pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

BUREAU OF CLEAN WATER

PENNSYLVANIA DEP FIELD PROTOCOL: PERIPHYTON STANDING CROP AND SPECIES ASSEMBLAGES

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Introduction

Benthic algal communities are significant primary producers in wadeable streams and can be utilized as indicators of environmental conditions (Barbour et al. 1999). Periphyton standing crop and community composition are influenced by nutrient levels, light, hydrologic condition, temperature, substrate type, and herbivore grazing (Stevenson, 1996). Accrual of benthic algal standing crop depends primarily on the level of two resources: nutrients and light (Biggs, 1996). Generally, filamentous chlorophytes become abundant sometimes creating nuisance conditions when high nutrient levels and adequate sunlight are provided. Studies have shown that phosphorous concentrations of only 25 to 50 µg/l orthophosphate are needed to maintain maximum benthic algal biomass in streams (Borchardt, 1996). Nuisance levels of algae and periphyton community shifts associated with high nutrients may cause water quality impairment (e.g. low dissolved oxygen), negative impacts to macroinvertebrates and fish, and/or aesthetic impairments for water uses (Dodds and Welch, 2000).

The purpose of this document is to provide a statewide standard field protocol for the collection of data that may be considered in conjunction with other chemistry and biological data to assess nutrient impairment as part of the Department's Instream Comprehensive Evaluations and provide nutrient and biological response data to support Pennsylvania's nutrient criteria development. Field methods described include: 1) epilithic periphyton sampling for standing crop, community assemblage, and cellular nutrient concentration, 2) periphyton field description, and 3) physicochemical site conditions at the time of sampling.

Epilithic Periphyton Sampling

Epilithic periphyton sampling is conducted using a **P**ennsylvania **E**pilithic **P**eriphyton Sampler (PEP Sampler). The PEP sampler provides a fixed circular sample area (17.8 cm^2 , 2" to 1.5" PVC Step Down Fitting) with a foam gasket seal (toilet seal) that is clamped to rock substrates (large gravel, cobble, small boulder) using Trex^R clamping boards that can be tightened with quick release 5-star knobs (Rockler.com) or wing nuts on threaded rods (Figure 1).

Select a riffle that is visually representative with regard to substrate size, shading, and periphyton standing crop of riffle habitat at the station you are sampling. Riffles are the priority richest targeted habitat for quantitative sampling described in USGS's Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program (Moulton et al. 2002). If riffles are absent, a run should be sampled. Visually stratify the riffle/run into thirds (upper, middle, lower), and randomly place one flag marker along the bank of the stream in each segment. Markers denote the location of three sampling transects perpendicular to the stream and should be placed without looking closely at the streambed or periphyton condition to help ensure unbiased sampling. The collector should not walk through the areas to be sampled prior to rock collection and should work downstream to upstream to ensure the periphyton community is undisturbed prior to sampling.

Three rocks are randomly collected for each transect. Three random numbers (0.00 to 0.99) are generated for each transect using two different colored 10-sided die (ex. die #1 = 5, die #2 = 8, percentage = 0.58). Wetted width for each transect is measured (left bank to right bank looking downstream). The wetted width is divided by 3 to stratify substrate collection locations into left, center, and right 1/3 of the channel along the transect. One third of the channel width is multiplied by the first random number to determine collection location 1; 1/3 channel width is

multiplied by the second random number and 1/3 channel width is added to determine collection location 2; 1/3 channel width is multiplied by the third random number and 2/3 channel width is added to determine collection location 3. The three rocks along each transect are composited (Figure 2). When retrieving rocks the collector should reach down at the collection location and pick a rock by touching it lightly with a finger while refraining to look at the streambed. If the rock is a suitable size for periphyton sampling (will accept a 2 inch diameter seal) it is placed in an empty dishpan (for transport to the PEP sampler. If the substrate is too small to obtain an adequate PEP sampler seal or too large to remove (large boulder, bedrock), the collector should retrieve the closest rock to the selected rock that is of suitable size for sampling. Dishpans can be covered to protect samples from sunlight and desiccation prior to scraping.

