Alliance for the Chesapeake Bay

Quality Assurance Project Plan

Volunteer Monitoring Support for Macroinvertebrate Sampling to Fill Chesapeake Bay Program Data Gaps

Project ID: CBT 18880

Effective Date: March 1, 2021

EPA Document Control Number (DCN): 210081

501 6th Street
Annapolis, MD 21403

A PROGRAM MANAGEMENT

A1. Approval Sheet

Concurrence

Preparer/Author

Name: Emily Bialowas Title: Chesapeake Monitoring Outreach Coordinator Organization: Izaak Walton League	Signature: Date:
Name: Julie Vastine Title: Director Organization: Alliance for Aquatic Resource Monitoring	Signature: Date: 2/23/21

Management/Supervisor

Name: Liz Chudoba Title: Water Quality Monitoring Initiative Director Organization: Alliance for the Chesapeake Bay	Signature: Date: 2/22/21
Name: Title: Organization:	Signature: Date:

Quality Assurance Officer

Name: Drew Siglin Title: Chief Operating Officer Organization: Alliance for the Chesapeake Bay	Signature: Date: 2/22/2021

EPA Region 3

Name: Greg Allen Title: R3 Designated Project Manager Organization: EPA	Signature: Date: 2-24-2021

Approval

EPA Region 3

Name: Durga Ghosh Title: R3 Delegated Approving Official Organization: CBP / USGS	Signature: Date: 06/14/2021

Note: This approval action represents EPA's determination that the document(s) under review comply with applicable requirements of the EPA Region 3 Quality Management Plan [https://www.epa.gov/sites/production/files/2020-06/documents/r3qmp-final-r3-signatures-2020.pdf] and other applicable requirements in EPA quality regulations and policies [https://www.epa.gov/quality]. This

Revision History

This table shows changes to this controlled document over time. The most recent version is presented in the top row of the table. Previous versions of the document are maintained by Quality Manager.

Document Control Number	History/ Changes	Effective Date

This document has been prepared according to the United States Environmental Protection Agency publications – *The Volunteer Monitor's Guide to Quality Assurance Project Plans,* EPA 841-B-96-003, 1996*,* available at http://water.epa.gov/type/rsl/monitoring/qappcovr.cfm and *EPA Requirements for Quality Assurance Project Plans*, EPA QA/R-5, 2001, available at http://www.epa.gov/quality/qs-docs/r5-final.pdf.

A2. Table of Contents

Figures

Tables

Appendices

KEY ACRONYMS

ACB	Alliance for the Chesapeake Bay
ALLARM	Alliance for Aquatic Resource Monitoring (Dickinson College)
CBP	Chesapeake Bay Program
CEC	Chesapeake Environmental Communications, Inc.
CMC	Chesapeake Monitoring Cooperative

DIWG	Data Integrity Workgroup
EPA	Environmental Protection Agency
IAN	Integration and Application Network
INWG	Integrated Monitoring Networks Workgroup
IWLA	Izaak Walton League of America
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
QMP	Quality Management Plan
SOP	Standard Operating Procedure
STAR	Scientific, Technical Assessment and Reporting
UMCES	University of Maryland Center for Environmental Science

A3. Distribution List

Table A3-1. Distribution list for this Quality Assurance Project Plan.

Name	Phone	E-mail	Organization
Kate Fritz Liz Chudoba	443-949-0575 804-775-0951	kfritz@allianceforthebay.org lchudoba@allianceforthebay.org	Alliance for the Chesapeake Bay (ACB)
Julie Vastine Stephanie Letourneau	717-245-1135 717-254-8133	vastine@dickinson.edu letourns@dickinson.edu	Alliance for Aquatic Resource Monitoring (ALLARM)
Samantha Briggs Emily Bialowas	301-548-0150 301-548-0150	sbriggs@iwla.org ebialowas@iwla.org	Izaak Walton League of America (IWLA)
William C. Dennison Caroline Donovan	443-496-0196 410-330-3330	dennison@umces.edu cdonovan@umces.edu	University of Maryland Center for Environmental Science Integration and Application Network (UMCES)
Terry Simpson	410-305-2739	simpson.terry@epa.gov	Environmental Protection Agency (EPA)
Durga Ghosh Peter Tango	410-267-5750 410-267-9875	dghosh@chesapeakebay.net ptango@chesapeakebay.net	United States Geological Survey (USGS)
Sarah Koser	410-974-2941	skoser@cbtrust.org	Chesapeake Bay Trust (CBT)

A4. Project/Task Organization

A4.1 Project Organization

The Chesapeake Monitoring Cooperative provides support, training, and guidance to volunteer and nontraditional monitoring groups sampling non-tidal and tidal portions of the Chesapeake Bay Watershed. The project is managed by the Alliance for the Chesapeake Bay (ACB) in partnership with the project coordinator at Izaak Walton League of America (IWLA), and includes partners at the University of Maryland Center for Environmental Science Integration and Application Network (UMCES) and Dickinson College's Alliance for Aquatic Resource Monitoring (ALLARM). These four organizations together are hereafter referred to as the "Project Team".

Within the Project Team, ACB, IWLA and ALLARM are the Certified Trainers and data collectors. These organizations will work with individual monitors or monitoring groups to ensure proper data collection, will coordinate sample transfer processes from the monitoring groups to Wheeling lab, and will upload data to the Chesapeake Data Explorer after it has been processed by the Wheeling Lab.

UMCES will provide a supporting role on the project team by producing the training materials for the project. This includes the program manuals branded with the CMC brand and training videos needed to onboard monitors.

Additionally, the Chesapeake Bay National Estuarine Research Reserve in Virginia with the Virginia Institute for Marine Science (CBNERR-VA/VIMS) manages the regional database, the Chesapeake Data Explorer (Data Explorer). Support for the Data Explorer is not provided through this project; however, the Data Explorer will be used to house all of the data collected through this project.

The CMC is partnering with the Chesapeake Bay Trust (CBT) and the Chesapeake Bay Program (CBP) in order to collect family level benthic macroinvertebrate samples that will fill in gaps in the CBP Stream Health Assessment. All samples for this project will be analyzed at the EPA Wheeling lab.

The personnel involved in the implementation of this project are listed in Table A4-1. The context of the participating organizations in the Chesapeake Monitoring Cooperative are shown in the organizational chart in Figure A4-1.

Table A4-1. Roles and individuals participating in this project.

Organization	Role in Project	Individuals Involved in Project
Alliance for the Chesapeake Bay (ACB)	Project Manager	Liz Chudoba, Water Quality Monitoring Initiative Director
Izaak Walton League of America (IWLA)	Project Coordinator	Emily Bialowas, Chesapeake Monitoring Outreach Coordinator

Alliance for the Chesapeake Bay (ACB)	Project Partner	Sophie Stern, Water Quality Monitoring Coordinator
Alliance for Aquatic Resource Monitoring (ALLARM) @ Dickinson College	Project Partner	Julie Vastine, Director Candie Wilderman, Science Advisor
Izaak Walton League of America (IWLA)	Project Partner	Samantha Briggs, Clean Water Director
University of Maryland Center for Environmental Science (UMCES)	Project Partner	Caroline Donovan
Chesapeake Bay National Estuarine Research Reserve in Virginia with the Virginia Institute for Marine Science (CBNERR-VA/VIMS)	Chesapeake Data Explorer	Dave Parrish
Certified Trainers	Trainer	Project Team – ACB/IWLA/ALLARM
Monitoring Groups	Data Collectors	See Appendix H for current list of participating groups
Chesapeake Bay Program (CBP)	Data Users	Peter Tango, Stream Health Workgroup
Chesapeake Bay Trust (CBT)	Grantor	Sarah Koser
EPA Wheeling Lab	Data Processor	Add contacts

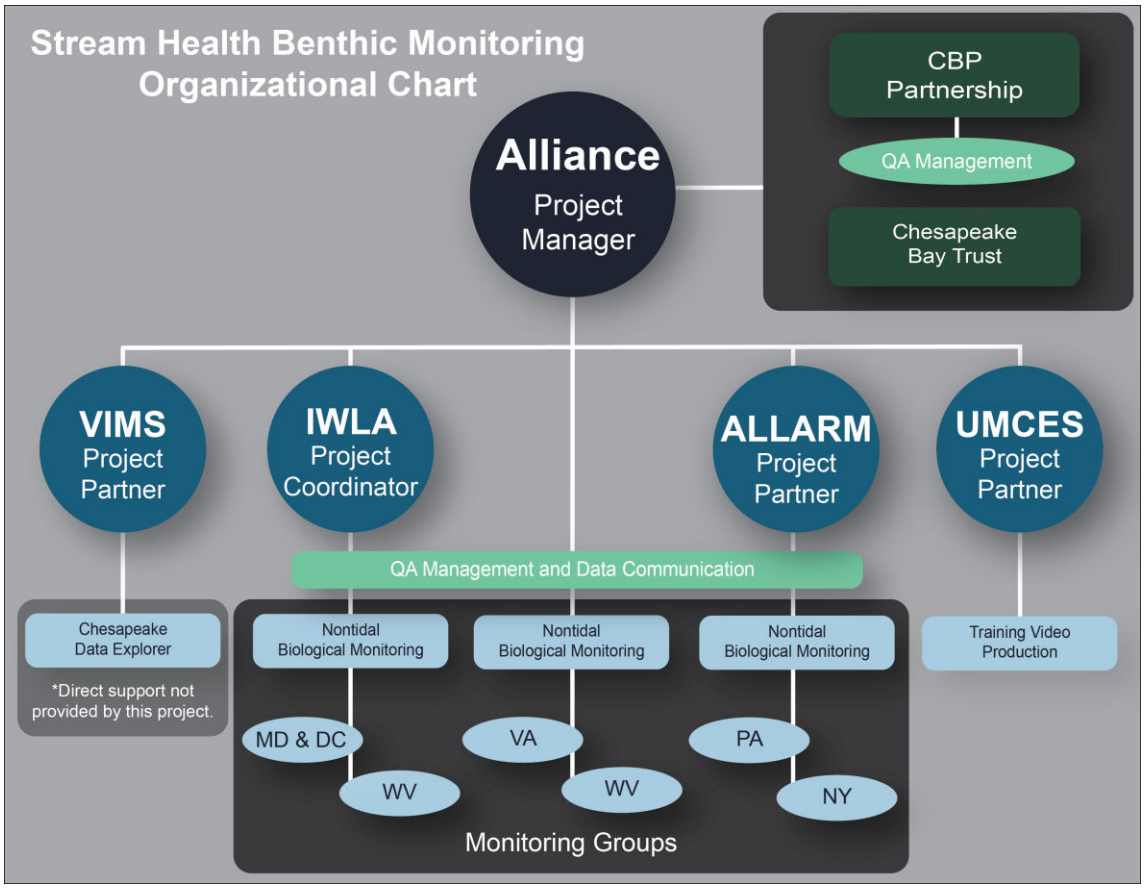


Figure A4-1. The CMC communication and QA organizational chart for this stream health benthic monitoring project.

The Project Team will train and support volunteer and nontraditional monitoring groups to collect macroinvertebrate data following this Benthic Macroinvertebrate Monitoring in Wadeable Streams (Tier II) Quality Assurance Project Plan (QAPP). ALLARM will coordinate the project's Macroinvertebrate Monitoring Program in the upper Chesapeake Bay Watershed (New York and Pennsylvania) and IWLA and ACB will coordinate the program in the lower portion of the watershed (Delaware, DC, Maryland, Virginia, and West Virginia).

The volunteer and nontraditional monitoring groups that participate in this project are spread throughout the Chesapeake Bay Watershed; however, the number or location of groups are not set and are always growing. To determine the universe of potential monitoring, the Project Team surveyed over 100 volunteer and nontraditional monitoring groups in the watershed (Figure A4-2). The groups that participate in the project currently include a subset of the groups surveyed.

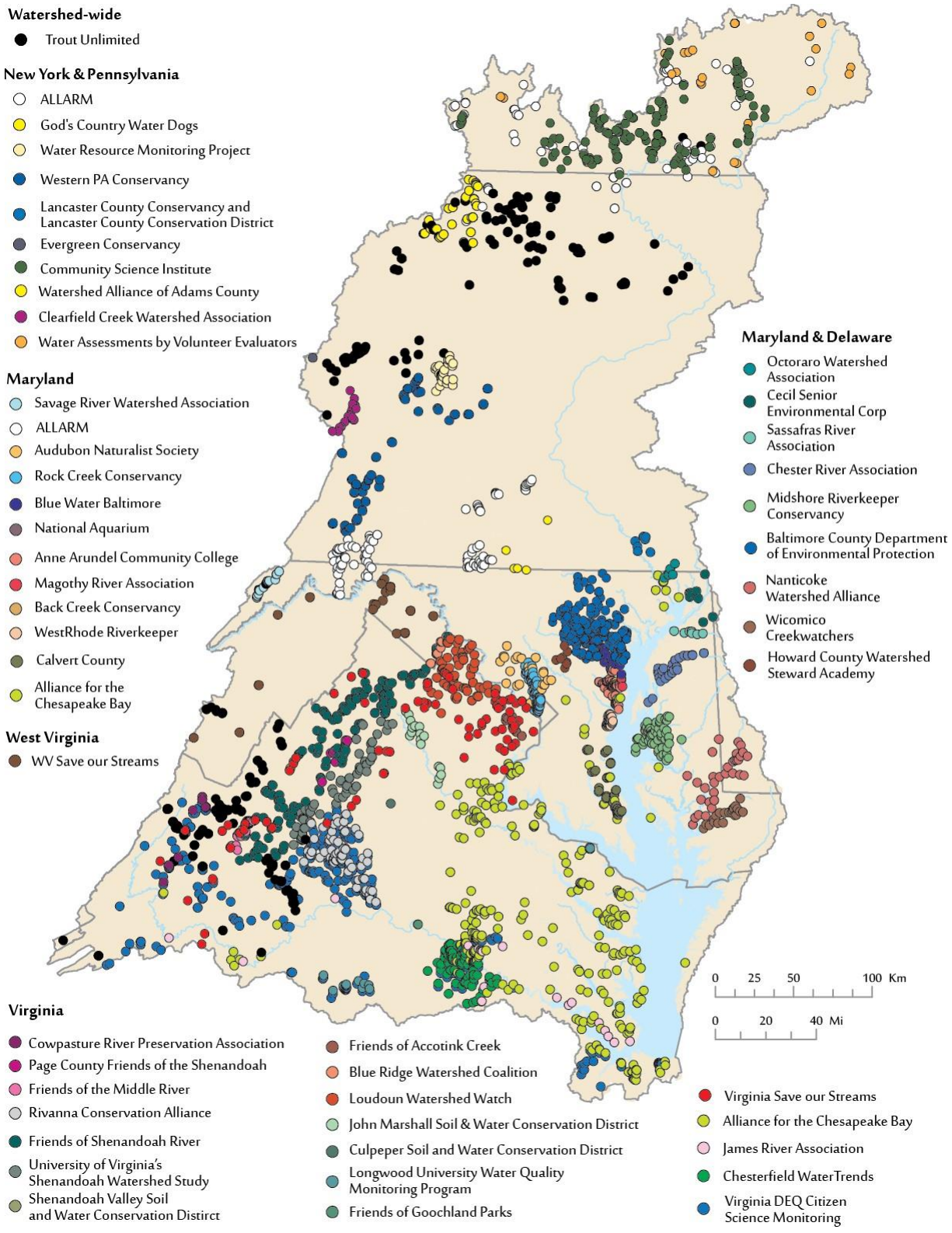


Figure A4-2. Sampling sites of volunteer and nontraditional monitoring groups in the Chesapeake Bay Watershed. *Note: all sites are not accounted for on map.*

A4.2 Roles and Responsibilities

Data collected through the CMC are categorized into three tiers based on the quality of the data (see A5.2 Data Use). The roles and responsibilities for the entire project are summarized below, however only those that pertain to Tier II macroinvertebrate data collection in wadeable streams are relevant to this Benthic Macroinvertebrate Monitoring in Wadeable Streams (Tiers I & II) QAPP.

Project Manager: Alliance for the Chesapeake Bay

a. Manages and provides support for the Project Team to ensure implementation of the Quality Management Plan (QMP), QAPPs, SOPs, and QA policies.
b. Annually reviews and updates the approved QMP, QAPP and SOP documents as needed. Any changes that impact the quality system are made to the QMP or QAPPs, the Project Manager will resubmit the QMP or QAPP to EPA for review and approval.
c. Acts as the liaison between the Project Team and Chesapeake Bay Program Workgroups, including attending workgroup meetings and calls with the STAR Team and the STAR Team's Stream Health Workgroup.
d. Submit water quality data published in the Data Explorer to the Chesapeake Bay Program's DUET system.
e. Provide CBT with all reporting documentation under the grant contract.

Project Coordinator: Izaak Walton League of America

a. Assists Project Manager in the coordination and supporting the Project Team to ensure implementation of the QMP, QAPPs, SOPs, and QA policies.
b. Assists the Project Manager in annual reviews and updates of the approved QMP, QAPP and SOP documents as needed.
c. Acts as the liaison between the Project Team and Chesapeake Bay Program Workgroups, including attending workgroup meetings and calls with the STAR Team and the STAR Team's Stream Health Workgroup.

QA Management – Alliance for the Chesapeake Bay, Alliance for Aquatic Resource Monitoring, Izaak Walton League of America, and Chesapeake Bay Program:

a. Reviews the project QAPPs and provides guidance to the Project Team for effective implementation of the QAPPs;
b. Reviews the QA/QC programs, practices, systems, training materials, and performance annually to ensure practices are in accordance with the QMP. Subsequently documents and responds to QA/QC needs and issues;
c. Assists with QA dispute resolutions (if/when needed); and
d. Assesses data management procedures for the monitoring programs and the project database to ensure they meet data quality objectives outlined in the QMP and QAPPs.

Project Team – Alliance for the Chesapeake Bay, Alliance for Aquatic Resource Monitoring, and Izaak Walton League of America:

a. Ensures that all monitoring groups adhere to the QMP and approved QAPP;
b. Ensures that all monitoring operations are covered by the appropriate documentation (i.e., SOPs, QAPPs, project plans);
c. Develops, reviews, updates, and approves SOPs for monitoring activities;
d. Conducts workshops and certifies monitors;
e. Coordinates with EPA Wheeling lab staff to get samples from monitors to the lab;
f. Continually assesses collected data and monitors performance through data QC, workshops, and re-certifications to identify QA compliance or deficiencies. All QA deficiencies will be properly documented and attempted to be resolved;
g. Assists monitoring groups in QAPP implementation;
h. Reviews and oversees QA policies and SOP's of monitoring groups and documents findings for CBP and project records;
i. Complies with findings and recommendations from QA reviews and audits; and
j. Resolves disputes regarding quality system requirements, QA/QC procedures, certifications, or corrective actions.

Supporting Role – University of Maryland Center for Environmental Science:

a. Produces the training materials for the project, including the program manuals branded with the CMC brand and training videos needed to onboard monitors.

Supporting Role – Chesapeake Bay National Estuarine Research Reserve with the Virginia Institute of Marine Science:

a. Manages and Maintains the Chesapeake Data Explorer, the home for all data collected in this project.

Monitoring Groups:

a. Adheres to SOPs and complies with QAPP guidelines;
b. Evaluates and reports QA issues to designated Project Team member, regional liaison, or QA Manager as they occur; and
c. Maintains certification as outlined in the project's QAPPs.

Stream Health Workgroup (SHWG) and Scientific, Technical Assessment and Reporting (STAR) Workgroup:

a. Stream Health Workgroup (SHWG) help to identify QA criteria for Tier II data collection and target watersheds for sample collection;
b. Scientific, Technical Assessment and Reporting (STAR) help to review and provide feedback on the Project Team's monitoring and training protocols, and quality assurance procedures.

A5. Problem Definition/Background

The Project Team has partnered to provide technical, logistical, and outreach support for the integration of citizen-based and nontraditional (e.g., non-agency) monitoring data into the CBP partnership. While the CBP has immediate access to agency (federal and state) data collected in the watershed, volunteer and nontraditional groups that collect stream data are scattered throughout the watershed, and their data are not compiled in one, easily-accessible platform. Macroinvertebrate samples that these groups can collect can be used to help answer many of the diverse questions asked by monitoring groups and the CBP and provide data to fill in gaps in the Chesapeake Basin-wide Index of Biotic Integrity (Chessie BIBI). The integration of data collected by volunteer and nontraditional monitors into the CBP monitoring network provide additional cost-effective information that supports shared decision-making and adaptive management by the CBP partners focused on restoration of the Chesapeake Bay and its watershed.

A5.1 Goals and Objectives

The 2014 Chesapeake Bay Watershed Agreement identified goals and outcomes related to the health and well-being of the Chesapeake watershed and community. These goals and outcomes are linked to measurable indicators. For the Stream Health Outcome, the Chesapeake Basin-wide Index of Biotic Integrity (Chessie BIBI) was developed using available benthic macroinvertebrate data from across the watershed. The data that the Chessie BIBI is based on have spatial gaps that can be filled in by samples collected by CMC monitoring groups that are monitoring in or near to those gaps. The Chessie Bibi Stream Indicator is applied over six- year periods, the last ones being 2006-11, and 2012-2017. The aim is to monitor at sites in HUC-12 watersheds that do not have adequate benthic data from these monitoring periods.

The objective of this project is to provide technical, logistical, and outreach support to nontraditional data collectors working in tributaries of the Chesapeake Bay Watershed, for them to provide macroinvertebrate samples that can be processed by the EPA Wheeling lab to family- level to fill the gaps in the Chessie BIBI dataset.

A5.2 Data Use

A *Tiered Framework for Data Collection and Integration for Nontraditional Monitoring* (*Tiered Framework;* Appendix B) has been developed by the Project Team to establish three categories of data based on data quality. The data quality requirements for each tier are largely dependent on the methodology used and the quality assurance procedures implemented by the monitor. The Project Team categorized the methods and QA procedures used by volunteer and nontraditional monitoring groups into three tiers based on comparability testing, manufacturer's specifications, experience, and how they are classified by other water quality monitoring programs. The data collected through this project will be stored in the project database along with the metadata needed to inform the data users of the specific quality of the data. The *Tiered Framework* suggests potential uses of the data;

however, the uses can extend beyond what is suggested, and it is ultimately the decision of the data users to choose the data which are appropriate for their specific uses given the metadata supplied in the database.
Table A5-1 lists the three data tiers along with the potential data uses and requirements.

