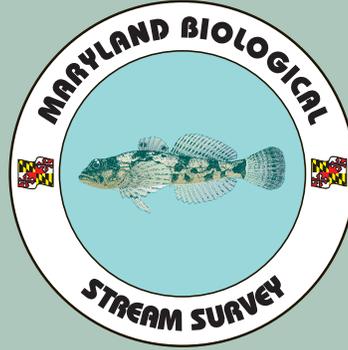


MARYLAND BIOLOGICAL STREAM SURVEY



Sampling Manual : Field Protocols



MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

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



Martin O'Malley
Governor

Anthony G. Brown
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Tawes State Office Building
580 Taylor Avenue
Annapolis, Maryland 21401

Toll free in Maryland: 1-(877)- 620-8DNR ext. 8610

Out of state call: 410-260-8610

TTY via Maryland Relay: 711 (within MD)

800-735-2258 (out of state)

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Martin O'Malley, Governor
John R. Griffin, Secretary

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Thank you for your interest in Maryland Biological Stream Survey (MBSS) field sampling protocols. The Monitoring and Non-Tidal Assessment Division of DNR provides this document, which details the sampling protocols being used for the Round Three MBSS. This manual is provided to persons attending training on MBSS field sampling methods. Documents describing protocols for Water Chemistry and Benthic Macroinvertebrate Laboratory Procedures, as well as The Survey Design and Site Selection are also available and can be provided upon request.

The Field Sampling Protocols manual is based on a modified and updated version of the MBSS Sampling Manual prepared by Paul Kazyak for the MBSS Round Two. This Round Three version has been updated by Scott Stranko, Dan Boward, Jay Kilian, Christopher Millard, Andy Becker, Rachel Gauza, Ann Schenk, Ann Roseberry-Lincoln, and Michelle O'Connor. Questions or comments about the contents of this document can be addressed to Scott Stranko (410-260-8603; sstranko@dnr.state.md.us).

Scott Stranko

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Foreword

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

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

Purpose of Manual

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

Maryland Biological Stream Survey Goal and Objectives

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

SECTION 3

Field Sampling Protocols

3.0 Introduction

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

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

3.1 Personnel and Crew Qualifications

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

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

To ensure comparability among data collected by different sampling crews, all crews must also commit to regular field audits (see chapter 3.5, Quality Assurance). Typically audits are performed at a minimum of two sites sampled by each crew. However, additional audits may be required depending on the experience of the crew, performance on previous audits, and intended use of collected data. Audits can

only be performed by a qualified MBSS QC officer. A qualified MBSS QC officer has had extensive MBSS crew leader experience, has extensive experience in conducting MBSS training, and is familiar with the intended use of MBSS data by the crew being audited. The QC officer should also be familiar with aspects of the MBSS other than field data collection (e.g. laboratory protocols, IBI calculation, data management).

3.2 Health and Safety

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

3.2.1 Training and Qualifications

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

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

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

3.2.2 Duties and Responsibilities

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

3.2.3 Field Crew Supervisor

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

3.2.4 Crew Leader

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

- instruction and supervision of the survey team such that sampling and travel at a given site are done in a manner which minimizes health and safety risks;
- reporting to the Field Crew Supervisor or his/her designee any unusual health and safety conditions, emergencies, or accidents encountered during the deployment of the crew. In the case of accidents or emergencies, the Crew Leader should, as soon as the situation permits, notify the Field Crew Supervisor or his/her designee by direct phone contact;
- ensuring that vehicles and sampling equipment are in safe operating condition prior to and during field deployments;
- ensuring that all members of the survey team are fully aware of any potentially hazardous materials used as part of sampling. Examples include preservatives for biological and chemical samples;
- determining whether sampling conditions are safe and appropriate;
- informing the survey team of any situation-specific dangers involved at a given site;
- ensuring that vehicles are operated in a safe manner; and
- ensuring that samples and sampling equipment are safely stored prior to vehicle operations.

3.2.5 Field Crew Members

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

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

- 2. Electric Shock.** Failure to observe appropriate safety precautions when using backpack electrofishing gear could result in electric shock. Under worst case conditions, this shock could result in cardiac arrest and loss of life. To minimize risks associated with electrofishing during the MBSS, the following measures shall be taken:
 - only personnel designated by Field Crew Leaders should operate the backpack electrofishing unit;

- to minimize the amount of body surface area potentially exposed to electric shock, normal wading gear for the MBSS should be chest waders. only non-leaking wading gear should be used during electrofishing-- if a leak is discovered, wading gear should be changed and the leaking gear repaired or replaced prior to the next use;
- bare wire portion of the cathode (rattail) or the anode should never be touched while the unit is in operation;
- electrofishing should only be conducted when a minimum of three persons are present at a site. In the event of electric shock, this provides for one person to administer CPR while another seeks medical assistance. Use of a portable phone is also recommended as an effective means to summon emergency medical care if necessary;
- if the Crew Leader determines that stream conditions at the time of the site visit present an abnormal risk of electric shock, he or she will determine that the site is not sampleable and sampling will be conducted at an alternate site or canceled in that reach;
- prior to each use, electrofishing gear should be verified to be in safe working condition by the Crew Leader. This verification should include an examination of external wiring and electrical connections; and
- in cases where two electrofishing units are used or barge shocking is employed at a site, extra care should be taken to ensure that unit operators maintain an awareness of all personnel in the water. In addition, unit operators should maintain adequate spacing between units to minimize the risks of shock from both electric fields in the event a crew member slips or falls into the water, or the discharge of one anode completing the switch circuit for another unit.

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

- when necessary, the Crew Leader will make a determination that access to the sampling site is not possible and the site will be deemed unsampleable;
- when traveling over any extensive distance, appropriate footwear should be worn instead of waders or hip boots;
- equipment should be distributed equitably among crew members for transport from the vehicle to the site; if determined to be necessary by the Crew Leader, more than one trip to transport equipment should be made;
- to the greatest extent possible, travel between the vehicle and the sample site should occur during daylight hours; and
- only in unusual circumstances (as determined by the Crew Leader) should a crew member travel alone over hazardous terrain.

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

- prior to sampling, the Crew Leader should ensure that all crew members who are to enter the stream are physically fit to do so and are aware of any specific sampling risks at the site;
- prior to sampling, the Crew Leader should make a determination as to whether the site can be sampled by wading without undue risks. If a negative determination is reached, the site should be revisited at another time or not sampled;
- all field crew members should wear chest waders outfitted with waist belts and felt soles or cleats should be used in rocky areas.

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 degree of 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; and all wading gear should have felt soles and/or cleats.

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

- all field survey personnel should receive training in field identification, avoidance of, and first aid for dangerous plants and animals which may be encountered during the MBSS;
- crew members should inform their Crew Leader of any known allergies and keep appropriate medical relief in the field 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 as they are noted. Similarly, field crew members should inform the Crew Leader as soon as they are discovered; and
- All crew members should be informed of the risks of lyme disease and should check each after conducting field work for ticks that may have become attached to the body.

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

- during development of the itinerary, the Crew Leader should examine the list of NPDES discharge permits and investigate through MDE any known pollution problems in the watershed being sampled. Using this information, a determination should be made as to whether special safety precautions are necessary;
- prior to entering the stream, the Crew Leader should make note of any evidence of high bacterial levels and inform the field crew;
- the use of gloves should be maximized during the sampling process;
- open wounds should not be exposed to contact with stream water; and
- after exposure to stream water, all crew members should wash their hands in isopropyl alcohol and clean water prior to consuming any food or drink.

