4.2 Maine DEP Biological Sampling Methods and Field Sheets
Protocols for Collecting Water Grab Samples in Rivers, Streams, and Freshwater Wetlands

Tom Danielson, Maine Department of Environmental Protection
17 State House Station, Augusta, ME, 04333
(207) 287-7728 Thomas.J.Danielson@maine.gov

December 2004
DEPLW0637
1. **Applicability.** This standard operating procedure (SOP) applies to the collection of water grab samples for water chemistry analysis [generally for nutrients and metals, see section C (1), below] from wadeable rivers, streams, and freshwater wetlands in Maine.

2. **Purpose.** The purpose of this SOP is to provide standardized methods for collecting water grab samples from wadeable rivers, streams, and freshwater wetlands in Maine.

3. **Definition.** A water grab sample is a sample of river, stream or freshwater wetland water collected for the purpose of analyzing its constituent water chemistry.

4. **Responsibilities**

   A. **Program Leader (variable, depending on program collecting samples):**
      1. Manage contract with laboratory performing analyses [e.g., Health and Environmental Testing Laboratory (HETL) in Augusta, ME].
      2. Manage grant funds.
      3. Purchase and maintain supplies not provided by laboratory performing analyses.
      4. Update SOPs.
      5. Coordinate with the Rivers Unit, Division of Watershed Management, and other partners during selection of sampling locations and scheduling of field teams.
      6. Coordinate and provide training opportunities for field study teams.
      7. Participate as a member the project team, including field studies.
      8. Manage database (generally Microsoft Access or Excel)
      9. Analyze and disseminate data.

   B. **Others (as appropriate):**
      1. Assist in procurement of programmatic funds.
      2. Provide technical guidance in regards to sample methods, data analysis, and selection of sampling locations.
      3. Participate as a member of a field study team as time allows.

5. **Guidelines and Procedures**

   A. **Sampling period and location.** Variable with project for which samples are collected.
B. Supplies
   (1) Water samples
      (a) Water quality kits from HETL, which include containers for all sample
          parameters [see section C (1) below] and preservatives as required
      (b) Disposable gloves (for sampling trace metals)
      (c) Long-handled plastic dipper (for wetland sampling)
      (d) Large sampling container or wide-mouth plastic mixing jug with lid
      (e) HETL chain of custody sheets
   (2) Miscellaneous supplies
      (a) Permanent marker
      (b) Pencil
      (c) Cooler with ice

C. Collecting Water Grab Samples in Field
   (1) Water samples for the river and stream or the wetland program are collected for
       all or a subset of the following parameters: Total P (TP), Soluble Reactive
       Phosphorus (SRP-P); Total Kjeldahl-N, Nitrate/Nitrite-N, Ammonia-N; Sulfate;
       Dissolved and Total Organic Carbon; Chlorophyll \( a \); Total Suspended Solids,
       Total Dissolved Solids; Chloride; Cadmium, Chromium, Copper, Iron, Lead,
       Magnesium, Manganese, Nickel, Zinc, Calcium, Potassium, Sodium; Silica;
       Alkalinity (as CaCO\(_3\)); pH; Specific Conductance; and True Color.
   (2) Record HETL sample kit number on program-specific Data Sheet.
   (3) Collect water samples before stirring up the stream or wetland bottom, or collect
       samples upstream of agitated water. For rivers and streams, collect samples
       (choosing OPTION 1 or 2, below, as appropriate) while standing on edge of water
       or on a rock. If this is not possible, reach upstream as far as possible to avoid
       collecting stirred up water. For wetlands, collect samples (using OPTION 3,
       below) by canoeing or carefully wading into the wetland if possible.
   (4) If sampling trace metals, wear disposable gloves.
   (5) Avoid touching the inside or lip of the sample bottles or caps.
   (6) OPTION 1 (recommended for hard-substrate and regular-flow streams)
      (a) Rinse sample containers (excluding Erlenmeyer flask for sampling TP and
          cubitainer for sampling Total Suspended Solids) in stream water three times.
      (b) Hold uncapped bottle upside down and submerge it.
      (c) Tip bottle upright and allow water to fill bottle.
      (d) Remove bottle from water and screw on cap.
   (7) OPTION 2 (recommended for soft-sediment and low-flow streams)
      (a) Use large, clean container to collect water.
      (b) Rinse container in stream water three times.
      (c) Collect stream water.
      (d) Rinse sample containers (excluding Erlenmeyer flask for sampling TP and
          cubitainer for sampling Total Suspended Solids) three times with small
          amount of sample water.
(e) Fill smaller containers with water from large container. To ensure even mixing of sample water, gently swirl water in large container each time before water is decanted into smaller container.

(8) OPTION 3 (wetlands)
(a) Use a clean long-handled plastic dipper and wide-mouth plastic mixing jug to collect water from a standing position or from canoe.
(b) Thoroughly rinse mixing jug and dipper three times with sample water.
(c) Fill mixing jug using long-handled dipper to collect water from just below the surface. Avoid collecting floating organic material by carefully clearing an opening in any surface film using the closed end of the dipper. Replace cover of mixing jug and transport back to truck in upright position.
(d) Back at the truck, mix large container once thoroughly with the lid on, then rinse sample containers (excluding Erlenmeyer flask for sampling TP) three times with small amount of sample water.
(e) Fill smaller containers with water from mixing jug. To ensure even mixing of sample water, gently swirl water in mixing jug each time before water is decanted into smaller container.

(9) If sampling trace metals, dispose of gloves in regular garbage.
(10) Store and transport samples in cooler with ice.
(11) Complete HETL chain of custody sheet.
(12) Drop off samples at HETL at end of day or early the next morning (store samples in refrigerator overnight) with HETL chain of custody sheet.

D. Quality Control
(1) At the beginning of each field season, all MDEP staff and field personnel who will collect water grab samples will have a training/refresher session to (re)familiarize themselves with the contents of this SOP.
(2) Field: for every 10 water grab samples collected for laboratory analysis, 1 duplicate sample must be collected at a random station and processed by the same laboratory.
(3) Laboratory: quality control samples analyzed in the laboratory are specified in the respective SOPs and generally include duplicate, spiked, and blank samples.

6. References. HETL Standard Operating Procedures as follows:

A. Analysis of Total Phosphorus
B. Analysis of Ortho Phosphorus (used for the analysis of Soluble Reactive Phosphorus)
C. Analysis of Total Kjeldahl Nitrogen in Waters
D. Analysis of Nitrate+Nitrite in Groundwater, Surface Water, and Wastewater
E. Analysis of Ammonia in Water
F. Analysis of Sulfate
G. Determination of Total and Dissolved Organic Carbon in Water)
H. Chlorophyll (Note: this is not a HETL SOP but they use this method for the analysis of Chlorophyll $a$)
I. Analysis of Total Suspended Solids
J. Analysis of Total Dissolved Solids
K. Analysis of Chloride in Waters
L. Analysis of Trace Metals in Water/Wastewater
M. Dissolved Silica Preparation Step
N. Analysis of Total and Bicarbonate Alkalinites
O. The Analysis of pH in Drinking Water, Wastewater, Groundwater, and Surface Water
P. Analysis of Conductivity
Q. Analysis of Color in Waters
## Addendum - List of edits to existing SOP

<table>
<thead>
<tr>
<th>SOP section</th>
<th>Old text</th>
<th>New text</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Guidelines and Procedures, C. Collecting Water Grab Samples in Field, subsection (1)</td>
<td>Water samples for the river and stream or the wetland program are collected for all or a subset of the following parameters: Total P (TP), Ortho-phosphate, soluble reactive Phosphorus (PO₄³⁻); Total Kjeldahl-N, …</td>
<td>Water samples for the river and stream or the wetland program are collected for all or a subset of the following parameters: Total P (TP), Soluble Reactive Phosphorus (SRP-P); Total Kjeldahl-N, …</td>
</tr>
<tr>
<td>5. Guidelines and Procedures, C. Collecting Water Grab Samples in Field, subsection (2)</td>
<td>Record sample kit number and HETL tracking number on program-specific Data Sheet.</td>
<td>Record HETL sample kit number on program-specific Data Sheet.</td>
</tr>
<tr>
<td>6. References, D.</td>
<td>Analysis of Nitrate+Nitrite in Drinking Water, Groundwater, Surface Water, and Wastewater</td>
<td>Analysis of Nitrate+Nitrite in Groundwater, Surface Water, and Wastewater</td>
</tr>
<tr>
<td>6. References, G.</td>
<td>TOC-SM505A (total and dissolved organic carbon)</td>
<td>Determination of Total and Dissolved Organic Carbon in Water</td>
</tr>
<tr>
<td>6. References, L.</td>
<td>Analysis of Trace Metals in Drinking Water</td>
<td>Analysis of Trace Metals in Water/Wastewater</td>
</tr>
<tr>
<td>6. References, N.</td>
<td>Analysis of Total Alkalinity</td>
<td>Analysis of Total and Bicarbonate Alkalinites</td>
</tr>
</tbody>
</table>
Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands

Tom Danielson, Maine Department of Environmental Protection
17 State House Station, Augusta, ME, 04333
(207) 287-7728 thomas.j.danielson@maine.gov

