# Standard Operating Procedure for Collection of Benthic Algae from Natural and Artificial Substrates

**SOP-WR-W-37**

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Title: Standard Operating Procedure for Collection of Benthic Algae from Natural and Artificial Substrates
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## 1. APPLICABILITY

This SOP applies to all Office of Water Resources (OWR) staff involved in collecting benthic algae in wadeable streams from natural and artificial substrates. Exemption from the use of this SOP for project work shall be allowed for reasons of inapplicability determined by management discretion.

## 2. PURPOSE

This SOP establishes a standardized method for performing quantitative field collection of benthic algae in wadeable streams from natural and artificial substrates. It sets a consistent protocol to ensure the quality of OWR's data collection—resulting in improved uniformity, reproducibility, verifiability, and defensibility of the data, as well as increased program credibility.

## 3. DEFINITIONS

3.1 RIDEM – Rhode Island Department of Environmental Management

3.2 OWR – RIDEM Office of Water Resources

3.3 SOP – Standard Operating Procedures

3.4 Benthic algae – Micro- and macroalgae growing on the bottom of a stream or lake.

3.5 Periphytometer – A piece of equipment designed to hold glass slides for colonization of benthic algae

3.6 Artificial substrate – Any substrate not naturally occurring in streams, such as clay tiles, glass slides, trash, or human-made structures.

3.7 Natural substrate – Any substrate that naturally occurs in streams, such as logs, rocks, or aquatic vegetation

3.5 QA – Quality Assurance refers to a systematic process to ensure production of valuable, accurate, reliable, reproducible and defensible environmental data

3.6 QC – Quality Control refers to the activities performed to affirm production of valuable, accurate, reliable, reproducible and defensible environmental data

3.7 QI – Quality Improvement refers to any act or process performed to enhance the value, accuracy, reliability, reproducibility or defensibility of environmental data collected by RIDEM OWR

## 4. RESPONSIBILITIES

### 4.1 TRAINING

Any RIDEM/OWR personnel collecting benthic algae for a RIDEM project or program should have completed RIDEM's Quality System Awareness Training Program with appropriate documentation from the Quality Assurance Manager. This training ensures the field analyst recognizes the importance of proper data collection and management and he/she comprehends the significance of the environmental decisions that may be made with the data. It is suggested that field analysts have also completed the USEPA Water Quality Standards Academy Basic Course and Supplemental Topic Modules online, but it does not require any additional special training or certification.

### 4.2 RESPONSIBILITIES OF FIELD ANALYST

To properly collect benthic algae, the field analyst must be familiar with and comply with the data collection techniques stated in this SOP. The field analyst is required to read and understand this SOP. The field analyst should complete and submit any required training forms and/or field assessments for project and/or program QAPPs to document proficiency with this procedure. Any field analyst not familiar with the collection of benthic algae should be assisted by OWR staff who are accustomed to collecting benthic algae.

The field analyst is responsible for checking the required equipment in the Sampling Center at the beginning of deployment and retrieval of artificial substrates and collection from natural substrates. The field analyst is responsible for verifying that the periphytometers are in proper operating condition prior to use (i.e. floats are properly attached; glass slides not cracked and locked into place) and communicating to the project manager when equipment is in need of repair or replacement. The field analyst is also responsible for ensuring that all supplementary equipment (trays, brushes, waders, hip boots, etc.) is present and in working condition. The field analyst is responsible for cleaning and storing the field equipment before and after deployment and before winter storage.

The field analyst is also responsible for using best professional judgment to determine if site conditions are safe for performing the procedure. The field analyst is accountable for employing proper measurement procedures and data recording in accordance with this SOP.

### 4.3 RESPONSIBILITIES OF PROJECT OR PROGRAM MANAGER

The project or program manager is responsible for providing the materials, resources, and/or guidance necessary to perform the measurements in accordance with this SOP. The project manager is responsible for ensuring that the field analyst collects benthic algae correctly in accordance with this SOP and that any additional, project-specific requirements are communicated to the project team.

The project manager is responsible for ensuring the periphytometers are maintained in proper operating condition annually. This includes ensuring the floats are properly attached to the periphytometers, glass slides are cleaned and

not cracked, and the supplementary equipment is present. The project manager is also responsible for repairing the periphytometers or reordering equipment when necessary.

The project manager will determine and communicate with field analysts what procedures and the order of procedures during deployment and retrieval of artificial substrates and collection from natural substrates. The project manager will determine the dates of deployment and retrieval and communicate the schedule to the field staff. The project manager will also monitor stream gages in the area during deployment to determine the schedule for retrieval of the periphytometers. The project manager will communicate with other OWR field staff sampling the stream segment about the potential for high flows. The project manager will communicate with other OWR staff, contractors, and departments the location of deployed substrates. Further, the project manager shall ensure annual review and periodic updates to this SOP as necessary to reflect current needs and standards as well as revise this SOP every five years.

## 5. GUIDELINES AND PROCEDURES

### 5.1 PROPER COLLECTION OF BENTHIC ALGAE

#### 5.1.1 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Datasheet or field notebook printed on waterproof paper (Figure 1; paper similar to Grainger Item Number 3XFR7)
- Clipboard
- Pencil or Rite in the Rain Pen (similar to Forestry Suppliers Item Number 49237)
- Waders or hip boots
- Periphytometers (Figure 2, similar to Wildco model 156-D30)
- 10% buffered formalin (similar to Fisher item 23245684)
- Disposable dropper (similar to Grainger item 3TRD2)
- Backpack containing (Figure 3):
    - Periphyton brush (similar to Wildco model 156-F40)
    - Sample sorting tray (similar to Wildco model 182-F20)
    - 2 - 250ml amber HDPE Nalgene® bottles per site (similar to Fisher item 02 923 103), pre-labeled with each the site name, date, collectors, and time.

