

**Development of a Comprehensive State Monitoring and
Assessment Program for Wetlands in Massachusetts**

Appendix H

Revised December 30, 2009

**Standard Operating Procedures: Assessment of Wetland
Communities**

Phase 2c: 2009

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Standard Operating Procedures: Assessment of Wetland Communities

1. Scope and Application

This SOP establishes a standard set of procedures to be followed for data collection toward the development of a Site Level Assessment Method (SLAM) for MA freshwater forested wetlands and to validate/calibrate the Conservation Assessment and Prioritization System (CAPS) as a mechanism for a landscape level analysis (Level 1) of ecological integrity. This project will focus on assessment of wetland biological community condition in forested wetlands.

Described below are the procedures that will be followed in collecting data on algae, macroinvertebrates, vascular plants, bryophytes, epiphytic macrolichens and habitat characterization (e.g. water chemistry, hydroperiod, etc.) to serve as a basis for development of a SLAM, which will incorporate the use of Indices of Biological Integrity, for freshwater forested wetlands.

2. Summary

This SOP is applicable for freshwater deciduous/coniferous forested wetlands that have the hydrogeomorphic (HGM) classification of a slope or flat throughout Massachusetts (hereafter referred to as forested wetland). Data collection for phase 2c will focus on forested wetland communities in the Miller's and Concord (Sudbury-Assabet-Concord) Watersheds, however this SOP can be applied to all forested wetland communities. Sampling sites will be selected via a stratified random process. Field data collection will involve sampling of several biotic communities to determine if 1) there is a dose-dependent response in various attributes of the biological community to stressors within the landscape and 2) to validate/calibrate the ecological integrity metrics that are utilized in the CAPS model. Characterization of the wetland and assessment of its biological condition will be conducted in the field by assessing habitat, algae, macroinvertebrates, vascular plants, bryophytes, epiphytic macrolichens and habitat characterization.

3. Safety Considerations

- Fieldwork will not be conducted during heavy rain events or unsafe conditions such as electrical storms or high wind events. Practice "safety first".
- If there is no safe access to a plot point, the field sampling will not be conducted for that site.
- Private property will be respected using the following guidelines.
 - If property is in close proximity to buildings or other heavily used areas, landowner permission will be sought
 - Posted property will not be accessed without permission of the landowner

- Otherwise, sampling will proceed without any special effort to gain landowner permission
 - If asked to leave private property by the landowner, samplers will discontinue work and leave.
- Each field technician will carry a personal first aid kit and a wilderness first aid guide
- Field personnel will not access sites alone without the instruction of a field manager
- No chemicals (other than ethanol) will be handled by personnel in the field

4. Sample Collection, Preservation, and Handling

Macroinvertebrates collected using the stovepipe sampler will be preserved in 95% ethyl alcohol solution. 70% ethanol will be used to preserve macroinvertebrates collected in the emergence traps. Macroinvertebrates collected in the pitfall traps will be preserved initially in a 50:50 propylene glycol/water solution and a drop of dishwashing liquid soap. The samples will be rinsed with tap water in the lab and transferred to a 70% ethyl alcohol solution. Samples will be labeled with the plot ID, date, surveyor, and collection method. They will be sorted and identified to order in the lab. Samples will be preserved and held in the lab until resources are available to identify the macroinvertebrates to genus and species (if possible).

Earthworms will be collected into 70% isopropyl alcohol and kept cool until transfer to the lab for permanent preservation in 10% formalin. Samples will be labeled in the field with plot ID, data, and name of surveyor. Transfer of worms into formalin will occur in a fume hood using safety glasses and gloves. Worms will remain in formalin for at least 24 hours before being permanently stored in 70% isopropyl alcohol. Tentative species IDs and counts may be made in the field. Official counts and IDs will be made in the lab using a dissecting microscope. Earthworm species identifications will follow Schwert (1990) and Reynolds (1977).

Algae will be collected and labeled with the plot ID, date, surveyor, and collection method. Algae samples will be preserved with M3 fixative (Potassium Iodide, Iodine (optional), glacial acetic acid, formalin) and stored until resources are available to identify them to genus and species.

Vascular plant, bryophyte and lichen collections will be limited to species that cannot be identified in the field. For species that cannot be positively identified in the field samples will be collected for lab identification and photographed for digital preservation. Taxonomic identification at the species level (preferred) or genus level (if species

identification is not possible) will be achieved in the laboratory through the use of field guides, technical keys, and reference to regional herbaria housed at research universities such as UMass. Samples will be labeled in the field with the plant ID (e.g., “unknown sedge #1”) site location, date, and person who collected the sample, and assigned a code in the laboratory for use in digital preservation.

