New Mexico Environment Department (NMED) Surface Water Quality Bureau (SWQB)

Standard Operating Procedure (SOP) for

BENTHIC MACROINVERTEBRATE SAMPLING

Approval Signatures

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Kristopher Barrios
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Date

1.0 Purpose and Scope

The purpose of this document is to describe the process for conducting benthic macroinvertebrate sampling. This includes sample collection techniques in wadeable streams/rivers and large river systems.

2.0 Personnel Responsibilities

The Program Manager coordinates with the Project Manager(s), Technical Staff, and the QAO as applicable to ensure quality data is collected, verified, and validated to support program commitments. The Program Manager will provide input on the scope and intent of the SOP as it pertains to the program's goals and objectives. The Program Manager will review SOP every two (2) years after revision by SME and/or Quality Assurance Officer.

Project Manager is responsible for organizing and planning data collection activities and ensuring monitoring activities adhere to applicable SOPs and Field Sampling Plans.

The Quality Assurance Officer (QAO) is involved in the development and revision of this SOP to ensure the SOP meets the requirements of the SWQB's Quality Assurance Project Plan. The QAO, along with the Subject Matter Expert and Program manager will determine if any revisions to this SOP are needed at a minimum of every two (2) years in accordance with SOP 1.1 for the Creation and Maintenance of SOPs (NMED/SWQB 2020). Pending the review and approval of the document, the QAO will ensure the SOP is accessible through the SWQB's website.

The SME reviews SOP every two (2) years and updates the SOP as the procedure or equipment changes in coordination with the QAO and Program Manager.

Personnel who conduct benthic macroinvertebrate sampling and data validation and verification activities or who supervise those who do must be familiar with this SOP.

3.0 Background and Precautions

3.1 Background

SWQB utilizes a modified EPA EMAP macroinvertebrate sampling method for wadeable streams and rivers (Peck et al., 2006) by collecting a reach-wide composite sample from 9 equidistant transects along the stream reach. This protocol uses a 30 cm wide D-frame kick net with a mesh size of 500 μm and a sample area of 0.09 m^2 (1 ft^2) per individual sample (0.81 m^2 total sample area). SWQB also utilizes the EMAP macroinvertebrate sample collection procedures for boatable rivers (Lazorchak et al., 2000) however, instead of 9 equidistant transects the SWQB utilizes 11 equidistant transects for boatable rivers .

3.2 Procedural Precautions

Benthic Macroinvertebrate samples should be taken prior to the pebble count to ensure that there is no disturbance within the transect.

If used in conjunction with SOP 5.0, Benthic macroinvertebrate samples should be collected at least six weeks after a scouring flow event.

Site conditions or project-specific data collection objectives may necessitate the use of alternative field procedures not included in this SOP. The use of field methods other than those presented in this SOP must be approved by the Program Manager and alternative methods must be accurately documented.

When collecting benthic macroinvertebrate samples in large rivers, it may be necessary to exit the boat to make collecting the sample easier. This process is unique to New Mexico due to large rivers in New Mexico being much more shallow than other rivers in the US.

3.3 Safety Precautions

Wading across a stream bed can be very dangerous depending on flow and substrate conditions. Do not attempt to wade into a stream if the depth (in ft) multiplied by velocity (in ft/s) equals or exceeds 10 square feet per second (ft^2/s). For example, a stream that is 2 ft deep, and has velocities of 5 ft/s or more, should be considered too dangerous to wade. If you unknowingly start to take measurements and discover part of the way across that you will violate this rule ("the rule of ten"), return to the nearest bank and note "too fast/deep to measure" on the field form. Some wadeable streams may be unwadeable during spring runoff but will be wadeable when base flow resumes.

Some channels have quicksand-like areas, deep holes, sharp rocks, excessive fallen logs, etc., that can lead to foot entrapment, injury, or falls. A wading rod, surveyor's rod, or stick can be used for stabilization and to probe the streambed when conditions are uncertain. Staff should use best professional judgement to assess risks involved with data collection.

