stream Survey Data Form or spreadsheet. Additionally, regional relationships that relate drainage area to bankfull channel dimensions developed for the Coastal Plain region of the mid-Atlantic (McCandless 2003) or for urban streams in Anne Arundel County (AADPW 2002) can be used to validate the dimensions associated with features of interest. For guidance on selecting the bankfull indicator in the Coastal Plain region, the reader is referred to McCandless (2003).

8. Record all measurements on the Stream Survey Data Form or in the Reference Reach spreadsheet.

Pertinent QA and QC Procedures:
Before the survey instrument is put away, the Field QC Officer must make sure that all the measurements have been recorded in the field. The following field measurements must be taken:

- All features as described above in Item 7.

The Field QC Officer must perform QC checks on data sheets or spreadsheets. All field data sheets should be filled out as accurately, neatly, and completely as possible. If an error is made, mark through the error with a single line and enter date and initials beside the marked-through information.

Appendix J: Abbreviated Stream Longitudinal Profile Measurement

Standard Operating Procedure KCI-SOP-GEO-005

Abbreviated Stream Longitudinal Profile Measurement

Prepared by:	Name: Megan Crunkleton	Title:	Environmental Scientist
	Signature:	Date:	12/09/2010
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	12/10/2010

Scope and Applicability: This method is to be used to determine the elevation of various features in a stream and to determine the overall slope of an assessment reach.

Responsibility and Personnel Qualifications: Any person who has received training in performing stream longitudinal profile measurements may use this procedure. One of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below:

- Provides oversight of daily operations, monitors QC activities to determine conformance, and conducts performance and systems audits of the procedures.
- Checks the measurement data as they are recorded on the Stream Survey Data Forms (Attachment).
- Verifies the completeness of every Stream Survey Data Form to ensure that these forms are filled out as accurately and completely as possible.
- Ensures that all field data sheets are filed properly.

Ensures that all survey equipment is properly calibrated and is in good working order.

References: McCandless, T.L. 2003. *Maryland Stream Survey: Bankfull Discharge and Channel Characteristics of Streams in the Coastal Plain Hydrologic Region.* Prepared by the U. S. Fish and Wildlife Service, Chesapeake Bay Field Office, Annapolis, MD, in cooperation with the Maryland State Highway Administration and the U.S. Geological Survey. 29 pp. plus Appendices.

U.S. Department of Agriculture Forest Service. 1994. General Technical Report, RM-245, *Stream Channel Reference Sites: An Illustrated Guide to Field Technique*. Rocky Mountain Research Station.

Equipment/Materials:

Stream Survey Data Forms (electronic or paper)
Field logbook and pencils
Aluminum forestry tags
Laser or optical level, or total station
Top-setting survey rod (25-foot or 4 meter) and rod level
Bank pins
Hammer and aluminum nails
GPS Unit
100 meter or 300 foot measuring tape
20 meter or 50 foot measuring tape
Compass
Flagging
Bright-colored (Day-Glo) spray paint

Precautions and Notes:

1. Ensure that survey equipment is in good working order and that field crews are trained in survey techniques.
2. All language referring to bank locations within the reach are oriented in the downstream direction. For example, if a step is called for involving the left bank, ***then the location of the left bank is determined by facing downstream.***
3. Data can be collected in SI or English units, but should be reported in English units.

Procedure:

1. If sampling as part of biological assessment work, measure a 75-meter section that is consistent with the desired sampling area, flagging the upstream and downstream 75-meter locations as detailed in the SOP entitled *"Establishing and Marking a Random Site" (KCI-SOP-BI-005).*
2. Determine a representative section of the reach for the cross-sectional measurements. If part of biological assessment work, the cross section should be placed as close to the midpoint of the sampling reach as possible, following guidance found in the SOP entitled "*Stream Cross Sectional Measurement" (KCI-SOP-GE-004).*
3. Complete the top two lines of the Stream Survey Data Form.
4. Once the sampling reach and its cross section location have been established as per the above-referenced SOPs, distance and elevation measurements are taken as close as possible to the end and beginning of the sampling reach (0 and 75 meters). At a minimum, the following points are collected: bankfull elevation, water surface elevation. Coupled with the measurements taken during the cross section survey, you will have three points for any given reach with which to characterize its slope.
   Notes:
   - When you have entrenched channels in disturbed landscapes, deciding on the bankfull indicator can be challenging. When in doubt, multiple calls for bankfull can be made on the Stream Survey Data Form. Additionally, regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to validate the locations associated with features of interest. For guidance on selecting the bankfull indicator in the Coastal Plain region, the reader is referred to McCandless (2003).
   - If the instrument is moved during this work, make sure proper survey work (back shots, height of instrument measurements, etc) is completed in order to tie in the cross section values to the elevation values from the long pro.
   - Water surface slope should be collected on the same type of stream feature at each end of the reach (i.e.—riffle to riffle, pool to pool, etc). This is true even if you collected the water surface slope measurement on a riffle at the cross section.
5. Record all measurements on the Stream Survey Data Form.

Pertinent QA and QC Procedures:

1. The Field QC Officer must make sure that all the measurements have been recorded in the field. The following field measurements must be taken:

    - All features as described above in Item 4.

2. The Field QC Officer must perform QC checks on data sheets. All field data sheets should be filled out as accurately, neatly, and completely as possible. If an error is made, mark through the error with a single line and enter date and initials beside the marked-through information.

Appendix K: Physical Habitat Assessment (MBSS Method)

Standard Operating Procedure KCI-SOP-BI-008

Physical Habitat Assessment (MBSS Methods)

Prepared by:	Name: Andrea Poling	Title:	Environmental Scientist
	Signature:	Date:	6/8/09
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist / Assistant Division Chief NRM
	Signature:	Date:	6/9/09

Scope and Applicability: This procedure is for use performing physical habitat assessments (MBSS methods).

Responsibility and Personnel Qualifications: Individuals performing physical habitat assessments should have recently attended Maryland Biological Stream Survey training and should have hands-on training from senior staff experienced in performing this work.

References:

MD DNR. 2003. A Physical Habitat Index for Freshwater Wadeable Streams in Maryland. CBWP-MANTA-EA-03-4. Published by the Maryland Department of Natural Resources, Annapolis, MD.

MD DNR. 2010. Maryland Biological Stream Survey Sampling Manual: Field Protocols. Revised January 2010. CBWP-MANTA-EA-07-01. Published by the Maryland Department of Natural Resources, Annapolis, MD. Publication # 12-2162007-190.

General Notes:

- The MBSS physical habitat assessments are designed such that some parameters are assessed in the spring sampling index period (March 1 to April 30), while others are assessed in the summer sampling index period (June 1 to September 30). If only one spring site visit is expected, complete the entire form in conjunction with macroinvertebrate sampling in the spring.
- The left and right banks are determined facing upstream unless otherwise noted on the assessment form.
- Habitat assessments are subjective and should be completed with the input of all field crew members.

Equipment/Materials:

Habitat Assessment Sheets or digital data forms	Pencils
Field Computer (optional)	First Aid Kit
Waders	All weather field notebook
300 ft Tape Measure	Digital camera

Procedure:

1. The habitat assessment will focus only on areas within or visible from the 75-meter sampling reach unless otherwise stated.

2. Photos facing upstream and downstream should be taken at the bottom, middle and upstream portions of the sampling reach and noted on the data sheet or in the field notebook.

