QUALITY ASSURANCE PROJECT PLAN FOR THE ANNE ARUNDEL COUNTY BIOLOGICAL MONITORING AND ASSESSMENT PROGRAM

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This document has been produced in accordance with U.S. EPA's Guidance for Quality Assurance Project Plans (EPA/240/R-02/009, U.S. EPA Office of Environmental Information, Washington, DC, December 2002) to ensure that biological and physical monitoring data acquired 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

BIBI	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	Ephemeroptera, Plecoptera, Trichoptera
FIBI	Fish Index of Biotic Integrity
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
TMDL	Total Maximum Daily Load
WPRP	Watershed Protection and Restoration Program

DISTRIBUTION LIST

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1.0 PROJECT/TASK ORGANIZATION

The purpose of this document is to present the Quality Assurance Project Plan (QAPP) for collecting, analyzing, and integrating environmental data from streams and watersheds in order to support a long-term biological monitoring program (Program) for the Anne Arundel County, Maryland, (County) Department of Public Works, Watershed Protection and Restoration Program Division (WPRP). This revised QAPP has been updated to reflect changes following completion of Round 1 (2004-2008) and Round 2 (2009-2013), which will be implemented during Round Three (2017-2021), as currently planned. The QAPP 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 plans to utilize a professional consulting firm under contract to 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 (BIBI), Fish Index of Biotic Integrity (FIBI) 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 quality assurance (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 (including calculations of data 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 Table 1; it reflects the relationships and lines of communication among all participants and data users. The responsibilities of these persons are described below.

The Contractor will provide project oversight for this study through 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 QAPP.
- 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.
- Ensuring that field audits are performed.

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

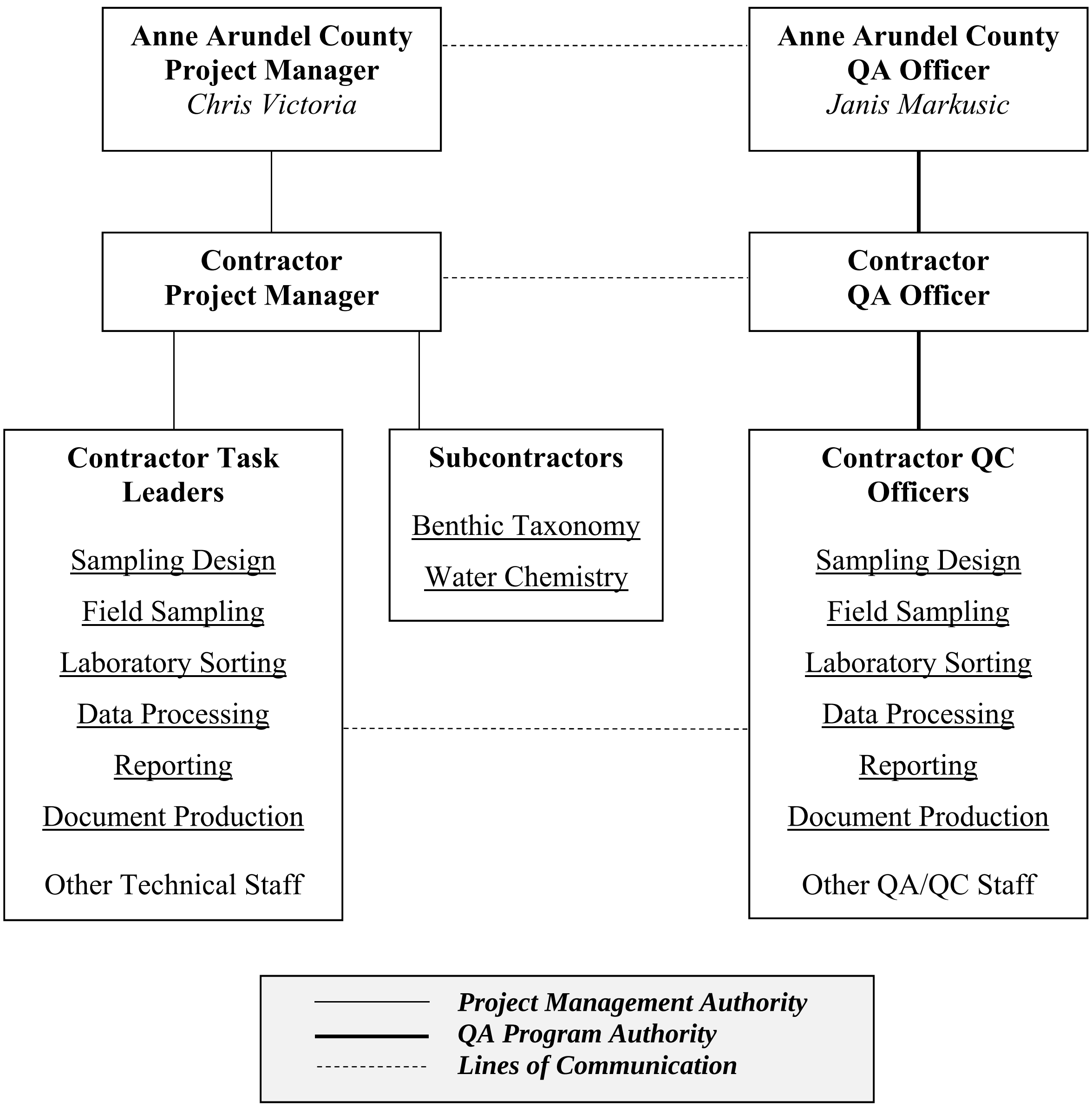

Figure 1. Project Organization

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

- Providing support to the PM in the preparation and distribution of the QAPP.
- Reviewing and approving the QAPP.
- 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.* performing field sampling to obtain biological, chemical water quality, physical habitat, and geomorphological data, processing benthic 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 role of the Field Sampling Task Leader, one of the several Contractor Task Leaders, will be of particular importance. QA/QC may be performed correctly throughout subsequent stages of the project; however, if field data are not collected correctly and according to protocol, calculations and assessments based on those data will still be erroneous. Additionally, while errors in calculations may be corrected at various points during the project, the nature of biological sampling and the sampling index periods makes corrections *(i.e.* re-sampling of benthic macroinvertebrates or fish) difficult and costly. 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 QAPP.
- 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; these QC officers 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 QAPP 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 County specifications.

For this project, the processing of water chemistry samples will be performed by a Subcontractor. Benthic macroinvertebrate sample processing and taxonomic identifications will be provided by a Subcontractor or by Contractor staff. The Contractor PM will oversee work performed by the Subcontractor or Contractor staff to verify that all work assigned 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. The benefits of sampling fish include detection of non-native, invasive species, stressors related to fish health, and barriers to movement.

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 healthy biota. Changes in the quality of the water resource are reflected as changes in the structural and functional attributes of the macroinvertebrate and fish assemblages. 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 and these data are frequently used to guide and support watershed management policies and practices.

Urbanization, which is present throughout much of the County, involves changes to land cover and an increase in impervious surfaces. These impervious surfaces impact the flow regimes of local streams and rivers, leading to decreased infiltration and groundwater reserves, as well as an in increase in flashy storm flows. Such changes in flow lead to alterations in channel shape and stability, which increase stream sediment loads that smother aquatic habitats and benthic species. Unstable stream channels may expose buried utilities and threaten nearby infrastructure. Geomorphic assessments allow for the classification of stream channel type and evaluation of a channel's connection to its floodplain.

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 enhance the characterization of Anne Arundel streams, the 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 Countywide. 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 the 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), continued during Round 2 (2009-2013), and will continue in Round 3 (2017-2021). Previous rounds focused on benthic macroinvertebrate sampling. Round 3 is structured to include sampling for fish, crayfish, mussels, and herpetofauna. 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 its streams. By sampling biology and stream habitat at approximately 384 sites over a 5-year period (an increase from 240 sites each in Rounds 1 and 2, in order to include sampling of smaller streams, not previously addressed) and integrating the biological sampling program results with chemical, hydrologic, physical habitat, geomorphological, and land use data, the County will be able to provide a comprehensive characterization of stream condition.

Questions regarding the County's streams can be asked in three scales: Countywide, 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 Rounds 1 and 2 to those in Round 3?

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 12?

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 certain targeted streams on a Countywide 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 QA requirements and requires oversight by a trained staff person. They can also be divided into a number of tasks, each requiring management and QA by qualified personnel.

Task 1: Develop Sample Design and Select Sites

This task has already been completed for Round 1 (2004-2008), Round 2 (2009-2013), and Round 3 (2017-2021). The sampling frame and site selection process was revised for Round 3 (Southerland et al., 2016; Appendix B and Appendix C). Beginning with Round 3, the sample frame includes two types of streams: first, those shown on the 1:100,000 scale map (same as employed in Rounds 1 and 2), and second, streams that are part of the County's more detailed stream mapping but not on the 1:100,000-scale map. An outline of the site-selection process is included here for future monitoring rounds.

(a) Develop a list of the population of potential sites (i.e., sampling segments) segregated into 24 primary sampling units (PSUs). Each PSU will contain two sampling strata: one stratum being the larger streams (from 1:100,000-scale map) and the other being the smaller streams (occurring on a more-detailed map but not on the 1:100,000-scale map). Eight sites from each stratum will be sampled in each of the 24 PSUs.

(b) The new survey design includes partial replacement of random sites with repeat sites from previous rounds (for the larger stream stratum only). Specifically, two sites in each Round 3 PSU will be randomly chosen from the 10 Round 1 sites, and two sites will be randomly chosen from the 10 Round 2 sites; the remaining four sites in each PSU will be new, randomly selected sites. The final list of sampling segments will be denoted by type (repeat vs. new random), by PSU, and by 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 the County and the Contractor PM.

Task 3: Conduct Sampling

The Contractor will conduct all sampling in accordance with MBSS methods guidance and the approved QAPP (this document). Sampling will occur during the spring index period for benthic sampling (March–April) and summer index period for fish (June-September) of each sampling year, though sampling activities will vary between the two sampling strata (Table 1 and Table 2).

Prior to the start of each year's sampling, the Contractor will review with the County that year's SOPs and field datasheets.

The spring 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 approximately 75 stream sites and 8 duplicate QC sites each year. Sampling will include acquiring latitude and longitude via portable global positioning system (GPS) units; taking site photographs; collecting of water samples for laboratory analysis; *in situ* measurement of 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 95% ethanol, and transported to the Contractor or Subcontractor's laboratory for processing and identification.

The summer sampling team will consist of four to seven persons, depending on the width of the stream, one of whom will be designated as the Field Sampling Task Leader and another designated as the QC Officer. Sampling will include taking site photographs; *in situ* measurement of pH, DO, water temperature, specific conductance, and turbidity; double-pass, quantitative electrofishing (catch and release); assessing physical habitat; and conducting crayfish, mussel, and herpetofauna searches.

Table 1. Sampling Performed for Large Streams During Each Index Period

Spring (March through April)	**Summer** (June through September)
Physical habitat: • MBSS Spring Habitat Assessment • Quantitative geomorphologic assessment • RBP Habitat Assessment	Physical habitat: • MBSS Summer Habitat Assessment • RBP Habitat Assessment
Water quality: • *In situ* • Laboratory sample	Water quality: • *In situ*
Biological monitoring: • Benthic macroinvertebrates • Vernal pools	Biological monitoring: • Fish • Crayfish • Mussels • Herpetofauna

Table 2. Sampling Performed for Small Streams During Each Index Period

Spring (March through April)	**Summer** (June through September)
Physical habitat: • MBSS Spring Habitat Assessment • Quantitative geomorphologic assessment • MBSS Summer Habitat Assessment • RBP Habitat Assessment	Physical habitat: • N/A
Water quality: • *In situ* • Laboratory sample	Water quality: • N/A
Biological monitoring: • Benthic macroinvertebrates • Incidental Crayfish • Incidental Mussels • Incidental Herpetofauna • Vernal pools	Biological monitoring: • N/A

Task 4: Conduct Laboratory Processing

Water samples will be collected, put on ice, and transferred to the laboratory within 36 hours of their collection. Processing at the Subcontractor water quality laboratory will occur within 48-hours for a standard suite of nutrient, metal, and other parameters:

- Turbidity
- Total Nitrogen
- Total Phosphorus
- Ammonia-N
- Total Kjeldahl Nitrogen (calculated)
- Nitrate-Nitrogen
- Nitrite-Nitrogen
- Dissolved Organic Carbon
- Orthophosphate
- Total Organic Carbon
- Total Copper
- Total Lead
- Total Zinc
- Chloride
- Total Hardness

These parameters will address most of the County's Total Maximum Daily Loads (TMDLs).

The benthic 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% 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 County's Watershed Management Tool.

Task 6: Analyze Data

The MBSS BIBI and FIBI will be calculated for large streams (1:100,000-scale map) using the most up-to-date version of the Coastal Plain BIBI, and site assessments performed. At the completion of Round 3 sampling, the County will develop a BIBI for small streams (those not included on the 1:100,000-scale map) using appropriate, small-stream reference conditions. Physical habitat scores will also be calculated, using the Rapid Bioassessment Protocols (RBPs) for Low Gradient streams and MBSS's Physical Habitat Index (MPHI). 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 for each year that sampling is completed. The report will present all physical, chemical, and biological data and assessments, aggregated to watershed scales, with conclusions and recommendations based on evaluation of the results from all watersheds. Individual site assessment summaries will also be provided as an Appendix to each annual report.

Implementation of the monitoring program during each year will proceed with several milestones as presented in Table 3. They include selection of sampling sites, initiation of sampling, fieldwork, laboratory and data analyses, and annual reports. Sites have been selected for Round 3 and initial sampling will begin with the 2017 spring index period (March 1- April 30). If tasks are added to address the monitoring program during additional years, addenda to this QAPP (e.g., sampling and analysis plans) will be prepared and distributed to participating staff.

Table 3. 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 QAPP (January)	—											
Logistical Arrangements & Scheduling for Spring (January – February)	—	—										
Field Sampling (Mar. 1 – Apr. 30; June 1 – Sept. 30)			—	—		—	—	—	—			
Laboratory Processing & Sample Taxonomy (March 15 – July 31)			—	—	—	—	—					
Draft Report to County (early October)								—	—			
County Report Comments to Contractor (early Nov.)										—		
Final Report (mid-December)											—	—

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 members of the Technical Review Committee for Round 3 of the County's Program are in Table 4.

Table 4. Contacts for Members of the Technical Review Committee for Round 3 Anne Arundel County Biological Monitoring Program

Contact/Agency	Program/Area of Coverage/Activities
Scott Stranko Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division	Acting Division Director, Resource Assessment Service, Monitoring & Non-tidal Assessment Division
Matt Stover Maryland Department of the Environment	Head, Water Quality Standards Section
Matt Baker University of Maryland, Baltimore	Associate Professor, Geography and Environmental Systems
Raymond Morgan Frostburg State University	Professor Emeritus, Ecology and Fisheries
Scott Lowe McCormick Taylor	Senior Environmental Scientist
Don Weller Smithsonian Environmental Research Center	Quantitative Ecologist and Senior Scientist
Dennis Whigham Smithsonian Environmental Research Center	Senior Botanist

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 a program. The process of establishing DQOs involves identifying the allowable uncertainty of a dataset which may lead to two types of error: *false positives* (Type I error: a difference is found to exist when in fact it does not) and *false negatives* (Type II error: a difference 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 dataset 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 the Program encompass aspects of field, laboratory analysis, and database management to reduce sources of errors and uncertainty in the use of the data. Data required for the Program are listed in Table 5.

The Program is being undertaken to identify the condition of streams in order 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 the County's 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 (resulting in 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) that have 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 BIBI and FIBI for each site based on multiple metrics and by comparing them 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 indices to be used for the large stream stratum (1:100,000-scale map) were developed by the MBSS (Southerland et al., 2007) and are specifically calibrated to the physiographic regions of Maryland. The County will use the Coastal Plain versions of the BIBI and FIBI to classify its streams. The County will be seeking to develop a BIBI for the small streams (not included on the 1:100,000-scale map) after the completion of Round 3 sampling.

Table 5. 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 [°C]
Specific conductance	microSiemens per centimeter [μS/cm] at 25ºC
Turbidity	nephelometric turbidity units (NTU).
Total Nitrogen	mg/l
Total Phosphorus	mg/l
Ammonia-N	mg/l
Total Kjeldahl Nitrogen (calculated)	mg/l
Nitrate-Nitrogen	mg/l
Nitrite-Nitrogen	mg/l
Dissolved Organic Carbon	mg/l
Orthophosphate	mg/l

Data Type	Measurement Endpoint(s) or Units
Total Organic Carbon	mg/l
Total Copper	µg/l
Total Lead	µg/l
Total Zinc	µg/l
Chloride	mg/l
Total Hardness	mg/l
Biological Parameters	
Benthic macroinvertebrates	number of each taxon
Fish	number of each taxon, aggregate biomass, gamefish lengths, anomalies
Herpetofauna	number of each taxon, life stages observed
Mussels	number of each taxon, life state (live vs. dead)
Crayfish	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 a scale of 0-20
Stream cross sectional measurement, water surface slope, and reach sinuosity	survey of channel dimensions, water depth, feature elevation, and distance along sampling reach, 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 scores and ratings, 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 the Program are:

- What is the status of a particular stream site (based on BIBI and FIBI scores)?
- What is the status of a particular watershed (based on the mean BIBI and FIBI scores, or proportion of BIB and FIBI score meeting a specific threshold)?

The index scores obtained for each site will be compared with thresholds 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, for example, 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 and fish 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 performed for Round 3 are explained in more detail in Appendix B.

The null hypothesis to be tested for Maryland's BIBI and FIBI nonimpaired thresholds (i.e., 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 accepted 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 BIBI for a site indicates that it is nonimpaired and the true value of the BIBI 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 BIBI is below the impairment threshold, then failing to implement actions to improve the stream condition based on acceptance of this value could have negative 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 dataset analysis. The power analysis showed that investigation of a specific impact type would require approximately eight sites affected by the impact, to detect a 30% change in IBI 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* multiparameter water quality instrument, while turbidity will be measured using the multiparameter water quality instrument or 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, a measure of the acidity (hydrogen/hydroxide ion concentration) in surface water. Most aquatic organisms have a preferred range of pH between 6.0 and 9.0. Water temperature will be measured for use in taking temperature-dependent measurements such as pH and specific conductance. Additionally, some aquatic organisms are thermally sensitive and DO concentrations are related to water temperature. Specific conductance is an indirect, aggregate 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 that is influenced by suspended particles and organisms, for example clay or silt sediments and phytoplankton. Many 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 or as per manufacturer's specifications.

Water quality samples will be collected during the spring index period, immediately placed on ice, and transported to a water quality laboratory within 36 hours of their collection (or sooner, depending upon holding time requirements specified by the Subcontractor laboratory). Processing of all samples will take place within 48 hours of their collection. The samples will be processed for a standard suite of analytes (listed in Table 5). All water quality processing will follow approved U.S. Environmental Protection Agency (EPA), or other commonly used, standard methods.

Biological Parameters

The list of candidate metrics describing the benthic macroinvertebrate communities (to be used to calculate the BIBI) and fish communities (to be used to calculate the FIBI) 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 6) which ensures linkage of the data analysis and resultant interpretation to project quality objectives. Samples are assessed by calculating the metrics for 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. 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.

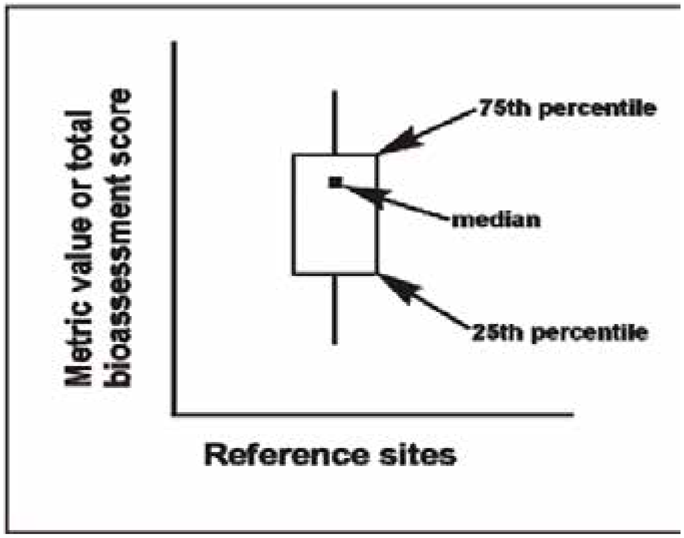

Figure 2. **Box-and-Whisker Plot Showing the Upper and Lower Percentiles and the Median of Individual Metric Value Distribution among Reference Sites**

The project quality objectives for the benthic macroinvertebrate 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 double entry of all values by two data technicians (data entered once by each individual).

The project quality objectives for the fish biological component are:

- Accuracy of field data will be ensured by call and response between the taxonomist and recording crewmember of all taxa counts, and by having a second crewmember double-checking all of the tallies of the recording crewmember.
- Accuracy of data entry will be ensured by double entry of all values by two data technicians (data entered once by each individual).

Table 6. 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 timeframe and geographic scale; sensitive to the 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 impacts.
Programmatic Considerations	
Relevance	Relevant to desired goal, issue, or agency mission (e.g., fish consumption advisories for human health protection, management of species having 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 could be expected.
Understandable	Indicator is or can be transformed into a format that target audience can understand (e.g., nontechnical for public).

Physical and Geomorphological Parameters

Physical habitat assessment will be performed at each sampling location using U.S. EPA's RBP approach and the MBSS spring and summer protocols, 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 perturbations of underlying physical and hydrologic processes, the physical habitat structure tends to become less complex, and thus has a diminished capacity to support biota and withstand destabilizing, erosive 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 and scoring of a series of instream, channel shape, and riparian characteristics. RBP parameters scores are summed for an overall physical habitat quality score. MBSS parameter scores are used in the PHI calculation.

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 affect aquatic communities. An increase in fine-sized sediments can alter the biochemical conditions of the stream; 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 via 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.
- All physical habitat data collected will be reviewed by at least two crewmembers prior to leaving the site to ensure accuracy and completeness, and to address any inconsistencies that should be addressed while still in the field.

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 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 County's 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. As detailed in Hill and Pieper (2010), where appropriate, each step in the sampling process is evaluated for 1) precision, which is a measure of measurement reproducibility, 2) accuracy, which is an evaluation of measurement "truth," 3) bias, which measures skew away from uniformity of effort, 4) representativeness, a determination of how representative a sample is of the population, and 5) completeness, or wholeness of a particular collection of data. Not all of these evaluative factors, however, are appropriate for each aspect of the sampling process.

4.2.1 Precision

4.2.1.1 Biological Assessments

a. Field Sampling

The nature of the quantitative electrofishing survey protocol used for this Program, where all of the fish within a sampling segment are disturbed, makes it difficult, if not impossible, to complete a duplicate sample. While it is possible to find similar rootwads in two adjacent segments of stream, finding two 75-meter segments with pools of similar structure and depth, or riffles with similar complexity and development, is more challenging and would make the appropriate QC segments challenging to find. The time and effort required to sample an adjacent segment, as well as the number of segments required for proper QC, is also cost-prohibitive. This would make the typical calculations of precision difficult for fish sampling; therefore, no MQOs are presented here for fish sampling precision. Proper training and adherence to field protocols is likely to enhance the precision of the fish sampling (more details regarding certification and training provided in Section 5.0).

Benthic macroinvertebrate QC samples only require that two sampling segments contain 20 square feet of comparable habitat for benthic macroinvertebrate sampling. The replicate benthic samples (QCs) are combined and used to calculate several measures of precision for individual metrics and the final index (BIBI). 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 1 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 BIBI scores. CV is calculated from RMSE by:

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

where 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 7. Measurement quality objectives (MQO) for evaluating field sampling precision of benthic macroinvertebrate sampling represented by median relative percent difference (RPD), root mean square error (RMSE), and coefficient of variation (CV) (From Hill and Pieper 2010).

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
BIBI	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 (PTD). 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% taxonomic disagreement. 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 (BIBI). 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 BIBI median RPD should be <20% 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

In order for the metrics and IBIs developed for the MBSS to accurately reflect stream condition, the field data must be collected in the manner for which the metrics and IBIs were designed and calibrated (*i.e.* according to protocol). For this reason, DNR provides annual training and certification. The Field Crew Task Leader should obtain all appropriate certifications for field sampling. At a minimum, all other field staff should attend the appropriate MBSS index period training, depending on the sampling activities they will be supporting. An exception to this requirement may be made for electrofishing larger sites (requiring more than five crewmembers). The additional crew members may provide field support; however, their role will be limited to activities where they are actively supervised by the Field Crew Task Leader or another experienced crewmember (defined as active participation during at least three sampling seasons).

Benthic Macroinvertebrate Sampling Certification requires the crewmember to pass a written exam and a field audit, both administered by MBSS staff. This certification is good for three years, provided the crewmember attend the annual MBSS Spring Training each year.

Fish Crew Leader Certification requires the crewmember to pass a written exam and a field audit, both administered by MBSS staff. This certification is good for three years, provided the crewmember attend the annual MBSS Summer Training each year.

b. Laboratory Sorting and Subsampling

As noted above, the manner in which data are collected is an important step in accurately assessing stream condition. This also applies to the laboratory procedures used for the sorting and subsampling of benthic macroinvertebrate samples. For this reason, laboratory procedures require QA/QC measures to demonstrate their ability to achieve MQOs. During actual sample processing, sorting efficiency is checked for 10% of the samples by examining the sorted remains for any missed organisms. The MQO for laboratory sorting efficiency is ≥90%. If this MQO is not achieved by the sorter, then the remaining grids will be checked until the sorter consistently passes.

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). Option 3 is not feasible, nor considered necessary, for this project. The certifications that are required for these biological assessments are a critical component in ensuring the accuracy of the biological dataset. In order to obtain certifications, taxonomists must demonstrate their expertise and meet a high threshold of accuracy with their identifications.

All benthic macroinvertebrate taxonomy in this project will be completed using up to date technical literature and performed by a taxonomist who has been certified by the Society of Freshwater Science. At least 10% of the subsamples will be sent to an independent taxonomic laboratory for re-identification and enumeration of the organisms. Per the guidance found in Hill and Pieper (2010), the maximum acceptable PTD between laboratories for each sample is 15%. All samples with disagreements should be examined to determine the reasons for the disagreement, but corrective actions are only required if the 15% threshold is exceeded. BIBI scores should be calculated for both taxa lists and compared. Per the guidance found in Hill and Pieper (2010), the median RPD between the BIBI for duplicate samples should be less than 20% and the RMSE should not exceed 0.6 between sample pairs. Finally, 95% of all organisms in each sample should be identified to target taxonomic levels.

Fish taxonomy will be performed only be crewmembers who have obtained the MBSS Fish Taxonomy Certification. This requires the crewmember to pass the Fish Taxonomy Test (requires a score ≥ 90%) and a field audit (requires 100% accuracy in identification, though crewmembers are allowed to not identify some specimens and collect photovouchers for identification by MBSS staff at a later time), both administered by MBSS staff.

Additionally, in keeping with the protocol, any fish that the crew leader cannot identify with confidence should be fixed in plastic jars with a 10% buffered formalin solution, followed by long-term storage in 70% ethanol (see MBSS Round 4 Manual for the complete preservation process; DNR 2017). These specimens will be submitted to MBSS staff for positive identification. Upon final identification, project data sheets should be updated, and any correspondence with MBSS staff added to the project files.

d. Enumeration

For benthic macroinvertebrates, at least 10% of the subsamples will be sent to an independent taxonomic laboratory for re-identification and enumeration of the organisms. Regarding enumeration, the difference between laboratories should be less than 10% for each sample (Hill and Pieper 2010). Quality objectives for re-identification have been described previously.

The nature of identifying fish, which includes the use of wet hands while handling live specimens to avoid damage to them, necessitates that the taxonomist rely on a second crewmember for recording data and tallying the various counts. Identification takes place outdoors with a significant amount of ambient noise. To minimize errors in recording fish counts, the taxonomist should employ the “call and response” technique, where the recorder repeats back every species and count by the taxonomist. Afterwards, the recorder will add up all of the tally marks and write a final count for each species on the Fish Crib Sheet. A second member will then add the tally marks independently and record their own final count. If there are any differences between the two summations, a third crewmember will add up the tally marks and determine the correct count. Once all final counts are correct, the totals will only then be recorded on the MBSS Fish Data Sheet.

e. Data Entry

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. The best solution to this problem is to use double entry. Once sampling activities for a season are completed, all biological and physical habitat data will be entered by two individuals into a database. This database will contain duplicates of each data table, one set for the first data entry technician and one set for the second. The data entry forms should mirror the layout of the data sheets to avoid confusion about where particular data are to be entered. Throughout the database, when possible, restrictions are placed on fields so that only possible data values can be entered. The two sets of tables are then electronically compared within the database. Discrepancies are then checked against the original data sheets and errors within one or both of the databases are rectified. The corrected databases are then compared a final time to ensure that all data entry errors have been resolved. This process will be reiterated until there are no errors (*i.e.* data are 100% accurately entered from the field data sheets). This final dataset is then used for all assessment, analysis, and calculations.

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 calculations. 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 BIBI and FIBI 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 BIBI is the classification efficiency (CE) (Stribling et al. 1998, MDEQ 2003). The accuracy of the Coastal Plain BIBI is a CE of 96 and the accuracy of the Coastal Plain FIBI is a CE of 83 (Southerland et al. 2007).

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 2017). 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 2017). This format allows the sampler to obtain the maximum number of individual organisms while still sampling 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 also for the overall project or "lot" of samples. The calculation of sorting efficiency is presented in Appendix S. The laboratory sorting/subsampling MQO for this project is to have a dataset *where ≤ 10% of the samples overall have a sorting efficiency of <90%*. Individual sorters will consistently attain a 90% or greater sorting efficiency. Metrics and a final biological index are not calculated if the entire sample (all 100 grids) are sorted and fewer than 60 organisms are recovered.

c. Taxonomy

This type of error in taxonomy (benthic macroinvertebrate or fish) would be problematic if there were consistent misinterpretation of technical keys, misunderstanding of morphological features, or 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

Benthic macroinvertebrate taxonomy and enumeration is dependent on specimens that are whole or sufficiently intact for positive identification. Species that are fragile and break apart easily are both more difficult to pick out during sorting (may be in smaller pieces easily missed amongst sortate) and/or identify, thus creating a bias towards hardier genera and increasing the counts of those organism relative to more fragile organisms. Care must be taken during all steps of benthic macroinvertebrate sampling, from collection to sorting to identification, to avoid physical damage to the organisms.

Fish taxonomy and enumeration is dependent on the capture of the fish for identification. Species, such as eel and lamprey can be more resistant to being stunned, and therefore more difficult to catch. Additionally, some bottom species, such as sculpin, are stunned while lying among gravel and cobble; it may be difficult to net them from between rocks and can be difficult to spot due to their coloration. The bias in enumeration can be towards species that are each easier to capture. In order to avoid this bias, crewmembers will use the appropriate style of polarized sunglasses for the stream conditions present, crews will be taught the appropriate techniques for netting species that are difficult to capture, and trailing netters and bucketers will stay behind the initial line of electrofishers in order to spend more time observing the substrate to find fish that are more difficult to spot.

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 a high CE, then 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 IItraining, 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 descending order of stability and productivity (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 75-meter 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.

Fish field sampling will include equal effort on both electrofishing passes and the sampling of all habitats within the transect.

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 subsampling tray, and, second, the randomization process for original selection of grids for sorting. The first grid is randomly selected for sorting and must be sorted to completion, even if more than 120 organisms are found. If less than the target number of organisms is found in the first grid, subsequent grids will be randomly selected and sorted to completion until the target is achieved. An important aspect of subsampling representativeness would be those samples where the 100-organism goal was attained in a low number of grids. 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 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.

Cross section locations are sited to be representative of overall channel conditions observed within the sample reach. Additionally, the Rosgen classification system specifies that cross section data be collected in a particular location in the assessment reach. Under ideal circumstances, the cross section is established on a riffle transition between two meander bends. If a riffle is not present, then a run or glide between two meanders or within a straight reach is chosen. Meander bends should be avoided when siting a cross section; they tend to be more geomorphically active than other areas of a stream channel and can provide a poor representation of overall channel stability (*i.e.* every unstable stream channel will likely have an actively eroding meander bend, but not every channel with an actively eroding meander bend is unstable).

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:

$$\%C = \frac{V}{T} X100$$

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

4.2.6 Comparability

Two datasets are considered 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 the appropriate spring or summer index periods.

Comparability in field data will also be improved by maintaining consistent crew leadership throughout a sampling year. Two crew leaders may be necessary to complete spring fieldwork within the index period. These crew leaders should spend at least one day in the field together, for calibration purposes, and maintain communication throughout the season to ensure consistent data collection. Summer sampling will ideally occur under the leadership of a single crew leader, unless there are extenuating circumstances that make that impossible (*e.g.,* crew leader unable to continue due to injury or illness, no longer employed with the Contractor, etc.).

5.0 SPECIAL TRAINING REQUIREMENTS/CERTIFICATION

This QAPP and other supporting materials will be distributed to all relevant personnel. All field, laboratory, and data analytical personnel have training and/or experience in performing all duties for which they are responsible.

Prior to initiation of field work, all field team members and alternates will attend an orientation session to review the QAPP 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 (who has obtained the appropriate MBSS certifications), or qualified designee, who will direct the measurement and/or sampling effort, and a QC Officer, who will ensure strict adherence to project protocols. Field teams will also attend the MBSS spring and summer training sessions. At least one member of the field crew performing the geomorphic work will have undergone Rosgen training through Level II.

Sample handling, sorting/subsampling, and primary taxonomic identifications will be performed by personnel with extensive experience and who have obtained the appropriate certifications. All samples picked by sorters- in-training will undergo a QC check until the sorter-in-training consistently passes the 90% sorting efficiency criterion. A summary of the required trainings and certifications by index period are included in Table 8.

Table 8. Summary of Trainings and Certifications by Index Period

Index Period	Training	Certification
Spring (March 1 – April 30)	• MBSS Spring Training *(every year)* • Rosgen Level II Training *(minimum level, one-time training)*	• MBSS Benthic Macroinvertebrate Sampling *(every 3 years)* • Society for Freshwater Science (SFS) Certification *(every 5 years; Group 2 – EPT genera & Group 3 – Chironomidae genera)*
Summer (June 1 – September 30)	• MBSS Summer Training (*every year*)	• MBSS Fish Crew Leader *(every 3 years)* • MBSS Fish Taxonomy *(every year)*

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.

If data are recorded using paper data sheets, each datasheet will be reviewed carefully by two crewmembers for completeness and accuracy. Each page will then be initialed by both reviewers after resolving any issues. If data are recorded in the field directly into a geodatabase, digital data forms will be 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. As with paper datasheets, the electronic data should be reviewed for accuracy by two crewmembers, whose initials are recorded and attached to the records reviewed.

Chemical Parameters

Field water quality measurements will be logged within the sampling device (as appropriate) (e.g., YSI). Turbidity may be an exception to this if a separate turbidimeter is used, in which case turbidity will be recorded on the appropriate datasheet or electronic data form on the field computer. Additionally, a Calibration Log Book will be used for recording multiparameter water quality instrument 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
- RBP Low Gradient Habitat Assessment Form
- MBSS Spring Habitat Data Sheet
- MBSS Summer Habitat Data Sheet

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.
- MBSS Spring Index Data Sheet.
- 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 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 Contractor PM. After identification, the laboratory manager will be responsible all subsamples will be assembled and maintained in the benthic laboratory for seven years. The laboratory manager will have primary responsibility for the maintaining the subsamples.

Collection and processing of fish will be documented in writing using the following forms and labels:

- Crib Sheet
- MBSS Fish Data Sheet
- MBSS Game Fish Length Data Sheet
- MBSS Photo Data Sheet (used to record photovouchers).
- An identification label to accompany each preserved specimen (for later identification by MBSS staff), one on the outside of the container and one placed inside with the preserved fish specimen

The Contractor 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
- QAPP
- 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 (or original electronic versions) and chain-of-custody forms 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 the County Project Manager. If required, 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 QAPP 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 QAPP.

All written records relevant to the sampling and processing of samples will be maintained by the Contractor PM with copies submitted to the County PM. Records will be maintained by the Contractor for a minimum of 5 years following project completion unless other arrangements are made with the County.

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 Appendices A and B. Water samples, habitat data, crayfish data, mussel data, herpetofauna data, and benthic macroinvertebrate samples will be collected from each of the approximately 384 stream locations in this project over the course of five years (Table 1 and Table 2). Fish will be assessed at 192 of those sites during the five year round. The County will use the MBSS spring index period, March 1 - April 30, for benthic macroinvertebrate sampling and the summer index period, June 1 – September 30, for fish sampling. These index periods are designed to limit seasonal variability in biological community composition during sampling.

GPS coordinates will be followed to and recorded at each site, physical habitat and geomorphological assessments will be performed at all sites, as well the collection of water samples, and *in situ* measurements of pH, dissolved oxygen, temperature, specific conductance, and turbidity.

Three types of sites will be monitored in this program to address Anne Arundel County's goals: probability, QC, and reference. Table 9 presents several design elements relative to sampling and site assessment.

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 "an estimated 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 selection 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 and 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 Update of the Anne Arundel County Biological Monitoring Program (Appendix B), 24 PSUs have been delineated in the County. Within each of these PSUs, two sampling strata were defined: one stratum being the larger streams (from 1:100,000-scale map) and the other being the smaller streams (occurring on a more-detailed map but not on the 1:100,000-scale map). Eight sites from each stratum will be sampled in each of the 24 PSUs (16 sites total per PSU). In each year of the monitoring program, approximately 75 sites will be sampled. After five years, a total of 384 sites (16 sites per PSU) will have been sampled, completing one round.

Quality control sites are duplicate reaches that are sampled at 10% of the total sites (i.e. one to two sites 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 75-meter 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 DPW-WPRP-CBMP-FO-001 "Selecting a QC Site for Duplicate Sampling" (Appendix E). 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 with buffers from adjacent land use).

Table 9. 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 — ~ 75 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, Summer: June 1 – September 30, in accordance with MBSS guidelines
Sampling Method Benthic macroinvertebrates: 20-ft² sample, multihabitat, randomized 100-organism subsamples, 500-540 µm mesh. Double-pass electrofishing, using blocknets at the upstream and downstream ends of sampling segment, anodes spacing of 1-3 meters across width of stream channel. Capture by hand or net (herpetofauna, crayfish, mussels) or as incidental by-catch from other sampling methods
Taxonomic Level Benthic macroinvertebrates - genus for most taxa, unless immature or damaged, then next higher classification level Fish – species Herpetofauna – species Crayfish – species Mussels - species
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
Fish Metrics 1. Abundance per Square Meter 2. Number of Benthic Species (adjusted for catchment size) 3. % Tolerants 4. % Generalists, Omnivores, Invertivores 5. % Round-bodied Suckers 6. % Abundance of Dominant Species

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 by MBSS using metric value distributions from multiple CP sites (Table 10). 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 10. Bioassessment Scoring Criteria for Biological Metrics

Benthic Metrics	**5**	**3**	**1**
Total Taxa	≥ 22	21 - 14	< 14
Number of EPT Taxa	≥ 5	4 - 2	< 2
Number Ephemeroptera Taxa	≥ 2	1	< 1
% Ephemeroptera	≥ 11	10.9 – 0.8	< 0.8
% Intolerant Urban	≥ 28	27 - 10	< 10
Number of Scraper Taxa	≥ 2	1	< 1
% Climbers	≥ 8.0	7.9 – 0.9	< 0.9
Fish Metrics	**5**	**3**	**1**
Abundance per Sq. Meter	≥ 0.72	0.71 – 0.45	< 0.45
Number of Benthic Species (Adjusted)	≥ 0.22	0.21 – 0.1	0
% Tolerants	≤ 68	69 - 97	> 97
% Generalists, Omnivores, Invertivores	≤ 92	93 - 99	100
% Round-bodied Suckers	≥ 2	1	0
% Abundance of Dominant Species	≤ 40	41 - 69	> 69

(Southerland et al. 2005)

Assessment of individual streams and watersheds 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 possible until the end of each round of sampling. It should be noted that targeted sites will not be used for Countywide or watershed-scale assessment.

8.0 SAMPLING METHODS

The protocols used for this project will generally follow the MBSS Sampling Manual (DNR 2017; Appendix D). However, there are some differences, so specifics for sampling methods are detailed in County-specific SOPs (Appendices E through S). Lists of equipment and materials are provided in each SOP. Prior to the start of Round 3 sampling, the Contractor will update the QAPP SOPs, particularly those regarding particular pieces of equipment, according to guidance provided by the County PM. The Contractor will review the SOPs annually, and update them according to any changes made in equipment used or procedures followed (changes must first be approved by the County PM and QA Officer).

Site Information Parameters

Site establishment is detailed in DPW-WPRP-CBMP-FO-002 "Establishing and Marking a Random Site (Appendix F). GPS coordinates will be obtained in accordance with DPW-WPRP-CBMP-FO-003, "Use of GPS" (Appendix G).

Chemical Parameters

The instream chemical data (pH, temperature, DO, specific conductance, and turbidity) will be collected by the field sampling team using a multiparameter water quality instrument in accordance with the appropriate SOP (DPW-WPRP-CBMP-FO-004 "Use of Water Quality Instrumentation"; Appendix H). A separate, portable turbidimeter may alternatively be used to collect turbidity measurements, in which case, details for the use and calibration of that meter will also be included in Appendix H. Water quality samples will be collected using triple-rinsed bottles, and stored on ice. The complete protocol for the handling of samples is available in accordance with DPW-WPRP-CBMP-FO-005 "Water Quality Sample Collection and Processing" (Appendix I).

Biological Parameters

Benthic macroinvertebrate sampling will be conducted according to DPW-WPRP-CBMP-FO-006 "Aquatic Macroinvertebrate Sampling in Non-tidal Freshwater Streams" (Appendix J). Samples will be logged in the field on the Chain-of-Custody Record, according to DPW-WPRP-CBMP-AO-007 "Benthic Sample Chain-of-Custody Completion" (Appendix K).

Fish sampling will be conducted according to DPW-WPRP-CBMP-FO-008 "Fish Sampling in Non-tidal Freshwater Streams" (Appendix L). Mussel, herpetofauna, and crayfish sampling will be conducted by the field sampling team following the methods outlined in the MBSS Sampling Manual (Appendix D; DNR 2017).

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 each parameter for the 75-meter sampling segment, including about 25 meters upstream of the sampling segment. Conditions are rated as being in one of four categories along a continuum, represented as optimal, suboptimal, marginal, and poor (Figure 3). A 20-point scale is used for each parameter with 0 being poor and 20 optimal (DPW-WPRP-CBMP-FO-009 "Physical Habitat Assessment of Low Gradient Streams (RBP Method)" (Appendix M).

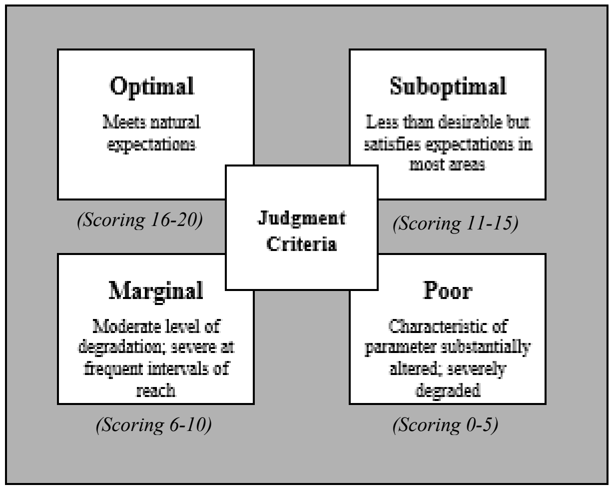

Figure 3. Narrative Attributes for Judging the Quality of Habitat Parameters along a Continuum of Conditions

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 (Appendix D; DNR 2017). The Contractor will conduct these habitat assessments following the procedures in DPW-WPRP-CBMP-FO-010 "Physical Habitat Assessment (MPHI Method)" (Appendix N).

