APPENDIX A

Letter of Agreement between the EPA and VADEQ Dated November 4, 2013

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

NOV 04 2013

Ms. Melanie Davenport, Director
Division of Water Quality Programs
Virginia Department of Environmental Quality
629 E. Main Street
P.O. Box 1105
Richmond, Virginia 23218

Dear ~~Ms. Davenport~~ Melanie:

This letter follows up a series of discussions between staff from the U.S. Environmental Protection Agency Region III (EPA) and the Virginia Department of Environmental Quality (DEQ) regarding our agencies' agreement to work cooperatively to develop a quantitative, repeatable, and scientifically valid methodology for evaluating algal growth in Virginia's non-tidal (free-flowing) waters.

While algae naturally occurs, excessive algal growth can have adverse impacts to water quality and the aquatic ecosystem, including smothering of streambeds, etc. Levels of algal growth frequently are associated with excessive instream levels of plant nutrients, but also may vary with other factors. In light of the foregoing, EPA and DEQ have agreed to work cooperatively to develop a methodology that would allow DEQ to evaluate algal growth in Virginia waters. The remainder of this letter summarizes what we believe to be our mutual understanding of a path forward. Your concurrence on this letter indicates DEQ's agreement with this understanding.

To the extent authorized by law, EPA intends to provide support to a pilot study, including field observation and data collection, of algal growth in the Shenandoah River Basin, with the goal of developing a methodology that will be science-based and repeatable in other Virginia waters. EPA and DEQ anticipate that the pilot study will consist of two phases: data collection (including collection methodology development) and a user survey of Virginia residents to identify levels of algal growth that could be considered excessive.

As part of the pilot study and to the extent authorized by law, EPA and DEQ have agreed to work with the Interstate Commission on the Potomac River Basin (ICPRB) on the pilot study. EPA and DEQ anticipate that ICPRB will create a work plan for development of a systematic methodology to evaluate spatial and temporal extent of algal growth. DEQ anticipates that it will work with ICPRB to review a draft work plan and to provide comment on both the work plan and on sampling location selection.

The first phase of the pilot study will consist of data collection and is anticipated to last approximately 14 months, with completion expected in January 2015. EPA and DEQ anticipate that ICPRB will develop, with input from EPA and DEQ as lawful and appropriate, a methodology for data collection, including data on both the extent of algal growth and water quality. EPA and DEQ anticipate that ICPRB will utilize the Filamentous Algae Monitoring methods developed by the West Virginia Department of Environmental Protection as a model for the data collection methodology. It is understood that the pilot study is intended to make use of citizen monitors to collect data utilizing the data collection methodology in a manner that could serve as a model for future use of citizen data regarding algal growth. Best efforts, including outreach, will be undertaken to ensure that data is collected from a broad array of citizen monitors representing all citizen groups in the Shenandoah River Basin. EPA and DEQ anticipate that ICPRB staff will train citizen monitors, and work with citizen monitors to develop a Quality Assurance Project Plan (QAPP) with a goal of being consistent with Virginia's citizen monitoring program. It is anticipated that ICPRB will perform field audits of citizen monitors to ensure good data quality, and that personnel from EPA and DEQ may participate in such audits. EPA and DEQ anticipate that ICPRB will produce a final report summarizing the data collection efforts.

EPA and DEQ anticipate the timeline for the first phase of the pilot study as follows:

- EPA,VADEQ and ICPRB review and finalize scope of work – November 2013 conference call
- Development of draft field methodology by ICPRB, opportunity for review and comment by EPA and DEQ, completion of field methodology – December 2013
- Development of citizen monitor QAPP – December 2013-March 2014
- Outreach to citizen monitor groups – January 2014 – March 2014
- Train citizen monitors how to distinguish filamentous algae from submerged aquatic vegetation and periphyton, and performance of field data collection methods (field training depends on onset of algal blooms)– March 2014 - July 2014
- Collection of algal presence/absence and percent cover data, QA/QC of citizen monitors by ICPRB - March – September 2014 (This will be coordinated as much as possible with VADEQ ambient water quality monitoring program)
- Data analysis – October December 2014
- Final report by ICPRB – Expected January 2015

It is anticipated that the second phase of the pilot study will consist of a user survey of Virginia residents to identify levels of algal growth that could be considered excessive.

. Results of the pilot study will be shared with Virginia DEQ staff to provide information on algal growth in Virginia's non-tidal (free-flowing) waters for their review and consideration as part of the public participation process for Virginia's 2016 Integrated Report.

During the study period, Virginia will continue to implement ongoing programs which will improve local water quality in the Shenandoah basin, including: nutrient removal upgrades at treatment plants to achieve Chesapeake Bay TMDL requirements , installation of best management practices to reduce pollutant loads from agriculture and urban runoff sources, and implementation of TMDLs already completed for local water quality concerns.

As required by the Antideficiency Act, 31 USC 1341 and 1342, any commitments made by EPA in this letter are subject to the availability of appropriated funds and Agency budget priorities. Nothing in this letter in and of itself obligates EPA to expend appropriations or to enter into any contract, assistance agreement, interagency agreement, or incur other financial obligations. EPA and DEQ may provide the public with information regarding the terms of this letter. It is important to note that the Clean Water Act and its implementing regulations contain the legally binding requirements. This letter does not substitute for those provisions or regulations and is not itself a regulation. Any decisions or other actions that may be taken in regard to any waterbody will be based on the applicable statutes, regulations, and case law. This letter agreement does not create any new right or benefit, substantive or procedural, enforceable by law or equity against EPA or DEQ, or any of their officers or employees, or any other person. EPA reserves any rights and remedies available to it under all federal laws or regulations for which EPA has jurisdiction. EPA also reserves the right to commence an action against any person in response to any condition which EPA determines may present an imminent and substantial endangerment to the public health, public welfare, or the environment.

Thank you for your cooperation as our agencies work together toward our common goal of better understanding algal growth in Virginia's waters. Feel free to contact me or contact Evelyn MacKnight, Associate Director of the Office of Standards, Assessment, and TMDLs, at 215-814-5717 if you have any questions.

Sincerely,

Jon M. Capacasa

Jon M. Capacasa, Director
Water Protection Division

Concurrence on this 19 day of November 2013:

Melanie D. Davenport

Melanie D. Davenport
Water Division Director
Virginia Department of Environmental Quality

Appendix B

Table of Algae and Periphyton Programs Across the United States

Current and in-development algal bioassessment protocols, by state. (Data assembled from the California swamp standard operating procedures for collecting stream algae samples and associated physical habitat and chemical data for ambient bioassessments in California, 2009).

