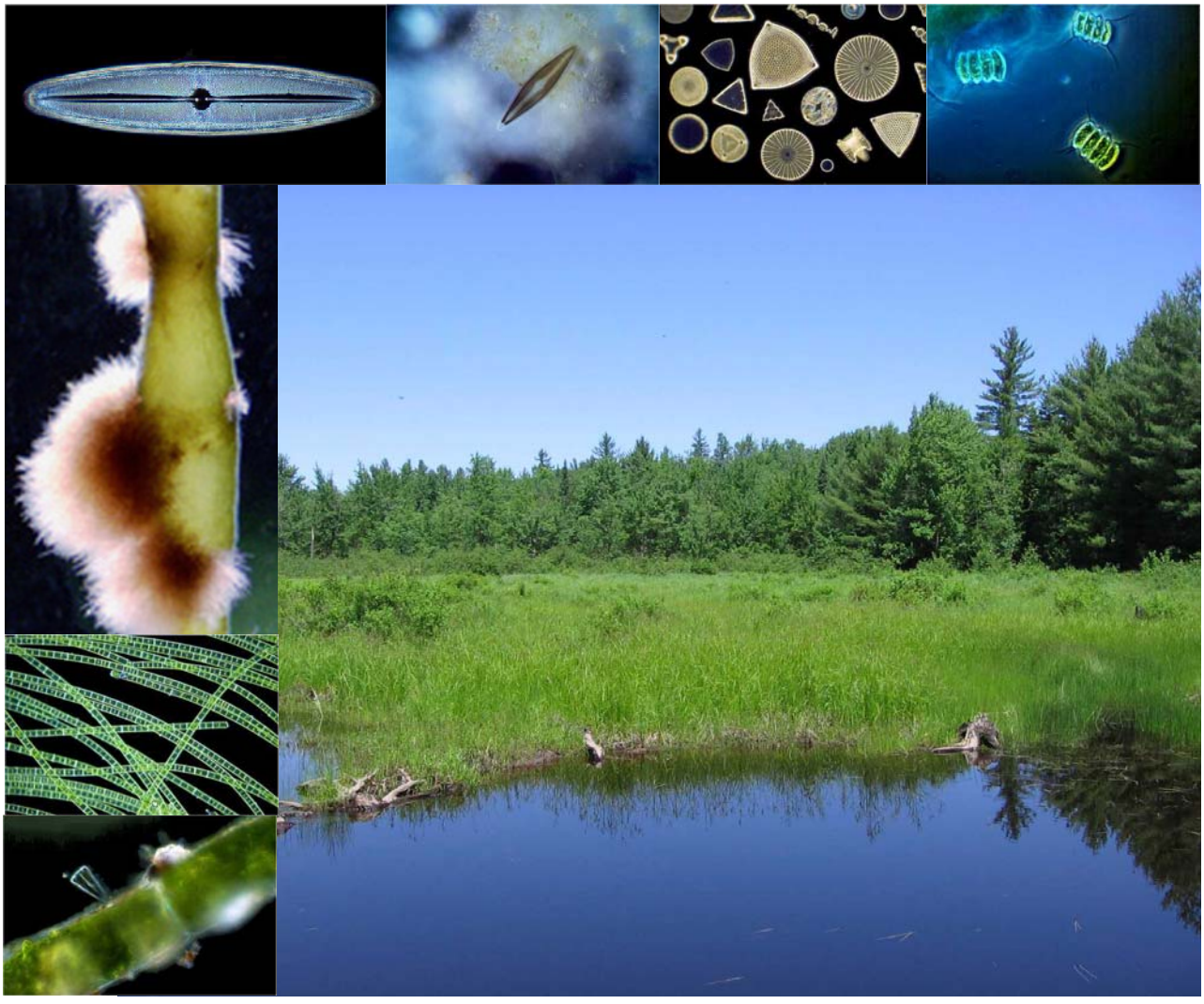
Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands



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Bureau of Land and Water Quality
Division of Environmental Assessment
Biomonitoring Program

Standard Operating Procedure
Methods for Sampling Stream and Wetland Algae

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Cover photographs – wetland by Jeanne DiFranco and algae by Micrographia

1. **Applicability.** This standard operating procedure (SOP) applies to the collection of benthic algae and phytoplankton from rivers, streams, and freshwater wetlands in Maine. This SOP describes the collection of (1) qualitative biomass data using a viewing bucket survey, (2) quantitative biomass and species composition data using artificial and natural substrates, and (3) quantitative phytoplankton data.

