



**Standard Operating Procedure for Measurement of
Benthic Algae and Non-Vascular Plant Cover by Viewing Bucket and Modified
Pebble Count**

SOP-WR-W-36

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Title: Standard Operating Procedure for Measurement of Benthic Algae and Non-Vascular Plant Cover by Viewing Bucket and Modified Pebble Count

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TABLE OF CONTENTS

1. APPLICABILITY	3
2. PURPOSE	3
3. DEFINITIONS	3
4. RESPONSIBILITIES.....	4
4.1 TRAINING.....	4
4.2 RESPONSIBILITIES OF FIELD ANALYST.....	4
4.3 RESPONSIBILITIES OF PROJECT OR PROGRAM MANAGER.....	5
5. GUIDELINES AND PROCEDURES	5
5.1 REQUIRED MATERIALS.....	5
5.2.1 RECORDING PARAMETER UNITS.....	9
5.3 FIELD MEASUREMENT PROCEDURES.....	9
5.3.1 DETERMINE FIELD PROCEDURE SCHEDULE.....	9
5.3.2 ESTABLISH TRANSECTS.....	9
5.3.3 ESTABLISH SAMPLING POINTS.....	10
5.3.4 TAKING SUBSTRATE AND BENTHIC ALGAE MEASUREMENTS WITH THE VIEWING BUCKET	11
5.3.5 MEASUREMENT OF AVAILABLE WOODY AND ROCKY SUBSTRATE WITH THE VIEWING BUCKET	12
5.3.6 MEASUREMENT OF MACROALGAE COVER AND MAXIMUM LENGTH WITH THE VIEWING BUCKET	12
5.3.7 MEASUREMENT OF MICROALGAE COVER AND RANK OF GROWTH WITH THE VIEWING BUCKET	12
5.3.8 COMPLETING THE VIEWING BUCKET MEASUREMENTS AT ALL SAMPLING POINTS AND UPSTREAM TRANSECTS.....	13
5.3.9 TAKING SUBSTRATE AND BENTHIC ALGAE MEASUREMENTS WITH THE MODIFIED PEBBLE COUNT METHOD	13
6. QUALITY CONTROL	16
6.1 QUALITY CONTROL.....	16
6.2 QUALITY ASSURANCE PLANNING CONSIDERATIONS.....	16
7. REFERENCES	16

Standard Operating Procedure for Measurement of Benthic Algae Cover by Viewing Bucket and Modified Pebble Count

1. APPLICABILITY

This SOP applies to all Office of Water Resources (OWR) staff involved in collecting benthic algae and non-vascular cover measurements in shallow, wadeable stream reaches using a viewing bucket and modified pebble count. Exemption from the use of this SOP for project work shall be allowed for reasons of inapplicability determined by management discretion.

2. PURPOSE

This SOP establishes a standardized method for performing semi-quantitative field measurements of benthic algae and non-vascular plant coverage in wadeable streams using a viewing bucket and modified pebble count. It sets a consistent protocol to ensure the quality of OWR's data collection—resulting in improved uniformity, reproducibility, verifiability, and defensibility of the data, as well as increased program credibility.

3. DEFINITIONS

3.1 RIDEM – Rhode Island Department of Environmental Management

3.2 OWR – RIDEM Office of Water Resources

3.3 SOP – Standard Operating Procedures

3.4 Benthic algae – Micro- and macroalgae growing on the bottom of a stream or lake

3.4.1 Macroalgae – Algae that have either a large colonial structure or a plant like structure visible to the naked eye

3.4.2 Microalgae – Algae that are either unicellular or colonial without structure visible to the naked eye

3.5 Non-vascular plants – Plants lacking vascular tissue to transport water and materials, which limits their size to less than 20 cm. Plants appear leafy but lack true stems, roots, and leaves. Includes mosses, liverworts, and hornworts.

3.6 Wadeable stream – Perennial streams 1st through 3rd order draining a watershed area of at least 0.5mi² and with a maximum depth less than or equal to 1.0m.