	Diatoms	**Diatoms + Softbodied Algae**	**Chlorophyll or Density**	**Chl a**	**AFDM**	**Chl a + AFDM**	**Other**	**Rapid Bioassessment Periphyton Method**	**Transects, Point Intercept (Y/N Presence)**	**Rough Visual Estimate**
	Taxonomic			***Biomass***				***Percent Cover***		
Alabama	X			X				X		
Alaska										
Arizona	X									X
Delaware		X				X				
Florida		X					X		X	
Idaho	X									
Indiana			X			X				
Kentucky								X		X
Maine		X						X		
Massachusetts		X								
Missouri	X									X
Montana		X		X						
New Jersey	X					X		X		
New Mexico	X			X					X	
New York	X			X						X
Oklahoma			X	X				X		
Pennsylvania		X				X		X		
South Dakota		X				X			X	
Tennessee			X					X		
Virginia		X				X				X
West Virginia	X				X	X				X
Wisconsin	X				X			X		
Wyoming		X		X						X

APPENDIX C

The WVDEP Filamentous Algae Monitoring Standard Operating Procedure (SOP)

Chapter VIII. 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 122 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 122. 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 stretched 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 II. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES

2014 V0.1 SOP FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. Guidelines for Completing the Stream Assessment Forms on page 41

Chapter III. WATER COLLECTION PROTOCOLS starting on page 138

Chapter XIV. Section A. Field Blanks and Duplicates starting on page 437

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 flow 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 stretching 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 123 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.

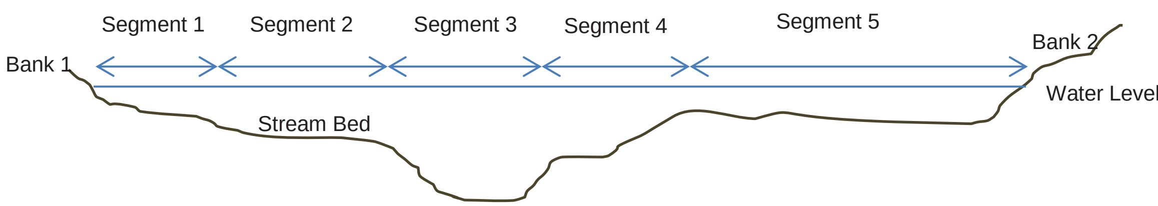


Figure 123. Hypothetical example of stream segments across a filamentous algae transect

> **NOTE: This concept of isolating differing sections laterally across a stream channel is used in the Flow measurement methodology in Chapter IV. STREAM FLOW MEASUREMENT PROTOCOLS starting on page 196 and longitudinally in the Dominant Substrate/Reach Characterization in Chapter II. 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 64.**

Documentation using the Filamentous Algae Monitoring Form

Record the site documentation information on the Filamentous Algae Monitoring Form as outlined in **Chapter II. 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 42.**

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 124 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 124. 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 125 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 125. Example of the additional Canopy Density (Densiometer) Readings Section on Page 2

- Page 3: Filamentous Algae Measurements (***see Figure 127 on next page and Taking Filamentous Algae Measurements on page 297***)

- 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 126 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 126. 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 127. Example of Page 3 of the Filamentous Algae Monitoring Form

<u>Taking Filamentous Algae Measurements</u>

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.

   > **<u>IMPORTANT</u>:** ***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. <u>Water depth (WD_i)</u> is determined by evaluating a given transect segment for a representative depth measurement.

   b. The <u>percent submerged aquatic vegetation (SAV)</u> and <u>percent filamentous algae cover (AC_i)</u> 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 128 to Figure 140 for photo examples of measured percent filamentous algae cover***.

Figure 128. 3% Filamentous Algae Cover

Figure 129. 4 % Filamentous Algae Cover

Figure 130. 12% Filamentous Algae Cover

Figure 131. 15% Filamentous Algae Cover

Figure 132. 20% Filamentous Algae Cover (Short Strands)

Figure 133. 20% Filamentous Algae Cover (Long Strands)

Figure 134. 26% Filamentous Algae Cover

Figure 135. 35% Filamentous Algae Cover (Short Strands). Note that this is the same location as Figure 136.

Figure 136. 38% Filamentous Algae Cover (Long Strands). Note that this is the same location as Figure 135.

Figure 137. 39% Filamentous Algae Cover

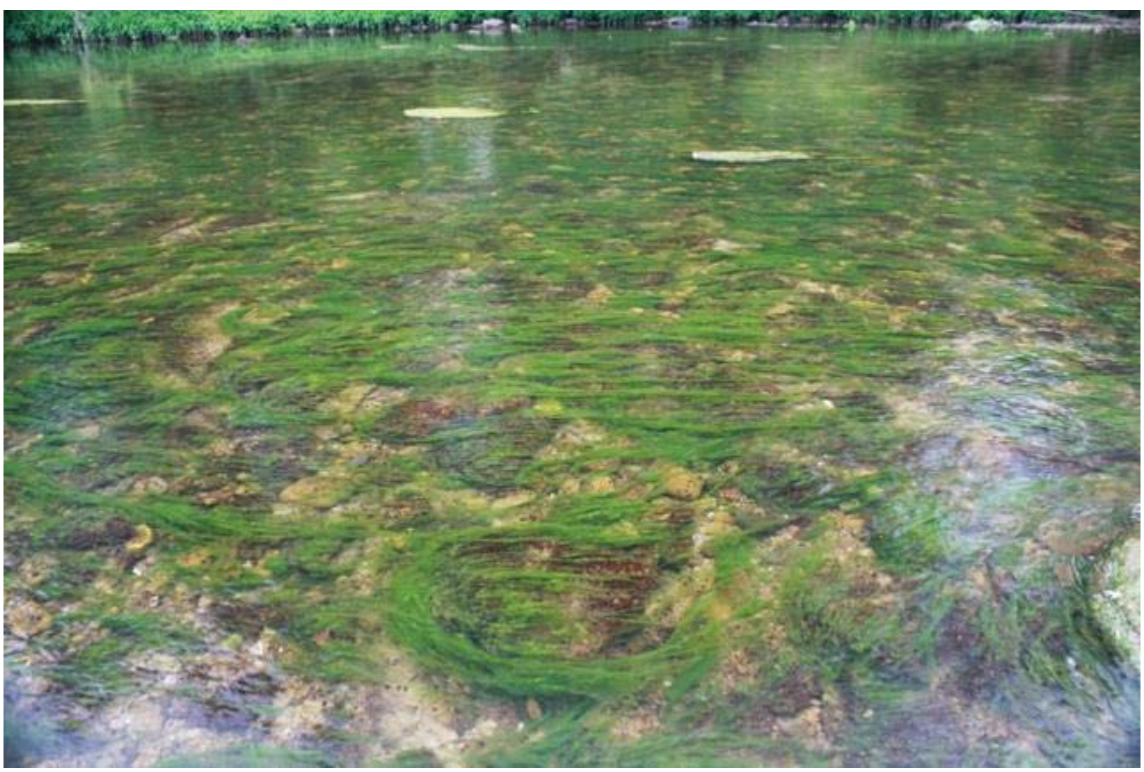


Figure 138. 47% Filamentous Algae Cover

Figure 139. 65% Filamentous Algae Cover

Figure 140. 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 127 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 141 on next page***.