Field staff should exercise their professional judgement regarding all sampling conditions and whether to begin or continue traveling to a field site or conduct sampling. Field staff should not risk traveling to remote sites during weather events such as severe monsoon rainstorms or snowstorms and should not sample during nearby thunderstorms. Sampling should be rescheduled or delayed to accommodate unsafe conditions. Field staff should alert their supervisor on their sampling plan for the day and carry a cellphone at all times in case of emergency. Field staff should carry driving directions, a GPS, and a map of the area surrounding the site for navigating.

When sampling in large (non-wadeable) rivers, staff should not exit the boat in water deeper than 1 meter. Boat must be secure and stable before staff collect a benthic macroinvertebrate sample while in the boat.

Refer to SWQB's Job Hazard Analysis (JHA) for further safety precautions when conducting field work.

4.0 Definitions

Benthic macroinvertebrates – Animals without backbones, living in or on sediments or other substrates, of a size large enough to be seen by the unaided eye, and which can be retained by a US Standard No. 30 sieve (0.595-mm openings).

D-frame dip net – Dimensions of frame are 0.3 m width and 0.3 m height and shaped as a "D" where frame attaches to handle. Net is cone or bag-shaped for capture of organisms. Can be used in a variety of habitat types and used as a kick net, or for "jabbing", "dipping", or "sweeping".

Program Manager – An individual within the SWQB that manages a program such as the Monitoring, Assessment and Standards Section (MASS), Watershed Protection Section (WPS) or Point Source Regulation Section (PSRS). The Program Manager may be the same individual as the Subject Matter Expert.

Project Manager – An individual responsible for a specific project. This individual, in most cases, holds a different title within the organization. The Program Manager and Project Manager are not necessarily synonymous. The Project Manager may be the same individual as the Subject Matter Expert.

Quality Assurance (QA) – An integrated system of management activities involving planning, implementation, documentation, assessment, reporting, and quality improvement to ensure that a process, item, or service is of the type and quality needed and expected by the SWQB.

Quality Control (QC) – The overall system of technical activities that measures the attributes and performance of a process, item, or service against defined standards to verify that they meet the stated requirements established by the SWQB; operational techniques and activities that are used to fulfill requirements for quality.

Quality Assurance Officer (QAO) – An individual within the MASS that is responsible for overseeing the development and implementation of all quality assurance procedures and processes within the SWQB including those projects that receive support or funding from the SWQB. The QAO is also responsible for documenting the validation and verification of data sets for potential use in assessment of surface waters.

Quality Assurance Project Plan (QAPP) – A formal planning document for environmental data collection activities that describes the data collection procedures and the quality assurance and quality control activities that must be implemented to ensure that the results are sufficient and adequate to satisfy the stated performance criteria.

Standard Operating Procedure (SOP) – A document that lists the steps that should be completed when performing a task.

Subject Matter Expert (SME) – A person who is familiar with the purpose and procedure for accomplishing a task. The SME may hold another title within the organization.

5.0 Equipment and Tools

- Field Form
    - Habitat and Biota Work Cover Sheet (wadeable)
    - NRSA Sample Collection Form (non-wadeable)
- 30 cm wide D-frame kick net with a mesh size of 500 μm
- Sieve (500 μm mesh size)

- Bucket
- Forceps
- 500-1000 mL sample container
- 95% ethanol (EtOH)
- Soft bristle brush
- Squirt/rinse bottle
- Water resistant paper
- Pencil(s)

6.0 Sampling Process Description

Staff use two different methods to sample benthic macroinvertebrates: (1) large river method and (2) reach wide multi-habitat method. If boatable EMAP monitoring is being conducted, then the large river method is used. Otherwise, a reach wide multi-habitat method is applied. There are two parts to each of these methods. The first step is laying out the reach to determine the location where the samples will be collected. The second step is using the sampling technique appropriate for each location.

6.1 Wadeable Streams/Rivers

For each sampling point, record the dominant substrate and habitat type at each transect on the HABITAT AND BIOTA FIELD WORK COVER SHEET (Field Form). Use the appropriate sampling method for each habitat type to collect benthic macroinvertebrates (*Section 6.1.1 and Section 6.1.2*). All macroinvertebrate samples collected at a stream or river site are combined into a single composite sample to characterize the reach.

