Department of Ecology EIM Environmental Information Management

EIM Help – Periphyton Counts

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What is Periphyton?

Periphyton is a complex assemblage of microorganisms including algae and cyanobacteria intermixed in a matrix of heterotrophic microbes and detritus. Periphyton is found attached to submerged surfaces in most aquatic ecosystems, often forming slimy coatings.

Periphyton and Watershed Health

Periphyton communities are helpful for assessing watershed health. Like other biological communities such as macroinvertebrates, periphyton respond to environmental stressors. The assemblage of periphyton species within a particular stream reach reflects the types of environmental conditions at both that reach and the watershed level. Data collected from relatively pristine reaches can be used to build models for assessing water quality at reaches that have experienced greater man-made impacts. Although we do not yet have periphyton models for Washington streams, we are working toward that. These models will help determine the degree of biological impairment in Washington's streams, in accordance with the State's Water Quality Assessment.

Periphyton and EIM - Overview

We enter the raw periphyton count data into EIM. Count data can be downloaded and used to perform calculations or metrics.

Do not enter calculated data or metrics for periphyton into EIM.

Cell Density is a common calculation that uses periphyton count data. Cell Density estimates the type and number of algae present in a particular stream reach. The Periphyton Cell Density section below has examples of how these counts and calculations are performed using Ecology protocols, including what supporting metadata you need. The EIM Data Entry section below shows you how to enter your raw periphyton count data and associated metadata into EIM.

Depending on study objectives, you might also have collected samples for Periphyton Areal Biomass (expressed in terms of chlorophyll *a* or ash free dry weight (AFDW)). See our Areal Biomass for Periphyton & Macrophyte Data help document if you are entering these types of data. You might also have collected samples for metals analyses, which will be addressed in an upcoming EIM help document.

Sample Collection

Periphyton is scraped with a small brush from submerged surfaces, such as rocks, woody debris, or the stream substrate, into a sample container using a known amount of filtered stream water. If you collected periphyton from different areas of the stream reach and combined them into a single sample container, it is considered a composite sample. A sample preservative is added to the sample bottle, which is sent to the lab for periphyton identification and counts. Final volume of scraped material, rinsate and preservative in composite sample should be kept to less than 500 ml. The final volume is recorded.

Make sure you record this information when sampling:

- Surface area you sampled or scraped.
- Total sample volume at the time of collection (or have your lab determine it for you).
- Whether or not your sample is a composite.

Laboratory Analyses

The processing lab removes a small portion of the sample for taxonomic identification and counts. The entire sample is not counted because there can be millions of cells in one sample. The lab count takes place in two steps: (1) **Wet Count** and (2) **Diatom Count**. These are detailed in the Periphyton Cell Density section below.

Make sure you also get the following data from your lab: (1) The **Volume of the Total Sample** you sent to the lab and (2) the **Volume of the Counted Sample** (subsample the lab used for identification and counting). You need these values to calculate the percent of the sample the lab counted. Your lab might even calculate this for you. These are also crucial values for calculating Periphyton Cell Density.

How to Enter Periphyton Data into EIM

In addition to EIM's standard required fields described in the Result Help Document, follow this guidance for entering your data into your EIM Result data entry template:

Field Collection Type (D): Enter "*Sample"*

Field Collection Comment (J): If your sample was a composite, which most periphyton count samples are, explain how the sample was composited (*e.g. "Sample is a composite of 8 randomly selected transects in the reach")*

Field Collection Area (K): Enter the surface area of the substrate you scraped to collect your sample (e.g. "352"). If you composited multiple samples into a single sample container, enter the summed area for all the samples. This information is needed to calculate Periphyton Cell Density and AFDW.

Field Collection Area Units (L): Enter the units for your Field Activity Area value (e.g. "cm^2").

Sample ID (R): Enter your lab's sample number or ID.

Sample Composite Flag (V):

- Enter "**Y**" - If you collected periphyton from different areas of the stream and combined them into a single sample container. Most periphyton count samples are composites.
- Enter "***N***" - If you collected periphyton from one general area of the stream (even if periphyton was collected from multiple rocks at that one location).

