GUIDANCE FOR NUTRIENT SURVEYS OF STREAMS AND RIVERS

New Mexico Environment Department
Surface Water Quality Bureau

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Definitions

Algae are non-vascular plants with no true roots, stems, or leaves. They are mostly aquatic and range from tall stalks of kelp to fuzzy growths of green filamentous algae to microscopic silica encased diatoms.

Assessment Unit (AU) is a length of stream defined by SWQB and used to assess waterbodies for the Integrated 303(d)/305(b) List.

Biological Sampling Index Period (BSIP) the time of year in which biological (fish , macroinvertebrates, periphyton) samples are collected for stream biological assessment.

Filamentous algae is algae that grow as fibers or strands of cells forming filaments or mats that are attached to substrate. The filaments can either be branched or unbranched.

Macrophyte is a general term that applies to many types of aquatic vegetation including flowering vascular plants, mosses, and ferns. Four categories of macrophytes are defined by their relationship to the air, water, and substrate: emergent, floating-leaved, submerged, and freely floating (USEPA 2000).

> **Emergent macrophytes** grow on the banks of rivers and streams in depths of water generally less than a meter and are typically rooted in the sediment with a portion of the plant in the water and part extending into the air. Common emergent macrophytes include plants such as reeds (*Phragmites* spp.*),* bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.).
>
> **Floating-leaved macrophytes** are rooted to the river bottom with leaves that float on the surface of the water, such as waterlilies (*Nymphaea* spp.) and spatterdock (*Nuphar* spp.).
>
> **Submerged macrophytes** are a diverse group that grow completely underwater and include mosses (*Fontinalis* spp.*),* stoneworts (*Nitella* spp.) and numerous vascular plants, such as various pondweeds (*Potamogeton* spp. and *Elodea canadensis*), tape-grass (*Vallisneria* spp.), and exotic species including hydrilla and Eurasian watermilfoil.
>
> **Free-floating macrophytes** typically float on or just under the water surface with their roots suspended in the water column. These unattached macrophytes range in size from small duckweeds (*Lemna* spp.) and water ferns (*Salvinia* spp.) to larger surface floating plants such as water hyacinth (*Eichhornia crassipes*).

Periphyton is an assemblage of organisms that grow on underwater surfaces and includes algae, bacteria, fungi, protozoa, and other organisms.

Phytoplankton are small (often microscopic) aquatic plants that are suspended in water.

Rapid Bioassessment Protocol (RBP) is the protocol developed by EPA for collecting, processing, and assessing fish, benthic macroinvertebrates, and periphyton from wadeable streams (Barbour et al 1999).

Introduction

The state of New Mexico currently has a narrative nutrient criterion which states that, "Plant nutrients from other than natural causes shall not be present in concentrations which will produce undesirable aquatic life or result in a dominance of nuisance species in surface waters of the state" (NMAC 2005). This narrative nutrient criterion is challenging to assess. To meet this challenge, SWQB developed a nutrient assessment protocol. This assessment protocol uses a tiered system and a weight of evidence approach. The tiered system is used to screen for potential nutrient impairment, then collect more data from reaches with suspected impairment. The weight of evidence approach uses a number of nutrient variables with threshold values to assess for nutrient impairment. This document describes the procedures for monitoring the nutrient variables used to assess rivers and streams. Generally, streams refer to wadeable systems and rivers are those systems that have drainage areas greater than 2,300 square miles and cannot be monitored effectively with the biological and habitat methods developed for wadeable streams.

Level I Nutrient Survey: Qualitative Measures and Data Review

Level I is a screening level survey that includes collecting on-site observations and measurements of chemical parameters as needed. It is intended to provide the data needed to conduct the Level I Nutrient Assessment (NMED 2005). The assessment should be conducted just prior to the biological sampling index period (BSIP) (August 15 – November 15) to allow sites that do not pass the Level I assessment to be surveyed more intensively during the index period. The Level I Nutrient Assessment uses a qualitative determination of algal biomass in addition to a number of chemical parameters including dissolved oxygen (D.O.) concentration, percent D.O. saturation at the local elevation, and pH as well as total phosphorus and total nitrogen. Typically, all of these parameters are measured as part of the general water quality surveys. The method for monitoring the nutrient variables are described in the New Mexico Surface Water Quality Bureau Standard Operating Procedures for Sample Collection and Handling (SOP) (NMED/SWQB 2004). There is no form for the Level I Nutrient Survey as the data are recorded on the general water quality field sheets and compiled in the SWQB Water Quality Database.

Periphyton and Algae

A qualitative algal biomass survey is part of the Level I Nutrient Survey and is conducted by field crews as part of regular water quality surveys. The information is recorded on the second page (back side) of the field sheets. This survey should be conducted at each sample site and completed once per season, i.e. in spring, summer, and fall. These seasons refer not to the calendar, but to the character of the hydrograph for a given waterbody. Typically in New Mexico, spring is characterized by higher flows resulting from snow melt, while summer and fall are characterized by low flow conditions interrupted by spates from seasonal rain storms. During the summer nutrient survey, the study lead should note which, if any, sites might need to be assessed using a reference approach due to the apparent **naturally high** productivity. This will allow for scheduling of appropriate Level II Nutrient Surveys to be conducted at the test site and a suitable reference site during the BSIP.