5. Equipment/Apparatus

Before leaving for the field the Field Manager will confirm the following equipment is available:

- Backpack sprayer
- Beaker
- Bleach solution (1/2 cup bleach per gallon tap water)
- Clipboard
- Compasses
- Cooler with ice
- Data sheets
- Deionized water
- Digital camera w/extra batteries
- Dip net, small, 500 micron mesh
- Dishwashing soap solution Emergence traps
- Ethanol (95%, 70%)
- Field notebook
- Flagging
- Forceps
- GPS (Global Positioning System)
- Hand lens
- Hanna ph/conductivity meter
- Hip chain
- HOBO Pendant Temperature/Light Data Logger
- iButtons
- Isopropyl alcohol
- Labels for algae samples
- Labels for earthworm samples
- Labels for macroinvertebrate samples
- Labels for vascular plant, bryophyte & lichen samples
- Lids, closed
- Liquid dish soap or hand soap (phosphate-free and biodegradable)
- Location maps
- Meter stick
- Meter tape
- M3 preservative
- Nalgene bottle (500ml)
- Palm Tungsten E2 Handheld (PDA)
- Pencils

- Permanent markers
- pH/CON 10 pH/Conductivity/C^o Meter
- Plastic collecting bags
- Plastic cups
- Plastic containers (32 oz and 16 oz)
- Plastic amber bottles (100 ml-250 ml)
- PVC pipe (2 1/2" diameter)
- Rite-in-rain paper and pen
- Scissors or jack knife
- Screens
- Stakes
- String
- Soil auger
- SOP
- Spoonulet
- Squirt bottle
- Standard solutions for calibration of pH/Conductivity/Temp meter
- Stovepipe sampler
- Tap water
- Trowel or bulb planter
- Turkey baster (large Pipette)
- Vials
- Water/detergent solution
- White bowl

6. Reagents

- Bleach solution (1/2 cup bleach per gallon tap water)
- Deionized water
- Ethanol
- Formalin solution (10%) *
- Glacial acetic acid *
- Isopropyl alcohol
- Liquid dish soap or hand soap (phosphate-free and biodegradable)
- Potassium Iodide *
- Propylene glycol/water solution
- Standard solutions for calibration of pH/Conductivity/Temp meter
- Tap water
- * M3 solution

7. Calibration & Training

Equipment calibration procedures for the GPS units, Oakton pH/CON 10 pH/Conductivity/C^o Meter, Hanna portable pH/EC/TDS/Temperature Meter, Thermocron ibutton, and HOBO Pendant Temperature/Light Logger will be done according to the manufacturers' recommendations. See section 2.6 of the QAPP for details.

Field crew members will have sufficient previous training and experience to reliably conduct field data collection or they will receive training from the UMass QA Manager and/or other project scientists with relevant expertise. The QA Manager will ensure that all field crew members receive specific training on macroinvertebrate sample sorting and identification (to order), plant identification, and delineation of a Bordering Vegetated Wetland.

All Field Managers and Field Scientists will receive training from the QA Manager on appropriate QA/QC procedures.

8.0 Procedures

Sampling will occur between May 11 and September 30, to ensure adequate assessment of the targeted wetland biotic communities. Forested wetlands in the Millers and Concord Watersheds will be identified using the MassDEP Wetlands Mapping data (1:12,000 based on photography from 1993 and 1999).

Sample locations will be randomly stratified across deciles of buffer zone insults (one of the landscape metrics used in CAPS) and deciles of ecological integrity (results from CAPS analysis) from the CAPS assessment of 2009. This will create 100 buffer zone insults x IEI bins. Up to five random points that fall within deciduous or mixed forested wetlands (as depicted in MassDEP wetlands; 1:12,000 based on photography from 1993 and 1999) will be selected for each bin. Samples within 100 m of a fourth order or larger stream will be excluded to avoid areas that might potentially be floodplain forests. All points will be separated by at least 500 meters. The 150 (75 in each watershed) sampling plots will be selected randomly from among the 100 bins. Within each bin, potential plots are ordered. If a plot needs to be dropped, the next-higher plot in the same bin will be used. Note that some bins will have fewer than five points or may be entirely empty because some combinations of IEI and wetland buffer insults are rare or absent in the landscape.

A random identifier will be assigned to each bin to obscure the IEI/wetland buffer insults class that each bin represents. Field personnel will not have access to the original classes, thus sampling will be blind with respect to CAPS predictions.

Plots will be compared to aerial photographs (1:5000, 2005 Color Orthophotos available from MassGIS) and GIS data for hydrography (MassGIS, 2005), Potential Vernal Pools (NHESP, 2000) and Certified Vernal Pools (NHESP, 2008). Plots that fall within 30 m of potential or certified vernal pools, dominated by conifers, or fall within 30 m of a 3rd order stream or greater will be dropped. Areas in close proximity to vernal pools and larger (> 2nd order) streams will be dropped to avoid sampling invertebrates too close to areas characterized by longer hydroperiods than our target wetland community. Likewise, areas dominated by conifers will be avoided because they do not match the target wetland community (freshwater deciduous/coniferous forested wetlands that have the hydrogeomorphic (HGM) classification of a slope or flat).

GPS navigation will be used to locate each wetland plot. GPS precision must be 10 m or less and the navigator will stop and establish the plot once the distance to plot center is 0m. In the case of GPS interference from tree-canopy or atmospheric effects two procedures may be followed. The first is to wait 10 minutes for satellite reception to improve. If a dense forest canopy appears to be the problem use triangulation to locate the plot. We will approach the plot from three different locations where the canopy is mainly deciduous. Using compass and distance measurements provided by the GPS (precision must be 10 m or less), the plot will be located.

It will not be necessary to hit the plot exactly (since it's randomly selected) it just needs to be selected without bias. However, a reasonably precise GPS point is needed of where the plot actually ends up. The strategy is (1) do the best we can when locating the plot and (2) take a precise location (precision ≤ 10 m RMS) once the plot has been established. Field workers will be on the plot for 2-3 hours and will be able to keep trying until they get good GPS coverage.