The protocols established in this QAPP are designed to produce Tier II benthic macroinvertebrate data. Data collected from this program is intended for use in the Chessie BIBI but can also be used by the CBP and other data users for the purpose of environmental health screening, environmental health report cards, targeting management actions, and education. Data can also be shared and used at the local level by local governments, community stakeholders, and residents to increase awareness and address environmental concerns. Using the data to inform local practices helps to meet the CBP goal of improving citizen engagement and the health of the Chesapeake Bay Watershed. The data can also provide a baseline of current watershed conditions to compare to if/when changes in the watershed occur.

Data are stored in the Chesapeake Data Explorer accompanied by the appropriate metadata to allow data users (both traditional and nontraditional) to determine appropriate end uses. The Chesapeake Data Explorer was developed by Greenfin Studios in coordination with the CBP Data Center staff.

Table A5-1. Summary of the three CMC data tiers from the *Tiered Framework*.

Tier	Potential Data Uses	Minimum Data Requirements
Tier I	Education, environmental health screening	Documentation showing that procedures outlined in this QAPP for collecting and producing Tier I data have been followed. All items must be reviewed and approved by a member of the Project Team: a. Written study design b. Documented monitoring methodology c. Documented site location(s) (with coordinates)
Tier II	Tier I uses plus environmental health report cards, targeting of management actions	State or federal government-approved EPA volunteer monitoring QAPP or written agreement to follow the procedures outlined in this QAPP to collect and produce Tier II data. All items must be reviewed and approved by a member of the Project Team: a. Written study design b. Documented monitoring methodology using field or laboratory standard operating procedures with defined levels of precision and accuracy c. Documented site location(s) (with coordinates) d. Acquired and maintained Tier II certification

Tier III	Regulatory assessment of water quality standards attainment	a. EPA or CBP approved QAPP b. EPA or CBP approved field/lab standard operating procedures c. Participation in DIWG field and lab audits

A6 Project/Task Description

A6.1 Project Timeline

This Quality Assurance Project Plan is designed to ensure that new samples collected for the CMC Macroinvertebrate Monitoring Program will be done in an approved, quality-controlled, and standardized way. Monitoring groups collecting Tier II data will sample benthic macroinvertebrates in wadeable streams within the Chesapeake Bay Watershed (Figure A6-1) at least one time within a six-year period, but more frequent, annual or every other year samples can also occur. The entire grant project period is December 2020 through December 2021 (Table A6-1).

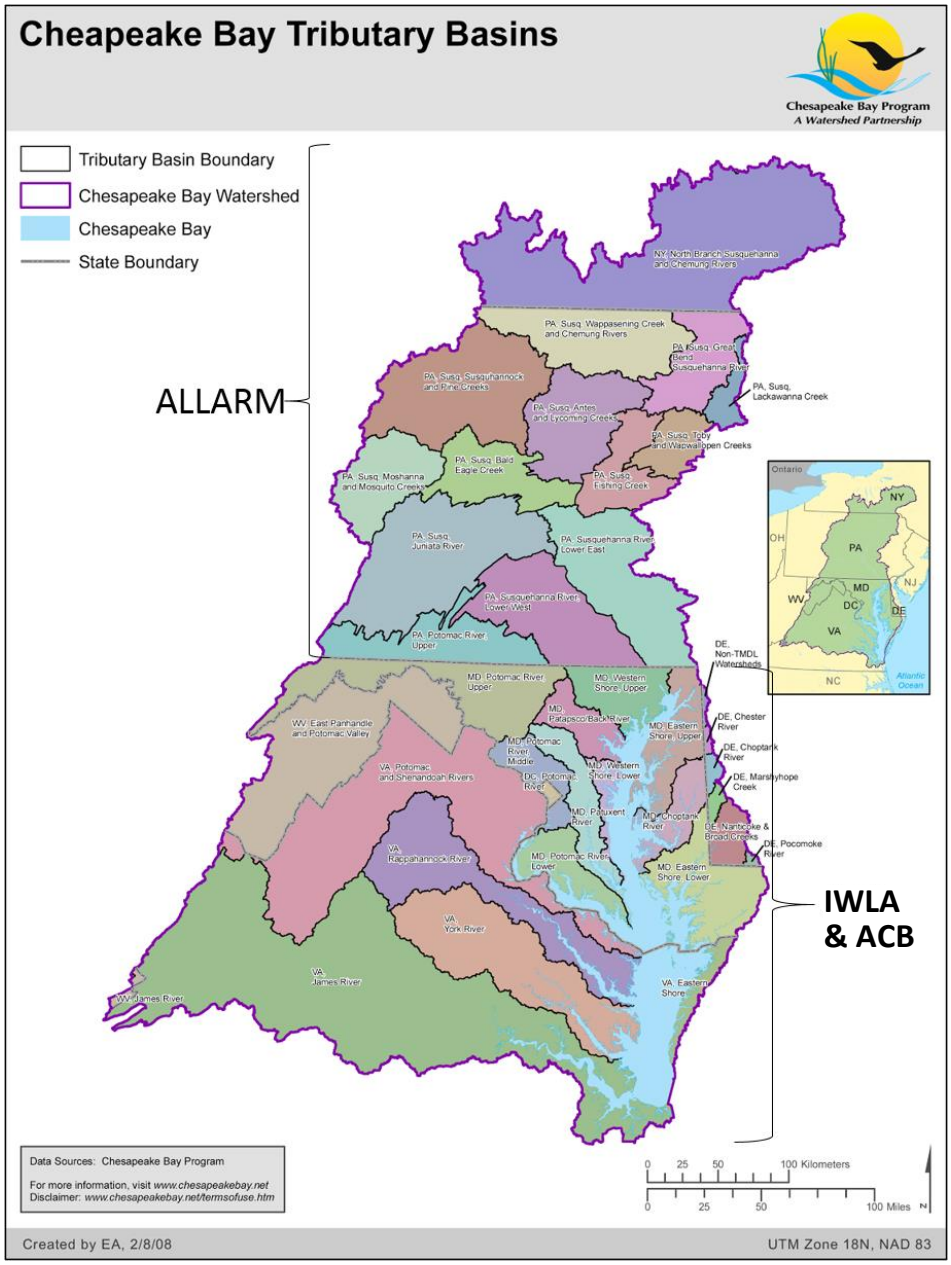


Figure A6-1. Areas of coordination by project partners (ALLARM, IWLA & ACB).

Table A6-1. Project timeline for 13- month grant period.

Report #/ reporting period	Project Deliverables	Date of Delivery	Amount
Report #1 12/1/20 - 12/31/20	Step 1 deliverables include: • Meeting minutes and summary of initial meeting (word). • List of gaps in watershed sampling and target areas to fill data gaps and to enhance robustness of the model used to fill gaps in unsampled regions (word). • List of volunteer teams and monitoring groups that may want to collect and contribute samples (word).	12/31/20	$0 – NFWF Match
Report #1 12/1/20 - 12/31/20	Step 2 deliverables include: • Draft QAPP (word). • Respond to comments, update the draft document and submit for final approval. • Final and approved QAPP (word and PDF).	12/31/2020	$0 - NFWF Match
Report #2 12/1/20 – 4/1/21	Step 3 deliverables include: • Draft and final list of field equipment and supplies (word). • Financial documentation of purchased equipment and supplies (pdf).	4/1/2021	$20,000
Report #3 12/1/20 – 12/31/21	Step 4 deliverables include: • Draft and final materials for training (word and PDF). • List of all attendees that attended the training (excel).	12/31/2021	$20,000
Report #3 12/1/20 – 12/31/21	Step 5 deliverables include: • Final training video (video file).	12/31/2021	$2,500
Report #3 12/1/20 – 12/31/21	Step 6 deliverables include: • Draft and final written procedures (word and PDF).	12/31/2021	$2,500
Report #3 12/1/20 – 12/31/21	Step 7 deliverables include: • Final training video (video file).	12/31/2021	$2,500
Report #3 12/1/20 – 12/31/21	Step 8 deliverables include: Summary of quarterly meetings attended (word).	12/31/2021	$0 - NFWF Match
Report #3 12/1/20 – 12/31/21	Step 9 deliverables include: • Final GIS data layers and metadata. Final training videos (two) on the CMC website.	12/31/2021	$0 - NFWF Match

A6.2 Site Selection

This project aims to fill in data gaps of the Chessie BIBI. For the purposes of this project as a pilot, the Project Team is focusing on rocky bottom stream sites. To achieve the goal of this project, the CMC has worked with CBP staff and the Stream Health Workgroup to identify those gaps on a HUC-12 watershed level (Figure A6-2). By identifying existing CMC monitoring locations in those gaps, the Project Team can begin to fill in gaps with tier II benthic data for the Chessie BIBI. Additionally, for the initial development of the CMC, the Project Team surveyed over 100 volunteer and nontraditional monitoring groups and collected information about their sampling sites (Figure A4-2). The Project Team also met with the CBP, state agencies, and key stakeholders to hear about their data needs. The information gathered by the Project Team from these entities, including a list of priority areas where data gaps exist, is summarized in the *Prioritization Report: How volunteer and nontraditional monitoring can help fill data gaps in the Chesapeake Bay Watershed; Appendix B*, and can help inform site selection beyond the monitoring groups currently integrated into the CMC.

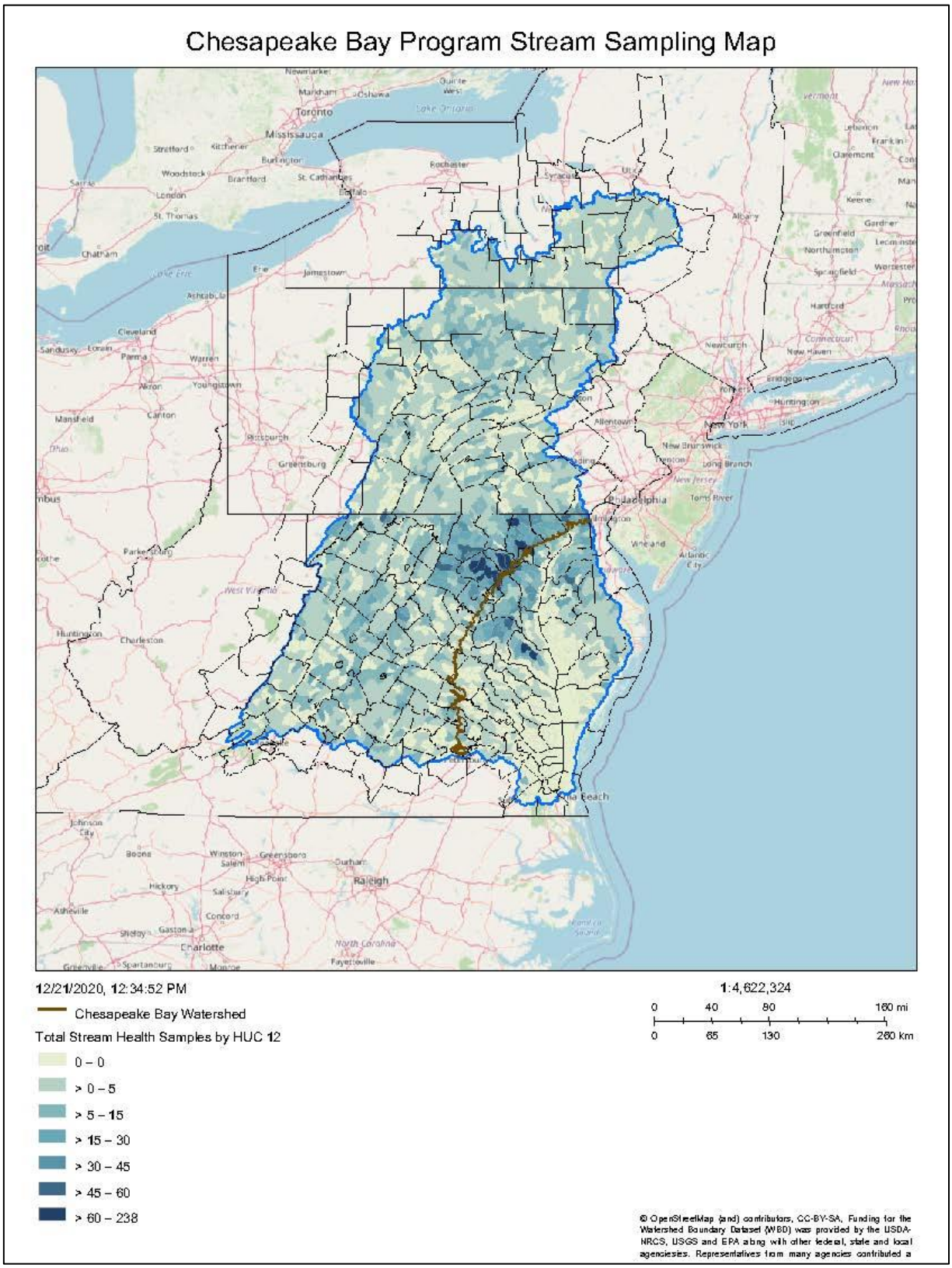


Figure A6-2. Map highlighting data gaps in stream sampling at the HUC-12 watershed level in the Chesapeake Bay watershed.

The Project Team select their sampling site(s) and work with nearby monitors to sample the location. The process for choosing sampling sites is outlined below:

1. Use CBP Stream Health Sampling map to identify HUC-12 watersheds with 5 or fewer samples between 2006 and 2017 that are located above the Fall line (i.e. are likely to have rocky bottom streams).
2. Identify existing CMC monitoring locations in those HUC-12 watersheds or identify sites that were included in the prioritization report in those HUC-12 watersheds.
3. Establish new sites if needed in watersheds that are missing data.

Once a sampling site is assigned by the Project Team, the monitor will:

1. Visit their site to confirm that it is safe and easy to access.
2. Use the Property Owner Permission and Liability Release Agreement (Appendix E) form to obtain written permission allowing them to access the stream, if the sampling site is on private property.
3. Obtain the proper permitting for scientific collection for the state they are sampling in.
4. Record a description of the site and the latitude and longitude coordinates using GPS with the North American Datum of 1983 (NAD83).
5. Send the sampling site description and coordinates to ALLARM/IWLA/ACB for verification using Google Maps and/or the USGS National Hydrography Dataset (NHD) in ESRI ArcMap.

A6.3 Data Management

All data collected through this project will be archived in a centralized database, the Chesapeake Data Explorer, and accompanied by the appropriate metadata to allow data users to determine appropriate uses for the data and to use them as they see fit. The overarching goal of the Data Explorer is to provide a centralized location where all volunteer and nontraditional data can be housed, accessed, and then used by stakeholders to better understand the quality of the Chesapeake Bay Watershed. Benthic Macroinvertebrate data can be evaluated for different purposes by different stakeholders, and the Data Explorer not only provides access to all of the data collected throughout the watershed for this project, but also allows data visualization through mapping and graphing applications. The query function on the Data Explorer allows users to search and find data that fit their specific needs, and a dataset with corresponding metadata detailing the methods and QA measures followed, can be downloaded for use.

Monitors will use field data sheets provided by CMC to record their sampling collections. All samples will be processed by the EPA Wheeling lab and provided to the CMC Project Team. All data will be uploaded to the Chesapeake Data Explorer by the CMC Project Team. Once data are uploaded into the Chesapeake Data Explorer it must be published by the Project Team, which certifies that the data have passed the QA checks required to be Tier II data as established in this QAPP.

All published data in the Data Explorer is publicly accessible for download on the homepage and will be uploaded to the Chesapeake Bay Program's DUET system annually. Data uploaded to DUET is automatically uploaded to EPA's Water Quality Exchange (WQX).

A7. Measurement Quality Objectives

The Chesapeake Monitoring Cooperative is designed to collect macroinvertebrate (and water quality) data that can be used to assess the health of the Chesapeake Bay Watershed. Therefore, the most effective means of ensuring that the data quality objectives are met, is to establish quality goals for the individual measurements that will be utilized to assess Chesapeake Bay Watershed health. Assessment of the quality for the various measurements obtained for the project can be expressed in terms of representativeness, completeness, comparability, accuracy, and precision.

A7.1 Data Precision, Accuracy and Measurement Range

ALLARM/IWLA/ACB/Certified Trainer will train monitors to properly collect and preserve macroinvertebrate community samples. Monitors collecting Tier II data will send their samples to the EPA Wheeling lab for family-level macroinvertebrate identification. Taxonomists verifying the identification of the benthic macroinvertebrate samples will be responsible for analytical accuracy and precision.

A7.2 Representativeness

A7.2.1 Selection of Sampling Sites

Each sampling site will be selected by the Project Team based on existing monitoring locations of CMC monitoring groups in watersheds with identified data gaps. Final site selection will occur after a discussion between the Project Team and the monitoring group. Sampling sites will be selected with the expectation that they will provide an adequate representation of the macroinvertebrate community of the stream segment being sampled.

Monitors will use a GPS, most likely on their smartphone, to determine the latitude and longitude coordinates of the sampling site, the accuracy of which is approximately within 10 meters. Monitors will take this measurement at the most downstream point of their sampling site. The Project Team will verify the site coordinates using Google Maps or the USGS National Hydrography Dataset (NHD) in ESRI ArcMap using the North American Datum of 1983 projection to ensure that the coordinates overlay the stream on the map and that the mapped sampling location matches the physical site description provided by the monitor.

Sites being monitored will be added to Appendix G.

A7.2.2 Sample Collection

Sample data shall be representative of the actual conditions present at the stream site. Sample collection, handling and sampling design, as well as preservation, are interactive factors that directly affect field sample representativeness. Monitors will follow standard operating and QA procedures found in their macroinvertebrate monitoring manual to ensure that representative data are collected. These techniques combined with external validation will ensure that the minimum standards of field representativeness are met.

A.7.2.3 Number of Sites

The Project Team has a goal of approximately 100 samples collected in the next 3-5 years, with approximately 20 samples collected per year. The goal is for samples to be collected in undersampled watersheds at least once, with some sites being revisited annually. The number of sites any individual group samples will vary by group depending on the group's location and available resources.

A.7.2.4 Sampling Timelines

The Chessie BIBI stream health indicator is assessed in six-year blocks, the next one being 2018-2023. For the purposes of this project, sites should be monitored at least once in that six-year period and in either the spring (March-June) or in the fall (August-November). Groups will determine their own monitoring schedule in coordination with the Project Team. The Project Team recommends monitors to sample during normal flow conditions when possible and to reschedule sampling events when unsafe conditions such as high water or strong storms occur.

A7.3 Data Comparability

This project is comprised of numerous monitoring groups spread throughout the Chesapeake Bay Watershed, which highlights the need for data comparability. The Project Team will develop and implement workshops to train certified monitors to follow comparable methods to collect and preserve macroinvertebrates. Certified monitors will follow the standard operating procedures and QA/QC requirements outlined in the macroinvertebrate monitoring manual, which will help to ensure comparability throughout the region. Certified monitors for this project will be required to attend workshops and maintain their Tier II data collection certification. Each monitoring group will have at least one certified monitor.

A7.4 Completeness

Groups collecting Tier II data are helping to fill in data gaps within a sampling design where benthic data are analyzed at least once over a six-year period. It is recommended that groups collect and preserve benthic samples at least once every six years from a monitoring location, but annual or biennial collections would also be welcomed for lab analysis.

A8. Training Requirements and Certification

The Project Team will hold trainings and field events and will check in with monitors on a regular basis. Workshop facilitators will have a thorough understanding of benthic macroinvertebrate sampling methods and QA protocols implemented by this project. They will also have experience working with volunteer and nontraditional groups and leading training events.

A8.1 Tier II Macroinvertebrate Monitoring Training

New monitors will be required to attend a Tier II Macroinvertebrate Monitoring Training in their region before they begin collecting macroinvertebrate samples for this project. If volunteers are interested in identifying order-level benthic macroinvertebrates or learning streamside sampling protocols, they can also attend a CMC Macroinvertebrate Monitoring Workshop that ALLARM or IWLA hold for Tier I macroinvertebrate monitors.

The Project Team will provide the trainings on an as-needed basis. At the Macroinvertebrate Monitoring Training, the Project Team will present the following information to the participants:

- Goals and objectives of the project
- Required permits and permissions for macroinvertebrate collection
- Macroinvertebrate collection and preservation methods
- Importance of safety when monitoring

The Project Team will train participants to:

- Clean, use, store, and maintain monitoring equipment
- Collect, sort, and preserve macroinvertebrates
- Accurately fill out and complete field data sheets
- Transport samples to the Project Team
- Follow quality assurance and quality control procedures

Participant performance will be evaluated at the workshop during the training activities. The Project Team will work closely with the participants during the hands-on training exercises to be sure that they achieve the goals of the exercises. Monitors will have to pass a field checklist to be certified (Appendix D). It is expected that each participant will be able to collect and preserve macroinvertebrate samples and appropriately store and transport samples after attending the Tier II Macroinvertebrate Monitoring Training.

Monitors will be able to borrow monitoring equipment after their completion and certification of the Tier II Macroinvertebrate Monitoring Training. They will also receive copies of the workshop materials, a taxonomic key, and a Tier II Macroinvertebrate Monitoring Methods Manual. The Methods Manual contains the information they learned at the workshop, standard operating procedures for collecting and preserving samples, recording field data sheets,

external QC forms, and references of how and where to access regional resources to supplement and reinforce what they learned at the workshop.

A8.2 Certified Monitors

There must be at least one certified monitor within each monitoring group who is responsible for the proper collection of macroinvertebrate samples. The Certification will be administered by the Project Team using the Monitor Certification Checklist (Appendix D) and includes:

1. In-stream observation – This portion of the test will be open-book and can be completed as a team with other monitors attempting certification. Each monitor must participate in collecting, processing and preserving an entire sample.
2. Understanding of equipment maintenance- This portion of the test will be open-book and can be completed as a team with other monitors attempting certification. Each monitor must demonstrate checking nets for holes.
3. Filling out field datasheet – Each monitor must demonstrate filling out a field datasheet correctly.

Monitors can maintain their certification by being field audited by the Project Team or a Certified Trainer biennially. Certified monitors and their groups will be listed in Appendix H.

A9. Documentation and Records

A9.1 Field Data Sheets

Monitors will fill out and complete field data sheets for every sampling collection event (Appendix C). The original data sheets will be submitted to the Project Team archived for at least seven years after the sampling date, and copies will be shared with the EPA Wheeling lab. The project will also maintain electronic (digital) records of the data within the Chesapeake Data Explorer.

A9.2 Other Documentation and Records

All documentation from trainings will be available at Project Team offices for a minimum of seven years as requested.

SECTION B – DATA GENERATION AND ACQUISITION

B1. Sampling Process Design

The CMC Project team will work with the Stream Health Workgroup to determine sampling site locations and work with monitoring groups to collect samples from those sites. Once the sampling site locations are determined, monitors will visit the site(s) to identify the specific stream reaches they will sample. Monitors will record the GPS coordinates of the stream reach, record a description of the reach, and confirm that it is safe and easy to access. If the stream reach is adjacent to private property, monitors will use the Property Owner Permission and Liability Release Agreement form to obtain permission from the property owner to access the reach prior to sampling. Monitors will also acquire the state-required permits to collect macroinvertebrates prior to sampling.