3. Instream water quality measurements are recorded at the downstream, middle and upstream portions of the sampling reach. Be sure that the sampling probe is set to use the correct units for each parameter or if different mark the units on the sampling form. Note any problems with the sampling equipment or any environmental parameter that may affect water quality measurements (i.e. previous rainfall, the stream was disturbed which may have led to high turbidity measures). Record all measurements on the Physical Habitat Assessment and Water Quality Data Sheet or comparable digital data forms.

4. The stream character portion of the assessment should be filled out using “A” (absent), “P” (present) or “E” (extensive) for each of the parameters listed under stream character. Bar formation, bank erosion and benthic macroinvertebrate habitat sampled is also noted.

5. Rootwads and woody debris (not living) are counted along the assessment reach. Woody debris (logs, dead tree trunks, etc) must be at least 10cm in diameter and greater than 1.5m long and in contact with the wetted portion of the stream. Rootwads are on live trees with a chest high trunk diameter (DBH) of at least 15cm. Instream and dewatered woody debris and rootwads are counted and noted separately. Only dewatered woody debris and rootwads that are likely to become wetted during high flows and dewatered rootwads that provide some bank stability are counted.

6. Complete the Stream Habitat Assessment using the guidance provided on the sheet next to each parameter and on the supplemental guidance sheet. Most parameters are scored on a scale of 1 -20 with the exception of embeddedness and shading which are scored as percentagess. Bank stability is scored for the left and right bank separately and then combined for a final score.

Pertinent QA and QC Procedures:

1. The field crew leader or field QC officer should check all data sheets/forms to ensure that all areas have been filled out completely and accurately.
2. Follow procedures outlined in KCI's current Quality Management System Manual section 3.7 Reviews and Checking Policy.

Physical Habitat Assessment and Water Quality Data Sheet

Site ID: _____
Subwatershed: _____
Length of Reach: _____ Date: _____
Team Members: _____Time: _____

Stream Character

- ☐ Meandering
- ☐ Braided
- ☐ Riffle
- ☐ Deep Pool >.5m
- ☐ Shallow Pool <.5m
- ☐ Gravel
- ☐ Boulder >2m
- ☐ Concrete/Gabion
- ☐ Undercut Bank
- ☐ Overhead Cover
- ☐ Effluent Discharge
- ☐ Emergent Vegetation
- ☐ Floating Vegetation
- ☐ Channelized
- ☐ Straight
- ☐ Run/Glide
- ☐ Silt/Clay
- ☐ Sand
- ☐ Cobble
- ☐ Boulder <2m
- ☐ Bedrock
- ☐ Rootwad
- ☐ Storm Drain
- ☐ Human Refuse
- ☐ Beaver Pond

A=Absent P=Present E=Extensive

Bar Formation

- ☐ None
- ☐ Minor
- ☐ Moderate
- ☐ Extensive

Bank Erosion (see guidance sheet)

	Left Bank	Right Bank	*Severity*
Extent (m)	_____	_____	
Severity	_____	_____	*0=none*
Avg. Height	_____	_____	*1=min*
			2=mod
Maximum stream depth (cm) _____			*3=severe*

Benthic Habitat Sampled (20 total)	**Sq. ft.**
Riffle	_____
Rootwad/Woody Debris	_____
Leaf Pack	_____
Submerged Vegetation	_____
Undercut Banks	_____
Other:_____	_____

Rootwads/Woody Debris

# Instream Woody Debris	_____
# Dewatered Woody Debris	_____
# Instream Rootwads	_____
# Dewatered Rootwads	_____

Stream Gradient

Location (ft)	Height (ft)	Water Depth (ft)
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Weather
Current: _____
Past 24hrs: _____
Past 48hrs: _____

Parameter	Down-stream	Mid-stream	Up-stream
Description			
pH			
Temperature (°C)			
DO (mg/l)			
Conductivity (uS/cm)			
TDS (mg/l)			
Turbidity (NTU)			

Notes: _____

Photographs/Observations:

MBSS Physical Habitat Assessment Sheet

Stream Habitat Assessment Guidance Sheet

Habitat Parameter	Optimal 16-20	Sub-Optimal 11-15	Marginal 6-10	Poor 0-5
1. Instream Habitat[a] SCORE_____	Greater than 50% of a variety of cobble, boulder, submerged logs, undercut banks, snags, rootwads, aquatic plants or other stable habitat	30-50% of stable habitat. Adequate habitat	10-30% mix of stable habitat. Habitat availability less than desireable	Less than 10% stable habitat. Lack of habitat is obvious
2. Epifaunal Substrate[b] SCORE_____	Preferred substrate abundant, stable, and at full colonization potential (riffles well developed and dominated by cobble; and/or woody debris prevalent, not new, and not transient	Abund. of cobble with gravel and/or boulders common; or woody debris, aquatic veg., undercut banks common but not prevalent/suited for full colonization	Large boulders and/or bedrock prevalent; cobble, woody debris, or other preferred surfaces uncommon	Stable substrate lacking; or particles are over 75% surrounded by fine sediment or flocculent material
3. Velocity/Depth Diversity[c] SCORE_____	Slow (<0.3m/s), deep (>0.5m); slow, shallow (<0.5m); fast (>0.3m/s), deep; fast, shallow habitats all present	Only 3 of the 4 habitat categories present	Only 2 of the 4 habitat categories present	Dominated by 1 velocity/depth category (usually pools)
4. Pool/Glide/Eddy Quality[d] SCORE_____	Complex cover/&/or depth >1.5m; both deep (>0.5m)/shallows (<0.2m) present	Deep (>0.5m) areas present; but only moderate cover	Shallows (<0.2m) prevalent in pool/glide/eddy habitat; little cover	Max depth <0.2m in pool/glide/eddy habitat; or absent completely
5. Riffle/Run Quality[e] SCORE_____	Riffle/run depth generally >10cm, with maximum depth greater than 50 cm (maximum score); substrate stable (e.g. cobble, boulder) & variety of current velocities	Riffle/run depth generally 5-10cm, variety of current velocities	Riffle/run depth generally 1-5cm; primarily a single current velocity	Riffle/run depth <1cm; or riffle/run substrates concreted
6. Bank Stability[g] (facing downstream.) Score each bank LB SCORE ____ RB SCORE ____	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected. 10 9	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. 8 7 6	Moderately unstable; 30-60% of bank in reach as areas of erosion; high erosion potential during floods. 5 4 3	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars. 2 1 0
7. Embeddedness SCORE_____%	Percentage that gravel, cobble, and boulder particles are surrounded by fine sediment or flocculent material			
8. Shading[f] SCORE_____%	Percentage of segment that is shaded (duration is considered in scoring). 0% = fully exposed to sunlight all day in summer; 100% = fully and densely shaded all day in summer			
9. Riparian Buffer Zone Width (m)[k] LB_____ RB_____	Zone width in which human activity is not evident. 50m (164ft) is the maximum recorded value.			
10. Remoteness[i] SCORE_____	Roads greater than 400 meters (0.25 mi) from stream	Roads within 400 meters (0.25 mi) of stream; stream NOT accessible by trail.	Roads within 400 meters (0.25 mi) of stream; stream accessible by trail.	Roads adjacent to stream.
11. Aesthetic (trash) Rating[m] SCORE_____	Little or no human refuse visible from stream channel or riparian zone	Refuse present in minor amounts	Refuse present in moderate amounts	Refuse abundant and unsightly
12. Number of Woody Debris and Rootwads[n] SCORE_____	Count only woody debris and rootwads within the wetted portion of the sampling segment. Count only woody debris >10cm (4in) diameter and >1.5m (5ft) long and rootwads with trunk diameter (at chest height) >15cm (6in) that are functional habitat within the wetted portion of the stream.			