  - Algae sampling frame (made from LDPE plastic similar to Grainger item 1YZU4, with circle cut out measuring 2.5 inches in diameter)
  - 8 Whirl-Pak® bags per site; labeled with the site name/ID, location(s) and a letter A-H
  - Rope (similar to Grainger item 2ELD3)
  - Multi-tool with knife (similar to Grainger item 3FRA8)
  - Black electrical tape
  - Tape measure
  - GPS or ArcPad
  - Infrared thermometer (similar to Forestry Suppliers item 89642)
- Secchi disk attached to tape measure
- Bricks or concrete blocks
- Bleach
- Acetone (90%)
- Pressure sprayer filled with hot tap water
- 2.5 L jug filled with distilled water
- Graduated cylinder

### 5.1.2 COLLECTION OF BENTHIC ALGAE IN THE FIELD

For most purposes, benthic algae collection will be completed in the field with samples taken from artificial or natural substrates in streams. This method does require sample containers and preservation.

### 5.1.3 RECORDING PARAMETER UNITS

The following units should be used when recording measurements taken with the artificial samplers and plastic algae frame:

Area sampled.....$cm^2$

## 5.2 FIELD MEASUREMENT PROCEDURES

### 5.2.1 DETERMINE FIELD PROCEDURE SCHEDULE

Prior to departure, the project manager will communicate with the field analysts what procedures should be accomplished for each sampling event to the sampling location and the order in which the field procedures should be completed. Prior to performing these analyses, the field analyst should ensure the benthic algae collection is completed in the correct order. This procedure may disrupt fish and microscopic organisms, such as benthic macroinvertebrates, fish, and algae. This disruption can interfere with other field procedures and sample collections in streams. Furthermore, this procedure can dislodge sediment, which can interfere with water quality sample collections. Benthic algae collection should preferably be completed on days when these samples are not being collected. If other sampling activities must occur on the same day, benthic algae collection should be undertaken after other water quality sampling has been completed. This procedure will typically take place late July through September to capture low flow conditions and maximum algal growth. Other seasons may be sampled as dictated by project goals. This will also highlight a time period in Rhode Island when streams may go dry. It is important that this procedure take place in streams that have continuous flow throughout the deployment of the artificial substrates.

### 5.2.3 BENTHIC ALGAE COLLECTION

Depending on the individual project goals, benthic algae collection can be taken from natural and/or artificial substrates. This method describes the procedure for collecting from both types of substrates. After collection of the artificial substrates and natural substrates in the field, all samples should be kept on ice and out of the light to prevent degradation of the samples. After compositing of samples, the samples will be stored in amber bottles that prevent light penetration. Any further preparation of the samples for preservation, shipping, or analysis should prevent exposure to light and hot temperatures.

### 5.2.4 ARTIFICIAL SUBSTRATE PREPARATION

Prior to departure from the sampling center, the field analyst will prepare the appropriate number of periphytometers for placement in the stream, as communicated by the project manager. If the periphytometers and concrete blocks have been deployed in previous years and not cleaned, the field analyst will need to scrub the artificial substrate equipment with warm, soapy water prior to the field season. Artificial substrates are sprayed with bleach prior to winter storage, so this wash will remove any bleach residue.

A scrubbing pad or toothbrush can be gently used on the periphytometers and deployment equipment to dislodge any remaining debris or biological

growth. The artificial substrate equipment will then need to be rinsed free of soap and allowed to dry. New rope should be used every year. Once dry, the periphytometers are prepared by sliding open the locking pieces on the top of the sampling tray (Figure 2).

Prior to the sampling event, the field analyst will prepare the periphytometers for deployment in the sampling center. The field analyst will then attach a rope on both sides of the periphytometer to the cement blocks, which will act as anchors. The field analyst will tie a rope around a concrete block or brick. The block should be tied so that the rope is looped around the block twice, with one end extending a little more than 1.5 feet to allow for manipulation of the concrete block placement to ensure the periphytometer is below the surface of the water (Figure 4B, 5). The field analyst will repeat this with a second concrete block or brick. The field analyst will securely tie the rope from one block to one ring on the periphytometer (Figure 4C). The field analyst will then use black electrical tape to secure the loose end of rope (Figure 4C). The field analyst will tie the other block to the ring on the other side of the periphytometer, securing the end with black electrical tape (Figure 4D). The field analyst will place the required number of periphytometers in separate boxes to prevent any contamination from other field equipment in the vehicle.

### 5.2.5 ARTIFICIAL SUBSTRATE PLACEMENT CONSIDERATIONS

Artificial substrates should be placed in the stream at least 3 weeks prior to collection to allow for maximum colonization and growth of benthic algae. The periphytometer will be left at the location for at least 3 weeks, preferably 4 weeks. Previous research has shown that maximum accrual in enriched and unenriched streams is reached at 4 weeks (Biggs 1988), but the potential for sloughing of materials from high flows and maximum growth could become an issue near the end of the deployment. The project manager will determine the pick up schedule.

The field analyst will observe the stream and canopy conditions at the sampling location. Several factors should be considered when determining the location of the periphytometer placement:

- The artificial substrate should be preferably placed in an area with continuous flow. The field analyst should make sure that the flow is not back flow (upstream flow) or from backwaters of the main channel. To maintain similar flow conditions across the periphytometer, it should not be placed in bends of the stream where flow will be directed in an arc across the side of the periphytometer.

- The periphytometer should be placed in an area where light is penetrating to the bottom of the stream.

    - The field analyst will ensure that light is penetrating to the bottom by lowering a Secchi disk to the bottom of the stream. The field analyst will say aloud whether the Secchi disk is visible on the

stream bottom, and this data will be recorded on the field data sheet (Figure 1) by the recording field analyst.

- The periphytometer should not be placed in areas with excessively turbulent flow conditions (i.e. areas with large amounts of spray-off from flow striking rocks or other substrate).

- The periphytometer should be placed in an area that receives some sunlight at some point through the day.