After thorough visual assessment of channel characteristics found in the assessment reach is made, a representative location, based on the best professional judgment of the crew leader, is selected for cross section establishment. One cross section is measured within the assessment reach and bankfull channel, slope, sinuosity, and other features are measured or calculated. Cross sectional measurements will be performed following the procedures in DPW-WPRP-CBMP-FO-011 "Stream Cross Section Measurement" (Appendix O). 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 DPW-WPRP-CBMP-FO-012 "Abbreviated Longitudinal Profile Measurement" (Appendix P). 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 DPW-WPRP-CBMP-FO-013 “Modified Wolman Pebble Count” (Appendix Q). 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 R) will be completed at the time of sample collection to accompany the samples to the Contractor's laboratory for storage prior to laboratory 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 DPW-WPRP-CBMP-AO-014 "Benthic Sample Log-in Procedures" (Appendix R). Prior to shipping the samples to the benthic laboratory for processing (if a subcontractor is used), samples will be logged onto a Chain-of-Custody Record following DPW-WPRP-CBMP-FO-007, "Benthic Sample Chain-of-Custody Completion" (Appendix K).

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 following MBSS subsampling procedures (Boward and Friedman, 2000). An SOP regarding the quality control of the benthic macroinvertebrate sample processing will be provided by the Contractor or Subcontractor laboratory. The secondary or final phase processing of the samples 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. SOPs for taxonomic identifications and the quality control of the taxonomic identifications will be provided by the Contractor or Subcontractor laboratory.

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 seven 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). In this case, the specimens identified will be maintained in such a manner that they are available for future examination, but with no special arrangement or organization beyond that used during the initial sample processing and identification. 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 11.

Table 11. 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*. Sixth 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.* Fifth ed. Holden-Day, Inc., San Francisco, CA.
Peckarsky, B. L., P. Fraissinet, M. A. Penton, and D. J. Conklin, Jr. 1990. *Freshwater Macroinvertebrates of Northeastern North America.* Cornell University Press, Ithaca, NY.
Pennak, R. W. 1989. *Fresh-Water Invertebrates of the United States: Protozoa 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.

Fish Taxonomy

Fish taxonomy will only be performed by crewmembers who have received Fish Taxonomic Identification Certification through MBSS.. Any specimens that cannot be positively identified will be preserved and sent to MBSS staff for positive identification.

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 metrics is assigned the appropriate bioassessment rating score according to the reference condition and its metric scoring criteria (Table 6). The rating scores for the metrics are averaged to an overall bioassessment score for each site. The overall score for a site is assigned to a narrative category:

- 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., 2007). 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 10th and 50th (median) percentile values for reference sites. For each positive metric, values below the 10th percentile are scored as 1; values between the 10th and 50th percentiles are scored as 3; and values above the 50th percentile are scored as 5. Scoring for negative metrics is reversed (e.g. values below the 50th percentile are scored as 5; values above the 90th 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 WPRP 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. The Field Sampling or Laboratory QC Officer will ensure that samples are taken according to the established protocols and that all forms, checklists, 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

All data will be entered into the database twice, by two separate individuals, and electronically compared. All errors will be corrected before the dataset is considered final and ready for use in analysis. 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 person who recorded the data. 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, and water quality samples for chemical parameters described in Table 3), 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 (multiparameter water quality instrument): The accuracy of DO sensors and methods used in this project will be determined at 100% solubility. The solubility concentration is determined in water that has been saturated with air. The actual concentration of DO at 100% solubility is determined internally by the unit from measurements of temperature and barometric pressure.

pH sensors (multiparameter water quality instrument): The accuracy of pH sensors used in this project will be checked using certified pH 4.0 and pH 7.0, buffer solutions traceable to National Institute for Standards and Technology (NIST) Standard Reference Material.

Conductivity sensors (multiparameter water quality instrument): The accuracy of conductivity sensors used in this project will be checked using a standard solution (i.e., 1,000 µS/cm or 1413 μS/cm) and by making the appropriate corrections for nonstandard specific conductance measurement.

Turbidity sensor (multiparameter water quality instrument or portable turbidimeter): The accuracy of the nephelometric turbidimeter used in this project will be checked using calibration standards ranging from 0 NTU, 20 NTU, 100 NTU (and any additional standards required for unit's particular calibration).

Digital scale: The accuracy of a digital scale is checked using a set of calibration weights, ranging from 2 grams to 4,000 grams (or the upper limit of the scale).

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 Coastal Plain, 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. Fish sampling is focused on the entire 75-meter sampling segment, and thoroughly fishing all habitats and areas of the stream. This allows for the characterization of the stream as a whole, by capturing species that prefer different stream depths, velocities or habitat types.

Comparability

Comparability is a description of the confidence with which one dataset 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 crew leader (who may be the Field Crew Task Leader or else is overseen by the Task Leader), who has successfully completed MBSS training and been certified by MBSS as appropriate.

- 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 period.
- Fish sampling will be conducted within one index period: summer (June 1 – September 30). The summer index period ensures seasonal comparability with other Maryland

counties, the MBSS, and the State of Delaware, Department of Natural Resources and Environmental Conservation (DNREC), which all sample during the same period.

- In Anne Arundel County, all benthic macroinvertebrate samples will be taken with the 20 "jab" multihabitat method (DNR 2017, 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 fish sampling will be double-pass electrofishing, using a spacing of no more than three meters apart between anodes. The upstream and the downstream ends of the sampling segment will be completely sealed off with blocknets.
- 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 Field Sampling Task Leader (if not also acting as the crew leader for this project) or the Contractor PM (or the appropriate designee with a minimum of five years of field experience with stream protocols, at least three of which were spent as a crew leader) to ensure comparability of methods and methods application. An additional audit may be performed by the County PM (or another appropriate County staff representative) at any time – this may include the first year of the Round 3 sampling, or each time a new crew leader is assigned to the project. Assignment of new crew leaders within a field season is to be avoided, if at all possible.

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 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,
- The randomly selected site is intermittent (however, the fact that the stream is dry is valuable information on that stream),
- The stream reach where the site was placed is fully contained within a closed storm drain system, or

- The site is judged not sampleable for other reasons described in MBSS documentation.

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. An MBSS Spring or Summer Index Data Sheet should be filled out at unsampleable sites and site photographs should be taken documenting the reason for unsampleablility.

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, block nets, D-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 vehicle. If small tears are found, they may be sewn; larger holes require replacement of 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 weekly during the sampling period. Laser levels will be checked for accuracy and/or calibrated on an annual basis. Factory/manufacturer long-term maintenance guidelines should be followed, as well. Critical spare parts (*e.g.*, dipnets) and backup equipment (*e.g.,* backpack electrofishing units) will always available in the event that equipment malfunctions while in the field or needs to be returned to the manufacturer for repair. One field crewmember will be designated to gather and inspect all equipment on the equipment supply list 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

Field calibrations will be conducted weekly and recorded in the Calibration Log Book; calibration values must be within 90% of the standards, otherwise replacement of sensors, repair, or substitution of the multiparameter water quality instrument will be required. The multiparameter water quality instrument will be calibrated with standard buffers for pH (4.00 and 7.00) and conductivity (*e.g.*, standard of 1,000 µS/cm or 1413 µS/cm). The DO calibration will be performed using percent air saturation. Turbidity standards used for calibration will depend on the equipment used (e.g., 2-point, 3-point or 4-point calibration capability).

Additional weekly checks will be performed for other equipment and the results recorded in the Calibration Log Book. Digital scale checks should include testing the scale with a set of calibration weights. A variety of weights should be checked, ranging from 10 grams to near the upper limit of the scale. At least three different weights should be used for calibration checks. If scale readings are off by more than 2 grams, the scale should be re-calibrated according to the manufacturer's instructions.

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 the 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 Field Sampling Task 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 multiparameter water quality instrument 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 bench sheets 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 Contractor 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. Upon completion of sampling, data files will be transferred to the project directory for QC checks and processing. Samples will be transported to the Contractor or Subcontractor laboratory and logged in using the COC Record (Appendix K). Samples will be stored appropriately until processing begins. Sample processing (sorting and subsampling) records will be recorded in the "sample processing" notebook in the benthic 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 S) will be completed to record the number of grids sorted in order to record the level of effort and quality control.

Taxonomic bench sheets provided by the benthic laboratory 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 or Microsoft Access, 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 at least five years subsequent to project completion (unless otherwise directed by the County PM). Data will be maintained in an ESRI Personal Geodatabase; however, manipulations and statistical analyses may be performed in other software packages 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 May 2016 (note that a message on the website indicates this webpage will be moving from the Ohio Department of Natural Resources to the Department of Agriculture): http://water.ohiodnr.gov/portals/soilwater/data/xls/Reference_Reach_Survey_4_3_L.xls

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 QAPP. 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 onsite. 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 <90%, the 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 Task 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 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 completed 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 QAPP. The Director 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 QAPP are being implemented. A system audit will not be conducted during the Anne Arundel County project unless additional or specific 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 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 7 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 will be subject to strenuous review and rejected from the dataset 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 Contractor 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 7 will be identified and uncontrolled sampling error investigated.

Results of the verification and validation processes will be reported to the 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 it is possible to draw the correct conclusions for the project with the remaining data. Limitations in the dataset 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 or QA Officer. Any discrepancies in the records will be reconciled with the appropriate associated field personnel and special cases that cannot be resolved will be reported to County PM.

The chemical, biological, and physical/geomorphological parameters measured and results of calculations performed for this project (i.e., metrics, BIBI, and FIBI 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 watershed 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 County PM and the County 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 inhabiting a given area.

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

Ecoregion: a geographic area that is 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

DESIGN OF THE BIOLOGICAL MONITORING AND ASSESSMENT PROGRAM FOR ANNE ARUNDEL COUNTY, MARYLAND

Design of the Biological Monitoring and Assessment Program for Anne Arundel County, 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 $\geq$ 6
ANC $\geq$ 50μeq/l
Dissolved $O_2 \geq$ 4 ppm
Nitrate-N $\leq$ 4.2 mg/l
Urban land use $\leq$ 20% of catchment area
Forested land use $\geq$ 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.

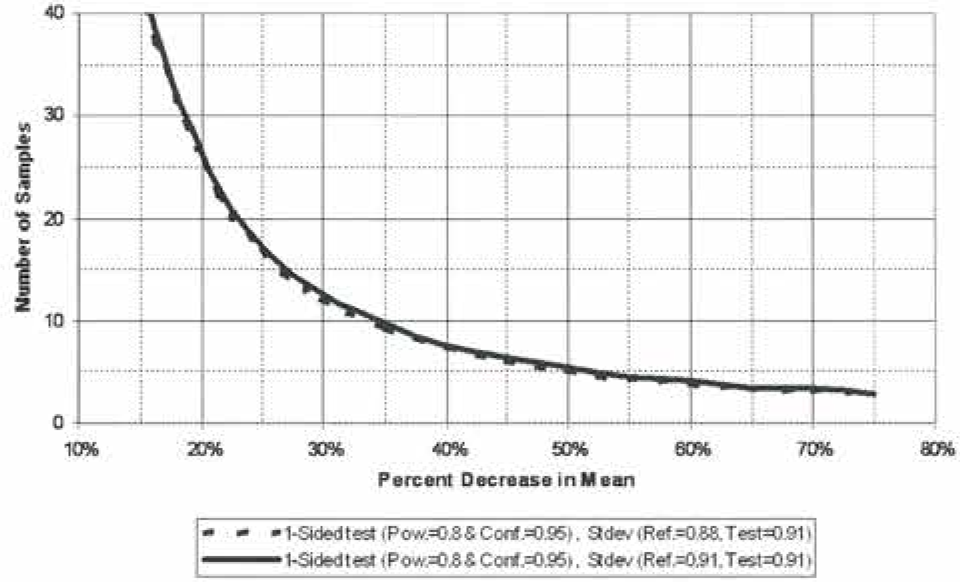

Figure 1. Sample size calculations for reference and test statistics.

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

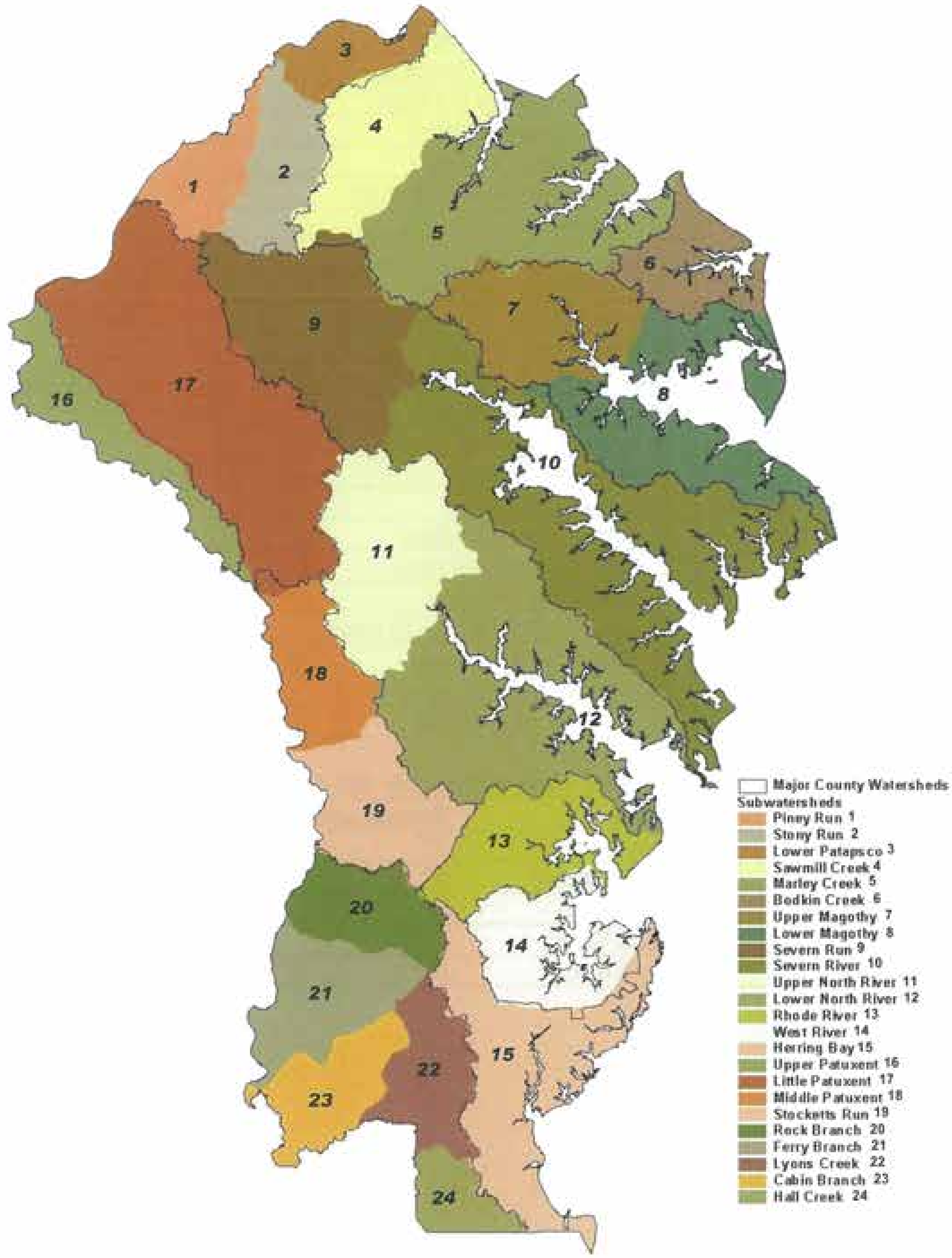

Figure 2. Map of the 24 subwatershed primary sampling units.

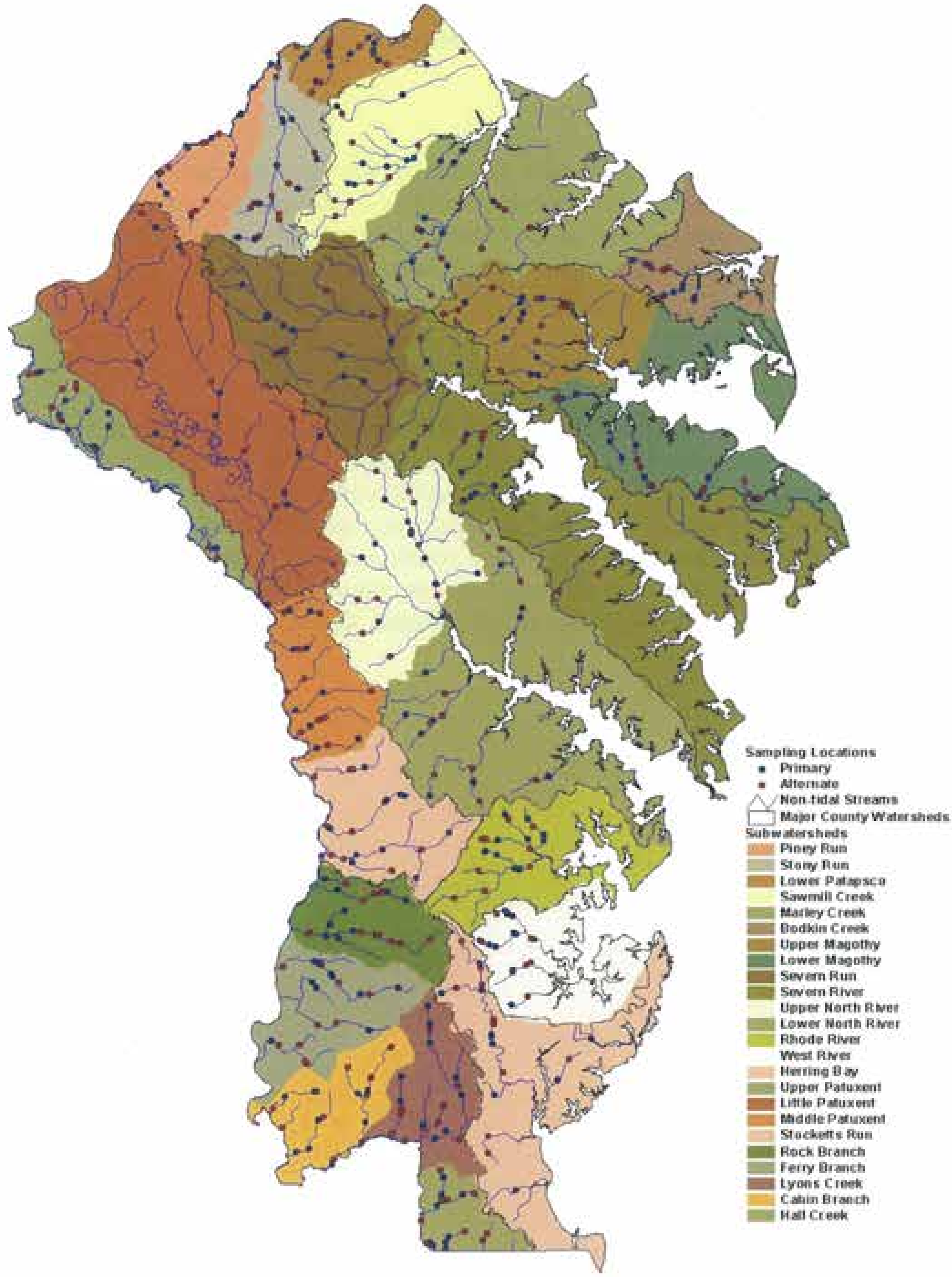

Figure 3. Map of randomly selected sampling locations in Anne Arundel County.

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
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	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
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	Primary	1	18-08	38.9703033712	-76.6892540906
	Primary	1	18-09	38.9523831269	-76.6738253507
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	Alternate	1	18-11A	38.9581573474	-76.6835861398
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	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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	Alternate	1	21-15A	38.8393808375	-76.6748180075
	Alternate	1	21-16A	38.8155249066	-76.6667311622
	Alternate	1	21-17A	38.8274092296	-76.6495054073

21 Ferry Branch	Alternate	1	21-18A	38.8321484022	-76.6659128791
	Alternate	2	21-19A	38.8385777800	-76.6955183072
	Alternate	2	21-20A	38.8062304016	-76.6978564886
22 Lyons Creek	Primary	1	22-01	38.7735410508	-76.6208020835
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	Primary	1	22-03	38.8150127270	-76.6172633494
	Primary	1	22-04	38.7932023060	-76.6321769793
	Primary	1	22-05	38.7829956967	-76.6330494388
	Primary	1	22-06	38.8225026178	-76.6184980902
	Primary	1	22-07	38.7927166452	-76.6033426659
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	Primary	2	22-10	38.7706029613	-76.6088553803
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	Alternate	1	22-13A	38.8124357260	-76.6173405241
	Alternate	1	22-14A	38.7742425568	-76.6127604455
	Alternate	1	22-15A	38.7796416415	-76.6325903292
	Alternate	1	22-16A	38.7813643865	-76.6103594607
	Alternate	1	22-17A	38.7697138055	-76.6372936705
	Alternate	2	22-18A	38.7670563144	-76.6304197412
	Alternate	2	22-19A	38.7890463150	-76.6024959415
	Alternate	2	22-20A	38.7672475170	-76.6015960829
23 Cabin Branch	Primary	1	23-01	38.7753484611	-76.6765156183
	Primary	1	23-02	38.7621629771	-76.6885269441
	Primary	1	23-03	38.7860967535	-76.6551049089
	Primary	1	23-04	38.7872083836	-76.6503566821
	Primary	1	23-05	38.7874146273	-76.6502740352
	Primary	1	23-06	38.7636736037	-76.6819142604
	Primary	1D	23-07	38.7872325228	-76.6777428380
	Primary	1D	23-08	38.7851846370	-76.6944769045
	Primary	2	23-09	38.7746186846	-76.6513795881
	Primary	3	23-10	38.7567933911	-76.6694265516
	Alternate	1	23-11A	38.7928408654	-76.6488626079
	Alternate	1	23-12A	38.8054527537	-76.6377744339
	Alternate	1	23-13A	38.7724995558	-76.6958114694
	Alternate	1	23-14A	38.7852970380	-76.6509153031
	Alternate	1	23-15A	38.7970035998	-76.6611246947
	Alternate	1	23-16A	38.7766105700	-76.6735326803
	Alternate	1D	23-17A	38.7802580149	-76.7007357957
	Alternate	1D	23-18A	38.7872191551	-76.6788819102
	Alternate	2	23-19A	38.7660870949	-76.6538082615
	Alternate	3	23-20A	38.7591251887	-76.6689505704
24 Hall Creek	Primary	1	24-01	38.7222632796	-76.6095327972
	Primary	1	24-02	38.7516691209	-76.6101998360
	Primary	1	24-03	38.7419418048	-76.6102283114
	Primary	1	24-04	38.7444403109	-76.6001071976

24 Hall Creek	Primary	1	24-05	38.7270560497	-76.6074674535
	Primary	1	24-06	38.7445966360	-76.6113430873
	Primary	1	24-07	38.7248927210	-76.6094361590
	Primary	2	24-08	38.7399582357	-76.6137979146
	Primary	2	24-09	38.7248550370	-76.6178117108
	Primary	2	24-10	38.7234016108	-76.6159305743
	Alternate	1	24-11A	38.7292478189	-76.5992292669
	Alternate	1	24-12A	38.7419210574	-76.6112570665
	Alternate	1	24-13A	38.7284077616	-76.5881882095
	Alternate	1	24-14A	38.7545883543	-76.6117128482
	Alternate	1	24-15A	38.7421972329	-76.6083841875
	Alternate	1	24-16A	38.7450232331	-76.5949804767
	Alternate	1	24-17A	38.7256053245	-76.5901239993
	Alternate	2	24-18A	38.7245838919	-76.6190307358
	Alternate	2	24-19A	38.7337515126	-76.6223760468
	Alternate	2	24-20A	38.7246364203	-76.6196248073

APPENDIX B

DESIGN UPDATE OF THE ANNE ARUNDEL COUNTY BIOLOGICAL MONITORING PROGRAM

DESIGN UPDATE OF THE ANNE ARUNDEL COUNTY BIOLOGICAL MONITORING PROGRAM

Prepared for

Anne Arundel County
Department of Public Works
Watershed Protection and Restoration Program
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Scott Stranko, Maryland Department of Natural Resources
Matt Stover, Maryland Department of the Environment
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Background

In 2003, Anne Arundel County initiated the design of a Countywide Biological Monitoring Program (Program) (Hill and Stribling 2004). From 2004 to 2013, the County implemented the Program in two, five-year sampling Rounds, which provided a statistically robust baseline assessment of the County's non-tidal streams. Over the ten years of program implementation, significant changes in the regulatory environment have occurred associated mostly with the County's NPDES municipal separate storm sewer system (MS4) compliance, especially in addressing Total Maximum Daily Loads (TMDLs) implementation. To meet these and other challenges, the County has revised the Program to increase its robustness and utility through the following efforts:

- Ensuring all regulatory requirements are met
- Enhancing the existing program to the latest scientific standards
- Increasing the efficiency and cost-effectiveness of the program
- Integrating the program with other watershed management and MS4 monitoring

The County, its consultants, and a Technical Review Committee (TRC) comprised of experts in stream and watershed assessment evaluated the following potential changes to the program in order to meet the County's current goals. This report details the specific analyses conducted and the changes adopted.

1. Redesign the biological survey to address trends in condition, provide a more even geographic coverage, and reduce local variability

2. Change to a more detailed stream network with implications for survey design and comparability with previous rounds

3. Combine the monitoring in the biological survey with monitoring in the watershed assessments using representative analysis

4. Add additional MBSS or other parameters (e.g., fish, amphibian, geomorphic, and water quality parameters) to the biological survey

5. Improve stressor identification at local scales

6. Redevelop IBIs or reference conditions to characterize the more detailed County stream network

7. Expand monitoring to include tidal waters

The report includes discussion of both planned changes to the current program (survey revisions) and potential future changes that cannot be implemented at this time.

Summary of Design Changes

1. Institute a partial replacement survey design that includes 50% fixed sites from previous rounds and 50% new random sites in each watershed (primary sampling unit or PSU)

2. Reduce the number of sites to be sampled in each watershed from 10 to 8 based on power analysis that used more data and showed less variability in western Coastal Plain reference streams

3. Add sampling on smaller streams (using the more detailed stream network developed by Anne Arundel County that more than triples the number of stream miles) as separate stratum, therefore, increasing the number of sites per watershed two-fold

4. Add sampling for the following components using Maryland Biological Stream Survey (MBSS) methods:
   - Larger suite of water quality parameters (from 5 to 18 parameters total)
   - Fish assemblages
   - Crayfish and mussel species
   - Amphibians, reptiles, and vernal pools in the riparian area

5. Develop a new Benthic Index of Biotic Integrity (B-IBI) for small streams that will be added with the more detailed County stream network

1 Redesign biological survey to address trends in condition, provide a more even geographic coverage, and reduce local variability

Currently, Anne Arundel County samples approximately 240 stream sites (75-m segments) over a 5-year round, countywide. Ten sites each are randomly allocated among the 24 watershed primary sampling units (PSUs). Within each PSU, sites are allocated by stream order in proportion to their occurrence. Round 1 was conducted during 2004-2008; Round 2 covered 2009-2013. Going forward, the biological survey design could be modified to provide better trend detection, a more even geographic coverage, or less local variability. The incremental benefits of each modification and implications for comparability with past data are described below.

1.1 Design options for improving trend detection

The current survey design provides trend detection through comparison of area wide estimates among rounds. There are three options for improving trend detection by reducing the among site variability that comes with selecting new random sites each round.

- Creating a fixed site network (either of reference condition or of a gradient of condition) in addition to the random survey
- Partial replacement design (combination of random and fixed sites selected each round)
- Using only repeats of sampled sites going forward (i.e., repeating sites sampled in earlier rounds)

The TRC supported the idea of fixed sites to detect trends over time. There was no consensus on whether this network should be included in the countywide design or separate. The pros and cons of the three options are as follows:

Fixed site network separate from the random survey. The MBSS currently maintains a sentinel site network of 29 sites identified as among the least disturbed and most protected in the state. These sites were selected from random sites previously sampled by the MBSS. The goal of this network is to evaluate changes resulting from natural factors (e.g., weather and climate change) as opposed to changes from local anthropogenic stressors. Currently the MBSS sentinel site network includes seven western Coastal Plain sites (none in Anne Arundel County), which should be adequate for determining trends in least disturbed sites. A network of sentinel sites that addresses the gradient of disturbance would be an extensive and expensive addition to the Program. Since the number of sites in the current Program is the minimum required for adequate power, a separate fixed-site network of any size would entail additional costs in the number of sites in the network.

Partial replacement design. This is a hybrid design where the next round of sampling includes both repeat sampling of sites sampled in previous rounds and new random sites selected for the next round. This design improves trend detection by reducing the among site variability by the proportion of repeat sites. The inclusion of new random sites ensures that assessments continue to approximate area wide conditions and are not overly constrained to the subset of sites selected originally. Sampling theory (e.g., Cochran 1977) indicates that a design using 25-50% repeat sites is the ideal balance of trend detection and representative assessment.

Conversion to fixed-site network. In this design, all sites sampled in subsequent rounds would be repeat sites selected from previously sampled random sites, creating a fixed site network going forward. This greatly reduces the among-site variability by eliminating new random sites (Naiman et al. 2001); however, the lack of new random sites reduces our confidence that the original set of random sites is representative of overall conditions over time. The MBSS is currently employing this design for its Round 4 because the focus of Round 4 is trends detection rather than additional condition assessment.

1.2 Evaluate adding stratification using smaller watersheds to provide more even geographic coverage

Random sampling creates a more variable density of sample sites across the landscape than grid sampling, but is easier to implement and analyze than grid sampling, especially over a stream network. A more even distribution of sample sites along a stream network can be obtained by allocating sites to smaller strata, such as subwatersheds. For Anne Arundel County, sample sites could be allocated to subwatersheds smaller than the current 24 watersheds sampled as PSUs to more evenly distribute the sites. Estimates of stream condition would not be calculated for the subwatershed strata, as each would contain too few sites (unless sampling effort was increased). Estimates would require calculation of stream miles by each subwatershed to properly weight the stream values.

Figure 1-1 illustrates that the distribution of Anne Arundel County random sites for both rounds provides generally good coverage of all parts of the County. In addition, as future rounds of random sampling are completed, the areas without samples will become fewer.

Of greatest concern, however, is whether the areas without sites are caused by the absence of sampling permissions, which can bias the assessment when the sites denied permission are different from the sites sampled. Table 1-1 shows that very few sites were denied permission in the monitoring program years of 2004-2013—only 44 sites were denied permission compared to the 480 sampled (9.2%). The new County requirement to obtain active permissions will likely increase the number of sites that cannot be sampled, so the distribution of non-permission sites among land use types should be evaluated in the future to identify any potential bias.

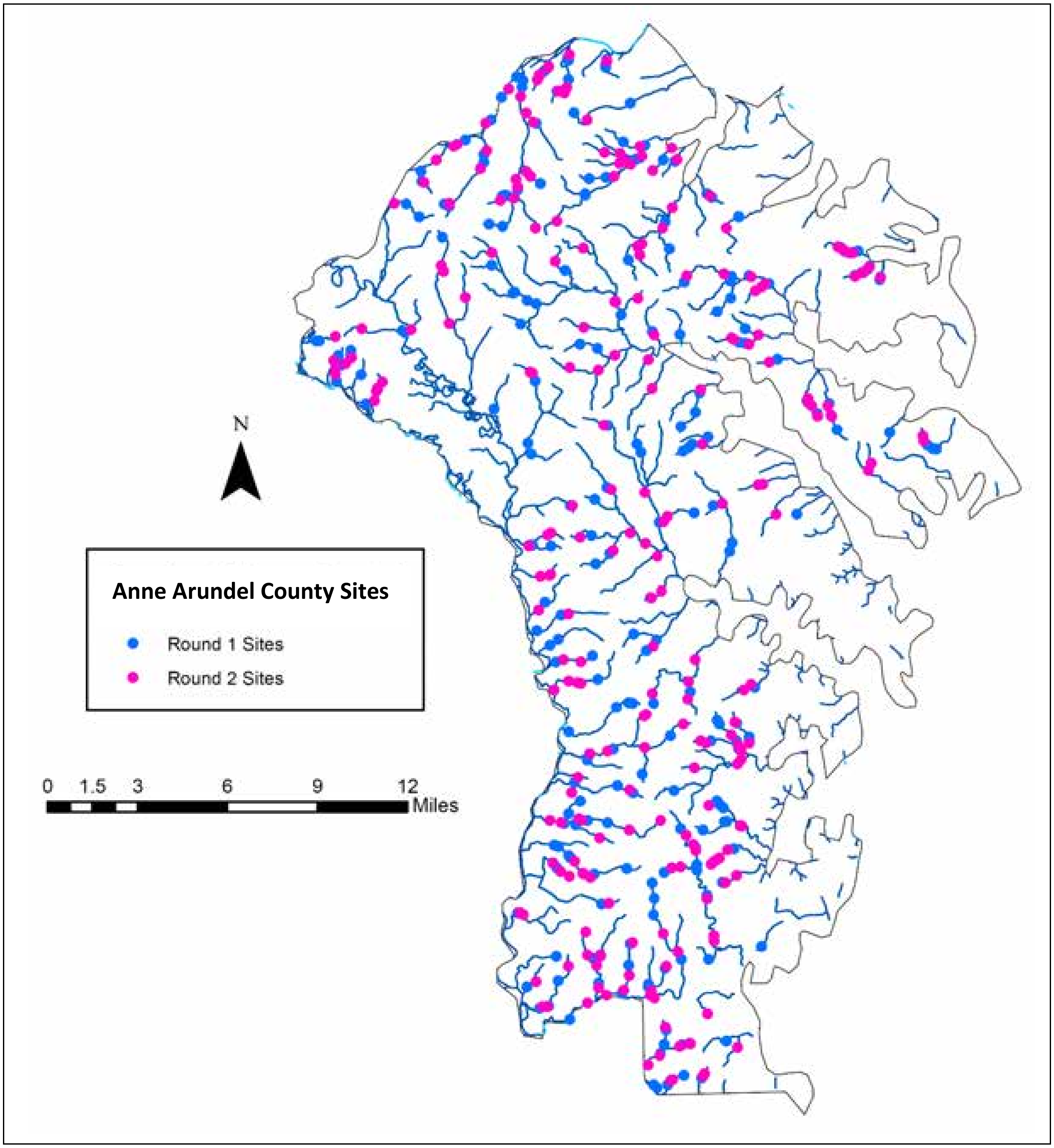

Figure 1-1. The distribution of random sites sampled during Rounds 1 and 2 of the Program, indicating generally good coverage of all parts of the County. The unsampled area along the central, western boundary of the county is Fort George G. Meade.

Table 1-1. Number of sites where permissions were denied by year and watershed, for the years 2004-2013

Year	Number of Sites with Permissions Denied	Watershed
2004	0	None
2005	0	None
2006	1	Hall Creek
2007	1	Piney Run
	2	Little Patuxent River
2008	1	Cabin Branch
	3	Rhode River
2009	1	Lower North River
	1	West River
	5	Rock Branch
2010	1	Stony Creek
	1	Sawmill Creek
	2	Herring Bay
	1	Middle Patuxent River
	3	Ferry Branch
2011	1	Bodkin Creek
	1	Upper Magothy River
2012	9	Rhode River
	2	Hall Creek
2013	1	Cabin Branch
	1	Lower Magothy River
	2	Stocketts Run
	4	Lyons Creek

1.3 Evaluate adding stratification by stream type (e.g., braided/wetland or slope, stream order or size, geology, ephemeral versus perennial if more detailed stream network is used), using analysis of MBSS data for western Coastal Plain

More important than stratifying the survey for more even geographic coverage is the issue of stratifying to address naturally different stream types, i.e., stream types for which the reference condition should be different. One of the challenges of large-area biological surveys is to develop and apply indicators of stream condition that are both practical and realistic—given that every stream is, in some sense, unique. The MBSS carefully considered the number of strata needed for the statewide survey and developed indicators for reference conditions in each of three geographic strata: Highlands, Eastern Piedmont, and Coastal Plain. However, the number of MBSS strata was limited by resources and practicality; finer strata would be beneficial but would provide only diminishing returns on the investment for a statewide survey.

All Anne Arundel County streams fall within the MBSS Coastal Plain stratum. Additional strata of stream types would undoubtedly be beneficial, though they would not necessarily exceed the costs required in indicator development and additional sampling. The finer differences in the geology of Anne Arundel County streams are not well enough known to warrant developing different reference conditions. Therefore, additional stratification would be limited to an evaluation of whether natural biological conditions vary with size or slope of streams in Anne Arundel County as described in Section 6.

1.4 Confirm/revise the number of sites per PSU that are needed to maintain the ability to detect 30% change, 80% of time, with 95% confidence (currently 10 sites per PSU). This will depend on whether the strata are being used for assessment or more even geographic coverage.

The original stream survey design for Round 1 of the Anne Arundel County biological monitoring program was based on power analysis that determined that 10 random sites per PSU would detect a 30% change in IBI scores, 80% of the time, with 95% confidence. Like the MBSS, this power analysis was based on the variability in "reference and similar" site IBI results found in the MBSS data from 1995-1997. Both power analyses at that time determined that 10 sites per PSU would achieve the desired power.

A new power analysis with the same 30-80-95 objective was completed using all MBSS sites sampled through 2014. The many more sites available also allowed us to use only true reference sites for this new power analysis (290 for Coastal Plain and 137 for Western Coastal Plain), rather than including the "similar" sites (i.e., sites did not fully meet the reference threshold) that were used in the original analysis to increase the number of sites for analysis. The number of available reference sites also allowed us to determine the variability (and power) in sampled sites for two different categories of Coastal Plain sites relevant to Anne Arundel County: (1) all MBSS Coastal Plain reference sites and (2) MBSS western Coastal Plain sites. In each analysis, only the most recent sampling at each site was used to eliminate pseudoreplication. This new power analysis indicates that less than 10 sites per PSU will meet the 30-80-95 objective (see Figure 1-2). Nine (9) sites per PSU are indicated by the entire Coastal Plain data, while 7 sites are indicated by the western Coastal Plain data. A second power analysis was completed on 31 "surrogate reference" sites in Anne Arundel County, since only 7 true reference sites occur in the county. A criterion of greater than 60% forest cover at the site was used to define these surrogate references, though it should be noted that most of these sites have stressors present that prevent them from being true reference sites (resulting in greater than natural variability). This power analysis indicates that 10 sites are needed to meet the 30-80-95 objective (see Figure 1-3). Based on the quality of the reference sites (i.e., closeness to natural variability) and geography similar to Anne Arundel County, we conclude that the western Coastal Plain power analysis is likely to be the most accurate (i.e., 7 sites meet the 30-80-95 objective), but choose to use 8 sites as a conservative number for survey design.

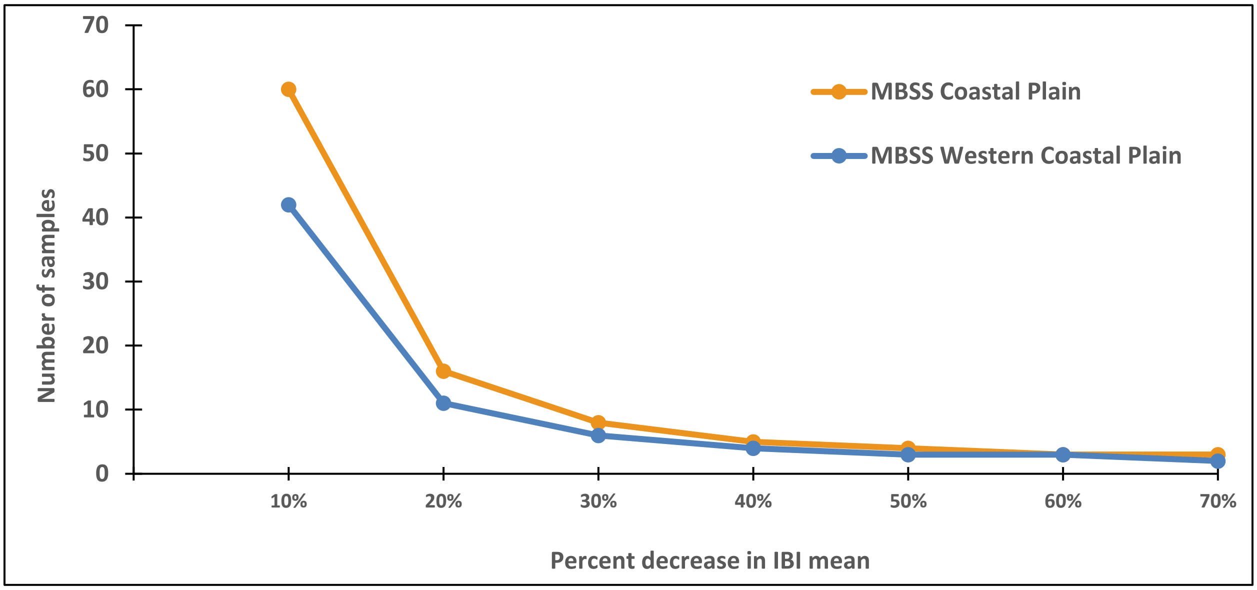

Figure 1-2. Power analysis of MBSS reference site data (for 290 Coastal Plain sites and 137 Western Coastal Plain sites relevant to Anne Arundel County) showing the number of sites per PSU that are needed to detect a 30% change in IBI scores, 80% of the time, with 95% confidence.

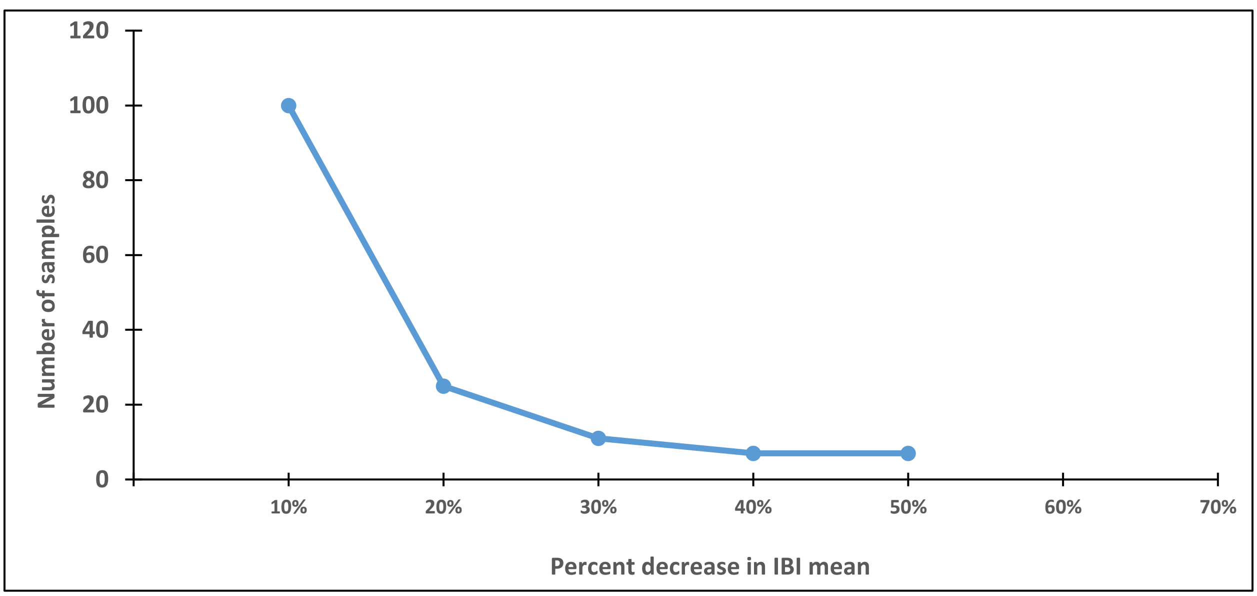

Figure 1-3. Power analysis of 31"surrogate reference" Anne Arundel site data (based on 60% or greater forest cover) showing the number of sites per PSU that are needed to detect a 30% change in IBI scores, 80% of the time, with 95% confidence.