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

- prior to commencement of field sampling, existing information (through MDE and EPA) about known or probable hazardous waste sites in Maryland in relation to MBSS sample sites should be reviewed. After review of available hazardous site information, the crew should be informed of any hazardous waste sites in areas designated to be sampled. Any such areas identified will be sampled by a crew that has received OSHA hazardous waste safety training (as specified in 29 CFR 1910.120);
- all sampling at hazardous waste sites will be conducted in accordance with site health and safety plans and only after proper advance notice has been given to authorities on site;
- if sampling is to be conducted in an area where hazardous waste is known to be present, MBSS personnel who participate in sampling should participate in a Medical Monitoring Plan established by the Contractor for the hazardous site sampling crew. Medical Monitoring should include baseline, yearly, and exit examinations;
- after sampling at or in the vicinity of hazardous waste sites, all exposed equipment should be thoroughly rinsed, including waders and any exposed personal equipment and;
- no food should be consumed at known hazardous waste sites and following sampling, food will only be consumed after thorough hand washing.

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

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

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

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

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

3.2.7 First Aid

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

3.2.8 Emergencies

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

3.2.9 Precautions for Minimizing Ecological Risk

An increasing potential exists for transferring non-native and invasive organisms (including those that cause serious diseases to native stream dwelling fauna) from one stream to another while conducting monitoring.

Whirling disease (a protist, *Myxobolus cerebralis*), rock snot (an alga, *Didymosphenia geminata*), and amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) are examples of such organisms. In addition, avian influenza can be transferred among farms simply by walking in the chicken litter that came from infected individuals and then walking in another area with chickens. It is important to properly clean all 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. Beginning in June 2007, all MBSS field crews will be required to disinfect all field equipment and waders that come in contact with stream or wetland (e.g. vernal pool) water following sampling at each stream site. This procedure should also be applied to all equipment that comes in contact with chicken litter.

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

Avoid skin and eye contact with bleach solution as it can be severely irritating. Thoroughly rinsing all equipment with freshwater also minimizes risk of skin and eye irritation.

3.3 QUALITY ASSURANCE

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

3.3.1 Introduction

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

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

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

3.3.2 Population of Interest

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

3.3.3 Comparability and completeness

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

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

3.3.4 Documentation

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

3.3.5 Field Audits

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

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

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

3.3.6 Training Requirements

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

3.3.7 Equipment Maintenance and Calibration

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

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

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

3.3.8 Field Information Management

Each MBSS site is assigned a unique identification code. The code is recorded at the top of all MBSS data sheets. The unique code is made up of four parts. 1) Watershed code. The appropriate four letter code indicating the eight digit watershed containing the site (watershed codes are found on page 49). 2) Segment. Three numbers are used to designate the segment. These three letters begin with the stream order and the next two letters refer to the order in which the site was selected. For random sites, the order in which the sites were collected can be important as sites lower in order being sampled indicate less probability of bias (i.e. in being representative of watershed conditions) compared to having many sites with higher order sampled. 3) Type. A one letter code is used to designate the site type. Site type codes that were used during the Round Two MBSS and are likely to be used during the Round Three MBSS include “R” for random sites, “S” for sentinel sites, “X” for special study sites and “T” for targeted sites. 4) Year. The last four digits in the site identification are the calendar year during which sampling occurred.

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

To ensure that all field data for the MBSS are collected and recorded in a usable manner, all data should be printed in the units specified on the MBSS data sheets. No writeovers are permitted on data sheets. The

incorrect entry should be lined out and the correct entry written in an obvious location next to the line out. Data sheets for a given site must be consecutively labeled so that the total number of data sheets generated for each site is known. Recorded data must be reviewed at the point of entry and the Crew Leader and one other member of the crew must review and initial all data sheets prior to departure from the site.

Each sample collected as part of the MBSS will be assigned a sample number. The sample number will contain several unique identifiers to minimize the possibility of misidentification. In addition, chain-of-custody forms (Pages 56-58) should be maintained for all water sample, benthic macroinvertebrate, herpetofauna, crayfish, mussel, and fish collections.

3.3.9 Data Quality Assessment

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

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

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

3.3.10 Duplicate Samples

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

3.3.11 Taxonomic Identification

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

During the Round Three MBSS, photographic vouchers will be accepted in lieu of preserved specimens.

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

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

3.4 Preparation for Sampling

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

3.4.1 Equipment

Prior to each field sampling trip, the Crew Leader should ensure that all necessary sampling equipment is prepared for sampling. A list of equipment for sampling during the Spring Index Period is shown on page 47, and equipment for the Summer Index Period is listed on page 48.

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

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

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

3.4.2 Schedule

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

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

Table 4-1. DNR Freshwater Fisheries Regional Managers			
Region	Counties	Biologist	Telephone Number
Western	Garrett, Allegany	Alan Klotz	(301) 334-8218
Central	Montgomery, Howard, Baltimore, Harford, Washington, Frederick, Carroll	Charlie Gougeon	(410) 442-2080
Southern	Anne Arundel, Prince Georges, Charles, Calvert, St. Marys	Mary Groves	(301) 888-2423
Eastern	Cecil, Kent, Queen Anne's, Talbot, Caroline, Dorchester, Wicomico, Somerset, Worcester	Rick Shaffer	(410) 275-9921

Table 4-2. DNR Natural Resources Police		
Region	Counties	Telephone Number
Headquarters	N/A	1-800-628-9940 410-260-8880 410-260-8888
Central	Baltimore, Howard, Montgomery, Harford, Carroll	410-356-7060 410-356-7061
Western Region	Frederick, Washington, Allegany, Garrett	301-777-7771 301-777-7645
Southern Region	Anne Arundel, Prince Georges, Charles, Calvert	301-888-1601
Upper Eastern Shore	Queen Anne's, Kent, Cecil, Talbot, Caroline	410-758-2890
Lower Eastern Shore	Dorchester, Wicomico, Worcester, Somerset	410-548-7070

3.5 Sample Collection

3.5.1 Introduction

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

3.5.2 Index Periods

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

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

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

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

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

3.5.3 Site Location and Length

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

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

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

3.5.4 Determination of Sampleability

To ensure that a site can be safely and effectively sampled, the Crew Leader will examine the stream prior to the initiation of any sampling. General criteria for determining sampleability include: safety, landowner permission, ability to electrofish effectively, and non-tidal status. No sampling should take place under dangerous conditions. If the site has non-wadeable areas that can be safely sampled using a combination of long-handled anodes and/or dipnets, the site should be considered sampleable. Examples of conditions which could deem a site unsampleable include: a dry stream bed, obvious tidal influence, and unsafe velocities/depths. The determination of Sampleability for benthic macroinvertebrates, spring physical habitat assessment, vernal pools, and spring water chemistry should be noted on the Spring Index Period Data Sheet. Sampleability for electrofishing, summer physical habitat assessment, water chemistry, herpetofauna, mussels, and crayfishes should be noted on the Summer Index Period Data Sheet. A description of how to determine sampleability for each of these is included with the description of sampling methods for each. A list of codes for sampleability are provided on page 49.

- 1. Culverts.** It should be noted that some sites may still be sampleable even though they include underpasses, beaver dams, large culverts, and dry sections. In the case of small culverts which can not be electrofished, the length of the culvert should be measured and recorded on the data sheet and the length added to the original 75 m site. If the culvert occurs in the first half of the site, the additional distance should be added to the downstream end of the site. Similarly, the additional distance should be added to the upstream end, if the culvert is within the upper half of the original site. If the culvert can be sampled completely, no change should be made to the original 75 m site.
- 2. Moving Sites.** The location of a site (even a randomly selected site) can be changed to ensure that a sample is collected as close as possible to the location originally chosen for sampling. This may be important to avoid any bias that may come from sampling large numbers of replacement sites. However, the maximum distance that a site should be moved is 75 meters. It is imperative that a randomly selected site be moved as little as possible. Sites (especially randomly selected sites) should only be moved after every attempt has been made to sample the site in its originally chosen location.

3.5.5 Marking Sites

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

3.5.6 Photodocumentation

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

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

3.5.7 Water Chemistry for Laboratory Analysis

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

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

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

Water must be collected prior to, or upstream of, any disturbance to the stream caused by site sampling or access. Stepping in the stream upstream of the location where water is being collected should be avoided until after all of the water has been collected. Collecting water at the upstream end (75 m) of the site can ensure that other sampling can occur coincident with the collection of water samples.