Clamp rocks to the PEP sampler. Prior to sampling, check to ensure that water is ponding around the gasket indicating a tight seal. Attached algae is removed by repeated scrubbing with a modified grout brush, rinsing with distilled or deionized water and transferring the slurry to a plastic sample container (approx. 1 liter) with a modified wide bore disposable pipette or turkey baster. Removal of filamentous green algae (ea. *Cladaphora*) may require cutting/scraping with an Exacto knife. Scrub the area for a minimum of 60 seconds until the area is visibly devoid of algae. Rinse water will appear clear after sampling unless substrates are easily erodable (ea. shale, sandstone). Slurry from the three transects (3 rocks each) are initially composited in separate sample containers. Total sample volumes are determined by pouring the algal slurries into a 500 ml volumetric cylinder. A funnel should be used for transfers to minimize spillage. Slurries can be processed in the field or returned to a regional laboratory for processing.

Samples containing filamentous macroalgae require blending utilizing a blender or food processor to homogenize the sample by breaking apart filaments. Well-mixed subsamples (shaking a wide-mouth bottle, or blending) from each transect composite are taken for 1) Chlorophyll-a and Phaeophytin analysis, 2) cellular carbon and nitrogen, and 3) cellular phosphorous. Subsamples for filtration are taken using a Hensen-Stempel pipette (2ml) or a conventional “to deliver” pipette with a modified wide bore tip. Chl-a samples are filtered onto glass fiber filters (Whatman EPM 2000, 0.3 μm, 47mm) using a filter apparatus and vacuum pump ($\leq$ 10 psi). Chl-a filter volumes are dependent upon algal slurry concentrations but should be in the range from 2ml (eutrophic, unshaded) to 10 ml (oligotrophic,shaded). After releasing the vacuum, dry filters are removed from the filter apparatus using flat-ended filter forceps. Filters are quartered and wrapped in aluminum foil. Cellular carbon and nitrogen samples are filtered onto glass fiber filters (Whatman EPM 2000, 0.3 um, 47mm). NC Filter volumes are dependent upon algal slurry concentration but should range from 10ml to 30ml. Cellular P samples are filtered onto polycarbonate filters (Millipore Polycarbonate, 0.2um, 47mm). P Filter volumes are dependent upon algal slurry concentration but should range from 10 ml to 30ml. Cellular P filtration is a time limiting step because of the slow filtration rate. If available, two filter apparatuses will help shorten processing time. As with the Chl-a filters, NC and P filters are quartered and wrapped in aluminum foil. Care should be taken to only handle the cellular nutrient filters with forceps to avoid potential contamination. Filters for each station should be stored on ice (if field filtered) in separate bags and frozen upon returning to the lab.

The remaining transect algal slurries are composited and a well-mixed subsample is taken for algal identification and enumeration. A 100 ml subsample is transferred to a 125ml Nalgene bottle and preserved with 3.5% Lugols (3.5ml) for algal identification and enumeration.

Labels for all processed samples should include station ID, date, subsample type (Chl-a, NC, P, Algal ID) and subsample volume. Total surface area for each transect (ea. 3 rocks x 18.1 cm^2 = 54.3 cm^2), total transect volumes, chl-a subsample volumes, total composite volume, CN and P subsample volumes and algal ID volumes are recorded on the Periphyton Survey Data Sheet (Appendix 1).

Samples for the WQN/Nutrient Criteria Study or TMDL data collection should be shipped to the Penn State University Phycology laboratory. Filtered samples should be shipped frozen (dry ice) and algal ID samples should be shipped at room temperature. Chl-a, CN, and P samples should be shipped within 14 days of sampling and analyzed within 30 days of sampling. A shipping/chain-of-custody form will be made available to collectors.

QA – One replicate subsample will be collected for Chl-a, CN, P (transect A,B, or C) and Algal ID (composite) for every 10 stations sampled (10%) to examine subsample variability. Replicate information should be on the field form to the right of the routine volume information and marked with an R.

Figure 1. Pennsylvania Epilithic Periphyton Sampler.