June, 2006
DEPLW0634
# Bureau of Land and Water Quality
## Division of Environmental Assessment
### Biomonitoring Program

Standard Operating Procedure  
Methods for Sampling Stream and Wetland Algae

## TABLE OF CONTENTS

1. Applicability ...................................................................................................................................... 3  
2. Purpose ............................................................................................................................................... 3  
3. Definitions........................................................................................................................................... 3  
4. Responsibilities ................................................................................................................................... 4  
5. Guidelines and Procedures.................................................................................................................... 4  
   A. Sampling Period................................................................................................................................. 4  
   B. Supplies............................................................................................................................................. 4  
   C. Site Visit - Streams ............................................................................................................................ 6  
   D. Site Visit - Wetlands.......................................................................................................................... 7  
   E. Viewing Bucket Survey ...................................................................................................................... 7  
   F. Artificial Substrate Sampling ........................................................................................................... 9  
   G. Natural Substrate Sampling - Rocky Substrate .............................................................................. 11  
   H. Natural Substrate Sampling - Soft Bottom ..................................................................................... 13  
   I. Phytoplankton Sampling .................................................................................................................. 16  
   J. Processing Samples in the Lab ......................................................................................................... 17  
   K. Equipment Maintenance ................................................................................................................. 17  
6. References............................................................................................................................................ 18  

Appendix 1 – Field Data Sheets

*Cover photographs – wetland by Jeanne DiFranco and algae by Micrographia*
1. **Applicability.** This standard operating procedure (SOP) applies to the collection of benthic algae and phytoplankton from rivers, streams, and freshwater wetlands in Maine. This SOP describes the collection of (1) qualitative biomass data using a viewing bucket survey, (2) quantitative biomass and species composition data using artificial and natural substrates, and (3) quantitative phytoplankton data.

2. **Purpose.** The purpose of this SOP is to provide standardized methods for collecting algae from rivers, streams, and freshwater wetlands in Maine.

3. **Definitions.**

   A. Algae – algae included in analysis include the following groups:
      (1) Cyanophyta – blue-green algae, cyanobacteria
      (2) Chlorophyta – green algae
      (3) Bacillariophyta – diatoms
      (4) Rhodophyta – red algae
      (5) Chrysophyta – chrysophytes, chrysomonads
      (6) Tribophyceae – yellow-green algae
      (7) Euglenophyta – euglenoids
      (8) Pyrrophyta – dinoflagellates
      (9) Cryptophyta – cryptomonads

   B. Macroalgae – algae that form macroscopic or plantlike morphologies with a thallus structure that is recognizable with the naked eye (Wehr and Sheath 2003).

   C. Microalgae – unicellular algae or colonies that are microscopic.

   D. Benthic Algae – microalgae and macroalgae that grow on the bottom substrate of a waterbody (e.g., rocks, logs, mud).

   E. Periphyton – microscopic algae, bacteria, and fungi that grows on the bottom substrate (e.g., rocks, logs) of a stream or river. Does not include macroscopic algae, such as Cladophora, Spirogyra, Chara, and Vaucheria (Stevenson et al. 1996).

   F. Growth habits – several terms are used to describe the microhabitats provided by different substrates (Stevenson et al. 1996).
      (1) Epilithic algae grow on hard relatively inert substrata, such as gravel, pebble, cobble, and boulder, that are bigger than most algae.
      (2) Epiphytic algae grow on plants and larger algae, which provide relatively firm substrata that are bigger than the epiphytic algae, but can be highly active metabolically and a great source of nutrients.
      (3) Epipsammic algae grow on sand, which is hard, relatively inert, and has relatively little surface area. Few algae live in sand among sand grains, because the sand is too unstable and may crush them.
      (4) Epipelic algae grow on inorganic or organic sediments that are smaller than most unicellular algae. Epipelic algae are typically large motile diatoms, motile filamentous blue-green algae, or larger motile flagellates like Euglena.
      (5) Phytoplankton – microscopic algae that are suspended in the water column.
G.  HETL – Health and Ecological Testing Laboratory, Augusta, ME

4.  Responsibilities.

A.  The Stream Algae Program Manager and has the following responsibilities associated with this SOP:
   (1) Manage contract with algal taxonomist and deliver samples to taxonomist.
   (2) Purchase and maintain supplies and field equipment.
   (3) Update SOP.
   (4) Coordinate with the Rivers Unit, Division of Watershed Management, and other partners during selection of sampling locations and scheduling of field teams.
   (5) Coordinate and provide training opportunities for field teams.
   (6) Participate as a member of a field team.
   (7) Coordinate wetland algae sampling with Wetlands Program Manager.

5.  Guidelines and Procedures.

A.  SAMPLING PERIOD
   (1) Sampling of stream algae should occur between June 15 and July 31 unless there are extenuating circumstances (e.g., prolonged high flows). The sampling window may be extended in the northern part of the state when appropriate. This period was selected for the following reasons:
      (a) This is roughly centered on the longest day of the year.
      (b) Stream and river flow should no longer be influenced by spring snowmelt.
      (c) Appears to be period of peak algal growth in many streams before the algal mats begin to senesce.
   (2) Sampling of fresh water wetland algae should occur during June and early July. This period was selected for the following reasons:
      (a) Wetlands are less likely to dry down during this period compared with later in the summer.
      (b) Overlap with stream algae and stream macroinvertebrate sampling is minimized.

B.  SUPPLIES
   (1) Tackle box.
      (a) permanent marker
      (b) pencils
      (c) knife or scissors for cutting rope
      (d) razor blades or utility knife for scraping algae off of microscope slides
      (e) garden shears for clipping plant stems
      (f) flat-head screwdriver
      (g) miscellaneous supplies
      (h) cooler with ice
      (i) bottle of bottled water
      (j) squirt bottle with bottled water
(k) pack baskets

(2) Water samples
(a) water quality kits from HETL, which include bottles for all or a subset of the following: Total P, Orthophosphate, soluble reactive Phosphorus (PO₄); Total Kjeldahl-N, Nitrate/Nitrite-N, Ammonia-Nitrogen; Sulfate; Dissolved Organic Carbon; Chlorophyll a; Total Suspended Solids; Chloride; Cadmium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Nickel, Zinc, Calcium, Potassium, Sodium, Silica; Alkalinity (as CaCO₃); pH; Specific Conductance; and True Color.
(b) HETL chain of custody sheets.

(3) Periphytometer.
(a) periphytometer
(b) microscope slides
(c) lightweight nylon rope
(d) rebar (not always used)
   1. approximately 3ft long
   2. 1 per periphytometer
(e) mallet

(4) Natural substrate sample.
(a) toothbrushes
(b) metal chemistry tool for scraping rocks
(c) large white sample trays
(d) Bottle of M3 preservative (Table 1)
(e) Pipette and bulb for measuring M3
(f) 250ml beaker
(g) widemouth, brown nalgene bottles (125ml or 250ml)
(h) garden shears
(i) whirl-paks
(j) 12 inch ruler

(5) Rapid periphyton survey.
(a) viewing bucket
(b) 6 inch ruler marked with millimeters and has markings at 5mm and 2cm with permanent marker
(c) meter stick

(6) Field sheets (Appendix 2).
(a) EPA Physical Characterization/Water Quality Field Data Sheet (Barbour et al. 1999)
(b) EPA Habitat Assessment Field Data Sheet – High Gradient (Barbour et al. 1999)
(c) EPA Habitat Assessment Field Data Sheet – Low Gradient (Barbour et al. 1999)
(d) ME DEP Stream Algae Field Data Sheet
(e) ME DEP Qualitative Benthic Algae Survey Data Sheet
(f) ME DEP Epiphytic Algae Data Sheet

(7) Electronic equipment and accompanying SOPs
(a) digital camera and diskettes
(b) Global® stream velocity meter

### Table 1: M3 Preservative

- 5 g Potassium Iodide
- 10 g Iodine (optional)
- 50ml glacial acetic acid
- 250ml formalin
- Bring up to 1 liter with distilled water
- Add 1 ml per 50ml sample
(c) Hanna® pH/conductivity/TDS meter
(d) Hanna® dissolved oxygen meter

C. SITE VISIT - STREAMS

(1) Identifying Stream Reach.
   (a) If possible, sample locations should be scouted out ahead of time to identify
       appropriate reaches and to determine what kinds of substrate are available.
   (b) Sample reaches should ideally have the following characteristics. Streams that do
       not have these characteristics can still be sampled at the discretion of the project
       manager.
       1. Located in areas of open or partly open canopies (>50%).
       2. Located in areas of riffles and runs, not pools. Runs are preferred.
       3. Located in areas with moderate water velocity (between 10 and 60 cm/sec). Try
          to avoid areas with little or excessive water velocity.
   (c) Rocky substrates are preferred over soft substrates. However, we do not currently
       have methods appropriate for sampling ledge. If rocks are not available, then
       periphytometers could be deployed and the slides and alternative natural substrate
       should be sampled on a second visit. Substrates should be selected in the following
       order or preference:
       1. Rocks (Section F)
       2. Branches/Logs (Section G.2)
       3. Epiphytes (Section G.3)
       4. Mud/Sand (Section G.4)

(2) Natural Substrate Only – The following activities must be completed.