In the context of this guidance, periphyton refers to the slime layer or biofilm growing on substrate. It is composed primarily of microscopic organisms while the algae, noted in the percent coverage field, are mainly macroalgae. Algae refers to the visible growth of non-rooted aquatic vegetation attached to the stream substrate. Percent algal coverage and a rating of periphyton growth is a qualitative indicator of algal biomass. An estimate of percent macrophyte coverage will also be recorded. Macrophytes are primarily the large aquatic vegetation rooted in the substrate, although they are also represented by small leaves with roots floating on the surface (duckweed and water fern).

Observations will be made in and around a riffle unless none are present or accessible or the velocity is so great that it limits algal growth (i.e. velocities exceed 1.75 ft/sec) and more algae are found in glides than in riffles. Riffle habitat was selected to help partition the variability in algal biomass and because benthic algal growth is stimulated by moderately high velocities (Barbour et al 1999, Biggs and Kilroy 2000). The Rapid Bioassessment Protocol (RBP) states that "single habitat sampling should be used when biomass of periphyton will be assessed" and "the recommended substrate/habitat combination is cobble obtained from riffles and runs with current velocities of 0.33 - 1.6 ft/sec." (Barbour et al 1999). If the velocity appears to be limiting algal growth, then select a glide (a length of stream with intermediate velocity) to survey. If access to the reach is limited, use a transect across the stream at the sample location. However, effort should be made to find a representative riffle whenever possible.

The methods for examining and rating algal and periphyton cover are described below and include surveys of both river and stream systems. As the aquatic vegetation observations are conducted, check under stones and in depositional areas for the presence of an anoxic layer. Anoxic conditions are characterized by a black color and rotten egg smell associated with hydrogen sulfide.

1) Select a suitable riffle near the water quality sample site.

2) Starting at either the top or bottom of the riffle, visualize a line running diagonally through the riffle. Select a landmark on the far bank that corresponds to the end of the diagonal transect. Note the midpoint, ¼, and ¾ distance. Five observations will be made along this diagonal: near the left bank, at ¼, ½, and ¾ the width, and near the right bank. In this manner, locations across the length and cross-section of the riffle will be observed.

3) From your starting point (on the bank at either the top or bottom of the diagonal through the riffle) observe the transect across the stream and estimate the percent algal cover in the wetted width. Look for strands of non-rooted aquatic vegetation attached to the substrate.

4) Take a step into the stream, reach down and pick up the cobble or large gravel nearest the front of your foot. Observe the particle and note the periphyton thickness and associated rating (see below).

5) Walk along the diagonal, stopping at ¼, ½, and ¾ the width, and near the far bank and make observations of both the algal cover across the wetted width and periphyton thickness on the substrate. If you cannot safely wade across the entire

riffle, go as far as possible then turn back and continue observations on a diagonal back to the bank at the opposite end of the riffle from where you started. For reaches that you cannot wade halfway across, pick up and/or examine the substrate in 5 to 10 location that you can reach and rate the algal and periphyton cover as described below. The observations should be made over an area that is approximately as long as the river is wide.

6) Once you reach theend of the transect or make 5 to 10 observations, record the average percent algal coverage and periphyton rating on the back of the field sheet (example shown below).

7) Note where in the stream growth is occurring (e.g., in low flow areas, only near seeps, on fine substrate, only on large stable substrate, etc). The physical characteristics of the substrate can influence the amount of algal coverage. As some substrates are too soft or unstable for algae to anchor to, the overall percent algal coverage may reflect the amount of stable substrate in the stream rather than the potential biomass.

Periphyton Rating: Look for the presence of slime on the coarse substrate (cobbles and gravel). Rate the periphyton thickness on the substrate using the following scale (Stevenson, 1996):

- 0 indicates substrate is rough with no apparent growth;
- 1 indicates a thin layer of microalgae is visible (tracks can be drawn in the film);
- 2 indicates accumulation of microalgae to a thickness of 0.5-1 mm;
- 3 indicates accumulation of microalgae from 1 mm to 5 mm thick;
- 4 indicates accumulation of microalgae from 5 mm to 20 mm and
- 5 indicates layer of microalgae is greater than 20 mm.

Percent algal cover : <25% 25-50% 50-75% >75% Location:
0 - rough with no apparent growth, **1** - thin layer of periphyton is visible, **2** - 0.5 to 1 mm thick, **3** - 1 mm to 5 mm thick, **4** - 5 mm to 20 mm, **5** - >20mm **Rating of the periphyton on coarse substrate:** 0 1 2 3 4 5
Anoxic layer present in fall: Yes No Location:
Field Notes:

Water Chemistry

Measurements of dissolved oxygen concentration (D.O.), percent D.O. saturation at the local elevation, and pH will be collected during water quality surveys. The assessment will be made with data exported from the NMED database. If the Assessment Unit (AU) being surveyed is not part of a current water quality survey, take measurements of D.O. concentration, percent D.O. saturation at the local elevation, and pH as described in the ***Field Measurements*** / *Instantaneous Field Measurements* section of the SOP.