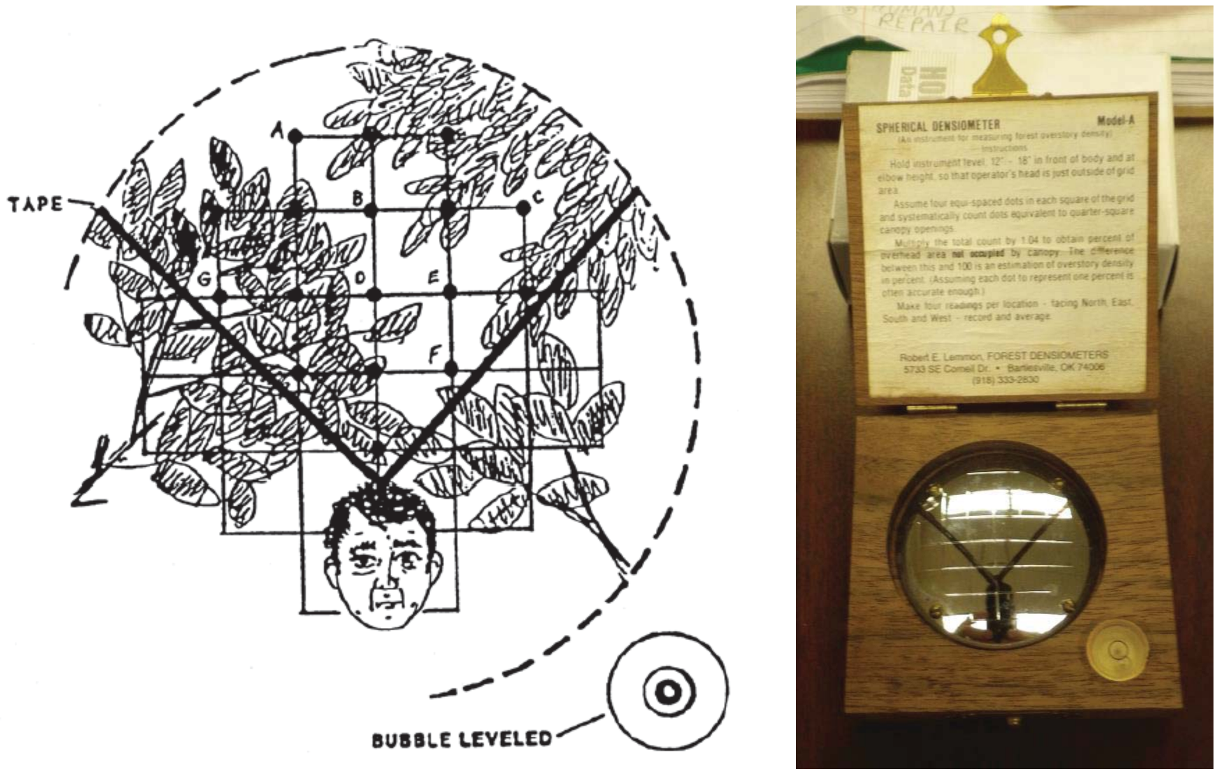


Figure 141. Examples of Modified Convex Spherical Canopy Densiometer (from Mulvey et al. 1992)

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 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 127 on page 296 for data used in the example calculations.***

<u>Calculating Percent Algae Cover</u>

1. Calculate the transect wetted width (WW) by subtracting $\mathrm{Bank_1D}_{End}$ from $\mathrm{Bank_2D}_{End}$.

$$\mathrm{WW} = \mathrm{Bank_2D}_{End} - \mathrm{Bank_1D}_{End}$$

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

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.

$$\mathrm{AHW} = (\mathrm{Bank_2D}_{End} - \mathrm{Bank_1D}_{End}) - \sum(\mathrm{Dry_1}, \mathrm{Dry_2}, \mathrm{Dry_3}, \ldots, \mathrm{Dry}_i)$$

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

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.

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

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

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

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

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.

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

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

<u>Calculating Percent Water Column Filled</u>

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.

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

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

<u>NOTE</u>: 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.

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

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

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 XIV. Section A. Field Blanks and Duplicates starting on page 437 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.

APPENDIX D

The WVDEP Filamentous Algae Monitoring Program Field Form

Filamentous Algae Monitoring Form

Hey you monkey, pay attention!						**Reviewers Initials**			
Stream Name (with location)									
AN-Code		**Date**		**Time**		**Geo**		**Bio**	
Basin		**County**		**Quad**					
GPS Type		**EPE**		**XY's Proofed**				**By**	
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**						
Sampled	**☐ Yes ☐ No**	**If not, why?**	**☐ No Access-Physical Barrier (☐ *Permanent* / ☐ *Temporary*)** **☐ No Access-Landowner Denial (☐ *Verbal Denial* / ☐ *Posted* / ☐ *Fenced* / ☐ *Private*)** **☐ Too Deep (☐ *Permanent-Not Wadeable* / ☐ *Temporary*) ☐ Dry ☐ Filled ☐ Impounded** **☐ Other:**						
Sample Type	**☐ YSI ☐ Fecal ☐ AMD ☐ Nutrients ☐ Acid Rain ☐ Orthophosphate ☐ Flow ☐ Other:**								
Duplicate type	**☐ None ☐ Lab ☐ Fecal**	**Duplicate WQ ID**		**Was site moved?**	**☐ Yes ☐ No**				
Explanation?									
Directions To Site									

Sketch of Assessment Reach and Comments: **Indicate North with (↑), indicate flow direction, indicate water sample (wq), indicate lat and long site with (X). Be sure to draw any houses, camps, or public access points in the area. Draw the sketch with a fine resolution to give a specific idea of the location of the transect.**

Notes		**Single WQ Sample ID**	

Reviewers Initials		FIELD WATER >>

WQ Sample Location	☐ Mid-Stream ☐ Bank (☐ Left ☐ Right) ☐ Thalweg (☐ Left ☐ Middle ☐ Right) ☐ Left Channel ☐ Right Channel ☐ Other:	WQ Type	☐ Single ☐ Profile ☐ Composite ☐ Other:
Sonde Method	☐ Grab ☐ Sample Tube ☐ Bucket	Lab Water Method	☐ Grab ☐ Sample Tube ☐ Bucket

Flag	Physicochemical Parameters (for a <u>Single</u> Water Quality Sample)		Seasonal Water Level		Water Odors		Surface "Oils"		Turbidity	
		Temperature °C		Below Normal		Normal		None		Clear
		pH (std. Units)		Normal		Sewage (Not Septic)		Flecks		Slightly Turbid
		Dissolved Oxygen (mg/L)		Above Normal		Petroleum		Sheen		Moderately Turbid
		Conductivity (μmhos/cm)		Flooding		Chemical		Globs		Highly Turbid
Sonde I.D. #:________ If any problems occur with the Water Meter or any readings are suspect, record notes in the space to the right.			Notes:			Anaerobic (septic)		Slick	Water color:	
						Other:				
					Foam/Suds (Rate 0-4 or NR)					

ABOVE: Record readings in box for corresponding physicochemical parameter. Insert a √ in the box for other categories.

