1 PROJECT MANAGEMENT

1.1 Title of Plan and Approval

Quality Assurance/ Quality Control Protocol Virginia Save Our Streams Program

Eastern Biomonitoring Method for Muddy Bottom Streams January 2024

The Virginia Save Our Streams Program (VA SOS)

A program of the Virginia Chapter of the Izaak Walton League of America

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1.3 Distribution List

The following groups and people will receive copies of the VA Save Our Streams (VA SOS) quality assurance plan for sampling muddy bottomed streams with the VA SOS Eastern Biomonitoring Method for Muddy Bottom Streams:

VA Save Our Stream Staff:

- Margaretta Dombroski, Coordinator
- Matthew Kierce, Coordinator
- Samantha Briggs, Clean Water Program Director
- Other appropriate personnel to be determined

VA Department of Environmental Quality Personnel:

- Quality Assurance Coordinator- Reid Downer
- Biological Monitoring Coordinator- Rick Browder
- Other appropriate personnel to be determined

VA Department of Wildlife Resources

• Shirls Dressler, Wildlife Permit Specialist

US Environmental Protection Agency

• Appropriate personnel to be determined Groups using VA SOS methods

VA SOS Regional Trainers

The quality assurance plan will also be provided to anyone requesting it, and will be made available on the VA SOS website (www.vasos.org).

1.4 Project/Task Organization

Virginia Save Our Streams Program Coordinator or Designee

- Provides training and follow-up testing to volunteers
- Trains additional regional trainers and quality assurance auditors
- Acts as quality assurance auditor when necessary
- Develops and maintains partnerships with groups and agencies across the state
- Assists in site selection
- Assist volunteers who have failed quality assurance procedures to correct problems
- Database manager Reviews all incoming data, assesses for inclusion in database, makes all updates to database, makes the data available through reports and on the Clean Water Hub (www.cleanwaterhub.org) and the Chesapeake Data Explorer (www.cmc.vims.edu)
- Maintains databases of trained, certified, regional trainers, and quality assurance auditors
- Ensures field sheets and training materials are up to date

- Identifies, analyzes, and stores incoming quality assurance samples
- Identifies incoming unknown specimens for volunteers
- Develops and maintains reference and testing collections

VA SOS Regional Trainers

- Locally trains and certifies volunteers
- Maintains equipment needed to train volunteers

VA SOS Regional Coordinators

- Does initial review and updates of local data and sends it to VA SOS Coordinator or designee in a timely fashion
- Makes sure volunteers in their area are progressing to certification and doing their sampling in a timely manner
- May maintain database of local monitoring data and volunteer monitors
- May purchase and maintain approved sampling equipment for volunteer monitors
- May assist in site selection.
- May develop and maintain reference and testing collections

VA SOS Quality Assurance Auditors

- Periodically goes into the field with volunteers to review their equipment, procedures and macroinvertebrate identification
- Sends results of these observations to VA SOS Coordinator or designee in a timely fashion

VA SOS Volunteers

- Attends the proper training and passes the certification test
- Purchases and maintains approved sampling equipment
- Monitors adopted site(s) at least two times a year or assist in the monitoring of other VASOS monitoring locations.
- Follows proper procedure for maintaining certification status

VA SOS Data Users

There are a wide variety of data users for this statewide program. These users include the Virginia Department of Environmental Quality (DEQ), the Virginia Department of Conservation and Recreation (DCR), the Chesapeake Bay Program, local Soil and Water Conservation Districts, localities, planning commissions, and universities. The VA SOS data is available to any interested party at vasos.org on the Clean Water Hub (www.cheanwaterhub.org), the Chesapeake Data Explorer (www.cmc.vims.edu) or by request.

Virginia Save Our Streams recommends that all potential data users contact the VA SOS Coordinator to discuss the use of the volunteer collected data and the appropriate uses of this data.

1.5 Problem Definition/Background

1.5.1 Problem Statement

With the passage of the Clean Water Act in the early seventies, there has been a focus on cleaning up our nation's waterways. Great strides have been made in reducing point source pollution, or that pollution that enters the stream through a specific known source, such as a discharge pipe. Discharging parties must obtain permits and are regulated to prevent too much pollution from entering our waterways.

While our waterways have greatly improved since these efforts were implemented, there are still steps to be taken. In the last ten years, there has been a shift in thinking to include non-point source pollution in addition to the point sources. Non-point source pollution is hard to regulate, as it comes from a broad area rather than one easily located source. Non-point source pollution includes nutrient additions and erosion from livestock in streams, runoff of fertilizer from agricultural fields and suburban lawns, and stormwater runoff carrying not just large pieces of litter but also all the oils and chemicals on our roadways and parking lots. It takes a broader monitoring plan to detect these types of pollution and to determine their origin.

This means that already overburdened state agencies must increase the monitoring they must do throughout the state. There are thousands of miles of streams in Virginia that must be monitored, and agencies have very limited resources with which to monitor all these streams. With current workloads and limited resources, it is not feasible that the majority of these streams are monitored on a regular basis. This is where the Virginia Save Our Streams Program helps.

1.5.2 Intended Usage of Data

The Virginia Save Our Streams Program has monitors across the state collecting large quantities of benthic macroinvertebrate data. The data collected under this quality assurance plan will be used in DEQ water quality assessment reports including the 305(b)/303(d) Integrated Report. It will be used to identify waters were agency scientists will conduct follow-up monitoring to identify if the water should be classified as impaired on the 303(d) report. VA SOS data will not be used to list streams on the 303(d) report. Instead, it can be used to identify pollution incidents when immediate agency response is required to mitigate the pollution event. VA SOS data may also be used in the development and implementation of Total Maximum Daily Load (TMDL) plans.

Data collected as part of VA SOS within the Chesapeake Watershed is also included in the Chesapeake Monitoring Cooperative's database (www.cmc.vims.edu) where it passes along to the Chesapeake Bay Program for use in their status and trends of stream health. In addition, the data collected by VA SOS volunteers can be used locally by Soil and Water Conservation Districts when looking at the effectiveness of implemented best management practices (BMPs). It can also help determine where future BMPs should be implemented. Localities can also use the volunteer data in evaluating current land use practices, to create an integrated water quality management approach to land use development, and to identify pristine conditions so that future developments do not degrade local streams.

1.6 Project/Task Description and Schedule

Throughout the year, monitors attend VA SOS training and certification sessions. This program

continues year after year. These sessions are held on an as needed basis.

The VA SOS volunteers monitor the benthic macroinvertebrate populations and the habitat of their adopted stream at least two times a year, fall and spring, using a method developed for the VA SOS program by Randolph Macon College scientists (Gowan, 2004). This method is outlined in the Sampling Methods Requirements section of this document (Appendix O). The samples are analyzed in the field using a multimetric index developed as part of the Randolph Macon study. Additional information about the analysis can be found in the Analytical Methods Requirements section of this document. This method was also approved by the Chesapeake Bay Program to be used through the Chesapeake Monitoring Cooperative. The field analysis gives a water quality score to let the volunteer know if the ecological conditions of the stream are acceptable or unacceptable.

VA SOS volunteers are also asked to record general site conditions and fill out a streamside visual assessment sheet.

Data is submitted and reviewed by regional coordinators and the VA SOS Coordinator or designee biannually. Data is compiled in a database that is kept current. Reports are made to interested parties whenever requested, and data is updated to the Clean Water Hub and the Chesapeake Data Explorer annually.

1.7 Quality Objectives and Criteria for Measurement Data

1.7.1 Data Precision, Accuracy, Measurement Range

The VA SOS modified method was developed and tested by scientists at Randolph Macon College (Gowan 2004), to accurately represent the stream condition and compare favorably with the results VA Department of Environmental Quality professional biologists would find when sampling the same sites. This method compared favorably with agency findings, and was found to be a good method for volunteers to use to determine the condition of their streams (Gowan 2004).

1.7.2 Data Representativeness

For the VA SOS program, representativeness depends largely upon site selection. Volunteers are requested to select sites that are representative of the stream and the conditions that are influencing the stream (see Appendix M). However, volunteers are asked not to monitor below permitted discharges. In selecting a monitoring site, volunteers survey the stream section to determine the most appropriate and representative stream segment. Also, more than one sample (jab) in the stream segment is collected. The jabs are combined into a single sample. The sample for the stream is then sub sampled and the results are composite into the final score.

1.7.3 Data Comparability

VA SOS ensures comparability requiring all volunteers to use the protocol designed by scientists at Randolph Macon. This protocol includes taxonomic keys to identify macroinvertebrates correctly. VA SOS also maintains several sets of reference collections for use by volunteers in the field.

1.7.4 Data Completeness

VA SOS does not apply rigorous completion standards to the volunteers collecting data. VA SOS expects each monitoring site to be monitored at least two times during the course of a year, in the spring and the fall. The completion of these monitoring events during the year is hampered by several factors: the need for the site (as identified by the monitor or regional coordinator) may have changed during the course of the year or the volunteer may have dropped from the program, drought conditions may prohibit monitoring, a volunteer may be sick, or conditions at the site may have changed. We do instruct volunteer monitors that monitoring over an extended period of time and during the same approximate times per year provides the most useful data. Some more established volunteer groups may begin a rotating sampling program, capturing data at a site in the spring and fall of the calendar year and rotating to another site the following year.

1.8 Special Training Requirements/Certification

As the VA SOS program has a hierarchy of volunteers to help administer the program, different training and certification requirements may apply.

VA SOS Volunteer

Persons interested in becoming a VA SOS volunteer must attend at least one training session given by VA SOS staff or a certified regional trainer. This training session includes information about the program and basic watershed education, safety information, instruction in methods of collection and analysis, instruction in macroinvertebrate identification, and hands on field experience with the methods (Appendix B). After this training event, the volunteer then has up to 24 months to practice the method and identification before becoming certified. This practice can be done alone, with other volunteers, or at other official training sessions. If it has been over 24 months since the volunteer last attended an official training session, they must attend another session before becoming certified. The volunteer cannot be certified during their initial training session. If a volunteer conducts aquatic insect studies as their profession, they may be able to skip the macroinvertebrate identification training session and just take the certification test.

The certification process includes an in-stream observation and a macroinvertebrate identification test. VA SOS staff or a regional trainer may administer the certification procedure. The in-stream observation consists of the volunteer completing an entire sampling session (collecting and processing an entire sample and completing the habitat assessment), while the person doing the certification fills out an observation report (Appendix C). This portion of the test is open book and can be completed as a team with other volunteers attempting certification. If a larger group is being trained, a trainer or VASOS staff may follow up with an online protocol test instead of filling out an observation report (Appendix C).

The identification portion of the process can be taken as a written test with a VA SOS staff or volunteer trainer (Appendix C). There are 24 lettered, unidentified vials containing preserved representatives of groups used in the VA SOS method. The volunteer must identify at least 21 vials correctly in order to pass. Volunteers have up to 90 minutes to complete this test. The identification portion of the process can also be taken online through IWLA's online quiz. Volunteers must identify 38/42 photos correctly or higher to pass the online macroinvertebrate identification quiz.

Within two months of successfully completing both parts of the certification process, the volunteer receives a certificate indicating they are a VA SOS monitor. If the volunteer continues to pass further quality assurance measures (see Quality Control Requirements), they will remain a certified volunteer. If the volunteer misses sampling for two consecutive calendar years, they will lose their certification status and must go through the certification process again.

Quality Assurance Auditor

Volunteers wishing to become quality assurance auditors must have been a certified volunteer for at least 6 months and have completed at least two monitoring events. During these two monitoring events, the volunteer must have demonstrated their ability to follow the method by completely and accurately filling out the data forms for all monitoring events.

If the interested volunteer meets these requirements, they will attend a training session with VASOS staff that teaches him or her how to conduct an audit of a volunteer. During this session, equipment needs and condition is covered, as are proper methods. How to complete the audit checklist used during the audit is covered (Appendix D).

The auditor must complete at least two audits every two years to remain an auditor, and must send the audit forms to the VASOS coordinator within three weeks of completion. Incoming audits are reviewed by the Coordinator or designee. If the audit form is not be filled out properly, the Coordinator or designee works with the auditor to improve their auditing performance. Should the auditor fail to properly complete the forms on more than twice in a year, they are required to attend another auditor training session or will lose their auditing status.

Regional Coordinator

As this is a local organization position, no additional training is required to be a regional coordinator. However, the VA SOS staff will remain in close contact with the regional coordinators and will act as a resource to these volunteers. In addition, the Coordinator or designee will remain in close contact with these volunteers to help them learn to assess the incoming data for completeness and how to respond to incomplete data forms.