Monitors will follow the methods and QA procedures outlined in this QAPP and provided in their Tier II Macroinvertebrate Monitoring Manual to collect macroinvertebrate samples at their sites when directed by the CMC Project Team.

B2. Sampling Method Requirements

Each group will work with the Project Team to determine their sampling locations and schedule and follow the protocols outlined in this QAPP. It is recommended that macroinvertebrate samples be collected at the sampling sites at least once in a six-year period.

Monitors will receive a copy of the Tier II Macroinvertebrate Methods Manual at the Macroinvertebrate Monitoring Workshop. The manual will contain standard operating procedures (SOPs) for sample collection and preservation, field data sheets, and QA/QC procedures. Method Manuals will also be available online.

ALLARM will coordinate, train, and certify monitors in the Upper Chesapeake Bay Watershed (New York and Pennsylvania) and IWLA and ACB will coordinate, train, and certify monitors in the Lower Chesapeake Bay Watershed (south of Pennsylvania border) using the Tier II Macroinvertebrate Monitoring Methods outlined in this QAPP.

B2.1 Choosing Where to Sample Within the Streams

B2.1.1 Rocky Bottom Stream

Volunteers should not monitor within 72 hours of a severe rain event. Volunteers should select a rocky bottom stream site upstream of a bridge or culvert by 30 meters with at least two large riffles containing cobbles within a 100-meter reach. Within the reach, volunteers should subsample a variety of velocities with different stone sizes as these microhabitats are important to different species. Kicks on the 100-meter reach should be distributed and

representative of the riffle-run habitat (slow flowing shallow riffles and fast-flowing deeper riffles). Kicks should also be conducted throughout the width of the stream to include left-descending, middle and right-descending areas that are typical of the stream. The work from these kicks will be composited as a single sample.

B2.2 Sampling Method

Monitors will sample high-gradient streams that have riffles/runs and substrates composed of gravel and/or cobble and will follow the Tier II Macroinvertebrate Monitoring Procedure (Appendix A).

Monitors place a 500-micron D-net perpendicular to the flow of water immediately downstream of the 1 ft^2 area in the riffle they have selected to sample. The bottom edge of the net should be pressed tightly against the stream bottom.

The monitor samples the targeted area for 40 seconds, by lifting and rubbing underwater all large rocks in the sample area to dislodge any clinging organisms as well as all exposed surfaces of rocks in the sampling area that are too large to lift. Then for 20 seconds the monitor agitates the small rocks and sediments on the streambed in order to dislodge any burrowing macroinvertebrates (that can be done with boots, hands, or a tool such as a hand rake). The monitor repeats this kick one foot upstream from where they started. This process is repeated at 5 other locations in the stream for a total of 6 locations at the sample site.

Monitors transfer the contents of the net into a sieve bucket after every 2-3 kicks or if the water cannot easily run through the net anymore. To transfer the contents, the sieve bucket should be partially submerged, and the net rinsed into the bucket. The net should be turned inside out and rinsed to catch any missed part of the sample.

B2.3 Processing and Preservation Method

B2.3.1 Unpicked Method

Large debris such as stones and large sticks should be removed as well as fish, mussels, salamanders, turtles. Large packs of leaves should be sorted through and checked for macroinvertebrates before being removed. Monitors will transfer the remaining contents of the sieve bucket into a sample collection jar. All remaining contents of the bucket will be washed with ethanol into the sample jar, including leaves, detritus, and macroinvertebrates stuck to the sides of the sieve bucket. Monitors will add ethanol until there is double the volume of the solids alone. Monitors will include a pencil written label inside the jar with the monitor's name, date and time, location, and site name.

B2.3.1 Picked Method

Large debris such as stones and large sticks should be removed as well as fish, mussels, salamanders, turtles. Monitors will follow methods modified from the Macroinvertebrate Sample Subsampling SOP provided by the EPA Wheeling Lab (Appendix F). Only the organisms sorted with the subsampling procedure will be transferred into a sample collection jar. Monitors will add ethanol until there is double the volume of the solids alone. Monitors will include a pencil written label inside the jar with the monitor's name, date and time, location, and site name.

B2.4 Field Data Sheets

Monitors will fill out and complete all sections of their field data sheet (Appendix C) at each sampling event.

B2.5 Tier II Classification

As noted by the *Tiered Framework* in section A5.2, the Project Team categorizes the data collected based on the quality of the data. Monitors participating in the CMC Tier II Macroinvertebrate Monitoring Program will follow the procedures outlined in this Benthic Macroinvertebrate Monitoring in Wadeable Stream (Tier II) QAPP. The *Rubric for Tier Determination and Inclusion of Data in the CMC Database* outlines the minimum requirements that must be met for the data to be included in the project database and assigned a designation of tier II. The guidelines of this project fall into the tier II designation with these criteria:

1. Program
    a. Approved, written study design
    b. Verified sampling location(s) with coordinates
2. Sampling methods
    a. Sample during normal flow conditions
    b. Use appropriate sampling method for stream type and follow procedures exactly
        i. Choose area with appropriate (if applicable)
            1. hydrology – water depth and flow
            2. substrate type and size
            3. habitat type
        ii. Equipment type and specification
        iii. Number of samples and/or organisms collected
3. Equipment maintenance
    a. Inspect equipment and materials each time before use
    b. Clean and store equipment and materials appropriately
4. Certified Monitor
    a. Attend a Tier II Macroinvertebrate Monitoring Training
    b. Become certified and maintain biennial certification
    c. Fill out field data sheets completely and accurately
5. Identification
    a. Samples are processed by a lab to at least family-level

Data collected under this program can be categorized as Tier II, as long as other Tier II criteria are met. In addition to the minimum requirements listed above, other specific criteria used to determine the tier designation within in the CMC are listed in Table B2-2.

Table B2-2. Specific criteria used to determine tier designation of macroinvertebrate data within the CMC. All data collected for this project is classified at Tier II - family taxonomic level identification, collected by certified monitors, and identification completed by Wheeling Laboratory taxonomists.

Requirement	Tier I	Tier II
Taxonomic level of identification	Order	Family
Monitor certification	All monitoring must be done under the supervision of a certified monitor or ALLARM/IWLA/ACB/Certified Trainer	All monitoring must be done under the supervision of a certified monitor or ALLARM/IWLA/ACB/Certified Trainer
Identification	Completed by monitors trained to order level	Completed by EPA Wheeling Laboratory taxonomists

B3. Sample Handling and Custody Procedures

Monitors are responsible for collecting and processing the macroinvertebrate samples and preserving them for lab identification. All macroinvertebrate samples will be collected in a clean sample jar, filled with 95% ethanol, and labeled inside and out with the monitor's name, site location, and date and time. Monitors will store samples in a cool dry place away from direct sunlight or any flammable material. The preserved sample and field data sheet will be delivered to the Project Team within one year of collection. The Project Team will transfer preserved samples and copies of their field data sheets to the Wheeling Lab within calendar year of sample collection (Appendix A).

Sample custody procedures are an integral part of laboratory and field operations. Since the data generated from this project are not used for legal purposes, formal Chain of Custody (COC) procedures are not required. However, when a monitor collects a sample to be analyzed by a certified laboratory, and the laboratory requires a COC procedure, the procedure will be outlined in the monitoring group's tailored sampling plan to ensure the integrity of the samples received by the laboratory.

Once samples have been received, the laboratory will assume all sample custody responsibility. Detailed procedures for custody procedures at Wheeling lab should be made available upon request by the laboratory.

The laboratory receiving the samples may reject the analysis of any macroinvertebrate sample under any of the following conditions:

a. Sample label is not inside;
b. Collection information is not included;
c. Sample label and collection information does not exactly match and the issue cannot be resolved.

B4. Analytical Methods Requirements

Macroinvertebrate samples collected by monitors will be processed and analyzed by the certified taxonomists to family level at the EPA Wheeling lab. See Appendix I for details.

B5. Quality Control Requirements

A goal of this project is to collect data that can be used to assist local and regional decisions affecting the Chesapeake Bay and its watershed, so it is essential that a high level of QA/QC be maintained. All members of the project will follow established procedures to ensure data accuracy, precision, representativeness, comparability and completeness necessary for a successful program.

B5.1 Field QC Checks

B5.1.1 Monitor Certification

Monitors can maintain their certification by attending another Tier II Macroinvertebrate Monitoring Training or being field audited by a certified trainer or the Project Team biennially. Monitors will be observed in the field as they collect and process an entire sample. The certification will help to verify the quality of the data collected by this project by validating that monitors are collecting, processing, and preserving macroinvertebrates correctly.

B5.2 Laboratory QC Checks

Certified taxonomists at the Wheeling Lab provide quality control to the collected Tier II data. Laboratory QC procedures are developed by the individual laboratory. The quality control procedures for Wheeling Lab are provided in Appendix I, sections 11.1-11.3.

B5.3 Data Entry QC Checks

All data will be sent to ALLARM/IWLA/ACB after analysis at the Wheeling Lab and entered into the Chesapeake Data Explorer via bulk upload. Procedures are outlined in Appendix I, section 10.1.

B6. Equipment Testing, Inspection, and Maintenance Requirements

Monitors will maintain their equipment and inspect it prior to each sampling event to ensure that all materials are clean and working properly (Table B6-1). Any problems found during inspection will need to be addressed prior to sampling.

Table B6-1. Equipment inspection requirements for macroinvertebrate sampling.

Equipment Type	Inspection Frequency	Type of Inspection
Net (kick, D-frame)	Before each sampling event	Visual: • Cleanliness – debris, sediment, or dead organisms • Small rips or holes
Other sampling equipment (buckets, waders, sorting trays and utensils)	Before each sampling event	Visual: • Cleanliness – debris, sediment, or dead organisms • Small rips or holes

After sampling, monitors will decontaminate their equipment, rinsing it with water and getting rid of any debris and drying in the sun for a few days. If monitors are going to a second site, they should decontaminate with biodegradable soap and a scrub brush before changing location. Equipment should be stored in a secure, dry area when not in use.

The Project Team will provide monitoring groups with the equipment they need to collect and preserve macroinvertebrates or provide them with information on where to purchase the materials.

B7. Instrument Calibration and Frequency

Instruments requiring calibration are not utilized in this sampling procedure.

B8. Inspections/Acceptance Requirements for Supplies

ALLARM/IWLA/ACB will obtain and recommend monitoring equipment and supplies from reputable field/laboratory supply companies. The Project Team will inspect purchased and constructed equipment and broken or defective items will be sent back to the supplier. Equipment will be distributed to monitors at the Tier II Macroinvertebrate Monitoring Training or afterwards as needed. The equipment must meet the specifications outlined in this QAPP and will be inspected and verified by the Project Team during the Certification Training or field audit.

B9. Data Acquisition Requirements

The Chesapeake Monitoring Cooperative will acquire data from the EPA Wheeling lab. The Project Team will enter the benthic data into the Chesapeake Data Explorer.

B10. Data Management

Monitors will record their sampling information onto field data sheets supplied by the Project Team. Monitors will complete all sections of their field data sheets and then send them to the Project Team along with their macroinvertebrate samples to be kept by the team for seven years.

The macroinvertebrate results will be entered into the Chesapeake Data Explorer by the Project Team after samples have been processed by the EPA Wheeling Lab. Monitors who collected samples will be notified that their data is available in the Chesapeake Data Explorer.

It is recommended that the Project Team contact monitors within three months of the Tier II Macroinvertebrate Monitoring Training to answer questions and review the sampling and data management methods. Digital data files will be maintained and backed up regularly by Greenfin Studios. Anyone who accesses the Chesapeake Data Explorer will be able to download and use the data for analysis.

SECTION C – ASSESSMENT AND OVERSIGHT

C1. Assessment and Response Actions

The Project Team will use four categories of assessment to ensure the integrity of the data:

1. Laboratory
2. Program
3. Field Sampling
4. Validation and Reporting

C1.1 Laboratory Assessments

It is the responsibility of the EPA Wheeling Lab to maintain the Society of Freshwater Scientists taxonomy certification, and the Project Manager may ask the laboratory to supply a copy of the certification if needed.

The audit procedures for the EPA Wheeling Lab are internal and are outlined in Appendix I, section 11.5.

C1.2 Program Assessments

QA Management will perform an annual assessment of the QA/QC procedures of the Tier II Macroinvertebrate Monitoring Program to determine if the data collected meet the program's objectives and are of known quality. If QA Management discovers any QA issues within the program, they will work to resolve the issue(s). It will be the responsibility of the Project Team to follow up on any issues. Data submitted to the Chesapeake Data Explorer during this time will be flagged until the Project Team has verified that the issues have been eliminated.

C1.3 Field Sampling Assessments

The Project Team is responsible for ensuring that all certified monitors attend a Certification training or are field audited every two years. The Certification Training serves as an audit or proficiency test for the monitors and their equipment. If a monitor demonstrates a faulty sampling technique during the Certification Training, the Project Team will retrain the monitor and will not renew the monitor's certification until the monitor can demonstrate that the sampling technique has been mastered, which is crucial in meeting data quality objectives. Any equipment determined to be faulty during a Certification Training or field audit will be replaced. Data quality objectives will be reviewed at the Certification Workshop, including:

a. Sample collection method
b. Preservation method
c. Field documentation
d. QA procedures
e. Equipment inspection

C1.4 Validation and Reporting Assessments

The Chesapeake Data Explorer has been developed to incorporate a data validation and

reporting system that is supported by the Project Team. These procedures will be reviewed as a part of the annual assessment conducted by QA Management.

C2. Reports to Management

QA Management will create an annual assessment report of the QA program that will be circulated to the Project Team, CBP, and EPA QA staff. All Project Team members are required to submit a quarterly report to the Project Manager of all project activities, including workshops, certifications, and QA problem resolution. In addition, the Project Manager is required to submit 2 progress reports to the Chesapeake Bay Trust (6/1/21 and 9/1/21) detailing of all project activities. Additionally, the Project Manager is required to submit 3 invoice and deliverable reports (12/31/20, 4/1/21 and 12/31/21) to the Chesapeake Bay Trust.

SECTION D – DATA VALIDATION AND USABILITY

D1. Data Review, Validation, and Verification Requirements

The macroinvertebrate results will be entered into the Chesapeake Data Explorer by the Wheeling Lab staff. Spreadsheets will be compared to the macroinvertebrate identification sheets by Wheeling Lab staff prior to upload. Laboratory QC procedures are available in Appendix I. The CMC project team will compare the results shared from the EPA Wheeling Lab with the field data sheets, doublechecking site locations and other metadata. Field data sheets will be retained by the Project Team for at least seven years after the sampling date.

D2. Validation and Verification Methods

The Project Team will work with monitors to ensure that their data are validated and verified by reviewing field data sheets. The Project Team will provide advice and technical assistance to monitors to ensure that they follow the procedures properly and review their data sheets before they submit them to the Project Team with their samples. Monitors will submit their data sheets to the Project Team along with their samples after every sampling event.

Certified taxonomists at the Wheeling Lab provide quality control for the collected Tier II data. Taxonomists recount and check taxonomic accuracy of at least 10% of samples per project. Laboratory QC procedures are available in Appendix I.

D3. Reconciliation with Data Quality Objectives

This QAPP is applicable to data quality objectives defined by Tier II criteria. Classifications for data use and quality objectives are summarized in Table A5-2 and further described in Section B2.8. If Tier II data are found to not meet the QA checks when performing family level identification, the data may be recommended for Tier I designation if applicable.

D3.1 Representativeness

The representativeness of the sample will be evaluated during the Certification Workshop. The Project Team will verify that the site sampled is representative of the conditions in the area. If the site location is not representative or the stream was not sampled in a representative manner, the Project Team will help the monitor reconcile the issue.

D3.2 Comparability

ALLARM/IWLA/ACB/Certified Trainer will evaluate monitors' adherence to the SOPs biennially at a Certification Training or at a field audit.

D3.3 Completeness

Monitors collecting Tier II data will sample their sites at least once every six years in the spring (March- June) or fall (August- November). This will be considered a complete sample set. The Project Team will investigate any issues that cause monitors to not meet the data completeness goals and will work with them to remedy the issue.

Appendix A:

Standard Operating Procedure – Benthic Sampling for Stream Health Indicator Protocol

Draft December 2020

Background: The following protocol is meant to support a special study project that will contribute to the ongoing assessment of Chesapeake Bay Stream Health. The current watershed-wide assessment utilized a 2006-2017 Index of Biotic Integrity (IBI) dataset and identified many areas of the watershed that have little or no data. The purpose of this project is to fill these data gaps by targeting the areas of the watershed with little or no data for macroinvertebrate sample collection. Your sample collection and the results produced from this special study on macroinvertebrate diversity will help extend the Chesapeake Bay watershed area coverage of the Stream Health assessment. Sampling at single sites in target locations is of interest, however, multiple sites in these catchments (2 or more) would be welcome. Adopting one station to sample for enhanced diversity assessment each year would provide added insights on trends in the response of our watershed to changing management and climate conditions over time.

The sampling process requires the collection and retention of the bugs in the sample. The entire sample (not picked) will be preserved for later processing in a lab. Importantly, these samples will be evaluated down to the family level (at a minimum) in order to be included in the IBI stream health assessment used by the Chesapeake Bay Program partnership. The family identification results from your sample will be shared with you within a year for each site. The final data will become part of the history documented in the CMC Chesapeake Data Explorer database.

Equipment –

- D-net with 500 µm mesh
- A sieve bucket with 500 µm mesh or DIY equivalent (link to James Beckley video for DIY option.)
- A collection container (to store preserved sample) –
- 95% Ethanol preservative
- Spray or squirt Bottle (for cleaning the D-net into the jar)
- Forceps
- Tape/gallon plastic bags (secondary containment, to make sure the jars don't leak)
- Waterproof labels for sample jars
- Data sheet (need to create)
- Waterproof pen/pencil/marker
- Boots (no felt soles!) to get into the stream
- Safety gear such as gloves, sun tan lotion, bug spray, and first aid kit.
- Flagging tape
- Measuring tape to measure a site.
- Sampling manual

Site selection – Chesapeake Bay Program Stream Health Benthic Sampling Map, targeting anywhere within watersheds that are white (zero samples), or very light blue (0-5 samples). Sampling first to fourth order headwater streams.
Target period – TBD, First cut might be during the month of October 2020

Sample Process:
Habitat to sample – riffle run areas, STABLE HABITAT conditions.
Upon arrival at a sampling site determine the 100 meters of area from which you will be sampling and determine sampleability of the site:

- Make sure the water is wadeable with riffles in your sampling area where you will be able to kick the substrate or rub cobbles with your hands. Your sampling area should preferably be upstream of the nearest bridge or culvert by 100 feet.
- Consider the entire 100 meters for your sampling area, but you can sample from within at least two riffles in that length of stream.

Now, assuming there are no significant issues, everything is all systems go for the day!

- Check your net and bucket, no holes or tears in the mesh.
- Establish the lower end of your sampling area, perhaps a transition spot between riffle-run habitat and a pool.
- Start at downstream end of your site and work upstream collecting your sample.
- Subsample a variety of velocities with different stone sizes as these microhabitats are important to different species. –
    - kicks on the 100 meter reach should be distributed and representative of the riffle-run habitat (slow flowing shallow riffles and fast-flowing deeper riffles). Kicks must also be conducted throughout the width of the stream to include left-descending, middle and right-descending areas.
- What constitutes a "kick"
    - A single kick consists of disturbing the substrate an area the width of the net and at most two net widths upstream, and getting 2-4 inches down into the substrate. Rub by hand any large sticks and/or stones from the disturbed area to dislodge and tightly-clinging organisms for 40 seconds, then kick to disturb lower rocks and substrate for 20 seconds.

Complete 4-6 double wide "kicks" (two widths of the net) for 60 seconds total each.

- These kicks should be done in at least two different riffle areas and should cover the left, middle, and right of the stream, as well as slow and fast moving riffles.

When you have completed your sample collection that is now all resting in your net:

- Partly submerge your sieve bucket.
- Empty contents of the net into the bucket after every 2-3 kicks or if the water cannot easily run through the net anymore.
- Clean the net into the bucket:
    - Sit the bucket into the stream so that it is half full of water
    - Rinse the net into the bucket
    - Turn the net inside out and rinse any missed part of the collection into the bucket
    - Wash out net into the sieve bucket to get any last clinging part of the sample
    - While the sample is in the sieve bucket, all large stones, debris, leaves etc., should be carefully washed inspected for organisms and discarded. Try to put as little material as possible into the sample bucket (remove rocks and sticks, and pick through leaf pack and remove most leaves).
- Remove fish, mussels, salamanders, turtles, large sticks, stones over 3 cm, large leaves, etc. **Crayfish stay IN the sample**.
- Gently wash sample up and down in the bucket.
- Empty contents of bucket into ONE sample jar.
    - Minimize water in sample to avoid overdilution of the preservative.
    - Empty ALL bucket contents available here into the sample jar.

- Add Ethanol until you have doubled the volume of the solids alone. Wear gloves if you are going to put your hands in the ethanol.
- Fill out label in pencil and place IN the sample on the liquid.
    - (We might add an external label too)
- Close lid well. (Add tape and place in ziplock bag?)
- Fill out data sheet.
- Decontaminate your equipment and boots before you go if you move on to a second stream location.

You can store sample for up to one year until you can coordinate for transfer to the CMC team. Please keep samples stored in a cool dry place away from direct sunlight or flammable materials. Bring your sample(s) to a collection event or coordinate pick-up with a CMC coordinator within the calendar year of sample collection.

Appendix B:
Tiered Framework

Tiered Framework for Data Collection and Integration for Nontraditional Monitoring

Tiered Framework for Data Collection and Integration for Nontraditional Monitoring

Introduction

The Alliance for the Chesapeake Bay (Alliance), Izaak Walton League of America (League), Dickinson College's Alliance for Aquatic Resource Monitoring (ALLARM), and the University of Maryland Center for Environmental Science Integration and Application Network (UMCES IAN) (referred to as the "Project Team" in this document) are partnering to provide technical, logistical, and outreach support for the integration of citizen-based and non-traditional (i.e., non-agency) monitoring data into the Chesapeake Bay Program (CBP) partnership. The integration of these data into the CBP monitoring networks will provide additional cost-effective data and information that supports shared decision-making and adaptive management by the CBP partners focused on restoration of the Chesapeake Bay and its watershed.