SURVEY REVISION

Anne Arundel County will revise the survey design to include partial replacement of random sites with repeat sites from previous rounds. Specifically, two sites in each Round 3 PSU will be randomly chosen from the 10 Round 1 sites, and two sites would be randomly chosen from the 10 Round 2 sites; the remaining four sites (as dictated by the new power analysis) in each watershed would be new, randomly selected sites. This will achieve an ideal balance of 50% fixed and 50% random sites in the round. Future rounds would retain the same fixed sites (for optimal trend detection) and select new random sites each round.

The revised design would retain the proportional allocation to stream order (1st, 2nd, and 3rd). The allocation in proportion to the number of stream miles in each stream order retains the assumption that sites are selected within watersheds in a simple random manner, without the need for weighting by stream order. The repeat sites will be selected at random and the new random sites chosen from each stream order as needed to retain the proportional allocation to the extent practical. Selected sites may deviate from the exact stream order proportions because of small numbers of stream miles in a stream order, but will remain randomly selected sites.

Calculations of areawide condition (equations used to determine significant differences in IBIs) will remain the same. Comparisons of areawide change over time will be more precise because among site variability will be less, i.e., the power analysis using only random sites is conservative for this design. The replacement of fixed sites that are unavailable owing to lack of permissions or access, with random sites will only have a small effect on reducing the ability to detect trends. Comparisons of change in the fixed sites alone will have the greatest power.

Modifying the survey design to include allocation of sites by subwatershed strata for more even geography is not warranted, given the fairly even distribution of random sites which will increase with future rounds.

As described in Section 2, the survey will also be revised to include the additional stratum of small streams on the more detailed County stream network, so that areawide condition assessments can be conducted for both small and large streams, separately, within each watershed. This requires that the number of sampling sites be increased proportionally. Therefore, as the number of sampling units will be increased from 24 watersheds to 48 categories of watershed-specific small streams and watershed-specific large streams, the number of sites that must be sampled to retain the same power will double from 8x24 or 192 sites to 8x48 or 384 total sites (compared to the 240 sites sampled in each of Rounds 1 and 2).

2 Change to a more detailed stream network with implications for survey design and comparability with previous rounds

Currently, Anne Arundel County uses the National Hydrography Dataset (NHD) at the 1:100,000 scale to identify and assess streams in the biological sampling program. The County is also developing a field-verified stream map from its watershed assessments that is finer than the topographic 1:24,000-scale map. This more detailed stream network map is complete for about 80% of the county and will be finalized when the remaining watershed assessments are completed. This map is derived from field investigations by a variety of consultants and includes some variability in the extent of the stream network by watershed. It should be noted, however, that no map is a perfect representation of on-the-ground features, and this more detailed map better captures the true stream network than previous maps.

The importance of these small streams is evidenced by the continued debate on the breadth of "Waters of the United States (WOTUS)" as summarized in Meyer et al. (2003) and U.S. EPA (2015a). The new federal rule on WOTUS mandates inclusion of smaller waterbodies in protection efforts. In addition, using the more detailed map is important for understanding the continuity of the stream network, even if some small streams are not sampled (Matt Baker, TRC).

While the benefits of sampling at this finer scale are considerable, the costs and complications are significant:

- The more detailed stream network will need to be quality assured and it will not have the attributes and modeling capability of the NHD.
- It will be more likely that many of these streams will be dry at the time of sampling, especially those streams designated as ephemeral or intermittent. While all the streams smaller than the 1:100,000-scale network are included in this sampling design, the County could choose to delete those stream segments designated as ephemeral or intermittent from the more detailed County stream layer. This would reduce the number of new small streams added by about one-third.
- To allow backward comparison of stream assessments, the number of sample sites will be twice that of a single stratum (in this case, two strata of 8 sites each in each watershed per the new power analysis, so 1.6 times more sites than sampled in Rounds 1 and 2)
- A new B-IBI for these smaller streams will need to be developed, as it is very likely that natural differences dictate a separate reference condition (see Section 6)

2.1 Stream miles that would be added to the monitoring program by converting to the more detailed County stream network

Figure 2-1 shows the extent of the more detailed stream network overlain on the current 1:100,000-scale network. The 1:100,000-scale map includes 422 stream miles; the more detailed Anne Arundel County map includes 1,448 stream miles. Note that 5% more small streams may be added with completion of the remaining watershed assessments (as about 20% more stream miles than the planimetric base map are added in the field verification), resulting in about

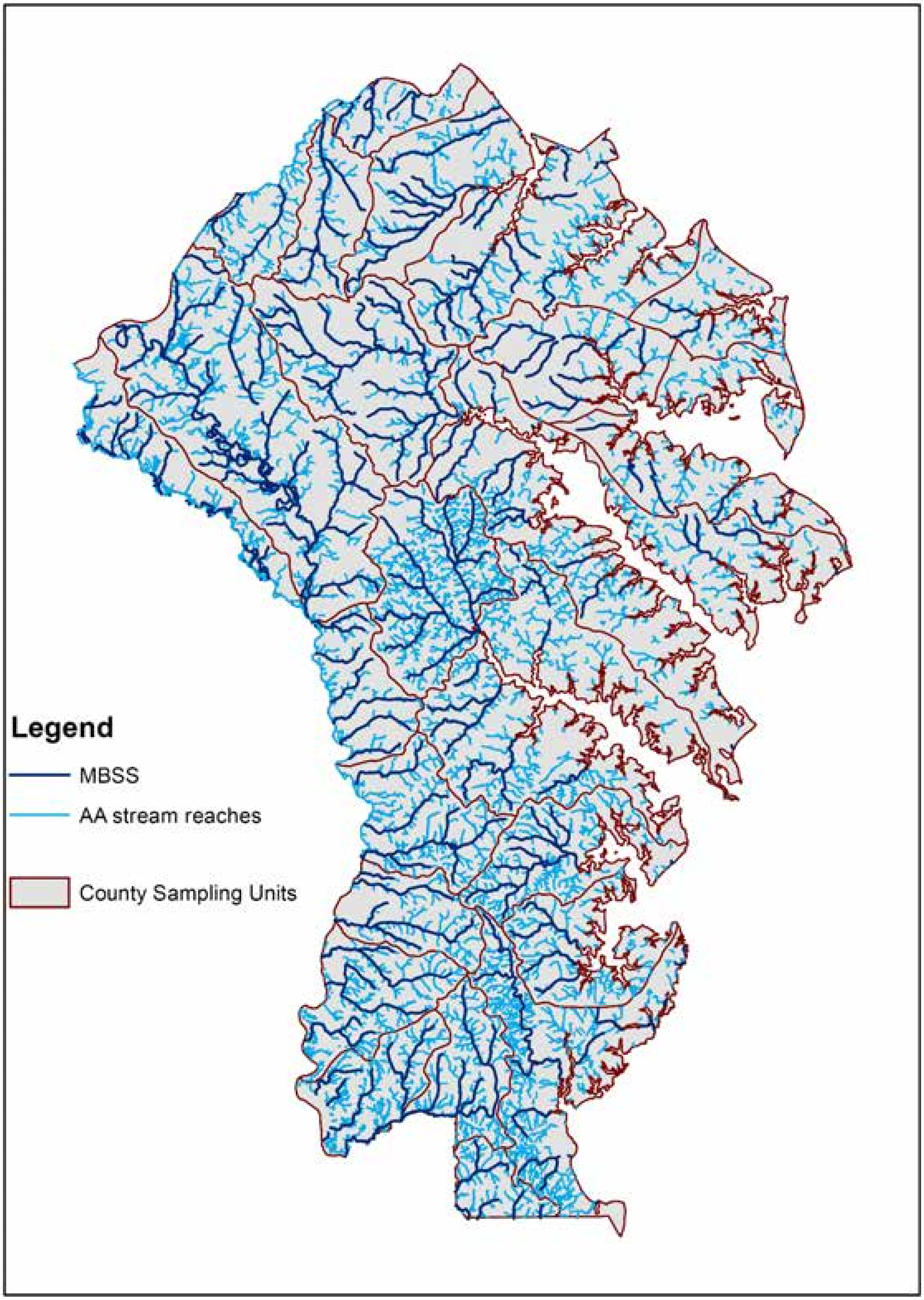

Figure 2-1. Comparison of the more detailed County stream network (not yet complete) and current 1:100,000-scale stream network, showing an increase from 422 to 1,448 stream miles.

1,500 stream miles. While there are some 1:100,000-scale stream reaches that do not appear on the more detailed stream network, the vast majority of the difference are the additional stream miles on the more detailed network compared to the current 1:100,000-scale network. Analysis done on the MBSS 100,000-scale stream network compared to the 1:24,000-scale network used by Montgomery County (Roth et al. 2002; Volstad et al. 2003) revealed that 4% of total stream miles were only on the 1:100,000-scale while 60% were only on the 1:24,000-scale (with 36% common to both maps).

The additional stream miles on the more detailed County stream network constitutes an approximately 250% increase in the number of stream miles (3.5 times more stream miles). These new stream reaches are, by definition, nearly all smaller in Strahler order and size of the catchments that drain to them.

2.2 Increase in sites needed to maintain 30-80-95 power (currently 10 per PSU) for comparing back to subset of larger 1:100,000-scale streams from previous rounds

The new power analysis completed for this project is described in Section 1.4. It concludes that eight sites per PSU will meet the 30-80-95 objective (see Figure 1-2). Section 1 also notes that eight sites per PSU will be needed for each population of streams to be assessed.

A key aspect of adding more, smaller stream miles to the survey design by switching to the more detailed County stream network is the need to continue to compare only the streams on the 1:100,000-scale network in future rounds with the results of Rounds 1 and 2. It is not statistically valid to compare the complete new stream network to Rounds 1 and 2, because the new network comprises a different population of streams, one that includes smaller streams not sampled in Rounds 1 and 2. Therefore, to retain the 30-80-95 power goal, eight random sites must continue to be sampled on the 1:100,000-scale stream network in future rounds. To attain an assessment of the smaller streams (those on more detailed map but not on the 1:100,000-scale map), another eight random sites per PSU must be sampled in future rounds.

We considered whether the smaller streams added from the more detailed County stream network would be more naturally variable and require more sites to be sampled to attain the same 30-80-95 power goal. To evaluate this, we conducted a power analysis of 31 MBSS reference sites that are small streams (draining catchments of less than 575 acres) sampled in the Western Coastal Plain. The 575-acre threshold represents the smallest 50% of streams in Anne Arundel County. This analysis indicates that these smaller streams are significantly less variable than all Western Coastal Plain reference sites, so that sampling more than eight sites per stratum are not needed (see Figure 2-2). Therefore, to obtain the desired 30-80-95 power with the more detailed Anne Arundel County stream network, the number of sites sampled would double from 192 to 384.

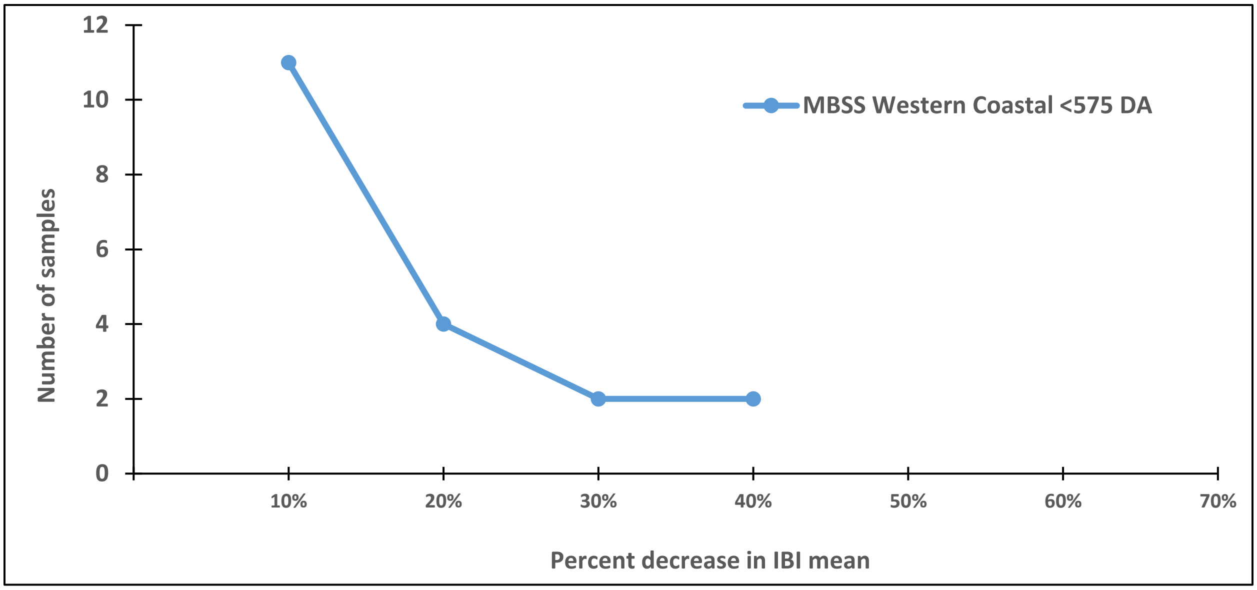

Figure 2-2. Power analysis of 31 MBSS reference small stream sites (draining catchments of less than 575 acres) sampled in the Western Coastal Plain., showing the number of sites per PSU that are needed to detect a 30% change in IBI scores, 80% of the time, with 95% confidence.

2.3 Evaluate stream size differences in B-IBI that might indicate the need for a new B-IBI for smaller streams added with more detailed County map (e.g., identify variation in metric scores at reference sites with different drainage areas)

In addition to the need to sample more sites when converting to the more detailed County stream network, the appropriateness of using the existing indicators (i.e., the MBSS Benthic Index of Biotic Integrity or B-IBI) on smaller streams must be considered.

The exact comparison of B-IBI differences between the 1:100,000-scale stream network and the more detailed stream network cannot be calculated because no sampling has been done on the smaller streams. As a surrogate, we looked at the analysis in Section 6 where Figure 6-6 shows the difference in mean B-IBI scores for county streams draining small (< 575 ac) versus large (> 575 ac) catchments. This difference would be greater, and perhaps much greater, when considering the smaller streams on the more detailed stream network. Some of the streams on the more detailed stream network are ephemeral and intermittent, and have significantly different ecological character.

Another critical concern with converting the sampling frame to the more detailed Anne Arundel County stream network is the need to quality assure and finalize the network. At present, there are inconsistencies in the density of streams identified in different watersheds (perhaps an effect of different sampling teams under the watershed management program), as well as the attributes ascribed to each stream reach. This is a substantial effort for any new sampling frame.

SURVEY REVISION

Anne Arundel County will use the more detailed stream network to assess watershed conditions in Round 3 and future rounds. The survey will treat the larger streams (from 1:100,000-scale map) as one stratum and the smaller streams (occurring on the more detailed map but not on 1:100,000-scale map) as a second stratum within each watershed. The survey will sample eight sites per stratum (as indicated by the new power analysis), equaling 16 sites in each of the 24 PSUs, so that both larger and smaller stream populations can be compared across all rounds where they were sampled. The County will attempt to develop a small-stream B-IBI using appropriate small-stream reference conditions. Note that this will increase the overall sampling effort for the Program from the current level of 240 sites per round (10 per PSU) to 384 sites (16 per PSU).

3 Combine the monitoring in the biological survey with monitoring in the watershed assessments using representative analysis

The County has assessed the biological condition (among other factors) of streams under a watershed management program that targets stream sites on downstream and mid-watershed reaches. In contrast, the Program monitors randomly selected stream sites stratified by watershed and stream size over multiple rounds. Because the watershed management program assessments are nearly complete, the biological monitoring program will not be revised to address integrating these targeted and random assessments.

Nonetheless, it is valuable to compare the biological condition results obtained by both work efforts. Figure 3-1 shows the percentage of sites scoring less than 3 on the Benthic Index of Biotic Integrity (B-IBI) within each of the 24 PSUs in the Program. A score of less than 3 indicates the site has a degraded biological condition (i.e., is rated Poor or Very Poor). The percentage of random (Round 1 and Round 2) sites is a valid estimate of the percentage of degraded stream miles in the PSU; the percentage of targeted sites may not be unbiased but may, nonetheless, produce similar results. The results show that, in one-third of the assessed watersheds, the percentage of degraded targeted sites fell between the percentages of degraded random sites in the two different random rounds. Where the targeted sites percentages were different, they were nearly equally higher (in six watersheds) or lower (in six watersheds). This indicates that the targeted biological assessments are not producing an identifiable bias in the results by watershed. In general, the extent of difference in the percentage of degraded targeted sites was similar to the difference between the two random site rounds, which can be attributed to temporal effects such as weather.

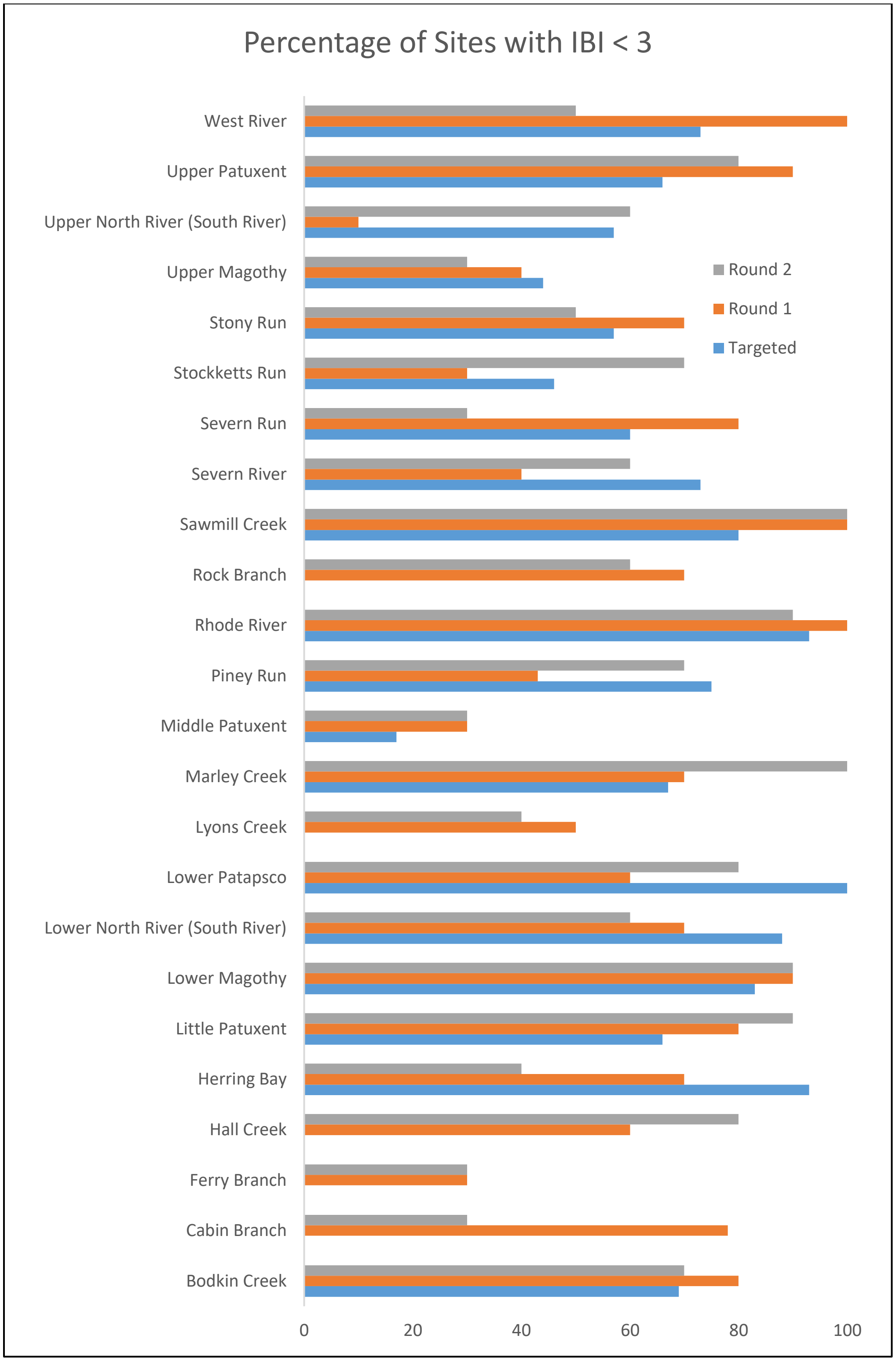

Figure 3-1. Percentage of sites scoring less than 3 on the Benthic Index of Biotic Integrity (B-IBI) within each of the Program's 24 PSUs from two random rounds of sampling (Round 1 and Round 2) and one targeted (non-random) program of sampling

Should the County wish to combine the results of the random (probability-based) and targeted (non-probability-based) sites in the future, this may be possible through representativeness analysis. A summary of this approach is provided below.

3.1 Summarize the literature on using non-random "found" samples to augment random surveys

A common problem that plagues long-term water quality monitoring programs is the change of survey structure and data collected over time as various monitoring programs can develop, diminish, or abruptly change over time. Often researchers are left with the challenge of how to link "found" data, or data from a nonprobability-based survey, with observations from a true statistical sample (p-sample) and attempt to retain the advantages for estimation inherent in the statistical sample. Overton et al. (1993) proposes a framework to integrate probability sampling and found samples by grouping found data into similar subsets. Two methods for integrating found data are outlined—a pseudo-random approach and a calibration approach.

The pseudo-random approach is taken when the variables of interest have been measured in both the found data and the random (p-sample) dataset, so that combined they increase the effective sample size. For pseudo-random approach, the p-samples are stratified into homogenous subsets that represent corresponding subpopulations. Found samples then are assigned to the p-sample subsets and are assumed to be representative of the corresponding subunits. The calibration method is used when the found sample contributes information that is unique and has not been measured in the p-sample. This approach requires the development a predictor equation. This equation estimates the variable of interest from a regression based on the characteristics of the p-sample sites. These estimations of the variable of interest can then be used to supplement the p-sample dataset.

Through this data supplementing approach, Overton et al. (1993) report a decrease in the standard error of the parameter estimates and an increase in precision when the found sites were assumed representative of the assigned subpopulations. Importantly, Overton highlights the most important use of found data, the ability to extend inferences to attributes not available in the p-sample.

For creating successful long-term monitoring studies, Overton and Stehman (1996) suggest design strategies that allow for adaptations to changes while maintaining capacity to detect trends over time. Three primary sampling features that they emphasize are (1) subpopulation estimation, (2) post-stratification from conditional design, and (3) sample restructuring. In addition, Overton and Stehman warn against using data from complex designs where the designs limit the data's suitability for other studies.

SURVEY REVISION

Because the watershed management program assessments are nearly complete, the biological monitoring program will not be revised to address integrating these targeted and random assessments. Should the County wish to combine the results of the Program and the targeted watershed management program sites in the future, the Overton approach can be used.

4 Add additional MBSS or other parameters (e.g., fish, geomorphic, and water quality parameters) to the biological survey

Although the Program was patterned after the MBSS in 2003 and revised in 2009, the MBSS has evolved during this time, as have the County goals, especially related to TMDLs. Currently the Program samples for five water quality parameters, benthic macroinvertebrates, and five physical and geomorphic parameters.

4.1 Water quality – Anne Arundel County currently only samples for dissolved oxygen, pH, temperature, specific conductance, and turbidity as part of the biological monitoring program.

The County will add sampling for additional water quality parameters to improve detection of local stressors, especially those related to TMDLs, and to comport with Federal and State monitoring programs. Of particular interest are total nitrogen, which is a better indicator of condition during baseflow monitoring than total phosphorus, which is bound to sediments that move in storm flows (Don Weller, SERC, TRC), and chloride which will soon become a water quality standard in Maryland (Matt Stover, TRC). Chloride from road salt is an emerging major stressor to streams for which EPA has established a new benchmark for drinking water standards in the Coastal Plain of 250 mg/L. Scott Stranko and Ray Morgan stated that Tim Fox of MDE has a method for determining the proportions of different constituents such as chloride in the ion matrix when at least one ion is measured along with conductivity (which is already sampled by the County). The TRC considered a phased approach that would only monitor additional parameters where conductivity is high, but the County determined that 93% of all sites sampled in Rounds 1 and 2 had conductivity values above 100 µS/cm and that more than 40% of sites had values above 250 µS/cm. Therefore, the County will add a standard suite of the nutrient and metals parameters typical of other monitoring programs. Sampling for pesticides will not be conducted as laboratory analysis was judged too expensive the Program to implement. Oil and grease is another useful parameter, but only when sampled in storm flows, which are not captured by the Program. The TRC also agreed that sampling for bacteria is of limited value without microbial source tracking (MST) to differentiate between human, pet, livestock, and wildlife sources; MST techniques are becoming increasingly accurate but were considered too expensive for a the Program to implement.

Table 4-1 lists the parameters sampled by the MBSS and some other Maryland counties. The prices shown are those charged by the UMCES-Appalachian Laboratory. Different prices may be available from different vendors. A per sample processing fee is typically an additional cost charged by analytical laboratories.

Table 4-1. Per site cost for chemical parameters sampled by MBSS and certain Maryland Counties (Katie Kline, UMCES-Appalachian Laboratory). The 48 sites/year annual cost is comparable to the size of the current annual chemistry monitoring conducted by the County.

Parameter	Cost per Sample	Cost per year (assuming 48 sites/year)
Turbidity	$5.00	$240.00
Total Nitrogen	$14.00	$672.00
Total Phosphorus	$14.00	$672.00
Ammonia-N	$7.00	$336.00
TKN (calculated)	$0.00	$0.00
Nitrate-Nitrogen	$15.00	$720.00
Nitrite-Nitrogen	$10.00	$480.00
Dissolved Organic Carbon	$15.00	$720.00
Orthophosphate	$15.00	$720.00
Total Organic Carbon	$10.00	$480.00
Copper	$12.00	$576.00
Lead	$12.00	$576.00
Zinc	$12.00	$576.00
Chloride	$15.00	$720.00
TOTAL	**$156.00**	**$7488.00**

4.2 Geomorphology – Anne Arundel County currently performs a Rosgen Level II geomorphic assessment, including a qualitative physical habitat assessment (0-20 scale), modified Wolman pebble count, stream cross section, water surface slope, and reach sinuosity measurements. Maryland DNR has recently developed geomorphic methods for MBSS core sampling (less intensive) and Trust Fund sampling (more intensive). Research on the relationships between geomorphologic and biological results is still inconclusive.

Attempts to correlate biological condition (B-IBI) in County streams with current geomorphology results (Rosgen Level 2 assessments) have met with very limited success (Chris Victoria, Anne Arundel County, TRC). To date, few strong relationships between geomorphic parameters and biology have been demonstrated; however, a useful metric may remain to be discovered somewhere between the coarse level perspective that humans see and the fine level that benthic invertebrates see (Scott Lowe, TRC). In addition, geomorphology is context dependent, meaning that (1) geomorphology may not be a controlling factor when degraded water quality is present and (2) Coastal Plain streams are relatively homogeneous in their geomorphic characteristics.

While the exact magnitude of influence varies, it is clear that the physical characteristics of stream channels influence the biological community found in these systems (see, for example, Myers and Resh 2000 or Schwartz and Herricks 2008). Consequently, it is important to have some understanding of these variables in a biological monitoring program beyond qualitative habitat assessments like those of the RBP or MPHI.

The MBSS has initiated geomorphic assessments for Round 4 sampling begun in 2014. Investigations into MBSS biology-geomorphology relationships are only recently underway, but indicate that substrate assessments may improve on previous correlations. Upon completion of the analysis from MBSS Round 4 analyses, the MBSS will continue to monitor the most promising geomorphology parameters in future years.

Given the TRC was not able to propose a geomorphic assessment method with a stronger relationship to overall biological condition, the County will continue Rosgen Level II assessments and participate in further analysis with MBSS before other parameters are added.

4.3 Fish, Amphibians, or Other Organisms – The County does not currently sample fish or amphibians as part of the program, but believes that these organisms may be good indicators for wetland-stream complexes within the county. Round 4 of the MBSS is sampling for salamanders and hopes to apply a streamside salamander IBI, though likely not in the Coastal Plain. MBSS also samples other herpetofauna, crayfish, mussels, and vernal pools.

Fish. Sampling stream fishes is an important part of assessing and restoring watershed health (Scott Stranko, TRC). They are the component of stream biota of most interest to the public, both commercially and aesthetically. The electrofishing technique employed by the MBSS gives a nearly complete census of the community which allows for robust estimates of the density and abundance of individual species, estimates that are not obtainable under current sampling methods for benthic macroinvertebrates. These estimates can be useful for identifying species that are common, rare, or in need of conservation measures to secure populations within the County. Accurate abundance and distribution information is particularly useful for managing recreational fishes in the County, such as various perch, bass, pickerel, and trout. Also, the distribution of American eel is of special importance for its commercial value and influence on stream communities.

Additional benefits of including fish sampling in the Program are the ability to detect non-native and invasive fishes, stressors related to fish health, and barriers to movement. By sampling for fish, the County will be able to detect and track the spread of invasive species within the county. Some of the species with the potential to affect County stream communities are the northern snakehead, blue catfish, and oriental weather loach. Fish sampling can also monitor stream conditions using long-lived fish, such as suckers and catfish that can live for 10 or more years. Long-lived species also develop fish tumors that can help identify potential stressors. A Countywide survey of fish would also identify gaps in stream connectivity, because, unlike stream invertebrates that have winged adult dispersal, fish rely upon stream connectivity to disperse and found populations in un-colonized streams.

Analysis of MBSS datasets has found low correlations between benthic invertebrate IBI scores and fish IBI scores, which likely indicates that these two indices of biological condition are responding to different environmental stressors and habitats. This is similar to findings in other regions that fish metrics tend to respond to reach-scale, geomorphology, and water chemistry effects, while macroinvertebrate metrics tend to respond to larger-scale land use effects (Johnson and Ringler 2014). By assessing both fish and macroinvertebrate conditions, the County will obtain a more complete picture of watershed health and will be able to identify and react to wider range of environmental conditions (Freund and Petty 2007).

The cost of adding fish sampling using the MBSS methods will likely be about $4,500 per site (Stranko, Maryland DNR, TRC).

Amphibians and Reptiles. The Stream Salamander Index of Biotic Integrity (SS-IBI) is the latest biological indicator developed by the MBSS (Southerland et al. 2004). Stream salamander sampling in underway as part of Round 4 of the MBSS and results will be used to validate the SS-IBI so that it can be used in Round 4 reporting. It is anticipated that MBSS will not apply the SS-IBI to Coastal Plain streams, because the number of species and abundance of individual salamanders are too low for effective IBI development.

The MBSS has sampled all amphibians and reptiles using either incidental or areawide searches of the riparian area during the four rounds of the MBSS. There is an increasing relationship between the number of amphibian and reptile species found at each site and B-IBI scores in Anne Arundel County (Figure 4-1). Table 4-2 and Figure 4-2 show the number of each species of amphibian and reptile sampled in Anne Arundel County by the MBSS from 1995-2013.

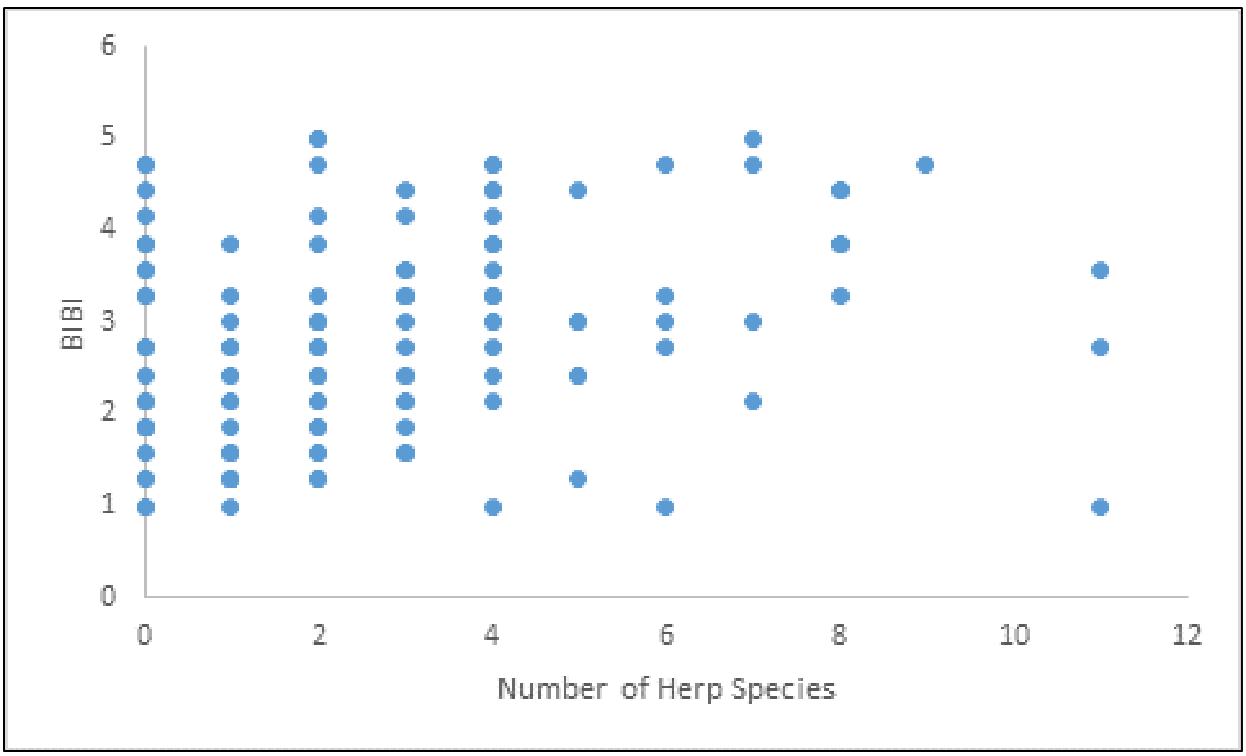

Figure 4-1. Relationship between B-IBI and number of amphibian and reptile species sampled at MBSS sites in Anne Arundel County from 1995-2013

Table 4-2. Amphibian and reptile species in Anne Arundel County found by the MBSS from 1995-2013	
Amphibian and Reptile Species	**Number of Occurrences**
American Bullfrog	58
Broad-Headed Skink	1
Common Five-Lined Skink	10
Cope's Gray Treefrog	4
Eastern American Toad	20
Eastern Box Turtle	16
Eastern Cricket Frog	11
Eastern Gartersnake	2
Eastern Mud Salamander	1
Eastern Painted Turtle	1
Eastern Red-Backed Salamander	2
Eastern Smooth Earthsnake	1
Eastern Snapping Turtle	6
Eastern Wormsnake	2
Fowler's Toad	11
Gray Treefrog	6
Marbled Salamander	1
Northern Dusky Salamander	2
Northern Green Frog	104
Northern Red Salamander	2
Northern Red-Bellied Snake	1
Northern Ring-Necked Snake	3
Northern Spring Peeper	17
Northern Two-Lined Salamander	38
Northern Water Snake	5
Pickerel Frog	51
Queen Snake	2
Red-Spotted Newt	3
Ring-Necked Snake	1
Southern Leopard Frog	13
Spotted Salamander	3
Stinkpot	3
Wood Frog	8

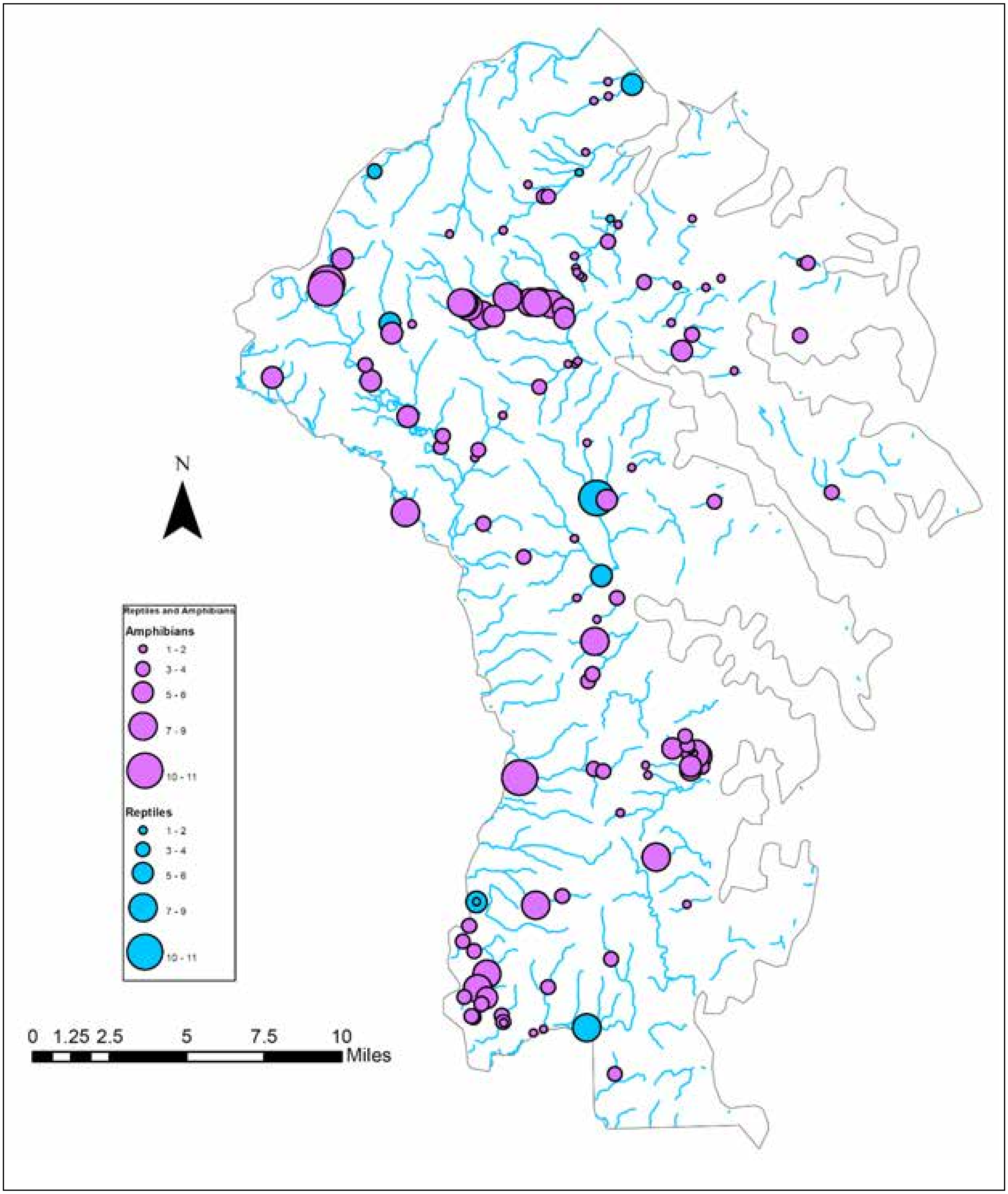

Figure 4-2. Locations of MBSS sites where amphibians and reptiles were found by the MBSS in 1995-2013. Size of circle indicates number of species.

The cost of the adding herpetofauna to the county survey is variable. Under current MBSS protocols for Round 4, herpetofauna searches last up to 60 minutes.

Crayfish and Mussels. The MBSS has also sampled crayfish and mussels for periods of the MBSS. Table 4-3 and Figure 4-2 show the number of each species of crayfish and mussels sampled in the County by the MBSS from 1995-2013 (2007-2013 for crayfish). Only 10 sites recorded mussels, eight of which were the non-native Asian clam. Crayfish were more common, totaling 34 records, only five of which were non-native.

Table 4-3. Crayfish and mussel species in Anne Arundel County found by the MBSS from 1995-2013 (2007-2013 for crayfish).

Crayfish Taxa	**Number of Occurrences**
Devil Crayfish	15
Eastern Crayfish	14
Virile Crayfish	4
White River Crayfish	1
Mussel Taxa	**Number of Occurrences**
Alewife Floater	1
Asian Clam	8
Eastern Floater	1

Vernal pools. Vernal pools are seasonal habitats for aquatic species including mole salamanders, wood frogs, fairy shrimp, and others. These pools are depressions in the landscape that fill with water during the fall and winter months, but which become dry over the summer. The inability of fish populations to survive in vernal pools makes them important for species that cannot survive in aquatic habitats with fish. While vernal pools are found throughout the landscape, many are found in the floodplains surrounding streams.

The MBSS has been sampling for vernal pools at their stream sites since 2007. All vernal pools observed in the 50-m riparian area are measured and recorded. A total of 23 vernal pools have been found in Anne Arundel County at 17 MBSS sites (some sites had more than one vernal pool). Figure 4-4 shows the number and location of vernal pools in the County by the MBSS from 2007-2013.

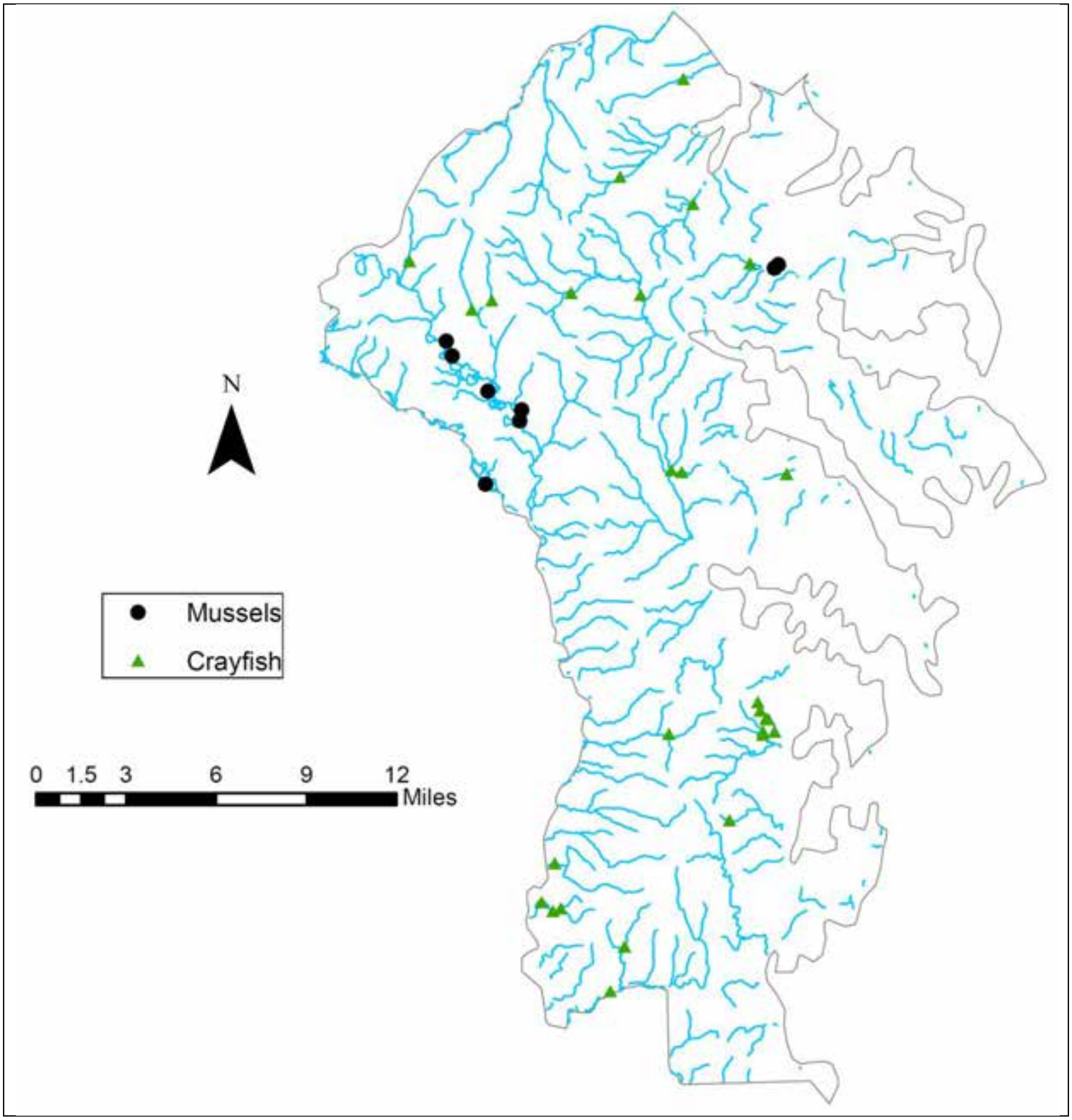

Figure 4-3. Crayfish and mussel species in Anne Arundel County found by the MBSS from 1995-2013 (2007-2013 for crayfish).