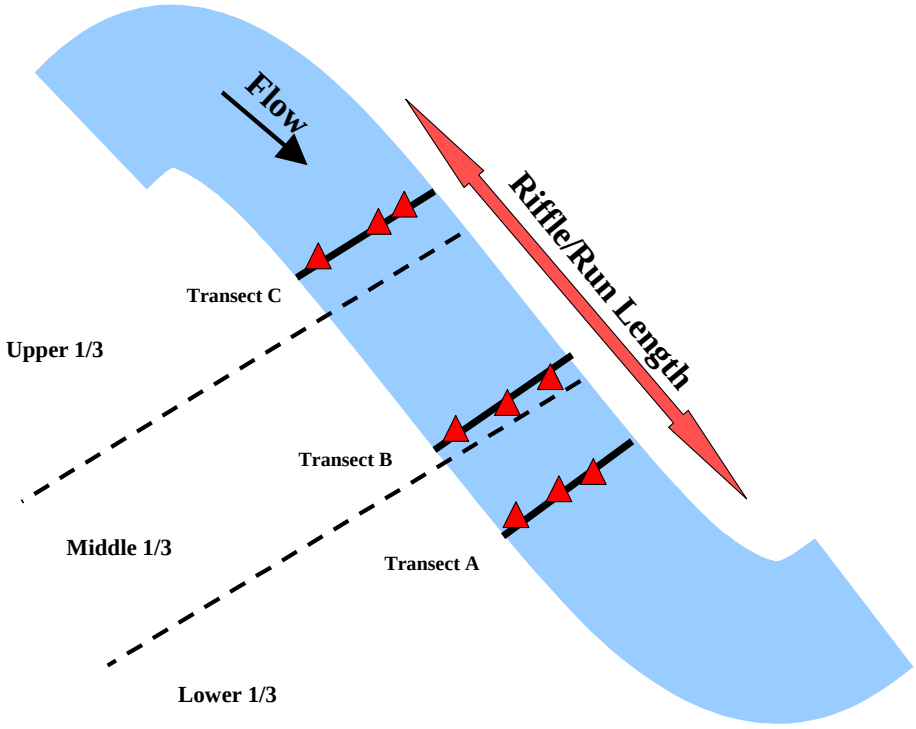

Figure 2. Schematic of nine rock, 3 transect composite sample in riffle/run.

Periphyton Field Description (Optional)

Biggs et al (2000) and Stevenson (1999) describe methods for rapid assessment protocols to quantify types of periphyton coverage. The procedure described here simplifies and borrows from Biggs (2000) Rapid Assessment Method 2 to provide a qualitative field description (% cover by type, color, growth form) of periphyton assemblages on substrates sampled for standing crop and species assemblage. This visual estimate of coverage will provide some description without significantly increasing sampling effort at each station. Visual descriptions may provide an inexpensive screening tool if they are found to correlate with periphyton standing crop. After collecting the three rock periphyton samples for a transect the collector estimates the percent coverage by algal type (to the nearest 10%) and dominant color of the attached algae based upon the periphyton field identification chart found in Appendix 2 (https://www.niwa.co.nz/our-science/freshwater/tools/shmak/identification). Macrophyte coverage (ea. moss or river weed) should be noted. In addition to the visual description, photographs of representative rocks with the station and date identified on a 3X5 index card is also recommended to document periphyton conditions.

Physicochemical Site Conditions

Water Chemistry – Field measurement of Dissolved Oxygen, % DO, temperature, specific conductance, and pH should be conducted in the thalweg. Meters shall be calibrated as outlined in the Department's Surface Water Collection Protocol (DEP 2013). Samples for nutrient analyses should be collected in the thalweg at mid-depth. One 500 ml Nalgene unfixed and one 125 ml Nalgene fixed with H_2SO_4 (pH < 2) are required for total phosphorous, (holiding time = 28 days), total nitrogen (holding time = 28 days), and alkalinity

(holding time = 14 days). Standard analysis code (SAC) 047 can be used for this parameter suite. Chemistry sampling should be conducted upstream of any disturbance associated with algal sampling.

