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State of Washington

Standard Operating Procedure EAP111, Version 1.14

Periphyton Sampling, Processing, and Identification in Streams and Rivers

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Purpose of this document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

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Standard Operating Procedures for Periphyton Sampling, Processing, and Identification in Streams and Rivers

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SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
12/21/16	1.0	Converted the original appendix for to the SOP format	All	Meghan Rosewood-Thurman
1/5/17	1.1	Added footers, general edits	All	Meghan Rosewood-Thurman
3/27/17	1.2	Included periphyton metals sampling information	all	Meghan Rosewood-Thurman
12/27/17	1.3	Reviewed and added detailed sampling information	All	Brian Engeness
12/28/17	1.4	Accepted changes, fused old periphyton SOP with new version	All	Meghan Rosewood-Thurman
1/4/18	1.5	Updated references	All	Meghan Rosewood-Thurman
1/5/18	1.6	Made minor changes so the document was consistent throughout	All	Brian Engeness
1/11/18	1.7	Incorporated edits from authors. Added eform sample tracking info.	All	Meghan Rosewood-Thurman
2/15/18	1.8	Added BIO, EM, SEN, WHM, with Study IDs Added epidendric, epilithic, epipsammic epipelic, epiphytic habitats Changed title to include rivers	3, Title page	Glenn Merritt
3/5/18	1.9	Accepted changes from reviewers and authors, removed MSDSs and provided link to SDSs	All	Meghan Rosewood-Thurman
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10/18/18	1.14	Minor changes	All	Meghan Rosewood-Thurman
2/8/19	1.14	Formatting and accessibility updates.	All	Ruth Froese

1.0 Purpose and Scope

1.1 This document is one of two Standard Operating Procedures (SOPs) available in the Environmental Assessment Program (EAP) for the collection of periphyton in freshwater rivers and streams. See SOP EAP085 *Standard Operation Procedure (SOP) for Collection and Processing of Periphyton Samples* (Anderson et al., 2016) for targeted habitat periphyton collection and TMDL modeling studies.

1.2 This SOP discusses the collection of periphyton in wadeable streams (< 25 m average bankfull width) and larger rivers (≥ 25 m average bankfull width or too deep to wade).

1.3 The standard methods in this SOP provide the minimum requirements for collecting, processing and preserving periphyton samples (taxonomy, chlorophyll-a, tissue metals), as well as guidance for taxonomic identification

2.0 Applicability

2.1 The procedures outlined here are used by EAP staff when collecting periphyton during a data collection event (DCE) from rivers and streams in Washington State.

2.2 The methods in this SOP are used by at least four studies in EAP for monitoring rivers and streams. These are:

2.2.1 Watershed Health Monitoring (**WHM**).

2.2.2 Ambient Biological Monitoring (**BIO**).

2.2.3 Effectiveness Monitoring (**EM**), and

2.2.4 Sentinel Site Monitoring (**SEN**).

2.3 See SOP EAP085 (Anderson et al., 2016) for more-directed biological assessments, such as targeted habitat periphyton samplers, or TMDL modeling studies.

3.0 Definitions

3.1 BIO: The Ambient Biological Monitoring Program. Its EIM Study ID begins with *WHM_BIO*.

3.2 DCE: The *Data Collection Event* is the sampling event for the given protocol. Data for a DCE are indexed using a code which includes the site ID followed by the year, month, day, and the time (military) for the start time of the sampling event. For example: WAM06600-000222-DCE-YYYY-MMDD-HH:MM. One DCE should be completed within one working day, lasting four to six hours, on average.

3.3 EAP: Environmental Assessment Program

3.4 Ecology: The Washington State Department of Ecology

3.5 EIM: The Environmental Information Management System (EIM) is the Department of Ecology's main database for environmental monitoring data. EIM contains records on physical, chemical, and biological analyses and measurements. Supplementary information about the data (metadata) is also stored, including information about environmental studies, monitoring locations, and data quality. The "Search by map" feature enables plotting coordinates over orthophotographic imagery.

3.6 EM: The Effectiveness Monitoring Program. Its EIM Study IDs begin with *WHM_EFF*.

3.7 Epidendric habitat: surfaces of coarse woody debris.

3.8 Epilithic habitat: surfaces of coarse gravel or larger rocks

3.9 Epipelic habitat: surfaces of silt/clay.

3.10 Epiphytic habitat: surfaces of emergent or submerged/macrophyte vegetation.

3.11 Epipsammic habitat: surfaces of fine gravel/sand

3.12 Intermediate container: A temporary sampling container used to directly sample water and transfer it to the primary container.

3.13 LAR: *Laboratory Analysis Required* form. This is a chain-of-custody form that is delivered to the Manchester Environmental Laboratory along with samples from a Data Collection Event (DCE). See SOP EAP095 (Hartman, 2017) for a completed LAR form.

3.14 Lugol's Solution: A solution of iodine and potassium iodide in distilled water and glacial acetic acid that is used as a preservative and microscopic stain.

3.15 Major Transect: One of 11 equidistant transects across the length of a site. These transects run perpendicular to the thalweg and are labeled as follows: A (furthest downstream), B, C, D, E, F, G, H, I, J, and K (furthest upstream).

3.16 MEL: Manchester Environmental Laboratory.

3.17 mL: milliliter

3.18 mm: millimeter

3.19 Narrow Protocol: The set of Watershed Health Monitoring SOPs that describe data collection at wadeable sites with an average bankfull width of less than 25 m at the index station.

3.19.1 Narrow protocol sampling stations: Sampling occurs in a zig-zag sequence (Table 1) when moving upstream.

Table 1. Pre-determined station locations on each transect of a Standard Stream Site.

Station	% Transect Distance Left to Right
1	25
2	50
3	75
4	50
5	25
6	50
7	75
8	50

3.20 Oz: ounce

3.21 Protocol: A collection of SOPs used to accomplish a DCE. Watershed Health Monitoring uses two protocols: The *Narrow Protocol* is used for sampling wadeable streams that are less than 25 m average bankfull width. The *Wide Protocol* is used for rivers or streams that are wider than 25 m average bankfull width or too deep to wade.

3.22 QA: Quality Assurance

3.23 QC: Quality Control

3.24 Reach-wide Composite Sample: The reach wide sample is composited from eight stations (Table 1). Each station is located on a separate randomly-selected transect and selected without regard to whether it is in a pool, riffle, or other habitat type. Sampling from multiple dispersed locations provides a representative sample.

3.25 SDS: Safety Data Sheets (previously Material Safety Data Sheets or MSDS) provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. An SDS includes information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment and spill/leak procedures.

3.26 Sealing laboratory film: A plastic paraffin film with a paper backing used in laboratories. It is commonly used for sealing or protecting vessels.

3.27 SEN: The sentinel site monitoring component of Watershed Health Monitoring. Its EIM Study ID begins with *WHM_SEN*

3.28 SOP: Standard Operating Procedure

3.29 Station: Any location within the site where an observation is made or part of a sample is collected. For SOP EAP073 (Larson, 2018) and SOP EAP111, 8 out of the 11 transects are randomly selected for Periphyton and Macroinvertebrate sampling. Table 1 defines the sampling path within the stream or river.

3.30 Substrate – the material that rests on the bottom of the stream.

3.31 Transect: A straight line along which observations are made or measurements are taken. This line spans the stream channel and is perpendicular to the direction of flow

3.32 WHM: Watershed Health Monitoring, a status and trends monitoring program within the Environmental Assessment Program at the Washington State Department of Ecology. Its EIM Study ID begins with *WHM_WAM0*

3.33 Wide Protocol: The set of WHM SOPs that describes the sample and data collection at non-wadeable sites or sites wider than 25 m bankfull width. It is an abbreviated version of the Narrow Protocol and is typically accomplished by use of rafts.

3.34 Wide protocol stations: For the wide protocol, sampling at each of the 8 transects occurs on the side of the stream/river where habitat is also surveyed. At each of the selected transects, a sample is collected from a representative portion (as much as practical) of a littoral zone extending 10 m into the stream/river from the wetted bank and 10 m upstream and downstream, respectively from the transect. The sample should also be collected in an area shallow enough to collect substrate and in an area away from backwaters, eddies, or other edge habitat.

