Standard Operating Procedures for the Collection of Streams Periphyton Samples

Water Quality Control Division • Watershed Section

1.0 Introduction

This Standard Operating Procedure (SOP) will address the standardized collection of benthic algae or periphyton samples in wadeable streams in Colorado. The collection of streams periphyton results in the laboratory analysis of chlorophyll α, ash-free dry mass and taxonomic identification and counts.

The objective of collecting periphyton is to quantitatively measure algal biomass and composition in order to evaluate attainment or impairment of existing or future water quality standards within the Water Quality Control Commission's regulatory and policy framework and develop biological indicator tools to support the aforementioned regulatory decisions.

2.0 Rationale

Periphyton is defined as the community of micro-organisms such as bacteria, protozoa, fungi and algae that grow attached to submerged surfaces (cobble, woody debris, macrophytes, etc.) or swim, creep, and lodge on the attached formations.

They are primary producers and an important foundation of many stream food webs. These organisms also stabilize substrata and serve as habitat for many other organisms. Because periphyton assemblages are attached to substrate, their characteristics are affected by physical, chemical, and biological disturbances that occur in the stream reach during the time in which the assemblage developed (Barbour et al 1999).

Diatoms in particular are useful ecological indicators because they are found in abundance in most lotic[1] ecosystems. Their abundant numbers provide multiple, sensitive indicators of environmental change and the specific conditions of their habitat (Barbour et al 1999). For this reason, there exists a clear advantage to using periphyton in assessing water quality because they are fast-growing and respond very markedly to pollution.

3.0 Sampling Restrictions

Periphyton samples will be sampled at times of normal, stable flows and when the benthic algal community has peaked for the season. The optimal sampling season is mid-summer to early fall. Earlier sampling may be performed at lower elevations, but only to the extent that normal flow conditions are present and algae is in a state of growing or has already matured.

In the event of light flooding or scouring, sampling shall be delayed for a minimum of one week to allow recolonization. Studies have shown recovery after high discharge can be as rapid as seven days if the scouring event was less severe (Stevenson 1990).

[1] Flowing waters

Sampling shall be delayed for three weeks following severe, bottom-scouring flows to allow for recolonization and succession to a mature periphyton community. This is based on a recommendation by Peterson and Stevenson (1990).

4.0 Procedure

This method will be considered the primary standard operating procedure for all Water Quality Control Division (WQCD) field activities.

4.1 Equipment

- 100' tape measure
- 10" galvanized nails
- White plastic or enamel pan
- Medium-sized hand towel
- Spray bottle
- 1-liter wide-mouth Nalgene® bottle with graduated increments
- Bottle cap (area = 0.785 in^2)
- PVC ring (inside area = 0.785 in^2)
- Stiff bristled toothbrush, suction bulb, disposable pipettes, spatula, stainless steel spoon, putty knife, and tweezers
- Pruning shears or small saw
- Nalgene® hand-operated PVC vacuum pump and siphon hose
- 47 mm polysulfone filter funnel and holder (see Figure 1)
- 47 mm Whatman® Glass Microfiber filters
- Falcon® 50 ml conical centrifuge tubes
- Aluminum foil
- Preservative (10% buffered formalin)
- Sample container labels, such as barcodes
- Pencils and indelible marker
- WQCD's Physical Characterization Field Form
- Chain of custody form
- Medium-sized ice chest
- Dry ice blocks

Figure 1. Filter Funnel

4.2 Establish Reach

A length of stream will be sampled corresponding to a sampling point (site). This length of stream is referred to as a "reach". The reach is where all biological, physical and chemical sampling will be conducted while at the site. All periphyton collections will be performed within this reach.

The extent of the reach is determined by multiplying the average wetted channel width by 40. The steps to establish a reach are detailed below:

- Use a tape measure to determine the wetted width of the channel at 3 to 5 locations considered to be of typical width within approximately 5 channel widths upstream of the sample site. Average the readings together and round to the nearest 0.5 ft mark. If the average width is less than 11.5 ft, use 500 ft as the minimum reach length.

If a braided channel is encountered during the reach determination phase then measure the individual wetted widths along the cross-section and add them up. A braided channel is characterized by numerous sub-channels with no obvious dominant channel (i.e. unvegetated bars).