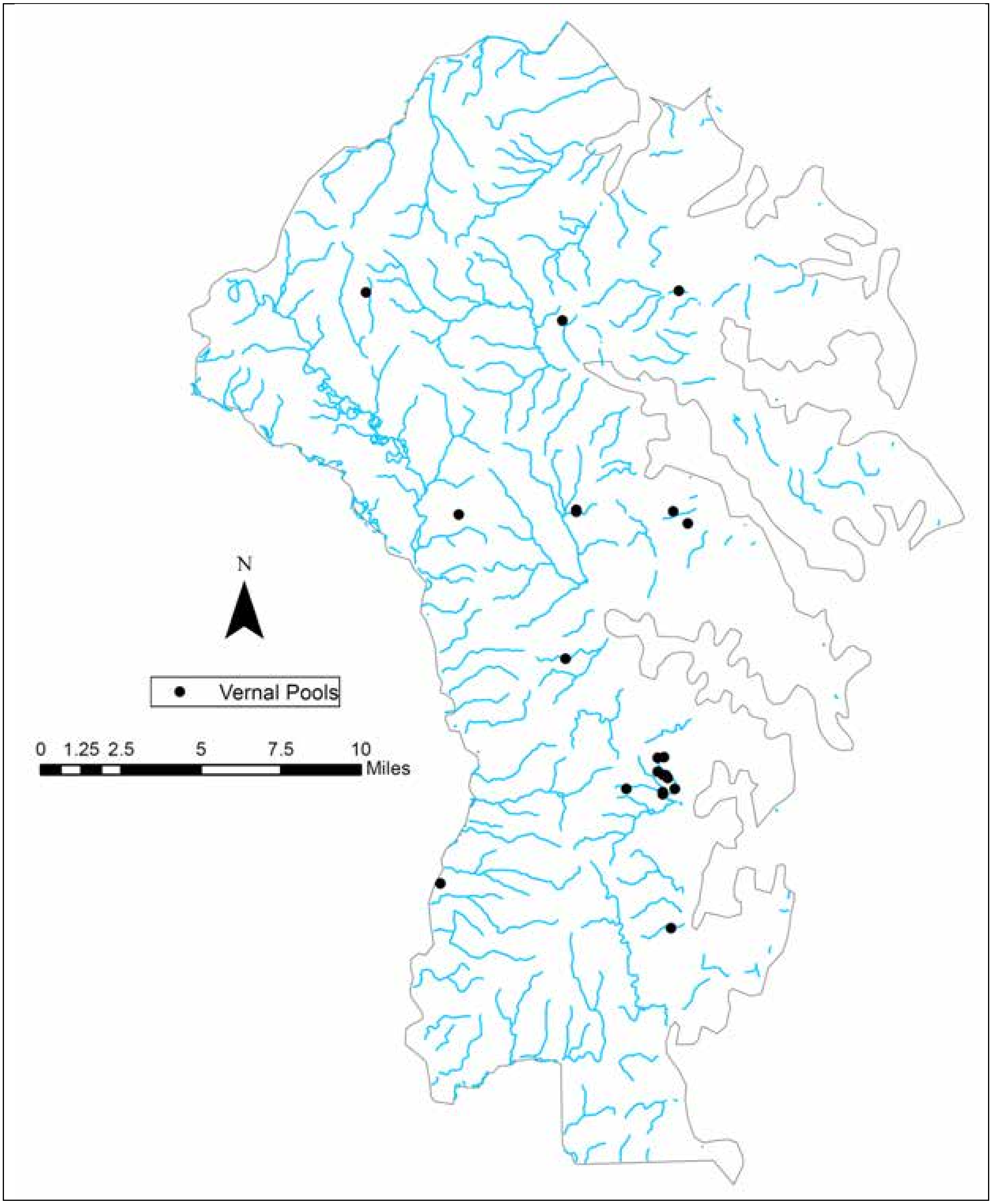

Figure 4-4. Vernal pools in Anne Arundel County found by the MBSS from 2007-2013

Periphyton. DNR has sampled for periphyton using EPA protocols (standard area scrape from in-situ rocks) at 50 sites over two years. Periphyton is also sampled in the EPA National Surveys. Periphyton have also been monitored by others for acid mine drainage, nutrient criteria, and identifying sensitive taxa. Attempts to use periphyton in nutrient criteria have been problematic, owing to variability and confounding factors such as shading (Matt Stover, TRC). While periphyton sampling is promising, especially as an additional indicator for nutrients which are subject to regulation in County TMDLs, the County and TRC decided to wait to adopt periphyton until locally validated indicators are available from another source.

SURVEY REVISION

The County will add the standard suite of the nutrient and metal parameters (addressing most TMDLs) typical of other monitoring programs, by sampling for the MBSS parameters in Table 4-1. This would include chloride, but not pesticides or bacteria.

Given the current uncertainty associated with geomorphology-biology relationships, but recognizing the need to continue trying to characterize such relationships, the County will not change its geomorphology sampling. Rosgen Level II assessments will continue in Round 3. The County will participate in further analysis with MBSS to determine if other parameters should be added. At that time, the County would continue the Rosgen Level II assessments for at least one additional year at sites that were sampled previously to (1) evaluate change over time and (2) investigate relationships between the Rosgen and new MBSS geomorphology parameters.

The County will add fish sampling as the best means of improving assessments of ecological condition of County streams. The County will adopt the MBSS electrofishing protocols at estimated cost of $4,500 per site.

The County will also add sampling for crayfish and mussels using MBSS protocols. The County will not add stream salamander sampling using the MBSS protocols as stream salamander communities in the Coastal Plain are depauperate compared to other parts of the state (MBSS SS-IBI will not apply to Coastal Plain streams). The County will also add herpetofauna searches and vernal pool sampling within the 50-m riparian area using MBSS methods. This sampling will improve the assessment of floodplain ecosystems and add more components of biodiversity to produce a more holistic ecosystem assessment.

5 Improve stressor identification at local scales

Assessment efforts like the Program and MBSS can produce robust characterizations of stressor extent and severity at the large watershed scale, depending on the parameters sampled. Stressor identification at finer scales, however, is needed for management decisions.

The County investigated four issues related to stressor identification: increase in sample parameters related to stressors, intensification of sampling in target areas, extrapolation of MS4 pollutant load monitoring to other areas, and effect of legacy land use.

5.1 Additional sample parameters

The County will add the MBSS suite of water chemistry parameters to Program as described in Section 4.1. These parameters will be used to flag potential stressors (where parameter values exceed a threshold of concern) for more intensive study. Typically, diagnosis of stressors affecting streams requires a specific "detective" method such as the U.S. EPA Causal Analysis Diagnosis Decision Information System (EPA 2015b).

5.2 Intensification of random samples in certain areas of the County

The TRC concluded that targeted sampling for stressors is more effective than intensifying random samples, so intensification of the survey design will not be implemented. One program enhancement that might provide information of the effects of stressors Countywide is the monitoring of fixed (or sentinel) sites along a gradient of imperviousness. This "sentinel" network would not evaluate the effect on annual variation in weather (or climate change) on reference sites, but would rather improve our understanding of how development (and correlated stressors) affect stream condition. This enhancement will not be pursued at this time, but may be investigated as combined activity with other counties or the State.

5.3 Evaluate which countywide biological survey parameters should be included in restoration and stressor monitoring to study possible surrogate parameters for intensive monitoring

The County already conducts restoration monitoring related to TMDL and other concerns and requirements. As stated above, targeted sampling for stressors is more effective than intensifying random samples, so intensification or stratification of the survey design for stressor identification will not be implemented. However, there are potential benefits of measuring the parameters sampled in the random survey as part of the intensive site monitoring for pollutant reduction performance and TMDL compliance. By doing this, it may be possible to identify relationships between random survey parameters and pollutant performance. One example relationship might be between simple geomorphological measurements and sediment reduction. Such relationships could be used to extrapolate pollutant reduction to larger areas where only random survey parameters were sampled.

5.4 Legacy impacts from previous land uses

Legacy land use is an important topic for interpreting monitoring results and determining the limits of restoration potential in Anne Arundel County. Harding et al. (1998) and Maloney and Weller (2011) describe and quantify the effects of legacy land use, including potential differentiation of biogeography (latitude) versus land use (elevation) effects. The TRC concluded that legacy impacts from previous land uses are another issue better addressed through targeted site monitoring. As with the gradient of impervious monitoring, this study could be undertaken in conjunction with other counties or the State and would not be a core component of the Program.

SURVEY REVISION

As described above, Anne Arundel County will add the MBSS water chemistry parameters to (1) flag potential stressors at random sites for further investigation and (2) provide estimates of stressor extent and intensity at the PSU scale.

The County will not implement a network of fixed sites along a gradient of imperviousness to better understand how development affects streams. This may be pursued in the future as a combined activity with other counties or the State, but would not be a core component of the Program.

The County will not intensify the random survey in presumed areas of high stressors, but rather add the parameters measured in the random survey (e.g., MBSS parameters) to all intensive stressor and restoration monitoring (if not already included), so that lessons learned in restoration monitoring can be applied to interpretation of the Program data.

6 Redevelop IBIs or reference conditions to address County stream types

While the Coastal Plain B-IBI is generally an effective indicator of stream condition throughout the Coastal Plain landscapes of the State, local environmental settings in Anne Arundel County can influence natural reference conditions. This is especially important at finer scale assessments. Streams near the Fall Line, such as the northwest part of the County, may have natural Piedmont characteristics. Small streams, which will become more numerous as the Program potentially incorporates the County stream network into the sampling effort, have different natural conditions than larger streams. Low-relief streams, in general, take on more anastomosed (braided) forms that have ecological implications and may justify different reference conditions.

Two kinds of analysis were performed to determine how stream conditions vary naturally based on (1) region (eastern vs. western shore), (2) size (drainage area), or (3) slope (gradient):

- comparisons of B-IBI scores at MBSS reference sites
- comparisons of B-IBI scores at Anne Arundel County sites with upstream catchments that are at least 60% forested.

The forested Anne Arundel County sites are surrogate reference sites since the parameters needed to apply MBSS reference criteria were not sampled.

Figure 6-1 shows that the distribution of stream sizes in Anne Arundel County has no distinct break point between large and small sizes with 90% of sites draining less than 5,000 acres and 50% draining less than 575 acres. Figure 6-2 also shows that the distribution of stream gradients in Anne Arundel County has no distinct break point between low and high gradient, with 90% of sites having gradients of less than 1% (with gradient calculated as rise over run distance, as a percentage)) and 50% of sites having gradients of less than 0.6%.

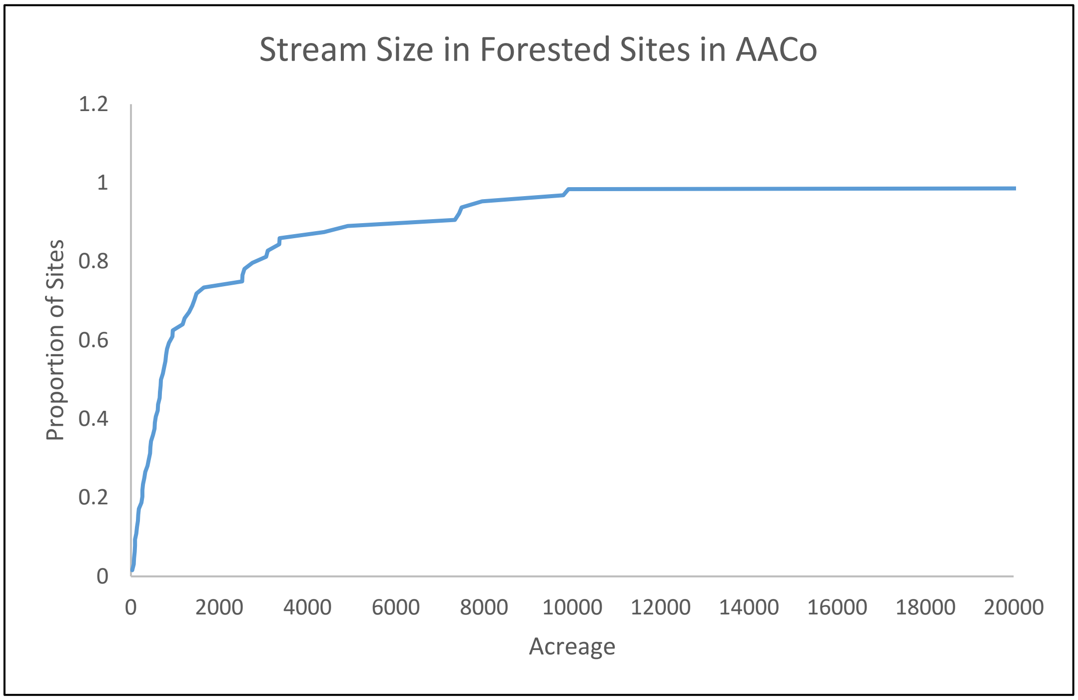

Figure 6-1. Distribution of stream sizes in Anne Arundel County showing that 90% of sites drain less than 5,000 acres and 50% drain less than 575 acres

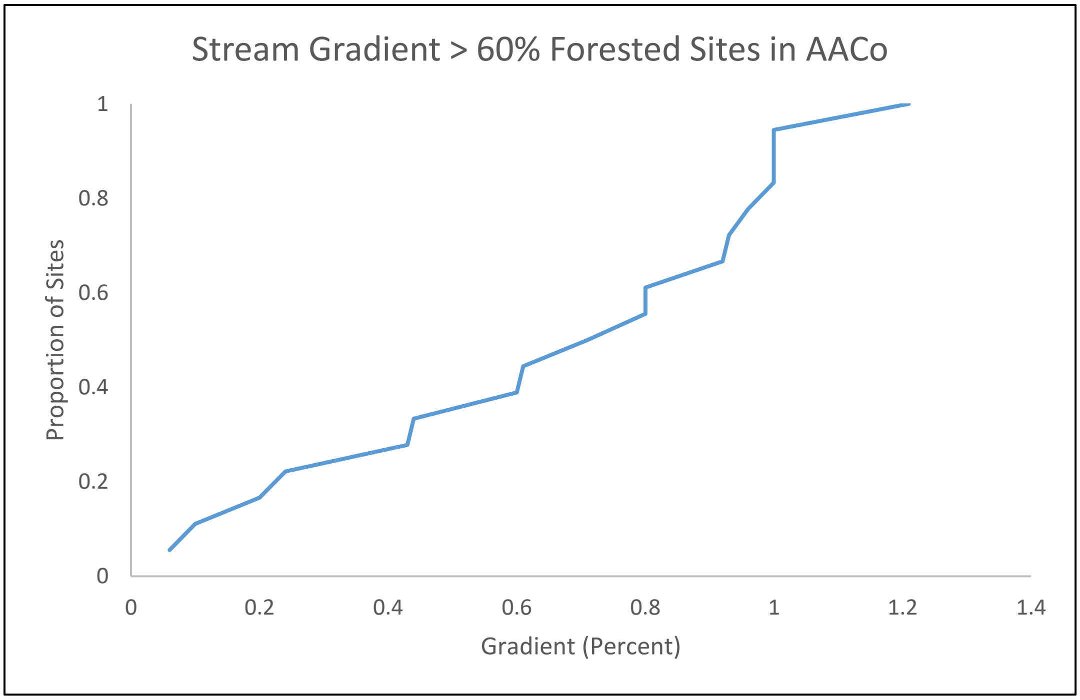

Figure 6-2. Distribution of stream gradients in Anne Arundel County showing that 90% of sites have gradients of less than 1% and 50% of sites have gradients of less than 0.6%

6.1 Are MBSS B-IBI results different between the western and eastern Coastal Plain (CP) which have a single MBSS B-IBI?

All MBSS reference sites in the Coastal Plain were assigned either to the western or eastern shore and the distribution of the B-IBI was investigated using box plots. The difference in the B-IBI scores for all sites was not significant (Figure 6-3), indicating that a separate western Coastal Plain B-IBI does not need to be developed.

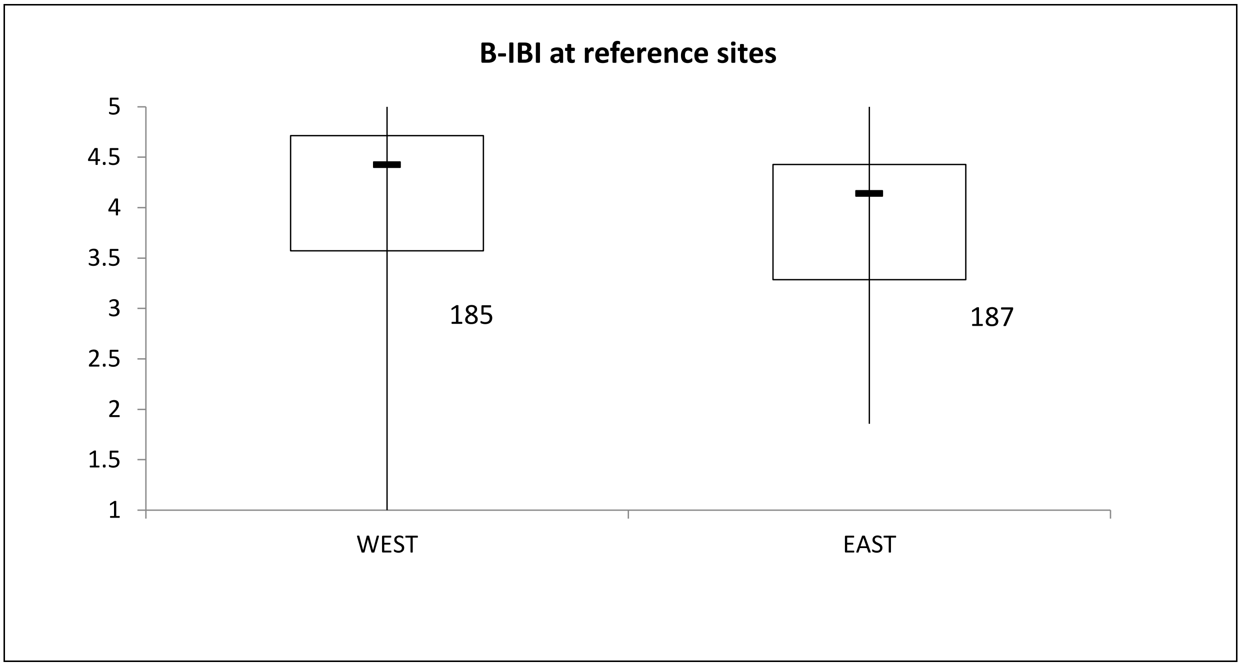

Figure 6-3. Comparison of B-IBI scores at MBSS reference sites in western and eastern Coastal Plain

Next we compared B-IBI scores at MBSS reference sites in the entire western Coastal Plain to those found in Anne Arundel County alone (Figure 6-4). In this case, there was a difference, though it was not statistically significant, as there were only seven MBSS reference sites in Anne Arundel County, including one with a B-IBI score of 1.00. Therefore we also compared western Coastal Plain and Anne Arundel B-IBI scores using surrogate reference sites based on 60% forested catchments (Figure 6-5). This comparison indicates that Anne Arundel surrogate reference sites have significantly lower B-IBIs than surrogate reference sites in the entire western Coastal Plain (not unexpected as these surrogate sites are not true reference sites).

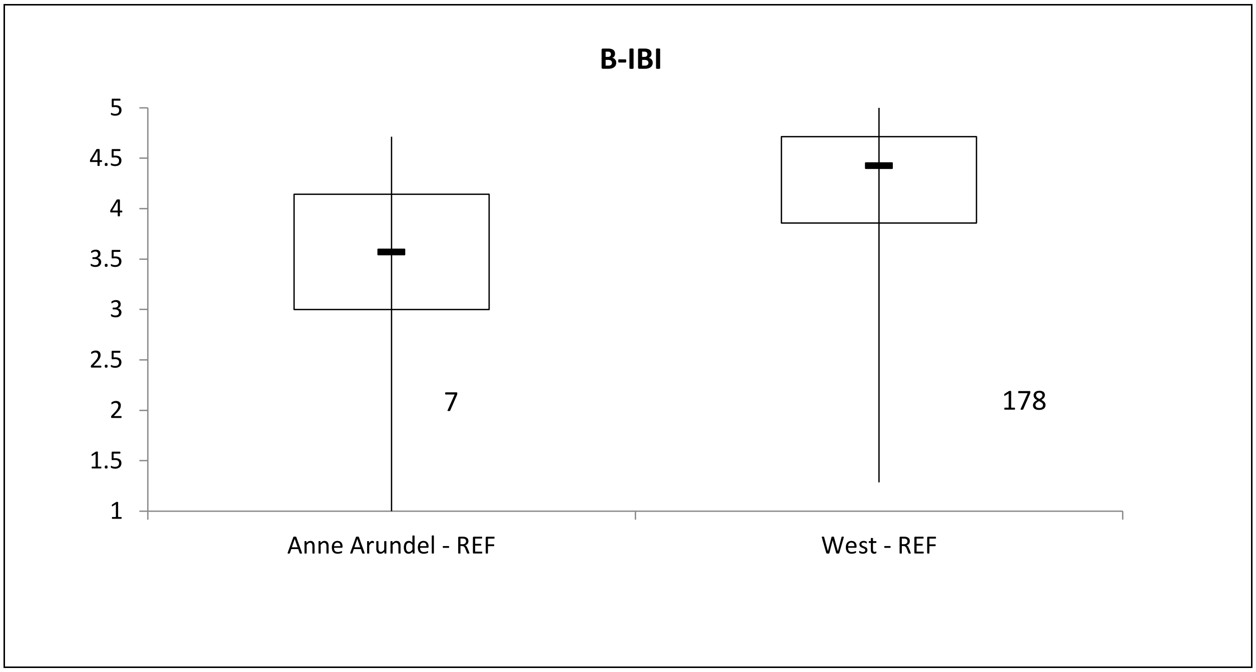

Figure 6-4. Comparison of B-IBI scores at MBSS reference sites in Anne Arundel County and the entire western Coastal Plain, which suggests a difference but is not statistically significant (may be driven by single outlier with B-IBI of 1.0)

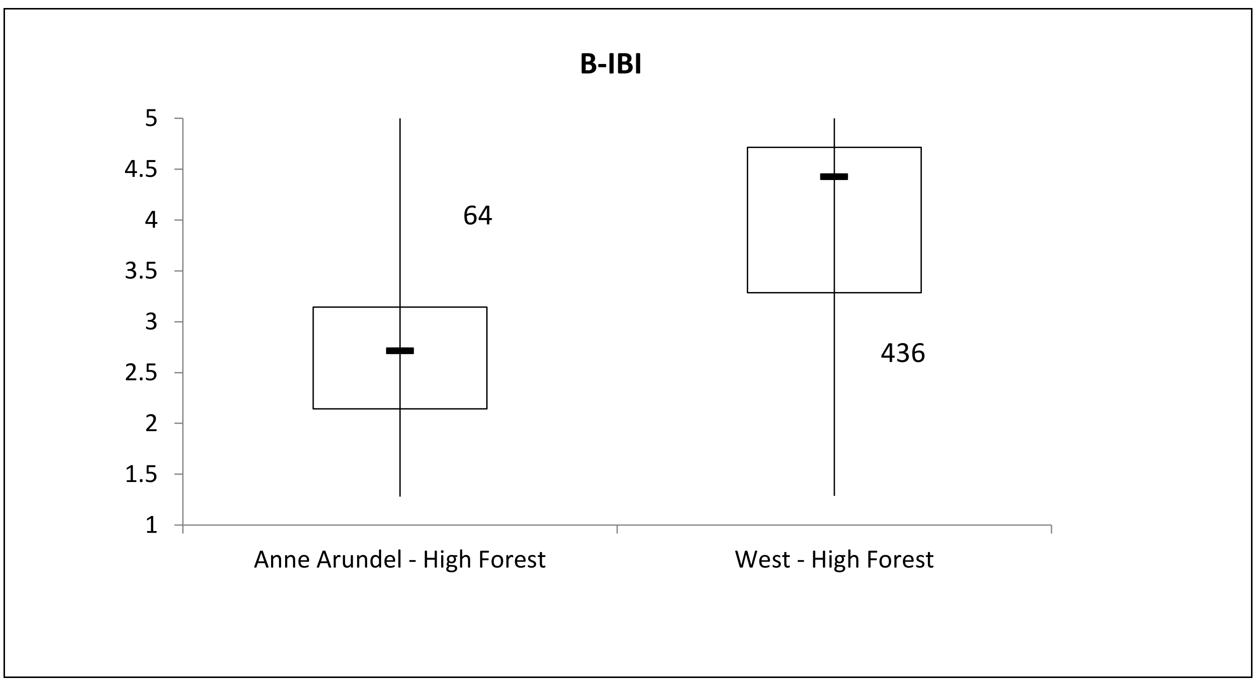

Figure 6-5. Comparison of B-IBI scores at surrogate (60% forested catchment sites) reference sites in Anne Arundel County and the entire western Coastal Plain, which is significantly different ($p < 0.001$). This may indicate that water quality or other non-land use stressors are degrading forested Anne Arundel streams.

6.2 Evaluate differences in MBSS B-IBI data by stream type (e.g., braided/wetland or slope, stream order or size, geology) for western Coastal Plain

To determine whether County streams of different sizes or gradients vary naturally in biological condition, we calculated the mean B-IBIs for each size and gradient class using surrogate reference sites based on sites draining catchments with at least 60% forest land use. These reference sites represent the top 20% of sites sampled by the County in terms of natural (forested) land use. It was necessary to use this surrogate approach because the sampling data from the Program do not include all the attributes needed to define reference using MBSS reference criteria and there are only seven MBSS-sampled references in the County.

Figure 6-6 shows that B-IBI scores for smaller Anne Arundel County streams (draining less than 575 ac) are significantly lower than scores for larger streams (draining more than 575 ac).

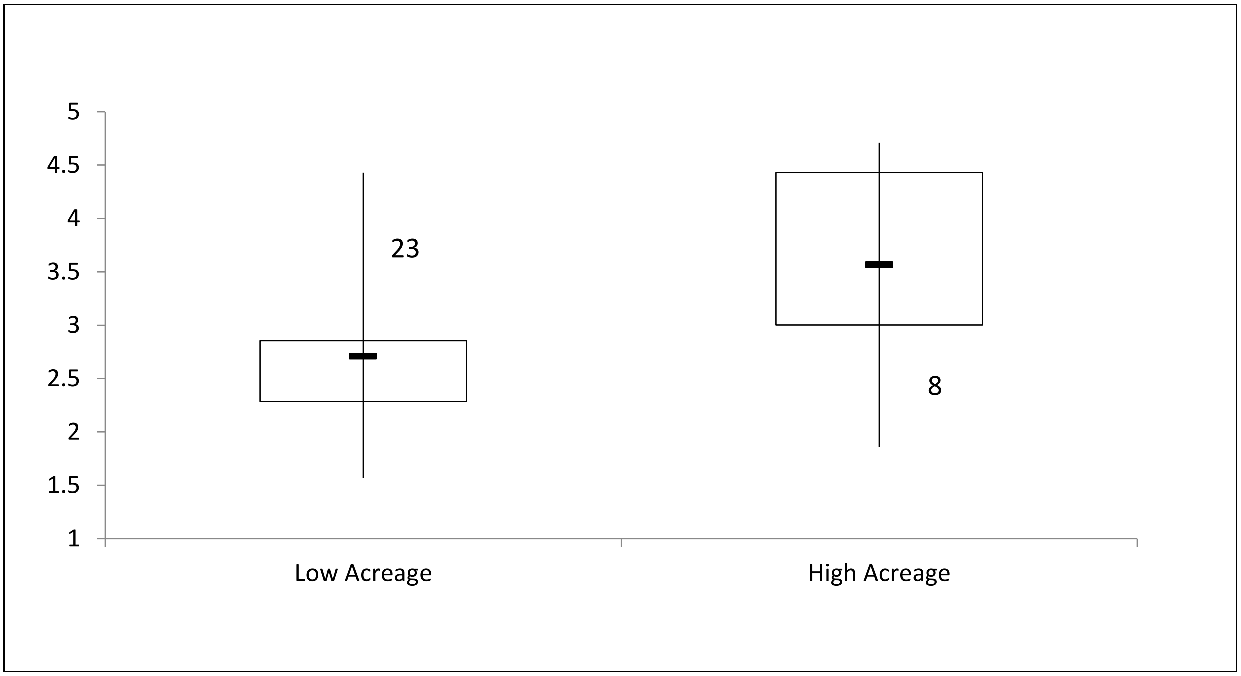

Figure 6-6. Comparison of B-IBI scores of small (draining < 575 ac) and large Anne Arundel County streams using surrogate (60% forested catchment sites) reference sites, which is significantly different ($p < 0.02$)

Figure 6-7 shows that B-IBI scores are not significantly different between low (< 0.6) gradient and high gradient County streams.

These results indicate that natural stream conditions likely differ among small and large County streams, but not among low and high gradient streams. It is possible that the low gradient cutoff for analysis did not capture wetland-type streams, which should be analyzed separately.

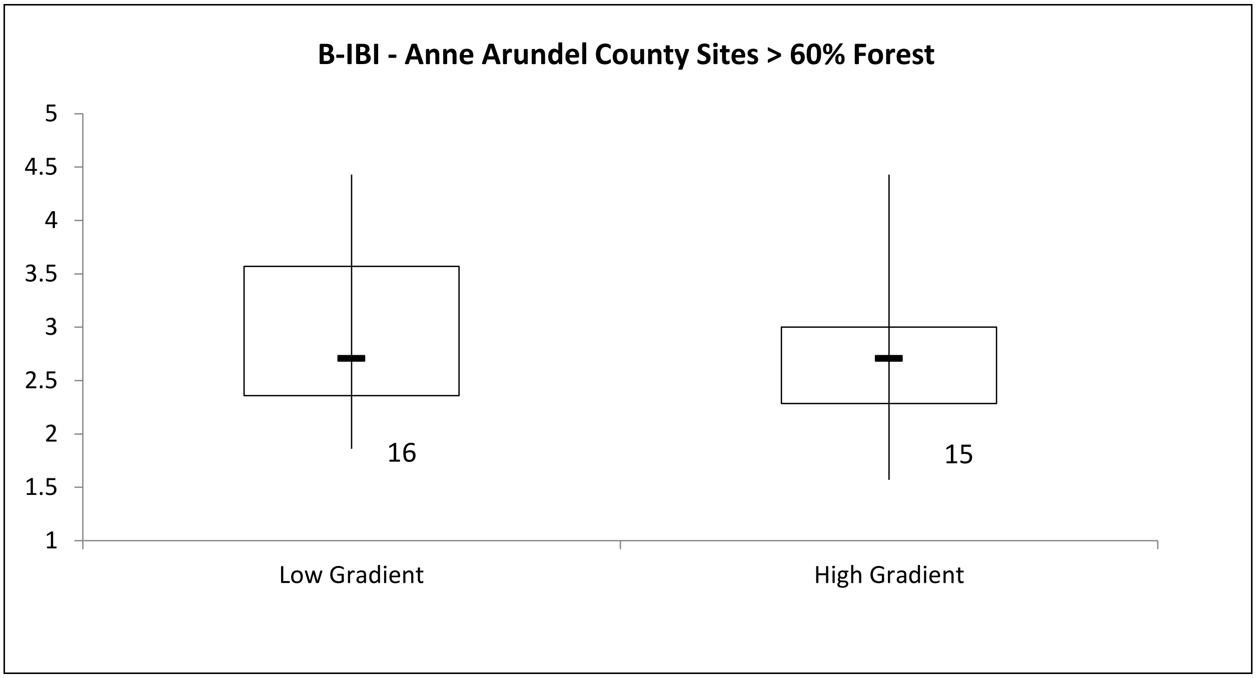

Figure 6-7. Comparison of B-IBI scores between low (< 0.6) gradient and high gradient Anne Arundel County streams, using surrogate (60% forested catchment sites) reference sites, which is not significantly different (16 low gradient, 15 high gradient sites)

Natural variation may extend to the interaction of size and gradient (e.g., four potential types of streams) with the relevant types in County being low gradient-large streams and low gradient-small wetlands (Matt Baker, TRC). Different land uses may confound the ability to distinguish these stream types, especially given the greater development found in coastal watersheds.

6.3 Evaluate stream size differences in B-IBI and component metrics that might indicate the need for new B-IBI for smaller streams added with the more detailed County stream network (e.g., identify variation in metric scores at reference sites with different drainage areas)

As described in Section 2.3, it is not possible to evaluate the difference in B-IBI scores between streams on the 1:100,000-scale map versus the more detailed County map, because the smaller streams on the County map have not been formally sampled in the Program. Nonetheless, the comparison of B-IBI scores in the larger and smaller sampled streams (< 575 ac on the 1:100,000-scale map) shown above indicates that inclusion of even smaller streams from the more detailed stream network would certainly require development of a new "small stream" B-IBI.

Both small streams and direct drainage streams typically have depauperate fish assemblages, because of their isolation and propensity to go dry (Scott Stranko, TRC). Therefore, the County will not sample for fish in the smaller streams, so only a new B-IBI will be developed.

6.4 Describe implications of implementing a new B-IBI for (1) affecting only the smaller streams added with the more detailed stream network and (2) affecting all sites which would complicate backward comparisons (though new B-IBIs could be calculated on old data)

Developing and implementing a small stream B-IBI for streams only present on the more detailed County stream network would not affect calculating areawide stream condition as this new B-IBI would be comparable to the existing B-IBI (albeit with different reference conditions). Countywide and watershed-wide stream conditions can be reported for small streams, large streams, and combined.

Backward comparison of stream condition, however, can only be done between the large streams on the 1:100,000-scale stream network. Including the smaller streams would result in comparing different populations of streams and might lead to erroneous results (e.g., showing a change in improvement in stream conditions overall when in reality the result was caused by adding in small streams in better condition, or conversely showing a decline in overall conditions if adding streams in worse condition). Going forward, the combined condition of small and large streams can be reported, but should include a caveat about comparing this result with previous rounds that only include larger streams.

RECOMMENDATION: Analyses indicate that streams in Anne Arundel County may differ naturally from streams in the entire western Coastal Plain (though the western and eastern Coastal Plain streams do not differ significantly based on MBSS data). Stream biological conditions in the County may naturally vary with size (catchment area) but not with slope (gradient), though wetland streams were not assessed directly.

These results indicate that development of new B-IBIs to address both regional and stream size differences would provide more accurate assessments of County stream condition. Therefore, a new B-IBI will be developed for the smaller streams that would be added by sampling on the more detailed County stream network. Because fish assemblages are generally depauperate in the smallest streams, sampling for fish in streams only on the more detailed stream network will not be conducted. Therefore, a new small stream Fish IBI will not be needed.

Development of the new B-IBI for smaller streams will require sampling of small streams outside of the random survey in Round 3. Because reference streams in Anne Arundel County are so uncommon, and therefore unlikely to be sampled randomly, the minimum 10 and ideally 40 reference sites needed for B-IBI development will be identified using GIS analysis. Once candidate reference sites are identified, they will be added to the sample sites for Round 3 to obtain information on stressors needed to confirm that they meet reference site criteria. Should distinct reference conditions be identified within the small stream references sites (e.g., between wetland streams in low gradients and gully streams in high gradients), more than one B-IBI should be developed.

7 Expand monitoring to include tidal waters

The County has a wealth of tidal waters that are not currently monitored as part of the Program. In the past, Maryland DNR has been interested in developing methods for integrating the tidal and non-tidal monitoring of the state, specifically for "filling the gap" between those programs in freshwater tidal and nearshore shallows. Baltimore County has recently incorporated tidal sampling into their biological monitoring program, allocating one-third of the sampling effort to tidal waters using the protocols of Chesapeake Bay Long-Term Benthic monitoring program. A similar sampling effort could be designed for Anne Arundel County in the future.

RECOMMENDATION: Add a tidal component to the Anne Arundel County biological program monitoring program in the future when budget is available.

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APPENDIX C

ROUND 3 SITE SELECTION PROCEDURE

Update to the Anne Arundel County Biological Monitoring Program: Round 3 Site Selection Procedure

In 2016, Anne Arundel County updated the design and methodology of its Countywide biological monitoring program prior to the start of Round 3 sampling (Southerland et al. 2016). The changes to the existing program were developed to enhance the program's ability to detect trends, through the re-sampling of sites from Rounds 1 and 2, and to better assess overall watershed condition, through the addition of smaller streams to the sampling frame. A total of eight random sites within each of the 24 Primary Sampling Units (PSUs, defined by watershed boundaries) are required for both the large stream stratum (MBSS 1:100,000 modified stream reach layer) and the small stream stratum (Anne Arundel County stream layer modified to exclude streams already accounted for in the MBSS reach layer), totaling 384 sites. Sampling at any given site is dependent upon permission being granted by the owner(s) of the parcel(s) where the site is located and those parcels that need to be crossed to access a site. Permission is not guaranteed for any given site and field crews may encounter conditions that render a site unsampleable (*e.g.,* dry channel, safety issues, etc.); therefore, additional sites should be identified to ensure sampling targets are met.

A variety of data layers were assembled into a geodatabase for use in Round 3 site selection. One feature dataset includes the large stream stratum, small stream stratum, and the 24 PSUs. The other feature dataset includes the following feature classes: randomly selected sites on large streams, randomly selected sites on small streams, randomly chosen sites from Round 1 for re-sampling, and randomly chosen sites from Round 2 for re-sampling.

Sampling Frame Development

Primary Sampling Units

The County has divided its watershed areas into 24 Primary Sampling Units (PSUs), which for the most part are nested within the larger watersheds used for restoration planning. These PSUs were used as the basis for random site selection. There are some areas within the County where the small streams stratum used disagrees with the PSU boundaries (*e.g.,* a small tributary crosses a PSU boundary, when it properly belongs in a single PSU). For this reason, it is recommended that the PSU boundaries be updated to reflect the more detailed, higher resolution streams contained in the small stream stratum.

Large Stream Stratum

The large stream stratum was created from the streams layer used as the sample frame for the MBSS, which is a modified version of the National Hydrography Dataset (NHD) 1:100,000 stream network. Each stream reach was assigned to a PSU based on the location of the line's center point. Manual cleanup was required to correct the sampling unit designations for some areas where the streams did not align with the PSUs, primarily along the County boundary.

Small Stream Stratum

The small stream stratum was created from the Anne Arundel County streams layer. Stream reaches that were already included in the large stream stratum were removed. The original County layer was the further modified to include only sampleable stream types (Table 1). Each stream reach was assigned to a PSU based on the location of the line's center point. The County's stream layer appears to be of a higher resolution than the County's PSU watershed layer. For this reason, there are areas where some stream reaches and the PSU boundaries disagree. In those cases, stream reaches were manually re-assigned to the PSU where the stream reach outlet point was located (Figure 1).

Table 1. Stream Types Included in Small Stream Stratum

Stream Type
Floodway
Main Stem
Not Assessed
Perennial
Perennial Mainstem
Pipe
Single Stream (centerline)
Single Stream Hidden (centerline)
Wetland
Wetland/Marsh

Note: Features where the stream type value was null or blank were also included in the stratum.

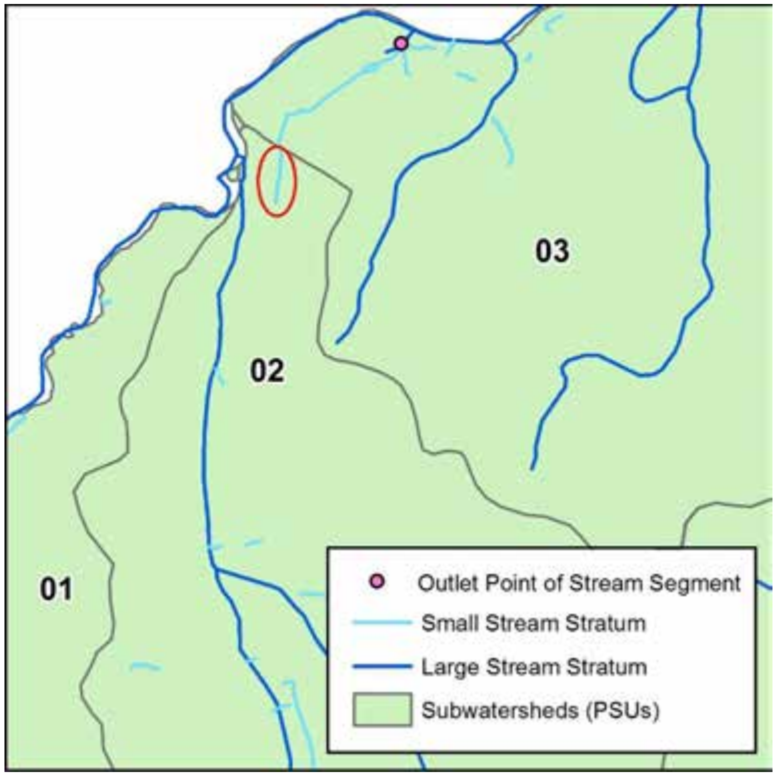

Figure 1. Example of PSU assignment. The reach circled in red was assigned to PSU "03" and not "02" because of where the stream segment outlet point is located.

Site Selection

Models were developed in ESRI ArcGIS to generate new random site locations for the large and small stream strata. The large stream model excluded reaches with a tidal designation and reaches that are larger than third order. Then the model grouped the stream reaches in a stratum by PSU. The ArcGIS tool "Create Random Points" was applied to the stream strata to identify site locations for each set of PSU stream reaches; the number of points was set to the desired size of the pool of potential sites (n = 50 per PSU) and the minimum distance allowed was set to reflect the length of a sampling site and the distance required between points so that sites would not overlap (d = 75m). When a PSU contained an insufficient length of stream for 50 random sites at the 75-meter minimum spacing, the maximum number of random sites meeting this minimum distance requirement were generated (this was only the case in PSU "03", Lower Patapsco, where only 32 potential sites were identified). Because the placement of points along a stream feature is linear, the model randomly reorders the points using a Python script so that the location and ordering of the sites is fully randomized. The random number seed (x = 2016) is set inside the model environment. The model spatially joins the potential sites with their respective stream layers to retain relevant information about the stream reach, and saves a set of sites for each PSU. For each stratum, the model output was manually merged into a single feature class, assigned site names, and saved within the geodatabase provided to the County.

In order to determine which sites to re-sample from Rounds 1 and 2, all of the sites within each

PSU for each round were assigned random numbers and then ranked accordingly. Two sites from each round will be sampled in each PSU, with the highest ranked sites (*i.e.* lowest numeric value) being considered the priority for sampling. In cases where landowner permission or sampleability is an issue at a particular site, the next highest ranked site(s) will be sampled.

Assignment of Sampling Year to PSUs

Another component of the randomization within the sampling design is the sampling schedule for the various PSUs. Each PSU was assigned a random value (1-24), sorted accordingly, and assigned a sampling group. Five PSUs are to be sampled in each year of Round 3 (2017-2021), except for 2020, when four PSUs will be sampled. This could not be avoided when trying to assign 24 PSUs during a five-year sampling round.

Table 2 - Sampling Unit Key and Sampling Order for Round 3

PSU ID	*PSU Name*	*Sampling Group*	*Sampling Year*
09	Severn Run	Year 1	2017
11	Upper North River (South River)	Year 1	2017
13	Rhode River	Year 1	2017
06	Bodkin Creek	Year 1	2017
10	Severn River	Year 1	2017
19	Stocketts Run	Year 2	2018
05	Marley Creek	Year 2	2018
03	Lower Patapsco	Year 2	2018
08	Lower Magothy	Year 2	2018
01	Piney Run	Year 2	2018
16	Upper Patuxent	Year 3	2019
12	Lower North River (South River)	Year 3	2019
18	Middle Patuxent	Year 3	2019
17	Little Patuxent	Year 3	2019
04	Sawmill Creek	Year 3	2019
07	Upper Magothy	Year 4	2020
02	Stony Run	Year 4	2020
14	West River	Year 4	2020
20	Rock Branch	Year 4	2020
23	Cabin Branch	Year 5	2021
15	Herring Bay	Year 5	2021
22	Lyons Creek	Year 5	2021
21	Ferry Branch	Year 5	2021
24	Hall Creek	Year 5	2021

Note: The rows shaded in blue were forced to the bottom of the list, despite their position in the random order, as a result of stream walks not yet being completed for these PSUs. However, the random order among these six PSUs was maintained in relation to which PSU would be sampled in Year 4 vs. Year 5.