    - NOTE: Some sites may have extremely dense canopy cover. Every effort should be made to locate the periphytometer in a place with some sunlight to keep light from becoming the limiting factor to growth. If a lighted area is not available at the sampling location, the field analyst should alert the recording field analyst to make a note on the datasheet or appropriate field notebook.

- To minimize disturbance and vandalism, the periphytometer should be placed in areas that are inconspicuous (away from public roadways, bridges, or walking paths).

    - NOTE: The periphytometer will be submerged just under the surface of the water to mimic light conditions of natural substrate, which should also assist in minimizing disturbance and vandalism.

5.2.6 ARTIFICIAL SUBSTRATE PLACEMENT

Both analysts should wear gloves throughout the entire procedure to minimize the possibility of contact with the glass slides. Using gloves, the field analyst will handle the edges of the slides and place a single slide in each of eight (8) slots (Figure 4A). It is important to wear gloves because skin contact with the glass slides can inhibit the growth of the algae due to oils that naturally occur on the skin. The field analyst will slide the locking piece closed to prevent the slides from slipping out.

The artificial substrate slides will be used to composite one sample for chlorophyll *a* analysis and one sample for diatom identification from each stream site.

- Once the placement location has been selected, the recording field analyst will record the sampling station name and number, date, time, and collectors at the top of the datasheet or field notebook (Figure 1). The recording field analyst will note any observations about stream condition, riparian area, benthic algae growth, or sampling trip and record this information on the datasheet. (Figure 1).

- To minimize disruption of the sediment, both analysts will enter the stream at a point downstream of the selected placement location. Sediment can obscure the view and coat the slides with a source of nutrients other than the flowing water. The recording field analyst will assist with carrying and handing materials to the field analyst. The recording field analyst will carry the backpack containing the supplies.

The field analyst will carry the concrete blocks or bricks and the attached periphytometer.

- Both analysts will travel upstream to the selected location of the artificial substrate placement.

- The field analyst will hand one concrete block or brick connected to the periphytometer to the recording field analyst.

- The field analyst will observe the direction of flow and orient the flow guard of the periphytometer to face into the direction of flow.

    - The flow guard is the clear, curved plastic piece in between one of the floats and the plastic case containing the glass slides on the periphytometer (Figure 2).

- The field analyst will hold the periphytometer in one hand and the brick or concrete block in the other hand. Using the rope tied to the brick or concrete block, the field analyst will gently lower the brick or concrete block to the bottom of the stream. The field analyst will continue to hold onto the periphytometer with the other hand.

- The field analyst will then slowly lower the periphytometer to a depth 0.2 feet below the surface of the water. The recording field analyst will take their concrete block and stretch the length of rope until it is gently taut. The recording field analyst will slowly lower the concrete block to the bottom of the stream bed using the rope. The periphytometer should remain at least 0.2 feet below the surface of the water. If the periphytometer is less than 0.2 feet below the surface of the water, the recording field analyst should grasp the rope of the concrete block and bring it upstream. The recording field analyst will then move the other concrete block downstream until the 0.2 feet depth is achieved.

- Using the tape measure attached to the Secchi disk, the field analyst will measure the depth of periphytometer from the stream substrate to the top of the plastic tray (Figure 6) and read the measurement aloud to the recording field analyst, who will record the value on the datasheet or field notebook.

    - NOTE: The field analyst should make sure the depth is measured from the stream substrate and not the top of the brick or concrete block.

- The field analyst will measure the depth of periphytometer from the water surface to the top of the plastic tray (Figure 6). The field analyst will read the measurement aloud to the recording field analyst, who will record the value on the datasheet or field notebook.

- The recording field analyst will hand a GPS unit or ArcPad to the field analyst. The field analyst will take a waypoint at the location of the periphytometer. The recording field analyst will retrieve the GPS unit

or ArcPad and record the location of the waypoint. The recording field analyst will also note any major landmarks or features on the datasheet or field notebook to identify the location of the periphytometer.

- The recording field analyst will hand the infrared thermometer to the field analyst. The field analyst will point the thermometer at the water surface and press and release the gray button on the front. The field analyst will read the measurement aloud to the recording field analyst, who will record the value on the datasheet or appropriate notebook.
    - NOTE: A water quality sonde may also be used to take this reading.
- Both analysts will exit the stream at the periphytometer location or another location that is safe for them to exit.
- The field analyst should communicate the location of the periphytometer with project manager. Other field personnel can avoid disturbing the equipment and also provide notification if the equipment is damaged or missing. This will also ensure that field staff are not injured by becoming entangled in the ropes attached to the concrete blocks or bricks.
- The project manager will check any available stream gages (http://ri.water.usgs.gov/) in the area and communicate with other field staff sampling area regarding the potential for high flows. The project manager will communicate with the field analyst and recording field analyst when the periphytometers should be retrieved from the sampling location.

5.2.7 RETRIEVING THE ARTIFICIAL SUBSTRATE

- Using the GPS location and the major landmarks or features, the analysts will return to the location of the periphytometer. Both analysts should wear gloves to retrieve the periphytometers to avoid contamination of the samples.
    - NOTE: If the periphytometer is not located, the recording field analyst should note this on the datasheet. Section 5.2.7 will not be completed, and the analysts should continue with Section 5.2.8 Sampling the Natural Substrate. The field analyst will notify the project manager if any periphytometers were not recovered.
    - NOTE: If site conditions have deteriorated (i.e. high flows, bank erosion) significantly since placement of the periphytometer, the field analysts should not retrieve the periphytometer or sample the natural substrate. Photographs of the site conditions should be taken to document the issue for the project manager. The field analyst will communicate with the project manager any lost equipment or inaccessible sites. The project manager will

determine any follow-up action to retrieve the artificial substrates or sample the natural substrates.

- To minimize sediment disruption, the analysts will enter the stream at a location downstream of the periphytometer and travel upstream to the location of the periphytometer. The recording field analyst will assist with carrying and handing materials to the field analyst. The recording field analyst will carry the backpack containing the supplies.