8.1 Establishing Sampling Area

A 30 m radius plot will be used to sample the wetland point (Figure 1). A reserved 5 m radius area will be established in the center of the plot. Eight 25 m transects will be run from plot center at 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° compass bearings. Vascular plants and bryophytes will be surveyed on transects run at, 45°, 135°, 225°, and 315°. Plant transects (transects 2, 4, 6, 8) and bryophyte plots will be denoted to prevent trampling, by flagging the transects and marking them on the Plot Information A form (Appendix L). The plot will be subdivided into 4 quarters, A-D.. They will be established in a clockwise direction beginning with transect 1 (Quarter A between the N and E transect, etc.)

Figure 1.

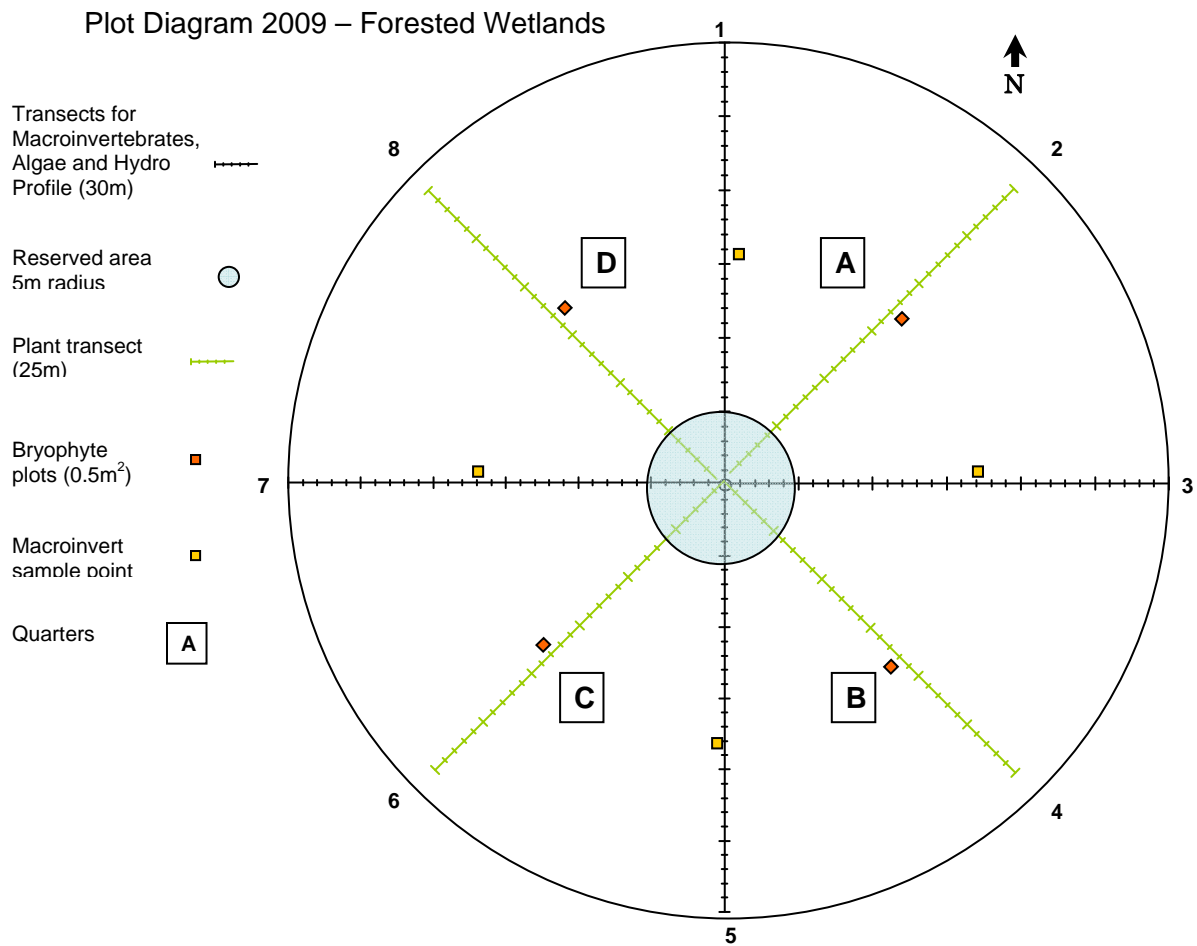


Diagram of sampling area. Eight 25 m transects run at 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° compass bearings. The location for all samples (algae, water chemistry, etc.) will be noted on the plot diagram.

A sampling point will be moved if any of the following conditions are encountered.

- The dominant tree cover in the plot area is <30% as determined by visual estimation

- Any transect length is <15 m, as may occur in narrow wetlands (e.g. fingerlike projections, narrow bands of wetland along streams)
- Plot area is inundated due to beaver dams
- Point falls within 30 m of a mapped 3rd order stream (or larger)

The sampling point will be moved to the nearest location that does not violate the previously stated conditions, but no greater than 30 m away. If a suitable sampling point cannot be found within 30m of the original point the site will be dropped and another sampling point from the same bin selected.

