**Department of Environmental Protection**

**Division of Watershed Management**

627 Main Street, Second Floor

Worcester, MA



# Standard Operating Procedure

**Title:** CN: 35.0 Percent Cover and Periphyton Collection Determinations

**Date:** May 2,2012

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SOP Percent Cover and Periphyton Collection Determinations-2012

1. SCOPE AND APPLICATION

Massachusetts Department of Environmental Protection has over the years, developed a database on macroinvertebrate communities particularly in rivers and streams. This information has been used to evaluate and assess aquatic life and to make determinations regarding organic and nutrient enrichment. MADEP is working to establish a data base using the biomass of periphyton (attached algae) i.e. chlorophyll and some algal identification work. The attached algae take up their nutrients from the water column not substrates which makes them a good indicator of nutrient enrichment as both the species present as well as the amount of biomass present can be affected by nutrient concentrations. Since we do not have established methods for periphyton yet, this gave us an opportunity to establish a Pilot Program. The objectives of this Pilot Program are:

* To develop/refine standard methods for measuring periphyton in wadeable streams (suitable for quantifying algal biomass in high-gradient with hard substrates or low-gradient streams with unconsolidated substrates)
* To research how to analyze periphyton results for assessment purposes
* To define the resources/funding that are required to support periphyton monitoring in the future
* To develop a database of chlorophyll from benthic algae as well as percent algal cover at locations being assessed for water quality and aquatic macroinvertebrate communities in Massachusetts streams and rivers.
* To establish a database on periphyton from reference conditions

This Pilot Program has been in effect for the past two years. In 2011, we began contracting out samples for diatom identifications, counts and biovolumes. Because diatoms are sensitive to changes in nutrients as well as being a much more diverse community than the ‘soft algae’ i.e. the greens, blue-greens and reds, much can be learned about the diatom communities at different sites from this addition.

1. BACKGROUND

The benthic algae, both micro and macroscopic forms live on or attached to the substrata. Field studies result in collections of both micro and macroscopic forms. The attached microcommunity constitutes the periphyton. It is composed of algae, bacteria, fungus held within a matrix of polysaccharides. In the literature, the terms periphyton and benthic algae are used interchangeably; the same is true for the descriptions included in this SOP.

Benthic algae are useful biological indicators of water quality because they do not have roots so their entire nutrient and mineral uptake is from the water column and not the sediments. Also, they are sessile and fast growing. Periphyton are important primary producers in streams and rivers and are critical in oxygen production as well as carbon dioxide use. They provide, along with macroinvertebrate, fish and habitat assessment, another biological community to help evaluate the condition of aquatic life as well as the impacts from toxicity or nutrient enrichment.

Benthic algae/periphyton can be used to assess above and below a point source or nonpoint source to look for nutrient impacts or toxicity issues. Changes in the community assemblage of the algae are examined as well as differences in algal biomass that are evaluated through determination of percent cover, chlorophyll a and/or ashfree dry mass from artificial substrates and natural substrates.

Along with community assemblage, evaluation of periphyton should also include information on the type of substrate, growth form, percent area within a reach covered with visible periphyton (macroalgae) and microalgae (primarily diatoms) and biomass covering the substrates. Nutrient availability and physical characteristics such as flow, available light and temperature all affect biomass. Biggs (2000) analyzed data from 30 sites to develop a relationship between mean and monthly maximum chlorophyll *a* as a function of soluble nutrient concentrations and found that the variation in stream-water nutrients explained 12-22.6 % of the variation in mean monthly chlorophyll a values and 29.5-32.5% of the variation in maximum chlorophyll a among sites. If days of accrual are also considered with chlorophyll a, then 43.7-48.8% of the variation in mean monthly chlorophyll a and 72.1-74.1% of the variation in maximum chlorophyll a is explained. Biggs and Close (1989) looked at streams with predominantly gravel substrates and found that the hydrological regime as well as nutrients are critical in development of the periphyton community. Not all studies are in agreement concerning the factors influencing algal biomass in stream. Dodds et al. (1997) examined other factors besides nutrients influencing benthic algal biomass including latitude, temperature, stream gradients, discharge and light, but they found only TN or TP were useful predictors of stream chlorophyll a-not physical factors found by other researchers or DIN or SRP.

