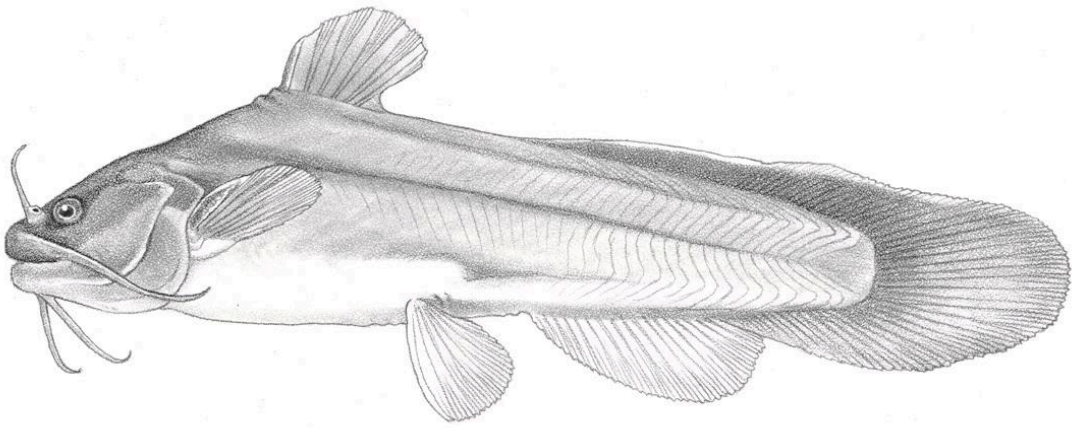
The Maryland Biological Stream Survey:

Field Sampling Manual



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Department of Natural Resources
Resource Assessment Service

MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

Maryland

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Foreword

This document titled "The Maryland Biological Stream Survey: Field Sampling Manual" was prepared by staff in the Maryland Department of Natural Resources (DNR), Monitoring and Non-Tidal Assessment (MANTA) Division. It provides written standard operating procedures for all aspects of Maryland Biological Stream Survey (MBSS) sampling. To facilitate data comparability, we encourage the use of this manual by anyone collecting stream ecological data using MBSS protocols, especially those seeking certification in MBSS benthic macroinvertebrate, fish, or physical habitat sampling protocols. This version was updated in 2024 to include protocols used by the MBSS since 2021.

Purpose of Manual

This document was prepared to support the ongoing efforts of the MBSS. It is imperative that the standard protocols used for every aspect of the MBSS be provided to guide the collection of consistent high-quality data and to ensure that the goals and objectives of the MBSS are met. These written protocols also provide information that standardize procedures used by the MBSS, which ensures comparability of data and results generated by the MBSS. All persons working on the MBSS or generating reports using MBSS protocols should be familiar with the information provided herein.

Certification in certain MBSS sampling protocols was first offered in 2012. This manual describes protocols for four aspects of MBSS sampling for which certification is offered, including Benthic Macroinvertebrate Sampling, Fish Crew Leader, Fish Taxonomy, and Physical Habitat Assessment. This manual should be the primary reference for detailed descriptions of these protocols.

This manual also provides detailed descriptions of all other field sampling protocols to be followed by DNR MANTA staff as part of the MBSS and other practitioners of MBSS protocols. Additional information regarding the MBSS certification process, other aspects of the MBSS for which certification is being offered, and requirements for attaining certification can be found on this Maryland Department of Natural Resources website (www.dnr.maryland.gov/streams/Pages/mbsstraining.aspx).

Maryland Biological Stream Survey Goal and Objectives

The goal of the MBSS is to provide the best possible information for ensuring the protection and restoration of Maryland's stream ecological resources. There are four objectives that the MBSS pursues to attain this goal: 1) Assess, with known confidence, the current condition of ecological resources in Maryland's streams and rivers; 2) Identify causes of adverse effects (stressors) to ecological resources; 3) Provide an inventory of biodiversity in Maryland's streams; and 4) Document changes (improvements and degradation) over time in Maryland's stream ecological conditions and biodiversity status.

The fourth objective – "document changes (improvements and degradation) over time in Maryland's stream ecological conditions and biodiversity status" - is achieved through repeated "rounds" of sampling throughout the state. There have been a total of five rounds of MBSS sampling since 1995. The first three rounds of the MBSS provided estimates of statewide stream conditions at varying scales. The fourth round of MBSS sampling, completed in 2018, was designed to assess changes in statewide stream condition between the preceding rounds by re-sampling many of the same sites visited previously. The fifth round of MBSS sampling, initiated in 2021, targets a different randomly selected site annually in each of the 84 Primary Sampling Unit watersheds in Maryland. These sites are selected from a 1:24,000 scale stream reach file which includes smaller streams and more comprehensively represents Maryland's varied stream

resources. This design allows statewide estimates of stream condition annually, as well as the ability to include several years of sampling to improve resolution in conditions estimates.

Introduction

Data and results generated by the MBSS have been widely used for making management decisions. Examples include Maryland's Integrated Report of Surface Water Quality, as well as identification of Tiered Aquatic Life Uses and aquatic biodiversity priority areas. Additionally, data from the long-term monitoring of reference (Sentinel) sites in Maryland are being used to explore both natural variability and variability that may be attributable to climate change. The quality, usefulness, and availability of MBSS data are also exemplified by their use in numerous peer-reviewed scientific publications. We anticipate that MBSS data will also continue to be useful for examining potential changes in Maryland's stream conditions over time.

For MBSS data to be useful in evaluating changes in stream health over time, it will be important that these data remain comparable with data collected during previous rounds of the survey. Thus, it is important to make sure that the protocols used for collecting data between years and rounds are as similar as possible. To this end, changes to protocols for collecting stream data including fish, benthic macroinvertebrates, water chemistry, and physical habitat in the field have been minimal since the program's inception in 1995.

There have been no notable changes to the protocols for fish and benthic macroinvertebrate sample collection since 1995. These are the core of this biological survey, and consistency has been maintained to ensure comparability of data throughout the duration of the survey.

Protocols for water chemistry grab samples have not changed since 1995. There have, however, been changes to the parameters analyzed. During Round One, six water chemistry variables (pH, ANC, sulfate, nitrate, conductivity, and DOC) were analyzed from spring water grab samples. At the beginning of Round Two (in 2000), six additional parameters (chloride, total nitrogen, nitrite, ammonia, total phosphorus, and orthophosphate) were analyzed from water samples to provide better assessments of nutrient concentrations. During Round Four, four additional parameters (copper, zinc, magnesium and calcium) were analyzed from water samples. Currently, all previously analyzed water chemistry parameters continue to be retained.

While the protocols for collecting in situ water chemistry measurements have not changed, the periods when these samples have been collected and the parameters recorded have changed. During Round One and Round Two, in situ measurements of pH, temperature, conductivity, and dissolved oxygen were recorded. At the beginning of Round Three and continuing through Round Four, in situ summer measurements of pH, temperature, conductivity, and dissolved oxygen were no longer being taken because all of these (except for dissolved oxygen) were available from the spring water sample or temperature loggers. At the beginning of Round Five, summer in situ dissolved oxygen measurements were resumed.

The major elements of MBSS physical habitat monitoring; the physical habitat assessment, assessments of riparian buffers and stream channel modifications, and measurements of bank erosion; have remained largely consistent throughout the survey. This ensures data comparability in this core habitat assessment over time

and between rounds. Beyond this core suite of habitat-related variables, various other procedures have been added and removed over the years. These have included assessments of stream character, flow, and geomorphology. Beginning in 2021, other channel modification types were added including the extent of bank regrading, the presence of intentionally placed woody structures, and the presence of erosion control matting installed within the sampled reach.

In addition to fish and benthic macroinvertebrates, the MBSS has maintained a consistent interest in other faunal groups, including stream salamanders, as well as other reptiles and amphibians, crayfishes, and freshwater mussels. These groups have been a part of the MBSS since Round One, though the exact data collected, and the intensity of survey efforts employed, has varied.

1.0 Personnel and Crew Qualifications

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

Along with having qualified persons in each of these positions, all MBSS field Crew Members and the Crew Leader must have received training in MBSS protocols during each season and year they are serving as a part of the MBSS. Additionally, the field crew must be made up of persons who collectively passed all MBSS taxonomy tests for any taxonomic groups that the crew plans to collect and identify in the field. For example, the fish taxonomy test must be passed by at least one person on the crew for that crew to identify fishes, and that person must conduct all the fish taxonomic identifications for their crew. Since benthic macroinvertebrates are identified in the laboratory, no one on the MBSS field crew is required to pass a 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 3.5; Quality Assurance). Typically, audits are performed annually at a minimum of one site sampled by each crew by the MBSS QC Officer. However, additional audits may be required depending on the experience of the crew, performance on previous audits, and intended use of collected data. Audits can only be performed by a qualified MBSS QC Officer. This individual has had extensive MBSS crew leader experience, has extensive experience in conducting MBSS training, and is familiar with the intended use of MBSS data by the crew being audited. The QC Officer should also be familiar with aspects of the MBSS other than field data collection (e.g., laboratory protocols, IBI calculation, data management).

2.0 Health and Safety

The purpose of this chapter is to provide recommendations for health and safety aspects to persons involved in MBSS field collections. Suggested training and qualifications are described, along with

general safety procedures, sampling hazards, provision of first aid, and emergency situations. The ultimate responsibility for health and safety of field crews lies with the parent organization for each field crew.

2.1 Training Requirements

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 for all field survey personnel:

- Recent (within 2 year) physician's approval to conduct rigorous physical work
- Recent (within 2 year) CPR certification
- Recent (within 2 year) Red Cross or equivalent first aid training
- Complete a satisfactory interview about health and safety aspects of the MBSS with the Field Crew Supervisor, including routine safety precautions and a discussion of actions to be taken in an emergency.

In addition, Crew Leaders should have adequate field sampling experience under rigorous conditions.

2.2 Duties and Responsibilities

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

Field Crew Supervisor

The Field Crew Supervisor has overall responsibility for health and safety aspects of the MBSS.

Crew Leader

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

- Instructing and supervising the survey crew such that sampling at and travel to a given site are done in a manner that 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 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.

Field Crew Members

All personnel involved in field sampling or field observations (e.g., QA/QC inspections) should be aware of the risks involved with the routine aspects of MBSS. When unsafe or hazardous conditions are observed, crew members should inform the Crew Leader at the earliest opportunity. In addition, crew members should notify the Crew Leader if, for any reason, they cannot perform an assigned task in a safe manner. Examples include sickness, physical limitations, or uncertainty about proper operation of the sampling equipment. Field crew members should also inform the Crew Leader of any allergies or medical conditions (e.g., diabetes, asthma, allergies) and any special needs (e.g., inhaler, epinephrine pen) the crew member has. It is the responsibility of each crew member (not the Crew Leader) to make sure he or she has any special needs medicine or equipment and that the Crew Leader knows about that special need.

2.3 Sampling Hazards and Procedures for Minimizing Risk

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

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

    - An inspection of the sampling vehicle should be performed by the Crew Leader or a designee prior to sampling departure. This inspection should include tire condition and operability of wipers, defroster, etc.;
    - During sampling activities, any potentially unsafe vehicle condition should be reported to the Field Crew Supervisor and corrected as soon as is practical;
    - If, in the judgment of the Crew Leader, the sampling vehicle is not safe to operate, the vehicle should not be operated until the condition is rectified; 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 driver's license.

2. Electric Shock. Failure to observe appropriate safety precautions when using 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 electrofishing units;
    - 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. The use of rubber gloves is highly recommended due to the danger of electric shock that could occur from contact with water being sampled;
    - The bare wire portion of the cathode (rattail) or the anode of an electrofishing unit 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. The use of a cell 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; and
- 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.

In cases where multiple electrofishing units (or an electrofishing barge with multiple anodes) are used to sample 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 multiple electric fields in the event a crew member slips or falls into the water, or the discharge of one anode completing the switch circuit for another unit.

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

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

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

- Prior to sampling, the Crew Leader should ensure that all crew members who are to enter the stream are physically fit enough to do so and are aware of any specific sampling risks at the site;
- Prior to sampling, the Crew Leader should make a determination 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; and
- Field Crew Members should wear chest waders outfitted with waist belts and sticky rubber soled wading boots and/or cleats should be used in rocky areas. Felt soled boots are banned throughout the State of Maryland and should not be worn to avoid the

transmission of harmful aquatic organisms.

5. Slippery Substrate. During the MBSS, sampling at some sites will be hazardous due to slippery substrates. 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 Crew Leader should factor the slipperiness of the substrate into decisions as to whether a site can be sampled and any extra precautions to be taken by the field crew. To improve traction, all wading gear should include boots with textured rubber outsoles and/or cleats.

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

   - All field survey personnel should receive training in field identification, avoidance of, and first aid for dangerous plants and animals which may be encountered during the MBSS;
   - Crew members should inform their Crew Leader of any known allergies and keep appropriate medical relief in the first aid kit (at a minimum, each crew should keep an emergency supply of Benadryl – gel caps or liquid are preferred because they enter the bloodstream more quickly than tablet form);
   - The Crew Leader should make all crew members aware of site- or situation-specific dangers. Similarly, field crew members should inform the Crew Leader as soon as they are discovered; and
   - All crew members should be informed of the risks of Lyme disease and should check themselves thoroughly for ticks after conducting field work.

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 hazards 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.
   - Prior to entering the stream, the Crew Leader should make note of any evidence of high bacterial levels (e.g., strong sewage odor; solids visible in stream; off-color water). Using this information, a determination should be made whether the site can be safely sampled. Sites where high levels of bacteria contamination is confirmed should NOT be sampled. If bacteria contamination is suspected, the use of gloves should be maximized during the sampling

process. Open wounds should not be exposed to contact with stream water. After exposure to stream water, all crew members should clean their hands using an alcohol-based hand sanitizer and/or antibacterial soap 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. Sites where contamination with hazardous waste is suspected or confirmed should NOT be sampled. Any suspected hazardous waste contamination discovered while sampling (or attempting to sample) should be reported to MDE. If contact with hazardous waste is suspected, all exposed equipment should be thoroughly cleaned, including waders and any exposed personal equipment. No food or water should be consumed at known hazardous waste sites and following sampling should 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 emergency 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. One of the most prevalent risks to MBSS sampling crews is the risk of dehydration. Fresh water 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.

12. Snags and Hanging Branches. Working at any site in a wooded area could expose the crew to standing or leaning snags or hanging branches. Any seemingly unstable trees or hanging/loose woody debris should be pointed out to the Crew Leader and the other crew members, especially under windy conditions. Additional caution should be employed around the unstable structure, if the vicinity of the structure cannot be avoided altogether.

2.4 First Aid

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

2.5 Emergencies

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

2.6 Precautions for Minimizing Ecological Risk (Decontamination)

An increasing potential exists for transferring non-native and invasive organisms (including those that cause serious diseases to native stream dwelling flora and fauna) from one stream to another while conducting monitoring. Whirling disease (a protist, *Myxobolus cerebralis*), Didymo (an algae, *Didymospenia geminata*), and amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) are examples of such organisms. In addition, avian influenza can be transferred among farms simply by walking in the poultry litter that came from infected poultry and then walking in another area with poultry. It is important to properly clean all footwear or other equipment that may have contacted contaminated water or substrates.

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

The disinfection procedure consists of soaking or rinsing all equipment that has come in contact with water (or poultry litter) in a 10% bleach or 1% Virkon (https://virkon.us/virkon-aquatic/) solution for at least one minute. Equipment with a smooth surface (e.g. buckets, sides – but not soles - of waders) can be sprayed with bleach or Virkon solution. After soaking and scrubbing have been completed, all equipment must be rinsed with freshwater to remove the bleach or Virkon solution. Avoid skin and eye contact with decontamination solutions as they can be severely irritating. Thoroughly rinsing all equipment with freshwater minimizes risk of skin and eye irritation, as well as degradation of equipment. Decontamination should occur away from surface waters to avoid polluting them.

3.0 Quality Assurance

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

3.1 Introduction

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

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

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

3.2 Stream Population of Interest

The current population of interest for the MBSS includes all non-tidal, 1st through 5th order stream reaches represented on the 1:24,000 map of streams in the State of Maryland, excluding reservoir-like impoundments which substantially alter the lotic nature of the reach.

3.3 Comparability and Completeness

Comparability of data between field crews is maximized by providing standardized training in MBSS techniques prior to each sampling period. Training is mandatory for all persons involved with MBSS data collection.

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

3.4 Documentation

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

3.5 Field Audits

For the field data collection component of the MBSS, the QC Officer is primarily responsible for conducting field audits. At least one site sampled by each MBSS crew annually during each index period should be subject to audit. However, additional audits may be required depending on the experience of the crew, performance on previous audits, and intended use of collected data. Field audits include, but are not limited to: checking for consistency and accuracy in taxonomic identification, site location confirmation, verification of calibration and maintenance of equipment, confirmation of adherence to established protocols, review of required record keeping, and prompt identification of necessary remedial or corrective actions.

For taxonomic identification, the QC Officer may designate someone who is an expert in a particular taxonomic group to verify accurate taxonomic identification.

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

3.6 Training Requirements

An important aspect of the MBSS QA program is the training program for field personnel, which will be conducted prior to each spring and summer sampling period. Training helps to ensure consistent implementation of required procedures and attainment by each person of a minimum level of technical competency. All participants in MBSS field sampling must receive MBSS training. Additionally, the field crew must be made up of persons who collectively passed all MBSS taxonomy tests for any taxonomic groups on which the crew plans to collect field data and identify organisms to species in the field (e.g., at least one member of each field crew must pass the fish taxonomy test every year for the crew to be qualified to collect MBSS fish data). See section 3.11 for details regarding taxonomic identification as it pertains to MBSS field sampling. Since benthic macroinvertebrates are identified in the laboratory, no one on a MBSS field crew is required to pass a benthic macroinvertebrate taxonomy test to collect benthic macroinvertebrates.

3.7 Equipment Maintenance and Calibration

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

For each item of equipment used as part of the MBSS, a bound logbook or electronic record for calibration

and maintenance must be maintained. Entries in the log must be made for all calibration and maintenance activities. Documentation includes detailed descriptions of all calibrations, adjustments, and replacement of parts, and each entry must be signed and dated.

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

3.8 Field Information Management

Each MBSS site is assigned a unique identification code that is recorded on all MBSS data sheets. This code has four parts:

1. Watershed code. The appropriate four letter code indicating the eight digit watershed containing the site (watershed codes are found in Appendix C).

2. Segment. Three numbers are used to designate the stream segment obtained from the appropriate reach file. These three letters begin with the stream order and the next two letters refer to the order in which the site was selected. For random sites, the order in which the sites were collected can be important as sites lower in order being sampled indicate less probability of bias (i.e. in being representative of watershed conditions) compared to having many sites with higher order sampled.

3. Type. A one letter code is used to designate the site type. Examples of site type codes include “R” for random sites, “S” for sentinel sites, “X” for special study sites and “T” for targeted sites.