8.2 Overview of Wetland Biotic Community and Habitat Assessment

Each point will be sampled for algae, macroinvertebrates, vascular plants, bryophytes and epiphytic macrolichens. Samples will be taken within a 30 m radius plot. Samples will be analyzed to determine if the attributes of the biotic communities show a dose-dependent response to anthropogenic stressors in the landscape as measured by CAPS metrics. In addition a habitat assessment will be conducted to characterize the assessment area. A detailed description of the plot (includes hydrology, anthropogenic disturbance, etc.) will be recorded in a field notebook by each surveyor. Data will be recorded with a PDA and paper forms. Tungsten E2 Handheld PDAs will be used to record vegetation, bryophyte and lichen data in the field. Paper data sheets will also be completed to serve as backups. Data from the PDAs will be downloaded to the master database on a daily basis.

8.2.1 Habitat Assessment

(a) Topographic complexity

Topographic complexity will be determined to assist in the characterization of the wetland. Each odd numbered transect will be walked to observe and record variations in slope/elevation.

From the center point of the plot walk four 30 m transects and count the number of micro-topographic depressions (“pits”) at least 1 m² in size encountered along each transect. Counts will be recorded on a data sheet Topographic Complexity form (Appendix L) Depressions will only be counted if they are sufficiently obvious that they could be recognized even if groundcover vegetation is dense. If a pit is divided along the transect line by a mound it will be counted as two separate pits. A mound is defined as $\geq 15\text{cm}$ in height relative to the base of a pit and has the development of soil. Vegetation (e.g. tussock sedge) will not count as a mound. Topographic complexity will be expressed as the number of micro-topographic depressions per 100 m of transect length.

(b) Hydrology

Hydroperiod

A HOBO Pendant temperature/light data logger will be placed in the water for the duration of the study period (about 4 months) to determine the relative hydroperiod of the wetland surface water. The HOBO will record temperature at two hour intervals.

Place the data logger in a location within the plot that is judged by the field manager likely to remain inundated longest whether or not there is any standing water at the time. Place the logger inside a plastic white container to protect it from direct sunlight. Holes will be drilled into the sides of the cup to allow water to flow through. The cup will be held flush to the surface of the ground with a plant stake with a metal ring at the top to keep the cup from moving. Label the container with the serial number of the HOBO. Measure the depth of the water where the HOBO is placed at each plot visit (7 measurements) and record on the Hydrological Characterization form (Appendix L).

An ibutton will be hung against the North side of the closest tree to the location of the HOBO. The ibutton will record ambient air temperature every two hours in sync with the HOBO. The ibutton will also be protected by direct sunlight with a white plastic container and holes will be drilled to allow air passage. Label the container with the serial number of the ibutton.

Record the placement location and the serial number of the loggers on the Plot Information A form. Collect data loggers upon the completion of the biotic community assessment.

The temperature data from the loggers will be uploaded following procedures according to the manufacturer's instructions (See QAPP Appendix J). The temperature data will be used to determine the relative hydroperiod (i.e. the duration of the sampling period). The coefficient of variation (CV) in temperature for each 24 hour period will be calculated for both the ambient air temperature (AAT) and water temperature (WT). The assumption is the ratio of $AAT(CV)/WT(CV)$ will approach 1 as the depth of the water decreases. This will be verified with the recorded water depth of the HOBO location (recorded at 7 dates throughout the sampling period). This relationship will be used to estimate the depth of water for each day based on the temperature data. This data will be used to characterize the relative hydroperiod of surface water for each plot (method to be determined).

Hydrologic Profile/Characterization

A hydrologic profile along odd numbered transects will be taken using a point intercept method each time a site is visited (eg. trap deployment, trap collection, etc.) The profile will be used to characterize the surface hydrology during the field season.

At the first site visit, odd numbered transects will be flagged every 5m. At each 5m point intercept along the transect, the presence of saturated soil, surface water (>2.5cm), or dry surface will be recorded on the Hydrologic Characterization form. The percent cover of each category will be determined for each visit and for the duration of the field season.