Total phosphorus, Total Kjeldahl Nitrogen, and Nitrate + Nitrite are also monitored during water quality surveys. Total Nitrogen is calculated by adding Total Kjeldahl Nitrogen plus Nitrate + Nitrite. In the event that Nitrate + Nitrite or Total Kjeldahl Nitrogen are below the detection limit, a value of one half the detection limit will be used (Gilbert 1987). The assessment will be made with data exported from the NMED database. If the AU being surveyed is not part of a current water quality survey, collect water samples for analysis of Total phosphorus, Total Kjeldahl Nitrogen and Nitrate + Nitrite as described in the "Nutrients" portion of the ***Surface Water Sample Collection*** / *Routine Water Chemistry Sample* section of the SOP. Also take flow measurements as described in ***Measuring Flow, Discharge, and Water Levels*** / *Surface Water Flow and Velocity Measurements* section of the SOP if there are no gages in the AU.

Level II Nutrient Survey: Quantitative Measures

The Level II survey involves quantitative measurement of selected indicators. It will be conducted if the Level I Assessment indicates that nutrient impairment may be occurring in an AU or if the AU was previously listed for nutrient impairment. This survey should be conducted during the BSIP. Normally, during this time there is the potential to have higher concentrations of plant nutrients in the stream and to see the effects of higher water temperatures and maximum solar gain from the summer conditions. Higher temperatures tend to enhance algal growth, photosynthesis, and respiration resulting in greater variation in diurnal DO and pH values (Fisher and Grimm 1983). The survey should be conducted at least 3 weeks after a scouring high flow event (Biggs and Kilroy 2000). Look for evidence of high flows on site and consult regional gage data.

All data from the survey should be recorded on the Level II Nutrient Survey Form found in Appendix A. Examples of tables found on this form are shown throughout this guidance document for discussion purposes.

Before selecting a location for the survey, note the character of the stream while driving to the site and walk a couple of hundred meters of the stream to ensure that the sampling station is representative of the reach being characterized. Collect the following data from each study site.

Location and Photographs

Describe the site location and record a GPS reading for latitude, longitude, and elevation. Take photos of the reach being studied, including the stream, substrate, and riparian area. Include the date, time, and personnel as well as description of photo location and content.

Site description

Note features that influence the supply, transport, and cycling of plant nutrients (Fisher and Grimm 1983). The shaded areas in the following table can be filled in after the field visit from the habitat and field forms, if a habitat survey is conducted, otherwise estimate values. Fill in the data from the riffle or other habitat where the periphyton sample was collected, not the whole reach. Circle either the "m" or "e" to indicate whether the value is measured or estimated. Note the presence and location of watercress in the stream as it can indicate the presence of springs that may be a natural source of nutrients. Watercress is an emergent macrophyte that can be identified by the following (USDA 1988): (1) it has small whitish flowers with four petals, several arranged closely, (2) has roots from stem nodes, and (3) has pinnately divided leaves with very large terminal leaflets on

mature leaves (see photos). Note the location of springs, wetlands, and upwelling areas. Upwelling areas may be indicated by patches of dense algal growth. Observe the color of the water as this may affect the availability of light in the system or indicate the presence of phytophankton, effecting primary production occurring in the water column. Observe the riparian area, noting the height, density, and type of streamside vegetation. Also, note areas where riparian vegetation has been removed. Note the current flow condition and look for evidence of recent high flow events. Evidence of recent high flows includes flood debris on the bank or in riparian vegetation and vegetation on the banks that is bent over in the direction of flow. Also, look at gage data where available.

Watercress present: Yes No Location:		
Location of springs, wetlands, and upwelling areas**:**		
Turbidity (ntu): m e	Water Color:	Average Depth of riffle: m e
Describe Riparian Corridor:		
Average % canopy cover:	Range of velocities:	
Evidence of recent scouring or deposition: Yes No		
Time since last high (≥bankfull) flow: < 1 month 1-3 months >3 months		
Current flow conditions: 0 1 2 3 4 5		
0 - dry channel (no surface or shallow subsurface water apparent) **1** - no flow (interrupted pools with no obvious shallow, subsurface flow between isolated pools) **2** - low flow (little surface or shallow subsurface flow between isolated pools) **3** - moderate flow (obvious flow, but substantially below bankfull) **4** - high flow (water level is at or near bankfull) **5** - flood flow (water level is above bankfull)		

Diurnal Cycles

A multiparameter meter (sonde) should be deployed for at least seven days and set to record hourly D.O., pH, specific conductance, temperature, and turbidity values.

Where conditions allow, the sonde should be placed upright in the stream in a location with significant flow, placed in PVC pipe, and secured to a T-post, with nylon straps or hose clamps (see photo). The PVC pipe should have holes in it to protect the sonde from debris, while allowing water to flow past the probes. The sonde should not be touching the bottom of the stream. In addition, the sonde should be further secured with a chain or cable and padlocked to a tree or other stable object on shore, and should be placed where it is not easily detectable. If the sonde cannot be placed upright it may be laid on the substrate in the PVC pipe and secured to a stable object. If the sonde cannot be safely deployed due to potential vandalism or likely high flow events, it should not be deployed for long term unattended logging.

Fill in a sonde deployment/retrieval field sheet (Appendix A) completely.