2. **Purpose.** The purpose of this SOP is to provide standardized methods for collecting algae from rivers, streams, and freshwater wetlands in Maine.

3. **Definitions.**

   A. Algae – algae included in analysis include the following groups:
      (1) Cyanophyta – blue-green algae, cyanobacteria
      (2) Chlorophyta – green algae
      (3) Bacillariophyta – diatoms
      (4) Rhodophyta – red algae
      (5) Chrysophyta – chrysophytes, chrysomonads
      (6) Tribophyceae – yellow-green algae
      (7) Euglenophyta – euglenoids
      (8) Pyrrophyta – dinoflagellates
      (9) Cryptophyta – cryptomonads
   B. Macroalga – algae that form macroscopic or plantlike morphologies with a thallus structure that is recognizable with the naked eye (Wehr and Sheath 2003).
   C. Microalga – unicellular algae or colonies that are microscopic.
   D. Benthic Algae – microalgae and macroalgae that grow on the bottom substrate of a waterbody (*e.g.*, rocks, logs, mud).
   E. Periphyton – microscopic algae, bacteria, and fungi that grows on the bottom substrate (*e.g.*, rocks, logs) of a stream or river. Does not include macroscopic algae, such as *Cladophora*, *Spirogyra*, *Chara*, and *Vaucheria* (Stevenson et al. 1996).
   F. Growth habits – several terms are used to describe the microhabitats provided by different substrates (Stevenson et al. 1996).
      (1) Epilithic algae grow on hard relatively inert substrata, such as gravel, pebble, cobble, and boulder, that are bigger than most algae.
      (2) Epiphytic algae grow on plants and larger algae, which provide relatively firm substrata that are bigger than the epiphytic algae, but can be highly active metabolically and a great source of nutrients.
      (3) Epipsammic algae grow on sand, which is hard, relatively inert, and has relatively little surface area. Few algae live in sand among sand grains, because the sand is too unstable and may crush them.
      (4) Epipelic algae grow on inorganic or organic sediments that are smaller than most unicellular algae. Epipelic algae are typically large motile diatoms, motile filamentous blue-green algae, or larger motile flagellates like *Euglena*.
      (5) Phytoplankton – microscopic algae that are suspended in the water column.

G. HETL – Health and Ecological Testing Laboratory, Augusta, ME

4. **Responsibilities**.

A. The Stream Algae Program Manager and has the following responsibilities associated with this SOP:
(1) Manage contract with algal taxonomist and deliver samples to taxonomist.
(2) Purchase and maintain supplies and field equipment.
(3) Update SOP.
(4) Coordinate with the Rivers Unit, Division of Watershed Management, and other partners during selection of sampling locations and scheduling of field teams.
(5) Coordinate and provide training opportunities for field teams.
(6) Participate as a member of a field team.
(7) Coordinate wetland algae sampling with Wetlands Program Manager.

5. **Guidelines and Procedures**.

A. SAMPLING PERIOD
(1) Sampling of stream algae should occur between June 15 and July 31 unless there are extenuating circumstances (*e.g.*, prolonged high flows). The sampling window may be extended in the northern part of the state when appropriate. This period was selected for the following reasons:
(a) This is roughly centered on the longest day of the year.
(b) Stream and river flow should no longer be influenced by spring snowmelt.
(c) Appears to be period of peak algal growth in many streams before the algal mats begin to senesce.
(2) Sampling of fresh water wetland algae should occur during June and early July. This period was selected for the following reasons:
(a) Wetlands are less likely to dry down during this period compared with later in the summer.
(b) Overlap with stream algae and stream macroinvertebrate sampling is minimized.

B. SUPPLIES
(1) Tackle box.
(a) permanent marker
(b) pencils
(c) knife or scissors for cutting rope
(d) razor blades or utility knife for scraping algae off of microscope slides
(e) garden shears for clipping plant stems
(f) flat-head screwdriver
(g) miscellaneous supplies
(h) cooler with ice
(i) bottle of bottled water
(j) squirt bottle with bottled water

(k) pack baskets

(2) Water samples
- (a) water quality kits from HETL, which include bottles for all or a subset of the following: Total P, Orthophosphate, soluble reactive Phosphorus (PO_4); Total Kjeldahl-N, Nitrate/Nitrite-N, Ammonia-Nitrogen; Sulfate; Dissolved Organic Carbon; Chlorophyll *a*; Total Suspended Solids; Chloride; Cadmium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Nickel, Zinc, Calcium, Potassium, Sodium, Silica; Alkalinity (as $CaCO_3$); pH; Specific Conductance; and True Color.
- (b) HETL chain of custody sheets.

