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

Standard Operating Procedure (SOP) for

PERIPHYTON SAMPLING

Approval Signatures

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For Lynette Guevara
Program Manager - Monitoring, Assessment and Standards Section

Date

1.0 Purpose and Scope

The purpose of this SOP is to describe the sample collection techniques, preservation requirements, and equipment associated with periphyton sampling in lotic environments.

2.0 Personnel Responsibilities

The Monitoring, Assessment and Standards Section (MASS) Program Manager coordinates with Monitoring Team Supervisor, Project Managers, and the Quality Assurance Officer (QAO) as applicable to ensure quality data is collected, verified, and validated to support program commitments.

The 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; the MASS Program Manager; and SWQB subject matter experts (e.g., field staff scientists) will determine if any revisions to this SOP are needed at a minimum of every two (2) years in accordance with the most current SOP 1.1 for the Creation and Maintenance of SOPs. Pending the review and approval of the document, the QAO will ensure the SOP is accessible through the SWQB's website.

All SWQB personnel who collect periphyton samples in lotic environments or process periphyton samples collected from lotic environments are responsible for implementing procedures as prescribed in this SOP. SWQB staff who conduct procedures described under this SOP are required to sign the SOP acknowledgement statement for Periphyton Sampling prior to conducting procedures detailed in this SOP.

3.0 Background and Precautions

3.1 Background

Currently, the State of New Mexico has a narrative criterion to determine nutrient impairment, which states,

> *"Plant nutrients from other than natural causes shall not be present in concentrations which will produce undesirable aquatic life or result in a dominance of nuisance species in surface waters of the state"* (NMAC 2025).

This narrative criterion can be challenging to assess because the relationships between nutrient levels and impairment of designated uses are not defined, and distinguishing nutrients from "other than natural causes" is difficult.

To address this challenge, the SWQB has developed a nutrient listing methodology for perennial wadeable streams in New Mexico. For monitoring and assessment purposes, the SWQB typically distinguishes rivers from streams by defining systems that cannot be monitored effectively with the biological and habitat methods developed for wadeable streams. These rivers also generally meet the Simon and Lyons (1995) definition of great rivers as those having

drainage areas greater than 2,300 square miles (mi^2). While methods for monitoring and assessing wadeable perennial streams are well established, methods for large rivers are less advanced. To that end, the SWQB collects causal and response variables, including periphyton, to inform the development of future nutrient assessment protocols for New Mexico's large rivers.

3.2 Procedural Precautions

It is recommended to contact a staff member from the laboratory(s) and/or section lead prior to sampling to ensure analyses can be carried out within holding times.

Lugol's solution is needed for the preservation of diatom identification, enumeration, and biovolume samples. Procedures for making Lugol's solution are outlined in SWQB SOP 12.1 Lake Sampling. All reagent containers and chemicals must be properly stored, labeled, and properly disposed.

Site conditions or project-specific data collection objectives may necessitate the use of 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 or QAO for the project prior to use of modified field procedure or equipment. The change will need to be documented (e.g., email correspondence with Program Manager or QAO) and saved in the applicable survey folder on the SWQB network shared folders.

3.3 Safety Precautions

Do not attempt to wade into a stream if the depth (in ft) multiplied by the velocity (in ft/s) equals or exceeds the "rule of ten" or 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. Do not attempt to wade a stream if you feel it is unsafe, regardless of the outcome of the "rule of ten." Some channels have quicksand-like areas, deep holes, sharp rocks, excessive fallen logs, etc., that can lead to foot entrapment, injury, or falls. Staff should use best professional judgment to assess risks involved with data collection. Use gloves when working in waters suspected of having high bacterial contamination or elevated contamination of pollutants identified in 20.6.4 NMAC. Refer to SWQB's JHA for further safety precautions when conducting field work.

All personnel operating and working aboard inflatable watercraft in lotic environments must complete a Swiftwater Training Safety, ideally an American Canoe Association Level 2 Safety and Rescue training (or equivalent). Do not operate inflatable watercraft in hazardous weather conditions such as thunderstorms or high winds/rough water. If weather conditions become hazardous while out on water sampling must cease and you must proceed to a take-out or shore as safely as possible. All personnel must wear US Coast Guard type lll personal flotation devices (PFD) at all times while on board watercraft.