4.0 Personnel Qualifications/Responsibilities

4.1 For collection of the sample, personnel should at a minimum review the Quality Assurance Monitoring Plans for the status and trends monitoring programs. Alternatively they may receive formal training from staff having themselves been formally trained.

4.2 For taxonomic analysis of the sample, the personnel should be certified for identification of Western United States taxa to the Genus or Species level by the Society for Freshwater Science (http://www.nabstcp.com/). Sample identification and enumeration should be to the lowest practical level as outlined in: Quality Assurance Monitoring Plan: Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton

4.3 All staff must comply with the requirements of the EAP Safety Manual (Ecology, 2017). A full working knowledge of the procedures in Chapter 1 is expected.

4.4 All staff must be familiar and comply with the requirements of Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan (Ecology, 2018).

4.5 Field staff must be annually trained to minimize the spread of invasive species. See SOP EAP070 (Parsons et al., 2018).

4.6 Read this standard operating procedure and discuss any questions with your supervisor or task team leader.

4.7 Read the Safety Data Sheets (SDS) for Lugol's solution (glacial acetic acid, iodide crystals and potassium iodide) before beginning the sample processing procedures.

4.8 Binders containing SDSs can be found in all field vehicles, vessels, Ecology buildings, or other locations where potentially hazardous chemicals may be handled. EAP staff following Ecology SOPs are required to familiarize themselves with these SDSs and take the appropriate safety measures for these chemicals. Use proper protective clothing and equipment as indicated.

4.9 Read the Safety Data Sheets (SDS) for ethanol before beginning the sorting/taxonomic procedures. The SDS are available in the Ecology Headquarters benthic laboratory. Use proper protective clothing and equipment as indicated.

5.0 Equipment, Reagents, and Supplies

5.1 Buchner funnel (e.g., Scienceware® 9.0 cm, supplier No. 146030000).

5.2 Filtered native stream water into spray bottle using Grade 4 filter paper 20-25 µm (e.g. Whatman 9.0 cm, supplier No. 1004-090).

5.3 Spray bottle (500 mL) with gradations marked every 62.5mL (Figure 1).

Figure 1. Periphyton spray bottle to hold filtered stream rinsate.

5.4 Delimiters: 7.5 cm diameter and 15 cm diameter (optional) for sampling epilithic (coarse substrates) (Figure 2). For **WHM** and **EM** studies, a 7.5 cm delimiter is used to collect periphyton at four locations at each transect with coarse substrate. One 15 cm delimiter may be used if large substrate exists. For **SEN** and **BIO** studies, one 7.5 cm delimiter is used to collect periphyton at each transect with coarse substrate. Note: more surface area is scraped with WHM and EM studies due to sampling for tissue metals analysis.

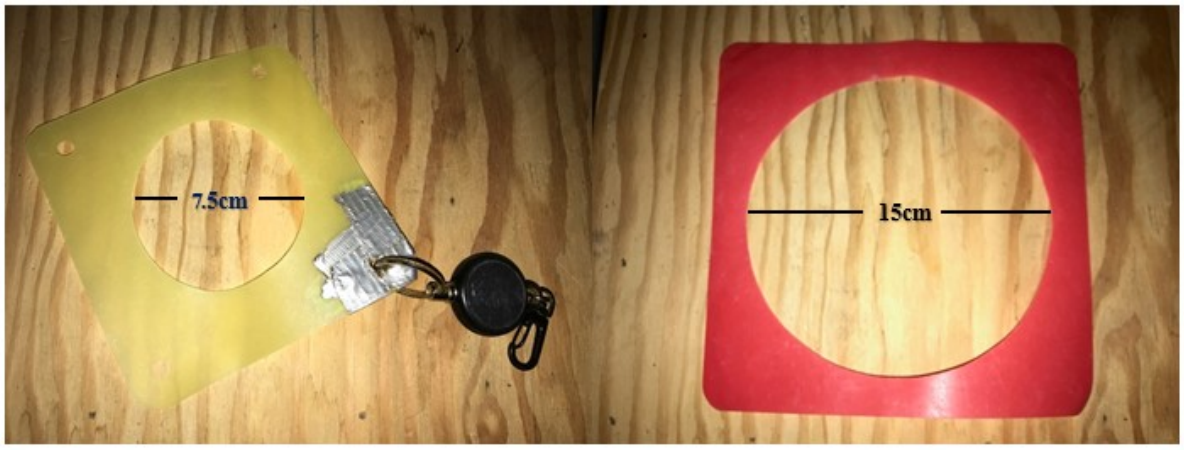

Figure 2. Template delimiters for sampling epilithic (coarse substrates).

5.5 Petri dishes (47 mm diameter) for sampling epipsammic (fine gravel/sand) and epipelic (silt/clay) habitats.

5.6 Hard, flat plastic spatula for sampling epipsammic and epipelic habitats.

5.7 Small kitchen strainer (e.g. 18/10 stainless-steel, approximately 6″diameter) for sampling epiphytic (submerged vegetation) habitats.

5.8 Digital Caliper (To measure the length and diameter of each cleaned stem/leaf section when performing epiphytic (emergent or submerged vegetation) habitat periphyton sampling).

5.9 Firm bristled toothbrush

5.10 Large plastic bowl or tray to catch rinsate

5.11 (2) Sterile 16 ounce HDPE wide mouth natural straight sided jars w/LDPE Lined closure, one ‘coarse jar’ and one ‘fines/macrophyte jar’ (Figure 3). See table 2 for details.

Figure 3. Sterile 16 ounce HDPE jars, one ‘coarse jar’ for epilithic, epidendric, or emergent epiphytic samples and one ‘fines/macrophyte jar’ for epipelic, epipsammic, or submerged epiphytic samples. See table 2 for details.

5.12 Filtration apparatus: filter base with rubber stopper, filtration flask, hand-operated vacuum pump with pressure gage, tubing (chlorophyll-a analysis)

5.13 47 mm 0.7 micron glass microfiber filters (chlorophyll-a analysis)

5.14 10 mL graduated cylinder

5.15 1 L graduated cylinder

5.16 Small test tube containing 90% Acetone (chlorophyll-a analysis; provided by Manchester Lab)

5.17 Centrifuge tube (50 ml)

5.18 Sample preservative (1-3% Lugol’s solution)

NOTE: Prepare Lugol's solution by dissolving 20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid. Store Lugol's solution in an opaque plastic bottle. Pre-made Lugol's solution can also be ordered from Manchester Lab or purchased from scientific suppliers.

5.19 Forceps

5.20 Sample tags and waterproof labels for chlorophyll-a, taxonomy, and metals/ associated parameters (if applicable)

5.21 Deionized water (DI)

5.22 Plastic funnel

5.23 Aluminum foil (to cover chlorophyll-a acetone vial)

5.24 Soft-lead pencils/ permeant markers

5.25 Clear tape

5.26 Electrical tape

5.27 Sealing laboratory film (to seal chlorophyll-a acetone vial)

5.28 Re-sealable snack bags (to store chlorophyll-a acetone vial)

5.29 Bubble wrap to protect glass acetone vials

5.30 Pocket knife/scissors

5.31 Chain of Custody Lab Analysis Request (LAR) Form

5.32 Cooler with Ice

5.33 Wading gear

6.0 Summary of Procedure

6.1 Multi-habitat Sampling

6.1.1 Periphyton sampling should be performed on-site along with macroinvertebrate sampling after site verification and layout of the sampling reach. Site verification and reach layout procedures are described in Ecology SOP EAP106 (Merritt, 2017).

6.1.2 Once the reach has been laid out, collect samples at each of eight randomly selected major transects. Collect substrate for the periphyton and macroinvertebrate samples one meter upstream, or downstream, from each transect.

6.1.3 Samples are to be collected in close proximity to (but not within) the randomly selected macroinvertebrate sample locations. Macroinvertebrate sampling procedures are described in Ecology SOP EAP073 (Larson, 2015).