The amount of time and resources needed to measure periphyton in wadeable streams depends on the objectives of the study. The lowest level of sampling effort is currently performed as part of the EPA’s Rapid Bioassessment for wadeable streams and rivers. Identifications are made from natural substrate scrapes. The samples are brought to the laboratory for identification, which is typically to genus level. The information is used for qualitative comparisons of the community assemblage and abundance to that of the reference station.

Other survey types include determination of percent cover and chlorophyll a analysis. Both of these surveys may require the establishment of transects while the chlorophyll sampling also may require the collection of several samples and cleaning of substrates in the field. Both of these survey types are time consuming, labor-intensive, and will required specialized crews. When possible, macroinvertebrate, habitat assessment and periphyton sampling should be done as part of a single survey to make efficient use of personnel and other resources. .

Periphyton sampling is primarily conducted during the summer growth period when community response to excessive nutrients is most pronounced (June to September). Based on experience obtained from our first two years of sampling, we will try to focus our sampling from late July-September. MassDEP monitoring program is based on a 5-year cycle where each watershed in the Commonwealth is monitored and assessed. The 2012 sampling locations were chosen based on probabilistic design. A subset of these stations may be included in the periphyton sampling. Samples may also be collected from reference sites determined using the Human Disturbance Index (Meek 2011).

Sampling locations for inclusion in the pilot study are not as narrowly defined as in earlier years. In previous years, sites were chosen that had runs/riffles, at least 30 % cobble substrates and were not too deep or fast flowing to be safely sampled. While sites with at least a partial canopy opening (50 % or greater) were considered to be the best for sampling because they provided excellent growth conditions for filamentous green algae, sites that do not meet this criterion will not be automatically eliminated. In part this is because diatoms will be collected at each site and diatoms can grow at lower light levels compared to green algae.

1. SUMMARY

Benthic algal surveys include at a minimum scrapes of the dominant substrate for taxonomic identifications and estimates of density as well as visual estimates of percent cover of the algae within the reach. The estimates are part of the Rapid Bioassessment Protocols which include macroinvertebrate and habitat assessment. Identifications are done to determine the community assemblage; to learn if the taxa indicate nutrient enrichment or perhaps some other environmental impact. All samples are collected in the riffle/run of each stream allowing this habitat to be compared site-to-site. Identifications have previously only being done of the “soft-bodied” algae and not the diatoms. The “soft-bodied” algae, when present in excessive amounts, can cause nuisance conditions in-stream and can eventually destroy the benthic habitat for invertebrate interstitial organisms as well as affect the aesthetics of a reach. This year, diatom sampling will be included at a subset of the sites chosen for examination. They will be sent to a contract laboratory for identifications and counts.

It is also important to determine the percent cover of the algal material and the dominant forms of algae within the habitat being sampled. Information obtained from the algal identifications and relative abundance in the riffle zone is determined by finding microscopically the genera that appear most frequently in the algae samples. This information is combined with habitat assessment, in particular, canopy cover and percent algal cover. Where potential problem areas are found based upon percent algal cover and abundance, these locations are noted. The relative abundance can be used by assessment personnel in determination of whether the uses of the rivers are impaired, in particular, aesthetics and aquatic life support.

In addition, to these bioassessment measures, DWM will be evaluating benthic algal biomass for possible inclusion in our Recreation criteria and/or Aquatic Life criteria Either visible filamentous periphyton (40 percent coverage of the streambed in more than one site visit during a prescribed time period), or mean benthic algae chlorophyll (shall not exceed 200 mg/m2 in more than one site visit during a prescribed time period ) are response measures that may ultimately become part of the MassDEP water quality standards.