<table>
<thead>
<tr>
<th>Complete During Only Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>*EPA Habitat Assessment Field Data Sheet – High Gradient or</td>
</tr>
<tr>
<td>*EPA Habitat Assessment Field Data Sheet – Low Gradient</td>
</tr>
<tr>
<td>ME DEP Stream Algae Data Sheet</td>
</tr>
<tr>
<td>water grab samples</td>
</tr>
<tr>
<td>flow measurement</td>
</tr>
<tr>
<td>pH, conductivity, temperature, and dissolved oxygen readings</td>
</tr>
<tr>
<td>Viewing Bucket Survey (Section E)</td>
</tr>
<tr>
<td>Natural Substrate Sampling (Section G &amp; H)</td>
</tr>
</tbody>
</table>
(3) Artificial substrate only or both natural and artificial substrates sampled

<table>
<thead>
<tr>
<th>Complete During First Visit</th>
<th>Complete During Second Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ME DEP Stream Algae Data Sheet</em> parts related to first visit</td>
<td><em>ME DEP Stream Algae Data Sheet</em> parts related to second visit</td>
</tr>
<tr>
<td><em>EPA Habitat Assessment Field Data Sheet – High Gradient</em> or <em>EPA Habitat Assessment Field Data Sheet – Low Gradient</em></td>
<td>water grab samples (Section D)</td>
</tr>
<tr>
<td>flow measurement</td>
<td>flow measurement</td>
</tr>
<tr>
<td>pH, conductivity, and DO readings</td>
<td>pH, conductivity, and DO readings</td>
</tr>
<tr>
<td></td>
<td>Viewing Bucket Survey (Section D)</td>
</tr>
<tr>
<td></td>
<td>Artificial Substrate Sampling (Section E)</td>
</tr>
<tr>
<td></td>
<td>Natural Substrate Sampling (Section F &amp; G)</td>
</tr>
</tbody>
</table>

D. SITE VISIT – WETLANDS

(1) For wetlands, where rocks are not readily available, substrates should be selected in the following order or preference:
   (a) Branches/Logs (Section G.2)
   (b) Epiphytes (Section G.3)
   (c) Mud/Sand (Section G.4)

(2) Natural Substrate Only – The following activities must be completed.

<table>
<thead>
<tr>
<th>Complete During Only Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ME DEP Wetland Bioassessment Field Data Form</em></td>
</tr>
<tr>
<td>water grab samples</td>
</tr>
<tr>
<td>pH, conductivity, and DO readings</td>
</tr>
<tr>
<td>Natural Substrate Sampling (Section G &amp; H)</td>
</tr>
</tbody>
</table>

E. VIEWING BUCKET SURVEY (Streams only)

(1) Fill in top of *ME DEP Qualitative Benthic Algae Survey Data Sheet*
(2) Establish transects across the habitat being sampled (preferably riffles or runs in the reach in which benthic algal accumulation is readily observed and characterized).
   (a) Normal Situation – identify 3 transects perpendicular to the flow through the designated reach and then haphazardly select 3 locations along each transect with one near the right bank, one near the middle, and one near the left bank.
(b) Narrow Stream – identify 3 transects diagonally across the reach and then select 3 locations along each transect OR walk upstream and select at least 3 locations through the reach.
(c) The transects should be equally spaced within the reach unless channel morphology makes it necessary to adjust the distance between transects.
(d) The transects should not overlap transects for sampling natural substrates.
(3) Have one person (viewer) conduct survey and one person (recorder) record data (Figure 1).
(4) At each location, record the transect and sample number (e.g., 1-1, 1-2, 1-3, 2-1, 2-2, 2-3, 3-1, 3-2, or 3-3)
(5) At a location, the viewer should immerse the viewing bucket in the water (Figure 2).
(a) 35 dots
(b) 4 cm between dots
(6) While viewing through the bucket, identify points on the stream bottom below the upper left dot and the lower right dot to help keep the bucket in the same area.
(7) To minimize glare, it is sometimes helpful to put a little water inside the viewing bucket.
(8) Measure the longest filament of algae. If you can identify the filamentous algae, record the names of the taxa on the field sheet.
(9) Start with the upper left dot and systematically proceed by observing the algal growth below each dot in the top row. Then proceed row by row to the bottom row.
(10) At each dot, the viewer should call out one of the following to characterize the algal growth below the dot. The viewer should use the 6-inch ruler to distinguish categories 2-5.
(a) unsuitable – unconsolidated substrate such as sand or mud
(b) plant – an aquatic plant
(c) moss – a moss
(d) Crust – a crust-forming algae (may be black, red, or green)
(e) Macro 1 - a filament or other macroalga that is between 1 and 5 cm long
(f) Macro 2 – a filament or other macroalga that is ≥ 5 cm and < 15 cm long
(g) Macro 3 – a filament or other macroalga that is ≥ 15 cm long
(h) 0 – substrate rough or slightly slimy with no visible algae
(i) 1 – a thin layer of algae is visually evident, underlying rock is still visible.
(j) 2 – periphyton mat from 0.5-1 mm thick is evident, underlying rock is covered and can no longer be seen
(k) 3 – periphyton mat between 1-5 mm thick is evident
(l) 4 – periphyton mat between 5 mm – 2 cm thick is evident
(m) 5 – periphyton mat > 2 cm thick is evident
(11) If there is a mixture of decomposing filaments, microalgae, and silt, then treat it as being periphyton mat and not a filament.
(12) The recorder should use hash marks to record the observations on the field sheet in the appropriate boxes.
(13) Before the viewer moves the viewing bucket, the recorder must add up the number of hash marks for each category and write down the number in the little boxes in the lower right corner of each rectangle. (See ME DEP Viewing Bucket Survey Data Sheet, Appendix 2)
(14) The recorder must add up the number of hash marks in the row to make sure that there were 35 observations. If not, then the viewer should make additional observations or subtract the most recent observations to get a total of 35 dots.

F. ARTIFICIAL SUBSTRATE SAMPLING (Streams only)

(1) Periphytometers should be deployed for 14 days ! 2 days.
(2) Maine DEP uses two types of periphytometers
   (a) Wildco® Periphytometer (Figure 3)
      1. They hold 8 standard microscope slides.
      2. They have two sliding plastic pieces that lock slides in place (Figure 4).
   (b) Durasampler® Periphytometer (Figure 5).
      1. They hold 20 standard microscope slides.
      2. Only 8 standard microscope slides should be installed in the sampler.
      3. Place slides in the slots marked with red dots (slots 2,4,6,8,10, 11, 13, 15, 17, & 19).
(3) Microscope slides
   (a) Use standard, non-frosted microscope slides.
   (b) Use new slides. If new slides are not available follow protocols in Section J for cleaning slides.
   (c) Only hold the edges; avoid touching the slide surfaces as it can effect colonization.
(4) Placement of samplers in the field
   (a) Sunlight
      1. Maine DEP standardizes sampling by putting samplers in areas with minimal canopy cover.
      2. If possible, samplers should receive sunlight for all or most of the day.
   (b) Flow
1. Periphytometers should be placed in areas with at least some visible flow.
2. Avoid putting periphytometers in backwaters or eddies.
3. Avoid putting periphytometers in excessively turbulent eddies that might limit algal colonization.

(c) Installation
1. Periphytometers should be secured with lightweight nylon rope to a metal ring or metal part of sampler.
2. The periphytometers should be secured so that the slides are parallel to stream flow (Figures 5 & 6).
3. The length of the rope will vary depending on stream flow, but the rope should be long enough to allow the periphytometer to sway slightly in the current, but short enough so the periphytometer does not drift into eddies or slow sections along the bank.
4. The other end of the rope should be securely tied to a boulder, log, woody vegetation, or piece of rebar that has been hammered into the substrate deep enough to prevent it from coming lose during high flows.

(d) Retrieving samplers and processing slides.
1. Care should be taken to avoid touching the flat sides of the microscope slides. Handle the slides by holding the edges.
2. Pick up periphytometer by holding the edges.
3. Slide the two plastic pieces (Figure 4) so the microscope slides can be removed.
4. Grasp slides along the edges and remove them from the periphytometer. Be careful to avoid disturbing the surfaces of the slides or other slides in the periphytometer.
5. Chl \( a \) slides
   i. Place 1 slide (3\(^{rd}\) from the left) into a whirl-pak with some bottled water.
   ii. Using a permanent marker, write down the date, stream name, town, sample location, Chl \( a \), and number of slides on the whirl-pak.
   iii. Place the sealed whirl-pak into a cooler and bring back to the lab for Chl \( a \) filtering (Section I.1).
   iv. Record the number of slides collected for Chl \( a \) on the field sheet.
6. Processing periphytometer slides for taxonomic analysis.
   i. Carefully pour slides and water into a graduated beaker.
   ii. Using a razor blade or utility knife, carefully scrape the other 7 microscope slides. Scrape only the flat surface, not the edges.
iii. Using a squirt bottle filled with bottled water, squirt the razor blade and slides and collect the sample into the graduated beaker.

iv. Add bottled water until there is a multiple of 50ml (e.g., 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.

v. Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 125ml or 250ml in size).

vi. Record the number of slides scraped for taxonomic analysis

vii. Record surface area:

- 1 slide (both sides) = 17.25cm$^2$
- 7 slides (both sides of each) = 241.5cm$^2$

viii. Label the bottle with the following information:

- date
- bottle number
- stream name
- town
- location
- type of sample (species)
- type of sample (slides)
- number of slides (e.g., 7) and sides (e.g., 14)
- volume of sample

ix. Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

x. Carefully clean razor blade/utility knife and beaker.