Appendix L: Physical Habitat Assessment (RBP Method)

Standard Operating Procedure KCI-SOP-BI-009

Physical Habitat Assessment of Low Gradient Streams (RBP Method)

Prepared by:	Name: Colin Hill	Title:	Environmental Scientist
	Signature:	Date:	2/25/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/16/10

Scope and Applicability: This procedure is for use performing physical habitat assessments on low gradient streams, such as those found on the Coastal Plain physiographic province following the methods described by the U.S. EPA's Rapid Bioassessment Protocols (RBP).

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in habitat assessment procedures for low gradient (Coastal Plain) streams. A second qualified field staff member must be present when a habitat assessment is performed to discuss the habitat assessment scores in order to minimize individual bias in the results. Additionally, one of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below.

- Provides oversight of daily operations, monitors QC activities to determine conformance, and conducts performance and system audits of the procedures.
- Verifies the completeness of the field data sheets or electronic data forms to ensure that they are filled out as accurately and completely as possible.

References:

Barbour, M.T., J. Gerritsen, 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.

General Notes:

- The left and right banks are determined facing downstream unless otherwise noted on the assessment form.
- Habitat assessments are subjective and should be completed with the input of all field crew members.

Equipment/Materials:

Habitat Assessment Sheets or electronic forms Pencils
Waders
300 ft Tape Measure

Procedure:

1. The habitat assessment will focus only on areas within or visible from the 75-meter sampling reach unless otherwise stated.
2. Conduct the habitat assessment using the guidance provided on the Habitat Assessment data sheet. Most parameters are scored on a scale of 1 -20 with the exception of parameters that are scored for each bank. For these parameters, the right bank and left bank are determined while facing in the downstream direction. The following steps provide guidance for evaluating each of the 10 parameters.
3. Epifuanal 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/nursing activities, or feeding. A wide variety and/or abundance of submerged structures in the stream provides macroinvertebrates and fish with a large number of niches, thus increasing habitat diversity. In low gradient streams, Snags and submerged logs are among the most productive habitat structure for macroinvertebrate colonization and fish refugia.
4. Pool substrate characterization evaluates the type and condition of bottom substrates found in pools. Firmer sediment types (e.g., gravel, sand) and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock and no plants. In addition, a stream that has a uniform substrate in its pools will support far fewer types of organisms than a stream that has a variety of substrate types.
5. Pool variability rates the overall mixture of pool types found in streams, according to size and depth. The 4 basic types of pools are:
    1) large-shallow,
    2) large-deep,
    3) small-shallow,
    4) small-deep.

    A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitat to support a diverse aquatic community. General guidelines are any pool dimension (i.e., length, width, oblique) greater than half the cross sectional width of the stream for separating large from small and 1 m depth separating shallow and deep.
6. Sediment deposition measures the amount of sediment that has accumulated in pools and the changes that have occurred to the stream bottom as a result of deposition. Deposition occurs from large-scale movement of sediment. Sediment deposition may cause the

formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increase in size as the channel is diverted toward the outer bank) or shoals, or result in the filling of runs and pools. Usually deposition is evident in areas that are obstructed by natural or manmade debris and areas where the stream flow decreases, such as bends. High levels of sediment deposition are symptoms of an unstable and continually changing environment that becomes unsuitable for many organisms.

7. Channel flow status determines the percent of the channel that is filled with water. The flow status will change as the channel enlarges (e.g., aggrading stream beds with actively widening channels) 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, the amount of suitable substrate for aquatic organisms is limited. In low-gradient streams, the decrease in water level exposes logs and snags, thereby reducing the areas of good habitat. Assess the wetted width of the channel in relation to the width of the channel from bottom of bank elevation.
8. Channel alteration is a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened, or diverted into concrete channels, often for flood control or irrigation purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present 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 and bridges are present; and when other such changes have occurred.
9. Channel sinuosity evaluates the meandering or sinuosity of the stream. A high degree of sinuosity provides for diverse habitat and fauna, and the stream is better able to handle surges when the stream fluctuates as a result of storms. The absorption of this energy by bends protects the stream from excessive erosion and flooding and provides refugia for benthic invertebrates and fish during storm events. To more accurately evaluate this parameter in low gradient streams, a longer segment or reach than that designated for sampling may be incorporated into the evaluation. In some situations, this parameter may be rated from viewing accurate topographical maps.

   For the final three parameters, each bank is evaluated separately and the cumulative score (right and left) is used for this parameter. Right and left banks are determined while facing in the downstream direction. These parameters should be assessed while taking into consideration the conditions occurring within one to two adjacent reach lengths.

10. Bank stability Measures whether the stream banks are eroded (or have the potential for erosion). Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks, and are therefore considered to be unstable. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil. Eroded banks indicate a problem of sediment movement and deposition, and suggest a scarcity of cover and organic input to streams.

11. Bank vegetative protection measures the amount of vegetative protection afforded to the stream bank and the near-stream portion of the riparian zone. The root systems of plants growing on stream banks help hold soil in place, thereby reducing the amount of erosion that is likely to occur. This parameter supplies information on the ability of the bank to resist erosion as well as some additional information on the uptake of nutrients by the plants, the control of instream scouring, and stream shading. Banks that have full, natural plant growth are better for fish and macroinvertebrates than are banks without vegetative protection or those shored up with concrete or riprap. The value of exotic vegetation to the quality of the habitat structure and contribution to the stream ecosystem should be considered in this parameter.
12. Riparian vegetative zone width measures the width of natural vegetation from the edge of the stream bank out through the riparian zone. The vegetative zone serves as a buffer to pollutants entering a stream from runoff, controls erosion, and provides habitat and nutrient input into the stream. A relatively undisturbed riparian zone supports a robust stream system; narrow riparian zones occur when roads, parking lots, fields, lawns, bare soil, rocks, or buildings are near the stream bank. The presence of "old field" (i.e., a previously developed field not currently in use), paths, and walkways in an otherwise undisturbed riparian zone may be judged to be inconsequential to altering the riparian zone and may be given relatively high scores.

Pertinent QA and QC Procedures:

1. The Field QC Officer must check all data sheets or data forms to ensure that all areas have been filled out completely.
2. Digital field forms have been programmed to have a range between zero (0) and 20 (0 to 10 for individual bank parameters), so that values outside of the acceptable range cannot be recorded. Physical habitat parameter fields are programmed as mandatory fields so that no field can be left blank without receiving a prompt warning.
3. Variability should be minimized through proper training of field members, discussing habitat parameters together, and conducting evaluations as a team. Only team members who have been trained by experienced field personnel in conducting RBP habitat assessments for low gradient streams should conduct such evaluations.
4. Follow procedures outlined in KCI's current Quality Management System Manual section 3.7 Reviews and Checking Policy.

Example of RBP Field Data Sheet:

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (FRONT)

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

Parameters to be evaluated in sampling reach

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

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (BACK)

Parameters to be evaluated broader than sampling reach

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

Appendix M: Modified Wolman Pebble Count

Standard Operating Procedure KCI-SOP-GE-003

Modified Wolman Pebble Count

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	3/16/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	5/5/10

Scope and Applicability: This method is to be used to characterize the particle size distribution of a stream reach in conjunction with longitudinal profile and cross-sectional measurement procedures while performing a Rosgen Level II geomorphic stream assessment on a 75 meter survey reach. This method provides a particle size distribution that is collected proportionally, or weighted, to the streams bed features (riffles, pools, runs, glides).

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in modified Wolman pebble count procedures. One of the field staff members must take on the role of Field Quality Control (QC) Officer. The roles and responsibilities of the Field QC Officer are described below.

