CHAPTER 7. PERIPHYTON COLLECTION PROTOCOLS

Periphyton Overview

Periphyton are algae, diatoms, fungi, bacteria, protozoa, and associated organic matter associated with stream channel substrates (*i.e.,* they grow on the exposed surfaces of rocks and other submerged objects). Phytobenthic (photosynthesizing, bottom-dwelling) periphyton is usually the dominant primary producers in stream ecosystems (especially small stream systems). Because they are attached to the substrate, the phytobenthic periphyton community integrates physical and chemical disturbances to a stream. They are useful indicators of water quality because they respond rapidly and are sensitive to a number of human disturbances, including habitat destruction, contamination by nutrients, metals, herbicides, and acids. Another advantage of using periphyton in water quality assessments is that the periphyton community contains a naturally high number of species, making data useful for statistical and numerical applications to assess water quality. Response time of the periphyton is rapid, as is recovery time, with recolonization after a disturbance often more rapid than for other organisms. Diatoms, in particular, are useful indicators of biological integrity because they are ubiquitous; at least a few can be found under almost any conditions. Most diatoms can be identified to species by experienced biologists and tolerances or sensitivities to specific changes in environmental conditions are known for many species. By using algal data in association with macroinvertebrate data, the biological integrity of stream ecosystems can be better ascertained.

Materials and Supplies

1. Support Ring – A piece of PVC pipe (1 cm long & 4 cm inside diameter) to delimit the sample area on rocks (12.56 cm^2 of area inside ring
2. Scraping Tool - microspatula
3. Small brush - toothbrush that is replaced at least weekly
4. Sample container - 4 oz. “specimen jar”
5. 10 % Formalin - for sample fixing/preservation
6. Cooler w/ Wet Ice - for sample storage/preservation
7. Electrical Tape -for sealing lids of sample jars
8. Labels - labels are to be placed inside sample container & on outside
9. Clear Tape - to affix label to container
10. Squirt Bottle

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.,* glass, metal, wire, *etc*.). They should also be worn during sample preservation or at any time while handling formalin, a known carcinogen.

Part 1. Field Sampling Procedures

Collect periphyton at benthic sampling sites (*e.g.,* reference, random, TMDL Bio Sites, targeted sites) or as directed on the stream list. Periphyton may also be collected at big streams (large rivers where benthic macroinvertebrate collection is not practical (*e.g.*, Elk River near mouth), and at streams that are too deep for benthos collection (*i.e.,* water over the net) but not too deep to reach in and grab cobble to sample periphyton as a biological indicator for the site.

Ideally, samples should only be collected during stable flow conditions. After extremes of flooding or drought, a two-week period is required for adequate recolonization. Because sampling tends to be conducted within a short index period (random sites), periphyton will be collected when streams are not turbid (*i.e.,* the substrate is visible).

1. Label sample container with Stream Name, AN-Code, date, collector, and "w/ formalin".

2. To be consistent, samples will only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying periphyton communities (brown vs. green) and intensities from throughout the reach. This includes rocks with just green or brown algae, rocks with both intermixed, rocks with long stringy algae, and rocks with a layer of periphyton growing on top of a thick layer of silt or sand (which tend to be motile species). Even a seemingly clean rock will have an unseen or undetectable community of periphyton that can be quantified. If there are no rocks available from the reach, collect periphyton from removable wood (same technique as for rocks), documenting on the field sheet exactly what was sampled. These riffle/run areas should roughly coincide with the areas where benthic macroinvertebrates are collected (if benthos collected) to avoid sampling above and below a source. **The most important thing is to be representative of the site when picking the five rocks for the sample!!!**

3. Rinse the PVC ring, toothbrush, microspatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from prior sampling of subsequent collections.

Figure 7-1. Microspatula being used to scrape algae and periphyton from upper surface of

Figure 7-2. Toothbrush being used to scrub remaining algae and periphyton from upper surface of rock.

4. Using the PVC ring to delimit the sample area (12.56 cm^2), use the microspatula to scrape **all algae** from **upper surface** of rocks into the sample jar (***see Figure 7-1 above***). Use the toothbrush to loosen any remaining periphyton (***see Figure 7-2 above***). In some cases, if the mineral content of the rock is just right, you may notice that you are removing a significant layer of the rock material along with the periphyton. In such a case, it is probably safe not to use the toothbrush after scraping since it is doubtful that any periphyton remains in the scraped area unless the rock is excessively fissured and rough.

5. Remove sampler and rinse loosened algae into the sample jar using clear stream water collected from that site in the squirt bottle (***see Figure 7-3 below***). Repeat Step 5 until all of the periphyton from the five rocks (representing 62.8 cm^2 of sampled area) is composited into one sample jar.

6. Rinse the microspatula, toothbrush, and PVC ring into the sample, removing as much of the lingering periphyton as possible. Snap the labeled lid onto the container.

7. Rinse the PVC ring, toothbrush, microspatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections.

Figure 7-3. Loosened algae and periphyton being rinsed into the sample jar.

8. A guideline for preservation is as follows: Assuming the sample jar is about 3/4 (120 ml) full, preserve with an adequate amount (a “plop”) of 10% formalin from the squeeze bottle) for sparse to normal periphyton amounts. Add more for samples

with heavy amounts of green algae. **The specimens cannot be over preserved.** The specimen cups are graduated (ml) so adding the proper amount of formalin can be measured. **Take extra care when preserving, as formalin is a known carcinogen.** Note: Samples do not need to be preserved immediately. It may be easier to preserve all periphyton samples collected in a given day at one time – upon returning to office or hotel parking lot. Whether samples are fixed immediately or not, they should be placed in a cooler with wet ice. Sample jars should be taped by sealing the rim of the lid with electrical tape to minimize the chance of spillage or cross-contamination.

9. Record the number of rocks "scraped" from each of the varying habitats (riffle vs. run and sunlight exposure classes). For example, 2 rocks in riffle, 3 in run/3 rocks with full exposure, 1 with partial shade, 1 with partial exposure. The yes/no questions and comments box on the Habitat Assessment Form (***see CHAPTER 2. Section C. Part 1. PAGE 9-Periphyton Collection Information starting on page 2-103***) will be used to aid in interpreting data from scoured or drought affected reaches.

Part 2. Laboratory Methods

Periphyton identification and biomass determinations are performed by a private contractor. The contractor is required to have a degreed biologist on staff that performs the actual identifications. The contractor must adhere to the following protocols.

A. **"Soft" Algae (Non-Diatoms) –** Relative and abundance are to be determined as follows:

Homogenize sample in a blender and pipette a subsample into a Palmer counting cell. Dilute the sample if cells overlap too much for accurate counting. Identify and count 300 non-diatom algal units to the lowest taxonomic level at 400X magnification. Colonial species are to be counted as individual cells, when appropriate. Filamentous species should not be counted as individual cells, but as cell units of 10 micrometers in length. The number of "live" diatoms observed should also be recorded (identification will be done under a separate procedure). Record the numbers and species of "soft" algae on the bench sheet.

B. **Diatoms** – Diatoms are to be analyzed after the "soft" algal identifications are complete, as the clearing process will destroy soft tissue. Procedures are as follows:

3) Clear the diatom frustules of organic material using either nitric acid or hydrogen peroxide/potassium dichromate oxidation.
4) Prepare slides and identify diatoms to species or lowest taxonomic level possible.
5) Record all taxa encountered on the bench sheet to create a species list prior to enumeration. Continue identification until no new taxa are found after a 2-3 minute scan. To obtain quantitative data, count a minimum of 600 valves and record the taxa and number encountered on the bench sheet.