(3) Periphytometer.
- (a) periphytometer
- (b) microscope slides
- (c) lightweight nylon rope
- (d) rebar (not always used)
  1. approximately 3ft long
  2. 1 per periphytometer
- (e) mallet

(4) Natural substrate sample.
- (a) toothbrushes
- (b) metal chemistry tool for scraping rocks
- (c) large white sample trays
- (d) Bottle of M3 preservative (Table 1)
- (e) Pipette and bulb for measuring M3
- (f) 250ml beaker
- (g) widemouth, brown nalgene bottles (125ml or 250ml)
- (h) garden shears
- (i) whirl-paks
- (j) 12 inch ruler

Table 1: M3 Preservative

- 5 g Potassium Iodide
- 10 g Iodine (optional)
- 50ml glacial acetic acid
- 250ml formalin
- Bring up to 1 liter with distilled water
- Add 1 ml per 50ml sample

(5) Rapid periphyton survey.
- (a) viewing bucket
- (b) 6 inch ruler marked with millimeters and has markings at 5mm and 2cm with permanent marker
- (c) meter stick

(6) Field sheets (Appendix 2).
- (a) *EPA Physical Characterization/Water Quality Field Data Sheet* (Barbour *et al*. 1999)
- (b) *EPA Habitat Assessment Field Data Sheet – High Gradient* (Barbour *et al*. 1999)
- (c) *EPA Habitat Assessment Field Data Sheet – Low Gradient* (Barbour *et al*. 1999)
- (d) *ME DEP Stream Algae Field Data Sheet*
- (e) *ME DEP Qualitative Benthic Algae Survey Data Sheet*
- (f) *ME DEP Epiphytic Algae Data Sheet*

(7) Electronic equipment and accompanying SOPs
- (a) digital camera and diskettes
- (b) Global® stream velocity meter

(c) Hanna® pH/conductivity/TDS meter
(d) Hanna® dissolved oxygen meter

C. SITE VISIT - STREAMS

(1) Identifying Stream Reach.
(a) If possible, sample locations should be scouted out ahead of time to identify appropriate reaches and to determine what kinds of substrate are available.
(b) Sample reaches should ideally have the following characteristics. Streams that do not have these characteristics can still be sampled at the discretion of the project manager.
1. Located in areas of open or partly open canopies (>50%).
2. Located in areas of riffles and runs, not pools. Runs are preferred.
3. Located in areas with moderate water velocity (between 10 and 60 cm/sec). Try to avoid areas with little or excessive water velocity.
(c) Rocky substrates are preferred over soft substrates. However, we do not currently have methods appropriate for sampling ledge. If rocks are not available, then periphytometers could be deployed and the slides and alternative natural substrate should be sampled on a second visit. Substrates should be selected in the following order or preference:
1. Rocks (Section F)
2. Branches/Logs (Section G.2)
3. Epiphytes (Section G.3)
4. Mud/Sand (Section G.4)

(2) Natural Substrate Only – The following activities must be completed.

Complete During Only Visit
EPA Habitat Assessment Field Data Sheet – High Gradient **or** *EPA Habitat Assessment Field Data Sheet – Low Gradient*
ME DEP Stream Algae Data Sheet
water grab samples
flow measurement
pH, conductivity, temperature, and dissolved oxygen readings
Viewing Bucket Survey (Section E)
Natural Substrate Sampling (Section G & H)

(3) Artificial substrate only **or** both natural and artificial substrates sampled

Complete During First Visit	Complete During Second Visit
ME DEP Stream Algae Data Sheet parts related to first visit	*ME DEP Stream Algae Data Sheet* parts related to second visit
EPA Habitat Assessment Field Data Sheet – High Gradient **or** *EPA Habitat Assessment Field Data Sheet – Low Gradient*	water grab samples (Section D)
flow measurement	flow measurement
pH, conductivity, and DO readings	pH, conductivity, and DO readings
	Viewing Bucket Survey (Section D)
	Artificial Substrate Sampling (Section E)
	Natural Substrate Sampling (Section F & G)

D. SITE VISIT – WETLANDS

(1) For wetlands, where rocks are not readily available, substrates should be selected in the following order or preference:
  (a) Branches/Logs (Section G.2)
  (b) Epiphytes (Section G.3)
  (c) Mud/Sand (Section G.4)

(2) Natural Substrate Only – The following activities must be completed.