4. Year. The last four digits in the site identification are the calendar year during which sampling occurred.

Currently, all MBSS data is recorded using an electronic datasheet on a handheld tablet computer. The tablets are outfitted in rugged waterproof cases, and care is taken to prevent them from overheating due to solar exposure. To ensure that all appropriate data are collected at each site, spreadsheets are set up prior to each field season and they include labeled cells corresponding to all of the prescribed fields on traditional MBSS paper datasheets. Screenshots of standard blank MBSS electronic datasheets for the spring and summer index periods are presented in Appendices H and I, respectively.

If data are to be recorded on paper, data sheets should be printed on waterproof paper to facilitate data recording during inclement weather. Paper versions of spring and summer datasheets that are similar to the versions last used by the MBSS in 2017 but have been updated to include all the parameters currently recorded electronically, are presented in Appendices J and K, respectively. These may be useful for those not equipped for electronic data collection or as a backup in case of electronic data collection equipment failure.

All data should be backed up daily. Electronic data sheets should be backed up using an external thumb drive or a cloud-based system that may be accessed via WiFi or a cellular data plan. Digital photographs should be labeled appropriately with site identification and backed up, either on an external hard drive or using a cloud-based system. Paper data sheets should have physical copies made and stored in a safe location, or have scanned electronic copies stored on an external hard drive or using a cloud-based system.

To ensure that all field data for the MBSS are collected and recorded in a usable manner, all data should be recorded in the units specified on the MBSS data sheets. Paper data sheets for a given site, if used, 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 should be clearly marked with the unique identification code for the site where it was collected to minimize the possibility of misidentification. In addition, chain-of-custody forms should be maintained for all water and benthic macroinvertebrate samples (Appendix F), as well as herpetofauna, crayfish, mussel, and fish voucher specimens.

3.9 Data Quality Assessment

Assessment of data quality against established data quality objectives will be conducted to determine the overall performance of the QA program, identify potential limitations to use and interpretation of the 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. This is accomplished and summarized through QA data and reports.

Some level of variability is unavoidable when sampling, but steps can be taken to minimize its effects on data quality. Site conditions and sampling efficiency may vary with geography and/or physiographic province. Field crews may have differing levels of sampling efficiency, experience, and knowledge of protocols, especially when utilizing contractual and/or temporary workers. Additionally, it is likely that sampling efficiency increases over time as crews gain experience. In all cases, field crews should contain as many experienced personnel as possible and any inexperienced crew members should be thoroughly trained. Crew leaders and experienced crew members will each have completed multiple seasons of MBSS sampling and should be familiar with the range of sampling conditions and requirements that must be met to collect quality data throughout the state. Making sure experienced staff lead and are a part of all sampling crews will help ensure that established standards of data quality are met. The QC Officer, who will have more experience than even the crew leaders, will oversee all survey crews and ensure all are operating at the same level and meeting the same standards.

3.10 Duplicate Samples

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

3.11 Taxonomic Identification and Specimen Vouchering

The MBSS is recognized as providing the highest quality biological data. This is due primarily to the QA/QC

requirements for taxonomic identification. The following taxa are identified to species (or sub-species in some cases) in the field: fishes, reptiles, amphibians, crayfishes, and freshwater mussels. The crew conducting MBSS sampling must consist of members who, collectively, have passed identification tests for all of these taxonomic groups. Only those on the field crew who have passed the test for the taxonomic group should conduct identification in the field.

During each calendar year of MBSS sampling, each field crew should maintain a voucher collection of at least five specimens of each fish, herpetofauna, mussel, and crayfish species encountered (as long as five were collected). The voucher collection can consist of photographs and/or preserved specimens of each species.

Photographic vouchers of fish, herpetofauna, mussel, and crayfish species are accepted in lieu of preserved specimens, as long as the diagnostic features for identifying the specimens photographed can be seen clearly. Dead mussel shells can be retained. Live mussels should be photographed and released. Specimens of crayfishes should be preserved in lieu of photographs when possible. Any rare, threatened, or endangered (see Appendix E) species encountered should be photographed and not preserved. Nuisance species (Appendix E) may be photographed or preserved. Care should be taken while taking photographs to avoid harming live specimens. Photographs must clearly show the appropriate features necessary for identifying the species. With the exception of rare, threatened, or endangered species, specimens that are too small to provide photographs that can be used to verify identifications (or with diagnostic features that do not show up well in photographs) should be preserved for verification. Please see Appendix A for detailed fish fixation and preservation procedures.

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

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

3.12 Legibility

If paper data sheets are to be used, data must be recorded on data sheets legibly to ensure accurate transfer of information from hard copy data sheets to the MBSS database. If the handwriting of certain individuals is deemed illegible by the crew leader, then those individuals should not record data on data sheets.

4.0 Preparation for Sampling

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

4.1 Equipment

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

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 (electronic or paper), preservatives, sample bottles, etc., needed for the next day are identified.

4.2 Vehicles

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

5.0 Sample Collection

5.1 Introduction

The purpose of this chapter is to describe, in detail, the specific procedures that must be followed during sampling for the MBSS, including water quality, benthic macroinvertebrate, fish, reptile, amphibian, crayfish, mussel, 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.

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. Primary activities conducted during the Spring Index Period include: benthic macroinvertebrate sampling, collecting water chemistry samples for laboratory analysis, select physical habitat variable sampling, and deploying temperature loggers. During the Summer Index Period, primary activities include: fish sampling and additional select physical habitat sampling, as well as recording in situ dissolved oxygen levels and sampling other faunal groups including reptiles and amphibians, stream salamanders, freshwater mussels, and crayfish. 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. Temperature loggers deployed during the Spring Index Period are typically retrieved during a third site visit near or after the end of the Summer Index Period.

The time period for the Spring Index Period was determined based on the preferred period for benthic macroinvertebrates sampling and because studies in Maryland have demonstrated that chemistry samples taken during this time can better represent acid and nutrient conditions in streams.

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 encompasses all days within these two months.

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

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

5.3 Site Location

Depending on when they were sampled by DNR, MBSS sites may be new randomly selected sites, revisits of sites that were sampled during previous rounds, or targeted sites used to answer important management questions. Stream reach files with different scales have been used to select sites in the past (1:250,000 in Round One, 1:100,000 in Rounds Two and Three) , but the current MBSS efforts beginning in 2021 have included smaller streams (and more comprehensively represent Maryland's streams) by utilizing a 1:24,000 scale stream reach file to select site locations.

The complete list of randomly selected sites to be sampled each year as part of DNR's statewide survey will be provided to the crew leader with a priority number assigned to each site by watershed. This list will include extra sites so that there are backups in the event that the initial visit to a site leads the crew leader to determine that the original site is un-sampleable. Crew leaders should attempt to sample the lowest number (corresponding to the highest priority) sites on the list with landowner permission first, by watershed, until the target number of sites in each watershed has been sampled. The entire list of targeted sites used to answer management questions or monitor the impacts of stream restorations (i.e., sites that are not part of the random statewide survey) should be sampled in the given year. There are not typically backups or replacements for these sites, and they are not ranked by priority- all should be sampled.

Maps showing landowner properties (usually tax maps) should also be consulted to ensure that the site is located on a property or properties where landowner permission has been acquired. Permission to use any

landowner's property for access to or sampling of any MBSS site is required. In extreme cases, where landowner permission or other sampleability issues prohibit sampling a site in the exact location where the site was chosen, the site may be moved up or downstream no more than one site length distance (75 m) from the original location and substantial documentation must be provided in the comments section of the Spring Index Period Data sheet to justify the location change. This option should be used only after all other options have been exhausted by the Crew Leader. A map showing the location of the site must also be included with data sheets, as well as proper landowner permission information.

Each site is determined from a set of geographic coordinates. The coordinates represent the midpoint of the 75 m long site (37.5 m from both the downstream and upstream ends of the site). If arrival at these coordinates occurs and the location is not on a stream, the mid-point of the site should be designated as the point that is reached using the shortest distance to the stream from the location indicated by the GPS. No matter how the site location for sampling during the current Round is determined, new geographical coordinates from the actual marked mid-point of the site should be recorded on the Spring Habitat Data Sheet. A copy of the applicable 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 accessed.

All sites consist of the watered portion of the stream and an area 50 meters perpendicular (on both sides) to the stream. Each site is 75 m in length. All sites consist of four transects that are 0, 25, 50, and 75 meters upstream of the most downstream point in the site.

It is important to sample any site that is a repeat as closely as possible to locations previously sampled. Crew leaders should use geographic coordinates and previous site location descriptions (as recorded on previous datasheets) to relocate sites. Extant flagging tape should always be considered the more precise mark of transect locations. If markings from previous sampling visits are found while re-visiting a site, those previous markings should be given priority over geographic coordinates for locating the current survey. Any additional flagging needed to mark transects should be added and flagging in poor condition should be replaced. If, based on reasonable effort, the markings for a previously-sampled site are not found (either during an initial revisit during the spring or during the subsequent summer visit), then the site location should be determined based on geographic coordinates and, when available, site location descriptions recorded during previous visits.

5.4 Site Selection and Determination of Sampleability

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

the spring visit, a Spring Index Period Data Sheet should be filled out indicating the reason the site was not sampleable. The next highest priority number site, with permission to sample, should then be sampled in place of the unsampleable site. If a site is unsampleable during the summer visit, it should be marked as such using the appropriate code(s) on the Summer Index Period Data Sheet and not sampled. Another site should not be sampled in its place.

Some notes on special situations:

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 cannot be electrofished, the length of the culvert should be measured and recorded on the data sheet and the length added to the original 75 m site. If the culvert occurs in the first half of the site, the additional distance should be added to the downstream end of the site. Similarly, the additional distance should be added to the upstream end, if the culvert is within the upper half of the original site. If the culvert can be sampled completely, no change should be made to the original 75 m site.

2. Moving Sites. The location of a site (even a randomly selected site) can be changed to ensure that a sample is collected as close as possible to the location originally chosen for sampling. However, the maximum distance that a site should be moved is 75 meters. Additionally, the moved site may not be on a different stream reach than the original site. 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 establish sampleability at the site in its originally chosen location.

3. Braided Streams. When a site is located on a braided stream (i.e., a stream with multiple parallel and/or interwoven channels), all the braids should be sampled. Each braid should be measured and marked along with the main channel (if a “main” channel is discernible) and included in any sampling considerations (e.g., number and size of block nets, number of anodes required). Each braid should be included in habitat assessments, electrofishing surveys, and surveys for herpetofauna, crayfish, and freshwater mussels. Water chemistry, temperature, and pebble counts should only be sampled in the main channel, or in a single representative channel if a “main” channel is not discernible. Care should be taken to differentiate braids from tributaries. It may be necessary to consult topographic maps or satellite imagery, or to engage in physical reconnaissance to determine whether multiple channels at a site are tributaries or parts of a braided system. Unlike braids, tributaries should be considered separate and are not to be sampled.

5.5 Marking Sites

The 75 meters 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. Marking of all four transect locations (0 m, 25 m, 50 m, and 75m) needed to complete Summer Index Period habitat sampling is recommended during the spring index period. Typically, orange spray paint (where permitted) and flagging are used to mark the 0 m and 75 m locations and flagging is used to mark the 25 m and 50 m locations. Effort should be made to adequately mark the site in the spring so it can be found again during the summer, with consideration to the aesthetics of such marks and any requests by landowners. 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 gray during the summer visit.

5.6 Photographic Documentation

Photos should be taken at all MBSS sites using a digital camera. 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 during either index period 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, riparian tree cutting, or other forms of habitat alteration. Many different 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.

In addition, MBSS field crews should also be taking photographic vouchers of all species of fish, herpetofauna, crayfish, and freshwater mussels encountered while sampling. These photos should clearly show the diagnostic features required to identify the species being vouchered. All voucher photos should show the left side of the specimen. It may be necessary to take more than one photograph if pertinent features cannot be captured in a single frame (e.g., photographing both the lateral and ventral sides of the same fish to depict the necessary diagnostic features). Field crews should take photographic vouchers of five specimens of each species encountered annually, if possible.

All photographs should be recorded on the appropriate section of either the Spring or Summer MBSS data sheet (as determined by when the photo was taken). The digital file name (as determined by the camera) should be recorded, along with a description of the photograph, the photo type (i.e., “Fish”, “Site Midpoint”, “Habitat Alteration”, “Herpetofauna”, “Crayfish”, “Mussel”, “Field Work”, “Salamander”, “Other”), and whether or not the photograph is to be used as a voucher. All files should be appropriately backed up throughout the index period. At the conclusion of the index period, all photograph files should be renamed using a combination of the site code where the photograph was taken and the description of the photograph content:

Example file name: MBSS-123-R-2023_photo-description.JPG

All renamed photograph files from each survey crew should be archived annually. In addition, all voucher photographs should be sent to an appropriate independent taxonomic expert for verification.

5.7 Water Chemistry for Laboratory Analysis

Select water quality variables are measured based on grab samples collected during the Spring Index Period (1 March to 30 April). These analytes provide information about acidification, nutrients, and specific ions. They include:

- Acid Neutralizing Capacity
- Ammonia
- Bromide
- Calcium
- Chloride
- Conductivity
- Copper
- Dissolved Organic Carbon
- Magnesium
- Nitrate
- Nitrite
- Orthophosphate
- pH
- Potassium
- Sodium
- Sulfate
- Total Nitrogen
- Total Phosphorus
- Zinc

Approximately 1.5 L of unfiltered stream water in bottles and an additional 50 mL of unfiltered stream water in a closed syringe are needed to provide data for the majority of the MBSS laboratory water chemistry parameters. Select dissolved trace metals are analyzed from an additional 125 mL sample of stream water that is filtered to eliminate particulate matter that might contain suspended trace metals.

The basic protocols used to collect samples in spring specify that 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.

Sampling during turbid conditions or just after heavy rains should be avoided. 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 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, provided that the stream was not disturbed upstream of the 75 m transect while accessing the site. When possible, the area from which water is taken should be near the center of the stream channel, in flowing water, and where adequate depth is present to completely submerge the water sampling bottles.

Each water sample container 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).

Wearing clean nitrile gloves and using care to avoid potential sample contamination from handling, fill the pre-leached 0.5 and 1 liter sample bottles to half-full, rinse, and discard. Repeat the process twice (so that the bottle has been filled and rinsed a total of three times). Then fill the sample containers such that no or

a minimum of air space exists in the neck of the bottle. Check to ensure that the seals on both sample bottles are tight. Consider wrapping the bottle seals with electrical tape to help ensure that lids remain closed during shipping.

Remove the syringe from its sealed factory packaging streamside and affix a Luer Lock valve on the end. 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 rise upward toward the Luer Lock, then use the plunger to expel the air. Install a clean 0.45 micron polyethersulfone (PES) syringe filter onto the Luer Lock fitting and push approximately 5 mL of the sample through the filter onto the bank or downstream to rinse the filter. Open the plastic bag containing the 125-mL trace metals sample bottle and carefully remove the bottle lid. Use the bag and/or clean nitrile gloves to keep the sample bottle and lid as clean as possible. Using the syringe, push the remaining sample volume through the filter into the sample bottle. Repeat until the bottle is full. Change filters as necessary when they become too clogged, but be sure to rinse each filter with about 5 mL of sample before continuing to fill the bottle. Place the cap tightly onto the bottle and reseal the plastic bag.

Fill the syringe a final time to approximately the 60 ml mark. Hold the syringe in a vertical position and gently tap it until all bubbles rise upward toward the Luer Lock. Use the plunger to expel the air and release 5 to 10 ml of sample. When the volume in the syringe is 50 to 55 ml, and while still discharging water, carefully close the Luer Lock valve. Syringes should not contain more than 55 ml of sample to minimize the possibility of plunger dislodgement during shipping, or less than 50 ml to provide sufficient water to determine the pH effectively.

Place samples on ice inside a closed lid cooler to maintain samples at 4°C until laboratory analysis is performed.

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

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

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

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.

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

5.8 In Situ Water Chemistry

In addition to laboratory water chemistry sampling during spring, in situ measurements of dissolved oxygen (mg/L and % Saturation) will be taken using a water quality meter 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 dissolved oxygen) during the Summer Index Period. These should be recorded in the "In-Situ Measurements" section on the "Summer Temp Loggers" page of the MBSS summer electronic data sheet. Other water quality parameters of interest, such as pH and specific conductance may also be measured in situ, but they are not a required part of current MBSS protocols.

In general, the manufacturer's instructions should be followed for whatever meter is used to collect summer water chemistry measurements. Prior to conducting in situ water chemistry sampling, all necessary meters must be calibrated and in working order. The Crew Leader should ensure that all calibrations are performed no less than once each week and are recorded in a calibration log. An extra meter 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, in situ water chemistry measurements must be recorded 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 recorded should be avoided until after all measurements have been completed. Recording these measurements 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, provided that the stream was not disturbed upstream of the 75 m transect while accessing the site. When possible, the area from which readings are recorded should be near the center of the stream channel, in flowing water, and where adequate depth is present to completely submerge the meter probe(s).

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 manufacturer's specifications. An instrument that is unstable or that did not pass calibration should not be used.

Readings should not be recorded until they have stabilized. After in situ measurements have been completed, necessary caps for probes should be replaced and the instruments carefully disassembled and stored for transport.

5.9 Physical Habitat

Physical habitat assessments conducted by the 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 physical habitat in Maryland's streams. MBSS habitat assessment protocols are based on a combination of metrics modified and adapted from US EPA'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 the nearest road to the site, width of the riparian buffer, width, depth, velocity, culvert width and length, extent and height of eroded bank, numbers of woody debris and root wads, extent of stream channel modifications, 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 is based on the observation (or not) of certain conditions such as buffer breaks, land use types, and evidence of channelization. Based on observations in the field, the absence, presence or extensive presence of stream features that include macro habitats, stream characteristics, substrates, and aquatic vegetation 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 the need to optimize the time required to collect usable information.

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

For MBSS physical habitat assessment variables, in all cases where it is necessary to differentiate the left bank of the stream from the right bank, the left and right are determined while facing upstream.

Only persons who have attended MBSS training and have received MBSS physical habitat assessment certification 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.

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 electronic datasheet on the “SpringHabitat'' page as well as the “Modifications” page. All spring habitat data should be recorded on these pages. The methods used to determine exactly what should be recorded for each variable are described, by variable, below. Data 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 the spring physical habitat assessment, this should be noted on the “SiteInfo” page of the MBSS Spring electronic data sheet. Codes designating the reason(s) that a stream could not be sampled are provided in Appendix C.

1. Trash Rating. The trash rating is scored on a 0-20 scale based on criteria found on the Stream Habitat Assessment Guidance Sheet (Appendix D). This rating is recorded on the “SpringHabitat” page of the MBSS Spring electronic datasheet.

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. This distance is recorded on the "SpringHabitat" page of the MBSS Spring electronic datasheet.