G. NATURAL SUBSTRATE SAMPLING – ROCKY SUBSTRATE (streams only)

(1) Sampling will focus on Epilithic algae.
(2) Fill in ME DEP Stream Algae Data Sheet
(3) Clean sample trays, brushes, and other equipment with tap or stream water.
(4) Establish transects through riffles or runs
  (a) If possible, 18 rocks must be collected from the reach
  (b) Normal Situation – identify 6 transects perpendicular to the flow through the designated reach and then select 3 locations along each transect (e.g., stratified random locations on right bank, middle, and left bank).
  (c) Wide River – identify 3 transects perpendicular to the flow through the designated reach and then select 6 locations along each transect.
  (d) Narrow Stream – identify 6 transects diagonally across the reach and then select 3 locations along each transect OR walk upstream and select 18 locations through the reach.
(5) At each location, collect a cobble or boulder-sized rock for a total of 18 rocks.
(6) Back at the stream bank, store the rocks in a large, white sample tray.
(7) Pick up a rock and hold it over a second sample tray that is clean.

(8) Place rubber sampling device (Figures 7 & 8) over the top of the rock and hold firmly in place to define surface area to be sampled. Alternatively, the neoprene washer with 1” diameter hole can be used by itself.

(a) Sampler is constructed by cutting a segment of mountain bike inner tube lengthwise and uncurling.

(b) Epoxy glue a neoprene washer with a 1” diameter hole to the outer surface of tubing.

(c) After the glue dries, flip the sampler over and cut away the tubing within the 1” circle. Cutting from the back reduces strain on the epoxy glue.

(9) Brush the area within the circle vigorously with a stiff bristled brush while holding rock over collection pan (note, you may need to scrape the area with a metal scraping tool first if the algae is very thick) (Figure 9).

(10) Rinse tools and sample area on rock with a squirt bottle filled with bottled water and collect sample in the large, white sample tray. The collector must hold the rock upside down and spray upward to minimize the chance of washing off algae from another part of the rock.

(11) The goal is to collect all algae from within the circles and none of the algae from outside of the circles.

(12) Repeat process for other rocks and composite all rock-scrapings into a graduated beaker. (rinse the tray and equipment to ensure all algae are in the beaker).

(13) Add bottled water until there is a multiple of 50ml (e.g., 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.

(14) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).

(15) Record the number of rocks scraped for taxonomic analysis.

(16) Record surface area:

(a) 1” circle = 5.067 cm²

(b) 18 circles = 91.027 cm²

(17) Label the bottle with the following information:

- date
• bottle number
• stream name
• town
• location
• type of sample (species)
• type of sample (rocks)
• number of rocks (e.g., 18)
• volume of sample

(18) Thoroughly clean all equipment, especially brush bristles, in water before leaving stream. Discard brushes if they get too grimy or difficult to clean.

(19) Add 1 ml of M3 for each 50 ml of sample in the brown bottle (refer to the field sheet to determine the amount).

H. NATURAL SUBSTRATE SAMPLING – SOFT BOTTOM (streams and wetlands)

(1) There are several options for sampling soft bottom streams, including the following methods listed in order of preference.
   (a) Epilithic algae from log scrapings.
   (b) Epiphytic algae from plant clippings.
   (c) Epipsammic and Epipelic algae from soft substrate.

(2) Epilithic algae from log scrapings.
   (a) Fill in ME DEP Stream Algae Data Sheet
   (b) Clean large, white sample trays, toothbrushes, and metal scraping tools.
   (c) Find logs or branches within the reach that can be lifted from the water.
   (d) Using the following methods, collect up to 18 log scrapings
      1. Pick up a log/branch and hold it over a large, white sample tray.
      2. Place rubber sampling device (Figure 7) over the log/branch and hold firmly in place to define surface area to be sampled.
      3. Brush the area within the circle vigorously with a toothbrush and wash down brush and log/branch with a squeeze bottle into a collection pan (note, you may need to scrape the area with a metal scraping tool first if the algae is very thick)
      4. Rinse tools and sample area on log/branch with a squirt bottle filled with bottled water and collect sample in the large, white sample tray.
      5. Repeat process for other logs/branches or other parts of long logs/branches and composite all scrapings into a graduated beaker. (rinse the tray and equipment to ensure all algae are in the beaker).
   (e) Add bottled water until there is a multiple of 50 ml (e.g., 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
   (f) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).
(g) Record the number of logs/branches scraped for taxonomic analysis.
(h) Record surface area:
   1. $1''$ circle = $5.067 \text{ cm}^2$
   2. 18 circles = $91.027 \text{ cm}^2$
(i) Label the bottle with the following information:
   - date
   - bottle number
   - stream name
   - town
   - location
   - type of sample (log/branch)
   - number of logs/branches (e.g., 18)
   - volume of sample
(j) Thoroughly clean all equipment, especially brush bristles, in water before leaving stream. Discard brushes if they get too grimy or difficult to clean.
(k) Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

(3) Epiphytic algae from plant clippings.
   (a) Fill in *ME DEP Stream Algae Data Sheet* and/or the *ME DEP Epiphytic Algae Data Sheet*
   (b) Clean scissors and large, white sample trays.
   (c) Identify a stream reach
      1. Identify a stream reach that is 10x the stream width or 20 m in length, whichever is less.
      2. Locate the stream reach in an area with runs and pools.
   (d) Select 3 locations in runs within the reach that have emergent vegetation (e.g., cattails, sedges).
   (e) In wetlands, do not define reach, just select 3 areas with emergent vegetation.
   (f) At each location, select plants that have at least 10cm underwater.
   (g) Clip plant stems near their base or at least 10cm underwater and trim off any parts that are above water.
      1. If plants are thin (e.g., 1 mm across - Soft Rush (*Juncus effusus*)), then clip 5 stems per location.
      2. If plants are thick (e.g., >5 mm across - Cattail (*Typha* sp.)), then clip 2 stems per location.
      3. If plants have intermediate thickness, then clip 3 or 4 stems per location.
   (h) Trim stems to approximately 10 – 15cm in length.
   (i) Place the stems into a whirl-pak, add a little bottled water, remove most of the air within the bag, and seal the whirl-pak.
   (j) Massage the plant stems to remove epiphytic algae.
   (k) Rinse each stem with bottled water as remove from whirl-pak.
(l) Pour the contents into a graduated beaker and store set aside the cleaned stems for measurement.

(m) Add bottled water until there is a multiple of 50ml (e.g., 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.

(n) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).

(o) Label the bottle with the following information:
   - date
   - bottle number
   - stream or wetland name
   - town
   - location
   - station location number
   - volume of sample

(p) Estimate surface area of each clipped stem, either in the field or back at the office.
1. Complete ME DEP Epiphytic Algae Data Sheet
2. Make the measurements that are appropriate for the stem shapes and enter the measurements on the field sheet.
3. Calculate the surface area for each stem using the formulas provided on the field sheet.
4. Add the surface areas together and record on the field data sheet.

(q) Thoroughly clean all equipment in water before leaving stream.

(r) Add 1 ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

(4) Epipsammic and Epipelic algae from soft substrate.
   (a) This method is appropriate for mucky bottom streams and wetlands. This should not be used with sandy bottom streams. Shifting sand is unsuitable because of its small grain size and unstable nature of the substratum. Epilithic algae from log scrapings (Section H.2) or phytoplankton samples should be used as alternatives.

   (b) Fill in ME DEP Stream Algae Data Sheet

   (c) Clean petri dish, spatula, and beaker.

   (d) Identify a stream reach that is 10x the stream width or 20 m in length, whichever is less.

   (e) Locate the stream reach in an area with runs and pools or locate area of wetland with mucky bottom.

   (f) Select 3 areas within sampling area suitable for sampling.

   (g) At each location, hold a petri dish (5cm diameter) upside down and press it lightly into the substrate.

   (h) Slide an unslotted spatula underneath the petri dish and carefully remove the petri dish and its core sample from the water.
(i) Composite the core samples in a graduated beaker.
(j) Add bottled water until there is a multiple of 50ml (e.g., 100ml, 150ml) and record the amount on the field sheets. For example, if the sample is 130ml, then add 20ml of bottled water. Having a multiple of 50ml will make it easier to determine how much preservative to add.
(k) Pour the sample from the beaker into a brown, wide-mouth, nalgene bottle (typically 250ml or 500ml in size).
(l) Record the number of core samples collected as well as the diameter (5cm) and depth of the petri dish.
(m) Record surface area.
   1. 1 petri dish (5cm diameter) = 19.635 cm²
   2. 3 replicates = 58.91 cm²
(n) Label the bottle with the following information:
   - date
   - bottle number
   - stream or wetland name
   - town
   - location
   - sample location number
   - type of sample (petri dish core samples)
   - number of core samples
   - volume of sample
(o) Thoroughly clean all equipment in water before leaving stream.
(p) Add 1ml of M3 for each 50ml of sample in the brown bottle (refer to the field sheet to determine the amount).