6.1.4 The objective is to collect a composite sample that is representative of the periphyton assemblage present within the reach.

6.1.5 **Epilithic (coarse substrate) composite samples are preferred** because they provide a more accurate estimate of surface area. However sampling from coarse substrate is not always possible. Methods to collect epipelic (silt/clay) habitats, epipsammic (fine gravel/sand), epidendric (coarse woody debris) and epiphytic (emergent and submerged macrophytes) substrates are also included in this SOP.

6.1.6 While on site, use each of two separate filtrate collection jars when many substrate types are encountered (Figure 3).

6.1.6.1 While on site, the composite sample jar (coarse substrate) jar is used for rinsate from transects with cobble/boulders.

6.1.6.2 The fines/vegetation jar is used for rinsate and material from transects with gravel, fines or submerged macrophytes (transects for which jar contents will need shaking).

6.1.7 Document the types of samples collected for multiple habitat methods (Table 2) onto the sample jars with a pencil.

6.1.8 If filamentous algae is observed within the reach, collect a small portion of algae by hand in proportion to its relative abundance in the reach and place it into the composite sample bottle (this will be somewhat subjective, but provides an opportunity for filamentous algae to be documented as occurring at a site if present).

Table 2. Habitat substrate types and the approximate surface area collected for each of eight transects across the reach-wide stations. Coarse substrate areas in table are listed for WHM and EM studies.

Habitat	Substrate type	Approximate surface area (1 transect)	Approximate surface area (8 transects)***	Field Rinsate Jar	Method Description Section
Epilithic*	Coarse	176.71 cm^{2****}	1413.72 cm^{2*****}	Coarse Jar	6.3
Epipelic**	Silt/Clay	17.4 cm^2	139 cm^2	Fines/ Macrophyte Jar	6.4
Epipsammic**	Fine gravel/sand	17.4 cm^2	139 cm^2	Fines/ Macrophyte Jar	6.4
Epidendric	Coarse woody debris	Variable, see method description	Variable, see method description	Coarse Jar	6.5
Epiphytic	Emergent vegetation	Variable, see method description	Variable, see method description	Coarse Jar	6.6
Epiphytic	Submerged vegetation	Variable, see method description	Variable, see method description	Fines/ Macrophyte Jar	6.7

*176.71 cm^2 is the area of one 15 cm ring or four 7.5 cm rings
**17.4 cm^2 is the area of one 4.7 cm petri dish
*** Values in this column reflect sampling area required for tissue metals sampling.
**** Value for surface area per transect for **BIO** and **SEN** projects is 44.18 cm^2.
***** Value for surface area at eight transects for **BIO** and **SEN** projects is 353.44 cm^2.

6.2 Filtering Native Stream Water for Rinsate

6.2.1 Prior to periphyton sampling, filter stream water through grade 4 filter paper (particle retention 20-25 µm) using a Buchner funnel. Collect the filtered stream rinsate into a 500 mL spray bottle that has been marked with graduations for eight transects; use rinsate sparingly.

6.3 Sampling Method for Epilithic (Coarse) Substrate Habitats

6.3.1 Epilithic (coarse) substrate is likely to be the most frequently encountered habitat sampled for periphyton.

6.3.2 Carefully remove rocks from each randomly selected sample location, retaining the rock's orientation as it occurred in the stream to avoid loss of periphyton. Rocks should be relatively flat and similar in size, depth and exposure to sunlight.

6.3.3 Collect one to four rocks per transect. Scrape the necessary amount of surface area needed for the appropriate Watershed Health Program:

- The **WHM** and **EM** studies scrape four 7.5 cm delimiter circles or one 15 cm delimiter circle, for a total area of 176.71 cm^2 at each coarse transect.
- The **BIO** and **SEN** studies scrape one 7.5 cm delimiter circle for a total area of 44.18 cm^2 at each coarse transect.

6.3.4 Without disturbing the periphyton on top, gently rinse the bottom of the rock(s) over the stream by holding the rock a few inches above the water and brushing the bottom with a wet hand. Rinse and repeat a few times to dislodge fine sediment and detritus.

6.3.5 Gently place the rock(s) in a plastic tray; do not stack them upon one another. Transport the tray to a convenient sample-processing area.

6.3.6 Rinse the plastic tray prior to processing the rock(s) if fine sediment or detritus exists.

6.3.7 Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

6.3.8 Use an appropriate delimiter size option (7.5 cm or 15 cm) to collect periphyton from the upper surface of the rock(s). Using the delimiter as a template, hold the delimiter steady and avoid disturbing the non-sample area. Do not brush the sides or bottom of rocks. Scrub the upper surface of each rock with a firm-bristled toothbrush using a circular motion. Use the spray bottle to move the periphyton into the rinsate tray.

6.3.9 Remove any filamentous algae and mosses within the template area by scraping with a knife and place into plastic rinsate tray. Use a knife or scissors to cut algal filaments or moss into roughly 2 to 3 mm segments.

6.3.10 Gently brush other larger plant material that may be attached to the rocks but do not collect the plants. Rinse the sampled rock, attached plants and toothbrush bristles. Use rinsate sparingly, but be thorough.

6.3.11 Collect rinsate in the plastic tray containing any filamentous algae or mosses and repeat for all substrate.

6.3.12 Pour the rinsate into a Sterile 16 ounce HDPE Wide Mouth Natural Straight Sided Jar. Take care not to splash or spill the rinsate contents.

6.3.13 Repeat steps in section 6.0 for a total of eight transects for the dominant substrate type. Take note of each type of substrate sampled and record for records management in section 7.0.

6.3.14 Process the composite sample following steps described in section **6.8 Subsample Processing Procedures** to measure the total volume, extract subsamples for chlorophyll-a analysis, taxonomy and (optional) periphyton metals/associated parameters. Document and record substrate types and areas for section **7.0 Records Management and taxonomic identification**.

6.4 Sampling Method for Epipsammic (Fine gravel/Sand) and Epipelic (silt/clay) Habitats

6.4.1 Periphyton samples are collected from the upper 5 - 7 mm layer of epipsammic (fine gravel/sand) and epipelic (silt/clay) habitat in organically-rich depositional areas of the reach.

6.4.2 At each sampling location, hold the lid of a plastic Petri dish (47 mm diameter) upside down in the water; gently stir/shake the lid to remove air bubbles without disturbing the substrate.

6.4.3 With the lid still submerged, turn the inside of the lid toward the substrate that will be sampled without disturbing the substrate.

6.4.4 Carefully and slowly press (in cookie cutter fashion) the lid into the substrate until the substrate fills the lid.

6.4.5 Slide the spatula under the lid to enclose the discrete collection. Holding the Petri dish firm against the spatula, carefully wash extraneous sediment from the spatula and lift out of the water.

6.4.6 Transport the Petri dish and spatula to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

6.4.7 Invert the lid and remove the spatula over the sampling tub. Be careful not to lose any of the discrete sample still adhering to the spatula.

6.4.8 Rinse the substrate from the lid and spatula into the sampling tub with the rinsate. Pour the solution containing fine sediment into a second 16 oz. HDPE jar.

6.4.9 Spray the sampling tub with the rinsate again to move all the fine sediment into the jar. **Do NOT use the same 16 oz. HDPE sample jar that is being used for coarse substrate.**

6.4.10 After all eight transects have been sampled, take the 16 oz. HDPE sample jar used for fine sediment and shake vigorously for 30 seconds.

6.4.11 Let the sediment settle to the bottom of the fines jar for approximately 3-5 minutes, then pour off the water into the coarse sample jar, leaving the sediment behind.

6.5 Sampling Method for Epidendric (Coarse Woody Debris) Habitats

6.5.1 Sampling submerged wood is appropriate only when it is not possible to sample other preferred targeted habitats (e.g., epilithic, epipsammic, epipelic) due to their absence or rare occurrence in the reach.

6.5.2 Collecting quantitative microalgal periphyton samples from epidendric habitats presents a challenge because they generally have an irregular surface and are difficult to remove without loss of periphyton biomass.