3. Adjacent Land Use. While at the site, a visual survey of the surrounding area for land use types is conducted. For each land use type listed under "Adjacent Landuse" on the "SpringHabitat" page of the MBSS Spring electronic datasheet, 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".

4. Riparian Buffer Width. The average width of the vegetated riparian buffer should be estimated to the nearest meter on each side of the stream, beginning at the water's edge, up to a maximum of 50 m. The average buffer width should be recorded for each bank separately, with the left and right banks of the stream determined while facing upstream. 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 7 below). The maximum allowable measurement for riparian buffer width is 50 m, even if the actual width exceeds 50 m. Riparian Buffer Width is recorded on the "SpringHabitat" page of the MBSS Spring electronic datasheet.

5. Adjacent Land Cover. The type of land cover immediately adjacent to the riparian buffer should be recorded using the codes for adjacent land cover types found in Appendix C at the back of this manual. If the buffer width is more than 50 m, the recorded code should describe the buffer itself. If the buffer is 50 m or less, the recorded code should describe the land cover where the buffer ends. Example:
   - The left bank of a stream has an 85 m forested buffer between the water's edge and a paved road. Adjacent land cover on this bank should be recorded as "FR" (forest). Riparian buffer is measured to a maximum of 50 m, and as this buffer extends beyond 50 m, the adjacent land cover is the buffer itself. The paved road in this case is irrelevant because it is outside the 50 m maximum buffer width.
   - The right bank of a stream has a 20 m forested buffer between the water's edge and a corn field. Adjacent land cover on this bank should be recorded as "CP" (cropland). Crops are not considered a buffer type. The buffer ends where the forest transitions to crops, and "CP" best describes the land cover adjacent to the 20 m buffer.

   Adjacent Land Cover is recorded on the "SpringHabitat" page of the MBSS Spring electronic datasheet.

6. Riparian Vegetation. Using the codes for vegetation types (Appendix C) the dominant vegetation types present within the 50m buffer of the 75 m site should be recorded. As many as four types can be recorded. The vegetation types are recorded in order of their dominance within the buffer, with the most dominant recorded first ("Veg Type 1"). Stem density and canopy density should both be taken into consideration for determining dominance. However, stem density should take precedence over canopy density. Riparian vegetation is recorded on the "SpringHabitat" page of the MBSS Spring electronic datasheet.

7. 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 on either bank, then "Yes" should be selected in the "Buffer Breaks Present" box on the "Modifications" page of the MBSS Spring electronic datasheet. If no buffer breaks are observed on either bank, then "No" should be selected in the

"Buffer Breaks Present" box on the MBSS Spring "Modifications" page. This box should not be left blank or as "None".

8. 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 "Modifications" page of the MBSS Spring electronic datasheet.

9. 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 "Modifications" page of the MBSS Spring electronic datasheet. Length and width of road culverts recorded here provide a measure of stream character. Details on culvert material and its impacts on the stream banks and bottom are captured in the "Channel Modifications" section of the "Modifications" page of the MBSS Spring electronic datasheet.

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 Fishing and Boating Services (FABS) keeps track of all known barriers to fish migration. The MBSS has provided the locations of many man-made barriers to fish migration to FABS 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 encountered while sampling a MBSS site should have the height (to the nearest 0.1 m) and location (latitude and longitude in decimal degrees) recorded on the "Modifications" page of the MBSS Spring electronic datasheet. The type of blockages should also be recorded. Codes for blockage types are provided in the back of the manual. Obvious blockages such as dams on major rivers need not be recorded, but if there is any doubt about whether or not to record a blockage, recording the blockage is recommended.

11. Channel Modifications. The site should be inspected for any evidence of channel straightening, dredging, or other modifications. If evidence of channel modifications is observed anywhere within the 75 m site, the linear extent of the modification should be recorded to the nearest meter on the "Modifications" page of the MBSS Spring electronic datasheet. Channel modifications along each bank and along the stream bottom should be measured separately and recorded in the appropriate portion of the "Channel Modifications" table. If channel modifications are observed at a site with a braided stream channel, the total extent of the stream channel that is modified should be recorded. Using this method, it is possible (when multiple channels are present) for the total modified extent of the left bank, right bank, or stream bottom to exceed 75 m. Since the objective of this measurement is to determine the total length of stream channel that is modified, this is acceptable. Explanations of each modification type are included below:

- *Concrete*: Linear extent of poured and formed concrete along the left bank, right bank, and the stream bottom, measured in meters. This may include the vertical walls or horizontal floor of box culverts. Aggraded bed material within a box culvert may prevent portions of either bank or the stream bottom from being included in this measurement (i.e., only record linear extents of exposed concrete in contact with the stream water).
- *Gabion*: Linear extent of gabion baskets (stones contained in pre-formed metal cages) along

the left bank, right bank, and the stream bottom, measured in meters.

- *Pipe Culvert*: Linear extent of exposed cylindrical piping (concrete or metal) that forms the left bank, right bank, or bottom of the stream channel, measured in meters. Aggraded bed material within the pipe may prevent portions of either bank or the stream bottom from being included in this measurement (i.e., only record linear extents of exposed pipe wall material in contact with the stream water).
- *Earthen Berm*: Linear extent of formed earthen berm constructed along the left bank and right bank, measured in meters.
- *Dredge Spoils Offchannel*: Linear extent of dredge spoils deposited along the left bank and right bank, measured in meters.
- *Bank Regrading*: Linear extent of stream bank that was intentionally regraded to achieve a less steep angle and/or restore floodplain connectivity, measured along the left bank and right bank in meters.
- *Riprap*: Linear extent of riprap material (loose placed stones or concrete rubble) along the left bank, right bank, and the stream bottom, measured in meters.
- *Erosion Control Fabric*: Linear extent of erosion control fabric matting placed along the left bank, right bank, and stream bottom, measured in meters.
- *Woody Alteration Structure*: Linear extent, measured in meters, of wood intentionally placed on the left bank, right bank, and the stream bottom to stabilize banks, control erosion, or provide habitat. May include beaver dam analogs.

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 electronic datasheet on the "SummerHab1", "SummerHab2", "Transects", and "Debris" pages. All summer habitat data should be recorded on these pages. The methods used to record each variable are described 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. Disturbing the area within and upstream of the site should be avoided, to the extent possible, prior to sampling so that visibility is not affected by resulting turbidity. All areas of the stream bottom containing physical habitat features within the 75 m site should be visible if a habitat assessment is to be conducted. Exceptions are sites with persistent, chronic turbidity problems, or where the water is stained dark from natural organic sources (e.g., tannins leached from leaves; blackwater streams). If a stream has insufficient water clarity for physical habitat assessment during the early part of the Summer Index Period or following a rain event, the stream should be revisited later in the Index Period or during a drier period to reassess sampleability. If return visit(s) indicate that the stream has persistently poor water clarity, a physical habitat assessment should be attempted regardless. Whether or not the entire stream bottom is clearly visible in all portions of the site should be recorded in "Habitat Notes" on the "SummerHab2" page of the Summer MBSS electronic datasheet. Although sampling can occur in chronically turbid or blackwater streams when visibility is relatively limited, sampling should not

occur in a blackwater stream that is also turbid due to recent precipitation or disturbance.

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 (due to water depth, turbidity, or any other reason), this should be noted on the "SiteInfo" page of the MBSS Summer electronic datasheet. Codes designating reasons that a stream could not be sampled are available in drop-down menus in the "Sampleability" section of the "SiteInfo" page of the MBSS Summer electronic datasheet and are also provided in the back of this manual.

1. Habitat Assessment Metrics. Five metrics: instream habitat, epifaunal substrate, velocity/depth diversity, pool/glide/eddy quality, and riffle/run quality are rated on a scale of 0-20 using criteria provided on the Habitat Assessment Guidance Sheet in Appendix D at the back of this manual. The scores for each of these metrics are meant to characterize a distinct aspect of stream habitat. All five of these metrics are recorded on the "SummerHab1" page of the MBSS Summer electronic datasheet.

   - *Instream Habitat*: Rated based on perceived quality 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 iron floc 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 usable by fish, a score of zero is assigned.
   - *Epifaunal Substrate*: Rated based on the amount and variety of stable substrates usable by benthic macroinvertebrates. Because they inhibit colonization, flocculent materials or fine sediments surrounding otherwise good substrates are assigned low scores. Certain scores are also reduced when substrates are less stable.
   - *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). This metric may result in lower scores in low-gradient streams but will provide statewide information on the physical habitat found in Maryland streams.
   - *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.
   - *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.

2. Extent of Pool/Glide/Eddy and Riffle/Run Habitat. The extent of Pool/Glide/Eddy and Riffle/Run habitat within the 75 m site should be recorded on the "SummerHab1" page of the MBSS Summer electronic datasheet. Each meter of stream habitat across the entire wetted width of the stream must be assigned to one category or the other, such that the total extent between both categories sums to

75 m. The only instance where extents of Riffle/Run and Pool/Glide/Eddy habitats may sum to more than 75 m is at sites with braided channels where the total linear extent of stream channel sampled is greater than 75 m.

3. Embeddedness. The percent of coarse riffle substrates surrounded by fine material, such as sand, silt, or flocculent material, is recorded based on visual estimation. Substrate particles that are examined should be located in the area with the fastest flow in the survey site, typically within riffle or run habitats. If no riffle or run habitat is present within the 75 m site, embeddedness can be rated based on the closest available riffle or run located on the same reach as the site (but not more than 75 m away from the upstream or downstream end of the site). Several coarse substrate particles (e.g., pebbles, cobbles) should be examined to estimate how much each one is embedded in fine substrate (i.e., what percent of the larger piece of coarse substrate is embedded, lodged or buried in the finer surrounding material). These individual particle assessments should then be combined to estimate the approximate average condition at the site. Substrates should be examined for embeddedness prior to disturbances (such as walking or netting) that are likely to dislodge fine materials from around larger substrates.
   - Embeddedness is scored as 0% if the substrate in the fastest riffle habitat is bedrock, concrete, or hardpan clay.
   - Embeddedness is scored as 100% if the substrate in the fastest riffle habitat is entirely fine sediment, sand, or flocculent material.

   Embeddedness is recorded on the "SummerHab1" page of the MBSS Summer electronic datasheet.

4. 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. This should be estimated for the entire day, taking into account changing angles of the sun over time. 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. Shading is recorded on the "SummerHab1" page of the MBSS Summer electronic datasheet.

5. 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 is counted as "instream woody debris". The number of large woody debris in the stream channel or immediate riparian area, but not in the wetted portion of the stream, is counted as "dewatered 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. Woody debris is recorded on the "Debris" page of the MBSS Summer electronic datasheet.

6. Root Wads. For the MBSS, root wads that are part of live trees with a chest high trunk diameter (DBH) of at least 16 cm should be counted. These should be counted along both banks of the stream within the 75 m site. Those root wads in direct contact with the wetted portion of the stream channel

(instream) are counted separately from those not in contact with the wetted portion of the stream channel (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. For consistency in counting dewatered root wads, the MBSS considers a tree's canopy to have a spread approximately equal to that of its roots. Thus, a live tree not in contact with the wetted portion of the stream channel, but whose canopy extends to the edge or over top the wetted portion of the stream channel, is counted as a dewatered root wad. Root wads are recorded on the "Debris" page of the MBSS Summer electronic datasheet.

7. Inventory. The stream Inventory on the "SummerHab2" page of the MBSS summer electronic datasheet records the status (i.e., "Absent", "Present", "Extensive") of various Macrohabitats, Stream Characteristics, Substrates, and Aquatic Vegetation types within the site. For each of these features, there is a drop-down menu used to indicate whether the feature is absent, present, or extensive within the 75 m survey site. All of the features included in this Inventory include:

Macrohabitat:

- *Braided Channel*: Presence of stream braiding (i.e., multiple parallel and/or interwoven channels)
- *Riffle*: Presence of stream riffles (i.e., moving water with a broken surface and/or aeration).
- *Run/Glid*e: Presence of runs and glides (i.e., moving water without a broken surface).
- *Deep Pool*: Presence of pool habitat with water ≥ 50cm deep.
- *Shallow Pool*: Presence of pool habitat with water < 50cm deep.

Stream Characteristics:

- *Undercut Bank*: Presence of stream banks that extend laterally over the water's surface
- *Overhead Cover*: Presence of structures or materials extending over top of the stream channel to provide shading. Typically this is vegetation but may also be anthropogenic (e.g., bridges, tunnels, culverts).
- *Beaver Pond*: Presence of beaver pond(s) within the stream channel. This should be habitat alteration due to impounded water upstream of beaver dams. Evidence of beaver activity (e.g., chewed sticks, stumps, felled trees) would not be counted if it did not accompany a dam and impoundment.

Substrate:

- *Large Boulder*: Presence of rocks/stones measuring 1024-4096 mm across their intermediate axis.
- *Small Boulder*: Presence of rocks/stones measuring 256-1023 mm across their intermediate axis.
- *Cobble*: Presence of rocks/stones measuring 64-255 mm across their intermediate axis.
- *Gravel*: Presence rocks/stones measuring 2-63 mm across their intermediate axis.
- *Sand*: Presence of small rocks/stones/silica-based particles measuring 0.062-1.999 mm across their intermediate axis.

- *Silt/Clay*: Presence of fine, inorganic particles measuring < 0.062mm across their intermediate axis, or so fine as to have no discernible texture from individual particles.
- *Bedrock*: Presence of rocks/stones measuring >4096 mm across their intermediate axis, or otherwise a part of an immovable, exposed mass of rock in the stream channel or stream bank.

Aquatic Vegetation:

- *Submerged Aquatic Vegetation*: Presence of vascular plants growing submerged beneath the water's surface (e.g., Curly Pondweed, Wild Celery, Eelgrass, Hydrilla).
- *Emergent Aquatic Vegetation*: Presence of vascular plants rooted in the water and growing above the water's surface (e.g., Pickerelweed, Common Reed, Cattails).
- *Floating Aquatic Vegetation*: Presence of vascular plants floating on the water's surface (e.g., Duckweed, Yellow Pond-Lily, Common Water Hyacinth).
- *Didymo*: Presence of invasive *Didymosphenia geminata* algae.
- *Didymo Voucher*: If Didymo is present, an indication of whether or not a voucher sample was collected.
- *Orange Flocculent*: Presence of iron-oxidizing bacteria producing orange or rust-colored flocculant, sludge, or surface film.
- *Filamentous Algae*: Presence of colonial algae forming mats or long strands on the water's surface or on submerged substrates.

8. Maximum Depth. The maximum depth of the MBSS site is considered the deepest area found anywhere within the 75 m sampled reach. Maximum depth is recorded to the nearest centimeter on the "Transects" page of the MBSS summer electronic datasheet.

9. Transect Measurements. The wetted width and thalweg depth are measured at four transects within the 75 m MBSS site. The four transects are located at the 0 m, 25 m, 50 m, and 75 m portions of the MBSS site (beginning with 0m at the downstream-most end of the site). Wetted width is measured from bank to bank (perpendicular to the direction of the stream flow) to the nearest centimeter and includes only the wetted portion of the stream. Islands or depositional bars should not be included in the measurement of wetted width. If there are multiple braided channels at a given transect, the recorded wetted width should be the sum of the individual wetted widths of each channel, excluding any dewatered space between those channels. Thalweg depth is the depth (in centimeters) of the deepest part of the stream channel at each transect. If there are multiple braided channels at a given transect, only the deepest thalweg measurement among the channels at that transect is recorded. These measurements are recorded on the "Transects" page of the MBSS summer electronic datasheet.

In cases where the stream was dewatered at any or all of the transects, the stream widths and thalweg depths should be recorded as 0 m at each dewatered transect. The average width and depth is calculated using the four transects, and includes any dewatered observations. These transect measurements are also used to estimate the stream area for a site, which is a necessary component of the Fish Index of Biotic Integrity.

If the stream is not watered throughout the 75 m reach, consisting instead of one or more standing pools, the length, width, and depth of each standing pool should be recorded in "Alternative Measurements for Standing Pools" on the "Transects" page of the MBSS summer electronic datasheet. In cases where the majority of the stream channel is dewatered, measurements of standing pools are used to determine stream area for each site, based on the total observed length and average width, which is a necessary component of the Fish Index of Biotic Integrity.

10. Flow. Flow is not a current part of MBSS sampling protocols, but may still be recorded in some situations. If these measurements that can be used to calculate flow (often referred to as discharge) are taken, they can be recorded on the "Discharge" page of the MBSS summer electronic datasheet. A transect that is suitable for taking these measurements should approximate a "U" shaped channel to the fullest 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 "Discharge" page of the MBSS summer electronic datasheet. 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 lateral location (distance from one stream bank or the other) of each measurement should be recorded along with the depth and velocity.

11. Alternative Discharge Measurements. If flow is to be recorded, but stream flows are so low that they cannot 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. These data can be recorded on the "Discharge" page of the MBSS summer electronic datasheet.

12. Bank Erosion. The length and average height of erosion on both banks of the stream within the 75 m site, if present, should be recorded, along with the severity of erosion, on the "SummerHab1" page of the MBSS Summer electronic datasheet. 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, and both banks are included, at the site, this is acceptable.

13. Bar Formation and Substrate. The severity and composition of any depositional bars within the 75 m site should be recorded on the "SummerHab1" page of the MBSS Summer electronic datasheet. The "Bar Severity" drop-down menu contains options for "No bar formation", "Minimal", "Moderate", and

"Severe" formation of depositional bars. Check-boxes should be selected to denote the composition of extant bars between "Cobble", "Gravel", "Sand", and "Silt/Clay". More than one particle type can be selected. However, particles comprising only a minor part of the substrate should not be selected. Dominance of particle types should be determined based on the proportion of the bar consisting of each type. Severity of bar formation is relative, and is determined by comparing the size of the bar(s) to the size of the stream channel.

- No bar formation: Depositional bars are absent from the stream channel.
- Minimal: Depositional bars comprise only a small portion of the stream channel.
- Moderate: Depositional bars comprise a large portion of the stream channel.
- Severe: Depositional bars comprise the majority of the stream channel.

5.10 Geomorphology Sampling

Geomorphology sampling at MBSS sites is limited to conducting a representative riffle pebble count at each site. Recommended equipment for the riffle pebble count includes:

- Riffle pebble count data sheet printed on waterproof paper or a tablet computer loaded with the MBSS Summer electronic datasheet.
- Metric ruler/meter stick (with mm markings) (1 per surveyor) or Gravelometer (1 per surveyor)
- Sand gauge reference cards (1 per surveyor)
- Metal hand tally counter (clicker) (1 per surveyor)
- Digital camera
- Hand shears, machete or other clearing device

1. Locating the Pebble Count. The riffle pebble count should be conducted within a representative riffle within the 75m site. If no riffle is present within the 75 m site, the pebble count can be performed in 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). If no suitable riffle can be located, the pebble count may be performed in a pool/glide, but only if there are no riffles present. If the pebble count is performed in a pool/glide this should be noted on the datasheet along with a habitat description.