Before launching the boat, make sure that the following safety equipment is present onboard watercraft:

- First aid kit
- Throw rope
- Safety knife
- Whistle

4.0 Definitions and Acronyms

For common definitions and acronyms not defined in this SOP, refer to the most up to date SWQB Quality Management Plan for Environmental Data Operations.

EMAP – Environmental Monitoring & Assessment Program

NRSA – National River and Stream Assessment

Periphyton - an assemblage of organisms that grow on underwater surfaces and includes algae, bacteria, fungi, protozoa, and other organisms.

Cobble – for this method cobble refers to any substrate particle that is large enough to sample with the delimiter, i.e. at least 2.4 cm across.

Woody Snag – any log or branch that is submersed in the water providing substrate for periphyton growth.

5.0 Equipment and Tools

- 12 cm^2 area delimiter (3.8 cm diameter pipe, 3 cm tall)
- Stiff-bristle toothbrush – bent back 90° and bristles trimmed to about 6 mm
- tray – to carry rocks and wood
- Large funnel (15-20 cm diameter)
- 500 mL graduated plastic sample container for the composite sample with marked volume graduations
- Scalpel or knife
- Scissors
- 1 L wash bottle for DI
- Ruler
- Pruning shears or saw
- 60 mL plastic syringe with tip removed, and length of tubing (20 mL)
- Cooler (small soft-sided preferred)
- Timer or stopwatch
- Wet ice
- Dry ice

6.0 Collection of Periphyton Samples (PROCESS DESCRIPTION)

Two different methods are used to sample periphyton depending on whether the reach is non-wadeable (large river) or wadeable. There are three parts to both methods. The first step is laying out the reach to determine transects where the samples will be collected. The second step is determining the type of substrate to be sampled at each site along the transect. The third step is using the prescribed sampling technique appropriate for the specific substrate. Although a single transect can contain multiple substrate types, each sample point along a transect must contain a single type of substrate. While hard substrate is preferred (cobble and wood), sand may be sampled when specific conditions and sampling criteria are met.

6.1 Substrate Sampling Methods

6.1.1 Sampling Procedure for Non-Wadeable Large Rivers

This sample method is used on large rivers where boatable National Rivers and Streams Assessment (NRSA) methods are conducted. Establish 11 equidistant transects by using the following protocols in the NRSA 2023-2024 Field Operations Manual Non-Wadeable for periphyton sampling procedure in large rivers (USEPA 2023a). Locating the X-Site and Transect A are completed in the office before sampling commences.

1) Using GIS or aerial photography, locate the X-site (as shown in **Figure 1)** using the coordinates associated with the site.
2) Determine the average wetted width of the channel at the X-site using GIS, available maps, and/or aerial photographs. To get an average, determine the wetted width of the channel at five places of "typical" width within approximately five channel widths upstream and downstream from the X-site. Average the 5 readings together and round to the nearest 1 m.
3) Multiply the average wetted width by 40 to determine the reach length. If the average width is <4 m, use 150 m as a minimum reach length. If the average width is >100 m, use 4 km as a maximum reach length.
4) From the X-site, measure a distance of one-half the reach length upstream using GIS. Be careful to measure all of the bends of the river/stream; do not artificially straighten out the line of measurement. The upstream endpoint is marked as Transect A which is the start point of the sampling reach.
5) Assign the sampling station at Transect A randomly (e.g., use the seconds display on a digital watch or other random number selection method to select the initial sampling station: 0 - 4 = Left Bank, 5 – 9 = Right Bank). From here, three stations will be on the first (randomly selected) side of the river, then two on the other, then two on the first side, and so on through Transect K (as shown in **Figure 1**). Note that left and right sides of the stream are determined while you are facing downstream.
6) After sampling at Transect A, measure 1/10 of the reach length downstream from Transect A using a laser range finder. This location is Transect B. Continue measuring in this method, marking Transects C-K, in increments of 1/10 of the reach length.