I. PHYTOPLANKTON SAMPLING
   (1) This method is used for collecting a water sample that will be used to determine the presence of phytoplankton
   (2) Follow the procedures in the Biomonitoring’s SOP for Collecting Water Grab Samples (DEPLW0637). Use a 1 L or 500 mL bottle.
   (3) Label the bottle with the following information:
      - Date
      - wetland name
      - town
      - location
      - sample location number
      - type of sample (phytoplankton sample)
      - volume of sample
   (4) Preserve the sample with 1 mL of M3 for every 50 mL of sample
J. PROCESSING SAMPLES IN LAB

(1) Chl \(a\) filtering from slides
   (a) Store whirl-paks containing Chl \(a\) slides in the refrigerator until ready to process
       (within 24 hrs of collection).
   (b) Pour content of whirl-pak (slides and water) into beaker.
   (c) Scrape algae off both sides of the slides with a razor blade and collect algae in
       beaker.
   (d) Rinse slides and razor blade with bottled water and collect water in beaker.
   (e) Using tweezers, place Chl \(a\) filter (0.45 microns) on to vacuum apparatus and
       attach container.
   (f) Pour contents of beaker into attached container.
   (g) Add 1 drops of magnesium carbonate per 50ml of sample.
   (h) Open airway under attached container and close airway on unused receptacles.
   (i) Label a glassine envelope with a pencil and include surface area scraped (lab
       standardizes to m\(^2\), not cm\(^2\)):
       1. 2 sides of a slide = 0.00345 m\(^2\)
       2. 4 sides (2 slides) = 0.0069 m\(^2\)
   (j) When filtering is complete, remove attached container.
   (k) Remove filter by grabbing edge of filter with tweezers, fold filter in half, and place
       filter in labeled glassine envelope.
   (l) Place glassine envelope in desiccant jar in freezer.

(2) Chl \(a\) filtering from rock scrapings
   (a) Pour contents of whirl-pak or bottle into attached container.
   (b) Using tweezers, place Chl \(a\) filter (0.45 microns) on to vacuum apparatus and
       attach container.
   (c) Pour contents of beaker into attached container.
   (d) Add 1 drop of magnesium carbonate per 50ml of sample.
   (e) Open airway under attached container and close airway on unused receptacles.
   (f) Label a glassine envelope with a pencil and include surface area scraped (lab
       standardizes to m\(^2\), not cm\(^2\))
       1. 1 rock = 0.000507 m\(^2\).
       2. more than one rock - - - # rocks * 0.000507 m\(^2\).
   (g) When filtering is complete, remove attached container.
   (h) Remove filter by grabbing edge of filter with tweezers, fold filter in half, and place
       filter in labeled glassine envelope.
   (i) Place glassine envelope in desiccant jar in freezer.

K. EQUIPMENT MAINTENANCE

(1) Periphytometers
   (a) Scrub periphytometers with warm, soapy water prior to the field season.
   (b) Use scrubbing pads and toothbrushes to clean as many surfaces as possible.
(c) Spray the periphytometers with a dilute bleach solution.
(d) Do not rinse or dry off solution; allow periphytometers to air dry.

(2) Periphytometer slides
(a) Use new slides when possible.
(b) Scrub with warm, soapy water.
(c) Soak in acetone.
(d) Rinse in water and dry slides.
(e) Be careful to not touch the slide surfaces. Oils from fingerprints can potentially alter algal colonization.

6. References
Protocols for Sampling Aquatic Macroinvertebrates in Freshwater Wetlands

Jeanne DiFranco
Maine Department of Environmental Protection
312 Canco Rd.
Portland, ME 04103
(207) 822-6359 Jeanne.L.Difranco@maine.gov

May, 2006 DEPLW0640
1. **Applicability.** This standard operating procedure (SOP) applies to the collection of aquatic macroinvertebrate samples from freshwater wetlands in Maine, including wetlands associated with low-gradient rivers and streams and lake littoral zones. It describes the collection of semi-quantitative species composition data using a D-frame net or stovepipe sampler, and qualitative macroinvertebrate data using a screening level multi-habitat method. This SOP also applies to the collection of related habitat and land use data. Methods for collecting associated water samples and physical/chemical field measurements are detailed in separate Maine DEP SOPs for water grab sampling and use of electronic meters.

2. **Purpose.** The purpose of this SOP is to provide standardized methods for collecting aquatic macroinvertebrate samples and related data from wetlands in Maine.

3. **Definitions.**
   
   A. **Aquatic Macroinvertebrates** – aquatic animals without backbones that can be seen with the naked eye. Generally, this includes animals that are retained by a 600 micron mesh screen. Examples of aquatic macroinvertebrates include aquatic insects (such as mayfly, dragonfly and caddis fly larvae), aquatic worms, amphipods (scuds), leeches, clams and snails.

   B. **Aquatic Macrophytes** – aquatic plants that can be seen with the naked eye. Examples include water lilies, pond weeds, and bladderwort.

   C. **Emergent Vegetation** – rooted plants with lower portions typically growing beneath the surface of the water, but having aerial leaves, stems and reproductive structures. Emergent plants often grow in shallow waters including marshes, lakeshores and river and stream margins. Examples include cattails, sedges, rushes and pickerel weed.

4. **Responsibilities.**

   A. The Program Manager of the Maine DEP Biomonitoring Section in the Division of Environmental Assessment has the following responsibilities:
(1) Assist in procurement of programmatic funds.
(2) Provide technical support related to biological assessment.
(3) Participate as a member of a field team as time allows.

B. The Wetlands Subsection Leader has the following responsibilities:
   (1) Write proposals and manage grant funds.
   (2) Manage contracts for seasonal staff and assist with contracts for macroinvertebrate sample sorting and taxonomic identification.
   (3) Purchase and maintain supplies and equipment.
   (4) Update wetland SOPs.
   (5) Coordinate with other DEP programs and partners during selection of wetland sampling locations and scheduling of field teams.
   (6) Train and oversee wetland monitoring field teams.
   (7) Supervise seasonal wetland program staff.
   (8) Participate as a member of a field team.

C. The Rivers and Streams Subsection Leader has the following responsibilities:
   (1) Manage contracts for macroinvertebrate sample sorting and taxonomic identification.
   (2) Supervise macroinvertebrate sample sorting contractors.
   (3) Purchase and maintain supplies and equipment.
   (4) Provide technical support related to biological assessment.
   (5) Participate as a member of a field team as time allows.

D. The Stream Periphyton Subsection Leader has the following responsibilities:
   (1) Supervise macroinvertebrate sample sorting contractors.
   (2) Purchase and maintain supplies and equipment.
   (3) Provide technical support related to biological assessment.
   (4) Participate as a member of a field team as time allows.

5. Guidelines and Procedures.
   A. Sampling Period
      (1) Sampling of wetland macroinvertebrates must occur during June and July, except for special studies that require sampling at other times (i.e. studies to identify seasonal variation, impact assessments from events occurring outside the normal sampling period, etc.). Scientific justification for departing from the normal sampling period must be provided, and interpretation of results must include professional judgement to ensure that seasonal differences in macroinvertebrate assemblages are considered.
      (2) This period was selected for the following reasons:
(a) Aquatic invertebrate taxa of interest have developed sufficiently to be identified.
(b) Wetlands are less likely to dry down during this period compared with later in the summer.
(c) Overlap with stream algae and stream macroinvertebrate sampling is minimized.

B. Supplies (see supply list, Appendix 1)

C. Selecting Macroinvertebrate Sampling Locations
   (1) Collect aquatic macroinvertebrates within one of the following preferred habitats if present at the site:
       (a) Areas having emergent vegetation
       (b) Aquatic macrophyte beds consisting of floating and/or submerged plants.
       (c) Sampling locations may include similar areas within or adjacent to other habitat types, for example pockets of emergent, floating or submerged vegetation occurring within a scrub-shrub wetland.
   (2) Other representative inundated habitats may be sampled as appropriate for monitoring wetlands where emergent and aquatic bed vegetation do not occur, provided all other selection criteria are followed.
   (3) Water depth in all locations sampled must be less than 1 meter.
   (4) Locations selected for all replicate samples collected at a site must be as similar to each other as possible with regard to water depth, vegetative community structure and substrate type.

D. Recording Site Characterization, Habitat and Land Use Data
   (1) Complete all applicable sections of Wetland Bioassessment Field Data Form (see Appendix 2).
   (2) Complete Human Disturbance Ranking Form for Biological Assessment of Wetlands (see Appendix 3).
   (3) Take one to several representative digital photos of the site to be monitored. Record the photo number on the field data form.
   (4) Record GPS waypoint for the sampling site, using the designated station number to name the waypoint. (Refer to Draft SOP How to Use Garmin ETrex GPS Receiver, for use of hand held GPS unit.)

E. Recording Physical/Chemical Measurements in the Field (Refer to Protocols for Using the Hanna Dissolved Oxygen and Specific Conductance/pH Meters in Rivers, Streams, and Freshwater Wetlands, DEPLW0636.)

F. Collecting Water Samples (Refer to Protocols for Collecting Water Grab Samples in Rivers, Streams, and Freshwater Wetlands, DEPLW0637.)
G. Collecting and Preserving Algae Samples (Refer to Draft Protocols for Sampling Benthic Algae in Rivers, Streams, and Freshwater Wetlands, DEPLW0634.)