Regional Trainers

A thorough understanding of benthic macroinvertebrate collection and identification methods and QA/QC procedures implemented by this project and their individual monitoring project. This can be achieved through prior knowledge and experience (as deemed appropriate by the VA SOS Coordinator) or by being a Certified Monitor for at least six months and completing two macroinvertebrate sampling events. During these two monitoring events, the volunteer must have demonstrated their ability to follow the method by completely and accurately filling out the data forms for all monitoring events. The potential trainer must also have observed at least two training sessions implemented by VA SOS staff or regional trainers. The initial training session a volunteer attended to become a monitor may count as one of these sessions. They should also help coordinate one training session before they can be certified as a trainer. In addition, the volunteer must feel comfortable talking in front of a group, and must remember that they are representing the VA SOS program while training volunteers so must accurately and correctly represent the goals and opinions of the VA SOS program.

Should the volunteer meet these requirements, they must go through an additional training session administered by the VA SOS staff before training other volunteers. This training includes a discussion of what is involved in a training session. A checklist of these items will be given to each regional trainer during this training session (Appendix B). In addition, the training session will cover how to be an effective trainer, frequently asked questions, reference collections, and the certification process. The potential regional trainer must complete the macroinvertebrate identification portion of the certification process again, but must receive a 100% in order to become a trainer. (The same form will be used for both the certification process and the regional trainer process – Appendix C).

Once the regional trainer successfully completes the training requirements, they will enter an observational period. VA SOS staff must observe the regional trainer's teaching abilities and demonstration of the protocol for review and comment on the trainer's performance. A training observation report will be completed at that time and a copy will be returned to the trainer within three weeks of the training (Appendix E). The regional trainer must complete at least one training session and certify at least one volunteer per year in order to remain a trainer. In addition, the trainer must undergo an observation by VA SOS staff in person or by video once every two years.

1.9 Documents and Records

Volunteer Field Sheets

All volunteers complete a field sheet packet at each sampling event (Appendix A). The packet includes a front informational sheet, which includes date, location, sampling team, and some basic physical stream information. The second sheet contains raw macroinvertebrate counts, the third sheet has individual metric calculations, and the forth sheet is a multimetric index calculation. The fifth sheet is a habitat assessment form.

The volunteer saves a copy of these forms and/or sends either hard copy or electronically, to their regional coordinator. The volunteer or the regional coordinator will submit their data electronically using the VA SOS Database (www.vasosdata.org). The volunteer or regional coordinator will save a hard copy or digital copy of each datasheet for 5 years.

Electronic data submissions will be reviewed by the Coordinator or designee and uploaded to the Clean Water Hub (https://www.cleanwaterhub.org/), where they are permanently saved. Back-up copies of the database are housed permanently elsewhere outside of the main VA SOS office.

Training and Certification Forms

A liability and photo-release form should be completed at each training session, whether it be for volunteers, quality assurance auditor, or regional trainer training (Appendix F). Once a volunteer completes all of their certification requirements, the regional trainers or coordinators complete a Monitor Report Form and send a digital copy of these sheets to the VA SOS office within three weeks of the training session, and retain a copy for themselves. The Coordinator or designee will maintain a permanent database of all volunteers. Back-up copies of this database are housed at the main VA SOS office. Digital copies of Monitor Report Forms will be kept on file in the VA SOS offices for a minimum of five years, and then recycled.

Quality Assurance Forms

A copy of forms filled out by the quality assurance auditor should be sent to the Coordinator or designee within three weeks of the audit (Appendix D). The pass/ fail status of each volunteer will be recorded in the database of volunteers. A copy of the audit will be sent to the volunteer(s) in question, and a copy will be kept on file for a minimum of five years at the VA SOS offices.

All samples preserved for quality assurance purposes (See Quality Control Requirements) must be properly labeled with a sample submittal form (Appendix D). This form will be kept with the sample at all times. After these samples have been identified, the laboratory record sheet (Appendix G) will be housed in the VA SOS records for a minimum of five years, then recycled. The pass/fail status will be recorded in the database of volunteers, and a copy of this status will be sent to the volunteer(s) in question. Preserved samples will be archived for a minimum of two years, then the organisms will be used in reference collection development or donated to a school, college, or university.

The results of the quality assurance audit and identification check will be sent to the volunteer(s) in question within three months of the audited monitoring event.

Unknown Specimen Submittal

All unknown specimens needing identification by the Coordinator or designee should be photographed and emailed to VASOS staff. After identification, the form will be completed by the Coordinator or designee. A copy of the form will be filed in the VA SOS offices for a minimum of five years, and a copy of the form and the unknown specimen will be returned to the volunteer. Submitted data that is quality assured should not have more than 5 unknowns in the sample.

2 DATA GENERATION AND ACQUISTION

2.1 Sampling Design

Volunteers collect macroinvertebrate samples and complete the VA SOS Field Sheets at least twice per year, in the fall and spring. While sampling can occur any time during a season, it is recommended that sampling occur between March 1st – May 31st and September 1st – November 30th, on a regular basis (Appendix N). Descriptive location information and latitude and longitude identify each monitoring site.

Most volunteers have a specific stream they wish to monitor. Often, this stream is located in close proximity to their home, or they spend time on the stream for recreational purposes. To promote continued interest and involvement in the VA SOS program, it is important for the volunteers to be allowed to monitor these locations. Some monitors do not have a specific spot in which they are interested, but rather wish to monitor somewhere in their watershed of interest. In such a case, the VA SOS staff, together with representatives from DEQ and DCR and the help of GIS maps and the Clean Water Hub, assess where current volunteer and agency monitoring is occurring, and helps the volunteer choose the site where they can be most effective. Site selection will also take in consideration potential uses of the data (background information, assess effectiveness of BMPS, monitor land use changes, etc). All sites must be located on public property, or the volunteer must obtain written permission if they choose to monitor private property. Sites are added to the program as often as new volunteers are trained. Sites may also be changed if the need for the monitoring site has changed. For example, if a volunteer chooses a site below a construction site to evaluate potential impacts, once the construction is complete, the volunteer may choose to abandon the site.

Volunteers are not to conduct their normal sampling within one week of heavy rainfall (approximately more than 1 inch of rainfall in rural areas or ½ inch of rainfall in urban areas). Rather, they should sample the stream during its average conditions for that season, and can use the USGS stream gauge website as a guide (https://waterwatch.usgs.gov/?m=real&r=va).

If the volunteer is not going to be able to complete their sampling for a season, they should alert their regional coordinator or the VA SOS staff, and assist them in locating a substitute volunteer for that season.

DWR must be notified of streams that are to be sampled prior to the sampling events. As soon as volunteers know where and when they will be sampling, or at least 48 hours in advance, volunteers must notify DWR by emailing CollectionPermits@dwr.virginia.gov with the sampling date, station ID, and permit number (provided by VA SOS). Before monitoring at a new site, volunteers should confirm the location with VA SOS. VA SOS staff will confirm that the site is not in proximity to threatened or endangered species as listed on the DWR website at: https://vafwis.dgif.virginia.gov/fwis/.

Should there be heavy rain, the sampling must be postponed allowing the stream to return to normal conditions.

If the volunteer is not going to be able to complete their sampling for a season, they should alert their regional coordinator or the VA SOS staff, and assist them in locating a substitute volunteer for that

season.

2.2 Sampling Methods

Required equipment includes a d-frame net with a mesh size between 500 to 650 microns, wash bucket or other seining device with a mesh size between 500 to 650 microns, a white sheet to place under the net, forceps, a plastic container in which to sort bugs, collection jars and alcohol for collecting unknown specimens, a magnifying glass, pencils, stream shoes, field sheets and a simple calculator. Volunteers are responsible for purchasing and maintaining their own equipment. When funding allows, VA SOS may be able to provide equipment reimbursement for approved items. The VA SOS program provides volunteers with a list of needed equipment and approved vendors found on the IWLA (https://www.vasos.org/monitor-page/equipment-list/) and VASOS websites (http://www.vasos.org/monitor-page/equipment-list/)

Choosing where to sample within the stream

Volunteers identify habitat areas within the stream. The habitat areas are: woody snags, banks, submerged aquatic vegetation, and riffle areas (cobble-stone sized rocks). These habitat areas will be sampled in proportion to their abundance in the stream segment sampled.

How to Sample

A single sample of macroinvertebrates consists of collecting 20 jabs in productive habitats. A single sample is what is recorded on the data sheets.

A single jab consists of aggressively thrusting the net into the target habitat for a distance of approximately 1 meter; i.e. the distance the net can be swept while standing in one place. This initial "jab" is followed by 2-3 sweeps of the same area to collect the dislodged organisms. The following techniques are recommended for sampling the four major productive habitats in coastal plain streams.

- 1. Woody snags snags or submerged woody debris, are sampled by jabbing in medium sized snag material (sticks and branches). Large material (e.g., logs) may be sampling by scraping the net along the surface. Woody debris may be picked up, held in the net, and rubbed by hand.
- 2. Banks Stream banks with roots and snag material are sampled similar to snags. Vegetated banks are preferred over unvegetated banks. If the bank is undercut, be sure to jab back under the bank, drawing the net from the stream bottom to the top of the undercut bank.
- 3. Submerged aquatic vegetation submerged macrophytes are sampled in deep water by drawing the net through the vegetation from the bottom to the surface of the water. Macrophytes in shallow water are sampled by bumping the net along the bottom in the macrophyte bed.
- 4. Riffle areas should be sampled by placing the net firmly along the bottom of the stream and using your hand or foot to "rub" around the cobbles in the riffle.

The sample is transferred to the sieve bucket (or other seining device) by banging the net over

the bucket opening or by inverting the net into a partially submerged bucket. Contents of the net are transferred into the sieve bucket after each jab

Processing the Sample

Thoroughly mix the sample in the sieve bucket by swishing it around in shallow water. Be sure to keep the entire sample in the bucket!

Empty the contents of your sieve bucket onto a flat, light colored surface, such as a white sheet, or table. This makes the organisms easier to see. Spread the sample across a square portion of your surface (as large an area as needed so that the material is not clumped into piles). Using a stick as a guide, divide your sample into 4 grids to make 4 squares of the same size. Randomly select one of these squares to start your picking and identification.

Using tweezers or your fingers, gently pick all the macroinvertebrates from selected grid and place them in your collecting container. Carefully look on both sides of any debris in the grid, as many insects will cling to any available litter. You may want to use a squirt bottle filled with water from the stream to wash away some of the mud that might hide organisms. Any moving creature is considered a part of the sample. Look closely for very small organisms and take your time. It is important to thoroughly pick all the organisms from the grid.

As you are picking the grid, separate the organisms into look-alike groups. Use primarily body shape and number of legs and tails, since the same family or order can vary considerably in size and color. Use the tally sheet and macroinvertebrate key to aid in the identification process.

Record the number of individuals you find in each taxonomic group on the tally sheet. Our tally sheet and metric calculations should be based on a sample size of at least 100 organisms. COUNT THE SCUDS FOUND IN YOUR SAMPLE BUT DO NOT COUNT THEM TOWARDS THE 100 ORGANISMS REQUIRED! In other words, you need at least 100 non-scud organisms for your sample. If you did not pick 100 organisms from the grid. You must select another grid to pick. The second grid must be picked in its entirety.

Record the number of individuals in each taxonomic group on the tally sheet for the second grid. Again, we are looking for 100 organisms. If you do not have 100 organisms after you have picked the second grid, continue onto the 3rd and pick that grid in its entirety. Continue picking grids in their entirety until you have at least 100 organisms **OR** you have picked the entire sample.

Volunteers use the tally sheet (Appendix A), the macroinvertebrate identification card (Appendix J), and the macroinvertebrate key in the <u>Save Our Stream's Monitor's Guide to Aquatic Macroinvertebrates</u> (Kellogg 1994), or any other resource to aid in the identification process.

Volunteers record the number of individuals they find in each taxonomic group on the tally sheet. When identification and recording are completed, samples are returned to the stream unless the quality assurance audit is occurring (See Quality Control Requirements). All equipment should be thoroughly rinsed at this time so as not to contaminate future samples.

For more detailed information about how to process the sample, reference the VA SOS Volunteer Water Quality Monitoring Manual

Habitat Analysis

Volunteers complete a qualitative streamside visual analysis that assesses the general conditions in the stream (Appendix A) every time the conduct a biomonitoring session. Some parameters require volunteers to pick the most representative description for their sites, while other parameters require volunteers to determine percentages present at their site. Guidelines for completing the habitat analysis are available to the volunteers on the VA SOS website (www.vasos.org) or in the Save Our Stream's Monitor's Guide to Aquatic Macroinvertebrates (Kellogg 1994). These data are used to gain perspective on the macroinvertebrate data collected from the same site.