References:

Harrelson, Cheryl C; Rawlins, C. L.; Potyondy, John P. 1994. Stream channel reference sites: an illustrated guide to field technique. Gen. Tech. Rep. RM-245. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 61 p.

Rosgen, D.L. 1994. Applied River Morphology. Published by Wildland Hydrology, Pagosa Springs, CO.

General Notes:

- The procedure can be modified to characterize the local cross-section particle size distribution by conducting steps 3-5 in the immediate upstream and downstream vicinity of the cross-section.

Equipment/Materials:
Pebble Count Field Data Sheet or Reference Reach Spreadsheet (.xls file)
Field computer (optional)
Pencils
Metric ruler with millimeter increment
Sand-gauge

Procedure:

1. Estimate the distribution of channel features (i.e., riffles, pools, runs, glides) within the 75 meter survey reach.
2. Proportionally distribute a total of 10 transects throughout the survey reach based on the percentage composition of each feature type in relation to the overall reach. For example, a 100 foot survey reach that has 30 feet of pools (30%), 30 feet of runs (30%) and 40 feet of riffles (40%), will have transects allocated so that 3 transects will occur in pools, three 3 in runs, and four 4 in riffles.
3. Measure a total of 10 particles along each transect, spanning the width of the bankfull channel. Count a total of 100 particles throughout the study reach.
    a. Particles should be selected at random from equally spaced intervals across the transect. To avoid bias, the person performing the pebble count should not look down toward the stream bottom when selecting a particle. They should reach forward with their index finger extended and measure the first particle their finger encounters. This process will be repeated until all 10 particles are measured across the transect and all 10 transects are assessed.
    b. The number of particles sampled from the bank surface (the area between the bottom of bank and the bankfull elevation) should be proportional to the amount of bankfull width surface that is comprised by the bank surfaces. For example, if each bank surface is 1 ft high at bankfull stage (2 feet total for both banks) and the bankfull width is 20 ft, then the bank surface is estimated as 10% of the bankfull channel width and 10% of samples, or one particle per transect, should be sampled from the bank surface. Unless the bank surface is estimated to be 20% or more of the bankfull width, the first and last particles of each transect should not be collected from the bank surfaces.
    c. Particles are measured to the nearest millimeter along the intermediate axis using a metric ruler. Sand is classified into categories based on grit. The person performing the pebble count should call out each measurement to be recorded on the Pebble Count Field Data Sheet by the person recording the data. The person recording the data should also note the feature type (riffle, run, glide, pool) on the form for each transect.
4. If a survey reach is comprised entirely of sand materials, 2 transects should be performed at locations that are representative of the entire reach to determine the percentage of sand types present. These percentages can then be applied to the entire survey reach without having to sample all 100 particles.
5. Record all measurements on the Pebble Count Field Data Sheet or electronic data form. Transcribe data to the lower table prior to data entry if using a paper form.

Pertinent QA and QC Procedures:

1. The Field QC Officer must make sure that 100 particles have been measured in the field.

2. The Field QC Officer must perform QC checks on data sheets/electronic data forms. All field forms should be filled out as accurately and completely as possible. Any errors found should be corrected prior to leaving the site.

3. Upon entering the data digitally a Project Checklist is completed to track entry and QC of the data.

4. Follow procedures outlined in KCI's current Quality Management System Manual section 3.7 Reviews and Checking Policy.

PEBBLE COUNT FIELD DATA SHEET

STATION NAME _____ LOCATION _____
STATION # _____ STATION TYPE _____ PROJECT NAME _____
LAT _____ LONG _____ RIVER BASIN _____
COUNTY _____ ECOREGION _____
INVESTIGATORS _____
FORM COMPLETED BY _____ DATE _____ TIME _____ (MILITARY)

		Grabs									
Transect	**Feature Type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

Abbreviations:

Silt/Clay	= SC	Sand - Coarse	= C
Sand - Very Fine	= VF	Sand - Very Coarse	= VC
Sand - Fine	= F	Boulder	= B
Sand - Medium	= M		

Feature Types:
Riffle
Run
Glide
Pool

After recording transects above, transcribe data into table below. Usually done by data entry person.

Size Class		**Size (mm)**	**Feature**	**Number**	**Feature**	**Number**	**Feature**	**Number**	**Total (for all features)**	**Cumulative Total (for all sizes)**
Silt/Clay		< 0.062								
Sand	Very Fine	0.062-0.125								
	Fine	0.125-0.25								
	Medium	0.25-0.50								
	Coarse	0.50-1.0								
	Very Coarse	1.0-2.0								
Gravel	Very Fine	2-4								
	Fine	4-6								
		6-8								
	Medium	8-12								
		12-16								
	Coarse	16-24								
		24-32								
	Very Coarse	32-48								
		48-64								
Cobble	Small	64-96								
		96-128								
	Large	128-192								
		192-256								
Boulder	Small	256-384								
		384-512								
	Medium	512-1024								
	Large - Very Large	1024-4096								
Bedrock		> 4096								

Appendix N: Sample Log-in Procedure

Standard Operating Procedure KCI-SOP-BI-006

Completing Benthic Sample Log-In

Prepared by:	Name: Megan Crunkleton	Title:	Environmental Scientist
	Signature:	Date:	02/15/10
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	3/10/10

Scope and Applicability: The primary objective of the sample log-in procedure is to create a written record that can be used to verify that a sample was collected, transported to the lab, and received by laboratory personnel at KCI.

Responsibility and Personnel Qualifications: Field crews and laboratory personnel must follow written log-in procedures for collecting and transporting samples.

References:

U.S. EPA. 1995. Generic Quality Assurance Project Plan Guidance for Programs Using Community Level Biological Assessment in Wadeable Streams and Rivers. EPA 841-B-95-004. Office of Water, Washington D.C.

Equipment/Materials:

KCI Benthic Sample Log-In Chain-of-Custody (COC)
Pencils

Procedure:

1. Information in columns 1-4 of the Benthic Sample Log-In COC will be recorded on-site immediately following collection of a sample. Information in columns 5-10 will be recorded once the field crew delivers samples to laboratory personnel at KCI.
2. Once the macroinvertebrate sample has been collected, transferred to the sample container(s), filled with 95% ethanol, and all labels (internal and external) are completely filled in and placed in and on the sample container (please see KCI-SOP-BI-001 or KCI-SOP-BI-002 for more detail), the sample should then be logged into the Benthic Sample Log-In COC.
3. With a pencil, record the date the sample was collected (DD/MM/YY) in the first column and in the second column record the site ID. Be sure to include the entire site ID to ensure that replicate sites are identified properly.

4. There may be times when the sample at a particular site exceeds the capacity of one sample bucket due to the amount of debris (leaves, twigs, sand, and detritus) retained in the D-net during sample collection. In this instance, two (or more) buckets must be used to preserve the entire sample. In the third column, record the number of containers used for the sample collected at this site.
5. Record the sampler's initials in the fourth column.
6. The Benthic Sample Log-In will be delivered to the KCI laboratory with the samples.
7. Once samples are delivered to the KCI laboratory by the field crew, KCI laboratory personnel will thoroughly inspect the sample containers and note the condition of the sample and any damage or breakage in the "Condition of Sample/Notes" column of the Log-In COC.
8. Laboratory personnel will review the Benthic Sample Log-In and verify that each sample, in its entirety, is accounted for in the delivery (i.e. if it is recorded that a site consists of two buckets, personnel will verify that bucket 1 of 2 and bucket 2 of 2 of the sample are accounted for).
9. Laboratory personnel will record the date received, site name, and the number of containers for each sample collected and recorded by the field crew in columns 5-7 of the Log-In COC.
10. For each sample, laboratory personnel will review the internal and external labels to assure that the site name and additional site information match for both labels—recording "yes" or "no" in the Internal and External Labels Match column. If any problems are found with the samples, laboratory personnel will contact the field crew and document what steps were taken to remediate the issue in the "Condition of Sample/Notes" column.
11. Laboratory personnel will initial the Log-In COC upon completion of sample inspection.