QA – One field replicate chemical sample will be taken for every 10 stations (10%) sampled to examine sampling precision. Samples should be collected in a stream water rinsed 500 ml Nalgene bottle, mixed well, and subsampled by pouring into stream water rinsed sample and replicate bottles. One field blank chemical sample will be submitted to the lab for every 20 stations (5%) sampled to examine potential bottle contamination and lab errors.

Flow – If the sampling station is located at a USGS gaging station, stream discharge can be recorded from USGS gage data for the appropriate date and time. The instream discharge measurement methods described here are modified from Gorden et al (1992). A straight reach is chosen having relatively uniform depth, width, velocity and slope. Sites having back eddies, dead water zones or extreme turbulence should be avoided. A measuring tape is strung across the flow measurement transect (left bank to right bank) perpendicular to the flow and the stream is divided into subsections within which velocity and depth are measured along verticals at the midpoint. Depending on width, the stream can be divided into 0.5, 1, 2, 3, 4, or 5 foot subsections in order to obtain a minimum of 15 verticals. Subsection midpoint velocities are measured at four-tenths of the depth (0.4D) from the streambed for water depths less than 1.6 ft. Velocities are measured at 0.2D and 0.8 D when water depths are greater than 1.6 ft. Length of the subsection (ft) is multiplied by depth (ft) and velocity (ft/sec) to calculate the subsection discharge (cfs). Subsection discharges are summed to calculate stream discharge. Velocity measurements should be taken with the rod held vertically, the meter facing upstream, and the observer standing to the side of the meter and downstream.

Hydrologic Condition – High flows and resultant scour can significantly reduce periphyton standing crop in nutrient enriched streams. Nutrient / chl-a standing crop relationships have the potential to be obscured if periphyton is sampled directly after scour events. The collector will need to monitor regional weather and flow conditions and sample during extended dry periods. Biggs (2000) indicates that as much as four weeks of stable flow may be necessary for periphyton communities to regain biomass and species diversity that was present prior to a flood which caused bed load movement and subsequent scour. Biggs estimates scour inducing flows as 5 to 6 times the average flow for a given time period. Collectors can refer to USGS's PA gaging station web site which provides real time flow conditions that include average and median flow statistics (http://waterdata.usgs.gov/pa/nwis/current/?type=flow&group_key=basin_cd). Collectors should attempt to sample periphyton following at least 14 days of stable low flow conditions (dry weather). The collector should estimate and record the number of stable low flow condition days prior to sampling.

Stream Size – Two simple measures of stream size should be recorded. Determine the Strahler Stream Order for the sampling station from the Pennsylvania streams GIS layer or topographic maps. Measure the wetted width of the stream at each sampling transect and calculate an average width. Drainage area data is also available for WQN stations.

% Inorganic Substrate – Visually estimate (to the nearest 5%) the percentage of inorganic substrate sizes found in the sampled riffle/run. Assign a percentage to each of the seven substrate sizes so that they sum to 100%.

Canopy Closure / Canopy Density – In small streams, riparian leaf canopies can intercept 95% of incident solar radiation (Borchardt, 1996). Benthic algal standing crops, as chlorophyll-a, can be 4 to 5 times higher at open canopy sites than closed canopy sites (Hill, 1996). Additionally, during summer conditions, East/West oriented streams can receive significantly more light than North/South oriented streams with similar canopy.

Stream orientation and canopy closure/density are measured with a compass and a Model C Concave Spherical Densiometer respectively. Facing downstream at midreach, midchannel, site the compass along the stream direction and orient the compass with magnetic north. Record the azimuth (horizontal direction in degrees) on the data sheet.

Methods for estimating canopy closure and canopy density follow Platts et al. (1987). Vegetative canopy closure is the area of the sky bracketed by vegetation, whereas canopy density is the amount of sky blocked by vegetation. Canopy closure is measured during leaf-off and canopy density is measured during leaf-on. The densiometer's concave mirror surface has 37 grid intersections forming 24 squares. To eliminate bias from overlap, only 17 of the 37 grid intersections are used as recording points. The 17 grid intersections to be used are delimited by taping a right angle on the densiometer (Figure 3).