Water Chemistry

Water samples should be collected for analysis of Total Suspended and Total Dissolved Solids (TSS/TDS), total phosphorus, and nitrogen concentrations. The primary inorganic forms of nitrogen are ammonium (NH_4+), nitrate (NO_3-), and nitrite (NO_2-). Kjeldahl nitrogen is the nitrogen in the form of organic proteins or their decomposition product ammonia, as measured by the Kjeldahl Method. Dissolved nutrients may also be collected. Orthophosphate (PO_4^{-3}) is the soluble, reactive form of phosphorus that is readily available for plant uptake. See the sections on TSS/TDS and Nutrients of the SOP for sample collection and preservation methods (NMED/SWQB 2004).

Algal Bioassays

If stream observations indicate that algal biomass is a problem in the stream or an NPDES permit is being written for the Assessment Unit, a limiting nutrient analysis and algal growth potential test may be performed (USEPA 1975 and 1978). Two one-gallon samples of water (no acidification) should be collected and immediately stored on ice. Deliver samples to UNM Biology Department within two days of collecting the samples. Please call Dr. Barton @ 505-277-2537 before taking the samples to his lab. Attach results to the survey form when received.

Algal Sampling

Observe the stream substrate and note the dominant and subdominant size classes (e.g. silt/clay, sand, cobble, etc.). Collect a sample of benthic algae from a known area of substrate for analysis of Chlorophyll *a* concentration, Ash Free Dry Mass (AFDM), and community composition. Record the sampling information in the following table found on the survey form. It is important to accurately measure and record the area and volumes so the final concentration per area of substrate may be calculated. The methods for collecting and handling periphyton samples are described in Appendix B (NMED/SWQB 2004). In rivers an algal biomass sample may also be collected from the water column by the methods described in the **Protocols For The Limnological Evaluation and Water Quality Assessment of New Mexico Lakes and Playas/** Methods for Collecting Water Quality Data on Chlorophyll

Habitat Sampled:	**Number of replicate filters:**	**Number and Type (or area) of delimitor:**
Dominant Substrate:	**Subdominant Substrate:**	
Total volume (mL):	**Volume filtered (mL):**	**Volume for ID (mL):**
Field Notes(observations on sample collection)**:**		

As the periphyton sample is collected, a qualitative survey of aquatic vegetation and periphyton may be conducted (Stevenson, 1996). Each rock that is picked up for periphyton collection is rated for periphyton thickness and macro-algae cover. The transect where the periphyton sample was collected is rated for macrophyte cover. At the sample location, an estimate of water depth and a general rating of the velocity is recorded. The ratings are recorded on the Aquatic Vegetation Evaluation Sheet (Appendix A). Rate the periphyton, macro-algal, and macrophyte cover separately using the following scales:

Periphyton:

- 0 indicates substrate is rough with no apparent growth;
- 1 indicates a thin layer of microalgae is visible (tracks can be drawn in the film with the back of your fingernail);
- 2 indicates accumulation of microalgae to a thickness of 0.5-1 mm;
- 3 indicates accumulation of microalgae from 1 mm to 5 mm thick;
- 4 indicates accumulation of microalgae from 5 mm to 20 mm and
- 5 indicates layer of microalgae is greater than 20 mm.

Macro-algal and macrophytes:

- 0 indicates no macrophytes or macro-algae present;
- 1 indicates some (but < 5% coverage) macrophytes or macro-algae present
- 2 indicates 5-25% cover of substratum by macrophytes or macro-algae
- 3 indicates 25-50% cover by macrophytes or macro-algae
- 4 indicates 50-75% cover by macrophytes or macro-algae and
- 5 indicates >75% cover by macrophytes or macro-algae

Benthic Macroinvertebrates

Samples of benthic macroinvertebrates should be collected from the reach being characterized and a suitable reference site, if possible. Record the sampling information and name of recommended reference site on the following table. Methods for collecting and handling benthic macroinvertebrate samples are described in the *Macroinvertebrate Sampling* section of the SOP (NMED/SWQB 2004). These methods describe techniques for sampling in wadeable and non-wadeable waterbodies as well as those with low gradient and fine substrate. The benthic community should be assessed using the currently accepted NMED assessment protocol.

Date:	**Sample method:**
Reference site:	
Field Notes:	

Landscape Features

If a site appears to be impaired, estimate the extent of the impacted area (i.e. the distance of the stream that is impaired). Note where indicators of nutrient enrichment change and regional geology and landscape patterns that may influence nutrient loading and cycling (e.g., an alluvium to bedrock transition, open basin to canyon transition, etc.).

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Appendix A: Level II Nutrient Survey Forms

Level II Nutrient Survey Form

Photographs: take photos of the stream, substrate, and riparian area.