Complete During Only Visit
ME DEP Wetland Bioassessment Field Data Form
water grab samples
pH, conductivity, and DO readings
Natural Substrate Sampling (Section G & H)

E. VIEWING BUCKET SURVEY (Streams only)

(1) Fill in top of *ME DEP Qualitative Benthic Algae Survey Data Sheet*

(2) Establish transects across the habitat being sampled (preferably riffles or runs in the reach in which benthic algal accumulation is readily observed and characterized).
  (a) Normal Situation – identify 3 transects perpendicular to the flow through the designated reach and then haphazardly select 3 locations along each transect with one near the right bank, one near the middle, and one near the left bank.

(b) Narrow Stream – identify 3 transects diagonally across the reach and then select 3 locations along each transect OR walk upstream and select at least 3 locations through the reach.
(c) The transects should be equally spaced within the reach unless channel morphology makes it necessary to adjust the distance between transects.
(d) The transects should not overlap transects for sampling natural substrates.

(3) Have one person (viewer) conduct survey and one person (recorder) record data (Figure 1).
(4) At each location, record the transect and sample number (*e.g.*, 1-1, 1-2, 1-3, 2-1, 2-2, 2-3, 3-1, 3-2, or 3-3)
(5) At a location, the viewer should immerse the viewing bucket in the water (Figure 2).
  (a) 35 dots
  (b) 4 cm between dots
(6) While viewing through the bucket, identify points on the stream bottom below the upper left dot and the lower right dot to help keep the bucket in the same area.
(7) To minimize glare, it is sometimes helpful to put a little water inside the viewing bucket.
(8) Measure the longest filament of algae. If you can identify the filamentous algae, record the names of the taxa on the field sheet.
(9) Start with the upper left dot and systematically proceed by observing the algal growth below each dot in the top row. Then proceed row by row to the bottom row.
(10) At each dot, the viewer should call out one of the following to characterize the algal growth below the dot. The viewer should use the 6-inch ruler to distinguish categories 2-5.

Figure 1: Using viewing bucket.

Figure 2: Viewing bucket for qualitative benthic algae

  (a) **unsuitable** – unconsolidated substrate such as sand or mud
  (b) **plant –** an aquatic plant
  (c) **moss –** a moss≤
  (d) **Crust –** a crust-forming algae (may be black, red, or green)
  (e) **Macro 1 -** a filament or other macroalga that is between 1 and 5 cm long
  (f) **Macro 2** – a filament or other macroalga that is ≥ 5cm and < 15 cm long
  (g) **Macro 3 –** a filament or other macroalga that is ≥ 15 cm long
  (h) **0** – substrate rough or slightly slimy with no visible algae
  (i) **1** – a thin layer of algae is visually evident, underlying rock is still visible.
  (j) **2** – periphyton mat from 0.5-1 mm thick is evident, underlying rock is covered and can no longer be seen
  (k) **3** –periphyton mat between 1-5 mm thick is evident

(l) **4** – periphyton mat between 5 mm – 2 cm thick is evident
(m) **5** – periphyton mat > 2 cm thick is evident

(11) If there is a mixture of decomposing filaments, microalgae, and silt, then treat it as being periphyton mat and not a filament.
(12) The recorder should use hash marks to record the observations on the field sheet in the appropriate boxes.
(13) Before the viewer moves the viewing bucket, the recorder must add up the number of hash marks for each category and write down the number in the little boxes in the lower right corner of each rectangle. (See ME DEP Viewing Bucket Survey Data Sheet, Appendix 2)
(14) The recorder must add up the number of hash marks in the row to make sure that there were 35 observations. If not, then the viewer should make additional observations or subtract the most recent observations to get a total of 35 dots.

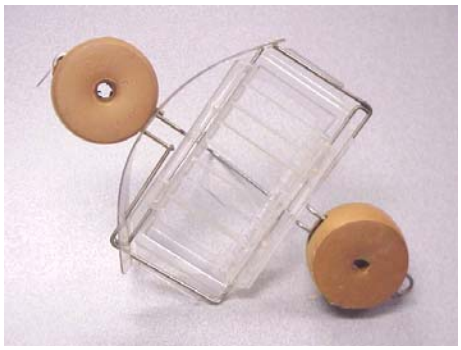

Figure 3: Wildco® 16-slide Periphytometer

F. ARTIFICIAL SUBSTRATE SAMPLING (Streams only)

(1) Periphytometers should be deployed for 14 days ! 2 days.
(2) Maine DEP uses two types of periphytometers
  (a) Wildco® Periphytometer (Figure 3)
    1. They hold 8 standard microscope slides.
    2. They have two sliding plastic pieces that lock slides in place (Figure 4).
  (b) Durasampler® Periphytometer (Figure 5).
    1. They hold 20 standard microscope slides.
    2. Only 8 standard microscope slides should be installed in the sampler.
    3. Place slides in the slots marked with red dots (slots 2,4,6,8,10, 11, 13, 15, 17, & 19).