For smaller streams (Strahler Order 1 to 4) the densiometer is held in the hand on the midreach transect line, perpendicular to the right wetted edge of the stream, 1 foot from the shoreline and 1 foot above the water surface. The leveled (bubble level) densiometer is pointed toward the bank (taped right angle points toward the recorder) with the observer's head reflection near the top grid line. The number of line intersections (maximum of 17) that are surrounded by vegetation (canopy closure during leaf-off) or intercepted by vegetation (canopy density during leaf-on) are counted. This same procedure is repeated at midchannel looking upstream, midchannel looking downstream, and along the left bank shoreline. Canopy closure or density scores are summed and divided by 68 to calculate a percent closure or density. Right and left bank designations are determined when looking downstream.

For larger streams (Strahler Order 5 to 7) the same procedure is used except eight recordings are made so that shore/bank recordings do not over influence mid channel readings. In addition to right bank, left bank, and mid channel (upstream and downstream) recordings, recordings are also made at the ¼ and ¾ interval across the transect. Canopy closure or density scores are summed and divided by 136 to calculate a percent closure or density.

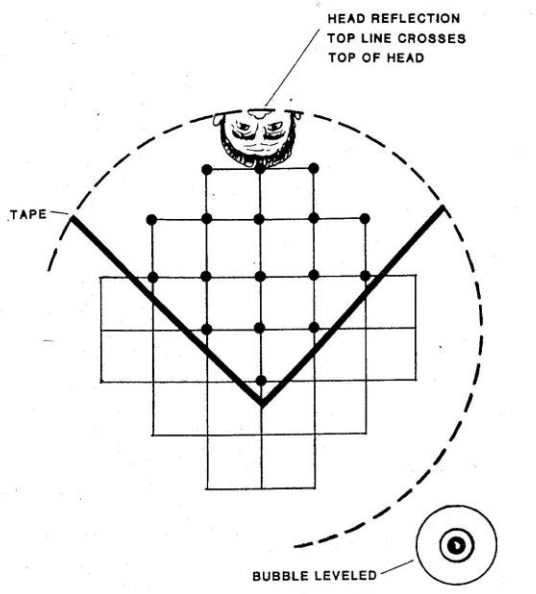

Figure 3. Concave spherical densiometer with placement of head reflection, tape and 17 observation points. From Platts et al. (1987).

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APPENDIX 1

FIELD DATA FORMS

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

PA DEP Periphyton Survey Data Sheet

Site Information			
Station ID		Latitude (NAD 83)	
Chem ID		Longitude (NAD 83)	
Date			
Time		Days of stable low flow conditions prior to sampling.	
Personnel			

Rock Substrate Sampling Information										
	Transect A			Transect B			Transect C			Composite
Transect Width (ft)										
Random Percentages	1.	2.	3.	1.	2.	3.	1.	2.	3.	
Collection Location	1.	2.	3.	1.	2.	3.	1.	2.	3.	
Surface Area (cm^2)										
Total Volume (ml)										
Chl-a Volume (ml)										
Cellular P Volume (ml)										
Cellular N, C Volume (ml)										
Algal Id Volume										

Physicochemical Site Conditions				
Stream Order			Water Temperature	
% Inorganic Substrate			Sp. Conductivity	
Bedrock			pH	
Boulder		>10 in	DO	
Cobble		2.5-10 in	%DO	
Gravel		0.1-2.5 in		
Sand		0.06-2mm		
Silt		0.004-0.06 mm		
Clay		<0.004 mm		

Stream Orientation	

Spherical Densiometer	Canopy Closure Leaf-Off	Canopy Density Leaf-On	
Right Bank			
1/4 Interval Upstream			> 4th order
1/4 Interval Downstream			> 4th

			order
1/2 Interval Upstream			
1/2 Interval Downstream			
3/4 Interval Upstream			> 4th order
3/4 Interval Downstream			> 4th order
Left Bank			
Total			

Divide total by 68 (small streams-4 recordings) or 136 (large streams-8 recordings) to calculate percent canopy closure or density.

