MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

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MARYLAND DEPARTMENT OF NATURAL RESOURCES

MARYLAND BIOLOGICAL STREAM SURVEY

QUALITY ASSURANCE PROJECT PLAN

July 2024

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Quality Assurance Project Plan for the Maryland Department of Natural Resources Maryland Biological Stream Survey

May 2024

Prepared by

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(Modified from a previous approved version prepared by Dan Boward)

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A.1 Introduction

This Quality-Assurance Project Plan (QAPP) describes quality-assurance goals and measures for the Maryland Biological Stream Survey (MBSS), operated by the Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division (hereafter referred to as DNR). It was produced under the guidance provided by EPA's Guidance for Quality Assurance Project Plans (EPA QA/G-5).

The more than 16,000 miles of streams and rivers in Maryland represent a vital natural resource to the people of the State because of the direct influence of tributaries on Bay water quality and their importance as habitat for fish and other living resources (Versar 2011). In spite of this importance, the current status of most of these waters is unknown and the relationships between biological conditions and environmental factors are poorly understood. To fill this information need, Maryland designed the MBSS to assess the fishability and biological integrity of streams and rivers in Maryland. The survey will assist decision makers in identifying the geographical distribution of biological resources, prioritizing environmental issues of concern within Maryland's flowing waters, and specifying regions where protection or mitigation activities are warranted. These issues have become and will likely continue to be of increasing concern as the focus of the Chesapeake Bay Program shifts further into tributaries.

One objective of the MBSS is to assess with known confidence the current status of the biological resources in non-tidal streams and rivers in Maryland. Biological resources are evaluated on a local, regional, and statewide basis using two endpoints, fishability and biological integrity. Another objective of the MBSS is to monitor indicators of pollution exposure and habitat condition to identify local, regional, or statewide causes of adverse effects, including acid deposition, point source discharges, and others. Additional objectives of the survey are to provide an inventory of biodiversity in Maryland's streams and rivers and a means to focus protection and restoration activities. Information from the MBSS is used to provide statistical summaries and interpretive reports on ecological status and fishability to decision-makers and the public.

A.2 Distribution List

Durga Ghosh, USGS/Chesapeake Bay Program
Kaylyn S. Gootman, EPA/Chesapeake Bay Program
David Goshorn, MDNR
Kristen Fidler, MDNR
Richard Ortt, Jr., MDNR
Renee Karrh, MDNR
Scott Stranko, MDNR
Jay Kilian, MDNR
William Harbold, MDNR
Tomas Ivasauskas, MDNR
Neal Dziepak, MDNR

A.3 Project/Task Organization

To ensure that adequate responsibility and accountability for MBSS data are maintained, an organizational structure defining the responsibilities for MBSS key personnel was prepared (see Figure 1). Adherence to the chain of authority and responsibility is especially important to the MBSS Quality Assurance/Quality Control Program. The responsibilities of each of these personnel are described below:

Unit Director: Richard A. Ortt, Jr., Resource Assessment Service, DNR.
richard.ortt@maryland.gov
Responsibilities: The unit director is responsible for overseeing the administrative aspects of the program including fiscal management, coordination among other DNR managers and coordination with cooperating agencies and institutions.

Division Director: Scott Stranko, Monitoring and Non-Tidal Assessment Division, DNR.
scott.stranko@maryland.gov
Responsibilities: The division director and principal investigator is responsible for overseeing the administrative aspects of the program including fiscal management, coordination among other DNR managers and coordination with cooperating agencies and institutions.

Project Officer: Jay Kilian, Monitoring and Non-Tidal Assessment Division, DNR.
jay.kilian@maryland.gov
Responsibilities: The MBSS Project Officer has overall responsibility for successful completion of the MBSS. Specific duties of the Project Officer include selection of project staff, direction and approval of training activities, contractor oversight, liaison with the public and resource agencies, document review, and peer review solicitation. The Project Officer is also responsible for maintaining and updating the Quality Assurance Project Plan.

Training Officer: William Harbold, Monitoring and Non-Tidal Assessment Division, DNR.
william.harbold@maryland.gov
Responsibilities: The Training Officer is responsible for training all field sampling personnel. At the direction of the Project Officer, the Training Officer coordinates with the QC Officer and the Field Crew Supervisor to implement remedial or additional training deemed necessary between MBSS field sampling periods.

Quality Control Officer (William Harbold) - The QC Officer is responsible for implementation of all aspects of the MBSS QA program, including inspection of field crews, data validation, taxonomic verification, site confirmation, calibration and maintenance of equipment, adherence to established protocols, and prompt identification of necessary remedial or corrective actions. The QC Officer is also responsible for oversight of laboratory QA managers to ensure that all MBSS laboratory activities meet MBSS QA/QC requirements.

Data Management and Analysis Officer (Dr. Tomas Ivasauskas) - The Data Management and Analysis Officer is responsible for receiving, reviewing, and signing off on the original electronic or paper data sheets, as well as supervising and verifying data entry.

Field Crew Supervisor (William Harbold) - The Field Crew Supervisor is responsible for day-to-day communication with Crew Leaders, coordination and approval of sampling schedules and itineraries, and other activities designated by the Project Officer.

Crew Leaders (Gregory Mathews, Kyle Hodgson, Mary Genovese) - The Crew Leaders are responsible for crew safety, sample scheduling, equipment maintenance and calibration, and performance of all sample collection activities in accordance with procedures and QA/QC requirements specified in the MBSS sampling manual.

Field Sampling Crew - Members of the sampling crew are responsible for carrying out the instructions of the Crew Leader and informing the Crew Leader of any unsafe conditions, equipment failures, or other problems observed that could jeopardize the health and safety of the crew or the quality of sample collections.

Benthic Macroinvertebrate Laboratory Manager (Neal Dziepak) – The DNR benthic macroinvertebrate laboratory manager is responsible for specific Quality Controls implemented for all MBSS samples processed, including sample check-in and Chain-of-Custody, processing (i.e., subsampling), taxonomy, data entry, and sample archiving.

Wet Laboratory Manager (Dr. Keith Eshleman) – Housed at the University of Maryland's Appalachian Laboratory, the MBSS water chemistry laboratory is responsible for Chain-of-Custody, instrument calibration and analyses, data entry and reporting for all MBSS water chemistry samples.

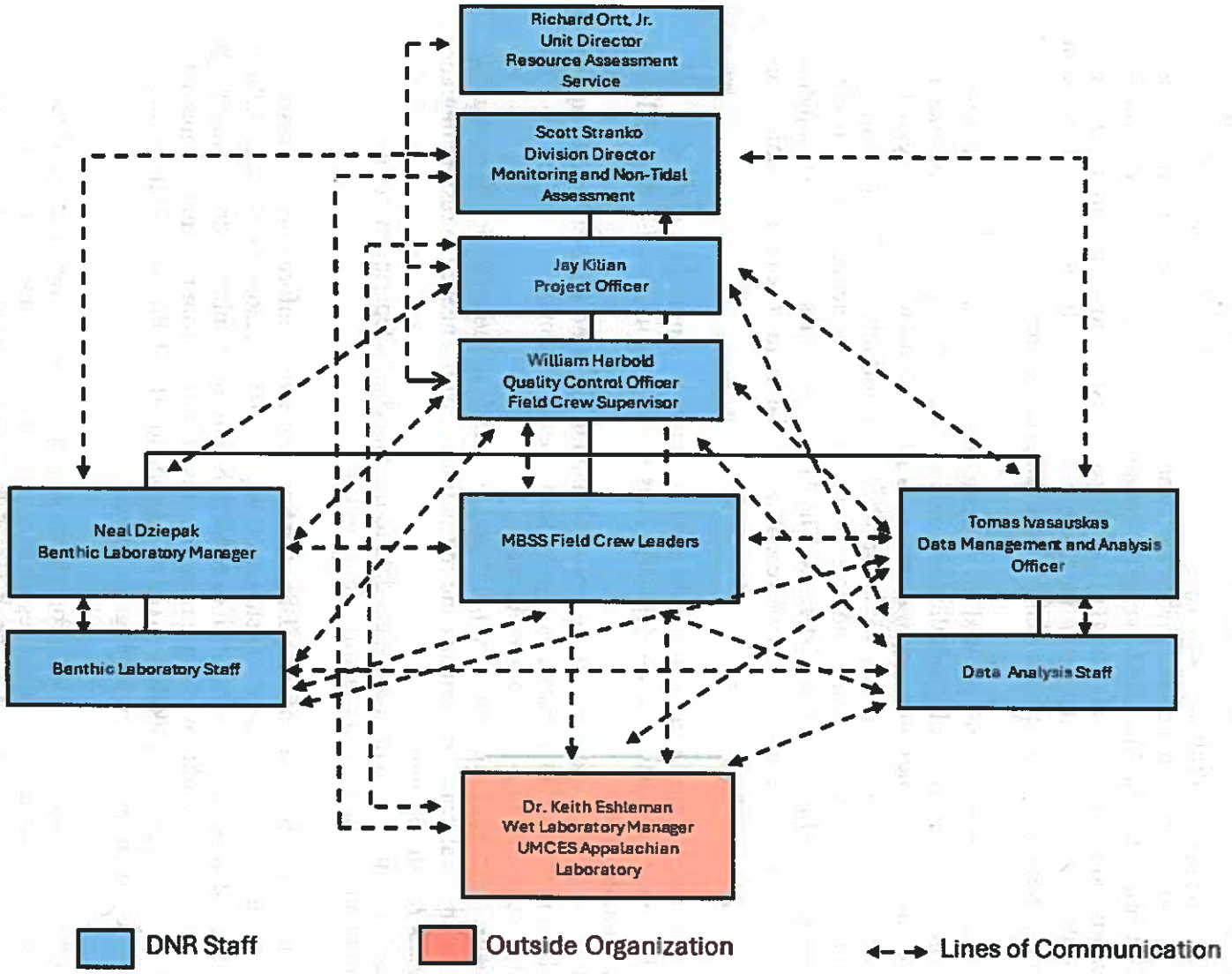

Figure 1. Organizational chart for the Maryland Biological Stream Survey

A.4 Problem Definition/Background

In the 1980s, the Maryland Department of Natural Resources recognized that atmospheric deposition was one of the most important environmental problems resulting from the generation of electric power. The link between acidification of surface waters and acidic deposition resulting from pollutant emissions was well established and many studies pointed to adverse biological effects of low pH and acid neutralizing capacity (ANC) and elevated levels of inorganic aluminum. To determine the extent of acidification of Maryland streams resulting from acidic deposition, DNR conducted the Maryland Synoptic Stream Chemistry Survey (MSSCS) in 1987. The MSSCS estimated the number of streams affected by or sensitive to acidification statewide, concluding that the greatest concentration of fish resources at risk may be in streams throughout the Appalachian Plateau and Southern Coastal Plain physiographic provinces.

While the MSSCS demonstrated the potential for adverse effects on biota from acidification, little direct information was available from the field on the biological responses of Maryland streams to water chemistry conditions. For this reason, in 1993, DNR created the Maryland Biological Stream Survey (MBSS) to provide comprehensive information on the status of biological resources in Maryland streams and how they are affected by acidic deposition and other cumulative effects of anthropogenic stresses. The MBSS continues to help environmental decision-makers protect and restore the natural resources of Maryland. The primary objectives of the MBSS are to

- assess the current status of biological resources in Maryland's non-tidal streams;
- quantify the extent to which acidic deposition has affected or may be affecting biological resources in the state;
- examine which other water chemistry, physical habitat, and land use factors are important in explaining the current status of biological resources in streams;
- provide a statewide inventory of stream biota;
- establish a benchmark for long-term monitoring of trends in these biological resources;
- target future local-scale assessments and mitigation measures needed to restore degraded biological resources, and
- provide high-quality stream ecological data to the Maryland Department of the Environment for use in water quality regulations.

To meet these and other objectives of the MBSS, the Survey provides information to answer questions that fall into three categories: (1) characterizing biological resources, physical habitat, and water quality (such as the number of fish in a watershed or the number of stream miles with $pH < 5$); (2) assessing the condition of these resources (as deviation from minimally impaired expectations); and (3) identifying likely sources of degradation (by delineating relationships between biological conditions and anthropogenic stresses).