Sample Matrix (X): Enter "*Solid/Sediment*"

Sample Source (Y): Enter "*Freshwater Taxonomy*"

Sample Collection Method (AA): Enter the appropriate method code:

Sample Collection Method Code	Sample Collection Method Description
PeriphytonCoarse	Periphyton Sampling Method for Epilithic (Course Substrate) Habitats
PeriphytonFine	Periphyton Sampling Method for Epipsammic (Pea gravel/Sand) and Epipelic (Silt) Habitats
PeriphytonCoarseFine	Periphyton Sampling Methods for composite sample containing material collected from Course Substrate + Pea gravel/Sand or Silt Habitats
PeriphytonVeg	Periphyton Sampling Method for Epiphytic (Submerged or Emergent Vegetation) Habitats
PeriphytonCoarseVeg	Periphyton Sampling Methods for composite sample containing material collected from Course Substrate + Emergent Vegetation or Submerged Vegetation Habitats
PeriphytonFineVeg	Periphyton Sampling Methods for composite sample containing material collected from Pea gravel/Sand or Silt Habitats + Emergent Vegetation or Submerged Vegetation Habitats
PeriphytonWoody	Periphyton Sampling Method for Epidendric (Woody Snag) Habitats
PeriphytonMultiple	Periphyton Sampling Methods for composite sample containing material collected from multiple (more than two) kinds of habitats
from Mathieu, et al. (2013) and Adams (2010)	
PeriphytonAS	Periphyton collected by in situ Artificial Substrate Sampler
from Anderson, et al. (2016)	

Percent Sample Sorted (AG): Calculate this value before entering your data into EIM. It will be a very small number. The Volume of the Counted Sample is provided by your lab. The Volume of the Total Sample is the volume of the original sample that you collected.

$$\text{Percent Sample Sorted} = \frac{\text{Vol Counted Sample (mL)}}{\text{Vol Total Sample (mL)}}$$

Result Parameter Name (AH): Enter "*Number of Individual Organisms*"

Result Reported Value (AM): Enter the count provided by your lab for each taxa.

Result Value Units (AN): Enter "*Count*"

Result Method Code (AY): Enter the method your lab used to count. The method must distinguish if it was an algae wet count or a diatom count. Below are counting methods based on Ecology protocols (Adams, 2010). If you need a different method entered into EIM, contact us and we will add it for you.

Result Method Code	Result Method Description
COUNT-ALGAE-PPHYTON	Soft-bodied algae wet count by Palmer-Maloney counting chamber
COUNT-DIATOM-PPHYTON	Diatom cell count by slide

Result Additional Comment (BA): If you are entering corresponding data into Ecology's Watershed Health Monitoring (WHM) database, enter the WHM Data Collection Event (DCE) name here for each result. It is a 35 character name containing the EIM Location ID + "DCE"+ date (YYY-MMDD) + time (24 hour) (e.g. "*BIO06600-AUST02-DCE-2010-0818-16:50*").

Result Lab Name (BC): Enter the name of the lab that processed your sample. These are the most common. If you need a different lab entered into EIM, contact us and we will add it for you.

Result Lab Name
Rhithron Associates Inc, Missoula MT
EcoAnalysts Inc, Moscow ID

Result Taxon Name (BE): Enter the scientific taxonomic name here. See our Taxon Reference Table for valid values. If you cannot find a particular taxon in EIM, contact us and we will add it for you.

Result Taxon Unidentified Species (BG): If your lab cannot identify a particular taxon, fill out this field. This will also affect what you enter for the Result Taxon Name. Refer to our Entering Unidentified Species Data help document for instructions.

Periphyton Cell Density

Cell Density is a common calculation used to estimate the number of periphyton present in a stream reach. We do not enter Cell Density values into EIM because they are calculated and not everyone calculates them the same way. However, we do enter the information needed to calculate Cell Densities. That way, all the information needed to calculate Cell Densities is available when the data is downloaded from EIM.