Precipitation Status and History					
Current		Past 24 Hours (If Known)		Major Rain Event in past week?	☐ Yes ☐ No

If it is raining or has rained recently, which of the following best describes the peak runoff (flush) condition of the stream at the site when water samples were collected? If the runoff condition is in response to snowmelt, please indicate as such above.

N/A		< 1 Hour		1 to 4 Hours		4 to 12 Hours		12 to 24 Hours		1 to 2 Days		2 to 4 Days		4 to 7 Days		Unknown	

Is the stream level in the process of rising or falling at the time of visit?	☐ Baseflow ☐ Rising ☐ Falling
<u>No Flow?:</u> If a flow was scheduled for the site and not performed, then indicate if one of the following applies	☐ Dry ☐ Low Flow ☐ Too Deep/Too Fast ☐ Instrument Failure ☐ Frozen/Ice ☐ Safety

Field Water Notes & Precipitation Comments:

Indicate abundance of each: 0=None, 1=Low, 2=Moderate, 3=High, 4=Extreme, NR=Not Rated	Periphyton (*Brown-slick; Diatoms*)		Filamentous Algae (*Green; Long*)		Blue-Green Algae (*Blueish-Green Slime; Not Long*)		Aquatic Mosses	
	Submerged Aquatic Plants (*e.g., Stargrass, Hydrilla*)		Emergent Aquatic Plants (*e.g., Water Willow*)		Floating Aquatic Plants (*e.g., Lily Pads, Duckweed*)		Total Aquatic Plants	

Periphyton/Algae/Aquatic Plants & Mosses Notes:

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	

FILAMENTOUS ALGAE MEASUREMENTS>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>		**Reviewers Initials**	
AN-Code		**Date**	
Measurer		**Time**	

Location & Bank	D_{Begin}	D_{End}	% SAV Cover	% Algae Cover (AC_i)	Water Depth (WD_i)	Algae Thickness (AT_i)	Segment Dominant Substrate Type	Segment Measurement Notes
Bank$_1$								LDB ☐ RDB ☐
Segment 1								
Segment 2								
Segment 3								
Segment 4								
Segment 5								
Segment 6								
Segment 7								
Segment 8								
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$_2$			DS Channel Orientation/Bearing		^{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

Reviewers Initials		*LANDOWNER/STAKEHOLDER INFORMATION & PHOTOS >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>*

Landowner/ Stakeholder Name		Landowner/ Stakeholder Name	
Address		Address	
1ºPhone #	( )	1ºPhone #	( )
ALT #	( )	ALT #	( )
Stream Data Requested [ ]	WS Report Requested [ ]	Stream Data Requested [ ]	WS Report Requested [ ]
Other Pertinent Landowner Information (e.g., email) & Comments:		Other Pertinent Landowner Information (e.g., email) & Comments:	

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:

Photography Log >>>>>>>>>>>>>>>>	Camera Type		Camera Number	

#	Photo ID (office)	Disk Photo # (field)	Stream Name and/or AN-Code	Photo Description (Use Key Words)	Date	Photographer
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

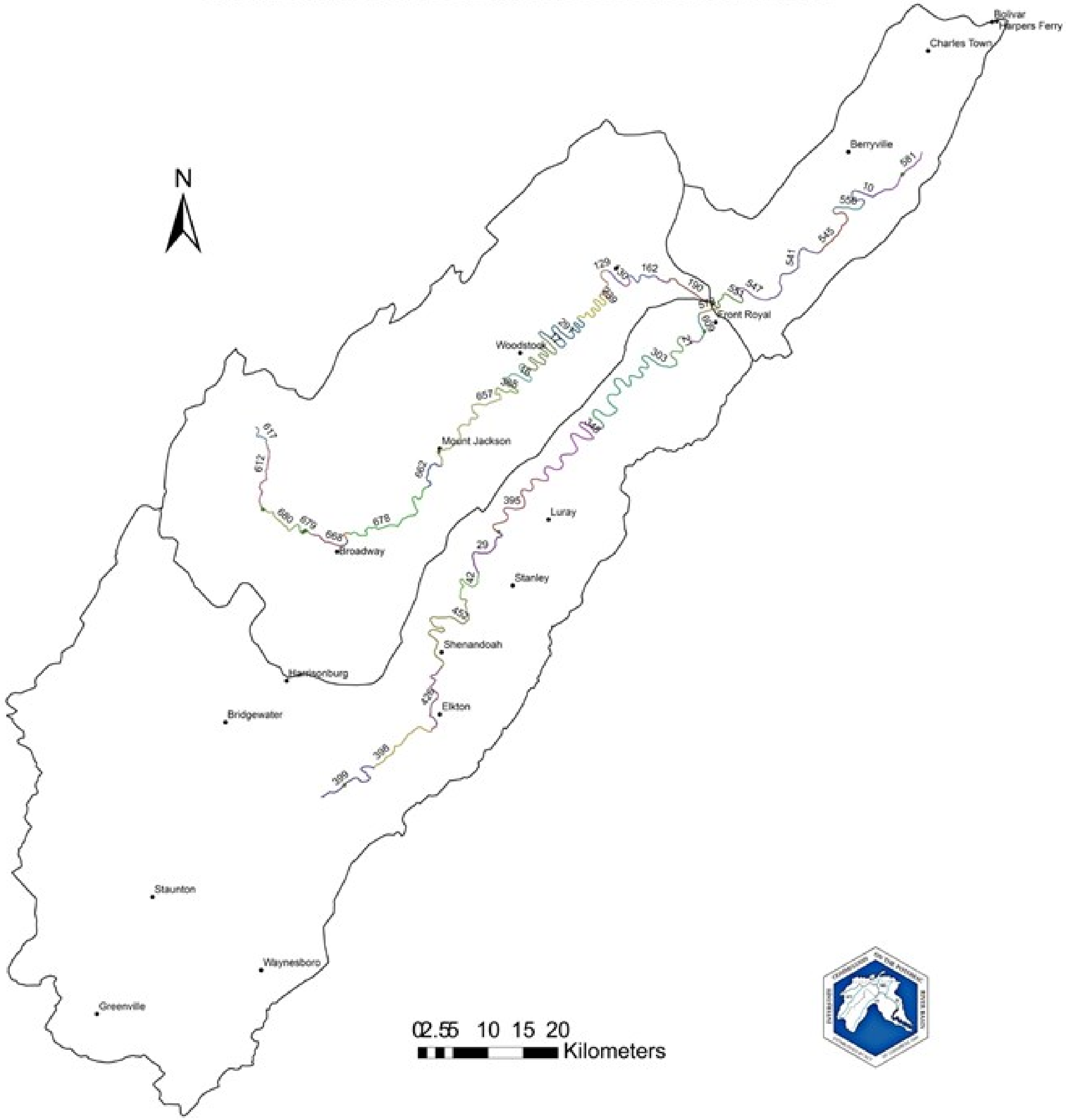
Appendix E

Virginia Shenandoah River Assessment Units and Estimated Wadeable Reaches

Virginia Assessment Units (AUs), their lengths, relative widths and estimated wadeability. A Y* indicates, that while the lateral width is not problematic, deep pools or glides may wake wading problematic or impossible.

