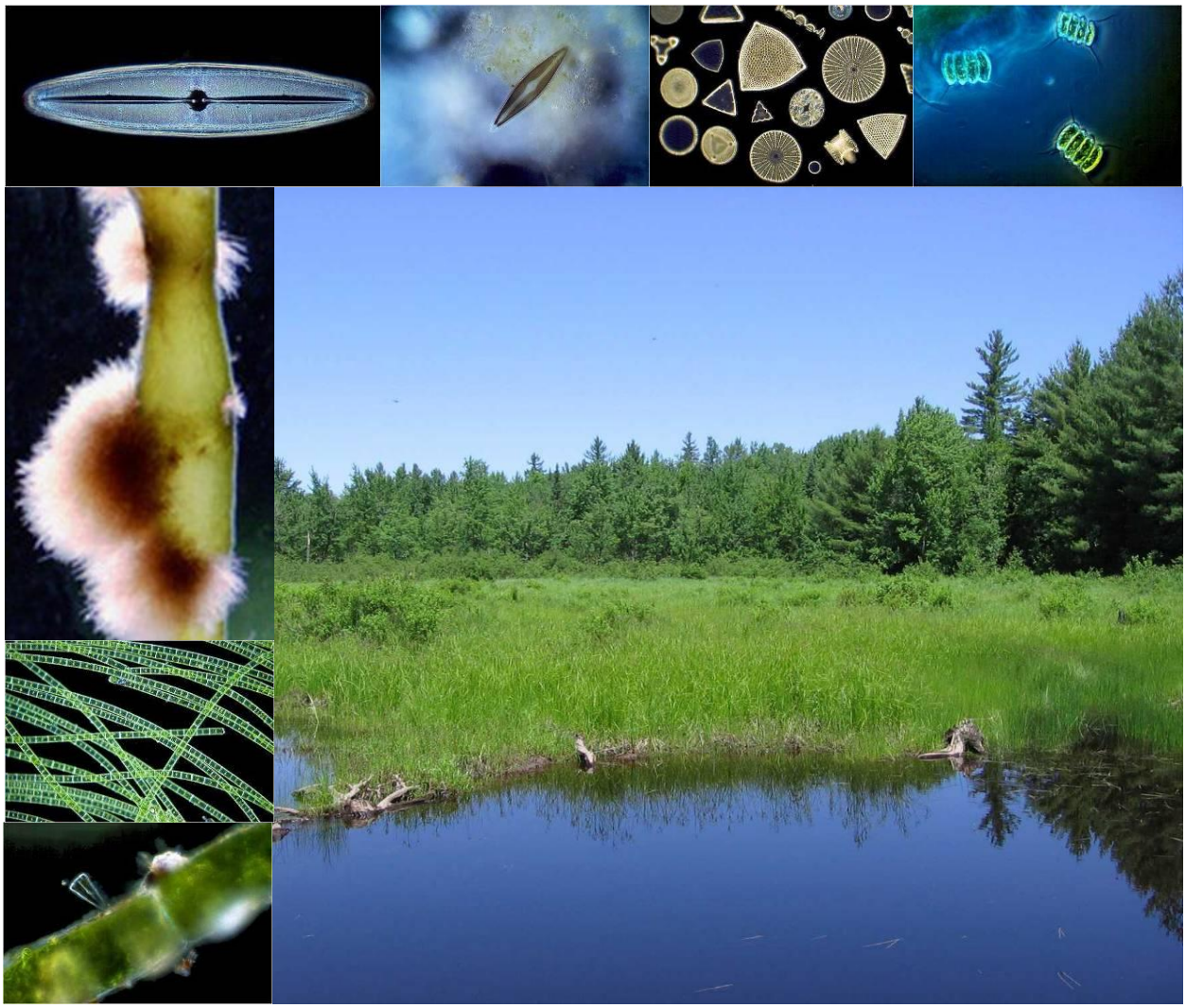
Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands



Thomas J. Danielson
Maine Department of Environmental Protection
17 State House Station, Augusta, ME, 04333
thomas.j.danielson@maine.gov

April 2014, Updated May 2019
DEPLW-0634B-2014

Bureau of Water Quality
Division of Environmental Assessment
Biomonitoring Program

Standard Operating Procedure
Methods for Sampling Stream and Wetland Algae

TABLE OF CONTENTS

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 grow on the bottom substrate (*e.g.*, rocks, logs) of a waterbody. 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.