2.3 Sample Handling and Custody

Unknown Specimens

Individual organisms that volunteers collect but cannot identify should either be preserved and sent to the VA SOS office for identification (see instructions below) or alternatively, a picture or video of the organism may be taken for identification.

If the organism is preserved, place organism in a vial and fill with rubbing alcohol (available at a local drugstore), label properly (Appendix H), and sent to the VA SOS office for identification or delivered to VA SOS employee at an appropriate time. The label should be written in permanent ink or pencil and placed inside the sample container. The volunteer is responsible for all costs associated with delivering the sample to the VA SOS office. The VA SOS program will return the identified sample to the volunteer for future reference.

If the organism is photographed, take as many photographs as possible to document the number of legs/appendages (if any), the head and mouth features, the thorax and abdomen (top and bottom if possible), any tail features, and other distinguishing characteristics. In addition, a photo with another object (like a ruler) in the picture for scale purposes is helpful. If taking video shots of the organism is possible (e.g. smartphone), record the organism as it moves around the container. Send photos and video to the SOS Coordinator at vasos@iwla.org

2.4 Analytical Methods Requirements

Volunteers use a multimetric index based on four individual metrics to analyze their macroinvertebrate data. Scientists at Randolph Macon developed this index for the VA SOS volunteers (Gowan 2004). Volunteers complete the index by following the steps in four tables found on pages three and four in the field sheet packet (Appendix A). The results of the multimetric index are calculated to determine if stream condition is acceptable or not. There is no real analytical procedure for analyzing the results of the streamside visual analysis. Rather, the results from this analysis are used to help the data users understand the scores obtained by the macroinvertebrate samples.

2.5 Quality Control Requirements

There are four quality control requirements that VA SOS maintains for its monitoring program.

Training and Certification

All Virginia SOS volunteers must attend an initial training session and complete a subsequent certification test. See the Training Requirements/ Certification section for details on these quality assurance efforts. Upon the completion of these requirements a volunteer is considered a certified monitor. Certified monitors go through the rigors outlined in this quality assurance plan and provide data for the state water quality agencies. If a certified monitor does not collect and submit data to the VA SOS office during the two year period after their initial certification, they are considered inactive and must go through the training and certification process again. VA SOS monitors are those who routinely monitor their sites (at least twice a year) are considered active certified monitors and must maintain their quality assurance status by participating in the field and lab audits as outlined below.

Reference Collection

VA SOS staff and regional trainers and/or coordinators have a complete reference collection of macroinvertebrates for volunteers to use during the course of their sampling. VA SOS staff is responsible for maintaining these reference collections.

Field and Lab Audits

All certified monitors must undergo periodic quality assurance audits. The quality assurance audit will occur once during the two years after the initial certification and at least every four years in subsequent years for active monitors (those who conduct sampling at least twice a year). The quality assurance audits involve a field visit by a quality assurance auditor or VASOS staff. The auditor reviews all volunteer materials to check that the proper equipment is used and is functioning properly. In addition, the auditor watches the volunteers collect and process their sample. The auditor uses a checklist (Appendix D) to assure the volunteers are correctly completing their sampling event. The completed auditing forms are sent to VA SOS staff. The forms are reviewed by VA SOS staff. Should the volunteers fail their audit, the VA SOS staff will work with the volunteer to update their equipment and/or collection and processing methods. The volunteers must have each sampling event audited until they pass. Once a volunteer fails an audit, their certification is revoked until they successfully complete an audit. Should the volunteer fail three audits in a row, they must attend a training session with an official trainer to refresh their sampling methods.

The auditor will identify and tally the volunteer-processed sample in the field once the volunteers' identification process is complete. The auditor will submit their field audit identification sheet (Appendix G) along with the data sheet of the group they just audited. Should the volunteer fail to correctly identify a significant portion of the sample (over 10%), their certified status will go on hiatus. The VA SOS staff will work closely with the volunteer to help him or her learn troublesome organisms. The volunteer must successfully complete the macroinvertebrate identification test (See Training and Certification) in order to re-instate their certified status. The volunteer must preserve their next sample after their certification status is re-instated for review by the Coordinator or designee. Should the volunteer fail that identification check, they must go

through a training session with an official trainer and must once again go through the certification process in order to be a certified volunteer.

Method Evaluation

As requested, VA SOS staff will make VASOS data available for comparison with DEQ data taken in the same sampling sites for evaluation of VASOS methods.

2.6 Instrument/Equipment Testing, Inspection, and Maintenance

Each VA SOS volunteer will be responsible for maintaining their own equipment. Prior to each monitoring event, the volunteer should check their net and wash bucket for cleanliness and for any small rips or holes. A sewing repair kit should be included in each kit, and small holes and rips should be repaired prior to sampling. If the hole or rip is of substantial size (irreparable), the volunteer is responsible for obtaining a new net prior to sampling. The sheet for under the net should also be cleaned and repaired as needed prior to sampling.

In addition, each volunteer is responsible for keeping the rest of their equipment up to date, clean, and in good condition. The volunteer is responsible for repairing or replacing all necessary equipment. The volunteer is also responsible for having the proper field sheets with them, either by making copies or downloading the from the VA SOS website (www.vasos.org). The volunteer should have the most current, up to date field sheets available.

The quality assurance officer will review all equipment and supplies during the field audit.

The VA SOS program will assist volunteers in keeping current, functioning supplies by providing volunteers recommendations as to where to purchase equipment on the IWLA (https://www.iwla.org/conservation/water/save-our-streams/biological-monitoring-equipment-and-forms) and VASOS websites (http://www.vasos.org/monitor-page/equipment-list/). The VA SOS program will keep all necessary documents current on the website, and will supply copy masters of these documents to those volunteers without Internet access.

2.7 Instrument Calibration and Frequency

No calibration is needed for macroinvertebrate collection/processing equipment. However, the quality assurance officer will review all equipment during their visit with the volunteer.

2.8 Inspection and Acceptance Requirements for Supplies

All equipment must meet specifications for VA SOS macroinvertebrate collection. D-frame dip nets must have mesh size of 500-650 microns. These nets can be purchased from an approved supplier (Appendix I) or the VA SOS program. The sample wash bucket must have a mesh size of 500 to 650 microns. All other supplies may be obtained from a local supply store or through catalogs. All supplies and equipment are subject to review during the quality assurance officer's

regular visit.

The VA SOS program encourages its volunteers to be innovative in order to improve the collection and analytical process. However, all innovations must be reviewed by the VA SOS state office either in person, by mail, or through photographs prior to their use in data collection.

2.9 Data Acquisition Requirements

The VA SOS uses collection and analytical methods for benthic macroinvertebrates developed for the program by Randolph Macon scientists (Gowan 2004). Google Maps and the Clean Water Hub are used for site selection and land use data. Google Maps is used to determine the latitude and longitude of a volunteer's site. Current stream conditions can be obtained at https://waterwatch.usgs.gov/?m=real&r=va. Forecasted rainfall intensity can be obtained either at www.wunderground.com or https://www.wpc.ncep.noaa.gov/qpf/day1-3.shtml. An almanac of previous rainfall levels can be obtained at www.wunderground.com.

Some VA SOS volunteers also collect chemical parameter data. When this information is reported to the VA SOS database manager, it is included in the master database. However, their chemical data is not covered by this QAPP. Those volunteers collecting chemical data should create and submit their own quality assurance plan for that monitoring.

2.10 Data Management

Field sheets (Appendix A) are filled out completely by the volunteers in the field. The volunteer should review their data sheets from each sampling event to make sure they are filled in as completely and accurately as possible. The volunteers have four weeks to submit their data hardcopy or electronically, keeping a copy of the data themselves.

Where available, field sheets are sent to the regional coordinators, who review the data for completeness. Should there be any data gaps, the regional coordinators contact the volunteers to fill in the missing information as much as possible. The regional coordinators must send their region's data to the VA SOS staff electronically (or hardcopy if necessary) within three weeks of obtaining all of that season's monitoring reports for their area. Again, the regional coordinators keep a copy of all data forms. Where no regional coordinator is available, the VA SOS Coordinator or designee acts as first reviewer of data.

The VA SOS Coordinator or designee reviews all data coming to the state office. Should there still be missing or incorrect information, the Coordinator or designee works with the volunteers, regional coordinator, and maps if necessary to fill in the gaps. VA SOS staff has final say over whether the data is complete enough to be entered in the state database by VA SOS staff. The VA SOS Coordinator or designee also maintains a database of all volunteers and their certification status, so can appropriately mark data as certified or not. The database will contain all data from all years. Hardcopy forms will be filed and kept by monitors and regional coordinators for a minimum of five years from its collection. After this time, the data forms will be recycled.

Monitoring data will be delivered in electronic database form to the Department of Environmental Quality every other year, or when requested. The database is reviewed and manipulated as needed by the DEQ Quality Assurance Coordinator, who works closely with the VA SOS Coordinator or designee to correct any problems found in the database.

Other organizations requesting the data are responsible for reviewing the database in accordance with their data needs.

The VA SOS staff will also keep data available for easy review by all interested parties on the Clean Water Hub and in the CMC Data Explorer. The data on the website will have gone through reviews by the VA SOS Coordinator or designee, and will be updated biannually. Data request needs that cannot be met by the internet data retrieval site should be made in writing. Data will be label with the following: "This data is intended for uses outlined in our most recent Letter of Agreement with state and federal natural resource agencies."

3 ASSESSMENT AND OVERSIGHT

3.1 Assessment/Oversight and Response Actions

A quality assurance auditor will review the field performance and equipment of all certified volunteers once during the two years after the initial certification and at least every four years in subsequent years for active monitors. For a discussion of this procedure, please see the Quality Control Requirements section. In addition, the volunteer's identification skills will also be reviewed by auditors in conjunction with a monitor's quality assurance audit (see Quality Control Requirements). Corrective actions, if necessary, will be taken and are discussed in detail in the Quality Control Requirements section.

All field sheets will be reviewed for completeness and anomalies by the collecting volunteer, regional coordinator, and VA SOS Coordinator or designee. Should any problems be detected, the involved parties will work together to fix the problem and assure future field sheets will be complete and meet quality assurance standards. Should the problem be irreparable, the VA SOS Coordinator or designee may decide not to include the data in the statewide public database.

3.2 Reports and Management

The data collected by the VA SOS volunteers will be available to anyone interested on the Clean Water Hub (www.cleanwaterhub.org) and the CMC Data Explorer (www.cmc.vims.edu). The websites are updated biannually, and contains highlights of the data from each site. Those parties interested in seeing the full data from any site can request such from the VA SOS program or from the data portals listed above. A full report will be made to the requesting group within three weeks of said request.

Reports, in terms of the full database from the last five years, are made to the VA DEQ every other year or when requested. Should other information, such as information about passage of quality assurance audits and identification passage, be required, it will be delivered upon request. Data collected when a volunteer has failed to pass a quality assurance check will be marked as uncertified when submitted to the DEQ.

As the database of volunteer data will be marked appropriately with certification status, the "raw" results of the quality assurance tests will not be available unless requested, and specific names will only be provided to the Department of Environmental Quality and other appropriate agencies, and to the regional coordinators. The names of volunteers having quality assurance troubles will not be made public to any other interested parties. However, statistics such as percentage passed in each watershed will be available by request and on the VA SOS website.

4 DATA REVIEW AND USABILITY

4.1 Data Review, Validation and Verification Requirements

All data sheets are reviewed by the collecting volunteer, the regional coordinator where appropriate, and the VA SOS Coordinator or designee. In addition, the DEQ Citizen Monitoring Coordinator reviews the database once every other year. The decision to accept or reject data is made by the VA SOS Coordinator or designee.

Data entry is checked for errors as it is entered. Data will be entered into a spreadsheet set up to calculate metrics and final scores. Should the scores in the spreadsheet be different from those calculated by the volunteers, the data will be reviewed for accurate entry. If data fields were entered incorrectly, they can be edited by the user. Habitat assessments are mainly ranges of scores, and these will be reviewed at the time of entry.

4.2 Validation and Verification Methods

The data will be reviewed for any inaccuracies and gaps and will be updated as described in the Data Management Section. Data will be updated as available. The VA SOS Coordinator or designee makes the final decision as to whether or not the data is complete and accurate enough to include in the database.

All quality assurance data will also be reviewed and recorded by the Coordinator or designee, as described in the Quality Control Requirements section. Any problems will be dealt with as described in that section by the VA SOS staff.