- At the stream bank, the recording field analyst will remove 8 Whirl-Pak® bags from the backpack, each labeled with the site location and a letter A-H.

- The field analyst will observe the location and condition of the periphytometer. The field analyst should relay to the recording field analyst any unusual circumstances of the periphytometer (plants caught on the periphytometer, periphytometer is out of the water, etc.). The recording field analyst should record this information on the datasheet or appropriate field notebook (Figure 1).

- Using the tape measure attached to the Secchi disk, the field analyst will then measure the depth of periphytometer from the stream substrate to the top of the plastic tray (Figure 6) and read the measurements aloud to the recording field analyst, who will record the value on the datasheet or field notebook. The field analyst will then measure from the water surface to the top of the plastic tray (Figure 6) and read aloud the recording field analyst, who will record the value on the datasheet or appropriate field notebook.

    - NOTE: The field analyst should make sure the depth is measured from the stream substrate and not the top of the brick or concrete block.

- The field analyst will carefully grasp the rope of the upstream cement block or brick just under the periphytometer. The field analyst will gently hold the periphytometer by a float or plastic sides in the other hand. The field analyst will then gently pull on the rope attached to the brick or concrete block. The field analyst should pull the rope until the brick or concrete block is exposed from the water. The recording field analyst will repeat this with the downstream concrete block or brick.

    - NOTE: Do not use the periphytometer to pull up the brick or concrete block. This risks ripping off the floats or cracking the plastic tray holding the glass slides.

    - NOTE: Depending on the site conditions, the field analysts may cut the ropes to retrieve the periphytometer. The field analysts will then need to retrieve the concrete blocks or bricks by pulling on the floating rope.

- Both analysts will move to the stream bank. The field analyst will place the upstream concrete block or brick on the stream bank near the recording field analyst. The recording field analyst will then place the downstream concrete block or brick on the stream bank. The field analyst will then hand the periphytometer to the recording field analyst.

- The recording field analyst will slide open the locking mechanism to remove the glass slides from the periphytometers (Figure 2). Carefully avoiding touching the face of each slide, the recording field analyst will remove a single slide from each slot and place one slide in each of the 8 Whirl-Pak® bags. The recording field analyst will add some distilled water to each of the Whirl-Pak® bags. The recording field analyst will roll the top of the bag and close with the imbedded twist-tie.

- The field analyst will observe the amount of aquatic macrophyte and duckweed (*Lemna* sp) and/or watermeal (*Wolffia* sp) growth in the visible 25m reach of stream upstream and 25m downstream of the periphytometer location. If necessary, the field analyst can hike or wade around overhanging vegetation or bends in the stream. If this cannot be accomplished (due to deep water or impassable vegetation), the recording field analyst will estimate how far they can see, and record that visible distance on the field sheet (Figure 1).

- The field analyst will estimate and say aloud the percent cover of all macrophyte growth and duckweed and/or watermeal. The recording field analyst will circle the percent cover of macrophytes and duckweed and/or watermeal growth on the datasheet or appropriate field notebook.

5.2.8 SAMPLING THE NATURAL SUBSTRATE

The field analyst will typically collect two composite samples. One sample will be analyzed for chlorophyll *a*, and the second sample will be sent to a contractor for diatom identification. During the collection of the natural substrates, the field analyst will need to keep 2 amber Nalgene® HDPE bottles in their wader pocket.

- Following retrieval of the artificial substrates, the field analyst will observe the location of natural substrates in the stream. Natural substrate will need to be completely submerged in the water. The natural substrate should be fixed at the location but easy to remove for sampling. Natural substrate will be collected with the following decreasing preference:

    1. Rocky substrate (>2cm – 25cm in diameter)

    2. Woody substrate (branches or sticks greater than 2cm in diameter or surface area)

3. Aquatic vegetation (such as wild celery (Figure 7)) or other broad leafed vegetation with some portion under the water)

    - NOTE: Do not sample any vegetation that is skin irritant, such as poison ivy or stinging nettle (Figure 7).

    - NOTE: Aquatic vegetation should only be used when rocky or woody substrate is not available. Broad-leafed vegetation can be sampled in the process described below, but the field analyst will need to scrub gently to avoid rupturing the cells of the vegetation.

    - NOTE: It is important to sample the same species of vegetation or a species of the same growth type. The recording field analyst should record on the datasheet or field notebook when a growth form other than broad-leafed vegetation is used.

- The field analyst should observe the amount of growth in the stream. The field analyst will use best professional judgment to select substrates that are representative of the benthic algal growth conditions. The field analyst will attempt to only sample the algal growth when possible. The field analyst will avoid heavy non-vascular plant growth

    - For example, in a stream with a single green rock or branch the field analyst should not sample the only rock or branch with growth, or in the case of a stream with large amounts of growth, the field analyst should not sample the only clean rock and woody substrate.

- The field analyst will randomly collect seven pieces of natural substrate representative of algal growth and bring them to a relatively flat surface. The field analyst will attempt to get a mix of different types of substrate.

    - For example, the field analyst should collect 5 rocks and 2 branches or sticks. The chlorophyll *a* analysis will use 2 rocks and 1 stick and diatom taxonomy will use 3 rocks and 1 branch.

- The field analyst will retrieve the backpack of sampling materials from the recording field analyst. The field analyst will remove the periphyton brush, sample sorting tray, algae sampling plastic, and wash bottle filled with distilled water.

- Using DI water, the field analyst will rinse the bottom of the substrate to be scraped over the ground to ensure that only scrubbed material is rinsed into the sample. This rinse will remove large debris adhered to the bottom of rocks.

- The field analyst will sit on the stream bank with their feet directly in front of them and knees slightly bent to make a 45º angle. The field analyst will place the sampling tray on their thighs with the pour spout closest to their body or in a position to not spill the contents while

scraping (Figure 8). All rinse water should be collected in the sampling tray. The field analyst should also take care to minimize the amount of rinse water to avoid overfilling the bottles for processing and shipment.