Site Naming Convention

Each potential site is named according to a set formula; the site naming convention was designed to convey multiple pieces of information about a particular site in a clear and concise manner. The different components of each site name are also stored in individual fields, which allows for easy querying and analysis of the monitoring program dataset based on various groupings (*e.g.*, by PSU, by year sampled, etc.). The explanation for the example site name is included in

Table 3.

Example Site Name: 01-R3M-03-18
(Site 03 is a random, MBSS sample frame site sampled in 2018 during Round 3, and located in PSU 01)

Table 3. Naming Convention for Biological Monitoring Sites

Position	***Domain***	***Description***
1-2	01 - 24	PSU ID as 2 digit numeric
3	-	-
4	L / R	Long Term (L) or Random/Regular (R)
5	1-3	Round of sampling during which the site was first sampled. Will be 3 for this round's Random (R) sites, but 1 or 2 for Long Term (L) sites
6	M / S	MBSS Large Stream Stratum (M) or Small Stream Stratum (S)
7	-	-
8-9	01-50	Random site selection order - 01-08 with up to 42 additional sites available
10	-	-
11-12	17-21	Assigned year (YY) in sampling schedule

Sampling Targets by PSU

For the small stream stratum, there are no sampling targets beyond the requirement to sample eight sites per PSU. Stream order is not readily available for the small stream stratum, so there is no additional partitioning for these sites.

For the large stream stratum, sampling targets (i.e., for the number of sites allocated) were developed based on stream order. Eight sites per PSU will be sampled, with the number of sites by stream order allocated in proportion to the number of "large stratum" stream miles in that PSU (Table 4). If a given stream order makes up more than 0.5% of stream miles in a PSU, it will be allocated a site. For each PSU, two sites each were randomly selected from Round 1 and Round 2 to be Long Term sites. These will be combined with four Random sites to meet the sampling target for each stream order (Table 5).

For example, based on the percentage of stream miles in each stream order, PSU 01 requires the sampling of four first-order sites, one second-order site, and three third-order sites. The first two sites randomly chosen as Long Term sites from Round 1 are first and third order; the first two sites chosen from Round 2 are also first and third order. This leaves two first-order, one second-order, and one third-order site to be sampled from the Round 3 Random sites.

Countywide, approximately 77% percent of large stream sites will be located on first-order streams, 17% on second-order streams, and 6% on third order streams. This distribution is roughly consistent with that of both Round 1 and Round 2.

Table 4. Percentage of Stream Miles in MBSS Streams by Stream Order, by PSU

PSU	*First Order*	*Second Order*	*Third Order*	*TOTAL*
01	45.66	12.93	41.41	100.00
02	65.06	34.94	0.00	100.00
03	94.47	5.53	0.00	100.00
04	80.40	12.55	7.05	100.00
05	89.20	7.43	3.37	100.00
06	100.00	0.00	0.00	100.00
07	94.59	5.41	0.00	100.00
08	100.00	0.00	0.00	100.00
09	66.37	17.30	16.33	100.00
10	93.25	6.72	0.03	100.00
11	78.69	15.90	5.41	100.00
12	75.68	24.32	0.00	100.00
13	77.11	17.95	4.94	100.00
14	82.31	17.69	0.00	100.00
15	71.04	28.96	0.00	100.00
16	81.78	17.16	1.06	100.00
17	78.62	21.38	0.00	100.00
18	94.85	5.15	0.00	100.00
19	80.09	19.91	0.00	100.00
20	88.77	11.23	0.00	100.00
21	77.39	22.61	0.00	100.00
22	63.51	36.49	0.00	100.00
23	69.19	10.17	20.64	100.00
24	72.88	27.12	0.00	100.00
Overall	78.66	16.81	4.53	100.00

Table 5. Number of Sites on MBSS Streams, by Stream Order, by PSU

PSU	***First Order***	***Second Order***	***Third Order***	***Total***
01	4	1	3	8
02	5	3	0	8
03	7	1	0	8
04	6	1	1	8
05	6	1	1	8
06	8	0	0	8
07	7	1	0	8
08	8	0	0	8
09	5	2	1	8
10	7	1	0	8
11	6	1	1	8
12	6	2	0	8
13	6	1	1	8
14	7	1	0	8
15	6	2	0	8
16	6	1	1	8
17	6	2	0	8
18	7	1	0	8
19	6	2	0	8
20	7	1	0	8
21	6	2	0	8
22	5	3	0	8
23	5	1	2	8
24	6	2	0	8
TOTAL	148	33	11	192

During the landowner permission process the following decision rules that will be employed:

Round 3 new Random sites

- If permission is not granted for a Random Round 3 site, the next site of that same stream order (on the randomized list of Round 3 sites in that PSU) will be selected as a replacement (for example, if permission is denied for a second-order site, the next second-order site in that PSU will be selected).
- If there are no more candidate sites of that stream order in that PSU, a site of a different stream order will be selected.

Long Term sites from Round 1 or 2:

- If permission is not granted for one of the Long Term sites, the next site of that same stream order (on the randomized list of sites in that PSU, for that same Round) will be selected as a replacement (for example, if permission is denied for a second-order site from Round 1, the next second-order Round 1 site in that PSU will be selected).

- If there are no more candidate sites of that stream order in that same Round, a site from the other previous Round may be selected (for example, if permission is denied for a third-order site from Round 1, and there are no more Round 1 third-order sites in that PSU, a Round 2 third-order site may be selected, if available).

- If there are no more candidate sites of that stream order from either Round 1 or Round 2, a site of a different stream order would be selected, preferably within the same Round as the initial site for which permission was denied. In this case, the target of sampling four Long Term sites takes precedence over stream order distribution.

Note that if the initial target distribution of sites by stream order cannot be met within the Long Term sites, the distribution of sites by stream order among new Round 3 sites will be adjusted to meet the original targets.

ADDENDUM TO APPENDIX C

TASK: In Round Three, the Program is attempting to evaluate smaller streams not included in the Round One and Round Two assessment work. The Large Stream Coverage is the Round One and Two MBSS 1:100,000 coverage while the Small Stream Coverage is the 1:2,400 (approximate) County stream coverage as updated during stream walks performed as part of comprehensive watershed assessment work done by WPRP. The County stream coverage was not fully updated during the Program redesign, so six PSUs must have sites selected from the Small Stream Coverage before sampling can commence. Eight random sites and 10 backup sites must be selected for each PSU. Before site selection can begin, the Small Stream Coverage must be created for these PSUs. To ensure proper random site selection outcomes, overlapping Large Stream Coverage (MBSS Streams at the 1:100,000 scale) segments must be removed from the Small Stream Coverage. Appendix C of the QAPP describes the procedure to be used for this process. This Addendum provides additional details on how the Small Stream Coverage was crafted in the other PSUs:

1. Display both the Large Stream Coverage and the Small Stream Coverage.

2. Delete any Small Stream Coverage reach that is co-located with a Large Stream Coverage segment using the following guidelines:

- Use County-provided aerial imagery and a fine resolution shaded relief map to determine landscape position of the stream reach of interest in the Large Stream Coverage.
- Most braided channels and wide floodplains will be visible in the imagery, while headwater stream locations typically follow closely along the low spots in a shaded relief map.
- It should be noted that the Large Stream Coverage segments were rarely clean matches with the shaded relief, but the one can determine what lines were representative of what features. Generally, the evaluation is best started at the downstream end of a sampling unit. Follow the Large Stream Coverage line upstream and delete the any co-located segments from the Small Stream Coverage.
- Additionally, large order segments (e.g.- 4th and 5th order) and shorelines will also be removed from the Large Stream Coverage.

3. Continue with random site selection procedures described in Appendix C.

APPENDIX D

MARYLAND BIOLOGICAL STREAM SURVEY – ROUND FOUR SAMPLING MANUAL

Maryland Biological Stream Survey

Round Four Sampling Manual

MARYLAND
DEPARTMENT OF NATURAL RESOURCES

2017

Larry Hogan
Governor

Boyd Rutherford
Lt. Governor

A message to Maryland's citizens

The Maryland Department of Natural Resources (DNR) seeks to preserve, protect and enhance the living resources of the state. Working in partnership with the citizens of Maryland, this worthwhile goal will become a reality. This publication provides information that will increase your understanding of how DNR strives to reach that goal through its many diverse programs.

Mark Belton
Secretary

MARYLAND
DEPARTMENT OF
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Maryland Biological Stream Survey: Round Four Field Sampling Manual

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Foreword

This document titled "The Maryland Biological Stream Survey: Round Four Sampling Manual" was prepared by staff in the Maryland Department of Natural Resources (DNR), Monitoring and Non-Tidal Assessment Division, with input from Versar Inc. and the University of Maryland Appalachian Laboratory staff. It provides written standard operating procedures for all aspects of the Round Four Maryland Biological Stream Survey (MBSS) sampling. The primary users of this manual are DNR and University of Maryland field crews collecting data as part of Round Four (2014 – 2018) of the MBSS. Additionally, to facilitate data comparability and sharing, we encourage the use of this manual by anyone collecting stream ecological data during Round Four, especially those seeking certification in certain aspects of MBSS benthic macroinvertebrate or fish sampling protocols.

Purpose of Manual

This document was prepared to support the Round Four MBSS. It is imperative that the protocols used for every aspect of the MBSS be provided to help guarantee the collection of consistently high-quality data throughout Round Four 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 and 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. Certification in certain MBSS sampling protocols was first offered in 2012. This manual describes protocols for three aspects of MBSS sampling for which certification is offered, including Benthic Macroinvertebrate Sampling, Fish Crew Leader, and Fish Sampling. This manual should be the primary reference for detailed descriptions of these protocols. The manual also provides detailed descriptions of all other field sampling protocols to be followed by DNR MANTA staff and anyone else sampling as part of the Round Four MBSS. Additional information regarding the MBSS certification process, other aspects of the MBSS for which certification is being offered, and requirements for attaining certification can be found on this Maryland Department of Natural Resources web site (www.dnr.maryland.gov/streams/Pages/mbsstraining.aspx).

Maryland Biological Stream Survey Round Four Goal and Objectives

The original goal and objectives of the MBSS from previous rounds are still germane to Round Four. 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 that the MBSS pursues 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.

The fourth objective – "document changes (improvements and degradation) over time in Maryland's stream ecological conditions and biodiversity status" - is the primary focus of Round Four. To optimize the chance for detecting changes, the Round Four design consists of re-sampling sites that were randomly-selected and sampled during Rounds One (20 years later) and Two (14 years later). More details regarding the Round Four sampling design are available in Appendix A.

Round One (1995 – 1997) provided Maryland's first statewide assessment of non-tidal stream 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, different stream maps were used as a basis for selecting sites during these two rounds. Thus, the data generated from these different maps are not directly comparable for detecting changes in stream conditions. The same stream map was used during Rounds Two (2000-2004) and Three (2007-2009). Data generated from randomly-selected sites sampled during these two Rounds, over approximately a seven year interval, did not show significant differences in estimated statewide stream conditions. The Round Four design optimizes the ability to compare stream conditions over time by sampling the same sites (thus decreasing the variability introduced by comparing different sets of randomly-selected sites) that were sampled previously and compares conditions over the longest possible time intervals (20 and 14 years). Although the condition of Maryland's individual 8-digit watersheds will not be provided from Round Four, statewide and basin (6-digit Maryland watershed) assessments of stream ecological conditions will be available and can be compared to results from Rounds One and Two.

Introduction

Data and results generated by the MBSS 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. Additionally, data from the long-term monitoring of reference (Sentinel) sites in Maryland are being used to explore potential natural variability and variability in stream ecology that may be attributable to climate change. The quality, usefulness, and availability of MBSS data are also exemplified by their use in a large number of peer-reviewed scientific publications. We anticipate the Round Four data being useful for examining potential changes in Maryland's stream conditions over time.

To achieve this objective, examine for potential changes over time, it will be important to be able to compare data collected during Round Four with data from Rounds One and Two. Thus, it is important to make sure that the protocols used for collecting data between Rounds are as similar as possible. The protocols for collecting benthic macroinvertebrate and fish data in the field have not changed since the first year of the MBSS (1995). The only change has been to eliminate the examination of each fish collected for external anomalies after the completion of Round One. During Round Four, fish will be examined for external anomalies at sites that were previously sampled during Round One so that anomaly data can be compared over the 20 year period between Rounds One and Four.

Protocols for collecting water chemistry samples have also remained largely consistent since 1995 and laboratory methods have not changed at all. During Round One, six water chemistry variables (pH, ANC, sulfate, nitrate, conductivity, and DOC) were measured from spring water grab samples. At the beginning of Round Two (2000), six parameters (chloride, total nitrogen, nitrite, ammonia, total phosphorus, and orthophosphate) were added to provide better assessments of nutrient concentrations. At the beginning of Round Three, in situ summer measurements of pH, temperature, conductivity, and dissolved oxygen were no longer being taken because all of these (with the exception of dissolved oxygen) are available from the spring water sample or temperature loggers. The water chemistry sampling for Round Four will follow the Round Three procedure, with the addition of copper, zinc, magnesium and calcium.

Most physical habitat assessment measurements and assessments have remained consistent since the first year of the MBSS. However, a few procedures were added at the beginning of Round Two and a few were modified. During Round Four, we will continue to use the methods employed at the beginning of Round Two. However, we will add several assessments conducted using Round One protocols to the repeated Round One sites. These include assessments of bank stability, minimum buffer width, channel flow status, and remoteness. In Round Four, the "Presence, Absence, or Extensive Presence of orange floc" was added to the "Stream Character" portion of the MBSS Summer Habitat Data Sheet. Along with the physical habitat assessment, during Round Four, geomorphological measurements will be taken at MBSS sites. These measurements will consist of a cross section, pebble count, and facies mapping.

Freshwater mussel, crayfish, and stream salamander data collection differed by MBSS Round. Round Four methods for sampling freshwater mussel and crayfish will be the same as the methods used during Round Three. However, additional effort will be employed for detecting and quantifying stream salamander abundance during Round Four. This extra effort is deemed necessary for testing the use of stream salamanders in a stream salamander index of biotic integrity.

The MBSS Round Four protocols are designed to optimize comparability with Rounds One and Two and add important information for assessing Maryland's streams into the future. Detailed protocol descriptions are provided in the remainder of this document. Persons conducting MBSS Round Four sampling should follow these descriptions and have this document with them while conducting Round Four sampling.

1.0 Personnel and Crew Qualifications

Persons responsible for field collection of MBSS data fit into one of three positions: Crew Supervisor, Crew Leader, or 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 less stringent; 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 during each year they are field Crew Members or a Crew Leader. 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 data and identify live organisms in the field. For example, the fish taxonomy test must be passed by at least one person on the crew to identify fishes, and that person must conduct the fish taxonomic identifications. Since benthic macroinvertebrates are identified in the laboratory, no one on the MBSS 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 by the MBSS QC Officer. 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. This individual 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).

2.0 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 ultimate responsibility for health and safety of field crews lies with the parent organization for each field crew.

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 2 year) physician's approval to conduct rigorous physical work
- Recent (within 2 year) CPR certification
- Recent (within 2 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.

2.2 Duties and Responsibilities

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

2.2.1 Field Crew Supervisor

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

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:

- Instructing and supervising the survey crew such that sampling at 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.

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. Field crew members should also inform the Crew Leader of any allergies or medical conditions (e.g., diabetes, asthma, allergies) and any special needs (e.g., inhaler, epinephrine pen) the crew member has. It is the responsibility of each crew member (not the Crew Leader) to make sure he or she has any special needs medicine or equipment and that the Crew Leader knows about that special need.

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 contain 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 are banned throughout the State of Maryland and 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 MBSS sites 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. Poison sumac, which occurs in boggy areas, should also be avoided. 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 materials 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.

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.

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.

2.6 Precautions for Minimizing Ecological Risk (Decontamination)

An increasing potential exists for transferring non-native and invasive organisms (including those that cause serious diseases to native stream dwelling fauna and fauna) from one stream to another while conducting monitoring. Whirling disease (a protist, *Myxobolus cerebralis*), Didymo (an algae, *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 poultry litter that came from infected poultry and then walking in another area with poultry. It is important to properly clean all footwear 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. Since 2007, all MBSS field crews have been 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 poultry litter.

The disinfection procedure consists of soaking or rinsing all equipment that has come in contact with water (or poultry litter) in a 10% bleach or 2% Virkon solution for at least one minute. Equipment with a smooth surface (e.g. buckets, sides – but not soles - of waders) can sprayed with bleach or Virkon solution. After soaking and scrubbing have been completed, all equipment must be rinsed with freshwater to remove the bleach or Virkon 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. Decontamination should occur away from surface waters to avoid polluting them.

3.0 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.

3.1 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 sampling crews and to data collected previously by the 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/parameters 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 and annual 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.

3.2 Stream 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 Comparability and Completeness

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

3.4 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.

3.5 Field Audits

For the field data collection component of the MBSS, the QC Officer is primarily responsible for conducting field audits. At least one site sampled by each MBSS crew during each index period 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 location 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 a particular taxonomic group 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 field audits. 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.

3.6 Training Requirements

An important aspect of the MBSS QA program is the training program for field personnel, which will be conducted prior to each spring and summer sampling period. Training helps to ensure 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 and identify organisms to species in the field (e.g., at least one member of each field crew must pass the fish taxonomy test every year to be qualified to collect MBSS fish data). See section 3.3.11 for details regarding taxonomic identification as it pertains to MBSS field sampling. Since benthic macroinvertebrates are identified in the laboratory, no one on a MBSS field crew is required to pass a benthic macroinvertebrate taxonomy test to collect benthic macroinvertebrates.

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 item 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.

3.8 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 in Appendix D). 2) Segment. Three numbers are used to designate the stream segment obtained from the appropriate reach file. 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. Examples of site type codes 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 should be maintained for all water and benthic macroinvertebrate samples (Appendix G), as well as herpetofauna, crayfish, mussel, and fish voucher specimens.

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 individual sites are potentially confounded by crew 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.

3.10 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.

3.11 Taxonomic Identification and Specimen Vouchering

The MBSS is recognized as providing the highest quality biological data. This is due primarily to the QA/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 identification tests for all of these taxonomic groups. Only the person(s) on each field crew who has passed the test for the taxonomic group should conduct identification in the field.

During the Round Four MBSS, each field crew should maintain a voucher collection of at least five specimens of each fish, herpetofauna, mussel, and crayfish species encountered (as long as five were collected). The voucher collection can consist of photographs and/or preserved specimens of each species. Photographic vouchers will be accepted in lieu of preserved specimens, as long as the diagnostic features for identifying the specimens photographed can be seen clearly. Dead mussel shells should be retained. Live mussels should be photographed. Specimens of crayfishes should be preserved in lieu of photographs when possible. Any rare, threatened, or endangered (see Appendix F) species encountered should be photographed and not preserved. Nuisance species (Appendix F) should be photographed or preserved. Care should be taken while photographing to avoid harm to the specimen. Photographs must clearly show the appropriate features necessary for identifying the species. With the exception of rare, threatened, or endangered species, specimens that are too small to provide photographs that can be used to verify identifications (or with diagnostic features that do not show up well in photographs) should be preserved for verification. Please see Appendix B for detailed fish fixation and preservation procedures.

To facilitate record keeping of vouchered specimens and QA/QC verification of species identifications, each MBSS field crew leader should maintain a list of all specimens vouchered (photographed or preserved) during the MBSS Spring and Summer Index periods. All vouchered specimens should be recorded on the MBSS Specimen Tracking Data Sheet (Appendix G). All preserved and photographed specimens will be reviewed by an expert in taxonomy for each taxonomic group and results will be kept on record. The MBSS Specimen Tracking Data Sheet will serve as a chain-of-custody form between field crew leaders and taxonomic experts.

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 one site per crew.

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 handwriting of certain individuals is deemed illegible by the crew leader, then those individuals should not record data on data sheets.

4.0 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.

4.1 Equipment
Prior to each field sampling trip, the Crew Leader should ensure that all necessary sampling equipment is prepared for sampling. Equipment lists for sampling during the Spring and Summer Index Periods are provided in the back on this manual.

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.

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.

5.0 Sample Collection

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 Four MBSS, including water quality, benthic macroinvertebrate, fish, reptile, amphibian, crayfish, mussel, invasive plant, physical habitat, and geomorphology 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.

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, eight primary activities are conducted: fish, reptile and amphibian, stream salamander, mussel, crayfish, invasive plant, select physical habitat, and geomorphology 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 4.5°C. 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 (440°C for Coastal Plain and 1050°C 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 Four 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 are taken during this time; therefore, they represent limiting conditions for these organisms.

5.3 Site Location

Sites sampled during Round Four will consist of sites that were sampled previously during Round One and Round Two, as well as sites in specific areas that are being used to answer important resource management questions (targeted sites). Sites previously sampled during Round Two are on non-tidal 1st – 4th order (Strahler) streams based on a 1:100,000 scale stream reach file. Sites previously sampled during Round One are on 1st – 3rd order (Strahler) streams based on a 1:250,000 scale stream reach file. Sites from Round One or Two to be re-sampled are randomly chosen from the complete list of sites sampled previously. The complete list of sites sampled previously will be provided to the crew leader with a priority number assigned to each site by watershed. Crew leaders should sample the lowest number (corresponding to the highest priority) sites on the list with permission to sample, by watershed, until the target number of sites in each watershed has been sampled.

All sites consist of the watered portion of the stream and an area 50 meters perpendicular (on both sides) to the stream. Each site is 75 m in length. It is important to sample Round One and Two sites as closely as possible to locations previously sampled. Crew leaders should use geographic coordinates and previous site location descriptions (as recorded on datasheets in previous Rounds) to re-locate sites. In Rounds One and Two, the 0, 25, 50, and 75 meter transects of these sites were marked with flagging tape and the approximate locations of the 0 and 75 m transects were marked with orange paint on the nearest tree. Note that the flagging tape more precisely marked the transect locations. If markings from Round One or Two are found while accessing the site, the site should be sampled based on those markings. Any additional flagging needed to mark transects should be added and flagging in poor condition should be replaced. If, based on reasonable effort, the markings are not found, then the site location should be determined based on geographic coordinates and, when available, site location descriptions recorded when the site was originally sampled. The coordinates represent the mid-point of the 75 m long site (37.5 m from the downstream end of the site). If arrival at these coordinates occurs 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. No matter how the site location for Round Four sampling was determined, new geographical coordinates from the mid-point of the site should be recorded on the Spring Habitat Data Sheet; even if they are identical to the coordinates used to find the site. 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. Permission to use any landowner's property for access to or sampling of any MBSS site is required. 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 or downstream no more than one site length distance (75 m) from the original location and substantial documentation must be provided in the comments section of the Spring Index Period Data sheet to justify the location change. This option should be used only after all other options have been exhausted by the Crew Leader. A map showing the location of the site must also be included with data sheets as well as proper landowner permission information.

5.4 Site Selection and 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, 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 in Appendix D. If a Round One or Two repeat site is deemed unsampleable during the spring visit, a Spring Index Period Data Sheet should be filled out indicating the reason the site was not sampleable. The next higher priority number site, with permission to sample, should then be sampled in place of the unsampleable site. If a site is unsampleable during the summer visit, another site should not be sampled.

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. 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.

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.

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.

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, dissolved organic carbon, copper, zinc, calcium, and magnesium) 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, degree of organic loading, and specific ions known to influence stream biota. 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 and the Western Maryland Watershed Mitigation Study. 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 (so that the bottle has been filled and rinsed a total of three times). 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 inside a closed lid cooler 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 Appendix G) 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.

5.8 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.

For MBSS physical habitat assessment variables, 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.

Round Four sites that were previously sampled during **Round One** will have four extra parameters assessed - bank stability, minimum buffer width, channel flow status, and remoteness –using the same methods used during Round One. These can be assessed during the Summer Index period. Procedures for their assessment are described in section 3.5.9.2 below (numbers 13-16). Habitat data for Round One repeat sites should be entered on the Round One Repeat Habitat Data Sheet (Appendix H).

5.8.1 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 in Appendix D.

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

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, beginning at the water's edge. 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 (found in the back of this manual), 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 (Appendix D) 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 since Round Two at MBSS sites.

    The calibration and proper functioning of the instrument that is used for determining stream gradient must be verified at least once every week 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 from 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.

5.8.2 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 in the back of this manual.

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 (in the back of this manual). 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 coarse 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 16 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 particle type(s) that make up the bars in the site. More than one particle type can be selected. However particles comprising only a minor part of the substrate should not be selected. Dominance of particle types should be determined based on the proportion of the bar consisting of each type.

13. **Round One Repeat of Bank Stability. Bank stability as it was assessed during Round One consists of scoring on a 0-20 scale, as with many of the other MBSS physical habitat assessment metrics. Guidance for scoring comes from the MBSS Round One sampling Manual as follows: A score of 16 – 20 if the upper bank is stable, 0-10% of banks with erosional scars and little potential for future problems; A score of 11 – 15 if banks are moderately stable, 10 – 30% of banks with erosional scars, mostly healed over, with slight potential for problems in extreme floods; A score of 6 – 10 if banks are moderately unstable, 30-60% of banks with erosional scars and high erosion potential during extreme high flows; A score of 0 – 5 if the banks are unstable, raw areas frequent along straight sections and bends and side slopes > than 60o common.**

14. **Round One Repeat of Channel Flow Status. Channel Alteration as it was assessed during Round One consists of scoring on a 0-20 scale, as with many of the other MBSS physical habitat assessment metrics. Guidance for scoring comes from the MBSS Round One sampling Manual as follows: A score of 16 – 20 if there is little to no enlargement of islands or point bars, no evidence of channel straightening or dredging, 0-10% of stream banks artificially armored or lined; A score of 11 - 15 if bar formation is mostly from coarse gravel and/or 10-40% of stream banks are artificially armored or obviously channelized; A score of 6 – 10 if recent but moderate deposition of gravel and coarse sand is on bars and/or embankments on both banks, and/or 40-80% of banks are artificially armored (or channel lined in concrete); A score of 0 – 5 if there is heavy deposition of fine material, extensive bar development, OR recent channelization or dredging evident, or over 80% of the banks are artificially armored.**

15. **Round One Repeat Remoteness. Round One Repeat of Remoteness as it was assessed during Round One consists of scoring on a 0-20 scale, as with many of the other MBSS physical habitat assessment metrics. Guidance for scoring comes from the MBSS Round One sampling Manual as follows: A score of 16 – 20 if the site is more than ¼ mile from the nearest road, access difficult and little or no evidence of human activity; A score of 11- 15 if the site is with ¼ mile of (but not immediately accessible to) the road and is accessed by trail and the site has moderately "wild" character; A score of 6 – 10 if the site is within ¼ mile of road and accessible by trail and human activities are readily evident; a score of 0 – 5 if the site is immediately adjacent to a road with obvious human activities evident.**

16. **Round One Repeat of Minimum Buffer Width. The minimum buffer width for repeated Round One sites should be assessed the same way as number 3 under section 3.5.9.1 above, except that the minimum width of the buffer for each bank should be recorded. Rather than recording "buffer breaks", a break in the buffer indicates no buffer at all. Adjacent land cover types and buffer vegetation should be recorded using the same codes as referenced in section 3.5.9.1 number 3.**

5.9 Geomorphology Sampling

5.9.1 Recommended Equipment – MBSS Geomorphology Sampling

Cross-section datasheets printed on Rite-in-the-Rain paper
Riffle pebble count datasheet printed on Rite-in-the-Rain paper
Regional curve derived bankfull parameters
Clipboard
Pencil
Tripod
Self-leveling laser level and audible laser receiver
Top-setting, telescoping survey rod marked with hundredths of a foot
300-foot measuring tape marked with tenths of a foot
Silvey Stakes
Pin flags
Metric ruler/meter stick (with mm markings) (1 per surveyor) or Gravelometer (1 per surveyor)
Sand gauge reference cards (1 per surveyor)
Metal hand tally counter (clicker) (1 per surveyor)
Digital camera
Hand shears, machete or other clearing device

5.9.2 Physical Stream Channel Measurement

The goal of physical stream channel measurement at MBSS sites is to collect channel dimension measurements and particle size in a representative riffle or straight reach at each site. The measurements will be taken using standard surveying and pebble count techniques. Cross-section and pebble count data will be collected during the Summer Index Period.

5.9.2.1 Cross-Section Survey Protocol

1. **Survey Site Selection.** Within the site, choose a representative riffle area to set up the cross-section. The area should be free from direct anthropogenic alterations and reflective of local geology such that the stream is able to adjust its banks under its current flow regime. The riffle location should be chosen along a relatively straight stretch of the stream when possible. Avoid transverse riffles, riffles located at bends, riffles directly influenced by tributaries/confluences, etc. Locate the cross-section within the top-third of the riffle, below the riffle crest (peak). The riffle crest is defined as the highest elevation within that specific riffle. Establish the cross-section perpendicular to the direction of flow. If no riffle is present within the site (e.g., some Coastal Plain streams), choose a cross-section within a relatively straight portion of the stream that is most representative of the site conditions and where flow is present. Mark on the Cross-Section Data Sheet whether or not the cross-section is located in a riffle. If no riffle is present in the site but present outside of the site, it is acceptable to use the out-of-site riffle as long as the crew has permission along both banks and there are no tributaries between the MBSS site and the out-of-site riffle. Mark on the Cross-Section Data Sheet if the selected riffle is located outside of the 75m MBSS site by placing a "Y" in the appropriate box.

2. **Cross-Section Surveying.** Stretch the tape (marked in tenths of feet) across the channel (zero on right bank facing upstream) making sure the tape is perpendicular to the direction of flow. The tape should be taut and should extend away from the stream for one channel width from the top of each bank. Use Silvey stakes or something similar to secure each end of the tape. Channel width will be measured from top of the right bank to top of the left bank. The minimum width of the cross-section will be three channel widths. In many cases, this will cover the flood-prone area (area bordering the stream that will be covered by stream waters at a flood stage of twice the thalweg bankfull depth). The flood-prone area width will be measured after the cross-section has been surveyed following the directions given in 5.9.1.2.3 below.

   Setup the surveying instrument in a location where the entire cross-section can be viewed. Vegetation may be pruned to allow the entire cross-section to be surveyed without moving the laser and tripod. The instrument should be placed at an elevation higher than the highest feature (e.g., flood-prone elevation) required for the survey. Ideally, only one instrument setup will be required to survey the entire cross-section; however, determining the width of the flood-prone area may require multiple instrument setups if foliage is dense.

   Use the pre-determined mean bankfull depth value obtained from a regional curve within the riffle cross-section to identify the bankfull stage on left and right banks. Additional field determinations of bankfull may be recorded if the field crew observes obvious bankfull features. Field bankfull determination is optional.

   Survey the elevation of station zero on the right bank looking upstream. Station 0 will be approximately one channel width upland from the top of the right bank. Place the rod firmly on top of the ground and hold it as steady and vertical/plumb as possible while moving the receiver up/down until the audible tone indicates a proper reading. Once the elevation is determined, the person holding the rod should call out to the person recording data the reading from the rod in hundredths of feet. Record this as station zero and the corresponding elevation in hundredths of feet on the datasheet.

   Continue surveying across the cross-section obtaining rod readings at major breaks in bed elevation (Figure 1). These readings will all be recorded on the Cross-Section Data Sheet. Typically, 15 to 20 points are necessary including key features such as top of right bank (TOB-R), right bankfull (RBF), right edge water (REW), Thalweg (THL), left edge water (LEW), left bankfull (LBF) and top of left bank (TOB-L). Other significant depositional features or breaks in slope should also be surveyed. Record the distance on the tape (station), the corresponding rod height and feature notes on the cross-section datasheet. Record station measurements in tenths of feet and rod heights in hundredths of feet.

The final measurement of the cross-section survey will be a second elevation at station zero. This second measurement of station zero should be used as a QA/QC check of the survey data.

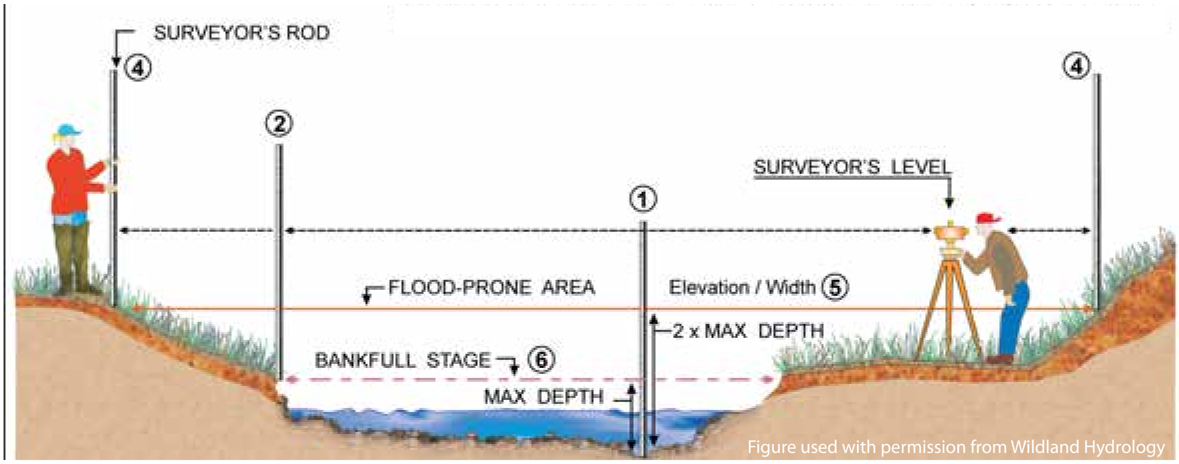

Figure 1 - Surveying a Cross-section.

If banks are severely undercut or slumping, an additional measuring device (e.g., measuring rod or yard stick equipped with a small level) can be used as a base for the main surveying rod. One person should hold the second rod horizontally against the bank at the first location of undercut, and perpendicular to the main survey rod, keeping it as level and steady as possible. A second person should rest the main survey rod on top of the second rod, making note of the distance of the base of the main rod along the second rod (reading 1) and the stationing of the main rod on the survey tape where they cross (reading 2). Move the receiver on the main rod up/down until the audible tone indicates a proper reading and record the elevation. Then determine stationing for the undercut by subtracting reading 1 from reading 2. Then move the entire setup down to the next point of measurement on the undercut bank and repeat until the bank is no longer undercut.

3. **Determining Flood-Prone Area.** Find the station on the cross-section that has an average water depth for that cross-section and take an elevation of the streambed. The elevation at that station will be entered on the Flood-prone Area Data Sheet. The mean bankfull depth value from the regional curve will be entered next on the Flood-prone Area Data Sheet and subtracted from the elevation at average water depth. This results in the bankfull elevation for this cross-section.

   The thalweg elevation from the cross-section should be transferred from the Cross-Section Data Sheet onto the Flood-prone Area Data Sheet. The bankfull elevation will be entered next and subtracted from the thalweg elevation. This results in the maximum bankfull depth for this cross-section. The maximum bankfull depth should be multiplied by two and entered into the 2x Max Bankfull Depth box.

   The thalweg elevation should be entered again on the Flood-prone Area Data Sheet. The 2x max bankfull value should be transferred into the next box and subtracted from the thalweg depth. This results in the flood-prone area elevation.

   The flood-prone area elevation should be used to determine the width of the flood-prone area of the cross-section. If this elevation is captured on both sides of the stream in the cross-section then the survey is complete. If the cross-section on neither side, or only one side contains the flood-prone elevation, then two additional survey points need to be collected.

The surveying rod should be set to the calculated flood-prone elevation. The person holding the rod should then traverse away from the stream, staying in line with the cross-section, until the preset surveying rod measures the flood-prone area elevation when set on the ground. This location is marked with a pin flag. This process is completed again on the opposite side of the stream. A tape is stretched between the two pin flags and the resulting distance is recorded as the width of the flood-prone area.

If the flood-prone elevation is greater than 50m from the edge of the stream, it is marked as greater than 50m on that side of the stream on the Flood-prone Area Data Sheet and the width is not measured in the field.

4. **Photodocumentation.** Take four photographs of each cross-section and record the photograph number, time stamp, and location information on the datasheet. Take one photograph from upstream of the cross-section looking downstream, one from downstream of the cross-section looking upstream, one from the left bank looking at the right bank, and one from the right bank looking at the left bank.

5. **Field QA/QC.** While the measuring tape is still deployed and level is still set up, the field crew leader must make sure that all measurements have been recorded on a data sheet and photographs taken. Recorded data must be reviewed at the point of entry. The crew leader and one other member of the crew must review and initial all field datasheets. Any errors found should be corrected before leaving the site.

5.9.2.2 Riffle Pebble Count Protocol

1. **Locating the Pebble Count.** The riffle pebble count should be conducted within the riffle or other section of stream channel used for the riffle cross-section survey.

2. **Riffle Pebble Count Protocol.** Ten transects (perpendicular to flow) within the riffle should be sampled for pebbles. Each transect will start at the wetted edge of the riffle on one bank and continue across the wetted portion of the riffle, ending at the edge of the opposite bank. Distribute transects for the riffle pebble counts evenly along the entire riffle feature. Sample each transect moving perpendicular to the stream banks until 10 random particles equally spaced along that transect have been measured. Repeat this procedure until 10 random particles at each of 10 different transects have been measured for a total of 100 particles. A metal hand tally counter/clicker can be used to help keep track of transect counts. To avoid bias of selecting larger particles, the observer should look away from the channel bed and select the first particle touched by the tip of index finger at observer's toe. While traversing across a transect, it is possible to select the same large piece of substrate more than one time. It is acceptable to measure the same large piece of substrate more than once.

3. **Measuring the Particle.** Measure the length of the intermediate axis in millimeters and mark a dot in the correct column and row on the data sheet. (The intermediate axis is neither the longest nor the shortest of the three mutually perpendicular sides of the particle). If the particle is linear-shaped, average the axes. If the particle is very small and a measurement cannot be taken (e.g., sand or silt), sand gauge reference cards can help the surveyor classify the particle appropriately. Do not measure organic material or trash/refuse. If the first particle touched is fine sediment forming a thin veneer on a larger particle, the larger particle is what is measured. The intent is to measure the particles that are defining the roughness of the stream channel.

4. **Field QA/QC.** Tally up counts in each cell of the datasheet to ensure that a total of at least 100 particles were measured and recorded. 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 field datasheets.

5.9.3 Facies Mapping

The objective of facies mapping at MBSS sites is to characterize substrate type and composition at each site.

Facies mapping data will be collected during the Spring Index Period and recorded on the MBSS Spring Facies Map Data Sheet.

5.9.3.1 Facies Mapping Protocol

1. **Creating the cells.** Divide the MBSS site into 6 cells, based on the 0M, 25M, 50M, and 75M markings, and the center of the wetted channel as follows:

2. **Classifying the substrate.** Record the dominant and subdominant substrate within each cell using the classifications provided on the MBSS Spring Facies Map Data Sheet. The dominant substrate is the category which covers the largest percentage of the cell's area. The subdominant substrate is the category which covers the second largest percentage of the cell's area. A meter stick can be used to measure the intermediate axis of several substrate particles to help classify the size of the substrate.

3. **Classifying stream depth.** Estimate the category of average water depth within each cell using the classifications provided on the MBSS Spring Facies Map Data Sheet. If the average depth is less than 0.5 m

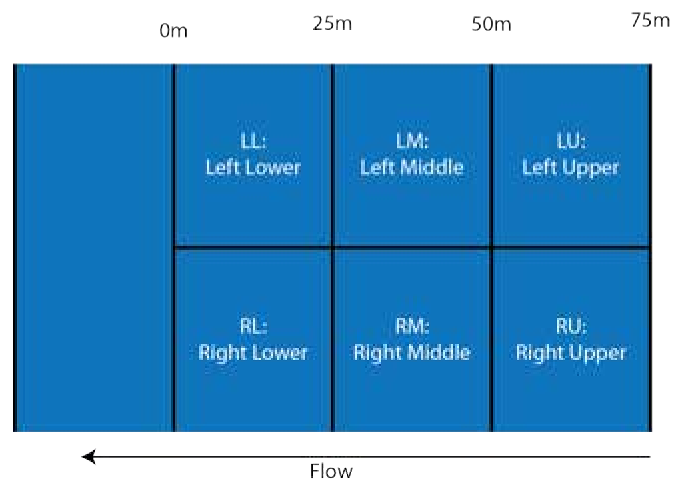

Figure 2 - Left and right are defined while facing upstream.

the category is Shallow; between 0.5 m and 1.0 m the category is Moderately Deep; greater than 1.0 m the category is Deep. A meter stick can be used to take multiple measurements in the cell to obtain an average stream depth for that cell.

4. **Classifying stream water velocity.** Estimate the average stream velocity within each cell. Record the velocity category on the MBSS Spring Facies Map Data Sheet using the categories provided on the data sheet. If the average water velocity is between 0 and 0.3 m/s, it is in the Slow velocity category. If the average water velocity is greater than 0.3 m/s, it is in the Fast velocity category.

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 in the back of the manual. 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.

5.11 Temperature Loggers

Temperature loggers should be deployed to measure water and air temperature at all MBSS sites. The loggers 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. Water 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. Temperature loggers deployed to record air temperature should be as close as possible to the location of the temperature logger deployed to record water temperature. The air logger should be at least 3 feet off the ground and no more than 20 meters from the wetted edge of the stream. The air logger can be affixed to a tree or other stable structure. 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.

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. Since the beginning of Round Three (2007), 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 m2), 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 in the back of the manual. 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.

5.13 Biological Sampling

Biological sampling has always been the primary focus of the MBSS. During Rounds One and Two, the 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 continue to be collected during Round Four. 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 that will be used during the Round Four MBSS for the collection of biological variables.

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 a MBSS site, benthic macroinvertebrate data collected by MBSS are used to calculate the MBSS benthic macroinvertebrate IBI. A D-net (540μ mesh; 10 inch "D" height), 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 landowner permission is denied. The appropriate code for unsampleability should be recorded on the Spring Index Period Data Sheet.

2. **Habitats to be Sampled.** Sampling should be conducted at a combination of habitats that support the most diverse macroinvertebrate community within a 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 that are most stable and in moving water are preferred to those that are unstable and 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 (Appendix G) 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 commences.

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 ft2 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 ft2 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. Rub by hand any large stones and sticks from within the one foot square area to dislodge any organisms that may be clinging to these substrates. Place these rubbed substrates off to the side. 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 gentler 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 and 4th order streams), a sufficient amount of potentially productive habitat may not be present within the 75 m site to collect a 20 ft2 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 ft2 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. All crayfish should remain in the sample regardless of size. 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.

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 placed 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. Corbicula should remain in the benthic sample.

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 (Appendix G) 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.

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.

During Round One, each individual fish (up to 100 individuals of each species per site) was inspected for external anomalies. Sites that were sampled during Round One and are being repeat sampled during Round Four will have up to 100 individuals of each species per site inspected individually for external anomalies. Please see number 18 below.

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 do not escape 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 landowner 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 a rain event, the stream should be visited later in the Index Period or during a drier 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 sites narrow enough to be sampled using only one anode, at least one dip net should accompany the anode for the length of the site. At wider sites, a minimum of one dip net for every two anodes should be used. 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 m 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 captured 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 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. Providing water 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 capturing 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 than 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 Appendix H 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 Appendix I. The MBSS fish key should be used as needed for positive verification during field identifications. Only persons who have passed the MBSS fish taxonomy 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.