Part 3. Periphyton Data Assessment

An assessment of biological integrity can be made based on the periphyton data. The goal is to categorize water quality as excellent, good, fair, or poor and to determine the degree and cause of aquatic life use impairments in fair or poor streams.

Biological indices represent mathematical models of community changes. Changes in water quality will affect resident biota, and indices that reflect these changes in a particular community are useful biological indicators of water quality. The periphyton community, especially diatoms, is a useful biological indicator because:

- They are attached to the substrate and, therefore, subjected to any immediate or prolonged disturbances;
- Diatoms are ubiquitous, with at least a few individuals found under almost any aquatic conditions;
- Total number of taxa at any given site is usually high enough for use in calculating various metrics;
- Diatoms, especially the most abundant species, are identifiable to species by trained professionals;
- Tolerance of or sensitivity to changes (autecological requirements) is known or suspected for many species or assemblages of diatoms; and
- Periphyton communities, especially diatoms, have a rapid response and recovery time because of their relatively short lifecycle (as compared to fish or macroinvertebrates) and their ability to quickly recolonize formerly disturbed (impacted) sites.

Several metrics have been used successfully to assess water quality conditions using periphyton. Some have the diagnostic ability to indicate the type of impact (nutrient enrichment, toxicity, acidity, salinity, sewage (organic) pollution, and siltation).

Periphyton Quality Assurance and Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for residual algal material, rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Periphyton will be collected along with other activities at the designated duplicate WAB sites. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been depleted by the first duplicate. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-

training. ***See CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1 for additional information.***

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of periphyton samples is included. In the field, biological sampling teams will consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. Individuals who are more experienced in collecting periphyton will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

CHAPTER 8. FILAMENTOUS ALGAE MONITORING

Filamentous Algae Overview

Since 2007, the Watershed Assessment Branch (WAB) has devoted much effort and resources to evaluating the causes, locations, and severity of filamentous algae blooms in West Virginia's streams and rivers (***see Figure 8-1 below***). As part of that effort, WAB has measured the development of filamentous algae blooms at various locations and reported the results as "percent algae cover" and occasionally "percent water column fill". Percent algae cover is the percent of the stream bottom covered by filamentous algae at a measured transect of the stream, and percent water column fill is the percent of the water column filled in a cross-sectional view of the stream at a given transect location. It should be noted that neither of these measurements have a longitudinal component; *i.e.*, these two measurements do not account for the length of stream reach impacted by filamentous algae.

Figure 8-1. A filamentous algae bloom on the Tygart River upstream of Norton, June 2012.

WAB has developed a method for measuring filamentous algae in streams based on the methods described in the following:

Morgan, A.M., T.J. Royer, M.B. David, and L. Gentry. 2006. Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. *Journal of Environmental Quality* **35**: 1110-1117.

Schaller, J.L., T.V. Royer, and M.B. David. 2004. Denitrification associated with plants and sediments in an agricultural stream. *Journal of the North American Benthological Society* **23**: 667-676.

At a given stream transect, a measuring tape is str*etc*hed across the stream; wetted width and portions (or segments) of the stream covered by filamentous algae are recorded. On occasion, WAB may also make an additional measurement of the filamentous algae thickness along the transect segments so that the amount of water column impact, not just stream cover, can be calculated.

This method was first used as a basis for filamentous algae measurement in West Virginia beginning in the fall 2008 in four priority river watersheds: Greenbrier River, Cacapon River, Tygart Valley River, and South Branch of Potomac River. In 2014, additional components to document submerged aquatic vegetation, canopy cover, and stream bearing were added.

Filamentous algae blooms tend to appear mostly during the summer months due to several factors including: flow induced high nutrient concentrations (*e.g.*, low flow), higher air temperatures, and longer photoperiods (*i.e.*, day lengths). Because of this, sampling has typically been constrained to this time period. However, filamentous algae blooms can appear under other conditions (*e.g.*, in the Early Spring due to a lack of competition for light before full canopy leaf-out and increasing air temperatures).

The procedure below represents a summary and explanation of the method used by WAB to determine the “percent algae cover” and “percent water column filled” by filamentous algae. It is set forth both to serve as WVDEP’s recommended standard measuring method for future measurements of filamentous algae blooms, both by WAB and by others wishing to make measurements comparable to those made by WAB.

The Filamentous Algae Monitoring Program utilizes some of the same sampling protocols of other Watershed Assessment Branch programs. Specifically, the SOP sections that apply to this program are as follows:

CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. Guidelines for Completing the Stream Assessment Forms on page 2-28

CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1

CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1

Materials and Supplies

IMPORTANT: ***The unit of measurement can be either in feet (marked in tenths of a foot) or meters, but the units must be the same for both the distance and depth.***

1. 100 meter Tape Measure – Units can be in either tenths of a foot (rather than inches) on one side and meters on the other. It is recommended to use some sort of stakes or pins (*e.g.*, steel rebar) to easily anchor each end of the tape.
2. Vertical Measurement Device – To be used only if calculating the "percent water column fill. A stiff vertical device such as a wading rod, thalweg pole, or surveyor rod marked in either tenths of a foot or meters. Other non-flexible measuring instruments (such as a yard stick with feet marked in **inches**) can be used if absolutely necessary, but the measurements will need to be converted to tenths of a foot before calculating any of the formulas below. Experience has shown that the use of more flexible metal measuring tapes are not suitable to measure water depth whenever there is significant water velocity in the stream.
3. Camera – to take photos of the transect area's algae cover
4. Filamentous Algae Measurement Form

Field Safety Precautions

Since this procedure often occurs on large streams and rivers, deep and swift water safety precautions (like the use of personal floatation devices) should be observed.

Part 1. Field Sampling Procedures

Site Selection

Basic guidelines for site selection.

1) Site should be wadeable with some depth, but not too deep.
If possible, select a stream measurement transect that may be waded comfortably and safely. Streams varying from one to three feet deep are ideal. Streams which are too deep to comfortably and safely wade may result in

measurement inaccuracy because so much effort is required to simply stay on one's feet. Also, deeper water decreases light penetration into the water, which tends to inhibit the development of algae growth. This may skew results if the measurer is looking for trends at different locations along a given stream.

2) **Site should have rocky substrate with minimal transient material (*i.e.*, fine sediments like silt, sand, and fine gravel).**
Growth of filamentous algae also tends to be inhibited by the lack of rocky substrate. So again, if possible, select measurement sites with little or no sediment accumulation to avoid skewing results in comparative studies.

3) **Sites in narrower streams should have a moderately open canopy.**
For narrow streams, shading from the tree canopy overhead can have an inhibitory effect on algae growth similar to that of streams that are too deep.

4) **Avoid sites that will potentially have a dry bar during low flow situations**. This may not easily be determined during higher flows, but any attempt to avoid this will make the calculations easier later.

Transect Selection

Basic guidelines for transect selection within a site.

1) **Level of algae growth**. Due to factors discussed above and other physical variables, the level of algae growth may vary considerably at a given location. When correlating the level of algae growth with stream chemistry, select a transect location that is representative of the general site location. If possible, avoid areas with fast currents or deep pools as this may interfere with algae growth.

2) **Return visits**. If the site is intended to monitor changes in algae growth over a period of time, the same transect location should be used for each visit. The exact transect location should be documented (drawing, photo, marked in the field or on the bank in some semi-permanent fashion) so that it can be consistently repeated for comparability.

Establishing the Transect

Determine the wetted width of the stream by str*etc*hing the tape measure across the stream perpendicular to the stream banks. Secure the tape on each stream bank, pulling it tight enough to minimize sag (*i.e.*, a sag in the tape will give a wider wetted width measurement than really exists). Wide rivers may necessitate variations of measuring instrument and logistics, such as using an accurate laser range finder or pulling a measuring tape across the river in sections at a time. However, the basic concept will remain the same.