All data reported to users will have undergone all reviews and will have passed all completeness and accuracy tests prior to reporting.

4.3 Reconciliation with Data Quality Objectives

Precision and Accuracy

The precision and accuracy of the VA SOS monitoring program is evaluated during the quality assurance audits and at the time the method is evaluated. If a volunteer fails the quality assurance audits, they must go through corrective action as outlined in Element 14, Quality Control Requirements.

Representativeness

The representativeness of the sample will be evaluated during data entry and during the field portion of the quality assurance audits. VA SOS will evaluate the site sampled during data entry (or data review) to make sure the site is representative of the conditions in the area. During the data review, VA SOS staff will also make sure that more than 100 organisms were selected and that the correct number of jabs was sampled. The quality assurance auditor will make sure the volunteer chooses the most appropriate habitat areas in the course of the field audit and that the habitat area is sampled appropriately. If either course indicates the site location is not

representative or the habitat areas were not sampled in a representative manner, corrective actions as outline in the Element 14, Quality Control Requirements, will be taken.

Comparability

Adherence to the VA SOS protocol will be evaluated periodically as outlined in the quality assurance audit section. At the same time the ability to correctly identify the macroinvertebrates will be determined through a field audit. If the volunteer does not successfully complete either element, corrective actions as identified in Element 14, Quality Control Requirements, will be taken.

The VA SOS Method will also be evaluated upon request by the Department of Environmental Quality to ensure comparability. During the method evaluation process, if the VA SOS method does not correlate with the DEQ order level ID 90% of the time, the VA SOS method will not be considered comparable and will undergo scientific evaluation and validation to make any necessary changes to the actual collection method or the metrics that are calculated.

Completeness

VA SOS will continue to encourage its volunteers to conduct sampling at their sites at least 2 times a year. This will be considered a complete sample set. No corrective action will be taken if a volunteer fails to monitor their site 2 times during a year, but the data may not be considered as useful by VA SOS or data users.

5 REFERENCES

Chesapeake Data Explorer. www.cmc.vims.edu.

Clean Water Hub. www.cleanwaterhub.org.

Engel, S.R. 2000. The effectiveness of using volunteers for biological monitoring of streams. Masters Thesis, Department of Entomology, Virginia Polytechnic Institute and State University.

Gowan, C. 2004. Research on Virginia Save Our Streams Eastern Method development (not yet published).

Kellogg, L. 1994. Monitor's guide to aquatic macroinvertebrates. The Izaak Walton League of America, Gaithersburg, Maryland.

Save Our Streams Equipment List. www.iwla.org/conservation/water/save-our-streams/biological-monitoring-equipment-and-forms.

Virginia Save Our Streams Equipment List. www.vasos.org/monitor-page/equipment-list/.

Appendix A: Field sheets for macroinvertebrate and habitat assessment







Biological Monitoring Data Form for Muddy Bottom Method

Name of Stream:	Sta	ation ID:	
Name of Certified Monitor(s)			
	Number of Participants:		
Latitude:	Longitude:		
County/State:			
Survey Date:	Start Time:	End Time:	
Description of Site Location:			
MUDDY BOTTOM SAMPI	_ING		
The Standard Control of Control o	CANADA MATERIAL TURNO TAMBAT IN TARRAMAN NO AND SANDA MATERIAL VANDA SANDA SANDA MATERIAL MATERIAL SANDA SANDA	ype (20 jabs total). Total jabs taken from a e overall percentage of the habitat type in the	
Banks	Woody S	Snags	
Riffles (Cobble Areas)	Submerg	ged Aquatic Vegetation	
PHYSICAL CONDITIONS	(check all that apply)		
Yesterday: 🗌 Sun	ny \square Overcast \square Interm	mittent Rain Steady Rain Heavy Rain Snov mittent Rain Steady Rain Heavy Rain Snov mittent Rain Steady Rain Heavy Rain Snov	
Water Temperature:	C°	Avg. Stream Width ft.	
		Avg. Stream Depth in.	
OTHER COMMENTS			
OTHER COMMENTS			
BIOLOGICAL MONITORING DA	 TA FORM FOR MUDDY BO	OTTOM STREAMS	

MACROINVERTEBRATE COUNT

Macroinvertebrate	Tally	Count	Macroinvertebrate	Tally Co	Count
Worms OS			Alderflies, Fishflies, and Hellgrammites		
Flat Worms			Common Netspinning Caddisflies		
Leeches			Most Caddisflies (not Netspinning)		
Crayfish			Beetles		
Sowbugs (\$\$)					
			Midges		
Scuds			Black Flies		
Shrimp (Freshwater)			True Bugs		
Stoneflies			True Flies		
Mayflies ()			Gilled Snails		
			Lunged Snails		
Dragonflies (not Gomphidae) and Damselflies			Clams		
Comphidae (clubtail)			Other benthic macroinvertebrates		
Dragonfly			Total number of organisms in the sample (include "other" category)		
BIOLOGICAL MONITORING	BIOLOGICAL MONITORING DATA FORM FOR MUDDY BOTTOM STREAMS	STREAMS		,	2

INDIVIDUAL METRICS

	Organism Groups	Number of Organisms		Total Number of Organisms in the Sample		Percent (This is your value for this metric.)
Metric 1	Mayflies + Stoneflies + Most Caddisflies (not Common Netspinning)		÷		Multiply by 100	%
Metric 2	Gomphidae (clubtail) Dragonflies		÷		Multiply by 100	%

Metric 3: Tolerant

Organism Groups	Number of Organisms
Black Flies	
Clams	
Dragonflies and Damselflies	
Flatworms	
Leeches	
Lunged Snails	
Midges	
Scuds	
Sowbugs	
Worms	
Total Tolerant	
÷	
Total number of organisms in sample	
Multiply by 100	
Percent (This is your value for Metric 3.)	%

Metric 4: Non-Insect

Organism Groups	Number of Organisms
Clams	
Crayfish	
Flatworms	
Gilled Snails	
Leeches	
Lunged Snails	
Scuds	
Sowbugs	
Worms	
Total Non-Insect	
÷	
Total number of organisms in sample	
Multiply by 100	
Percent (This is your value for Metric 4.)	%

MULTIMETRIC INDEX (STREAM HEALTH SCORE)

	Metric Organism	Your Metric Value	6	3	0
Metric 1	Mayflies + Stoneflies+ Most Caddisflies		Greater than 7.8	0.85 - 7.8	Less than 0.85
Metric 2	Gomphidae (clubtail) Dragonflies		Greater than 0.5	0 - 0.5	0
Metric 3	Tolerant		Less than 63	63 - 85	Greater than 85
Metric 4	Non-Insects		Less than 27	27 - 70	Greater than 70
			Total # of 6s:	Total # of 3s:	Total # of Os:
			Multiply by 6:	Multiply by 3:	Multiply by 0:
		SUBTOTALS			

Add the three subtotals to get the Save Our Streams Multimetric Index Score:	_
Acceptable Ecological Condition (Greater than 14)	
Ecological conditions cannot be determined at this time/Grayzone (8 - 14)	
Unacceptable Ecological Condition (0 - 7)	

STREAM CONDITIONS

Fish water quality indicators:	Barriers to fish movement:	Surface water appearance:	Streambed deposit (bottom):
scattered individuals scattered schools trout (pollution sensitive) bass (somewhat sensitive) catfish (pollution tolerant) carp (pollution tolerant)	beaver dams man-made dams waterfalls (> 1 ft.) none other	clear clear, but tea colored colored sheen (oily) foamy milky muddy black grey other_	grey orange/red yellow black brown silt sand other
Odor: musky oil sewage other none	Stability of streambed (bed sinks beneath your feet in): no spots a few spots many spots	Algae color: light green dark green brown coated matted on stream bed hairy	Algae located: _ everywhere _ in spots _ % covered
Stream channel shade: full (more than 75%) high (50% - 74%) moderate (25% - 49%) slight (1% - 24%) none			
Indicate whether the followir moderate (M), slight (S), or no Oil & gas drilling Housing developments Forestry Logging	(N) potential impact to	the quality of your stream. ng lots, highways, etc.)	g site have a high (H), _Agriculture (type:) _Trash dump _Fields _Livestock pasture _Other
COMMENTS: Describe the potential future threats to the potential future threats the potential future th	e stream's health.	nline at www.vasos.org. If you have any c	questions about this protocol, please

Appendix B: Training Session Checklist

Training Agenda: Initial VA SOS Training

- 1. Introduce myself and the VA SOS program
- 2. Describe the VA Division of the Izaak Walton League of America
- 3. Describe SOS method
 - Explain what a watershed is
 - Describe point source vs. non-point source pollution
 - Explain difference between chemical and biological monitoring
 - Explain macroinvertebrates
 - Types of pollution
 - o Toxic
 - Sediment
 - o Nutrients
 - o Bacteria Health hazard not readily identifiable with macroinvertebrate biomonitoring
- 4. Safety Stress especially with children
 - Wash hands gastro-intestinal problems
 - Cuts and scrapes use peroxide
 - Sample in pairs
 - Watch for glass
- 5. Discuss critters and their identification individually
- 6. Discuss the importance of uniformity of method QA/QC issues
- 7. Demonstrate metric calculation and multimetric calculation
- 8. Demonstrate and describe method
 - Evaluate stream to determine stream habitat areas and percentages
 - Inspect net
 - Approach from downstream
 - Collect correct number of "jabs" in each habitat area
 - Release vertebrates
 - Thoroughly mix sample in wash bucket
 - Place sample on flat surface divide into 4 equal quadrants
 - Count need 100 non-scud organisms
- 9. Demonstrate the habitat assessment (tips at end of Monitor's Guide)
- 10. Show reference collection

- 11. Demonstrate Books, Resources, Discuss Partners
 - DEQ
 - DCR
 - DWR
 - Dept. of Forestry
 - SWCDs & NRCS
 - IWLA Chapters
 - Local Colleges
 - Regional Trainers
 - VA SOS staff
- 12. Cooperate with state and local decision makers
- 13. Why do we need to monitor?
- 14. What happens to the data & how to choose sites (contact DEQ so don't duplicate efforts)
- 15. Establish monitoring councils & join watershed roundtables encourage diverse participation. Everyone has a skill to contribute even if they don't want to be a "front line monitor"
- 16. What volunteers should do next
 - Get certified
 - Monitor & report data to VA SOS
 - Become a Regional Trainer or Quality Assurance Auditor

Appendix C: Certification Tests







VA SOS Macroinvertebrate Identification Practical Exercise

Trainer Name:	Training Date:			
Monitor Name:	itor Name:Score:			
Using the macroinvertebrate groupings found on you organisms in the lettered vials. You may use whatev not discuss the organisms with a friend during this pot pass. Depending upon the specimen set, some may not be used.	er printed resources you wish. However, you may rocedure. You must get at least 21 out of 24 correct			
A.	M.			
B.	N.			
C.	О.			
D.	P.			
E.	Q.			
F.	R.			
G.	s.			
н.	т.			
Ī.	U.			
J.	v.			
K.	w.			
L.	X.			







VA SOS Muddy Bottom Protocol Observation Checklist

Trainer Name:	Observation Date:
Monitor Name:	Score:
	field collection skills of monitors in the Virginia Save Our out by official Virginia Save Our Streams Program ceived in order to pass.
1. Monitor selected a representative section of the	stream to monitor? Y N
2. Monitored accurately identified 3 habitat areas?	Y N
3. Monitor adequately assigned percentages to ea	ch habitat area? Y N
4. Monitor disturbed sample area prior to monitori	ng? Y N
5. Monitor correctly sampled woody debris habitat	? Y N
6. Monitor correctly sampled submerged aquatic v	egetation habitat? Y N
7. Monitor correctly sampled undercut/vegetated by	panks? Y N
8. Monitor correctly emptied each jab into the siev	e bucket? Y N
9. Monitor discarded any jab that was had too mu	ch sediment or debris? YN
10. Monitor correctly placed sample on the table a	nd divided sample? Y N
11. Monitor quickly picked all organisms from the	net and sheet? YN
12. Monitor showed adequate field identification s	xills? Y N
13. Monitor correctly filled out field sheets?	Y N

Izaak Walton League of America Online Muddy Bottom Protocol Quality Assurance Test

The following quiz is designed to help you determine your understanding of the Izaak Walton League of America Muddy Bottom Protocol. You may refer to your written materials, but you may not ask a fellow monitor for help. You must receive a score of 17 out of 19 to pass.