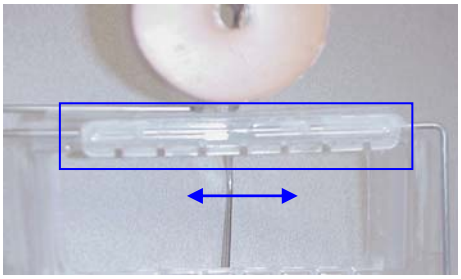

Figure 4: Movable plastic piece that locks slides in place.

(3) Microscope slides
  (a) Use standard, non-frosted microscope slides.
  (b) Use new slides. If new slides are not available follow protocols in Section J for cleaning slides.
  (c) Only hold the edges; avoid touching the slide surfaces as it can effect colonization.
(4) Placement of samplers in the field
  (a) Sunlight
    1. Maine DEP standardizes sampling by putting samplers in areas with minimal canopy cover.
    2. If possible, samplers should receive sunlight for all or most of the day.
  (b) Flow

1. Periphytometers should be placed in areas with at least some visible flow.
2. Avoid putting periphytometers in backwaters or eddies.
3. Avoid putting periphytometers in excessively turbulent eddies that might limit algal colonization.

(c) Installation

1. Periphytometers should be secured with lightweight nylon rope to a metal ring or metal part of sampler.
2. The periphytometers should be secured so that the slides are parallel to stream flow (Figures 5 & 6).
3. The length of the rope will vary depending on stream flow, but the rope should be long enough to allow the periphytometer to sway slightly in the current, but short enough so the periphytometer does not drift into eddies or slow sections along the bank.
4. The other end of the rope should be securely tied to a boulder, log, woody vegetation, or piece of rebar that has been hammered into the substrate deep enough to prevent it from coming lose during high flows.

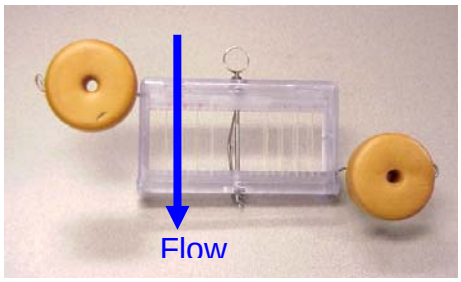

Figure 5: Durasampler® 40-slide Periphytometer

(d) Retrieving samplers and processing slides.

1. Care should be taken to avoid touching the flat sides of the microscope slides. Handle the slides by holding the edges.
2. Pick up periphytometer by holding the edges.
3. Slide the two plastic pieces (Figure 4) so the microscope slides can be removed.
4. Grasp slides along the edges and remove them from the periphytometer. Be careful to avoid disturbing the surfaces of the slides or other slides in the periphytometer.

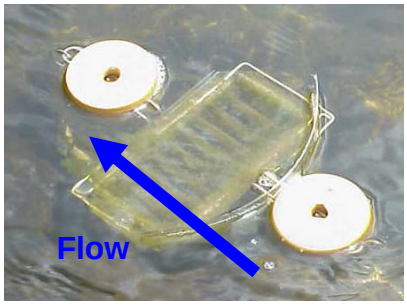

Figure 6: Direction of flow for Wildco® Periphytometer

5. Chl *a* slides
   i. Place 1 slide (3rd from the left) into a whirl-pak with some bottled water.
   ii. Using a permanent marker, write down the date, stream name, town, sample location, Chl *a*, and number of slides on the whirl-pak.
   iii. Place the sealed whirl-pak into a cooler and bring back to the lab for Chl *a* filtering (Section I.1).
   iv. Record the number of slides collected for Chl *a* on the field sheet.
6. Processing periphytometer slides for taxonomic analysis.
   i. Carefully pour slides and water into a graduated beaker.
   ii. Using a razor blade or utility knife, carefully scrape the other 7 microscope slides. Scrape only the flat surface, not the edges.