4. **Responsibilities**.

A. The Project Manager (variable, depending on program collecting samples) has the following responsibilities associated with this SOP:
(1) Purchase and maintain supplies and field equipment
(2) Update SOP
(3) Coordinate with other DEP programs and outside partners during selection of sampling locations and scheduling of field teams
(4) Coordinate and provide training opportunities for field teams
(5) Participate as a member of a field team

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 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) Dry tackle box (stream sampling)
(a) permanent markers
(b) lab tape
(c) pencils
(d) knife or scissors for cutting rope
(e) razor blades or utility knife for scraping algae off of microscope slides
(f) garden shears for clipping plant stems (optional)
(g) flat-head screwdriver
(h) 500 mL whirl-paks
(i) plastic metric ruler
(j) spherical densitometer
(k) compass
(2) Wet tackle box (stream sampling)

(a) 3 squirt bottles
(b) 2 or more 50 mL plastic beakers
(c) 3 or more Neoprene, flexible washers with 1" diameter opening
(d) 3 or more stiff-bristled brushes (metal bristles work better)
(e) metal tool for scraping algae off of rock

(3) Pack baskets
(4) 1 gallon bottle for collecting stream water
(5) 1 gallon bottle with tap water for refilling squirt bottles
(6) 3 or more large, white plastic trays
(7) Water samples
  (a) water quality kits from laboratory performing analyses
    1. minimum desired parameters for stream samples- total Kjeldahl nitrogen (TKN), nitrate plus nitrite-N (NO3+NO2-N), total phosphorus (TP), orthophosphate-P (OPO4-P), and alkalinity (if funding allows)
    2. additional parameters for wetland sampling – Chlorophyll a, Chloride, Dissolved Organic Carbon (DOC), Dissolved Silica, True Color (if funding allows)
  (b) Chain of custody sheets
  (c) Cooler with ice
(8) 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
  (f) pipe clamp for attaching rope to rebar
  (g) screwdriver for tightening clamp
(9) Preservative
  (a) bottle of M3 preservative (Table 1)
  (b) pipette and bulb for measuring M3
  (c) disposable nitrile gloves
  (d) assortment of widemouth, brown nalgene bottles (250 mL, 500 mL, or 1 L)
  (e) pre-printed preservative labels ("sample preserved with M3")
(10) Viewing bucket survey
  (a) viewing bucket
  (b) 6 inch ruler marked with millimeters and has markings at 5 mm and 2 cm with permanent marker
  (c) meter stick
(11) 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)

Table 1: M3 Preservative

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

(c) *EPA Habitat Assessment Field Data Sheet – Low Gradient* (Barbour *et al*. 1999)
(d) *ME DEP Stream Algae Field Data Sheet*
(e) *ME DEP Viewing Bucket Survey Data Sheet*
(f) *ME DEP Epiphytic Algae Data Sheet*
(g) Maine DEP Canopy Cover Sheet

(12) Electronic equipment and accompanying SOPs
(a) digital camera
(b) stream velocity meter
(c) pH/SPC/TDS meter
(d) dissolved oxygen/temperature 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) The stream reach should be approximately 10 x stream width, up to a maximum of 30 m.
(c) Sample reaches ideally should 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 (>25% open canopy).
2. Located in areas of riffles or 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.
(d) 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. Logs or branches > 1” in diameter (Section H.2)
3. Plant stems (Section H.3)
4. Mud/Sand (Section H.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
water velocity measurement
pH, specific conductance, temperature, and dissolved oxygen readings
Viewing Bucket Survey (Section F)
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
water velocity measurement	water velocity measurement t
pH, specific conductance, temperature and DO readings	pH, specific conductance, temperature and DO readings
Viewing Bucket Survey (Section D)	Viewing Bucket Survey (Section E)
	Artificial Substrate Sampling (Section F)
	Natural Substrate Sampling (Section G & H)

D. SITE VISIT – WETLANDS

(1) For wetlands, where rocks are not readily available, substrates should be selected in the following order or preference:

(a) Plant stems (Section H.3)
(b) Logs and branches >1” in diameter (Section H.2)
(c) Mud/Sand (Section H.4)

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

Complete During Only Visit
ME DEP Wetland Bioassessment Field Data Form
ME DEP Epiphytic Algae Data Sheet
ME DEP Wetland Human Disturbance Assessment
water grab samples
pH, specific conductance, temperature and DO readings
Natural Substrate Sampling (Section H)

E. VIEWING BUCKET SURVEY (Wadeable 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 9 locations through the reach.
  (c) Large rivers – at the discretion of the Project Manager, the number of locations along each transect can be increased (e.g., doubled) for better spatial coverage
  (d) The transects should be equally spaced within the reach unless channel morphology makes it necessary to adjust the distance between transects.
  (e) 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

Figure 1. Using viewing bucket.

Figure 2. Viewing bucket for qualitative benthic algae

(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.
   (a) **Macro 1 -** a filament or other macroalga that is between 1 and 5 cm long (filaments <1 cm long are counted as part of the periphyton mat, such as Mat 2 or Mat 3)
   (b) **Macro 2** – a filament or other macroalga that is ≥ 5 cm and < 15 cm long
   (c) **Macro 3 –** a filament or other macroalga that is ≥ 15 cm long
   (d) **Mat 0** – substrate rough or slightly slimy with no visible algae
   (e) **Mat 1** – a thin layer of algae is visually evident, underlying rock is still visible.
   (f) **Mat 2** – periphyton mat from 0.5-1 mm thick is evident, underlying rock is covered and can no longer be seen (may include filamentous algae <1 cm long)
   (g) **Mat 3** –periphyton mat between 1-5 mm thick is evident
   (h) **Mat 4** – periphyton mat between 5 mm-2 cm thick is evident
   (i) **Mat 5** – periphyton mat > 2 cm thick is evident
   (j) **Sand/Clay/Mud** – unconsolidated substrate such as sand or mud
   (k) **Plant –** an aquatic plant or plant-like macroalga, such as *Batrachospermum* or *Lemmanea*
   (l) **Moss –** a moss
   (m) **Crust –** a crust-forming algae (may be black, red, or green)
   (n) **Sewage fungus –** a filamentous bacteria, such as *Sphaerotilus*. Does not include iron or manganese bacteria. Try to bring a sample back for verification.
   (o) **Sponge** – a freshwater sponge
(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 viewer-recorder team has two options for recording observations
   (a) Count the number of dots in each category and record the amounts in the appropriate boxes. This method is acceptable when the algal cover within the area of the 35 dots only has a few categories and is fairly uniform.
   (b) Use tally dots or tally lines to record each of the 35 observations in the appropriate categories. Sum the tally marks in the smaller boxes after completing the 35 dots. This method is preferable when there are many categories and the algal cover within the area of the 35 dots varies a lot.
(13) After completing the 35 observations, add the category subtotals to confirm there were 35 observations. If not, then the viewer can make additional observations or subtract

the most recent observations to get a total of 35 observations. It is acceptable to have more than 35 observations if it is not clear what to remove.

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).
(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 oil from fingerprints can affect colonization.
(4) Placement of samplers in the field
  (a) Sunlight
    1. If possible, samplers should be placed in a part of the stream reach with maximum light availability.

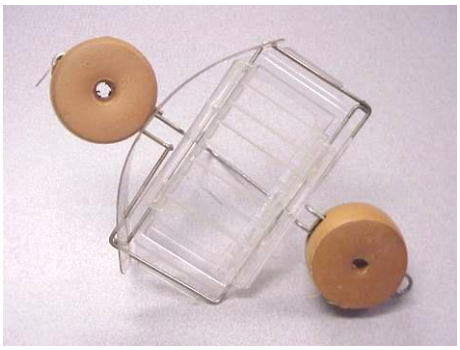


Figure 3. Wildco® 16-slide Periphytometer

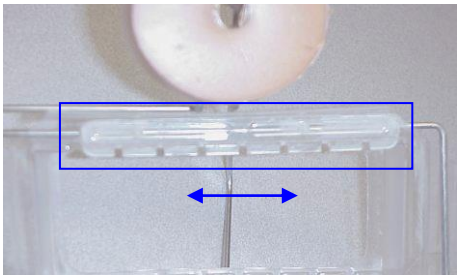


Figure 4. Movable plastic piece that locks slides in place.