1. SAFETY CONSIDERATIONS

The safety concerns for field collection of samples are discussed in DWM SOP CN 1.0 “Grab Collection Techniques for DWM Water Quality Sampling”. Care should also be taken in handling the knife for the algal scrapes. Samples collected for chlorophyll a analysis need to be put into acetone. The safety considerations for the acetone are outlined in the Chlorophyll a Standard Operating Procedure: CN 3.0.

1. SAMPLE COLLECTION, PRESERVATION AND HANDLING

Method for Percent Cover of Algal Coverage-2012

Equipment:

* Field kit
* First aid kit
* tape measure (30 m)
* marking tape
* field sheets
* clip board
* vials
* camera-underwater if available
* cell phone
* GPS
* viewing bucket(s)-2
* small metric ruler
* small plastic bags for algae collection
* cooler with ice
* boots
* bug spray
* hand sanitizer
* wash bottle
* tools for algal scrapes
* compass
* straps for algal scrapes
* wide-mouth amber jars for chlorophyll/diatom samples
* Lugol’s solution for fixing diatom samples

Procedure:

The sampling reach should be approximately 300 meters long.

* Select a site at the downstream end of the riffle/run zone within the area defined by the macroinvertebrate samplers as a reach to establish a transect
* Four transects will be established informally through at least two riffle/run areas. At least 5 meters should be present between transects. An effort should be made to have some habitat uniformity among the transects. If the reach varies a lot from top to bottom then transects 1 and 2 should be similar and 3 and 4 should be similar.
* Measure and record the wetted width of each transect as well as the depth of the thalweg. For percent cover measurements, diatom sample collection or for chlorophyll a analysis divide the width of the stream into 3 points (near left bank, mid stream, right bank) beginning near the waters’ edge where flow is discernable. Use the viewing bucket to determine percent cover of the bottom or to collect suitable substrates for analysis.
* For the percent cover surveys, have one person (viewer) conduct the survey and one person (recorder) record data (Figure 1).
* At a location, the viewer should immerse the viewing bucket in the water (Figure 2). There are 35 dots, 4 cm between dots.

Figure 1: Viewing bucket for qualitative benthic algae survey



* 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.
* To minimize glare, it is sometimes helpful to put a little water inside the viewing bucket.
* Before beginning the view bucket procedure, it helps to expedite things if you pick up a few rocks and become familiar with categories of film or algal cover that are present on them
* 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.
* If filamentous algae are present measure the longest filament under the view bucket. If you can identify the filamentous algae, record the names of the taxa on the field sheet. Samples of the algae can be brought back to the laboratory for identification and added to the field sheet later.
* At each dot, the viewer should call out one of the following to characterize the algal growth below the dot.
  + - unconsolidated substrate such as sand or mud
    - plant – an aquatic plant
    - moss – a moss
    - Macro 1 - a filament or other macroalga that <2 cm long
    - Macro 2 – a filament or other macroalga that is ≥ 2 cm and < 15 cm long
    - Macro 3 – a filament or other macroalga that is ≥ 15 cm long
    - 0– substrate rough or slightly slimy with no visible algae. A thin layer of algae may be present, but not visible.
    - 1–thin layer of algae is visually evident (green or opaque type covering on surface)
    - 2– periphyton mat from 0.5-1 mm thick is evident-material can be scraped with fingernail
    - 3–periphyton mat between 1-3 mm thick is evident
    - 4- periphyton mat >3 mm thick
    - 5-periphyton mat thickness >2 cm
    - If there is a mixture of decomposing filaments, microalgae, and silt, then treat it as being periphyton mat and not a filament.
    - The recorder should use hash marks to record the observations on the field sheet in the appropriate boxes.

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. (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.

The viewer should use the 6-inch ruler to distinguish categories 2-5. Macroalga – algae that form macroscopic or plantlike morphologies with a thallus structure that is recognizable with the naked eye (Wehr and Sheath 2003).