	Name (First and Last):
∠.	Date Trained:
	Name three conditions that make it unsafe to monitor at a particular site or at a particular time:
	a
	b c
sli	nswers can include: water is above the knee, water is rushing too fast, banks are too steep or ippery, thunderstorm with lightning, it is posted that the stream is unsafe for human contact or it oks or smells very polluted (sewage smell, etc.)
4.	How does one determine the flow rate of the stream?
	a. By comparing the streams flow to other waterways in the area
	a. By comparing the streams flow to other waterways in the areab. By comparing the current streams flow with past knowledge of the sample streams flow
5.	b. By comparing the current streams flow with past knowledge of the sample streams flow
5.	b. By comparing the current streams flow with past knowledge of the sample streams flow
5.	b. By comparing the current streams flow with past knowledge of the sample streams flow What are the four habitat areas that make up a muddy bottom stream?
5.	b. By comparing the current streams flow with past knowledge of the sample streams flow What are the four habitat areas that make up a muddy bottom stream? a
5.	b. By comparing the current streams flow with past knowledge of the sample streams flow What are the four habitat areas that make up a muddy bottom stream? a. b.

Answers are: woody snags; submerged aquatic vegetation; banks; cobble (riffle) areas

- 6. Which seasons should you sample your site? Check all that apply.
 - a. Spring
 - b. Summer
 - c. Fall
 - d. Winter
- 7. What kind of net should you use for sampling?
 - a. Seine net
 - b. Gill net
 - c. Cast net
 - d. D net
- 8. How do you take a sampling jab?
 - a. Take several scoops of water from the surface above target habitat.
 - b. Aggressively thrust the net approximately one meter into target habitat and perform 2-3 sweeps of the area.
 - c. Use your feet to disturb the habitat and perform 2-3 sweeps of the area.

d. Us	se the	sieve	bucket to	take 2-3	scoops	of substrate	from the	habitat.
-------	--------	-------	-----------	----------	--------	--------------	----------	----------

- 9. How many jabs do you perform in a sampling event?
 - a. Perform 20 total jabs over an area of 100 meters.
 - b. Perform 10 total jabs over an area of 50 meters.
 - c. Perform 10 total jabs over an area of 100 meters.
 - d. Perform 20 jabs over an area of ½ mile.
- 10. When sampling one or more habitats, you should start work downstream and head upstream.
 - a. True
 - b. False
- 11. Why?

Answer: to avoid disturbing habitat, avoid double sampling

- 12. After collection is completed, how many grids should you divide the sample into?
 - a. 2
 - b. 3
 - c. 4
 - d. 6
- 13. The metric calculations are based off of a sample size containing at least _____ organisms?
 - a. 100 organisms
 - b. 200 organisms
 - c. 300 organisms
 - d. It doesn't matter how many are collected
- 14. When sampling, one should count the total number of scuds found and: (Check all that apply)
 - a. Should count them towards the 100 organisms required
 - b. Should not count them towards the 100 organisms required
 - c. Should include scuds in the total organism count for metric calculations
- 15. There is one type of dragonfly that is counted separately. What is it called?

Answer: *Gomphidae*

- 16. If you don't reach the number of organisms needed for a water quality rating after identifying the organism in all grids, you should run the metrics calculations on your sample and report your data anyway.
 - a. True
 - b. False
- 17. You may find organisms like fish or salamanders in your sample that are not part of the Muddy Bottom survey count.
 - a. True
 - b. False

18.	An undetermined	or "grayzone"	score is between	and	

Answer: 8 and 14

- 19. What should you do with your boots or waders after sampling?
 - a. Wash with biodegradable soap
 - b. Leave in full sun for several days
 - c. Let them dry completely before sampling another site
 - d. All of the above
- 20. How many certified monitors must be present at each collection in order for the data to be approved??

Answer: 1

21. How do you keep your certification up to date?

Answer: by submitting data to VASOS at least once every two years.

Appendix D: Quality Assurance Audit Documents

Virginia Save Our Streams Pro Quality Assurance Audit	ogram ()
Date:	
Name(s) and address(es) of volunteer(s) be	ing audited:
Equipment - check for completeness, clean Were there any problems (circle one, explai	
Please circle any missing equipment:	in in commence in year.
Net with poles White sheet Sorting containers Current field sheets ID card	Monitor's Guide book Magnification Thermometer Calculator Forceps
Please circle any parts of the method that vo Chose the most appropriate habitat areas Endownstream of sampling area All organisms were collected from sheet and	Monitor took the proper number of nets A habitat assessment was completed
Comments (continue on back if needed):	
Ouality Assurance Auditor:	



VA Save Our Streams Program
Izaak Walton League of America
707 Conservation Lane
Gaithersburg, MD 20878
301-548-0150 www.vasos.org

Virginia Save Our Streams Program Quality Assurance Sample Submittal Identification Check

lame(s) and address	s(es) of volunteer(s) being che	cked:		
ample Information:				
•		Station	County	
	Longitude	Descripti	on of Site Location	
	etely and preserve your sample to your quality assurance aud ample.			
or office use: ate received by VA	SOS:	Date	orocessed:	
ate results mailed t	o volunteers: curned until VA SOS has receiv		-	
		ed Heid Sheets.		
-	ntification check? Y N			
:	l out in pencil and include in			
Date				
Name(s)	f samplers:			
Stream_		Station	County	
Latitude_	Longitude			
Location	(please be specific)			:



VA Save Our Streams Program Izaak Walton League of America 707 Conservation Lane Gaithersburg, MD 20878 301-548-0150 www.vasos.org

Appendix E: VA SOS Observation of Regional Trainer Form

Virginia Save Our Streams Program

Methods Please check the area the regional trainer did not accepted and program □	ed: dequately Analy analy analy befive in the	y cover in t ysis of Metl Habitat Conduc Referer Resour Cooper Establis What to	che training se hods t Assessment cted in-strean nce collection rces/Books/P ration with de shing monito o do next	ession and ex n event artners ccision maker		omments s
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views of SOS						
Comments (continue on back if needed):						
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Appendix F: Event Sign In-Sheets

Facilitator Notes:

Registration, Liability Waiver & Photo Release *PLEASE READ THE WAIVER BELOW PRIOR TO SIGNING* Save Our Streams

I he used part part part abov my cont right I acknowledge that I am voluntarily participating in a Save Our Streams training, certification and/or monitoring activity. I understand as a volunteer that I will not be paid for my services, that I will not be covered by any medical or other insurance coverage provided by the Izaak Walton League of America, and that I will not be eligible for any Workers Compensation benefits.

an orany providing, recordings, interviews, violentes, interior pretices, or similar visual recording or the analysis in railing interiors.	
em or any process apris, recordings, interviews, viacotapes, motion pretares, or similar visual recording or my raining members.	
thorized by them of any photographs, recordings, interviews, videotanes, motion pictures, or similar visual recording of me and/or my family members	thorized by them of any
to Jhts. I am signing this document of my own free will. I further consent to the unrestricted use by the Izaak Walton League of America and/or person(s)	hts. I am signing this d
ntents. I am aware that this is a release of liability and a legal contract between me and the Izaak Walton League of America and that it affects my legal	ntents. I am aware that
y participation in Save Our Streams. I have carefully read this assumption of risk and general liability release agreement, and I fully understand its	y participation in Save
ove-named parties. This release is intended to be broad in its effect. I hereby agree to accept any and all risks of injury, illness or death in connection with	ove-named parties. This
rticipation in Save Our Streams, including any such claims which allege negligent acts or omissions of the Izaak Walton League of America and/or other	rticipation in Save Our S
ed for Save Our Streams, or any of the volunteer workers, for the injury or death to me or damage to my property, however caused, arising from my	ed for Save Our Stream
irtner organizations or contractors, or either of their officers or directors collectively or individually, or the supplier of any materials or equipment that is	rtner organizations or co
hereby agree that I, and anyone else claiming through me, will not make a claim against the Izaak Walton League of America, any of its affiliated and	nereby agree that I, and

Signature

Print Name

Address

Phone

Chapter/Org

Appendix G: Field Sheets for Identification of Quality Assurance Samples

Virginia Save Our Streams Program Quality Assurance Project Plan Virginia Save Our Streams Program Field Audit Identification Sheets

January 2023

am		oncounty	
tudeLongitu	de		
ation (please be specific)			
a of Idantification.		Field Auditor:	
of Identification: Organism	Number in	Number volunteer	
organism	Sample	found	" Misibea
Worms	1		
Flatworms			
Leeches			
Crayfishes			
Sowbugs			
Scuds			
Stoneflies			
Mayflies			
Dragonflies & Damselflies			
Hellgrammites, Fishflies,			
& Alderflies			
Common Netspinners			
Most Caddisflies			
Beetles			
Midges			
Black Flies			
Most True Flies			
Gilled Snails			
Lunged Snails			
Clams			
Other			

 \sqcup (<10%) Yes \sqcup (>10%) No



VA Save Our Streams Program Izaak Walton League of America 707 Conservation Lane Gaithersburg, MD 20878 301-548-0150 www.vasos.org

Appendix H: Unknown Specimen Submittal Form and Label







Unknown Sample Submittal Form

Sample Information:	
Survey Date:	Station ID:
County/State:	
Latitude:	Longitude:
Location (be specific):	
Please describe the physical chara movements):	cteristics of this organism (including any identifiable
Do you have any thoughts on what	this organism might be?
If you would like to send the VA S organism to assist with identificat the information below in pencil ar preservation jar or vial.	SOS office this tion, please fill out nd include in your Izaak Walton League of Amer ATTN: VA SOS Coordinato 707 Conservation Lane Gaithersburg, MD 20878
If you would like to send the VA S organism to assist with identificat the information below in pencil ar preservation jar or vial.	SOS office this Izaak Walton League of Amer tion, please fill out ATTN: VA SOS Coordinator nd include in your 707 Conservation Lane Gaithersburg, MD 20878
If you would like to send the VA Sorganism to assist with identificat the information below in pencil arpreservation jar or vial. Date Collected:	SOS office this tion, please fill out nd include in your Izaak Walton League of Amer ATTN: VA SOS Coordinato 707 Conservation Lane Gaithersburg, MD 20878

Appendix I: Virginia Save Our Streams Safety Recommendations

VASOS Safety Recommendations

- Always monitor in at least pairs.
- All kits should contain some sort of waterless hand sanitizer and/or peroxide. These should be used frequently, especially before touching face or eyes and before eating.
- Be careful of glass. If a site has known glass, use a garden rake to dig up substrates and consider purchasing neoprene gloves to help protect hands. Should a volunteer get cut, they should clean the cut immediately.
- Be sure to have plenty of water and sunscreen in the summer, and wear plenty of clothing in the winter. In the winter, consider purchasing neoprene gloves to help keep hands warm, and bring plenty of towels to stay dry.
- Be cautious about ticks and Lymes disease. Precautions should be taken as necessary for area conditions.
- Monitoring sites should be conducted in wadable sections of streams. The depth of the stream should be no deeper than 3 feet (the height of the net).
- If high waters are present at the site, this should be noted on the front page of the field sheet and the site should not be monitored at this time.
- Never allow children (16 or younger) to go to the stream alone. When monitoring with children, stress that they should not come back to the stream without an adult present.

Appendix J: Macroinvertebrate Identification Card

Stream Insects and Crustaceans ID Card

Lines under picture indicate the relative size of organisms



Aquatic Worm: Class Oligocheata

\frac{1}{4}" - 2", can be very tiny;
thin, wormlike body, tolerant of
impairment



Flat Worm: Family Planaridae Up to ‡", soft body,

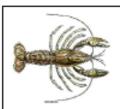
may have distinct head with eyespots, tolerant of impairment



Leech:

Order Hirudinea

1" - 2", segmented body, suction cups on both ends, tolerant of impairment



Crayfish: Order Decapoda

Up to 6", 2 large claws, 8 legs, resembles a small lobster, somewhat tolerant of impairment



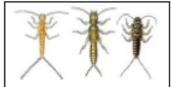
Sowbug: Order Isopoda

1 - 1", gray oblong body wider than it is high, more than 6 legs, long antennae, somewhat tolerant of impairment



Scud: Order Amphipoda

‡", white to gray, body higher than it is wide, swims sideways, more than 6 legs, resembles small shrimp, somewhat tolerant of impairment



Stonefly: Order Plecoptera

 $\frac{1}{2}$ " – 1 $\frac{1}{2}$ ", 6 legs with hooked tips, antennae, 2 hair-like tails, no gills on abdomen, very intolerant of impairment



Mayfly: Order Emphemeroptera

¹/_{*} - 1*, plate-like or feathery gills on abdomen, 6 hooked legs, 2 or 3 long hair-like tails, tails may be webbed together, very intolerant of impairment



*

1

Beetles: Order Coleoptera

¹/₄" - 1", disk-like oval body with 6 small legs and gill tufts on underside OR small black beetle crawling on streambed OR comma-like brown "crunchy" body with 6 legs on upper 1/3 and possibly gill tuft on back end, OR (miscellaneous body form - rare), somewhat tolerant of impairment



Hellgrammite, Fishfly, and Alderfly: Order Megaloptera

‡" - 4", 6 legs, large pinching jaws, 8 pairs of feelers along abdomen, 2 hooks on tail end OR 1 single spiky tail, somewhat tolerant of impairment



Common Netspinners: Family Hydropsychidae

Up to ‡*, 6 hooked legs on upper 1/3 of body, 2 hooks at back end, underside of abdomen with white tufts of gills, somewhat tolerant of impairment



Most Caddisfly: Order Trichoptera

Up to 1", 6 hooked legs on upper 1/3 of body, may be in stick, rock or leaf case, no gill tufts on abdomen, intolerant of impairment

Illustrations from: Voshell, J. R., Jr. 2001. Guide to the Common Freshwater Invertebrates of North America. MacDonald and Woodward Publishing Co. With permission of the author.