Site Location:	
Date of survey:	Investigators:
Description of photo locations and content:	

Site description: note feature that influence the response variables, shaded areas can be filed in after the field visit from the habitat and field forms

Watercress present: Yes No Location:		
Location of springs, wetlands, and upwelling areas**:**		
Turbidity (ntu): m e	Water Color:	Average Depth of riffle: m e
Describe Riparian Corridor:		
Average % canopy cover:	Range of velocities:	
Evidence of recent scouring or deposition: Yes No		
Time since last high (≥bankfull) flow: < 1 month 1-3 months >3 months		
Current flow conditions: 0 1 2 3 4 5		
0 - dry channel (no surface or shallow subsurface water apparent) **1 -** no flow (interrupted pools with no obvious shallow, subsurface flow between isolated pools) **2 -** low flow (little surface or shallow subsurface flow between isolated pools) **3 -** moderate flow (obvious flow, but substantially below bankfull) **4 -** high flow (water level is at or near bankfull) **5 -** flood flow (water level is above bankfull		

Nutrient sources: Fill out Pollutant Source Documentation form and identify possible sources of plant nutrients to the survey reach.

Field Notes

Sonde: deploy Sonde set to take readings for seven days (3 to 14 days acceptable) fill out and attach Sonde Deployment Form.

Field Notes:

Water Chemistry: collect samples for analysis of total and dissolved plant nutrients.

Algal Sampling: collect samples of benthic algae for analysis of Chlorophyll a concentration, Ash Free Dry Mass, and community composition.

Habitat Sampled:	**Number of replicate filters:**	**Number and type (or area) of delimitors:**
Dominant Substrate:	**Subdominant Substrate:**	
Total volume (mL):	**Volume filtered (mL):**	**Volume for ID (mL):**
Field Notes(observations on sample collection**:**		

Benthic Macroinvertebrates: Collect benthic macroinvertebrates from the reach being characterized and a suitable reference site.

Date:	**Sample method:**
Reference site:	
Field Notes:	

Algal Bioassays: Collect 2 gallons of water for limiting nutrient analysis and algal growth potential tests. Attach results when received.

Date collected:	**Date to UNM:**
Field Notes:	

Aquatic Vegetation Evaluation Form

Site Location:_____

Step	Depth (cm)	Velocity Rank (0-3)	Rock Diam. (mm)	Macro-Algae (0-5)	Periphyton (0-5)	Macro-phyte (0-5)	Anoxic Layer (Y or N)

Type of macrophytes and comments:

Velocity rating:
0 : minimal flow, 1 : moderate, 2 : fast, and 3: very fast

macrophytes and macro-algae
0 : none present
1 : < 5% coverage
2 : 5-25% cover
3 : 25-50%
4 : 50-75%
5 : >75%

Periphyton
0 : rough with no apparent growth
0.5 : slimy, but biofilm is not visible
1 : thin layer of periphyton is visible
2 : thickness of 0.5-1 mm
3 : 1 mm to 5 mm thick
4 : 5 mm to 20 mm
5 : >20 mm

Nutrient Survey Equipment Checklist

Water Quality

- ❑ Clipboard
- ❑ Pencils
- ❑ Sharpies
- ❑ Site list/directions
- ❑ Access authorization documents
- ❑ Field sheets
- ❑ Lab forms
- ❑ Stickers
- ❑ Water carboy (w/DI water)
- ❑ Wash bottles

- ❑ Cubitainers (liters, gallons)
- ❑ Sulfuric acid
- ❑ Pipetters (and repair kit)
- ❑ Pipetter tips

- ❑ Turbidity standard
- ❑ Conductivity standard
- ❑ pH buffers and storage solution
- ❑ Sonde, data logger, cable, & manual
- ❑ Barometer (for sonde deployment)
- ❑ Sonde maintenance kit
- ❑ D.O. probe repair kit
- ❑ Kemwipes
- ❑ Deployment PVC pipe
- ❑ Chains & locks
- ❑ T-post & driver

Habitat

- ❑ Measuring tape
- ❑ Survey Staff
- ❑ Bank pins
- ❑ Hammer
- ❑ Ruler(s)
- ❑ Counter
- ❑ Densiometer
- Clinometer
- Flow meter
- Forms (RGA, RBP, Pebble, X-section, sonde)

General

- ❑ Coolers
- ❑ Extra batteries (AA &C)
- ❑ Cell phone
- ❑ Camera
- ❑ Field notebook
- ❑ Maps
- ❑ Tool box
- ❑ Dry ice (for overnight trips)

Periphyton

- ❑ Tray or basin
- ❑ Toothbrush (bent)
- ❑ Scalpel or knife
- ❑ Delimiter
- ❑ Wash bottle
- ❑ Funnel
- ❑ Sample containers
- ❑ Graduated cylinder

- ❑ Pipetter and tips
- ❑ Glass fiber filters
- ❑ Aluminum foil and sm ziplocks
- ❑ Homogenizer (hand-held blender)
- ❑ Vacuum pump
- ❑ Filter flask
- ❑ Filter funnel and clamp
- ❑ Forceps
- ❑ Formaldehyde

Benthic Macroinvertebrates

- ❑ Hess sampler
- ❑ Surber sampler
- ❑ Kicknet
- ❑ Bucket
- ❑ Sample containers
- ❑ Sieve
- ❑ Ethanol
- ❑ Forceps
- ❑ Labels

Personal/Optional

- ❑ Rain gear
- ❑ Boots/waders
- ❑ Neoprene gloves
- ❑ Hat/sun protection
- ❑ Sunglasses/polarized glasses
- ❑ NMED ID/business cards
- ❑ Binoculars
- ❑ Field guides
- ❑ Flashlight

Sonde Deployment/Retrieval Field Sheet

Sonde #: _____ **Model:** _____

Staff at deployment: _____

Assessment unit: _____

Water Quality Standards segment: **20.6.4.** _____

Station name/STORET ID: _____

Location description:

Lat/Long: _____ **GPS: Y / N**

Elevation: _____

Date/time deployed: _____

Sonde file name: _____

Channel depth at sonde: _____ at deployment _ at retrieval

Velocity at sonde: _____ at deployment _ at retrieval

Absolute barometric pressure: _____ at deployment _ at retrieval

Staff at retrieval: _____

Date/time retrieved: _____

Additional comments:

Archive file name: _____

Cross section sketch: **Plan view sketch:**

Write good directions, draw a good site map, and note triangulation distances.