Pertinent QA and QC Procedures:

1. A second member of KCI's laboratory staff should check the log-in records to be sure they are complete and correct.

Example of Benthic Sample Log-In COC:

BENTHIC SAMPLE LOG-IN									
In Field				In Lab					
Date Collected	Site ID	Number of Containers	Collected By (initials)	Date Received	Site ID	Number of Containers	Internal and External Labels Match?	Received By (initials)	Sample Condition / Notes

Appendix O: Benthic Macroinvertebrate Sample Processing

Standard Operating Procedure KCI-SOP-BI-004

Benthic Macroinvertebrate Sample Processing – 100 Organism Subsample

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	9/4/09
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist / Assistant Division Chief NRM
	Signature:	Date:	11/23/09

Scope and Applicability: This method is to be used to process and subsample benthic macroinvertebrate samples collected from wadeable streams in Maryland. This procedure is specific to a 100-organism subsample, resulting in between 100 and 120 organisms for taxonomic identification.

Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in processing and subsampling benthic macroinvertebrate samples. A laboratory staff member qualified to perform quality control (QC) checks, a QC Officer, must be present when samples are processed by an inexperienced (unverified) individual, or when QC checks are needed for 10% of an experienced (verified) sorter's samples. The qualifications of this individual include achieving ≥90% sorting efficiency. The roles and responsibilities of the QC Officer are described below.

1. Provides oversight of daily operations and sample processing, monitors QC activities to determine conformance, and conducts performance and systems audits of laboratory procedures.

2. Checks all grids sorted by "unverified" laboratory personnel (i.e., those who have not achieved a ≥90% sorting efficiency) for missed organisms and records the number of missed organisms in the appropriate blank of the Laboratory Bench Sheet.

3. Determines the sorting efficiency for each sample and sorter, and checks 10% of samples sorted by "verified" laboratory personnel (i.e., those who have demonstrated the ability to achieve a sorting efficiency of ≥90% on five or more consecutive samples).

4. Performs evaluations to ensure that QC is maintained throughout the laboratory sorting and subsampling procedure.

References:

Caton, L.W. 1991. Improved sub-sampling methods for the EPA "Rapid Bioassessment" benthic

protocols. Bulletin of the North American Benthological Society 8(3):317-319.

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

Tetra Tech, Inc. 2006. Random subsample routine spreadsheet. Developed by Erik W. Leppo of Tetra Tech, Inc., Owings Mills, MD

Precautions:

1. The QC checks in the Pertinent QA and QC Procedures section must be performed only by qualified personnel (QC Officers).
2. Sorting equipment should be thoroughly cleaned and free of organisms prior to sorting the next sample.

Equipment/Materials:

Forceps
70% ethanol
95% ethanol
Standardized gridded screen (595-µm mesh, 30 grids 6cm X 6cm)
6-cm^2 metal "cookie cutter" dividing frame
12" diameter plastic sieve; U.S. #30 (595-µm mesh)
Large and small petri dishes
Scissors
100-mL polyethylene vials
Halogen desk lamp
500-ml squirt bottles
Metal scoop
Plastic bucket or sorting tray
Dissecting microscope
Sample labels
Laboratory bench sheet

Procedure:

<u>Subsampling</u>

1. Before processing any sample, carefully check all labels (internal and external) to ensure that the correct sample is being sorted, and to identify if there is more than one bucket that both are from the same sample before homogenizing the sample. Record the Project No., Sample ID, and Date sorted at the top of the Laboratory Bench Sheet.

2. In a large sink, pour the contents of one or more buckets into a U.S. #30 (595µm mesh) sieve over a large container to collect the ethanol for later use, and gently rinse the contents of each lid and bucket into the sieve. Thoroughly wash the contents of the sieve under running water until the odor from the ethanol preservative is no longer noticeable, taking care not to

damage any organisms. Large objects (e.g., sticks, leaves, rocks) can be cleaned and inspected for attached organisms, then discarded if none are present. Any organisms found on large debris should be removed and placed back into the sieve. If the sample was stored in more than one container, the contents of all containers for a given sample should be mixed thoroughly to homogenize the sample.

3. Transfer sample to the subsampling tray and spread as evenly as possible across the entire tray. Rinse the sieve with a squirt bottle to ensure that all organisms have been transferred to the subsampling tray. If there are multiple buckets resulting in a large amount of material, the sample can be spread over two trays. If two trays are used, this should be recorded on the bench sheet.

4. Using a random number table or random number generator, select 4 pairs of numbers corresponding to 4 individual grids within the gridded tray. Once the numbers are selected, record this on the Bench Sheet along with the initials of the person who will be sorting each grid.

5. Place the metal "cookie cutter" dividing frame over the grid location selected for processing. Using a metal scoop, forceps, or spoon, carefully remove all material (organisms and debris) from within the "cookie cutter'' and place in plastic bucket or sorting tray. If necessary, use a pair of scissors to cut around the edges of the "cookie cutter" to allow removal of larger detritus that is contained within more than one grid. Inspect the screen for remaining organisms and place any found into sorting tray or bucket. The following rules should be applied when dealing with organisms than span across multiple grids:

    a. An organism belongs to the grid containing its head
    b. If it is not possible to determine the location of the head (i.e. worms), the organism is considered to belong to the grid containing most of its body.
    c. If an organism's head lies on the line between 2 grids, organisms along the top and right side of a grid belong to that grid.

6. Add a sufficient amount of water to the sorting tray or bucket to "float" the organisms out of the detritus. If, through a cursory count or observation, there appear to be greater than 120 organisms (cumulative of 4 grids; e.g., more than 30 organisms per grid), then the contents of the 4 grids will be re-spread in a second gridded pan for subsampling, as described in steps 4 and 5. Otherwise, continue to step 7.

<u>Sorting</u>

7. Under a halogen desk lamp, closely inspect the contents of each selected grid, and using forceps, remove any organisms clearly visible or floating on the water surface and place in a small petri dish filled with 70% ethanol. After a brief period of initial inspection, pour small amounts of the suspended material into a large petri dish for closer inspection under the lamp. Any organisms found should be placed into the small petri dish. Continue inspecting

the material, one petri dish at a time, until all organisms are removed and the entire grid has been sorted.

a. Once a grid has been sorted to completion (i.e., all organisms have been removed) all sorted material (i.e., sortate) should be retained for QC inspection. First, drain the water from the sortate by pouring it into a U.S. #30 (595µm mesh) sieve. Then transfer the sortate into a clean container and preserve with recycled 95% ethanol (from step 2). Label the container with both external and internal labels to indicate "Sorted Sample Remains", as well as including the sample ID, grid number, date sorted, and initials of the person who sorted the grid/sample.