1. Establish 5 equidistant transects following protocols in the *Reach Layout* section of SOP 5.0 - Physical Habitat Measurements (NMED/SWQB 2019). Figure 1 in this SOP illustrates the reach layout for biological collections. For this modified EMAP method, only 9 samples are collected, one at each of the 5 transects (A-E) and one at the midpoints between transects (A.5-D.5). As you proceed upstream from transect to transect, combine all samples into a bucket or similar container, regardless of whether they were collected using the "riffle/rapid" or "pool/glide" procedure.

2. Using the appropriate sampling method for each habitat type encountered (riffle/rapid or pool/glide), start sampling on the LEFT side of Transect A (left when looking downstream) and follow the pattern of LEFT-CENTER-RIGHT as you move upstream to each consecutive sampling point. That is to say, collect a sample at 25% of the wetted width (LEFT) along Transect A, at 50% of the wetted width (CENTER) along Transect A.5, and at 75% of the wetted width (RIGHT) along Transect B. Repeat this pattern for the remaining 6 transects (B.5 – E). If you cannot collect a sample at the designated point because of deep water or unsafe conditions, relocate to another random point on the same transect. Record sample collection information on the Field Form.

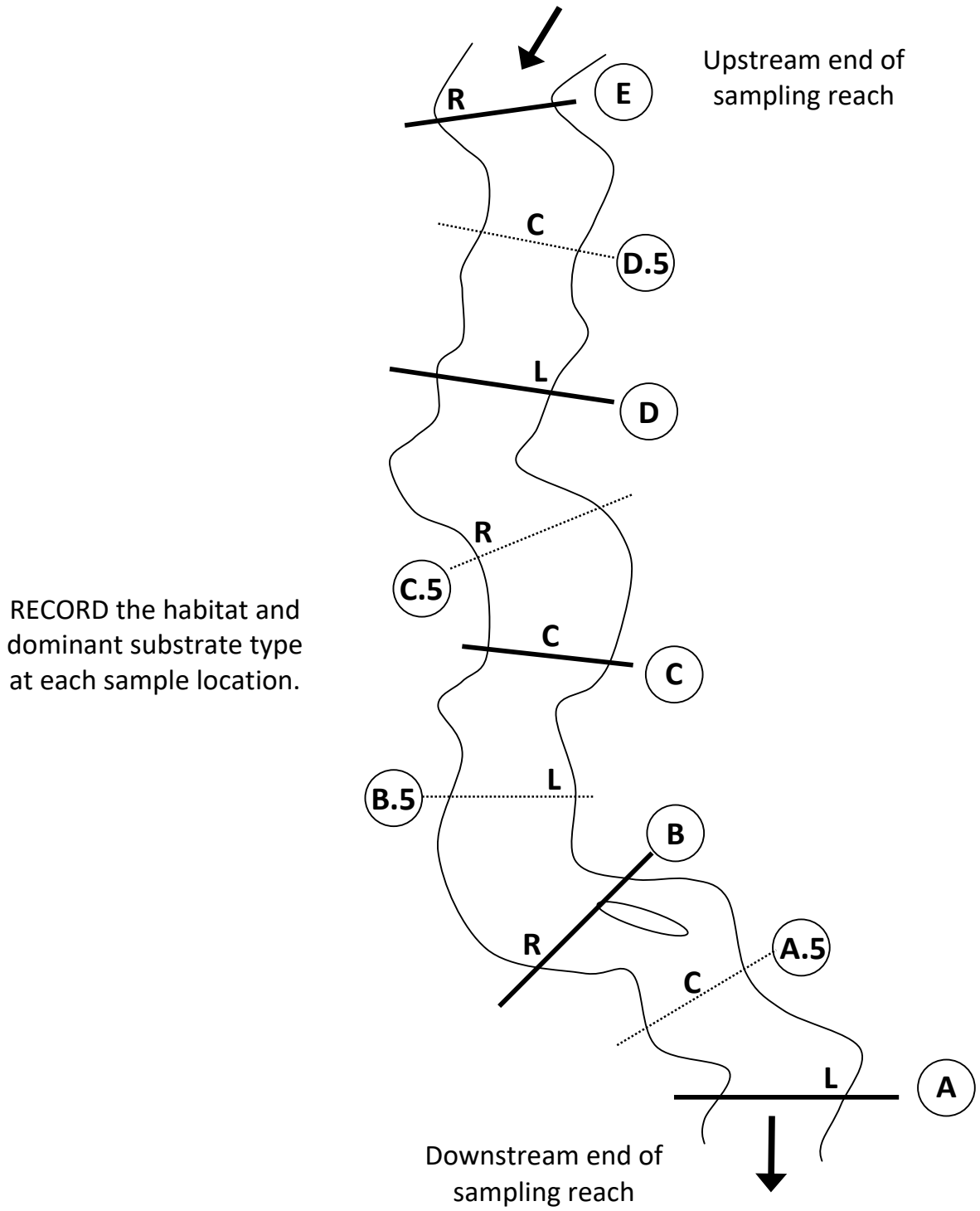
NOTE: If the net cannot be used, hand-pick a sample for 30 seconds from about 1 square foot (ft^2) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the vegetation within a 1-ft^2 quadrat for 30 seconds. Place this hand-picked sample directly into the sample container. Note which transect(s) required the modified collection procedure in the comments section of the Field Form.