6.5.3 Select pieces greater than one cm in diameter that are submerged but not smothered by bottom sediments.

6.5.4 If small enough to fit, carefully remove the coarse woody debris and place in a plastic tub. Transport the tub to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

6.5.5 Scrub the entire surface of the woody section with a firm-bristled toothbrush.

6.5.6 Follow steps 6.3.11 – 6.3.14 listed above.

6.5.7 Measure and record the diameter and length of each woody section for later calculation of sampling surface areas. Calculate the total sampled surface area by using the following formula (assumes a cylinder) (Figure 4). Document the total area for section 7.2.1

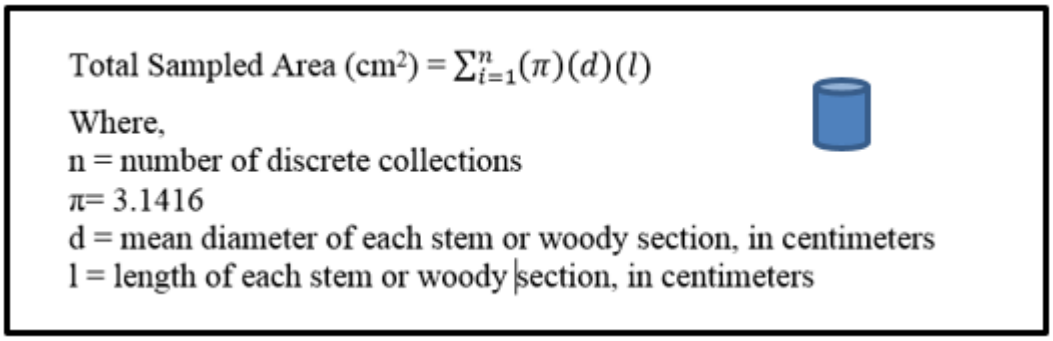

Figure 4. This formula can be used to calculate area sampled from cylindrical stems/branches.

6.5.8 Alternatively, a foil template can be used for irregularly shaped coarse woody debris sections.

6.6 Sampling Method for Epiphytic (**Emergent Vegetation**) Habitats

6.6.1 Sampling emergent macrophytes is appropriate only when it is not possible to sample other preferred targeted habitats (e.g., epilithic, epidendric, epipsammic, and epipelic) due to their absence or rare occurrence in the reach. Collecting quantitative periphyton samples from emergent vegetation presents a challenge because of varying sizes and shapes in vegetation and the care needed to remove the vegetation without loss of periphyton biomass.

6.6.2 All samples should be collected from live specimens of the same emergent species or group of closely-related emergent species that are common in the reach to facilitate sampling of the same species/group in the future. This will allow for representative comparisons of periphyton data over time. Groups of closely-related emergent plants such as *Carex* (sedges), *Juncus* (rushes), *Polygonum* (smartweed) and *Typha* (cattails) are all suitable.

6.6.3 Record the genus or group of closely-related species that will be sampled on the field datasheet.

6.6.4 Select a total of three sections of emergent vegetation from each transect where emergent vegetation is the dominant habitat. Each section represents a stem or leaf (no roots); however, all three sections must be of the same type.

NOTE: Do not cause unneeded disturbance to the emergent vegetation or excessive wave-action when wading; epiphytic periphyton are often loosely attached and easily dislodged.

6.6.5 Prior to sampling a section, remove the un-submerged portion of the selected emergent vegetation with pruning shears or a scissor at water level and discard.

6.6.6 Select a large diameter/width section of stem or leaf from the submerged portion of the emergent vegetation just below water level. Select sections that have been submerged for its growing season to allow for sufficient periphyton colonization. Avoid sections that have been smothered by bottom sediments.

6.6.7 Carefully remove a 10 - 20 cm long section of each stem or leaf and place in a plastic tray. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

6.6.8 Gently brush the entire surface of the stem or both sides of a leaf section with a soft-bristled toothbrush. If algal filaments are found, follow instructions in 6.3.9.

6.6.9 Rinse the toothbrush and the stem or leaf section with rinsate. Set the stem or leaf section aside. Repeat for the remaining stem or leaf sections.

6.6.10 Follow steps 6.3.11 – 6.3.14 listed above.

6.6.11 After sample processing is complete, measure and record the total sampled area.

6.6.11.1 For cylindrical-shaped stem or leaf samples, use a digital caliper to measure the length and diameter (take an average of three diameter measurements) of each cleaned stem/leaf section and calculate the total sampled surface area by using the formula (assumes a cylinder) in Figure 4 for cylindrical stems and branches.

6.6.11.2 For samples with ≥ 10 cylindrical-shaped leaves, take an average of the length and width measured from each of the randomly-selected leaf segments. Use these mean values and the above equation to obtain a surface area and then multiply by the total number of leaf segments in the sample. This alternative method may also be used to calculate the area for cylindrical-shaped branches when the sample contains ≥ 10 branches. In all cases, the stem must be measured in its entirety.

6.6.11.3 For triangular stem samples, use a digital caliper to measure the width of all three sides (measure the width of each side and take an average) and length for each cleaned stem section. Calculate the total sampled surface area by using the following formula in Figure 5.

6.6.11.4 For samples with ≥ 10 non-cylindrical or broad-shaped leaves, randomly select three leaf segments and estimate area for each leaf using an appropriate shape approximation (e.g., circle or triangle). Record the total number of leaves on the datasheet. Take the average surface area from the three measured leaf segments and multiply by the total number of leaf segments in the sample. Document the total area for section 7.2.1.

Total Sampled Area (cm^2) = $\sum_{i=1}^{n} 3(w)(l)$

Where,
n = number of discrete collections
w = mean width of each triangular stem section, in centimeters
l = length of each triangular stem section, in centimeters

Figure 5. This formula can be used to calculate area sampled from triangular leaves

6.6.11.5 For circular shaped leaves use the formula in Figure 6.

Total Sampled Area (cm2) = $\sum_{i=1}^{n} (\pi)(r)(r)$

Where,
n = number of discrete collections
π = 3.1416
r = mean radius of circle-shaped leaf section, in centimeters

Figure 6. This formula can be used to calculate area sampled from circular leaves.

6.6.11.6 For irregular shaped leaf samples, place each cleaned leaf section on a sheet of aluminum foil. With a permanent marker or pen, trace the shape of the leaf section to match the area sampled. For large leaf sections, either trim the foil with a knife or fold the foil to match the area sampled. Mark "X 2" on each leaf template to note that both sides of the leaf section were sampled. Place the marked or trimmed/folded foil templates into a labeled collection envelope and attach to the field data sheets.

6.7 Sampling Method for Epiphytic (**Submerged Vegetation**) Habitats

6.7.1 Sampling submerged macrophytes is appropriate only when it is not possible to sample other preferred targeted habitats (e.g., epilithic, epidendric, epipsammic, epipelic) due to their absence or rare occurrence in the reach.

6.7.2 Collecting quantitative periphyton samples from submerged macrophytes is a challenge because several submerged macrophytes have small or finely dissected leaves and calculating the surface area of periphyton colonization is tedious. Furthermore, care is needed to remove the vegetation without the loss of periphyton biomass.

6.7.3 All samples should be collected from live specimens of the same submerged species or group of closely-related submerged species that are common in the reach to facilitate sampling of the same species/group in the future. This will allow for representative comparisons of periphyton data over time. Groups of closely-related submerged plants such as *Ceratophyllum* (coontail), *Myriophyllum* (milfoil), *Najas* (water-nymph), and *Potamogeton* (pondweed) are all suitable.

6.7.4 Record the genus or group of closely-related species that will be sampled on the field datasheet.

6.7.5 Select a total of three samples of submerged vegetation strands from each transect throughout the reach where epiphytic habitat is dominant. Each sample should consist of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip. Submerged leafless stems should not be included.

6.7.6 Select sections that have been submerged for its growing season to allow for sufficient periphyton colonization. Avoid sections that have been smothered by bottom sediments.