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

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

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

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

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

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

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

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

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

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

3.5.8 In Situ Water Chemistry

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

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

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

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

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

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

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

3.5.9 Physical Habitat

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

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

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

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

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

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

3.5.9.1 Spring Index Period Physical Habitat Assessment

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

If the stream cannot be sampled for spring physical habitat assessment, this should be noted on the Spring Index Period Data Sheet. Codes designating reasons that a stream could not be sampled are provided on page 49.

1. **Trash Rating.** The trash rating is scored on a 0-20 scale based on criteria found on the Stream Habitat Assessment Guidance Sheet (Page 50).
2. **Distance of Nearest Road to Site.** This variable should be measured when practical with a tape measure or GPS to the nearest meter. If it is not practical to measure this distance, it can be estimated to the nearest 10 m.
3. **Riparian Buffer Width.** The riparian buffer width should be measured to the nearest meter on each side of the stream. The left and right banks of the stream are determined while facing upstream. The average width of the buffer should be recorded. Buffer breaks should not be considered when estimating the average buffer width as buffer breaks are recorded in a different portion of the data sheet (see number 6 below). If the average buffer width is greater than or equal to 50 meters, enter 50 for the buffer width.
4. **Adjacent Land Cover.** Using the codes for adjacent land cover types (page 49), the type of land cover immediately adjacent to the stream buffer should be recorded. If the buffer is 50 m or more, then the same code that was recorded for the buffer should be recorded for the adjacent land cover.
5. **Riparian Vegetation.** Using the codes for vegetation types (page 49) the dominant vegetation types present within the buffer of the 75 m site should be recorded. As many as four types can be recorded. The vegetation types are recorded in order of their dominance within the buffer, with the most dominant recorded first (in the left most box under the bank where the buffer is being recorded). Stem density and canopy density should both be taken into consideration for determining density. However, stem density should take precedence over canopy density.
6. **Buffer Breaks.** Both banks of the stream for the entire 75 m site should be examined for buffer breaks. For each bank of the stream, if any buffer breaks are observed, then a Y should be placed in the box on the MBSS Spring Habitat Data Sheet next to the words "Buffer Breaks (Y/N)". If no buffer breaks are observed, write an N in the box.

7. **Buffer Break Types.** If a buffer break is observed while examining the stream banks, the severity of the buffer break should be noted and recorded as M (minor) or S (severe) in the box alongside the most appropriate buffer break type listed on the Spring Habitat Data Sheet.
8. **Channelization.** The site should be inspected for any evidence of channel straightening or dredging. If evidence of channel straightening or dredging are observed anywhere within the 75 m site, the linear extent of the channelization should be measured to the nearest meter. Channelization along each bank and the stream bottom should be measured separately and recorded in the appropriate portion of the Spring Habitat Data Sheet, where the type of channelization is listed. If channelization is observed at a site with a braided stream channel, the total extent of stream channel that is channelized should be recorded. It is possible (when multiple channels are present), using this method, for the total extent of left bank, right bank, or stream bottom channelized to be more than 75 m. Since the objective of this measure is to determine the total length of stream channel that is channelized, this is acceptable.
9. **Land Use.** While at the site, a survey of the surrounding area for land use types is conducted. For each land use type listed on the Spring Habitat Data Sheet mark a Y or N indicating whether or not the land use type is present near the site. Any land use that can be observed while in or alongside the stream at the site should receive a Y and any that cannot be observed should receive an N.
10. **Stream Gradient.** The intent of this is to measure the slope of the stream over 75 m. This is achieved by recording the difference in water surface height from the 0 m to the 75 m locations of the MBSS site as compared to a level plane. A levelometer was used during the Round Two MBSS to measure stream gradient. Laser levels and other techniques may provide similar results, sometimes with increased precision. Any technique used to measure stream gradient should provide data accurate to at least the nearest 5 centimeters to be comparable to data collected during the Round Two MBSS.

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

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

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

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

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

3.5.9.2 Summer Index Period Physical Habitat Assessment

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

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

Many of the summer physical habitat assessment measures require sufficiently clear water to observe the stream bottom throughout the majority of the 75 m site. If conditions do not allow sufficient visibility to see all of the features that must be observed, or if conditions are unsafe for wading, the site should be considered unsampleable for physical habitat. In many cases, the stream may be sampleable during a return visit when the water level is lower. However, if the stream cannot be sampled for summer physical habitat assessment, this should be noted on the Summer Index Period Data Sheet. Codes designating reasons that a stream could not be sampled are provided on page 49.

- 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 (page 50). The scores for each of these metrics are meant to characterize a distinct aspect of stream habitat. The instream habitat metric primarily addresses habitat for fishes and epifaunal substrate is meant to rate the suitability of habitat for benthic macroinvertebrates. The general quality of riffle and pool habitats are rated based primarily on the prevalence of sufficient depth and extent of these habitats. Velocity/depth/diversity provides a measure of the how well fast, slow, deep, and shallow areas are represented in the stream.
- 2. Embeddedness.** The percent of riffle substrates surrounded by fine substrates, such as sand and silt, is recorded based on visual observation. The 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 materials.
- 3. Shading.** The percent of the wetted area of the 75 m site that is shaded by overhanging vegetation or other structures is approximated based on a visual assessment. If clearing of vegetation was conducted to facilitate electrofishing, or for any other reason, shading should be rated based on the condition prior to clearing.

4. **Woody Debris.** For the MBSS, large woody debris are defined as any natural woody structures (e.g. logs, snags, dead tree trunks), with the exception of live trees that are at least 10 cm in diameter and more than 1.5 m long. The number of large woody debris, located in the wetted portion of the 75 m stream site (instream woody debris), is counted. The number of large woody debris in the stream channel or immediate riparian area, but not in the wetted portion of the stream (dewatered woody debris) are counted separately from instream woody debris. Only those dewatered woody debris from the immediate riparian area that (in the opinion of the evaluator) are likely to become wetted during high flows, or fall into the stream channel should be counted.
5. **Root Wads.** For the MBSS, root wads that are on live trees with a chest high trunk diameter (DBH) of at least 15 cm should be counted. These should be counted along both banks of the stream within the 75 m site. Those root wads that are in the water (instream) are counted separately from those not in the stream (dewatered). However, only those dewatered root wads that provide stability to the stream bank or that are likely to become wetted during high flows should be counted.
6. **Stream Character.** The Stream Character portion of the MBSS Summer Habitat Data Sheet lists 15 stream features. For each feature, an A, P, or E should be recorded in the box next to the feature indicating whether the feature is absent, present, or extensive respectively in the 75 m stream site.
7. **Maximum Depth.** The maximum depth of the MBSS site is considered the deepest area found anywhere within the 75 m. Maximum depth is recorded to the nearest cm.
8. **Wetted Width, Thalweg Depth, and Thalweg Velocity.** The wetted width, thalweg depth and thalweg velocity are measured at four transects within the 75 m MBSS site. The four transects are located at the 0m, 25m, 50m, and 75m portions of the MBSS site (beginning with 0m at the downstream-most end of the site). Wetted width is measured from bank to bank (perpendicular to the direction of the stream flow) to the nearest 0.1 m and includes only the wetted portion of the stream. Islands or other large features in the stream that would not be covered by water during higher base-flow should not be included in the measurement of wetted width. Features that would be covered by water (during higher base-flow should be included in the wetted width measurement. Thalweg depth is the depth (in cm) of the deepest part of the stream at each transect. Thalweg velocity is the stream current velocity (in m/sec) in the deepest part of the stream at each transect.
9. **Flow.** Measurements that can be used to calculate flow (often referred to as discharge) are recorded on the MBSS Summer Habitat Data Sheet. A transect that is suitable for taking these measurements should be located. A suitable transect approximates a "U" shaped channel to the extent possible. The most useful measurements are acquired by avoiding transects with boulders or other irregularities that create backflows and cross flows. The stream channel can be modified to more closely approximate a "U" shaped channel and provide laminar flow with adequate depth for taking velocity measurements. Unless the stream is very small (less than 0.5 m wide), a minimum of 10 measurements should be taken. As many as 25 measurements can be recorded on the MBSS Summer Habitat Data Sheet. In general, more measurements are required in larger streams. The measurements consist of depth (to the nearest 0.5 cm) and velocity (to the nearest 0.001 m/sec) and should be recorded at regular intervals. Velocity measurements should be taken at 0.6 of the distance from the water surface to the bottom (measured from the surface), making sure to orient the sensor to face upstream and taking care to stand well downstream to avoid

deflection of flows. Depth and velocity measurements should be taken at the exact same locations. The Lat Loc on the MBSS Summer Habitat Data Sheet refers to the distance from one stream bank (either left or right) where each depth and velocity measurement is taken.