Take photos from 2 angles (have someone point to or stand next to the sonde):

PHOTO 1 Description _____

PHOTO 2 Description _____

Appendix B: PERIPHYTON SAMPLING AND HANDLING METHODS

PERIPHYTON SAMPLING IN LOTIC WATERS

A Richest Targeted Habitat (RTH) approach will be used (Moulton et al 2002). Selection of the appropriate sampling method is based on habitat availability and study objectives. Selection of the habitat where samples are collected is generally based on the following priority:

A. Riffles in shallow streams with coarse-grained substrates (eplithic habitat);

B. Woody snags in streams with fine-grained substrates (epidendric habitat);

C. DTH (Depositional Targeted Habitat): organically rich, clay, silt, or sandy depositional areas (episammic and epipelic habitats) in streams where coarse-grained substrate, and woody snags are absent.

Select a representative riffle. Where riffles are present, samples will be collected in a riffle unless the velocity is so great that it limits algal growth, (i.e., velocities exceed 1.75 ft/sec) and more algae are found in glides than in the riffles. If the velocity appears to be limiting algal growth or no riffles are present, then select a glide (a length of stream with intermediate velocity). Generally five locations are sampled. Starting at either the top or bottom of the riffle, visualize a line running diagonally through the riffle. Select a landmark on the far bank that corresponds to the end of the diagonal transect. Note the midpoint, ¼, and ¾ distance. Collect one cobble from each of five locations along this diagonal: near the left bank, at ¼, ½, and ¾ the width, and near the left bank. If the study design stipulates that other then 5 locations be sampled, divide the transect by the required number of locations and sample at those points. In this manner, locations across the length and cross-section will be observed. Bend down to lightly touch the bed sediments without looking at what is there. Pick up the first stone that you touch. If it is too big to retrieve, then take the nearest one that can be picked up. If you touch a small silty, sandy, or gravely patch among the cobbles, then also take the nearest stone that can be picked up. However, if you must take more than 3 paces to find a suitable cobble among finer sediment, then use then use the appropriate method to sample the substrate at that location, i.e. use the Petri dish, suction, or gravel sampler methods. The sample from each of the locations is composited into a single sample. If the stream width is less than 3 feet, the sample may be collected from 2 or 3 separate riffles by collecting 2 to 3 samples from each riffle. If you cannot safely wade across the entire riffle, go as far as possible, then turn back and continue to collect samples on a diagonal back to the bank at the opposite end of the riffle from where you started. For reaches that you cannot wade halfway across, collect periphyton samples in 5 to 10 location that you can access. Use the targeted riches habitat approach in selecting substrate to sample, i.e. collect cobble or gravel if present, if not then woody debris or finer substrate. The samples should be collected over an area that is approximately as long as the river is wide.

1) Sampling methods for epilithic habitats

Epilithic (cobble and gravel) habitats are sampled using one of two methods depending on the types of rock substrate being sampled. The ring method is used when sampling cobble surfaces. A gravel sampler is used to sample periphyton attached to gravel substrates when only gravel is present (i.e., no > 2-inch diameter substrate is present in riffles or runs to allow use of the ring method).

Using the ring method to sample epilithic habitats:

A. Gather equipment:
delimitor – section of 2- inch diameter PVC pipe, about 1 inches long
toothbrush – bent back 90° and bristles trimmed to about 6 mm
tray –- to carry rocks
funnel
sample container
scalpel or knife
wash bottle

B. Select a representative riffle. Starting at either the top or bottom of the riffle, visualize a line running diagonally through the riffle. Divide the transect by the required number of locations and collect a cobble at each of those points. Place stones in a plastic tray, top side facing up (i.e., in the same orientation that they were found), and transport them to the bank to collect periphyton. Place the ring on top of the stone to define a circle in the center, or as near as possible to the center, of the stone.

C. Use a knife or scalpel to scrape off as much periphyton growth as possible from within the ring and rinse off the scalpel into an appropriately labeled sample container.

D. Scrub the defined area for ~30 seconds with a modified toothbrush. Remove the slurry from within the circle by removing the ring, holding the rock at an angle over a funnel placed in the sample bottle and rinsing with a wash bottle of stream water. Thoroughly rinse the area but use a minimal amount of water. Thoroughly rinse the brush into the container. (Note: only use small amounts of wash water to avoid running out of space in the sample container making homogenizing the sample more difficult).