8. Using a dissecting microscope, visually inspect all organisms in the small petri dish prior to transferring to a pre-labeled, 100-ml vial filled with 70% ethanol. Do not transfer or count empty snail or bivalve shells, specimens of surface-dwelling or strict water column arthropod taxa (e.g., Collembola, Veliidae, Gerridae, Notonectidae, Corixidae, Cladocera, or Copepoda), or incidentally-collected terrestrial taxa. Do not count fragments such as legs, antennae, gills, or wings, or specimens that do not have heads. If the head cannot be clearly seen, the specimen can be added to the vial but not counted. Also, for organisms that appear to be damaged and may not be identifiable to the target level, the specimen can be added to the vial but not counted. For Oligochaeta, attempt to remove and count only whole organisms and fragments that include the head; also, do not count fragments that do not include the head.

9. Count each organism as they are transferred from the petri dish to the vial. Note this number on the Bench Sheet along with the sorters initials and time required to sort each grid. If the total number of organisms removed from the first four grids is equal to or greater than 100, subsampling is complete for the entire sample. If not, repeat the above process for single successive randomly-chosen grids until at least 100 organisms have been subsampled. **NOTE: Each grid chosen must be sorted to its entirety, even if it results in the total specimen count exceeding 120 organisms.**

    a. For samples where the total number of individuals counted exceeds 120, all organisms should be spread evenly in a 3.5" square petri dish (subdivided into 36 equal sized grids) for further subsampling (i.e., secondary subsampling). Grid numbers will be randomly selected as described above and each individual contained within those chosen grids will be counted and transferred back into the specimen vials until at least 100 (but less than 120) organisms are selected, however, each grid must be counted to completion. The number of grids secondary subsampled and total number of individuals counted and transferred into the vial should be recorded on the bench sheet.

10. Once the target number of organisms is tallied (between 100 and 120 organisms), note the number of grids sorted on the Bench Sheet. If the sample was split and subsampled twice, make a note of the number of grids sorted to get the target number of organisms.

11. Clearly label 100-ml specimen vials with internal and external labels. Labels should include information such as project name, sample ID, date sorted, sorter initials. Caps on the sample vials should be securely fastened with black electrical tape to minimize the potential for leakage during transfer to the lab for identification.

12. After subsampling is complete, transfer the remaining sample material back into the laboratory sieve, allowing the water to drain. Transfer the sample material back into the original sample bucket (or buckets), revise the external and internal labels to indicate "Unsorted Sample Remains", and preserve with 95% ethanol (from step 2) or recycled 95% ethanol (from step 2).

13. Samples incidentally containing more than 120 organisms after taxonomic identification will be subsampled using a spreadsheet-based rarefaction method (Tetra Tech, 2006). This post-processing method randomly subsamples the identified organisms to the desired target number for the sample (e.g., 110 organisms).

Pertinent QA and QC Procedures:

1. Initially, a QC Officer will check all sorted grids from the first five samples processed by a sorter to ensure that all organisms were removed from the detritus. This will not only apply to new sorters, but also to those with previous sorting experienced who have not been "verified". Verification will only occur when a sorter has achieved ≥90% sorting efficiency for five consecutive separate grids or samples.

2. If the sorting efficiency for each of these five consecutive samples is ≥90% for a particular individual, this individual is considered verified and the QC Officer need only check 10% of his or her samples. In the event that an individual fails to achieve ≥90% sorting efficiency, they will be required to sort an additional five samples/grids 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 five samples, whereby they acquire the ≥90% sorting efficiency, the QA Officer may, at his/her discretion, consider this individual to be verified.

3. After individuals have become verified, 10% (1 out of 10) of their samples will be randomly checked by the QC Officer.

4. If a verified individual fails to maintain a ≥90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a ≥ 90% sorting efficiency is achieved again.

5. If a subsampled organism count is ≥100 but the final count of identified organisms falls below 80, verify that the correct subsampling and taxonomic enumeration procedures were implemented. If the correct procedures were followed and the discrepancy is due to a large number of unidentifiable organisms (>20), the sample should be re-spread and an additional grid (or grids) should be picked to obtain a minimum of 80 identified organisms. However, if all 30 grids were already sorted, no further steps are necessary. If numerous discrepancies are observed between subsample and taxonomic identification counts, additional training of

sorters may be required to ensure that only identifiable organisms are counted, or at the very least, fewer than 20 “questionable” organisms are included in a subsample.

6. Follow procedures outlined in KCI’s current Quality Management System Manual section 3.7 Reviews and Checking Policy.

Benthic Macroinvertebrate Laboratory Bench Sheet

Project Number ________________________

Sample ID ________________________ Date Sorted_______________

Grid No.	Sorter	Time Spent	# Found by Sorter	# Found in QC Check	# Found in Subsampling	Total # Found
	Total Time				Total # Orgs.	

Appendix P: Benthic Macroinvertebrate Sorting Quality Control

STANDARD OPERATING PROCEDURE
Macroinvertebrate: Sorting Quality Control

KEY WORDS

APPROVALS
APPROVED BY:_____DATE:_____
Senior Project Manager / QAC

1.0 INTRODUCTION

1.1 Purpose The purpose of this SOP is to provide a methodology for assuring the quality of macroinvertebrate sample sorting. For each project Environmental Services & Consulting undertakes, a percentage of the project's samples will be re-sorted for quality assurance purposes.

1.2 Definitions

1.2.1 Quality Assurance: The steps taken to ensure the proper quality of a product. In this case the steps taken to ensure that a minimum percentage of macroinvertebrates have been sorted from the benthic sample.

2.0 MATERIALS

2.1 Sample: The sample will need to be rinsed and prepared before sorting. Refer to the SOP INVERT001.01 for this procedure.

2.2 Bottle for invertebrates: The sorted macroinvertebrates are stored in a small, labeled container with a lid. The bottle is filled with the appropriate preservative.

2.3 Tweezers: A pair of tweezers are used to sort through the sample.

2.4 Water: Water is used to dilute the sample, making it easier to sort.

2.5 Preservative: Preservative is used to store the invertebrates and prevent decomposition. A premixed solution of 70% ethanol is used unless otherwise noted.

2.6 Flat Dish: A flat dish is used to separate out a small portion of the sample. This allows for easier sorting.

2.7 Counter: A counter is used to count the number of invertebrate sorted.

2.8 Magnification Lamp (optional): A magnification lamp provides light and a magnified viewing area.

3.0 PROCEDURES

3.1 Quality Control of a Trainee: If the employee whose work is being inspected is a trainee, then the person is checked on every tray that they sort.

3.1.1 After the employee finishes sorting the tray he/she is currently working upon, they are to signal to the supervising biologist. The supervising biologist visually inspects they tray with the employee present and shows the trainee and organisms that were missed. This process is continued until the trainee consistently demonstrates the ability to remove over 90% of the organisms present in every tray.

3.1.2 Once the employee demonstrates the ability to sort over 90% of the organisms in a tray, he/she is then reviewed by a sample basis instead of having every tray checked.

3.2 Quality Control of a Trained Employee: If the employee whose work is being inspected is a trained employee, then the sample is re-sorted after the sample has been completed by the employee.

3.2.1 A sample is randomly chosen by the lab manager or approved employee and sorted following the procedures outlined in SOP INVERT002.01.

3.2.2 The sorted organisms are placed in a separate macroinvertebrate bottle labeled with the lab identification number and "QC". If the sample fails the 90% QC standard after identification then the employees other samples are also re-sorted and evaluated. Disciplinary actions are taken on an individual basis.

Appendix Q: Benthic Macroinvertebrate Identification

STANDARD OPERATING PROCEDURE
Macroinvertebrate: Identification

KEY WORDS

APPROVALS
APPROVED BY:_____DATE:_____
Senior Project Manager / QAC

STANDARD OPERATING PROCEDURE
Macroinvertebrate: Identification

1.0 INTRODUCTION

1.1 Purpose The purpose of this SOP is to provide a methodology for identification of macroinvertebrates.

1.2 Definitions:

1.2.1 Lowest Practical Taxon: Defined as the lowest practical taxonomic level an organism maybe identified given a reasonable amount of time and a readily available key. Typically this refers to Genus level, but there are exceptions (Appendix A).