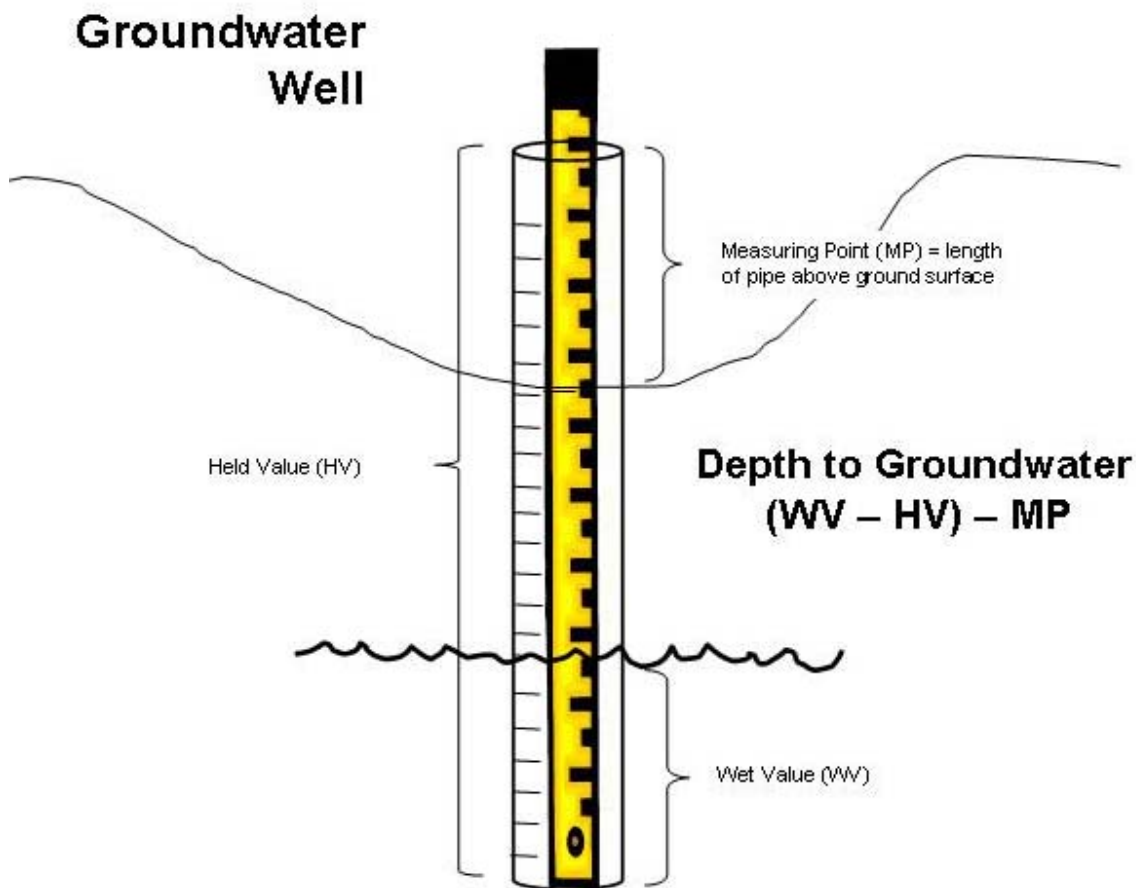
Hydrologic features such as a single channel or braided stream channel that is located in the plot will be described (direction of flow, etc.) and recorded on the Plot Information A form.

Groundwater

Groundwater will be monitored using shallow groundwater monitoring wells to determine the fluctuation in the water table throughout the field season. Readings will only be taken 6 or 7 times and will not be monitored daily. This information will provide information to characterize the influence of groundwater to the wetland point.

A PVC pipe, 1.2 m in length and 6.35 cm in diameter, will be installed to monitor groundwater (Fig. 2). A single pipe will be installed at the lowest point in the wetland, based on topography and depth of surface water. This will be determined after setting up the hydrologic profile transects and walking around the plot. The hole for the pipe will be dug using a soil auger. 0.90 m will be placed below the surface. Slits will be cut every 4.8 cm along the length of the pipe on each side through about a quarter of the pipe. The slits will allow the passage of water while preventing the soil from entering the pipe. The bottom of the pipe will be capped with a water tight seal. A 4.8 cm diameter cap will cover the top of the pipe for ease of removal to take water measurements. A meter stick lined with chalk will be used to measure the depth to groundwater. First determine the measuring point (MP) by measuring the length of the pipe above the surface. Insert the meter stick lined with chalk above the well and record when it crosses into the pipe (held value). Remove the stick and note where the chalk is wet (wet value). To determine the depth to groundwater first subtract the wet value from the held value to determine the water level below MP. Then subtract MP to determine the level below the land surface. (*personal correspondence*, R. S. Socolow, USGS) Measurements will be taken each time the site is visited. The data will be recorded on Hydro Profile form.

Figure 2. Groundwater well measurements.



(c) Water geochemistry

Conductivity, temperature and pH will be measured for surface water (if present) using a portable pH/Conductivity meter at 4 locations in the plot.

Take readings from surface water closest to the midpoint of each of the odd numbered transects running in cardinal directions (location of algae samples). If there is no standing water present along a transect move in a clockwise direction to find the closest area with standing water. If there is no standing water present within the quarter plot keep moving clockwise until readings are collected from four locations within the plot. The minimum distance between readings must be 3 m. Take a reading from any major stream channel in the plot if present. Note on the Plot Information A form the transects and/or quarters from which readings were taken. Record pH, conductivity, and temperature on the Plot Information B form.

(d) Human disturbance

Visual observations of human disturbance to the wetland will be noted. Surveyors will note the following activities in the field notebook, describing the type and extent of each disturbance.

Walk the four odd numbered transects running in cardinal directions and record in the field notebook the type and extent of disturbance for each of the following.

- Water control structures (culvert, dam, weir, storm water input, fill (road/railroad), ditching, channelization, beaver dam, and other human activity affecting the hydrology of the site
- Soil disturbance (filling, plowing, grading, grazing, dredging, sedimentation, vehicle use.
- Obvious spills.
- Direct point or nonpoint source discharge from agricultural operations, septic or sewage treatment systems, or storm water affecting water quality of the site
- Walking trails, horse trails, logging roads, ATV trails, old cart paths, and roads (excluding wildlife trails)
- Evidence of mowing, burning, or timber harvesting.
- Presence of trash/litter.
- Presence of garbage dumping.

Also record any of these indicators of disturbance when encountered while implementing other elements of the SOP.

(e) Soils

A soil pit will be used to characterize the soil for each plot.

Select a location for the soil pit within 5 m of the groundwater well and 1 m distant from tree stems, animal holes, or other disturbances. Using a spade dig a soil pit 12 inches in diameter to a minimum depth of 16 inches; increase depth if more information is needed to characterize the soil. Dig a second soil pit for plots lacking uniform topography, where a change in the soil may be present. Conduct work only when the light allows for accurate color classification of the soil and its features.