A minimum of five algae measurement segments should be recorded for each transect

across the stream channel. For example, a stream that is 200 feet wide would require at least five 40 foot (5 x 40 = 200) segments across the stream where the algae growth is measured. As you traverse the stream channel while setting up the tape measure, make some notes about where the minimum five segments should be placed. Segments should be placed to document and define areas with or without algae mats, significant changes (> 0.5 feet or 0.15 meters) in amount of algae growth (both in percent cover and thickness) on the stream bed, and/or sudden changes in depth. For example, if the 200 foot stream in the previous example had a flow (or thalweg) channel that was only 10 or 15 feet wide and deeper than the rest of the stream, the flow channel should be treated as a separate segment in the stream transect as it is most likely conveying water all year long and will represent a fixed segment in trend measurements (***see Segment 3 in the Figure 8-2 below***). Conversely, segments can be lengthened in areas where the water depth and algae growth are more uniform. Additional segments may be added to the transect while taking measurements and the stream bottom is more thoroughly investigated.

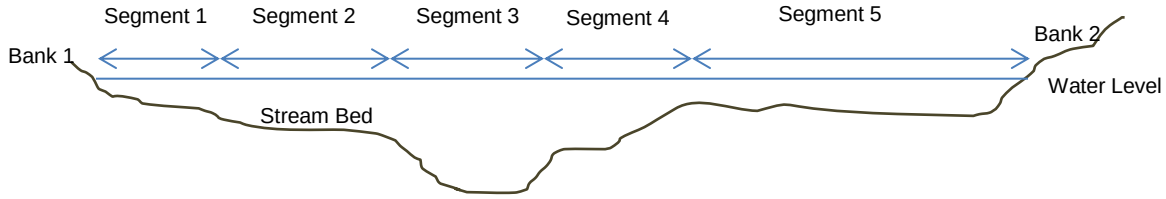

Figure 8-2. Hypothetical example of stream segments across a filamentous algae

<u>NOTE</u>: This concept of isolating differing sections laterally across a stream channel is used in the Flow measurement methodology in CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS starting on page 4-1 and longitudinally in the Dominant Substrate/Reach Characterization in CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. PAGE 3. Dominant Substrate Type and Reach Characterization on page 2-51.

<u>Documentation using the Filamentous Algae Monitoring Form</u>

Record the site documentation information on the Filamentous Algae Monitoring Form as outlined in **CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C.**

PAGE 1**. Site Verification on page 2-30.**

Information includes:

- PAGE 1: Stream name, ANCode, and Location (*e.g.,* Greenbrier River at I-64 East bridge.), Coordinates of the waters sample location (x-site) and, if the stream is excessively wide, coordinates where the transect ends (***see Figure 8-3 below***), Site Map, *etc*.

Field Lat X-site	**N**	**Field Lon X-site**	**W**
LDB Transect Lat	**N**	**LDB Transect Lon**	**W**
RDB Transect Lat	**N**	**RDB Transect Lon**	**W**

Figure 8-3. Example of additional Transect LDB and RDB Coordinate Boxes on PAGE 1

- PAGE 2: Field Water, Periphyton/Algae/Aq. Plant Info, Canopy Density. An additional section to document the canopy density along the stream transect (***see Figure 8-4 below***).

Canopy Density (Densiometer) Readings: 0-17 Possible per reading; Hold 1ft (0.3 m) above surface of water									
Left		**Left Middle**		**Center**		**Right Middle**		**Right**	
Up		**Up**		**Up**		**Up**		**Up**	
Left		**Left**		**Left**		**Left**		**Left**	
Down		**Down**		**Down**		**Down**		**Down**	
Right		**Right**		**Right**		**Right**		**Right**	

Figure 8-4. Example of the additional Canopy Density (Densiometer) Readings Section on PAGE 2

- PAGE 3: Filamentous Algae Measurements (***see Figure 8-6 on next page and Taking Filamentous Algae Measurements on page 8-8***)

- PAGE 4: Landowner/Stakeholder Information, Recon, & Photos. Additional options to document Houses, Campgrounds/Campsites, Public and Private Stream Access are provided under the Recon/Accessibility section in this version of the form (***see Figure 8-5 below***).

Discuss the accessibility to the site including accessibility, posted property, fenced, beside road, long walk over treacherous terrain, hike length, 4 x 4 needed, get key from landowner, etc.	
Check all that apply:	☐ Easy Access ☐ Difficult Access ☐ Public Property ☐ Private Property ☐ Posted ☐ Fenced ☐ Gated ☐ Get Key from Landowner ☐ Beside Road ☐ Short Hike ☐ Long Hike ☐ 4x4 Needed ☐ Houses ☐ Campgrounds/Campsites ☐ Public Stream Access ☐ Private Stream Access ☐ Other (explain)
Recon/Accessibility Notes:	

Figure 8-5. Example of the expanded Recon/Accessibility Section on PAGE 4

FILAMENTOUS ALGAE MEASUREMENTS>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>							Reviewers Initials	
AN-Code	PSB-(12.1)						Date	5/30/2014
Measurer	John Doe					Time	1625	
Location & Bank	D_{Begin}	D_{End}	Water Depth (WD_i)	% SAV Cover	% Algae Cover (AC_i)	Algae Thickness (AT_i)	Segment Dominant Substrate Type	Segment Measurement Notes
$Bank_1$		0.5						LDB ■ RDB □
Segment 1	0.5	2.6	0.5	0	25	0.2	CB	
Segment 2	2.6	4.9	1.1	5	50	0.4	CB	
Segment 3	4.9	8.9	0.4	5	50	0.3	BL	
Segment 4	8.9	12.5	0	0	0	0	BR	
Segment 5	12.5	14.2	0.5	0	75	0.4	FG	
Segment 6	14.2	18.2	1.5	5	75	0.4	CB	
Segment 7	18.2	22.6	1	15	45	0.2	FG	
Segment 8	22.6	24.5	0.4	10	35	0.1	SA	
Segment 9								
Segment 10								
Segment 11								
Segment 12								
Segment 13								
Segment 14								
Segment 15								
Segment 16								
Segment 17								
Segment 18								
Segment 19								
Segment 20								
$Bank_2$		24.5	DS Channel Orientation/Bearing		319^0	Measurement Units Feet ■ Meters □		LDB □ RDB ■

Substrate Particles	Particle Codes	Size Class	Substrate Particles	Particle Codes	Size Class
Bedrock	BR	Smooth surface rock/hardpan (>4000 mm –bigger than a car)	Fine Gravel	FG	Ladybug to marble (>2-16 mm)
Boulder	BL	Basketball to car (>250-4000 mm)	Sand	SA	Gritty – up to ladybug (>0.06-2 mm)
Cobble	CB	Tennis ball to basketball (>64-250 mm)	Silt & Fines	ST	Fine – not gritty (<0.06 mm)
Coarse Gravel	CG	Marble to tennis ball (>16-64 mm)	Clay	CL	Slick/ hard clay or hard-pan clay

Page 3 WVDEP WAB Filamentous Algae Monitoring Form (5/30/2014)

Figure 8-6. Example of PAGE 3 of the Filamentous Algae Monitoring Form

Taking Filamentous Algae Measurements

Fill out PAGE 3 of the Filamentous Algae Measurements Form using the following steps:

1. Determine the downstream channel orientation by taking a bearing measurement (*e.g.*, 0^0 being a stream flowing due North; 90^0 being a stream flowing due East; 180^0 being a stream flowing due South; 270^0 being a stream flowing due West; 315^0 being a stream flowing Northwest). Record this value near the bottom center of the page.