Table 1. Sampling habitat types

HABITAT	CHARACTERISTICS
Pool	Still water; low velocity; smooth, glassy surface; usually deep compared to other parts of the channel
GLide	Water moving slowly, with smooth, unbroken surface; low turbulence
RIffle	Water moving, with small ripples, waves, and eddies; waves not breaking and surface tension is not broken; "babbling" or "gurgling" sound
RApid	Water movement is rapid and turbulent; surface with intermittent "white water" with breaking waves; continuous rushing sound

Table 2. Substrate types

SUBSTRATE	CHARACTERISTICS
Fine/sand	Non-gritty to gritty particles (silt/clay/muck up to ladybug sized;<2 mm diameter)
Gravel	Fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm diameter)
Coarse	Cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm diameter)
Other	Bedrock (larger than car sized; > 4000 mm diameter); hardpan (firm, consolidated fine substrate); wood of any size; aquatic vegetation, etc. Note the type of "other" substrate in comments section of field form.



RECORD the habitat and dominant substrate type at each sample location.

Figure 1. Reach layout for biological collections in wadeable streams

6.1.1 Riffle/Rapid Habitats

1. Position the net directly downstream of each transect with the opening facing upstream, quickly position the net securely on the stream bottom and eliminate gaps under the frame. Avoid large rocks that prevent the net from seating properly on the stream bottom.

2. Holding the net in position on the substrate, visually define a quadrat that is one foot wide and one foot long immediately upstream of the net opening. The area within this quadrat is 1 square foot.

3. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands or a scrub brush to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball-sized or larger and which are at least 50% into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.

4. Hold the D-net securely in position. Starting at the upstream end of the quadrat, vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch).

NOTE: If there is insufficient water to collect the sample with the D-net, randomly pick up 10 rocks from the riffle and wash the organisms off into a bucket which is half full of water.

NOTE: For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually these types of vegetation are lying flat against the substrate due to current. Use a knife or scissors to remove only the vegetation that lies within the quadrat (i.e., not entire strands that are rooted within the quadrat) and place it into the net.

5. Pull the net up out of the water. Immerse the net in the stream flow (being careful not to disturb the stream bottom) several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any detritus or substrate enter the mouth of the net during this step.

6. Process the composite sample as described in Section 6.3 Sample Processing.

6.1.2 Pool/Glide Habitats

1. Start downstream of each transect, visually define a quadrat that is one foot wide and one foot long at the sampling point. The area within this quadrat is 1 square foot.

2. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands or a scrub brush to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball-sized or larger and which are at least 50% into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.

3. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net at all times so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds (use a stopwatch).

NOTE: For vegetation-choked sampling points, sweep the net through the vegetation for 30 seconds.

4. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

5. Process the composite sample as described in Section 6.3 Sample Processing.

6.2 Large Rivers (non-wadeable)

If non-wadeable EMAP monitoring is being conducted, then the modified large river method described below is used (USEPA, 2019). Benthic macroinvertebrate sample locations are shown in Figure 2 and accessed by boat. Benthic macroinvertebrate samples are collected from the two most dominant habitat types that are found within a 10m x 15m randomly selected sampling station at 11 equidistant transects along the reach (Figure 2). Two 1-meter sweeps are collected from the dominant habitat and a third sweep is collected from the secondary habitat (i.e. 3 sweeps from the 10m x 15 m station at each transect. Sampling should be conducted from upstream to downstream along the reach (from Transect A progressively down to Transect K). All macroinvertebrate samples are combined into a single composite sample to characterize the reach. Sample collection information is recorded on the NRSA Sample Collection Form (USEPA, 2019).