Answering these questions has required a progression of steps in the implementation of the Survey, including (1) devising a sampling design to monitor wadeable, non-tidal streams throughout the state and allow area-wide estimates of the extent of the biological resources, (2) implementing sampling protocols and quality assurance/quality control procedures to assure data quality and precision, (3) developing indicators of biological condition so that degradation can be evaluated as a deviation from reference expectations, and (4) using a variety of analytical methods to evaluate the relative contributions of different anthropogenic stresses.

A.5 Project/Task Description

In creating the MBSS, DNR implemented a probability-based sampling design as a cost-effective way to characterize statewide stream resources. By randomly selecting sites, the Survey can make quantitative inferences about the characteristics of the more than 16,000 miles of non-tidal streams in Maryland. The EPA has encouraged the use of random sampling designs to assess status and trends in surface water quality. The Round One MBSS design began with the MSSCS sample frame and was modified during the 1993 pilot and 1994 demonstration phases to provide answers to the questions of greatest interest. That design allowed robust estimates at the level of stream size (Strahler orders 1, 2, and 3), large watersheds (17 river basins), and the entire state. Estimates by other categories, such as counties or smaller watersheds (137 in Maryland), were possible depending on the number of sample points in each unit. Round Two of the MBSS had a slightly different design that allows estimates at the level of smaller watersheds (84 individual or combined Maryland 8-digit watersheds); to achieve the necessary sample density at the available level of effort, Round Two took five years to complete (rather than the three years in Round One). Round Three was designed at the same scale as Round Two but allowed only statewide and major tributary (12 in Maryland) basin estimates of stream condition. Round Three took three years to complete. Round Four was designed to assess change over time at various scales between Round One and Round Four and Round Two and Round Four. Round Four data collection was completed at the end of calendar year 2018 and analyses are currently underway. Round Five MBSS was initiated in 2021 and employs a finer scale map than used previously, thus providing assessments for more streams. During Round Five, a different randomly selected site is chosen for sampling annually in each of the 84 Primary Sampling Unit watersheds in Maryland.

DNR recognized that the utility of the MBSS sampling Round estimates depended on accurately measuring appropriate attributes of streams. The Survey focuses on biology for two reasons: (1) organisms themselves have direct societal value and (2) biological communities integrate stresses over time and are a valuable and cost-effective means of assessing ecological integrity (i.e., the capacity of a resource to sustain its inherent potential). Inevitably, overall environmental degradation is tied to a failure of the system to support biological processes at a desired level. It is equally important to recognize that the natural variability in biota requires that several components of the biological system be monitored. Fish are an important component of stream integrity and one that also contributes substantial recreational values. The Survey collects quantitative data for the calculation of population estimates for individual fish species (both game and nongame). These data can also be used to evaluate fish community composition, fish anomalies, and the geographic distribution of commercially important, rare, or non-indigenous fish species. Benthic (bottom-dwelling) macroinvertebrates are another essential component of streams and they constitute the second principal focus of the Survey. The Survey uses rapid bioassessment procedures for collecting benthic macroinvertebrates; these semi-quantitative methods permit comparisons of relative abundance and community composition, and have proven to be an effective way of assessing biological integrity in streams. The Survey also records the presence of amphibians and reptiles (herpetofauna), freshwater mussels, and crayfishes. The Survey has established rigorous protocols for each of these sampling components, as well as training and auditing procedures to ensure that data quality objectives are met.

Although the MBSS sampling design and protocols provide exceptional information for characterizing the stream resources in Maryland, the designation of degraded areas and

identification of likely stressors require additional activities. Assessing the condition of biological resources (whether they are degraded or undegraded) requires the development of ecological indicators that permit the comparison of sampled segment results to minimally impacted reference conditions (i.e., the biological community expected in watersheds with little or no human-induced impacts). The Survey has used its growing database of information collected with consistent methods and broad coverage across the state to develop and test indicators of individual biological components (i.e., fish and benthic macroinvertebrates). These two indices are the basis for estimating the number of stream miles in varying degrees of degradation (good, fair, poor, and very poor condition) and mapping the locations of sites by their condition. Each of these indicators consists of multiple metrics using the general approach developed for the Index of Biotic Integrity (IBI) and the Chesapeake Bay Benthic Restoration Goals. The fish and benthic IBIs (which combine attributes of both the number and the type of species found) are widely accepted indicators that have been adapted for use in a variety of geographic locations. These indices are developed, tested, and verified using standard index periods. All taxa counts are calibrated to a regional reference condition. The Survey currently reports a composite fish and benthic indicator (Combined Biotic Index, or CBI), as well as fish and benthic indicators separately. Details on the development and application of MBSS IBIs, including the rationale for index period selection, can be found in Southerland et al. (2005).

In addition to using reference-based indicators, the Survey applies a variety of analytical methods to the question of which stressors are most closely associated with degraded streams. This involves correlational and multivariate analyses of water chemistry, physical habitat, land use, and biological information (e.g., presence of non-native species). The water chemistry parameters chosen for the Survey are among the most widely-used indicators of stream degradation stemming from atmospheric deposition and land disturbance – two of the most widespread sources of stress to stream ecosystems. A complete list of MBSS chemical parameters can be found in Harbold et al. (2024).

The biological information also provides an unusual opportunity for evaluating the status of biodiversity across the state; the distribution and abundance of species previously designated as rare only by anecdotal evidence can be determined and unique combinations of species at the ecosystem and landscape levels can be identified. Land use and other landscape-scale metrics also play an important role in identifying the relative contributions of different stressors to the cumulative impact on stream resources. Ultimately, the Survey seeks to provide an integrated assessment of the problems facing Maryland streams that will facilitate interdisciplinary solutions.

Among other findings, Round One collected 83 fish species, including a number of rare species. According to the fish IBI, 45% of stream miles fell into the range of good to fair, while 49% fell into this range according to the benthic IBI. Statewide, 28% of stream miles were acidic or acid sensitive, indicating a slight improvement since the 1987 MSSCS. Acidic deposition was by far the most common source of stream acidification, dominating 19% of stream miles. Statewide, 59% of stream miles had nitrate-nitrogen concentration greater than 1.0 mg/l, indicating anthropogenic sources. Nearly all sites with greater than 50% urban land use had IBI scores indicative of poor to very poor biological condition. These and other results have and continue to be used by Maryland DNR to target resource management efforts and to reevaluate state designations of rare, threatened, and endangered species. MBSS Round One results were used to support Maryland's Unified Watershed Assessment and other components of the Federal Clean Water Action Plan, the Maryland Tributary Strategy Teams plan to reduce nutrient contributions

to the Chesapeake Bay, and the Maryland Department of the Environment's water quality standards program that lists impaired waters and develops total maximum daily loads (TMDLs). Rounds Two and Three of the Survey continued to contribute to these activities and others, by refining the assessment of statewide stream conditions. Round Four evaluated change over time in ecological conditions over 20 and 14-year intervals. Round Five uses a more detailed map (1:24,000 scale), thus affording the opportunity for more Maryland streams to be assessed.

A.6 Data-Quality Objectives and Criteria for Measurement Data

The data quality objectives and criteria for measurement data described below pertain largely to water chemistry, benthic macroinvertebrate and fish data collected by the Maryland Biological Stream Survey.

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 the use and interpretation of the field collected data, and provide information for other data users regarding the utility of the data for other purposes.

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

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

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

The MBSS is recognized as providing the highest quality biological data possible. This is due primarily to the QA 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 testing and/or certification requirements for all of these taxonomic groups. Only the person(s) on each crew who has demonstrated proficiency through annual testing and/or certification for the taxonomic group should conduct identification in the field.

During MBSS Rounds 3 and 4, photographic vouchers were accepted in lieu of preserved specimens. Photographs of at least five specimens of each fish, reptile, amphibian, and crayfish species encountered by each crew each year during Round 3 and 4 (as long as five were collected) should be photographed. In addition, any rare, threatened, or endangered species encountered should be photographed, as long as the photograph can be taken without causing any harm to the specimen. Photographs must clearly show the appropriate features necessary for identifying the species. The Maryland Department of Natural Resources' Monitoring and Non-Tidal Assessment Division kept all photographs taken during Round 3 and 4 sampling. With the exception of rare, threatened, or endangered species, specimens that are too small to provide photographs that can be used to verify identifications should be preserved for verification. Photographs were reviewed and verified by an expert in taxonomy for each taxonomic group and results will be kept on record.

Fish taxonomic experts (or a designee assigned by the taxonomic expert) will also audit field identification of stream fishes. Field audits will be conducted at at least one site per crew per year. Photographs and/or preserved specimens of herpetofauna, crayfishes, and freshwater mussels taken by each field crew will be verified for accuracy by a taxonomic expert for each group.

Precision: The precision of the MBSS results is determined by measuring the agreement among individual measurements of the same property, under similar conditions. Precision is assessed through the analysis of laboratory duplicates or splits. The degree of agreement between replicates can be expressed as the percent relative standard deviation (RSD):

$$\text{Percent RSD} = \frac{SD}{\bar{x}} \times 100$$

Accuracy: Accuracy is defined as a measure of the closeness of an individual measurement to the true or expected value. Analyzing a reference material or quality control check solution (QCCS; water chemistry samples) of known concentration is a method of determining accuracy. QCCSs are independently made and analyzed after calibration, at specified intervals during sample analysis and at the conclusion of sample analysis, to ensure accurate measurement throughout analysis.

Laboratory Blanks: Deionized water blanks serve as a check of laboratory-induced contamination. Laboratory blanks will be analyzed at predetermined intervals as outlined in the standard operating procedures for each analyte.

Sample Spikes: Sample spikes will be used with most of the analytical techniques to determine whether the sample matrix affect analytical accuracy. A known concentration of analyte will be added to about 15% of the samples. Both the spiked and unspiked samples will then be analyzed. Percent recovery will be calculated using the following equation:

$$\text{\% Spike recovery} = \frac{\text{spiked sample - routine sample}}{\text{spike amount (mg/L)}} \times 100$$

Percent recovery calculated for sample spikes should be within 15% of 100%.

Collection and Analysis of Natural Audit Sample: Natural audit samples are another useful part of a comprehensive quality assurance assessment. Because they are collected from streams,

they are more representative of the actual sample matrix than a manufactured calibration check solution. The audit samples are stored in the dark at 4 °C over a period of several days and are analyzed periodically for all analytes except closed pH. Although there are no actual correct or incorrect results for any of the analytes, as when a known QCCS is measured, variations in analyte concentration can help determine or diagnose any sources of analytical error. They are especially useful as a diagnostic tool when there are any changes in the operating conditions of an instrument (i.e., column or electrode replacement).

Field Duplicates: Field duplicates are typically obtained from about 5% of all sites. Precision of the duplicate samples is determined by measuring the Relative Percent Difference (RPD). Lower RPDs indicate greater precision

$$RPD = (|X1-X2|*100)/((X1+X2)/2)$$

A.7 Special Training/Certification

An important aspect of the MBSS QA program is the mandatory training of all field personnel that is conducted prior to sampling. The goal of the training is to ensure consistent implementation of required procedures and attainment of a minimum level of technical competency by each MBSS participant. This standardized training helps to maximize the comparability of data among field crews. In addition to crew training, Crew Leaders are given additional instruction and guidance to maximize consistency in decision-making. To meet the program's QA objectives for training, crew leaders must successfully pass examinations administered during annual training and subsequent field audits.

For personnel involved in sampling during the Spring Index Period, training includes water quality, select habitat assessment metrics, and benthic macroinvertebrate sampling using MBSS procedures (Harbold et al. 2024). For personnel involved in sampling during the Summer Index Period, training includes fish and herpetofauna sampling, habitat assessment, and a laboratory examination concerning the identification of Maryland fishes, crayfishes, freshwater mussels, and herpetofauna. These taxonomy tests may involve the identification of preserved specimens and may underestimate the ability of the individual to identify live specimens in the field.