Basin	AU #	AU Code	Length (Km)	Wadeable?	Minimum Width	Maximum Width
South Fork	399	VAV-B33R_SSF01A00	12.69	Y	30	60
	398	VAV-B35R_SSF02A10	11.41	Y	30	80
	426	VAV-B35R_SSF0`1A00	12.82	Y	60	100
	452	VAV-B37R_SSF02A10	22.17	N	30	140
	42	VAV-B37R_SSF01A00	8.02	N	80	140
	29	VAV-B38R_SSF02A10	9.63	Y*	30	80
	395	VAV-B38R_SSF01A00	16.84	N	40	180
	348	VAV-B40R_SSF02A00	21.85	N	60	180
	303	VAV-B40R_SSF01A00	30.80	N	50	200
	21	VAV-B41R_SSF04A00	7.20	N	65	85
	591	VAV-B41R_SSF03A00	2.49	N	70	100
	609	VAV-B41R_SSF02A00	3.95	N	60	100
	578	VAV-B41R_SSF01A00	2.98	N	60	100
North Fork	617	VAV-B42R_NFS01A00	4.17	Y		<25
	612	VAV-B43R_NFS01A00	10.70	Y		<25
	680	VAV-B44R_NFS01A00	6.79	Y		<25
	679	VAV-B45R_NFS04A04	3.26	Y		<25
	668	VAV-B45R_NFS03A00	4.72	Y		<25
	659	VAV-B45R_NFS02C10	1.50	Y		<25
	646	VAV-B45R_NFS02B08	1.70	Y		<25
	678	VAV-B45R_NFS02A00	18.59	Y	20	55
	662	VAV-B45R_NFS01A00	5.78	Y	10	45
	657	VAV-B48R_NFS01A00	19.72	Y	20	60
	388	VAV-B50R_NFS04A00	6.91	Y*	30	70
	16	VAV-B50R_NFS03A00	8.28	Y*	50	85
	17	VAV-B50R_NFS02A00	19.11	Y*	30	65
	291	VAV-B50R_NFS01A00	19.82	Y*	35	60
	239	VAV-B51R_NFS07A00	11.29	Y*	35	60
	129	VAV-B51R_NFS06A00	9.44	Y*	40	55
	130	VAV-B51R_NFS05A00	2.63	Y*	35	55
	14	VAV-B51R_NFS04B10	1.96	Y*	40	55
	162	VAV-B51R_NFS04A00	6.09	Y*	40	75
	180	VAV-B51R_NFS03A00	2.07	N	40	75
	190	VAV-B51R_NFS02A00	7.58	N	40	75
Shenandoah	551	VAV-B55R_SHN02A00	5.90	N	85	140
	547	VAV-B55R_SHN01B10	7.12	N	100	190
	541	VAV-B55R_SHN01A00	13.71	N	100	125
	545	VAV-B57R_SHN03A00	8.69	N	75	140
	556	VAV-B57R_SHN02A00	5.33	N	75	150
	557	VAV-B57R_SHN01A00	4.39	N	75	160
	10	VAV-B58R_SHN02A00	4.33	N	50	150
	581	VAV-B58R_SHN01A00	8.40	N	50	120

Shenandoah Basin Assessment Units



APPENDIX F

Field Observations of Percent Algal Cover
North Fork Shenandoah River - September 15, 2014

(see report for additional details)

Place and time data

Site-Section ID	DATE	TIME	GPS_TYPE	GPS EPE (ft)	START LAT	START LONG	FINISH LAT	FINISH LONG
NF_SHEN_DEER_1	9/15/2014	10:20	Garmin	14	38.95456	78.3751	38.9525	78.36675
NF_SHEN_DEER_2	9/15/2014	10:50	Garmin	14	38.95253	78.36677	38.95622	78.36755
NF_SHEN_DEER_3	9/15/2014	11:20	Garmin	13	38.95622	78.36755	38.96155	78.37686
NF_SHEN_DEER_4	9/15/2014	12:30	Garmin	13	38.96155	78.37686	38.96575	78.37578
NF_SHEN_DEER_5	9/15/2014	12:40	Garmin	13	38.96575	78.37578	38.97691	78.38075
NF_SHEN_DEER_6	9/15/2014	12:55	Garmin	12	38.97691	78.38075	38.98	78.38377
NF_SHEN_DEER_7	9/15/2014	13:09	Garmin	13	38.98	78.38377	38.98611	78.38158
NF_SHEN_DEER_8	9/15/2014	13:25	Garmin	13	38.98611	78.38158	38.97306	78.35155

Water quality data (collected with YSI meter)

Site-Section ID	WTEMP	pH	DO	SPCOND
NF_SHEN_DEER_1	18.72	8.25	9.87	420
NF_SHEN_DEER_2				
NF_SHEN_DEER_3	18.88	8.28	11.16	417
NF_SHEN_DEER_4				
NF_SHEN_DEER_5				
NF_SHEN_DEER_6	19.54	8.11	10.55	426
NF_SHEN_DEER_7				
NF_SHEN_DEER_8				

Qualitative assessment of aquatic vegetation in segment

Types of vegetation: FGA = filamentous green algae (Chlorophyta), BGA = filamentous blue-green algae (Cyanobacteria), MOSS = aquatic mosses, PERI = periphyton, EAV = emergent aquatic vegetation, FAV = floating aquatic vegetation, SAV = submerged aquatic vegetation, TOTAL = all vegetation

Qualitative scores: 0 = Absent, 1 = Rare, 2 = Common, 3 = Abundant, 4 = Extreme

Site-Section ID	FGA	BGA	MOSS	PERI	EAV	FAV	SAV	TOTAL	Decision to perform lateral transect measurements
NF_SHEN_DEER_1	2	2	0	2	1	0	2	2	N*
NF_SHEN_DEER_2	1	1	0	2	1	0	1	1	N
NF_SHEN_DEER_3	3	1	0	2	2	0	1	1	Y
NF_SHEN_DEER_4	1	2	0	2	2	0	3	3	N
NF_SHEN_DEER_5	2	2	0	2	2	0	2	2	N
NF_SHEN_DEER_6	2	1	0	2	2	0	0	1	N**
NF_SHEN_DEER_7	1	1	0	1	1	0	2	1	N
NF_SHEN_DEER_8	1	2	0	2	2	0	1	1	N

*Algae was immediately encountered, and it was assumed that other chances to measure in this section would present themselves, but they did not. **Section was not wadeable and visibility was obscured.