NOTE: Do not cause unneeded disturbance to the emergent vegetation or excessive wave-action when wading; epiphytic periphyton are often loosely attached and easily dislodged.

6.7.7 Carefully remove the sample with pruning shears or scissors and gently place in a plastic tray. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

6.7.8 Use a knife or scissors to cut the sample's stem and branches into 10 - 20 cm segments, preferably at the plant nodes. Do not cut through the leaves.

NOTE: For samples with small or finely dissected leaves, the sampler has the option of discarding the leaves and only processing the stems and branches. If leaves are discarded, sampler must note this on the datasheet. Use a knife or scissor to cut any algal filaments or moss into roughly 2 - 3 mm segments and place in a separate plastic tray. Repeat for remaining samples. Within the plastic tray, separate the sample segments from the algal/moss segments.

6.7.9 Collect all epiphytic samples encountered across the 8 transects into the macrophyte/fines16-oz HDPE sample jar.

6.7.10 Fill the sample jar with approximately 200 mL of filtered stream water and replace the cap. Shake the jar vigorously for 30 seconds to dislodge attached periphyton.

6.7.11 Open the jar, and pour algal slurry into the composite coarse sample jar using a funnel and a small kitchen strainer placed above the funnel to capture individual macrophyte segments. Rinse macrophyte segments with filtered stream water. Use rinse water sparingly, but be thorough; rinsate should flow through the strainer and into the composite jar.

6.7.12 After sample processing is complete, measure and record the total sampled area, following steps 6.6.11.1 – 6.6.11.6 listed above.

6.8 Composite Sample Processing Procedures.

6.8.1 **Overview of processing**

6.8.1.1 Using the 1L graduated cylinder, measure the total volume. See section 7.0 for records management.

6.8.1.2 Always homogenize the composite sample prior to extracting an aliquot by shaking vigorously for 30 seconds.

6.8.1.3 Extract aliquots for sections 6.8.2, 6.8.3 and 6.8.4 (if applicable).

6.8.1.4 Sterilize all sampling equipment with ethanol or peroxide after processing to avoid cross contamination between sampling locations.

6.8.2 **Chlorophyll-a Subsample**

6.8.2.1 In an area out of direct sunlight, assemble the filtration apparatus by attaching the filter base with rubber stopper to the filtration flask. Join the flask and a hand-operated vacuum pump (with pressure gage) using a section of tubing.

6.8.2.2 Place a 47 mm, 0.7 micron glass microfiber filter (for example, Whatman® GF/F) on the filter base and wet with deionized, distilled, or filtered stream water. NOTE: Wetting the filter will help it adhere to the base in windy conditions. Attach the filter funnel to the filter base.

6.8.2.3 Extract one 10 mL aliquot of homogenized composite sample using a disposable serological volumetric pipette and dispense onto the middle of the wetted glass microfiber filter.

6.8.2.4 Filter the aliquot with the vacuum pump using 7 - 10 psi.

NOTE: For composite samples with abundant organic material and/or fine sediment, filtration of a 10 mL aliquot may not be possible. In these circumstances, filter one 5 mL aliquot. If no difficulties were apparent when filtering the first 5 mL aliquot, proceed with filtering a second 5 mL aliquot add both filters to one acetone vial and make a note on the label/LAR form.

6.8.2.5 The filtered aliquot(s) represent the chlorophyll-a subsample. Determine the number of aliquots filtered and record the chlorophyll-a subsample volume on the datasheet. For example, 2 aliquots x 5 mL/aliquot = 10 mL subsample volume.

6.8.2.6 Rinse the sides of the filter funnel with deionized, distilled, or filtered stream water and allow the water to be vacuumed completely before releasing the vacuum from the filtering apparatus.

6.8.2.7 Using forceps, fold the filter into quarters with the filtered biomass inside. Remove the filter from the funnel base with forceps and place it in a small test tube containing 90% acetone (provided by the Manchester Lab).

6.8.2.8 Seal the test tube with sealing laboratory film and wrap the vial in aluminum foil to shade it from light. Wrap the sealed vial in bubble wrap to protect the sample then label the sample according to Figure 7, located in section 7.0 Records Management.

6.8.2.9 Repeat the aliquot extraction and filtration processes if necessary for quality control duplicates.

6.8.2.10 Insert the labeled test tube in a re-sealable plastic bag and place in a cooler containing ice. Coolers should be shipped within a few days after the subsamples have been prepared, as the chlorophyll-a samples have a 25 day holding time limit.

6.8.3 **Taxonomic Identification Subsampling**

6.8.3.1 For **WHM** and **EM** studies, extract a 30 mL aliquot of homogenized composite sample and dispense into a clean 50 mL centrifuge tube.

6.8.3.1.1 Preserve the ID sample with Lugol's solution. A minimum of 1 – 3 mL Lugol's stock solution per 100 mL sample is needed (1 – 3% of sample volume) but exceeding this amount is fine. Add 1 ml of Lugol's to the 30 mL aliquot. Make note of the added preservative volume and record this value into Figure 8, located in section 7.0 Records Management.

6.8.3.2 For the **SEN** and **BIO** studies (after the chlorophyll-a subsample) preserve the remaining composite sample for taxonomic identification. For an average sample, add approximately 10 mL of preservative solution. Make note of the added preservative volume and record this value into Figure 8, located in section 7.0 Records Management.

6.8.3.3 Fill out a label with site name, sample ID, collection date (mm-dd-yyyy), collection time (24 hour), and total taxonomic identification sample volume (mL) [identification sample + preservative volumes] and initial composite sample volume (Figure 8). Use electrical tape to seal the lid and packing tape to secure the label. Store sample in a dark location until sent to the lab for processing.

6.8.4 **Metals and Associated Parameters Subsampling (WHM and EM)**

6.8.4.1.1 Label the remaining composite sample with site name, sample ID, collection date (mm-dd-yyyy), collection time (24 hour), and volume of sample (mL). Store on ice until additional subsampling.

6.8.4.1.2 Deliver the labeled composite sample to Ecology Headquarters (HQ) in Lacey, for further processing. For **EM** and **WHM** sampling, the delivery schedule is consistent with that for OC delivery of water samples (SOP EAP095 (Hartman, 2017)). See section 6.9 for further processing instructions.

6.9 Additional Subsampling for **WHM** and **EM** Studies

6.9.1 **Metals and associated parameters** (Table 3)

Table 3. Metals and associated parameters derived from samples prepared at Ecology

Parameter	Units	Analysis Method	Container for MEL
AFDW	mg/kg/dw	SM10300C	125 mL poly brown jar
TOC	%	EPA440.0	50 mL centrifuge tube
Total Solids	ug/L	SM2540B	125 mL poly brown jar
Total Nitrogen	%	EPA440.0	50 mL centrifuge tube
Total Phosphorus	mg/kg/dw	EPA6020A	50 mL centrifuge tube
Al	mg/kg/dw	SW6010D	50 mL centrifuge tube
As	mg/kg/dw	SW6020B	50 mL centrifuge tube
Cd	mg/kg/dw	SW6020B	50 mL centrifuge tube
Cu	mg/kg/dw	SW6020B	50 mL centrifuge tube
K	mg/kg/dw	SW6010D	50 mL centrifuge tube
Fe	mg/kg/dw	SW6010D	50 mL centrifuge tube
Mn	mg/kg/dw	SW6020B	50 mL centrifuge tube
Ni	mg/kg/dw	SW6020B	50 mL centrifuge tube
Pb	mg/kg/dw	SW6020B	50 mL centrifuge tube
Zn	mg/kg/dw	SW6020B	50 mL centrifuge tube

6.9.2 **Ash Free Dry Weight and Percent Total Solids Subsampling**

6.9.2.1 Prior to extracting aliquot, homogenize the remaining composite sample by blending or vigorously shaking for 30 seconds.

6.9.2.2 Extract a 30 mL aliquot and dispense into a clean 125 mL wide mouth polypropylene brown bottle.

6.9.2.3 Label the sample bottle with site name, sample ID, collection date (mm-dd-yyyy), collection time (24 hour), analysis type, and volume of sample (mL). Store at 4 - 6 °C until sent to the laboratory for analysis.