A.8 Documentation and Records

The MBSS maintains an exhaustive system of data and report documentation. Reports and data are made available to management and all interested users in several ways including the DNR website, custom data requests, the Maryland Stream Health Website (see www.maryland.streamhealth.gov), hard copy and electronic reports, and fact sheets

B. Data Generation and Acquisition

B.1 Experimental Design

This section describes the design for the Maryland Biological Stream Survey from 1995 to present. The core MBSS is a statewide, probability-based survey that uses random sampling to draw inferences about stream condition throughout watersheds and larger regions. The design of the core MBSS facilitates the assessment of average stream condition over a multi-year period for (1) the entire state and, depending on the level of effort expended in each round, for other

areas of interest such as (2) ecological regions, (3) Maryland counties, (4) Maryland 6-digit drainage basins, and (5) Maryland 8-digit watersheds. The MBSS also samples specific areas, using a mix of random and targeted designs, to address other assessment needs as part of the non-core survey.

Since its inception, the MBSS has completed four statewide surveys, or 'Rounds'. Each Round differed slightly in its main objectives and in the level of survey effort (number of site sampled per year). There were also changes made in the resolution of the stream reach maps used for random site selection. For Round 1, a stream reach map at the 1:250,000 scale was used for random site selection. Rounds 2, 3, and 4 used a 1:100,000 scale map. Round 5 is currently using a 1:24,000 scale map. Further details of each round are described below.

Round 1 of the MBSS was conducted in 1995-1997 to provide comprehensive statewide coverage and estimates of stream condition at the scale of large drainage basins (18 in Maryland). Round 2 of the MBSS in 2000-2004 was the first 'biocriteria" round that obtained estimates of stream condition on the scale of Primary Sampling Units (PSUs) comprising single or multiple Maryland 8-digit watersheds (84 in Maryland; see map below). Maryland 8-digit watersheds are the scale at which the state 303(d) list of biologically impaired waters is prepared using MBSS data. Round 3 of the MBSS was a non-biocriteria round that included core random sampling stratified among the 84 PSUs over the three years, 2007-2009. Specifically, 252 sites were sampled in the core survey with three sites allocated to each of the 84 PSUs. Additional Round 3 sampling supported non-core objectives such as (1) augmenting random sites in small or variable 8-digit watersheds, (2) delineating the extent of high-quality waters, and (3) refining rare species distributions. Results from Round 3 MBSS can be found at https://dnr.maryland.gov/streams/Pages/r3reportintro.aspx

Sampling for MBSS Round 4 was completed from 2014 to 2018. This round of the MBSS optimized DNR's ability to detect changes in Maryland's stream conditions and thus help to inform management decisions in the future. Since stream ecosystems are incredibly complex and variable, there is a need to know how they are responding to management efforts in the face of continued impacts. Over the last 20 years, the population of Maryland has increased, putting stress on streams. At the same time, stream restoration activities and conservation (including additional state and local regulations and policies) have increased. Round 4 provided data for assessing how stream ecological conditions have changed which could lead to recommendations on the success of these activities and whether management actions should be adapted to become more effective.

Round 4 involved re-sampling a subset of randomly-selected stream sites that were sampled previously - a widely accepted design. The sampling process took five years. Sites that were sampled in 1995, 1996, and 1997 were re-sampled 20 years later (in 2015, 2016, and 2017). A separate set of sites that were sampled in 2000, 2001, 2002, 2003, and 2004 were sampled beginning in 2014 — 14 years later. At the end of 2018, DNR collected sufficient information to compare statewide and river basin estimates of stream condition over 20 and 14 year intervals. certain variables (e.g., electrofishing effort; benthic taxonomy) required normalization to ensure comparability between sampling time periods. Results from Round 4 can be found at https://dnr.maryland.gov/streams/Pages/publications.aspx.

Round 5 of the MBSS utilizes a finer-scale (higher resolution) stream map (1:24,000) than has been used previously. This map provides a framework for more comprehensive assessments of

Maryland's more than 16,000 stream miles – including more small headwater streams - than assessed during previous rounds. As with some previous MBSS sampling rounds, sites are randomly selected for sampling. Unlike other MBSS rounds during which a subset of watersheds was sampled annually, the Round 5 design includes sampling a site in each of the 84 primary sampling unit watersheds each year. Multiple years of sampling data will be combined to assess the condition of Maryland's streams. As with previous rounds, targeted and fixed sampling will be combined with random sampling to help answer important management questions.

Round 5 Objectives:

- Assess the ecological condition of the more than 16,000 Maryland's streams and rivers on a 1:24,000 scale stream map
- Assess the ecological condition of streams mapped at the 1:100,000 scale used during Rounds Two and Three for historical comparison
- Identify indicators of potential stressors to stream ecological conditions such as climate change, atmospheric deposition, and other factors
- Provide an inventory of biodiversity in streams mapped at the finer (1:24,000) scale
- Estimate the distributions and abundance of rare and imperiled stream species to help inform conservation status
- Detect emerging invasive species and continue to document the distributions of non-native species
- Assess the efficacy of management actions intended to improve or conserve stream conditions
- Continue to build a long-term database and document changes over time in Maryland stream conditions and biodiversity status
- Communicate results to the scientific community, the public, and policymakers

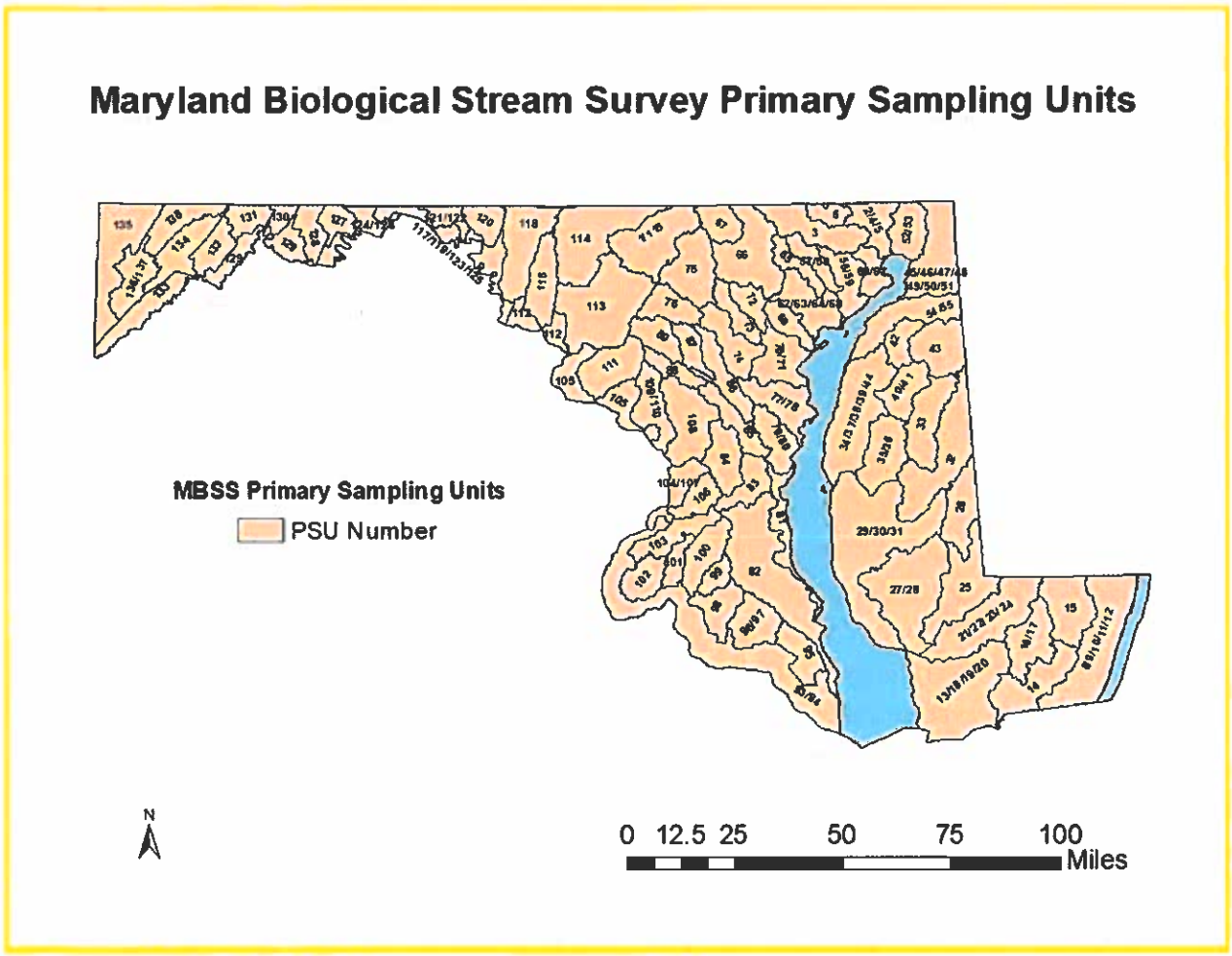

Figure 2. MBSS primary sampling units

PSU Number	PSU Abbreviation	PSU Name
1/115	COCR/DOUB	Conewago Creek/Double Pipe Creek
2/4/5	LSUS/OCTO/CDAM	Lower Susquehanna River/Octoraro Creek/Conowingo Dam Susquehanna River
3	DEER	Deer Creek
6	BROA	Broad Creek
8/9/10/11/12	ASSA/ISLE/SINE/NEWP/CHIN	Assawoman Bay/Isle of Wight Bay/Sinepuxent Bay/Newport Bay/Chincoteague Bay
13/18/19/20	PCSO/TANG/BANN/MANO	Pocomoke Sound/Tangier Sound/Big Annemessex River/Manokin River
14	LOPC	Lower Pocomoke River
15	UPPC	Upper Pocomoke River
16/17	DIVI/NASS	Dividing Creek/Nassawango Creek
21/22/23/24	LOWI/MONI/WICR/WIRH	Lower Wicomico River/Monie Bay/Wicomico Creek/Wicomico River Head
27/28	FISH/TRAN	Fishing Bay/Transquaking River
29/30/31	HONG/LICK/LOCK	Honga River/Little Choptank River/Lower Choptank River
34/37/38/39/44	EAST/KENA/LOCR/LANG/KEIS	Eastern Bay/Kent Narrows/Lower Chester River/Langford Creek/Kent Island Bay
35/36	MILE/WYER	Miles River/Wye River
40/41	CORS/SEAS	Corsica River/Southeast Creek
45/46/47/48/49/50/51	LOEL/BOHE/UELK/BACR/LIEL/BELK/CHRI	Lower Elk River/Bohemia River/Upper Elk River/Back Creek/Little Elk Creek/Big Elk Creek/Christina River
52/53	NEAS/FURN	Northeast River/Furnace Bay
54/55	SASS/STIL	Sassafras River/Stillpond-Fairlee
56/59	BUSH/BYNU	Bush River/Bynum Run
57/58	LWIN/ATKI	Lower Winters Run/Atkisson Reservoir
60/61	ABPG/SWAN	Aberdeen Proving Ground/Swan Creek
62/63/64/68	GUNP/LOGU/BIRD/MIDD	Gunpowder River/Lower Gunpowder Falls/Bird River/Middle River-Browns
70/71	BODK/BALT	Bodkin Creek/Baltimore Harbor

PSU Number	PSU Abbreviation	PSU Name
74	PATL	Patapsco River Lower North Branch
75	LIBE	Liberty Reservoir
76	SBPA	South Branch Patapsco River
77/78	MAGO/SEVE	Magothy River/Severn River
79/80	SOUT/WEST	South River/West River
81	WCHE	West Chesapeake Bay
82	PAXL	Patuxent River Lower
83	PAXM	Patuxent River Middle
84	WEBR	Western Branch
85	PAXU	Patuxent River Upper
86	LPAX	Little Patuxent River
87	MPAX	Middle Patuxent River
88	RKGR	Rocky Gorge Dam
89	BRIG	Brighton Dam
93/94	PRLT/PRMT	Potomac Lower Tidal/Potomac Middle Tidal
96/97	BRET, STCL	Breton Bay/St. Clements Bay
98	WICO	Wicomico River
99	GILB	Gilbert Swamp
100	ZEKI	Zekiah Swamp
101	PTOB	Port Tobacco River
102	NANJ	Nanjemoy Creek
103	MATT	Mattawoman Creek
104/107	PRUT/OXON	Potomac Upper Tidal/Oxon Creek
105	PRMO	Potomac River Montgomery County
106	PISC	Piscataway Creek
108	ANAC	Anacostia River
109/110	ROCK/CABJ	Rock Creek/Cabin John Creek
112	PRFR	Potomac River Frederick County
113	LMON	Lower Monocacy River
114	UMON	Upper Monocacy River
116	CATO	Catoctin Creek
117/119/123/235	PRWA/MARS/TONO/LTON	Potomac River Washington County/Marsh Run/Tonoloway Creek/Little Tonoloway Creek
118	ANTI	Antietam Creek
120	CONO	Conococheague Creek
121/122	LCON/LIKG	Little Conococheague Creek/Licking Creek
124/126	PRAL/SIDE	Potomac River Allegany County/Sideling Hill Creek
127	FIMI	Fifteenmile Creek
128	TOWN	Town Creek
129	PRLN	Potomac River Lower North Branch
130	EVIT	Evitts Creek
131	WILL	Wills Creek
132	GEOR	Georges Creek
133	PRUN	Potomac River Upper North Branch
134	SAVA	Savage River
135	YOUG	Youghiogheny River
136/137	LYOU/DCRL	Little Youghiogheny River/Deep Creek Lake

B.2 Sampling Methods

A full description of MBSS field sampling methods is beyond the scope of this document. A summary of MBSS field sampling protocols is below. Details can be found in Harbold et al. (2024).