H. Dip Net Measured Sweep
   (1) The dip net measured sweep is the primary method used to collect aquatic macroinvertebrates in wetlands.
   (2) Conduct dip net sweep in an area where the bottom has not yet been disturbed and approach selected area slowly, in order to minimize accidental disturbance.
   (3) Using a 600 micron D-frame net, sweep through the water column for a distance of one meter. Measure the sweep distance using a meter stick held slightly above the surface of the water by a second person to avoid disturbing aquatic organisms.
   (4) Bump the net against the bottom substrate three times (at the beginning, the middle, and the end of the one meter sweep) to dislodge and collect organisms from the sediment. The net should remain submerged during the entire sweep.
   (5) At the end of the sweep, turn the net so the opening is facing the surface of the water and lift the net out of the water, so no organisms are lost out of the opening.
   (6) If the net becomes significantly clogged or if branches, rocks, or other obstructions prevent the net from properly contacting the wetland substrate, discard the sample and resample in another undisturbed location.
   (7) Perform the measured sweep as quickly as possible to prevent aquatic organisms from escaping out of the net. The sweep should be completed within approximately 3 seconds.
   (8) Transfer all material collected in the net into a 600 micron sieve bucket by placing the bucket half way into the water and turning the net inside out inside the bucket. Place material in and on the net into the water in the bucket. Visually inspect the net and remove any clinging organisms.
   (9) Examine, wash and discard large pieces of vegetation, woody debris, stones, etc., making sure to remove and retain any aquatic invertebrates observed. Retain all finer plant and material and detritus.
   (10) Drain water out of sieve bucket and transfer all material collected into a wide mouth quart sized canning jar. Use additional jars as needed for each sample so that none of the jars are more than approximately 1/2 full. Place sample material into each jar loosely (not packed), to ensure adequate space for alcohol.
   (11) Collect three replicate samples in areas of emergent or aquatic bed vegetation, if present, or in other representative inundated habitats as appropriate for the wetland type sampled. Collect each replicate sample in an undisturbed location. Space replicate samples so that samples are spread out to the extent possible across each site (but generally within 100 meters.
of each other), to account for potential uneven spatial distribution of macroinvertebrate communities. For sites where appropriate available sampling habitat is limited or patchy, spacing of replicate samples may be adjusted as necessary based on professional judgement.

(12) Preserve samples in 95% ethyl alcohol for later sorting and taxonomic analysis in the laboratory.

I. Stovepipe Sampler

(1) The stovepipe sampler is an alternative method used to collect macroinvertebrate samples in areas where it may be difficult to use a standard D-frame net. This may include sites having particularly dense vegetation and/or little standing water.

(2) A stovepipe sampler is a five-gallon plastic bucket with the bottom removed. The bottom diameter is 25 cm. The sampler is used to enclose fixed-area plots to restrict the movement of organisms.

(3) Press the stovepipe sampler firmly into the wetland substrate and hold in place while a second person performs the remaining sample collection steps.

(4) Be sure to select a site where water depth does not overtop the stovepipe sampler.

(5) Using one hand, gently agitate the area within the sampler for approximately 10 seconds to dislodge organisms from vegetation and sediment. Shoulder length rubber gloves are recommended for this procedure.

(6) Remove vegetation, coarse woody debris, and approximately the top 1 cm of sediment enclosed within the sampler and place it into a 600 micron sieve bucket.

(7) Sweep the area within the sampler 10 times with a small 500 micron mesh hand net, starting from the bottom of the sampler and moving up through the water column. Between sweeps, transfer all material collected in the hand net into the sieve bucket by placing the bucket half way into the water and turning the net inside out inside the bucket. Place material in and on the net into the water in the bucket. Visually inspect the net and remove any clinging organisms.

(8) Examine, wash and discard large pieces of vegetation, woody debris, stones, etc., making sure to remove and retain any aquatic invertebrates observed. Retain all finer plant material and detritus.

(9) Drain water out of sieve bucket and transfer all material collected into a wide mouth quart sized canning jar. Use additional jars as needed for each sample so that none of the jars are more than approximately 1/2 full. Place sample material into each jar loosely (not packed), to ensure adequate space for alcohol.

(10) Collect three replicate samples in areas of emergent or aquatic bed vegetation, if present, or in other representative inundated habitats as appropriate for the wetland type sampled. Collect each replicate sample in
an undisturbed location. Space replicate samples so that samples are spread out to the extent possible across each site (but generally within 100 meters of each other), to account for potential uneven spatial distribution of macroinvertebrate communities. For sites where appropriate available sampling habitat is limited or patchy, spacing of replicate samples may be adjusted as necessary based on professional judgement.

(11) Preserve samples in 95% ethyl alcohol for later sorting and taxonomic analysis in the laboratory.

J. Multi-Habitat Sampling

(1) Multihabitat sampling is a qualitative method that may be used as a screening tool for assessing aquatic invertebrates.

(2) Using a 600 micron D-frame net, sample all inundated microhabitats at each site by jabbing the net into the wetland substrate and quickly sweeping upward to the water’s surface. Examples of habitats to be sampled include areas of emergent vegetation, aquatic macrophyte beds, pools, channels and areas between vegetation hummocks.

(3) Between jabs, transfer all material collected in the net into a 600 micron sieve bucket by placing the bucket half way into the water and turning the net inside out inside the bucket. Place material in and on the net into the water in the bucket. Visually inspect the net and remove any clinging organisms. All material collected at a given site is composited into a single sample.

(4) Examine, wash and discard large pieces of vegetation, woody debris, stones, etc., making sure to remove and retain any aquatic invertebrates observed. Retain all finer plant material and detritus.

(5) Transfer a small amount of the composite sample from the sieve bucket into a large white picking tray.

(6) Using a forceps, separate organisms from detritus and place one to several organisms representing each different taxon observed into a vial of alcohol. Continue until no different taxa are observed and discard remaining material contained in the picking tray.

(7) Repeat steps 5 and 6, working with small amounts of the composite sample at a time, until entire sample has been picked.

K. Labeling Macroinvertebrate Samples in the Field

(1) Label quart jars using opaque tape and a fine-tipped permanent marker. Required labeling information and format are described in paragraph 3 below.

(2) Label multihabitat sample vials by placing a strip of heavy paper inside each vial. Record required information using a number 2 or darker pencil only. Do not use other types of writing utensils, as alcohol used to preserve the sample may cause ink to run.
Include the following information on the label for each sample:

(a) Sample collection date (day/month/year). Example: 6/10/03

(b) Sample identification number. Example: DN-2003-001

1. The sample identification number is unique for each sample collected, and consists of the method abbreviation, 4-digit year (i.e. 2003), and 3 digit station number, separated by hyphens.


3. Wetland invertebrate samples are generally collected only once per year at a given site for the ambient monitoring program. If a special project involves multiple samples collected in the same year at the same site, and the same collection method is used, an additional 2-digit sequential code must be included in the sample identification number based on the order in which samples were collected. Example: DN-2003-001-01, DN-2003-001-02, DN-2003-001-03, etc.

(c) Station name. Example: Meadow Brook

(d) Town. Example: New Gloucester

(e) Replicate number. Example: DN#1

1. The replicate number consists of the method abbreviation plus the number assigned to each replicate sample for a particular site (generally 1, 2, or 3).

2. A replicate number is not required for multihabitat samples.

(f) Container number. If a single sample must be divided between two or more sample containers, additional information must be recorded on the label to indicate the container number and the total number of containers for that sample. Examples: Jar 1 of 2, vial 2 of 3, etc.

(g) The following is an example of a completed label:

6/10/03 DN-2003-001
Meadow Brook, New Gloucester
DN#1 Jar 2 of 3

L. Preserving Macroinvertebrate Samples in the Field

(1) Procedures for wetland macroinvertebrate sample preservation follow methods described in Davies and Tsomides 2002.

M. Decontaminating Sampling Gear

(1) The DEP Division of Environmental Assessment (DEA) uses standardized methods for cleaning and disinfecting all sampling equipment to prevent the spread of invasive species and disease pathogens which threaten amphibians and other wildlife in Maine. These methods are described in Protocols for Decontaminating Biomonitoring Sampling Equipment, DEPLW0641.
N. Laboratory Procedures for Macroinvertebrate Samples
   (1) Laboratory procedures for wetland macroinvertebrate sample sorting, preservation, labeling, subsampling and taxonomy follow methods described in Davies and Tsomides 2002.

6. References


Wetland Sampling Steps to Success: Collecting Macroinvertebrates Using a Dip Net Measured Sweep

**Standard Sampling Season:** June and July

**Other information collected at each site:**
- Physical/chemical water characteristics using hand-held meters
- Water grab samples for analysis at the lab
- Algae samples (phytoplanktonic and epiphytic)
- Description of the site and its surrounding habitat and land uses

**Macroinvertebrates**
- Collect from areas not disturbed by other sampling
- Complete all sweeps in areas of emergent vegetation or macrophyte beds having similar habitat representative of the overall site.