  - NOTE: Do not sit with knees bent in more than a 45º angle. This can promote spilling of the rinse water.

- The field analyst will place the plastic algae sampling frame on the surface of the natural substrate exposed to sunlight. If the circle cut into the plastic is not filled by the surface of the natural substrate, the field analyst will need to observe and estimate the amount of the circle filled.

- Using the periphyton brush, the field analyst will scrub the surface of the natural substrate over the sorting tray. The field analyst will remove the plastic algae sampling frame and place it next to the natural substrate. The field analyst will use a small amount of water to rinse the scrubbed circle on the substrate, and if necessary, any debris on the frame. The field analyst will repeat the scrubbing and rinsing until a clear circle is apparent on the surface of the natural substrate (Figure 9).

  - NOTE: If the circle was not filled by the surface being scrubbed, the field analyst will select another location on the surface and scrub the appropriate area to complete the surface area encompassed by the circle.

- After the circle is scrubbed and rinsed clean, the field analyst will rinse any debris remaining on the plastic algae sampling frame into the tray. The field analyst will select another substrate and repeat the scrubbing and rinsing of the surface until a clear circle is apparent on the surface of the natural substrates.

  - NOTE: Again, at least one of the selected natural substrates should be different than the other selected natural substrates (i.e. 1 rocks, 3 sticks or 3 rocks, 1 stick). The preferred division is 2 rocks and 1 stick for chlorophyll *a* and 3 rocks and 1 stick for diatom taxonomy, but if this is not possible at a site, the field analyst will sample the same natural substrates.

- When the field analyst has scrubbed the preferred number of natural substrates, the field analyst will rinse the periphyton brush and plastic algae sampling frame until a clear rinse has been achieved. If spray from scrubbing the samples has gotten on the field analyst's hands, the field analyst will then rinse their hands, with a small amount of DI water, into the sorting tray.

- The field analyst will then take and open 1 of the amber Nalgene® HDPE bottles and place it at the bottom of the pour spot. The field analyst will then pour the rinse water into the Nalgene® bottle and

rinse the entire sampling tray into the bottle. The field analyst will then tightly replace the lid.

- The field analyst will announce to the recording field analyst the types of substrate sampled for the first sample. The recording field analyst will record this information on the appropriate datasheet or field notebook.

- The field analyst will then rinse the sampling tray, periphyton brush, and plastic algae sampling frame with distilled water. This is to ensure that all debris from scraping has been rinsed clean.

- The field analyst will then select another substrate. The field analyst will again place the sampling tray on their thighs at a 45º angle with the pour spout closest to their body or in a manner to not spill the contents. Using the periphyton brush, the field analyst will scrub the surface of the natural substrate over the sorting tray. The field analyst will remove the plastic algae sampling frame and place it next to the natural substrate. The field analyst will use a small amount of water to rinse the scrubbed circle on the substrate, and if necessary, any debris on the frame. The field analyst will repeat the scrubbing and rinsing until a clear circle is apparent on the surface of the natural substrate.

    - NOTE: If the circle was not filled by the surface being scrubbed, the field analyst will select another location on the surface and scrub the appropriate area to complete the surface area encompassed by the circle.

- After the circle is scrubbed and rinsed clean, the field analyst will rinse any debris remaining on the plastic algae sampling frame into the tray. The field analyst will select another substrate and repeat the scrubbing and rinsing of the surface until a clear circle is apparent on the surface of all the natural substrates.

    - NOTE: Again, at least one of the selected natural substrates should be different than the other selected natural substrates (i.e. 1 rocks, 3 sticks or 3 rocks, 1 stick). The preferred division is 2 rocks and 1 stick for chlorophyll *a* and 3 rocks and 1 stick for diatom taxonomy, but if this is not possible at a site, the field analyst will sample the same natural substrates.

- When the field analyst has scrubbed the preferred number of natural substrates, the field analyst will rinse the periphyton brush and plastic algae sampling frame until a clear rinse has been achieved. If spray from scrubbing the samples has gotten on the field analyst's hands, the field analyst will then rinse their hands, with a small amount of DI water, into the sorting tray.

- The field analyst will then take and open 1 of the amber Nalgene® HDPE bottles and place it at the bottom of the pour spot. The field analyst will then pour the rinse water into the Nalgene® bottle and

rinse the entire sampling tray into the bottle. The field analyst will then tightly replace the lid.

- The field analyst will announce to the recording field analyst the types of substrate sampled for the first sample. The recording field analyst will record this information on the appropriate datasheet or field notebook.

- The field analyst will then rinse the sampling tray, periphyton brush, and plastic with deionized water. This is to ensure that all debris from scraping has been rinsed clean.

- The analysts will exit the stream at the periphytometer location or another location that is safe to exit.

- The benthic algae samples collected will be placed in a cooler on ice.

- Upon return to the vehicle, the field analyst will spray the sampling equipment with pressurized hot tap water to minimize potential transfer of contaminants and invasive species. It will also ensure that sampling equipment is clean between sites to minimize cross-contamination of samples.

- Samples for diatom taxonomy will need to be preserved upon return to the Sampling Center. See Section 5.2.10. All chlorophyll *a* will need to remain in a cooler or refrigerator until filtering.

5.2.9 PROCESSING THE ARTIFICIAL SUBSTRATES IN THE SAMPLING CENTER

The compositing of samples should be done within 24 hours of collection, preferably immediately upon return to the sampling center after retrieval or the artificial substrates. Four slides will be composited for analysis of chlorophyll *a*. The other 4 slides will be composited for diatom taxonomic identification and analysis. All parts of the artificial substrate processing should be done wearing gloves.

- Once back in the sampling center, the field analyst will remove one set of artificial substrate samples from one site for a total of 8 Whirl-Pak® bags. The field analyst will then place two amber Nalgene® bottles on the counter. The field analyst should attempt to minimize light in the sampling center, but the field analyst should not dim the lights to a point where safety will be a concern.