Each MBSS site was visited three times. During the spring index period (March 1 – April 30), sites were located and marked, benthic macroinvertebrates were sampled, water chemistry samples were collected, herpetofauna and other fauna were listed, water temperature loggers were deployed, and select physical habitat parameters were measured. Summer sampling (June 1 – September 30) consisted of fish sampling, physical habitat evaluations, and more faunal searches.

In the spring, single-grab samples of water were collected and analyzed for analytes such as pH, acid-neutralizing capacity (ANC), sulfate, nitrate-nitrogen, conductivity, total phosphorus and dissolved organic carbon (DOC) in the laboratory. These variables primarily characterize the sensitivity of the streams to acid deposition, and to other anthropogenic stressors to a lesser extent. In 2014, additional ions (Calcium, Bromide) and metals (Copper, Magnesium, Zinc) were added to the suite of water chemistry parameters collected in the spring of each year. In 2017, additional ions including Sodium and Potassium were added.

Temperature loggers were deployed during the spring index period. Loggers deployed at this time are set with a delayed launch date of June 1. Loggers (both air and water) were set to record at 20-minute intervals to provide adequate characterization of the diurnal flux during the critical period. Air loggers were deployed in any structure (tree, shrub, fence, etc) adjacent to the stream that provides shade and protection to the logger. Both loggers were retrieved after September 1 of each year and data were downloaded shortly thereafter. Details can be found at http://dnr.maryland.gov/streams/Publications/QA_TemperatureMonitoring.pdf.

Benthic macroinvertebrates were collected in the spring using D-frame dip nets in the most productive habitat(s) (e.g., riffles, rootwads, aquatic vegetation) available in the 75 m segment. About 2 m^2 of stream substrate are sampled at each site and pooled. Preserved samples are subsampled (100 organisms) in the laboratory and identified to genus (if possible).

Certain habitat assessments were conducted in the spring and summer sampling visits using metrics largely patterned after EPA's Rapid Bioassessment Protocols and Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) in the designated 75 m stream segments. Riparian habitat measurements are based on the surrounding area within 50 m of the segment. Other qualitative measurements include (1) aesthetic value, based on evidence of human refuse; (2) remoteness, based on the absence of detectable human activity and difficulty in accessing the segment; (3) land use, based on the surrounding area immediately visible from the segment; (4) general stream character, based on the shape, substrate, and vegetation of the segment; and (5) bank erosion, based on the kind and extent of erosion present. Quantitative measurements at each segment include flow, depth, wetted width, and velocity.

Fish and herpetofauna were sampled during the summer index period using quantitative, double-pass electrofishing of the 75 m stream segments. Block nets were placed at each end of the segment, and one or more direct-current, backpack electrofishing units were used to sample the entire segment using double-pass depletion. All fish captured during each electrofishing pass were identified, counted, and weighed in aggregate. All gamefish captured were also measured for length. Any amphibians, reptiles, crayfish and freshwater mussels either in or near the stream segment were identified.

Data collected during Rounds 1 - 4 from each sample site have been used to develop statewide and watershed-specific estimates of totals, means (or averages), proportions, and percentiles for the parameters of interest. The amount of variability (or margin of error) associated with any estimate of a total, mean, proportion, or percentile is determined by calculating a standard error, a statistic that measures the reliability of an estimate. A standard error also provides a statistical basis for deciding if the observed changes in any parameter of interest over time or space are significantly different or simply due to chance alone.

For all phases of the Survey, there is an ongoing, documented program of quality assurance/quality control (QA/QC). The QA/QC program used by the Survey allows for the generation of data with known confidence.

B.3 Sample Handling and Custody

For MBSS water chemistry samples, samples were counted, observed for potential problems (melting, broken containers, etc.), and placed on ice until analysis upon arrival at the laboratory. Sample information and date of arrival are recorded on a log sheet, and entered in an ACCESS file for internal tracking. Benthic macroinvertebrate samples are preserved appropriately, double-labeled, tracked on a Chain-of-Custody sheet, and delivered to the respective laboratory within two to three weeks after collection. The same protocols are followed for fish, crayfish, and herpetofauna specimens. These are delivered to taxonomic experts for verification at the end of the summer of each year. Detailed MBSS sample handling procedures can be found in the MBSS Sampling Manual (Harbold et al. 2024).

B.4 Water Sample Analytical Methods

Chemical analyses for the MBSS are performed by the University of Maryland's Appalachian Laboratory (AL). Information on specific analytes, acceptance criteria, corrective action needed for QC procedures, and the Data Quality Indicator each QC sample is testing for can be found in Rogers (2013).

Laboratory instrumentation required for MBSS sample analysis includes:

- Lachat QuikChem 8000 Flow Injection Analyzer (four channel),
- Teledyne Tekmar Fusion Total Organic Carbon Analyzer,
- Dionex DX-120 Ion Chromatograph, and
- Brinkmann Automated Titration System.

PC Computers with complete spreadsheet packages are used for data reduction and management.

Below are chemical and physico-chemical parameters routinely measured by the MBSS as well as an overview of laboratory analytical protocols.

Specific Conductance

Specific conductance is a numerical expression of the ability of an aqueous solution to carry an electrical current. This ability depends on the presence of ions, their total concentration, mobility, valence, and relative concentrations, as well as on the temperature of measurement. Conductivity can be used to generate a synthetic ion balance which can be used as a check of measured ionic concentrations.

The specific conductance of samples is measured using a conductance meter and conductivity cell. The meter and cell are checked using potassium chloride standards of known conductivity. Standards and samples are analyzed at or corrected to 25°C (equivalent to room temperature).

Instrumentation

Digital specific conductance meter
Conductivity cell

Sample Collection, Preservation, and Storage

Raw samples are collected in clean, polyethylene containers. Specific conductance should be determined as close to sample collection time as possible. The maximum holding time is seven days. Samples should be kept in the dark at 4°C until analysis.

Interferences

Temperature variations represent the major source of potential error in specific conductance determinations. To minimize this error, check standards and samples should be measured at the same temperature. Use of a temperature-controlled water bath is recommended.

Natural surface waters contain substances which may build up on the conductivity cell. Such a buildup interferes with the operation of the cell and should be removed periodically, following the cell manufacturer's recommendations.

Operating Procedures

1. Perform electronics check on meter with resistors.
2. Measure DI water blank.
3. Perform conductivity cell calibration check.
4. Measure working standard solutions, rinsing cell 6 times with DI water between each standard and sample.
5. Measure samples.
6. Correct all values to 25°C.
7. Re-measure working standards.
8. If conductance of any samples are greater than the highest working standard (147 µS/cm), prepare a higher concentration standard and measure.

Closed pH

pH is measured in a closed system on a sample collected without exposure to the atmosphere to prevent gaseous exchange between the samples and the atmosphere. The measurement is performed by attaching a sample syringe to the pH sample chamber, injecting the sample, and determining the pH using a pH meter and electrode.

Instrumentation

Orion Model 611 pH meter, or equivalent
Combination pH electrode
pH sample chamber
60-mL polyethylene syringes and Luer-Lok valves

Sample Collection, Preservation, and Storage

Samples are collected and sealed without air bubbles in 60-mL polyethylene syringes. They are stored at 4°C in the dark until analysis. Analysis should be performed as close to the time of collection as possible, generally within seven days.

Operating Procedures

1. Replace electrode filling solution.
2. Perform two-point temperature calibration.
3. Perform two-point pH standardization (pH 7 and 4).
4. Measure and record pH of pH 10.0 buffer.
5. Rinse and fill a syringe with pH 5.00 QCCS (Quality Control Check Solution). Attach syringe to inflow tubing of pH sample chamber. Inject enough sample to fill chamber. Rinse electrode by swirling for 15 to 30 minutes and drain chamber. Repeat.
6. Fill chamber a third time. Loosely insert electrode and inject it with an additional 5 to 10 mL of solution to expel all air bubbles. Seal electrode into chamber.
7. Start stopwatch, record initial pH, temperature, and time.
8. Wait until the readings seem fairly consistent and then note the time and pH values. If the pH does not vary by more than 0.02 pH units in one direction throughout a 1-minute interval, the reading is considered stable. Record the stable pH, temperature readings, and total elapsed time in logbook.
9. Inject a 5-mL portion of solution into the chamber. Repeat step 7. Record the first stable pH reading, temperature and time in the logbook.
10. Inject a second 5-mL portion of solution into the chamber. Repeat step 7 and record the data.
11. Check the pH values obtained from steps 8 and 9. If they agree within $\pm$ 0.03 pH units, sample measurement is completed. If they do not agree, continue to inject 5-mL portions of solution into the chamber and record the first stable pH, temperature, and elapsed values until two successive stable pH readings agree within $\pm$ 0.03 units.
12. If measured QCCS pH value, continue with analysis.
13. Rinse the sample chamber and electrode copiously with DI water.
14. Repeat steps 4 through 10 for a DI water blank and samples.
15. Re-analyze QCCS every 5 samples.
16. Copiously rinse sample chamber and glassware with DI water.
17. Remove electrode from sample chamber. Cover fill hole of electrode and place in storage solution.

18. Set meter to stand-by mode.

Acid Neutralizing Capacity

Sample open pH is determined prior to the start of sample titration. While pH is monitored and recorded, samples are titrated with standardized acid.

ANC is determined by analyzing the titration data using a modified Gran analysis technique. The Gran analysis technique defines the Gran Function F_1, which is calculated from sample volume, acid volume added, and constants. The Gran function is calculated for several titration data pairs (volume of titrant added, resulting pH) on either side of the equivalence point. When the Gran function is plotted versus volume of titrant added, a linear curve is obtained. The equivalence point can be interpolated from where the line crosses the volume axis.

Instrumentation

pH/mV Meter - Digital pH/mV meter capable of measuring pH to $\pm$ 0.01 pH unit, potential to $\pm$ 1 mV, and temperature to $\pm$ 5°C is required. The meter should also have automatic temperature compensation capability.

pH Electrodes - High-quality, low-sodium glass pH and reference electrodes should be used. Gel-type reference electrodes should not be used.

Buret - A microburet capable of precisely and accurately delivering 10 to 50 µL is required (relative error and standard deviation less than 0.5 percent) or a titration system equipped with the appropriate meters, burets, electrodes, and software to perform the titration (Brinkmann ANC titrator).

Sample Collection, Preservation, and Storage

The sample for which ANC and pH are to be determined is raw sample (not filtered or chemically preserved) stored in at least a 500-mL polyethylene bottle. The bottle should be completely filled to eliminate headspace. Only deionized water-washed containers should be used to collect and store the sample. Store in the dark at 4°C and minimize atmospheric exposures. Samples that are to be measured for ANC and open pH should be analyzed within seven days. The holding time for ANC analysis alone is 14 days.