The Project Team, using their background, expertise, and knowledge with the nontraditional monitoring community, are working with CBP STAR (Scientific, Technical Assessment and Reporting Team) to: 1) establish institutional structures and procedures, such as the tiered data use framework; 2) facilitate development of consistent monitoring and training protocols, technical guidance, data gathering tools, quality assurance mechanisms, and data analysis and communication tools; 3) inventory, prioritize and recruit monitoring groups; and 4) provide training and technical support to monitoring entities. This comprehensive approach will ensure a consistent submittal of known quality data to the CBP.

Purpose of the Framework

The Tiered Framework for Data Collection and Integration for Nontraditional Monitoring identifies recommended categories of data quality and their associated end uses. Broad data quality requirements for each category are identified. This framework also provides recommendations of existing resources to inform data production protocols.

For the development of this framework and associated data collection and management protocols, the Project Team is working with experienced nontraditional monitoring programs, state agency programs, and the STAR Data Integrity workgroup to incorporate best practices and lessons learned. Additionally, the Project Team has examined thirteen states' volunteer monitoring programs, and identified five states to best inform the development of this tiered framework. The Project Team will seek adoption of the tiered data use framework, monitoring protocols, and Quality Assurance Project Plans (QAPPs) by the CBP.

The framework is meant to be a guiding document that will be subject to change and refinement once the Project Team receives data from a watershed monitoring census (to document the most commonly used monitoring techniques in the Bay Watershed) which will inform equipment testing and the development of corresponding monitoring method manuals and QAPPs. Once those key monitoring

tools are established, the framework document will be updated (Fall 2016) to reflect the monitoring that is and will be taking place in the watershed.

Monitoring Questions

Non-traditional monitoring entities typically develop study designs, in part to identify their research questions and objectives. Most non-traditional monitoring entities have been monitoring for water quality status and trends using three lines of evidence:

- Water quality/chemistry
- Biological – macroinvertebrates and submerged aquatic vegetation
- Physical – habitat and stream bank assessments

Although the issues addressed are almost always locally-based, the data collected can also be utilized, along with other Bay-wide data, to address the status and trends of waterway health in the Chesapeake Bay watershed. Some examples of Bay-wide priority research questions that local non-traditional monitoring data can inform include:

- What is the effectiveness of management actions?
- What are the relationships in space and time between watershed health and bay health?
- What are the effects of emerging contaminants and climate change on the status and recovery of bay and watershed health?
- Where should natural resource managers prioritize restoration efforts?
- How does the inclusion of citizen science data change individual behaviors and increase environmental stewardship?

Once the data are organized and entered into a database, CBP may use the non-traditional data to help answer these and additional questions.

Intended Data Use

TIERS	Intended Data Use
TIER 1	Education, Environmental Health Screening
TIER 2	Environmental Health Report Cards, Environmental Health Screening, Targeting of Management Actions
TIER 3	Chesapeake Bay Watershed trends and assessments to help inform policy and management decisions

Tier Descriptions and Framework for Determining Tiers

There are diverse motivations for monitoring and diverse projects where non-traditional data are collected. In the aquatic citizen science field/volunteer monitoring, most organizations developing monitoring programs answer the question “how do they intend to use their data” prior to identifying parameters, appropriate techniques, and corresponding quality assurance measures. This process is done with the goal to match the data quality with the intended use. For the integration of non-

traditional data into the Bay program, the Project Team has identified Tiers for data use. If data do not meet the data requirements of the different tiers, those data will not be included in this project.

Tier 1 – Education and Environmental Health Screening:

Definition: Tier 1 data include programs whose data do not meet the requirements of Tier 2 and Tier 3 but are of known quality, have written study designs, documented quality assurance/quality control measures, and as a result still contribute to understanding of the health of the Bay watershed.

Data Uses:
These data can be used to:

- Provide location information on where monitoring is taking place;
- Provide on-the ground information for future site development;
- Indicate potential pollution hot spots;
- Prioritize sites for follow-up monitoring;
- Target restoration projects;
- Inform sub watershed report cards; and
- Highlight local, community projects that are implemented to improve the health of the Bay watershed.

Data Requirements: Clearly documented monitoring methodology, site locations, and written study designs.

Tier 2 - Environmental Health Report Cards, Environmental Health Screening, Targeting of Management Actions:

Definition: Tier 2 data are data with clearly defined and approved methodology (using the volunteer monitoring EPA QAPP guidelines) but do not meet Tier 3 data requirements.

Data Uses: These data will:

- Be used for Bay Program report cards;
- Be used to help target stream segments for water quality standards attainment assessments and Clean Water Act 305(b) reports;
- Be used for screening for Clean Water Act 303(d) stream segments;
- Target new priority agency sites;
- Track the performance of Total Maximum Daily Load (TMDL) implementation projects; and
- Be used for all uses identified in Tier 1.

Data Requirements: Program, at minimum, has an approved volunteer monitoring Quality Assurance Project Plan (http://www.epa.gov/sites/production/files/2015-06/documents/vol_qapp.pdf). Data collected, uses approved field or laboratory standard operating procedures with defined levels of precision and accuracy for the measurements, or

program can be participating in an umbrella monitoring initiative that has an approved QAPP or field/lab standard operating procedures.

Tier 3 - Chesapeake Bay Watershed trends and assessments to help inform policy and management decisions:

Definition: Tier 3 data are regulatory, decision-making, legally defensible data.

Data Uses: These data can be used for:

- Attainment purposes, Clean Water Act 305 (b) reports, Clean Water Act 303 (d) listing and delisting; and
- All uses identified in Tier 1 and Tier 2.

Data Requirements: United States Environmental Protection Agency (EPA) or CBP approved QAPP and field/lab standard operating procedures.

The Project team will be refining data requirements (Fall 2016) criteria after monitoring method manuals and QAPPs are developed to add additional information on types of monitoring techniques, their precision, accuracy, and sensitivity as well as quality assurance measures.

Examples of Non-traditional Data Contributor Success Stories within Each Tier

There a number of existing success stories that highlight the diverse ways that nontraditional data can be used to inform education, screening of pollution problems, long-term trend analysis, and water quality standards attainment.

Tier 1 - Education:

Data collection is inherently educational for participants. Beyond the educational development of the data collector/analyzer, a typical goal for watershed organizations and programs is to use the data collected to educate municipal officials, community members, and other stakeholders about water quality in their community. Most data-collecting entities use the stories found in their data for local education.

One successful case study in Pennsylvania is the work accomplished by the Antietam Watershed Association (AWA). When AWA developed their study design with technical support from ALLARM, they were primarily concerned with the effect of non-point source runoff in the watershed; agricultural runoff was the primary issue in the West Branch of the Antietam and stormwater runoff was the primary issue in the East Branch of the Antietam. Three years into their baseline data collection, there were three large farms that were sold in the West Branch for housing subdivisions. Using the data they collected, AWA was able to illustrate the impact of agricultural runoff on the West Branch as well as the impacts of stormwater runoff in the East Branch. As a result, AWA was able to work with the local municipality, Washington Township, to develop a buffer ordinance for the new housing subdivision.

The South Anna Monitoring Project is a citizen water quality monitoring volunteer group that operates under a VA DEQ-approved QAPP to monitor water quality parameters at designated sites along the

South Anna Creek and its tributaries in Louisa County, Virginia. Volunteers have been collecting data and noting land use changes in the upper portion of the watershed for the past 10 years. With this data, an educational report was developed to illustrate land use change impacts.

Tier 2 - Screening, Report Cards, Targeting:

A common product of watershed monitoring activities are reports and report cards that outline findings as well as recommendations on data use, sites for further investigation, and additional questions to answer in the watershed.

The Shermans Creek Conservation Association (SCCA) was a watershed group located in Perry County, Pennsylvania (one of Pennsylvania's more rural counties). SCCA formed in 1998 and conducted baseline chemical, biological, and physical stream monitoring from 1999 – 2008 with technical support and mentoring from ALLARM. Throughout their nine years of water quality data collection they went through three rounds of data interpretation and data use. The first watershed report on Shermans Creek was published in 2004 and it was the impetus and primary content used for the development of a Rivers Conservation Plan. As a primarily agricultural county, the watershed data were particularly useful in identifying locations for best management practices to be installed to address a variety of issues from faulty manure storage facilities to lack of riparian buffers. Another result of the 2004 report was a petition to the Pennsylvania Department of Environmental Protection (PADEP) to upgrade the stream designation of a portion of the main stem of Shermans Creek, based on the citizen-collected data. The SCCA data which were submitted to the state helped the state target their own monitoring to inform the designated use upgrade process.

The Reedy Creek Coalition (RCC), a watershed group in Richmond, Virginia, with training and technical assistance from the Alliance, has been collecting water quality data to help identify pollution hotspots and potential sources. Through regularly monthly monitoring along the creek and streamwalks, the Coalition has identified several illicit discharges over the span of the monitoring program. In 2011, during a streamwalk, a dry weather discharge was detected at a large stormwater pipe, along with a strong sanitary sewer odor and bacterial growth. This, along with follow-up testing from Randolph Macon College students which showed very high levels of *E. coli*, prompted the City of Richmond to investigate. They discovered a damaged sanity sewer line nearby and repaired it. Also, in 2012, the water quality monitors identified foul odors and elevated *E. coli* counts at a monitoring site on Crooked Branch, a tributary of Reedy Creek. The RCC notified the City of Richmond's Department of Public Utilities (DPU) regarding their observations and the DPU Pretreatment Program began an investigation. Their monitoring confirmed the volunteers' findings and they traced the contamination to a blocked sanitary sewer line. This was fixed, and follow up sampling showed much lower concentrations of *E. coli.*

Tier 3/Tier 2 - Water Quality Standards Attainment:

Typically for monitoring programs interested in attainment, there is a strong reliance upon state-approved protocols and certified laboratories for data analysis. However, there are success stories of nontraditional data being used to inform Clean Water Act violations as well as the listing and delisting of streams.

The Codorus Creek Watershed Association was formed in 1998 to implement watershed assessments. One of the group's concerns centered on the Glatfelter Paper Plant, whose discharge led to the community nickname of the Codorus as the "inky stinky." Upstream of the plant's effluent the Codorus is classified as a High Quality Cold Water Fishery (the second highest designated use in PA). As a result

of the temperature and color of the plant's discharge the creek downstream only met criteria for a Warm Water Fishery. Using two parameters, temperature and color, the group produced data that illustrated the plant was in violation of the Clean Water Act and the Pennsylvania Chapter 93 code. The Pennsylvania Department of Environmental Protection then sued the plant, which resulted in $2.5 million in penalties and required the plant to install $32 million worth of new equipment to improve the clarity and temperature of the discharge.

Existing Tools

There are a number of existing tools to help identify appropriate chemical water quality monitoring procedures that will be helpful for this project:

- To inform non-tidal monitoring procedures, the Project Team will use the VA DEQs Virginia Citizen Water Quality Monitoring Program's Methods Manual and the Mid Atlantic Tributary Assessment Coalition Nontidal Protocols http://www.deq.state.va.us/Portals/0/DEQ/Water/WaterQualityMonitoring/CitizenMonitoring/Citmon_Manual.pdf;
- To inform tidal monitoring procedures, the Project Team will use the Mid Atlantic Tributary Assessment Coalition Tidal Protocols; and
- To inform attainment data use, the Project Team will use the Chesapeake Bay Program's Recommended Guidelines for Sampling and Analysis as well as the 2015 Technical Addendum for Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity, and Chlorophyll a for the Chesapeake Bay and Its Tidal Tributaries.

Areas for Development and Consideration

For chemical data, the VA DEQ methods will have to be examined and the Project Team will have to confirm that those tiers fit in appropriately with this project.

The questions that nontraditional data will help answer are expansive and will require integrative data. One consideration here is how the program will diversify the information inputs into the tiered framework to better integrate additional parameters such as benthic macroinvertebrates, physical habitat, and submerged aquatic vegetation.

Metadata Requirements

As a part of the tiered approach, data producers will need to submit accompanying metadata alongside their monitoring data. All data of known quality are valuable as long as the end use matches the data quality; metadata are crucial to ascertain the quality of data.. The metadata provide additional information as to how the measurements were obtained and the level of precision and accuracy. Typically metadata includes, but is not limited to: equipment and materials used, storage methods, holding times, and analysis methods.

There are a number of approaches to determining what metadata is needed, including relying on existing tools and frameworks, such as:

- EPA Volunteer Monitoring QAPP Development guidelines http://www.epa.gov/sites/production/files/2015-06/documents/vol_qapp.pdf;
- VA DEQ established metadata protocols for their databases; and
- Conversations with the Chesapeake Bay Program teams that are discussing required metadata for different data uses.

Below is an example of metadata from a Pennsylvania Watershed Group's Study Design:

Parameter	Equipment	Holding Container	Storage	Maximum Holding Time	Method
Temperature	LaMotte Hg-Free Thermometer	Measured at stream	N/A	Immediate	Field Thermometer
Conductivity	LaMotte Tracer PocketTester	500 ml Nalgene	N/A	Immediate	Field meter
pH	EMD Millipore ColorpHast pH strips	Measured at stream	Refrigerate	2 hours	pH strips
Dissolved Oxygen	LaMotte Kit #5860	60 ml glass container	N/A	Fixed at streamside, titrate within 8 hours	Winkler Titration
Water Clarity	LaMotte Transparency Tube			Immediate	Visual
Ortho-Phosphates	Hach Kit #PO-19	500 ml Nalgene	Refrigerate	Within 48 hours	Ascorbic Acid
Nitrate- Nitrogen	Hach Kit #NI-14	500 ml Nalgene	Refrigerate	Within 48 hours	Cadmium Reduction
Benthic Macro-invertebrates	Kick net or D-net with 500-micron mesh	Identify at stream side; OR Preserve in wide mouth 1 liter plastic screw cap container	Preserved in at least 70% ethanol	Indefinite	EASI or VA SOS protocol
Streamwalk	Field data sheet, camera	N/A	N/A	N/A	Adaptation of Tier I of USDA Visual Assessment Protocol
Stream Reach Survey	Field data sheet, camera	N/A	N/A	N/A	Adaptation of EPA Volunteer Stream Monitoring Protocol
Heavy Metals	Professional lab	500 ml container	Preserve with nitric acid to a pH < 2		Atomic Absorption Spectroscopy or Inductively Coupled

					Plasma Mass Spectrometry

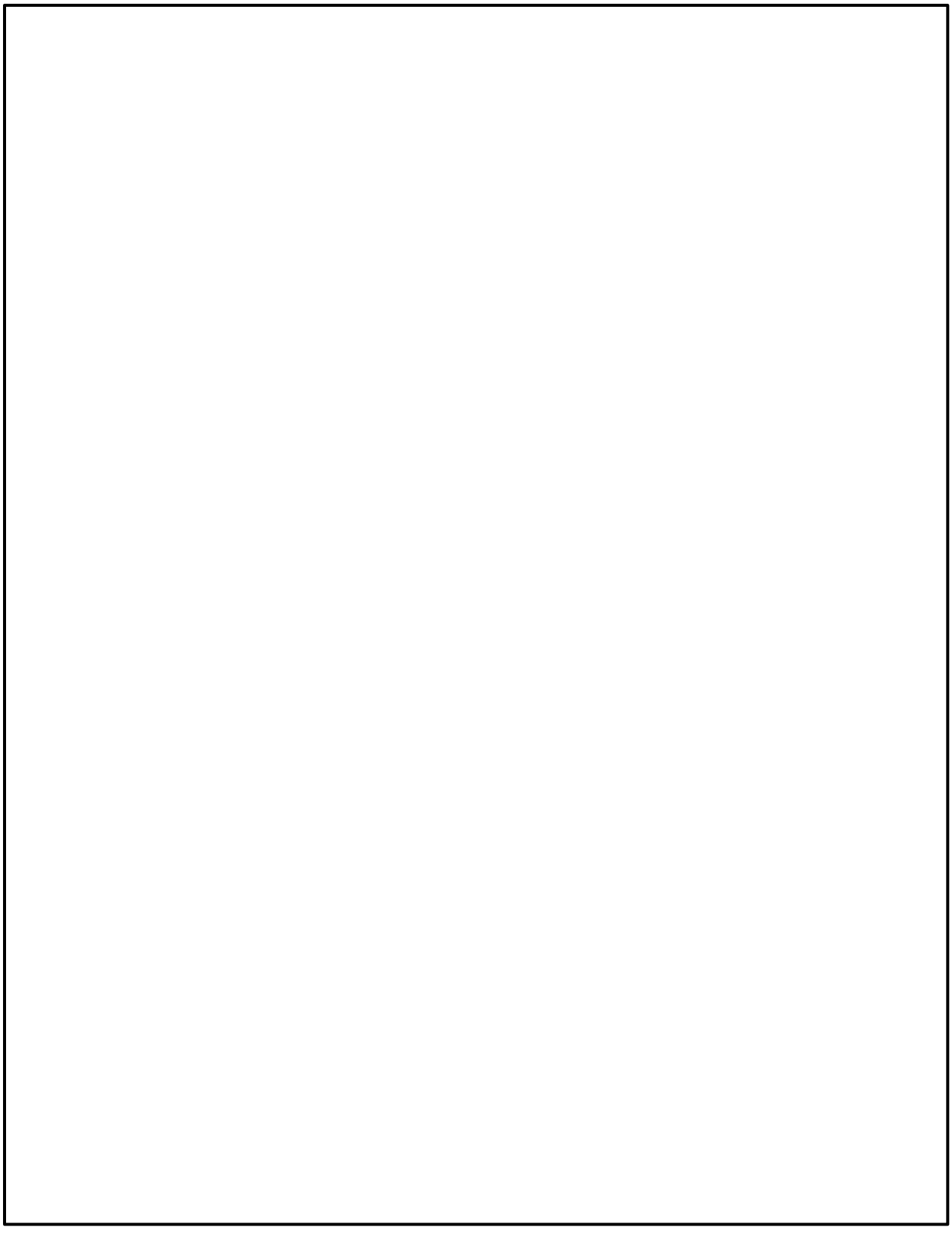
Appendix C:
Field Data Sheet

Tier II Benthic Sampling Field Data Sheet

Sampling Team: **Certified Monitor:**	
Station ID:	**Date:** m/d/yy
Latitude: **(decimal degrees):**	**Longitude:** **(decimal degrees)**
Start Time: AM/PM	**End Time:** AM/PM
Additional Comments:	

	Field Readings
Water Temperature (nearest tenth)	_____ . _____ °C
Length of reach sampled (meters) All kicks should be taken within a 100m stretch of stream.	_____ M
Number of Riffles Sampled Target 2-3 riffles if possible.	
Number of kicks Should be between 6 and 12 depending on the area covered with each kick	

Draw a schematic of your stream sampling locations. They should be throughout the stream sample reach from at least 2 riffle zones. Try to get samples from the left and right side of the stream and in fast and slower flowing riffle areas. Check them off as you go.



Appendix D:
Monitor Certification Checklist

CMC Tier II Certification Checklist

Monitor Name:_____

Date:_____

This form has been designed for reviewing the field collection skills of monitors performing CMC Tier II Macroinvertebrate sampling. A minimum score of eleven must be received in order to pass.

Monitor selected a representative section of the stream to monitor?	Y N
Monitor inspected net and sieve bucket for holes before use in the stream?	Y N
Monitored accurately identified habitat areas and sampled only riffle areas?	Y N
Monitor sampled 2+ riffles where possible?	Y N
Monitor disturbed sample area prior to monitoring?	Y N
Monitor correctly emptied each jab into the sieve bucket?	Y N
Monitor discarded any jab that had too much sediment or debris?	Y N
Monitor removed debris and picked through leaves before emptying sample into sample jar (if doing unpicked method)?	Y N
Monitor correctly filled out field sheets?	Y N
Monitor correctly labeled samples inside and outside of sample jar?	Y N

Test administered by:

_____

Appendix E:
Landowner Permission Form

Property Owner Permission and
Liability Release Agreement

PROPERTY OWNER PERMISSION AND LIABILITY RELEASE AGREEMENT

_____is participating in a program to collect baseline data to
(organization/volunteer name)

monitor the condition of local streams and ensure that water quality and stream habitat are properly maintained. As part of the program, trained local volunteers collect macroinvertebrate samples 1 – 4 times per year at specific sites. Volunteers will be at the site for approximately 30 minutes, collecting and processing the sample. This agreement is intended to grant permission to volunteers to access private property for data collection, as well as to release and hold harmless the property owner from liability arising from that access.

I,_____, hereby grant permission to
_____,
(property owner name) *(organization/volunteer name)*

its volunteers, and necessary program partners, to enter my property located at

_____
,

beginning_____until_____, for the sole purpose of
(start date) *(end date or program completion)*

site-access and sample collection that takes place on or near my property to gather baseline data on the adjacent stream. I agree that my permission is granted on a voluntary basis and I have neither received or expect to receive any form of compensation in exchange for my permission. I agree to hold the organization listed above, its volunteers, and necessary project partners, harmless from and forever discharge them from any and all liability for damages, injury, or loss which may be sustained as a result of their entry into the private property described in this agreement. In addition, the organization listed above holds harmless and forever discharges me, the property owner, from any and all liability for any damage, injury, or loss which may be sustained as a result of their entry into the private property described in this agreement.

Property Owner_____Date: _____

Organization_____ Date:_____

Appendix F:
Macroinvertebrate Sample Subsampling

Standard Operating Procedure

U.S. Environmental Protection Agency, Region 3
Laboratory Services & Applied Sciences Division
Field Services Branch

Standard Operating Procedure

Macroinvertebrate Sample Subsampling

Effective Date: 2021
Doc. Control Number (DCN): R3QA1002.4

Preparer / Author
Name: Kelly Krock Signature/Date:
Title: Biologist
Division/Branch: LSASD/FSB

Technical Reviewer

Name: Greg Pond Signature/Date:
Title: Biologist
Division/Branch: LSASD/FSB

Quality Assurance Officer
Name: Kelly Krock Signature/Date:
Title: Quality Assurance Officer
Division/Branch: LSASD/FSB

Branch Chief

Name: Jennifer Fulton Signature/Date:
Title: Branch Chief
Division/Branch: LSASD/FSB

Revision History

This table shows changes to this controlled Standard Operating Procedure (SOP) over time. The most recent version is presented in the top row of the table.

Document Control Number and Version	History	Effective Date
R3QA1002.4	Put in new template, updated location names, added photos	2021
R3QA1002.3		February 9, 2006

While this SOP may be informative, it is not intended for and may not be directly applicable to operations in other organizations. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

Table of Contents:

1.0 Scope and Applicability

The purpose of this SOP is to describe the process by which benthic macroinvertebrate samples are subsampled and by which the organisms in the subsample are separated from sample debris (e.g. leaves, sticks, gravel, sand). This SOP applies to all macroinvertebrate samples processed by Wheeling, WV USEPA staff.