- The length of the reach is calculated by multiplying the average wetted channel width by 40.

- Record the reach length on the WQCD Physical Characterization Field Form.

- Determine if the reach needs to be adjusted around the sampling site due to confluences, impoundments (i.e. lakes, reservoirs or ponds), physical barriers, or because of access restrictions to a portion of the reach.

  If a confluence, barrier or access restriction is present, note the distance and mark the restriction as one endpoint of the reach. Move the other endpoint of the reach an equivalent distance from the sampling point. This results in a reach length that remains the same but slides up or down around the sampling point.

  > **Note:** *Do not adjust the reach to avoid man-made obstacles such as bridges, culverts, rip-rap or channelization.*

  The reach is now set.

4.3 Establish Transects

Five transects will be setup within the overall reach. The method is as follows:

- Determine a representative stream length of 50 to 100 meters that contains at least one riffle or run habitat. This may not always be achievable, so as an alternative, choose 3 to 5 riffles and/or runs, so that 5 transects or cross-sections can be established.

- Beginning at the most downstream position, extend a tape measure from one wetted edge to the other being careful not to disturb the substrate beneath or immediately downstream of the tape measure. Fasten the tape measure to each bank edge using 10" galvanized nails so the measuring tape is taut and does not dip into the stream. An illustration depicting this may be found in Figure 2.

- Moving in an upstream direction, establish the remaining four transects at equidistant locations within the selected stream length or at each chosen riffle/run.

  > **Note:** *Periphyton is typically collected in chorus with a pebble counting procedure. Transects may be shared between the two procedures but caution must be taken to prevent agitation to the substrate immediately below the tape measure.*

- Illustrate the area between the first and fifth transects on the site sketch section of the WQCD Physical Characterization Field Form.

4.4 Collection

This procedure has been adapted to two different types of substrate common to Colorado. The first procedure is best applied to streams with pre-dominantly hard-bottomed substrate, such as cobble, pebble and gravel (herein referred to as "rocks").

The second type is applied to sandy, shifty bottom streams, as found in lower elevations of the Plains bioregion or the far western Xerics bioregion of Colorado.

4.4.1 Hard-Bottomed Streams

The following procedure applies to hard-bottomed streams:

- Note the distance from one wetted edge to the other along the tape measure at the first transect. Collect three rocks from the first transect at the ¼, ½ and ¾ points along the tape measure. See Figure 2.

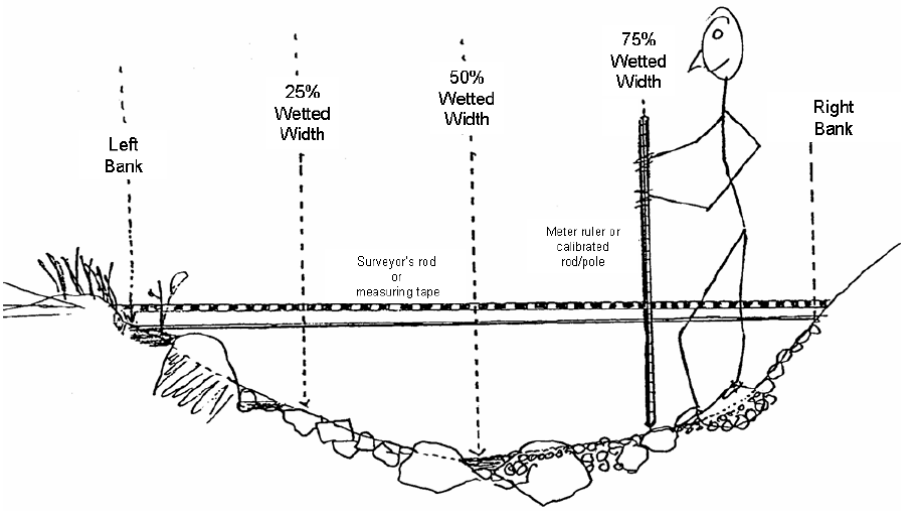

Figure 2 Illustration of sampling points along a cross-section transect.

- Place the cobble facing upwards in the plastic pan. Cover the rocks with a moist medium-sized hand towel to prevent exposure to sunlight.