2. Indicate what the selected measurement units will be (*i.e.*, Feet or Meters) near the bottom center of the page.

3. On the first or starting Bank ($Bank_1$), record the measurement at the stream channel's or wetted edge as the initial point ($Bank_1$ D_{End}). Check in the note field whether this is the left descending bank (LDB) or right descending bank (RDB). This same measurement for the bank ($Bank_1$ D_{End}) should also be recorded as the beginning measurement for the first cross segment (1 D_{Begin}). The D_{End} measurement for each segment will be the D_{Begin} measurement for the next segment.

4. Wade into the stream observing the water depth, submerged aquatic vegetation, filamentous algae percent cover or depth, and algae mats. If there are significant changes in any of these conditions before reaching the approximate predetermined segment width, define the end of the segment and record the distance on the tape measure that corresponds to the end of the first segment (1 D_{End}). Again, the D_{End} for each segment then becomes the D_{Begin} for the next segment.

5. Within that segment, record the representative water depth (WD_i), estimated percent stream bottom submerged aquatic vegetation (SAV) & filamentous algae cover (AC_i), the representative algae thickness (AT_i) (if necessary), and the Dominant Substrate Type.

> **IMPORTANT:** ***Remember that large variations in water depth (>0.5 feet or >0.15 m) or algae growth should cause the start of a new segment.***

   a. Water depth (WD_i) is determined by evaluating a given transect segment for a representative depth measurement.

   b. The percent submerged aquatic vegetation (SAV) and percent filamentous algae cover (AC_i) for the segment can only be visually estimated. Carefully observe the stream bottom (both the stream substrate and SAV/filamentous algae present) in a **one unit wide** area (centered on the transect tape measure either in feet or meters) for the entire length of the segment.

Estimate and record the percent of the stream bottom in the segment that is covered with submerged aquatic vegetation and filamentous algae growth respectively. "Covered" means that SAV or algae are present and visually obscuring the stream bottom. The SAV or algae may not necessarily be attached to the stream bottom within the one foot wide area. For example, algae may only be attached to 40% of the stream bottom in the one foot wide segment, but long strands of algae may extend down from upstream and cover 80% of the stream bottom within the transect area. ***See Figure 8-7 to Figure 8-19 for photo examples of measured percent filamentous algae cover***.

Figure 8-7. 3% Filamentous Algae Cover

Figure 8-8. 4 % Filamentous Algae Cover

Figure 8-9. 12% Filamentous Algae Cover

Figure 8-10. 15% Filamentous Algae Cover

Figure 8-11. 20% Filamentous Algae Cover (Short Strands)

Figure 8-12. 20% Filamentous Algae Cover (Long Strands)

Figure 8-13. 26% Filamentous Algae Cover

Figure 8-14. 35% Filamentous Algae Cover (Short Strands). Note that this is the same location as Figure 8-15.

Figure 8-15. 38% Filamentous Algae Cover (Long Strands). Note that this is the same location as Figure 8-14.

Figure 8-16. 39% Filamentous Algae Cover

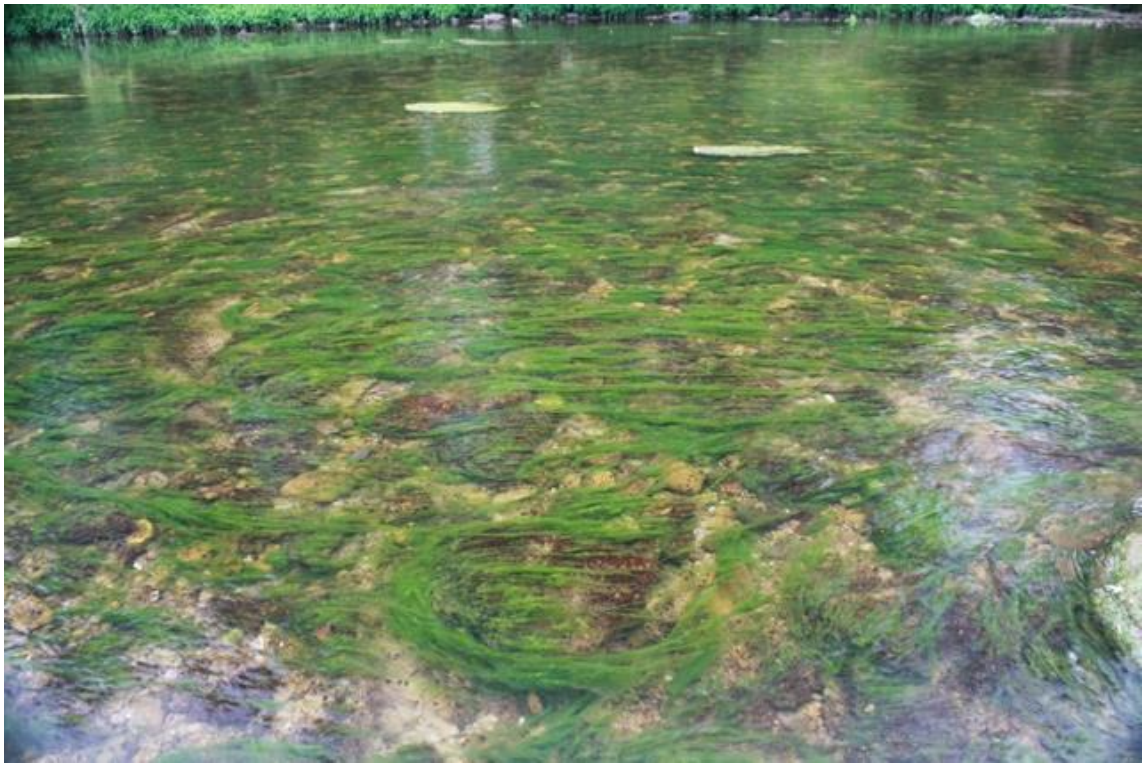

Figure 8-17. 47% Filamentous Algae Cover

Figure 8-18. 65% Filamentous Algae Cover

Figure 8-19. 98% Filamentous Algae Cover

c. Algae Thickness (AT_j) is determined by evaluating the given transect segment for a representative algae thickness measurement. Thickness measurements

are made to determine how much of the water column is filled by filamentous algae in relation to the water depth. Length of the filaments is not a primary concern here; only how much of the vertical water column is filled with algae. If algae mats are present, the recorded algae depth is determined by adding the depth of the algae mat to the depth of the algae growing on the stream bottom. Mats may need to be moved out of the way to observe the bottom layer of algae attached directly to the stream bottom. REMEMBER: Algae Thickness should never be more than the water depth.

d. Dominant Substrate Type is determined by observing the predominant substrate size particle for the segment and using the classification table at the bottom of PAGE 3 ***(Figure 8-6 above)***.

6. Repeat steps two and three with each progressing segment of the transect.

7. If you encounter a mid-stream dry bar (not underwater) then document the bar width as its own segment (using the wetted edges of the bar as D_{Begin} and D_{End} for the segment) and make a note on the form. This area will be excluded from the calculation later.

8. When the far bank ($Bank_2$) is reached, record the final tape measurement distance at the wetted edge ($Bank_2$ D_{End}) and note LDB or RDB in the note field.

Measuring Canopy Density with a Densiometer

Canopy Density is measured based on a method used in the USEPA's National Rivers and Streams Assessment (NRSA), which is an element of the National Aquatic Resources Survey (NARS). Measurements are made using a spherical densitometer (model A-convex type) that is modified with a permanent marker or tape ***exactly as shown in Figure 8-20 on next page***.