    For sites previously sampled during Round One, up to 100 individual fishes of each species should be inspected for any external anomalies. The number of individuals, by species, with any external anomaly, by anomaly type, should be recorded on the MBSS Fish Data Sheet. The following codes should be used on the data sheet for anomalies:

    DI = Discoloration
    CL=Fin Cloudiness
    BL=Black Spot
    UL=Ulcertations/Lesions
    FI= Fin Erosion
    DV=Deformities of the Vertebral Column
    AN=Swelling of the Anus
    RE=Red Spot
    OT=Other (define in comments on the data sheet)
    FD=Fin Deformed or Missing
    IK=Ich
    LE=Leeches
    EC=Eye Cloudiness
    PO=Exopthalmia (pop eye)
    NO= Eye Missing
    HM= Hemorrhaging
    CL= Raised Scales
    GR=Growths/Cysts
    EP= Visible External Parasites
    FU= Fungus
    DM=Deformities of the Mandible
    SC= Scale Deformities
    HK=Hooking Injury
    BS=Body Shape (NOT BLACK SPOT)
    CT=Cut
    AW=Anchor Worm
    FU=Fungus
    HE=Eye Hemorrhage
    OR=Depression Into the Orbits
    CA= Cataract

19. **Voucher Specimens.** For the Round Four 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, amphibian, and crayfish species encountered during Round Four (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.

20. **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 one site 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 stream flow through live cars and/or using battery-operated bubblers.

21. **Gamefish.** During counting and identification of the fish sample for each pass, each gamefish species collected 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.

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, particularly stream salamanders, have been shown to be excellent indicators of MBSS site conditions. During Round Two, only incidental observations of herpetofauna were recorded for MBSS sites, with no focused search conducted. Round Three emphasized searching for stream salamanders and spending a minimum of fifteen minutes searching available habitat for herpetofauna. In Round Four, stream salamander sampling will follow a protocol shown to adequately support population estimates and use of the stream salamander Index of Biotic Integrity (SS-IBI). Incidental observations will also continue to be recorded. The life-history stage of reptiles and amphibians should also be recorded (egg, larva, or adult).

1. **Incidental Collection.** During Round Four, 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 Fauna Data Sheet or Summer Fauna Data Sheet, respectively.

2. **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. Stream salamander sampling consists of electrofishing in aquatic habitat and bank searches in terrestrial habitat. All stream salamanders found incidentally, during the bank search, or while electrofishing should be counted by species and life history stage (e.g., larva or adult).

    Electrofishing protocols are described in the fish sampling portion of this manual. Salamanders should be collected coincident with fish collection during electrofishing. Bank searches focused on finding stream salamanders will be conducted during the Summer Index Period visit, along both banks of the 75-m site. Although the bank search will be conducted entirely outside the wetted portion of the stream, the bank search should focus on cover objects (including cobbles, small boulders, logs, or other objects) where sufficient moisture is present to support stream salamanders. In streams where seeps or small tributaries enter the stream, these habitats should also be searched to a distance 10 m from the main channel. Cover objects in places that are completely dry have a lower likelihood of supporting salamanders and do not need to be searched. During the search, all suitable cover adjacent to the site should be carefully flipped over and then returned as closely as possible to the original position. In some Maryland streams, such suitable cover can extend several meters from the edge of the stream before the habitat is completely dry and not suitable.

Recognizing that a small proportion of MBSS sites will have high abundances of salamanders, salamander transect searches will be limited to 60 minutes. If all stream salamander habitat is searched in less than 60 minutes, then searching will stop. It is important that both stream banks are searched at a consistent, steady pace. In those unusual cases where all stream salamander habitats (i.e., the cover objects described above) have not been sampled in 60 minutes, the crew should estimate the remaining amount of habitat (including seeps) as a proportion of the entire site. For example, the crew might estimate that of the 100% of stream bank with cover objects, only 80% was searched in 60 minutes and 20% remains after sampling stopped. The percentage of habitat remaining (unsearched) should be recorded on the data sheet. Research has shown that 97% of all stream salamander species are sampled in the first 60 minutes, so the percentage of unsampled, remaining habitat will only be used to improve estimates of salamander abundance.

3. **Sampleability.** Bank searches can be conducted in some streams even when electrofishing cannot (e.g., dry stream channel). However, bank searches may be precluded by certain stream conditions. As with other aspects of MBSS sampling, the sampleability codes provided on Appendix D should be used to indicate sampleability for stream salamanders.

4. **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 Four MBSS.

5. **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.

6. **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.

7. **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.

5.13.4 Crayfish Sampling

1. **Sampleability.** If the site can be safely accessed and with landowner 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.

2. **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.

3. **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.

4. **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.

5. **Crayfish Vouchers.** All specimens that cannot be positively identified in the field should be retained for further inspection by a regional crayfish expert. Field identification of *Procambarus acutus* and *P. zonangulus* is difficult and unreliable. All specimens of these two species should be recorded on the datasheet as *Procambarus acutus/zonangulus* and vouchered to allow for species-level identification in the laboratory. Retention of all specimens which cannot be positively identified is mandatory. For Round Four MBSS, one to five individuals of each species collected by each MBSS field crew should be retained so that taxonomic identifications can be verified. 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.

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 scientific 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 placed 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.

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.

Appendices

Appendix A: MBSS Round Four Design Summary

Appendix B: Fish Fixation and Preservation Procedures

Appendix C: Equipment Lists

Appendix D: Codes and Abbreviations

Appendix E: MBSS Stream Habitat Assessment Guidance Sheet

Appendix F: Species Names

Appendix G: Chain of Custody Forms

Appendix H: MBSS Voucher Label

Appendix I: MBSS Summer Data Sheets

Appendix J: MBSS Spring Data Sheets

Appendix K: MBSS Geomorphology Data Sheets

Appendix A: MBSS Round Four Design Summary

1. *Introduction*

The Maryland Department of Natural Resources (DNR) will begin Round Four (R4) of the Maryland Biological Stream Survey (MBSS) in the spring of 2014. The MBSS is a stratified-random, probabilistic survey that provides essential information on the ecological condition of Maryland streams for the State's natural resource decision makers. The primary goal for R4 of the MBSS is to document changes in stream conditions over time. R4 will also provide status information at the large watershed (basin) and statewide scales.

2. *Survey Design*

R4 will include sampling over the 5-year period of 2014-2018. The R4 sites will be sites previously sampled during Rounds One and Two of the MBSS. These "repeat" R4 sites will be randomly selected from the originally randomly selected sites in Round One (R1) during 1995-1997 and Round Two (R2) during 2000-2004. The R1 repeat sites will be sampled in 2015-2017, following the same annual allocation of sites so that the period between sampling will be 20 years for each site. The R2 repeat sites will be sampled 2014-2018, following the same annual allocation of sites so that the period between sampling will be 14 years for each site.

The following number of repeat sites will be sampled each year using the design of the original round:

Year	R1 Sites	R2 Sites	R4 Core Sites	Targeted R4 Sites*
2014	--	48 sites in 16 PSUs	48	7
2015	54 sites in 6 basins	51 sites in 17 PSUs	105	7
2016	54 sites in 6 basins	51 sites in 17 PSUs	105	TBD
2017	45 sites in 5 basins	51 sites in 17 PSUs	96	TBD
2018	--	51 sites in 17 PSUs	51	TBD
Total			405	TBD

*This final number of sites has not been determined for certain. The final number will most likely consist of repeat sampling all MBSS sites that were sampled during R1 and R2 in certain "special interest" watersheds. An example of this targeted sampling is Mattawoman Creek – described below. Other targeted sampling sites and watersheds will be added to each year's effort after determination of the priority "special interest" watersheds.

This totals 153 sites from R1 and 252 sites from R2 for 405 core repeat sites in R4.

The sample design for the repeat sites from R1 will follow the R1 design of random selection of stream reaches from strata defined by basin and stream order, specifically equal probability of selection in 1st, 2nd, and 3rd order streams within each of 17 basins statewide. 3 repeat sites will be randomly selected from the R1 sites in each stratum, where possible (some strata have low numbers of R1 sites to select from).

The sample design for the repeat sites from R2 will follow the R2 design of random selection of stream reaches from Primary Sampling Units (PSUs) that generally equate to Maryland 8-digit watersheds (which are combined when they are small to make 84 PSUs statewide). three repeat sites will be randomly selected from the R2 sites in each PSU.

Given that R1 and R2 used different sample designs and stream network map scales (R1 sites were selected from the 1:250,000-scale map and R2 sites were selected from the 1:100,000-scale map), the inter-round comparison of repeated samples will be conducted separately for each round. The R1 comparison will be a 20-year comparison; the R2 comparison will be a 14-year comparison. Each of these inter-round comparisons will have the ability to detect a change of 0.20-0.25 BIBI units with 80% probability.

3. *Watershed Intensification*

To increase the density of samples in certain watershed of special interest, all sites sampled in these watersheds in R1 and R2 will be repeat sampled in R4. For example, the 7 sites sampled for R1 in the Mattawoman Creek watershed during 1995 (some of these may be included in the 3 repeat sites for the R4 Lower Potomac sampling) will be repeat sampled in 2015, while all 10 sites sampled for R2 in 2000 will be repeat sampled in 2014 (these 10 sites include the 3 core R4 samples and 7 samples for additional density). The complete list of special interest watersheds will be reviewed each year during Round Four.

4. *Bookkeeping and Logistics*

It should be noted that some of the repeat sites selected randomly from R1 or R2 may have been designated as MBSS sentinel sites and sampled annually since they were first designated. If one or more of these sites are selected randomly for R4, their annual sentinel sampling results in the appropriate year will be included in R4 (a separate record denoting membership in R4 should be created in the database).

To obtain site access permissions, landowners for the first 10 sites per strata randomly selected for R4 from R1 and R2 will be identified and letters requesting permission will be mailed. The goal will be to obtain permissions for twice the number of sites to be sampled per stratum (i.e., 2x3=6) and to provide them to DNR.

5. *Additions and Modification to Sampling and Assessment Protocols*

The R4 MBSS will sample the same parameters sampled during R3, with one addition and one modification. Geomorphological assessments will be added. This will consist of a rapid assessment of geomorphology at randomly-selected R4 sampling sites (exact protocols to be determined) and detailed geomorphological assessments (consisting of cross-sections, longitudinal profiles, and pebble counts) at all MBSS Sentinel Sites. By increasing the time and area searched, the stream salamander sampling protocol has been modified from R3 to provide a greater likelihood of collecting stream salamanders. The goal is to support the development of a salamander index of biotic integrity, a biological indication that will be especially useful in small headwater streams with naturally depauperate fish assemblages. More detailed descriptions of these R4 sampling protocols will be included in an updated version of the MBSS sampling manual, scheduled for completion by February 2014.

Appendix B: Fish Fixation and Preservation Procedures

Fixation disables proteins such as enzymes that cause cell lysis and thus stops the cellular degradation. However, fixation also breaks up DNA so is not good for genetic samples.

* fixation - use 10% formalin for juveniles and adults; 5% for larvae. Formaldehyde gas in 40% aqueous solution can be diluted 9:1 with water to make 10% formalin. The volume of fishes in the sample themselves must be taken into account upon fixation, so the initial solution should be stronger than 10% (e.g., 20%). Make a one-inch cut in larger specimens (>150 mm) on the right hand side of the fish above and in front of anus. **Put fish in a large screw-top container with a fluid to fish ratio of at least 2:1 and ideally 5:1.** Try to get fish to lie straight - laying the bottle on its side for a while helps. You can begin transfer of fish to a storage fluid after at least 48 hours in formalin.

Storage fluids are used to maintain fixed or preserved fish for long periods. For fixed fish, formalin is rinsed off by soaking specimens in water for at least two days and up to one week. During this period, **water should be changed at least four times. Note that formalin and formalin-fixed specimens are considered toxic wastes and must be disposed of properly.** Decanted formalin can be saved for reuse in proper concentration. Rinsed fish are then transferred into 70% ethanol or 45-50% isopropanol.

Preservation tries to stop tissue degradation by removing liquid water. This can happen with freezing, using salts, or alcohol. The typical field approach is to use 95% ethanol, which allows recovery of DNA, but the fish's morphology is altered making them difficult to work with for morphology.

* preservation - use 95% ethanol. Make a one-inch cut in larger specimens (>150 mm) on the right hand side of the fish above and in front of anus. **Put fish in a large container with a fluid to fish ratio of about 5:1.** For best results, decant after 24 hrs and replace with fresh ethanol. Preserved fish are usually stored in 70-90% ethanol.

Detailed instructions for fixation and preservation can be found in Kelsh and Shields (1996).

References

Maryland Natural Heritage Program. 2010. Rare, threatened, and endangered animals of Maryland. Maryland Department of Natural Resources, Wildlife and Heritage Service, Annapolis, MD.

Kelsh, S.W. and B. Shields. 1996. Care and handling of sampled organisms. Pages 121-144 in B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, MD.

Stauffer, J.R., J. Karish, and T.D. Stecko. 2001. Guidelines for using digital photos as fish vouchers for Pennsylvania Fishes. The Pennsylvania State University and Natural Park Service

Appendix C: Equipment Lists

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	Chest waders
Road maps and itinerary	Wader repair kit
Site list/maps	G.P.S. unit
Spring Habitat Data Sheets	Compass
Spring Index Period Data Sheets	Ice
Vernal Pool Data Sheets	Bubble wrap, packing material, packing tape
Permanent markers	Clear label tape
Pencils	Deionized water for blanks
Taxonomic Keys (reptiles and amphibians, crayfish, freshwater mussels)	Water quality sample bottles- 1 liter
Machete or other clearing tools	Water quality sample bottles- 500 mL
Digital camera	Syringes and valves
First aid kit	Tripod, level, and stadia
Spray paint	Pre-printed adhesive outside labels and inside labels
Flagging	Spare batteries
100 m measuring tape	Chain-of-custody forms
540 micron mesh D-net (frame dimensions 12” W x 10” H)	Ziplock bags
Spare net bag for D-net	Pump sprayer or decontamination solution container
540 micron sieve bucket	Rinse water
Benthic sample containers (86 oz or larger)	Decontamination solution – 10% bleach or Virkon
EtOH (2 liters per site)	Extra drinking water
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	

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	Pruning tool
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
Summer Photo Data Sheets	Spare netting/cable ties for anode ring nets
Habitat Guidance Sheet	25 liter buckets
Clipboards	Dip nets
Pencils	Block nets
Sample jars	Live cars
Pre-printed voucher labels	Machete
Taxonomic keys (reptiles and amphibians; fish, freshwater mussels, crayfishes)	Calibration log
Voucher lists	Waders and wading boots
Preservatives (alcohol and formalin)	Cellular phone
100 m measuring tape	Backpacks
Flagging	Measuring board
Digital camera	Meter sticks
G.P.S. unit	Tool box
Compass	Wader repair kit
Disinfectant lotion	Polarized Glasses
Drinking water	Pump sprayer or decontamination solution container
First aid kit	Rinse water
Foul weather gear	Decontamination solution – 10% bleach or Virkon
Extra drinking water	

Appendix D: Codes and Abbreviations

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

Appendix E: MBSS Stream Habitat Assessment Guidance Sheet

MBSS Stream Habitat Assessment Guidance Sheet				
Habitat Parameter	**Optimal 16-20**	**Sub-Optimal 11-15**	**Marginal 6-10**	**Poor 0-5**
1. Instream Habitat(1)	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(2)	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., under-cut 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(3)	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(4)	Complex cover/&/or depth ≥1.5m; 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(5)	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(6)	Percentage that gravel, cobble, and boulder particles are surrounded by line sediment or flocculent material.			
7. Shading(7)	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(8)	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

1. **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.

2. **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.

3. **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.

4. **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.

5. **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.

6. **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.

7. **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.

8. **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.

Appendix F: Species Names

Common Names of Maryland Fishes

Common Names of Maryland Reptiles and Amphibians

Scientific Names of Maryland Crayfishes

Scientific Names of Maryland Freshwater Bivalves

Common Names of Invasive Plant Species

Names are listed as they should be recorded on MBSS data sheets. Letters in parentheses next to a species name correspond to the state status of the species from the Rare, Threatened, and Endangered Animals of Maryland (April 2010) and from the Maryland Aquatic Species Matrix Team. E = Endangered, T = Threatened, I = In need of Conservation, X = Presumed Extirpated, and N = Nuisance.

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 (N)
Brown bullhead
Channel catfish
Flathead catfish (N)
Margined madtom
Stonecat (E)
Tadpole madtom
White catfish
Yellow bullhead

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

KILLIFISH:
Banded killifish
Mummichog
Rainwater killifish
Striped killifish

STICKLEBACKS:
Fourspine stickleback
Threespine stickleback

MINNOWS:
Eastern 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
Golden shiner
Goldfish
Grass carp
Ironcolor shiner (E)
Longnose dace
Mimic shiner (N)*
Pearl dace (T)
Redside dace
River chub
Rosyface shiner
Rosyside dace
Sand shiner*
Satinfin 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
Walleye
Yellow perch

SUNFISHES:
Banded sunfish
Black crappie
Blackbanded sunfish (E)
Bluegill
Bluespotted sunfish
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
Eastern mosquitofish
Northern snakehead (N)
Oriental weatherfish (N)
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
Cambarus carinirostris
Cambarus diogenes
Cambarus dubius
Cambarus monongalensis
Cambarus thomaii
Fallicambarus fodiens
Orconectes limosus
Orconectes obscurus
Orconectes rusticus
Orconectes virilis
Procambarus clarkii
Procambarus acutus/zonangulus

Scientific Names of Maryland Freshwater Bivalves

Alasmidonta heterodon (E)
Alasmidonta undulata (E)
Alasmidonta varicosa (E)
Anodonta implicata
Corbicula sp.
Dreissena bugensis
Dreissena polymorpha
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

Appendix G: Chain of Custody Forms

MBSS Water Quality Chain of Custody Sheet

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

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

MBSS Specimen Tracking Data Sheet

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

_______ oC

Analyze For:

1-L Grab: DOC, TDP, TDN, Cl, NO2, NO3, PO4, SO4, 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 410-260-8605.

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

Site ID	Number of Buckets	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 ______________________________

Mbss Specimen Tracking Data Sheet

Crew Leader		
Date Delivered MM/DD/YYYY		
Delivered By		
Received By		

Species	Date Collected MM/DD/YYYY	Site ID	Voucher Type Photo or Specimen	Image no. (s) or No. (s) of specimens	Comments

Appendix H: MBSS Voucher Label

Maryland Biological Stream Survey

SITE ID ______________________________

Cat. No. ______________ Family _________________

Species ____

Basin ________________ Date ____________________

State _________________ County _________________

Locality ____

Lat __________________ Long ___________________

Col. By ____

Det. By _______________ No. Specimens ___________

Appendix I: MBSS Spring Data Sheets

MBSS Spring Index Period Data Sheet

MBSS Spring Faunal Data Sheet

MBSS Vernal Pool Data Sheet

MBSS Spring Habitat Data Sheet

MBSS Spring Facies Map Data Sheet

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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 NAME: ____________________

LOCALITY: ____________________

TIME ☐☐☐☐ (Military)

SAMPLEABILITY

☐ Benthos ☐ Facies Mapping

☐ Habitat Assessment

☐ Water Quality

☐ Vernal Pool ☐ Present (Y/N)

Other: ____________________

SITE ACCESS ROUTE: ____________________

SAMPLE LABELS

Verified by: ____________________

QC LABELS

Watershed Code ☐☐☐☐ Segment ☐☐☐ (Letters Only) Type ☐ Year ☐☐☐☐

Dup. (D) or Blank (B): ☐ Verified by: ____________

TEMP. LOGGERS

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

LOCATION: ____________________

PHOTODOCUMENTATION

Number	PHOTODOCUMENTATION	Voucher (Y/N)
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐
☐☐☐☐	____________________	☐

BENTHIC HABITAT SAMPLED

☐☐ Riffle ☐☐ Leaf Pack ☐☐ Undercut Banks

☐☐ Rootwad/Woody Debris ☐☐ Macrophytes ☐☐ Other: ____________

SAMPLING CONSIDERATIONS: (NUM. ANODES) ____________________

STREAM WIDTH (m) ☐☐☐.☐ 0 m ☐☐☐.☐ 75 m

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MBSS SPRING FAUNAL DATA SHEET

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: First ______ / Second ______

HERPETOFAUNA

☐ None Observed

Species	Lifestage: Adult	Lifestage: Larval	Lifestage: Egg	SEEN (Y/N)	HEARD (Y/N)	Number Retained	Number Photos Taken

MUSSELS

☐ None Observed

Species	LIVE	DEAD	Number Retained	Num. Photos Taken

Corbicula — LIVE ☐ DEAD ☐ NONE ☐

CRAYFISH

☐ None Observed

Species	Number Retained

Crayfish Burrows (A,P,E) ☐

COMMENTS: ____________________

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MBSS VERNAL POOL DATA SHEET

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

Fish Observed? (Y/N): ☐

Anostraca Observed? (Y/N): ☐

Predominant Surrounding Landuse: ☐☐ ☐☐ ☐☐

Distance From Pool →

HERPETOFAUNA

☐ None Observed

Species	Lifestage: Adult	Larva	Egg	Seen (Y/N)	Heard (Y/N)	# Ret.	# Photos Taken

PHOTODOCUMENTATION

Number		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

Fish Observed? (Y/N): ☐

Anostraca Observed? (Y/N): ☐

Predominant Surrounding Landuse: ☐☐ ☐☐ ☐☐

Distance From Pool →

HERPETOFAUNA

☐ None Observed

Species	Lifestage: Adult	Larva	Egg	Seen (Y/N)	Heard (Y/N)	# Ret.	# Photos Taken

PHOTODOCUMENTATION

Number		Voucher (Y/N)

COMMENTS: ____________________

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MBSS SPRING HABITAT DATA SHEET

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

RIPARIAN VEGETATION

(facing upstream)

	LEFT BANK	RIGHT BANK
Width (50m max)	☐☐	☐☐
Adj. Land Cover	☐☐	☐☐
Veg Type	☐☐☐☐	☐☐☐☐
Buffer Breaks (Y/N)	☐	☐

BUFFER BREAKS

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

Buffer Break Types (M = Minor; S = Severe)

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	☐☐	☐.☐☐
	☐☐	☐.☐☐

CHANNELIZATION

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

TYPE	EXTENT (m) LEFT BANK	BOTTOM	RIGHT BANK
Concrete	☐☐	☐☐	☐☐
Gabion	☐☐	☐☐	☐☐
Rip-Rap	☐☐	☐☐	☐☐
Earthen Berm	☐☐	☒☒	☐☐
Dredge Spoil Off Channel	☐☐	☒☒	☐☐
Pipe Culvert	☐☐	☐☐	☐☐

Actual Site Midpoint Coordinates

(Taken at Time of Sampling)

Lat	☐☐.☐☐☐☐☐☐
Long	☐☐.☐☐☐☐☐☐

Stream Blockages

Stream Block Ht. (m)	☐.☐
Stream Block Type	☐☐
Lat	☐☐.☐☐☐☐☐☐
Long	☐☐.☐☐☐☐☐☐

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MBSS SPRING FACIES MAP DATA SHEET

SITE: Watershed Code ☐☐☐☐ | Segment ☐☐☐ | Type ☐ | Year ☐☐☐☐

Reviewer: First _____ / Second _____

DATE: Year ☐☐ | Month ☐☐ | Day ☐☐

	L	Center R
75m		
	Dominant Substrate ☐	Dominant Substrate ☐
	Subdominant Substrate ☐	Subdominant Substrate ☐
	Depth ☐	Depth ☐
	Velocity ☐	Velocity ☐
50m		
	Dominant Substrate ☐	Dominant Substrate ☐
	Subdominant Substrate ☐	Subdominant Substrate ☐
	Depth ☐	Depth ☐
	Velocity ☐	Velocity ☐
25m		
	Dominant Substrate ☐	Dominant Substrate ☐
	Subdominant Substrate ☐	Subdominant Substrate ☐
	Depth ☐	Depth ☐
	Velocity ☐	Velocity ☐
0m		

Classifications for **Dominant** and **Subdominant Substrate** Categories

Y = Silt/Clay (< .062mm)
S = Sand (.062 - 2mm)
G = Gravel (2 - 64mm)
C = Cobble (64 - 256mm)
B = Boulder (256-4096mm)
K = Bedrock (> 4096mm)

Classifications for Average Stream **Depth** Categories

1 = Shallow (< 0.5 m)
2 = Moderately Deep (0.5 m - 1.0 m)
3 = Deep (> 1.0 m)

Classifications for Average Stream **Velocity** Categories

1 = Slow (0-0.3 m/s)
2 = Fast (> 0.3 m/s)

COMMENTS

Appendix J: MBSS Summer Data Sheets

MBSS Summer Index Data Sheet

MBSS Summer Habitat Data Sheet

MBSS Summer Fauna Data Sheet

MBSS Stream Salamander Data Sheet

MBSS Fish Data Sheet

MBSS Game Fish Length Data Sheet

MBSS Photo Data Sheet

MBSS Round 1 Repeat Data Sheet

Crib Sheet

v. 2014

MBSS SUMMER INDEX DATA SHEET

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: First _____ / Second _____

DATE — Year ☐☐ Month ☐☐ Day ☐☐

CREW: ______________________________

____

TIME ☐☐☐☐ (Military)

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 = Landowner Permission Denied
8 = Unsafe (Describe in Comments)
9 = Beaver
10 = Other: ________________

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

EXOTIC PLANTS

☐ None Observed

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

TEMPERATURE LOGGERS

Air Logger Present? (Y/N) ☐

Water Logger De-watered? (Y/N) ☐

AQUATIC VEGETATION

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

DIDYMO

(A,P, or E) ☐ Voucher (Y/N) ☐

PHOTODOCUMENTATION

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

COMMENTS: ______________________________

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v. 2014

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 = severe

- 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	Gravel	Boulder >2m
Riffle	Sand	Boulder <2m
Run/Glide	Silt/Clay	Beaver Pond
Deep Pool (>= 0.5m)	Cobble	Overhead Cover
Shallow Pool (< 0.5m)	Bedrock	Undercut Bank
		Orange Floc

A = Absent P = Present E = Extensive

Woody Debris

- 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: ____

v. 2014

MBSS SUMMER FAUNA DATA SHEET

Page ☐ of ☐

SITE	Watershed Code	Segment	Type	Year	Reviewer: First / Second
					/

STREAM SALAMANDERS

☐ None Observed

Species	Electrofishing Catch: Adult	Electrofishing Catch: Larva	Transect Catch: Adult	Transect Catch: Larva	Number Retained	Number Photos Taken

OTHER HERPETOFAUNA

☐ None Observed

Species	Lifestage: Adult	Lifestage: Larva	Lifestage: Egg	Number Retained	Num. Photos Taken

MUSSELS

☐ None Observed

Species	Live	Dead	Number Retained	Num. Photos Taken

Corbicula LIVE ☐ DEAD ☐ NONE ☐

CRAYFISH

☐ None Observed

Crayfish Burrows (Absent, Present, Extensive) ☐

Species	Incidental Catch? (Y/N)	1st Pass Catch (Total)	2nd Pass Catch (Total)	Number Retained

COMMENTS: ______________________________

v. 2014

MBSS STREAM SALAMANDER DATA SHEET

	Watershed Code	Segment	Type	Year
SITE	☐☐☐☐	☐☐☐	☐	☐☐☐☐

Reviewer: First ______ / Second ______

	Year	Month	Day
DATE	☐☐	☐☐	☐☐

Seeps Present? (Y/N) ☐

Habitat Composition (%)

☐☐☐ Stream Corridor

☐☐☐ Seeps

	Stream Corridor	Seeps
All Available Habitat Sampled? (Y/N)	☐	☐
Time Searched (Max 60 min)	Minutes ☐☐ : Seconds ☐☐	Minutes ☐☐ : Seconds ☐☐
Available Habitat Left Unsampled (%)	☐☐	☐☐

COMMENTS

____

____

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____

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____

☐ None Observed

SPECIES	ADULTS Abundance Stream Corridor	ADULTS Abundance Seeps	ADULTS Num. Photos Taken	LARVAE Abundance Stream Corridor	LARVAE Abundance Seeps	LARVAE Num. Photos Taken
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐
	☐☐☐	☐☐☐	☐	☐☐☐	☐☐☐	☐

v. 2014

MBSS FISH DATA SHEET

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) ☐

	Unit ____	Unit ____	Unit ____	Unit ____	Unit ____	Unit ____
Anodes/Unit	☐	☐	☐	☐	☐	☐
Begin 1st P.	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Begin 2nd P.	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
End	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Voltage	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐

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

Aggregate Fish Biomass ☐☐☐☐ ☐☐☐☐ (g)

v. 2014

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)

v. 2014

MBSS PHOTO DATA SHEET

Page ☐ of ☐

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: First ______ / Second ______

Number	PHOTODOCUMENTATION	Voucher (Y/N)

v. 2014

MBSS ROUND 1 REPEAT DATA SHEET

Page ☐ of ☐

	Watershed Code	Segment	Type	Year
SITE				

Reviewer: First ____ / Second ____

Habitat Assessment

Channel Alteration (0-20)...............

Bank Stability (0-20).......................

Remoteness (0-20).........................

Minimum Buffer Zone Width (m) ....

Buffer Type ....

Adjacent Land Cover ....

Fish Anomalies

Species w/ Anomalies	Number of Affected Individuals (max. 100 per species)					
	Anomaly Type	Anomaly Type	Anomaly Type	Anomaly Type	Anomaly Type	Anomaly Type

Anomaly Types

Body Surfaces and Fins
- AN = Swelling of the Anus
- AW = Anchor Worm
- BL = Black Spot
- BS = Body Shape
- CL = Fin Cloudiness
- CT = Cut
- DI = Discoloration
- DM = Deformities of the Mandible
- DV = Deformities of the Vertebral Column
- EP = Visible External Parasites
- FD = Fin Deformed or Missing
- FI = Fin Erosion
- FU = Fungus
- GR = Growths/Cysts
- HK = Hooking Injury
- HM = Hemorrhaging
- IK = Ich
- LE = Leeches
- OT = Other (define in comments section)
- RE = Red Spot
- RS = Raised Scales
- SC = Scale Deformities
- UL = Ulcerations/Lesions

Eyes
- CA = Cataract
- EC = Eye Cloudiness
- EH = Eye Hemorrhage
- NO = Eye missing
- OR = Depression into the Orbits
- PO = Exopthalmia (pop eye)

Comments

v. 2014

CRIB SHEET

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

_____ PASS

Species	Tally	Anomalies

_____ PASS

Species	Tally	Anomalies

Appendix K: MBSS Geomorphology Data Sheets

MBSS Geomorphology Cross-Section Data Sheet

MBSS Flood-Prone Area Data Sheet

MBSS Cross-Section Pebble Counts Data Sheet

MBSS GEOMORPHOLOGY CROSS-SECTION DATA SHEET

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: First ______ / Second ______

Cross-Section Within Site? (Y/N) ☐ Cross-Section Within Riffle? (Y/N) ☐
(riffle may not be present on Coastal Plain)

	Distance, Point or	Back-Sight	Fore-Sight	COMMENTS:
	STATION	B S	F S	
Item	ft	ft	ft	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

v. 2014

MBSS FLOOD-PRONE AREA DATA SHEET

Page ☐ of ☐

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: First ______ / Second ______

1.)

(A) ☐☐.☐☐ ft

– (B) ☐☐.☐☐ ft

(C) ☐☐.☐☐ ft

A = Elevation at average water depth (from Cross-Section Data Sheet)

B = Mean bankfull depth (from regional curve)

C = Bankfull elevation (A-B)

D = Thalweg elevation (from Cross-Section Data Sheet)

E = Max bankfull depth (D-C)

F = 2x max bankfull depth (E x 2)

G = Flood-prone area elevation (D-F)

2.)

(D) ☐☐.☐☐ ft

– (C) ☐☐.☐☐ ft

(E) ☐☐.☐☐ ft

3.)

(E) X 2 = (F) ☐☐.☐☐ ft

4.)

(D) ☐☐.☐☐ ft

– (F) ☐☐.☐☐ ft

(G) ☐☐.☐☐ ft ← FLOOD-PRONE ELEVATION

Flood-Prone Elevation >50m from edge of water? (Y/N) ☐

FLOOD-PRONE AREA WIDTH ☐☐☐.☐ ft

Comments/Notes:

v. 2014

MBSS CROSS-SECTION PEBBLE COUNTS DATA SHEET

Page ☐ of ☐

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐

Reviewer: ______ / ______ (First / Second)

		Range (mm)	Particle Tally	Total
SILT/CLAY	Silt/Clay	<.062		
SAND	Very Fine	.062 - .125		
	Fine	.125 - .25		
	Medium	.25 - .50		
	Coarse	.50 - 1.0		
	Very Coarse	1.0 - 2		
GRAVEL	Very Fine	2 - 4		
	Fine	4 - 6		
	Fine	6 - 8		
	Medium	8 - 11		
	Medium	11 - 16		
	Coarse	16 - 22		
	Coarse	22 - 32		
	Very Coarse	32 - 45		
	Very Coarse	45 - 64		
COBBLE	Small	64 - 90		
	Small	90 - 128		
	Large	128 - 180		
	Large	180 - 256		
BOULDER	Small	256 - 362		
	Small	362 - 512		
	Medium	512 - 1024		
	Large	1024 - 2048		
	Very Large	2048 - 4096		
BEDROCK	Bedrock	>4096		
			Grand Total	

APPENDIX E

DPW-WPRP-CBMP-FO-001:
SELECTING A QA SITE FOR DUPLICATE SAMPLING

Standard Operating Procedure DPW-WPRP-CBMP-FO-001

Selecting a QC Site for Duplicate Sampling

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This procedure is provided to detail the steps involved in selecting a Quality Control (QC) Duplicate site at randomly selected biological sampling sites in Anne Arundel County for QA/QC purposes. The goal is to identify and sample an adjacent QC Duplicate site with similar immediate and upstream drainage area characteristics and with an absence of observed potential stressors that may be unique to each site. A total of 10% of all sites in each stream stratum sampled in a given sampling year will have QC Duplicate sites installed (i.e. – typically, four in MBSS-modified 1:100,000-scale Large Stream stratum and four in the County's Small Stream stratum). Fixed sites in the Large Scale stratum will also be included as potential candidates for QC Duplicate site establishment and will be evaluated each year of the Round along with that year's proposed random sites.

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 Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

References:
None

Precautions:
1. All QC Duplicate sampling reaches are established upstream of the original probability site reach.

Equipment/Materials:
Computer
GIS mapping software
Recent orthophotography and appropriate shapefiles

Procedure:
This method consists of two parts: a desktop review followed by a field assessment. Each is discussed below.

Desktop Review

1. Plot the current Round's sampling points using GIS software such as ArcMap to display all potential sampling sites (primary and alternate sites) and add all necessary data layers to assist with locating potential stressor sources (e.g., orthophotography, County stream layer, MBSS-modified 1:100,000 stream layer stream layer, stormwater pipes, roads, utilities, etc.).

2. Review each point to determine which sites may be good candidates for a quality control site. Consider the following factors:

   a. If there appears to be a marked difference (increase or decrease) in potential stressors between the random site or adjacent 75-meter reach upstream (e.g., number of road crossings or tributaries, number of stormwater pipe outfalls or other point source discharges, inconsistent buffers from adjacent land use), the site should be excluded as a QC Duplicate site location.

   b. If two random sites are located in close proximity to one another such that there is not sufficient length of stream between them to fit a duplicate reach (75 meters), the downstream site should be excluded as a QC Duplicate site location.

   c. If a random or fixed site is located less than 150 meters downstream from a man-made impoundment, the site should be excluded as a QC Duplicate site location.

3. Annotate the site list appropriately so that sites judged as having possible locations for QC Duplicate sites will be evaluated in the field.

Field Review and QC Duplicate Site Establishment

1. Of the remaining random sites where establishing a QC Duplicate site remains viable after the desktop review, **evaluate the first site visited** following the criteria provided below. Final in-field identification of a valid QC Duplicate site is based solely on observations and visual comparison of the sampled reach and the adjacent 75 meter reach upstream. Habitat assessment evaluations or biological sampling should not be conducted at the potential QC Duplicate site before a determination of similarity 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 that is absent in the other. 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.

2. 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. Geomorphic procedures (e.g.—cross section monumenting, pebble counts, longitudinal profile, etc.) are not performed at QC Duplicate sites, but geomorphic characteristics can be used in the evaluative process for final site selection. Water quality sample field duplicates can be collected at these sites, if the site is judged appropriate for such QA/QC sample collection.

3. Continue with this procedure until the required number of sites has been achieved.

Pertinent QA and QC Procedures:

1. The Task Leader must ensure that the most up to date GIS datasets available for the desktop review.

2. Ensure that all at least primary sites and secondary sites are evaluated for installation of a QC Duplicate site.

3. The Task Leader is responsible for making the final determination that comparisons made in the office valuation are valid and has final responsibility for deciding if a QC Duplicate site is appropriate.

APPENDIX F

DPW-WPRP-CBMP-FO-002:
ESTABLISHING AND MARKING A RANDOM SITE

Standard Operating Procedure

DPW-WPRP-CBMP-FO-002

Establishing and Marking a Random Site

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 the 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. While all field crew members should assist in determining a site's location, the Field Sampling Task Leader (Task Leader) will make the final determination on where a particular site is ultimately established. This SOP must be read completely by the Task Leader and the Field Crew before sample site determinations are made.

References:
DNR. 2016. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2016. Publication # 12Resource Assessment Service-3142014-700. 78 pp.

Precautions:
Because these sites are randomly placed, field crews should be prepared for potentially long hikes over difficult terrain to reach them.

Equipment/Materials:
Field computer with appropriate GIS layers, or paper maps of the sites
100 meter or 300 foot tape measure
Spray paint
Surveyor's flagging
Hammer
Aluminum nails
Permanent marker
Foil tree tags
GPS Receiver
Site List with Preliminary Drainage Areas and Bankfull Hydraulic Geometry Relationships

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 the Task Leader 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 by the Task Leader, 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, which represents the potential mid-point of the 75 meter sampling reach. 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, MBSS-modified 1:100,000 stream layer). Consult pre-printed paper maps of the site and its surrounding land use if digital mapping products are not available.

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), proceed to step 3.

b. If the GPS places the site near the confluence of (or topographically between) two stream channels, determine the stream order of the reach where the site is supposed to be placed and refer to list of drainage areas preliminarily assigned to all the sites. Typically, your site will be the larger (i.e., higher order) stream carrying the most flow. Additionally, regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to determine and validate that the correct site is chosen based on the site's pre-determined drainage area.

For example, suppose you have to choose between two channels where one has a bankfull width of 20 feet and the other has a bankfull width of three feet. If the target site is supposed to be on a 2nd order stream with 10 square miles of drainage, you should choose the site with a bankfull width of 20 feet as those channel characteristics best fit with the targeted site's drainage area.

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 stream line (on the MBSS-modified 1:100,000 stream layer or the County layer, depending on the site) does in fact represent the nearby stream channel. Navigate to a location on the "intended" channel that approximates the preliminary drainage area calculated for the site. Once you reach the "intended" location, proceed 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.

a. The Task Leader must determine if the site can be sampled 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.

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. The Task Leader is responsible for making this decision regarding sampleability in these cases.

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 can be 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 Task 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 coordinates of this location (in decimal degrees, to the nearest 0.00001 degree), which becomes 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. Measure a distance of 123 feet (37.5 meters) downstream from the mid-point following the thalweg (i.e. - the deepest part) of the channel. This becomes the downstream extent of the sampling reach, or 0 meter mark. Mark this location with survey flagging and/or spray paint, clearly indicating the '0 m'. If possible, locate the nearest tree or shrub and attach surveyor's flagging. Flagging should be labeled with the site ID and station (e.g., 09-R3M-06-17 0m or 09-R3M-06-17 75m). At the mid-point attach a tree tag, with the site ID and its station along the sampling reach if not the midpoint, to the closest tree perpendicular to the stream channel.

b. Using a survey pin, secure the end of the measuring tape at the 0 m mark. Following the thalweg (of the channel, measure off 246 feet (75 meters) in the upstream direction. This becomes the upstream extent of the sampling reach. Follow the aforementioned procedures for marking the upstream end (75 m mark) of the reach.

c. For sites on the large stream network that will be revisited in the summer, mark the 25 meter station (82 feet) and 50 meter station (164 feet) with surveyor's flagging and/or spray paint. Flagging only needs to be labeled 25m and 50m, respectively.

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.

3. A separate person other than the original data recorder will review the data forms upon completion to check for errors or blank spaces prior to leaving the site.

APPENDIX G

DPW-WPRP-CBMP-FO-003:
USE OF GPS

Standard Operating Procedure DPW-WPRP-CBMP-FO-002

Establishing and Marking a Random Site

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 the 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. While all field crew members should assist in determining a site's location, the Field Sampling Task Leader (Task Leader) will make the final determination on where a particular site is ultimately established. This SOP must be read completely by the Task Leader and the Field Crew before sample site determinations are made.

References:
DNR. 2016. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2016. Publication # 12Resource Assessment Service-3142014-700. 78 pp.

Precautions:
Because these sites are randomly placed, field crews should be prepared for potentially long hikes over difficult terrain to reach them.

Equipment/Materials:
Field computer with appropriate GIS layers, or paper maps of the sites
100 meter or 300 foot tape measure
Spray paint
Surveyor's flagging
Hammer
Aluminum nails
Permanent marker
Foil tree tags
GPS Receiver
Site List with Preliminary Drainage Areas and Bankfull Hydraulic Geometry Relationships

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 the Task Leader 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 by the Task Leader, 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, which represents the potential mid-point of the 75 meter sampling reach. 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, MBSS-modified 1:100,000 stream layer). Consult pre-printed paper maps of the site and its surrounding land use if digital mapping products are not available.

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), proceed to step 3.

   b. If the GPS places the site near the confluence of (or topographically between) two stream channels, determine the stream order of the reach where the site is supposed to be placed and refer to list of drainage areas preliminarily assigned to all the sites. Typically, your site will be the larger (i.e., higher order) stream carrying the most flow. Additionally, regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to determine and validate that the correct site is chosen based on the site's pre-determined drainage area.

   For example, suppose you have to choose between two channels where one has a bankfull width of 20 feet and the other has a bankfull width of three feet. If the target site is supposed to be on a 2nd order stream with 10 square miles of drainage, you should choose the site with a bankfull width of 20 feet as those channel characteristics best fit with the targeted site's drainage area.

   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 stream line (on the MBSS-modified 1:100,000 stream layer or the County layer, depending on the site) does in fact represent the nearby stream channel. Navigate to a location on the "intended" channel that approximates the preliminary drainage area calculated for the site. Once you reach the "intended" location, proceed 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.

a. The Task Leader must determine if the site can be sampled 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.

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. The Task Leader is responsible for making this decision regarding sampleability in these cases.

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 can be 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 Task 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 coordinates of this location (in decimal degrees, to the nearest 0.00001 degree), which becomes 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. Measure a distance of 123 feet (37.5 meters) downstream from the mid-point following the thalweg (i.e. - the deepest part) of the channel. This becomes the downstream extent of the sampling reach, or 0 meter mark. Mark this location with survey flagging and/or spray paint, clearly indicating the ‘0 m’. If possible, locate the nearest tree or shrub and attach surveyor’s flagging. Flagging should be labeled with the site ID and station (e.g., 09-R3M-06-17 0m or 09-R3M-06-17 75m). At the mid-point attach a tree tag, with the site ID and its station along the sampling reach if not the midpoint, to the closest tree perpendicular to the stream channel.

b. Using a survey pin, secure the end of the measuring tape at the 0 m mark. Following the thalweg (of the channel, measure off 246 feet (75 meters) in the upstream direction. This becomes the upstream extent of the sampling reach. Follow the aforementioned procedures for marking the upstream end (75 m mark) of the reach.

c. For sites on the large stream network that will be revisited in the summer, mark the 25 meter station (82 feet) and 50 meter station (164 feet) with surveyor’s flagging and/or spray paint. Flagging only needs to be labeled 25m and 50m, respectively.

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.

3. A separate person other than the original data recorder will review the data forms upon completion to check for errors or blank spaces prior to leaving the site.

APPENDIX H

DPW-WPRP-CBMP-FO-004:
USE OF WATER QUALITY INSTRUMENTATION

Standard Operating Procedure DPW-WPRP-CBMP-FO-004

Use of Water Quality Instrumentation

Prepared by:	Name: Colin Hill, KCI	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This method is to be used to calibrate and deploy the YSI Professional Plus (Pro Plus), YSI Professional Digital Sampling System (Pro DSS), and Hydrolab Quanta multiparameter meters, as well as the Hach 2100P Turbidimeter. This electronic instrumentation is used to measure dissolved oxygen, pH, temperature, conductivity, and turbidity *in situ* using specialized sensors, or probes. Proper training in the operation of all water quality instruments is required before use, and the procedure(s) should be reviewed before each deployment.