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

substrate more than one time. It is acceptable to measure the same large piece of substrate more than once.

3. Measuring the Particle. Measure the length of the intermediate axis in millimeters and record the tally in the appropriate bin on the data sheet. The intermediate axis is neither the longest nor the shortest of the three mutually perpendicular sides of the particle:

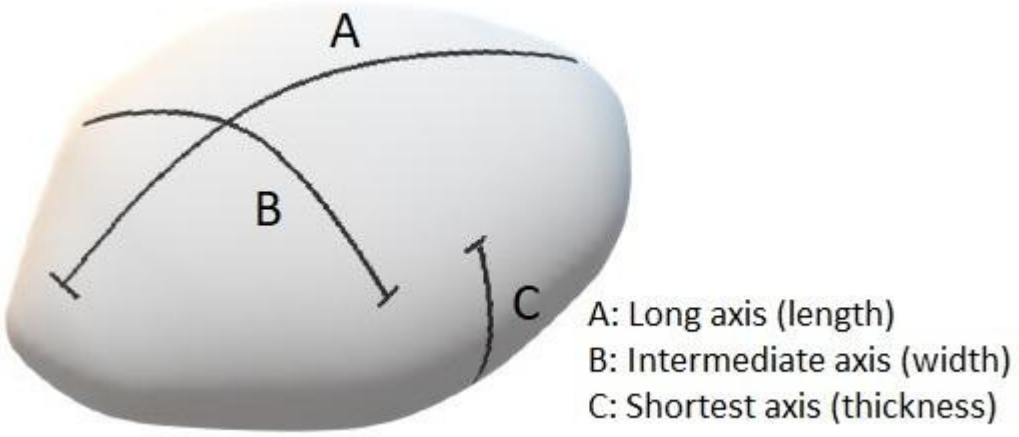


Photo illustration by W. Harbold 21 December 2023

If the particle is linear-shaped, average the axes. If the particle is very small and a measurement cannot be taken (e.g., sand or silt), sand gauge reference cards can help the surveyor classify the particle appropriately. Record counts but not measure organic material or trash/refuse. If the first particle touched is fine sediment forming a thin veneer on a larger particle, the larger particle is what is measured. The intent is to measure the particles that are defining the roughness of the stream channel.

4. Field QA/QC. Tally up counts in each cell of the datasheet to ensure that a total of at least 100 particles were measured and recorded. Recorded data must be reviewed at the point of entry and the crew leader and one other member of the crew must review and initial all field datasheets.

5.11 Temperature Loggers

Temperature loggers should be deployed to measure water and air temperature at all MBSS sites where temperature data is to be recorded. The loggers should be programmed to record temperatures from 1 June to at least 31 August. Each logger should be set to record temperature during a predetermined interval. The MBSS uses a 20-minute interval between temperature measurements.

Ideally, temperature loggers should be deployed within the limits of the 75 m site. If a suitable location for deployment is not available within the site, temperature loggers can be deployed outside of the site as long as they are still located on the same reach. Water temperature loggers should be secured to a well anchored tree root, gabion, rebar, or other stable structure. Care should be taken when selecting the deployment location for water temperature loggers to ensure that the logger is not in an area where high

flows could cause the logger to be lost, that it is placed at a sufficient depth to ensure that it will remain submerged during base flows, and is not likely to be buried in sediment. Loggers deployed to record air temperature should be as close as possible to the location of the temperature logger deployed to record water temperature. The air temperature logger should be at least 3 feet off the ground, no more than 20 meters from the wetted edge of the stream, and not in direct sunlight when possible. The air temperature logger can be affixed to a tree or other stable structure. Care should be taken to ensure that temperature loggers are deployed inconspicuously if the survey site is located where theft or vandalism may be a concern.

The serial number of the temperature logger deployed at each site should be recorded on the "TempLoggers" page of the MBSS Spring electronic datasheet along with a description of the location(s) where the loggers were deployed. When each temperature logger is retrieved, the time and date of retrieval should be recorded. Verifying that the serial number for the logger that was retrieved matches the serial number entered on the Spring Index Period Data Sheet is recommended. It is often useful (and recommended) to attach a flag or piece of tape to the logger with the site identification, date, and time of retrieval.

5.12 Vernal Pools

Vernal pools are small, temporary bodies of water that provide vitally important habitat for many amphibians and aquatic invertebrates. To qualify as a vernal pool, a waterbody must be less than one acre (4,000 m^2) and not directly connected to a flowing stream. There is no minimum size requirement. Despite their importance, very limited information is currently available on Maryland's vernal pools.

The MBSS has collected data on vernal pools during the Spring Index Period since 2007. Early surveys (during Round Three) involved a targeted search for vernal pools in the riparian area and the collection of information on the location and size of vernal pools encountered, listing of herpetofauna associated with the pool(s), and recording select physical habitat information. Since 2021, the MBSS has only recorded the presence or absence of vernal pools at stream survey sites. Any herpetofauna found associated with the pool are recorded as part of the fauna data for the site.

Any vernal pools encountered within the area 50 m perpendicular to the 75 m site on both sides of the stream should be recorded as part of sampling visits during the Spring Index Period. If vernal pools are observed anywhere within the area 50 m perpendicular to the 75 m site on either side of the stream, "Yes" should be selected from the Vernal Pool dropdown menu under "Adjacent Landuse" on the "SpringHabitat" page of the MBSS Spring electronic datasheet. If vernal pools are not observed, "No" should be selected from the dropdown menu. Any herpetofauna observed in association with a vernal pool that is within the area 50 m perpendicular to the 75 m site on both sides of the stream should be recorded on the "Fauna" page of the MBSS Spring electronic datasheet.

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 the information described above can be conducted without wading into the water. Wading in potential vernal pools should be avoided.

5.13 Biological Sampling

Biological sampling has always been the primary focus of the MBSS. During Rounds One and Two, the MBSS focused primarily on fish and benthic macroinvertebrates. Indices of Biological Integrity (IBIs) for these groups were developed using Round One and Round Two MBSS data. These IBIs are now the basis of biocriteria in Maryland and have been extensively used to represent the ecological condition of streams and rivers. 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. This section describes the current protocols used by the MBSS for the collection of biological variables.

5.13.1 Benthic Macroinvertebrates

Benthic macroinvertebrate sampling for the MBSS is conducted within the 75 m survey site during the Spring Index Period (1 March to 30 April). The intent of benthic macroinvertebrate sampling is to provide a representative sample of the community composition and relative abundance of benthic macroinvertebrates in favorable habitats (those supporting the greatest benthic diversity) within the site. In addition to representing the diversity at a MBSS site, benthic macroinvertebrate data from these samples are used to calculate the MBSS benthic macroinvertebrate IBI. Required equipment includes a "D-net" (≤ 600 micron mesh; 10 inch high hoop with a 12" wide flat bottom and round top), sieve bucket (≤ 600 micron mesh sieve), and a sample bucket to store the sample.

1. Sampleability. Before sampling benthic macroinvertebrates, the crew leader 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. Sites that can not be waded safely in their entirety can still be sampled for benthic macroinvertebrates if the suitable benthic habitats are shallow enough to be waded safely. It is up to the crew leader to make this determination.

   Effective sampling for benthic macroinvertebrates is difficult in water where visibility is reduced. Disturbing the area within and upstream of the site should be avoided, to the extent possible, prior to sampling so that visibility is not affected by resulting turbidity. All areas of the stream bottom containing suitable benthic habitats should be visible if benthic macroinvertebrate sampling is to be conducted. Exceptions are sites with persistent, chronic turbidity problems, or where the water is stained dark from natural organic sources (e.g., tannins leached from leaves; blackwater streams). If a stream has insufficient water clarity for benthic macroinvertebrate during the early part of the Spring Index Period or following a rain event, the stream should be revisited later in the Index Period or during a drier period to reassess sampleability. If return visit(s) indicate that the stream has persistently poor water clarity, benthic macroinvertebrate sampling should be attempted

regardless. Whether or not the entire stream bottom is clearly visible in all portions of the site should be recorded in "Sample Notes" on the "Samples&Labels" page of the Spring MBSS electronic datasheet. Although sampling can occur in chronically turbid or blackwater streams when visibility is relatively limited, sampling should not occur in a blackwater stream that is also turbid due to recent precipitation or disturbance.

Other situations that may preclude sampling include dry streams, marshes, impoundments such as those produced by beaver dams that are too deep to sample, tidally influenced streams, and areas where landowner permission is denied. The appropriate code for sampleability should be recorded on the "SiteInfo'' page of the MBSS Spring electronic datasheet.

2. Habitats to be Sampled. Sampling should be conducted at a combination of habitats that support the most diverse macroinvertebrate community within a site. These habitats often include riffles when they are 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 may include gravel, broken peat, clay lumps and detritus or sand in runs. Note that, among all the habitats listed above, those that are most stable and in moving water are preferred to those that are unstable and in still water.

3. Benthic Macroinvertebrate Sampling Protocols:

- Twenty square feet of habitat should be surveyed at each site with collected material pooled into one sample bucket. Conveniently, the hoop on the standard D-net used for MBSS sampling is 12" wide with ≤ 600 micron mesh. Collecting the sample as twenty individual scoops or jabs, each encompassing a square foot section of habitat with dimensions equal to the width of the net, allows for an easy approximation of the necessary 20 ft^2 sample.

- Immediately before sampling benthic macroinvertebrates at each site, inspect the D-net to ensure that there are no holes larger than ≤ 600 micron and no remnants of prior samples. The net must be clean, and holes must be repaired before sampling commences. If the net cannot be repaired, a backup replacement net must be used.

- Walk the entire site to locate the most productive benthic macroinvertebrate habitats as listed above. 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 (e.g., If 75% of the productive benthic habitat is riffle and 25% is rootwads the sample should come from 15 ft^2 of riffles and 5 ft^2 of rootwads). 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. The habitats sampled for benthic macroinvertebrates should be recorded on the "Samples&Labels" page of the MBSS Spring electronic datasheet. Sampling procedures that should be used in each habitat type are described below:

    - *Riffles*: Start at the downstream edge of the riffle and place the net firmly in the substrate with the opening facing into the current. Rub by hand any large stones and sticks from within the 1 ft^2 area upstream of the net (an area bounded by the

12” width of the net and 12” estimated along the stream bottom upstream of the net opening) to dislodge any organisms that may be clinging to these substrates. Remove these cleaned objects from the sample area and place them off to the side. Aggressively disturb the substrate with your hands and/or foot to a depth 5 to 8 cm below the substrate surface. Rub by hand any additional large sticks and/or stones dislodged from within the disturbed substrate to release any organisms that may be clinging to them. Allow the stream flow to wash any suspended debris and/or organisms into the net before lifting the frame off the substrate. Repeat as necessary until the desired number of square feet have 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.

- *Rootwad/Woody Debris*: 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 to catch any dislodged debris or organisms. The D-net should be used in a jabbing or sweeping motion to dislodge organisms from root mats, taking care to allow the stream flow to wash any suspended debris and/or organisms into the net. Alternatively, clumps of roots can be enveloped within the net, and shaken, rubbed, or otherwise disturbed by hand to release organisms and allow them to be trapped within the net. The irregular surface area of logs, snags, and clumps or mats of roots should be estimated to account for the required square feet in each sample.

- *Leaf Packs*: Only leaf packs composed of older, decomposing leaves in areas of moving water should be targeted for sampling. Care should be taken to avoid filling the D-net with excessive numbers of leaves. When sampling a leaf pack, clumps of leaves can be held within the net and shaken, rubbed, or otherwise disturbed by hand to release organisms and allow them to be trapped within the net. Once the leaf pack has been adequately cleaned of organisms within the net it can be discarded back into the stream. Surface area of leaf pack can be difficult to estimate. For the purposes of consistency, the MBSS considers a “hearty handful” of leaf pack to be equivalent to 1 ft^2.

- *Macrophytes*: The D-net should be used in a jabbing or sweeping motion to dislodge organisms from clumps of submerged macrophytes, allowing time for the stream flow to wash any suspended debris and/or organisms into the net. Alternatively, clumps of macrophytes can be enveloped within the net, and shaken, rubbed, or otherwise disturbed by hand to release organisms and allow them to be trapped within the net. The irregular surface area of macrophytes should be estimated to account for the required square feet in each sample.

- *Undercut Banks*: Undercut banks included in a benthic macroinvertebrate sample should be targeted only in the absence of other preferred habitats and should be those that are comprised of soil, clay, and/or rock, without abundant roots or vegetation (an undercut bank that contains an abundance of roots or vegetation would be more appropriately sampled and counted as a rootwad or macrophyte).

To sample this habitat, the D-net should be used in a jabbing or sweeping motion to dislodge organisms from the bank, allowing time for the stream flow to wash any suspended debris and/or organisms into the net.

- *Other Habitat Types*: Other habitat types (e.g., gravel, peat, clay, detritus, sand, silt, stable refuse) should only be sampled in the absence of the other more productive habitats listed above. Sampling techniques may vary based on the material. In soft substrates, the net motion should be gentler to minimize the collection of detritus. In all cases the D-net should be placed downstream of the sampled substrate following jabbing and sweeping to make sure that dislodged organisms are carried into the net.

- In some rare cases (e.g., some large 3rd and 4th order streams) there may not be enough productive habitat present within the 75 m site to collect a 20 ft^2 sample. If this is the case, moving out of the sample site in an upstream direction to find habitat that can be sampled using a D-net is permissible. This should only be done if it is not possible to collect a sufficient sample within the 75 m site and should only be done within the same reach. If sampling is conducted upstream of the 75 m site, a description of the habitats sampled and distance from the upstream end of the 75 m must be recorded in the "Sample Notes" section of the "Samples&Labels" page of the MBSS Spring electronic datasheet.

- When a complete 20 ft^2 sample has been obtained, or when the D-net becomes filled to the point that water does not pass easily through it, the net should be washed into a sieve bucket that is partially submerged and in a shallow portion of a run or pool. While the sample is in the sieve bucket, all large stones (i.e., those greater than 3 cm in diameter), debris, leaves, etc., should be carefully washed, inspected for organisms, and discarded. If necessary, use forceps to remove any animals remaining on the net. All vertebrates (e.g., herpetofauna and fish) should be removed from the sieve bucket at this time. All crayfish should remain in the sample regardless of size. Any herpetofauna or crayfish collected or encountered during the benthic macroinvertebrate sample should be recorded on the "Fauna" page of the MBSS Spring electronic datasheet.

- To remove fine sediments from the sample, the sieve bucket may be gently placed in the stream and lifted up and down while the bottom of the bucket is submerged. Do not rotate the sieve bucket quickly during this process, as this action may damage many soft-bodied macroinvertebrates, potentially rendering them unidentifiable. After processing the sample in the sieve bucket, the benthic net should be rinsed carefully in stream water to make sure that no benthic macroinvertebrates remain that may be transported to the next sample site.

- Any unionid mussel or *Corbicula sp.* (Asiatic clam) collected as part of the benthic macroinvertebrate sample should be recorded on the "Fauna" page of the MBSS Spring electronic datasheet. *Corbicula sp.* should remain in the benthic sample. Any live unionid mussels should be promptly removed from the sample and placed as closely as possible to where they were collected, or into the appropriate habitat if unsure where the specimen was collected. The mussel should be gently placed partway into the substrate with the anterior end pointing down.

4. Preservation. The processed composite sample should be transferred from the sieve bucket to an appropriate 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 by repeatedly inverting the sample container, taking care to ensure that the lid is properly sealed.

5. Labeling. 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 "SiteInfo" page of the MBSS Spring electronic datasheet. Verify the information on each label and indicate who has verified the labels on the "Samples&Labels" page of the MBSS Spring electronic datasheet. The external label should be covered with clear plastic tape to prevent smudging and/or label loss. Internal labels must be printed on waterproof paper. Both labels should be filled in with pencil. Benthic sample Chain-of-Custody forms (Appendix F) should also be filled out with the name of the sampler, date, time, and sample site number.

6. Delivery to the Laboratory. A Benthic Macroinvertebrate Chain-of-Custody Sheet (Appendix F) 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.

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 1-September 30). 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 section 2.3 in this manual, where hazards and procedures for minimizing risk for electric shock, prior to conducting electrofishing are described.

2. Sampleability. Prior to conducting electrofishing, the crew leader must determine if the site can be sampled safely and effectively. Factors that may impact safe and effective electrofishing may include water depth and velocity, water clarity, overhanging vegetation, and beaver dams.

   Electrofishing can only be conducted if the site being sampled can be waded safely. If the depth or current velocity precludes safe wading, then the site should be considered unsafe for electrofishing. If a stream is determined unsampleable due to depth or velocity during the early part of the Summer Index Period or following a rain event, the stream should be revisited later in the Index Period or during a drier period to reassess sampleability. If return visit(s) indicate that the stream is continuously too deep or fast, it should not be electrofished and the appropriate sampleability code should be recorded on the "SiteInfo'' page of the Summer MBSS electronic data

sheet. However, if the margins of deep areas can be safely waded and fish can be effectively captured using long handled dip nets and anodes, electrofishing should be attempted as long as all other sampleability considerations are met.

After safe depth and velocity for wading, the most predominant effective sampleability consideration for electrofishing is water clarity. Effective MBSS electrofishing is difficult in water where visibility is reduced. Disturbing the area within and upstream of the site should be avoided, to the extent possible, prior to electrofishing so that visibility is not affected by resulting turbidity. All areas of the stream bottom should be visible if electrofishing is to be conducted. Exceptions are sites with persistent, chronic turbidity problems, or where the water is stained dark from natural organic sources (e.g., tannins leached from leaves; blackwater streams). If a stream has insufficient water clarity for electrofishing during the early part of the Summer Index Period or following a rain event, the stream should be revisited later in the Index Period or during a drier period to reassess sampleability. If return visit(s) indicate that the stream has persistently poor water clarity, electrofishing should be attempted regardless. Whether or not the entire stream bottom is clearly visible in all portions of the site should be recorded on the "Fish" page of the Summer MBSS electronic datasheet. Although sampling can occur in chronically turbid or blackwater streams when visibility is relatively limited, sampling should not occur in a blackwater stream that is also turbid due to recent precipitation or disturbance.

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

Beaver dams may prevent sampling due to excessive depth in the resultant impoundment. In some cases, a beaver dam may be removed or breached, draining the impoundment and lowering the stream to a sampleable depth. If, in the crew leader's estimation, a beaver dam can be removed or breached to improve sampleability, block nets should be set and physical habitat measurements should be recorded first, prior to altering the dam. This will ensure that the data recorded is an accurate representation of the stream prior to being influenced by sampling.

Other situations that may preclude sampling include dry streams, marshes with no defined channel, impoundments, tidally influenced streams, and areas where landowner permission is denied. If any of these conditions are encountered at a site, the stream should not be sampled and the appropriate sampleability code (found on the "SiteInfo '' page of the Summer MBSS electronic data sheet) should be recorded. As these conditions are not likely to change later in the Index Period, a repeat visit to reassess sampleability is typically not necessary.