Qualitative notes about segments

Site-Section ID	NOTES
NF_SHEN_DEER_1	Bedrock dominated. FGA/BGA in channel - up to 40% coverage. BGA in shade. Pondweed sp? / Riverweed, Stargrass. FGA 5-40% where present.
NF_SHEN_DEER_2	Clean section. Segment ends at next algae occurrence.
NF_SHEN_DEER_3	Several measurements taken in segment (19.94%, 40.52%) and one estimate of over 90%. FGA is abundant and consistently 40-90% coverage.
NF_SHEN_DEER_4	Sago Pondweed/Riverweed? Abundant. FGA reduced. BGA increased. Up to 80% SAV coverage.
NF_SHEN_DEER_5	Segment begins at ledge where algae replaces SAV. Large sponge colonies observed. At 38.97277 -78.37380, transition back to grasses.
NF_SHEN_DEER_6	Deep run section. Bottom dominated by dead and dying algae. Creek input on river-left. Segment ends at island.
NF_SHEN_DEER_7	Riverweed, Stargrass, Coontail, sponges, and FGA observed. Cobble dominated.
NF_SHEN_DEER_8	*Chara* dominant. Bedrock, sand substrate. Small trib enters with algae at mouth. BGA increased - heavy at 38.97380 -78.35343.

Lateral transect measurements made in Segment NF_SHEN_DEER_3 (n=3)

Distance-weighted cover is the transect segment width multiplied by the segment's percent algal cover.

Transect 1

	Distance (ft)	Transect Section Width (ft)	Percent Algal Cover in Section	Distance-Weighted Cover
Total width	**124**			
Lateral section 1	100	24	1%	0.24
Lateral section 2	75	25	35%	8.75
Lateral section 3	50	25	50%	12.50
Lateral section 4	25	25	75%	18.75
Lateral section 5	0	25	40%	10.00
			Sum:	**50.24**

Percent of algal cover across lateral transect 1 is: 50.24 / 124 = **40.52%**

Transect 2

	Distance (ft)	Transect Section Width (ft)	Percent Algal Cover in Section	Distance-Weighted Cover
Total width	**238**			
Lateral section 1	194	44	1%	0.44
Lateral section 2	163	31	17%	5.27
Lateral section 3	119	44	40%	17.6
Lateral section 4	50	69	35%	24.15
Lateral section 5	0	50	0%	0
			Sum:	**47.46**

Percent algal cover across lateral transect 2 is: 47.46 / 238 = **19.94%**

Transect 3

The visual estimate of percent algal cover across this lateral transect was estimated to be **>90%**. An image of this transect can be seen in Report Figure 4. The lateral transect protocol deems single visual estimates of the entire transect to be sufficient at very low (< 10%) and very high abundance (>80%). Moderate amounts of algae (10% – 80%) require the observer to make multiple measures of homogeneous sections of the transect. The data sheets also have small “E” and “M” symbols in the appropriate boxes, helping to remind the observer when each estimate method is appropriate.

APPENDIX G

Subsample Method Field Form

FILAMENTOUS ALGAE SUBSAMPLE FORM	Page	_1_ of _2_	Reviewers Initials	
Reach Name	SH_SF_006	Date	9/16/2015	

Sample Location	Rater 1 ______			Rater 2 ______			Dominant Substrate	Water depth at X-point	Channel Location	Segment Measurement Notes (SAV species, other observations)
	% FGA Cover	% BGA Cover	% SAV Cover	% FGA Cover	% BGA Cover	% SAV Cover				
Segment 1	25	0	20	35	0	30	BR	0.3	C	Vallisneria
Segment 2	20	5	12	32	2	20	BR	0.4	CR	Stargrass
Segment 3	5	25	5	0	45	3	BR	0.2	R	
Segment 4	65	1	20	45	0	17	BR	0.3	L	Stargrass with attached FGA
Segment 5	5	0	50	5	0	40	CB	0.6	LC	
Segment 6										
Segment 7										
Segment 8										
Segment 9										
Segment 10										
Segment 11										
Segment 12										
Segment 13										
Segment 14										
Segment 15										
Segment 16										
Segment 17										
Segment 18										
Segment 19										
Segment 20										

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

APPENDIX H

Field Observations of Percent Algal Cover
South Fork Shenandoah River - September 24, 2014

Percent cover data recorded by the three pilot observers implementing the subsample method on the South Fork of the Shenandoah River on September 24, 2014.

		Percent SAV Cover				Percent Algae Cover				PERCENT Cyanobacteria Cover			
Segment	**Location**	**Obs 1**	**Obs 2**	**Obs 3**	**AVG**	**Obs 1**	**Obs 2**	**Obs 3**	**AVG**	**Obs 1**	**Obs 2**	**Obs 3**	**AVG**
Segment 1	C	15	12	20	15.67	1	1	5	2.33	1	3	3	2.33
Segment 2	CR	10	10	20	13.33	1	1	8	3.33	5	2	2	3.00
Segment 3	R	30	40	35	35.00	5	10	10	8.33	55	65	20	46.67
Segment 4	L	20	30	8	19.33	1	0	2	1.00	3	2	3	2.67
Segment 5	CL	20	23	35	26.00	1	1	2	1.33	5	3	4	4.00
Segment 6	C	5	8	5	6.00	1	1	2	1.33	3	3	1	2.33
Segment 7	CR	12	12	15	13.00	1	1	1	1.00	2	3	4	3.00
Segment 8	R	15	7	10	10.67	50	40	35	41.67	2	3	3	2.67
Segment 9	L	45	45	50	46.67	1	1	5	2.33	45	45	25	38.33
Segment 10	CL	35	35	20	30.00	40	35	13	29.33	1	1	1	1.00
Segment 11	C	20	15	15	16.67	70	70	85	75.00	2	0	0	0.67
Segment 12	CR	55	60	50	55.00	90	80	70	80.00	1	1	0	0.67
Segment 13	R	15	15	25	18.33	30	35	60	41.67	20	35	5	20.00
Segment 14	L	1	0	15	5.33	2	4	1	2.33	1	3	4	2.67
Segment 15	CL	7	7	3	5.67	5	4	2	3.67	0	0	1	0.33
Segment 16	C	20	30	20	23.33	25	35	25	28.33	0	0	0	0.00
Segment 17	CR	12	20	10	14.00	20	32	5	19.00	5	2	0	2.33
Segment 18	R	5	3	2	3.33	5	5	0	3.33	25	45	15	28.33
Segment 19	L	20	17	18	18.33	65	45	30	46.67	1	0	0	0.33
Segment 20	CL	50	40	35	41.67	5	5	5	5.00	0	0	0	0.00
Segment 21	C	25	35	20	26.67	1	1	1	1.00	1	0	0	0.33
Segment 22	CR	50	50	40	46.67	55	65	45	55.00	0	0	0	0.00
Segment 23	R	7	3	25	11.67	0	1	10	3.67	80	65	20	55.00
Segment 24	L	20	25	20	21.67	70	80	60	70.00	0	1	0	0.33
Segment 25	CL	60	55	50	55.00	5	5	8	6.00	2	1	0	1.00
Segment 26	C	70	85	60	71.67	0	0	2	0.67	0	0	0	0.00
Segment 27	CR	20	18	5	14.33	5	10	1	5.33	2	2	0	1.33
Segment 28	R	8	7	10	8.33	10	3	4	5.67	1	1	1	1.00
Segment 29	L	65	70	50	61.67	0	0	1	0.33	8	3	3	4.67
Segment 30	CL	45	40	15	33.33	0	0	5	1.67	15	8	5	9.33
Segment 31	C	95	98	60	84.33	45	47	60	50.67	0	0	2	0.67
Segment 32	CR	55	65	25	48.33	80	87	60	75.67	0	0	1	0.33
Segment 33	R	10	5	10	8.33	40	35	15	30.00	40	45	15	33.33
Segment 34	L	5	5	3	4.33	1	1	0	0.67	0	0	0	0.00
Segment 35	CL	10	15	8	11.00	30	45	30	35.00	0	0	0	0.00
Segment 36	C	0	0	1	0.33	65	65	50	60.00	0	0	0	0.00
Segment 37	CR	0	0	2	0.67	1	2	1	1.33	0	0	0	0.00
Segment 38	R	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00
Segment 39	L	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00
Segment 40	CL	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00