6.9.3 **Metals, Total Organic Carbon, and Nutrients Subsampling**

6.9.3.1 The remaining sample volume after subsampling in 6.8.4 will be split between two 50 mL acid-washed polyethylene centrifuge tubes. One centrifuge tube will be used for metals and total organic carbon (TOC) and the other will be for nutrients.

6.9.3.2 Once filled, centrifuge the samples at 4000 rpm for 10 minutes. After the 10 minutes lapses remove the centrifuge tubes and decant the supernatant (water) leaving the pellet at the bottom.

6.9.3.3 Refill and centrifuge the samples until sufficient periphyton biomass pellet is obtained (>1 g) or the original composite sample is gone.

6.9.3.4 After centrifuging is completed, weigh and record the centrifuge tube with the pellet. Calculate and record the final sample weight (g).

6.9.3.5 Label each sample bottle with site name, sample ID, collection date (mm-dd-yyyy), collection time (24 hour), analysis type, and weight of sample (g). Freeze the samples until sent to the laboratory for analysis.

7.0 Records Management

7.1 Chlorophyll-a Sample Labeling and Tracking

Figure 7. A completed sample tag.

7.1.1 Label chlorophyll-a samples according to Figure 7.

7.1.2 Send chlorophyll samples to MEL according to the schedule for submission of water samples collected on the same date (e.g. Hartman, 2017). For **WHM**, these are typically returned to the Operations Center cooler on the return date.

7.1.1.2 Complete the appropriate section of the Laboratory Analysis Request (LAR) form, indicating any additional comments.

7.2 Taxonomy Sample Labeling and Tracking

7.2.1 Label the samples according to the example provided in Figure 8.

Periphyton Composite Sample Jar Label (1 of 1 composite jars for HQ)		
Stream Description Deep Creek		
DCE WAM06600-000000-DCE-07-25-2017-10:56		
Who collected Bob Keeshan		
	Circle one/row (count)	Area scraped (cm2)
Transects as coarse	1 2 3 4 (5) 6 7 8	883.6
Transects as fines/sediment	(1) 2 3 4 5 6 7 8	17.4
Transects as wood	(1) 2 3 4 5 6 7 8	676.0
Transects as vegetation	(1) 2 3 4 5 6 7 8	630.0
	Total Area (cm2)	2207.0
Chl-a volume filtered (ml)	10	MEL ID (chlor-a):
ID Volume (ml)	30	
ID Lugol's added (ml)	1	1707022-04
Remainder for HQ (ml)	460	
Collector's Notes: Total volume in ID tube is 31 ml including Lugol's. Thick slurry; needed to filter 5mm twice for Chl-a		

Figure 8. Periphyton taxonomy jar label.

7.2.2 Enter the periphyton total volume measured in section 6.8 into the *samples* tab on the eforms (Figure 9).

7.2.2.1 For **WHM** and **EM**, the total volume would be the sum of your aliquots: 10mL (Chlorophyll-a) + 30mL (ID Volume) + 460mL (Remainder for periphyton Metals) = 500mL.

7.2.2.2 For **BIO** and **SEN**, record the total volume sent to the taxonomic ID lab with preservative: 500mL (Total sample) – 10mL (Chlorophyll-a) + 10mL (Lugol's preservative) = 500mL

7.2.3 Perform a general visual assessment across the entire length of the site. Select a value on the periphyton scale that most closely resembles the periphyton abundance within the stream:

1 – Very little periphyton observed

2 – Noticeable brown film/ coloration on rocks

3 – Noticeable brown film on rocks with occasional appearance of filamentous green algae

4 – Considerable accumulation of material on rocks

5 – Very thick coating on rocks, including abundant filamentous algae

7.2.4 Select the most accurate value on the *samples* tab under periphyton scale (Figure 9).

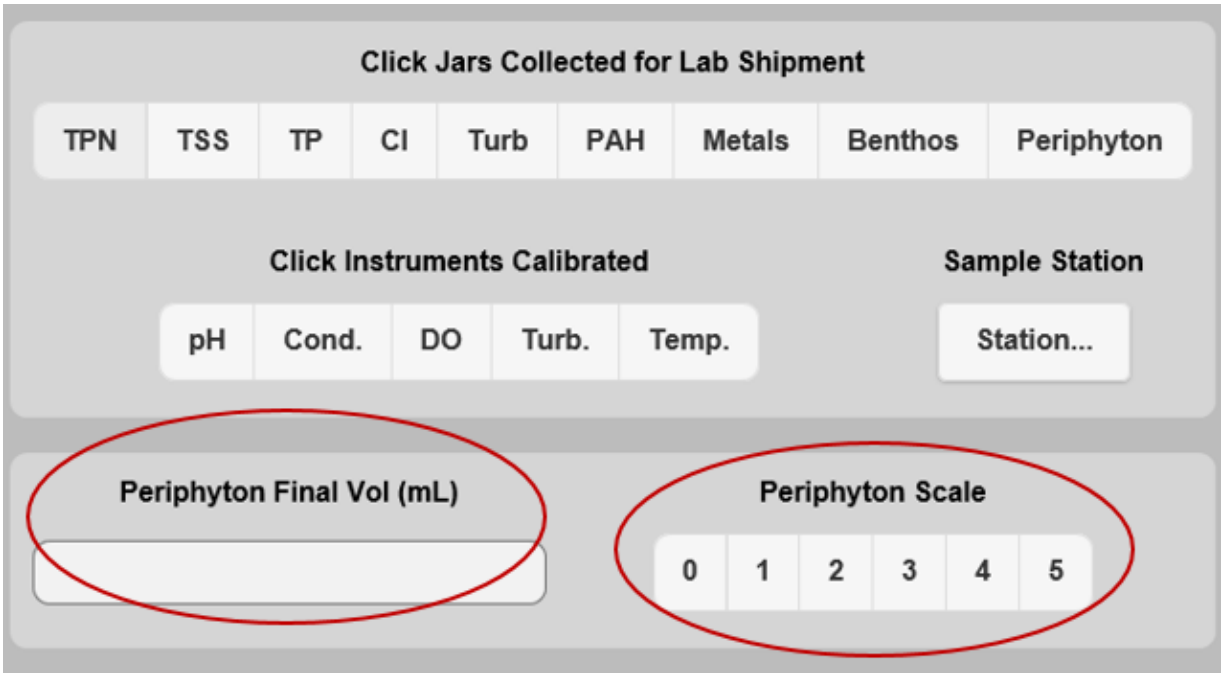

Figure 9. Periphyton Final Volume and Periphyton Scale values, located on the Samples tab in the WHM eforms.

7.2.5 Make a note in the *samples* tab on the eforms of the periphyton sample collection method for The Environmental Information Management System (EIM):

- PeriphytonCoarse (Coarse Substrate)
- PeriphytonFine (Fine gravel/sand)
- PeriphytonCoarseFine (Coarse Substrate + Fine Gravel/Sand)
- PeriphytonVeg (Emergent and/or Submerged Vegetation)
- PeriphytonCoarseVeg (Coarse Substrate + Emergent/Submerged Vegetation)
- PeriphytonFineVeg (Fine gravel/sand + Emergent/Submerged Vegetation
- PeriphytonWoody (Coarse Woody Debris)
- PeriphytonMultiple (More than 2 collection types)

7.2.6 Return labeled, sealed centrifuge tubes (with Lugol's) to the EAP Operations Center. Keep all taxonomy samples for the project/season together in an appropriate hazard cabinet.

7.2.7 Photograph labels for periphyton after each sampling event, and keep the label with the centrifuge tube. Secure with a rubber band, or seal the information together inside a re-sealing snack bag.