Stream Insects and Crustaceans ID Card

Lines under picture indicate the relative size of organisms



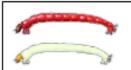
Dragonfly and Damselfly: Order Odonata

 " - 2", large eyes, 6 hooked legs, large protracting lower jaw, 3 broad oar-shaped tails OR wide oval to round abdomen, somewhat tolerant of impairment



Dragonfly: Family Gomphidae

¹/₂ " - 2", large eyes, 6 hooked legs, large protracting FLAT lower jaw, wide oval to round abdomen, short stubby antennae that are parallel to each other, intolerant of impairment



Midges

Family Chironomidae

Up to ‡", distinct head,
worm-like segmented body,
2 leg-like projections on
each side, often whitish to
clear, occasionally bright
red, tolerant of
impairment



Black Fly: Family Simuliidae Up to $\frac{1}{4}$ ", and of body wider (like bowling pin), distinctive head, sucker on end, tolerant of



Most True Flies: Order Diptera

‡" - 2", bodies plump and maggotlike, may have caterpillar like "legs" along body, may have lobes or conical tails on end, tolerant of impairment



Gilled Snails: Class Gastropoda

Up to ‡", shell opening covered by a thin plate called an operculum, with helix pointed up shell opens to the right, intolerant of impairment



impairment

Lunged Snails: Class Gastropoda Up to ‡", no operculum, with helix pointed up shell opens to the left,

tolerant of impairment



Clams: Class Bivalvia

Up to ‡", fleshy body enclosed between two clamped together shells (if clam is alive, shells cannot be pried apart without harming clam), somewhat tolerant of impairment



Abdomen

none v



Tails: There are many different kinds of macroinvertebrate tails. The thin threadlike tails found on stoneflies and mayflies are called cerci. The oar-shaped tails found on a damselfly are not really tails - they are actually gills called caudal lamellae!



VA Save Our Streams Program

VA Division of the Izaak Walton League of America P.O. Box 8297 Richmond, VA 23226 (804) 615-5036 www.vasos.org These sheets are modified from the National Izaak Walton League of America SOS Program Stream Insects & Crustaceans ID Card.

http://www.iwla.org/505/index.html

Illustrations from: Voshell, J. R., Jr. 2001. Guide to the Common Freshwater Invertebrates of North America. MacDonald and Woodward Publishing Co. With permission of the author.

Appendix K: Validation Studies

Stream Health Monitoring By Citizens: New Field and Analytical Methods Suitable for Virginia's Coastal Plain.

Ryan Knisley, Lauren Grimmer and Charles Gowan Environmental Studies Program Randolph-Macon College Ashland, Virginia

August, 2003

Abstract

Citizen volunteers are essential for monitoring health of streams in the Chesapeake Bay watershed, relying primarily on analysis of benthic macroinvertebrates to make assessments. But, methods suitable for citizens working in coastal plain streams are not available, and so we developed a new Save Our Streams Coastal Plains ("SOS") method. In a preliminary test conducted in 2002, SOS scores were strongly correlated to those from a professional method (the Mid-Atlantic Coastal Streams or MACS method), but all sample sorting and organisms identification were conducted by professionals in the laboratory. The goal for the SOS method is for citizens to conduct assessments in the field. In this study, eighteen streams in Virginia's coastal plain were sampled in spring and again in summer using new methods for sorting and identifying macroinvertebrates in the field. To further evaluate the reliability of the SOS method, we calculated MACS scores for each stream and compared them to SOS scores using linear regression. We found that the relationship between SOS and MACS scores was statistically significant (P<0.001) and strong (r = 0.84); the relationship was stronger in spring (r=0.87) than in summer (r=0.73).

Identifications by citizens in the field and those by professionals in the lab showed good agreement, and SOS field and lab scores were strongly correlated (r=0.93, P<0.001). In addition, citizens successfully sorted macroinvertebrates in the field, except that there was some bias against finding smaller organisms. Despite this, SOS scores based on field sorting were strongly correlated to those based on the entire sample being sorted in the lab under magnification (r=0.91, P<0.001). We compared spring-to-summer scores to evaluate seasonal variation; the correlation was relatively weak (r=0.69), indicating that samples taken in different seasons within the same year may not yield similar results. Finally, we compared SOS scores from spring and summer 2003 to SOS scores from the same streams generated in spring and summer 2002 to assess annual variability. Comparisons between years were variable, with summer-to-summer scores being only weakly correlated (r=0.25), but spring-to-spring ones strongly so (r=0.84). We conclude that citizens are capable of making reliable stream health assessments using SOS methods when identification and sorting occurs in the field, and citizens should adopt the SOS method for the coastal plain of Virginia. Sampling should be repeated seasonally within the same year, and monitoring should extend across years to detect trends in stream health.

Appendix L: Reference Materials for Virginia Save Our Streams Volunteer Monitors

Barbour, M.T., J. Gerritsen, and B. Synder. 1999. Rapid bioassessment protocols for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates, and fish, 2nd edition. EPA 841-B-99-002 Office of Water, Washington, D.C.

Engel, S.R. 2000. The effectiveness of using volunteers for biological monitoring of streams. Masters Thesis, Department of Entomology, Virginia Polytechnic Institute and State University.

Kellogg, L. 1994. Monitor's guide to aquatic macroinvertebrates. The Izaak Walton League of America, Gaithersburg, Maryland.

United States Environmental Protection Agency. 1997. Volunteer stream monitoring: A methods manual. EPA 841-B-97-003 Office of Water, Washington, D.C.

Voshell, J. Reese. 2002. A guide to common freshwater invertebrates of North America. Illustrated by Amy Bartlett Wright. The McDonald & Woodward Publishing Company. Blacksburg, Virginia.

Appendix M: Virginia Save Our Streams Site Selection Guide

Selecting a Monitoring Location

Selecting representative sites is one of the most important elements in designing a monitoring program. Before selecting monitoring sites, you should determine two things: where and what kind of monitoring is already being done in your watershed and what question would you like your monitoring to answer. The answers to both of these questions will help you map out the most effective monitoring locations.

Site locations will depend on the goal of your monitoring program. If you want to know what the water quality is of a particular stream, you might select a site close to the mouth of the stream. If you want to know the water quality at a particular fishing spot, you might want to select a site within that fishing spot. If you want to know if a development is impacting a stream you might want to have one site upstream of the development and one site downstream of the development. If you want to collect data to assist the state in developing water quality assessment reports, you might want to select a site within a watershed that is not currently monitored.

Virginia Save Our Streams can help you locate your sites by:

- determining which streams are currently monitored in your watershed
- finding out the natural resource questions professionals would like to have answered in your watershed
- providing a map with natural resource characteristics to assist in developing a monitoring plan
- making a site visit to potential monitoring sites to evaluate access and habitat

Your monitoring site should have good access and you should always get landowner permission (unless in a public right of way).

Defining Monitoring Stations

Monitoring should be done at one station, defined as a single stretch of stream 100 meters long. If you wish to assess a longer section of a stream, select two monitoring stations at the top and bottom of the stretch, or multiple sites along the length of the stretch at quarter-mile or greater intervals. Be sure to revisit the same station each time so that your results will be comparable. Carefully record the location of your monitoring station on your VA SOS Stream Survey form. If you do not know the latitude and longitude coordinates when you monitor, use an accurate description of the site (i.e. Site located on north side of route 660, 1 mile east of route 607) that enables you or another monitor to return to the same location. The regional coordinator or VA SOS staff will help you identify the coordinates at a later date.

Documenting Monitoring Stations

Stations should be properly documented by including the stream name, county, and location. The location should be specific and should allow someone to find the property using a Google Maps. For instance the site location could be: East side of route 630 bridge, 2 miles north of route 29. This location is easy to find for anyone using Google Maps. The following is a poor example of location: at northwest corner of Mr. Earl's property. Unless you know Mr. Earl, you will not be able to find the site! Include latitude and longitude if possible. If you have more than one site on a stream, identify the sites with a station number and always use the same station number for a site! If you cannot remember site number, consider using a descriptive name for the site such us "downstream", "upstream", or "route11".

Appendix N: Recommended Sampling Seasons for Virginia Save Our Streams

Recommended Sampling Seasons for Virginia Save Our Streams

The Virginia Save Our Streams program recommends monitoring two times a year, once in the spring and once in the fall. While volunteers may go during any time of the season, recommended times are in bold in the below table.

Winter	Spring	Summer	Fall
	March, April , May		September, October,
			November

Appendix O: Biological Monitoring Protocol for Muddy Bottom Sampling





Biological Monitoring Instructions for VA SOS Stream Monitors

Surveying stream macroinvertebrates provides information about the health of your stream. Many stream-dwelling organisms are sensitive to changes in water quality. Their presence or absence can serve as an indicator of environmental conditions.

Before selecting a site to monitor, please follow these rules:

- Check with state and county agencies to make sure you are not disturbing a survey area used by government agencies (over-monitoring may harm the stream).
- · Contact local landowners before monitoring to make sure you are not trespassing.
- Ask for permission if you need to cross private land. Most landowners will give permission for your study and may even want to help you conduct your survey.

Monitoring should be conducted at the same station (location) each time you sample during the year. If you want to monitor several stations on your stream, make sure the stations are no closer than one-quarter mile. This means, for example, that if you want to monitor a one-mile segment of a stream, you can have a maximum of four monitoring locations. If the stations are spaced more closely, the monitoring activity may become the main impact on water quality.

Carefully record the location of your monitoring station on your Biological Monitoring Data Form. Include roads, bridges, and significant landmarks. Use your smart phone's GPS functionality to determine your longitude and latitude.

THINGS TO CONSIDER

If you are monitoring more than one station, begin monitoring downstream and move upstream. This will prevent macroinvertebrates disturbed by the first test from washing downstream and being captured in your net a second time. Each survey should record only the organisms present at that particular location and time.

Monitoring should be conducted two times per year at each station, in spring and fall. This monitoring will accurately record the yearly life cycle in the stream. Less frequent monitoring, while still useful, will not give the complete picture of stream life.

When scheduling monitoring events, remember that excessive monitoring can become a major threat to stream health because each monitoring event disturbs the streambed and dislodges macroinvertebrates. In general, monitoring stations should have two months to recover from a monitoring event. It is crucial to the integrity of your data that you do not over-monitor your stations. There is some flexibility in this rule.

For example, if an oil spill occurs, you might want to monitor your stream, even if you have done your two surveys for the year. The data you collect might be the only data available on the immediate impacts of the spill.

The methods described in these instructions are for use in wadable streams. To be wadable, the water level in the stream must not exceed the height of your knees. When planning monitoring sessions for younger people, please keep in mind that knee height varies greatly between adults and children.

Safety is critical when monitoring a stream. Do not enter a stream if the water is flowing abnormally fast or high, if the banks are steep or unstable, or during a thunderstorm. If the water smells of raw sewage, do not enter the water; contact state environmental authorities immediately. Monitors in urban-area streams should wear gloves to protect against glass or metal that may be buried in the streambed. Finally, always sanitize your hands and equipment after each monitoring session to avoid bacterial infection.

There are two sampling methods available to collect aquatic macroinvertebrates, Muddy Bottom Sampling is used in streams that do not have riffles, a streambed feature with cobble-sized stones between 2 to 10 inches in diameter where the water bubbles over the rocks. If your stream has riffles, please refer to the Rocky Bottom Sampling section.