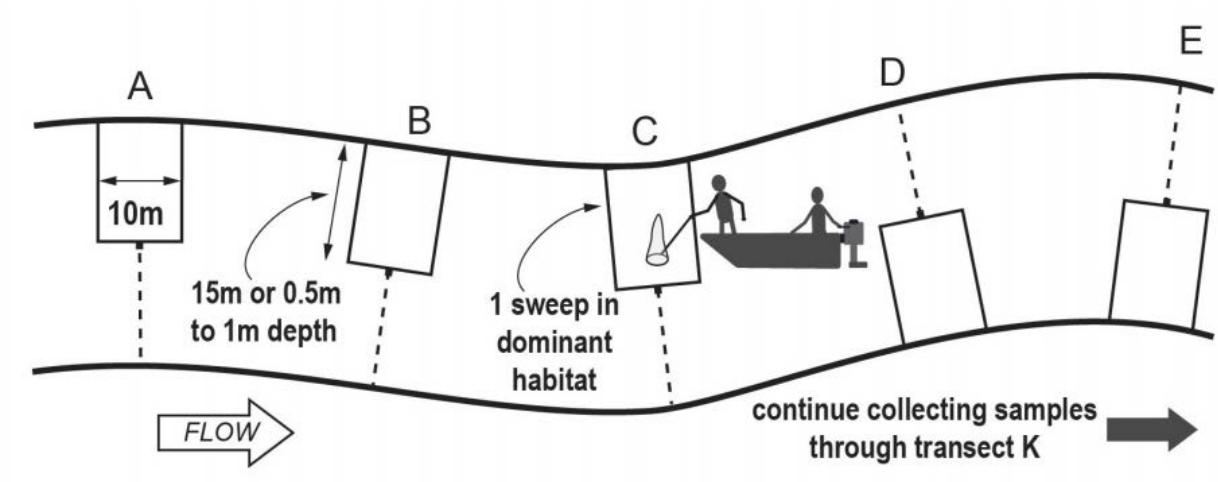


Figure 2. NRSA Sampling Design Benthic Macroinvertebrate Large Rivers

NRSA sampling design for reach wide benthic macroinvertebrate collection in large rivers. Modified to two sweeps in dominant habitat and one sweep in sub-dominant (or secondary) habitat.

Refer to the National Rivers and Streams Assessment 2018/19: Field Operations Manual Non-Wadeable Benthic Macroinvertebrates sampling procedure.

1. After locating the sampling station site, identify the two dominant habitat type within the 10X15m station as described in Figure 2:

    - Rocky/cobble/gravel/large woody debris: fine to coarse gravel; tennis ball to car sized; and/or woody material with a small end diameter of at least 10 cm (4 in.) and a length of at least 1.5 m (5 ft).
    - Organic fine mud or sand: material less that or equal to 2 mm in size.
    - Macrophyte beds: groups of aquatic plants growing in or near water. They may be either emergent, submerged, or floating.
    - Leaf pack: leaves that fall into streams and accumulate behind branches, rocks, and other obstructions in the stream, forming “packs”.

    Record the side of the river on which the sampling station is located (left or right, facing downstream).

2. Use the D-frame dip net to sweep through 1 linear meter of the most dominant habitat type at two locations within the 10m x 15m sampling station (i.e., take 2 sweeps of the dominant habitat type), making sure to disturb the substrate enough to dislodge the organisms.

    - If the dominant habitat is rocky/cobble/large or woody debris, it may be necessary to exit the boat and disturb the substrate (e.g., overturn rocks, logs) using your feet while sweeping the net through the disturbed area.
    - Because a dip-net is being used for sampling, the maximum depth for sampling will be approximately 0.5 m. In cases in which the depth of the river quickly drops off, it may be necessary to sample in the nearest several meters to the shore.

3. After completing the first 1-meter sweep, remove organisms and debris from net and place them in a bucket or sample container.