APPENDIX I

Instructions and Best Practices for Installing and Operating ImageJ

1. Install and ImageJ algae abundance protocol

The ImageJ software download link can be found at, http://ImageJ.nih.gov/ij/download.html.

Download the "**Bundled with 64-bit java**" option.

ImageJ has free macros that have been developed by other users and shared for a myriad of photo processing needs. For algae photo processing you will need the **Threshold_Colour** macro (Link below. It will download immediately).

Threshold_Colour: http://www.mecourse.com/landinig/software/thresholdcolour.zip

In order to run the macro, ImageJ must be closed. Open the downloaded zip-file and select all files (shift + select top and bottom of file list). Drag and drop selection onto ImageJ icon on the desktop.

2. A guide to processing photographs

OBJECTIVE: Highlight as much of the algae in the image as is possible by adjusting the histogram slide bars in the **Threshold_Colour** macro. The user will ultimately be able to quantify their selection of algae in the image as a *% cover* estimation.

a. Select photo of interest (It can be dragged and dropped onto the ImageJ icon or opened manually within the program).
b. Click **Plugins** from the menu bar and click **Threshold_Colour.**
c. Adjust the **Hue** to capture algae in the image.
    i. Click the **Pass** button to the right of the histogram.
    ii. There are two range selectors, bottom color range (top) and top color range (bottom).
        - The bottom color range will be between **35** and **65** to capture FGA (0-255 scale).
        - The value can be slid left and right (higher or lower) to capture the best representation of algae.
        - Wait a moment for the image to process the histogram adjustment. The program will remove parts of image as the histogram bars are adjusted.
        - The top range can be left at 255.

Note: If after you have made your HUE selection you are dissatisfied, you may click the "Original" button on the bottom left of the **Threshold_colour** display to revert the image back to its original appearance.

d. Adjusting **Brightness** to remove shadow.
    i. Click the **Pass** button to the right of the histogram.
    ii. Slide the **Brightness** low range to remove dark areas and shadows.
        - The bottom end range is variable depending on the image, and therefore has no set range. It will often be the lower 50% of the histogram curve. Brightness may not have to be adjusted at all.
    iii. Slide the **Brightness** high range to remove bright areas and highlights.

Note: If after you have made your BRIGHTNESS selection you are dissatisfied, you may click the "Original" button on the bottom left of the **Threshold_colour** display to revert the image back to its original appearance.

e. Highlighting algae
    i. Click **Select**, the program highlights you selection with a yellow border.
    ii. Take a moment to check that the selection made captures the algae.
    iii. If the selection does NOT adequately capture the desired areas, click **Original** to reset the image back to its original format. Readjust the histogram slide bars and click **Select** again.
    iv. When algae is selected to the user's desire, close the histogram window.
f. Removing Algae from image.
    i. In the menu heading click on **Edit**, and then **Clear**.
    ii. The FGA will be black. Only non-algae is visible.
    iii. Click **Edit**, click **Selection**, and then click **Select None.**
g. Changing the image type
    i. Click **Image**
    ii. Click **Type**
    iii. Click **8-bit**. This will turn the image grey scale allowing for image calculation.
h. Select image threshold
    i. Click **Image**
    ii. Click **Adjust**
    iii. Click **Threshold.**
    iv. Set the threshold for both top and bottom to 0.
    v. Click **Apply**.
i. Analyzing Algae.
    i. Click **Analyze** in the menu heading
    ii. Click **Analyze Particles**.
    iii. Set range: 5,000 pixels^2 to infinity
    iv. Check boxes: **Display Results**, **Clear Results**, **Summarize,** and **In situ Show.** Click **OK**.
j. The **Results** box that appears has numbered each cluster of algae. The **Summary** box will give you a **% Area** as well as **Total Area**, and a **Count** of the number of clusters present.

3. Post-processing for validation against original image.

1. **Save As** the analyzed black and white picture as a new file name.
    - Open the original color image as well as the recently saved processed photo.
2. In menu heading click **Process,** then click **Image Calculator**.
3. The original color image should go in the **Image 1** box. The **Operation** is **Average**. The **Image 2** box is the black and white filtered output picture file name. Click **OK**.
4. **Final Output**: There will be a single picture that shows the selected algae area in full color and the omitted area as a transparent grey.

4. Best practices for both above water and submerged photography of filamentous benthic algae.

Equipment:

- A circular polarizer should be applied to reduce incidental light (glare) on the surface of the water.
- The bright highlights that appear on the surface of the water within the image can later be corrected or removed. Any edited areas within the image were not analyzed, therefore reducing the total measurable area of the image.
- Glare can be removed as a variable if the image is taken submerged underwater.
    - The photographer should only attempt submerged photography when using a non-wadeable protocol due to the small field of view that is captured in water depths less than .5 meters.
    - The photographer should designate a camera to submerged photography. Changes in temperature and humidity often cloud the lens inside the mechanism of the camera and so cloud all digital images after the first emersion into the body of water.