Remove a clean slice of soil from the soil pit. If saturated conditions prevent a pit from being dug use an auger to sample the soil and collect the necessary data. Turn the auger no more than 4 times so that the core is not mixed and an accurate profile can be documented. Repeat this step until the profile reaches a depth of at least 16 inches.

Record on the Soils data form a description of the soil profile, including soil horizons, redoximorphic features and the associated colors. A Munsell Soil Color Chart (Munsell 2000) will be used as a guideline when describing the color and redoximorphic features of the soil pit. Soil Taxonomy, tenth edition (USDA 2006) will be used to define the soil horizons. Document on the data form additional

information useful for classification, such as hydric indicators (USDA 2002), texture, depth to groundwater, stoniness, and slope. This information will be analyzed in order to classify the soil using Keys to Soil Taxonomy, tenth edition.

8.2.2 Protocols for Sampling Biotic Communities

8.2.2.1 Algae

Algae will be sampled as a indicator of water quality, community composition, and ecosystem health. Algae are an integral component to the wetland community and are a primary food source to many macroinvertebrates. Samples will be collected in June before water draw down occurs. Four samples, each 50 ml, will be collected from each microhabitat within the wetland (benthic, including leaf litter and surface sediments, and surface water) for a total of 12 samples per site. Algae samples will be preserved in M3 fixative (Potassium Iodide, Iodine (optional), glacial acetic acid, 25% formalin). One ml of M3 will be added per 50 ml sample. All algae samples will be recorded on the algae sample login form before storage in the lab. Protocols for sampling algae were adapted from Danielson, 2006, Hawkins et al., 2003, and Vermont DEP, 2003.

(a) Benthic algae

Leaf litter samples will be collected. Leaf litter will be collected from areas within the plot with surface water present. In the absence of surface water, leaf litter will be collected from wet depressions.

Collect leaf litter from areas of standing water closest to the midpoint of odd numbered transects. If there is no standing water present along a transect move in a clockwise direction to find the closest suitable sampling location within the quarter plot. If standing water is lacking within a quarter plot collect leaves from a wet depression closest to the midpoint of the transect. If there are no suitable locations (surface water or wet depressions) present within a quarter keep moving through the plot until four samples have been collected. The minimum distance that samples must be spaced is 3 m. Note on the Plot Information A form the transects and/or quarters from which samples were taken and a description of the sampling location. Record the depth of the surface water if present on the Plot Information B form.

From each sampling location collect red maple leaves to cover the bottom of a small bowl (10.5 cm²). Scrape the leaf surfaces using a metal spoonulet to scrape off the algae. If red maple leaves are not available collect other deciduous leaves of similar size and make a note of the species used. Rinse each leaf with DI water after scraping. Collect all scrapings from the small bowl into a 50 ml vial. Keep rinsing the pan with DI water until there is 50ml in the vial. Add 1ml of M3 per 50ml of benthic leaf scrapings for preservation.

Clean the pan and spoonula with tap water after sampling.

(b) Water grab sample (adapted from ME DEP)

Water samples will be collected to sample algae.

Take samples from surface water closest to the midpoint of the four odd numbered transects. If there is no standing water present along a transect move in a clockwise direction to find the closest suitable sampling location. If there is no suitable location present within the quarter plot keep moving clockwise until samples are collected from four locations within the plot. The minimum distance between samples must be 3 m. Note on the Plot Information A form the transects and/or quarters from which samples were taken. Record the depth of the surface water on the Plot Information B form

Use a clean and dry 50 ml vial to collect sample. Submerge the water sampler to collect the surface water taking care to minimize the collection of organic material. Water samples will not be collected in areas where the leaf litter must be depressed in order to collect a sample. Add 1ml of M3 per 50ml of the water sample for preservation. Repeat for each transect.

(c) Surface substrate sampling

Surface substrate samples will be collected to sample algae.

Using a turkey baster (large pipette) collect a 50 ml sample of the surface substrate from areas with surface water at the same location as leaf samples (see (a) above). To collect the sample, stick the end of the baster into the substrate and suck up a sample from the surface. If necessary, loosen up the substrate by moving around the tip of the baster before taking a sample. Pour the 50 ml sample into a 50 ml vial. Add 1ml of M3 per 50ml of the water sample for preservation. Note on the Plot Information A form the transects and/or quarters from which samples were taken. Repeat for each transect. Record the depth of the surface water if present on the Plot Information B form.

Clean the turkey baster with deionized water after sampling.

8.2.2.2 Macroinvertebrates

Macroinvertebrates are will be sampled as an indicator of water quality and community composition, and ecosystem health. Macroinvertebrates will be sampled from June-August. Stovepipe sampler and emergence traps will be used in June; pitfall traps to collect epigeal macroinvertebrates and soil pits to collect earthworms will be conducted from July-August.

(a) Earthworms

Earthworms will be sampled in forested wetlands from August through November using a combination of liquid extraction and midden counts (Lawrence and Bowers 2002, Hale et al 2005):

For midden counts place 1m² sampling frame on soil surface at 15m along each odd-numbered transect and count number of middens inside the frame.