MUDDY BOTTOM SAMPLING

The Muddy Bottom Sampling method is intended for volunteers sampling streams that primarily do not have rocky bottoms or riffles. Muddy bottom streams are composed of muddy or sandy substrate, overhanging bank vegetation, and submerged woody and organic debris. This method enables sampling of streams where kick-seining techniques do not yield the best representative sample of macroinvertebrates or allow easy collection from the most productive aquatic

Monitoring is conducted using an aquatic D-frame or dip net with 500 micron mesh and a four-foot pole. The dip net is used to sample a wide variety of habitats and collect many different kinds of organisms.

Before you begin monitoring, familiarize yourself with the four main habitats that can exist along muddy bottom streams: woody snags, stream banks, riffles, and submerged aquatic vegetation. Search for these habitats along a 100-meter section upstream from the monitoring station.

Following are simple descriptions of the habitat types and collection techniques for each habitat.

Woody snags

Snags, or submerged woody debris, are areas where tree trunks or limbs have fallen into the stream. Leaves and debris may be collected or tangled in the snag. To sample woody debris, jab the medium-sized submerged material (sticks and branches), scrape along the submerged surface of large material (logs), or pick up and rub woody debris in the net by hand.

Stream banks

Stream banks are the edge of the stream. These may be vegetated, bare soil, undercut, or eroded. Stream banks are sampled in a bottom-to-surface motion, jabbing at the bank to loosen organisms. Each scoop of the net should cover one foot of submerged area.

Riffles

Riffles are shallow, fast-moving areas of water flowing over cobble-sized stones and rocks. To sample a riffle, place the net firmly along the bottom of the stream and use your hands or foot to rub around the cobbles.

Submerged aquatic vegetation

Submerged aquatic vegetation includes any plant growing under or out of the water of the stream. In deep water, plants are sampled by drawing the net through the vegetation from the bottom to the surface of the water. In shallow water, plants are sampling by bumping the net along the bottom of the bed of vegetation.

A single sample of macroinvertebrates consists of collecting 20 "jabs" in productive habitats. A single "jab" consists of aggressively thrusting the net into the target habitat for approximately one meter. This initial jab is then followed by two to three sweeps in the water of the same area to collect dislodged organisms. The sample is then transferred to the sieve bucket or seining device, by banging the net over the bucket opening or by inverting the net into a partially submerged bucket. Transfer sample contents to the sieve bucket after every jab.

Each habitat should be sampled in proportion to its abundance in the stream sample area. For example, if 50 percent of a sample area is woody debris, it should be sampled with ten jabs.

Thoroughly mix the sample in the sieve bucket by swishing it around in shallow water, being careful to keep the entire sample inside. Empty the contents of the bucket onto a flat,

MUDDY BOTTOM SAMPLING EQUIPMENT

- Biological Monitoring Data Form for Muddy Bottom Streams
- . One D-frame aquatic dip net with mesh of 500 microns
- · Portable table
- White sheet or table cover
- One screen-bottom bucket with a mesh of 1/32 inches
- "Field Guide to Aquatic Macroinvertebrates"
- · Aquatic thermometer
- Magnifying glass
- · Small magnifier boxes (optional)
- Spray bottle
- · Ice cube trays or specimen jars for sorting organisms
- · Tweezers or forceps
- Clipboard
- · Boot-footed waders or waterproof knee boots
- Neoprene gloves, hand, elbow or shoulder length (optional)
- · Additional identification resources

light colored surface, such as a white sheet or table. Spread the sample evenly across a square portion of the surface, such that the sample material is not clumped together. Using a stick, divide the sample into a grid with four equal quadrants. Randomly select a quadrant to start sorting and identification.

Using tweezers or your fingers, separate all the organisms from the surface and place them in your collecting container. Plastic ice cube trays filled with stream water are helpful when sorting samples. Sort organisms into similar groups as you separate your sample. Be sure to regularly wet the surface using a spray bottle, as the organisms will begin to dry out. See the "Identification" section for details on identifying the organisms in your sample.

Record the number of individuals you find in each taxonomic group on the tally sheet. Metric calculations should be based on a sample size of at least 100 organisms. Count the number of scuds found in your sample, but do not count them towards the 100 required organisms (in other words, you need at least 100 non-scud organisms for your sample).

If the first grid doesn't yield 100 organisms, move on to a second grid and sort it in its entirety. Record the number of individuals in each taxonomic group on the tally sheet for the second grid. If you do not have 100 organisms after you have picked the second grid, continue on to the third. Continue

sorting grids in their entirety until you have at least 100 organisms or you have sorted the entire sample.

ROCKY BOTTOM SAMPLING

The Rocky Bottom Sampling method is intended for volunteers sampling streams that have rocky bottoms or riffles. A kick-seine net – a finely meshed net with supporting poles on each side – is the best tool to use for collecting macroinvertebrates in rocky bottom streams. The VA SOS Rocky Bottom Sampling method recommends using a kick-seine net with 1/32-inch mesh. The 1/32-inch mesh net will provide you with a large sample because it captures younger, and therefore smaller, organisms of each species, and some state and local government agencies require use of the 1/32-inch mesh.

Select a riffle that is a shallow, fast-moving area of water with a depth of 3 to 12 inches and cobble-sized stones (2 to 10 inches) or larger. Before entering the stream, record observations about riffle composition on the back of the Biological Monitoring Data Form.

Place the kick-seine net at the downstream edge of the riffle. The net should be secured with rocks selected from outside the sample area. Rub the rocks to dislodge any macroinvertebrates outside of the sample area before placing on the bottom of the net, or use dry rocks from outside the stream. Don't allow any water to flow over the top of the net either — organisms can escape over the net. Also, if water is flowing over the top of the net, the water level is too high for safe monitoring.

Monitor a one-foot by one-foot area of the streambed directly in front of the net.

The sample site can be sampled up to four times in order to yield a total of 200 or more macroinvertebrates. It is important to have at least 200 invertebrates by the end of the sampling session.

The length of each sampling period can be adjusted depending on the number of macroinvertebrates found in each sampling period. Each sampling period must be between 20 and 90 seconds. For example, if 100 macroinvertebrates are found during one 30 second sampling period, you will likely only need to monitor for a second 30 second period. Do not do another sampling period once you have reached 200 organisms, if you have already sampled four times, or for longer than 90 seconds.

If you sample the maximum number of seconds for at least three nets and do not reach 200 organisms, you should still record your results and calculate the stream health score.

Once you have determined the length of the sampling period, calculate the amount of time you will spend rubbing rocks versus disturbing the substrate. You should spend 75% of the sampling period rubbing rocks, and the remaining

ROCKY BOTTOM SAMPLING EQUIPMENT

- Biological Monitoring Data Form for Rocky Bottom Method
- . Kick-seine with 1/32-inch mesh
- · Net poles
- · Portable table
- White sheet or table cover
- · "Field Guide to Aquatic Macroinvertebrates"
- · Aquatic thermometer
- · Magnifying glass
- Small magnifier boxes (optional)
- · Spray bottle
- · Ice cube trays or specimen jars for sorting organisms
- · Tweezers or forceps
- Clipboard
- · Boot-footed waders or waterproof knee boots
- Neoprene gloves, hand, elbow or shoulder length (optional)
- Additional identification resources

25% disturbing the substrate. For example, in a 30 second sampling period you will spend 22.5 seconds rubbing rocks and 7.5 seconds disturbing substrate.

Firmly and thoroughly rub your hands over individual cobbles within the sampling area, placing each rock outside of the sampling area when finished. Once you have reached 75% of the sampling period, disturb the sample substrate using a dry rock or garden tool. At the end of the sampling period, stop disturbing the substrate and let the water run clear.

Before removing the net, rub any rocks that you used to anchor the net to the stream bottom and remove the rocks from the bottom. Firmly grab the bottom of the net so that your sample does not fall from the net, and then remove it from the water with a forward-scooping motion. This will allow you to remove the net without allowing any insects to be washed under or off it.

Placing a white trash bag or white sheet under the net before separating the sample will catch any tiny organisms that may crawl through the net. Use a watering can or spray bottle to periodically water your net. The organisms will stop moving as the net dries out. Occasionally wetting the net will cause the organisms to move, making them easier to spot. Watering the net is especially important on hot, dry days. Place the net on a flat, bright area, out of direct sunlight. Using tweezers or your fingers, separate all the organisms from the net and place them in your collecting container, which should be full of water from the stream. Sort organisms into similar groups as you separate your sample. This will make your identification quicker when you are ready to record results. Plastic ice cube trays are helpful when sorting the catch. For example, put all organisms with legs in one section and all organisms with no legs in another section. Any organism that moves, even if it looks like a worm, is part of the sample. Look closely, since most aquatic macroinvertebrates are only a fraction of an inch long.

IDENTIFICATION

Once organisms are collected through either the Rocky Bottom or Muddy Bottom Sampling methods, they are sorted and identified. You can use IWLA's "Field Guide to Aquatic Macroinvertebrates" or A Guide to Aquatic Insects and Crustaceans, both of which can be purchased through links on the Save Our Streams equipment page on the League's website: iwla.org/sos. The League's free Aqua Bugs app provides easy-to-follow instructions to help you identify your macroinvertebrates. Search for it in the Apple Store and Google Play Store.

Izaak Walton League macroinvertebrate guides provide a general overview of the macroinvertebrate types found across the United States. The composition of macroinvertebrate populations varies depending on local geography and geology. Try contacting your local environmental protection agency or universities for more information about local macroinvertebrates. Local experts might be able to share additional field guides that are specifically designed for your area.

Not all organisms in your stream are listed in the guides. For instance, macroinvertebrates such as whirligig beetles, water striders, and predaceous diving beetles are not included on the survey sheet. They are surface breathers and do not provide any indication of water quality.

When beginning your identification, ask yourself the following questions:

- How large is the organism?
- . Is the body long and slender, round, or curved?
- · Does the organism have any tails? How many?
- · Does the organism have any antennae?
- · Does the organism have legs? How many? Where?
- Is the body smooth and all one section, or is it segmented (two or more distinct sections)?
- Does the organism have any gills (fluffy or plate-like appendages)?

- Where are the gills located? Sides, back, underside, under its legs?
- Does it have pinching jaws like a beetle larvae?
- Are any legs or antennae missing because they were broken off in the net?
- What color is the organism?
- Does the organism swim underwater or remain on the surface?

When using the macroinvertebrate guides, read the descriptions for each organism. Sizes are provided for reference. However, if you catch a young macroinvertebrate that has just hatched and has not yet reached full size, it may be smaller than indicated in the guides. Specimens can be put into magnifying boxes to ease identification. Return the organisms to the stream after sampling is completed.

METRICS

During identification of macroinvertebrates, record your results on the macroinvertebrate chart. Once you have counted all collected organisms, start calculating the Individual Metrics. Each Individual Metric is a percentage of various macroinvertebrate groups. Tally each indicated organism group and calculate the percentage to determine the Individual Metrics.

Use each Individual Metric to calculate the Multimetric Index Score (stream health score). Write each metric value from the Individual Metrics into the corresponding box under Your Metric Value. Determine the score based on the range for each metric value and indicate which score each Metric Value falls under. Follow the multiplication steps at the bottom of the table to determine your Save Our Streams Multimetric Index Score and determine whether the site has acceptable or unacceptable ecological conditions.

BIOLOGICAL MONITORING DATA FORM QUESTIONS

The Biological Monitoring Data Form also includes questions about the land and vegetation surrounding the stream. These questions help characterize the quality of stream habitat and its ability to support a healthy population of stream organisms. The land use information also paints a picture of the stream for other people who might review your data. Guidelines for correctly answering these questions are given below. Record the answers based on the area that is upstream from your monitoring site; generally, you should record the data for the area you can see. For land use information, include uses for one mile upstream from your site or the section of stream you have adopted. If necessary, take a walk or consult a map for this information.

Fish water quality indicators: Different fish have different tolerances to pollution. The type of fish present may indicate the type of water quality expected. If you collect fish but don't recognize the type, write a description of the fish on the data form or take a picture to use for later reference. You can find fish identification charts or experts to help with fish identification at local schools, agencies, libraries, or online.

Barriers to fish movement: The absence of certain fish types may be due to a dam or other large obstacle, not because of water quality. Note on your survey form if the dam is upstream or downstream from your monitoring site and how far away. Waterfalls should only be recorded if they are large enough that a fish could not reasonably jump over them or swim around them. Usually, waterfalls of a few feet or less are not impediments to the upstream movement of fish.

Surface water appearance: You may check more than one of the colors listed but not all of them. Note if strange colors are present throughout the stream or only in one section, such as immediately below a discharge pipe or highway culvert.