3. Sampling Considerations. The width of the stream, number of anodes needed to effectively electrofish, and any other fish sampling considerations should be recorded on the "SiteSpecs" page of the MBSS Spring electronic datasheet 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 (e.g., culverts, braided channels).

4. Number of Anodes. The appropriate number of anodes to cover the entire width of the MBSS site must be used. In all cases, there must be one anode for every three meters of stream width. More coverage may be necessary depending on the amount of habitat available within the stream site, deep areas, or other reasons to be determined by the crew leader. All anodes used by MBSS sampling crews are outfitted with ¼" mesh netting to facilitate fish capture. The netting on the anodes should not have any holes or tears greater than ¼". Since it is possible to use more than one anode simultaneously on the same electrofishing unit, the number of anodes for each unit is recorded on the "Anodes" page of the Summer MBSS electronic data sheet to accurately report electrofishing effort.

5. Dip Nets. It is standard MBSS practice that every crew member using an electrofishing anode also carries and uses a dip net to aid in collecting fish. At sites narrow enough to be sampled using only one anode, at least one additional crew member with a dip net should accompany the crew member operating the electrofisher for the length of the site. At wider sites, a minimum of one crew member with a dip net should accompany every two crew members with anodes. Dip nets used by MBSS sampling crews have ¼" mesh and should not have any holes or tears greater than ¼". Fish 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 and/or to preserve backpack electrofisher batteries. These units often employ splitters to run multiple anodes. When a barge is used with multiple anodes, care should be taken to ensure that the number of anodes is recorded accurately on the "Anodes" page of the Summer MBSS electronic data sheet so that the correct electrofishing effort is reported for the site.

7. Block Nets. MBSS fish sampling requires the use of block nets. Block nets for MBSS sampling should have ¼" or smaller mesh, be completely free of holes or tears larger than ¼", 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 fish from within the site and to prohibit entry of additional fish 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 into or out of the site. In braided streams, all braids should be blocked at the 0 m and 75 m locations and all braids should be sampled.

   If the MBSS site includes a culvert that is too small to sample through, block nets should be used to isolate the culvert from the site. The length of the culvert should then be added to the upstream or downstream end of the site so that the sampled section of stream is a total of 75 m long. If the culvert occurs in the first half of the site (i.e., between the 0 m transect and the site midpoint), the additional distance should be added to the downstream end of the site. Similarly, the additional distance should be added to the upstream end of the site if the culvert is within the upper half of the original site (i.e., between the site midpoint and the 75 m transect). If the culvert can be sampled completely, no change should be made to the original 75 m site.

Although block nets are typically outfitted with small lead weights along their bottom end, these weights are usually insufficient to keep fish 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. 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 allow fish to escape by jumping or swimming over the net. To prevent this, 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 to the stream bank so that they are not dislodged by the stream current or by floating debris during electrofishing. Maintaining tension on the anchor ropes at the ends of the net will help keep prop sticks or other devices in place.

8. Fish Movement. Prior to and during the installation of block nets, care must be taken to ensure that fish are not chased out of or into the MBSS site. Any observed movement of fishes into or out of the site should be noted on the "Fish" page of the Summer MBSS electronic data sheet.

9. Appropriate Voltage. The output voltage of the electrofishing unit should be adjusted to ensure that fish are effectively immobilized while still minimizing mortality or permanent injury. 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 on live fish downstream of the MBSS site, prior to use in the site (i.e., temporarily stunning both large and small fish without causing mortality), should be conducted. This is the best way to definitively be sure that the electrofisher is being effective.

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

11. Effort. The seconds of electrofishing (i.e., "effort") for each unit being used for sampling should be monitored and recorded for each electrofishing pass. On the "Anodes'' page of the Summer MBSS electronic datasheet, the time in seconds is recorded for each unit at the beginning of the first electrofishing pass, at the end of the first electrofishing pass, and at the end of the second pass. In some instances a third pass is utilized, and there is space for recording that time as well. Also recorded on this page are the electrofisher unit name, model, number of anodes attached to each unit, and voltage used. Typically, there is only one anode used per backpack electrofisher, but it is possible to employ a splitter and use two or more anodes with one electrofishing unit, especially when using an electrofishing barge. In the event that more than one anode is used on the same unit, it is imperative that the correct number of anodes is recorded so that electrofishing effort is reported accurately (i.e., total effort is the summation of each electrofishing unit's recorded time multiplied by the number of anodes in use with that unit).

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 with a total body length less than 30 mm need not be collected. When necessary to ensure capture of fish, the operator of the electrofishing unit should use the net on the anode ring. For the MBSS, continuous rather than intermittent electrofishing is used to avoid bias introduced by selective placement of the electrode and reduce sampling mortality.

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

    In fast water or where visibility is reduced, dip netters should keep their nets on the stream bottom as much as possible to increase the probability of capturing bottom dwelling individuals that may be difficult to see. Particular attention should be given to capturing small benthic fishes (darters, sculpins, and madtoms) in these habitats.

13. Block Net Checks. Upon completion of each electrofishing pass, the entire downstream block net must be examined for fish. Fish found trapped in the downstream block net after an electrofishing pass should be included in the counts for the electrofishing pass that immediately preceded their discovery.

    It is important to make sure that both block nets still effectively block the movement of fishes throughout all electrofishing passes. Accumulated debris is most common in the downstream net (having been stirred up by electrofishing activity) but it may collect in the upstream net as well, depending on the conditions in the stream. Too much debris will restrict water flow, and may cause the block net to lift off the bottom of the stream or to fall over in the current, rendering it ineffective. Accumulated debris should be removed from both block nets during and between electrofishing passes as needed to maintain adequate water flow and prevent net failure. Debris removed from the downstream net should be thoroughly checked for fish (to be included in counts for the most appropriate electrofishing pass) before being discarded below the site. Debris from the upstream net should be discarded on the bank to prevent it from drifting into the site and impacting the downstream net. Any fish collected with debris in the upstream net should be released above the site and not counted.

14. Delaying Second Pass. If water clarity in the site is reduced because of turbidity and substrate disturbance during a preceding electrofishing pass, a subsequent electrofishing pass must be delayed until 1) water clarity is restored OR 2) one hour elapses. Field crews may begin the subsequent electrofishing pass in less than one hour if the crew leader determines that water clarity has returned to levels observed prior to the preceding pass by that time. If water clarity has not been restored within one hour, the subsequent electrofishing pass should commence despite any reduced visibility. If second pass visibility is poorer than first pass visibility, it should be noted on the "Fish" page of the Summer MBSS electronic datasheet.

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

16. Biomass. Fish collected during each electrofishing pass are weighed in aggregate to the nearest gram. Separate weights are recorded for each electrofishing pass. Only fish should be weighed. Other organisms, rocks, sticks, leaves and other debris must be removed prior to weighing. Aggregate fish biomass for each electrofishing pass is recorded on the "Fish" page of the Summer MBSS electronic datasheet

17. Fish Counts and Identification. Only crew members who have passed the MBSS fish taxonomy test are permitted to identify fish. Using the MBSS fish key as needed for positive verification, all collected fish are identified to species and enumerated in the field. The numbers of fish by species are recorded separately for each electrofishing pass on the "Fish" page of the Summer MBSS electronic datasheet. A "Fish Crib Sheet" is provided in Appendix I to aid in counting by species.

    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 fish in covered, flow-through live cars and/or by using battery-operated bubblers and frequent water changes. Aside from American Eels, all individuals not retained as voucher specimens and/or for laboratory examination should be released as quickly as possible.

    The MBSS has observed that the American Eel is more likely than other fishes to move under a block net and re-enter a survey site between electrofishing passes. To prevent any individuals from being counted twice, all American Eels collected during one electrofishing pass should be held in a covered, flow-through live car downstream of the site until all subsequent electrofishing passes are complete.

    All individuals not clearly identifiable to species in the field should be retained for later inspection and identification in the laboratory, with the number of individuals retained indicated on the "Fish" page of the Summer MBSS electronic datasheet. Retention of all specimens that cannot be positively identified is mandatory.

    Retained specimens should be preserved promptly by placing them into plastic jars filled with a 10% buffered formalin solution. Individuals >150 mm should be slit on the lower abdomen of the RIGHT side prior to preservation in formalin. After a minimum of five days (but no more than a month) in formalin, the specimens should be rinsed by soaking three times in fresh water for 24-48 hours each time, changing the water after each rinse. After the third rinse, specimens can be transferred to a 70% ethanol solution for long term storage. All specimen jars should be identified with inside labels specifying the date, site number, and name of collector. An example of the MBSS Voucher Specimen Label is included in Appendix G.

18. Gamefish. During counting and identification of the fish sample for each pass, each gamefish species collected should be measured to the nearest mm (total length) and recorded on the

"Gamefish" page of the MBSS Summer electronic datasheet. Gamefish species for the MBSS include all black bass (*Micropterus spp*.), Striped Bass (*Morone saxatilis*), all trouts (Salmonidae), Walleye (*Sander vitreum*), and pikes and pickerels (Esocidae) with the exception of Redfin Pickerel (*Esox americanus*). If visual observations suggest that some individuals may be stocked fish (based on fin wear, fin size, etc.), indicate so in the "Note" field for those individuals on the "Gamefish" page of the MBSS Summer electronic datasheet.

19. Taxonomic Vouchers. DNR field crews are required to maintain voucher collections annually. Digital 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 species encountered by a field crew during each calendar year (as long as five were collected) should be taken. 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. Each photograph should have a filename that includes the site identification and species depicted. The Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division will keep all voucher photographs taken during MBSS sampling. With the exception of rare, threatened, or endangered species, specimens for which photographs cannot be used to verify identifications should be preserved. Both photographs and preserved specimens will be reviewed by an appropriate expert in taxonomy and results will be kept on record.

20. Taxonomic Experts. All fish vouchers (photographs and specimens) will be verified by a taxonomic expert or their assigned designee. The taxonomic expert or their designee will also audit field identifications. Field audits will be conducted at a minimum of one site per crew.

5.13.2 Reptile and Amphibian Sampling

Reptile and amphibian sampling has been an integral part of the Maryland Biological Stream Survey since 1994. Reptiles and amphibians, particularly stream salamanders, have been shown to be excellent indicators of MBSS site conditions. Effort has varied over time, but since 2021, MBSS protocols have required field crews to record incidental observations during any sampling visit as well as to record any species collected during electrofishing and spend fifteen minutes searching the best available habitat for herpetofauna, including stream salamanders, during the Summer Index Period visit. The life history stage of reptiles and amphibians should also be recorded (egg, larva, or adult). Reptile and amphibian information is recorded on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable.

1. Recording Observations. 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 "Fauna" pages of the MBSS Spring and Summer electronic datasheets, respectively. Incidental observations should be recorded during either index period. During the summer visit, any herpetofauna collected while electrofishing should be recorded on the "Fauna" page of the MBSS Summer electronic datasheet. Additionally, during the summer visit, survey crews should spend fifteen minutes searching the best available habitat for herpetofauna within the riparian area 50m perpendicular to the 75 m site on either side of the stream, recording any species encountered on the "Fauna" page of the MBSS Summer electronic datasheet.

2. Stream Salamanders. Stream salamanders include the following species in the family Plethodontidae; the Northern Red Salamander, Eastern Mud Salamander, Northern Spring

Salamander, Northern Two-Lined Salamander, Long-Tailed Salamander, Northern Dusky Salamander, Allegheny Mountain Dusky Salamander, and Seal Salamander. Stream salamander sampling consists of electrofishing in aquatic habitat (concurrent with electrofishing sampling for fish) and bank searches in terrestrial habitat during the 15 minute general herpetofauna search. All stream salamanders found incidentally, during the 15 minute search, or while electrofishing should be counted and recorded by species and life history stage (e.g., larva or adult) on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable.

Electrofishing protocols are described in the fish sampling portion of this manual. Salamanders should be collected coincident with fish collection during electrofishing. Bank searches focused on finding stream salamanders will be conducted during the Summer Index Period visit, along both banks of the 75-m site as a part of a 15 minute general herpetofauna search. Although the bank search will be conducted entirely outside the wetted portion of the stream, the bank search should focus on cover objects (including cobbles, small boulders, logs, or other objects) where sufficient moisture is present to support stream salamanders. In streams where seeps or small tributaries enter the stream, these habitats should also be searched as well. During the search, suitable cover adjacent to the site should be carefully flipped over and then returned as closely as possible to the original position. In some Maryland streams, such suitable cover can extend several meters from the edge of the stream before the habitat is completely dry and not suitable for stream salamanders.

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

4. Handling Care and Precautions. Live specimens 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 underneath the object on its own.

5. Taxonomic Identification. Only those members of the field sampling crew who have passed the MBSS reptile and amphibian taxonomy test are permitted to perform reptile and amphibian identification for the crew. The Key to the Reptiles and Amphibians of Maryland can be consulted to assist with identifications.

6. Frog Calls. A frog call test will be administered prior to the Spring Index Period. 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 during spring or summer sampling. Frogs that are heard calling can be recorded on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable, during spring and summer sampling visits.

7. Taxonomic 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, fewer than five vouchers are acceptable if less than five individuals were 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 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 MBSS field sampling.

8. Taxonomic Experts. All reptile and amphibian voucher photographs will be verified by a taxonomic expert or their assigned designee. The taxonomic expert or their designee may also audit field identifications. Field audits should be conducted at a minimum of one site per crew.

5.13.3 Crayfish Sampling

The MBSS collects crayfishes while sampling to record data on these important members of Maryland's stream ecosystems, as well as to track the spread of invasive crayfish species. Crayfish may be collected during both the Spring and Summer Index Periods.

1. Sampleability. If the site can be safely accessed and with landowner permission, sampling for crayfishes should be conducted. This includes sampling in dry streams. During the spring and summer index period, incidental findings should also be recorded on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable.

2. Electrofishing. An attempt should be made to capture all crayfish encountered during each electrofishing pass. Most stream-dwelling crayfishes are primarily nocturnal and reside 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 crayfish captured. All captured crayfish 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 block net is checked for crayfish. Identify and enumerate all adult (>15 mm carapace length) crayfishes caught during each electrofishing pass. The full scientific (Latin) name of each species and the number collected during each pass, as well as any incidental captures outside of the electrofishing passes, are recorded in the crayfish section of the "Fauna" page of the MBSS Summer electronic datasheet.

3. Burrows. The presence of crayfish burrows (often detected via their aboveground mud "chimneys") along stream banks or within the floodplain adjacent to the MBSS site is recorded on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable. The abundance of burrows is recorded as (P): Present, (A): Absent, or (E): Extensive. If time allows, an attempt can 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. Taxonomic Vouchers. All specimens that cannot be positively identified in the field should be retained for further inspection by a regional crayfish expert. Field identification of *Procambarus acutus* and *P. zonangulus* is difficult and unreliable. All Form 1 male individuals

should be vouchered for species-level identification in the laboratory. Form 2 male, female, and juvenile specimens of these two species should be recorded as *Procambarus acutus/zonangulus* and released. Five individuals of each species collected by each MBSS field crew should be retained annually so that taxonomic identifications can be verified. The preservation of Form I males is preferred, but females and Form II males may 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.

6. Taxonomic Experts. All crayfish vouchers (photographs and specimens) will be verified by a taxonomic expert or their assigned designee. The taxonomic expert or their designee may also audit field identifications. Field audits should be conducted at a minimum of one site per crew.

5.13.4 Freshwater Mussel Sampling

The MBSS records observations of live and dead freshwater mussels while sampling to record presence of these important members of Maryland's stream ecosystems, as well as to track the spread of invasive species. Freshwater mussel data may be collected during both the Spring and Summer Index Periods. Any native freshwater mussels (Family Unionidae), invasive Zebra Mussels (Dreissenidae), or Asiatic clams (*Corbicula sp.*) that are observed while sampling MBSS sites should be identified to species with their scientific names recorded.

1. Sampleability. If the site can be safely accessed and with landowner permission, sampling for freshwater mussels should be conducted. During the spring and summer index period, observations of freshwater mussels (or their shells) should also be recorded on the "Fauna" pages of both the MBSS Spring and Summer electronic datasheets, as applicable. While it is rare to find evidence of mussels in streams that are dry when visited during the Summer Index Period, they have the ability to withstand short periods of drought. Therefore, mussel sampling can be conducted in streams with standing pools or streams that have become dry.

2. Spring Index Period. Incidental observations of freshwater mussels should be recorded during the spring index period. There is no targeted search for freshwater mussels as a part of spring MBSS sampling protocols, but any unionid mussel or *Corbicula sp.* incidentally encountered while marking the site, recording habitat data, or collecting the benthic macroinvertebrate sample should be recorded. If live mussels are collected in the D-net during benthic macroinvertebrate sampling, they should be placed back in the stream 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.

3. Summer Index Period. During the Summer Index Period, suitable bivalve habitats within the sampling segment may be visually searched, with part of this effort focused on searching the stream bank for shells and animal middens. Live specimens that are encountered should be identified in the field, and then immediately returned as closely as possible to where they were collected. The mussel should be gently placed partway into the substrate with the anterior end pointing down.

4. Mussel Condition. If any species of unionid mussels or *Corbicula sp.* are encountered during sampling; their condition as:

- "Live": The mussel is alive. It was found properly oriented in the substrate with the foot and/or siphons extended or the shell is tightly closed.
- "Fresh Dead": The shell has an intact prismatic layer, an intact and possibly flexible hinge ligament, unstained nacre, little to no deterioration of periostracum, presence of abductor muscle tissue. Likely died within the past 24 hours.
- "Old Dead": The shell has no prismatic layer, an intact, but inflexible hinge ligament, minor staining of nacre, moderate deterioration and flaking of periostracum, no muscle tissue. Dead for weeks to a year or two.
- "Relict": The shell has no prismatic layer, an inflexible or no hinge ligament (i.e., one valve), stained nacre, heavy deterioration to lack of periostracum. Dead for at least several years.

Observations be noted in the "Bivalves" section of the "Fauna" page of either the MBSS Spring or Summer electronic datasheet, as applicable. If no *Corbicula sp.* or unionid mussels are encountered, it should be noted as "No Corbicula Observed" or "No Unionid Mussels Observed" in the "Bivalves" section of the "Fauna" page of either the MBSS Spring or Summer electronic datasheet, as applicable.

5. Taxonomic Identification. Only those members of the field sampling crew who have passed the freshwater mussel taxonomy test should perform freshwater mussel identification for the crew. If no crew members are qualified to identify freshwater mussels in the field, the crew leader should provide vouchers to a qualified expert for identification.

6. Taxonomic Vouchers. No live freshwater mussels should be vouchered. Digital pictures should be taken of live specimens for which the identification is uncertain. Photographs must clearly show characters necessary to confirm the identification. At a minimum, photo documentation will clearly show a lateral and a dorsal aspect of each specimen. Additional characters that may prove beneficial to identification include umbo/beak sculpture and posterior slope. Placing the specimen against a light-colored background for the picture may help produce a clear photograph. Each photograph should have a filename that includes the site identification and species depicted. The Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division will maintain all photographs taken during MBSS sampling.