  (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. One end of the nylon rope should be attached 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. A metal pipe clamp can be used to help secure the rope.

(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.
5. Chl *a* slides (optional)
   i. Place 2 slides into a whirl-pak with tap 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 remaining 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 50 mL (*e.g.*, 100 mL, 150 mL) and record the amount on the field sheets. For example, if the sample is 130 mL, then add 20 mL of bottled water. Having a multiple of 50 mL 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 250 mL or 500 mL in size).
   vi. Record the number of slides scraped for taxonomic analysis
   vii. Record surface area:

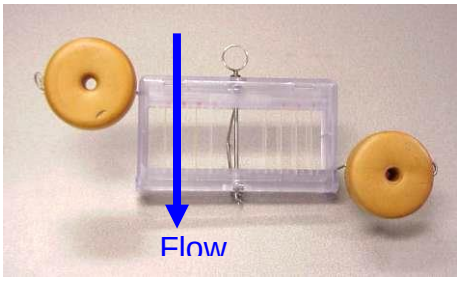


Figure 5. Durasampler® 40-slide Periphytometer

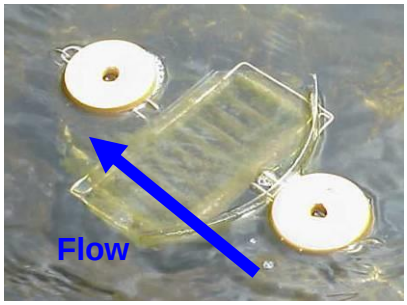


Figure 6. Direction of flow for Wildco® Periphytometer

- 1 slide (both sides) = 34.5 cm^2
- 2 slides(both sides) = 69.0 cm^2
- 6 slides (both sides) = 207.0 cm^2
- 7 slides (both sides) = 241.5 cm^2
- 8 slides (both sides) = 276 cm^2

viii. Label the bottle with the following information:

- date
- bottle number
- stream name
- town
- location
- 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 50 mL of sample in the brown bottle (refer to the field sheet to determine the amount).

x. Carefully clean razor blade/utility knife and beaker with tap water.

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 4 transects perpendicular to the flow through the designated reach and then select 4-5 locations along each transect, totaling 18 rocks.
  (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 streambank, 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 a neoprene washer with 1" diameter on the top of the rock. Alternatively, one may use a sampling device (Figures 7 & 8) over the top of the rock and hold firmly in place to define surface area to be sampled. The sampler is constructed by cutting a segment of mountain bike inner tube lengthwise and uncurling. Epoxy glue a neoprene washer with a 1" diameter hole to the outer surface of tubing. 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 50 mL (*e.g.*, 100 mL, 150 mL) and record the amount on the field sheets. For example, if the sample is 130 mL, then add 20 mL of bottled water. Having a multiple of 50 mL 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 250 mL or 500 mL in size, but sometimes 1 L).

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

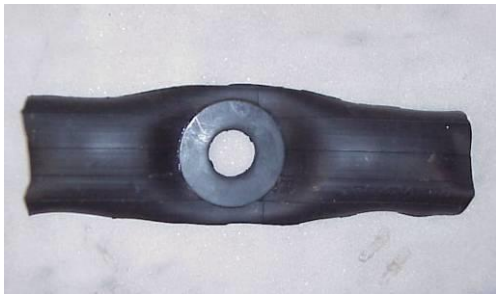


Figure 7. Natural substrate sampling device.

Figure 8. Wrapping sampling device around cobble

Figure 9. Collecting algae from cobble.

(18) Thoroughly clean all equipment, especially brush bristles, with stream and tap water before leaving stream. Discard brushes if their bristles are falling out or if the brushers are difficult to clean.
(19) Add 1 mL of M3 for each 50 mL 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 50 mL (*e.g.*, 100 mL, 150 mL) and record the amount on the field sheets. For example, if the sample is 130 mL, then add 20 mL of bottled water. Having a multiple of 50 mL 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 250 mL or 500 mL 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 50 mL 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) Select 3 locations in runs within the reach that have emergent or floating-leaved vegetation (*e.g.*, pickerel weed, water lilies, rushes, cattails,).