Operating Procedures

1. Replace electrode filling solution.
2. Perform two-point pH standardization (pH 7 and 4).
3. Measure and record pH of pH 5.00 QCCS.
4. Standardize titrant (0.01 N HCl) to determine actual normality.
5. Titrate QC solutions (QCCS and blank) and samples with 0.01 N HCl:
    a. Measure 50 mL of sample into titration vessel and add 300 µL of 2 N KCl to increase sample ionic strength.
    b. Add Teflon coated stir bar and place vessel on magnetic stirrer.
    c. Immerse electrode and measure pH.

d. Add titrant to lower sample pH to about 4.70.
e. Add increments of titrant to obtain at least 8 data pairs (pH and volume acid added) between pH 4.7 and 3.5. Wait for stable pH readings between each addition.
f. For each data pair, record pH and cumulative volume of acid added.
g. For each data pair, calculate the Gran function, F_{1a}:

$$F_{1a} = (V_s + V_a)[H^+]$$

where

V_s = total initial sample volume (mL)
V_a = total volume of acid titrant added.

h. Plot F_{1a} versus V_a. The data should be on a straight line with the equation $F_{1a} = a + bv$.
i. Perform a linear regress of F_{1a} on V_a to determine the correlation coefficient (r) and the coefficients a and b. The coefficient r should not exceed 0.999. If it does not, examine the data to ensure that only data on the linear portion of the plot were used in the regression. If any outliers are detected, repeat the regression analysis.
j. Calculate an initial estimate of the equivalence volume (V_1) by:

$$V_1 = -a/b$$

k. Calculate ANC by:

$$ANC = \frac{V_1 C_a}{V_{sa}}$$

where

C_a = concentration of acid titrant
V_{sa} = original sample volume

6. Rinse electrode copiously between samples.
7. Re-analyze QC solutions at the conclusion of analysis.
8. Cover fill hole of electrode and place in storage solution.
9. Set meter to stand-by mode.

Nutrients

Instrumentation

All of the nutrient techniques require the use of a flow analyzer such as the Lachat Quikchem 8000 Flow Injection Analyzer (FIA). Colorimetric reagents are continuously added in a specific sequence along a path of polyethylene tubing and mixing coils, called a manifold. Using a flow injection valve, sample is precisely injected onto the manifold and moved through the manifold by a carrier solution where analyte reacts to form a color change. The absorption of that color change is measured by the instrument. Reactions in flow injection analysis, as in other flow techniques, do not develop to completion as in manual methods, but reach identical stages of development within each sample since every sample follows the same path, timing and exposure to specific reagents.

Sampling and Storage

Collected water samples are filtered through Gelman Membrane filters (nominal pore size = 0.45 μm), placed in polyethylene bottles and frozen. All samples are routinely analyzed within 28 days.

Operating Procedures

The following describes step-by-step operating procedures for the Lachat QuikChem 8000 system.

1. Colorimeter - Turn power on and allow 10 minutes for warm-up.
2. Prepare appropriate reagents and standards related for analyte(s) of interest.
3. Computer - Turn power on and load software and select appropriate sample method and create sample table.
4. Sampler Water Reservoirs - Check and fill the deionized water reservoirs.
5. Pump - Connect pump tubes and attach platen to pump. Start pump with deionized water flowing through the system.
6. Check for leaks in tubes at connections and for smooth liquid flow through the manifold.
7. Set heaters to appropriate temperatures as needed.
8. Check injection valve timing with dye.
9. Allow reagents to pump through the system for at least 10 minutes. Place output lines in appropriate waste collection containers.
10. Open any column valves, if appropriate (e.g., cadmium column for nitrate+nitrite analysis)
11. Begin standard and sample analysis in Omnion software.
12. At completion of the run, close column valves, remove lines from reagents, and place tubes in deionized water.
13. Shut-down - Turn off computer and colorimeter. Wash system with DI water for 15 minutes, remove lines from DI water and pump air for 5 minutes.. Turn off pump, release proportioning platen and loosen pump tubes. Turn off colorimeter.

Orthophosphate

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample. This method covers the determination of phosphorus in drinking, ground and surface waters, and domestic and industrial wastes.

Instrumentation

Lachat QuikChem 8000 Flow Injection Analyzer

Interferences

- Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 30 mg/L would be required to produce a 0.007 mg P/L positive error in orthophosphate.
- Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interferences due to arsenates.
- Glassware contamination is a problem in low level phosphorus determinations. The use of dedicated glassware for phosphorus analysis is strongly recommended. All glassware should be washed with 1:1 HCl and rinsed with DI water (flasks, graduated cylinders, etc.). If detergents are needed, use special phosphate-free preparations for lab glassware.

Ammonium as Nitrogen

Ammonia in the sample reacts with hypochlorite ions which are generated in situ by alkaline hydrolysis of sodium dichloroisocyanurate. This reaction forms monochloramine, which then reacts with salicylate ions in the presence of sodium nitroprusside. A blue, indophenol-type compound is formed which is measured colorimetrically at 660 nm.

Ammonium ion reacts with hypochlorous acid and salicylate ions in the presence of nitroferricyanide ions -- actually their hydrolysis product, pentacyanoaquoferroate -- to form the salicylic acid analog of indophenol blue. The optimum pH for formation of this chromophore is about 13.4. The absorption maximum at this pH is about 660 nanometers. Tartrate ions are added to the highly alkaline reaction medium to prevent precipitation of Ca (II) and Mg (II) ions that would otherwise occur.

Instrumentation

Lachat QuikChem 8000 Flow Injection Analyzer

Interferences

- Magnesium interferes by forming a precipitate of magnesium hydroxide at high pH values (> 12) required for full color development. Trisodium citrate is used as a complexing agent to prevent this interference. At the concentration of trisodium citrate specified, the method will tolerate magnesium at concentrations normally

encountered in most non-saline waters. The tolerance of the chemistry to magnesium can be increased, to deal with partially saline waters, by increasing the concentration of trisodium citrate in the reagents (up to the limit of solubility, which is 390 g/L).

Nitrite as Nitrogen

Nitrite is determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color, which is read at 540 nm.

Instrumentation

Lachat QuikChem 8000 Flow Injection Analyzer

Total Nitrogen

This method is a persulfate technique for nitrogen which determines total nitrogen by oxidation of all nitrogenous compounds to nitrate. Total nitrogen is then analyzed by flow injection analysis for nitrate-nitrogen using cadmium reduction. Digested samples are passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite then is determined by diazotizing with sulfanilamide and coupling with N-1naphthylethylenediamine dihydrochloride to form a colored azo dye. Color is proportional to nitrogen concentration.

Instrumentation

Autoclave, hotplate, or pressure cooker capable of developing 100 to 110°C for 30 minutes
Lachat QuikChem 8000 Flow Injection Analyzer

Procedure

1. Measure 5 mL of sample into a 30 mL screw cap test tube and freeze. (Prepare all samples in duplicate.)
2. Measure 5 mL of each standard (3 replicates of each) into 30 mL screw cap test tubes and treat exactly as samples.
3. When ready to analyze, thaw samples at room temperature.
4. Add 10 mL of Oxidizing Reagent. Invert to mix and loosen cap.
5. Place test tubes in an autoclave at 100 - 110°C and 3 - 4 psi for 60 minutes. Bring back to atmospheric pressure over 1 hour.
6. Remove test tubes, tighten caps and cool to room temperature. Samples can be stored at this point.
7. Add 1 mL of Buffer Solution to each tube and shake. The pH of the sample should be 4 - 4.5 after the addition of the buffer solution.
8. Analyze samples for nitrite + nitrate using digested standards for calibration.

Total Phosphorus

Polyphosphates are converted to orthophosphate form by a sulfuric acid digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion reacts with ammonium

molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample.

Instrumentation

Autoclave, hotplate, or pressure cooker capable of developing 100 to 110°C for 30 minutes
Lachat QuikChem 8000 Flow Injection Analyzer

Procedure

1. Measure 50 mL of sample into a 100 mL screw cap test tube and freeze. (Prepare all samples in duplicate.)
2. Measure 50 mL of each standard (3 replicates of each) into 100 mL screw cap test tubes and treat exactly as samples.
3. When ready to analyze, thaw samples at room temperature.
4. Add 1 mL of Sulfuric Acid Solution.
5. Add 0.5 g solid potassium persulfate.
6. Invert to mix and loosen cap.
7. Place test tubes in an autoclave at 100 - 110°C and 3 - 4 psi for 60 minutes. Bring back to atmospheric pressure over 1 hour.
8. Remove test tubes, tighten caps and cool to room temperature. Samples can be stored at this point.
9. Analyze samples for orthophosphate using digested standards for calibration.

Anions (Chloride, Nitrate, Bromide, and Sulfate)

A small volume of filtered sample is introduced into the ion chromatograph (IC). The sample is pumped through a pre-column, separator column, suppressor column and conductivity detector. Anions are separated in the pre-column and separator column based on their affinity for resin anion exchange sites on the separator columns. The suppressor column converts the sample anions to their acid form. The separated anions are measured by the conductivity detector. The concentration of anions is determined by comparing peak areas of unknowns to a calibration curve generated from known standards.

Instrumentation

Thermo Fisher/Dionex Aquion Ion Chromatography System with Chromeleon 7.2.10 operating software on a computer running Microsoft Windows 10.

Procedure: Analytical Operations

1. Prepare at least two liters of eluent as outlined in Section 9.5 and add to eluent reservoir.
2. Check the level of the waste container and empty as needed. The waste can be dumped directly down the drain.
3. Check level of reagent water in the AS-AP diluent container and refresh as needed
4. Turn on nitrogen tank.
5. Turn on the ion chromatograph and autosampler.
6. Initiate the Chromeleon software.

7. Prime the pump for at least 500 seconds by opening the purge valve and choosing the prime option in Chromeleon. After 500 seconds, deselect the prime option in Chromeleon and close the purge valve.
8. Adjust the pump flow rate to 1.2 mL/minute and start the pump.
9. Adjust the suppressor current to 41 mA. Note: Running the pump without current applied to the suppressor can cause damage to the suppressor. Be sure to complete this step almost immediately after priming the pump.
10. To ensure suppressor operation, check for the presence of air bubbles in the waste stream from the suppressor.
11. Allow sealed samples and calibration standard solutions to come to room temperature for at least one hour on the lab counter.
12. Allow the system to come to equilibrium for at least 30 minutes and monitor the signal for a stable baseline. A stable baseline or background conductivity measurement indicates equilibrium conditions.
13. Record the background conductivity, which should be less than 20 µS (typically close to 16 µS), and the system backpressure in the logbook. Compare both values to values from previous runs to ensure stable operating conditions.
14. While system is equilibrating, set up sequence in Chromeleon.
15. Pour/prepare rinse, calibration standards, initial quality control samples (at a minimum) and start the sequence.
16. Following completion of the "rinse" sample (a mid-range standard run prior to any calibration standards), verify that Chromeleon has identified each peak correctly and make any needed adjustments to the integration and/or retention times for each analyte of interest.
17. Following completion of all six calibration standards, examine each calibration curve. Verify that the correlation coefficient for each analyte of interest is greater than 0.995. Verify that the calculated value for each standard for each analyte of interest is within ten percent of the expected value.

Procedure: Data Analysis/Clean-up

1. Verify that all initial quality control samples (QCS, ICV, and method blank) are within acceptance limits.
2. Make sure all peaks are marked completely and correctly.
3. With all Chromeleon windows closed except the main "Chromeleon Console," inside the sequence select standards 1-6 as "Calibration Standards." Standards 7-9 should be marked as "Unknown".
4. Open the "Studio" and click on "Data Processing," click "Results" at the top of the page, click "Summary" at the bottom of the page. Select each analyte individually, and copy the data into the IC Data Sheets Excel sheet.
5. Save and close the "Studio" window.
6. Open "Studio" again and click "Electronic Report" at the bottom left of the screen.
7. Click "Update" at top left to update the report. Open the dropdown labeled "Use report template" and select the option for "Anions with Dilution Factors Applied." Include "Integration" only, and click finish at the bottom left.
8. Once the update is complete, select "Print" and click "Microsoft Print to PDF" in the dropdown.
9. Save the PDF to the "IC Report Exports" folder titled with the run date formatted as "YYMMDD_LOW_CURVE_Your Initials."