**Dip Net Measured Sweep:**
- Using a 600 micron D–frame net, sweep through the water for 1 meter– measured using a yard stick held above the water’s surface
- Bump net against bottom substrate 3 times (beginning, middle, end), to dislodge and collect organisms from the sediment
- Keep the net submerged during the entire sweep
- Complete sweep in approximately 3 seconds
- At the end of the sweep, turn net so the opening is facing the surface of the water and lift the net quickly out of the water - so no organisms are lost out of the opening
- If net becomes clogged or if it was prevented from thoroughly contacting the bottom substrate - discard the sample and start again in an undisturbed location
- Transfer all material collected in the net into a 600 micron sieve bucket by placing the bucket halfway into the water and turning the net inside out into the bucket
- Place material in and on the net into the water in the bucket
- Visually inspect the net and remove any clinging organisms
- Examine, wash, and discard any large pieces of vegetation, woody debris, and stones– remove and retain any aquatic macroinvertebrates observed
- Retain fine plant material and detritus
- Drain water out of sieve bucket and transfer all material collected into 1 quart wide mouth canning jar - none of the jars should be more than half full
- Preserve samples in 95% ethyl alcohol for later sorting and taxonomic analysis in the laboratory
- Repeat process to collect a total of three replicate samples

For further information, please refer to the Biomonitoring Homepage

DEP-LWo877
Protocols for Decontaminating Biomonitoring Sampling Equipment

Beth Connors
Maine Department of Environmental Protection
312 Canco Road
Portland, ME, 04103
(207) 822-6300  Beth.Connors@maine.gov

April 2008
DEPLW-0919
1. **Applicability.** This standard operating procedure (SOP) applies to all equipment (sampling devices, nets, boats and trailers, etc.) used in the collection of biological samples from waters of the state of Maine that come in direct contact with the waterbody. This SOP applies to biomonitoring activities conducted by the Division of Environmental Assessment (DEA) in all freshwaters of the State of Maine, unless more stringent program or project decontamination protocols already exist. This SOP also applies to all non-DEA entities that conduct biomonitoring activities within the State in cooperation with DEA staff.

2. **Purpose.** The purpose of this SOP is to provide standardized methods for cleaning and disinfecting all equipment used to collect samples from waters of the state of Maine in order to prevent the spread and/or introduction of disease pathogens and invasive algae, plant and animal species. This SOP is designed to be consistent with the decontamination procedures of other entities conducting sampling in the State, such as the Department of Marine Resources, Inland Fisheries and Wildlife and Acadia National Park, as well as the procedures of the Vermont Agency of Natural Resources.

Examples of confirmed and potential disease pathogens of concern in Maine include, but are not limited to: Chytrid fungi, *Ichthyophonus*, Ranavirus, *Ribeiroia*, *Saprolegnia*, *Proteocephalus ambloplitis* and other fish pathogens. Examples of invasive aquatic plant and alga species of concern in Maine include, but are not limited to: Variable water-milfoil (*Myriophyllum heterophyllum*), Eurasian water milfoil (*Myriophyllum spicatum*), *Hydrilla* (*Hydrilla verticillata*), Curly-leaved Pondweed (*Potamogeton crispus*), and Didymo (*Didymosphenia geminata*).

3. **Definitions.**

   A. **Chytrid fungi.** Ubiquitous fungi that inhabit aquatic habitats and wet soils, infecting plants, algae, protists and invertebrates. *Batrachochytrium dendrobatidis* is a Chytrid fungus known to infect vertebrates, including amphibians.

   B. **Ichthyophonus.** A protozoan-like organism which affects marine and freshwater organisms. *Ichthyophonus* infects the skeletal muscle of amphibians, and has also been identified as the cause of herring die-offs.
C. Ranavirus. A common virus in New England that primarily affects wood frogs and spotted salamanders. This pathogen was associated with a large die-off of spring peepers in Acadia National Park.

D. Ribeiroia. A common pathogen which causes malformations and deformities in amphibians belonging to the genus Rana.

E. Saprolegnia. A pathogen which affects the eggs of many amphibians.

F. Proteocephalus ambloplitis. Fish pathogen, commonly known as bass tapeworm.

G. Variable water-milfoil (Myriophyllum heterophyllum). Invasive submerged aquatic plant, non-native to the Northeast U.S., that out competes native species and clogs shallow areas of lakes, making swimming and boating difficult.

H. Eurasian water milfoil (Myriophyllum spicatum). An invasive submerged plant with feather-like whorled leaves. It grows in extremely large dense mats in depths up to 15 feet. This plant produces emergent flower spike important for distinguishing Eurasian from Variable-leaf and other milfoils.

I. Hydrilla (Hydrilla verticillata). A prolific invasive weed that forms stems reaching up to 30 feet in length, dominating fresh water ecosystems quickly by way of winter buds, underground tubers, and surface runners. It is tolerant of low light levels, high or low nutrient levels, and freezing temperatures, posing fouling problems for swimmers and boaters.

J. Curly-leaved Pondweed (Potamogeton crispus). An invasive submerged aquatic plant from Eurasia. It is highly competitive plant, spreading and growing rapidly. This plant grows in cool waters and spreads by rhizomes, turions (vegetative buds) and fragmentation. Of particular concern are the turions, which are released from the plant in late spring and float until they sink to the bottom where they lie dormant until water temperatures cool enough for sprouting in the fall.

K. Didymo (Didymosphenia geminata). An invasive freshwater diatom species, also known as Rock Snot.

4. Responsibilities.

A. Training. It is the responsibility of the Project Manager to ensure that all individuals collecting samples have received training in and follow these decontamination procedures.

B. Determination of threat. It is the responsibility of the Project Manager to determine if a significant threat of contamination exists, which level of decontamination is necessary, at what point in the sampling process the decontamination should take place (before sampling, after sampling, or before and after sampling), and what disinfectant should be used.
C. Out of State Equipment. It is the responsibility of the Project Manager to ensure that all cooperating entities follow the Level 3 Cleaning and Disinfection procedures, see 5 C (3), below.

5. Guidelines and Procedures

A. Materials
   (1) Disinfectant. **For non-absorbent materials** (boats, rubber waders and other “hard-sided” objects), use either a 2% household bleach solution (3 oz bleach per gallon of water) or a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). **For absorbent materials** (nets, felt-soled waders, life jackets, sandals with fabric straps and other “soft-sides” objects), use a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). Note: an alternative disinfectant can be used, if approved by the Project Manager.
   (2) backpack sprayer, garden hose or other suitable applicator
   (3) scrub brush
   (4) liquid dish or hand soap (phosphate-free and biodegradable)
   (5) measuring cup (with cup and ounce increments marked)
   (6) plastic container(s) large enough to hold items that need to be soaked
   (7) plastic bucket (to rinse small items)
   (8) 5 gallon plastic container of tap water
   (9) rubber gloves (including an extra pair)
   (10) goggles
   (11) plastic apron (optional)

B. Precautions and limitations
   (1) Always wear gloves and safety goggles when using disinfectant, and avoid contact with exposed skin, clothing, vehicle upholstery and/or other fabric.
   (2) When using a backpack sprayer, keep it upright at all times to avoid spillage.
   (3) New bleach solution must be made up daily. New quaternary ammonia solution should be made up every 2-3 days, or as needed. Old solutions must be disposed of down a drain connected to a wastewater treatment system, accompanied by a large amount of water.
   (4) For safety and logistical reasons, only take one type of disinfectant into the field. It is up to the Project Manager to decide which one will be needed based on the types of equipment to be used in the field (absorbent vs. non-absorbent).

C. Procedures for inspecting, cleaning and disinfecting equipment. Level 1 decontamination should always be done. The necessity of decontamination beyond Level 1 is to be determined by the Project Manager.
   (1) Level 1 – Visual inspection
      (a) Applicable to all waters.
(b) Visually inspect all equipment having contact with the water (waders, nets, sieve buckets, canoe, boat trailers, etc.) for any plant fragments or other debris. If any plant material or associated mud is found, remove it and either throw it in a trashcan or dispose of it on high, dry ground. Do not put it back into the waterbody or along the shore. All plant fragments must be removed before equipment is transported to another waterbody.

(c) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.

(2) Level 2 (done in addition to Level 1) - Cleaning

(a) Applicable to: waters used for aquaculture activities, waters within a State owned Ecological Reserve, waters designated Statutory Class A or B, or as deemed necessary by the Project Manager.

(b) Visually inspect all equipment having contact with the water for any plant fragments or other debris, as outlined in C (1) (b), above.

(c) Designate a grassy area or other upland vegetated area, at least 100 feet from open water and remove mud and other debris, by washing with soap and water. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.

(d) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.

(3) Level 3 (done in addition to Levels 1 and 2) – Cleaning and Disinfection

(a) Applicable to: critical habitats (vernal pools, designated salmon rivers, waters designated Statutory Class AA), areas with a known infestation of Infectious Salmon Anemia virus (ISAV), areas with a known infestation of an invasive aquatic plant, areas susceptible to Didymo infestation, or as deemed necessary by the Project Manager.

(b) Visually inspect all equipment having contact with the water for any plant fragments or other debris, as outlined in C (1) (b), above.

(c) Designate a grassy area or other upland vegetated area, at least 100 feet from open water and remove mud and other debris, by washing with soap and water. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.

(d) Disinfect by thoroughly spraying all equipment with appropriate disinfectant. Bleach solutions are not recommended for absorbent materials due to ineffective penetration compared to Quaternary ammonia solutions.