3.6.1 Perennial stream – A stream with continuous flow year-round under typical conditions

3.7 Riffle – A section of stream characterized by shallow, fast-flowing water with the water surface broken by the presence of rocky substrate

3.8 Pool – A section of stream characterized by deep, slow-moving water with the surface not broken by the presence of rocky substrate

3.9 Run – A section of stream that is characterized by fast-flowing water with the surface not broken by the presence of rocky substrate

3.10 Riparian area – The area of land immediately adjacent to the stream

3.11 QA – Quality Assurance refers to a systematic process to ensure production of valuable, accurate, reliable, reproducible and defensible environmental data.

3.12 QC – Quality Control refers to the activities performed to affirm production of valuable, accurate, reliable, reproducible and defensible environmental data.

3.13 QI – Quality Improvement refers to any act or process performed to enhance the value, accuracy, reliability, reproducibility or defensibility of environmental data collected by RIDEM OWR.

4. RESPONSIBILITIES

4.1 TRAINING

Any RIDEM/OWR personnel collecting benthic algae and non-vascular plant cover measurements with a viewing bucket and modified pebble count for a RIDEM project or program should have completed RIDEM's Quality System Awareness Training Program with appropriate documentation from the Quality Assurance Manager. This training ensures the field analyst recognizes the importance of proper data collection and management and he/she comprehends the significance of the environmental decisions that may be made with the data. It is suggested that field analysts have also completed the USEPA Water Quality Standards Academy Basic Course and Supplemental Topic Modules online, but additional special training or certification is not required.

To properly employ the viewing bucket and perform the modified pebble count, the field analyst must be familiar with and comply with the data collection techniques stated in this SOP. The field analyst is required to read and understand this SOP. The field analyst should complete and submit any required training forms and/or field assessments for project and/or program QAPPs to document proficiency with this procedure. Any field analyst not familiar with the use of the viewing bucket or performing the modified pebble count should be assisted by OWR staff who are accustomed to using the equipment and performing the procedure.

4.2 RESPONSIBILITIES OF FIELD ANALYST

The field analyst is responsible for checking the required equipment in the Sampling Center at the beginning of the sampling event before taking measurements in the field. The field analyst is responsible for verifying that the

viewing bucket is in proper operating condition prior to use (i.e. no cracks in the acrylic sheet; white dot pattern apparent; silicon seal water-tight) and communicating to the project manager when equipment is in need of repair or replacement. The field analyst is also responsible for ensuring that all supplementary equipment (waders/hip boots, etc.) is present and in working condition. The field analyst is also responsible for using best professional judgment to determine if site conditions are safe for performing the procedure. The field analyst is accountable for employing proper measurement procedures and data recording in accordance with this SOP.

4.3 RESPONSIBILITIES OF PROJECT OR PROGRAM MANAGER

The project or program manager is responsible for providing the materials, resources, and/or guidance necessary to perform the measurements in accordance with this SOP. The project manager is responsible for ensuring that the field analyst operates the viewing bucket correctly and performs the modified pebble count in accordance with this SOP and that any additional, project-specific requirements are communicated to the project team. The project manager is responsible for ensuring the viewing bucket is maintained in proper operating condition annually. This includes ensuring the acrylic sheet is not cracked, the dot pattern is apparent, and the silicon seal is water-tight. The project manager is also responsible for repairing the viewing bucket or reordering equipment when necessary. The project manager will determine and communicate with field analysts what procedures and order of procedures are to be accomplished during each sampling event to a sampling location. Further, the project manager shall ensure annual review and periodic revisions to this SOP as necessary to reflect current needs and standards as well as renew this SOP every five years.

