



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L604

Algae / Periphyton Identification and Enumeration

Standard Operating Procedure

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1. Purpose

This SOP describes the laboratory procedures for identifying and enumerating benthic algae (periphyton) from stream bioassessment samples. Periphyton assemblages are sensitive indicators of water quality, nutrient enrichment, and physical habitat condition, and are a required component of the California SWAMP bioassessment protocol (Barbour et al., 1999; Ode et al., 2016). This SOP covers soft-bodied algae (green algae, cyanobacteria, and other non-diatom taxa) and diatoms, which are processed separately due to different preparation requirements (Charles et al., 2002; Stevenson & Bahls, 1996).

2. Scope

- **Applies to:** Identification and enumeration of benthic periphyton from composite epilithic, epiphytic, or epidendric samples collected per SWAMP protocols at EA Program stream sites
- **Does not apply to:** Phytoplankton (water column) identification; chlorophyll *a* extraction (see [SOP-L310](#)); algal culture or toxin analysis; molecular identification (eDNA metabarcoding)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve taxonomic protocols; arrange external QC
Trained Personnel	Process samples, identify and enumerate algae
New Students	Complete training before independent identification

4. Definitions

- **Acid washing (plant tissue):** Cleaning tissue with dilute HCl to remove surface contamination (dust, soil particles, fertilizer residues) before analysis for trace metals or nutrients; may be inappropriate for samples requiring analysis of acid-labile compounds.
- **Air drying:** Drying plant tissue at ambient temperature (typically 20–40 °C) to a stable moisture content; used as a preliminary step before oven drying; preserves enzyme activity and RNA better than direct oven drying.
- **Ball milling:** Size reduction by grinding samples between hardened steel or ceramic balls inside a rotating vessel; the preferred method for producing a homogeneous powder from fibrous plant tissue for EA-IRMS and elemental analysis.
- **Critical tissue concentration:** The minimum concentration of a nutrient required for maximum growth; below this level the plant is deficient; above it, growth is normal or the nutrient may be toxic at very high levels.
- **Lyophilization (freeze drying):** Removal of water from tissue under vacuum after rapid freezing; preserves cell structure, volatile compounds, and biological activity better than oven drying; required for enzyme, RNA, and some metabolite analyses.

- **Subsampling:** Drawing a representative small portion from a bulk dried, ground sample for analysis; requires thorough mixing and homogenization of the bulk powder before subsampling to ensure the subsample is representative.

5. Safety and Hazards

Hazards

- **Chemical:** Lugol's iodine solution (preservative) contains potassium iodide and iodine; it is a mild irritant and stains skin and clothing. Nitric acid (used in diatom cleaning) is corrosive; handle in the fume hood. The mounting medium Naphrax contains toluene, which is flammable and toxic by inhalation.
- **Physical:** Hot plates used for diatom cleaning and slide preparation pose burn risk. Coverslips and slides are fragile.
- **Biological:** Stream samples may contain pathogens. Wear gloves.

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ Fume hood (required for nitric acid cleaning and Naphrax mounting)

5.2. Emergency Procedures

For acid splashes, flush immediately with copious water for at least 15 minutes. For Naphrax/toluene spills, ventilate and absorb. Refer to [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Compound microscope (brightfield)	400x and 1000x (oil immersion) for diatoms
Inverted microscope (optional)	For soft-bodied algae in settling chambers
Palmer-Maloney counting cell	For soft-bodied algae enumeration
Hot plate	For diatom acid cleaning
Centrifuge	For concentrating cleaned diatom material
Slide warmer	For drying diatom mounts

6.2. Consumables

Item	Notes
Lugol's iodine solution	Preservative for soft-bodied algae
Concentrated HNO ₃ (trace metal grade)	For diatom frustule cleaning
30% H ₂ O ₂	Alternative oxidizer for cleaning
Naphrax mounting medium	High refractive index (n = 1.73); for permanent diatom slides
Coverslips (22 x 22 mm, No. 1)	
Glass microscope slides	
Immersion oil	

7. Procedure

7.1. Sample Processing Overview

Periphyton samples are processed in two parallel workflows:

1. **Soft-bodied algae:** Examined alive or preserved in Lugol's solution using a Palmer-Maloney cell or settling chamber at 400x magnification.
2. **Diatoms:** Cleaned with acid or peroxide to remove organic matter, mounted in Naphrax on permanent slides, and identified at 1000x (oil immersion).

7.2. Soft-Bodied Algae Identification

1. Homogenize the preserved sample by gently inverting the jar 20 times.
2. Pipette a known volume (typically 0.1 mL) into a Palmer-Maloney counting cell.
3. Allow cells to settle for 5 minutes.
4. Scan the entire cell at 400x magnification.
5. Identify and count a minimum of 300 natural units (cells, colonies, or filament lengths of 10 μm). A natural unit is the smallest entity that can be identified: a single cell for unicellular taxa, a colony for colonial taxa, or a 10 μm filament segment for filamentous taxa (Stevenson & Bahls, 1996).
6. Record counts by taxon. Identify to genus where possible; use higher-level groupings (family or order) for taxa that cannot be resolved without specialized preparation.
7. Calculate cell density:

$$\text{Cells/cm}^2 = \frac{N \times V_{\text{sample}}}{V_{\text{counted}} \times A_{\text{substrate}}}$$

where N = number of cells counted, V_{sample} = total sample volume (mL), V_{counted} = volume examined in the counting cell (mL), and $A_{\text{substrate}}$ = total area of substrate scraped in the field (cm^2).