iii. Using a squirt bottle filled with bottled water, squirt the razor blade and slides and collect the sample into the graduated beaker.
iv. Add bottled water until there is a multiple of 50ml (*e.g.*, 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
v. Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 125ml or 250ml in size).
vi. Record the number of slides scraped for taxonomic analysis
vii. Record surface area:
- 1 slide (both sides) = 17.25cm^2.
- 7 slides (both sides of each) = 241.5cm^2

viii. Label the bottle with the following information:
- date
- bottle number
- stream name
- town
- location
- type of sample (species)
- type of sample (slides)
- number of slides (*e.g.*, 7) and sides (*e.g.*, 14)
- volume of sample

ix. Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).
x. Carefully clean razor blade/utility knife and beaker.

G. NATURAL SUBSTRATE SAMPLING – ROCKY SUBSTRATE (streams only)

(1) Sampling will focus on Epilithic algae.
(2) Fill in *ME DEP Stream Algae Data Sheet*
(3) Clean sample trays, brushes, and other equipment with tap or stream water.
(4) Establish transects through riffles or runs
  (a) If possible, 18 rocks must be collected from the reach
  (b) Normal Situation – identify 6 transects perpendicular to the flow through the designated reach and then select 3 locations along each transect (*e.g.*, stratified random locations on right bank, middle, and left bank).
  (c) Wide River – identify 3 transects perpendicular to the flow through the designated reach and then select 6 locations along each transect.
  (d) Narrow Stream – identify 6 transects diagonally across the reach and then select 3 locations along each transect OR walk upstream and select 18 locations through the reach.
(5) At each location, collect a cobble or boulder-sized rock for a total of 18 rocks.
(6) Back at the stream bank, store the rocks in a large, white sample tray.

(7) Pick up a rock and hold it over a second sample tray that is clean.
(8) Place rubber sampling device (Figures 7 & 8) over the top of the rock and hold firmly in place to define surface area to be sampled. Alternatively, the neoprene washer with 1" diameter hole can be used by itself.
  (a) Sampler is constructed by cutting a segment of mountain bike inner tube lengthwise and uncurling.
  (b) Epoxy glue a neoprene washer with a 1" diameter hole to the outer surface of tubing.
  (c) After the glue dries, flip the sampler over and cut away the tubing within the 1" circle. Cutting from the back reduces strain on the epoxy glue.
(9) Brush the area within the circle vigorously with a stiff bristled brush while holding rock over collection pan (note, you may need to scrape the area with a metal scraping tool first if the algae is very thick) (Figure 9).
(10) Rinse tools and sample area on rock with a squirt bottle filled with bottled water and collect sample in the large, white sample tray. The collector must hold the rock upside down and spray upward to minimize the chance of washing off algae from another part of the rock.
(11) The goal is to collect all algae from within the circles and none of the algae from outside of the circles.
(12) Repeat process for other rocks and composite all rock-scrapings into a graduated beaker. (rinse the tray and equipment to ensure all algae are in the beaker).
(13) Add bottled water until there is a multiple of 50ml (*e.g.*, 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
(14) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).
(15) Record the number of rocks scraped for taxonomic analysis.
(16) Record surface area:
  (a) 1" circle = 5.067 cm^2
  (b) 18 circles = 91.027 cm^2
(17) Label the bottle with the following information:
  - date

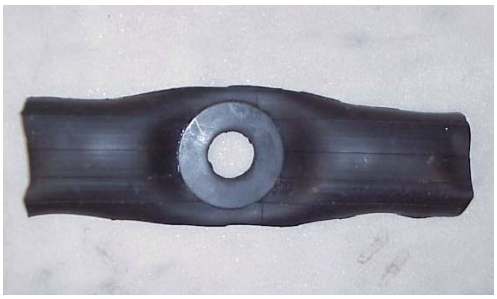

Figure 7: Natural substrate sampling device.

Figure 8: Wrapping sampling device around cobble

Figure 9: Collecting algae from cobble.

- bottle number
- stream name
- town
- location
- type of sample (species)
- type of sample (rocks)
- number of rocks (*e.g.*, 18)
- volume of sample

(18) Thoroughly clean all equipment, especially brush bristles, in water before leaving stream. Discard brushes if they get too grimy or difficult to clean.