Establish one earthworm sampling plots at the most suitable location (not standing water) within the assessment area. Place sampling frame (11' diameter or 613 cm²) on top of soil and carefully remove any vegetation from within frame. Collect any earthworms found on soil surface or in vegetation and place in small plastic sampling tray with lid. Count number of juveniles, adults, and middens within the plot. Push sampling frame into soil. Pour ½ gallon liquid mustard solution into sample area and begin collecting worms as they surface. Wait three minutes before pouring remaining ½ gallon into soil. Liquid extraction sampling time for each plot is 10 minutes.

Earthworms encountered during the excavation pit traps or soil pits will be collected and preserved.

Kill all worms in 70% isopropyl alcohol. Place worms into alcohol-filled vial labeled with plot ID, subplot ID, and date, and collector's name. Keep earthworms cool until transfer into 10% formalin solution for permanent preservation at the end of the field day.

(b) Aquatic macroinvertebrates: Stovepipe sampler (adapted from ME DEP)

Macroinvertebrates will be collected using a stovepipe sampler (5 gallon plastic bucket with the bottom cut off). Collections will be made in two locations dispersed within the plot where surface water and/or wet depressions are present.

Samples will be taken from two locations within the plot where surface water is most suitable for sampling based on water depth and areal extent of inundation. If surface water is not present within the plot, sample in locations (depressions) with the wettest substrate. If possible locate the sampling locations in diagonal quarters of the plot (e.g. quarters 1 & 3 or quarters 2 & 4). If suitable sampling conditions are not present in diagonal quarters try to use sampling locations in each of two adjacent quarters. If necessary place both sampling locations in the same quarter. The minimum distance between samples must be 3 m. Note on the Plot Information A form the transects and/or quarters from which samples were taken.

At each sampling location place the stovepipe sampler firmly into the substrate (few cm deep) and hold it in place. Agitate the water in the sampler for 10 seconds to dislodge organisms from the substrate and vegetation. If surface water (>1.27 cm) is present take five sweeps within the sampler with a 500 micron mesh hand net (10.5x12.5 cm). After each sweep, transfer all material into a 32 oz

collecting jar. Inspect the net, remove any clinging organisms and add them to the sample. The jar should only be filled halfway with sample material and additional jars may be used if necessary. Fill container with 95% ethanol. Record depth of surface water on the Plot Information B form.

For wet depressions (with little or no standing water) collect three, one-hand leaf litter grab samples from within the stovepipe. Distribute grabs evenly throughout the stovepipe area. Preserve the sample the same as for the dipnet samples. Record on the Plot Information B form. Label containers with site ID, date of collection, surveyor ID, and description of microhabitat. Samples will be strained and preserved with fresh ethanol within four months of collection. Containers will be stored in the lab for up to five years until they are processed.

(c) Insects: Emergence Traps

Four emergence traps per plot will be set and collected after 7 days. Emergence traps will be set on the water surface or on the surface of the soil in the wettest depressions in the absence of surface water. Site selection for trap placement will follow the protocol previously described for benthic algae, but will be placed 1m apart from areas that were disturbed while sampling for algae or using the stovepipe sampler.

Set emergence traps in areas of standing water closest to the midpoint of each transect. If there is no standing water present along a transect move in a clockwise direction to find the closest suitable sampling location within the quarter plot. If standing water is lacking within a quarter plot set the trap in a wet depression closest to the midpoint of the transect. If there are no suitable locations (surface water or wet depressions) present within a quarter keep moving through the plot until four trap locations are selected. The minimum distance that samples must be spaced is 3 m. Note on the Plot Information A form the transects and/or quarters where emergence traps are set.

Fill a jar (with funnel top) with 70% ethanol and place it upside down at the top of the emergence trap to collect emerging insects. Tie the traps with string to nearby vegetation or with stakes to prevent drifting. Make sure that there is enough slack in the string to ensure the trap will stay flush with the water surface if draw down or flooding occurs. Upon collection of the traps replace the jar lids with fully enclosed lids and add ethanol as needed. Samples will be kept separately. Label jars with site ID, start and end date of collection, surveyor ID, and description of microhabitat. If surface water is present record the depth at the time of placement and collection on the Emergence Trap Log form (Appendix L). In addition, record the setter and collector ID, microhabitat, condition of the trap, and the amount of ethanol in the jar when collected. Jars will be stored in the lab for up to five years until processed.

(d) Epigeal macroinvertebrates

Pitfall traps will be set out in July to collect epigeal macroinvertebrates. Traps will be 16 oz clear cups placed in the ground with the top of the cup flush with the ground surface. Cups will be filled with ~150ml of a 50:50 propylene glycol/water solution and a drop of dishwashing soap. A small screen made of hardware cloth (1x1 cm squares) will be placed inside the cups to prevent small vertebrates from entering the killing solution. A plastic plate held up with small stakes will be placed over the pitfall trap to serve as a roof.

Place eight pitfall traps, 2 on each transect at 10 and 15m. Place traps in areas where the chance of flooding by surface water (avoid pits) is reduced. Collect the contents of pitfall traps after 7 days. If the trap is >1/2 full of water it will be discarded. Each trap will be collected separately in a small container. Record the setter and collector ID, microhabitat, amount of water in the trap, and the condition on the Pitfall Trap Log (Appendix L). The samples will be rinsed with tap water in the lab (to remove the soap) and 70% ethanol will be added. Label jars with site ID and start and end date of collection. Samples will be stored for up to five years in the lab until they are processed.