2.0 Summary of Methods

This SOP describes the method by which macroinvertebrate samples are rinsed, randomly subsampled to obtain a fixed-count subsample, and picked or sorted from the debris. This SOP also describes the QC acceptance limits for picking efficiency (PE), and how to calculate pick efficiency.

3.0 Definitions

Fixed-count: a predefined number of organisms to be randomly sorted from the entire sample collected in the field (e.g., 100, 200, or 300 individuals). This is specified in the Quality Assurance Project Plan or by the client.

PE: the quality control parameter “pick efficiency”, which is a calculated as the number of organisms found by the second sorter divided by the total number of organisms in a subsample.

RBP: USEPA’s Rapid Bioassessment Protocol- EPA 841-B-99-002.

Sample:the entire sample collected in the field.

Subsample: portion of the sample actually sorted.

Sorting: Refers to the process of separating organisms from the debris. The term “picking” is also used to refer to this process.

Scan: Refers to a timed search (10 minutes) of large/rare taxa in the subsample, after completing subsampling.

4.0 Health and Safety Warnings

Any full sample container must be marked that it contains ethanol. For sample jars, internal labels note that the jar contains 70-100% ethanol and an overall sign is placed in the flammable cabinets with this same information. On benchtops, vial boxes must be labeled to say all vials contain 70-100% ethanol. Squeeze bottles with ethanol must be labeled with the approximate concentration of ethanol and dated and initialed when refilled.

During rinsing, exposure to the samples should be minimized by using eye protection and by rinsing the samples under a ventilation hood.

Care should be taken with unknown samples because the chemical composition of the water quality at ambient sampling locations is usually not well known. Precautions may include wearing chemically resistant gloves in addition to the regular eye protection and rinsing under a ventilation hood

If the samples are preserved with denatured ethanol, exposure to the samples should be minimized

(e.g., by using chemically resistant gloves and eye protection and by rinsing the samples under a ventilation hood). Samples collected by the Wheeling Freshwater Biology Team are preserved with non-denatured ethanol which is safer

It is advisable to cleanse exposed parts of the body with soap and water after contact with samples.

Before beginning any procedures, refer to the Wheeling Field Office Chemical Hygiene Plan (CHP) and Wheeling Field Office Occupant Emergency Plan (OEP) for general safety precautions and guidelines.

Material Safety Data Sheets (MSDS) for ethanol are stored in the sample processing area.

Small amounts of spilled ethanol should be wiped up immediately with paper towels, a sponge or spill pillows. In the event of a larger spill, follow the procedures in the CHP.

All applicable safety and compliance guidelines set forth by the EPA and by federal, state and local regulations must be followed during the performance of this SOP. In addition, all procedures outlined in the Wheeling Field Office Chemical Hygiene Plan (CHP) must be adhered to. Stop all work in the event of a known or potential compromise to the health and safety of any person and immediately notify the Safety, Health and Environmental Manager (SHEM) and other appropriate personnel as outlined in the CHP.

5.0 Cautions

Analysts should ensure the same sample identification information is written on all labels and matches the information in the log book. Labels for vials that contain organisms from a scan or second sort or containers with large organisms should be clearly marked to differentiate them from the initial subsample vial and also numerated to keep all vials together (i.e., 1 of 3, 2 of 3, etc.)

Pencil should be used with internal sample labels due to the use of ethanol; pen and marker bleed when in contact with ethanol. Care should be taken to not get ethanol on log book pages.

Do not use high pressure water to rinse the sample to avoid deterioration of organisms.

6.0 Interferences

The sorter must attempt to get an even distribution of the sample in the tray prior to subsampling.

The subsampling grids must be sorted randomly in order to obtain a representative subsample. Do not sort the subsampling grids visually as this tends to bias the selection toward the grid with larger organisms.

When done, wash the sieve, sorting tools and trays and pans thoroughly to avoid contaminating the next sample.

The subsample should be covered with plastic sheeting when not being actively sorted. The subsample should not be left out for more time than is necessary than to complete the sort. If the subsample cannot be finished in one work day, both the gridded tray and sorting pan can be covered with plastic and placed in the refrigerator overnight (ensure there is enough water so the sample does not dry out). The subsample should be re-preserved in a lidded plastic container with ethanol if the sample cannot be

sorted during sequential days so that the sample does not degrade.

7.0 Personnel Qualifications/Responsibilities

7.1 Demonstration of Capability

7.1.1 New and inexperienced analysts

For new and inexperienced analysts, an experienced macroinvertebrate analyst should perform a Quality Control (QC) pick of the sorted sample debris for every sample to provide a check on the sorting efficiency. An experienced analyst will review the missed organisms with the new sorter to point out what the new sorter missed. Our sorting efficiency goal is that less than 10% of the total organisms should be missed by the first person sorting the sample. A new sorter shall not be deemed capable and independent until they can consistently meet that goal.

The experienced analyst who performs these quality control checks is responsible for updating the appropriate laboratory notebooks and the spreadsheet that tracks macroinvertebrate QAQC statistics. The experienced analyst is responsible for checking the samples and for reporting to the QAO when the new analyst is deemed capable of subsampling and sorting independently. The QAO is responsible for updating the new analyst's Demonstration of Capability (DOC) file with a memo that indicates the new analyst is capable of performing sorting and subsampling independently, based on experience with the FSB.

7.1.2 New and experienced analysts

For new analysts that have prior experience subsampling and sorting macroinvertebrate samples, the new member is responsible for supplying the QAO with their resume and a summary of their relevant laboratory experience. The Team Leader and QAO are responsible for reviewing the new team member's experience and deciding whether they are capable of performing the activity independently without initial supervision. The QAO is responsible for updating the new analyst's Demonstration of Capability (DOC) file with a memo that indicates the new analyst is capable of performing sorting and subsampling independently, based on prior experience.

7.1.3 Ongoing Demonstration of Capability

For all analysts, after they have demonstrated capability, a second experienced analyst will repick only 10% of the samples to confirm ongoing demonstration of capability. The experienced analyst who performs these quality control checks is responsible for updating the appropriate spreadsheet that tracks macroinvertebrate QAQC statistics.

8.0 Equipment and Supplies

8.1 List of needed items

- 95% (200 proof) non-denatured ethanol
- Corrective Actions Log Book (sample page in attachment 1)
- Benthic Sample Log Book (sample page in attachment 2)
- USGS no. 30 (595 micron) sieve
- USGS no. 35 (500 micron) sieve
- sink
- vented hood

- glass beaker with volume marks and funnel for collecting used ethanol
- waste ethanol container with volume log
- subsampling tray with 32 gridded cells (outside dimensions are 20.5 x 12.5 inches)
- random numbers table
- cylindrical (approx. diameter 5.5 cm) or square “cookie cutters” (5 cm x 5 cm, 2.5 cm x 2.5 cm)
- white pan(s)
- spoon
- plastic pipette with bulb
- lighted tabletop magnifier (5X)
- fiber optic illuminator
- 2 and 4 dram glass vials with polyseal screw caps
- plastic lidded containers or larger vials (for larger organisms that won’t fit in the smaller vials)
- stereo microscope with petri dish
- forceps
- dental picks
- squeeze bottles with 95% non-denatured ethanol
- tally counter
- plastic sheeting
- rubber bands
- waterproof labels
- sharpie, pen and pencil
- cart to store subsampling equipment
- flammable storage cabinets
- hazardous waste stickers
- Paper towels and spill kit

8.2 Equipment Maintenance

The stereo microscopes must be properly adjusted to see the object image in three dimensions and to perceive heights and depths of the specimen. See the SOP for Macroinvertebrate Identification R3QA1003 for more details on microscope settings. Keep a dust cover on the microscope when not in use. Do not attempt to make adjustments to the internal optics or mechanics. If the microscope does not seem to be functioning properly, alert the equipment manager, and the scope will be sent to an authorized repair service for cleaning and repair.

Notify the equipment manager if fuses or light bulbs need replaced in the microscope, lighted tabletop magnifier or the fiber optic illuminator.

Replace worn or broken equipment as needed (e.g., pipette, spoon, etc.) and enhance the markings on the gridded tray with black sharpie if they fade.

9.0 Procedure

9.1 Choosing method

When working in a particular state or for a river basin commission, use their subsampling method to be consistent with the organization’s procedures for collection and sample

processing (unless otherwise noted in the project-specific QAPP). For example, adjust the fixed count of the subsample or the sieve mesh size to match the state's method. This modification ensures that the data generated are comparable to state data and that we can use the state bioassessment tools (e.g., multi-metric indices) or assessment methods (e.g., thresholds for determining impairment). See Table 1 for a summary of these methods.

Table 1. Summary of Methods used in US EPA Region 3						
State or River Basin Commission (RBC)	Sieve mesh size (microns)	Pan size	Number of Grids in Pan	Size of grid or "cookie cutter"	minimum subsample size (approx. proportion of total sample)	fixed count for subsample (e.g. 100, 200 300 organisms)
Delaware (DNREC)	600	36 cm x 30 cm	30	6 cm x 6 cm	1/30 or .033	200 ±20% (used to be two 100s ±20% and average scores, but moving to one 200 ±20%)
Maryland (MDDNR)	595	100 cm x 25 cm	100	5 cm x 5 cm	1/100 or .01	100 ± 20%
Pennsylvania Freestone and Low gradient, streams (PADEP)	500	14" x 8"	28	5 cm diameter cylinder (grid 2" x 2")	4/28 or 0.14	200±20%
Pennsylvania Limestone (PADEP)	500	14" x 8"	28	5 cm diameter Cylindrical (grid 2" x 2")	4/28 or 0.14	300±20%
Virginia	600	?	50	5 cm x 5 cm	4/50 or .08	110±10%
West Virginia (WVDEP)	600	40" x 10"	100	4 square inch	4/100 or .04	200±20%
Delaware RBC	250	N/A	N/A	2" x 2"	1/total # of grids in tray	500 ± 10%

Table 1. Summary of Methods used in US EPA Region 3						
					(Project dependent)	
Susquehanna RBC Large River Assessment	unknown	15”x10”	40	1/75”x1.75”	1/40 or .025	300 ± 20%
Susquehanna RBC Subbasin Survey	Unknown	15”x10”	40	1/75”x1.75”	1/40 or .025	200 ± 20%
Susquehanna RBC low flow monitoring and remote water quality monitoring network	Unknown	14x8	28	2” x 2”	4/28 or 0.14	200±20%

9.2 Step by step subsampling instructions

Unless otherwise noted in the QAPP or specified in state procedures, use the following procedure:

1. Dump the contents of the sample jar into the project-appropriate sieve, catching the used ethanol in a glass beaker with a funnel (note the volume). Transfer the waste ethanol to the larger “waste ethanol container” (see section 9.4) and record the volume on the log.

2. Rinse the sample bottle and lid to remove any debris or organisms adhering to the walls and then put aside. Rinse the internal paper label and put in vial with new ethanol.

3. Wash all large leaves, wood, and other large pieces of debris with water to rinse off organisms and dispose of this large rinsed debris in garbage when clean. Be thorough in this step since it is easier to sort the subsample later on if the sample is cleaned of large debris well in this step. Rinse the remaining sample to clear bacterial growth, iron precipitates, or other fine particulates from the sample.

4. Make sure the dimensions of the gridded trays and the cutters are appropriate for the project and use only that size gridded tray and cutter for one entire project. Dump the entire sample into the gridded sorting tray. Each gridded cell is uniquely numbered 1 to 32. Rinse sieve into tray to make sure all organisms make it into the tray and are not left on the sieve. Minimally add water to the tray as necessary. Ensure the sample is evenly distributed throughout the tray.

5. Randomly choose four numbers between 1 and 32 to select four cells in the tray, which constitutes about 1/8th of the sample. The numbers are chosen from a random number list generated by an analyst in Microsoft Excel and printed out. Use the random numbers in order as they are listed, crossing out each one as they are used; also bracket and note the sample ID for which they are used. Do not use the same random number twice for the same sample; mark the duplicate one out and continue to the next random number in the list. Do not sort a cell based on visual observation. Sorting cells based on visual observation biases the subsample toward cells with large, sometimes rare organisms. In some cases, three initial cells can be chosen if organism density is expected to be high. Any changes from an initial quantity of 4 grids must first be discussed and decided upon with a senior biologist.

6. Use a spoon and pipette to remove the contents of each of the initial four cells (the subsample) to a second white sorting pan. Inspect the selected cells in the gridded tray to make sure all organisms are removed. Redistribute debris in the second pan and add water to desired level.

7. Using the lighted tabletop magnifier and fiber optic illuminator, sort only identifiable organisms from the white pan and place in vial that contains the original sample label and 95% ethanol. Keep track of the number of organisms put in the vial with the tally counter. If needed, separate clumps of debris with dental picks or forceps to ensure no organisms are within the material. Do not pick aquatic pupae (unless working in Maryland), empty cases or shells or non-benthic organisms. If you have any question as to whether an organism qualifies, place the organism in a petri dish with ethanol and look at it under the stereo microscope; consult with one of the taxonomists as needed. If some organisms (e.g., crayfish) are too large to fit in the vial, use a lidded plastic specimen jar; put a duplicate label in the jar and write the sample information on the lid with a sharpie.

8. When all the organisms are sorted out of the white pan in its entirety, determine if the target fixed-count was met. The sorted number should be +/– 20% of the target fixed count (unless otherwise stated in project protocol, e.g., Maryland protocol is +/– 10%). For example, if the target fixed-count is 200 organisms, the sorted number of organisms can range from 160-240. The lower range is not preferable though since some organisms may not be identifiable by the taxonomist and the count may then fall below the minimum.

9. If the initial cells do not supply the target number of organisms, sort additional cells, in their entirety, until the target number is reached.

10. If the total number of organisms sorted exceeds the fixed count target, place all organisms into a second gridded tray and randomly select cells for removal of organisms until the target count is reached. Cells must be sorted entirely.

11. If the sample is to be sorted by a second sorter for a quality control check, the sample should be checked right away. The organisms comprising the second sort should be stored in a second vial with a label and attached to the first sort sample using a rubber band.

12. After the target fixed count is met, some state or project-specific protocols may require a 10-minute scan (time may vary by project protocol) of the remaining debris for any large or rare organisms that were not sorted initially. These organisms should be placed in a separate vial

with a label marked scan and the scan vial should be attached to the initial sample vial with a rubber band.

13. Empty any remaining debris into the sieve to remove water and then dispose of the waste debris in the garbage. Some projects have a requirement to save the remainder of the sample which was not subsampled. If so, throw away the subsampled portion and put the remaining debris that was not subsampled into the plastic lidded jar with a duplicate label and clearly indicate how much of the original sample it contains (e.g., 28 of a total of 32 grids or 7/8ths). The original sample label should be retained with the subsample vial. The remaining sample jar should be stored in the flammable storage cabinet.

14. Place subsample vial(s) in the designated vial box tray for the taxonomist.

15. Record all subsampling information in the "Benthic Sample Log Book" in the row for that sample.

- the number of organisms sorted by the first sorter (if sorted by a second sorter, add a note in this column that specifies how many were in the second sort; e.g., 198 & 2 in 2nd sort)
- the number of cells sorted
- If the total number of organisms sorted exceeded the fixed count target +20%, record the number of squares sorted from both the first pan and the second pan in the log book. For example, if 4 grids were originally removed from the first pan, but this subsample had to be further subsampled in the second pan to reach the target count by picking 8 grids from the second pan, record 4/8 in the log book.
- the date subsampled
- the initials of the first sorter to the left of the date and if applicable, the initials of the second sorter to the right of the date

16. Rinse all sieves, trays and pans, sorting tools, etc. and place back in appropriate location on subsampling cart or benchtop. Ensure all lights and microscopes are turned off. Put dust cover on microscope.

9.3 Pollution prevention and waste management

Small amounts of ethanol can be rinsed down the sink without an impact to water quality but care should be taken to minimize the volume.

To increase water conservation, personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use.

9.4 Waste management

Waste Type Code: Ethanol
Estimate Amount of Waste per Sample: 1000 ml
Describe any Treatment: Store ethanol in marked "waste ethanol container" in flammable storage cabinet in sample processing area

When the "waste ethanol container" is full, place a hazardous waste sticker label on it and place

the container and its completed log in the hazardous waste room in the designated flammable cabinet. Complete the accumulation date on the hazardous waste sticker label as the date it is placed in the hazardous waste room. Notify the facility manager or designee that it is ready for disposal. Waste management and disposal shall be in accordance with the Wheeling Field Office Chemical Hygiene Plan.

If a vial happens to break, it should be disposed of in the "glass only" waste container in the sample processing area.

10.0 Data and Records Management

The samples are logged in according to the SOP for Macroinvertebrate Sample Handling and Receiving (R3QA1001).

For purposes of constructing the final taxa list, and calculating metrics, the first sort and second sort results should be combined (see the SOP for Macroinvertebrate Identification R3QA1003). However, in the log book and on the bench identification sheet, these counts are kept separate so that statistics for "sorting efficiency" can be summarized and documented.

11.0 Quality Control and Quality Assurance

Frequency: For certain projects when specified in the project plan, and with inexperienced sorters-in-training, every subsample must be sorted by a second sorter. For most projects, and with experienced sorters, only 10% of the subsamples must be second sorted, as suggested in the RBP guidance (Barbour et al, 1999). Even with experienced sorters, at least 5% of the second sort checks should always take place early in each project to make sure the sorter is finding all of the smaller or rare organisms that might be specific to a project area or new to the sorter.

The number of organisms found in the second sort is used to check picking efficiency. The picking efficiency is calculated by determining the proportion of organisms that were found by the first sorter divided by the total number of organisms in the subsample. For example, if the first sorter found 198 organisms and the second sorter found 2 organisms, the PE would be 99% (i.e., [198÷200] x100%=99%). The PE is recorded in the macroinvertebrate "QA-QC tracking" electronic spreadsheet on FBT's Bioprojects drive.

Our target for picking efficiency is ≥90%, as suggested in the RBP protocol and other programs (for example the USGS's National Water Quality Program (USGS 1993)). The second sorter should review the organisms found in the second sort and confirm that they constitute less than 10% of the total number.

If more than 10% of the organisms in the subsample were missed by the first sorter, the sorter fails. Sorting checks early in the project will determine how long the sorting checks should continue. If early checks fail, second sorts will continue on every subsample until the sorter can reliably pass the check.

Corrective actions for sorters that fail will consist of a review by one of the senior biologists of the number and types of organisms that were missed by the first sorter. If a particular type of organism was missed, the biologist should review that with the first sorter using the magnifier and a stereoscope. Depending on the situation, the first sorter may be instructed to look more carefully for the types or sizes of organisms being missed, to take more time with the first sort of the subsample, or to spend

some time with a microscope reviewing the types and sizes of organisms missed to make sure they can identify them. Any corrective action discussions with the sorter should be documented in the "Correction Actions Log Book". Again, it is important that at least 3% of the second sort checks take place early in the project so that any systematic problems with the first sorter will be caught early.

12.0 References

Delaware Department of Natural Resources and Environmental Control, Division of Water Resources, Environmental Services Section, Laboratory Standard Operating Procedures

Delaware River Basin Commission, Standard Operating Procedures.

Guidelines for the Processing and Quality Assurance of Benthic Invertebrate Samples Collected as Part of the National Water Quality Assessment Program. USGS. Open File Report 93-407. 1993.

Maryland Department for Natural Resources, Maryland Biological Stream Survey, Laboratory Methods for Benthic Macroinvertebrate Processing and Taxonomy

Pennsylvania Department of Environmental Protection, Standardized Biological Field Collection and Laboratory Methods

Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic, Macroinvertebrates and Fish, Second Edition" by Michael T. Barbour, Jeroen Gerritsen, Blaine D. Snyder and James B. Stribling. EPA 841-B-99-02.

Susquehanna River Basin Commission, Standard Operating Procedures.

US EPA. Chemical Hygiene Plan, current revision. Wheeling Field Office, Wheeling, WV.

US EPA. Occupant Emergency Plan, current revision. Wheeling Field Office, Wheeling, WV.

US EPA. 2006. SOP R3-QA1001.4. Macroinvertebrate Sample Receiving and Handling. Effective Date 02/09/2006. US EPA Wheeling, WV.

US EPA. 2006. SOP R3-QA1003.4. Identification of Macroinvertebrates. Effective Date 02/16/2006. US EPA Wheeling, WV.

US EPA. 2006. SOP R3-QA1007.0. Field Bioassessments. Effective Date 02/17/2011. US EPA Wheeling, WV.

Virginia Department of Environmental Quality, Biological Monitoring Program, Standarnd Operating Procedures and Quality Assurance Project Plan for Wadeable Streams and Rivers.

West Virginia Department of Environmental Protection, Watershed Assessment Section, Standard Operation Procedures

13.0 Attachments

Attachment 1

Corrective Actions Log Book

Date	Action

Analyst's Initials following review of completed notebook page:_____

Page No: ____

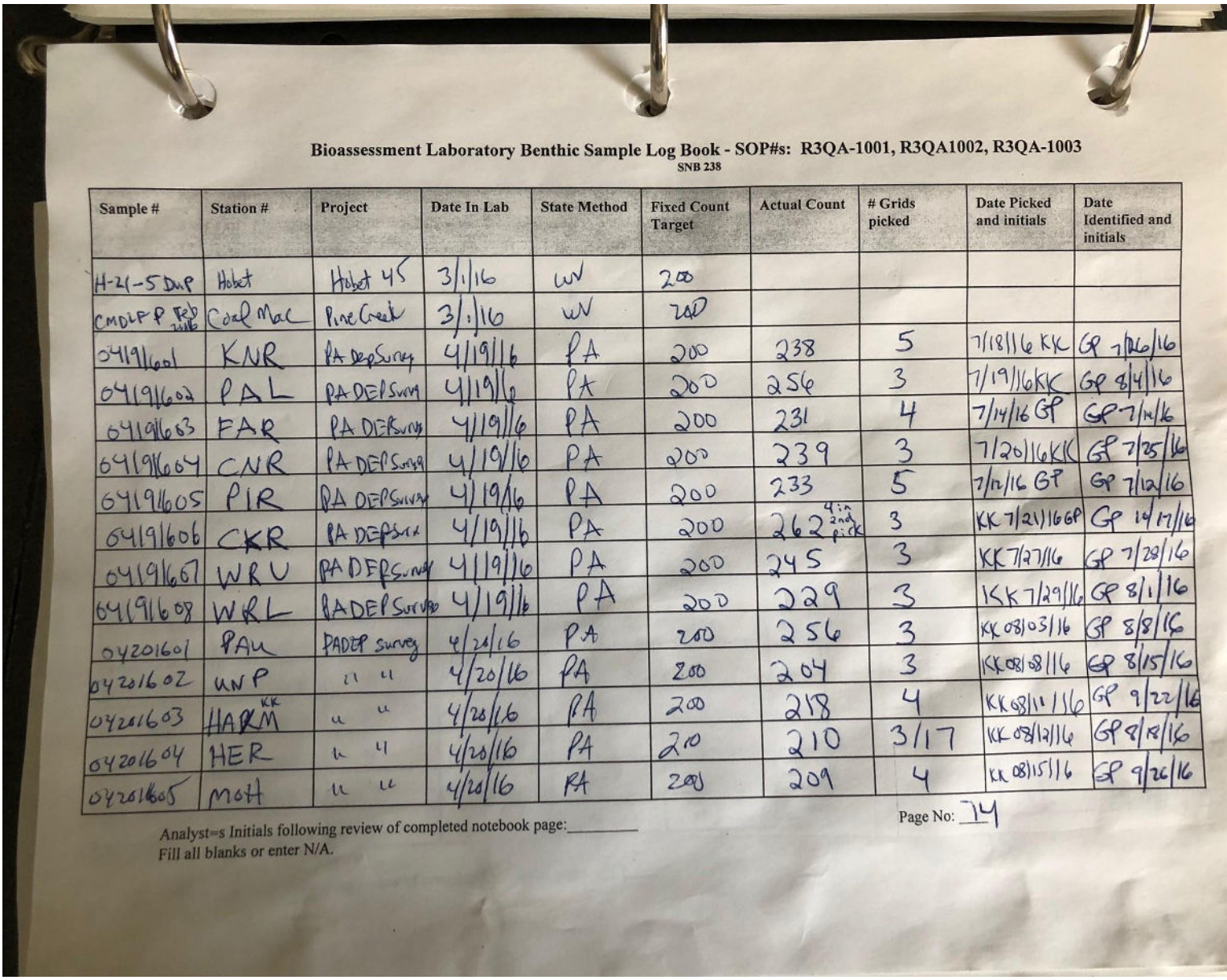
Attachment 2

Benthic Sample Log Book

	Station #	Project	Date In Office	State Method	Fixed Count Target	Actual Count	# Grids picked	Date Picked and initials	

Analyst's Initials following review of completed notebook page:_____ Page No: ____
Fill all blanks or enter N/A.