There are some conditions that may require sliding the reach about the X-site (i.e., the X-site is no longer located at the midpoint of the reach) to avoid features we do not wish to or physically cannot sample across. Reasons for sliding the reach may include lack of landowner permission, confluence with a higher order water body, impoundment, or impassable barrier. Sliding the reach involves noting the distance of the barrier, confluence, or other restriction from the X-site, and flagging the restriction as the endpoint of the reach. Add the distance to the other end of the reach, such that the total reach length remains the same, but it is no longer centered about the X-site. As much as possible, use GIS and aerial imagery to identify conditions that necessitate sliding the reach prior to sampling.

For braided channels, sum the wetted width of all the braids that fall within a measurement and use that as the width for calculating the 40 x channel width reach length. If there is any question regarding an appropriate reach length for the braided system, it is better to overestimate.

Record the Station ID, Transect A and K GPS coordinates, elevation at Transects A and K, Average Wetted Width, Field Crew, and Total Reach Length on *Boatable Periphyton Field Form*. At each of the 11 transects, collect samples from the near shore shallows at each sampling station assigned during the layout of the reach (Figure 1). Collect the substrate selected for sampling from a depth no deeper than 0.5 m. If you cannot collect a sample because the location is too deep, skip the transect. Collect one sample from each of the transects (if possible) and composite all discreet collections into one container to produce a composite sample for each stream reach. Record the number of transects sampled and the total volume of the composite sample on the field sheet.

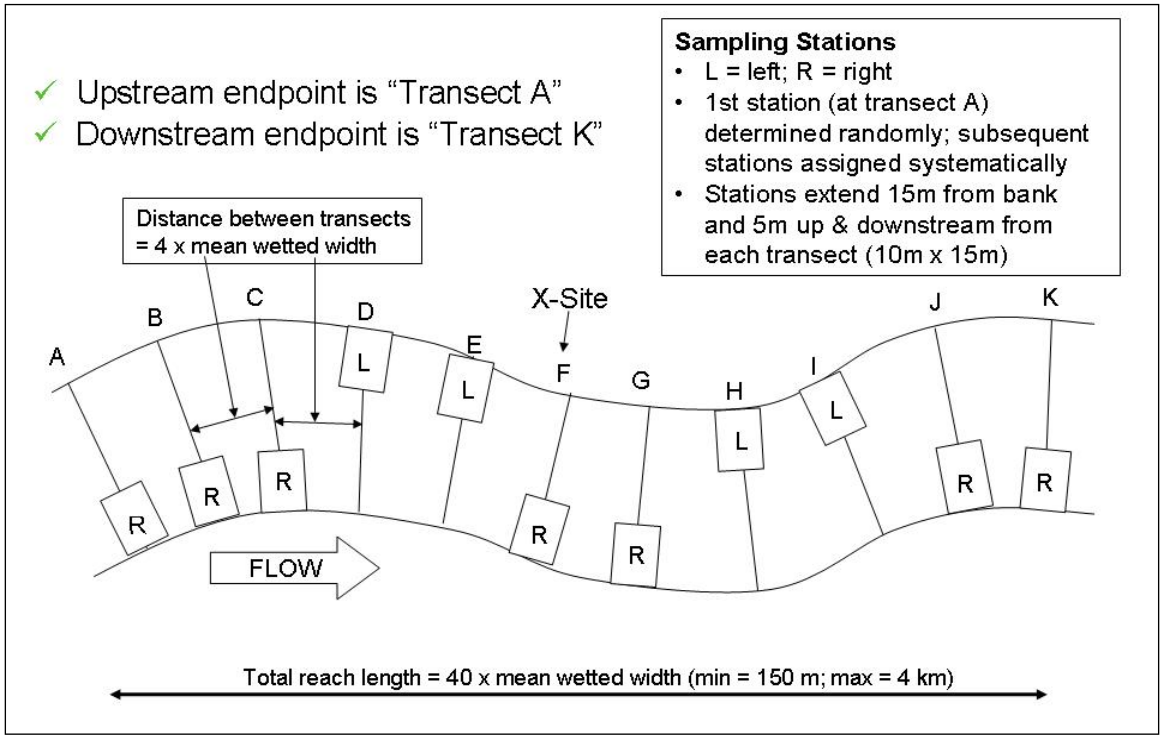

Figure 1. NRSA substrate sampling design for reach-wide periphyton collection in large rivers

Starting with Transect "A" (i.e., the most upstream transect):

1. Identify a sample of hard substrate (rock or wood) and collect a periphyton sample with the appropriate technique described in section 6.2.