- Continue to collect three rocks from the remaining four transects, as described in Section 4.3, carefully pulling rocks from the ¼, ½ and ¾ points along the tape measure and covering them with the hand towel as you proceed from transect to transect.

- Proceed to a shaded location on the stream bank to begin scraping periphyton from the rocks. See Section 4.5.

4.4.2 Soft-Bottomed Streams

Collecting periphyton in soft-bottomed streams allows periphyton from all available substrates and habitats to be sampled as long as they are representative of the overall reach established in Section 4.2.

The purpose of this section is to collect 15 subsamples, each with an area of 0.785 in^2, from submerged, removable habitats or loose sediment depositional zones present along or near each transect.

The following procedures apply to soft-bottomed streams:

Sampling Method for Rocks (Cobble), Woody Snags or Submerged Vegetation

- If rocks are not available or are limited at the ¼, ½ and ¾ points along the tape measure, then select the nearest woody snag (debris) or submerged vegetation (mosses, microalgae, vascular plants, and root masses).

- If submerged woody snags or other vegetation are large or flexible enough, can be lifted above the water line, and has a relatively smooth service then use the bottle cap method described in Section 4.5 making certain that scrapings and rinse water are flushed directly into the 1-liter Nalgene bottle.

  Otherwise, identify the part of the submerged woody snag or vegetation to be scraped later and carefully remove a 4-8 in. section with pruning shears or a small saw. Place the removed section into the plastic pan.

Suction Method for Loose Sediments

Loose sediment may be defined as sand, silt, clay or fine particulate organic matter.

- At sampling points where depth/velocity are low and have a depositional zone consisting of any of the loose sediments listed above, place a PVC ring on top of the sediment. Press the ring into the sediment to a depth of one-half inch.

- Use a suction bulb to remove the entire top layer of periphyton. It is acceptable to suction up some sediment in the process.

- Squirt this medium of material into the 1-liter Nalgene bottle.

Cover the rocks or other removable substrates and the 1-liter Nalgene bottle with the hand towel to prevent exposure to sunlight as you move from habitat to habitat in the stream channel.

Once completed, proceed to a shaded location on the stream bank to begin scraping periphyton from the remaining rocks or other removable substrates placed in the plastic pan. See Section 4.5.

> **Example:** *If 10 subsamples are collected via siphoning or by instream bottle cap method, then the remaining 5 subsamples must be removable substrate that are scraped on the stream bank later. All forms of subsamples must add up to 15.*

4.5 Scraping and Rinsing

This procedure applies to all objects that were removed from the stream and need to be scraped, but will be simply referred to as "rocks" in this section.

- Sit in a shaded location on the stream bank, within an arm's reach of the water's surface.

- Rinse twice and then fill the spray bottle with stream water. Ensure that the spray head mechanisms are thoroughly rinsed by pumping water through the spray head several times.

- Carefully pull back an edge of the hand towel and remove the first rock. Place the bottle cap ("cap") on any section of the upside aspect (e.g. where the attached algae is) of the rock. With the rock in hand, hold the cap firm with your thumb. With your free hand, vigorously scrape algae from the area not under but around the cap with the toothbrush. Rinse the rock, with the cap still firmly in place, and toothbrush bristles in the stream. Repeat the scrape and rinse process one more time.

  Note: *Based on the density of periphyton on the rock, you may have to use a spoon or putty knife to remove macroalgae from the area not under the cap.*

- Remove the cap and place to the side. Hold the rock directly above the 1-liter Nalgene bottle. Gently scrape the area that was under the cap with the toothbrush.

- Use the spray bottle to rinse the dislodged scrapings directly into the 1-liter Nalgene bottle. Ensure that the slurry of algal material and rinse water runs off or drips into the 1-liter Nalgene bottle. Keep the rinse water to a minimum, just enough to rinse off the scrapings.

- Scrape and rinse the area that was under the lid a second time.

- Discard the rock back into the stream and rinse the toothbrush (or applicable scraping device) in the stream.

- Repeat the scraping and rinse process for the remaining 14 rocks at sites with hard-bottomed substrate or the remaining number of rocks or removable objects pulled from soft-bottomed streams.