2.0 MATERIALS

2.1 Macroinvertebrate Bottle
2.2 Benthic Macroinvertebrate Laboratory Bench Sheet
2.3 Binocular Microscope
2.4 Forceps (curved or needlepoint jeweler forceps)
2.5 Probes (fine point insect pin with a wooden or plastic handle)
2.6 Petri Dishes (1 large, 1 small)
2.7 Squirt bottle with 75-100 percent Isopropyl or Ethanol alcohol
2.8 Reference Manuals (Appendix B)
2.9 Multi tab counter

3.0 PROCEDURES

3.1 Setting up: After a sample has been sorted and data entered into the LIMS, take the bottle of sorted organisms from the designated area and remove the internal label. (Note: Some samples will have more then one bottle of organisms.) Remove the internal label and view the label under the microscope to verify that no organisms are attached to it. Place any organisms found into the large petri dish. Set aside the clean label for later. Pour some of the alcohol from the macroinvertebrate bottle into the small petri dish. Verify absence of organisms in this dish and set aside for use as accessory dish. Empty the remaining alcohol and organisms from the macroinvertebrate bottle into the large petri dish. Organisms remaining in the bottle can be removed using the squirt bottle. When the macroinvertebrate bottle is empty, set it aside with a small amount of alcohol.

3.2 Identification of Organisms: Using the appropriate references, identify the organisms to the taxonomic level specified by the project (Family, Genus - Lowest Practical Taxon, and/or species). For incomplete specimens enumerate only the anterior ends (head and thorax), depending upon the taxon. Use at least two pieces of literature to

identify each taxon whenever possible. Once identified, note the organism on the bench sheet and on the counter, and placed back into the macroinvertebrate bottle. If all the tabs on the counter are taken, new organisms may be tallied using tick marks on the bench sheet. If more than one life stage of any organism is identified (Larva and Pupa), enumerate and note separately on the bench sheet. The smaller petri dish previously filled with alcohol may be used to partition larger samples for identification, or to clean smaller organisms clinging to larger ones. Remove any terrestrial organisms (including adult Diptera) and debris from the sample and disposed of them in an approved manner.

3.2.1 Unidentifiable Organisms: Immature or damaged organisms that cannot be identified to the required level should be identified to the lowest taxonomic level possible. (Ex: Family instead of Genus). If applicable, some of these may be grouped with previously identified organisms in the same sample (Ex. You have several Leptophlebiidae mayflies without gills, and you also have some Paraleptophlebia present. In the absence of any other Leptophlebiidae genera it may be acceptable to assume all are Paraleptophlebia).

3.2.2 Chironomidae and Oligocheata: For samples requiring identification of Chironomidae and/or Oligocheata to Tribe, Genus or Species level, count these organisms and set them aside in a small vial with an appropriate internal label for later identification via the appropriate Preparation/Identification SOPs.

3.3 Taxonomic Certainty Rating (TCR): A taxonomic certainty rating is written next to each taxon identified on the bench sheet. This is a rating of the taxonomist's comfort level with the specific identification. The range of this rating is from 1 to 5, with 1 being the most certain. For TCR ratings above 3, note why the identification is uncertain (immature organism, missing parts (legs, gills, cerci, etc).

3.4 Reference Organisms: When referencing an organism, either for the client, or for company use (if allowed by the client) the representative organism(s) is placed in a separate vial with an internal reference label. The label, written in pencil, contains the following information: sample number, station where sample was collected, the ID of the organism(s), collector's name or initials and date of collection, the taxonomist's name or initials, and the date of identification. Note the number of organisms removed for reference, and which collection they were removed for (Lab or Client) on the bench sheet.

3.5 Cleanup: Upon completion of the sample, check both petri dishes to make sure no organisms were missed. Some of the original alcohol may be siphoned from the petri dishes and reused in the macroinvertebrate bottle. Dispose of remaining alcohol in an approved manner. Rinse petri dishes and forceps with running water. In pencil, fill out the label previously removed from the macroinvertebrate bottle and return it to the container. Tightly secure the bottle lid and fill out the external label using a permanent marker. Place the macroinvertebrate bottle on the shelf designated for identified samples.

3.6 Recording Results: Finish filling out the bench sheet. Along with the taxonomist's name and date of identification, calculate the total number of viable organisms and report this number on the bottom of the bench sheet. Total the number of distinct taxa found in the sample, omitting duplicates due to multiple life stages, immature or damaged organisms and report this on the bottom of the bench sheet. Some samples have additional sort QC bugs in a separate macroinvertebrate bottle. To identify these organisms, follow the identification procedures in this SOP, but report these bugs separately on the bench sheet. On the back of the bench sheet fill in the time it took to identify the sample and post any additional comments here. Turn in the bench sheet for entry into the database.

Appendix A: Lowest Practical Taxon (LPT) Level

Here at ES&C, we define LPT as the lowest practical taxonomic level achievable with a reasonable amount of effort and readily available keys. In other words; **Genus** level on all Insecta larvae and aquatic adults (Ephemeroptera, Plecoptera, Odonata, Trichoptera, Megaloptera, Hemiptera, Coleoptera, Neuroptera). Other aquatic macroinvertebrates (Amphipoda, Isopoda, Cambaridae, Gastropoda, and Bivalvia) are also taken to Genus level, with exceptions listed below.

Exceptions include:

- Damaged or immature specimens will be backed to family or order level ID when reasonable doubt exists on genus level taxonomy.
- Chironomidae – family
- Female Crustaceans – family (genus level usually requires mature males)
- Collembola - order
- Bryozoan – super phylum
- Nemertea – phylum
- Nematoda – phylum
- Nematomorpha – phylum
- Cnidaria – phylum
- Rotifera – phylum
- Cladocera – class
- Ostracoda – class
- Turbellaria – class
- Oligochaeta - sub-class
    - Lumbriculidae – family
- Collembola – order
- Acari – order
- Hydrobiidae – family

Where it is possible to identify to a lower taxonomic level for most of these organisms, additional cost may occur due to time and/or supplies needed to do so. Please contact us for pricing for these organisms.

Appendix B: List of Taxonomic Keys Available to ES&C

GENERAL WORKS

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Appendix R: Benthic Macroinvertebrate Identification QA/QC

STANDARD OPERATING PROCEDURE
Macroinvertebrate: Identification Quality Analysis / Quality Control

KEY WORDS

APPROVALS
APPROVED BY:_____DATE:_____
Management

STANDARD OPERATING PROCEDURE
Macroinvertebrate: Identification Quality Analysis / Quality Control

1.0 INTRODUCTION

1.1 Purpose The purpose of this SOP is to provide a methodology QA/QC of identified samples to monitor taxonomist performance and provide feedback necessary to maintain acceptable performance levels. The QC checks will monitor two types of errors: 1) identification errors, 2) enumeration errors.

2.0 MATERIALS

2.1 Datasheets (from original Taxonomist and QC taxonomist)
2.2 Computer with a spreadsheet program (Excel, Quatro Pro, etc).

3.0 PROCEDURES

3.1 Re-Identification: Samples randomly chosen for QAQC should be re-identified completely by another skilled taxonomist following the appropriate Identification SOP.