4. Conduct a sweep of the most dominant habitat at a second location; again, remove organisms and debris from net and place them in a bucket or sample container.

5. Conduct a third and final sweep of the secondary habitat; again, remove organisms and debris from net and place them in a bucket or sample container. If only one habitat type is present, all 3 sweeps will be in that habitat.

6. Record the sampled substrate types (listed in Table 2) on the NRSA Sample Collection Form.

7. Identify the channel habitat type (listed in Table 1) where the sampling quadrat was located. Place an “X” in the appropriate channel habitat type box for the transect on the NRSA Sample Collection Form.

8. Proceed to the next sampling station and repeat steps 1-7. The organisms and detritus collected at each station on the river should be combined to create a single composite sample. After sampling at all 11 stations is completed, process the composite sample in the bucket according to procedures described in Section 6.3 Sample Processing.

6.3 Sample Processing

After collecting all of the samples, prepare one reach wide composite sample. Typically, the SWQB combines all samples taken in a 5-gallon bucket to sort organic matter from benthic macroinvertebrates samples. After all samples have been combined into a composite, place contents of the bucket into a sieve (500 μm mesh size) and wash off and discard large objects. Depending on how much debris was collected, this may need to be done in several steps, being careful not to overfill the sieve. Repeated rinsing should be done until rinse water is clear exiting the sieve. When large amounts of sediment are collected, thoroughly agitate the contents of the composite sample (in bucket) by swirling and pour off organic debris into the sieve. Refill the bucket with stream water to 1/4 – 1/2 full. Once organic debris has been removed, inspect large pebbles for attached macroinvertebrates (scrub off all that are present into sieve), and discard pebbles. Agitate the bucket contents once again by swirling, and immediately pour off the water into the sieve before lighter items in the water column can settle out. Be careful not to pour out large amounts of sediment. Repeat several times until you are confident that all macroinvertebrates have been removed and placed in sieve. Visually inspect any remaining sediment for snails, clams, mussels, and macroinvertebrate cases and tubes. Remove these items and place in the sieve or directly into the sample jar.

Using forceps and a rinse bottle, wash the contents of the sieve into the sample jar. Put as little water as possible into the sample container. Do not fill the sample container more than 1/2 full. Divide the sample into as many jars as needed and indicate the jar number on the sample label and the id of the jar (e.g., 1 of 3). Fill the jar with 95% ethanol (EtOH) leaving no head space. Tighten the cap on each jar and slowly tip the jar to a horizontal position, gently rotating the jar to mix the preservative. Do not shake the jar.

Place a sample request identification (RID) sticker on each sample container (RIDs must match for each container for a site). A sample label is required for all sample jars from a single composite sample. The label should be written on water resistant paper in pencil and contain the site name, RID number, and date that the sample was collected, as well as the jar number if multiple containers were used. Place the label(s) in the sample jar(s).

6.4 Sample Preservation

If processing or shipping of the samples will be delayed for more than a week, carefully pour the ethanol through a 500 μm sieve and dispose properly. Any organisms in the sieve must be placed back in the sample container. Add fresh preservative (95% ethanol (EtOH))to the sample jar so that there is no head space left. Invert the sample to ensure the sample has adequate contact with the preservative. This procedure should be repeated if the samples have not been processed or shipped after three months.

7.0 Data and Records Management

Enter all data from the hard copy Habitat and Biota Field Work Cover Sheet to an electronic version and save in the electronic project folder for wadable streams. NRSA Sample Collection Forms are hard copy only and must be filed in the physical project binder.

7.1 SQUID Benthic Macroinvertebrate Event Creation and Excel Data Upload Instructions.

7.1.1 Creating a Benthic Macroinvertebrate Event Within a Station

1. Navigate to the Project File in SQUID. Find the station you are adding macroinvertebrate metadata to.
2. Select "Add New Sampling Event."
3. Select "benthic macroinvertebrates" as the *Sampling Event Type* category.
4. Populate all of the fields in the metadata tab. Collection method is "EMAP-Transect," collection equipment is "D-Frame Net," mesh size is "500," and Ben. Sample ID/Activity ID is the same as the station ID for the probabilistic site.