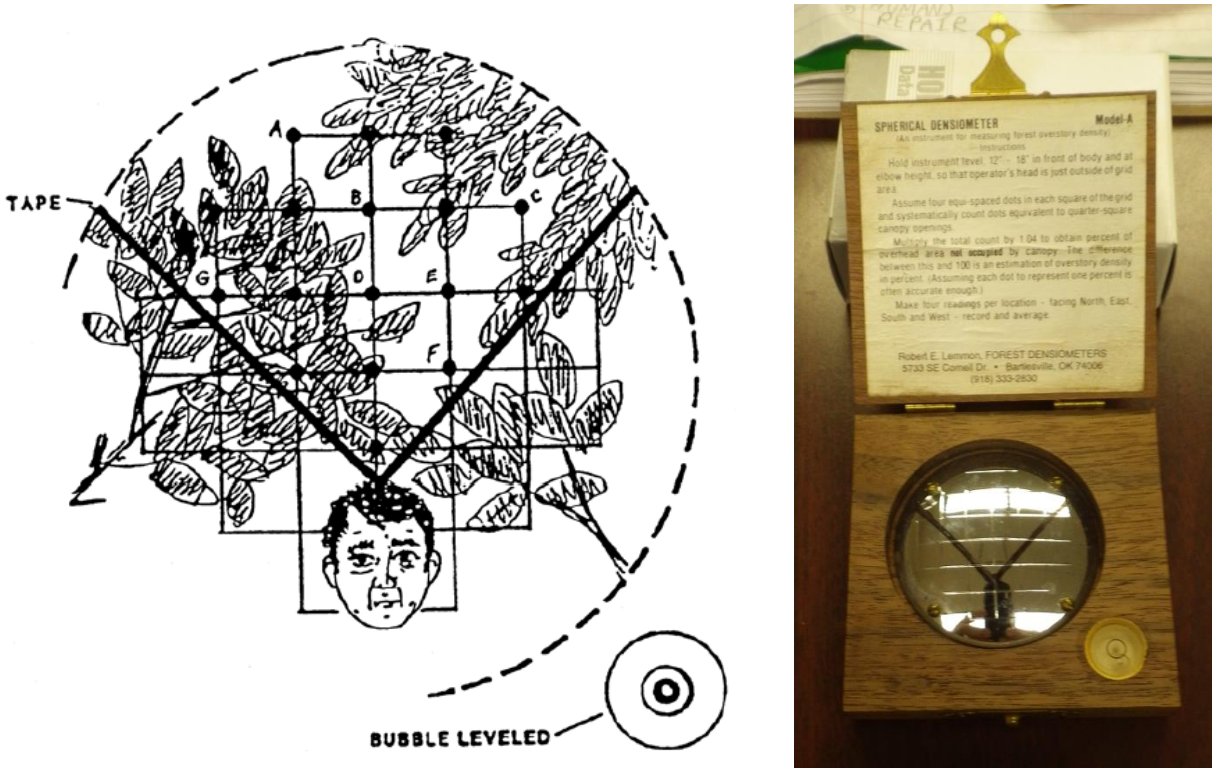

Figure 8-20. Examples of Modified Convex Spherical Canopy Densiometer (from Mulvey et al.

The markings or tape will limit the number of square grid intersections to 17 and densiometer readings can range from 0 (no canopy cover) to 17 (maximum canopy cover). Four measurements are obtained at each of 5 equidistant locations along the cross-section transect: Left (at the water's edge on the Left Descending Bank), Left Middle, Center, Right Middle, and Right (at the water's edge on the Right Descending Bank).

Fill out the bottom of PAGE 2 (***see Figure 8-4 on page 8-6***) of the Filamentous Algae Measurements Form using the following steps:

1. At the first location on the cross-section transect, stand in the stream and face upstream.
2. Hold the densiometer 0.3 m (1 ft.) above the surface of the stream. Level the densiometer using the bubble level. Move the densiometer in front of you so your face is just below the apex of the taped "V".
3. Count the number of grid intersection points within the "V" that are covered by a tree, a leaf, or a high branch. Record the value (0 to 17) in the Up field for the given location of the cross-section transect on the bottom of PAGE 2 under the Canopy Density section.
4. Face toward the left bank (left as you face downstream). Repeat Steps 2 and 3, recording the value in the Left field for the given location of the cross-section transect on the data form.

5. Repeat Steps 2 and 3 facing downstream and facing the right bank (right as you look downstream). Record the values in the Down and Right fields given location of the cross-section transect on the data form.
6. Repeat Steps 1 through 5 at for the remaining locations on the cross-section transect.

Part 2. Filamentous Algae Data Analysis

Use the following equations to calculate the Percent Algae Cover and Percent Water Column Algae Fill. ***See Figure 8-6 on page 8-7 for data used in the example calculations.***

<u>Calculating Percent Algae Cover</u>

1. Calculate the transect wetted width (WW) by subtracting $\mathrm{Bank}_1\mathrm{D}_{End}$ from $\mathrm{Bank}_2\mathrm{D}_{End}$.

$$\mathrm{WW} = \mathrm{Bank}_2\mathrm{D}_{End} - \mathrm{Bank}_1\mathrm{D}_{End}$$

$$e.g., \mathrm{WW} = 24.5 - 0.5 = 24.0$$

If any dry bars or islands were measured in the transect, add up their individual widths (Dry_i) and subtract from WW calculated above to get an algal habitat width of the stream transect.

$$\mathrm{AHW} = (\mathrm{Bank}_2\mathrm{D}_{End} - \mathrm{Bank}_1\mathrm{D}_{End}) - \sum(\mathrm{Dry}_1, \mathrm{Dry}_2, \mathrm{Dry}_3, \ldots, \mathrm{Dry}_i)$$

$$e.g., \mathrm{AHW} = (24.0) - 3.6 = 20.4$$

2. Calculate the width of each segment (W_i) by subtracting $\mathrm{D}_{Begin\,i}$ from $\mathrm{D}_{End\,i}$.

$$\mathrm{W}_i = \mathrm{D}_{End\,i} - \mathrm{D}_{Begin\,i}$$

$$e.g., \mathrm{W}_1 = 2.6 - 0.5 = 2.1$$

3. Calculate the distance weighted algal cover of each segment (wAC_i) by multiplying the width of each segment (W_i) by the estimated percent bottom algae cover of that segment (AC_i) and divide by 100.

$$\mathrm{wAC}_i = \mathrm{W}_i \text{ x } \frac{\mathrm{AC}_i}{100}$$

$$e.g., \mathrm{wAC}_1 = 2.1 \text{ x } \frac{25}{100} = 0.525$$

Any segment that was a dry bar (DB_i) should be calculated with the Algae Cover (AC_i) as 0.

$$e.g., \mathrm{wAC}_4 = 3.6 \text{ x } \frac{0}{100} = 0$$

4. Calculate the Estimated Percent Algae Cover (PAC) for this transect by dividing the sum of the distance weighted covers by the algal habitat width (AHW) and multiply by 100.

$$\mathrm{PAC} = \frac{\sum(\mathrm{wAC}_1, \mathrm{wAC}_2, \mathrm{wAC}_3, \ldots, \mathrm{wAC}_i)}{\mathrm{AHW}} \text{ x } 100$$

$$e.g., \mathrm{PAC} = \frac{0.525 + 1.15 + 2.0 + 0 + 1.275 + 3.0 + 1.98 + 0.665}{20.4} \text{ x } 100 = 51.9$$