- 10. Alternative Flow.** If flows are so low that they can not be measured with a flow meter, the stream should be constricted as much as possible in a 1 meter section of uniform width and depth. The speed of a floated object should be recorded three times as a substitute for velocity measured with the flow meter. Record on the data sheet the depth, width, and time (3 trials) for the floated object.
- 11. Bank Erosion.** The length and average height of erosion on both banks of the stream, within the 75 m site should be recorded along with the severity of erosion, on the MBSS Summer Habitat Data Sheet. In braided streams it is possible to have the total extent of eroded bank add up to more than 75 m. Since the objective of this measure is to determine the total area of erosion present at the site, this is acceptable.
- 12. Bar Formation and Substrate.** Boxes in this portion of the MBSS Summer Habitat Data Sheet should be filled in completely to indicate if the bar formation is absent (fill in the box next to “None”), minor, moderate, or extensive; and the dominant substrate type(s) that make up the bars in the site. More than one substrate can be selected. However substrates comprising only a minor part of the substrate should not be selected.

3.5.10 Stream Blockages

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

To continue to provide this useful information, any man-made stream blockages either at the MBSS site or en route to the MBSS site, should have the height (to the nearest 0.1 m) and location (latitude and longitude in decimal degrees) recorded on the MBSS Spring Habitat Data Sheet. The type of blockages should also be recorded. Codes for blockage types are provided on page 49. Well known and obvious blockages such as dams on major rivers need not be recorded, but if there is any doubt about whether or not to record a blockage, recording the blockage is recommended.

3.5.11 Temperature Loggers

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

matches the serial number entered on the Spring Index Period Data Sheet is recommended. It is often useful (and recommended) to attach a flag or piece of tape to the logger with the site identification, date, and time of retrieval.

3.5.12 Vernal Pools

- 1. Definition.** A vernal pool is a small, temporary body of water that provides vitally important habitat for many amphibians and aquatic invertebrates. Despite their importance, very limited information is currently available on Maryland's vernal pools. During Round Three, the MBSS has added the collection of information on the location and size of potential vernal pool habitats encountered, as well as a list of herpetofauna associated with the pool and minimal physical habitat information. Habitats that qualify as potential vernal pools are less than one acre (4,000 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. Only sites with the entire 50 m on both sides of the stream being searched are recorded as sampleable. Any vernal pool found in this 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 any habitat that meets the vernal pool definition above. At least one digital photograph of the vernal pool should be taken and the number of the photograph recorded. The approximate dimensions of the potential vernal pool (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 should be recorded using the codes provided on page 49. Up to three codes can be recorded. The codes should be entered in order, from left to right on the data sheet, starting with the closest land cover to the pool and ending with the land cover that is furthest away from the pool. The land cover types that are recorded should be the dominant types that are in the area that can be seen while standing next to the pool. The presence of fishes or fairy shrimp (order Anostraca) (observed while standing near the pool) should be recorded using a "Y" for presence and an "N" for not observed. It is not necessary to sample for fishes or other aquatic organisms in potential vernal pools. Any amphibians observed in or immediately adjacent to the potential vernal pool should also be recorded along with the life stage of the organism (egg, larval, juvenile, adult). Frogs that are heard calling from within or immediately adjacent to the potential vernal pool, but that are not

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

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

3.5.13 Biological Sampling

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

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

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

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

3.5.13.1 Benthic Macroinvertebrates

Benthic macroinvertebrate sampling is conducted within the same 75m site used for other MBSS sampling. MBSS benthic macroinvertebrate sampling must occur during the Spring Index Period (1 March to 30 April). The intent of benthic sampling is to provide a representative sample of the community composition and relative abundance in favorable habitat (habitats supporting the greatest benthic diversity) within the site. In addition to representing the diversity at an MBSS site, benthic macroinvertebrate data collected by MBSS are used to calculate the MBSS benthic macroinvertebrate IBI. A D-net (450 μ mesh), sieve bucket (450 μ mesh sieve), and sample bucket are needed to collect an MBSS benthic macroinvertebrate sample.

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

Effective MBSS benthic macroinvertebrate sampling requires inspection of suitable habitats. Although turbidity or darkly stained water should not prohibit benthic macroinvertebrate sampling, streams that are so turbid that benthic macroinvertebrate habitat cannot be seen at all should not be sampled. Exceptions are sites with persistent and excessive turbidity problems (based on many return visits none of the stream bottom is ever visible). In these cases a note describing the turbidity problem should be made in the comments section of the spring data sheet. Other situations that may preclude sampling include dry streams, marshes, impoundments such as those produced by beaver dams that are too deep to sample, tidally influenced streams, and areas where permission is denied. The appropriate code for unsampleability (page 49) should be recorded on the Spring Index Period Data Sheet.

- 2. Habitats to be Sampled.** Sampling should include a combination of habitats that support the most diverse macroinvertebrate community within a sample site. These habitats often include riffles when one is present. Other habitats, in order of preference, are root wads, root mats and woody debris and associated snag habitat; leaf packs; submerged macrophytes and associated substrate; and undercut banks. Other less preferred habitats include gravel, broken peat, clay lumps and detrital or sand areas in runs. Note that, among all the habitats listed above, those in moving water are preferred to those in still water.
- 3. Benthic Macroinvertebrate Sampling Protocols.** Benthic sample buckets must be labeled twice - on the external wall of the bucket and on the inside. The following information must be included on the label: date, time, and site identification code from the Spring Index Period Data Sheet. Verify the information on each label and indicate so on the Spring Index Period Data Sheet. The external label should be covered with clear plastic tape to prevent smudging and/or label loss. Internal labels must be printed on waterproof paper. Both labels should be filled in with pencil. Benthic sample Chain-of-Custody forms (page 57-58) should also be filled out with the name of the sampler, date, time, and sample site number.

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

Survey the site to locate the most productive benthic macroinvertebrate habitats as listed above. Twenty square feet of habitat should be sampled at each site and material collected for this 20 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. Aggressively disturb the substrate with hand and/or foot. Sampling typically disturbs riffle habitat about 5 to 8

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

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

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

In some rare cases, (e.g. some large 3rd-4th order streams) a sufficient amount of potentially productive habitat may not be present within the 75m 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 75m site. If sampling is conducted upstream of the 75m site, a description of the habitats sampled and distance from the upstream end of the 75m 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. To remove fine sediments from the sample, the sieve bucket may be gently “slapped” against the stream water surface and very slowly rotated while the bottom of the bucket is submerged. Do not rotate the sieve bucket quickly during this process, as this action may damage many soft-bodied macroinvertebrates potentially rendering them unidentifiable. After processing the sample in the sieve bucket, the benthic net should be rinsed carefully in stream water to make sure that no benthic macroinvertebrates remain that may be transported to the next sample site.