E. If the sampling point falls over a mat of filamentous algae, a slightly different approach is required for sample collection. Place the ring on the center of the rock. At the ring, cut any filaments passing into or out of the ring. Remove any loose filaments from outside the ring. Scrape off all of the filaments inside the ring and rinse it off the scalpel into sample container. Scrub the defined area for ~30 seconds with a toothbrush then remove the slurry from within the circle as described above.

F. Combine the discrete collections in a 500-mL sample bottle (i.e., composite the samples from the five or more stones).

G. Place the bottle on ice inside a cooler and keep in the dark if the sample is not processed immediately. The sample must be processed within 12 hours.

H. Note the area sampled.

Total Sampling Area = $n[(\pi)(r^2)]$
where,
n = number of discrete collections,
π = 3.1416, and
r = radius of the delimiter in centimeters (for PVC = 2.3 cm)

I. Process the periphyton sample following the steps described in “"Sample Processing Procedures.”"

Using the gravel sampler method to sample epilithic habitats:

A. Assemble the gravel sampler from a plumbing “"clean-out”" (7.6-cm diameter). Attach the threaded cap; bevel the bottom edge of the clean-out by using a grinding wheel to improve the coring capability of the sampler. Obtain a large masonry trowel wide enough to enclose completely the bottom of the sampler.
B. Select five to ten10 sampling locations along a diagonal transect through a riffle;, record number of locations sampled.
C. Press the beveled end of the sampler into the gravel substrate. After the sampler is in place, carefully remove the gravel surrounding the outside of the sampler and insert the masonry trowel.
D. Slide the sampler onto the trowel and carefully lift it out of the water.
E. Quickly invert the sampler to contain the gravel and water in the sampler cap.
F. Pour each discrete collection into a dishpan and rinse the sampler before taking another discrete collection.
G. Repeat these steps to complete five to ten10 discrete collections, which that form the composited sample. Record the number of collections on the field sheet.
H. Extract macroalgal filaments (if present) from the gravel with forceps and then cut them into fine pieces.
I. Brush and rinse (with dishpan water) the gravel. Recycle rinse water to keep the sample volume < 475 mL.
J. Pour the sample from the dishpan through a funnel into a 500-mL sample bottle. Place the bottle on ice inside a cooler and keep in the dark if the sample is not processed immediately.
K. Process the periphyton sample following the steps described in “"Sample Processing Procedures.”"

2) Sampling method for epidendric habitats

Collecting quantitative microalgal periphyton samples from epidendric (woody snags) habitats presents a challenge because they generally have an irregular surface and are difficult to remove without loss of algal biomass (Porter et al. 1993). If the woody snag can be removed from the water and has a smooth surface, it can be sampled in a similar manner to epilithic habitats, i.e. the ring method. Otherwise, periphyton is collected from woody snags by using the cylinder scrape method.

Using the cylinder scrape method to sample epidendric habitats:

A. Select one woody snag in each of five locations throughout the reach.
B. Identify the part of the woody snag that will be sampled for periphyton. Carefully remove a 10- to 20-cm long section with pruning shears or saw and place in a plastic dishpan.
C. Scrub the entire surface of each woody snag section in the dishpan with a stiff brush. Rinse the brush and each section in the dishpan. Recycle rinse water to keep the sample volume less than 475 mL.
D. Pour the sample from the dishpan through a funnel into a 500-mL sampling bottle.
E. Measure the length and diameter of each cleaned woody snag section and calculate the total sampling area by using the following formula (assumes a cylinder):

   Total Sampling Area = Σ n (π)(di)(li)
   where,
   n = number of discrete collections,
   π = 3.1416,
   di = diameter of each woody snag section, in centimeters, and
   li = length of each woody snag section, in centimeters.

F. Process the periphyton sample following the steps described in “"Sample Processing Procedures”".

3) Sampling method for epipsammic/epipelic habitats (DTH)

Quantitative microalgal periphyton samples are collected from the upper five- to seven-mm layer of epipsammic (sand) or epipelic (silt) habitat in depositional areas. If the depth and velocity are low, the suction method may be used.

Using the suction method for epipsammic or epipelic habitats:

A. In locations in the diagonal crossection that have a depositional zone consisting of either sand or silt substrates, place the delimitor (PVC ring) onto the sediment push into the sediment to a depth of 1-2 cm.
B. Use a turkey baster to remove the entire top 5-7 mm of sediment and deposit into the sample container.
C. Note the area sampled as described in the ring method section.
D. Process the sample following the steps described in “"Sample Processing Procedures.”

Using the inverted petri-dish method to sample epipsammic or epipelic habitats:

A. At locations in the reach that have a depositional zone consisting of either sand or silt substrates, hold a small plastic petri dish (about 47-mm diameter) upside down in the water; rub the inside of the lid to remove air bubbles.
B. Turn the inside of the lid toward the substrate that will be sampled without disturbing the sediment.