Following are examples of Ecology (Adams, 2010) / EPA (Stevenson and Bahls, 1999) protocols for periphyton counts and cell density calculations:

Wet Count

Typically, a minimum of 300 cells are counted using a 'wet mount' Palmer-Maloney counting chamber at 400× magnification. 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 "live" cells (cells with organic matter) per unit has been reached. Additionally, the number of empty diatom cells may also be counted by the processing lab, but are not used to estimate total density. For filamentous green algae and cyanobacteria that lack cell walls, one counting unit equals a 10-micron length of that filament in the field of view.

Estimating density requires that the processing lab reports the volume of sample counted.

This is the equation the lab uses to determine the volume of sample counted using a Palmer-Maloney counting chamber. The equation is provided only as a reference. The volume of the sample counted should be provided by your lab.

$$\text{Sample Volume Counted } (mL) = \left(\left(\left[No.\,of\ Fields\ of\ View\right] \times 0.307 \times 0.4\right) \times 0.001\right)$$

where: 0.307 = field of view area
0.4 = counting cell depth
0.001 – conversion from mm^3 to mL

Diatom Count

Diatoms are algae with transparent cell walls (frustules) of silica and innards of chloroplasts. Each frustule is made up of two valves. A minimum of 600 diatom valves are typically identified to the species level and counted by the processing lab. To do this, a portion of the algal slurry from a particular site/reach is processed to remove diatom chloroplasts, which also removes soft algae and other organic matter. A drop of solution containing cleaned/empty diatom frustules is placed on a coverslip and mounted on a slide with Naphrax™ mountant and heat-fixed. Cells are counted with an oil-immersed objective at 1000× magnification. Information from the count is used to calculate the relative proportion of diatoms belonging to each species (i.e. if total valves counted = 600 and valves of species *Achnanthidium minutissimum* = 300, then relative proportion of *A. minutissimum* in the sample = 300/600, or 0.5).

The relative proportion of 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 from the diatom count, and the remaining 50 diatoms cells would be split by the relative proportions of the remaining diatom species itemized in the diatom count.

EPA Method for Calculation of Cell Densities

Cell densities (cells per square centimeter) are determined by dividing the numbers of cells counted by the proportion of sample counted and the area from which samples were collected.

$$\text{Cell Density (cells/cm}^2) = \frac{\left(\text{Wet Cell Count} \div (Vol\ Counted\ Sample\ (mL) \div Vol\ Total\ Sample\ (mL))\right)}{\text{Surface Area Scraped (cm}^2)}$$

$$139{,}599.8\ \text{cells/cm}^2 = \frac{\left(324\ \text{cells} \div (0.0007368\ mL \div 35\ mL)\right)}{110.25\ \text{cm}^2}$$

References

Adams, K., 2010. Quality Assurance Monitoring Plan: Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton, August 2010, Appendix C-3. https://fortress.wa.gov/ecy/publications/summarypages/1003109.html

Anderson, P.D., N. Mathieu, and S. Collyard, 2016. Standard Operation Procedure (SOP) for Collection and Processing of Periphyton Samples. SOP No. EAP085, V 2.0 DRAFT. WA Department of Ecology, Olympia, WA.

Mathieu, N., S. Collyard, and T. Mohamedali, 2013. Standard Operating Procedure (SOP) for the Collection of Periphyton Samples for TMDL studies. Version 1.1. Washington State Department of Ecology, Olympia, WA. SOP Number EAP085. www.ecy.wa.gov/programs/eap/quality.html

Stevenson, R.J., and L.L. Bahls, 1999. Periphyton Protocols. Pages 6-1 through 6-22 *in* M.T. Barbour, J. Gerritsen, B.D. Snyder, and J.B. Stribling (eds.). Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd ed., Report EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC. http://water.epa.gov/scitech/monitoring/rsl/bioassessment/ch06main.cfm

Revision History

Revision Date	Revision No.	Summary of Changes	Reviser(s)
5/21/15	1.0	Original Document	C. Lee, C. Larson, CN
05/17/16	1.1	Added Collection Method PeriphytonAS	KC