Example Completed Version- Benthic Sample Logbook



Bioassessment Laboratory Benthic Sample Log Book - SOP#s: R3QA-1001, R3QA1002, R3QA-1003
SNB 238

Sample #	Station #	Project	Date In Lab	State Method	Fixed Count Target	Actual Count	# Grids picked	Date Picked and initials	Date Identified and initials
H-21-5 Dup	Hobet	Hobet 45	3/1/16	WV	200				
CMOLP P Reb 2016	Coal Mac	Pine Creek	3/1/16	WV	200				
04191601	KNR	PA DEP Survey	4/19/16	PA	200	238	5	7/18/16 KK	GP 7/26/16
04191602	PAL	PA DEP Survey	4/19/16	PA	200	256	3	7/19/16 KK	GP 8/4/16
04191603	FAR	PA DEP Survey	4/19/16	PA	200	231	4	7/14/16 GP	GP 7/21/16
04191604	CNR	PA DEP Survey	4/19/16	PA	200	239	3	7/20/16 KK	GP 7/25/16
04191605	PIR	PA DEP Survey	4/19/16	PA	200	233	5	7/21/16 GP	GP 7/12/16
04191606	CKR	PA DEP Survey	4/19/16	PA	200	262 4 in 2nd pick	3	KK 7/21/16 GP	GP 14/17/16
04191607	WRU	PA DEP Survey	4/19/16	PA	200	245	3	KK 7/27/16	GP 7/28/16
04191608	WRL	PA DEP Survey	4/19/16	PA	200	229	3	KK 7/29/16	GP 8/1/16
04201601	PAU	PADEP survey	4/20/16	PA	200	256	3	KK 08/03/16	GP 8/8/16
04201602	UNP	" "	4/20/16	PA	200	204	3	KK 08/08/16	GP 8/15/16
04201603	HARM KK	" "	4/20/16	PA	200	218	4	KK 08/11/16	GP 9/22/16
04201604	HER	" "	4/20/16	PA	200	210	3/17	KK 08/12/16	GP 8/18/16
04201605	MOH	" "	4/20/16	PA	200	209	4	KK 08/15/16	GP 9/26/16

Analyst=s Initials following review of completed notebook page:_____
Fill all blanks or enter N/A.

Page No: 14

Appendix G:

Tier II Benthic Macroinvertebrate Monitoring Sites

Tier II Benthic Monitoring Site Tracking Sheet

Site Name	HUC12 Watershed	Latitude	Longitude	Previously Monitored Site?	Site in a HUC12 that has been monitored since 2006?

Appendix H:

Certified Monitors

Certified Tier II Benthic Monitors

Name	Monitoring Group	Date Certified	Date of Last Sample

Appendix I:

Standard Operating Procedure: Identification of Macroinvertebrates

U.S. Environmental Protection Agency, Region 3
Laboratory Services & Applied Sciences Division
Field Services Branch

Standard Operating Procedure

IDENTIFICATION OF MACROINVERTEBRATES

Effective Date: May 13, 2021
Doc. Control Number (DCN): R3LSSOP004-20210513

Preparer / Author
Name: Greg Pond
Title: Biologist
Division/Branch: LSASD/FSB
Signature/Date: GREGORY POND Digitally signed by GREGORY POND Date: 2021.05.15 07:46:11 -04'00'

Technical Reviewer / Quality Assurance Officer
Name: Kelly Krock
Title: Biologist
Division/Branch: LSASD/FSB
Signature/Date: KELLY KROCK Digitally signed by KELLY KROCK Date: 2021.05.13 11:32:19 -04'00'

Branch Chief
Name: Norman Rodriguez
Title: Acting Branch Chief
Division/Branch: LSASD/FSB
Signature/Date: NORMAN RODRIGUEZ Digitally signed by NORMAN RODRIGUEZ Date: 2021.05.13 17:22:0 -04'00'

Revision History

This table shows changes to this controlled Standard Operating Procedure (SOP) over time. The most recent version is presented in the top row of the table.

Document Control Number and Version	History	Effective Date
R3LSSOP004-20210513	Current Version	5/13/2021
R3-QA1003.4	Previous version and format. Other versions before that in outdated software.	2/16/2006

While this SOP may be informative, it is not intended for and may not be directly applicable to operations in other organizations. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

Table of Contents:

1.0 SCOPE AND APPLICABILITY

The purpose of this SOP is to describe the process and methods by which benthic macroinvertebrate samples are identified. This SOP applies to all samples identified by Wheeling, WV, U.S. EPA staff.

2.0 SUMMARY OF METHODS

This method describes how the samples are identified, including rules on whether to include or exclude parts of organisms, how the counts are tallied, how to mount specimens (e.g., Chironomidae), how the voucher samples are preserved, how the reference collection is maintained, and how the Quality Control checks are performed and documented.

3.0 ACRONYMS/DEFINITIONS

CHIM: Chemical Inventory Management

CHP: Chemical Hygiene Plan

COC: Chain of Custody

DOC: Demonstration of Capability

EPT: Ephemeroptera, Plecoptera, Trichoptera

FSB: Field Services Branch located in the U.S. Environmental Protection Agency Region 3's Laboratory Services & Applied Sciences Division

FSB's network drive: EPA Agency network drive named H: - LSASD 3LS30. Backed up regularly and maintained through Agency policy.

JHA: Job Hazard Analysis

LS: Life Stage

Mounting medium / Mountant: any substance in which a specimen is suspended between a slide and a cover glass for microscopic examination

SDS: Safety Data Sheets

SNB: Supplemental Notebook

OEP: Occupant Emergency Plan Quality assurance or quality control check: reviewing data and procedures to ensure no quality issues

PDE: Percent Disagreement Enumeration

PNB: Primary Notebook

PTD: Percent Taxonomic Disagreement

QAO: Quality Assurance Officer

QAPP: Quality Assurance Project Plan

QAQC: Quality Assurance, Quality Control

SDS: Safety Data Sheets

SHEM: Safety, Health and Environmental Manager

4.0 HEALTH AND SAFETY WARNINGS

4.1 General Precautions and Guidelines

Before beginning any procedures, the Job Hazard Analysis (JHA) should be reviewed in Appendix A. Also, refer to the Wheeling Office Chemical Hygiene Plan (CHP) (current version) and Wheeling Office Occupant Emergency Plan (OEP) (current version) for general safety precautions and guidelines.

All applicable safety and compliance guidelines set forth by the EPA and by federal, state, and local regulations must be followed during the performance of this SOP. In addition, all procedures outlined in the Wheeling Office CHP must be adhered to. Stop all work in the event of a known or potential compromise to the health and safety of any person and immediately notify the Safety, Health and Environmental Manager (SHEM) and other appropriate personnel as outlined in the CHP.

4.2 Chemicals Management

For vials or other sample containers, internal labels should note that the jar contains 70-100% ethanol and an overall sign is placed in the specimen storage and flammable cabinets with this same information. Vial boxes must be labeled to say all vials contain 70-100% ethanol.

Bulk containers of ethanol should always be stored in flammable storage cabinets. Squeeze bottles with ethanol must be labeled with the chemical name, approximate concentration of ethanol, and dated and initialed when refilled.

If samples are preserved with denatured ethanol, exposure to the samples should be minimized. Samples collected by the FSB are preserved with non-denatured ethanol which is safer.

It is advisable to cleanse exposed parts of the body with soap and water after contact with samples.

Safety Data Sheets (SDS) for all reagents (ethanol, slide mounting media [CMC 9, CMC10]) are currently located in the sample processing area. SDS information is also available on the internet.

4.3 Chemical Spill Procedures

For chemical spills, follow the procedures outlined in the Wheeling Office OEP, the Hazardous Material Spills section. For minor spills (which can be handled by the analyst), wear safety glasses, protective coat, and gloves to clean up the material by wiping up immediately with paper towels, a sponge or spill pillows. For major spills, immediately contact the safety office and facilities manager.

4.4 Pollution Prevention and Waste Management

Small amounts of ethanol can be rinsed down the sink without an impact to water quality,

but care should be taken to minimize the volume.

If a vial happens to break, it should be disposed of in the "glass only" waste container in the sample processing area.

When any logged chemicals are depleted or disposed of, the analyst shall initial and date the Chemical Inventory Management (CHIM) notebook (SNB-277). When a bulk ethanol container is emptied, the container should be rinsed with water and retained to be used later for waste. The CHIM number can be crossed out with permanent marker.

If waste ethanol is present, place in marked waste ethanol container in flammable storage cabinet in sample processing area and record amount placed in container on associated log. When the waste ethanol container is full, place a hazardous waste sticker label on it and place the container and its completed log in the hazardous waste room in the designated flammable cabinet. Complete the accumulation date on the hazardous waste sticker label as the date it is placed in the hazardous waste room. Notify the facility manager or designee that it is ready for disposal. Waste management and disposal shall be in accordance with the Wheeling Office Chemical Hygiene Plan.

4.5 Safety Training

New analysts must take 24 hours of safety training before assuming sample processing duties. Training requirements are outlined in Agency policy. After the initial training, analysts are required to obtain 8 hours of refresher safety training annually.

5.0 CAUTIONS

5.1 Regional Identification

Taxonomists should ensure final identification of a specimen is regionally present in the sampled area and within the known distribution of the taxon as listed in primary reference, i.e. not identified to a Western Genus if the sample was from the Mid-Atlantic.

5.2 Sample Degradation

Samples in ethanol or water (whether in sorting trays or petri dishes) must not be allowed to dry out. A large watch glass can cover the working petri dish when temporarily away from the scope for a short time period. Microscope lights should also not be left on when the sample is unattended, since this can speed up drying and reduce the life of the bulb.

5.3 Damaged or Immature Specimens

When organisms are damaged or only pieces of the organism are present, analysts should follow the below rules to decide whether and how to identify the organism or part:

- Tails, abdomens, and other body parts (except as specified below) should not be counted, unless the identification can be positively made, and double counting is avoided (e.g. Hydropsychidae abdomens which do not have a matching head and thorax).

- Parts of organisms that could not be identified should be discarded with the debris and not added to the sample vial.

- Heads and attached thoraxes are always counted when the identification can be made.
- If the identification cannot be made at the required taxonomic level (e.g., family) due to the condition or size of the specimen, then the organism should be identified at the next highest level (e.g., order). Write in parentheses next to the record whether the taxon was damaged or immature. Standardized notes are recorded on the macroinvertebrate identification sheets when specimens cannot be identified to the target taxonomic level (see table 1). This note is later recorded in a comments field in the database.
- If there are any questions about the identity of an organism, consult with another analyst at the time of the identification.

Table 1. Standardized notes on macroinvertebrate identification sheets

Note	Description
dam.	Damaged- Specimen is too damaged/degraded to identify to target taxonomic level.
e.i.	Early instar- Specimen is not mature enough to identify to target taxonomic level; used sometimes interchangeably with imm. but only for arthropods.
imm.	Immature- Specimen is not mature enough to identify to target taxonomic level.
indet.	Indeterminate- Specimen cannot be identified to target taxonomic level due to indistinct characters, missing/damaged case, or habitat/ecological information.
nr.	Near- Specimen keys near to target but not identification not positive.

6.0 INTERFERENCES

Pencil should be used with internal sample labels due to the use of ethanol; pen and marker bleed when in contact with ethanol. Care should be taken to not get ethanol on log book pages.

Semiaquatic and terrestrial organisms are not counted unless instructed in the project plan or equivalent document.

To avoid contamination, analysts shall check that all vials, petri dishes, trays, and tools are clean from any debris or organisms when beginning each new sample.

Analysts shall ensure all samples are labeled properly and at all times. Analysts should ensure the same sample identification information is written on all labels and matches the information in log books.

If subsampling is needed (section 9.7), the analyst must attempt to get an even distribution of the sample in the tray prior to subsampling. The subsampling grids must be sorted randomly in order to obtain a representative subsample.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

7.1 General Responsibilities

Analysts shall be familiar with this SOP, safety procedures, and chemical handling procedures, and are responsible for properly logging identification results, securing samples, and managing data and records. Before starting to identify a sample, the analyst shall determine project/program requirements to know target taxonomic resolution, sample retention length, data reporting process, and final sample storage location. Analysts are responsible for maintaining, inventorying, and keeping records for equipment.

7.2 Taxonomic Changes

Analysts shall stay current on taxonomic changes through peer reviewed publications and professional organizations (e.g., Society for Freshwater Science). Experienced analysts shall also share knowledge of new taxonomic designation with other analysts and notify all analysts when commencement of the changes should start being used or if exceptions will exist for certain state protocols or projects.

7.3 Ongoing Demonstration of Capability

For all macroinvertebrate analysts, after they have demonstrated capability, a second experienced analyst will identify 10% of the samples to confirm ongoing demonstration of capability. The experienced analysts who perform the quality checks are responsible for documenting these checks by updating the appropriate spreadsheet that tracks macroinvertebrate QAQC statistics (Section 11.2).

7.4 Certification

The Society for Freshwater Science provides certification in macroinvertebrate taxonomy at family and genus level. Analysts are encouraged, but not required, to obtain these certifications. The analysts are responsible for arranging for the certification test and for providing the Quality Assurance Officer (QAO) with a copy of their certificates. The QAO is responsible for filing copies of the certifications in the analysts' Demonstration of Capability (DOC) file.

7.5 Qualifications for New, Inexperienced Analysts

For new analysts, an experienced analyst should perform a second identification of all samples to provide a check on the taxonomic and enumeration agreement. The Percent Taxonomic Disagreement (PTD) goal is that less than 10% of the total organisms should be identified differently by the two analysts. Our Percent Disagreement Enumeration (PDE) goal is that there should be less than 10% difference in the total numbers of organisms between the two analysts. The experienced analyst shall review all misidentifications with the new analyst. A new analyst shall not be deemed capable and

independent until they can consistently meet those goals. The experienced analyst who performs these quality control checks is responsible for updating the appropriate laboratory notebooks and the spreadsheet that tracks macroinvertebrate QAQC statistics. The experienced analyst is responsible for checking the samples and for reporting to the

QAO when the new analyst is deemed capable of performing macroinvertebrate identification independently. The QAO is responsible for updating the new analyst's DOC file with a memo that indicates the analyst is capable of performing macroinvertebrate identification, based on experience with FSB.

7.6 Qualifications for New, Experienced Analysts

Experienced analysts who are new may be deemed capable of identifying macroinvertebrates independently based solely on their education, formal training, or past experience. For experienced but new analysts, the Branch Chief and QAO are responsible for reviewing the new analyst's resume and deciding whether the new analyst is capable, based on discussions with the new analyst and other experienced macroinvertebrate analysts on staff. If the new analyst is deemed capable of independently performing macroinvertebrate identification, the analyst's resume detailing relevant education, course work and experience will serve as DOC. The QAO is responsible for updating the analyst's DOC file with a resume and memo that indicates the analyst is capable of performing macroinvertebrate identification, based on prior experience.

8.0 EQUIPMENT AND SUPPLIES

8.1 Items Needed

Table 2 identified items needed to perform macroinvertebrate identification. When equipment needs replaced or supplies need ordered, the analyst will follow Agency policy and division procedures for procuring items.

Table 2. Items Needed for Macroinvertebrate Identification

Chemicals	**Forms and Notebooks**
95% (200 proof) non-denatured ethanol (squeeze bottle and bulk container for refill) 70% non-denatured ethanol for storage of specimens (squeeze bottle and bulk container for refill) Waste ethanol in marked container Mounting medium (e.g., CMC 9, CMC10) Immersion oil for compound microscope	Macroinvertebrate identification sheets Random numbers sheet Benthic Sample Log Book (SNB-238) Freshwater Macroinvertebrate Results Log Book (PNB-160) Corrective Actions Log Book (SNB-241) Macroinvertebrate Reference Collection Log Book (SNB-239) Chemical Inventory Management Notebook (SNB-277) Individual hazardous waste disposal log and stickers
Equipment	**Supplies**
Dissecting stereo microscope Compound microscope Fiber optic illuminator Tally counter Dissecting forceps Dissecting needles 140 mm diameter petri dish with 154 gridded cells for subsampling Chironomidae Large watch glass Specimen storage cabinet(s) Flammable cabinet(s) Glass eye dropper Applicator sticks Taxonomic library Sink/water	Waterproof labels (i.e., Rite-in-the-Rain paper) Pens and pencils 2 and 4 dram glass vials with polyseal screw caps Plastic lidded containers or larger vials (for larger organisms that won't fit in the smaller vials) Plastic petri dishes with covers Frosted microscope slides Slide covers Slide boxes Rubber bands Clear finger nail polish (optional) Replacement light bulbs for illuminators and microscopes Replacement fuses for illuminators and microscopes Paper towels and spill kit

8.2 Microscope Adjustment

The stereo and compound microscopes must be properly adjusted to see the object image in three dimensions and to perceive heights and depths of the specimen. To achieve these height/depth effects, the images coming from the binocular eye pieces must be "fused" into a single image by the observer - this requires some practice and careful setting of the binocular body.

Move the eyepiece tubes in and out to find the place where the distance between the eyepiece centers matches the distance between your pupils. This is the "interpupillary distance" and will vary somewhat from operator to operator. When these distances are equal, one central image is seen.

It is necessary to adjust so focus remains sharp through the whole range of zoom magnification:

- Set microscope magnification to the highest power by turning the zoom control ring counter-clockwise.
- Focus sharply on the specimen.
- Set to the lowest magnification by turning the zoom control ring clockwise. Do not touch the focusing controls.
- Looking with the right eye through the right-hand eyepiece, turn the eyepiece's diopter adjustment ring until the image is precisely in focus.
- Do the same with the left eye and the left-hand eyepiece.
- The fused microscope image should now be uniformly sharp throughout the zoom range and without refocusing.
- Do not attempt to make adjustments to the internal optics or mechanics. If the microscope does not seem to be functioning properly, the scope will be sent to an authorized repair service for cleaning and repair.

8.3 Equipment Maintenance

Keep a dust cover on microscopes when not in use. Do not attempt to make adjustments to the internal optics or mechanics. If the microscope does not seem to be functioning properly, contact an authorized repair service for cleaning and repair in coordination with the Branch Chief, Agency purchasing policy, and Division procedures. Maintenance records should be scanned and saved electronically on the FSB network drive. Responsible analysts will update the equipment inventory system on the FSB Network Drive as new equipment is ordered or if old equipment is taken out of inventory.

Replace fuses or light bulbs in microscopes or the fiber optic illuminators as needed. Replace worn or broken equipment as needed (e.g., pipette).

When done using, rinse tools, trays, etc. in the sink thoroughly.

8.4 Mounting Media

CMC is a water based mounting medium, also referred to as mountant, for slides.

Material can be mounted in it from alcohol or water, and the medium does impart a clearing action. CMC 10 provides quick mounting, easy clearing action, can be thinned with alcohol, water, or another CMC medium (e.g., CMC 9) and the water base makes it easier to clean up old slides and other equipment. The medium is considered temporary, and should not be used for permanent archived specimens or reference collections. The slides can be ringed with clear finger nail polish in order to seal them better.

8.5 Taxonomic Library

Only taxonomic keys that have been peer-reviewed and are available to other taxonomists are used. A library of basic taxonomic literature is essential in aiding identification of specimens and should be maintained in the taxonomic library. The taxonomic references in common use should be stored in the sample processing area. These references are listed in the references section. When available, newly updated taxonomic references should be purchased if possible. Table 3 shows the primary keys to be used for the various taxonomic groups.

Table 3. Primary Literature/Keys for Identification by Taxonomic Group

Taxa Group	**Primary Key**
Chironomidae	Anderson et al. 2013, Epler 2001
Coleoptera	Epler 2010, Ciegler 2003
Crustaceans	Smith 2001, Rogers and Hill 2008
Ephemeroptera (E)	Morse et al. 2017, Merritt et al. 2019
Gastropods	Thorp and Covich 1991, Smith 2001
Insects in general	Merritt et al. 2019, Morse et al. 2017 (EPT)
Invertebrates in general	Thorp and Covich 1991, Smith 2001
Odonata	Needham et al. 2000, Westfall and May 1996
Plecoptera (P)	Stewart and Stark 2002, Morse et al. 2017
Trichoptera (T)	Wiggins 1998, Morse et al. 2017
Tipulidae	Gelhaus 2002, Merritt et al. 2019

8.6 Chemical Inventory

Analysts must log new chemicals into the CHIM Notebook (SNB-277), assign a CHIM number, and initial the dates that chemicals are brought into the office, opened, and disposed. Chemicals must be logged in regardless of their source or toxicity. The CHIM number consists of the year and the sequential number of the chemical. For example, 21-01 would be the first chemical brought into the office in the year 2021. The CHIM number should be written on the side of the chemical container. If the chemical does not have an expiration date assigned by the manufacturer, then the analyst assigns an expiration date that is 10 years from the date of receipt.