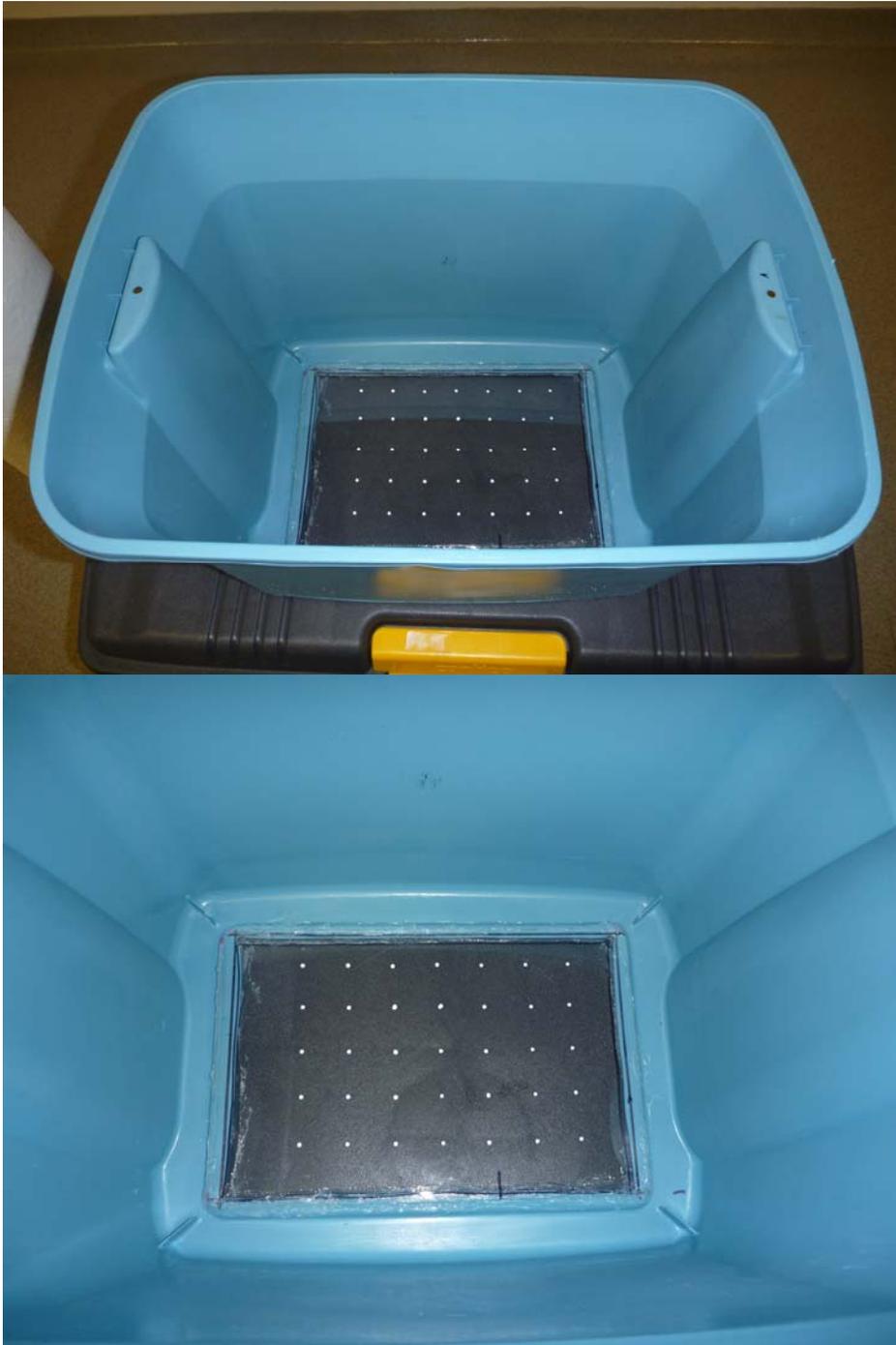
5. GUIDELINES AND PROCEDURES

5.1 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Viewing Bucket (Figure 1)
- Metric Ruler (Similar to Fisher Scientific Item S40641P)
- Datasheet (Figure 2)
- Clipboard
- Pencil or Rite in the Rain Pen (Similar to Forestry Suppliers Item 49237)
- Waders, hip or knee boots
- 2 - Handheld Tally Counter (Similar to Grainger Item 2PAU4)
- Arm-length puncture resistant gloves (Similar to Grainger 1AHG1)
- Tape Measure

Figure 1. Viewing Bucket



A Patterson

Figure 2. Viewing Bucket Datasheet for Monitoring Section Sampling Events
 Page 1