- Using a book or other equipment, the field analyst will set the sampling tray at a 45º angle on the counter.

- The field analyst will put on gloves and unwhirl Whirl-Pak® bag A from the site. The field analyst will carefully remove the glass slide, handling only the sides of each slide.

- Using a periphyton brush or razor blade, the field analyst will carefully scrub or scrape only the surface of each side of the glass slide over

the sampling tray. The field analyst will not scrub the edges of the slide. The field analyst will rinse the scrubbed area into the tray and repeat the scrubbing and rinsing until the slide surface is clean. The field analyst will then place slide A in a wash tub filled with warm, soapy water.

- The Whirl-Pak® bag A should then be rinsed with distilled water into the sampling tray. The Whirl-Pak® bag A should then be discarded in the trash.

- The field analyst will then unwhirl Whirl-Pak® bag B, C and D and repeat the above procedure.

- The field analyst will then rinse the periphyton brush or razor blade into the sorting tray until the rinse is clean. The field analyst will then rinse their hands if spray is apparent on the gloves. The field analyst will then place one of the empty, labeled amber Nalgene® bottles under the pour spout. The field analyst will then rinse the sorting tray into the amber Nalgene® bottle. The field analyst will then recap the bottle and place it back in the refrigerator.

- The field analyst will set the sampling tray at a 45º angle on the counter.

- The field analyst will then unwhirl Whirl-Pak® bag E for the site. The field analyst will carefully remove the glass slide, handling only the sides of the slides.

- The field analyst will carefully scrub only the surface of each side of the glass slide over the sampling tray using a periphyton brush or razor blade. The field analyst will not scrub the edges of the slide. The field analyst will rinse the scrubbed area and repeat the scrubbing and rinsing until the slide surface is clean. The field analyst will then place slide E in a wash tub filled with warm, soapy water.

- The field analyst will then then unwhirl Whirl-Pak® bags F, G, and H for the site. The field analyst will then rinse the periphyton brush until the rinse is clean. The field analyst will then rinse their hands if spray is apparent on the gloves. The field analyst will then place the second empty amber Nalgene® bottles for the site under the pour spout. The field analyst will then rinse the sorting tray into the amber Nalgene® bottle.

5.2.10 DIATOM TAXONOMY SAMPLE PRESERVATION AND MEASUREMENT

- In the hood, the field analyst will add 3 mL of 10% buffered formalin to each bottle being sent for diatom analysis.

    - NOTE: Most algal preservatives contains acid, which will interfere with the analysis of chlorophyll *a*. To ensure that chlorophyll *a* samples are not exposed to acid, preservative should only be

added at the end of compositing all sample sites processed at the end of the day.

- In the hood, the field analyst will gently swirl each bottle being sent for diatom analysis. The contents of the bottle will be poured into a graduated cylinder. The field analyst will note the volume of the sample on the chain of custody (Figure 10). The contents of the bottle will then be poured back into the amber sample bottle. The graduated cylinder will be rinsed with a very small amount of DI water. The bottle will then be placed in the refrigerator until shipping to the contractor.

5.2.11 EQUIPMENT MAINTENANCE

Periphytometers are designed to be reused over many years and sampling sites. In order to minimize cross-contamination of sites and years, the periphytometers and all equipment deployed in the stream must be cleaned and decontaminated after deployment. This process will use bleach, so the field analyst will need to wear clothes or a lab coat that can be exposed to bleach. The field analyst should also consult the MSDS and safety sticker on the bottle of bleach to determine whether safety glasses or other protective equipment is required. The field analyst should wear gloves when cleaning the glass slides.

- After deployment, the field analyst will need to prepare a bucket of warm, soapy water. The field analyst will use scrubbing pads and toothbrushes to gently scrub and clean any debris or growth from the periphytometers and deployment equipment.

- The field analyst will need to prepare a dilute solution of bleach (10%). The field analyst will spray the periphytometers with the bleach solution. The bleach should not be washed off to allow for all current growth to be killed and to discourage any growth over the winter. The periphytometer should be allowed to air dry then placed in the sampling center for winter storage.

- Discard any broken slides in the appropriate glass disposal container in the sampling center. It is preferable to use new slides, but slides that are going to be reused should be scrubbed in warm, soapy water. The slides should then be soaked in 90% acetone overnight. The slides will then be rinsed with distilled water and allowed to dry. The slides can then be stored in the Sampling Center for the winter.

## 6. QUALITY CONTROL

### 6.1 QUALITY CONTROL

Quality control of the artificial substrate procedure will be assessed by placing a second periphytometer at 10% of stream segments. Quality control of natural substrate procedure will be assessed by collection of a second set of bottles by the field analyst at 10% of stream segments. This will give a measure of precision for both procedures.

### 6.2 QUALITY ASSURANCE PLANNING CONSIDERATIONS

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. Unless specified otherwise in a site or project-specific work plan, Quality Assurance Project Plan (QAPP), Quality Assurance Program Plan (QAPP) or laboratory Quality Assurance Manual (QAM), all data collected following the protocols set forth in this document will be collected in accordance with the minimum QAQC requirements of Section 6.1. Further quality assurance requirements will be defined in project specific work plans and may include duplicate or replicate measurements or confirmatory analyses.

## 7. REFERENCES

Biggs, B.J.F. 1988. Artificial substrate exposure times for periphyton biomass estimates in rivers. New Zealand J. Marine Freshw. Res. 22:507-515.