Light:

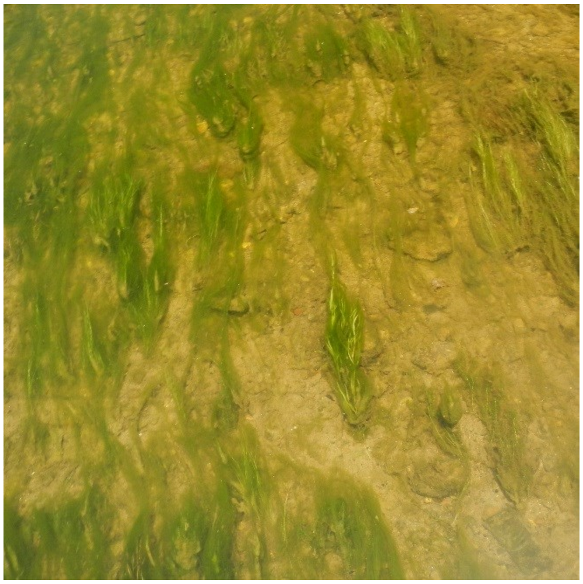
- The photographer should avoid cloudy days due to the increased amount of glare that is produced from indirect, dispersed light (Figure 1).
- On clear sunny days the photographer should shoot with the sun to their back, or ideally, within the shadow of a large structure such as a tree or high bank (Figure 2.).
- For small areas a dark colored umbrella or other shade making device was used to remove glare from the image. The increased bulkiness and additional gear that must be brought to the field make the artificial shade method cumbersome.

Weather:

- The photographer should avoid days where there is excessive surface disturbance such as wind or rain. Surface disturbances distorts the ability to define a clear edge of algae and substrate and therefore will give inaccurate estimations. Heavily disturbed water will also produce large amounts of glare which will result in non-analyzable images.

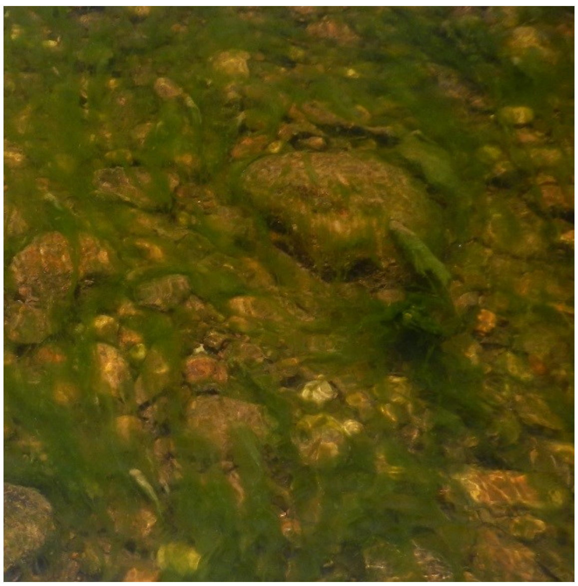
APPENDIX J

Eight Test Images for ImageJ Analysis



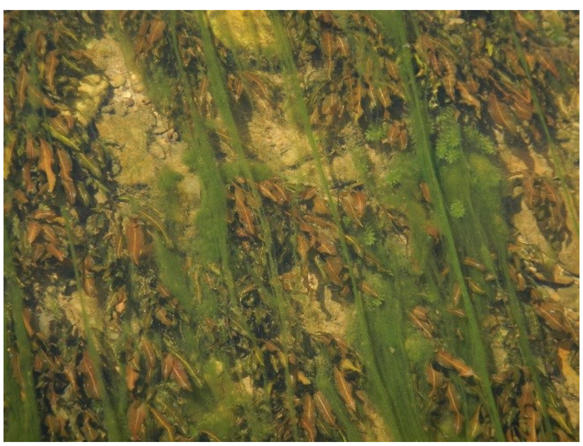
Test Image 1

Moderate to heavy filamentous green algae cover attached to Water Stargrass on a predominantly sandy substrate.



Test Image 2

Moderate to heavy filamentous green algae cover attached to a cobble substrate.



Test Image 3

Moderate cover of long green algae filaments attached to dense SAV on a sand and gravel substrate.

Test Image 4

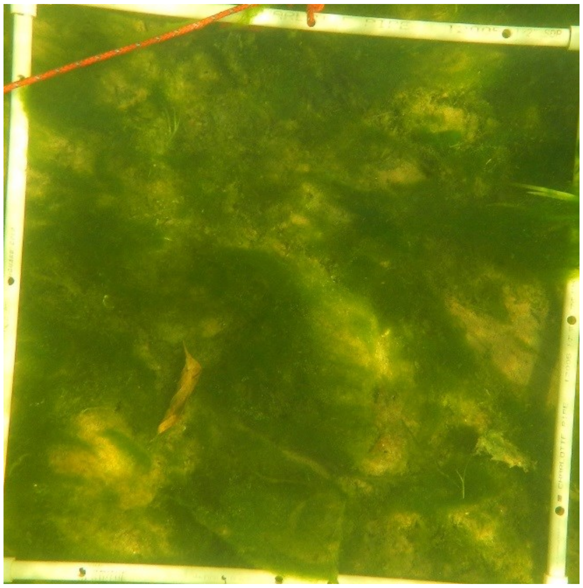
Light to moderate filamentous algae cover established on a cobble-sand substrate.

Test Image 5

Moderate periphytic algae cover on a mix of sand, gravel, and cobble substrate.

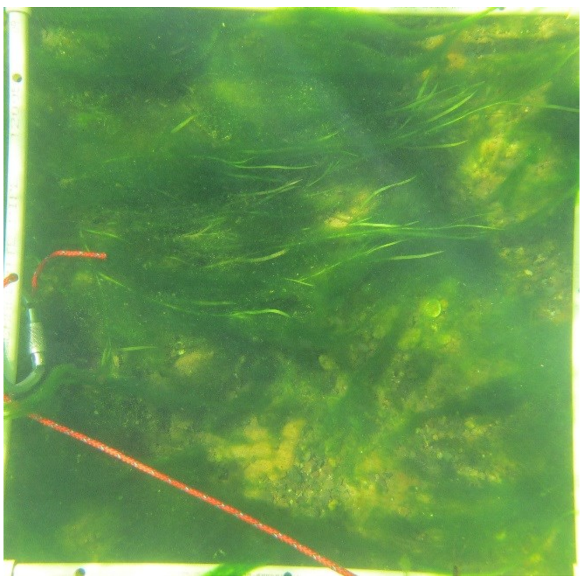
Test Image 6

Moderate cover by filamentous cyanobacteria cover and periphytic algae on a mix of sand, gravel, and cobble substrate, with SAV in the lower right corner.



Test Image 7

Heavy filamentous green algae cover on a mix of sand and gravel substrate. Standardized quadrat included.



Test Image 8

Heavy filamentous green algae cover attached to SAV and a sand and gravel substrate. Standardized quadrat included.