7.3. Diatom Cleaning

1. Transfer approximately 5 mL of well-mixed sample to a labeled glass beaker or test tube.
2. In the fume hood, add an equal volume of concentrated HNO_3 (or 30% H_2O_2 as a less hazardous alternative).
3. Heat on the hot plate at low-to-medium heat (approximately 80 °C) until the sample clears and all organic material is digested (30–60 minutes). Do not boil.
4. Allow to cool. Centrifuge at 1500 x g for 5 minutes and decant the supernatant.
5. Wash the pellet with DI water, centrifuge, and decant. Repeat the wash 3–5 times until the pH of the supernatant is near neutral.
6. Resuspend the cleaned frustules in a small volume of DI water (approximately 1–2 mL).

7.4. Diatom Slide Preparation

1. Pipette a drop of cleaned diatom suspension onto a clean coverslip. Allow to air-dry completely on the slide warmer.
2. Place a drop of Naphrax on a labeled glass slide (work in the fume hood; Naphrax contains toluene).
3. Invert the coverslip (diatom side down) onto the Naphrax drop.
4. Heat gently on the hot plate or slide warmer to drive off the toluene until no more bubbles appear.
5. Allow to cool. The slide is now a permanent mount.

7.5. Diatom Identification and Enumeration

1. Examine the slide at 1000x under oil immersion.
2. Count a minimum of 600 diatom valves, traversing random transects across the coverslip (Charles et al., 2002).
3. Identify each valve to the lowest practical taxonomic level (species where possible) using regional diatom floras and SWAMP reference keys (Krammer, 2000; Spaulding et al., 2021).
4. Record counts by taxon.

7.6. Data Entry and Metric Calculation

1. Enter soft-bodied and diatom counts into the standardized data entry spreadsheet.
2. Calculate summary metrics: total taxa richness, relative abundance of indicator taxa (e.g., pollution-tolerant vs. sensitive diatoms), and community-level indices.
3. If submitting to SWAMP/CEDEN, format data per SWAMP requirements.

8. Quality Control

- **Soft-bodied algae:** Re-count 10% of samples. Counts should agree within 15% RPD for dominant taxa.
- **Diatoms:** Have a second identifier verify 10% of slides. Taxonomic agreement should be greater than or equal to 85% at the species level.

- For SWAMP submissions, submit 10% of diatom slides to a SWAMP-certified taxonomist for external QC.
- Include a reference slide set in the laboratory for training and calibration.

9. Waste Disposal

Waste Type	Disposal Method
Nitric acid waste	Neutralize and collect in acid waste; EH&S pickup
H ₂ O ₂ waste	Dilute and drain with copious water
Naphrax / toluene waste	Collect in organic waste; EH&S pickup
Lugol's iodine waste	Collect in designated container; EH&S pickup
Used slides and coverslips	Standard sharps/glass waste

10. References

- Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and Wadeable rivers: Periphyton, benthic macroinvertebrates, and fish* (2nd ed.). U.S. Environmental Protection Agency, Office of Water. <https://www.epa.gov/cwa-methods/rapid-bioassessment>
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- Ode, P. R., Fetscher, A. E., & Busse, L. B. (2016). *Standard operating procedures for the collection of field data for bioassessments of California Wadeable streams: Benthic macroinvertebrates, algae, and physical habitat*. California State Water Resources Control Board, Surface Water Ambient Monitoring Program. https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html
- Spaulding, S. A., Potapova, M., Bishop, I. W., Lee, S. S., Gasperak, T. S., & Graeff, C. L. (2021). *Diatoms of North America: The freshwater flora*. <https://diatoms.org/>
- Stevenson, R. J., & Bahls, L. L. (1996). An assessment of the environmental and aesthetic quality of streams using diatom and soft algae assemblages. In R. J. Stevenson, M. L. Bothwell, & R. L. Lowe (Eds.), *Algal ecology: Freshwater benthic ecosystems* (pp. 493–525). Academic Press.

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials Handling	Acid and solvent safety
L310	Chlorophyll Extraction	Complementary biomass metric
L601	Wet Mount for Microscopy	General microscopy technique
F610	BMI Sampling	Co-collected bioassessment data

12. Training Record

Before identifying and enumerating algae independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) and [SOP-L003 Hazardous Materials Handling](#) training
3. Review the SDS for nitric acid and Naphrax
4. Study the diatom and soft-bodied algae reference collections and identification keys
5. Complete a supervised identification exercise (minimum 100 diatom valves and 50 soft-bodied algae natural units) with greater than 80% agreement with reference identifications
6. Demonstrate safe acid cleaning and Naphrax mounting under supervision
7. Be signed off by the PI or lab manager