Viewing Bucket Sampling Datasheet										
Stream Segment :				Town:						
Site Number:										
Date:				Military Time:			Collectors:			
Meter #						Pictures:				
Max Depth:				ft	Lat/Long					
Weather: (Circle)	Clear			Partly Cloudy			Overcast			
	Raining			Windy			Sunny			
Comments/Notes:										
VIEWING BUCKET										
	# of dots									
	1A	1B	1C	2A	2B	2C	3A	3B	3C	
Woody Substrate										
Rocky Substrate										
Total										
Macroalgae										
Macroalgae Length (1/sampling point)										

Page 2

Stream Segment: _____						
VIEWING BUCKET MICROALGAE						
Rank	Description	1A	1B	1C	2A	2B
0	No visual evidence					
1	Thin layer evident					
2	0.5 - 1mm thick					
3	1.01 - 5mm thick					
4	5.01mm - 2cm thick					
5	> than 2.01cm thick					
Rank	Description	2C	3A	3B	3C	
0	No visual evidence					
1	Thin layer evident					
2	0.5 - 1mm thick					
3	1.01 - 5mm thick					
4	5.01mm - 2cm thick					
5	> than 2.01cm thick					

5.2 PROPER USE OF VIEWING BUCKET AND PERFORMANCE OF PEBBLE COUNT

For most purposes, the viewing bucket are modified pebble count are used specifically for in situ benthic algae cover measurements taken directly in the field, in wadeable streams. This method does not require sample containers or preservation.

5.2.1 RECORDING PARAMETER UNITS

The following units should be used when recording measurements taken with the viewing bucket:

Macroalgae length.....	millimeter
Microalgae mat depth.....	rank tally
Non-vascular plant matter mat depth.....	rank tally
Suitable substrate.....	count of dots; rank tally
Macroalgal coverage.....	count of dots; rank tally

5.3 FIELD MEASUREMENT PROCEDURES

5.3.1 DETERMINE FIELD PROCEDURE SCHEDULE

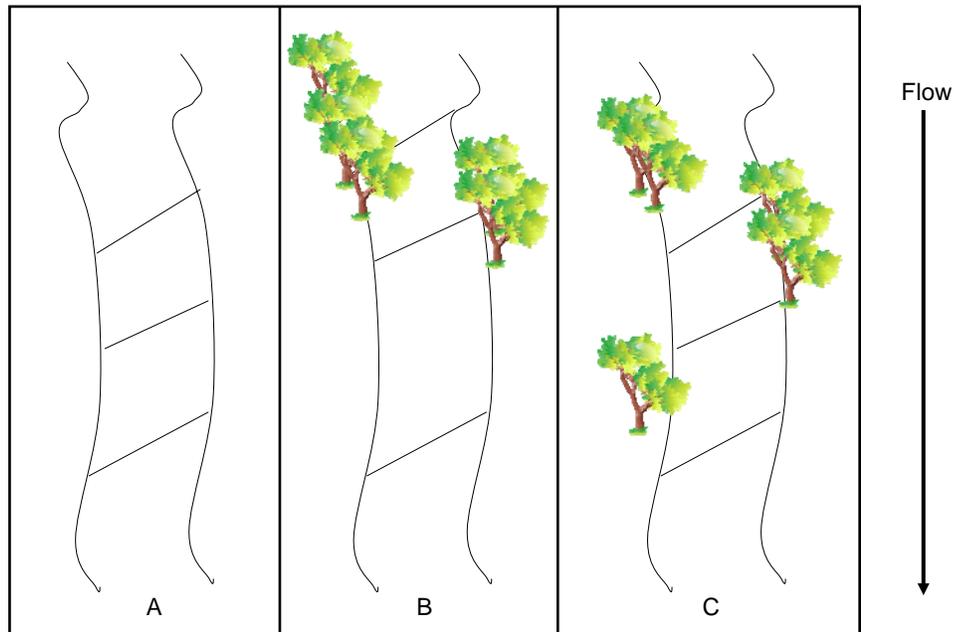
Prior to departure, the project manager will communicate with the field analysts what procedures should be accomplished for each sampling trip to the sampling location and the order the field procedures should be completed. Prior to performing this analysis, the field analyst should ensure the viewing bucket measurement is taken in the correct order. This procedure may disrupt sediment, fish and benthic organisms, which can interfere with other field procedures and sample collections in streams. Viewing bucket measurements should be measured after these samples have been collected. However, viewing bucket measurements should be taken before any sampling procedure or activity that may disturb bottom sediments to avoid increasing turbidity at the location. The field analyst should note any disturbance to the bottom sediment in the Comment/Notes section of the field datasheet (Figure 2) or appropriate field notebook.

