



# Protocols for Sampling Aquatic Macroinvertebrates in Freshwater Wetlands



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**Bureau of Water Quality  
Division of Environmental Assessment  
Biomonitoring Program**

Standard Operating Procedures  
Protocols for Sampling Aquatic Macroinvertebrates in Freshwater Wetlands

- 1. Applicability.** This standard operating procedure (SOP) applies to the collection of aquatic macroinvertebrate samples from inundated freshwater wetland habitats, including marshes, pools and other depressional wetlands, lacustrine and riverine fringe wetlands, low-gradient river and stream reaches, lakes and ponds. It describes the primary method to collect aquatic macroinvertebrate community composition data using a D-frame net. This method is required if data collected are to be used in the DEP wetland macroinvertebrate linear discriminant model to predict aquatic life use attainment. The use of a stovepipe sampler is also described as an alternative method to collect macroinvertebrate samples in areas where it may be difficult to use a D-frame net. In addition, this SOP describes how to collect qualitative macroinvertebrate data using a screening level multi-habitat method. This SOP also applies to the collection of related habitat and land use data. Protocols for completing the Wetland Human Disturbance Assessment are described in a separate Maine DEP SOP. Methods for collecting associated water samples, physical/chemical field measurements, and use of temperature logging devices are detailed in separate Maine DEP SOPs.
- 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 500 - 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, lake shores, and river and stream margins. Examples include cattails, sedges, rushes and pickerel weed.



#### **4. Responsibilities.**

- A. The section leader of the Maine DEP Biomonitoring Program 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 used for sample sorting.
  - (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 used for sample sorting.
  - (4) Provide technical support related to biological assessment.
  - (5) Participate as a member of a field team as time allows.
- D. The Stream Algae Subsection Leader has the following responsibilities:
- (1) Supervise macroinvertebrate sample sorting contractors, when Rivers and Streams Subsection Leader is unavailable.
  - (2) Provide technical support related to biological assessment.
  - (3) 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-leaved and/or submerged plants.
  - (c) Sampling locations may include similar areas within or adjacent to other habitat types, for example pockets of emergent, floating-leaved 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 Maine DEP Wetland Bioassessment Field Data Form.
- (2) Complete the Biological Monitoring Wetland Human Disturbance Assessment form (refer to Protocols for Completing the Biological Monitoring Wetland Human Disturbance Assessment, DEPLW-1259).
- (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. Follow manufacturer's directions in manual for GPS unit used.

#### 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, DEPLW0636A-2014).



- F. At some sites a water temperature logger may be deployed during the site visit and retrieved later in the sampling season. (Refer to Protocols for Measuring Continuous Water Temperature Using an Onset Data Logger, DEPLW0700A-2014).
- G. Collecting Water Samples (Refer to Protocols for Collecting Water Grab Samples in Rivers, Streams, and Freshwater Wetlands, DEPLW0637A-2014).
- H. Collecting and Preserving Algae Samples (Refer to Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands, DEPLW-0634B-2014).
- I. Manage all data collected in accordance with the Protocols for Managing Biomonitoring Data, DEPLW-1202A-2014.
- J. Dip Net Measured Sweep
  - (1) The dip net measured sweep is the primary method used to collect aquatic macroinvertebrates in wetlands. This method is required if data collected are to be used in the DEP wetland macroinvertebrate linear discriminant model to predict aquatic life use attainment.
  - (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 500 - 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 500 - 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.

#### K. 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) One person will 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 500 -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.

#### L. 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 500 - 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 500 - 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.

#### M. 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.

- (3) Include the following information on the label for each sample:
  - (a) Sample collection date (day/month/year). Example: 6/10/2013
  - (b) Sample identification number. Example: DN-2013-001
    1. The sample identification number is unique for each sample collected, and consists of the method abbreviation, 4-digit year (i.e. 2013), and 3 digit station number, separated by hyphens. Method abbreviations: DN = dip net measured sweep sample, SP = stovepipe sample, MH = multihabitat sample.
    2. 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-2013-001-01, DN-2013-001-02, DN-2013-001-03, 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:

6/10/13            DN-2013-001  
Meadow Brook, New Gloucester  
DN#1            Jar 2 of 3

#### N. Preserving Macroinvertebrate Samples in the Field

- (1) Wetland macroinvertebrate samples are preserved in 95% ethyl alcohol.