Analysts need to contact the Branch Chief and facility manager to report any possible new hazardous waste stream, before it is generated, so that a procedure can be put in place to

handle the waste. Any new procedures should be reviewed before adoption to make sure any hazardous waste issues are addressed.

9.0 PROCEDURE

9.1 Secure the sample.

For the sample chosen to be identified, get all sample vial(s) (typically grouped together by a rubber band) (e.g., 2nd sort specimens) and any other associated larger containers with specimens (e.g., large crayfish). Ensure labels are present and all match the same sample ID.

9.2 Determine the taxonomic resolution.

Determine the taxonomic level for identification determined by project or program requirements as specified in the Quality Assurance Project Plan or equivalent document. Typically, insects, mollusks, and crustaceans are identified to the Genus level whereas aquatic worms are identified to the Family or Class level (i.e., Oligochaeta) when possible. Other groups (e.g., flatworms and nematodes) are identified at higher taxonomic levels (e.g., Phylum). See Appendix B for typical taxonomic levels for Mid-Atlantic and regional organizations.

9.3 Adjust the microscope.

Properly adjust the stereo microscope to see the object image in three dimensions and to perceive heights and depths of the specimen as outlined in Section 8.2.

9.4 Prepare data sheet.

Get a blank macroinvertebrate identification sheet (see Appendix C).
Fill out the header information:

- project name
- sample number
- station number
- stream name
- location of monitoring site
- type of net
- total substrate area sampled (in field)
- replicate(yes or no)
- who collected sample
- who identified sample and date
- subsample portion and count (e.g., 1/8th of the total sample or a fixed count of 100 organisms).
- # of grids subsampled

Page number is completed when the identification is completed and placed in the primary notebook for final storage. Note that the macroinvertebrate identification sheet may vary with the project, i.e. state agency-specific sheets may be used.

9.5 Identify specimens.

Uncap and empty main vial(s) into petri dish, rinsing with ethanol as needed to make sure no specimens are remaining in the vial. Place under the stereo microscope with fiber optic illuminator and if desired, roughly sort specimens into higher taxa groups. Use dissecting forceps and needles to manipulate and move specimens as needed to aid identification, ensuring no to minimal damage is done to specimens. When organisms are damaged or only pieces of the organism are present, follow rules as outlined in Section 5.3 and make standardized notes on macroinvertebrate identification sheets as needed.

Identify specimens, using keys and other literature as necessary. On the macroinvertebrate identification sheet, record the identity in the column titled Organism and number of organisms in the 1st sort No. column. Use a tally counter for abundant organisms (Chironomidae, Hydropsychidae, etc.). Use hand tallies or slash marks next to the organism name on the macroinvertebrate identification sheets to keep track of more rare taxa. For projects, indicate life stage in the column 'LS' if applicable (e.g., P=pupae, A=adult). Also identify any large specimens if other sample containers are present. As needed for longer taxa lists, move to the second column to list organisms. For specimens that need mounted on a slide, use a compound microscope (e.g., Chironomidae) as discussed in step 9.8.

9.6 Identify 2nd subsampling specimens if applicable.

A portion of the original samples are sorted twice in order to estimate the subsampling error rate (see Macroinvertebrate Sample Subsampling SOP, R3QA-1002). When the subsample was checked by a second analyst and specimens were found in the 2nd check, there will be at least one vial identified as the results of the second sort with a separate label. T. Identify these specimens and record the results of the second sort into the column titled "2nd sort" (the counts from the first sort/original vial should be summarized in the column titled "1st sort"). These numbers must be kept separate on the macroinvertebrate identification sheet in order to calculate the subsampling error rate. However, later they are combined in the database for the final taxa list.

9.7 Subsample Chironomidae if needed.

Depending on the project, it is sometimes necessary or permitted to further subsample Chironomidae. The petri dish is approximately 12.1 cm in diameter and is divided into 154 10mm by 10 mm grids. The grids are numbered sequentially from left to right. Generate a random numbers table in Microsoft Excel (see Appendix G) and print out.

Place Chironomidae in the gridded plastic petri dish and spread out as evenly as possible. Start at the top of the list of random numbers and cross out each number as it used. All of the Chironomidae in that numbered grid are sorted and put in a separate petri dish. If any part of a larvae is in the chosen grid, it is sorted. This is repeated until the target

number of Chironomidae are chosen. The remainder of the Chironomidae in the gridded petri dish are returned to the sample vial with the rest of the identified organisms. Alternatively, the remainder of the Chironomidae can be stored in a small plastic vial which is placed in the larger glass vial. This makes it easier to review the unmounted midge, if necessary.

9.8 Mount specimens for identification if applicable.

If needed to reach the target taxonomic resolution (e.g., Chironomidae), mount the specimens on slides for identification. Label the slides with sample number, station number or stream name, and number of that slide out of the total number of slides prepared for that sample (e.g., slide 1 of 5). Use frosted slides and label the slide directly or use tape as a label on one side of the slide. Several slides can be lined up, taped, and then separated using a fine blade to affix several tape labels at once.

Apply a few drops of CMC mounting medium to the glass microscope slide using the glass eye dropper and applicator sticks as needed. Using fine forceps or a needle, transfer 6 to 8 larvae to the mountant on one side of the slide. Arrange the larvae so that they are ventral side up. If the larvae are large, detach the head and last few anal segments from the body. Tease out some of the larger bubbles that form in the mountant. If Chironomidae larvae are mounted whole, make sure the anterior parapods are not obscuring the head.

Take a clean cover slip and slowly lower it over the mountant at an angle. Push the cover from side to side, and gently press on the cover with tips of forceps or a pencil eraser. Try to keep the head ventral side up, and spread the mouthparts. Each slide can accommodate 2 cover slips; repeat steps above for second cover slip. Place under microscope and identify at 100-400x using published keys. For some characters (e.g., antennal segments), use immersion oil with the 100x objective (i.e., 1000x total magnification), when necessary. On the bottom of the macroinvertebrate identification sheet, indicate the number of slides associated with the sample.

9.9 Complete final taxa list.

After the sample is identified, total the tallies for each organism and record in the 1st sort column (and 2nd sort if applicable). Total all the 1st and 2nd sort counts and record in the number of organisms box. Count how many unique taxa and record in the total taxa box. Count all Ephemeroptera, Plecoptera, and Trichoptera taxa and record in the EPT Taxa box.

If applicable, calculate 2nd sort error percentage by determining the proportion of organisms that were found by the first sorter divided by the total number of organisms in the subsample. For example, if the first sorter found 198 organisms and the second sorter found 2 organisms, the sort error percentage would be 99% (i.e., [198÷200] x100%=99%). Record this calculation in the Sort Error (%) box. Also determine if any taxa were not previously identified by Wheeling taxonomists and if not, indicated the number in the No. New Taxa box.

Record the date of identification and initials in the Benthic Sample Log Book (Appendix D). Ensure all lights and microscopes are turned off. Put dust cover on microscope.

9.10 Measures biomass estimates if applicable.

For some projects, additional biomass estimate measurements are taken during the identification process for pre-determined taxa as outlined in the QAPP or equivalent document. Typically, either all specimens, or a random number of specific taxa are selected in a sample, depending on the project objectives. Measure the head capsule width and/or body length to the nearest µm with a dissecting scope and connected digital camera with measurement capabilities. For body lengths, graph paper with 1 x 1 mm squares can also be used for size class categorization. Record measurements on the macroinvertebrate identification sheet.

9.11 Arrange for secondary identification if applicable.

To check taxonomic and count accuracy as outlined in Section 11.2 (10% of samples), have an analyst not responsible for the original identifications reidentify and recount the entire sample, including the Chironomidae larvae. Clearly identify the second identification sheet as a quality check on the top of the sheet and staple it to the original sheet.

9.12 Prepare voucher sample.

Place the unmounted specimens from each sample in glass vial(s) and preserve with 70% ethanol. Include the original label in vial(s) so that the label can clearly be read. Place in sample storage cabinet, and if necessary, bind together multiple vials with a rubber band. Store mounted specimens in a slide box for each project.

If the sample contains a rare specimen that may not be in the reference collection, check the reference collection list. If the taxon is not represented in the reference collection, it should be added to the reference collection as described in Section 11.4. Preserve the specimen with 70% ethanol. Note on the macroinvertebrate identification sheet that this specimen was removed from the sample vial and resides in the reference collection. This is necessary in case the sample is randomly chosen for a quality check.

9.13 Enter data.

Enter identification results into a state or project specific database or spreadsheet, and record location, date, and initials on the macroinvertebrate identification sheet and on the FSB project checklist if an FSB project. For purposes of constructing the final taxa list, combine the first sort and second sort results when entering data. Proof the data entry (see Section 10.1) by comparing the original macroinvertebrate identification sheet to the electronic entries at a later time or ask another analyst to perform the check. Record data and initials of the check.

9.14 Analyze data.

Analyze macroinvertebrate data (e.g., calculating metrics and index of biological integrity score) as outlined in relevant Quality Assurance Project Plan or equivalent document. Save results in the project file on the FSB network drive. Conduct quality checks and record details, dates, and initials in the project file on the FSB network drive and/or on the FSB project checklist if an FSB project.

9.15 Complete records management.

Scan the macroinvertebrate identification sheet(s), save in the project file on the FSB network drive, and share with applicable project personnel. Place original identification sheets in the Freshwater Macroinvertebrate Results Log Book, adding the next sequential page number to the sheet.

Enter quality metrics (e.g., results from 2nd sort identification tallies, PDE, PTD) in the Microsoft Excel spreadsheet on the FSB's network drive in the QAQC folder, and in the project file as relevant as detailed in Section 11.1.

10.0 DATA AND RECORDS MANAGEMENT

10.1 Data Management

Data are recorded on the macroinvertebrate identification sheets with pen. Any corrections must be made with a single strike out and initialed with date. There should be no erasures.

When sample data are entered into either a state or project specific database or spreadsheet, it gets logged on the macroinvertebrate identification sheet and on the FSB project checklist if an FSB project. The analyst entering the data should initial the date when data were first entered. 100% of data entry is quality checked and must be done before data analysis occurs. Data are quality assurance checked by comparing the database raw data table or spreadsheet to the original macroinvertebrate identification sheets or other data sheets. Any mistakes are corrected in the database or spreadsheet. If mistakes are encountered on the original data sheet (e.g. misnumeration), the original data sheet should be corrected with a single strike through, initials and date. The date that the data were proofed is written on the original raw data sheet and initialed by the reviewing analyst.

Macroinvertebrate data are analyzed (e.g., calculating metrics and index of biological integrity score) as outlined in relevant Quality Assurance Project Plan or equivalent document. Typical quality checks performed on macroinvertebrate data analysis include visually checking the results of database queries, inspecting for missing values and anomalous output, hand calculating metrics to check query accuracy, and re-running the queries after fixing query syntax.

10.2 Records Management

Records should not be kept in personal files. Data sheets (e.g., macroinvertebrate identification sheets) are scanned and saved electronically in the relevant project folder on the FSB's network drive. Analysts may make hard copies of the data sheets for data entry or other purposes, but they shall be stamped or marked as copies. Original identification data sheets are returned to the PNB-160 notebook (current volume) for final storage and are hand-paginated based on the order placed in the notebook. In rare cases where analysts are generating data that is not recorded in a notebook, the analyst or project lead must file the original data sheets in the hard copy and electronic project records.

Records from taxonomic and count accuracy are recorded in a Microsoft Excel spreadsheet on the FSB's network drive in the QAQC folder, and scanned and saved in the project file as relevant as detailed in Section 11.2.

Analysts who perform data analysis are responsible for keeping accurate and sufficient records on the process used and results. Details, including dates/initials, for data analysis quality checks are recorded in the project file on the FSB network drive and/or on the FSB project checklist if an FSB project.

Analysts are required to get an appropriate signed Chain of Custody (COC) form for any samples that were collected by other agencies or individuals and submitted to FSB for storage or identification. If the individual or agency does not have a COC form, the analyst accepting the samples should provide one and make sure it is filled out correctly and signed by the individual relinquishing the samples. The Project Leader is responsible for filing these COC forms in the project file and logging the samples in correctly.

10.3 SOP Maintenance

Annotate small changes, additions, and edits in the margins of the working copy of the SOP, which resides in sample processing area, as the SOP is used and mistakes are found or changes are deemed necessary.

FSB SOPs generally follow the format detailed in the Guidance for Preparing Standard Operating Procedures (SOPs), EPA QA/G-6. The analyst and QAO will review the SOPs at least every three years to determine whether the SOP should be revised. The lead author should revise an SOP whenever a method changes significantly or when working copies contain excessive annotations. If the QAO and analyst agree that the SOP does not need revision, they will sign a review sheet to document that the review occurred. The QAO will file the original review sheet with the original SOP in the quality control records. The analyst will place a copy of the review sheet with the working copy of the SOP.

10.4 Notebook Management

New analysts should initial and write their name in the beginning of each notebook they use so the initials can be traced to the individual. There must be no erasures in notebooks. The analyst should strike out any mistakes with a single line, and initial and date the change. The analyst should not leave any blanks in notebooks. The initials NA (for not applicable) should be entered into blanks as appropriate.

Electronic copies of notebook templates are stored on the FSB network drive in the QAQC folder. If a staff member changes a notebook template, they must also annotate the hard copy SOP (that includes that notebook template as an appendix) to indicate that change. The lead author of the SOP must include the current notebook template in the corresponding SOP when the SOP is revised. Analysts should consult the affected staff on changes to notebook templates. If a notebook binder fills up, another volume can be created and the QAO should be notified.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Sample Accidents

If for any reason specimens are lost or damaged due to an accident (e.g., spilled vial), notes describing the accident, estimate of % sample lost, corrective actions performed, date, and initials should be recorded on the macroinvertebrate identification sheet and in the Corrective Actions logbook (Appendix F). The analyst(s), QAO, and project managers will determine if the final taxa list is valid or not for project/program purposes. Other alternatives can also be discussed such as subsampling again if the original field sample remains.

11.2 Taxonomic and Count Accuracy

For every project, at least 10% of the samples should be checked after the initial identification for taxonomic and count accuracy. An analyst not responsible for the original identifications should check the samples. The entire sample should be reidentified and recounted, including the Chironomidae larvae.

A separate macroinvertebrate identification sheet should be prepared with the second identifications and counts. This sheet should be clearly identified as a quality check on the top of the sheet and should be stapled to the original sheet. Records of these reviews should in the Microsoft Excel spreadsheet in the QAQC folder on the FSB network drive. The reviewer should record the date of the review, the sample number, the reviewer, and the original biologist. The reviewer should calculate the Percent Difference in Enumeration (PDE) and the Percent Taxonomic Difference (PTD). Any discrepancies between the original taxa list or counts and the quality check taxa list and counts should be resolved between the two analysts and recorded in the log book.

Any taxonomic mistakes that are discovered should be discussed with the biologist who performed the original identification immediately. When a taxonomic mistake is made

the original taxonomist and the reviewing biologist should review the taxonomic keys and characters. Any clear mistakes in counts or taxonomy should be corrected on the original macroinvertebrate identification sheet and should also be documented in the Corrective

Actions Log Book. These checks should occur before the data are entered into the database.

If the sample was identified and enumerated by a second analyst for an identification quality check, the following quality statistics should be calculated.

Percent Difference in Enumeration:

$$\mathrm{PDE} = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100,$$

where,
n_1 = the higher number of the counts by the two analysts
n_2 = the lower number of the counts by the two analysts

Percent Taxonomic Difference:

$$\mathrm{PTD} = \left(1 - \left[\frac{a}{N}\right]\right) \times 100,$$

where,
a is the total number of agreements (matches between Taxonomist 1 and Taxonomist 2) summed across all individuals and taxa and N is the total number of individuals identified in the larger of the 2 counts for a sample.

Calculations of PTD and PDE will be maintained in an Excel file on the FSB Network drive under the QAQC folder with cell-referenced equations built into the spreadsheet.

The goals for PDE and PTD are ≤ 10%. In other words, the taxonomic agreement and enumeration agreement should be at least 90%.

11.3 Voucher Collection

A voucher collection for projects should be retained for the period of at least two years after identification. However, the retention period may vary with project requirements. The voucher collection consists of all the samples that were identified for a project. The alcohol levels in the voucher samples must be checked periodically to ensure that adequate alcohol remains in the vial. If alcohol levels fall, replenish the alcohol with 70% ethanol. These periodic checks should be recorded in the Corrective Actions Log Book.

11.4 Reference Collection

FSB also maintains a reference collection with the list of taxa in Macroinvertebrate Reference Collection Log Book (SNB-239) (Appendix E). Any new taxa that are encountered during identification that are not already in the collection should be added to the collection. Each unique organism should be put in one vial. Specimen labeling should include both a locational label and a determination (det) label. The locational label should include the project name, the unique sample number, the state, the county,

a detailed location, the town, the date of collection, and the collector. The det label should include the taxon name, the person who made the identification, and the date of identification. The reference collection should be organized by order in the specimen cabinet. A list of all the reference taxa should be kept as a cross reference. This list should include the same type of information that is included on the locational and det labels, and should also be organized phylogenetically, by classes and orders.

The original macroinvertebrate identification sheet should clearly indicate that an organism has been removed from the voucher vial and placed in the reference collection. The new specimen should be first identified by one biologist, and then reviewed by a second independent biologist for verification. The reference collection list should clearly indicate the identity of the original and reviewing biologists. The new additions to the taxa list should be sent for outside verification as time and funds allow. Normally this occurs annually.

11.5 Audits

The QAO and optionally the Field Services Branch Chief perform periodic quality walkthrough of the sample processing areas. During the walkthrough, the QAO will review various aspects of activity including use, calibration and maintenance of support equipment, handling and storage of chemicals, handling and storage of samples, and general housekeeping. The QAO will also ensure that all support equipment is calibrated and maintained according to the manufacturer's schedule. During the walkthrough, the QAO will complete a checklist that documents any deficiencies or needed changes to the sample processing area or support equipment. Branch members will discuss the findings and work to correct deficiencies and make any necessary changes. The QAO will document the corrective actions on the checklist. The QAO retains the checklists and notes on corrective actions as quality control records. During the annual walkthrough, the QAO will also perform a review of the Quality Assurance Field Activities Procedure and Region 3 Field Operations Management System as it relates to Branch activities and note any Nonconformances or Concerns in a memo. The results of this review will be shared with all Branch members, and any corrective actions will be assigned, tracked, and documented as completed. The results and corrective actions will be filed by the QAO as an internal audit with the quality records.

The QAO and Branch members will work together to prepare for any internal or external audits. The QAO and Branch members will also work together to perform and document any corrective actions resulting from audits. The Branch Chief is responsible for ensuring corrective actions are completed. The QAO retains all audit materials as quality records.

12.0 REFERENCES

Anderson, T. P.S. Cranston, and J.H. Epler (editor). 2013. Chironomidae of the Holarctic Region. Keys and diagnoses, Part 1, Larvae. Entomological Scandinavica Supplement 66, 1-573.

Ciegler, Janet C. 2003. Water beetles of South Carolina: (Coleoptera: Gyrinidae, Haliplidae, Noteridae, Dytiscidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae, Limnichidae, Heteroceridae, Psephenidae, Ptilodactylidae, and Chelonariidae) (Biota of South Carolina). ISBN-10: 0971252777. Clemson University, Public Service Activities, Clemson, South Carolina, USA. 210 pages.

Epler, J. H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. Special Publication SJ2001-SP13.

Epler, J. H. 2010. The Water Beetles of Florida. An Identification Manual for the Families Chrysomelidae, Curculionidae, Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, and Scirtidae. Final report to State of Florida Department of Environmental Protection, Division of Environmental Assessment and Restoration, Tallahassee, FL

Gelhaus, J.K. 2002. Manual for the identification of aquatic Crane Fly larvae for Southeastern United States. Carolina Area Benthological Society, Durham, NC.

Merritt, R.W., K.W. Cummins, and M.B. Berg *(editors).* 2019. An Introduction to the Aquatic Insects of North America. 5th edition/revised edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Morse, J. C., W. P. McCafferty, B. P. Stark, and L. M. Jacobus, eds. 2017. Larvae of the Southeastern USA Mayfly, Stonefly, and Caddisfly Species (Ephemeroptera, Plecoptera, and Trichoptera). Biota of South Carolina. Vol. 9. Clemson University Public Service and Agriculture, Clemson University, Clemson, South Carolina, USA. 482 pp.

Needham, J.G., M.J. Westfall, and M.L. May. 2000. Dragonflies of North America. Scientific Publishers. Gainesville, FL.

Rogers, D. C. and M. Hill, 2008. Key to the Freshwater Malacostraca (Crustacea) of the Mid-Atlantic Region. EPA-230-R-08-017. US Environmental Protection Agency, Office of Environmental Information, Environmental Analysis Division, Washington, DC.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea. 4th edition. John Wiley & Sons, New York.

Stewart, K.W. and B.P. Stark. 2002. Nymphs of North American stonefly genera (Plecoptera). Second Edition. The Caddis Press, Columbus, OH. 510 pp.

Thorp, J.H. and A.P. Covich (eds.). 1991. Ecology and classification of North American freshwater invertebrates. Academic Press, New York, N.Y.

U.S. EPA Region 3. 2019 (or current version). Wheeling Office Occupant Emergency Plan. Wheeling, WV.

U.S. EPA Region 3. 2019 (or current version). Wheeling Office Chemical Hygiene Plan. Wheeling, WV.

Westfall, J.J., and M.L. May. 1996. Damselflies of North America. Scientific Publishers. FL. 650 p.

Wiggins, G.B. 1998. Larvae of the North American Caddisfly Genera (Trichoptera), Second Edition. University of Toronto Press. Canada. 457 p.