7.1.2 Uploading Excel Data to the Sampling Event

1. Copy and paste results from the lab into a new Excel spreadsheet.
2. Label three new columns "Status", "Exclude", and "Lab ID".
3. Enter "Final" into the "Status" column for every row with data.
4. Enter "N" into the "Exclude" column for every row with data.
5. Enter the name of the contract lab into the "Lab ID" column for every row with data.
6. Save file as a .csv
7. In SQUID, select "Data Management"
8. Select "Imports", then "Biological Results"
9. Choose the correct .csv file saved for importing.
10. Upload File
11. If all the data was successfully uploaded, click the upload button. If some data is not successfully uploaded, scroll over the error message and determine what is causing the data to not be uploaded.
12. Only upload data if all the data was accepted.

Tips and Tricks:

- If an error message appears, make sure life stage spelling matches the database (e.g. larva instead of larvae).
- Check the cell in the .csv file, ensure that there isn't a space in front or behind the data.

7.1.3 Viewing Uploaded Data in Excel

1. Select *Adhoc Report*.
2. Select *Project* and *Station*
3. From the *Reports* sections select the *Biological Data tab*.
4. Then select *Benthic Taxon Report*

8.0 Quality Assurance and Quality Control (QAQC)

The SWQB controls the quality of the benthic macroinvertebrate collections by using standardized methods that are documented in this SOP. All personnel who collect benthic macroinvertebrates must be familiar with these protocols, sign the acknowledgment form associated with this specific SOP and collect data in accordance with the procedures as they are defined in this SOP. In addition to standardized methods, proper training of field personnel represents a critical aspect of meeting the data quality objectives in order to fulfill the goals of the SWQB's QAPP (NMED/SWQB. 2018).

The SWQB implements QA/QC through the training of the procedures detailed in this SOP for benthic macroinvertebrate collections. Any SWQB personnel who conduct benthic macroinvertebrate collections are required to receive training in the field and be supervised by MASS monitoring staff for all aspects of benthic macroinvertebrate collections. Any MASS monitoring staff who conduct training in the field must have completed at least 1 field season of benthic macroinvertebrate collections. Once field training has occurred and all aspects of benthic macroinvertebrate collections have been observed as correct, personnel may conduct unsupervised benthic macroinvertebrate collections. The training will be documented and filed by Monitoring Team Supervisor. The trainee may request documentation of training from the Monitoring Team Supervisor if desired. The documentation of training may be requested up to 3 months after training, after 3 months it is at the discretion of Monitoring Team Supervisor. The trainee will be approved for benthic macroinvertebrate collections until next SOP revision. This process will ensure comparability and accuracy of data used for water quality assessments, refinement of water quality standards and TMDL development.

Assurance of field data collection for benthic macroinvertebrate collections are done through adherence to the process outlined in this SOP and oversight of the process by the QAO. If at any time the QAO determines this process is not being adhered to, the QAO has the authority to cease activities specific to this SOP with prior support and approval by the SWQB Bureau Chief and MASS Program Manager, until such a time that the issue can be resolved.

9.0 Related Forms

Habitat and Biota Field Work Cover Sheet, Physical Habitat Field Sheets SOP 5.0
NRSA Sample Collection Form - National Rivers & Streams Assessment 2018-19 Field Operations Manual Appendices, Appendix B Page 8, Boatable section. https://www.epa.gov/sites/production/files/2019-05/documents/nrsa1819_fom_appendix_version_1.2_may_2019.pdf
Bug, Habitat, and Nutrient Survey Equipment Checklist
Physical Habitat Measurements SOP 5.0

10.0 Revision History

Revision 0. Effective 2013.05.01. Original SOP.
Jodey Kougioulis, QAO; Seva Joseph, SME; Shelly Lemon, Acting Program Manager –MASS.

Revision 1. Effective 2020.06.24. Format change to SOP; Updated format to be consistent with SOP 1.1. Major updates to Personnel Responsibilities, Data and Records Management, Create New Sampling Event in SQUID, Uploading Excel Data to a Sampling Event, Viewing Uploaded Data in Excel, and Quality Assurance and Quality Control. Minor updates to Background and Precautions, Definitions, Equipment and Tools, Sample Process Description, Sample Processing, and References.

Miguel Montoya, QAO; Eliza Montoya, SME; Kris Barrios, Program Manager – MASS

11.0 References

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