Based upon:

Barbour, M*.* T., Gerritsen, J., Snyder, B. D. and J. B. Stribling. 1999. *Rapid Biological Assessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*. 2 nd edition. USEPA, Office of Water, Washington, D. C.

Danielson, T. (2006) *Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands*. Maine-DEP-Bureau of Land and Water Quality, Division of Environmental Assessment, Biomonitoring Program**.**18 p.

Fritz, K. M., Johnson, B. R. and D. M. Walters. 2006. *Field Operations Manual for Assessing the Hydrologic Permanence and Ecological Condition of Headwater Streams*. EPA/600/R-06/126. U.S. Environmental Protection Agency, Office of Research and Development, Washington DC.

Wehr, J.D. and R.G. Sheath. 2003. Freshwater Algae of North America: Ecology and Classification. Academic Press. New York.

Method for Chlorophyll a Analysis from Periphyton

Equipment:

* field sheet A (Appendix A)
* clip board
* vials
* camera
* GPS unit
* 2 white enamel trays
* strap for cobbles
* soft toothbrushes
* knife/razor blades, scalpel type implements
* wash bottles containing tap water/Poland Spring water
* 500 ml plastic graduated cylinder
* cooler
* boots
* bug spray
* hand sanitizer
* Fisher Scientific PowerGen 35 hand held tissue grinder
* Fisher Scientific Vortex Mixer
* Waring Blender, stainless steel

Procedure-Chlorophyll a from hard natural substrates

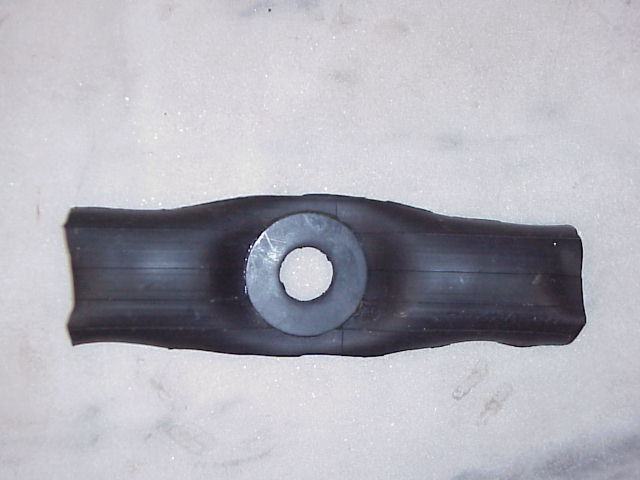
* Establish four transects through the riffle/run areas. A length of river of approximately 300 m should be examined before establishing transects. The sampling reach itself should include where possible at least two riffle/run areas. To accomplish this the transects do not have to be contiguous. Transects 1 and 2 should be 5 meters apart as well as 3 and 4. Setting up the transects before the sampling date will help save time.
* Take pictures upstream/downstream indicating vegetation instream and canopy cover.
* If the submersible camera is available include pictures of the substrates.
* The four chlorophyll a transects define the area to be sampled and include transects needed for percent cover calculations. If the stream is narrow, the samples can be located in a zig-zag pattern from one transect to the next.
* Along each transect three locations are randomly chosen facing downstream on the right side, mid-stream and left side of the stream. This is true for both percent cover and chlorophyll a. (**If macroinvertebrates are being collected periphyton samplers need to wait to enter the water until the macroinvertebrate samplers have gone further upstream).**
* **A**fter at least transects one and two are established, the chlorophyll samples can be collected by haphazardly removing a cobble from a site along the right shore, mid channel and left shore.
* Put the cobble stone into the white enamel pan, substrates can be collected from transects one and two or three and four at the same time and then brought to shore for processing.
* At this time, pictures can be taken of the cobble in white pan.
* To begin the chlorophyll sampling/diatom sampling, place the periphyton strap on the top surface of the substrate. The 1 inch opening in the washer is cleaned of periphyton by scraping or cutting long filaments with a knife or scalpel.
* Collect the scraped material into the white enamel tray containing ~100 mls of tap water. After the 6 substrates from two transects are scraped pour the scrapings into a labeled jar.
* Fill out qualitative field sheet B (Appendix B) and describe the micro and macro algae on the stone and collect samples for ID’s of soft-bodied algae.