(19) Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

H. NATURAL SUBSTRATE SAMPLING – SOFT BOTTOM (streams and wetlands)

(1) There are several options for sampling soft bottom streams, including the following methods listed in order of preference.
   (a) Epilithic algae from log scrapings.
   (b) Epiphytic algae from plant clippings.
   (c) Epipsammic and Epipelic algae from soft substrate.

(2) Epilithic algae from log scrapings.
   (a) Fill in *ME DEP Stream Algae Data Sheet*
   (b) Clean large, white sample trays, toothbrushes, and metal scraping tools.
   (c) Find logs or branches within the reach that can be lifted from the water.
   (d) Using the following methods, collect up to 18 log scrapings
      1. Pick up a log/branch and hold it over a large, white sample tray.
      2. Place rubber sampling device (Figure 7) over the log/branch and hold firmly in place to define surface area to be sampled.
      3. Brush the area within the circle vigorously with a toothbrush and wash down brush and log/branch with a squeeze bottle into a collection pan (note, you may need to scrape the area with a metal scraping tool first if the algae is very thick)
      4. Rinse tools and sample area on log/branch with a squirt bottle filled with bottled water and collect sample in the large, white sample tray.
      5. Repeat process for other logs/branches or other parts of long logs/branches and composite all scrapings into a graduated beaker. (rinse the tray and equipment to ensure all algae are in the beaker).
   (e) Add bottled water until there is a multiple of 50ml (*e.g.*, 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
   (f) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).

(g) Record the number of logs/branches scraped for taxonomic analysis.
(h) Record surface area:
1. 1" circle = 5.067 cm^2
2. 18 circles = 91.027 cm^2
(i) Label the bottle with the following information:
- date
- bottle number
- stream name
- town
- location
- type of sample (log/branch)
- number of logs/branches (*e.g.*, 18)
- volume of sample

(j) Thoroughly clean all equipment, especially brush bristles, in water before leaving stream. Discard brushes if they get too grimy or difficult to clean.
(k) Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

(3) Epiphytic algae from plant clippings.
(a) Fill in *ME DEP Stream Algae Data Sheet* and/or the *ME DEP Epiphytic Algae Data Sheet*
(b) Clean scissors and large, white sample trays.
(c) Identify a stream reach
1. Identify a stream reach that is 10x the stream width or 20 m in length, whichever is less.
2. Locate the stream reach in an area with runs and pools.
(d) Select 3 locations in runs within the reach that have emergent vegetation (*e.g.*, cattails, sedges).
(e) In wetlands, do not define reach, just select 3 areas with emergent vegetation.
(f) At each location, select plants that have at least 10cm underwater.
(g) Clip plant stems near their base or at least 10cm underwater and trim off any parts that are above water.
1. If plants are thin (*e.g.*, 1 mm across - Soft Rush (*Juncus effusus*)), then clip 5 stems per location.
2. If plants are thick (*e.g.*, >5 mm across - Cattail (*Typha* sp.)), then clip 2 stems per location.
3. If plants have intermediate thickness, then clip 3 or 4 stems per location.
(h) Trim stems to approximately 10 – 15cm in length.
(i) Place the stems into a whirl-pak, add a little bottled water, remove most of the air within the bag, and seal the whirl-pak.
(j) Massage the plant stems to remove epiphytic algae.
(k) Rinse each stem with bottled water as remove from whirl-pak

(l) Pour the contents into a graduated beaker and store set aside the cleaned stems for measurement.
(m) Add bottled water until there is a multiple of 50ml (*e.g.*, 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
(n) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).
(o) Label the bottle with the following information:
- date
- bottle number
- stream or wetland name
- town
- location
- station location number
- volume of sample

(p) Estimate surface area of each clipped stem, either in the field or back at the office.
1. Complete *ME DEP Epiphytic Algae Data Sheet*
2. Make the measurements that are appropriate for the stem shapes and enter the measurements on the field sheet.
3. Calculate the surface area for each stem using the formulas provided on the field sheet.
4. Add the surface areas together and record on the field data sheet.

(q) Thoroughly clean all equipment in water before leaving stream.
(r) Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

(4) Epipsammic and Epipelic algae from soft substrate.
(a) This method is appropriate for mucky bottom streams and wetlands. This should not be used with sandy bottom streams. Shifting sand is unsuitable because of its small grain size and unstable nature of the substratum. Epilithic algae from log scrapings (Section H.2) or phytoplankton samples should be used as alternatives.
(b) Fill in *ME DEP Stream Algae Data Sheet*
(c) Clean petri dish, spatula, and beaker.
(d) Identify a stream reach that is 10x the stream width or 20 m in length, whichever is less.
(e) Locate the stream reach in an area with runs and pools or locate area of wetland with mucky bottom.
(f) Select 3 areas within sampling area suitable for sampling.
(g) At each location, hold a petri dish (5cm diameter) upside down and press it lightly into the substrate.
(h) Slide an unslotted spatula underneath the petri dish and carefully remove the petri dish and its core sample from the water.