(c) In wetlands, do not define reach, just select 3 representative areas with emergent and/or floating leaved vegetation.

(d) At each location, select plants that have at least 10 cm underwater.

(e) Using clean garden clippers, clip plant stems near their base or at least 10 cm underwater and trim off any parts that are above water.

1. Clip 5 stems per location.
2. If plants are thick (*e.g.*, >2 cm across), then clip 3 stems per location.

(f) Place each stems into a whirl-pak and trim stems to approximately 10-15 cm in length.

(g) When all stems are collected, add a little bottled or tap water, remove most of the air within the bag, and seal the whirl-pak.

(h) Massage the plant stems to remove epiphytic algae.

(i) Rinse each stem with bottled or tap water as it is removed from the whirl-pak.

(j) Pour the whirl-pak contents into a graduated beaker and set aside the cleaned stems for measurement.

(k) Add bottled or tap water until there is a multiple of 50 mL (*e.g.*, 100 mL, 150 mL) and record the amount on the field sheets. For example, if the sample is 130 mL, then add 20 mL of bottled water. Having a multiple of 50 mL will make it easier to determine how much preservative to add.

(l) Pour the sample from the beaker into a brown, wide-mouth, Nalgene bottle (typically 250 mL or 500 mL in size).

(m) Label the bottle with the following information:

- date
- bottle number (if known at this time)
- stream or wetland name
- town

- location
- station location number
- volume of sample

(n) 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.

(o) Thoroughly rinse all equipment with water before leaving site.

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

(q) After M3 has been added, place a pre-printed preservative label ("sample preserved with M3") on the sample container.

(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) Locate the stream reach in an area with runs and pools or locate area of wetland with mucky bottom.

(e) Select 3 areas within sampling area suitable for sampling.

(f) At each location, hold a petri dish (5 cm diameter) upside down and press it lightly into the substrate.

(g) Slide an unslotted spatula underneath the petri dish and carefully remove the petri dish and its core sample from the water.

(h) Composite the core samples in a graduated beaker.

(i) Add bottled water until there is a multiple of 50 mL (*e.g.*, 100 mL, 150 mL) and record the amount on the field sheets. For example, if the sample is 130 mL, then add 20 mL of bottled water. Having a multiple of 50 mL will make it easier to determine how much preservative to add.

(j) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250 mL or 500 mL in size).

(k) Record the number of core samples collected as well as the diameter (5 cm) and depth of the petri dish.

(l) Record surface area.
1. 1 petri dish (5 cm diameter) = 19.635 cm^2
2. 3 replicates = 58.91 cm^2

(m) Label the bottle with the following information:

- date
- bottle number
- stream or wetland name
- town
- location
- station number
- type of sample (petri dish core samples)
- number of core samples
- volume of sample

(n) Thoroughly clean all equipment in water before leaving stream.

(o) Add 1mL of M3 for each 50 mL 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 Program's Protocols for Collecting Water Grab Samples in Rivers, Stream and Freshwater Wetlands (DEPLW0637A-2014). Use a 1 L or 500 mL brown, Nalgene bottle.

(3) Label the bottle with the following information:

- date
- stream or wetland name
- town
- location
- station number
- type of sample (phytoplankton sample)
- volume of sample

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

(5) After M3 has been added, place a pre-printed preservative label ("sample preserved with M3") on the sample container

J. PROCESSING SAMPLES IN LAB

(1) Chl *a* filtering from slides (alternatively, the lab could choose to extract chlorophyll pigments directly from the 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 50 mL 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
1. both sides of a 1 slide = 34.5 cm^2 or 0.00345 m^2
2. both sides of 2 slides = 69.0 cm^2 or 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 50 mL 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
1. 1 rock = 5.067 cm^2 or 0.0.000507 m^2
2. more than one rock - - - # rocks x 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) Rinse off soapy water and 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

- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- Stevenson, R.J., M.L. Bothwell, and R.L. Lowe. 1996. *Algal Ecology: Freshwater Benthic Systems*. Academic Press; Boston.
- Wehr, J.D. and R.G. Sheath. 2003. *Freshwater Algae of North America: Ecology and Classification*. Academic Press, Boston, MA, 918pp.