2. If hard substrate is not present at the Transect, use the area delimiter to confine an area of soft sediments and sample with the *Suction Method* described in Section 6.2.

3. Place the sample collected at each transect into a single sample container to produce the composite sample. Follow sample storage procedures described in Section 6.2.

4. Repeat Steps 1-3 for Transects B through K. Record sample collection information on the *Boatable Periphyton Field Form.* See Section 6.2 for data recording requirements.

6.1.2 Sampling Procedure for Wadeable Streams

A reach wide, multi-habitat composite will be collected using a modified EMAP method (Peck et al., 2006).

1. Establish 5 equidistant transects following protocols in the *Reach Layout* Section of the current SOP 5.0 - Physical Habitat Measurements. Record items listed on the *Habitat and Biota Worksheet.* 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 (Figure 2). Specifically, 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 *Habitat and Biota Worksheet.*

2. Use the appropriate sampling method for each substrate type encountered (*refer to Section 6.2*). Record sample collection information on the *Habitat & Biota Worksheet* including the Transect – Substrate/Habitat Table. Combine each sample into a single composite sample and process as described in Section 6.3.

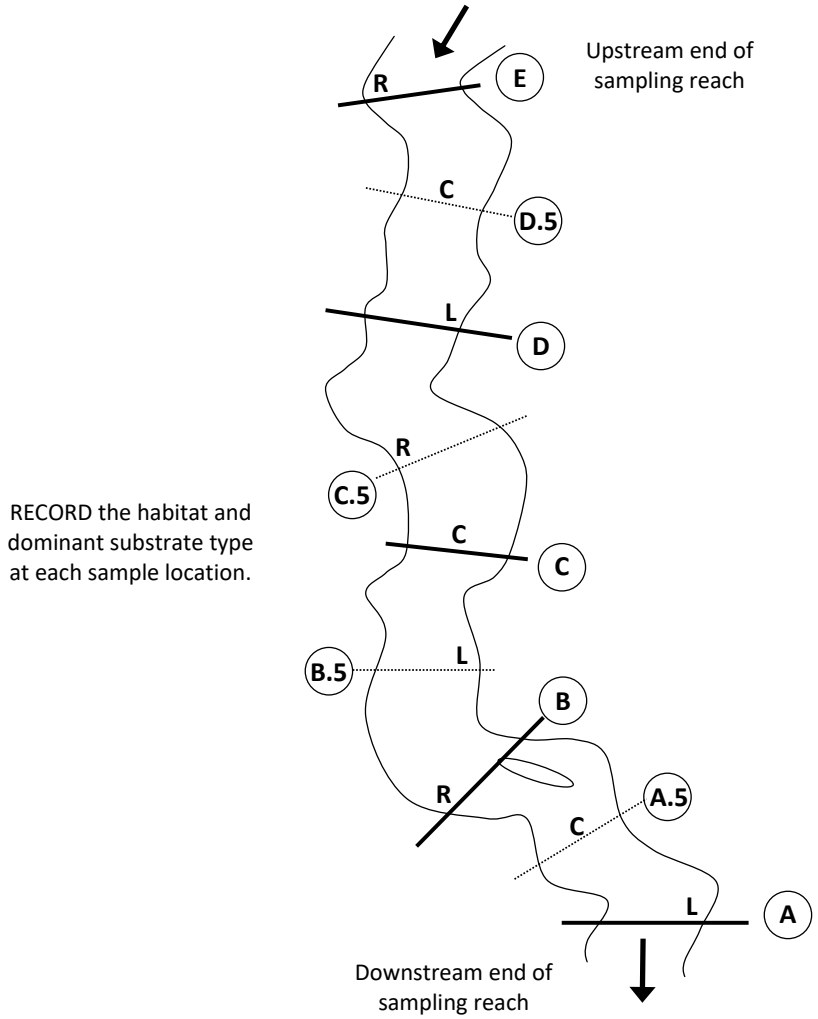

Figure 2. Substrate sampling design for reach-wide periphyton collection in wadeable streams

6.2 Periphyton Sampling Methods, Sample Storage and Clean Up

1) Starting with Transect A, collect a single sample from the assigned sampling station using the procedures below.