10. Close the studio page and change standards 7-9 to "Calibration Standards" in the "Main Console." Save the Sequence and open the "Studio," and repeat steps 4 - 9. Label the exported report in the format of "YYMMDD_HIGH_CURVE_Your Initials."
11. In the IC Data Sheets Excel file for this batch, make sure the conditional formatting covers all of the samples. This sheet will help to determine which curve to use for each analyte on each sample (e.g., Cl^- concentrations for the low curve ranges from 0.0310 mg/L to 25 mg/L and from 25 mg/L to 105 mg/L for the high curve). The sheet for the high curve will also mark an analyte as out of range if it requires a dilution. Dilutions may be performed on a subsequent batch. Due to an increase in the number of samples which exceed the low curve for chloride, nitrate-N, and sulfate, a high curve was constructed using standards 1-6 in conjunction with three higher concentration standards. Bromide is not included in the high curve since concentrations seldom require dilution.
12. Return samples to the appropriate location in the cooler.
13. Enter all QC data in Anions by ion chromatography in the QA/QC folder on the WCL google drive.
14. Inspect control charts for the blanks, QCS, lab duplicates, spikes, and FNBR audit sample.
15. Evaluate all QC results for % recovery, % relative standard deviation, and relative percent difference, and add any applicable data qualifiers.
16. Make clear notations and provide written explanations about any unusual occurrences with respect to the sample results or QA/QC results.

Trace Metals and Base Cations

Inductively coupled plasma-mass spectrometry (ICP-MS) is an analytical technique used to measure the concentration of trace metals and other elements in a wide range of concentrations (ppt-ppb). Samples are introduced as a liquid through a sample probe and line to the nebulizer. The sample is introduced to an argon fueled, high temperature plasma. Energy transfers from the plasma to the sample stream, and then, the target element atomizes, and ionizes. The resulting ions are filtered through the cones into the first and second differential vacuum stages, and extracted from the lenses and focused onto the octopole reaction system. There, interferences are removed via the octopole reaction system in helium collision mode. The ions are separated by their mass to charge (m/z) ratio by the mass spectrometer. The electron multiplier detector (ODS) counts the separated ions, and the software processes the resulting information.

Instrumentation

Agilent 7900 ICP-MS system with an autosampler, and computer with Masshunter software.

Procedure

Pre Start-up

1. Check the following utilities:
    a. Argon gas pressure: 500 – 700 kPa
    b. Cell gas (Helium): 90 – 130 kPa
    c. Exhaust duct

d. Cooling water: heat exchanger is on, water is not dirty, at correct level
e. Drain & rinse tanks (under instrument, beside printer): empty waste, fill rinse tank

2. If analyzing for "sticky elements" (Tl, Sb, Th, Ag) disconnect mixing tee sample line from the nebulizer to a waste container instead. Run 10% HNO3 : 10% HCl : 10% NH_4OH through the sample line and ISTD line at 0.3 rps for a minimum of 30 minutes. Follow with DI water through both lines for a few minutes at 0.1 rps.

Start-up

1. If the instrument is in ***Standby*** mode, the LED on the top right side of the instrument and the indicator in the ***Instrument Status Pane*** displays an orange light. If the instrument is in ***Shutdown*** mode, turn on the instrument and the computer.

2. Open the ***ICP-MS MassHunter Workstation software***. Select ***Instrument Control***. The Hardware pane will be displayed. To navigate to other panes in the software, select one of the *gadgets* on the ***Dashboard*** to display the corresponding task pane.

3. You can click on the Gadget dropdown arrow, to see further options; list the dropdown menu options available for each Gadget.

4. If the ***Instrument Status*** pane is not open in the ***ICP-MS MassHunter*** window, click ***View > Instrument Status*** from the menu bar to open.

    a. To change the 5 real-time meters displayed, click ***View > Meter*** from the menu bar. The ***Select Meter*** dialog box appears

5. Check the condition of the peristaltic pump tubing and replace if necessary. Ensure that they are correctly aligned and clamped into the peristaltic.
Open Rinses #1-3 in the back row of the autosampler starting next to the sample probe rinse port on the far left (vials 1, 2, 3), and the **1 ppb tuning solution** in the last position in the back row (vial 5).

6. If in ***Standby*** mode, click the ***Plasma*** gadget on the Dashboard or select **Startup > Startup Configuration** from the ***Plasma*** gadget dropdown.

    a. Under ***Hardware Settings***, ***Torch Axis, EM, Plasma Adjustment, Standard Lens Tuning, Resolution/Axis***, and ***Performance*** should all be ***check marked*** in the ***On*** column. The ***Vial #*** should be ***5*** for all of these hardware settings too.

7. Put the sample probe in one of the Rinse solutions by clicking the ***Auto Sampler*** gadget, and double click the ***Vial #*** or ***single click*** the ***Vial #*** > select ***"Go to"***.

    a. If new or cleaned front-end consumables (cones or lens assembly) have been installed: Put the sample probe in the "Cone Conditioning Solution" for the first 10 minutes of instrument warm-up, and then in one of the Rinse solutions for the last 8 minutes.

8. Put the **ISTD line** in the **2% HNO3**.

9. Double-click the ***Plasma*** gadget on the Dashboard to ignite the plasma. A confirmation dialog box is displayed.

    a. Click ***Yes***. The instrument goes to analysis mode and starts the hardware setting automatically.

10. Click the ***Queue*** gadget. The ***Queue*** pane appears and displays the progress of the hardware setting.

11. After the "Performance Report" is finished in the ***Queue*** pane, go to ***Mainframe***, and then click ***Performance Report*** to display the ***Performance Report*** table in the ***Hardware*** Pane.

    a. Check to make sure the performance report passes.

Creating a Batch

1. The samples to be analyzed need to be separated by percent total dissolved solids (%TDS). The template needed depends on the %TDS of the samples to be analyzed. The templates are as follows:
    a. Low Matrix – <0.1% TDS
    b. General Purpose – 0.1-0.2% TDS
    c. HMI-4 – 0.2-1.0% TDS
    d. HMI-8 – 1.0-3.0% TDS
    e. HMI-25 – >3%

2. Select ***Open Batch from Template...*** from the ***Batch*** gadget pull-down menu. Select the preferred ***template*** based on the samples %TDS, and click ***Open***.

    a. Name the batch by date, method, project (e.g., "180710 gen purp Bioswales").

Acquisition Method Tab

1. ***Acq Parameters Tab***

    a. Used to select or deselect elements to be acquired.

2. To add an element to acquire, click to select the element on the periodic table and mark the check boxes for the masses to acquire in the Mass table. To delete a mass, right-click the mass to delete.

3. If any elements are added to the batch, the ***Integration Time/Mass [sec]*** for elements needs to be ***increased*** from the auto-fill of 0.09.

4. ***PeriPump/ISIS Tab*** – Nothing needs to be done unless acquiring ***Ag***, then an ***Intelligent Rinse*** needs to be added with Rinses #1 and #2 increased to 45 from 30.

a. Check the box next to ***Intelligent Rinse***, and fill out accordingly: ***Numerator – 107***, ***Sample – 300000***, and ***Std – 300000***.

5. ***Tune Tab***

a. Move the sampling probe to the ***Batch Tuning Solution (10 ppb),*** select ***Start Signal Monitor*** on ***Tune*** toolbar, and wait until the signal stabilizes.

b. Select ***Stop Signal Monitor,*** and click on ***Tune*** toolbar.

i. The ***Start Auto Tune*** dialog box appears.
ii. Compare the ***Batch Tune Report*** to others in the "Tune Report" binder under the appropriate section for that batch (i.e., HMI-4) to ensure batch parameters are in the correct range.

Data Analysis Method Tab

1. Select ***FullQuant Analysis*** on the **Basic Information Tab** to perform quantitative analysis.

2. Select the analytes for quantitative analysis on the **Analytes Tab.**

3. To add or delete an analyte, ***right-click*** over the ***Analyte*** header on the ***Acquisition Parameters Tab***.

4. Set the standards parameters used for calibration and quantitative analysis on the **Full Quant Tab**.

5. Select QC outliers and actions on the **FullQuant Outlier Tab**.

6. Set each item for semi-quantitative analysis on the **Semi Quant Tab.**

Sample List Tab

1. Fill in the Standards information, and autosampler locations.

2. **Samples** to be **analyzed** will need to be added under ***"Unknown Samples"***

Main setting items for Unknown Samples (blank unless described)

Item	Description
Sample Type	Set a sample type- **Sample** for unknown samples.
Sample Name	Enter a sample name.
Comment	Enter a comment if necessary.
Vial Number	Set this item always when an autosampler is used. Click on the right end of the cell, and set a vial position.
Dilution	Leave blank unless a dilution of the original sample was performed before analysis.

Sublist	Select the project the sample is from unless analysis of all elements is preferred.

3. After you set up the acquisition method, data analysis method, and sample list, check whether the settings of the methods are correct or not by clicking the validate method button.

4. Fix errors before continuing.

Executing the Queue

Once the batch is created, and the Tune Report is passing, acquisition may be started.

1. The ISTD line needs to be taken out of the **2% HNO3**, and placed in the **ISTD solution**. Proceed when the ISTD solution has had time to make it through the mixing tee, and stabilize.

2. To start the acquisition, add a batch to the queue and execute it. Click on the ***Batch*** pane.

    a. When the acquisition starts, the ***ICP-MS Data Analysis*** window opens automatically

3. To check the progress status of the automatic acquisition, click the ***Queue*** gadget on the ***Dashboard***.

    a. The ***Queue*** pane displays the details and results of each task. Tasks can be suspended and deleted here too

NOTE: If ***Plasma Off at the End*** on the toolbar in the ***Queue*** pane is set to On, the plasma is turned off automatically after all tasks in the queue are completed, and the instrument goes to Standby mode.

Checking the Analysis Results

The analysis results can be checked on each pane in the ICP-MS Data Analysis window.

Quantitative Analysis

The ***ICP-MS Data Analysis*** window under the ***FullQuant Tab*** displays information such as the sample list, data analysis results, mass spectra, and calibration curves.

Batch Table Pane – Check the concentration and count for each element in the samples.

Spectrum Pane – Displays mass spectra. To zoom in on a spectrum, right-click on the spectrum and drag the mouse cursor around the desired mass number. The following operations can be completed:

- Change between log scale and linear scale
- Change the horizontal scale (1 row or 3 rows)
- Add a comment
- Identify unknown spectra against an element database
- Subtract background spectra
- Overlay multiple spectra
- Tabulate the spectral information

ISTD Stability Graph Pane – Check percent (%) recovery for each element in the samples.

QC Sample Stability Graph – Check concentration percent (%) recovery and stability for each analyte throughout the run.

Calibration Curve Pane – Check the calibration curves for each element in the samples.

- The calibration curve fit R minimum value is set to 0.99 in the templates. Outliers are displayed with a pink background.
- On this pane, you can:
  - Ignore a calibration curve level (standard)
  - Change the concentration of the calibration curve level
  - Change the calibration curve type
  - Change how the origin is handled
  - Change the weighting of calibration curve
- If a calibration curve is still an outlier (displayed in pink), then the calibration standards will have to be run again until there is a passing calibration curve.

Assessing and Exporting Batch Data

QA/QC Requirements

1. Any sample that is ***flagged*** in the ***Outlier Summary*** column must be checked for why it was flagged. It is best to keep up with the run to avoid having to rerun large sections of the batch.
   a. Calibration curves should be checked for outliers as described above before the first QC's and unknown samples are analyzed.
   b. Since each sample is replicated 3 times, one of the replicates for cps or concentration can be eliminated by element (analytes & ISTDs) and tune mode if it appears there was a stabilization error in one of the replicates due to an interference, such as, an air bubble.
      i. If there were more outliers flagged than one replicate, then the sample will need to be re-analyzed.