Streambed deposit (bottom): Record the over-all appearance of the stream bottom. If the streambed does not have any apparent coating, you may note it as "other" and write in "normal."

Odor: Note any unusual odors. Odors may come from natural processes or may indicate potential water quality problems.

Stability of streambed: An unstable streambed can mean that soil is eroding from the bottom of the stream and may indicate water quality problems. When standing in the stream, determine how frequently the bed sinks beneath your feet.

Algae appearance: Algae feels slimy. You will notice it as you rub rocks during monitoring. A great deal of algae may indicate too many nutrients in the water. Sometimes more algae will appear in the spring after snowmelt releases extra nutrients into the stream. However, take note of the percent and type of algae present in the stream to make sure it is not increasing over time.

Algae located: Estimate the percentage of stream bed that is covered by algae. Algae is often present in small quantities in healthy streams. Excess algae may indicate water quality problems.

Stream channel shade: Over the course of the day, estimate what percentage of the stream channel is shaded by stream-side trees, shrubs, and grasses. Shading helps keep water cool and can be beneficial for aquatic life.

Streambank composition: Remember to look at both sides of the stream's banks. When questions ask for a percentage, use the information for both the left and right bank and combine values. For instance, if one side of the bank is completely bare due to erosion while the other side is well vegetated, you should record the percent of bank coverage as 50 percent.

When recording total percentages of shrubs, grasses, and trees, you should also look at both sides of the bank. However, if one side has artificial structures such as rock riprap or concrete, you will have to account for such ground cover. For instance, if the left side of the bank is not vegetated, you cannot have more than 50 percent of shrubs, grasses, and trees total when those values are added together.

Streambank erosion: Again, look at both sides of the bank to determine the percentage of soil erosion.

Riffle composition: This question refers to the 3x3-foot area of the stream sampled for Rocky Bottom Sampling techniques with a kick-seine net. Do not fill out this question when using the muddy bottom sampling technique.

If you used a kick-seine to conduct the Rocky Bottom Sampling method, answer this question before you disturb the site. The organisms you collect are most abundant in riffles composed of predominantly cobble-sized stones (more than 70 percent cobbles is a good riffle habitat). Start with the largest rocks first when recording bed composition. If you don't have any boulders (rocks larger than 10 inches), record cobble-sized stones and continue until your percentages equal 100 percent. A typical riffle in a medium-gradient stream might be recorded as 5 percent boulders, 65 percent cobbles, 15 percent gravel, 10 percent sand, and 5 percent silt. Ranges are given on the survey form for the rock sizes. For the smaller rock sizes, remember that silt feels like talcum powder and sand feels gritty. If your riffle had 40 percent silt, 10 percent gravel, and no cobbles, you should either find another station to monitor or switch to the Muddy Bottom Sampling method.

Land uses in the watershed: The survey form asks if land use impacts within a one-mile radius of your sampling site are high (H), moderate (M), slight (S), or none (N). Although these questions are somewhat subjective, determining the impact is easy and straightforward.

- Note "H" for a land use if it:
 - Comprises the majority of land in the watershed and is polluting the stream, such as a stream traveling through land that is being strip mined for coal.

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BIOLOGICAL MONITORING INSTRUCTIONS FOR VA SOS STREAM MONITORS

- Has a severe impact on stream quality even though the land use does not utilize a great deal of land, such as a construction site that has caused the stream to be full of sit.
- Note "M" if the land use is definitely contributing to stream degradation, but is not the major cause for degradation (or is one of many causes). For example, parking lot runoff and trash from a shopping mall may contribute significantly to stream pollution, but they may not be the only causes of stream degradation.
- Note "S" for a land use if its impacts only slightly pollute the stream. For example, although a farm may be present, good farming practices and conservation measures may mean the pollution impact is negligible.
- Note "N" if the land use is present but causing no pollution.
- · If the land use is not present, do not write anything.

Take the time to drive or walk through your watershed before filling out this section to determine if these land uses are present and impacting the stream.

When considering land use as the controlling factor in stream quality, look not just at the area visible from the stream but at all the land draining into the stream – the watershed. If the stream collects water from an intensely developed or agricultural area, do not be surprised if no organisms are found. Should this be the case, consider visiting a forested stream of the same size in the same

watershed for sampling comparison. You might be surprised by the different types of organisms you find.

You can identify a pollution source by sampling the stream at quarter-mile intervals upstream from the initial sampling point (where a pollution impact is suspected) until quality improves. The pollution sources should be identified somewhere between the point where degraded conditions were first found and the point where water quality improves.

Comments: Use this space to record observations that are not noted elsewhere on the data form. This may include current and potential future threats to the stream's health.

STREAM PROBLEMS AND THEIR EFFECTS ON STREAM ORGANISMS

- Physical Problems may include excessive sediment from erosion, street runoff, or discharge pipes. Sediment can create poor riffle characteristics, contribute to excessive flooding, reduce flow, change water temperature, and smother aquatic life. The result is usually a reduction in the number of macroinvertebrates in the study area.
- Organic Pollution is from excessive human or livestock wastes or high nutrient enrichment from farm or yard runoff.
 The result is usually a reduction in the diversity of insects.
- Toxic Pollution includes chemical pollutants such as chlorine, acids, metals, pesticides, and oil. The result is usually a reduction in the number of insects.

Appendix P: Stations Table*

Appendix							Monitori	Monitori	
Station ID	Latitud e	Longitu de	City or County	Waterbody Name	Sample Site Desciption	Collecting Organizat ion	ng Purpose s	ng Frequen cy	Parameters Sampled at this Location
CC1	37.4585 57	- 77.3795 02	Henrico	Cornelius Creek	7580 Laurel Hill Ln Richmond	Henrico Area Water Quality Samplers (HAWQS)	General Stream Health Assessm ent, Public Education , Pollution Screening , Advocacy , CMC Integratio	Twice Per Year	Benthic macroinvertebr ates, temperature
CC1	57	02	Henrico	Creek	Richmond	(HAWQS)	General Stream	Per Year	temperature
P7-FC-01	37.55	-77.93	Powhatan	Fighting Creek	Between Powhatan Elementary School and Elizabeth Randolph Lewis Powhatan YMCA	VASOS	Health Assessm ent, Public Education , Pollution Screening , Advocacy , CMC Integratio n	Twice Per Year	Benthic macroinvertebr ates, temperature
							General Stream		
							Health Assessm ent, Public Education , Pollution Screening		
	00.0404	-		El . 6 . 1	Buffalo Springs		Advocacy , CMC	.	Benthic macroinvertebr
M6-FRB-01	36.6484	78.6611	Mecklenb urg	Flat Rock Branch	National Historic Site	VASOS Henrico Area	Integration General Stream Health Assessm ent, Public Education , Pollution Screening	Twice Per Year	ates, temperature
HC2	37.5987 76	- 77.4268 04	Henrico	Horse Creek	where thrush lane hits lark drive	Water Quality Samplers (HAWQS)	Advocacy , CMC Integratio n	Twice Per Year	Benthic macroinvertebr ates, temperature
P7JC1	37.5744	- 77.8038	Powhatan	Jones Creek	0.3 miles northeast of the end of Farmington Lane and 0.2 miles northwest of the end of Old Timber Way	James River Master Naturalists	General Stream Health Assessm ent, Public Education , Pollution Screening	Twice Per Year	Benthic macroinvertebr ates, temperature

	T	T	T		1	1		1	T
							Advocacy , CMC		
							Integratio		
							n		
							General Stream		
							Health		
							Assessm		
							ent,		
							Public Education		
					Between		, Pollution		
					where Park	Henrico	Screening		
					Ln and Libbie Lake	Area Water	, Advocacy		Benthic
		_			S St hit	Quality	, CMC		macroinvertebr
	37.5886	77.4936		Jordan	Staples Mill	Samplers	Integratio	Twice	ates,
JB1	1	1	Henrico	Branch	Rd	(HAWQS)	n	Per Year	temperature
							General Stream		
							Health		
							Assessm		
							ent, Public		
							Education		
							, Pollution		
					Noor		Screening		
					Near playground		, Advocacy		Benthic
		-		Little	area in		, CMC		macroinvertebr
WD4	37.5856	77.5494	Handa	Westham	Tuckahoe	1100000	Integratio	Twice	ates,
WP1	33	21	Henrico	Creek	Public Park	HAWQS	n General	Per Year	temperature
							Stream		
							Health		
							Assessm ent,		
							Public		
							Education		
							, Pollution		
							Screening .		
							Advocacy		Benthic
	37.6645	77.5500		Meredith	End of Lindsey		, CMC Integratio	Twice	macroinvertebr
MB1	69	24	Henrico	Branch 1	Lakes Dr	HAWQS	n	Per Year	ates, temperature
							General		,
							Stream		
							Health Assessm		
							ent,		
					Northwest		Public		
					edge of Reynolds		Education , Pollution		
					Community		Screening		
					college		, A di		Donth:-
		_			campus 300 feet south of		Advocacy , CMC		Benthic macroinvertebr
	37.6375	77.4775		North Run	E Parham		Integratio	Twice	ates,
NR1	19	2	Henrico	1	Rd	HAWQS	n	Per Year	temperature
							General Stream		
							Health		
							Assessm		
							ent, Public		
							Education		
					Off the end		, Pollution		5 4.
		_			of Boxwood Place		Screening		Benthic macroinvertebr
	37.6292	77.4767		North Run	Lakeside,		, Advocacy	Twice	ates,
NR2	7	9	Henrico	2	Va	HAWQS	, CMC	Per Year	temperature

							Integratio		
							n General		
							Stream Health		
							Assessm ent,		
							Public Education		
					Crooked		, Pollution Screening		
				Pohick	Creek Park 150 feet		, Advocacy		Benthic
		-	Fairfax	Creek - Rabbit	north of Commonwe		, CMC Integratio	Twice	macroinvertebr ates,
РОН9	38.8145	77.2892	(county)	Branch	alth Blvd	VASOS	n General	Per Year	temperature
							Stream Health		
							Assessm ent,		
					Mount Vernon		Public Education		
					District Park west of Fort		, Pollution Screening		
16-PL28-					Hunt Rd just south of the driveway to	Northern	, Advocacy , CMC		Benthic macroinvertebr
Quander- QC1	38.7695 75	77.0612 88	Fairfax (county)	Quander Creek	Westgrove Dog Park	Virginia SWCD	Integratio	Twice Per Year	ates, temperature
QUI	7.5	00	(county)	Oreck	Dog r ark	OWOD	General Stream	Terrear	temperature
							Health Assessm		
							ent, Public		
							Education , Pollution		
							Screening ,		
					At the end	James River	Advocacy , CMC		Benthic macroinvertebr
P7SC1	37.573	- 77.8006	Powhatan	Skunkwater Creek	of Old Timber Way	Master Naturalists	Integratio n	Twice Per Year	ates, temperature
							General Stream		
							Health Assessm		
							ent, Public		
					Straight line		Education , Pollution Screening		
					from where Windbluff Dr		, Advocacy		Benthic
	37.6181	- 77.5939		Stony Run	and Pepperhill		, CMC Integratio	Twice	macroinvertebr ates,
SRW1	52	27	Henrico	West	Ln intersect	HAWQS	n General	Per Year	temperature
							Stream Health		
							Assessm ent,		
							Public Education		
							, Pollution Screening		
					Where Vale		, Advocacy		Benthic
TD4	37.6011	77.4628	Honrine	Trumpet	St and Gillespie	Намос	, CMC Integratio	Twice	macroinvertebr ates,
TB1	1	81	Henrico	Branch	Ave cross	HAWQS	n	Per Year	temperature

TuckahoeCre ek1	37.6086 99	- 77.6369 59	Henrico	Tuckahoe Creek	Straight back from Brandon Rd behind Tuckahoe Village West Recreation	HAWQS	General Stream Health Assessm ent, Public Education , Pollution Screening , Advocacy , CMC Integratio n	Twice Per Year	Benthic macroinvertebr ates, temperature
UBCP1	37.6057 44	- 77.5458	Henrico	Upham Brook	In Cheswick Park	HAWQS	General Stream Health Assessm ent, Public Education , Pollution Screening , Advocacy , CMC Integratio	Twice Per Year	Benthic macroinvertebr ates, temperature
UBTB1	37.6081 14	- 77.4628 86	Henrico	Upham Brook/Trum pet Branch	Located at 6000 Club Road behind Belmont Park and adjacent to Belmont Golf Course	HAWQS	General Stream Health Assessm ent, Public Education , Pollution Screening , Advocacy , CMC Integratio n	Twice Per Year	Benthic macroinvertebr ates, temperature

^{*}New sites may be added as additional volunteers become certified.