Ring method for coarse substrate that can be removed from the water

i) Collect a sample of substrate (rock or wood) that is small enough (< 15 cm diameter) and can be easily removed. When removing the substrate from the water, note which side is facing up and use this side for sampling since the upper surface is where the periphyton is located. Place the substrate in or over a plastic funnel which drains into a 500 mL plastic bottle with volume graduations marked on it.

ii) Use the area delimiter to define a 12 cm^2 area on the upper surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter into the funnel by brushing with a stiff-bristled toothbrush for 30 seconds. Take care to ensure that the upper surface of the substrate is the surface that is being scrubbed, and that the entire surface within the delimiter is scrubbed.
iii) Fill a wash bottle with DI water. Using water from this bottle, wash the dislodged periphyton from the funnel into the 500 mL bottle. Use an amount of water (~45 mL) that brings the composite volume up to the next graduation mark on the bottle.
iv) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite).

Sampling method for coarse substrate that is too large to remove from the water (bedrock, large wood materials, boulders, etc.)

i) Use the area delimiter to define a 12 cm^2 area on the upper surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter using the clear tube, attached to the tip of the syringe, in a scraping motion.
ii) While dislodging periphyton with the tube, simultaneously pull back to 25 mL on the syringe plunger to draw the dislodged periphyton into the syringe. The 25 mL in the syringe combined with the 20 mL in the tube equals the target volume of 45 mLs.
iii) Empty the syringe into the same 500 mL plastic bottle as above. If the volume of the vacuumed sediment is not enough to raise the composite volume to the next graduation on the bottle (~45 mL), add additional DI water to the bottle to raise the level to the next graduation.
iv) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)

Suction Method for Sandy/Silty Habitat (Epipsammic/Epipelic Habitats)

i) Use the area delimiter to confine a 12 cm^2 area of soft sediments in a shallow area of the sampling station.
ii) Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60 mL syringe with attached clear tube up to the 25 mL line of the syringe.
iii) Empty the syringe into the same 500 mL plastic bottle as above. If the volume of the vacuumed sediment is not enough to raise the composite volume to the next graduation on the bottle (~45 mL), add additional DI water to the bottle to raise the level to the next graduation.
iv) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)

2) Record the substrate and habitat type encountered at each transect on the *Boatable Periphyton Field Form* or *Habitat and Biota Worksheet*. Tables 1 and 2 define the different substrate and habitat types.

3) Repeat Steps 1-2 for Transects B through K. Place the sample collected at each sampling station into the single 500 mL bottle to produce the composite index sample.

4) After samples have been collected from all 11 transects (or as many transects as possible), thoroughly mix the 500 mL bottle regardless of substrate type. Record the total volume of the composite sample in the periphyton section of the *Boatable Periphyton Field Form* or *Habitat and Biota Worksheet*. The graduations on the collection bottle are placed at 45 mL increments, so the total volume of the composite sample should be the number of transects sampled times 45. Additionally record the number of transects sampled and reason(s) for skipping any transects on the appropriate field form.

Table 1. Sampling habitat definitions

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.

6.3 Sample Processing, Handling, and Analysis

All samples should be processed immediately after collection. If field processing (see Sections 6.3.1 and 6.3.2) must be delayed, hold sample water on ice at 6°C and protect from exposure to light by using opaque bottles and storing in the dark until processing can begin or freeze samples as described below. Samples for periphyton chlorophyll a analysis must be either frozen or filtered and frozen at the end of each day, no more than 12 hours after collection. Place the labeled periphyton samples or filters on dry ice in latched coolers until transfer into a freezer. Samples or filters can be kept frozen for 28 days before analysis for chlorophyll a. Samples from acidic water (< pH 6) should be processed promptly after filtration to prevent possible chlorophyll degradation from residual acidic water on filter.