5.3.2 ESTABLISH TRANSECTS

The field analyst will establish three (3) transects running diagonal across the stream. The field analyst should observe the location of riffles, runs, and pools along the stream segment. The field analyst should locate transects in areas with runs and riffle, if present, and avoid locations with large pools.

The transects should be approximately at a 45° angle to the right bank (Figure 3A). The field analyst should observe the amount of shade and, using best professional judgment, locate the transects to capture the range of shade conditions available (Figure 3B,3C). The location of the transects should not overlap another transect on any part of the transect.

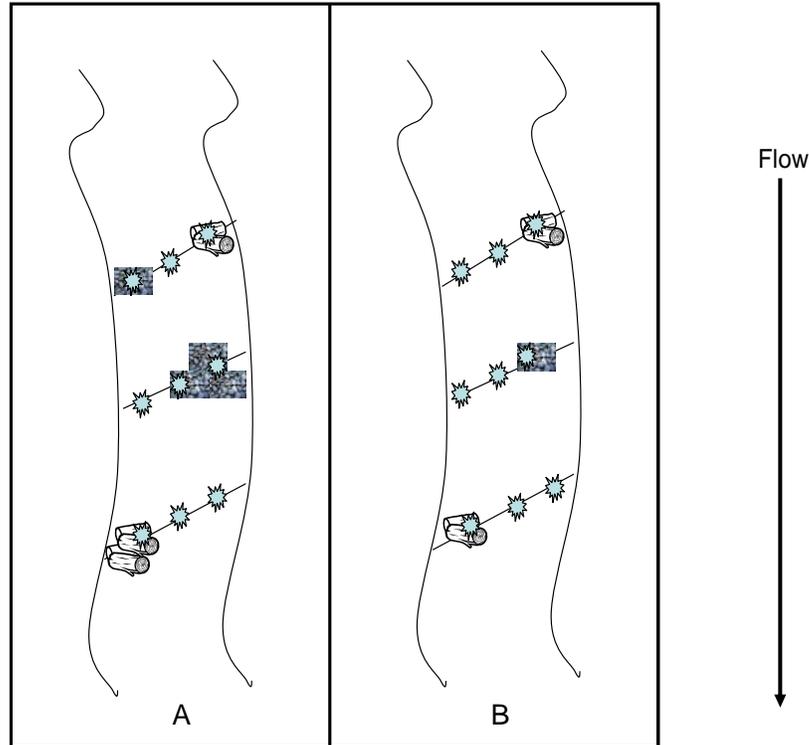
Figure 3. Appropriate establishment of transects



5.3.3 ESTABLISH SAMPLING POINTS

The field analyst will establish three (3) viewing bucket sampling points along each transect for a total of nine (9) viewing bucket sampling points for the stream reach. The field analyst should observe the different available habitat and stream conditions and, using best professional judgment, locate the viewing bucket sampling stations to capture the range of available habitats and stream conditions (Figure 4A, 4B).

Figure 4. Appropriate establishment of sampling points



5.3.4 TAKING SUBSTRATE AND BENTHIC ALGAE MEASUREMENTS WITH THE VIEWING BUCKET

The field analyst will take measurements of available rocky substrate, available woody substrate, amount of macroalgae cover, maximum length of macroalgae, and amount and rank of microalgae cover.

- Record the stream segment station name and number, date, time, and collectors at the top of the datasheet or field notebook. Note any observations about stream condition, riparian area, benthic algae growth, or sampling trip.
- Carefully enter the stream at the most downstream transect at the left bank. Locate the left bank sampling point. It is important to begin at the left bank, because it is the most downstream station. By starting at the most downstream sampling point, the possibility for disruption of sediment and obscuring the bottom of the stream will be minimized.
- Immerse the viewing bucket into the stream so that approximately 4 inches of the bottom of the bucket is underwater. The viewing bucket should be oriented with the longest length perpendicular to flow, and the field analyst should be downstream of the viewing bucket to

minimize sediment disruption obscuring visibility of the bottom. The field analyst should bend over or squat in the water to view the bottom of the stream without interference. If glare or floating is a problem, add a little water to the viewing bucket.