13.0 APPENDICES

Appendix A: Macroinvertebrate Identification Job Hazard Analysis

JOB HAZARD ANALYSIS

Hazard Types (HT)

1. Toxic Chemicals
2. Flammable Chemicals
3. Corrosive Chemicals
4. Environmental
5. Explosion (Chemical Reaction)
6. Explosion (Over pressurization)
7. Mechanical/Vibration
8. Electrical (Shock, Short Circuit)
9. Electrical (Fire)
10. Electrical (Static, ESD)
11. Electrical (Loss of Power)
12. Ergonomic (Overexertion)
13. Ergonomic (Human Error)
14. Vibration
15. Fall (Slips/Trips)
16 Fall (To a Different Level)
17. Excavation (Collapse)
18. Fire, Heat, Thermal, Cold
19. Noise
20. Radiation (Ionizing/Non-Ionizing)
21. Visibility
22. Weather
23. Caught (In, On, Between)
24. Struck (By, Against)
25. Driving
26. Confined Space
27. Other

Job Task: Macroinvertebrate Identification

Job Frequency/Duration:
30-100 days/year

Tools Used:
Microscope, Fiber optic illuminator, Tally counter, Dissecting forceps, Dissecting needles, 2 and 4 dram glass vials with polyseal screw caps, Plastic petri dishes with covers, Frosted microscope slides, Slide covers

Chemicals Used:
95% (200 proof) non-denatured ethanol, 70% non-denatured ethanol for storage of specimens, Mounting medium (e.g., CMC 9, CMC10), Immersion oil for microscope

CRITICAL TO SAFETY (CTS)

Risk Estimation Matrix

Probability of Occurrence of Harm	SEVERITY OF HARM			
	Catastrophic	Serious	Moderate	Minor
VERY LIKELY	Extreme	High	High	Medium
LIKELY	High	High	Medium	Low
UNLIKELY	Medium	Medium	Low	Negligible
REMOTE	Low	Low	Negligible	Negligible

* High = CTS tasks should receive engineering controls prior to assigning administrative or PPE controls.

Job Description: Personnel identify macroinvertebrate samples in the Wheeling, WV office.
Required Training H&S: 24 HR Health and Safety with 8 HR annual H&S refresher

Step #	Procedures (LOP Procedure Step)	Potential Hazards	HT	Check CTS	Recommended Safe Practice	PPE

1	Handling containers, sample vials and petri dishes of samples with ethanol throughout the process.	Ethanol exposure, flammability	2, 18	Low	For vials or other sample containers, internal labels should note that the jar contains 70-100% ethanol and an overall sign is placed in the specimen storage and flammable cabinets with this same information. Vial boxes must be labeled to say all vials contain 70-100% ethanol. Bulk containers of ethanol should always be stored in flammable storage cabinets. Squeeze bottles with ethanol must be labeled with the chemical name, approximate concentration of ethanol, and dated and initialed when refilled. Safety glasses and nitrile gloves should be worn when refilling smaller containers from bulk containers in case there is liquid splash. When a	When risk of liquid splash with ethanol (e.g., handling bulk containers or cleaning up large spills), wear safety glasses and nitrile gloves.
					bulk ethanol container is emptied, the container should be rinsed with water. If samples are preserved with denatured ethanol, exposure to the samples should be minimized. Samples collected by the Field Services Branch are preserved with non-denatured ethanol which is safer. It is advisable to cleanse exposed parts of the body with soap and water after contact with samples. Small amounts of ethanol can be rinsed down the sink without an impact to water quality, but care should be taken to minimize the volume. If a vial happens to break, it should be disposed of in the "glass only" waste container in the sample processing area. If waste ethanol is present, place in marked waste ethanol container in flammable storage cabinet in sample processing area and record amount placed in container on associated log. For minor spills (which can be handled by the analyst), wear safety glasses and gloves to clean up the material by wiping up immediately with paper towels, a sponge or spill pillows. For major spills, immediately contact the safety office and facilities manager.	
2	Using microscopes to identify samples.	Ergonomic injuries	12, 13	Medium	Limit time on microscopes as needed to ensure physical safety and health and engage in best ergonomic practices available. Properly adjust the microscopes for viewing.	N/A

3	Mount specimens on slides for identification if applicable.	Mounting Media and immersion oil	1	Low	Read the safety data sheet before handling. Cleanse exposed parts of the body with soap and water after contact. Wear gloves.	Nitrile gloves

HAZARDS –NOTE ALL POTENTIAL HAZARDS ASSOCIATED WITH THE JOB (CHECK ALL THAT APPLY)

Physical						
General	☐	thermal stress	☐	cold	☐	Noise
	☐	explosion	☒	fire	☐	Weather
	☒	fatigue	☐	violence	☐	illness/injury
Radiation	☐	ionizing	☐	microwave	☐	Light
Vehicles	☐	traffic	☐	heavy equip	☐	forklift
	☐	helicopter	☐	small aircraft	☐	boat
Boat Ops	☐	sediment sampling	☐	rapid water	☐	open water
	☐	diving	☐	electrofishing		
Industrial	☐	comp gas	☐	electricity	☐	confined space
	☐	equip	☐	moving parts		
Overhead	☐	obstruction	☐	falling objects		
Elevation	☐	roof	☐	scaffold	☐	ladder
	☐	stairs	☐	catwalk		
Slips/trips	☐	terrain	☐	debris	☐	slippery
	☐	trench	☐	pits/holes		
Other physical hazards:			☒	Ergonomics		

Biological						
Agriculture	☐	CAFO	☐	fish	☐	farm animals
Animals	☐	dogs	☐	feral animals	☐	snakes
Arthropods	☐	spiders	☐	mosquitoes	☐	wasp/hornet
	☐	bees				
Pathogens	☐	bloodborne	☐	sewage	☐	med/lab
Other Biological:	☐	Poisonous vegetation				

Chemical						
Containers	☐	ammonia	☐	chlorine	☐	other
VOCs	☐	solvents	☐	fuel	☒	oils
Wastes	☐	sewer	☐	landfill	☐	smoke/dust/fume
	☐	metals	☐	PCBs	☐	paints/surfacing
Particulates	☐	fibers	☐	diesel	☐	asbestos
Sampling	☐	acids	☐	bases		
Other Chemicals:	☒	Ethanol, mounting media				

PERSONAL PROTECTIVE EQUIPMENT (PPE) REQUIRED (CHECK ALL THAT APPLY)

Feet:	☐	safety boots	☐	steel-toe boots	☐	shank
	☐	rubber boots	☐	waders	☐	Other: Site specific
Gloves:	☐	leather	☐	cotton	☐	cut-resistant
	☒	chemical resist	☐	disposable		
Body:	☐	safety vest	☐	PFD	☐	harness
	☐	tyvek	☐	sarnex-tyvek	☐	coveralls
Eyes:	☒	safety glasses	☐	sunglasses	☐	goggles
Head:	☐	hard hat	☐	hearing protection	☐	respirator

OTHER REQUIRED SAFETY EQUIPMENT/TRAINING

☐	dosimetry	☐	communication	☐	decon
☒	first aid kit	☒	fire extinguish	☐	flares
☐	chains/studs	☒	eye wash/shower		

☐	24 hr HAZWOPER	☐	40 hr HAZWOPER	☐	HAZWOPER Annual Refresher
☐	TLD Program	☐	RPP Program	☒	Medical Surveillance
☐	1st Aid/CPR	☒	Other: 24 Hr Health & Safety and 8Hr H&S annual refresher		

COMMENTS:

For macroinvertebrate identification, a 2-4 dram glass vial containing benthic macroinvertebrates is emptied into a plastic petri dish. Additional ethanol is added to the dish from a squeeze bottle, and little exposure from ethanol occurs during this process. The squeeze bottle is refilled as needed from a bulk ethanol container and care should be taken when refilling as there can liquid splash or spills (safety glasses and nitriles gloves should be worn). The petri dish is placed under a microscope for identification. A lot of time is spent at the microscope so ergonomics should be enhanced to reduce fatigue and health hazards:

1. Adjust the microscope to you- Find your most comfortable working position by defining your "free space" zone. Find your most comfortable working position that minimizes leaning in at an awkward angle. Then re-adjust the microscope to fit you. Elevate, tilt, or move the microscope close to the edge of the counter to avoid bending your neck. Adjust your chair or microscope as needed to maintain an upright head position. Keep elbows close by your sides.
2. Optimize your working environment- Place tools, vials, and keys within an optimal working distance without cluttering or impeding workspace. Use a chair that provides good back support. Sit close to your work surface. Remove supplies from under the work area. Avoid leaning on hard edges.
3. Limit eye fatigue- Adjust the microscope according the R3 LSASD Field Services Branch Macroinvertebrate Identification Standard Operating Procedure. Spread microscope work throughout the day, if possible. Take breaks. Every 15 minutes close your eyes or focus on something in the distance. Every 30-60 minutes get up to stretch and move.

If specimens need mounted on microscope slides to aid identification, use mounting media to prepare slides and wear gloves while in contact with media.

CERTIFICATION OF HAZARD ASSESSMENT	
SUPERVISOR: NORMAN RODRIGUEZ **DATE:** Digitally signed by NORMAN RODRIGUEZ Date: 2021.05.13 17:36:05 -04'00'	**SAFETY/HEALTH REPRESENTATIVE: RACHAEL KANE** **DATE: 5/13/2021**

Personal Protective Equipment Recommendations

Where engineering and administrative controls are not feasible or sufficient for controlling hazards, PPE must be used to protect workers. The following PPE are recommended for the noted tasks above:

Eye and Face Protection			
☒	Safety glasses with side shields	☐	Reflective goggles/face shield
☐	Chemical splash goggles	☐	Cutting/brazing/welding eye protection
☐	Face shield	☐	Other:
Head Protection			
☐	Hard hat	☐	Helmet, cowl, hood
☐	Welding helmet/mask	☐	Other:
Foot Protection			
☐	Steel-toed safety shoes/boots	☐	Other:
☐	Chemical-resistant boots	☐	
Body Protection			
☐	Apron (splash, work)	☐	Head-reflective garments
☒	Lab coat	☐	Sleeves (cut-resistant)
☐	Coveralls (work, chemical-resistant) Hazard Type: Fire Resistant Type coverall: Coverall	☐	Other:
Respiratory Protection			
☐	Respirator	☐	Type of respirator:
Hand Protection			
☐	Rubber insulating gloves	☐	Rubber insulating sleeves
☐	Rubber insulating hoods	☒	Other: Nitrile gloves.

Other:

PPE Hazard Assessment Form

HEALTH AND SAFETY HAZARDS		
Chemical Hazards		**Description/Mitigation**
☐	Vapors/gases	
☒	Dusts/mists/fumes	Personnel may be potentially exposed to fumes from ethanol.
☒	Liquid splash	Although minimal, personnel may be potentially exposed to splash from refilling smaller ethanol containers from bulk containers and should wear eye protection.
☐	Other	
Comments:		

Physical Hazards		**Description/Mitigation**
☒	Ergonomics	Personnel may experience repetitive motions and prolonged postures. Adjust microscope to appropriate height or sit in adjustable stool for prolonged work.
☐	Heat — sparks, molten splash, high temperatures	
☐	Cold — cryogens, cold temperatures	
☐	Electricity	
☐	Noise	
☒	Fire/Explosion	Ethanol is highly flammable. All heat sources are to remain off or six feet away from chemical. If vapors accumulate in the area, stop work, and provide additional ventilation to the area.
☐	Slips/Trips/Falls	
☐	Elevation - Falls	
☐	Other –	
Biological Hazards		**Description/Mitigation**
☐	Animals/Insects	
☐		

Completed by: KELLY KROCK Digitally signed by KELLY KROCK Date: 2021.05.13 17:42:25 -04'00' **Date:**_____

SHEM Review: **Rachael Kane** **Date:** **5/13/2021**

Appendix B: Target Identification Levels for Mid-Atlantic and Close Regional Organizations

Entity	Sampling / assessment protocols	Target ID levels	Notes	Reference Web Site
PADEP	Smaller freestone riffle-run streams (< 25 - 50 sq. mi.) Limestone spring streams Low gradient, pool-glide streams	Genus, except Chironomidae, snails, clams mussels (fam); Nematoda, Nemertea, Bryozoa (phy); Turbellaria, Hirudenia, Oligochaeta (class); water mites (artificial)		https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Data-Collection-Protocols.aspx
WVDEP	Wadeable streams (WVSCI) Wadeable streams (GLIMPSS)	Family (all insects); only ID to family for Oligochaeta, Turbellaria, Hirudinea and Class for the Nemertea, Nematoda, Hydroida, Bryozoa Genus (all insects minus Collembola); only ID to family for Oligochaeta, Turbellaria, Hirudinea and Class for the Nemertea, Nematoda, Hydroida, Bryozoa		https://dep.wv.gov/WWE/watershed/Pages/WBSOPs.aspx
MDDNR	Maryland Biological Stream Survey (MBSS)	Genus (or lowest practical); crayfish and mussels identified to species (sometime sub-species?) in the field along with fish, reptiles, amphibians and some invasive plants		https://dnr.maryland.gov/streams/Pages/publications.aspx
DNREC	Biological Index, Piedmont Coastal Plain (CPMI)	Genus, or lowest practical		https://dnrec.alpha.delaware.gov/watershed-stewardship/assessment/
VADEQ	Coastal Plain (CPMI) Non-coastal Plain (VSCI)	Family Family	Identifications done to Genus level but IBIs calculated based on family level	https://www.deq.virginia.gov/water/water-quality/monitoring/biological-monitoring
CBP	CBP BIBI	Family		https://www.potomacriver.org/focus-areas/aquatic-life/chessie-bibi-stream-health-indicator/
ICPRB	Potomac BIBI	Family		https://www.potomacriver.org/focus-areas/aquatic-life/

Entity	Sampling / assessment protocols	Target ID levels	Notes	Reference Web Site
DRBC	DRBC Biomonitoring	Genus (or lowest practical)		https://nj.gov/drbc/programs/quality/biomonitoring.html
SRBC	All programs	Genus (or lowest practical)		https://www.srbc.net/our-work/programs/monitoring-protection/
NJDEP	New Jersey Impairment Score (NJIS)	Family		https://www.state.nj.us/dep/wms/bfbm/amnet.html
	High-gradient macroinvertebrate index (HGMI)	Genus		
	Coastal Plain Macroinvertebrate Index (CPMI)	Genus		
	Pinelands Macroinvertebrate Index (PMI)	Genus		
NYSDEC	The Biological Assessment Profile (BAP)	Genus, species level although target levels vary based on group (see their SOP).		https://www.dec.ny.gov/chemical/23847.html
OHEPA	Invertebrate Community Index (ICI)	Lowest practical		https://www.epa.state.oh.us/dsw/bioassess/BioCriteriaProtAqLife
ORSANCO	Non wadeable	Species level identifications are made wherever possible and practical. Generic or higher level classifications are made if specimens are damaged beyond identification, in those cases where taxonomy is incomplete or laborious and time-consuming, or where the specimen is an unidentifiable early instar.		https://www.orsanco.org/programs/macroinvertebrates/

Appendix C: Macroinvertebrate Identification Sheet Template

MACROINVERTEBRATE IDENTIFICATION SHEET

SOP # R3QA-1003, PNB 160 Page

PROJECT NAME:	TYPE OF NET: TOTAL SUBSTRATE AREA:
SAMPLE NO.:	REPLICATE (Y / N):
STATION NO.:	COLLECTED BY:
STREAM NAME:	IDENTIFICATION BY: ID DATE:
LOCATION:	SUBSAMPLE PORTION / TARGET COUNT: # OF GRIDS

	1ST SORT		2ND SORT			1ST SORT		2ND SORT	
ORGANISM	No.	LS	No.	LS	ORGANISM	No.	LS	No.	LS

ORGANISM	No.	LS	No.	LS		
					NUMBER OF ORGANISMS	Notes:
					TOTAL TAXA EPT TAXA NO. NEW TAXA	
					SORT ERROR (%) N/A___	
					PTD (%) PTE (%) N/A___	
					Data Entry/Proof: Initials_____/_____ Date _______/_______ Electronic Location	
LS = life stage: I= Immature; P = pupae; A = adult					Quality Metrics Entry: Initials______ Date ___________ Electronic Location	

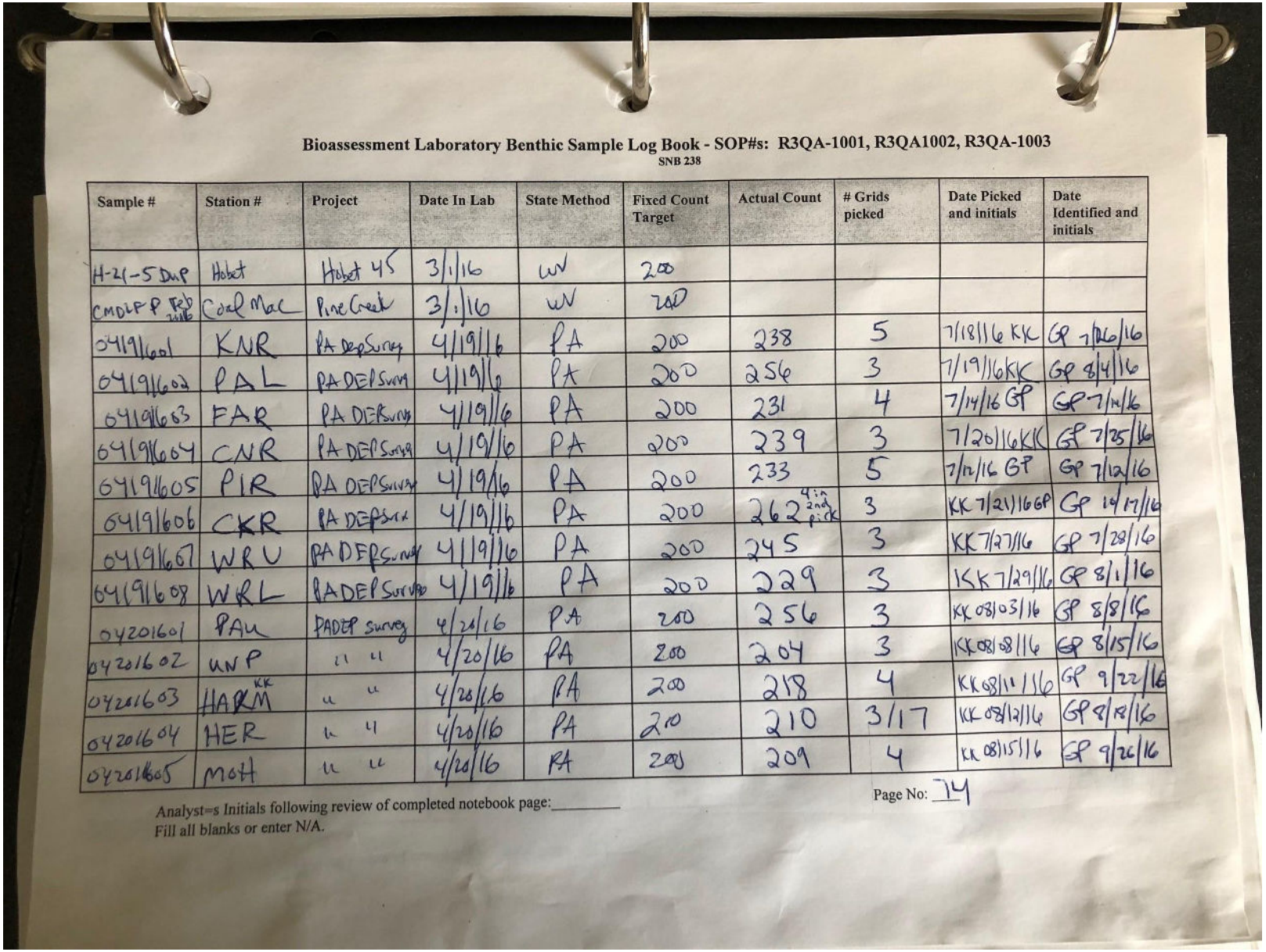
Appendix D: Benthic Sample Log Book

Sample #	Station #	Project	Date In Office	State Method	Fixed Count Target	Actual Count	# Grids sorted	Date Sorted and initials	Date Identified and initials

Analyst's Initials following review of completed notebook page:_____ Page No: ____

Fill all blanks or enter N/A.

Example Completed Version- Benthic Sample Logbook



Bioassessment Laboratory Benthic Sample Log Book - SOP#s: R3QA-1001, R3QA1002, R3QA-1003

SNB 238

Sample #	Station #	Project	Date In Lab	State Method	Fixed Count Target	Actual Count	# Grids picked	Date Picked and initials	Date Identified and initials
H-24-5 Dup	Hobet	Hobet 45	3/1/16	WV	200				
CMDLPP Feb 2016	Coal Mac	Pine Creek	3/1/16	WV	200				
04191601	KNR	PA DepSurvey	4/19/16	PA	200	238	5	7/18/16 KK	GP 7/26/16
04191602	PAL	PA DEP Surv	4/19/16	PA	200	256	3	7/19/16 KK	GP 8/4/16
04191603	FAR	PA DEP Surv	4/19/16	PA	200	231	4	7/14/16 GP	GP 7/14/16
04191604	CNR	PA DEP Surv	4/19/16	PA	200	239	3	7/20/16 KK	GP 7/25/16
04191605	PIR	PA DEP Surv	4/19/16	PA	200	233	5	7/12/16 GP	GP 7/12/16
04191606	CKR	PA DEP Surv	4/19/16	PA	200	262 4 in 2nd pick	3	KK 7/21/16 GP	GP 10/17/16
04191607	WRU	PA DEP Surv	4/19/16	PA	200	245	3	KK 7/27/16	GP 7/28/16
04191608	WRL	PA DEP Surv	4/19/16	PA	200	229	3	KK 7/29/16	GP 8/1/16
04201601	PAU	PADEP survey	4/20/16	PA	200	256	3	KK 08/03/16	GP 8/8/16
04201602	UNP	" "	4/20/16	PA	200	204	3	KK 08/08/16	GP 8/15/16
04201603	HARM KK	" "	4/20/16	PA	200	218	4	KK 08/11/16	GP 9/22/16
04201604	HER	" "	4/20/16	PA	200	210	3/17	KK 08/12/16	GP 8/18/16
04201605	MOH	" "	4/20/16	PA	200	209	4	KK 08/15/16	GP 9/26/16

Analyst=s Initials following review of completed notebook page:_____

Fill all blanks or enter N/A.

Page No: 14

Appendix E: Reference Collection Log Book

USEPA, Wheeling, WV
Macroinvertebrate Reference Collection
Master List
Additions

ORGANISM	PROJECT	SAMPLE NUMBER*	SITE	STATE: COUNTY	LOCATION	FAM ID	FAM VER	GEN ID	GEN VER	COMMENTS

Appendix F: Corrective Actions Log Book Template

Date	Action

Analyst's Initials following review of completed notebook page:_____

Page No: ____

Appendix G: Generating Random Numbers in Microsoft Excel

1. Open Excel.

2. Go to View>Page Layout.

3. Select all the columns above the page by clicking on the first column A and then draggingyour mouse through column F. While A-F are still selected, place cursor on any line between columns F and click and drag to widen columns until only A-F (i.e., 6 columns) are on a page. Space between columns allows for notes.

4. Add any desired header or footer information.

5. In the first cell, enter the formula:
   =RANDBETWEEN(1,154)
   where 154 represents the maximum number of grids in your Chironomidae subsamplingtray.

6. Copy this cell (ctrl + c).

7. Select all cells on multiple pages and then paste the first cells content (ctrl + v).

8. Print.