Responsibility and Personnel Qualifications:
This procedure may be used by any person who has received training in the operation of the YSI Professional Plus (Pro Plus), YSI Pro DSS, Hydrolab Quanta, and Hach 2100P Turbidimeter instruments. The Field Sampling Task Leader (Task Leader) shall ensure adherence to all relevant QA/QC procedures during the use of this instrument.

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

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

YSI ProDSS User Manual. 2014. Item #626973-01REF. YSI Incorporated, Yellow Springs, Ohio.

Hydrolab Quanta Operating Manual. 2002. Hydrolab Corporation, Austin, Texas.

Precautions:
1. Measurement of water quality parameters must precede any field activities that disrupt the water column or bottom sediments in the assessment reach.

Equipment/Materials:

All needed supplies and other equipment to ensure proper operation are listed in the tables below for each device.

Table 1. YSI Pro Plus Equipment/Materials

YSI Pro Plus instrument	Deionized water
C-cell alkaline batteries	Specific conductance standard (1413 µS/cm)
Weighted sensor guard	pH buffer standards (pH 4.0 & 7.0)
Calibration & storage cup	Dissolved oxygen sensor membranes
Calibration Log	Dissolved oxygen sensor electrolyte solution
ProPlus User Manual	Philips head screwdriver

Table 2. HACH 2100P Equipment/Materials

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

Table 3. YSI ProDSS Equipment/Materials

YSI ProDSS instrument	Specific conductance standard (1000 µS/cm)
Sensor guard	pH buffer standards (pH 4.0 & 7.0)
Calibration & storage cup	Turbidity standard (0 NTU, 12.4 NTU)
Calibration log	
ProDSS User Manual	
Deionized water	

Table 4. Hydrolab Quanta Equipment/Materials

Hydrolab Quanta instrument	Specific conductance standard (1000 µS/cm)
Sensor guard	pH buffer standards (pH 4.0 & 7.0)
Storage & calibration cup with cover	Turbidity standard (12.4 NTU)
Calibration log	C-cell alkaline batteries
Hydrolab Quanta User Manual	Dissolved oxygen sensor membranes
Deionized water	Dissolved oxygen sensor electrolyte solution

Procedure:

YSI ProPlus 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 2nd 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 3rd 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.

HACH 2100P Turbidimeter 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.

YSI ProDSS Calibration

1. Verify that all sensors, sensor guard, and calibration cup are clean.
2. Turn display unit on.
3. Verify that the built in barometer reading matches a local weather station. The barometer is factory calibrated and should not need to be adjusted. If barometer needs to be calibrated:
    a. Press **Cal** key, select Barometer and press enter.
    b. Select calibration value, enter the "true" barometric pressure from local weather station.
    c. Select Accept Calibration.
4. Calibrate the dissolved oxygen (DO) sensor using the following steps:
    a. Ensure no droplets are on the ODO sensor cap or temperature sensor.
    b. Partially screw calibration cup over sensor guard filled with a small amount (1/8") of clean water.
    c. Power on instrument and wait 5 to 15 minutes for the air in storage container to fully saturate with oxygen.
    d. Press **Cal** key, select ODO, select DO%.
    e. Observe readings and wait to stabilize for 40 seconds, then select Accept Calibration.
    f. Record both the value under 'Actual Readings' before calibration and the value displayed by the unit after calibration in the Calibration Log.

5. Calibrate the conductivity sensor using the following steps:
    a. Turn the display unit on and press the **Cal** button. Select Conductivity and press enter.
    b. Place the sensor into a conductivity calibration solution. The solution must cover the vent holes for the conductivity sensor (Figure 46 in manual).
    c. Gently rotate sensor to remove any bubbles from sensor.
    d. Press **Cal** key, select Conductivity, select Specific Conductance.
    e. Select Calibration Value then enter the value for the standard used, in this case 1,000 µS.
    f. Observe actual measurement readings and wait to stabilize for 40 seconds, then select Accept Calibration.
    g. 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.

h. 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.

6. Calibrate the pH sensor using the following steps:
   a. Turn the display unit on and press the **Cal** button.
   b. Fill calibration cup with pH 7 buffer solution.
   c. Immerse probe in calibration cup.
   d. Allow for one minute for temperature stabilization. During this time the Calibration Value will be automatically adjusted based on the selected buffer. Alternatively it can be manually entered.
   e. Observe readings to stabilize for 40 seconds, then select Accept Calibration. “Ready for cal point 2” will be displayed.
   f. Rinse sensor 2-3 times with small amount of pH 4 buffer solution.
   g. Rinse then fill calibration cup with pH 4 buffer solution.
   h. Immerse probe in calibration cup.
   i. Allow for one minute for temperature stabilization. During this time the Calibration Value will be automatically adjusted based on the selected buffer. Alternatively it can be manually entered.
   j. Observe readings to stabilize for 40 seconds, then select Accept Calibration. “Ready for cal point 2” will be displayed.
   k. After calibrating second point, select Finish Calibration.
   l. Record both the values under ‘Actual Readings’ before calibration and the values displayed by the unit after calibration in the Calibration Log.

7. Calibrate the turbidity sensor using the following steps:
   a. Turn the display unit on and press the **Cal** button. Select Turbidity and press enter.
   b. Place the sensor into a 0 NTU turbidity calibration solution. Distilled or deionized water is suitable.
   c. Press **Cal** key, select Turbidity.
   d. Select Calibration Value then enter the value 0.00.
   e. Observe actual measurement readings and wait to stabilize for 40 seconds, then select Accept Calibration. “Ready for cal point 2” will display.
   f. Rinse sensor 2-3 times with small amount of 12.4 NTU turbidity calibration solution.
   g. Rinse then fill calibration cup with 12.4 NTU solution.
   h. Immerse probe in calibration cup.
   i. Select Calibration Value then enter the value 12.4.
   j. Observe actual measurement readings and wait to stabilize for 40 seconds, then select Accept Calibration. “Ready for cal point 3” will display.
   k. Select Finish Calibration to complete 2 point calibration.
   l. Record both the value under ‘Actual Readings’ before calibration and the value displayed by the unit after calibration in the Calibration Log.

8. The temperature sensor does not require calibration because it has been factory-calibrated, and the accuracy and precision do not vary over time.
9. When finished calibrating, rinse sensors in clean water, then dry with soft cloth.
10. Fill calibration cup with 0.5" of any water and screw firmly on instrument.

Hydrolab Quanta 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 (Hydrolab, 2002), prior to calibration.
    b. Assemble the calibration cup around the probe housing.
    c. Turn the display unit on.
    d. Fill the calibration cup with deionized or tap water until the water is just level with the o-ring used to secure the membrane.
    e. Carefully remove any water droplets from the membrane with the corner of a tissue.
    f. Turn the black calibration cup cover upside down and lay it over the top of the calibration cup to cover the opening.
    g. Wait approximately 5 to 10 minutes for the storage container to become completely saturated and allow the sensor to stabilize.
    h. Use the arrow keys to navigate to the Calib menu.
    i. Highlight *DO %* and press Enter to confirm.
    j. Determine the barometric pressure (mm HG) and enter the value into the BP field.
    k. Press the enter key and ensure that the *Fail* message does not appear on the screen. Press Esc to exit the calibration menu.
    l. Record the barometric pressure and whether the calibration was successful in the calibration log.

2. Calibrate the conductivity sensor using the following steps:
    a. Turn the display unit on.
    b. Assemble the calibration cup around the probe housing.
    c. Fill the calibration cup with the 1,000 µS/cm standard up to the black turbidity sensor ring.
    d. Wait for the reading to stabilize for one to two minutes and then record the reading in the calibration log.
    e. Use the arrow keys to navigate to the Calib menu.
    f. Select *SpC* and press Enter. Specific conductance is a measure of conductivity reported as a temperature compensated value using a reference temperature of 25°C.
    g. Use the arrow keys to raise or lower the reading to match the calibration standard value.
    h. Press the enter key and ensure that the *Fail* message does not appear on the screen. Press Esc to exit the calibration menu.
    i. Record the original and new calibration values and whether the calibration was successful in the calibration log.

3. Calibrate the pH sensor using the following steps:

a. Turn the display unit on.
b. Inspect the pH sensor to ensure there is no sediment, biological growth, etc. is present and clean with tap water and a soft, non-scratching cloth, if necessary.
c. Assemble the calibration cup around the probe housing.
d. Fill the calibration cup with the 7.0 buffer standard up to the black turbidity sensor ring. Be sure to calibrate the 7.0 standard first, as this will be treated as the "zero".
e. Wait for the reading to stabilize for one to two minutes and then record the reading in the calibration log.
f. Use the arrow keys to navigate to the Calib menu.
g. Select *pH* and press Enter.
h. Use the arrow keys to raise or lower the reading to match the calibration standard value (7.0).
i. Press the enter key and ensure that the *Fail* message does not appear on the screen.
j. Rinse out the calibration cup with tap water.
k. Fill the calibration cup with the 4.0 buffer standard up to the black turbidity sensor ring.
l. Wait for the reading to stabilize for one to two minutes and then record the reading in the calibration log.
m. Use the arrow keys to navigate to the Calib menu.
n. Select *pH* and press Enter.
o. Use the arrow keys to raise or lower the reading to match the calibration standard value (4.0).
p. Press the enter key and ensure that the *Fail* message does not appear on the screen. Press Esc to exit the calibration menu.
q. Record the original and new calibration values and whether the calibration was successful in the calibration log.

4. Calibrate the turbidity sensor using the following steps:
   a. Turn the display unit on.
   b. Inspect the turbidity sensor ring to ensure there is no sediment, biological growth, etc. is present and clean with tap water and a soft, non-scratching cloth, if necessary.
   c. Assemble the calibration cup around the probe housing.
   d. Fill the calibration cup with the 12.4 FNU turbidity standard up to above the black turbidity sensor ring. Be sure to completely submerge the ring in standard.
   e. Ensure there are no bubbles around the sensor by gently agitating the calibration cup.
   f. Wait for the reading to stabilize for up to ten minutes and then record the reading in the calibration log.
   g. Use the arrow keys to navigate to the Calib menu.
   h. Select *Turb* and press Enter.
   i. Use the arrow keys to raise or lower the reading to match the calibration standard value (12.4).
   j. Press the enter key and ensure that the *Fail* message does not appear on the screen. Press Esc to exit the calibration menu.

k. Record the original and new calibration values and whether the calibration was successful in the calibration log.

5. The temperature sensor does not require calibration because it has been factory-calibrated, and the accuracy and precision do not vary over time.

YSI ProPlus/ProDSS 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.

YSI ProPlus/ProDSS 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.

Turbidity 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.

Turbidimeter Operation

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.

Hydrolab Quanta Deployment and Operation

1. The Hydrolab Quanta must be calibrated and working properly prior to operation/deployment.
2. Deployment of the Hydrolab Quanta must occur prior to any field activities that disrupt the water column or bottom sediments.
3. Press the power button to turn the unit on.
4. Remove the storage cup from the end of the Quatro cable assembly and attach the plastic sensor guard. Gently lower the sonde into the water 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. Ensure that the Screen menu is flashing at the bottom of the display. If not, use the arrow keys to navigate to the Screen menu.
6. Monitor the water parameter reading via the data display while allowing the unit to stabilize. 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.
7. When all measurements have been completed, unscrew the plastic sensor guard and replace the storage cup, ensuring that the storage cup contains water up to the *Storage Fill Level* line.

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. 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.
4. Be sure that StablCal Stabilized Standards have not expired and that the vials containing the standards are free of scratches.
5. If the instruments cannot be calibrated by following these procedure and the information in the instruction manual, making adjustments or replacing sensors if necessary, the unit should be sent to the manufacturer for repair and another unit should be used for the project until repairs are completed.
6. 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 I

DPW-WPRP-CBMP-FO-005:
WATER QUALITY SAMPLE COLLECTION AND PROCESSING

Standard Operating Procedure DPW-WPRP-CBMP-FO-005

Water Quality Sample Collection and Processing

Prepared by:	Name: Colin Hill, KCI	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This procedure describes field methods for the collection of water quality (WQ) samples at all probability and fixed stations and to verify that water quality samples were transported from KCI and/or Coastal Resources, Inc. (CRI) to University of Maryland Center for Environmental Science (UMCES), Appalachian Laboratory for processing and received by laboratory personnel. WQ Samples are collected during the Spring Index Period. The techniques used are similar to those employed by the Maryland Biological Steam Survey. Table 1 lists the parameters that will be analyzed for each sample.

Responsibility and Personnel Qualifications:
This procedure may be used by any person who has received MBSS training or who has experience in water quality grab sample collection. Additionally, one of the field staff members must take on the role of Field Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

References:
DNR. 2017. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2017. Publication # 12Resource Assessment Service-3142014-700. 78 pp.

Southerland, M., G. Rogers, N. Roth and D. Zaveta. 2016. Design Update of the Anne Arundel County Biological Monitoring Program. Prepared for the Anne Arundel County Department of Public Works, Watershed Protection and Restoration Program, Annapolis, Maryland. Prepared by Versar, Inc., Columbia, Maryland, and AKRF, Inc., Hanover, Maryland. 37pp.

Precautions:
1. Spare containers should be kept in case of accidental contamination or breakage.

2. Sample collection should precede any field activities that disrupt the water column or bottom sediments in the assessment reach. If the reach has been disturbed, the sample should be taken at the upstream end of the assessment reach.

3. Ensure necessary coordination with the contract laboratory takes place so that sample pick up/delivery occurs to ensure compliance with all parameter holding times.

Equipment/Materials:
1-L and 125-mL sample containers (pre-cleaned)
Nitrile gloves
Labels
Multiparameter Water Quality Instrument (*see "DPW-WPRP-CBMP-FO-004: Use of Water Quality Instrumentation" for details on the use this device)*
Cooler and Ice
Pencil or waterproof pen
Data Sheets
UMCES – Appalachian Lab Chain-of-Custody (COC) Record
Plastic resealable bag
Black or blue ballpoint pen
Large trash bags
Clear packing tape

Collection Procedure:
1. Locate an appropriate place in the assessment reach to collect these samples and to deploy the multiparameter water quality instrument (WQ meter). While the default location for collection of the sample is the approximate midpoint of the assessment reach, other factors must be considered. Sufficient depth must be present to submerge the bottles enough to collect the sample without disturbing bottom sediments. Find a suitable location as close to midpoint as feasible. However, if the reach has been disturbed by the commencement of sampling activities before acquiring the sample, then the sample will be collected at a suitable location at or near the upstream end (75 meter mark) of the assessment reach. The WQ meter deployment will mirror WQ sample collection locations.

2. All bottle labels should be completed before sampling begins. Use pencil or waterproof pen only. Each bottle label should include:

- Sample ID
- Date
- Military Time
- Sampler name

When completed, cover the labels with clear plastic before samples are collected to prevent smudging or damage during transport. Use a large enough piece of tape to ensure that all edges of the label are covered to prevent water from wicking under the tape. Overlap multiple pieces of tape if necessary.

3. Enter the stream just below the desired sample collection location and walk a couple of steps upstream. Doing this step minimizes the possibility of stirred up bottom sediments accidentally entering the sample.

4. Carefully remove the cap from bottle, taking care not to touch the inside as you remove it or as you hold it during subsequent steps. If the cap is set down, ensure that it is protected from contamination.

5. For the 1-liter bottle, dip the mouth of the bottle so it's just below the water surface. Avoid unusual surface conditions not typical of the site, floating vegetation or garbage, etc. Fill the bottle at least ½ full, then discard the contents downstream. Repeat the fill-and-rinse two more times so that the bottle has been rinsed a total of three (3) times. Rinse the cap thoroughly in the stream. Fill the bottle completely so that once the cap is screwed onto the bottle there is no headspace. The cap may be used to help scoop stream water into the sample bottle in shallow streams. Carefully place the cap on the bottle and tighten securely.

6. Before collecting the sample in the 125-mL bottle, put on nitrile gloves. Dip the mouth of the bottle so it's just below the water surface. Fill the bottle at least ½ full, then discard the contents downstream. Repeat the fill-and-rinse two more times so that the bottle has been rinsed a total of three times. Rinse the cap thoroughly in the stream. Fill the bottle so that the level of sample comes to the bottom of the neck of the bottle. This will allow sufficient space for laboratory personnel to acidify the sample once it is received by the lab. Carefully place the cap on the bottle and tighten securely.

7. Place on ice in cooler and hold at 4°C until all samples are collected.

8. Collect the information from the WQ instrument display and record on the data sheet. The following parameters are recorded:

pH (units)
Specific Conductivity (µS/cm)
Dissolved Oxygen (mg/L)
Temperature (°C)
Turbidity (NTU) (*Note: This parameter may be collected with a standalone meter. See "DPW-WPRP-CBMP-FO-004: Use of Water Quality Instrumentation" for details*.)

Shipping Procedure:

1. After water quality samples have been collected (please see the Water Quality Sample Collection and Processing SOP DPW-WPRP-CBMP-FO-005 for more detail), each sample is recorded under the "Site ID" column along with its corresponding date (YYMMDD) and time (0000) on the Appalachian Lab chain-of-custody form. The total number of 1L and 125 mL bottles in the cooler will be recorded in the "Cooler Contents" block on the chain-of-custody form.
2. Place one large trash bag inside of a second large trash bag and place both inside a clean, dry cooler. The water quality sample bottles will be placed inside the doubled trash bag inside of the cooler. After all sample bottles are placed in the double bag a sufficient amount of ice will be

added to the doubled trash bag and on top of the sample bottles to keep the samples at or below 4°C during shipping.

3. The interior most trash bag will be gathered up at the top and excess air will be removed by gently squeezing the top of the bag. Once as much excess air is removed as is practicable, the top of the bag will be twisted several times to seal the bag. The twisted top will be closed with clear packing tap to ensure that melted ice water cannot escape the bag.
4. The above sealing procedure will be followed for the second bag.
5. Any excess space in the cooler will be filled with bubble wrap or other suitable packing material. DO NOT use biodegradable packing peanuts, condensation within the cooler can cause these to disintegrate during shipping. Enough packing material should be used so that the contents of the cooler do not shift around when the lid of the cooler is closed.
6. KCI or CRI field personnel will sign next to “Cooler relinquished by” and enter the date (YYMMDD).
7. Place the Appalachian Lab COC in a plastic resealable bag, seal top, and place on top of the sealed garbage bag.
8. Close lid of cooler and seal with clear packing tape using two vertical bands of tape, one close to each end. One horizontal band of tape will cover the seam between the lid and the body of the cooler. Each band of tape should encircle the cooler at least three (3) times.
9. A shipping form provided by the chosen shipping carrier will be filled out with KCI/CRI’s office address in the “from” area, and UMCES Appalachian Lab address in the “to” area.
10. Choose the appropriate shipping type (e.g. overnight, overnight express) to ensure the cooler arrives at Appalachian Lab the next morning. Guidance from the shipping carrier may be needed to select the appropriate shipping type.
11. Drop off cooler at the nearest shipping location. Call the Appalachian Lab manager (Katie Kline 310-689-7122) to notify her of the number of samples which will arrive the next day.
12. Once the samples are delivered to Appalachian Lab, Appalachian Lab laboratory personnel will sign next to “Cooler received by” and record the date (DD/MM/YY) and time (0000) the samples were delivered by the shipping carrier. A copy of the signed form should then be scanned or photocopied and a copy retained for KCI’s records.
13. Appalachian laboratory personnel will inspect each sample container for damage, leakage, or any other problem and will note problems in the “Lab Comments” block 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. The Task Leader must ensure that sample collection procedures are being followed and that all data labels have been filled out completely and correctly.

2. At least one field blank should be submitted during the sampling season. Field duplicate samples should be submitted for 10% of the sites. To the extent feasible, these WQ duplicates should be collected at the QA duplicate sample reaches (*see "SOP-WPRP-CBMP-FO-001: Selecting a QC Site for Duplicate Sampling" for details on establishing these sties)*.

3. Ensure that all appropriate internal QA/QC procedures are being performed by the contract laboratory to ensure data quality.

Table 1. List of parameters for analysis.

Parameter	Maximum Reporting Limit*
Turbidity	1 NTU
Total Nitrogen	0.2
Total Phosphorus	0.05
Ammonia-N	0.2
TKN (calculated)	0.2
Nitrate-Nitrogen	0.05
Nitrite-Nitrogen	0.05
Dissolved Organic Carbon	0.5
Orthophosphate	0.05
Total Organic Carbon	1.0
Total Copper	0.004
Total Lead	0.002
Total Zinc	0.03
Chloride	0.02
Total Hardness	N/A

*All values in mg/L, except as noted.

4. A second member of the field crew will check the chain-of-custody form to ensure completeness and correctness prior to sealing the cooler.

5. A second member of the laboratory staff will check log-in records to ensure completeness and correctness.

APPENDIX J

DPW-WPRP-CBMP-FO-006:
AQUATIC MACROINVERTEBRATE SAMPLING IN NON-TIDAL FRESHWATER STREAMS

Standard Operating Procedure DPW-WPRP-CBMP-FO-006

Aquatic Macroinvertebrate Sampling in Non-tidal Freshwater Streams

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 the aquatic macroinvertebrate sampling must have successfully completed MBSS Spring Training and at least one crew member must have earned MBSS certification. Additionally, one of the field crew members must be designated the Field Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

References:

DNR. 2017. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2017. Publication # 12Resource Assessment Service-3142014-700. 78 pp.

Precautions:
1. The sampling net and sieve bucket should be inspected prior to use to ensure they are in good working order. Any holes in the netting or gaps around the net's frame must be repaired prior to sampling. The sieve bucket should have no holes in its mesh and be in sound condition.

2. Any instream water quality measurements or water quality sample collection should occur prior to sampling. See SOPs "DPW-WPRP-CBMP-FO-004 Use of Water Quality Instrumentation" and "DPW-WPRP-CBMP-FO-005 Water Quality Sample Collection and Processing" for details.

Equipment/Materials:
Standard aquatic dip net (D-Frame) with 500-540 µm mesh
Sieve bucket with 500-540 µm mesh
2 wash buckets
Ethanol (95%)

Sample containers and lids (allow at least 2 containers per sample to be collected)
Waders
Biological Assessment Forms or Laptop Computer with digital forms
Internal/External bucket labels
Clear packing tape
Pencils
First Aid Kit
300 ft. tape

Procedure:
1. It is assumed that the site has been located, determined to be sampleable, and established before beginning this procedure. See the SOPs "DPW-WPRP-CBMP-FO-002: Establishing and Marking a Random Site" and "DPW-WPRP-CBMP-FO-003: Use of GPS".

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.).

Riffles – Generally, these areas are gravel or sand/gravel areas between two meander bends or are found in steeper sections in straight channels where meanders might be lacking. The sampling procedure is the same regardless of location: Place the net firmly in the substrate. Forcefully disturb (to a depth of 5 to 8 cm) a one-square-foot patch of substrate on the upstream side of the net, allowing any organisms to flow into the net. For sand-dominated riffles, use less force to minimize the flow of sand into the D-net. Use the net to perform a final sweep through water column above the disturbed area to collect any organisms suspended in the water immediately following substrate agitation. Any large rocks or sticks should be rubbed by hand within the net and set aside to dislodge any organisms clinging to the object.

Root Wads/Root Mats/Woody Debris/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. Use the net to sweep through the disturbed area to collect any organisms suspended in the water immediately following the agitation/rubbing of the substrate.

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.

Submerged macrophytes – 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.

Undercut Banks. These are cohesive soil banks without obvious or protruding rooting material, which may or may not be present in the bank. To sample, gently rub one square foot of the bank with your hand or the net. Alternatively, the D-net can be bumped along the substrate. Immediately following the end of agitating the materials, use the net to sweep through water column above the disturbed area to collect any organisms suspended in the water. For clay banks, it is acceptable to scrap 0.5 to 1 cm of material into the net as it will wash through the mesh. Avoid this approach on sandy stream banks and use the approach outlined for sand dominated riffles.

Miscellaneous Sediment /Material Types in Runs – These might include broken peat, sand, clay lumps, or detritus. To sample, place the net downstream and gently agitate the substrate as described for a gravel or sand riffle area. Use the net to sweep through the disturbed area to collect any organisms suspended in the water. Take care to not capture too much sediment when sampling these materials.

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).

6. Once the sample has been cleaned, transfer it from the sieve bucket to sample containers. Sample containers should be no more than half full with material. 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). Clear tape is placed over the label to prevent smearing or damage during storage or transport. An additional label (also in pencil) is 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 to twice the material depth (i.e. - in a half full bucket, fill to the top with ethanol).

7. Complete the appropriate field data sheets. Be sure to note the habitats sampled and proportions sampled. Data sheets should be filled out with input from all field team members.

Pertinent QA and QC Procedures:

1. Field sampling QC involves collecting a replicate sample at 10% 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. See the SOP "DPW-WPRP-CBMP-FO-001: Selecting a QC Site for Duplicate Sampling" for details on selecting these sites. The replicate sample is collected in the same manner as the initial sample.

2. The Task Leader must check all data sheets or data forms to ensure that all areas have been filled out completely.

Attachment

Spring Index Period Data Sheet

Placeholder for Consultant-specific data sheet

APPENDIX K

DPW-WPRP-CBMP-AO-007:
BENTHIC SAMPLE CHAIN-OF-CUSTODY COMPLETION

Standard Operating Procedure DPW-WPRP-CBMP-AO-007

Benthic Sample Chain-of-Custody Completion

Prepared by:	Name: Colin Hill, KCI	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 and/or Coastal Resources, Inc. (CRI) to EcoAnalysts, Inc. for processing and received by laboratory personnel.

Responsibility and Personnel Qualifications:
All Contractor 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.

Precautions:
Site labels must be verified and checked against the chain-of-custody form before shipping.

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 DPW-WPRP-CBMP-AO-014 for more detail), KCI/CRI laboratory personnel will then document benthic samples before they are delivered to the EcoAnalysts laboratory for sorting and identification.
2. KCI or CRI office information is filled in on the 'Company' line. Beneath that, the total number of samples for the project should be entered, as well as the number of samples shipped per shipment.
3. For each sample, record the number of sample containers, stream (if known), site ID number, replicate (if applicable) device type [D-net], habitat [multi], collection date, and any relevant

notes.

4. KCI/CRI laboratory personnel will sign under “Relinquished by” and record the date (DD/MM/YY) and time (0000) the samples are relinquished from the laboratory.
5. The EcoAnalysts COC will then be placed in a plastic resealable bag and will be delivered with the benthic samples to the EcoAnalysts laboratory.
6. Once the samples are delivered to EcoAnalysts, EcoAnalysts laboratory personnel will sign under “Received by” and record the date (DD/MM/YY) and time (0000) the samples were delivered by KCI/CRI. A copy of the signed form should then be photocopied and a copy retained for KCI’s records.
7. EcoAnalysts 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 the laboratory staff will check all chain-of-custody forms to ensure completeness and correctness.

APPENDIX L

DPW-WPRP-CBMP-FO-008: FISH SAMPLING IN NON-TIDAL FRESHWATER STREAMS

Standard Operating Procedure DPW-WPRP-CBMP-FO-008

Fish Sampling in Non-tidal Freshwater Streams

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This procedure is for use performing quantitative fish sampling in freshwater streams using Maryland Biological Stream Survey (MBSS) methods.

Responsibility and Personnel Qualifications:
Individuals performing fish sampling must have successfully completed MBSS Summer Training and at least one crew member must have earned MBSS Fish Crew Leader and/or Fish Taxonomy Certification. Additionally, one of the field crew members must be designated the Field Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

References:

DNR. 2017. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2017. Publication # 12Resource Assessment Service-3142014-700. 78 pp.

Precautions:
1. The block nets should be inspected before the start of the field season and at the end of each sampling day to ensure there are no holes or tears in them. Any holes or tears must be repaired prior to sampling. The electrofishing units should be in good working order and tested prior to the field season; if any problems are detected, the unit should be returned to the manufacturer for repair or maintenance.

2. Any instream water quality measurements or water quality sample collection should be performed before sampling begins. See SOPs “DPW-WPRP-CBMP-FO-004 Use of Water Quality Instrumentation” and “DPW-WPRP-CBMP-FO-005 Water Quality Sample Collection and Processing” for details.

3. Per state regulations, felt-sole waders are not permitted for use in Maryland streams and rivers due to concerns regarding invasive species and pathogenic organism transfers. Only rubber sole waters are permitted.

Equipment/Materials:
Digital camera (additional memory cards or batteries as needed)
Sample jars
Pre-printed voucher labels
Taxonomic keys
Voucher lists
White tray for voucher photo backgrounds (optional)
Preservative (10% buffered formalin for field, 70% ethanol for long-term storage)
Clip board
Pencils
Biological Assessment Forms or Laptop Computer with digital forms
300 ft. tape
Permanent marker
Flagging
Digital scale
Machete and/or pruning tools
Work gloves
Backpack electrofishing units
Anode ring probes, fitting with 3/16" mesh
Electrofishing batteries
Polarized sunglasses (amber lenses for overcast conditions and green/brown/gray lenses for direct sunlight)
Spare netting
Cable ties
String for emergency net repairs
25 liter buckets
Dip nets3/16" mesh
Blocknets 3/16" mesh
Tent stakes
Live cars
Measuring boards
Aquarium nets
Virkon solution
Tubs for decontamination (large and deep enough to step in) and dip equipment
Tub of rinse water
Sprayer for Virkon solution
Insulated chest waders and Vibram-soled (or similar material) wading boots First Aid Kit

Procedure:

1. It is assumed that the site has been located, determined to be sampleable, and established before beginning this procedure. If recent rains have made the stream turbid, sampling should be re-scheduled for a later date. See the SOPs "DPW-WPRP-CBMP-FO-002 Establishing and Marking a Random Site".

2. In travelling to the site, care should be taken not to enter the stream segment or the areas up or downstream of the site to avoid 1) causing the movement of fish into or out of the sampling segment, and 2) stirring up bottom sediments, which may lead to turbidity and decrease visibility in the sampling segment.

3. After arrival at the site, blocknets should be deployed at both ends of the segment. The lead-lined bottom of the nets should be secured with rocks on the upstream side of the net. If no rocks are available, tent stakes may be used at intervals along the bottom. Care should be taken to secure the net so that the channel is completely blocked off, including areas beneath undercut banks. The top of the net should be secured well above the water surface (as material collects in the net and it is bowed out, the top of the net will move closer to the water surface, so it must initially be secured high enough that even after the bowing, it will not dip below the water surface). Long sticks are useful for propping and securing the center areas of the net.

If there is vegetation (multiflora rose, vines, etc.) is hanging over the stream channel and impeding passage or visibility, it should be cleared after block net deployment and the trimmed material removed from the segment using the machete, pruning tools, and work gloves. (Note you may need to acquire permission from the landowner, depending on the location of the site and the nature/extent of the clearing required).

If there are any tributaries entering the segment, blocknets should deployed at their confluence points with the sampling segment. If an unsampleable culvert is in the segment, the up and downstream ends of the culver should be secured with blocknets.

4. The number of anodes required for the site was determined for each site in the spring (generally anode spacing is one anode for every 1-3 meters of stream - depth or high flow velocities may require a closer spacing of the anodes). The appropriate number of electrofishing units should be set up with fresh batteries.

5. A live car should be left at the midpoint of the site.

6. The electrofishing units should be tested downstream of the 0-meter block net. Adjustments should be made to the unit voltage and settings until the unit set to the lowest voltage that allows for effective stunning of the fish (newer models may self-adjust). After testing and adjustments are complete, the times on the units should be set to 0. Buckets should be filled at least 2/3 full with stream water (preferably from an aerated section of the stream. Otherwise, two buckets may be used to pour water back and forth between them to provide additional oxygen to the water. Generally, there should be a netter and a bucket for every 1-2 anodes.

7. Crewmembers should wear the appropriate polarized sunglasses for the weather conditions (amber lenses for overcast, blue/green/brown for sunny days.

8. Electrofishing will begin at the 0-meter (downstream) block net. If the set-up activities created turbidity, the crew should wait until the stream has cleared to begin. The crew will move up the

stream, with the anodes sweeping from side to side and maintaining spacing, to the extent possible. Dip netters should keep their nets as flush as possible against the bottom of the stream to capture benthic species or fish that may stun and quickly float downstream with strong currents; fish may be caught that moved too quickly for the netter to see. (Note that is slower streams, crews may need to make sweeping motions with the dip net to prevent fish from drifting back out of the dip nets or being pushed out in eddies. All fish captured should be transferred to the buckets as soon as possible to avoid exposing then to the electric field for longer than necessary. A small aquarium net may be useful for removing small fish from the niche spaces between cobbles. Fish should be transferred from the buckets to the live car at the mid-point of the site (or sooner if there are a large number of fish captured and the buckets become over-crowded – note some sites may be too shallow for deployment of the live car, however, in those cases, generally there are fewer fish caught and the buckets are sufficient.

9. At the end of the first pass the shocker times should be recorded, and the live car transported to a location downstream of the 0-meter block net (preferably an area with enough flow to aerate the live car, but still allowing for stability of the live car during fish processing). Fish must be processed outside of the sampling segment.

10. The downstream block net should be cleared of debris and checked for any fish that may have floated downstream after being stunned. These fish should be added to the live car. Both nets should be cleared of debris and detritus to prevent the nets from blowing out.

11. Rocks, sticks and debris should be removed from the live car. The digital scale should be set up on a level, stable surface and tared. Water should be drained from the fish and they should be quickly placed in the scale bucket (make sure not to place the bucket on the ground after it is used for taring, as it may pick up sand, mud, etc. and add weight). The weight should be recorded and the fish quickly returned to the live car. If the weight of the fish is more than the maximum scale calibration, then the fish should be broken up and weighed in smaller batches and the aggregate biomass for the pass calculated by summing these weights.

12. All fish are identified and counted by the certified fish taxonomist(s). Each count and species are called out by the taxonomist and repeated out loud by the data recorder before adding tally marks to the crib sheet (large counts, for example 50 or 100 blacknose dace may simply be recording using numerals rather than tally marks). Fish may be released after they are positively identified - ensure that they are released outside of the sample reach if processing the first pass. Fish that are needed for photovouchering should be transferred to a separate bucket for photographing after identifications are complete. Gamefish (all bass, striped bass, trout, walleye, pikes, and pickerels [except for redfin] should be measured on the measuring board and their lengths recorded on the Game Fish Length Data Sheet. Eels and lamprey should be kept in a bucket until after the second pass, as they may wiggle through or under blocknets and make their way back into the site. Record any unusual anomalies. Photographs of five specimens of each species should be taken and recorded on the Photo Data Sheet. Voucher lists should be kept to help track of what species and how many of each species have been photovouchered to-date.

13. Fish that are not positively identified should be preserved for later identification by MBSS.

a.) Promptly place specimen into a plastic jar filled with formalin (specimens greater than 160 mm should be slit on the lower abdomen of the right side prior to being placed in the formalin). Add interior and exterior labels that indicate the site name, watershed, date, time, collector, # of specimens, and any other pertinent information. Make notes on the datasheet regarding how many fish were preserved for later identification.

b.) After 5-30 days, remove specimens from formalin and soak in water for 24-48 hours.

c.) Place specimens in 70% ethanol for long-term storage. Make sure that the labeling of all specimens is maintained throughout this process.

14. After fish processing is complete, if the stream is still cloudy and stream clarity is worse than when the crew arrived at the site, the crew should allow up to 1 hour to pass from the time when all crew exited the sampling segment before beginning second pass. If water is not as clear for the second pass, as for the first, this should be noted on the datasheet.

15. Equal effort should be made during the second pass, and all areas of the stream and habitat thoroughly sampled, even if no fish were present during the first pass. The number of anodes and netters should remain the same between the two passes. After second pass, the final shocker times should be recorded. The downstream block net should be checked again, and any fish found in it added to the live car.

16. The process of weighing, identifying, counting, and measuring the second pass fish is the same as the first pass. Care should be taken that all data recorded for the second pass are kept separate from the first pass data.

Pertinent QA and QC Procedures:

1. After fish processing is complete, the person who recorded data for the fish counts should add up the number of each species from each pass and record that number in a circle beside the species name. A second individual should repeat the addition and ensure that his or her count agrees with the data recorder's counts. Both crewmembers should re-count until they have agreement about the correct number. If the biomass for a pass needed to be broken into multiple batches for weighing, the addition of the weights should be checked by a second crewmember. The fish taxonomist should also review the crib sheet to ensure that all species names were accurately recorded (*e.g.* greenside darter rather than green sunfish).

2. After all counts and weights are verified correct, the data should be transferred from the Crib Sheet to the MBSS Fish Data Sheet. The full common name for each species should be recorded, the number captured during each pass, and any anomalies that were observed. Additionally, the aggregate biomass should be recorded for each pass. A second crew member should check that all species names and numbers were transcribed correctly from the Crib Sheet to the Fish Data Sheet.

Attachment

MBSS Fish Data Sheet

MBSS Game Fish Length Data Sheet

Crib Sheet

MBSS Photo Data Sheet

Placeholder for Consultant-specific data sheet

APPENDIX M

DPW-WPRP-CBMP-FO-009: PHYSICAL HABITAT ASSESSMENT OF LOW GRADIENT STREAMS (RBP METHOD)

Standard Operating Procedure DPW-WPRP-CBMP-FO-009

Physical Habitat Assessment of Low Gradient Streams (RBP Method)

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 minimum of two 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 Field Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

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.

Precautions:
1. **The left and right banks are determined facing downstream unless otherwise noted on the assessment form.**
2. Habitat assessments are subjective and will be completed with the input of all field crew members.

Equipment/Materials:
Habitat Assessment Sheets or Field Computer with Electronic Forms (optional)
Waders
300 ft Tape Measure
Pencils

Procedure:

1. The habitat assessment will focus only on areas within or visible from the 75-meter sampling reach unless otherwise stated. The entire assessment reach should be examined before scoring commences.

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.

Epifaunal Substrate/Available Cover. This metric evaluates 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 provide macroinvertebrates and fish with a large number of niches, thus increasing habitat diversity. In low gradient streams, snags/woody debris jams, submerged woody tree roots, and submerged logs are among the most productive habitat structures for macroinvertebrate colonization and fish refugia. It should be noted that features which are relatively new and fresh are scored less favorably than those that have been in place in the system for longer periods.

Pool Substrate Characterization. This metric measures 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. If a biological sampling is being done as part of this assessment, crew members should pay attention to pool substrate quality during the sampling work and use that knowledge to inform the scoring of this metric.

Pool Variability. Consisting of a rating of the overall mixture of pool types found in streams, according to size and depth, the four basic types of pools are:

- large-shallow,
- large-deep,
- small-shallow,
- 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.

Sediment Deposition. This metric assesses the amount and location of sediment—particularly fine sediment—in the assessment reach in two basic ways: 1) by looking at the accumulation in pools and 2) by evaluating the changes that have occurred to extent and newness of depositional features created by the stream in the assessment reach. Deposition occurs from large-scale movement of sediment. Excessive sediment deposition on a point bar may cause the opposite meander to increase in size by bank erosion as the channel is diverted toward the outer bank. Additionally, shoals or side bars may form or the excess sediment may result in the filling of runs and pools. Usually deposition may also be evident in areas that are obstructed by natural or manmade debris. High levels of sediment deposition are symptoms of an unstable and continually changing environment that and such levels are not typically associated with healthy biological communities.

Channel Flow Status. This metric scores 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. The metric is scored by assessing the wetted width of the channel as a percentage of the width of the channel from bottom of bank elevation. A bank to bank flow condition would result in a high score while flow confined to small pools only would result in a low score.

Channel Alteration. This parameter 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.

Channel Sinuosity. This metric 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. Finally, this metric can be directly measured. The assessment reach length (always 75 meters) can be divided by the straight line valley distance from the 0 meter point to the 75 meter point, which will give you the sinuosity value for the assessment reach.

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.

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 can indicate a variety of issues, including floodplain disconnection and excessive energy within the channel, poorly vegetated stream banks, or instability caused by excessive in-channel deposition, all of which can adversely impact aquatic biological communities.

Bank Vegetative Protection. This is a measure of 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. In addition, some possible insight on the potential for riparian area nutrient processing, the control of instream scouring, and the amount of stream shading is also realized. Banks that have full, natural plant growth provide better habitat for fish and macroinvertebrates than banks without vegetative protection or than 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.

Riparian Vegetative Zone Width. This is a measure of 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.

3. Each metric should be evaluated and discussed by the crew and a consensus score reached and recorded in the appropriate location on the data sheet. If the crew is unable to reach consensus, then the Task Leader has the responsibility to make the final call on a metric score.

Pertinent QA and QC Procedures:

1. The Task Leader must check all data sheets or data forms to ensure that all areas have been filled out completely.

2. Digital field forms will be 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.

4. Only team members who have been trained by experienced field personnel in conducting RBP habitat assessments for low gradient streams should perform this method.

5. To minimize inter-crew variability, all crews members assigned to perform this work will attend a mandatory field meeting (preferably at a previously assessed site) to calibrate metric scoring among crew members. This calibration meeting will occur before any Program sites are visited.

Attachment

Stream Survey Form

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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 N

DPW-WPRP-CBMP-FO-010:
PHYSICAL HABITAT ASSESSMENT
(MPHI METHOD)

Standard Operating Procedure DPW-WPRP-CBMP-FO-010

Physical Habitat Assessment (MPHI Method)

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This procedure is for use in scoring the metrics that comprise the Maryland Physical Habitat Index (MPHI), the stream physical habitat assessment method performed by the Maryland Biological Stream Survey (MBSS).

Responsibility and Personnel Qualifications:
Individuals performing the MPHI must have successfully completed MBSS Spring Training and at least one crew member must have earned MBSS certification. Additionally, one of the field crew members must be designated the Field Sampling Task Leader (Task Leader), whose roles and responsibilities are described in the QA/QC discussion below.

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.

DNR. 2017. Maryland Biological Stream Survey: Round Four Field Sampling Manual Field Protocols. Originally published January, 2014. Revised January 2017. Publication # 12 Resource Assessment Service-3142014-700. 78 pp.

Precautions:
1. Typically, the MPHI is performed 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). However, all parameters are scored in the Spring Index period if no visit is planned for the Summer Index period. If only fish assessment work is performed, then all metrics are scored in the Summer Index period. Finally, if fish and benthic macroinvertebrates are assessed, the method is scored in both index periods.

2. **The left and right banks are determined facing upstream unless otherwise noted on the assessment form.**

3. Habitat assessments are subjective and will be completed with the input of all field crew members.

Equipment/Materials:
Habitat Assessment Sheets or Field Computer with Electronic Forms (optional)
Waders
100 m (300 ft.) tape measure
Pencils

Procedure:
The habitat assessment will focus only on areas within or visible from the 75-meter sampling reach unless otherwise stated.

2. Complete the general information on the Physical Habitat Assessment (Attachment) data sheet (site ID, subwatershed/project name, length of reach, date, time, team members and pertinent weather conditions) or complete electronic form. 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.

3. The stream character portion of the assessment should be filled out using “A” (absent), “P” (present) or “E” (extensive) for each of the 25 parameters listed under stream character.
Bar formation, bank erosion and benthic macroinvertebrate habitat sampled are also noted.

4. 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 included in the MPHI calculation.

5. Complete the Stream Habitat Assessment using the guidance provided on the sheet next to each parameter and on the supplemental guidance sheet, all found in DNR (2016), or complete electronic form. Most parameters are scored on a scale of 1 -20 with the exception of embeddedness and shading, which are scored as a percentage.

Pertinent QA and QC Procedures:

1. The Task Leader must check all data sheets or data forms to ensure that all areas have been filled out completely.

2. Digital field forms will be programmed to have a range between zero (0) and 2, or 0 – 100% for percentage measures, 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.

4. Only team members who have been trained by experienced field personnel in conducting MPHI habitat assessments should perform this method.

5. To minimize inter-crew variability, all crews members assigned to perform this work will attend a mandatory field meeting (preferably at a previously assessed site) to calibrate metric scoring among crew members. This calibration meeting will occur before any Program sites are visited.