- For example: ISTD percent (%) recovery outlier, analyte CPS or concentration RSD greater than 5%, and/or concentration value is over the calibration range.

c. A **blank** and at least one **QC** (Ultra Check Low, Ultra Check High, NIST 1620a, Si QCCS, Cation Ultra Check) per analyte are analyzed directly following the calibration standards, are repeated every 15 unknown samples, and following the last unknown sample.

   i. If a **blank verify** is ***flagged*** for any analyte (concentration > 1 ppb for trace elements and > 1 ppm for major elements), then pause or stop the run until the source of contamination is determined.

   ii. If a **QC** is ***flagged*** for any analyte, then all samples measured since the last passing QC must be re-analyzed for the analyte(s). A flagged QC has a percent recovery greater than 115% or less than 85%.

d. **Lab duplicates** should be analyzed every ten samples at a minimum. Percent RSD between the original and the duplicate must be less than 10%. If a lab duplicate fails for any analyte, then all samples measured since the last passing lab duplicate must be re-analyzed for the analyte(s). Do not analyze the duplicate immediately after the original sample.

e. **Matrix spikes** should be prepared and analyzed every ~15 samples. The spiked amount should be at least 30% of the original sample concentration, and the percent recovery should be between 85 and 115%.

2. Once the batch is finished running, and the data has been examined for the above restrictions, then the ***batch table*** can be ***exported*** to Microsoft Excel by clicking , save as follows:

   Desktop > Exported Data Files > Project > File name (Format: date, project, mode; Ex: 180811 MDE gen purp.xlsx)

   a. The file should contain a sheet with each of the following:

      i. "All Data" – The entire run except for rinses, including analyte concentrations, and ISTD % recoveries.

      ii. "QC's" – All of the blank verifies and QC concentrations for each analyte throughout the entire run with the percent recovery calculated for all QC results.

      iii. "Lab Dup's & Spikes" – All of the original sample concentrations that were duplicated, the duplicate sample concentrations, and the calculated percent RSD between them. All of the original sample concentrations that were spiked, the matrix spiked sample

concentrations, and the calculated percent recovery of the matrix spikes.

iv. "Prep Blanks & Dup's" (only when digested samples analyzed) – All of the Prep Blank concentrations for each analyte throughout the entire run. All of the original sample concentrations that were duplicated during the digestion procedure, the duplicate (Prep Dup's) sample concentrations, and the calculated percent RSD between them.

v. "Reporting" – Only the analyzed unknown sample's final concentrations for all elements by project.

Plasma Off

1. Double-click the ***Plasma*** gadget on the Dashboard.
   A confirmation dialog box is displayed.
2. Click ***Yes***.
   The ***Plasma*** gadget indicates that the plasma is off.

Exiting the MassHunter Workstation

Use any of the following methods to exit MassHunter Workstation. Be sure to save any changes before you exit the program.

1. Double-click the Control Box Menu in the upper left corner of the ***ICP-MS MassHunter*** window, or click the Control Box Menu and then click ***Close*** in the pop-up menu that appears.

2. Click on the "close" button in the upper right corner of the ***ICP-MS MassHunter*** window.

3. From the menu bar in the ***ICP-MS MassHunter*** window, select ***File > Exit***.

A message appears that says "Save changes you made to batch?". Click ***Yes to exit*** MassHunter Workstation. Click ***No to return to the program***.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) is the fraction of TOC that passes through a 0.45 µm-pore-diameter filter. This technique uses the persulfate oxidation technique to oxidize the organic carbon to carbon dioxide by persulfate in the presence of ultraviolet light. The carbon dioxide produced is purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared (NDIR) detector. Before analysis, the inorganic carbon is removed from the sample by acidification and sparging.

<u>Instrumentation</u>

Teledyne-Tekmar Fusion Carbon Analyzer

Sample Collection, Preservation, and Storage

DOC samples are filtered and preserved (pH adjusted to less than 2 with concentrated phosphoric acid). The sample is stored at 4°C until analysis.

Procedure

1. Follow manufacturer's instructions for calibration and operation.
2. Allow the UV lamp to warm up for at least 10 minutes.
3. Leak check instrument.
4. Analyze at least 6 replicates of a reagent blank
5. Analyze 2 replicates of each standard and calibration blank.
6. Determine instrument response for each standard and blank.
7. Generate calibration curve by plotting standard organic carbon concentration against corrected instrument response.
8. Analyze samples with appropriate QC samples.
9. If any samples exceed the concentration of the highest standard, dilute and re-analyze.
10. Generate reports of analytical results.
11. Perform appropriate instrument shut down procedures.

B.5 Quality Assurance/Quality Control

Quality assurance and quality control are a significant component of the MBSS. Details of MBSS QA/QC procedures can be found in Stranko et al (2007) for Round 3. QA/QC for benthic laboratory processing can be found in Boward and Friedman (2000) and Dziepak et al. (2022), and water chemistry analytical QA/QC procedures can be found in Eshleman and Mentzer (2024). Results from Round 2 MBSS QA/QC analyses can be found in Roth et al. (2005). Results of Round 4 MBSS QA/QC analyses can be found in Stribling et al. (2017a,b).

Water Chemistry Laboratory

A constant consideration of the Appalachian Laboratory is assuring the quality of data generated by the procedures presented in this manual. Further, indication of data quality is accomplished by analyzing duplicates, spikes, standards-as-samples, standard reference materials; and participating in cross-calibration exercises. The table below provides an overview of the water chemistry laboratory QA/QC procedures.

QA/QC Procedures	Data Quality Indicator	Acceptable Range	Failure Action
Laboratory Duplicates	Precision	10% RSD*	Re-analyze batch back to last passing lab dupe.
Matrix Sample Spikes	Accuracy	±15% Recovery	Re-analyze sample and spike; if it still doesn't pass prepare spike again; if it still doesn't pass, re-calibrate instrument and re-analyze sample and spike
Quality Control Check Solutions (QCCS)	Accuracy	±10% Recovery	Re-analyze solution; if it still fails to pass, re-calibrate and re-analyze; re-run all samples to last passing QCCS
Blanks	Accuracy	< MDL+	Re-analyze blank; if it still fails to pass, re-calibrate and re-analyze
Natural Audit Samples	Accuracy	±15% of average value	Re-analyze sample; if it still fails to pass, re-calibrate and re-analyze
Laboratory Control Samples (LCS)	Accuracy	±10% Recovery	Re-analyze solution; if it still fails to pass, re-calibrate and re-analyze; re-run all samples to last passing LCS

* RSD = relative standard deviation
+MDL = method detection limit

Laboratory Duplicates

Approximately 5% of the total number of samples analyzed consist of laboratory duplicates. For dissolved analytes, after a sample is analyzed, the same sample container is placed farther along in the automatic sampler tray and re-analyzed. The mean of the two values is reported as the concentration for that sample. If a difference of >10% is observed between replicates, then all of the replicates for that particular analytical run are carefully reviewed. If only one of the duplicate pairs is in question, then only that sample is re-analyzed. If all show a similar trend, then instrumentation/reagent problems are suspected and the analytical run is halted until such time as the problem is resolved. This procedure is practiced for all dissolved analytes that are not consumed completely in the analytical procedure. For those that are completely consumed and for particulate analytes, duplicate analyses are actually duplicate samples processed in the laboratory and analyzed.

Values for each duplicate analyzed are recorded in a separate QA data file along with the sample number, sample collection date and analysis date. The mean concentration and standard deviation of the replicates are calculated in this data file.

Laboratory duplicates serve as an indicator of instrument stability, consistency in laboratory sample preparation and analysis, as well as an estimate of field proficiency.

Laboratory Spikes

Approximately 5% of the total number of samples analyzed consist of laboratory spikes. A spike is prepared by adding a known volume of standard to a known volume of pre-analyzed sample. Enough concentrated standard is routinely added to provide a significant response on the instrument that is distinguishable from the original concentration of the sample. This concentrated standard is used to minimize any possible change in sample matrix by the addition of spike.

The spiked sample is analyzed and its expected concentration calculated as the sum of the original concentration and the spike concentration, normalized for the constituent volumes. A comparison is made between the actual value and the expected value. These concentrations (original, expected and actual) are recorded in a separate QA data file along with sample number, sample collection date, analysis date and the amount of spike added.

If a value of >115% or <85% is observed for percentage recovery of the spike, then all of the spikes for that particular analytical run are carefully reviewed. If only one of the spikes is in question, then only that sample is re-analyzed. If all show poor recovery, then instrumentation/reagent problems are suspected and the analytical run is halted until such time that the problem is resolved.

Documentation of Slopes, etc.

A running record of the slopes of the standard curves (the so-called "F," "S" and "K" factors) is maintained for each analysis. Random up and down movement within a predetermined range as a

function of time indicates the analysis is under control. Consistent upward or downward trend of these factors indicates the analysis is out of control and requires immediate attention.

Limits of Detection

Limits of detection, the lowest concentration of an analyte that the analytical procedure can reliably detect, have been established for all parameters routinely measured by the Appalachian Laboratory. The limit of detection is three times the standard deviation of a minimum of seven replicates of a single low concentration sample. These values are reviewed and revised periodically.

Analytical methods, instruments, detection limits, and holding times for MBSS analyses as of 2023. * Indicates analyses that require filtration within 48 hours.

Analyte (units)	Method	Instrument	Detection Limit	Holding Time (days)
pH (units)	EPA (1987) Method 19	Orion pH meter	0.01	7
Acid neutralizing capacity (µg/L)	EPA (1987) Method 5	Brinkmann Automated Titration System equipped with customized software	0.01	14
Sulfate (mg/L)*	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.098	14
Nitrite-nitrogen* (mg/L)	EPA (1999) Method 354.1)	Lachat QuikChem Automated Flow injection Analysis System	0.0015	28 (frozen)
Nitrate-nitrogen* (mg/L)	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.0371	14
Total ammonia-nitrogen (mg/L)*	USGS (1993) NWQL I-2525	Lachat Quikchem Automated Flow Injection Analysis System	0.0049	28 (frozen)
Total Nitrogen (mg/L)	APHA (2005) 4500-N (B)	Lachat QuikChem Automated Flow Injection Analysis System w/ In-line Digestion module	0.003	28 (frozen)
Ortho-phosphate (mg/L)	APHA (2005) 4500-P (G)	Lachat Quikchem Automated Flow Injection Analysis System	0.0015	28 (frozen)
Total Phosphorus (mg/L)	APHA (2005) 4500-P (I)	Lachat QuikChem Automated Flow Injection Analysis System w/ In-line Digestion module	0.0056	28 (frozen)
Chloride (mg/L)	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.031	14

Bromide (mg/L)	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.027	14
Specific conductance (mg/L)	EPA (1987) Method 23	YSI Conductance Meter w/ Cell	0.1	7
Dissolved Organic Carbon (mg/L)	EPA (1987) Method 14	Dohrmann Phoenix 8000 Organic Carbon Analyzer	0.112	28
Magnesium (mg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.002	6 months
Sodium (mg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.021	6 months
Potassium (mg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.008	6 months
Calcium (mg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.004	6 months
Copper (mg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.02	6 months
Zinc(µg/L)*	APHA (2005) 3125	Agilent 7900 Inductively Coupled Plasma-Mass Spectrometer	0.77	6 months

Quality Control Check Solutions (QCCS)

Standards prepared from a second stock solution, or QCCSs, are analyzed as samples throughout the analytical run. This is an excellent means of checking for accuracy by evaluating instrument performance during the course of an analytical run. Standards as samples are analyzed every 12 - 20 samples, depending on the instrument and analyte.

Blanks

Deionized water blanks serve as a check of laboratory-induced contamination. Laboratory blanks are analyzed on a daily basis. Field sampling personnel also periodically collect deionized water blanks that are submitted for analysis. Reagent blanks are also prepared and analyzed, as outlined in the standard operating procedures for those analytes.