(i) Composite the core samples in a graduated beaker.
(j) Add bottled water until there is a multiple of 50ml (*e.g.*, 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
(k) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).
(l) Record the number of core samples collected as well as the diameter (5cm) and depth of the petri dish.
(m) Record surface area.
1. 1 petri dish (5cm diameter) = 19.635cm^2
2. 3 replicates = 58.91 cm^2
(n) Label the bottle with the following information:
- date
- bottle number
- stream or wetland name
- town
- location
- sample location number
- type of sample (petri dish core samples)
- number of core samples
- volume of sample

(o) Thoroughly clean all equipment in water before leaving stream.
(p) Add 1ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

I. PHYTOPLANKTON SAMPLING

(1) This method is used for collecting a water sample that will be used to determine the presence of phytoplankton
(2) Follow the procedures in the Biomonitoring's SOP for Collecting Water Grab Samples (DEPLW0637). Use a 1 L or 500 mL bottle.
(3) Label the bottle with the following information:
- Date
- wetland name
- town
- location
- sample location number
- type of sample (phytoplankton sample)
- volume of sample

(4) Preserve the sample with 1 mL of M3 for every 50 mL of sample

J. PROCESSING SAMPLES IN LAB

(1) Chl *a* filtering from slides
- (a) Store whirl-paks containing Chl *a* slides in the refrigerator until ready to process (within 24 hrs of collection).
- (b) Pour content of whirl-pak (slides and water) into beaker.
- (c) Scrape algae off both sides of the slides with a razor blade and collect algae in beaker.
- (d) Rinse slides and razor blade with bottled water and collect water in beaker.
- (e) Using tweezers, place Chl *a* filter (0.45 microns) on to vacuum apparatus and attach container.
- (f) Pour contents of beaker into attached container.
- (g) Add 1 drops of magnesium carbonate per 50ml of sample.
- (h) Open airway under attached container and close airway on unused receptacles.
- (i) Label a glassine envelope with a pencil and include surface area scraped (lab standardizes to m^2, not cm^2):
  1. 2 sides of a slide = 0.00345 m^2
  2. 4 sides (2 slides) = 0.0069 m^2
- (j) When filtering is complete, remove attached container.
- (k) Remove filter by grabbing edge of filter with tweezers, fold filter in half, and place filter in labeled glassine envelope.
- (l) Place glassine envelope in desiccant jar in freezer.

(2) Chl *a* filtering from rock scrapings
- (a) Pour contents of whirl-pak or bottle into attached container.
- (b) Using tweezers, place Chl *a* filter (0.45 microns) on to vacuum apparatus and attach container.
- (c) Pour contents of beaker into attached container.
- (d) Add 1 drop of magnesium carbonate per 50ml of sample.
- (e) Open airway under attached container and close airway on unused receptacles.
- (f) Label a glassine envelope with a pencil and include surface area scraped (lab standardizes to m^2, not cm^2)
  1. 1 rock = 0.000507 m^2.
  2. more than one rock - - - # rocks * 0.000507 m^2.
- (g) When filtering is complete, remove attached container.
- (h) Remove filter by grabbing edge of filter with tweezers, fold filter in half, and place filter in labeled glassine envelope.
- (i) Place glassine envelope in desiccant jar in freezer.

K. EQUIPMENT MAINTENANCE

(1) Periphytometers
- (a) Scrub periphytometers with warm, soapy water prior to the field season.
- (b) Use scrubbing pads and toothbrushes to clean as many surfaces as possible.

(c) Spray the periphytometers with a dilute bleach solution.
(d) Do not rinse or dry off solution; allow periphytometers to air dry.

(2) Periphytometer slides
(a) Use new slides when possible.
(b) Scrub with warm, soapy water.
(c) Soak in acetone.
(d) Rinse in water and dry slides.
(e) Be careful to not touch the slide surfaces. Oils from fingerprints can potentially alter algal colonization.

6. References

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- Wehr, J.D. and R.G. Sheath. 2003. *Freshwater Algae of North America: Ecology and Classification*. Academic Press, Boston, MA, 918pp.

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