7.2.8 Maintain a spreadsheet identifying all tracking information required by the contract taxonomy laboratory

7.2.9 Hand-deliver the samples to staff from the contract laboratory at a convenient time during the fall. Verify that all lines of the laboratory chain of custody form are accurate and submit it to the contract laboratory by email

7.2.10 For laboratory taxonomy processing, follow details in section 8.0 for results tracking

7.3 Metals and associated parameters (**WHM** and **EM**)

7.3.1 Ensure that all composite samples (16-oz HDPE sample jars) for each site are labeled completely (Figure 7). The label in Figure 8 remains with the periphyton taxonomy sample, however information from this tag is needed for processing the remaining composite. Photograph each label and keep records together in a file.

7.3.2 Ensure the samples are sealed, and contained in Ziploc bags in a cooler of fresh ice.

7.3.3 Return the cooler(s) of samples to the Headquarters prep lab at the schedule dictated by your project's quality assurance plan. Leave the samples with fresh ice in a cooler. Email the prep lab staff with a list of the samples collected including photographic images of all composite sample labels.

NOTE: The same label images can be provided to the contract taxonomy lab (see section 8.0)

7.3.4 Follow details of the periphyton tissue prep lab (Section 6.9)

NOTE: For WHM, the samples might sometimes exceed holding time for Ash Free Dry Weight. These data will be qualified.

8.0 Periphyton Taxonomic Lab Sampling Procedures

8.1 Summary of Procedure

8.1.1 Taxonomic identification is conducted by a lab that employs taxonomists with degrees in a related field and adequate training.

8.1.2 The taxonomist should have experience with the freshwater periphyton of the Pacific Northwest. A standardized level of identification is required to allow for valid comparison of periphyton data sets between sampling locations.

8.1.3 The standard level of identification required by Ecology is that diatoms be identified to species (or species variety, if possible), and non-diatoms (soft-bodied algae) be identified to genus.

8.2 Personnel Responsibilities

8.2.1 One person or more performs this activity. Staff performing this method must have been trained.

8.3 Sample Processing and Subsampling

8.3.1 Samples are homogenized in a blender to break up large colonies and filaments and to evenly distribute individual cells.

8.3.2 A sub-sample is transferred to a Palmer-Maloney counting chamber, and 300 live algae cells are counted at 400X.

8.3.3 The sub-sample is diluted or concentrated to achieve an optimum concentration of 10-20 cells per field.

8.3.4 For small filamentous cyanobacteria (e.g., *Phormidium*) and for coenocytic algae that lack cell walls (e.g., *Vaucheria*), the counting unit will be a 10-micron length of filament.

8.3.5 Diatoms are identified only as diatoms; soft (non-diatom) algae are identified to genus or to species if possible. The number of dead (empty) diatom frustules is also recorded on the bench sheet during the count of 300 live cells.

8.3.6 After the identification of soft algae, the homogenized sample is re-agitated and a portion of the raw sample is extracted to make duplicate diatom slides.

8.3.7 The diatom sub-sample is cleaned of organic matter using 70% Nitric acid and digested using a closed-vessel microwave digestion system (Milestone Ethos EZ), following the method developed by the Academy of Natural Sciences, Philadelphia (ANSP 2002).

8.3.8 Following several dilutions with distilled water, two permanent diatom slides are prepared from cleaned material using Naphrax, a high refractive index mounting medium.

8.3.9 At least 300 diatom cells (600 valves) are counted at random and identified to species, and variety if possible. Diatom naming conventions should follow those adopted by the Academy of Natural Sciences (Philadelphia) for USGS NAWQA samples (Morales and Potapova, 2000) as updated in 2005 (Morales and Charles, 2005) http://diatom.ansp.org/nawqa/Workshops.aspx.

8.3.10 Separate bench sheets will be used for algae and diatom counts, and these will provided to Ecology when samples are completed each year.

8.3.11 Counts of soft algae and diatoms will be conducted with compound research microscopes using brightfield transmitted light or differential interference contrast. Proportional counts will be performed using 100X oil immersion objectives and 10X oculars.

8.3.12 Permeant slides prepared by the taxonomic lab will be provided to Ecology at the end of the project.

8.3.13 Remaining sample volume will be re-preserved and stored by the contract laboratory. Contractor storage of sample fractions may be as long as six months.

8.3.14 Ecology may request that a subset of samples be returned for quality assurance purposes. Non-requested sample fractions will be appropriately discarded by the contractor.

8.4 Sample Submittal and Data Turn-Around

8.4.1 Ecology will collect samples between early July and late October. Ecology will ship samples to the contractor no later than December 1 of each year. Contracts between Labs and Ecology outline responsibility for costs of shipping samples and forms to the contract laboratory. The processing of samples and reporting of data will be completed within a reasonable amount of time, agreed upon between Ecology and the contract laboratory, usually 3-5 months of the receipt of the samples. Data reported to Ecology will consist of:

1. The original (signed) chain-of-custody form.
2. A taxonomic matrix for each sample based on the required level of identification. (Excel or Access format).
3. Metric calculations (Excel or Access format).
4. Copies of bench sheets used by the laboratory.
5. Copies of all QA/QC documentation.

8.5 Reporting Requirements

8.5.1 Instructions for submitting periphyton data to the Department of Ecology's Environmental Information Management (EIM) are outlined in the *Taxonomy, tissue and bioassay* header on this page: https://fortress.wa.gov/ecy/eimhelp/HelpDocuments) Refer to *Periphyton counts*.. Briefly, a taxa list for each sample should be reported with the associated count number for each taxon. The following should accompany this list:

1. Sample ID.
2. Surface area sampled (cm^2).
3. Volume of sample sent to laboratory, including preservative (mL).
4. Volume of sample counted by laboratory (mL).
5. Taxon name.

8.5.2 The contract laboratory will analyze the taxonomy matrix for each sample and summarize with a suite of periphyton metrics. In most cases, diatoms and non-diatoms will be analyzed separately with a few exceptions (combined metrics) outlined below. This list represents the minimum metric reporting requirements. The contractor is encouraged to provide additional metrics if available and relevant.

8.5.3 Combined metrics

1. Total number of algal divisions.
2. Total number of algal genera.
3. Total number of algal species.

8.5.4 Diatom metrics

1. Total number of diatom genera.
2. Total number of diatom species.
3. Shannon Diversity Index.
4. Pollution Index (Lange-Bertalot, 1979; Bahls, 1993; Barbour et al., 1999).
5. Siltation Index (% *Navicula* + *Nitzschia* + *Surirella*) (Bahls, 1993; Barbour et al., 1999).
6. Percent live diatoms (Hill, 1997).
7. Valves counted.
8. Cells counted.
9. Percent dominant species.
10. Pollution tolerance (% by category).
11. Disturbance index (Bahls, 1993; Barbour et al., 1999).
12. Percent rhopalodiales.
13. Percent aerophiles.
14. Percent centrics.
15. Motility index.

8.5.5 Van Dam diatom metrics (% in each category) (Van Dam et al., 1994)

1. pH.
2. Salinity.
3. Nitrogen uptake.
4. Oxygen demand.
5. Saprobity.
6. Trophic state.
7. Moisture.

8.5.6 Non-diatom metrics

1. Dominant phylum.
2. Percent 5 dominant genera.
3. Percent 10 dominant genera.
4. Total number of genera (within each non-diatom algal division).
5. Total number of non-diatom genera.

8.6 Instructions for estimating periphyton cell densities from samples collected using Ecology protocols

8.6.1 Information needed to estimate periphyton density (cells/cm^2)

8.6.1.1 # Cells counted in 'wet count' – this information is obtained from the processing lab and includes counts of 'soft algae' (i.e. green algae and cyanobacteria) and diatoms (number of diatoms counted only and not identified to genus/species).

8.6.1.2 Volume of sample collected (mL). See section 7.2.2 for this information.

8.6.1.3 Volume of sample counted (mL)* – this information is obtained from the processing lab and represents the volume of sample counted in a counting chamber. This volume depends on the counting chamber used and number of fields of view counted (see 8.6.3.1).

8.6.1.4 Surface area scraped (cm^2) – this is estimated by person(s) collecting the sample as outlined above (C-3).

8.6.2 Wet count:

8.6.2.1 A minimum of 300 cells are enumerated using a 'wet mount' using a Palmer-Maloney counting chamber at 400× magnification.