   Valves (shells) from as many dead specimens as practical for which the identification is uncertain should be retained. Valves collected from a single site can be placed in one zip-lock bag with a paper label containing site name, date, and collector. Voucher shells should be cleaned of all debris with a soft brush (e.g. toothbrush) in water.

7. Taxonomic Experts. All freshwater mussel vouchers (photographs and valves) will be verified by a taxonomic expert or their assigned designee. The taxonomic expert or their designee may also audit field identifications. Field audits should be conducted at a minimum of one site per crew.

List of Appendices

Appendix A: Fish Fixation and Preservation Procedures

Fixation

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

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

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

Preservation

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

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

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

References

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

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

Appendix B: Equipment Lists

Spring Index Period Equipment List

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

MBSS Sampling Manual	Chest waders
Road maps and itinerary	Wader repair kit
Site list/maps	G.P.S. unit
Compass	Ice
First aid kit	Bubble wrap, packing material, packing tape
Permanent markers	Clear label tape
Pencils	Deionized water for blanks
Taxonomic Keys (reptiles and amphibians, crayfish, freshwater mussels)	Water quality sample bottles- 1 liter
Machete or other clearing tools	Water quality sample bottles- 500 mL
Digital camera	Syringes, Luer Locks, and 0.45 micron syringe filters
Spray paint	Pre-printed adhesive outside labels and inside labels
Flagging	Spare batteries
100 m measuring tape	Chain-of-custody forms
≤ 600 micron mesh D-net (frame dimensions 12” W x 10” H)	Ziplock bags
Spare net bag for D-net	Pump sprayer or decontamination solution container
≤ 600 micron sieve bucket	Rinse water
Benthic sample containers (86 oz or larger)	Decontamination solution – 10% bleach or Virkon
EtOH (2 liters per site)	Extra drinking water
Foul weather gear	Backpack
Small cooler for transporting water samples from site to vehicle	Large cooler for keeping samples cool after collection and for shipping to laboratory
Temperature loggers	Datasheets, electronic or paper
Nitrile gloves	

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	Extra drinking water
Road maps	Spring or electronic scale
Site list and site maps	Calibration weights
Datasheets, electronic or paper	Pruning tool
Fish Data Sheets	Backpack electrofishing Unit(s)
Summer Habitat Data Sheets	Anode ring probe(s) (fitted with 3/16” mesh netting);
Gamefish Length Data Sheets	Electrofishing batteries
Summer Photo Data Sheets	Spare netting/cable ties for anode ring nets
Habitat Guidance Sheet	7 gallon buckets
Clipboards	Dip nets
Pencils	Block nets
Sample jars	Live cars
Pre-printed voucher labels	Machete
Taxonomic keys (reptiles and amphibians; fish, freshwater mussels, crayfishes)	Calibration log
Voucher lists	Waders and wading boots
Preservatives (alcohol and formalin)	Cellular phone
100 m measuring tape	Backpacks
Flagging	Measuring board
Digital camera	Meter sticks
G.P.S. unit	Tool box
Compass	Wader repair kit
Disinfectant lotion	Polarized Glasses
Drinking water	Pump sprayer or decontamination solution container
First aid kit	Rinse water
Foul weather gear	Decontamination solution – 10% bleach or Virkon

Appendix C: Codes and Abbreviations

Watershed Codes

Atkisson Reservoir	ATKI	Dividing Creek	DIVI	Lower Wicomico	LOWI
Aberdeen Proving Ground	ABPG	Double Pipe Creek	DOUB	Little Patuxent River	LPAX
Anacostia River	ANAC	Eastern Bay	EAST	Lower Susquehanna	LSUS
Antietam Creek	ANTI	Evitts Creek	EVIT	Little Tonoloway	LTON
Assawoman Bay	ASSA	Fifteen Mile Creek	FIMI	Lower Chesapeake Bay	LWCH
Atlantic Ocean	ATLA	Fishing Bay	FISH	Lower Winters Run	LWIN
Back River	BACK	Furnace Bay	FURN	Little Youghiogheny	LYOU
Back Creek	BACR	Georges Creek	GEOR	Magothy River	MAGO
Baltimore Harbor	BALT	Gilbert Swamp	GILB	Manokin River	MANO
Big Annemessex River	BANN	Gunpowder River	GUNP	Marsh Run	MARS
Big Elk Creek	BELK	Gwynns Falls	GWYN	Marshyhope Creek	MACK
Bird River	BIRD	Honga River	HONG	Mattawoman Creek	MATT
Bodkin Creek	BODK	Isle of Wight Bay	ISLE	Middle Chesapeake Bay	MDCH
Bohemia River	BOHE	Jones Falls	JONE	Middle Chester River	MICR
Breton Bay	BRET	Kent Island Bay	KEIS	Middle River-Browns	MIDD
Brighton Dam	BRIG	Kent Narrows	KENA	Miles River	MILE
Broad Creek	BROA	Langford Creek	LANG	Monie Bay	MONI
Bush River	BUSH	Little Conococheague Creek	LCON	Middle Patuxent River	MPAX
Bynum Run	BYNU	Liberty Reservoir	LIBE	Nanjemoy Creek	NANJ
Cabin John Creek	CABJ	Little Choptank	LICK	Nanticoke River	NANT
Casselman River	CASS	Little Elk Creek	LIEL	Nassawango Creek	NASS
Catoctin Creek	CATO	Little Gunpowder Falls	LIGU	Northeast River	NEAS
Chincoteague Bay	CHIN	Licking Creek	LIKG	Newport Bay	NEWP
Conowingo Dam/Susquehanna River	CDAM	Lower Monocacy River	LMON	Octoraro Creek	OCTO
Christina River	CHRI	Loch Raven Reservoir	LOCH	Oxon Creek	OXON
Conewago Creek	COCR	Lower Choptank	LOCK	Patapsco River Lower North Branch	PATL
Conococheague Creek	CONO	Lower Chester River	LOCR	Patuxent River Lower	PAXL
Corsica River	CORS	Lower Elk River	LOEL	Patuxent River Middle	PAXM
Deep Creek Lake	DCRL	Lower Gunpowder Falls	LOGU	Patuxent River Upper	PAXU
Deer Creek	DEER	Lower Pocomoke River	LOPC	Pocomoke Sound	PCSO

Watershed Codes, continued

Piscataway Creek	PISC	Savage River	SAVA	Upper Elk River	UELK
Potomac Allegheny County	PRAL	South Branch Patapsco	SBPA	Upper Monocacy River	UMON
Prettyboy Reservoir	PRET	Southeast Creek	SEAS	Upper Chesapeake Bay	UPCH
Potomac River Frederick County	PRFR	Seneca Creek	SENE	Upper Choptank	UPCK
Potomac River Lower North Branch	PRLN	Severn River	SEVE	Upper Chester River	UPCR
Potomac Lower Tidal	PRLT	Sideling Hill Creek	SIDE	Upper Pocomoke River	UPPC
Potomac River Montgomery County	PRMO	Sinepuxent Bay	SINE	West Chesapeake Bay	WCHE
Potomac River Middle Tidal	PRMT	South River	SOUT	Western Branch	WEBR
Potomac River Upper North Branch	PRUN	St. Clement Bay	STCL	West River	WEST
Potomac Upper Tidal	PRUT	Stillpond-Fairlee	STIL	Wicomico River	WICO
Potomac River Upper North Branch	PRUN	St. Mary's River	STMA	Wicomico Creek	WICR
Potomac Upper Tidal	PRUT	Swan Creek	SWAN	Wills Creek	WILL
Potomac Washington County	PRWA	Tangier Sound	TANG	Wicomico River Head	WIRH
Port Tobacco River	PTOB	Tonoloway	TONO	Wye River	WYER
Rocky Gorge Dam	RKGR	Town Creek	TOWN	Youghiogheny River	YOUG
Rock Creek	ROCK	Transquaking River	TRAN	Zekiah Swamp	ZEKI
Sassafras River	SASS	Tuckahoe Creek	TUCK		

Vegetation Types

Codes recorded to describe the vegetation types that are components of the riparian buffer during Spring Index Period habitat assessments.

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

Riparian Buffer Zone Adjacent Land Cover Types

Codes to describe the land cover type found adjacent to the riparian buffer during Spring Index Period habitat assessments.

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

FR, OF, EM, LN, and TG are buffer types. If the riparian buffer is > 50 m, one of these land cover codes should be recorded to describe the buffer that exists immediately beyond 50 m from the wetted edge of the stream channel. The remaining land cover types are not considered to be a type of buffer. If the riparian buffer is ≤ 50 m, one of these land cover codes should be recorded to describe the land cover outside the riparian buffer, adjacent to the buffer's edge.

Instream Blockage Codes

Codes to describe the type(s) of stream blockages encountered at MBSS sites during Spring Index Period habitat assessments.

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: Blockage height is measured in meters from the stream surface downstream of the structure to the stream surface upstream of the structure.

Sampleability Codes

These codes should be recorded in “SiteInfo'' to describe sampleability for each aspect of the survey. “S” denotes conditions conducive to sampling (“sampleable”). Codes 1-10 are used to describe reasons why either an entire site or a particular aspect of the survey were not sampleable.

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 (Describe in Comments)

Appendix D: MBSS Stream Habitat Assessment Guidance Sheet

This table provides guidance on assessing MBSS habitat variables that are rated on a 0-20 numerical scale, along with guidance for % embeddedness and % shading.

MBSS Stream Habitat Assessment Guidance Sheet				
Habitat Parameter	**Optimal 16-20**	**Sub-Optimal 11-15**	**Marginal 6-10**	**Poor 0-5**
Instream Habitat	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
Epifaunal Substrate	Preferred substrate abundant, stable, and at full colonization potential (riffles well developed and dominated by cobble; and/ or woody debris prevalent, not new, and not transient)	Abund. of cobble with gravel &/or boulders common; or woody debris, aquatic veg., under-cut banks, or other productive surfaces common but not prevalent /suited for full colonization	Large boulders and/or bedrock prevalent; cobble, woody debris, or other preferred surfaces uncommon	Stable substrate lacking; or particles are over 75% surrounded by fine sediment or flocculent material
Velocity/Depth Diversity	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)
Pool/Glide/Eddy Quality	Complex cover/&/or depth ≥1.5m; both deep (≥ .5 m)/shallows (< .2 m) present	Deep (≥0.5 m) areas present; but only moderate cover	Shallows (<0.2 m) prevalent in pool/ glide/eddy habitat; little cover	Max depth <0.2 m in pool/glide/eddy habitat; or absent completely
Riffle/Run Quality	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
Embeddedness	Percentage that gravel, cobble, and boulder particles are surrounded by line sediment or flocculent material.			
Shading	Percentage of site 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			
Trash Rating	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

Appendix E: Species Names

This appendix includes lists of species names including:

- Common Names of Maryland Fishes
- Common Names of Maryland Reptiles and Amphibians
- Scientific Names of Maryland Crayfishes
- Scientific Names of Maryland Freshwater Bivalves

Names are listed as they should be recorded on MBSS data sheets. Letters in parentheses next to a species name correspond to the state status of the species from the Rare, Threatened, and Endangered Animals of Maryland (November 2021) and from the MDNR Invasive Species Matrix Team.

- E = Endangered
- T = Threatened
- I = In need of Conservation
- X = Presumed Extirpated
- N = Nuisance

Common Names of Maryland Fishes

LAMPREYS:
American Brook Lamprey (T)
Least Brook Lamprey
Sea Lamprey

HERRINGS:
Alewife
American Shad
Blueback Herring
Gizzard Shad
Hickory Shad

CATFISHES:
Blue Catfish (N)
Brown Bullhead
Channel Catfish
Flathead Catfish (N)
Margined Madtom
Stonecat (E)
Tadpole Madtom
White Catfish
Yellow Bullhead

SUCKERS:
Eastern Creek Chubsucker
Golden Redhorse
Longnose Sucker* (X)
Northern Hog Sucker
Quillback
Shorthead Redhorse
White Sucker

KILLIFISH:
Banded Killifish
Mummichog
Striped Killifish

STICKLEBACKS:
Fourspine Stickleback
Threespine Stickleback

MINNOWS:
Blacknose Dace
Bluehead Chub (N)
Bluntnose Minnow
Bridle Shiner (X)
Central Stoneroller
Comely Shiner
Common Carp
Common Shiner
Creek Chub
Cutlip Minnow
Eastern Silvery Minnow
Emerald Shiner*
Fallfish
Fathead Minnow
Golden Shiner
Goldfish
Grass Carp
Ironcolor Shiner (E)
Longnose Dace
Mimic Shiner (N)*
Alleghany Pearl Dace (I)
River Chub
Rosyface Shiner
Rosyside Dace
Satinfin Shiner
Silverjaw Minnow
Spotfin Shiner
Spottail Shiner
Striped Shiner (I)
Swallowtail Shiner

PERCHES:
Banded Darter
Chesapeake Logperch (T)
Fantail Darter
Glassy Darter (T)
Greenside Darter
Johnny Darter
Maryland Darter (E)
Rainbow Darter
Shield Darter
Stripeback Darter (E)
Swamp Darter
Tessellated Darter
Walleye
Yellow Perch

SUNFISHES:
Banded Sunfish
Black Crappie
Blackbanded Sunfish (E)
Bluegill
Bluespotted Sunfish
Flier (I)
Green Sunfish
Largemouth Bass
Longear Sunfish
Mud Sunfish
Pumpkinseed
Redbreast Sunfish
Redear Sunfish
Rock Bass
Smallmouth Bass
Warmouth
White Crappie

SCULPINS:
Blue Ridge Sculpin
Checkered Sculpin
Mottled Sculpin
Potomac Sculpin

TEMPERATE BASSES:
Striped Bass
White Perch

TROUTS:
Brook Trout
Brown Trout
Cutthroat Trout
Rainbow Trout

PIKES:
Chain Pickerel
Muskellunge
Northern Pike
Redfin Pickerel

MISCELLANEOUS:
American Eel
Bowfin
Eastern Mosquitofish
Eastern Mudminnow
Freshwater Drum (N)
Inland Silverside
Longnose Gar
Northern Snakehead (N)
Pond Loach (N)
Pirate Perch
Trout-Perch (X)

*Historically from and potentially occurring in the Youghiogheny River basin

Common Names of Maryland Reptiles and Amphibians

SALAMANDERS:

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

FROGS:

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

SNAKES:

Common Gartersnake
Common Watersnake
Common Wormsnake
Dekay's Brownsnake
Eastern Copperhead
Eastern Hog-nosed Snake
Eastern Kingsnake
Eastern Milksnake
Eastern Ratsnake
Eastern Ribbonsnake
Mountain Earthsnake (E)
North American Racer
Northern Mole Kingsnake
Plain-bellied Watersnake
Queensnake
Rainbow Snake (E)
Red Cornsnake
Red-bellied Snake
Ring-necked Snake
Rough Greensnake
Scarletsnake
Smooth Earthsnake
Smooth Greensnake
Timber Rattlesnake

TURTLES:

Bog Turtle (T)
Diamond-backed Terrapin
Eastern Box Turtle
Eastern Mud Turtle
Eastern Musk Turtle
Northern False Map Turtle
Northern Map Turtle (E)
Northern Red-bellied Cooter
Painted Turtle
Red-eared Slider
Snapping Turtle
Spiny Softshell (I)
Spotted Turtle
Striped Mud Turtle
Wood Turtle
Yellow-Bellied Slider

LIZARDS:

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

Scientific Names of Maryland Crayfishes

Cambarus acuminatus (I)
Cambarus bartonii
Cambarus carinirostris
Cambarus dubius
Cambarus monongalensis
Creaserinus fodiens
Faxonius limosus
Faxonius obscurus
Faxonius rusticus
Faxonius virilis
Lacunicambarus diogenes
Lacunicambarus thomaii
Procambarus acutus/zonangulus
Procambarus clarkii

Scientific Names of Maryland Freshwater Bivalves

Alasmidonta heterodon (E)
Alasmidonta undulata (T)
Alasmidonta varicosa (E)
Atlanticoncha ochracea
Corbicula sp. (N)
Dreissena bugensis (N)
Dreissena polymorpha (N)
Elliptio complanata
Elliptio fisheriana
Elliptio lanceolata (T)
Elliptio producta (I)
Lampsilis cardium
Lampsilis cariosa
Lampsilis radiata
Lasmigona subviridis (E)
Pyganodon cataracta
Sagattunio nasutus
Strophitus undulatus (I)
Utterbackia imbecillis
Utterbackiana implicata

Appendix F: Chain of Custody Sheets

This appendix includes the chain of custody sheets used by the MBSS, along with related guidance for each sheet. This includes:

- MBSS Water Quality Chain of Custody Sheet
- MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet
- MBSS Specimen Tracking Data Sheet

Currently, the MBSS uses electronic chain of custody sheets that are filled out in the field as samples are collected. This appendix includes screenshots of those electronic forms, and may be used as guidance for creating paper sheets as needed.

MBSS Water Quality Chain of Custody Sheet

1	UMCES Appalachian Laboratory					Chain-of-Custody Water Chemistry Samples			
2	301 Braddock Road					Maryland Biological Stream Survey			
3	Frostburg, MD 21532					Spring Index Period			
4									
5	Date of Shipment:		Tracking Number:			Analyze for:			
6	YYMMDD					1-L Bottle: DOC, TP, TN, Anions, Nutrients			☐
7						0.5-L Bottle: ANC, Specific Conductance			☐
8			Cooler Temperature on Receipt:	_____ C		Syringe: Closed pH			☐
9						125-mL pre-acidified Bottle: Copper, Zinc, Magnesium, Calcium			☐
10	**Sample Identification**	Date	Time			**Sample Identification**	Date	Time	
11	Site ID	YYMMDD	Military			Site ID	YYMMDD	Military	
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24	Field Comments:		Total Number in Cooler:			Cooler Relinquished By:			
25			Syringes						
26			1-L Bottles				Print name:		
27			0.5-L Bottles						
28			125-mL Bottles				Signature:		
29									
30	Date and Time of Receipt at Laboratory:		Lab Comments:			Cooler Received By:			
31	YYMMDD	Military					Print name:		
32									
33							Signature:		
34									
35									

The MBSS water quality chain-of-custody sheet is recorded and submitted electronically using Google Sheets. The sheet can be filled out in the field on a phone or tablet and shared immediately with lab personnel, allowing the lab to prepare for the correct number of samples prior to their arrival. Some portions of this sheet are filled out by the lab upon receipt of samples. The portions of this sheet that are filled out by MBSS prior to shipping samples include:

- "Date of Shipment": Enter the date that the cooler is shipped to the lab.
- "Tracking Number": Enter the tracking number provided by the courier upon shipment.
- "Analyze for": Identify the parameters that shipped samples are to be analyzed for.
- "Sample Identification": Provide the Site ID, Date, and Time for the samples included in the shipment.
- "Field Comments": Record any pertinent comments regarding the delivered samples, including unusual circumstances.
- "Total Number in Cooler": Indicate the number of each type of sample vessel included in the shipment.
- "Cooler Relinquished By": Provide the name and signature of the individual who shipped the samples.