4. **Preservation.** The processed composite sample should be transferred from the sieve bucket to an externally labeled sample bucket and preserved in 95% ethanol. Place the internal label atop the sample material and ensure that the lid to the sample bucket is tight. Gently mix the sample material and preservative and ready the sample for transport.
5. **Delivery to Laboratory.** A Benthic Macroinvertebrate Chain-of-Custody Sheet (page 57-58) must accompany all samples taken to the benthic macroinvertebrate identification laboratory, which includes the sample identification codes for all samples being delivered, sampler name, date, and a signature from a laboratory representative upon transfer of samples to the laboratory.

During the spring visit, record in the comments section of the spring data sheet any herpetofauna (positive identifications only) observed or heard at the site, including those released from the sieve bucket during benthic macroinvertebrate processing. Maintain as vouchers any species not previously collected from the basin being sampled.

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

3.5.13.2 Fish Sampling

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

- 1. Electrofishing Safety.** All persons conducting electrofishing should be familiar with chapter 3.2.6, in which hazards and procedures for minimizing risk for electric shock, prior to conducting electrofishing are described.
- 2. Sampleability.** Prior to conducting electrofishing, the crew leader (with input from other crew members) must determine if the site can be sampled safely and effectively. Electrofishing can only be conducted safely if the site being sampled can be waded. If the depth or current velocity precludes safe wading, then the site should be considered unsafe for electrofishing. However, where the margins of deep areas can be safely waded and fish can be effectively captured (e.g. using long handled dip nets and anodes), as long as all other sampleability considerations are met, sampling should occur. The most predominant effective sampleability consideration is water visibility. Effective MBSS electrofishing cannot occur in water that is turbid. All areas of the stream bottom must be visible. The only exception to the visibility consideration is a stream that is stained dark from natural organic sources (tannins leached from leaves; blackwater streams). Although sampling can occur in blackwater streams when visibility is relatively limited due to a natural cause, sampling should not occur in a blackwater stream that is also turbid. Whether or not the entire stream bottom is clearly visible in all portions of the site is recorded on the MBSS Fish Data Sheet.

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

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

3. **Sampling Considerations.** The width of the stream, number of anodes needed to effectively electrofish, and any other fish sampling considerations should have been recorded on the Spring Index Period Data Sheet during spring sampling. In cases where spring sampling is not being conducted, site reconnaissance is recommended prior to the electrofishing visit to determine the number of anodes and length of block nets needed, as well as any other fish sampling considerations.
4. **Number of Anodes.** The appropriate number of anodes to cover the entire width of the MBSS site must be used. In all cases this number of anodes is at least one for every three meters of stream width. More than this number may be necessary depending on the amount of habitat available within the stream site, deep areas, or other reasons to be determined by the Crew Leader. All anodes used by MBSS sampling crews are outfitted with 1/4" mesh netting to facilitate fish capture. The netting on the anodes should not have any holes or tears greater than 1/4". As more than one anode can be used for each electrofishing unit, the number of anodes/unit is recorded on the MBSS Fish Data Sheet.
5. **Dip Nets.** At least one person with a dip net should accompany each person using an anode, even if the person with the anode also carries a dip net. Dip nets used by MBSS sampling crews have 1/4" mesh and should not have any holes or tears greater than 1/4". 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 1/4" or smaller mesh, be completely free of holes or tears larger than 1/4" and be long enough to block the entire width of the stream perpendicular to the flow and be high enough to reach from the bottom to above the surface of the stream. Block nets should be placed at the 0 m and 75 m ends of the MBSS site, so as to effectively prohibit the escape of fishes from within the site and to prohibit entry of additional fishes from outside the site. Any tributaries or seeps entering the site that will not be sampled must also be blocked with block nets to prohibit the movement of fish in or out of the site. In braided streams, all braids should be blocked at the 0 and 75 m locations and all braids should be sampled.

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

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

- 8. Fish Movement.** Prior to and during the installation of block nets care must be taken to ensure that fishes are not chased out of or into the MBSS site. Any observed movement of fishes in or out of the site should be noted on the MBSS Fish Data Sheet.

Disturbing the area within and upstream of the site should also be avoided, to the extent possible, prior to electrofishing so that visibility is not affected by resulting turbidity.

- 9. Appropriate Voltage.** The output voltage of the electrofishing unit should be adjusted to ensure that fishes are being recruited effectively. Proper adjustments of electricity output will vary according to the varying conductivity of the water in different streams. The conductivity should be used as a guide to determine the approximate voltage and frequency to be used. In addition most electrofishers are equipped with a signal that can be used to guide the adjustment of these settings. Regardless of the conductivity and any signals that the electrofisher provides, testing of the electrofisher's effectiveness downstream of the MBSS site, prior to use in the site, should be conducted, as this is the best way to definitively be sure that the electrofisher is being effective. Effective electrofishing for MBSS sites effectively stuns both small and large fishes without causing mortality.
- 10. Crew Requirements.** All persons participating in electrofishing must wear watertight chest waders. In rocky bottom streams felt 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 are captured. Using flow through live cars and bubblers will substantially increase survival of collected fishes compared to using closed systems without bubblers. Care should be taken to avoid electrofishing near any flow through containers as the fishes in these containers will be affected by the electricity.

In fast water areas 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 paid to sighting small bottom fishes such as 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 page 52 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% EtOH solution. Individuals > 160mm should be slit on the lower abdomen of the RIGHT side prior to preservation in formalin. All specimen jars should be labeled with inside labels specifying the date, site number, and name of collector. An example of the MBSS Voucher Specimen Label is included on page 68. The MBSS fish key should be used as needed for positive verification during field identifications. Only persons who have passed the MBSS fish identification test should identify fishes to species in the field.

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

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

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

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

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

3.5.13.3 Reptile and Amphibian Sampling

Reptile and amphibian sampling has been an integral part of the Maryland Biological Stream Survey since 1994. Reptiles and amphibians, and particularly stream salamanders, have been shown to be excellent indicators of MBSS site conditions. During Round Two, however, only incidental observations of herpetofauna were recorded for MBSS sites, with no focused search conducted. During Round Three, particular emphasis will be made on searching for stream salamanders and a minimum of fifteen minutes

will be spent searching available habitat for herpetofauna. Incidental observations will also continue to be recorded. The life-history stage of reptiles and amphibians should be recorded (egg, larvae, or adult).

- 1. Incidental Collection.** The full common name of any reptile or amphibian species that is encountered while sampling or accessing MBSS sites, during either spring or summer, should be recorded on the MBSS Spring Index Period Data Sheet or Summer Index Period Data Sheet, respectively.
- 2. General Herpetofauna Search.** Approximately fifteen minutes should be spent searching the best available habitat within the riparian area of the stream during the Summer Index Period for herpetofauna. In rare cases, a site may not have any suitable habitat. In these cases, less than fifteen minutes may be spent searching for herpetofauna. Searches should consist primarily of flipping cover objects such as rocks and logs, or even human refuse. The full common name of all reptile and amphibian species encountered should be recorded on the MBSS Summer Fauna Data Sheet.
- 3. Stream Salamanders.** Stream salamanders include the following species in the family Plethodontidae; the northern red salamander, eastern mud salamander, northern spring salamander, northern two-lined salamander, long-tailed salamander, northern dusky salamander, Appalachian mountain dusky salamander, and seal salamander. Searches focused on finding these species will be conducted, during the Summer Index Period, along a 25 by one meter transect paralleling the stream and can be immediately downstream of the 75 m site (but can also be upstream) so as not to interfere with electrofishing. The 25 m transect should be contiguous with, but not within, the 75 m MBSS site and be located so that it follows the wetted edge of the stream. During the search, all available cover objects (including cobbles, small boulders, logs, or other objects) within the transect should be carefully flipped over and then returned as closely as possible to the original position. Only one side of the stream needs to be searched. The side that is searched should be selected based on the availability of potential stream salamander habitat, with the bank containing the most potential habitat being the one that is searched.