3.2 Setting up spreadsheet: A spreadsheet will be put together with columns showing Sample number, Order, Family, Genus (and/or Species), along with the total identified by each taxonomist. Additional columns should include the number of agreements at the **Family** level, number of agreements at the **Genus** (or species) level, the number of organisms for which the hierarchical target was obtained by each taxonomist, and the number of differences broken up by **Straight, Heirachical and Missing** between the two taxonomists (Note: These last three columns will only be filled out after the Percent Taxonomic Disagreement (PTD) as been calculated, AND if the PTD is above 10%. There should be 13 columns total. (See Associated Spreadsheed: **IDQAQCExample.XLS**)

3.3 Setting up calculations: At the bottom of the spreadsheet, sum up each column with numeric data. In the following row, figure percent completeness for each taxonomist by taking the sum of the Heirarchical target and dividing by the total number of organisms identified by that taxonomist, multiplied by 100.

Percent Completeness (for T_n) = (HT_n / $Total_n$) X 100

Where HTn = Heirachical total for Taxonomist n
And Total n = The total number of organisms identified by Taxonomist n.

3.3.1 Percent Difference in Enumeration (PDE): Figure the PTD between the two taxonomists using the following equation.

PDE = ([n1 – n2] / (n1+n2)) X 100

Where n1 is the number of specimens counted by 1st taxonomist
And n2 is the number of specimens counted by 2nd taxonomist.

Enumeration error may contribute to uncertainty about the quality of the data, and will also affect the estimation of taxonomic precision (PTD). If a sample shows a high enumeration error, then steps should be taken to figure out why. Usual suspects include faulty counting equipment, different counting rules (ex: counting incomplete specimens (empty mollusk shells, exuvia etc), and/or missing specimens. Samples with high enumeration errors should be re-identified and tallied by one of the original taxonomists (or by a third) to ensure accuracy.

3.3.2. Percent Taxonomic Disagreement: between the two taxonomists or laboratories can be figured out by the following equation.

$PTD = [1-(COMP_{pos} / N)] X 100$

Where $COMP_{pos}$ is the number of agreements (positive comparisons)
And N = the total number of specimens in the larger of the 2 counts.

The PTD should be calculated both for agreements at the Family level, and for agreements at the target level (Genus).

The lower the PTD value, the greater the overall taxonomic precision indicating consistency in sample treatment between the two taxonomists. In samples with unreasonably high disagreement (PDT ≥ 10 %) corrective action should be taken. To help assess where disagreements are prevalent, in samples exceeding the 10% standard the number of straight, hierarchical, and missing errors will be figured out in the last 3 columns of the spreadsheet. By looking at the completed spreadsheet, the taxonomists may be able to reasonably assess significant disagreements on specific taxa – warranting a second look at the sample, and the same taxa from other samples in the series.

3.3.3. Percent Taxonomic Completeness: A measure of the number of valid data points gathered relative to the number of planned data points (Smith et al. 1988) can be obtained via the following equation.

Percent Taxonomic Completeness (abs diff) = $[HT_1 - HT_2]$

Where HT_n Refers to the Hierarchical Target Total for Taxonomist 1 and 2.

3.4. Groups: Where specific large groups are concerned (example – Chironomidae identified to Genus) the PDE and PTD calculations may be used on totals for that specific group to further isolate taxonomy problems from the rest of the sample. (See Associated Spreadsheet: **IDQAQC Example.XLS**).

4. Reconciliation:

4.1. **Significant enumeration** errors will be reconciled by re-enumeration of the sample a third time by the primary taxonomist, the QC taxonomist, or by a third taxonomist. The tally that comes closest to this third viewing of the sample will contain the data to be reported.

4.2. **Taxonomy errors** will be reconciled by reviewing available current literature until agreement between the two taxonomists is found – providing both reconciliation of the sample and education. Where agreement cannot be reached on mature specimens, they will be reviewed by a third taxonomist.

If a taxonomist consistently fails QC checks, all samples previously identified by that taxonomist will be re-identified by another, and the failing taxonomist will be removed from the project assignment.

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Appendix S: Selecting a QC Site for Duplicate Sampling

Standard Operating Procedure KCI-SOP-BI-010

Selecting a QC Site for Duplicate Sampling

Prepared by:	Name: Colin R. Hill	Title:	Environmental Scientist
	Signature:	Date:	2/23/11
Approved by:	Name: Michael Pieper	Title:	Senior Environmental Scientist
	Signature:	Date:	2/23/11

Scope and Applicability: This procedure is provided to detail the steps involved in selecting a QC site for duplicate sampling for randomly selected biological sampling sites in Anne Arundel County. The goal is to identify and sample an adjacent QC site with similar immediate and upstream drainage area characteristics and with an absence of observed potential stressors that may be unique to each site.

Responsibility and Personnel Qualifications: Personnel need to be trained or experienced in the use of mapping software (e.g., ArcMap) to properly review sampling sites for evaluation. The final field-based decision/selection of the QC site is at the discretion of the Field Quality Control (QC) Officer.

References:

Equipment/Materials:
Computer
GIS mapping software
Recent orthophotography and appropriate shapefiles

Procedure:

Desktop Review

1. Plot sampling points using GIS software such as ArcMap to display all potential sampling sites, and add all necessary data layers to assist with locating potential stressor sources (e.g., orthophotography, County stream layer, NHD stream layer, stormwater pipes, roads, utilities).
2. Review each point to determine which sites may be good candidates for a quality control site.
    a. If there appears to be a marked difference (increase or decrease) in potential stressors between the probabilistic site or adjacent 75-meter reach upstream (i.e., presence of road crossings or tributaries, presence of stormwater pipe outfalls or other point source discharges, inconsistent buffers from adjacent land use), the site should be excluded as a quality control site.

   b. If two probabilistic sites are located in close proximity to one another, and there is not a sufficient length of stream between them to fit a duplicate reach (75 meters), the downstream site should be excluded as a quality control site.
   c. If a probabilistic site is located less than 150 meters downstream from a man-made impoundment, the site should be excluded as a quality control site.
3. Of the remaining probabilistic sites not excluded as a potential quality control site, choose the first site visited following the criteria provided below. Final in-field identification of a valid QC site is based solely on observations and visual comparison of the sampled reach and the adjacent 75 meter reach upstream. Habitat assessment evaluations or geomorphological measurements should not be conducted at the potential QC site before the determination is made.
   a. Complete an in-field verification of the presence and absence of stressors as was completed in the desktop review. If there appears to be a marked difference (increase or decrease) in potential stressors between the probabilistic site or adjacent 75-meter reach upstream (i.e., presence of road crossings or tributaries, presence of stormwater pipe outfalls or other point source discharges, inconsistent buffers from adjacent land use), the site should be excluded as a quality control site.
   b. Sites that exhibit considerable differences in physical habitat or geomorphology based on visual observation and comparison should be avoided. This would include sites with a considerable influence from beaver impoundments. As a general rule of thumb, if it is determined that the sites would likely receive different physical habitat condition ratings and/or Rosgen Level I classifications, the site should be avoided.
   c. Sites should not be excluded if only minor differences in physical habitat (e.g., slight difference in the number of woody debris or riffles) or geomorphology (e.g., slight difference in bankfull width) exists.
4. Once a site has been selected that meets the aforementioned criteria, proceed to measure a 75 meter segment upstream of the probabilistic site and repeat the benthic macroinvertebrate sampling process, physical habitat assessment, and in situ water quality measurement after the downstream reach has been completed.

Pertinent QA and QC Procedures:

1. Best professional judgment should be used when evaluating whether or not the physical habitat and/or geomorphic conditions are comparable between reaches.
2. The most recent orthophotography and shapefiles should be used when conducting the desktop review.