***How to construct the sampler chlorophyll a for use on cobbles and larger stone substrates***

*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.*

Figure 7: Natural substrate sampling device.



*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.*

* The surface is scrubbed with the toothbrush and the bits are removed by spraying with the wash bottle by holding the cobble with the top surface facing down so that the wash material can slough off into the second enamel pan.
* The cobbles are cleaned either into a pan or directly into the 0.5 l sampling jar.
* The subsamples from transects 1 and 2 are composited as well as 3 and 4.
* A total of 12 samples should be collected.
* Repeat process for other rocks and composite transects 1 and 2 rock-scrapings into a graduated beaker. (rinse the tray and equipment to ensure all algae are in the beaker). Pour the sample from the beaker into an amber, wide-mouth, nalgene bottle (at least 500ml in size). Repeat for transects 3 and 4.
* Record the number of rocks scraped for chlorophyll analysis.

Record surface area:

1” circle = 5.067 cm2

18 circles = 91.027 cm2

|  |  |
| --- | --- |
| Label bottles with the following information | |
| Type of sample | location |
| Bottle number | Type of sample |
| Stream name | Number of rocks |
| Town | Volume of sample |

* Thoroughly clean all equipment, especially brush bristles, in water before leaving stream. Discard brushes if they get too grimy or difficult to clean.
* The samples are brought back to the lab, logged in and refrigerated until processed.
* The following AM (within 24 hours) the samples are taken from the refrigerator, the contents of the first sample emptied into the 1 liter plastic graduated cylinder and the volume brought up to 300 mls.
* The sample is poured into a beaker. Remove any pieces of moss present. If macroalgae long filaments) are present then either a stainless steel Waring blender or a Fisher Scientific PowerGen 35 hand held tissue grinder is used to cut up these fragments.
* After the sample is cut up and mixed a 5 ml aliquot is quickly removed using a disposable pipette. It is filtered onto a glass fiber filter, put into a plastic petri dish, labeled, covered with aluminum foil and put in the freezer until it can be ground and analyzed within 21 days of collection (SOP CN003.4 Draft Extracted Chlorophyll a Fluorometric Method).
* If diatom samples are being collected for the contract lab the remainder of the sample should be put into a sample bottle preserved with glutaraldehyde or Lugol’s at this time.

Based in part upon:

Biggs, B.J.F. and C. Kilroy. 2000. *Stream Periphyton Monitoring Manual*. New Zealand Ministry for the Environment. NIWA, Christchurch, NZ

Danielson, T. (2006) *Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands*. Maine-DEP-Bureau of Land and Water Quality, Division of Environmental Assessment, Biomonitoring Program**.**18 p.

Massachusetts Division Watershed Management. 2011. *SOP CN003.4 Draft Extracted Chlorophyll a Fluorometric Method*. Worcester, MA

Areas Lacking Suitable Hard Substrates for Chlorophyll a Samples

Equipment:

* field sheets
* clip board
* vials
* camera
* 12 tiles per site
* 12 “tile baskets” per site
* GPS
* toothbrush
* knife/razor blades
* white enamel tray
* wash bottle
* cooler
* boots
* tent pegs for anchoring baskets
* Mark out the starting point for the 4 transects.
* Percent cover is recorded as for hard substrates,
* After completing the percent cover transects place on the stream bed (right, left and mid stream locations) a plastic tray filled with ambient sediment or cobble, anchor it if needed using a steel tent peg and then place an unglazed clay terra cotta tile (two inch square) on top.
* Take a GPS reading and/or tie surveyors tape to a tree or plant to mark the location of the transect.
* The tiles are deployed for three weeks.
* When the tiles are to be retrieved, start at the downstream end of the sampling reach and retrieve each tile tray and carry to shore.
* At the shore the surface of each tile will be scraped and brushed into a small amount (50 mls) of filtered ambient water or bottled water. The scraped material will be composited and put in one container.
* Record how many tiles were retrieved and their dimensions.