Calculating Percent Water Column Filled

1. Calculate the volume of water in each segment (VW_i) by multiplying the width of each segment (W_i) (*from calculation 2 above*) by the water depth in in segment (WD_i). The third dimension of the volume is the one unit (foot or meter) wide area along the transect segment, effectively multiplying the equation by 1.

$$\mathrm{VW}_i = \mathrm{W}_i \text{ x } \mathrm{WD}_i = \mathrm{W}_i \text{ x } \mathrm{WD}_i \text{ x } 1$$

$$e.g., \mathrm{VW}_i = 2.1 \text{ x } 0.5 \text{ x } 1 = 1.05$$

NOTE: Any dry bar segments have a total volume (VW_i) of 0 since they have a water depth (WD_i) of 0 in this equation.

$$\mathrm{VW}_i = \mathrm{W}_i \text{ x } 0 = 0$$

$$e.g., \mathrm{W}_4 = 3.6 \text{ x } 0 = 0$$

2. Calculate the volume of algae in each segment (VA_i) by multiplying the width of each segment (W_i) (*from calculation 2 above*) by the algae thickness in that segment ($\underline{\mathrm{AT}_i}$) and by the estimated percent bottom algae cover of that segment (AC_i). Alternatively, you can multiply wAC_i (*from calculation 3 above*) by the algae thickness in that segment (AT_i).

$$\mathrm{VA}_i = \mathrm{W}_i \text{ x } \frac{\mathrm{AC}_i}{100} \text{ x } \mathrm{AT}_i = \mathrm{wAC}_i \text{ x } \mathrm{AT}_i$$

$$e.g., \mathrm{VA}_1 = 2.1 \text{ x } \frac{25}{100} \text{ x } 0.2 = 0.525 \text{ x } 0.2 = 1.05$$

Any segment that was a dry bar (DB_i) should be calculated with the volume of algae (VA_i) as 0.

$$e.g., \mathrm{VA}_4 = 3.6 \text{ x } \frac{0}{100} \text{ x } 0 = 0$$

3. Calculate the percent of the water column filled (PWCF) with algae by dividing the sum of the volumes of algae in each segment (VA_i) by the sum of the volumes of water in each segment (VW_i).

$$\mathrm{PWCF} = \frac{\sum(\mathrm{VA}_1, \mathrm{VA}_2, \mathrm{VA}_3, \ldots, \mathrm{VA}_i)}{\sum(\mathrm{VW}_1, \mathrm{VW}_2, \mathrm{VW}_3, \ldots, \mathrm{VW}_i)} \text{ x } 100$$

$$e.g., \mathrm{PWCF} = \frac{0.105 + 0.46 + 0.6 + 0 + 0.51 + 1.2 + 0.396 + 0.0665}{1.05 + 2.53 + 1.6 + 0 + 0.85 + 6.0 + 4.4 + 0.76} \text{ x } 100 = 19.4$$

Filamentous Algae Measurement Quality Assurance and Quality Control

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that all of the personnel who perform the filamentous algae measurements participate in duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training. ***See CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1 for additional information.***

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. Whilst a specific session on Filamentous Algae

Monitoring is not covered, other sessions (*e.g.,* site documentation and completing the stream assessment forms, sonde calibration, water collection protocols, stream flow measurement, field blanks and duplicates, *etc*.) are covered. In the field, individuals who are more experienced in Filamentous Algae Monitoring will be teamed up to give hands-on training to less experienced to assure reinforcement of training and accurate results before they are allowed to maintain these stations solo. This document is also provided to all program personnel for review and use in the field.

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CHAPTER 9. GOLDEN ALGAE COLLECTION PROTOCOLS

Golden Algae Overview

Golden Algae (*Prymnesium parvum*) is a common saltwater toxin-producing microscopic algae first identified in WV in the fall of 2009 on Dunkard Creek, a large stream that straddles the border between Pennsylvania and West Virginia. In the first week of September 2009, the first reports of an aquatic life kill (*i.e.,* fish, freshwater mussels, and amphibians) began to come into various state and federal regulatory agencies. By the end of the month, the aquatic life kill had exploded both in degree of severity and range within Dunkard Creek. The cause of the fish kill was eventually determined to be due to Golden Algae, specifically the toxin produced by the algae.

Golden Algae has become an invasive saltwater algae that is now being found in brackish (both natural and anthropogenic) inland waters in several states. The effects of the toxin produced by Golden Algae are dependent on several factors including: algal cell densities, availability of cations, temperature, pH, nutrient availability, and native freshwater algae which compete with Golden Algae for resources. In some cases, the algae can persist in streams completely unnoticed until one or more of the factors above change and force the release of toxin and consequently cause an aquatic life kill. The only viable means of controlling Golden Algae toxicity once the algae has become established in a water body is to control the factors that cause the release of the toxin.

Due to the great concern with the spread of Golden Algae to other waterbodies in the state, we have begun monitoring for the algae in target waters (*e.g.,* streams with conductivities greater than 1000) across the state that may be suitable for the algae to survive and act as a source for further spread. In addition, we continue to monitor the water chemistry and algae in Dunkard Creek in order to prevent the algae from causing further aquatic life kills.

Materials and Supplies

The following materials are required for filtering Golden Algae samples for qPCR analysis (***see Figure 9-1 on right***):

1. 1-liter cubitainer – Used as a sample container.
2. Dark or Black Plastic Trash Bag – Used to isolate the collected sample from any light source.
3. Cooler with ice – Used to keep the samples cool.
4. Filter Flask – Receptacle for the filtered water (at least 500 mL in size.)

Figure 9-1. Photo of the materials used to filter a Golden Algae sample.

5. Filter Funnel – Consists of three parts: A sterile Nalgene plastic 250 mL cup to hold the unfiltered sample, a small disposable plastic funnel that attaches below cup, and rubber stopper that affixes the filter cup to the flask.
6. Filters – *Whatman* Glass microfiber filters GF/F 47 mm diameter with 0.45 µm pore size.
7. Vacuum Pump – A variety of hand operated pumps are available. **Do not use Peristaltic Pump/Drill Apparatus.**
8. Vacuum Tubing – usually already attached to vacuum pump.
9. 10% Bleach Solution - for sterilization.
10. Distilled water and industrial grade deionized (DI) water (if available).
11. Sterilized stainless steel forceps or sterile plastic forceps.
12. 4" squares of clean aluminum foil (1 square of foil per sample).
13. Zip Lock Baggies – 1 sandwich size per sample and 1 gallon size to hold all samples per filtering session.
14. Sample Labels (***see Figure 9-2 on right***) – 1 paper label to be included with each foil pouch. Label should have:
    a. Volume filtered
    b. Number of filters
    c. Stream name
    d. AN-code
    e. Date collected
    f. Date filtered
    g. Collector
    h. Filtered by
15. Golden Algae Analysis Request forms with Chain-of-Custody (COC).

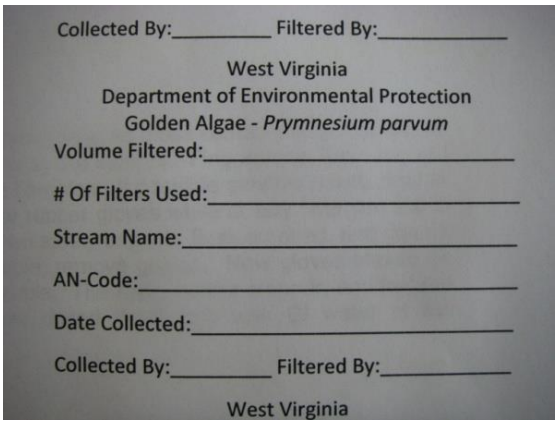

Figure 9-2. Photo of a Golden Algae label.

