Chapter VII. PERIPHYTON COLLECTION PROTOCOLS

Periphyton Overview

Periphyton is attached algae, *i.e.*, algae that grow on the exposed surfaces of rocks and other submerged objects. Phytobenthic (bottom-dwelling) algae are usually the dominant component of a periphyton community. Phytobenthic algae, the primary producers in the stream ecosystem, are sensitive indicators of change in lotic waters. Because it is attached to the substrate, the periphyton community integrates physical and chemical disturbances to a stream. 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 WVSCI is not applicable – 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.

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. Use the toothbrush to loosen any remaining periphyton. 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. 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.

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 II. Section C. Part 1. PAGE 9-Periphyton Collection Information starting on page 73*****)** 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:

1) Clear the diatom frustules of organic material using either nitric acid or hydrogen peroxide/potassium dichromate oxidation.
2) Prepare slides and identify diatoms to species or lowest taxonomic level possible.
3) 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 XII. Section A. Field Blanks and Duplicates starting on page 285 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 VIII. 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 64 on right***):

1. 1-liter cubitainer – Used as a sample container.
2. Filter Flask – Receptacle for the filtered water (at least 500 mL in size.)
3. 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.

Figure 64. Photo of the materials used to filter a Golden Algae sample.

4. Filters – *Whatman* Glass microfiber filters GF/F 47 mm diameter with 0.45 µm pore size.
5. Vacuum Pump – A variety of hand operated pumps are available. **<u>Do not use Peristaltic Pump/Drill Apparatus.</u>**
6. Vacuum Tubing – usually already attached to vacuum pump.
7. 10% Bleach Solution - for sterilization.
8. Distilled drinking water and industrial grade deionized (DI) water.
9. Sterilized stainless steel forceps or sterile plastic forceps.
10. 4" squares of clean aluminum foil (1 square of foil per sample).
11. Zip Lock Baggies – 1 sandwich size per sample and 1 gallon size to hold all samples per filtering session.
12. Sample Labels (***see Figure 65 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
13. Golden Algae Analysis Request forms with Chain-of-Custody (COC).

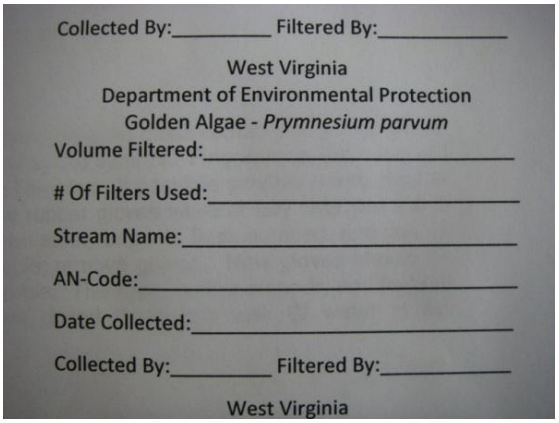

Figure 65. Photo of a Golden Algae label.

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 and lid twice with stream water.

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-loc 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 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.

Sample blanks are generally prepared and processed as below using either distilled drinking water from freshly opened gallon containers or industrial grade deionized 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* Error! Reference source not found. *n right***).

Figure 66. Photo of the insertion of rubber stopper into flask with plastic funnel already inserted through rubber stopper.

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 67*Error! Reference ource not found. *below***).

Figure 67. Photo of cup being removed to access the prepackaged filter for removal.

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

Figure 68. Photo of the GF/F filter being placed on top of support screen with forceps.

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 68 right***). The cross-hatch pattern side of the disk should be placed facing down against the support screen (***see Figure 69 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.

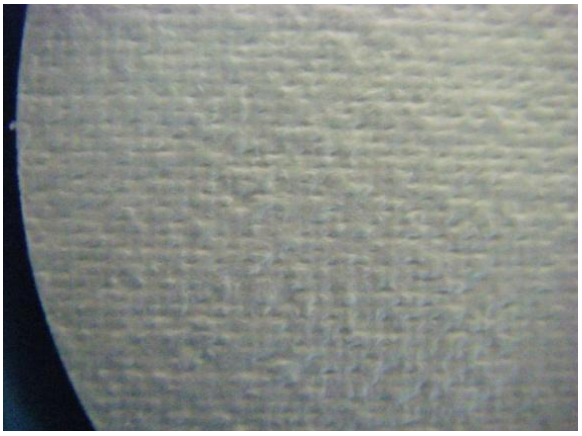

Figure 69. Photo of the gridded pattern of GF/F filter. This side placed down when filtering Golden Algae sample.

Figure 70. Photo of the pouring of a 250 mL sample into filter cup.

Figure 71. Photo of monitoring the pump pressure while filtering sample – keep pressure under 5 psi.

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 70 above***). Place remaining sample back into bag to prevent further UV exposure.

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 (<u>not to exceed 5 psi</u>) is used via the hand pump to pull sample through filter (***see Figure 71 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 72 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 72. 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, soak in a 10% bleach solution for 1 minute (***see Figure 73 on right***), and rinse with distilled drinking water (***see Figure 75 below***). A final rinse with industrial grade deionized water completes the cleaning process between samples (***see Figure 74 above***).

Figure 73. Photo of Forceps and small plastic funnel soaking in 10% bleach solution for 1 minute.

Figure 75. Photo of forceps being rinsed with deionized (DI) water.

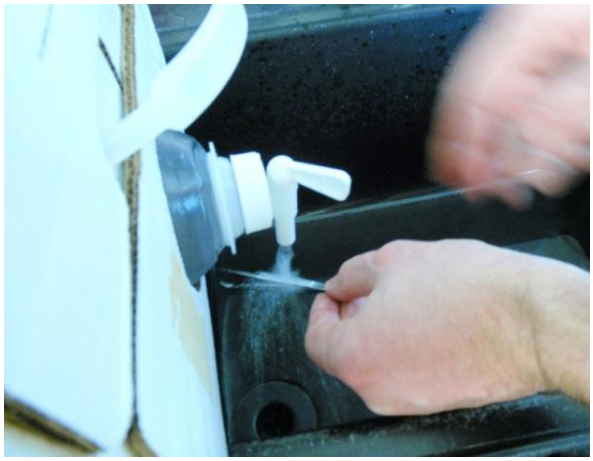

Figure 74. Photo of small plastic funnel being rinsed with distilled water.

NOTE: If desired volume of water (500 mL) will not go through filter due to clogging, start procedure from the beginning with a new cup and new filter with reduced sample volume in the cup. Do not remove any volume from the cup once the sample has been added or filtering has started, as this may cause contamination or loss of sample. The desired volume may be obtained by reducing the sample into smaller aliquots and filtering through a separate filter per aliquot. For example, it may be necessary to filter 250 mL through one filter and 250 mL through a second filter in order to obtain the desired 500 mL sample. If this is necessary, both filters should be folded and placed in separate sample foil packets. Both foil packets can be placed in 1 small zip-lock bag. However; when using the two filter method, a new cup must be used for each half and the funnel/forceps must be cleaned using step 14 above.

<u>Remember</u>: Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade deionized 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 76 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 76. 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 below. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Sample Blanks

Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade deionized 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.