6.3.1 Homogenizing the sample for chlorophyll a analysis

Representative sub-sampling is essential for accurate assessment of periphyton communities. To counter the natural settling that occurs when the composite sample bottle sits, gently invert the bottle several times to thoroughly mix before sub-sampling. Some periphyton samples will contain clumps of filamentous algae or diatoms that cannot be homogenized by hand. Homogenizing these samples with a hand-held blender can significantly reduce variability in sub-sample analyses, particularly in samples dominated by filamentous algae. The blending process generally does not greatly damage cells. Certain green filamentous algae do not break apart easily (e.g., *Cladophora* sp.) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs and Kilroy, 2000), otherwise the filaments can be removed from the blades and cut with scissors.

Equipment:

- Glass or plastic beaker
- Squirt bottle with DI water
- Kitchen or laboratory blender
- Graduated cylinder
- Small sharp scissors

1. If the sample was frozen, allow sample to thaw and liquefy prior to homogenizing. Mix the composite sample bottle thoroughly by gently inverting the bottle several times. If the composite sample contains fine sediment because one or more of the transects were sampled using the soft substrate method, allow the sample to settle for 10-20 seconds before measuring the aliquot to be filtered.

2. If an abundance of filamentous algae and diatoms prevents homogenizing the sample by hand, pour the contents of the sample container into a graduated cylinder. Rinse out any sample residue from the container and lid into the cylinder. Rinse off, but do not transfer, sand and gravel. DI water is preferred for rinsing and making up the sample volume, however, tap water can be used provided the water is not heavily chlorinated. Measure and record the total sample volume on the appropriate *Field Form*.

3. Pour the sample into a beaker (the width of the beaker should be only slightly greater than the width of the housing holding the blades of the blender).

4. Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample. Non-algae solids have the potential to buffer acids concentrations. The top 0.5 cm of substrate should go into the sample container but not into the homogenized sample. The heavy debris in the sample (i.e. sand and pebbles) should be shaken for one minute and rinsed but not added to the blending container. The exception is where there is a calcium precipitate that has been removed from the substrate. This should be blended, as it frequently contains abundant cyanophytes.

5. Ensure that there is enough water to fully cover the blender-blade housing.

6. Blend for about 30 seconds or until the mixture is completely homogenized and is free of obvious clumps of material. If the sample contains filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that have become caught on the blades or blender housing and cut into lengths less than 5 mm. The end result must be a homogeneous solution suitable for sub-sampling.

6.3.2 Diatom sub-samples

For diatom taxonomic identification, enumeration and biovolume samples, pour off a 45-mL sub-sample from the homogenized composite sample and preserve with enough Lugol's solution to turn the sample the color of black tea. Once preserved, place the sub-sample in a small cooler or other secondary containment device. Do **not** freeze this sub-sample. For diatom DNA sequencing samples, pour off a 25 – 50 mL sub-sample from the homogenized composite sample into a plastic sample container per EPA Office of Research and Development guidance. Keep the sample in the dark and in a cooler on wet ice or on dry ice if it is available. Place the sample in a freezer upon return to the lab. Record the RIDs and sample volume of each sample on the *Boatable Periphyton Form*.

6.3.3 Filtration for chlorophyll a analysis

Equipment:

- Vacuum filtration apparatus
- Handheld vacuum pump with pressure gauge
- Glass fiber filters, 47 mm Whatman GF/F
- Forceps/tweezers
- Wide aperture (~ 2 mm diameter) 5 mL pipette
- Squirt bottle with deionized or distilled water
- Aluminum foil and permanent marker
- Graduated cylinder

1. Set up filtering apparatus with a fresh Whatman GF/F filter.

2. Place a sub-sample in the filtering chamber. The sub-sample is made up of aliquots drawn from the homogenized sample. The volume of the aliquots depends on the concentration of the sample. For samples homogenized by hand, measure 25 mL aliquots of sample with a graduated cylinder. For samples that required blending, extract between 1 to 5 mL with a pipette. Pour the aliquot into the filtration unit.