- At this point, approximately 75-150 ml of scrapings and rinse water will be in the 1-liter Nalgene bottle. Remove the spray head from the spray bottle. Pour stream water from the spray bottle into the 1-liter Nalgene bottle until the mixture reaches the 500 ml graduated increment.

- If applicable, discard the remaining stream water from the spray bottle.

- Cap the 1-liter Nalgene bottle and invert several times to homogenize the composite of scrapings and rinse water.

- Record the composite material volume on the WQCD's Physical Characterization Field Form. See Figure 3.

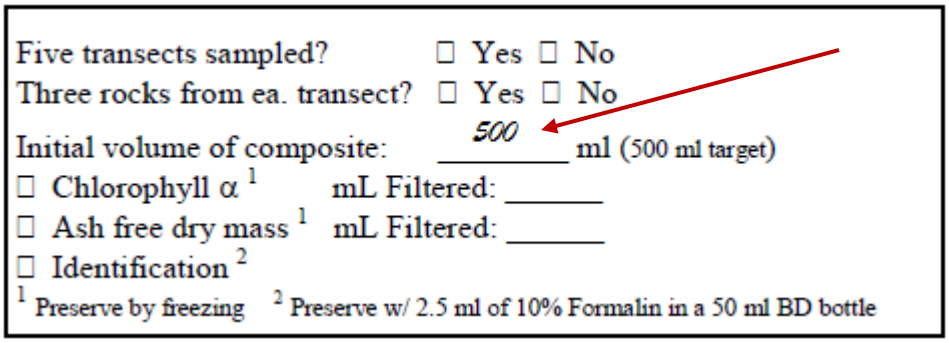

Figure 3. Documenting Composite Material Volume

4.6 Sample Preparation

Use the 500 ml of composite material gathered as described in Section 4.5 to prepare the three sample types in Sections 4.6.1 through 4.6.3. The three sample types may be prepared in any order. But care should be taken to retain the composite material until all three are prepared and preserved.

4.6.1 Chlorophyll α

- Rinse the filter funnel and holder in the stream. Using tweezers, center a single 47 mm Whatman glass microfiber filter directly on the filter funnel base. Then screw and tighten the filter funnel to the base.

- Using a cap-less 50 ml conical centrifuge tube, measure 20-50 ml of composite material. Be consistent in the volume selected. If 30 ml is measured for the chlorophyll α sample, then measure 30 ml for the ash-free dry mass sample, too.

- Pump the measured composite material through the filter using the hand-operated PVC vacuum pump. Do not let the vacuum pressure rise above 20 psi to prevent cell damage.

- Check the "Chlorophyll α" box and note the volume filtered on the WQCD's Physical Characterization Field Form, in the Periphyton section. See Figure 4.

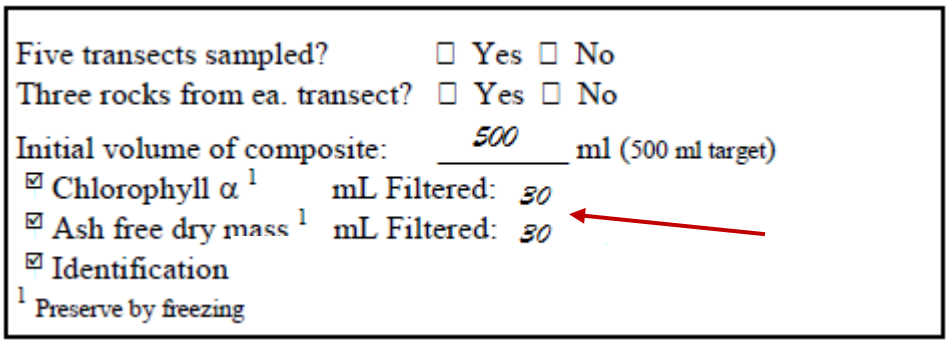

Figure 4. Documenting Volume Filtered

- Remove the cap from a pre-labeled 50 ml conical centrifuge tube. Use the tweezers to remove and gently fold in half the glass microfiber filter. Slide the folded filter into the centrifuge tube. Screw the cap back on.

- Wrap the centrifuge vial in a 6" x 6" sheet of aluminum foil. Fold close the ends in such a fashion to ensure the aluminum foil does not rip or come undone during transport to the laboratory.