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.,* glass, metal, wire, *etc*.).

Part 1. Field Sampling Procedures

Golden algae samples are collected in a 1 liter cubitainer from a slow run or preferably a pool at approximately elbow depth (12-18 inches). To achieve a completely subsurface sample, the sample container must be capped until it is at the desired depth. The main concern is to avoid the upper surface of the water since the *P. parvum* cells may be sensitive to direct UV-light.

1. Rinse the 1-liter cubitainer **three times** at least **one-half full** with sample water. During the rinse, secure the lid on the cubitainer, shake for 5 seconds, and then empty.

2. Cap the cubitainer and submerge to the desired depth.

3. Uncap the cubitainer and fill completely with no air space (*i.e.,* no head-space). Remove capped/sealed cubitainer from water, quickly shake off external water and place inside a black or dark disposable plastic bag to prevent exposure to UV-light. Ideally the sample can now be placed in gallon zip-lock bag labeled with AN-code and date.

4. Samples are then placed on wet ice inside a cooler (maintained at 4° C) to keep them in the dark until filtering is started. Ideally, samples should be filtered immediately. If this is not possible, unfiltered samples should be maintained at 4° C and kept in the dark until they can be filtered.

Part 2. Sample Preservation (Filtration & Holding)

If possible, samples should be filtered and frozen by the end of the day they were collected. If this is not possible, unfiltered samples should be maintained at 4° C and kept in the dark until they can be filtered.

It is very important to keep the filtering process **as clean as possible** to prevent contamination. Therefore, filtration should be done in as controlled of an environment as possible. This may range anywhere from the back of a clean enclosed vehicle, to a hotel room, to the laboratory at WVDEP depending on the needs of the project.

Be sure to handle all materials by their exterior or by the stopper. Fingerprints, dirt/dust, and liquid from other samples can contaminate samples. Store all filtration equipment, aluminum foil, and sample labels in sealed plastic baggies when not in use. While handling aluminum foil at any time (cutting, folding, or removing from plastic bag); always be sure your hands and any surfaces it comes in contact with are clean and sanitized. If possible sanitize hands prior to handling any of the above materials and/or wear disposable laboratory gloves any time you are in contact with the sample. New gloves should be used for handling each individual sample. The flask, rubber stopper, and steel forceps should be soaked in 10% bleach solution for one minute and rinsed thoroughly with DI or distilled water at the beginning of and following each filtering session. Additionally, steel forceps and rubber stopper should be rinsed in bleach solution between each sample.

Inspect all sterilized materials for traces of liquid. Any liquid should be removed from the flask vacuum nozzle or the vacuum tube.

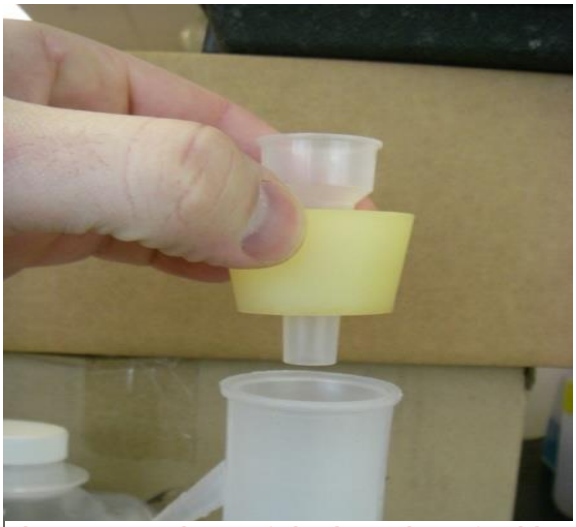

Figure 9-3. Photo of the insertion of rubber stopper into flask with plastic funnel already inserted through rubber stopper.

Sample blanks are generally prepared and processed as below using either distilled drinking water from freshly opened gallon containers or industrial grade DI water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described below. Two blanks should be prepared for each batch of samples. One of the blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered.

Filtration

1. Place plastic funnel snuggly into rubber stopper, and insert stopper snuggly into empty flask (***see Figure 9-3 on right***).

Figure 9-4. Photo of cup being removed to access the prepackaged filter for removal.

2. Attach vacuum tube and hand pump to flask.

3. Snap bottom of filtration cup onto plastic funnel then carefully remove top portion of the cup from its base revealing prepackaged nitrate seal/filter (***see Figure 9-4 below***).

Figure 9-5. Photo of the GF/F filter being placed on top of support screen with forceps.

4. Remove prepackaged nitrate seal/filter from cup base using sterilized forceps and place in a clean zip lock bag for potential use in other water quality monitoring projects.

5. Using sterilized forceps, place a new glass GF/F 47 mm filter in center on top of support screen found on the cup-base (***see Figure 9-5 right***). The cross-hatch pattern side of the disk should be placed facing down against the support screen (***see Figure 9-6 below***). Note: *Whatman brand filter disks are packaged with the cross hatch facing up – so, reversing them before placing on the support screen puts them in the proper position for filtering.*

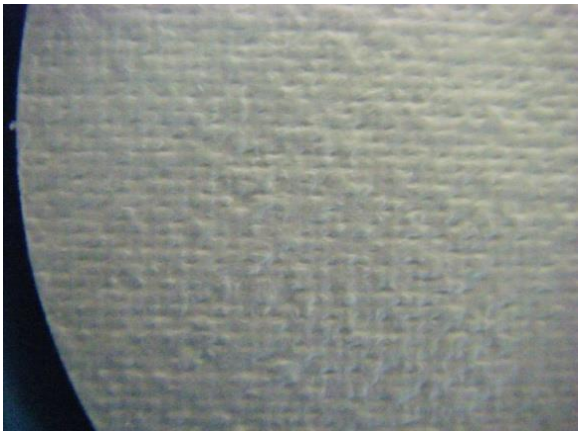

Figure 9-6. Photo of the gridded pattern of GF/F filter. This side placed down when filtering Golden Algae sample.

Figure 9-7. Photo of the pouring of a 250 mL sample into filter cup.

6. Replace top portion of cup straight down onto base with a snap to ensure seal. Make sure the filter disk is still centered.

7. Remove plastic lid from cup. Apply disposable laboratory gloves now before touching sample bag or bottle.

8. Mix sample thoroughly by inverting the cubitainer/sample bottle three times. Pour 250 mL of sample into filter cup and replace cup lid to ensure proper vacuum (***see Figure 9-7 above***). Place remaining sample back into bag to prevent further UV exposure.

Figure 9-8. Photo of monitoring the pump pressure while filtering sample – keep the pressure under 5 psi.

9. Filter 250 mL into the flask. Agitate the remaining sample in cubitainer again, remove cup lid, and pour an additional 250 mL into filter cup. This should be done without disassembly so that a total of 500 mL is filtered through a single filter. If more or less than 500 mL is filtered, be sure to record the actual amount as precisely as possible.

10. Minimal pressure/suction (not to exceed 5 psi) is used via the hand pump to pull sample through filter (***see Figure 9-8 above***). This is important because of the potential to break the filter and lose a portion of the algal sample into the flask. Turbid samples may take many minutes to filter so remain patient.

11. After full sample volume has been filtered, carefully remove filter cup from its base and discard. Filter disk will usually remain on support screen, but be prepared to remove from top portion of cup with sterile forceps if necessary. Save the remainder of unfiltered sample in the cubitainer by putting it a dark 4° C cooler or refrigerator. This unfiltered sample will be sent to the identifying laboratory to use for QA/QC

purposes. Now remove disposable plastic gloves carefully and dispose gloves in trash.