Danielson, T. 2006. Protocols for Sampling Benthic Algae in Streams, Wetlands, and Freshwater Wetlands. Maine Department of Environmental Protection. DEPLW-0634

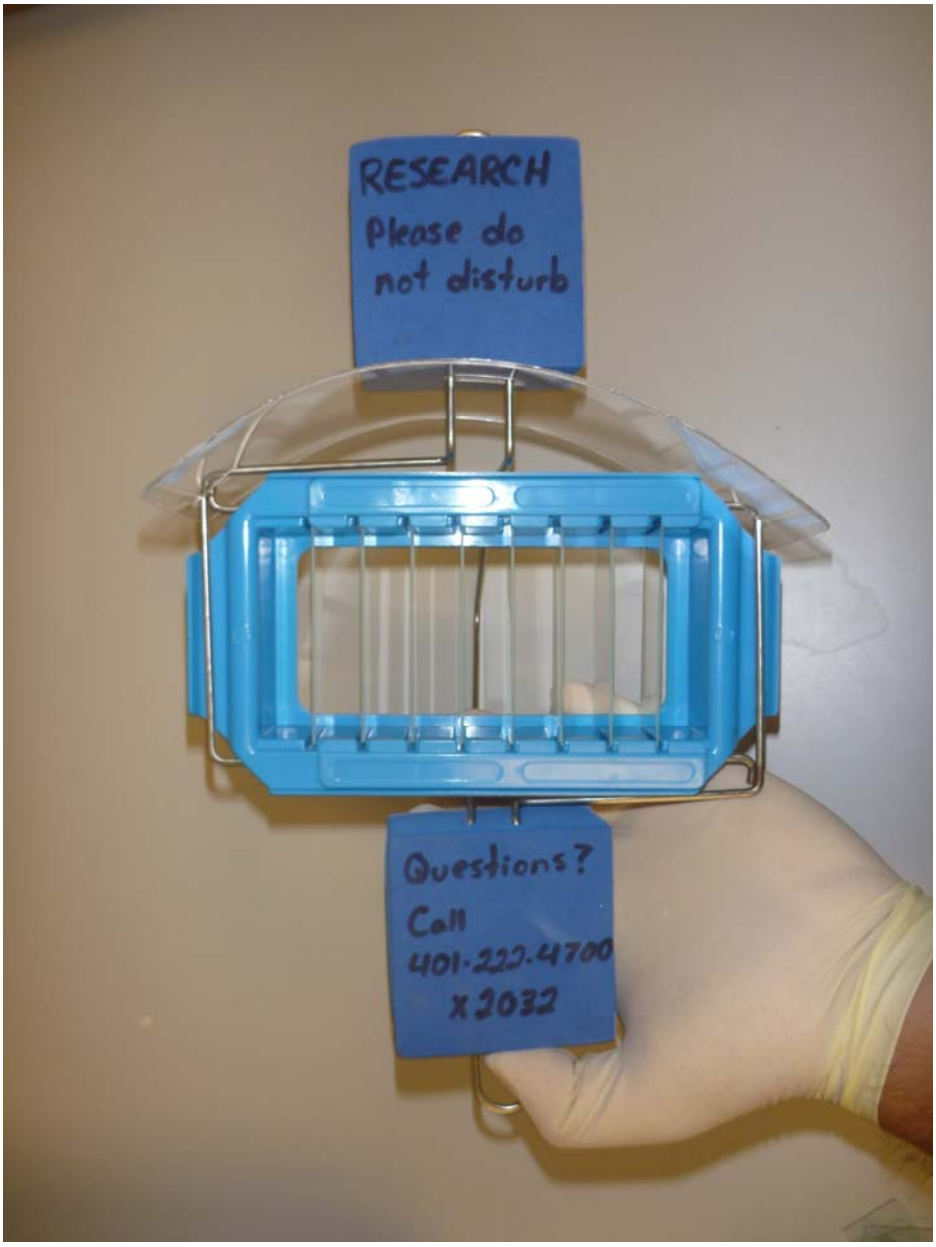
Danielson, T. 2009. Description of Nutrient Criteria for Fresh Surface Waters (Chapter 583). Maine Department of Environmental Protection. DEPLW-0974A.

Potapova, M. and D.F. Charles. 2005. Choice of substrate in algae-based water-quality assessment. J. N. Am. Benthol. Soc. 24:415-427.

**Figure 1. Benthic Algae Collection Datasheet for Monitoring Section Sampling Events**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Benthic Algae Collection Datasheet** | | | | | | |
| **Stream Segment :** | _____ | | | | **Town:** | _____ | |
| **Site Number:** | _____ | **Deploy** | **Stream Depth:** | _____ ft | **Periphytometer #** | | _____ |
| | | **Pick-up** | **Stream Depth:** | _____ ft | | | |
| **Deployment Date:** | _____ | **Time:** | _____ | **Pictures:** | _____ | **Collectors:** | _____ |
| **Retrieval Date:** | _____ | **Time:** | _____ | **Pictures:** | _____ | **Collectors:** | _____ |
| **Lat/Long of Art Sub:** | _____ | | | | **QA Site?** | **Yes** | **No** |
| **Lat/Long of Art Sub Dup:** | _____ | | | | | | |
| **Major Landmarks of Art Sub:** | _____ | | | | **Secchi?** | **Yes** | **No** |
| **Major Landmarks of Art Sub Dup:** | _____ | | | | **Secchi?** | **Yes** | **No** |
| **Comments/Notes:** | | | | | | | |
| **Percent Macrophyte Cover** (Circle 1) | 0 | 10 - 20 | 21 - 30 | 31 - 40 | 41 - 50 | 51 - 60 | |
| | 61 - 70 | 71 - 80 | 81 - 90 | 91 - 100 | | | |
| **Percent Duckweed and/or Watermeal** (Circle 1) | 0 | 10 - 20 | 21 - 30 | 31 - 40 | 41 - 50 | 51 - 60 | |
| | 61 - 70 | 71 - 80 | 81 - 90 | 91 - 100 | | | |
| | **Deploy** | **Pickup** | | | **Deploy** | **Pickup** | |
| **Art Sub Depth Below Surface** | _____ | _____ | ft | **Depth to Bottom** | _____ | _____ | ft |
| **Art Sub Depth Below Surface Dup** | _____ | _____ | ft | **Depth to Bottom** | _____ | _____ | ft |
| **Art Sub Retrieved?** | **Yes** | **No** | | **Intact Glass Slides** | | _____ | |
| **Art Sub Retrieved Dup?** | **Yes** | **No** | | **Intact Glass Slides Dup** | | _____ | |
| **# of Nat Sub Sampled (Chl)** | **Rocks** | _____ | **Wood** | _____ | **Vegetation Type:** | _____ | |
| **Total Area (Circles*31.6531)** | _____ | cm^2 | | | | | |
| **# of Nat Sub Sampled (Tax)** | **Rocks** | _____ | **Wood** | _____ | **Vegetation Type:** | _____ | |
| **Total Area (Circles*31.6531)** | _____ | cm^2 | | | | | |