  Note: *It is okay to cover the sample barcode. This process ensures that the label will remain fixed to the centrifuge vial rather than on top of the aluminum foil and will prevent loss of the label during transport.*

- Place the wrapped centrifuge tube(s) in an ice chest containing blocks of dry ice. For proper preservation, place the tubes in direct proximity to the dry ice.

- Discard the filtered extract from the filter funnel holder. Rinse the entire filtering apparatus.

4.6.2 Ash-Free Dry Mass

- Follow the same procedures as shown in Section 4.6.1. Again, note the volume filtered on the WQCD's Physical Characterization Field Form, in the Periphyton section. See Figure 4.

4.6.3 Algal Identification and Counts

- Homogenize the composite material by inverting the 1-liter Nalgene bottle several times.

- Remove the cap from a pre-labeled 50 ml conical centrifuge tube. Pour 30 ml of composite material into the centrifuge tube.

- Add 20 ml of 10% buffered formalin.

- Screw the cap back on. Homogenize the sample by gently inverting the centrifuge tube several times to ensure proper preservation.

- Check the "Identification" box shown in Figure 4.

  Note: *This liquefied sample does <u>not</u> need to be wrapped in aluminum foil or frozen on dry ice. Store the centrifuge tube in a safe location during transport.*

- Discard the remaining composite material into the stream and rinse out the 1-liter Nalgene bottle <u>only</u> if Sections 4.6.1 and 4.6.2 are completed.

Completing Sections 4.6.1 through 4.6.3 will yield a set of three periphyton samples per site. Two samples are preserved on dry ice and the third is preserved with liquid formalin.

5.0 Chain of Custody

The chain of custody (COC) documentation will identify all persons who have had custody of the periphyton samples from collection to laboratory analysis. This will include collection, custody, control, transfer and disposition of the samples.

For each set of three periphyton samples collected at a single site, complete a row on the WQCD Periphyton Sample Chain of Custody form shown below in Figure 5. The COC will accompany the samples at all times. The COC is attached to this SOP in Appendix A.

Sample Date	Site ID Number	Stream Name / Description	Type of Sample			Volume Filtered	Total Area Scraped[1]	Initial Volume[2]
			ID	AFDM (frozen)	Chlorophyll (frozen)			
8/26/2015	20166001001	Example Creek / at mouth	X	X	X	30	11.775	500

Figure 5. Chain of Custody Example

6.0 References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling, 1999, Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency: Office of Water. Washington, D.C.

Peterson, C. G. and R. J. Stevenson. 1990. Post-spate development of epilithic algal communities in different current environments. Canadian Journal of Botany 68:2092-2102.

Standard Operating Procedure for the Collection of Pebble Counts. Colorado Department of Public Health and Environment, Water Quality Control Division. May 2015.

Stevenson, R. J. 1990. Benthic algal community dynamics in a stream during and after a spate. Journal of the North American Benthological Society 9:277-288.

Water Quality Control Division. 2015. Standard Operating Procedure for the Collection of Pebble Counts

7.0 Document Version

2.0_122315

8.0 Approval Signatures

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Environmental Data Unit - Q&A Officer

Periphyton Sample Chain of Custody

Page ____ of _____

Sample Date	Site ID Number	Stream Name / Description	Type of Sample			Volume Filtered	Total Area Scraped [1]	Initial Volume [2]
			ID	AFDM (frozen)	Chlorophyll (frozen)			

[1] If 15 rocks are scraped then the *Total Area Scraped* is 11.775 in^2. If less than 15 rocks are scraped multiply the number of scraping areas by 0.785 in^2.
[2] Initial volume target is 500 ml (this includes a mixture of scrapings and rinse water).

Comments:

Sample Tracking:	Name/Agency:	Date/Time:	Additional Information:
Samples Relinquished by:	Name_____ Agency:_____	Date (MMDDYY):_____ Time (military):_____	Total Number of Samples:_____ Delivery Method:_____ Other Comments:_____
Samples Received by:	Name_____ Agency:_____	Date (MMDDYY):_____ Time (military):_____	Total Number of Samples:_____ Delivery Method:_____ Other Comments:_____