12. The filter disk is removed with sterile forceps, folded into a half-circle (still using only forceps) with sample-side in (grid side out for Whatman filters), and wrapped loosely in one 4"X4" aluminum foil square to prevent loss of algal cells via compression and smearing (***see Figure 9-9 on right***). The sample is then tagged with a completed sample label on outside of foil, placed in an individual sandwich-sized zip lock bag, and then placed in a freezer before shipping. If multiple samples are filtered, all of the individually packaged and bagged filters should be placed in a gallon-sized zip lock bag for storage and shipping to laboratory.

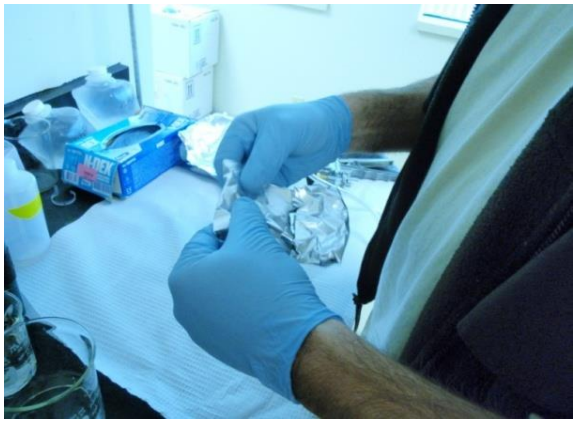

Figure 9-9. Photo of the filter disk being wrapped loosely in one 4"X4" aluminum foil square to prevent loss of algal cells via compression and smearing

13. After filter disk is properly packaged, labeled, and frozen, discard all water in flask by removing rubber stopper, and pouring out the remainder. Be sure not to allow water into flask nozzle or vacuum tube. If enough plastic funnels are available you can now dispose of used plastic funnel. If not replacements are available see directions below.

14. Between filtering samples, rinse the small plastic funnel and forceps with tap water, then soak in a 10% bleach solution for 1 minute (***see Figure 9-10 on right***).

Figure 9-10. Photo of Forceps and small plastic funnel soaking in 10% bleach solution for 1 minute.

15. A final rinse with industrial grade DI or distilled water completes the cleaning process between samples (***see Figure 9-11 below***).

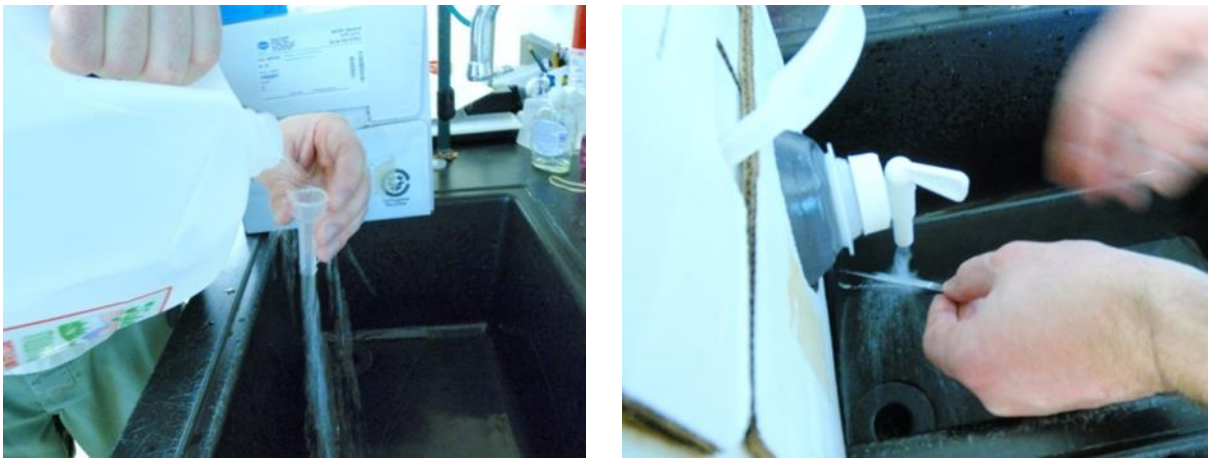

Figure 9-11. Left: Photo of small plastic funnel being rinsed with distilled water. Right: Photo of forceps being rinsed with industrial grade deionized water.

***IMPORTANT*: Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade DI water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described above. Two blanks should be prepared for each batch of samples. One of the blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered.**

Holding

If possible, samples should be filtered and the filter samples frozen by the end of the day on which they were collected. Freezing can be accomplished by placing the filter samples in a freezer or on dry ice (during field processing). Frozen samples are

shipped overnight in batches or individually on dry ice to appropriate contract identification lab. An Analysis Request Form with Chain-of-Custody (COC) designed for Golden Algae samples should be completed which includes all samples (field and blanks). ***See Figure 9-12 below for an example of a Golden Algae Analysis Request Form with COC***.

WV DEPARTMENT OF ENVIRONMENTAL PROTECTION - WATERSHED ASSESSMENT BRANCH

Golden Algae Analysis Chain of Custody Form, Rev. 2/10

Laboratory Name: _____

Preservation Method:
Unfiltered Sample: Store on ice at 4° C
Filtered Sample: Frozen

Stream Name	AN-Code	Collection Information			Filtration Information			
		By:	Date:	Time:	By:	Date:	Vol. (ml)	# of Filters

Relinquished by:	Date & Time:	Received By:	Relinquished by:	Date & Time:	Received By:

Mail Results to: ATTN: Janice Smithson, WVDEP, DWWM, Watershed Branch 601 57th Street SE, Charleston, WV 25304
Phone: (304) 926-0499 Ext. 1051, Fax. (304) 926-0496
Mail Invoice to: ATTN: Susan Rose, WVDEP, DWWM, Watershed Branch 601 57th Street SE, Charleston, WV 25304
Phone: (304) 926-0499 Ext. 1056, Fax. (304) 926-0496
On invoice, bill to Organization Unit 9481
Original COC Mailed to Laboratory, Photocopy retained by WVDEP

Figure 9-12. Example of a Golden Algae Analysis Request Form with COC

Golden Algae Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of Golden Algae samples is included. In the field, sampling teams will often consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in collecting water quality will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to collect Golden Algae samples solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Sample transfer to the lab shall be documented using the Chain-of-Custody (COC) portion of the Golden Algae Analysis Request Form.

The unfiltered portion of the original sample water is to be retained after filtering and may be sent to the contract identification laboratory with the filter samples. If the original filter sample appears to be contaminated, the contract lab may elect to replicate the filter sample using the original sample water as a mean of investigation.

Duplicate sampling and sample blanks must be performed at a minimum of 2.5% of our sites. To assure we meet these requirements, each team list will have a designated duplicate and sample blank. The sample blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented in ***CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1.***

Sample Blanks

Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade DI water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described above. Two blanks should be prepared for each batch of samples. One of the blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered. Laboratory analysis of the

sample blanks should indicate if there is any contamination originating from the filtering process.

Duplicate Samples

Both duplicates are collected at the same date and time and literally side by side by different individuals. If the sampling team consists of one person, as is often the case during a TMDL assessment, the duplicate is still performed by the one sampler. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been disturbed by the first duplicate. TMDL replicates are collected at any TMDL site with the full potential of parameters on the TMDL list. TMDL replicate sites are not specifically assigned; however, field crews should not repeatedly duplicate the same site.

Duplicates should be rotated to different sites each sampling event.

Results of the duplicates are compared and any samples not falling within an acceptable range are examined for sampling error. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training.

NOTE: If two people are involved in collecting a duplicate, each person should filter his or her own sample and not filter the other person's sample.