3. Apply vacuum and do not exceed 6in. Hg (20kPa) during filtration. Excessively long filtration times (>10 min) and high pressures may damage cells and result in a loss of chlorophyll (EPA 1997).

4. If there is not an obvious coloring from periphyton on the filter, more aliquots should be filtered.

5. Check for any fragments of leaves, mosses, invertebrates, etc. on the filter and remove these with forceps.

6. Record the volume filtered on the appropriate *Field Form*.

7. Remove the filter from the filtering apparatus, fold in half, place it in a petri dish labeled with the sample identification number, then wrap the petri dish in a sheet of aluminum foil.

8. Repeat steps 1 – 7 until the desired number of replicates of chlorophyll *a* sub-samples are filtered (generally 3).

Wrap and label (RID) filters and place in a re-sealable plastic bag. Freeze the wrapped, labeled filters on dry ice. Samples should be processed as soon as possible after collection; however, the wrapped filters can be frozen for up to 28 days before analysis, if necessary. Samples from acidic water (< pH 6) should be processed promptly after filtration to prevent possible chlorophyll degradation from residual acidic water on filter. If processing must be delayed, hold sample water on ice or at 6°C and protect from exposure to light by using opaque bottles and storing in the dark. If processing multiple chlorophyll-a samples at a site, record the sample RIDs on the *Boatable Periphyton Field Form*.

Notes:
- Always check that clumps of algae or leaf fragments, etc., have not blocked the intake to the pipette.
- If the sub-samples are taking a very long time to filter, or the filter is darkly colored or covered with a thick layer of material, the sample probably needs dilution or use a smaller volume aliquot. Ensure that you record the degree of dilution and aliquot volume on the appropriate *Field Form*, so that this can be used in the concentration calculations.

6.3.4 Clean up

After preparing laboratory samples, thoroughly clean each of the pieces of periphyton equipment (delimiter, brush, funnel, syringe, composite bottle, blender, beaker, graduated cylinder, and scissors) with a 10% bleach solution and rinse with tap or DI water. After processing chlorophyll a samples, rinse filtering apparatus with tap or DI water.

7.0 Quality Assurance/Quality Control

All staff conducting periphyton sampling will attend a training session on the sampling and processing of periphyton and the analysis procedures for chlorophyll *a*. Before leaving the sampling site, all field forms will be checked for completeness. SWQB staff who conduct procedures described under this SOP are required to sign the SOP acknowledgement statement for Periphyton Sampling prior to conducting procedures detailed in this SOP.

8.0 Field Forms

- *Boatable Periphyton Field Form (for non-wadeable)*
- *Habitat and Biota Worksheet (for wadeable)*

9.0 Revision History

Revision 4 (May 2025) – addressed inconsistencies with updated SOP format procedures; updated Purpose and Scope and Personnel Responsibilities with updated SOP format procedures; added current sampling priorities to Background and Precautions; updated training requirements for operating inflatable watercraft; added relevant acronyms; updated Equipment and Tools to reflect current sampling procedure equipment requirements; revised sampling procedures in Section 6.1.1 to reflect current boatable collection methods; updated and streamlined Periphyton Sampling Methods; added additional diatom sub-sample procedures; added equipment clean up procedures; removed chlorophyll a analysis section as the SWQB no longer performs these activities; updated references.

Revision 3 (September 2014) – clarification regarding sample location selection and suitable substrate, optional use of ethanol or Lugols solution dependent on laboratory requirements, and minor clarifying edits to language to reduce interpretation and increase clarity.

Revision 2 (March 15, 2013) – replaced the *Field Form* with the *Habitat & Biota Field Work Cover Sheet*; for health and safety reasons, sub-samples for diatom taxonomic identification are now being preserved with 95% ethanol instead of formaldehyde.

Revision 1 (May 16, 2012) – described and justified in better detail SWQB's modified chlorophyll extraction method using 90% ethanol in place of acetone; identified appropriate setting and time for centrifuge operation; clarified procedures (e.g. absorbance peaks) and reagents for spectrophotometric determination of chlorophyll *a*; referenced formula used to calculate chlorophyll *a* concentration including absorbance correction for ethanol; enhanced the quality assurance/quality control section.

Original (July 1, 2011).

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