Attachment

Physical Habitat Data Sheet

Placeholder for Consultant-specific data sheet

APPENDIX O

DPW-WPRP-CBMP-FO-011: STREAM CROSS SECTION MEASUREMENT

Standard Operating Procedure DPW-WPRP-CBMP-FO-011

Stream Cross Section Measurement

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
This method is to be used to measure the cross section established in a Countywide Biological Monitoring Program (Program) assessment reach. The resulting data are intended primarily for use in the application of the Rosgen stream classification method. This work should not begin until the 75 meter assessment reach has been established following procedures detailed in DPW-WPRP-CBMP-FO-001: Establishing and Marking a Random Site.

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 Sampling Task Leader (Task Leader), whose roles and responsibilities are described below:

- Provides oversight of daily operations of the field crew,
- Checks the measurement data 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 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.

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:

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 metric or English units, but should be reported in English units.

Equipment/Materials:

Stream Survey Data Form or Reference Reach Spreadsheet and Field Computer (optional)
Reference Reach Spreadsheet (optional)
Field Logbook or Field Computer (optional)
Pencils
Laser level
Top-setting survey rod (25-foot or 4 meter)
Survey pins
2-pound sledgehammer
Survey caps
½” diameter iron rebar (2-4 ft length)
Camera
Flagging
Hammer and aluminum nails
GPS Unit
100 meter or 300 foot measuring tape
20 meter or 50 foot measuring tape
Bright-colored (Day-Glo) spray paint
Compass
Aluminum forestry tags

Procedure:

1. Within the assessment reach, determine a representative section for the cross-sectional measurements. Use 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 meander bends in a relatively straight reach. DO NOT install the section on an outside meander bend. 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 anastomosed 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.

- An attempt should be made to install the cross section at the midpoint of the assessment reach, but if that location is unsuitable, move to the closet suitable location instead.

Additionally, the section should be located in an area free from direct anthropogenic alteration 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. See Rosgen (1994) for additional guidance.

Should the assessment reach be greater than 80% impacted by such anthropogenic or natural alterations (e.g.—the reach is greater than 80% wetland without a defined channel or is 80% beaver impoundment), then this assessment should not be performed. Note the reason(s) for not completing the assessment on the Stream Survey Data Form.

2. Complete the top two lines of the Stream Survey Data Form (attached) or its digital equilivent. Please use the abbreviations provided or provide explanations of the abbreviations used if different, as necessary.

3. 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 at least 3 inches (0.25 feet) above the ground surface.

Loosely attach a tape measure to the left monument 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 tape. Then install the right bank monument. Record the station along the sampling reach where the cross section tape crosses the assessment reach tape.

Spray paint the exposed end of each monument with a bright color (e.g., hunter orange) or wrap with flagging. Finish the installation by topping the rebar monuments with survey caps. **These caps must be installed before any survey data are collected.**

4. Record GPS location of each monument.

5. 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.). From the benchmark location, note the compass direction and/or distance to the most convenient monument on the Stream Survey Data Form. If the benchmark is a tree, label and affix an aluminum forestry tag to the tree using a nail.

6. After installing zero on the tape measure at the left bank monument, begin to collect survey data. For each survey point, you are collecting distance from the left monument and a relative elevation. Additionally, a notation of some sort should be made about each measurement (e.g.—SOG for "shot on ground", SIS for "shot in stream", or whatever feature you are calling at the location); blanks in the note field are not allowed. Data should be collected in feet in tenths or converted to these units in the office. 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.

7. Record all measurements on the Stream Survey Data Form.

8. Collect photographs of the left bank, right bank, downstream channel, and upstream channel at this location. The left and right bank photos must be taken perpendicular to the bank and capture the monument location and surrounding land use/forest cover. Bank photos that show overall bank conditions can be collected, but will not substitute for these direct photos of the banks at the cross section. All of these photos are in addition to any photographs called for in other SOPs to characterize the assessment reach.

Q: These stream reaches are in really poor condition and I'm finding it difficult to select bankfull indicators. Any advice?

A: When you have entrenched channels in disturbed landscapes, deciding on a bankfull indicator can be challenging. Regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to validate the dimensions associated with features of interest. In particular, McCandless (2003) has an excellent description of what typical bankfull indicators look like in local Coastal Plain streams. As drainage area is typically know ahead of time before this SOP is implemented, running that value through the appropriate regional equations can provide guidance you can use to locate the right feature. DO NOT, however, use these values to arbitrarily assign the location of the indicator in the cross section. Morphological evidence should be observed that validates your call.

In very difficult cases, multiple calls for bankfull can be made on the Stream Survey Data Form and a final selection can be made in the office based upon modeled discharge, relevant literature, or consultation with more experienced colleagues. Finally, in very disturbed or altered channels, no call might be the appropriate call.

Pertinent QA and QC Procedures:

Task Leader must make sure that all the measurements have been recorded in the field and checks the measurement data as after they are recorded on the Stream Survey Data Forms (Attachment) or in Reference Reach Spreadsheet. The following field measurements must be taken or attempted:

• All features as described above in Item 7 or an explanation why a particular measurement could not be collected.

2. Ensures that all survey equipment is properly calibrated and is in good working order.

3. Ensures that proper photographs representing the conditions at the cross section have been collected.

Attachment

Stream Survey Form

Stream Survey Data Form

Watershed Name:_________________ Stream/Reach ID:__________________
Drainage Area:_____mi^2/acres/ha
Observers:________________________ Date/Time:______/______ Lat: ________________
GPS [ ]Y [ ] N Differential Correction? [ ]Y [ ]N Positional Error:_____ft. Long:________________
Measurement Type: [ ] Cross Section [ ] Longitudinal Profile
Location:____
____
____

Station/Distance (ft)	Elevation/Depth (ft)	Remarks	Station/Distance (ft)	Elevation/Depth (ft)	Remarks

ABBREVIATIONS:

SOG=Shot on Ground
LB=Left Bank
RB=Right Bank
LTOB=Left Top of Bank
RPIN=Right Monument
BM=Benchmark
HI=Height of Instrument
TOP=Top of Pool

SIS=Shot in Stream
RTOB=Right Top of Bank
RBF=Right Bankfull
LBF=Left Bankfull
LPIN=Left Monument
FS=Fore Sight
TOR=Top of Riffle
BOP=Bottom of Pool

REOW=Right Edge of Water
LEOW=Left Edge of Water
TH=Thalweg
BPIN=Bank Pin
BS=Back Sight
BOR=Bottom of Riffle
WDJ=Woody Debris Jam

APPENDIX P

DPW-WPRP-CBMP-FO-012:
ABBREVIATED LONGITUDINAL PROFILE MEASUREMENT

Standard Operating Procedure

DPW-WPRP-CBMP-FO-012

Abbreviated Longitudinal Profile Measurement

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 the assessment reach. This tasks outlined in this SOP are performed only after the assessment reach is selected and the cross section has been located and installed. See the SOPs describing these procedures for more information.

Responsibility and Personnel Qualifications:
Any person who has received training in performing stream longitudinal profile measurements may use this procedure. To ensure high quality data collection, the Field Sampling Task Leader (Task Leader) is responsible for the following:

- Provides oversight of daily operations of the field crew,
- Checks the measurement data 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 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.

Precautions:
1. Ensure that the 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 metric or English units, but should be reported in English units.

4. 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 survey to this longitudinal profile.

Equipment/Materials:
Stream Survey Data Form or Reference Reach Spreadsheet and Field Computer (optional)
Field Logbook or Field Computer
Pencils
Laser Level and Target or other Survey Instrument approved by the County QC Officer
Top-setting survey rod (25-foot or 4 meter)
100 meter or 300 foot measuring tape

Procedure:
1. Complete the top two lines of the Stream Survey Data Form or its digital equalivent.

2. Set up the survey instrument, if possible, so that the entire assessment reach is available for survey work without having to move the instrument. Preferably, this work will be done using the same instrument set up as used for the cross section survey.

2. Working from the downstream end of the assessment reach, distance and elevation measurements are taken as close as possible to the end and beginning of the sampling reach (0 and 75 meters) and recorded on the Stream Survey Data Form. At a minimum, the following features are surveyed:

- Top of Bank
- Right or Left Bankfull
- Water Surface Elevation
- Thalweg
- If you moved the instrument from its location during the cross section survey, take an elevation measurement from at least one monument. This will allow for an easy tie in of needed values from the cross section work to this survey.

Coupled with the measurements taken during the cross section survey, you will have at least three points for any given reach with which to characterize its slope.

Water surface slope should be collected on the same type of stream feature at each end of the reach (e.g.—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.

3. Record all measurements on the Stream Survey Data Form or its digital equilivent.

Pertinent QA and QC Procedures:

1. Before the survey instrument is moved and put away, the Task Leader must make sure that all the measurements have been recorded in the field and that the elevation data make sense when compared with visual observations of the reach's slope. If the slope seems higher or lower than expected, new measurements should be taken before disturbing the survey equipment and used to verify or correct the original set of measurements. This data verification should be noted in the comments section of the datasheet. The following field measurements must be taken:

• All features as described above in Item 2.

2. The Task Leader 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.

Q: These stream reaches are in really poor condition and I'm finding it difficult to select bankfull indicators. Any advice?

A: When you have entrenched channels in disturbed landscapes, deciding on a bankfull indicator can be challenging. Regional relationships that relate drainage area to bankfull channel dimensions developed for the mid-Atlantic can be used to validate the dimensions associated with features of interest. In particular, McCandless (2003) has an excellent description of what typical bankfull indicators look like in local Coastal Plain streams. As drainage area is typically know ahead of time before this SOP is implemented, running that value through the appropriate regional equations can provide guidance you can use to locate the right feature. DO NOT, however, use these values to arbitrarily assign the location of the indicator in the cross section. Morphological evidence should be observed that validates your call.

In very difficult cases, multiple calls for bankfull can be made on the Stream Survey Data Form and a final selection can be made in the office based upon modeled discharge, relevant literature, or consultation with more experienced colleagues. Finally, in very disturbed or altered channels, no call might be the appropriate call.

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Attachment

Stream Survey Data Form

Watershed Name:_________________ Stream/Reach ID:__________________
Drainage Area:_____mi^2/acres/ha
Observers:_______________________ Date/Time:______/______ Lat: _______________
GPS [ ]Y [ ] N Differential Correction? [ ]Y [ ]N Positional Error:_____ft. Long:_______________
Measurement Type: [ ] Cross Section [ ] Longitudinal Profile
Location:____
____
____

Station/Distance (ft)	Elevation/Depth (ft)	Remarks	Station/Distance (ft)	Elevation/Depth (ft)	Remarks

ABBREVIATIONS:

SOG=Shot on Ground
LB=Left Bank
RB=Right Bank
LTOB=Left Top of Bank
RPIN=Right Monument
BM=Benchmark
HI=Height of Instrument
TOP=Top of Pool

SIS=Shot in Stream
RTOB=Right Top of Bank
RBF=Right Bankfull
LBF=Left Bankfull
LPIN=Left Monument
FS=Fore Sight
TOR=Top of Riffle
BOP=Bottom of Pool

REOW=Right Edge of Water
LEOW=Left Edge of Water
TH=Thalweg
BPIN=Bank Pin
BS=Back Sight
BOR=Bottom of Riffle
WDJ=Woody Debris Jam

APPENDIX Q

DPW-WPRP-CBMP-FO-013: MODIFIED WOLMAN PEBBLE COUNT

Standard Operating Procedure DPW-WPRP-CBMP-FO-013

Modified Wolman Pebble Count

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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. Properly applied, this method results in a particle size characterization that is collected proportionally to the distribution of particular streams bed features (e.g.--riffles, pools, runs, glides) found in the assessment reach.

Responsibility and Personnel Qualifications:
This procedure may be used by any person who has received training in modified Wolman pebble count procedures, either as part of Rosgen Level II training or as standalone training. One of the field crew members must take on the role of Field Sampling Task Leader (Task Leader). The roles and responsibilities of the Task Leader are described below.

- Provides oversight of daily operations of the field crew,
- Checks the measurement data recorded on the Data Forms (Attachment).
- Verifies the completeness of every Data Form to ensure that these forms are filled out as accurately and completely as possible.

References:
Rosgen, D.L. 1994. Applied River Morphology. Published by Wildland Hydrology, Pagosa Springs, CO.

Precautions:
1. To eliminate bias to the extent possible, be sure to not look down when selecting a particular particle for evaluation.

Equipment/Materials:
Pebble Count Field Data Sheet or Reference Reach Spreadsheet and Field Computer (optional)
Pencils

Gravelometer (optional)
Caliper or Ruler with Metric gradations
Sand Card or other fine material gage

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, 3 in runs, and 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 either measured to the nearest millimeter along the intermediate axis using a metric ruler, caliper, or gravelometer. Sand is classified into categories based on grit as determined using a sand card or other comparator. 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 visual survey of the reach indicates that the bottom is comprised entirely of fine (< 2 mm) materials, a minimum of three transects should be performed at locations that are representative of the entire reach to determine the percentage of fine material types present. These percentages can then be applied to the entire survey reach without having to sample all 100 particles. However, if any materials larger than sand are observed in the reach (no matter how minor a percentage such materials compose of the bottom), then a full pebble count must be performed.

5. Record all measurements on the Pebble Count Field Data Sheet or in the Pebble Count tab of the Reference Reach Spreadsheet.

Pertinent QA and QC Procedures:
The Task Leader will perform the following activities:

1. The Task Leader must make sure that 100 particles have been measured in the field.

2. The Task Leader 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.

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Attachment

Pebble Count Data Sheet

PEBBLE COUNT FIELD DATA SHEET

STATION ID ____________________________________ DATE/TIME _________________________________

MAJOR WATERSHED______________________ SUBWATERSHED _____________________ PROJECT NAME ________________________

LAT _________________ LONG ___________________INVESTIGATORS __________________________________

		Particles									
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 - Very Fine = VF
Sand – Fine = F
Sand – Medium = M
Sand – Coarse = C
Sand - Very Coarse = VC
Bedrock = B

Feature Types:
Riffle
Run
Glide
Pool

APPENDIX R

DPW-WPRP-CBMP-AO-014:
BENTHIC SAMPLE LOG-IN PROCEDURES

Standard Operating Procedure

DPW-WPRP-CBMP-FO-014

Benthic Sample Log-in Procedures

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

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 and/or Coastal Resources, Inc.

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.

Precautions:
None

Equipment/Materials:
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.
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 DPW-WPRP-CMBP-FO-006 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 home laboratory with the samples.
7. Once samples are delivered to the laboratory by the field crew, 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 the laboratory staff will check log-in records to ensure completeness and correctness.

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APPENDIX S

DPW-WPRP-CBMP-FO-015:
LABORATORY ANALYSIS: BENTHIC MACROINVERTEBRATE INDICATOR

STANDARD OPERATING PROCEDURES

for

Laboratory Analysis: Benthic Macroinvertebrate Indicator

Prepared by

EcoAnalysts, Inc.
LIFE IN WATER

1420 South Blaine Street, Suite 14
Moscow, Idaho 83843

March 2017

A1. TITLE AND APPROVAL SHEET

Document Title:
Quality Assurance Project Plan for Laboratory Analysis: Benthic Macroinvertebrate Indicator

Preparer:
EcoAnalysts, Inc., Moscow, Idaho

Address and Telephone Number:
1420 South Blaine Street, Suite 14, Moscow, Idaho 8343/ (208) 882-2588

Day/Month/Year
07/March/2017

EcoAnalysts, Inc. President/CEO, Project Manager: ____________________
Gary T. Lester / 07 March 2017

EcoAnalysts, Inc. Quality Assurance Manager: ____________________
Robert Bobier / 07 March 2017

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Table 1. Acronyms and Abbreviations

BMI	Benthic Macroinvertebrate
CEO	Chief Executive Officer
EPA	United States Environmental Protection Agency
DQO	Data Quality Objective
EcoAnalysts	EcoAnalysts, Inc.
LIMS	Laboratory Information Management System
MQO	Measurement Quality Objective
QA	Quality Assurance
QAM	Quality Assurance Manager
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
SOP	Standard Operating Procedure
US EPA	United States Environmental Protection Agency

DOCUMENT CONTROL

This document has been prepared according to the United States Environmental Protection Agency publication, *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R5, March 2001). This QAPP will be reviewed annually and updated as needed. Updated versions of this QAPP will bear a new (x + 1) revision number.

GROUP A: PROJECT MANAGEMENT

A3. DISTRIBUTION LIST

Each person listed on the Approval Signature Page and each person listed in Table 2 or his/her successor will receive a copy of the final approved version of this Quality Assurance Project Plan. A copy will also be made available to other persons taking part in the project and to other interested parties.

Table 2. QAPP for Laboratory Analysis: BMI Distribution List

Name	Title/Affiliation	Address	Phone/email
Gary Lester	CEO, Project Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 21 glester@ecoanalysts.com
Robert Bobier	QA Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 34 rbobier@ecoanalysts.com
Bill Lavoie	Freshwater Taxonomy Lead EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 80 pbarrett@ecoanalysts.com
Megan Payne	Lab Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 59 mpayne@ecoanalysts.com

A4. PROJECT/TASK ORGANIZATION

The primary responsibilities of the principals are as follows:

<u>EcoAnalysts Project Manager – Gary Lester, CEO</u>

- Provides overall coordination of the project and makes decisions regarding the proper functioning of all aspects of the project; and

- Makes assignments and delegates authority as needed, to other parts of the project organization.

EcoAnalysts QA Manager – Robert Bobier

- Oversees transfer of samples and related records for the benthic macroinvertebrate indicator;
- Ensures the validity of data for the benthic macroinvertebrate indicator;
- Interacts with EcoAnalysts Project Manager on issues related to sample processing and schedules for conduct of activities;
- Collects copies of all official forms, evaluation checklists and reports;
- Oversees and maintains records of laboratory operations, but is not part of laboratory operations; and
- Directs laboratory audits.

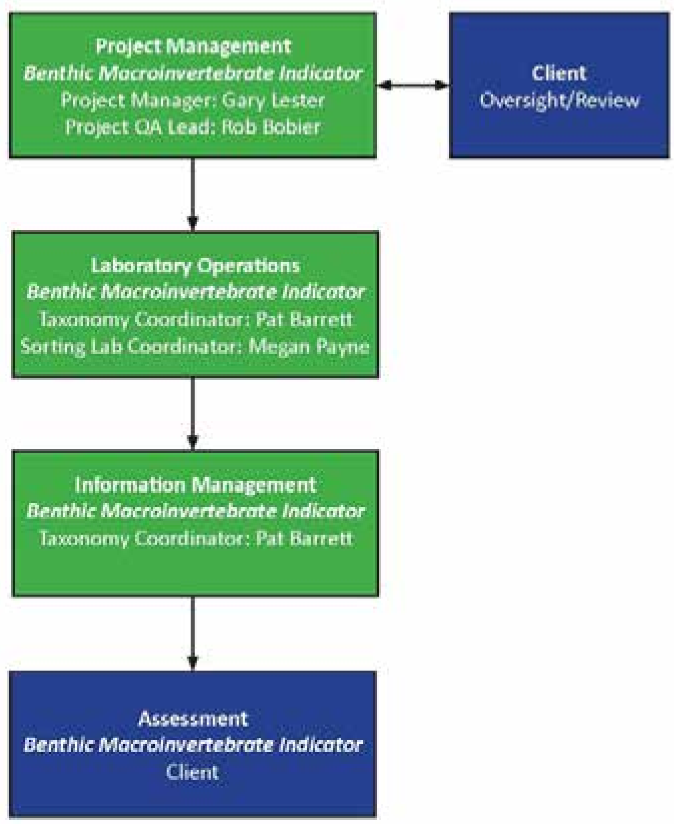
EcoAnalysts Laboratory Managers – Bill Lavoie and Megan Payne

- Oversee analysis of benthic macroinvertebrate samples; and
- Ensure the validity of data for the benthic macroinvertebrate indicator.

Table 3. Principal Contact List

Gary Lester CEO, Project Manager EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 Phone: 208-882-2588 ext. 21 Fax: 208-883-4288 glester@ecoanalysts.com	Robert Bobier QA Manager EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 Phone: 208-882-2588 ext. 34 Fax: 208-883-4288 rbobier@ecoanalysts.com
Bill Lavoie Freshwater Taxonomy Lead EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 Phone: 208-882-2588 ext. 80 Fax: 208-883-4288 pbarrett@ecoanalysts.com	Megan Payne Lab Manager EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 Phone: 208-882-2588 ext. 59 Fax: 208-883-4288 mpayne@ecoanalysts.com

Figure 1. Project Organization



The QA Manager is independent from project staff that generates data. The QA Manager, Robert Bobier, is responsible for managing this QAPP and is available to address project QA/QC problems and concerns.

A5. PROBLEM DEFINITION/BACKGROUND

This QAPP addresses the laboratory operations and analyses for benthic macroinvertebrate indicator samples. This plan describes elements of project management, data quality objectives, measurement and data acquisition, and information management for processing benthic macroinvertebrate samples.

This QAPP covers in scope the processing of benthic macroinvertebrate samples collected from all water body types including, but not limited to coasts and estuaries, wetlands, lakes, rivers, and streams.

A6. PROJECT/TASK DESCRIPTION

EcoAnalysts is well equipped and staffed to conduct highly specialized analyses related to the benthic macroinvertebrate indicator. EcoAnalysts complies with all methods, procedures, and QA/QC requirements as described in required laboratory methods manuals. Prior to initiation of task orders, EcoAnalysts' laboratory operations may be evaluated by EcoAnalysts' QAM.

Benthic macroinvertebrate samples will be sorted and identified at EcoAnalysts' laboratory to the lowest practicable level or level required. The sample will first be sorted into major taxonomic groups, which then will be identified to the required taxonomic level and counted. The sorting laboratory manager and taxonomy coordinator will oversee, and periodically review, the work performed by sorting technicians.

A7. QUALITY OBJECTIVES AND CRITERIA

Performance objectives as associated primarily with measurement error, are established (following USEPA Guidance for Quality Assurance Plans EPA240/R-02/009) for analyzing benthic macroinvertebrate indicator samples. The following sections describe approaches for evaluating benthic macroinvertebrate indicator sample analyses.

A7.1 Sorting Efficacy – Aliquot Method

To ensure each technician is sorting to a standard minimum level of sorting efficacy (90%), Quality Control Technicians will resort 100% of 1 out of every 10 samples from each individual.

The QC technician re-sorts 100% of the sorted fraction of the sample to check if at least 90% of the organisms have been removed. An estimated percent efficacy is calculated by dividing the number of organisms found in the original sort by the total number of organisms in the sorted material, using the following equation:

Equation 1. Sorting Efficacy

$$SortingEfficacy = \frac{OriginalCount}{OriginalCount + QC\ Count} * 100$$

Where:
OriginalCount = the number of organisms picked by the first sorter
QCCount = the number of organisms found in the Quality Control sort

Sorting efficacy is measured as the estimated percent of the total organisms found during the original sorting process. If the estimated percent sorting efficacy is 90% or greater, the sample passes the quality control check, and nine samples from that technician may be passed for QC. If the estimate is less than 90%, then a minimum of 3 other samples from that technician must be QC'd until 3 in a row are passed. In addition to calculating sorting efficacy, the QC technician also verifies label accuracy, information captured on the benchsheet, and the presence/absence of non-target organisms in the taxa vials.

A7.2 Taxonomic Precision and Accuracy

Taxonomic precision is quantified by comparing whole-sample identifications completed by independent taxonomists or laboratories. Accuracy of taxonomy is qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species) and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). To calculate taxonomic precision for benthic macroinvertebrate samples, 10 percent of the samples are randomly selected for re-identification by an independent taxonomist or laboratory. Comparison of the results of whole sample re-identifications provides a Percent Taxonomic Disagreement (PTD) calculated as:

Equation 2. Percent Taxonomic Disagreement (PTD)

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

where
$comp_{pos}$ = the number of agreements
N = the total number of individuals in the larger of the two counts.

The lower the PTD, the more similar taxonomic results are and the overall taxonomic precision is better. A Measurement Quality Objective (MQO) of ≤15% is recommended for taxonomic differences. Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, the reasons for disagreement investigated, and corrective measures taken where needed.

Where re-identification by an independent, outside taxonomist or laboratory is not practical, percent similarity will be calculated between each identifying taxonomist. Percent similarity is a measure of similarity between two communities or two samples (Washington 1984). Values range from 0% for samples with no species in common, to 100% for samples that are identical. It is calculated as follows:

Equation 3. Percent Similarity

$$PSC = 1 - 0.5\sum_{i=1}^{K}|a-b|$$

where:
a and b = for a given species, the relative proportions of the total samples A and B, respectively, which that species represents.

A MQO of ≥85% is recommended for percent similarity of taxonomic identification. If the MQO is not met, the reasons for the discrepancies between analysts should be discussed. If a major discrepancy is found in how the two analysts have been identifying organisms, the last batch of samples counted by the analyst under review may have to be re-identified.

Additionally, percent similarity should be calculated for re-processed subsamples. This provides a quantifiable measure of the precision of subsampling procedures. A MQO of ≥70% is recommended for percent similarity of subsamples. If a sample does not meet this threshold, additional subsamples should be processed from that sample until the MQO is achieved.

Sample enumeration is another component of taxonomic precision. Final specimen counts for samples are dependent on the taxonomist, not the rough counts obtained during the sorting activity. Comparison of counts is quantified by calculation of percent difference in enumeration (PDE), calculated as:

Equation 4. Percent Difference in Enumeration

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

An MQO of ≤5% is recommended. Individual samples exceeding 5% are examined to determine reasons for the exceedance.

A7.3 MQO Evaluation

For samples exceeding these MQOs, corrective actions can include defining the taxa for which re-identification may be necessary (potentially even by a third party), for which samples (even outside of the 10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems.

Taxonomic accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts. Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. Where necessary, the Integrated Taxonomic Information System (ITIS, http://www.itis.usda.gov/) will be used to verify nomenclatural validity and spelling. A reference collection will be compiled as the samples are identified.

A8. SPECIAL TRAINING/CERTIFICATION

Training of EcoAnalysts' project staff, when needed, is done internally through assistance from project operations staff. When appropriate, identifications are verified by taxonomists certified in the applicable area. As verification of EcoAnalysts' taxonomic expertise, Society for Freshwater Science (SFS, formerly NABS) certification information is also available on the Society's Taxonomic Certification Programme website: www.nabstcp.com.

Table 4. Taxonomist's SFS Certifications and Education

Name	Education	SFS Certifications
Chip Barrett	PhD – Zoology MS – Zoology BA – Zoology	Oligochaeta, 2016
Matt Hill	MS – Fishery Science BS - Entomology	EPT East, 2013 EPT West, 2013 General Arthropods East, 2013 General Arthropods West, 2013 Oligochaeta, 2013
Wade Hoiland	MS – Zoology BS – Secondary Education in Biology	EPT East, 2013 EPT West, 2013 General Arthropods East, 2016 General Arthropods West, 2016 Chironomidae East, 2015 Chironomidae West, 2015
William Lavoie	MS - Zoology and Physiology BS - Fish & Wildlife Resources	EPT East, 2013 EPT West, 2013 General Arthropods East, 2013 General Arthropods West, 2013 Chironomidae East, 2013 Chironomidae West, 2013
Anndrea Navesky	MS - Entomology BS - Biology	EPT East, 2013 EPT West, 2013 General Arthropods East, 2013 General Arthropods West, 2013
John Pfeiffer	MS - Entomology BS – Fisheries Resource Management AAS	EPT East, 2012 EPT West, 2012 General Arthropods East, 2012 General Arthropods West, 2012
Gregory Wallace	BS - Wildlife Conservation & Management	EPT East, 2016 Chironomidae East, 2009 Chironomidae West, 2009 Oligochaeta, 2013

A9. DOCUMENTATION AND RECORDS

All versions of the QAPP are retained by EcoAnalysts, Inc. EcoAnalysts retains sorting bench sheets indefinitely. Taxonomic data are entered into EcoAnalysts' custom Laboratory Information Management System (LIMS) by taxonomists during the identification process. Sample data are retained by EcoAnalysts indefinitely following completion of the project.

GROUP B: DATA GENERATION AND ACQUISITION

B1. SAMPLING DESIGN

The protocols for establishing sample and study design associated with different indicators are described in the benthic macroinvertebrate indicator-specific sections of the field QAPP or client field manual.

B2. SAMPLING METHODS

The protocols for the collection of samples associated with different indicators are described in the benthic macroinvertebrate indicator-specific sections of the field QAPP or client field manual.

B3. SAMPLE HANDLING AND CUSTODY

Immediately upon receipt of benthic macroinvertebrate samples, all containers are inspected for damage or leakage. Sample labels are checked against chain of custody forms and/or packing slips and any discrepancies are noted. Receipt records are reported to the client within one business day of sample receipt. Chain of custody logs are reported, throughout the project, according to timelines and methods requested by the client.

Samples are logged into the EcoAnalysts, Inc. custom LIMS database and assigned a unique sample tracking number.

B4. ANALYTICAL METHODS

B4.1 Sorting Benthic Macroinvertebrate Samples

A sample is checked out by a trained sorting technician via the LIMS. A sorting bench sheet is printed that contains all of the sample information and sorting protocols assigned to it. The sorter records the primary matrix type and estimates the volume of detritus in the entire sample prior to rinsing. The standard descriptors for the types of sample matrix are: Inorganic, Coarse Organic, Fine Organic, Vegetation, and Filamentous Algae.

The sample is prepped for subsampling procedures by emptying the matrix into a sieve of a specified mesh size to remove preservative and fine sediment. If the sample matrix is made up of a significant percentage of inorganic material, the organic material will be elutriated from the inorganic material prior to sorting.

For elutriation, the whole sample is washed into a shallow pan of water where any large pieces of organic material are rinsed and inspected thoroughly by another technician for attached invertebrates. The sample is agitated with water to separate any organic matter from inorganic sediments. After agitating the sample in water, the lighter organic material is poured back into the sieve. The inorganic portion of the sample remaining in the pan is repeatedly washed and decanted into the sieve until no more organic matter remains in the pan with the inorganic material.

The remaining inorganic sediments are inspected under a magnifying lamp (3X) to look for any invertebrates too heavy to have been elutriated (e.g. mollusks, snails, stone-cased Trichoptera, etc.). If there are significant numbers of heavy invertebrates in the inorganic material – too many to easily remove under the magnifying lamp – the inorganic and organic matrix is recombined into the sieve and entire sample matrix will be prepared for subsample. If there are not significant numbers of heavy invertebrates in the inorganic material, they are removed under the magnifying lamp and placed with the organic matrix. A second technician inspects the inorganic material for organisms until it is determined there are no more invertebrates in the inorganic fraction of the sample. Unless otherwise requested, the inorganic elutriate is discarded.

The organic material and other contents of the sieve are then evenly distributed into the bottom of a gridded sorting tray. The tray consists of 100 uniform grids (2.54cm^2), with a bottom constructed of 250-micron mesh. A grid is randomly selected, and its contents transferred to a Petri dish. The material in the Petri dish is sorted by naked eye and supplemental illumination. The benthic macroinvertebrates are counted as they are placed into vials containing 70% ethanol. Full girds will be sorted while aiming for 120 organisms, but due to finishing the grid the count may surpass 120. Physical subsampling will be performed on samples containing greater than 150 organisms following procedures described in SOP DPW-WPRP-CBMP-FO-016 Benthic Secondary Subsampling Procedure. No count less than 100 organisms will be submitted unless the sample is sorted in its entirety.

Sorters are trained to pick and count only benthic macroinvertebrates, with heads, that were alive during sampling and contain the attributes required for taxonomic identification. Organisms picked may include sub-aquatic organisms or other specified organisms according to the specific study design. Specimens rejected according to EcoAnalysts' standard include: Sub-aquatic Adults, Terrestrials, Vertebrates, Collembola, Copepoda, Zooplankton, Exuviae, and any organism without a head. This project will also reject surface dwellers. When the target count of organisms has been reached or the target percentage of the sample has been sorted but not fully sorted, a special large and rare protocol may be followed on any remaining unsorted material. Organisms deemed relatively large or rare to the sample (in comparison with the target taxa enumerated in the final count) are found by a naked eye scan in the unsorted sample remnants and are not counted but picked and placed in a separate vial.

Laser-printed labels containing the appropriate sample tracking information are placed in the vial(s). The total number of organisms removed (not including large and rare organisms), the number of grids sorted out of the total, the time spent sorting, and the final volume of the remaining sample volume are all recorded on the sorting bench sheet, as well as comments significant to the preparation, sorting, and/or condition of the sample.

To ensure every sample meets a standard minimum level of sorting efficacy, EcoAnalysts, Inc. standard sorting quality assurance is maintained by re-sorting a portion of the sorted material of every sample that is processed in the lab, and ensuring a minimum efficacy is reached (as required by the project). See Section A7.1 for sorting quality objectives.

B4.1 Taxonomic Identification of Benthic Macroinvertebrates

A taxonomist selects a sample for identification via the LIMS and empties it into a Petri dish. Under a dissecting and/or compound microscope, the invertebrates are to genus, if possible, except for Oligochaeta which are identified to family. Organisms not identifiable to these target taxonomic levels (due to small size or damaged) may be left at higher taxonomic levels but are not assumed to be unique taxa from those identified to target levels when they occur in the same sample. Taxonomic references used for the taxonomic analysis of samples may be provided upon request. The taxonomist enters each taxon directly into the project database using a unique taxonomic code (this is done while at the microscope). The number of individuals of each taxon is counted and entered into the database.

As the sample is being identified, the taxonomist enters data directly into the computer using a custom built LIMS database and user interface. The data entry program has several features built into it, including steps for entering taxon names, life stage information, taxonomic notes, etc. There is a visual cue at each step which prompts for a user confirmation. A running tally of invertebrates as well as the number and type of taxa in the sample are displayed on the screen. Therefore, a taxonomist can quickly look for low or high counts as a flag for major discrepancies. Note: With this process, we have successfully eliminated the need for handwritten bench sheets, thereby doing away with a secondary step of data entry and the errors associated with it.

A synoptic reference collection can be prepared, if requested, where at least one specimen (preferably 3-5 specimens) of each taxon encountered is placed into a 1-dram vial containing 70% ethanol and is properly labeled with identity and sample number. Chironomidae reference specimens are permanently slide mounted and labeled with the EcoAnalysts, Inc. sample number and taxonomic determination.

Depending on the requirements of the project, one or several reference collections can be made. Also, organisms can be vouchered by a specified taxonomic level, i.e. vouchered by each taxon per sample. If any synoptic reference collection is made, a second taxonomist examines the reference collection specimens to verify the accuracy of all taxa identified in the project.

If requested, a specified number of the samples are randomly selected for re-identification by a QC taxonomist. All specimens in those samples that were not set aside for the reference collection are re-identified. See Section A7.2 for taxonomic precision and accuracy measurement quality objectives. The final data is adjusted according to the recommendations of both taxonomists. If requested, reconciliation reports are written and delivered to the client as part of the overall Quality Assurance Report.

B5. QUALITY CONTROL

Each benthic macroinvertebrate sample is checked for quality control. See Sections A7.1 and A7.2 of this QAPP for quality objectives.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

All microscopes and laboratory equipment are inspected regularly according to manufacturer recommendations.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All microscopes and laboratory equipment, including digital imaging equipment, are calibrated regularly according to manufacturer recommendations. Calibration will be checked throughout the project and equipment will be recalibrated if necessary.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Supplies and consumables include alcohol and sample jars. Supplies and consumables are purchased only from reputable and reliable suppliers and are inspected for usability upon receipt.

B9. NON-DIRECT MEASUREMENTS

EcoAnalysts maintains a library of current taxonomic references. These are used for taxonomic identification purposes when such need arises. Taxonomists are responsible for using current references and publications.

B10. DATA MANAGEMENT

As described in section B4.1, data is directly entered into the custom built LIMS database and user interface. With several features built into it, including steps for taxonomic identification of a specimen, the number of specimens in each taxon, life stage information, taxonomic notes, etc., the data entry program successfully eliminates the need for handwritten bench sheets, the secondary step of data entry, and the errors associated with it. Additionally, a running tally of invertebrates and taxonomic groups are displayed on the screen, therefore allowing the taxonomist to quickly identify low or high counts as a flag for potential discrepancies.

Throughout the project and sample analysis, data entry is double checked for accuracy, and validated by the laboratory coordinator. Using our networked computer systems, the appropriate data are combined for each sample to obtain the sorting statistics and comprehensive taxa lists and counts.

Various metrics calculations are offered as output from the LIMS, with EcoAnalysts standard deliverables including (but not limited to) abundance, richness, and community measures.

Additional metrics calculations, including more detailed Benthic Invertebrate Indices, may be provided upon request. Other supplemental reports, such as QA/QC results and data analysis and/or interpretation, can be provided dependant on project requirements.

Data are delivered in an electronic format specified by the client and emailed to the technical contact(s). Hard copies and/or copies on compact disc can be mailed to the client upon request. The delivery schedule is agreed upon by the client and EcoAnalysts, Inc. in advance, specifying the sample lots, dates, and components. EcoAnalysts, Inc. retains all raw data files used and derived in our projects.

Quality assurance data sheet checks are part of the sample validation process, and include scanning for apparent entry errors, measurement errors, omissions, and anomalies. Suspect data are flagged and/or excluded from use. Data may be presented in table, graph, and chart format. Unusual data are rechecked to verify their accuracy.

GROUP C: ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

The project manager, Gary Lester, is responsible for all reporting, tracking, and overall project management including field activities, reviewing the data, reporting, and forwarding all data to the client for inspection. Megan Payne and Pat Barrett are responsible for laboratory operations involving processing benthic macroinvertebrate indicator samples for projects. Robert Bobier, EcoAnalysts QAM, is authorized to oversee all activities as required for quality assurance.

C2. REPORTS TO MANAGEMENT

Draft reports of project findings will be prepared for the client on a regular basis, as requested. Problems that arise during the project are corrected and reported to client and EcoAnalysts staff via this report. The project manager will submit a final report prior to the conclusion of the task order. All data are tracked through use of EcoAnalysts' LIMS. The data compiled during this project are incorporated into spreadsheets and sent to the client and, if requested, will be uploaded to the client's database.

GROUP D: DATA VALIDATION AND USABILITY

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

All raw data are transcribed into EcoAnalysts' LIMS. Any hard copies of raw data are organized and filed. Statistical analyses of replicate samples are recorded so that the degree of certainty can be estimated, when requested. All laboratory analytical results are cross-checked to ensure data are complete and error free. Data are archived using EcoAnalysts' LIMS on EcoAnalysts' servers, with multiple data backups in place.

D2. VERIFICATION AND VALIDATION METHODS

Project staff follows the EPA *Guidance on Environmental Verification and Validation* (EPA QA/G-8) whereby the data are reviewed and accepted or qualified by project staff.

D3. RECONCILIATION WITH USER REQUIREMENTS

Upon receipt of results of each sample group, calculations and determinations of precision and accuracy are made and, if needed, corrective action is implemented. If data quality does not meet project specifications, the deficient data are flagged and the cause of failure evaluated. For the data to be considered valid, data collection procedures, the handling of samples, and data analysis must be monitored for compliance with all the requirements described in this QAPP. Data are flagged and qualified if there is evidence of habitual violation of the procedures described in this QAPP. Any limitations placed on the data are reported to the data end user in narrative form. Any limitations on data use are detailed in the project reports and other documentation.

APPENDIX T

DPW-WPRP-CBMP-FO-016: BENTHIC SECONDARY SUBSAMPLING PROCEDURE

Standard Operating Procedure DPW-WPRP-CBMP-FO-016

Benthic Macroinvertebrate Secondary Subsampling Procedure

Prepared by:	Name: Christopher J. Victoria	Title:	Environmental Scientist
	Signature:	Date:	
Approved by:	Name: Janis Markusic	Title:	Program Manager
	Signature:	Date:	

Scope and Applicability:
Primary benthic macroinvertebrate subsampling is performed according to MBSS subsampling protocols using a 100-grid tray. However, when the total count of an initial subsample is greater than or equal to 150 organisms, a secondary physical subsampling procedure is performed prior to taxonomic identification. This process is performed to bring the subsample down to the 100-120 organism target count for final identification. If the secondary subsampling procedure results in a final sample size greater than the 100-120 organism target, a spreadsheet-based rarefaction method (Tetra Tech 2006) will be used to randomly subsample the identified organisms to the desired target number for the sample.

Responsibility and Personnel Qualifications:
Laboratory personnel must follow written secondary subsampling procedures as described herein.

References:
Tetra Tech, Inc. 2006. Random subsample routine spreadsheet. Developed by Erik W. Leppo of Tetra Tech, Inc., Owings Mills, MD

Precautions:
Make sure that all taxonomy component vials (generals, midges, worms) are included prior to subsampling.

Equipment/Materials:
36 grid subsampling petri dish
Random number generator or dice
Forceps
Subsampling bench sheet
Labeled vial
70% ethanol

Procedure:

1. Gather all taxonomy component vials (generals, midges, worms) and bench sheet for the sample.

2. Redistribute all initial subsample organisms into a 36 grid subsampling petri dish, combining the generals, midge and worm components.

3. Evenly distribute organisms across the entire petri dish. After distribution, do not to disturb the subsampling dish.

4. Randomly select a grid to begin the subsample, using either dice or a random number generator, and remove all organisms from that grid.

5. Continue randomly selecting grids and removing organisms until the count falls between 100 and 120 organisms. *Note: once a grid it started, all organisms in that grid must be removed.*

6. When the target count is reached, record the following information on the sorting subsampling bench sheet: number of subsampled grids, total number of grids in dish, and new counts for each of the three taxonomy components (generals, midges, and worms).

7. Any remaining *extra* organisms are placed in a separate labeled vial and preserved with 70% ethanol. These organisms are now considered part of the sort residue and either retained or discarded depending on client directions for handling sort residues.

Pertinent QA and QC Procedures:

1. A second member of the laboratory staff will check subsampling bench sheet to ensure completeness and correctness.

EcoAnalysts, Inc. LIFE IN WATER

Sort & QC Bench Sheet

7642 - KCI MD Anne Arundel County MBSS Benthos 2017-2021: (.03) 2019 Samples

7642 - 3 - 1

DATA ENTERED

Sample Information

Shelf Location:
Residue Loc.:
Jars: 2

Sorting Protocols

Target Count: 120
Reject Organisms: Standard MBSS
QC Type: 100% of 1/10 samples

Count Range: Target Count Minimum
Efficacy Minimum: 90%

Large/Rare: No
Mesh Size: 595 Micron
Worms (Mount/ID): Y/Family

Notes: Naked eye sort-Reject surface dwellers-Must sort Full Grids

Sorting Information

Technician	Sort Date	Grids: Sorted	Grids: Total	Counts: G	Counts: M	Counts: W	Counts: PNC	Time (hrs): Scope	Time (hrs): Non-Scope	L/R
Steven Little	4-24-19	11	100	34	87	0	0	5.25	.75	/

Counts total (circled): 121

Primary Matrix: Coarse Organic
Secondary Matrix: —

Sample Volume (liters): Initial Volume	Final Volume	Elutriate Check Technician	E Check Date	E Check Hours	Organics Retained	Inorganics Discarded	Post Sorting Jar Count
1,250	,450	—	—	—	N	N	2

Sort Comments:

QC 1 Information

QC Technician	QC Date	Grids: QC'd	Grids: Total	QC Counts: G	M	W	PNC	QC Time (hrs)	Est. Total Count	Est. % Efficacy	Stragglers
Ben Alexander	5/1/19	4	4	←				0	121	100%	N

QC 1 Comments:

Re-Sort 1 Information

Re-Sort Technician	Re-Sort Date	Re-Sort Counts: G	M	W	PNC	Re-Sort Time	Total Count	Target Count

Re-Sort 1 Comments:

QC 2 Information

QC 2 Technician	QC 2 Date	Grids: QC'd	Grids: Total	QC 2 Counts: G	M	W	PNC	QC 2 Time (hrs)	Est. Total Count	Est. % Efficacy	Stragglers

QC 2 Comments:

Subsample Information

Subsample Technician	Subsample Date	Grids: Picked	Grids: Total	Subsample Counts: G	M	W	PNC	Sub. Time (hrs)