Natural Audit Samples

Natural audit samples are another useful part of a comprehensive QA Plan. Because they are collected from streams, they are more representative of the actual sample matrix than a manufactured calibration check solution. As needed, a field natural audit sample is collected from Upper Big Run in the Savage River State Forest in order to establish an internal audit sample (FNBR). Approximately 50 liters of sample is filtered using a 0.45 µm filter capsule and a Masterflex pump. The sample is returned to the Appalachian laboratory where it is refrigerated for approximately 20 days and periodically checked for stability by analyzing sample ANC. Once the sample is stable, it is poured off into 500 ml aliquots. The audit samples are stored in the dark at 4°C and are analyzed periodically for certain analytes, including ANC, anions, DOC, and specific conductance. Although there are no actual right or wrong results for any of the analytes, as when a known QCCS is measured, variations in analyte concentration can help determine or diagnose any sources of analytical error. These results are especially useful as a diagnostic tool when any changes in the operating conditions of an instrument (i.e., column or electrode replacement). Results from analysis of the audit sample provide another indication of the precision and accuracy of the analytical techniques

Laboratory Control Samples (LCS)

Standard reference samples for ammonium, nitrite + nitrate, nitrite, orthophosphate, total nitrogen, total phosphorus, anions, cations, and DOC are purchased from Phenova, a certified provider of Proficiency Test Samples. The samples arrive in ampules and final concentrations are prepared to approximate typical surface water concentrations. The samples are then stored in pre-cleaned aliquots, preserved as required by each method, and analyzed on a daily basis.

The analysis of these frozen standard reference materials as a function of time also provides data on the effect of our preservation technique (freezing) on the integrity of the concentration of samples for nutrients. The US EPA recommends a holding time of 28 days for many of the parameters that we routinely analyze.

Cross Calibration Exercises

The Appalachian Laboratory has participated in many cross calibration exercises. Participation in such programs is an excellent means of determining accuracy of results. Examples of such cross

calibration exercises include the Chesapeake Bay Program Blind Audits and the Environment Canada Proficiency Test Program.

Field quality control is checked during random field audits. The QA Officer assures that samples are collected, labeled, and preserved according to standard operating procedures and that all sampling protocols are followed.

Field Collection

Below are descriptions of documentation procedures, responsibility and accountability of project personnel, training requirements, facilities, and equipment for MBSS field sampling. To achieve the objectives of the MBSS, it is imperative that all project personnel follow the procedures and guidance provided in this chapter.

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

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

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

Population of Interest

The current population of interest for the MBSS includes all non-tidal, 5th order and smaller stream reaches in the State of Maryland (map scale = 1:24,000), with the exception of reservoir-like impoundments which substantially alter the lotic nature of the reach.

Comparability and Completeness

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

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

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.

Field Audits

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

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

To ensure consistency in data collection, the QC Officer is required to fill out an extra set of MBSS electronic data sheets at sites sampled during QC visits. These electronic data sheets are to be filled out independently from those completed 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 electronic 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.

Training Requirements

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

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 prior to equipment use and anytime equipment problems are suspected. Preventative maintenance must be performed at intervals not to exceed the frequency recommended by the manufacturer. All equipment malfunctions must be fully corrected prior to reuse. For weighing scales, weekly checks must be conducted during field sampling using NIST standards or other accepted standards to demonstrate that instrument error is within limits specified by the manufacturer.

For each piece of equipment used as part of the MBSS, a bound logbook 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 requirements and maintained and calibrated based on equipment specifications, the QC Officer inspects the current calibration/maintenance logbook records kept by each crew leader during QC audits.

Field Information Management

Field recording of MBSS data was carried out using primarily paper datasheets in 1995-2017 and primarily digital datasheets in 2018-present. To facilitate data recording during inclement weather, data recording devices (two iPads per crew) are kept in waterproof cases. iPads are also kept from direct sun whenever possible and charged during transit between sites. Files saved to the iPads are backed up frequently using iCloud or Google Drive, which can be accessed via WiFi or Cellular Data.

Each sample collected as part of the MBSS is assigned a sample number. The sample number contains several unique identifiers to minimize the possibility of misidentification. In addition, Chain-of-Custody forms should be maintained for all water and benthic macroinvertebrate samples and fish vouchers.

Data Quality Assessment

Assessment of data quality against established data quality objectives will be conducted to determine the overall performance of the QA program, identify potential limitations to use and interpretation of the field collected data, and to provide information for other data users regarding usability of the data for other purposes. Examples of MBSS data quality reporting can be found in Rogers (2013) and Stribling et al. (2017a,b).

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

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

Duplicate Samples

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

Taxonomic Identification

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

Photographic vouchers are accepted in lieu of preserved specimens. Photographs of at least five specimens of each fish, reptile, amphibian, and crayfish species encountered by each crew per year (as long as five were collected) are to be photographed. In addition, any rare, threatened, or endangered species encountered are photographed, as long as the photograph can be taken without causing any harm to the specimen. Photographs must clearly show the appropriate features necessary for identifying the species. DNR/MANA keep a voucher library of all photographs taken during MBSS sampling. With the exception of rare, threatened, or endangered species, specimens that are too small to provide photographs that can be used to verify identifications should be preserved for verification. Photographs are reviewed by an expert in taxonomy for each taxonomic group and results will be kept on record.

Benthic Macroinvertebrate Laboratory

Repeated Subsampling

Using sequential Log Numbers, every 20th sample (if two buckets were required at a site, they should be treated as a single unit) is randomly chosen for re-subsampling and identification according to the following procedure:

- subsample and identify the sample as usual EXCEPT – identify Chironomids to Subfamily or Tribe (do not slide mount the larvae) and Oligochaetes to class.

- return the once-identified organisms to the original sample bucket containing the sortate and preservative, and re-subsample.
- identify the second subsample according to standard procedures (i.e., slide mount Chironomid larvae and Oligochaetes and identify them to genus and family, respectively, if possible).

- QC comparisons are made on the two taxa lists and benthic Index of Biotic Integrity (BIBI; see Southerland et al. 2005) values generated from the two subsamples (of the same sample).

Taxonomy

Questionable identifications are verified by consulting other DNR benthic taxonomists, regional experts, and regional keys for certain taxonomic groups.

Taxonomic Testing

Lab personnel must be certified by the Society of Freshwater Science in EPT East and North American Chironomidae. The certification provided by the Society of Freshwater Science lasts five years.

B.6 Instrument/Equipment Testing, Inspection, and Maintenance

Instrument probes are cleaned and thoroughly inspected between sampling events. If any probe is not functioning correctly, it is determined whether it is necessary to perform maintenance and/or replace (retire) the instrument.

Physical sampling gear is inspected before each use to assure that all parts are intact. Any gear that shows operational deficiency is not used until repairs can be made.

For a full description on this topic for MBSS water chemistry laboratory and field operations protocols, see Section B4 above and Harbold et al. (2024), respectively

B.7 Instrument Calibration and Frequency

Water chemistry laboratory

Analytical instruments are maintained on a regular basis and records are kept of hours of operation, scheduled maintenance, pump tube changes, etc. A critical spare parts inventory is maintained for each instrument. Instrument down-time is minimized by troubleshooting instrument problems by telephone with manufacturers and service representatives. Spare parts can be received within 24 hours via next-day air service.

Field physico-chemical meters

There are occasions when field crews continue to use state-of-the-art water quality sondes. The meters used to determine field parameters (e.g., dissolved oxygen, specific conductance, pH and water temperature) are calibrated weekly. Specific instructions for calibration are found in the operating manuals provided with the instrument. Fresh standards are available for calibration

prior to each sampling period. The field crew leader is responsible for ensuring that calibration occurs according to established protocols and recommendations from the sonde manufacturer.

A calibration record is maintained for each unit in a logbook. This log serves as documentation for pre- and post-calibration information for each parameter recorded. The log is useful in determining drift in a probe, which indicates that maintenance is necessary. The field crew leader remains aware of questionable performance of any instruments, and determines when it is necessary to perform maintenance and/or replace an instrument.

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

B.8 Inspection Acceptance Requirements for Supplies and Consumables

The crew leader routinely inspects equipment and supplies. The crew leader is responsible for determining when supplies and consumables should be discarded. Special attention is paid to the condition of syringes, caps and bottles to assure that they are uncontaminated. If contamination is suspected, the supplies are discarded. Any supplies that have exceeded their expiration date are disposed of.

B.9 Non-direct Measurements

Non-direct measurements used by the MBSS include land use/land cover data, watershed boundaries, meteorological data, and supporting environmental data used during the reporting process. Land use/land cover data are acquired from other DNR units as well as other Maryland State agencies such as the Maryland Department of Planning. These data are considered suitable for use in analyzing MBSS findings since these agencies have their own rigorous QA procedures. Hence these data should be of known and acceptable quality. Watershed boundaries are also produced by DNR's GIS Services and are of known and acceptable quality. Weather data are most often acquired from NOAA and other environmental data, such as soils, geology and topographical data are all acquired from sources of known data quality.

B.10 Data Management

Office

The MBSS Data Management and Analysis Officer oversees all aspects of data entry, Quality Assurance checks, and storage. Data are downloaded from field iPads or entered from paper data sheets into personal computers and stored in MS Access databases that serve as a "front end." Paper and digital copies of data sheets are kept indefinitely. Any and all MBSS staff have unfettered access to MBSS data sheets. Most databases are read only and available for edit only to the MBSS data manager.

All field-collected data are scrutinized by the QC officer for any potential issues within three months of collection. Review procedures include range checks, frequency distribution of coded variables, and other internal consistency checks. Questionable data are flagged and a determination of validity is made by the Data Management and Analysis Officer, the QC Officer, and the responsible Crew Leader. For all editing activities, full documentation of all changes is

mandatory. Corrections to paper datasheets are documented via notes and edits directly on the datasheet. Corrections to digital datasheets are documented via notes and edits that are saved within Adobe PDF files flagged for further attention.

Data entry (paper data sheets) used entry screens designed to emulate the data sheet format. All data were double-entered using two different data entry operators and compared for consistency. All MBSS paper data sheet data were entered twice and a "diff" program was used to highlight differences in data entered by two data entry personnel. All data entry file cells that contain different data were scrutinized and the differences were rectified.

Data recording using a digital datasheet is completed in the field as much as possible. Data from each site are saved to individual files in Apple's proprietary NUMBERS file format. Files are managed using the Apple iCloud interface, which enables conversion and export to PDF and CSV formats. All datasheets are initially exported in PDF format, for review by the Data Management and Analysis Officer and digital archival. Any corrections that were noted on the original PDFs are then rectified on NUMBERS file saved to the iCloud. Corrected site data are then exported as CSV files. An automated procedure programmed via the Visual Basic Code Module in Microsoft Excel is used to condense data from the various csv files into a single Excel page.

To verify and document that field and field preparation activities are carried out according to established protocols, field audits are necessary. At a minimum, the QC Officer will make one site audit per index period with each MBSS field crew, and on these visits the QC Officer will independently do a habitat assessment at each site sampled. The QC Officer may conduct other activities such as time and conditions permit, including capture efficiency checks, site location verification, spot checks of taxonomic identifications, etc. The QC Officer will retain on file the results of all field audit activities.

Data Analysis

MBSS data analyses are conducted by DNR personnel and by consultants working to support MBSS data reports. Analyses are conducted using several software packages including R, SAS, MS Access, Microsoft Excel, and PC Ord.

C. Assessment/Oversight

C.1 Assessment and Response Actions

For specific descriptions of assessment and response actions related to MBSS field, laboratory (benthic and water chemistry) and data management tasks, see the appropriate portions of Section B above.

D. Data Validation and Usability

D.1 Data Review, Validation, and Verification

Reported in Section B above. An overview is contained in the chart on the next page. Third party activities are included in benthic macroinvertebrate taxonomic rechecks (EPA Wheeling Laboratory and Tetra Tech, Inc.) and fish voucher identification (Frostburg State University). MD DNR does not have the funding available to involve third party performance in all aspects of the MBSS.

D.2 Validation and Verification Methods

Reported in Section B above. General validation and verification procedures were followed according to those outlined in EPA (1987) and EPA (1989).

D.3 Reconciliation with Data-Quality Objectives

MBSS data quality objectives are evaluated and reconciled in Quality Assurance Reports. In this report, recommendations are made to improve MBSS data quality given the limited resources available to operate the Survey. Examples of such reports can be found in Roth et al. (2005); Rogers (2013) and) Stribling et al. 2017a,b.

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