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

	A	B	C	D	E	F	G	H	I
1	MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet								
2	Site ID	# Buckets	Collector	Collection Date	Delivery Date	Relinquished By	Received By	Field Office Log #	Comments
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
32									
33									
34									
35									

The MBSS benthic macroinvertebrate sample chain-of-custody sheet is recorded and submitted electronically using Google Sheets. The sheet can be filled out in the field on a phone or tablet and shared immediately with lab personnel, allowing the lab to prepare for the correct number of samples prior to their arrival. Some portions of this sheet are filled out by the lab upon receipt of samples. The portions of this sheet that are filled out by MBSS prior to shipping samples include:

- "Site ID": Enter the site ID just as it appears on the field data form.
- "# Buckets": Enter the number of buckets the sample is contained in.
- "Collector": Print the name of the person who collected the benthic sample.
- "Collection Date": Enter the date the sample was collected (using DD/MM/YY format) just as it appears on the field data form.
- "Delivery Date": Enter the date the sample was delivered to the field office using DD/MM/YY format.
- "Relinquished By": Enter the name of the person relinquishing the sample to the appropriate field office staff member.
- "Comments": Record any pertinent comments regarding the delivered samples, including unusual circumstances.

MBSS Specimen Tracking Data Sheet

	A	B	C	D	E	F
1	Crew Leader			MBSS Specimen Tracking Data Sheet		
2	Date Delivered (MM/DD/YYYY)					
3	Delivered By					
4	Received By					
5	Species	Date Collected (MM/DD/YYYY)	Site ID	Voucher Type Photo or Specimen	Image no. (s) or No. (s) of specimens	Comments
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						
33						
34						
35						

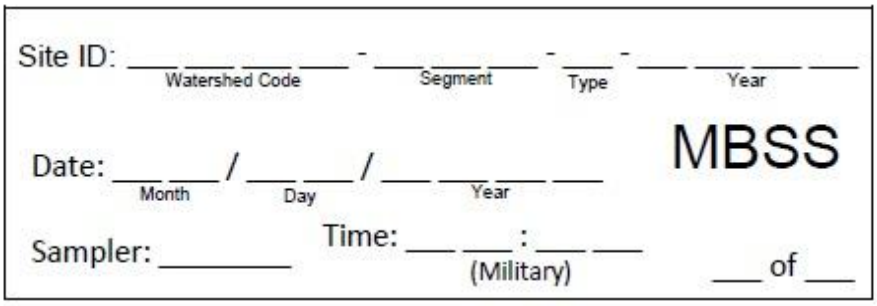
The MBSS Specimen Tracking Data Sheet is recorded and submitted electronically using Google Sheets. The sheet can be filled out in the field on a phone or tablet, and a shared document hosted online may allow multiple members of the field crew to make entries in the same file. The crew leader should maintain one sheet for each fauna group to track the number of vouchers (photographs or preserved specimens) kept throughout the field season and to accompany those vouchers when they are submitted to a taxonomic expert for review. The portions of this sheet that are filled out by MBSS prior to submitting vouchers include:

- "Crew Leader": Enter the name of the crew leader ultimately responsible for the collected vouchers.
- "Date Delivered": Enter the date that the voucher collection was turned over for review, if applicable.
- "Delivered By": Enter the name of the individual who delivered the vouchers for review.
- "Species": Enter the species being vouchered.
- "Date Collected": Enter the date the voucher was collected.
- "Site ID": Enter the site where the voucher was collected.
- "Voucher Type": Indicate whether the voucher is a photograph or a preserved specimen.
- "Image no. (s) or No. (s) of Specimens": Provide image file numbers or the number of preserved specimens for a vouchered species at a site.
- "Comments": Record any pertinent comments regarding the vouchers, including unusual circumstances.

Appendix G: Labels

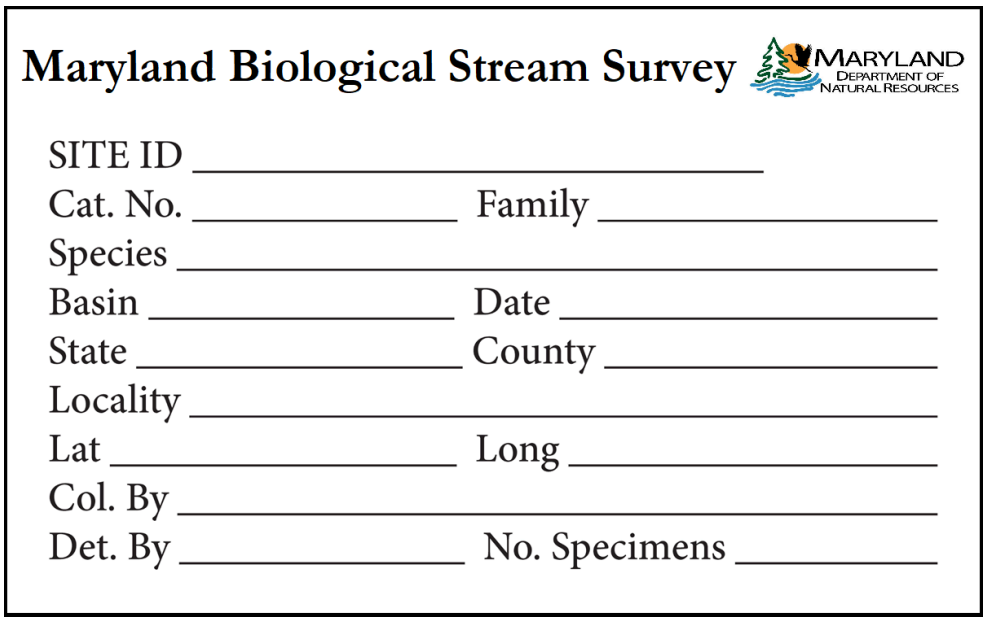
This appendix contains blank templates of the labels used by the MBSS.

MBSS Sample Label



This label (or one containing the same information) should be used with both water samples and benthic macroinvertebrate samples. The label should be filled out in pencil or indelible ink, with exterior labels covered with clear plastic tape. Water samples should have one label affixed to the exterior of each bottle/syringe. Benthic macroinvertebrate samples should have one label affixed to the exterior of each sample container and covered with clear plastic tape, with a duplicate label placed inside the container with the sample. Labels should be numbered in the lower right corner to indicate how many containers are expected for each sample.

MBSS Voucher Label



This label (or one containing the same information) should be used to identify preserved specimens kept as MBSS taxonomic vouchers. Photographic vouchers are preferred, but in instances where a specimen has been preserved, this label should be printed on waterproof paper and filled out using pencil or indelible ink and placed inside the jar(s) with each specimen.

Appendix H: MBSS Spring Electronic Datasheet

All MBSS data from the Spring Index Period is currently recorded using an electronic datasheet. This datasheet is divided into eight pages:

- SiteInfo
- SiteSpecs
- TempLoggers
- Samples&Labels
- SpringHabitat
- Modifications
- Fauna
- Photos

This appendix includes screenshots of the electronic datasheet pages, which may be used as guidance for creating paper datasheets as needed. If data is to be recorded on paper datasheets, those sheets should be printed on waterproof paper to facilitate data recording during inclement weather. Backup copies of all field data sheets (both electronic and paper) must be made.

SiteInfo

Site: (Spring)		
QC1:		⚠ Warning messages persist in datasheet
QC2:		
Site Info		
Site Code		⚠ Blank
Date		⚠ Blank
Time		⚠ Blank
Sampleability		
Benthos		⚠ Blank
Spring Hab Variables		⚠ Blank
Summer Hab Variables		⚠ Blank
Water Quality		⚠ Blank
Herpetofauna		⚠ Blank
Crayfish		⚠ Blank
Mussels		⚠ Blank
Index Comments		
Crew		
	Total number of people: 0	
1.) Crew Leader		⚠ Blank
2.)		↣Blank
3.)		
4.)		
5.)		
6.)		
7.)		
8.)		
9.)		
10.)		
11.)		
12.)		
13.)		
14.)		
15.)		
16.) write-in		
17.) write-in		
18.) write-in		
19.) write-in		
20.) write-in		

SiteSpecs

Site: (Spring)		
QC1:		⚠Warning messages persist in datasheet
QC2:		
Location Information		
Stream Name		
Locality		⚠Blank
Access Route		⚠Blank
Midpoint Location		
# Midpoint Photos	0	↣2 midpoint photos required
Field Coordinates Recorded?		
GPS Latitude		
GPS Longitude		
Sampling Considerations		
# Anodes Needed	0	
Stream Width: 0m	Record on 'Transects'	↣Input data on Transects sheet
Stream Width:75m	Record on 'Transects'	↣Input data on Transects sheet
Other Considerations		

TempLoggers

Site: (Spring)		
QC1:		⚠ Warning messages persist in datasheet
QC2:		
WQ Overview		
Loggers Deployed		⚠ Blank
In-Situ Measurements		⚠ Blank
Logger #1		
Type Deployed		
Time		
Serial Number		
Location/Notes		
Logger #2		
Type Deployed		
Time		
Serial Number		
Location/Notes		
Logger #3		
Type Deployed		
Time		
Serial Number		
Location/Notes		
Logger #4		
Type Deployed		
Time		
Serial Number		
Location/Notes		
In-Situ Measurements		
WQ Meter Identification		
Time		
Temperature (˚C)		
DO (mg/L)		
DO(% Sat.)		
pH		
Sp. Conductivity(µs/cm)		

Site: (Spring)

QC1:		⚠ Warning messages persist in datasheet
QC2:		

Benthic Habitat Sampled

Riffle	0	⚠ 20 habitats required, you sampled 0.
Rootwad/WoodyDebris	0	
Leaf Pack	0	
Macrophytes	0	
Undercut Bank	0	
Other (Specify)	0	
Description of 'Other'		
Sample Notes		

Sample Labels

Labels Verified By		⚠ Verify Sample Labels

QC Labels

QC Samples Taken		⚠ Blank
Duplicate or Blank		
Labels Verified By		
Code for QC Samples	*NO QC SAMPLE*	

SpringHabitat

Site: (Spring)

QC1:		⚠ Warning messages persist in datasheet
QC2:		

Habitat Assessor		⚠ Blank

Habitat:

Distance to Road (m)		⚠ Blank
Trash Rating	0	⚠ Blank

Adjacent Landuse:

Old Field		⚠ Blank
Deciduous Forest		⚠ Blank
Coniferous Forest		⚠ Blank
Wetland		⚠ Blank
Surface Mines		⚠ Blank
Landfill		⚠ Blank
Residential		⚠ Blank
Commercial/Industrial		⚠ Blank
Cropland		⚠ Blank
Pasture		⚠ Blank
Orch./Vinyd/Nursery		⚠ Blank
Golf Course		⚠ Blank
Riparian Planting		⚠ Blank
Vernal Pool		

Riparian Vegetation

	Left Bank	Right Bank	
Buffer Width	0	0	
Adjacent Land Cover			⚠ Blank
Veg Type 1			
Veg Type 2			
Veg Type 3			
Veg Type 4			

Modifications

Site: (Spring)

QC1:		⚠ Warning messages persist in datasheet
QC2:		

Buffer Breaks:

Buffer Breaks Present		⚠ Blank

Buffer Break Type	Left Bank	Right Bank	# Photo
Storm Drain			0
Tile Drain			0
Imperv. Drainage			0
New Construction			0
Orchard			0
Crop			0
Pasture			0
Gully			0
Dirt Road			0
Gravel Road			0
Railroad			0
Raw Sewage			0

Road Culvert:

Present in Segment		⚠ Blank
Sampleable?		
Width of Culvert (m)		
Length of Culvert (m)		
# Photos	0	

Stream Blockage:

Blockage Present?		⚠ Blank
Blockage Height (m)		
Blockage Type		
Latitude		
Longitude		
# Photos	0	

Channel Modifications:

Modifications Evident		⚠ Blank

Channelization Type	Extent (m)			# Photo
	Left Bank	Bottom	Right Bank	
Concrete				0
Gabion				0
Pipe Culvert				0
Earthen Berm				0
Dredge Spoils Offchannel				0
Bank Regrading				0
Rip-Rap				0
Erosion Control Fabric				0
Woody Alteration Structure				0

Fauna

Site: (Spring)

QC1:

QC2:

⚠ Warning messages persist in this dataset

Herpetofauna

	Species	Adult	Larva	Egg	Seen	Heard	# Photo	# Ret.	Comments	
1							0	0		⚠ Error, incomplete
2							0	0		
3							0	0		
4							0	0		
5							0	0		
6							0	0		
7							0	0		
8							0	0		
9							0	0		
10							0	0		

Crayfish

Burrows Present?

⚠ Blank

	Species	Incidental	# Photos	# Ret.	Comments	
1			0	0		⚠ Error, incomplete
2			0	0		
3			0	0		
4			0	0		
5			0	0		
6			0	0		
7			0	0		
8			0	0		

Bivalves

	Species	Live	Fresh Dead	Old Dead	Relict	# Photos	# Ret.	Comments	
Corbicula						0	0		⚠ Error, incomplete
Mussel 1						0	0		⚠ Error, incomplete
2						0	0		
3						0	0		
4						0	0		
5						0	0		
6						0	0		
7						0	0		

Photos

Photo	Photo ID # Camera only	Description	Photo Type	Photo Voucher?
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
			[Select	No

Appendix I: MBSS Summer Electronic Datasheet

All MBSS data from the Summer Index Period is currently recorded using an electronic datasheet. This datasheet is divided into fourteen pages:

- SiteInfo
- Summer Temp Loggers
- SummerHab1
- SummerHab2
- Transects
- Debris
- Fauna
- Anodes
- Fish
- Gamefish
- Pebble Count
- Photos
- Discharge

In addition to the electronic datasheet, MBSS field crews utilize a paper Crib Sheet to record fish species and counts during fish sample processing. Fish data from the Crib Sheet is reviewed by the crew leader before being transferred to the “Fish” page of the MBSS Summer electronic data sheet. Crib sheets are retained in a bound notebook during the index period. A copy of the paper Crib Sheet is included in Appendix K.

This appendix includes screenshots of the electronic datasheet pages as well as a copy of the paper Crib Sheet. The electronic datasheet screenshots may be used as guidance for creating paper datasheets as needed. If data is to be recorded on paper datasheets, they should be printed on waterproof paper to facilitate data recording during inclement weather. Backup copies of all field data sheets (both electronic and paper) must be made.

SiteInfo

Site: (Summer)		
QC1:		⚠ Warning messages persist in datasheet
QC2:		

Site Info

Site Code		⚠ Blank
Date		⚠ Blank
Time		⚠ Blank

Sampleability

Electrofishing		⚠ Blank
Spring Hab Variables		⚠ Blank
Summer Hab Variables		⚠ Blank
Water Quality		⚠ Blank
Herpetofauna		⚠ Blank
Salamanders		⚠ Blank
Crayfish		⚠ Blank
Mussels		⚠ Blank
Aquatic Plants		⚠ Blank

Index Comments	

Crew

	Total number of people: 0	
1.) Crew Leader		⚠ Blank
2.)		↦Blank
3.)		
4.)		
5.)		
6.)		
7.)		
8.)		
9.)		
10.)		
11.)		
12.)		
13.)		
14.)		
15.)		
16.) write-in		
17.) write-in		
18.) write-in		
19.) write-in		
20.) write-in		

Summer Temp Loggers

Site: (Summer)		
QC1:		⚠ Warning messages persist in datasheet
QC2:		
WQ Overview		
Loggers Previously Deployed		⚠ Blank
In-Situ Measurements		⚠ Blank
Water Logger Present		
Water Logger Dewatered		
Air Logger Present		
Logger Notes		
In-Situ Measurements		
WQ Meter Identification		
Time		
Temperature (˚C)		
DO (mg/L)		
DO(% Sat.)		
pH		
Sp. Conductivity(µs/cm)		

Site: (Summer)

QC1:

QC2:

Warning messages persist in this datasheet

SUMMER HABITAT METRICS

Habitat Assessor		Blank

Habitat Scores

Instream Habitat	0	Blank
Epifaunal Substrate	0	Blank
Velocity/Depth Diversity	0	Blank

»Attention: VD Types Blank (Optional)

	Shallow (<0.5 m)	Deep (≥0.5 m)
Slow (<0.3m/s)		
Fast (>0.3m/s)		

Pool/Glide/Eddy Quality	0	Blank
P/G/E Extent (m)	0	Blank
Riffle/Run Quality	0	
Riffle/Run Extent (m)	0	Blank
Embeddedness	0%	»Attention: 0% embedded/bedrock
Shading	0%	»Attention: Full sun exposure

Bar Formation

Bar Severity		Blank

Bar Composition

Cobble		Blank
Gravel		
Sand		
Silt/Clay		

Erosion

Erosion Present (Y/N):		Blank

	Left Bank	Right Bank
Erosion Extent (m)	0	0
Severity		
Avg. Height (m)		

SummerHab2

Site: (Summer)		
QC1:		⚠Warning messages persist in datasheet
QC2:		
Inventory		
Macrohabitat		
Braided Channel		⚠Blank
Riffle		⚠Blank
Run/Glide		⚠Blank
Deep Pool (>0.5m)		⚠Blank
Shallow Pool (<0.5m)		⚠Blank
Stream Characteristics		
Undercut Bank		⚠Blank
Overhead Cover		⚠Blank
Beaver Pond		⚠Blank
Substrate		
Large Boulder (>2m)		⚠Blank
Small Boulder (<2m)		⚠Blank
Cobble		⚠Blank
Gravel		⚠Blank
Sand		⚠Blank
Silt/Clay		⚠Blank
Bedrock		⚠Blank
Aquatic Vegetation		
Submerged Aquatic Veg.		⚠Blank
Emergent Aquatic Veg.		⚠Blank
Floating Aquatic Veg.		⚠Blank
Didymo		⚠Blank
Didymo Voucher		⚠Blank
Orange Floculent		⚠Blank
Filamentous algae		⚠Blank
Habitat Notes		
Habitat Comments		

Transects

Site: (Summer)		
QC1:		⚠ Warning messages persist in datasheet
QC2:		
Traditional Transect Measurements		⚠
Maximum Depth (cm)		⚠ Blank
0 Meters		
Stream Width (m)		⚠ Blank
Thalweg Depth (cm)		⚠ Blank
25 Meters		
Stream Width (m)		⚠ Blank
Thalweg Depth (cm)		⚠ Blank
50 Meters		
Stream Width (m)		⚠ Blank
Thalweg Depth (cm)		⚠ Blank
75 Meters		
Stream Width (m)		⚠ Blank
Thalweg Depth (cm)		⚠ Blank

Alternate Measurements For Standing Pools

	Length (m)	Width (m)	Depth (cm)	
Pool 1				
Pool 2				
Pool 3				
Pool 4				
Pool 5				
Pool 6				
Pool 7				
Pool 8				
Pool 9				
Pool 10				
Pool 11				
Pool 12				
Pool 13				
Pool 14				
Pool 15				

Site: (Summer)

QC1:

QC2:

⚠ Warning messages persist in this datasheet

Woody Debris

Tally Method

Select talley method

⚠ Select talley method

Count: 0-25	In-water	De-
Woody Debris:	0	0
Rootwads:	0	0

Count: 25-50	In-water	De-
Woody Debris:	0	0
Rootwads:	0	0

Count: 50-75	In-water	De-
Woody Debris:	0	0
Rootwads:	0	0

Select talley method

⚠ Select talley method

	In-water	De-
Woody Debris:	0	0
Rootwads:	0	0

Auto-Sum of Wood Count

	In-water	De-watered
Woody Debris:	0	0
Rootwads:	0	0

Fauna

Site: (Summer)

QC1:

QC2:

⚠ Warning messages persist in this dataset

Stream Salamanders

	Species	ELECTROFISHING # Adults	ELECTROFISHING # Larvae	INCIDENTAL # Adults	INCIDENTAL # Larvae	# Photos	# Ret.	Comments	
1		0	0	0	0	0	0		⚠ Error, incomplete
2		0	0	0	0	0	0		
3		0	0	0	0	0	0		
4		0	0	0	0	0	0		
5		0	0	0	0	0	0		
6		0	0	0	0	0	0		
7		0	0	0	0	0	0		
8		0	0	0	0	0	0		
9		0	0	0	0	0	0		
10		0	0	0	0	0	0		

Herpetofauna

	Species	Adult	Larva	Egg	Seen	Heard	# Photo	# Ret.	Comments	
1							0	0		⚠ Error, incomplete
2							0	0		
3							0	0		
4							0	0		
5							0	0		
6							0	0		
7							0	0		
8							0	0		
9							0	0		
10							0	0		

Crayfish

Burrows Present?