#### O. 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, DEPLW0919A-2014.

#### P. 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

Connors, B., 2019. *Protocols for Decontaminating Biomonitoring Sampling Equipment*. Maine Department of Environmental Protection, Augusta, ME. DEPLW0919A-2014.

Connors, B., 2019. *Protocols for Measuring Continuous Water Temperature Using an Onset Data Logger*. Maine Department of Environmental Protection, Portland, ME. DEPLW0700A-2014.

Connors, B., 2019. *Protocols for Managing Biomonitoring Data*. Maine Department of Environmental Protection, Portland, ME. DEPLW-1202A-2014.

Connors, B., 2019. *Protocols for Using Dissolved Oxygen and Specific Conductance/pH Meters in Rivers, Streams, and Freshwater Wetlands*. Maine Department of Environmental Protection, Portland, ME. DEPLW0636A-2014.

Connors, B. and J. L. DiFranco, 2019. *Protocols for Completing the Biological Monitoring Wetland Human Disturbance Assessment*. Maine Department of Environmental Protection, Portland, ME. DEP-LW1259.

Danielson, T. J., 2019. *Protocols for Collecting Water Grab Samples in Rivers, Streams and Freshwater Wetlands*. Maine Department of Environmental Protection, Augusta, ME. DEPLW0637A-2014.

Danielson, T. J., 2019. *Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands*. Maine Department of Environmental Protection, Augusta, ME. DEPLW-0634B-2014.

Davies, S.P., and L. Tsomides, 2002. *Methods for Biological Sampling and Analysis of Maine's Rivers and Streams*. Maine Department of Environmental Protection. Augusta, ME. DEP LW0387-B2002.



## **Appendix 1: Wetland Sampling Equipment List**

### Water Chemistry

D.O. meter  
Conductivity/pH meter  
extra batteries (C)  
screwdriver  
sample container kits (from lab)  
long handled dipper  
1 gallon plastic mixing jugs (2)  
cooler and ice

### Macroinvertebrates

600 micron D-frame nets  
600 micron sieve buckets  
meter stick  
canning jars/lids (wide mouth quarts)  
extra lids/rings  
extra label tape  
alcohol/field alcohol container

### Algae/Diatoms

wide mouth Nalgene containers  
M3 preservative  
M3 labels for sample bottles  
gloves  
Pipette  
bulb or rolling controller  
squirt bottles  
tap water  
plastic beaker  
whirlpak bags  
Epiphyte samples:  
    hand pruner  
    metric ruler

### Sedimented algae/diatoms:

    small plastic petrie dishes  
    spatula

### Decontamination gear

garden sprayer  
household bleach or quaternary ammonia  
5 gallon container of water for rinsing  
scrub brush  
liquid soap  
measuring cup  
rubber or nitrile gloves

Protocols for Sampling  
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goggles  
apron

### General

canoe  
paddles  
pfd's  
canoe wheels  
pack baskets  
boots/waders (non-felt bottomed preferred)  
wader repair kit  
hand wipes  
paper towels  
flagging  
extra rope  
duct tape  
packing tape  
freezer bags  
trash bags  
utility/work gloves  
tarp  
screw drivers (Philips and flathead)  
bug shirts/head nets  
field sheets/clipboards  
wetland maps  
DeLorme Gazetteer  
sharpies / pencils  
camera and charged spare battery  
GPS unit and spare batteries (AA)  
binoculars  
distance finder  
EZ pass

### First aid kit

with tick kit(s) and some type of water purifier  
(i.e. iodine tablets)

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