Discharge Measurement

Transect Width	

	Midpoint	Length (ft)	Depth (ft)	Velocity (f/s)	Discharge (cfs)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
				Total	

Periphyton Field Description (Optional)										
Periphyton Description	**Rock Number**									
	Peri. Score	**Transect A**			**Transect B**			**Transect C**		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**

Thin mat/film: green	7									
(under 0.5 mm) light brown	10									
black/dark brown	10									
Medium mat: green	5									
(0.5-3mm) light brown	7									
black/dark brown	9									
Thick mat: green	4									
(>3mm) light brown	4									
black/dark brown	7									
Filaments, short green	5									
(<2cm) brown/reddish	5									
Filaments, long green	1									
(>2cm) brown/reddish	4									
Other (Moss, River Weed)										
Total		100	100	100	100	100	100	100	100	100

Notes:

APPENDIX 2

FIELD IDENTIFICATION CHART

Reprinted from Biggs and Kilroy, 2000

Periphyton field identification chart. Part 1

(from the New Zealand Stream Health Monitoring and Assessment Kit)

For more information on each periphyton type, see page 45 in this Manual.

Thin mat or film (less than 0.5 mm thick)

Green

Light brown

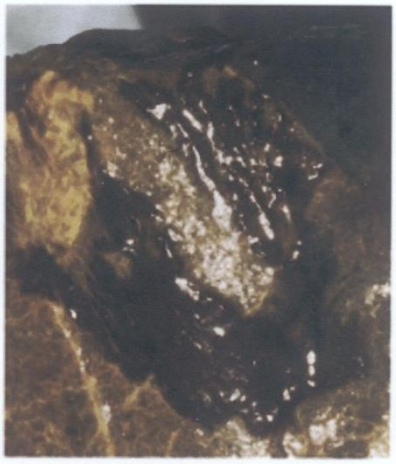
Black/dark brown

Medium mat (0.5 to 3 mm thick)

Green

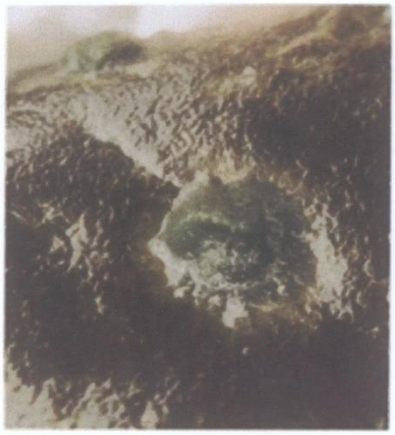
Light brown

Black/dark brown

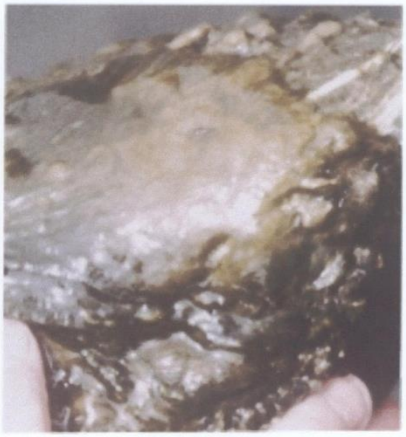


Thick mat (more than 3 mm thick)

Green



Light brown



Black/dark brown

Periphyton field identification chart. Part 2

(from the New Zealand Stream Health Monitoring and Assessment Kit)

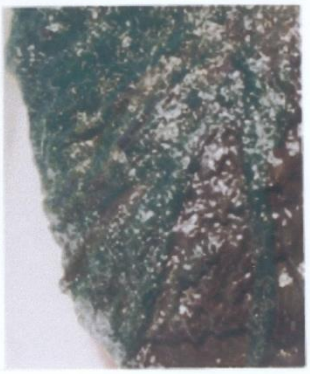
Short filaments (less than about 2 cm long)

Green

Brown/reddish

Long filaments (more than about 2 cm long)

Green



Brown/reddish