8.6.2.2 Soft algae (green algae and cyanobacteria) and diatoms (number of diatoms counted only and not identified to genus/species) are counted until a minimum of 300 cells/units has been reached.

8.6.2.3 For filamentous green algae and cyanobacteria, one unit is each 10 µm length of that filament in the field of view. Additionally, the number of empty diatom cells may also be counted by the processing lab, but are not used to estimate total density.

8.6.3 Estimating density requires that the volume of sample counted is given by the processing lab (example below).

8.6.3.1 *Equation used to determine mLs counted using Palmer-Maloney counting chamber:

(([FeildsOfView]*0.307*0.4)*0.001)

Where:

0.307 = area of field of view

0.4 = depth of counting cell

0.001 – to bring mm^3 up to mL

8.6.4 Diatom count:

8.6.4.1 Typically, a minimum of 600 diatom valves are identified to species and enumerated by the processing lab.

8.6.4.2 After a portion of the algal slurry from a particular site/reach has been processed to remove diatom chloroplasts (this process also removes organic matter including soft algae), a drop of solution containing cleaned/empty diatom frustules is placed on a coverslip, where they are heat fixed and mounted on a slide with Naphrax™.

8.6.4.3 Cells are enumerated with an oil objective at 1000× magnification. Information from this count of diatom valves will be used to calculate the relative proportion of diatoms attributed to each species (e.g. if total valves counted = 600 and valves of *Achnanthidium minutissimum* = 300, then relative proportion of *A. minutissimum* in the sample = 300/600, or 0.5).

8.6.4.4 Relative proportion for each diatom species is then used to estimate the number of diatom cells attributed to each species in the 'wet count'. For example, if 100 of 324 total cells counted in a Palmer-Maloney cell were diatoms, then 50 of them would be *A. minutissimum* using the value in the previous paragraph, and the remaining 50 diatoms cells would be split by the relative proportions of the remaining diatom species enumerated in the 'diatom count'.

8.6.5 EPA method for calculation of cell densities:

8.6.5.1 Cell densities (cells · cm^2) are determined by dividing the numbers of cells counted by the proportion of sample counted and the area from which samples were collected.

Example:

Total # cells/units counted in 'wet count' = 324

Volume (mL) of sample sent to processing lab = 35

Volume (mL) of sample counted = 0.0007368

Surface area scraped (cm^2) = 110.25

Total density (cells · cm^2) = (324/(0.0007368/35))/(110.25) = 139,599.8 cells · cm^{-2}

9.0 Quality Control and Quality Assurance Section

9.1 Sampling Methods-Field Procedures.

9.1.1 To account for sample variability, it is recommended that 10% of all collected composite samples must consist of duplicate composite samples (e.g., two duplicates for 11 - 20 samples, three duplicates for 21 - 30 samples, etc.) collected on the same day or repeat visits collected in the same sampling season. The first is a measure of spatial variability or sample precision while the latter is a measure of temporal variability. Study priorities will determine which approach should be applied, with sample precision being the approach likely to be of most interest.

9.1.2 Following the processes described under Subsample Processing Procedures, the sampler that collected the duplicate composite sample, extracts two chlorophyll _ subsamples from the duplicate composite sample. The remaining duplicate composite sample volume will be used for the duplicate ID subsample.

9.1.3 Duplicate composite samples are collected to check the variability between field samplers while the two duplicate chlorophyll _ subsamples provide an indication of precision and the quality of the duplicate composite sample homogenization. An illustration of the duplicate composite sample/subsample processes is provided in Figure 10.

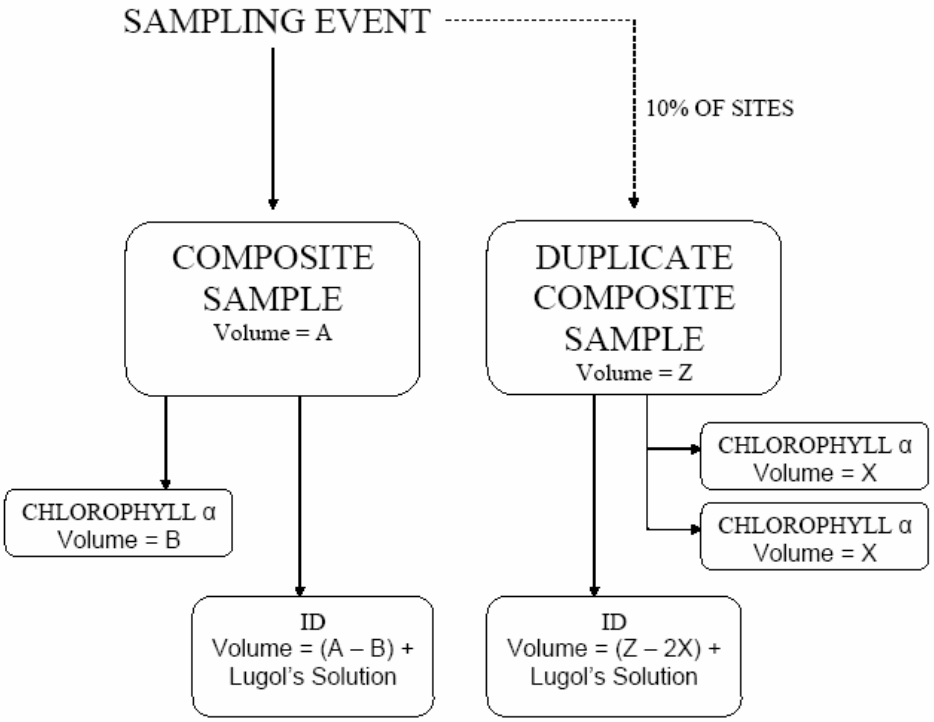

Figure 10. Flow chart for the duplicate composite sample/subsample process.

10.0 Safety

10.1 Safety is the primary concern when retrieving periphyton from streams and rivers. Review the Environmental Assessment Programs Safety Manual (Ecology, 2017) periodically to assist with these safety determinations.

10.2 A proper safety assessment of the sampling reach of the stream is extremely important given that the stream must be waded in order to perform these tasks.

10.2.1 Assess whether or not the velocity and depth of the stream are low enough to safely wade across it. As a rule-of-thumb: **Do NOT wade in flowing water when the product of depth (in feet) and velocity (in feet per second) equals 10 or greater. For example, if the stream is estimated to be 3 feet deep and have a velocity of 4 ft./s, do NOT wade across the stream. This is only a general rule, take extra precautions where the substrate is unstable (slippery or moving), water visibility is impaired (high turbidity or glare), or other challenges are present.**

10.2.2 Never wade across a stream **where: a) potentially fatal entrapments/obstacles are located downstream (such as strainers, dams, large boulders, frowning (upstream V) rapids, etc.); or b) medium to large debris pieces are floating down the stream.**

10.3 For a more complete list of river hazards see: https://paddling.com/learn/river-hazards/

10.4 ALWAYS **a) wear a PFD while wading in the stream, b) wear a tight wading belt with waders, c) wear wading specific boots with good traction, d) work with a partner, and e) have the non-wading partner carry a throw-bag (and make sure they know how to use it).**

10.5 **Use extreme caution when preparing Lugol's solution and handling reagents (See http://teams/sites/EAP/QualityAssurance/ChemicalSafetyDataSheets/Forms/AllItems.aspx for the latest SDS information):**

Caution! Potassium iodide may cause eye, skin, and respiratory tract irritation. May cause reproductive and fetal effects.

Danger! Glacial Acetic Acid causes severe eye and skin burns. Causes severe digestive and respiratory tract burns. **Flammable liquid and vapor.** May be harmful if absorbed through the skin. Glacial acetic acid solidifies below 62°F (17°C). Corrosive to metal.

Danger! Crystalline Iodine may cause allergic skin reaction. Harmful if swallowed, inhaled, or absorbed through skin. Causes burns by all exposure routes. Extreme inhalation exposure causes build-up of fluid in the lungs and can be fatal in extreme cases.

11.0 References

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