How surveys will be conducted

When possible, a crew of two will reconnaissance the stream segments before sampling is to occur. If possible, the periphyton station will be within the reach chosen for macroinvertebrate sampling. An effort will be made to locate sites with at least partially open canopy, riffle/runs, and cobble bottom, but professional judgment can be used when needed to allow stations that do not completely meet the criteria. The field crew will characterize the reach as being riffle/run dominated or pool/run by observations made either from road crossings (or other field observations), map work or Google earth interpretations. The crew will also determine if substrates are unconsolidated, cobble/gravel, boulder or bedrock. If unconsolidated silt and sand is dominant then unglazed tiles may be deployed.

The actual survey crew will require a minimum of 3 people, although 4 is preferred. If percent cover is done as part of the macroinvertebrate survey then field sheet Qualitative Periphyton Field Data Sheet B will be filled in. Sampling should include two riffle/run areas.

If natural substrates are available only one survey will be needed, but if only unconsolidated substrates are available then tiles will be deployed and will need to be recovered after 3 weeks.

Collection of Diatom Samples

Diatom samples will be collected as part of the periphyton analysis. Diatoms represent an important part of the periphyton community that can provide information on nutrient levels and other stressors in-stream. The sample of algae scraped from the hard substrates described above will be used for the diatom sample. After the 5 ml sample is drawn off for chlorophyll analysis, the sample will be remixed and then 300 mls will be poured into an amber Nalgene wide-mouth jar and either glutaraldehyde or Lugol’s added as a preservative.

References

Barbour, M. T., Gerritsen, J., Snyder, B. D. and J. B. Stribling. 1999. *Rapid Biological Assessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*. 2 nd edition. USEPA, Office of Water, Washington, D. C.

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Biggs, B.J.F. and M. E. Close. 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology*. 22:209-231.

Biggs, B.J.F. and C. Kilroy. 2000. *Stream Periphyton Monitoring Manual*. New Zealand Ministry for the Environment. NIWA, Christchurch, NZ

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Fritz, K. M., Johnson, B. R. and D. M. Walters. 2006. *Field Operations Manual for Assessing the Hydrologic Permanence and Ecological Condition of Headwater Streams*. EPA/600/R-06/126. U.S. Environmental Protection Agency, Office of Research and Development, Washington DC.

Meek, J. 2011. Human Disturbance Index. Massachusetts Division of Watershed Management. Worcester, MA.

### Oak_deplogoAppendix A: Massachusetts DEP Viewing Bucket Survey Data Sheet-A

Date: Viewer: Recorder:

Watershed: Waterbody: Location:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Filamentous or Other Macroalgae** | | | Periphyton Mat Includes microalgae, detritus, decaying macroalgae. | | | | | |
| **Transect/**  **Sample** | **Clay, Sand, or Mud** | Plant | **Moss** | **1**  < 2 cm long | **2**  2 cm and  < 15 cm long | **3**  ≥ 15 cm long | **0**  no visible layer/thin film-may be slippery, but underlying rock is visible | **1**  thin layer of algae-underlying rock not visible | **2**  0.5 - 1 mm thick-can be scraped with a fingernail | **3**  1-3 mm thick | **4**  >3 mm thick | **5**  >2cm thick |
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| **TOTAL** |  |  |  |  |  |  |  |  |  |  |  |  |
| **ID of plants/ Moss:** | | | | |  |  |  |  |  |  |  |  |