- The field analyst will observe a grid of white dots painted on the clear acrylic sheet in the bottom of the viewing bucket. The dots will be used as locations to estimate and measure the amount of benthic algae growth at the nine sampling stations as described in the following sections.

5.3.5 MEASUREMENT OF AVAILABLE WOODY AND ROCKY SUBSTRATE WITH THE VIEWING BUCKET

- Using the handheld tally-counter, the field analyst will count the number of dots under which suitable rocky substrate is present. Read aloud the number of dots to the recording field analyst. Reset the handheld tally-counter.
 - Suitable rocky substrate is >2cm in length
- Using the handheld tally-counter, the field analyst will count the number of dots under which suitable woody substrate is present. Read aloud the number of dots to the recording field analyst. Reset the handheld tally-counter.
 - Suitable woody substrate is woody branches or logs that are stationary.

5.3.6 MEASUREMENT OF MACROALGAE COVER AND MAXIMUM LENGTH WITH THE VIEWING BUCKET

- Using the handheld tally-counter, the field analyst will count the number of dots that occur over macroalgae growth. Read aloud the number of dots to the recording field analyst. Reset the handheld tally-counter (Macroalgae Examples Figure 6).
- Using the metric ruler, measure the length of the longest macroalgae growth. Read aloud the measurement to the recording field analyst.

5.3.7 MEASUREMENT OF MICROALGAE COVER AND RANK OF GROWTH WITH THE VIEWING BUCKET

- For microalgae, the field analyst should locate the lower left-hand corner of the viewing bucket. Beginning in the lower left-hand corner should allow the field analyst to minimize movement of viewing bucket for measurement, which will help to keep the viewing bucket over the sampling station.
- At each white dot, using the metric ruler, the field analyst will measure the depth of the microalgae layer, if one is present, on the available woody or rocky substrates. The field analyst should read aloud the

measurement to the recording field analyst. If no algae layer is present, the field analyst will say zero to the recording field analyst.

- Note: The recording field analyst should review the chart on the datasheet to rank the amount of growth (0-5) on the substrate based on the measurement taken by the field analyst, and make a tally mark in appropriate row on the chart (Figure 2).
- The recording field analyst will add up the number of tally marks. The recording field analyst will ensure that the number of recorded data for tally marks equal the total number of white dots.

5.3.8 COMPLETING THE VIEWING BUCKET MEASUREMENTS AT ALL SAMPLING POINTS AND UPSTREAM TRANSECTS

- The field analyst will move to the sampling point in the middle of the stream on the transect and repeat the counts and measurements of substrate, macroalgae growth, and microalgae growth described in Sections 5.2.5, 5.2.6, and 5.2.7.
- The field analyst will move to the sampling point at the right bank of the stream on the transect and repeat the counts and measurements of substrate, macroalgae growth, and microalgae growth described in Sections 5.2.5, 5.2.6, and 5.2.7.
- After completing all viewing bucket sampling points on the downstream transect, the field analyst will move to the next transect upstream. The field analyst will repeat the counts and measurements for all transect viewing bucket sampling points beginning at the left bank as described in Sections 5.2.5, 5.2.6, and 5.2.7. The field analyst will then move upstream to the next transect and repeat all counts and measurements at all transect viewing bucket sampling points beginning at the left bank as described in Sections 5.2.5, 5.2.6, and 5.2.7.
- After completing measurements on all transects, the recording field analyst will check that nine (9) viewing bucket sampling points have been assessed.
- The field analyst will exit the stream, if possible, at the final sampling station or another location that is accessible.