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

- 4. Sampleability.** General herpetofauna searches should be conducted at any MBSS site that can be safely accessed, even if electrofishing cannot be conducted for a number of reasons (e.g. due to dry stream bed, excessive depth, or beaver pond). The ability to perform stream salamander searches, however, may be precluded by certain stream conditions. As with other aspects of MBSS sampling, the sampleability codes provided on page 49 should be used to indicate sampleability for stream salamanders.
- 5. Photographic Vouchers.** Photographs should be taken of any rare, threatened, or endangered species (provided the photograph can be taken without harming the specimen). In addition, voucher photographs of at least five individuals (provided at least 5 individuals are encountered) of each species encountered should be taken to verify proper identification in the field. Photographs should show the anatomical features that are necessary for proper taxonomic identification and files for digital photographs should include the MBSS site identification. The best photographs have the site identification in the photograph, with the specimen. The Department

of Natural Resources, Monitoring and Non-Tidal Assessment Division will keep a photographic voucher collection for reptiles and amphibians. It is not necessary to preserve any reptile or amphibian specimens during the Round Three MBSS.

6. **Taxonomic Identification.** Only those members of the field sampling crew who have passed the reptile and amphibian taxonomy test should perform reptile and amphibian identification for the crew. The Key to the Reptiles and Amphibians of Maryland can be consulted to help with identifications.
7. **Frog Calls.** A frog call test will be administered during the Spring Index Period training. Field crew members who pass the frog call test can identify frogs as present in the vicinity of MBSS sites based on hearing the frog call. Frogs that are heard calling can be recorded on the Spring Index Period Data Sheet during spring sampling and on the Summer Index Period Data Sheet during summer sampling.
8. **Handling Care and precautions.** Live specimens that will be released should be handled as little as possible, while still ensuring sufficient observation to obtain accurate taxonomic identification. Animals should be released as closely as possible to where they were captured. If an animal was found under a cover object. The object should be returned to its original position and the animal should be placed next to the object and allowed to return to underneath the object on its own.

3.5.13.4 Crayfish Sampling

1. **Sampleability.** Sampling for crayfishes occurs during the Summer Index Period and coincides with stream fish sampling. Therefore, crayfish sampleability is determined following identical protocols used to determine sampleability of stream fishes.
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 (> 15mm 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 could not be positively identified in the field should be retained for further inspection by a regional crayfish expert. Retention of all specimens which cannot be positively identified is mandatory. For Round Three MBSS, one to five individuals of each species collected in each of the major river basins (6-digit) should be retained so that taxonomic identifications can be verified. For the major river basin collections, the preservation of Form I males is preferred. However, females and small males should be vouchered in the absence of Form I males. Specimens for preservation should be promptly placed into plastic jars filled with 70% ethanol solution. Label all specimen jars with an inside label specifying the date, site name, and name of collector. Release all crayfishes not retained for vouchers.

3.5.13.5 Mussel Sampling

Any freshwater mussels of the family Unionidae or exotic Asian clams (*Corbicula*) that are observed while sampling MBSS sites should be identified to species with species common names recorded. During the Summer Index Period, suitable Unionidae habitats within the sampling segment should be searched for the presence of freshwater bivalves, with part of this effort focused on searching obvious, bank side animal middens for shells. This search can be conducted in conjunction with the herpetofauna search and can take as little as five to ten minutes. Live specimens that are found should be identified in the field, and then returned as closely as possible to where they were collected. A pin flag should be used to mark the location where a live specimen is removed from the substrate for identification. After identification, the specimen should be returned to the location from where it was collected. The mussel should be gently placed partway into the substrate with the anterior end pointing down. The species encountered and whether specimens of that species were live (L), recent shell (R), or old shell (O) will be noted on the Summer Index Period Data Sheet in the appropriate area. If no freshwater mussels or *Corbicula* are encountered, it will be noted on the data sheet as none (N).

Any Unionidae or *Corbicula* encountered during the spring index visit should be noted in the comments section of the Spring Index Period Data Sheet. The data sheet should list the species as well as if the specimen that was found was live or dead shell. If live Unionidae are collected in the d-net during benthic macroinvertebrate sampling, they should be replaced 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 Unionid 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. 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. Any empty shell for which the identification is uncertain should be retained. Empty shells collected from a single site can be collected in one zip-lock bag with a completed label containing site name, date, and collector.

3.5.13.6 Invasive Plants

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

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

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

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

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

MBSS Drainage Basin Codes

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

Watershed Abbreviation

Aberdeen Proving Ground
 Anacostia River
 Antietam Creek
 Assawoman Bay
 Atkisson Reservoir
 Atlantic Ocean
 Back River
 Back Creek
 Baltimore Harbor
 Big Annesmesex River
 Big Elk Creek
 Bird River
 Bodkin Creek
 Bohemia River
 Breton Bay
 Brighton Dam
 Broad Creek
 Bush River
 Bynum Run
 Cabin John Creek
 Casselman River
 Catoctin Creek
 Conowingo Dam Susquehanna R
 Chincoteague Bay
 Christina River
 Conewago Creek
 Conococheague
 Corsica River
 Deep Creek Lake
 Deer Creek
 Dividing Creek
 Double Pipe Creek
 Eastern Bay
 Evitts Creek
 Fifteen Mile Creek
 Fishing Bay
 Furnace Bay
 Georges Creek
 Gilbert Swamp
 Gunpowder River
 Gwynns Falls
 Honga River
 Isle of Wight Bay
 Jones Falls
 Kent Island Bay
 Kent Narrows
 Langford Creek
 Little Conococheague
 Liberty Reservoir
 Little Choptank
 Little Elk Creek
 Little Gunpowder Falls
 Licking Creek
 Lower Monocacy River
 Loch Raven Reservoir
 Lower Choptank
 Lower Chester River
 Lower Elk River
 Lower Gunpowder Falls

ABPG
 ANAC
 ANTI
 ASSA
 ATKI
 ATLA
 BACK
 BACR
 BALT
 BANN
 BELK
 BIRD
 BODK
 BOHE
 BRET
 BRIG
 BROA
 BUSH
 BYNU
 CABJ
 CASS
 CATO
 CDAM
 CHIN
 CHRI
 COCR
 CONO
 CORS
 DCRL
 DEER
 DIVI
 DOUB
 EAST
 EVIT
 FIMI
 FISH
 FURN
 GEOR
 GILB
 GUNP
 GWYN
 HONG
 ISLE
 JONE
 KEIS
 KENA
 LANG
 LCON
 LIBE
 LICK
 LIEL
 LIGU
 LIKG
 LMON
 LOCH
 LOCK
 LOCR
 LOEL
 LOGU

Lower Pocomoke River
 Lower Wicomico
 Little Patuxent River
 Lower Susquehanna
 Little Tonoloway
 Lower Chesapeake Bay
 Lower Winters Run
 Little Youghiogheny
 Magothy River
 Manokin River
 Marsh Run
 Marshyhope Creek
 Mattawoman Creek
 Middle Chesapeake Bay
 Middle Chester River
 Middle River-Browns
 Miles River
 Monie Bay
 Middle Patuxent Rier
 Nanjemoy Creek
 Nanticoke River
 Nassawango Creek
 Northeast River
 Newport Bay
 Octoraro Creek
 Oxon Creek
 Patapsco River Lower North Br
 Patuxent River Lower
 Patuxent River Middle
 Patuxent River Upper
 Pocomoke Sound
 Piscataway Creek
 Potomac AL Co
 Prettyboy Reservoir
 Potomac River FR Co
 Potomac River Lower North Br
 Potomac Lower Tidal
 Potomac River MO Co
 Potomac River Middle Tidal
 Potomac River Upper North Br
 Potomac Upper Tidal
 Potomac WA Co
 Port Tobacco River
 Rocky Gorge Dam
 Rock Creek
 Sassafras River
 Savage River
 South Branch Patapsco
 Southeast Creek
 Seneca Creek
 Severn River
 Sideling Hill Creek
 Sinepuxent Bay
 South River
 St. Clement Bay
 Stillpond-Fairlee
 St. Mary's River
 Swan Creek
 Tangier Sound
 Tonoloway
 Town Creek
 Transquaking River
 Tuckahoe Creek
 Upper Elk River
 Upper Monocacy River
 Upper Chesapeake Bay
 Upper Choptank
 Upper Chester River
 Upper Pocomoke River
 West Chesapeake Bay
 Western Branch
 West River
 Wicomico River
 Wicomico Creek
 Wills Creek
 Wicomico River Head
 Wye River
 Youghiogheny River
 Zekiah Swamp