**Figure 2. <u>Periphytometer</u>**



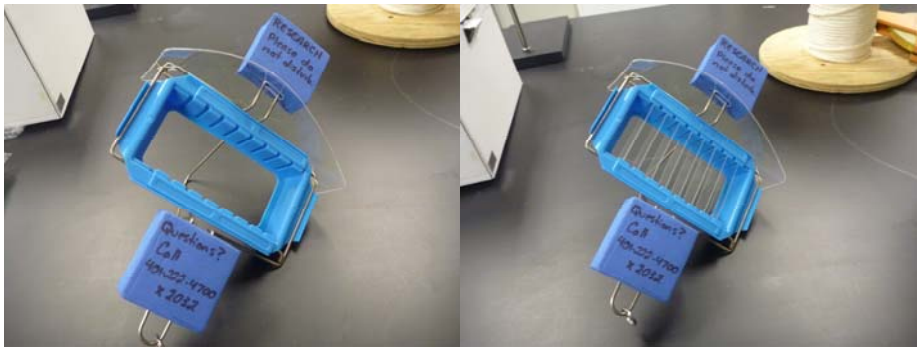

A Patterson

**Figure 3. <u>Supplementary Equipment</u>**

A Patterson

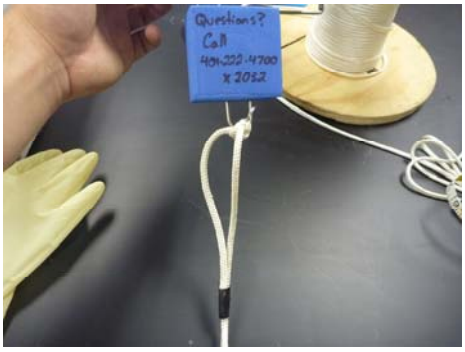
## Figure 4. Preparation of the Artificial Substrates

A




B

C




D

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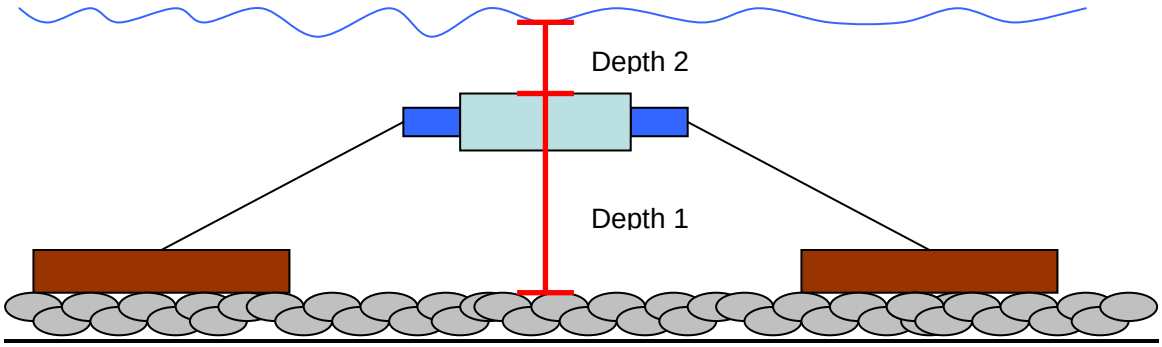
**Figure 5. Deployed Artificial Substrate**

**Figure 6. Measurements of Periphytometer Depths**



## Figure 7. Vegetation Pictures

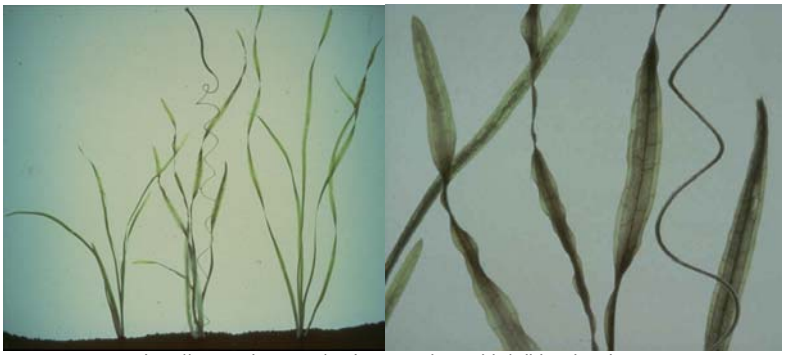
**Poison Ivy**
(*Toxicodendron radicans* (L.) Kuntze)

http://greatermd.bbb.org/watch-out-for-poison-ivy-/

**Stinging Nettle**
(*Urtica dioica* L.)

http://www.wildmanstevebrill.com/Plants.Folder/Nettle.html

**Wild Celery**
(*Apium graveolens* L.)



http://www.mlswa.org/underwaterplantguide/wild_celery.htm

## Figure 8. Cleaning of the natural substrate

## Figure 9. Example of clean scrubbed circle

## Figure 10. Diatom Taxonomy Chain of Custody

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**Periphyton Chain of Custody**

| Sample ID # | Sample Date Collected | Site Name/Waterbody | Station ID | # of Jars | Collection Procedure | Latitude | Longitude | Comments |
|---|---|---|---|---|---|---|---|---|
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|---|---|---|
| Project Name: | | |
| Contact Person: | Phone: | Email: |
| | | |
| Relinquished by: | Date: | |
| Received by: | Date: | |

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