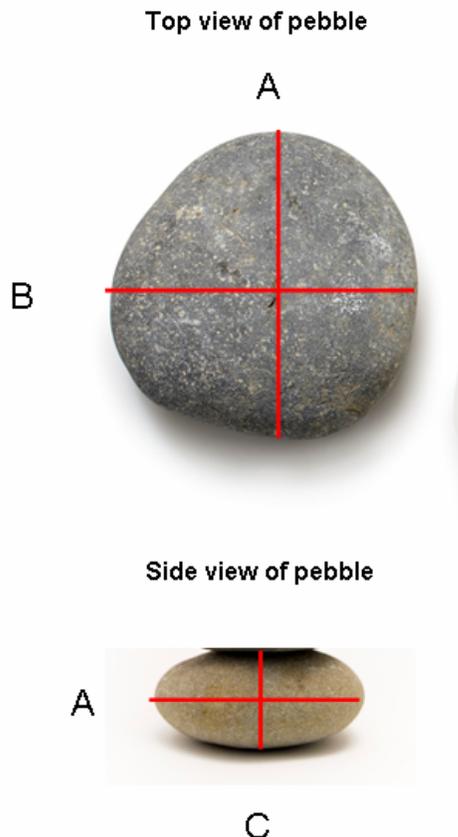
5.3.9 TAKING SUBSTRATE AND BENTHIC ALGAE MEASUREMENTS WITH THE MODIFIED PEBBLE COUNT METHOD

As the field analyst is traveling the transects established for the viewing bucket, the modified pebble count procedure will be executed. This procedure will only be completed if site conditions allow. Sites with sharp objects, especially trash, or particularly murky water will not be sampled using the modified pebble count method. The goal is to assess a minimum of 100 pebbles. If 100 pebbles are not encountered during the viewing bucket procedure, additional transects will be established

upstream using the guidelines in Section 5.3.2 and traveled until 100 pebbles are encountered and assessed. If 100 pebbles are not encountered after 50 m of the stream reach have been assessed, then the procedure will be discontinued. For every pebble measured by the field analyst, the recording field analyst will count the total number of pebbles assessed using the second handheld tally counter.

- After completing the first viewing bucket sampling point, the field analyst should take one pace upstream along the established viewing bucket transect.
- The field analyst should visually check the stream bottom for any sharp items or trash. If any dangerous items are encountered, the field analyst should move another pace upstream.
- If the stream bottom conditions are safe, the field analyst should avert their eyes, select a randomly-sized pebble from the stream bottom, and remove it from the stream bottom.
- The field analyst will measure the intermediate axis of the pebble with the ruler (Figure 5, Axis B). The field analyst will say aloud the measurement. The recording field analyst will mark a tally in the substrate size column.

Figure 5. Intermediate axis of pebble



- If the measurement is less than 2 cm, then the field analyst will take another pace, pick up another pebble, and repeat the measurement above. If the measurement is greater than 2 cm, then the field analyst will observe the growth of non-vascular growth (Figure 8), macroalgae, and microalgae.
- The moss and macroalgae will be ranked using the scale below. A separate rank for each type of growth will be observed by the field analyst and said aloud for the recording analyst to mark on the field sheet.
 - 0=no moss or macroalgae present
 - 1=some (<5% coverage) present
 - 2=5-25% coverage of the substratum
 - 3=>25% coverage of substratum
- The microalgae will be ranked using the scale below. The rank for will be observed by the field analyst and said aloud for the recording analyst to mark on the field sheet.
 - 0=substratum is rough with no apparent growth
 - 1=substrate slimy, but biofilm is not visible (tracks CAN NOT be drawn with fingernail or edge of ruler or greenish color to surface)
 - 2=thin layer visible (tracks CAN be draw in biofilm)
 - 3=accumulation to thickness of 0.5-1mm
 - 4=accumulation to thickness of 1-5mm
 - 5=accumulation to thickness of 5-20mm
 - 6=accumulation to thickness of >2cm
- After ranking the growth, the field analyst will take another pace upstream and repeat the above procedure along the entire viewing bucket transects.
 - NOTE-As the field analyst encounters a viewing bucket sampling point, the field analyst will stop to take the viewing bucket measurements as described in Sections 5.2.5, 5.2.6, and 5.2.7. Once the viewing bucket sampling point is complete, the field analyst will continue the modified pebble count.
 - NOTE-If the field analyst reaches 100 pebbles before all viewing bucket sampling points are completed, then field analyst will discontinue the modified pebble count but continue to conduct the viewing bucket survey.