C. Carefully and slowly press (in cookie cutter fashion) the lid into the sediment.
D. Slide a spatula under the lid to enclose the discrete collection. Holding the petri dish tight against the spatula, carefully wash extraneous sediment from the spatula, and then lift out of the water.
E. Invert the lid and remove the spatula.
F. Rinse the sediment from the lid with stream water into a 500-mL sample bottle.
G. Repeat this collection procedure at each additional sampling location in the reach.
I. Combine the discrete collections in a 500-mL sample bottle
J. Note the total area sample

   Total Sampling Area = n[(π)(r2)]
   where,
   n = number of discrete collections,
   π = 3.1416, and
   r = radius of the Petri dish in centimeters

L. Process the sample following the steps described in “"Sample Processing Procedures.”

SAMPLE TRANSPORT

All samples should be processed as soon as possible after collection. Samples must be either frozen or filtered and frozen at the end of each day, no more then 12 hours after collection. After collection, pack labeled periphyton samples in ice in coolers until processing. It is important to keep samples chilled and in darkness.

SAMPLE PROCESSING PROCEDURES

Collection and preservation of taxonomic identification sub-sample

Before homogenizing or freezing the composite sample, a 50mL sub-sample should be removed, labeled, and preserved with 2 mL of Formaldehyde for taxonomic identification. Periphyton and algal samples for taxonomic identification should be preserved in formaldehyde to enable the soft-bodied algae to be preserved. This preservative is active indefinitely.

Sample preparation

Most periphyton samples will contain clumps of filamentous algae or diatoms creating difficulties in sub-sampling. Representative sub-sampling is essential for accurate assessment of periphyton communities. Homogenizing the sample with a hand-held blender can significantly reduce variability in sub-sample analyses, particularly in samples dominated by filamentous algae. The blending process generally does not greatly damage cells. Certain green filamentous algae do not break apart easily (e.g., Cladophora) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs 1987). Filaments that remain after blending should be cut into five mm lengths with scissors. The sample is homogenized, then filtered for analysis of chlorophyll a and AFDM.

Equipment

- Glass or plastic beaker
- Squirt bottle with distilled water
- Kitchen or laboratory tissue blender
- Graduated cylinder
- Small sharp scissors

Procedure

A. Pour the contents of the sample container into a graduated cylinder. Rinse out any sample residue from the container and lid into the cylinder. Rinse off, but do not transfer, sand and gravel. Distilled or DI water is preferred for rinsing and making up the sample volume. However, tap water can be used providing the water isn't heavily chlorinated. Measure and record the total sample volume. Remember to add the 50 mL that was removed before freezing for taxonomic identification. Pour the sample into a beaker (the width of the beaker should be only slightly greater than the width of the housing holding the blades of the blender).
B. Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample.
C. Ensure that there is enough water to fully cover the blender-blade housing.
D. Blend for about 30 seconds or until the mixture is free of obvious clumps of material. If the sample contains much filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that have become caught on the blades or blender housing and cut into less than 5 mm lengths. You need to end up with a homogeneous solution suitable for sub-sampling.

Filtration for Chlorophyll a and AFDM analysis

Equipment

• Vacuum filtration apparatus.
• Hand held vacuum pump with pressure gage.
• Glass fiber filters, 47 mm Whatman GF/F.
• Forceps/tweezers.
• Wide aperture (~ two mm diameter) five mL pipette.
• Squirt bottle with deionized or distilled water
• Tin Aluminum foil and permanent marker

Procedure

A. Set up filtering apparatus with a fresh filter.
B. Place a sub-sample in the filtering chamber. The sub-sample is made up of 3 aliquots drawn from the blended sample. The volume of the aliquots depend on the concentration of the sample and vary from 1 to 5 mL. You want to have good color development on the filter but not so much that extensive dilution will be required after extraction. To take aliquots, shake the bottle of blended sample or

briefly turn on the blender and withdraw 1-5 ml with a pipette from halfway down the solution while the liquid is still agitated. Repeat this twice more to give the full sub-sample (i.e., the total sub-sample volume = 3-15 mL).

C. Apply suction pressure (be careful not to have a high pressure as this will rupture cells releasing the chloroplasts; <6 inches Hg vacuum is recommended).
D. If there is not an obvious coloring from periphyton on the filter, then you should filter more aliquots. AFDM analyses are not as sensitive as Chlorophyll *a* and require larger sub-samples where there is a low concentration of periphyton.
E. Check for any fragments of leaves, mosses, invertebrates, etc. on the filter paper and remove these with forceps.
F. Record the volume of sub-sample (i.e., number and volume of aliquots used).
G. Remove the filter from the filtering apparatus, fold in half, and place on a sheet of aluminum foil. Fold in the sides of the aluminum foil to seal in the filter. Label the wrapped filter with the location, date, and analysis to be preformed (Chl a or AFDM).
H. Repeat steps 1-8 until the desired number of replicate of Chlorophyll *a* and AFDM sub-samples are filtered.
I. The wrapped, labeled filters should be placed in a ziplock bag which has been label with the location, date, volume filtered, total volume, and number of replicates (1 of n). Freeze the wrapped, labeled filters. Samples should be processed as soon as possible after collection. However, the wrapped filters can be frozen for up to 6 months before analysis, if necessary.
J. Refreeze the remains of the sample for repeat analyses if needed.

Notes:

• Always check that clumps of algae or a leaf fragment, etc., hasn't blocked the intake to the pipette.

• If the sub-samples are taking a very long time to filter for the filter is darkly colored or covered with a thick layer of material, you probably need to dilute your sample or take a smaller volume aliquot. Ensure that you record the degree of dilution and aliquot volume so that this can be used in the concentration calculations concentration.