LOPC
 LOWI
 LPAX
 LSUS
 LTON
 LWCH
 LWINT
 LYOU
 MAGO
 MANO
 MARS
 MACK
 MATT
 MDCH
 MICR
 MIDD
 MILE
 MONI
 MPAX
 NANJ
 NANT
 NASS
 NEAS
 NEWP
 OCTO
 OXON
 PATL
 PAXL
 PAXM
 PAXU
 PCSO
 PISC
 PRAL
 PRET
 PRFR
 PRLN
 PRLT
 PRMO
 PRMT
 PRUN
 PRUT
 PRWA
 PTOB
 RKGR
 ROCK
 SASS
 SAVA
 SBPA
 SEAS
 SENE
 SEVE
 SIDE
 SINE
 SOUT
 STCL
 STILL
 STMA
 SWAN
 TANG
 TONO
 TOWN
 TRAN
 TUCK
 UELK
 UMON
 UPCH
 UPCK
 UPCR
 UPPC
 WCHE
 WEBR
 WEST
 WICO
 WICR
 WILL
 WIRH
 WYER
 YOUNG
 ZEKI

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

VEGETATION TYPES

G= Grasses /Forbes

MBSS Stream Habitat Assessment Guidance Sheet

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

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

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

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

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

e) **Riffle/Run Quality** Rated based on the depth, complexity, and functional importance of riffle/run habitat in the segment, with highest scores assigned to segments dominated by deeper riffle/run areas, stable substrates, and a variety of current velocities.

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

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

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

COMMON NAMES OF MARYAND FISHES

LAMPREYS:

American brook lamprey (T)
Least brook lamprey
Sea lamprey

HERRINGS:

Alewife
American shad
Blueback herring
Gizzard shad
Hickory shad
Threadfin shad

CATFISHES:

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

SUCKERS:

Black redhorse
Creek chubsucker
Golden redhorse
Longnose sucker (E)
Northern hogsucker
Shorthead redhorse
Quillback
White sucker

KILLIFISH:

Banded killifish
Mummichog
Rainwater killifish
Sheepshead minnow
Striped killifish

STICKLEBACKS:

Fourspine stickleback
Threespine stickleback

MINNOWS:

Blacknose dace
Bluntnose minnow
Bridle shiner
Bullhead minnow
Central stoneroller
Cheat minnow (E)
Common carp
Bridle shiner (E)
Common shiner

Comely shiner (T)
Creek chub
Cutlips minnow
Eastern silvery minnow
Fallfish
Fathead minnow
Goldfish
Golden shiner
Grass carp
Ironcolor shiner (E)
Longnose dace
Pearl dace (T)
Redside dace (E)
River chub
Rosyface shiner
Rosyside dace
Satinfin shiner
Silverjaw minnow
Spotfin shiner
Spottail shiner
Striped shiner (I)
Swallowtail shiner
Tench

SCULPINS:

Checkered sculpin (R)
Mottled sculpin
Potomac sculpin
Blue ridge sculpin

PERCHES:

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

SUNFISHES:

Banded sunfish (R)
Black crappie
Blackbanded sunfish (T)
Bluespotted sunfish
Bluegill
Flier (T)
Green sunfish

Mud sunfish (I)
Pumpkinseed
Redbreast sunfish
Redear sunfish
Rock bass
Longear sunfish

Warmouth
White crappie
Lepomis hybrid
*Smallmouth bass
*Largemouth bass

TROUTS:

*Brook trout
*Brown trout
*Cutthroat trout
*Lake trout
*Rainbow trout

PIKES:

*Chain pickerel
*Muskellunge
*Northern pike
Redfin pickerel

TEMPERATE BASS

*Striped bass
White perch

MISCELLANEOUS

Longnose gar
Mosquitofish
Pirate Perch
American eel
Trout-perch (E)
Rainbow smelt
Bowfin
Eastern mudminnow
Inland silverside
Atlantic menhaden
Northern snakehead

STATUS KEY:

(R)= RARE
(I) = IN NEED OF
CONSERVATION
(E) = ENDANGERED
(T) = THREATENED
(U) = UNKNOWN

* = Gamefish

COMMON NAMES OF MARYLAND REPTILES AND AMPHIBIANS

SALAMANDERS

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

FROGS

American Bullfrog
Northern Green Frog
Carpenter Frog (I)
Northern Leopard Frog
Southern Leopard Frog
Pickerel Frog
Wood Frog
Barking Treefrog (E)
Green Treefrog
Gray Treefrog
Cope's Gray Treefrog
Northern Spring Peeper
Mountain Chorus Frog (T)
New Jersey Chorus Frog
Upland Chorus Frog
Northern Cricket Frog
Eastern American Toad
Fowler's Toad
Eastern Narrow-mouthed Toad (E)
Eastern Spadefoot Toad

TURTLES

Bog Turtle (T)
Spotted Turtle
Wood Turtle
Eastern Box Turtle
Red-eared Slider
Eastern Painted Turtle
Midland Painted Turtle
Eastern River Cooter
Northern Red-bellied Cooter

Northern Map Turtle (E)
Northern Diamond-backed Terrapin
Stinkpot
Eastern Mud Turtle
Eastern Snapping Turtle
Eastern Spiny Softshell (I)

SNAKES

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

LIZARDS

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

STATUS KEY:

(R)= RARE
(I) = IN NEED OF CONSERVATION
(E) = ENDANGERED
(T) = THREATENED
(U) = UNKNOWN

SCIENTIFIC NAMES FOR MARYLAND CRAYFISHES
(NOT ALL SPECIES HAVE COMMON NAMES)

<i>Cambarus acuminatus</i>	<i>Procambarus clarkii</i>
<i>Cambarus bartonii bartonii</i>	<i>Procambarus zonangulus</i>
<i>Cambarus carinirostris</i>	<i>Procambarus alleni</i>
<i>Cambarus diogenes</i>	<i>Orconectes obscurus</i>
<i>Cambarus dubius</i>	<i>Orconectes limosus</i>
<i>Fallicambarus fodiens</i>	<i>Orconectes virilis</i>
<i>Procambarus acutus acutus</i>	<i>Orconectes rusticus</i>

COMMON NAMES FOR MARYLAND FRESHWATER BIVALVES

Alewife floater	Green floater (E)
Asian clam	Northern lance
Atlantic spike	Paper pondshell
Brook floater (E)	Plain pocketbook
Creeper/squawfoot	Susquehanna elktoe
Dwarf wedgemussel (E)	Tidewater mucket
Eastern elliptio	Triangle floater (E)
Eastern floater	Yellow lampmussel
Eastern lampmussel	Yellow lance
Eastern pondmussel	

INVASIVE PLANT SPECIES TRACKED BY MBSS

Autumn olive	Multiflora rose
Common reed (Phragmites)	Norway maple
Empress tree	Oriental bittersweet
English ivy	Porcelainberry
Garlic mustard	Privet
Giant hogweed	Purple loosestrife
Japanese barberry	Shrub honeysuckle
Japanese honeysuckle	Tree of heaven
Japanese hops	Wineberry
Japanese knotweed	Wisteria
Japanese stilt grass	
Kudzu	
Mile-a-minute	

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 _____

Cooler Temperature on Receipt _____ °C

Analyze For:

YYMMDD _____

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 Military _____

Field Comments:

Cooler Contents
 Total Number of:

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

Cooler relinquished by:

 (print name)

 (signature)

 (print name)

 (signature)

Date and Time of Receipt at Laboratory:
 YYMMDD Time (24hr) _____

Cooler received by:

Lab Comments:

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.
(Done by field office personnel) Enter the Benthic Sample Log-in number.
8. **Field Office Log-In Number**
9. **Comments** Place any pertinent comments regarding the delivered samples, including unusual circumstances, here. Examples include "label for sample from site HA-P-056-312 fell off - see label in container" or "some of sample for site HA-P-056-312 spilled while in transport".