6. QUALITY CONTROL

6.1 QUALITY CONTROL

Quality control will be assessed by the recording field analyst repeating the measurements of the entire procedure at 10% of stream segments. This will give a measure of bias for the procedure.

6.2 QUALITY ASSURANCE PLANNING CONSIDERATIONS

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. Unless specified otherwise in a site or project-specific work plan, Quality Assurance Project Plan (QAPP), Quality Assurance Program Plan (QAPP) or laboratory Quality Assurance Manual (QAM), all data collected following the protocols set forth in this document will be collected in accordance with the minimum QAQC requirements of Section 6.1. Further quality assurance requirements will be defined in project specific work plans and may include duplicate or replicate measurements or confirmatory analyses.

7. REFERENCES

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Danielson, T. 2006. Protocols for Sampling Benthic Algae in Streams, Wetlands, and Freshwater Wetlands. Maine Department of Environmental Protection. DEPLW-0634

Danielson, T. 2009. Description of Nutrient Criteria for Fresh Surface Waters (Chapter 583). Maine Department of Environmental Protection. DEPLW-0974A.

Stevenson, R.J. and L.L. Bahls. 1999. Periphyton protocols. In: Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition*. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

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Figure 6. Macroalgae Examples

Tolypella sp.



http://www.globaltwitcher.com/photo_info.asp?photoid=31466

Nitella sp.



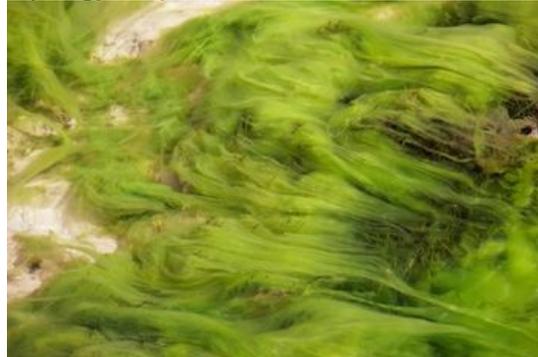
http://www.awc-america.com/plant_id_utility/plants/nit.html

Chara sp.



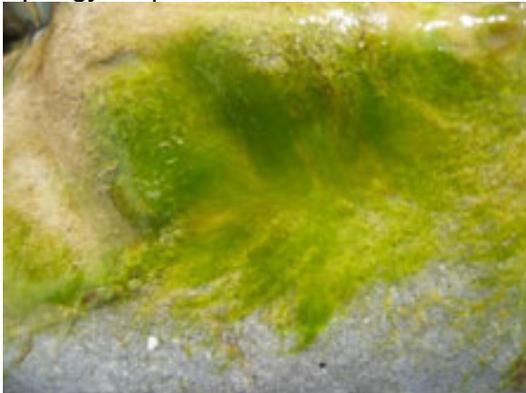
[http://images.mirasites.com/chara-\(alga\).html](http://images.mirasites.com/chara-(alga).html)

Spirogyra sp.



<http://www.buzzle.com/articles/what-is-spirogyra.html>

Spirogyra sp.



<http://www.doc.govt.nz/conservation/native-plants/freshwater-algae/>

Vaucheria sp.



http://www.keweenawalgae.mtu.edu/gallery_pages/xanthophytes.htm

Figure 7. Microalgae Examples

Didymosphenia geminata (INVASIVE-NOT FOUND IN RI)



<http://blogs.app.com/enviroguy/2012/05/03/damaging-rock-snot-infesting-the-delaware-river/>

Gomphoneis sp



<http://www.doc.govt.nz/conservation/native-plants/freshwater-algae/>

Microalgae



<http://www.darbynelson.com/blog/whats-in-your-lake-lake-ecology-101-periphyton/>

Figure 8. Examples of Non-vascular Plant Growth



<http://www.jimmccormac.blogspot.com>



<http://www.squirrelsview.blogspot.com>

Fontinalis sp.



<http://www.ecy.wa.gov/programs/wq/plants/plantid2/photopages/fontinalis.html>

Fontinalis sp.



http://www.aphotoflora.com/moss_fontinalis_squamosa_alpine_water_moss.html

Fontinalis sp.



http://www.aphotoflora.com/moss_fontinalis_squamosa_alpine_water_moss.html