⚠ Blank

	Species	Pass 1	Pass 2	Incidental	# Photos	# Ret.	Comments	
1		0	0		0	0		⚠ Error, incomplete
2		0	0		0	0		
3		0	0		0	0		
4		0	0		0	0		
5		0	0		0	0		
6		0	0		0	0		
7		0	0		0	0		
8		0	0		0	0		

Bivalves

	Species	Live	Fresh Dead	Old Dead	Relict	# Photos	# Ret.	Comments	
Corbicula						0	0		⚠ Error, incomplete
Mussel 1						0	0		⚠ Error, incomplete
2						0	0		
3						0	0		
4						0	0		
5						0	0		
6						0	0		
7						0	0		

Anodes

Site: (Summer)

QC1:

QC2:

⚠ Please have two people review this datasheet.

Unit Name	Gear-Type	Model	Num. Anodes	Voltage	Wave-form	Begin Pass 1	End Pass 1	End Pass 2	End Pass 3	Notes
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				
	[SELECT]	[SELECT]	0			0				

Fish

Site: (Summer)

QC1:				
QC2:			⚠Warning messages persist in datasheet	
Length of segment sampled (m)		⚠**Blank**	Fish Captured	
Fish move during net install		⚠**Blank**	Total # Sampled	0
Bottom visible entire segment		⚠**Blank**	# Gamefish	0
Same water clarity 2nd pass		⚠**Blank**	# Species	0
			Total Biomass	0
PASS 1 BIOMASS (g)			PASS 3	
PASS 2 BIOMASS (g)				

Species	PASS 1	PASS 2	PASS 3	Photos	Vouchers	Comments
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	
	0	0		0	0	

Gamefish

Site: (Summer)

QC1:		
QC2:		↣No gamefish sampled

Gamefish Talley		**Species Counts (Measured/Sampled, All passes)**		
Gamefish Sampled:	0	LMB: 0/0	BKT: 0/0	CHP: 0/0
Recorded:	0	SMB: 0/0	BNT: 0/0	
Measured:	0	STB: 0/0	RBT: 0/0	
These records match FishList				

Pass #	Species	TL (mm)	Weight (g)	DELT	Not measured	Note
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						
1						

Pebble Count

Site: (Summer)

QC1:	
QC2:	

⚠ 10 transects & 100 particles required

PEBBLE COUNT

Total Particles:	0	D16:	N/A
Transects:	0	D50:	N/A
Habitat:		D84:	N/A
Pebble Ct Notes:			

Material	Size Range (mm)	Count
silt/clay	>0 - 0.062	0
very fine sand	>0.062 - 0.125	0
fine sand	>0.125 - 0.25	0
medium sand	>0.25 - 0.5	0
coarse sand	>0.5 - 1	0
very coarse sand	>1 - 2	0
very fine gravel	>2 - 4	0
fine gravel	>4 - 6	0
fine gravel	>6 - 8	0
medium gravel	>8 - 11	0
medium gravel	>11 - 16	0
coarse gravel	>16 - 22	0
coarse gravel	>22 - 32	0
very coarse gravel	>32 - 45	0
very coarse gravel	>45 - 64	0
small cobble	>64 - 90	0
medium cobble	>90 - 128	0
large cobble	>128 - 180	0
very large cobble	>180 - 256	0
small boulder	>256 - 362	0
small boulder	>362 - 512	0
medium boulder	>512 - 1024	0
large boulder	>1024 - 2048	0
very large boulder	>2048 - 4096	0
bedrock	----------	0
clay hardpan	----------	0
detritus/wood	----------	0
artificial	----------	0

Total particles: 0

Photos

Site: (Summer)

Total Number of Photos Recorded:0

Photo	Photo ID # Camera only	Description	Photo Type	Photo Voucher?
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
Tap + to add images.			[Select photo type]	No
			[Select	No

Discharge

Site: (Summer)

QC1:

QC2:

⚠ Warning messages persist in datasheet

Traditional Discharge Measurements

Desired number of stations:	10
LB Value:	0.00
RB Value:	0.00

Width @ Transect ---- 0.00 m

To obtain 10 in-channel measurements, they must be taken every 0.00 m.

#	Suggested Lat. Location (m)	Measured Lat. Location (m)	Depth (cm)	Velocity (m/s)	
1	0.00		0.0	0.00	⚠ No measurements recorded
2	0.00				
3	0.00				
4	0.00				
5	0.00				
6	0.00				
7	0.00				
8	0.00				
9	0.00				
10	0.00				
11	0.00				
12	0.00				
13	0.00				
14	0.00				
15	0.00				
16	0.00				
17	0.00				
18	0.00				
19	0.00				
20	0.00				
21	0.00				
22	0.00				
23	0.00				
24	0.00				
25	0.00				

Alternate Discharge Measurements

Distance (cm)	⚠ Incomplete
Depth (cm)	
Width (cm)	
Time #1 (seconds)	
Time #2 (seconds)	
Time #3 (seconds)	

Average time:	⚠	s
Velocity:	⚠	m/s
Discharge:	⚠	m^3/s

Appendix J: MBSS Spring Paper Datasheet

This appendix contains a paper Spring datasheet similar to those used by the MBSS prior to the adoption of electronic datasheets. It has been modified to include all the same parameters recorded in the electronic datasheet, and may be useful for those not equipped for electronic data collection or as a backup in case of electronic data collection equipment failure. This datasheet is divided into three pages:

- Site Info and Samples
- Habitat and Water Chemistry
- Fauna

Site Info and Samples

MBSS SPRING INDEX PERIOD DATA SHEET

Page ☐ of ☐

SITE CODE ☐☐☐☐ (Watershed Code) ☐☐☐ (Segment) ☐ (Type) ☐☐☐☐ (Year)

Reviewer: First _____ / Second _____

DATE ☐☐ (Year) ☐☐ (Month) ☐☐ (Day)

CREW: _____

STREAM NAME: _____

LOCALITY: _____

TIME ☐☐☐☐ (Military)

SAMPLEABILITY

☐ Benthos ☐ Crayfish

☐ Habitat Assessment

☐ Water Quality

☐ Herpetofauna ☐ Mussels

Other: _____

SITE ACCESS ROUTE: _____

SAMPLE LABELS

Verified by: _____

QC LABELS

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

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

TEMP. LOGGERS

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

LOCATION: _____

PHOTODOCUMENTATION

Number		Voucher (Y/N)
☐☐☐☐	Midpoint looking upstream	N
☐☐☐☐	Midpoint looking downstream	N
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		

BENTHIC HABITAT SAMPLED

☐☐ Riffle
☐☐ Rootwad/Woody Debris

☐☐ Leaf Pack
☐☐ Macrophytes

☐☐ Undercut Banks
Other: _____

SAMPLING CONSIDERATIONS: (☐☐ NUM. ANODES)

STREAM WIDTH (m) ☐☐☐.☐ (0 m) ☐☐☐.☐ (75 m)

MBSS SPRING HABITAT DATA SHEET

Page ☐ of ☐

SITE — Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐ Reviewer: First _____ / Second _____

DATE — Year ☐☐ Month ☐☐ Day ☐☐ Habitat Assessor: _____

☐☐☐☐ Dist. from Nearest Road to Site (m)

☐☐ Trash Rating 0 - 20

LANDUSE (Y/N)

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

RIPARIAN VEGETATION
(facing upstream)

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

ROAD CULVERT

- Present in Segment? (Y/N) ☐
- Sampleable? (Y/N) ☐
- Width of Culvert (m) ☐☐
- Length of Culvert (m) ☐☐

In-Situ Water Chemistry

- WQ Meter ID ☐
- Time ☐
- Temperature (C) ☐
- DO (mg/L) ☐
- DO (% Sat.) ☐
- pH ☐
- Sp. Conductivity (uS/cm) ☐

BUFFER BREAKS

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

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

CHANNEL MODIFICATIONS ☐ (Y/N)

TYPE	EXTENT (m) LEFT BANK	BOTTOM	RIGHT BANK
Concrete	☐☐	☐☐	☐☐
Gabion	☐☐	☐☐	☐☐
Rip-Rap	☐☐	☐☐	☐☐
Earthen Berm	☐☐	☒☒	☐☐
Dredge Spoil Off Channel	☐☐	☒☒	☐☐
Pipe Culvert	☐☐	☐☐	☐☐
Bank Regrading	☐☐	☐☐	☐☐
Erosion Control Fabric	☐☐	☐☐	☐☐
Woody Alteration Structure	☐☐	☐☐	☐☐

Actual Site Midpoint Coordinates
(Taken at Time of Sampling)

Lat ☐☐☐☐☐☐☐☐

Long ☐☐☐☐☐☐☐☐

Stream Blockages

Present? (Y/N) ☐

Stream Block Ht. (m) ☐☐

Stream Block Type ☐☐

Lat ☐☐☐☐☐☐☐☐

Long ☐☐☐☐☐☐☐☐

Fauna

MBSS SPRING FAUNAL DATA SHEET

Page ☐ of ☐

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

Reviewer: First ______ / Second ______

☐ None Observed

HERPETOFAUNA

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

☐ None Observed

MUSSELS

Species	LIVE	FRESH DEAD	OLD DEAD	RELICT	Number Retained	Num. Photos Taken
	☐	☐	☐	☐	☐☐	☐
	☐	☐	☐	☐	☐☐	☐
	☐	☐	☐	☐	☐☐	☐
	☐	☐	☐	☐	☐☐	☐
	☐	☐	☐	☐	☐☐	☐
	☐	☐	☐	☐	☐☐	☐

Corbicula NONE ☐ LIVE ☐ FRESH DEAD ☐ OLD DEAD ☐ RELICT ☐

☐ None Observed

CRAYFISH

Species	Incidental? (Y/N)	Number of photos	Number Retained
	☐	☐☐	☐☐
	☐	☐☐	☐☐
	☐	☐☐	☐☐
	☐	☐☐	☐☐
	☐	☐☐	☐☐

Crayfish Burrows (A,P,E) ☐

COMMENTS: ______________________________

Appendix K: MBSS Summer Paper Datasheet

This appendix contains a paper Summer datasheet similar to those used by the MBSS prior to the adoption of electronic datasheets. It has been modified to include all the same parameters recorded in the electronic datasheet, and may be useful for those not equipped for electronic data collection or as a backup in case of electronic data collection equipment failure. This datasheet is divided into eight pages:

- Site Info
- Habitat
- Fauna
- Fish & Anodes
- Gamefish
- Pebble Count
- Photos
- Crib Sheet

Site Info

MBSS SUMMER INDEX DATA SHEET

Page ☐ of ☐

SITE CODE ☐☐☐☐ (Watershed Code) ☐☐☐ (Segment) ☐ (Type) ☐☐☐☐ (Year)

Reviewer: First _____ / Second _____

DATE ☐☐ (Year) ☐☐ (Month) ☐☐ (Day)

CREW: _____

TIME ☐☐☐☐ (Military)

SAMPLEABILITY

S = Sampleable
0 = N/A
1 = Dry Streambed
2 = Too Deep
3 = Marsh, no defined channel
4 = Excessive Riparian Vegetation
5 = Impoundment
6 = Tidally Influenced
7 = Landowner Permission Denied
8 = Unsafe (Describe in Comments)
9 = Beaver
10 = Other: _____

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

Water Chemistry

In Situ Measurements (Y/N) ☐

Water Quality Meter ID: _____

Time: ____ ____ : ____ ____

Temperature (C) : _____

Dissolved Oxygen

mg/L: _____

% Saturation: _____

pH: _____

Sp. Conductivity (us/cm): _____

Aquatic Vegetation (A, P, or E)

- Submerged Aquatic Vegetation ☐
- Emergent Aquatic Vegetation ☐
- Floating Aquatic Vegetation ☐

DIDYMO

- (A,P, or E) ☐
- Voucher (Y/N) ☐

Temperature Loggers

Loggers previously deployed? (Y/N) ☐

Water Logger Present? (Y/N) ☐

Water Logger Dewatered? (Y/N) ☐

Air Logger Present? (Y/N) ☐

Logger Notes: _____

COMMENTS: _____

MBSS SUMMER HABITAT DATA SHEET

Page ☐ of ☐

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

Reviewer: First _____ / Second _____

Habitat Assessor: _____

BANK EROSION

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

BAR FORMATION & SUBSTRATE

Severity ___ (0 = none, 1 = min, 2 = mod, 3 = severe)

- ☐ Cobble
- ☐ Gravel
- ☐ Sand
- ☐ Silt/Clay

HABITAT ASSESSMENT

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

FLOW

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

STREAM CHARACTER

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

A = Absent P = Present E = Extensive

Woody Debris

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

Maximum Depth (cm) ☐☐☐

	Wetted Width (m)	Thalweg Depth (cm)
0 m	☐☐.☐	☐☐☐
25 m	☐☐.☐	☐☐☐
50 m	☐☐.☐	☐☐☐
75 m	☐☐.☐	☐☐☐

Alternative Flow Measurements

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

COMMENTS: _____

MBSS SUMMER FAUNA DATA SHEET

Page ☐ of ☐

SITE CODE Watershed Code ☐☐☐☐ Segment ☐☐☐ Type ☐ Year ☐☐☐☐ Reviewer: First _____ / Second _____

STREAM SALAMANDERS

☐ None Observed

Species	Electrofishing Catch Adult	Electrofishing Catch Larva	Incidental Catch Adult	Incidental Catch Larva	Number Retained	Number Photos Taken

OTHER HERPETOFAUNA

☐ None Observed

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

MUSSELS

☐ None Observed

Species	Live	Fresh Dead	Old Dead	Relict	Num. Photos Taken	Number Retained

Corbicula	None	Live	Fresh Dead	Old Dead	Relict	# Photos	# Retained
	☐	☐	☐	☐	☐	☐☐	☐☐

CRAYFISH

☐ None Observed

Crayfish Burrows (Absent, Present, Extensive) ☐

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

COMMENTS: _____

Fish & Anodes

MBSS FISH DATA SHEET

Page ☐ of ☐

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

Reviewer: First _____ / Second _____

	(Y/N)	Unit ____	Unit ____	Unit ____	Unit ____	Unit ____	Unit ____
Fish Move. During Net Installation?	☐	Anodes/Unit ☐					
Bottom Visible in all Areas of Seg.?	☐	Begin 1st P.					
Same Water Clarity - 2nd Pass?	☐	Begin 2nd P.					
Length of Seg. Sampled (m)	☐☐	Begin 3rd P.					
		End					
Fish Captured? (Y/N) ☐ Gamefish? (Y/N) ☐		Voltage					

SPECIES	1st Pass Catch (Total)	2nd Pass Catch (Total)	3rd Pass Catch (Total)	Anomalies (Y/N)	Number Retained	Number of Photos	Comments

Aggregate Fish Biomass ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ (g)

MBSS GAME FISH LENGTH DATA SHEET

Page ☐ of ☐

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

Reviewer: First _____ / Second _____

Pass	SPECIES	LENGTH (TL; mm)	WEIGHT (g)	Pass	SPECIES	LENGTH (TL; mm)	WEIGHT (g)

MBSS RIFFLE PEBBLE COUNT DATA SHEET

Page ☐ of ☐

SITE CODE ☐☐☐☐ (Watershed Code) ☐☐☐ (Segment) ☐ (Type) ☐☐☐☐ (Year)

Reviewer: _____ (First) / _____ (Second)

		Range (mm)	Particle Tally	Total
SILT/CLAY	Silt/Clay	<.062		
SAND	Very Fine	.062 - .13		
	Fine	.13 - .25		
	Medium	.25 - .50		
	Coarse	.50 - 1.0		
	Very Coarse	1.0 - 2		
GRAVEL	Very Fine	2 - 4		
	Fine	4 - 6		
	Fine	6 - 8		
	Medium	8 - 12		
	Medium	12 - 16		
	Coarse	16 - 24		
	Coarse	24 - 32		
	Very Coarse	32 - 48		
	Very Coarse	48 - 64		
COBBLE	Small	64 - 96		
	Small	96 - 128		
	Large	128 - 192		
	Large	192 - 256		
BOULDER	Small	256 - 384		
	Small	384 - 512		
	Medium	512 - 1024		
	Large	1024 - 2048		
	Very Large	2048 - 4096		
BEDROCK		>4096		
CLAY HARDPAN		-------		
DETRITUS/WOOD		-------		
ARTIFICIAL		-------		
# TRANSECTS: _____		HABITAT: _____	NOTES: _____ Grand Total	

Pebble Count

Photos

MBSS PHOTO DATA SHEET

Page ☐ of ☐

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

Reviewer: First ______ / Second ______

Number	PHOTODOCUMENTATION	Voucher (Y/N)
☐☐☐☐	______	☐
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Crib Sheet

CRIB SHEET

Page ☐ of ☐

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

_____ PASS			_____ PASS		
Species	Tally	Anomalies	Species	Tally	Anomalies