(e) Allow all equipment to air dry and visually inspect again, repeating procedures if necessary.

(f) All equipment used to collect water samples (dipper, mixing jugs) must be rinsed 3 times prior to reuse. Rinse with clean water; either tap water or de-ionized water, as determined by the Project Manager.

(g) Sampling devices that are placed into a waterbody for an extended length of time (e.g. rock bags, periphytometers) will be decontaminated using one of the following methods, as determined by the Project Manager.
i. Air dried for several months, in direct sunlight for part of the time, if possible.
ii. Cleaned with hot soapy water, rinsed with hot tap water and air dried for several months in direct sunlight for part of the time, if possible.
iii. Immersed in a bleach solution bath for 30 minutes, rinsed with hot water and air dried for several months, in direct sunlight for part of the time, if possible.

(h) This disinfection procedure does not apply to electronic equipment such as dissolved oxygen and pH/conductivity meters. Rinse meter probes with clean water between sites, remove all plant fragments and other debris from meters and cables by hand (clean paper towels may be used if needed), and allow equipment to air dry. Meters and data sondes may be disinfected with an alcohol rinse, if deemed necessary by the Project Manager.

D. Whenever possible, use non-absorbent, “hard-sided” sampling equipment. The use of rubber-soled waders is preferred to using felt-soled waders.

E. Whenever possible, dedicate equipment to a single site for repeat sampling. Such equipment must be clearly labeled and kept clean, but does not require disinfection between uses.

F. Whenever possible, sample upstream sites first and work downstream to avoid potential introduction of pathogens or invasive species further up into the watershed. Uncontaminated sites must be sampled before sites having known disease or invasive species problems.

G. All equipment used in sites having known disease or invasive species problems must be decontaminated prior to reuse.

H. All used sampling equipment brought in from outside the state of Maine must be cleaned and disinfected (see C (3) above) before and after its use in Maine waters.

6. References.


Maine Department of Inland Fisheries and Wildlife. 2005. *Fisheries Staff Biosecurity and Disinfection Guidelines for Field Work (Draft).*


Addendum - List of edits to existing SOP

<table>
<thead>
<tr>
<th>SOP section</th>
<th>Old text</th>
<th>New text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Applicability</td>
<td>N/A</td>
<td>This SOP also applies to all non-DEA entities that conduct biomonitoring activities within the State in cooperation with DEA staff.</td>
</tr>
<tr>
<td>4. Responsibilities</td>
<td>N/A</td>
<td>C. Out of State Equipment. It is the responsibility of the Project Manager to ensure that all cooperating entities follow the Level 3 Cleaning and Disinfection procedures, see 5 C (3), below.</td>
</tr>
<tr>
<td>5. Guidelines and Procedures, A. Materials</td>
<td>(1) bleach solution (1/2 cup bleach per gallon tap water) Note: an alternate disinfectant can be used, if approved by the project manager.</td>
<td>(1) Disinfectant. <strong>For non-absorbent materials,</strong> use either a 2% household bleach solution (3 oz bleach per gallon of water) or a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). <strong>For absorbent materials,</strong> use a 2.5% Quaternary ammonia (Sani-Care Quat-128, etc.) solution (3.5 oz quaternary ammonia per gallon of water). Note: an alternative disinfectant can be used, if approved by the Project Manager.</td>
</tr>
</tbody>
</table>
Maine DEP Wetland Bioassessment Field Data Form (revised January 2008)

**Station Information**

Station #:______ Date:__________ Time:__________ Town:__________________________ County:___________________________

Name of wetland and/or associated waterbodies:_________________________________________________________________________

Trip ID: ________ Sample Location (boat, wading): ______________ Watershed Characteristics:____ flat ____rolling ____ hilly____ mountains

Detailed directions and description of sampling station (mark location on attached map): _____________________________________________

________________________________________________________________________________________________________________

________________________________________________________________________________________________________________

Project Manager and Sampling crew members:__________________________________________________________________________

GPS accuracy:  ___________________ WayPoint Name: ________________ Latitude: ___________ Longitude: ___________

Pictures (photo #s): ___________________________________________________________ (projection=NAD83; units=metric; north ref=magnetic)

Legislative Class:_______________ Biophysical Region:__________________________

**Macr0invertebrate Samples**: Record the following information for each habitat sampled. (Use habitat and substrate codes below.)

<table>
<thead>
<tr>
<th>Habitat Code</th>
<th>Sampling Method</th>
<th>Rep #</th>
<th># of jars</th>
<th>Water Depth (cm)</th>
<th>Substrate Code(s)</th>
<th>Dominant Plant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Habitat Codes
1. Open water – standing (ponds, marshes)
2. Open water – flowing (river/stream channels)
3. Aquatic macrophyte bed (floating/submerged vegetation dominant)
4. Emergent - non-persistent vegetation dominant (non-woody species not visible at certain seasons, such as pickerelweed)
5. Emergent - persistent vegetation dominant (non-woody species that remain standing until the beginning of the next growing season, such as grasses, cattails)
6. Scrub-shrub (dominated by woody vegetation < 6m tall)
7. Peatland (emergents, shrubs and trees < 30% cover)
8. Forested (dominated by woody vegetation > 6m tall)
9. Vernal pool
10. Other

Substrate Codes
1. sand (<1/8”)
2. gravel (1/8” – 3”)
3. rubble (3” – 10”)
4. silt/muck
5. clay
6. organic soil (well decomposed)
7. peat
8. boulders (>10”)
9. bedrock
10. detritus

**Algae Samples (check if collected)**: Phytoplankton (water sample) Epiphytes (submerged plant stems) Bottle # Bottle # Volume (mL) Surface Area (cm²)

**Physical/Chemical parameters**: Dissolved Oxygen Temp Conductivity pH
D.O. meter number: _______ Calibrated? Y / N Conductivity meter number: _______ Calibrated? Y / N

**Water Samples Collected**: Water samples______ Water field duplicates______ HETL #:_________________________ DUP HETL #:_________________________

Notes/comments (continue on back if necessary)___________________________________________________________________________

________________________________________________________________________________________________________________

____________________________________________________________________________________________________________________________________________________________________________________________________________________________________
<table>
<thead>
<tr>
<th>Plant Type (cattail, sedge, rush, grass)</th>
<th>Circular Stems</th>
<th>Flat Stems</th>
<th>Triangular Stems</th>
<th>Semicircular Stems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.14 * D * L Surface Area</td>
<td>2 * W * L Surface Area</td>
<td>avg side W * 3 * L Surface Area</td>
<td>[(1.57 * D) + D] * L Surface Area</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>2</td>
<td>3</td>
<td>1.57</td>
<td>0</td>
</tr>
</tbody>
</table>

**Totals**: 0.00 0.00 0.00 0.00

**TOTAL SURFACE AREA**: 0.00

**NOTE**: All measurements must be in centimeters.
### Human Disturbance Ranking Form for Biological Assessment of Wetlands

Station #:_________  Date:___________  Time:____________

Town:_______________________________________  County:______________________________________________

Evaluator(s):_______________________________________________________________________________________

Name of wetland and/or associated waterbodies____________________________________________________________

For each wetland station assessed, score all potential factors in the 5 categories below using the following scale:

<table>
<thead>
<tr>
<th></th>
<th>Not Observed</th>
<th>Minimal</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

The purpose of this ranking is to characterize the degree of human disturbance at a given wetland biomonitoring station, including the portion of the watershed immediately surrounding the station, relative to other stations sampled. (Note that the human disturbance ranking is not intended to serve as an impact assessment in the absence of biological data.)

<table>
<thead>
<tr>
<th>Category</th>
<th>Observations/Comments</th>
<th>Score</th>
<th>Section 1 Subtotal:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hydrologic Modifications to Wetland</td>
<td>man-made dikes or dams</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>causeways, roads or railroad bed crossings which impede water flow; inadequate or obstructed culverts</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ditching, draining or dewatering</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>filling or bulldozing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other hydrologic modifications not included in this section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Vegetative Modifications to Wetland</td>
<td>timber harvesting in wetland</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other clearing/removal of vegetation (roads, utility lines, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>plowing, mowing or grazing in wetland</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>evidence of herbicide use in wetland</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other vegetative modifications not included in this section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Evidence of Chemical Pollutants</td>
<td>discharge pipes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>oil, petroleum, chemicals observed, or chemical odor present</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>soil staining and/or stressed or dying vegetation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>trash, chemical containers, demolition debris, drums, etc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other evidence of chemical pollutants not included in this section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Impervious Surface in Watershed</td>
<td>residential development</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>commercial/industrial development</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>recreational development (campgrounds, picnic or boat launch areas, trails, docks, boardwalks, parking areas, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>additional roads, highways bridges</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other impervious surfaces not included in this section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Potential for NPS Pollution</td>
<td>excess sediment accumulation or unstable eroding soil from human activities (roads, construction or excavation sites, agriculture, forestry activities, etc.) observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>alterations to wetland buffer (within 100 feet of wetland edge)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>livestock, feedlots or manure piles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>evidence of fertilizer or pesticide use (lawns, golf courses, agricultural crops, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other NPS sources not included in this section</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Additional notes (use back if needed):**

Total:_________