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

MBSS SPRING INDEX PERIOD DATA SHEET

Page Of
 First Second

Watershed Code _____ Segment _____ Type _____ Year 2007

Reviewer: _____

Year Month Day
 DATE 07 _____

CREW: _____

STREAM: _____

TIME _____ (Military)

LOCALITY: _____

SAMPLEABILITY

Benthos

Habitat Assessment

Water Quality

Vernal Pool PRESENT/ABSENT

OTHER (SPECIFY) _____

SITE ACCESS ROUTE

SAMPLE LABELS

Verified by: _____

QC LABEL

Watershed Code _____ Segment _____ Type _____ Year _____

(Letters only)

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

TEMP. LOGGER

(Y/N)

WATER NUM. _____

AIR NUM. _____

LOCATION _____

PHOTODOCUMENTATION

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

None Observed

HERPETOFAUNA

	Lifestage				HEARD		
	Adult	Juv.	Larval	Egg	SEEN	Y/N	RETAINED
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						

BENTHIC HABITAT SAMPLED

Riffle

Rootwad/Woody Debris

Leaf Pack

Macrophytes

Undercut Banks

Other _____

SAMPLING CONSID.: (NUM. ANODES) _____

STREAM WIDTH (m) _____

0 m 75 m

MBSS SPRING HABITAT DATA SHEET

Page Of

Watershed Code _____ Segment _____ Type _____ Year 2007

First _____ Second _____
Reviewer: _____

Year _____ Month _____ Day _____
DATE 07 _____

_____ Dist. from Nearest Road to Site (m)

_____ Trash Rating 0 - 20

LANDUSE (Y/N)

- | | |
|--|---|
| <input type="checkbox"/> Old Field | <input type="checkbox"/> Residential |
| <input type="checkbox"/> Deciduous Forest | <input type="checkbox"/> Commercial/Industrial |
| <input type="checkbox"/> Coniferous Forest | <input type="checkbox"/> Cropland |
| <input type="checkbox"/> Wetland | <input type="checkbox"/> Pasture |
| <input type="checkbox"/> Surface Mine | <input type="checkbox"/> Orchard/Vineyard/Nursery |
| <input type="checkbox"/> Landfill | <input type="checkbox"/> Golf Course |

RIPARIAN VEGETATION (facing upstream)

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

ROAD CULVERT

Present in Segment? (Y/N) _____

Sampleable? (Y/N) _____

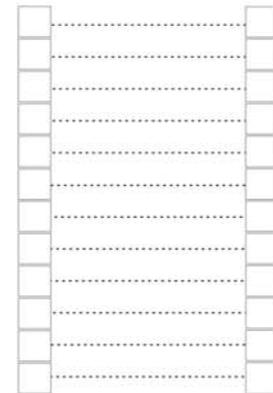
Width of Culvert (m) _____

Length of Culvert (m) _____

STREAM GRADIENT

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

- Storm Drain
- Tile Drain
- Impervious Drainage
- Gully
- Orchard
- Crop
- Pasture
- New Construction
- Dirt Road
- Gravel Road
- Raw Sewage
- Railroad



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

CHANNELIZATION

Evidence of Channel Straightening or Dredging (Y/N)

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

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

Lat _____

Lon _____

Stream Block Ht. (m) _____

Stream Block Type _____

Lat _____

Lon _____

MBSS VERNAL POOL DATA SHEET

Page of

Watershed Code _____ Segment _____ Type _____ Year 2007

First _____ Second _____
Reviewer: _____

Vernal Pool ID: V.P. _____ Lat _____ Long _____

Within transect? (Y/N):

Dimensions: m X m

Max Depth: cm

Landscape Setting: Upland or Floodplain

Predominant Surrounding Landuse:

Fish Observed (Y/N): Dist. From Pool →

Anostraca Observed (Y/N):

HERPETOFAUNA							
None Observed	Lifestage				Seen (Y/N)	Heard (Y/N)	Num. Retained
	Adult	Juv.	Larval	Egg			
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						

PHOTODOCUMENTATION				Voucher (Y/N)
Time	Number	Title		
_____	_____	_____	_____	
_____	_____	_____	_____	
_____	_____	_____	_____	

COMMENTS: _____

Vernal Pool ID: V.P. _____ Lat _____ Long _____

Within transect? (Y/N):

Dimensions: m X m

Max Depth: cm

Landscape Setting: Upland or Floodplain

Predominant Surrounding Landuse:

Fish Observed (Y/N): Dist. From Pool →

Anostraca Observed (Y/N):

HERPETOFAUNA							
None Observed	Lifestage				Seen (Y/N)	Heard (Y/N)	Num. Retained
	Adult	Juv.	Larval	Egg			
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						
_____	<input type="checkbox"/>						

PHOTODOCUMENTATION				Voucher (Y/N)
Time	Number	Title		
_____	_____	_____	_____	
_____	_____	_____	_____	
_____	_____	_____	_____	

COMMENTS: _____

MBSS SUMMER FAUNA DATA SHEET

Page Of

Watershed Code _____ Segment _____ Type _____ Year 2007

Reviewer: _____ / _____

None Observed

STREAM SALAMANDERS

Species	Number Retained?	Electrofishing Catch (Total)	General Catch (Total)	Transect Catch (Total)
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Habitat Types Sampled in 25m Transect

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

None Observed

OTHER HERPETOFAUNA

Species	Lifestage				Number Retained?
	Adult	Juv.	Larval	Egg	
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	└─┘

None Observed

MUSSELS

Species	Live/Dead	Number Retained?
_____	└─┘	└─┘
_____	└─┘	└─┘
_____	└─┘	└─┘
_____	└─┘	└─┘
_____	└─┘	└─┘

Corbicula (Dead Shell, Live, None)

None Observed

CRAYFISHES

Species	Number Retained?	1st Pass Catch (Total)	2nd Pass Catch (Total)
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>
_____	<input type="text"/>	<input type="text"/>	<input type="text"/>

Crayfish Burrows
(Absent, Present, Extensive)

COMMENTS

MBSS GAME FISH LENGTH DATA SHEET

Page Of

Watershed Code Segment Type Year 2007
 SITE

Reviewer: /

#	SPECIES	LENGTH (TL; mm)	#	SPECIES	LENGTH (TL; mm)								
1.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					31.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
2.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					32.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
3.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					33.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
4.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					34.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
5.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					35.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
6.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					36.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
7.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					37.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
8.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					38.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
9.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					39.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
10.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					40.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
11.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					41.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
12.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					42.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
13.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					43.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
14.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					44.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
15.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					45.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
16.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					46.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
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19.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					49.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
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22.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>					52.	_____	<table border="1" style="width: 100%; height: 20px;"><tr><td> </td><td> </td><td> </td><td> </td></tr></table>				
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MBSS VOUCHER SPECIMEN LABELS

<i>Maryland Biological Stream Survey</i>	
SITE ID	_____
Cat. No.	_____ Family: _____
Species:	_____
Basin:	_____ Date: _____
State:	_____ County: _____
Locality:	_____
Lat:	_____ Lon: _____
Col. By:	_____
Det. By:	_____ No.Specimens: _____