8.2.2.3 Vascular plants

Vascular plant data will be collected as an indicator of community composition and species diversity (proportion of native to invasive), will contribute to the understanding of the status of species of conservation concern (rare, endangered, or invasive), and provide useful information on potential threats to natural systems. Invasive plants named as such in this assessment are those currently regulated by the Commonwealth of Massachusetts (Somers et al 2006). Data collection will occur throughout the field season, June – September 2008.

- a. Estimate species abundance of all vascular plants in a 30 m radius plot using a point intercept method. Estimate percent cover as the proportion of the line directly intercepted by each species by vertical projection on four 25 m transects (excluding reserved area) placed in the four directions (even numbered transects). Tally each plant species that touches the transect line or is intercepted by a vertical projection from forest floor to canopy every 1m along the transect. Record tallies every 5 m to ensure an accurate count.
- b. Following transect sampling conduct a 20-minute walk around (within) the entire plot and list species not encountered on transects. Assign these additional species a percent cover class of <1%. Record data on the vascular plant data form.
- c. Estimate basal area using a wedge prism (10 or 15-factor). Stand near plot center, hold prism over plot center, view trees through prism at breast height (1.4 m) and tally trees, moving in a full circle starting north. List the species of each tallied tree.
- d. Assign a forested landcover class according to MassWildlife Landcover Mapping Decision Rules (March 1996) and a natural community type according to the

Massachusetts Natural Heritage & Endangered Species Program (Swain & Kearsley 1999).

- e. Collect unknown species for lab identification under dissecting scope. Place each species in a separate collecting bag labeled with plant ID (e.g., "Unknown #1, etc.), plot ID and date. Take digital photographs on site as needed. List PhotoID # next to unknown plant ID on the vascular plant form.
- f. Refer to resources on regional flora if necessary (Gleason & Cronquist 1991, Magee & Ahles 1999). Assistance from the herbaria and staff at the UMass herbarium will be requested as needed.

8.2.2.4 Epiphytic macrolichens

Epiphytic macrolichen data will be collected as an indicator of forest health, community composition, and species diversity.

Stand at center of established 30 m radius plot. Starting due north, use a 10 or 15-factor prism to select trees for lichen sampling. Identify and estimate percent cover for macrolichens on all trees and shrubs with a diameter at breast height (dbh) of four inches or greater. Estimate percent cover on the trunk in the area between from base of tree up to 2m from base. On the Epiphytic Macrolichens form number and list each tree, record the tree species and dbh, and list macrolichen species present. Estimate percent cover for each macro-lichen species using the following cover classes: 0.1=<1%, 1=1-5%, 2=6-25%, 3=26-50%, 4=50-75%, 5=>75%.

Collect samples as needed into paper herbarium packets labeled with plot ID, date, collector, and sample number. Mark any samples collected with a "V" for voucher on the data sheet next to its tentative name or as "Unknown #1, Unknown #2, " etc. Nomenclature will follow (Esslinger 2007).

8.2.2.5 Bryophytes

Bryophytes have important roles in mineral cycling, water dynamics (some species may hold 10 times their weight in water), regulation of microclimate, and provide food and habitat to a host of invertebrates. Many are sensitive to human disturbance including forest management, and bryophytes may comprise a major component of the biomass and net productivity in wetland systems. Ground-dwelling moss and liverwort data will be collected on 4-0.5 m² plots located in representative areas along the vascular plant sampling transects.

Estimate percent cover for each bryophyte species in each quadrat using the following cover classes: 0.1=<1%, 1=1-5%, 2=6-25%, 3=26-50%, 4=50-75%, 5=>75%. Follow quadrat sampling with a 20-minute walk around the plot and list additional species not found in quadrats; species documented during the walk around will be assign a percent cover of 0.01%. Collect a voucher specimen in herbarium packets for each species found

across all study plots. Nomenclature for mosses follows Anderson (1990) and Anderson et al (1990), for liverworts follows Schuster (1974).

8.7 Protocol for Decontamination of Field Equipment

Inspect all equipment for debris before leaving a site. Dispose of debris in a trash bag or on dry, high ground. When possible, leave equipment to air dry and inspect to remove any remaining plant fragments. Spray equipment with a bleach solution, scrub, and rinse with tap water to remove any additional debris. Clean the pH/conductivity meter according to manufacturer's recommendations.

9. Quality Control

Compliance with procedures in this SOP will be maintained through monthly internal reviews. Personnel will conduct periodic self-checks by comparing their results with similarly trained personnel working on the project. See sections 2.5 and 2.6 of the QAPP for details about QA/QC measures.

10. Interferences

Inclement weather (heavy rain) may interfere with our ability to collect representative data on a variety of parameters. Severe weather may delay field data collection due to safety concerns. Access may be a challenging aspect of data collection in more developed areas of the study area. Posted property or sites that are too difficult to access or unsafe to sample will be replaced with alternative sites from the same stratified sampling bin.

11. Preventative Maintenance

Field equipment will be inspected by the UMass Field Manager each day before going out to collect field data. At the field site equipment will be tested prior to data collection to ensure that it is working properly. Equipment will be subject to regular maintenance as needed and as recommended by the manufacturer. GPS accuracy will be assessed once a month by a check of any units used in the field with a known location. See section 2.6 of the QAPP for more detail.

11. Corrective Actions

Data quality control ensures high quality data, however we are prepared to re-measure any plots within the same season or period of monitoring which contain data anomalies. Any plots that contain anomalous data that cannot be resolved will be removed from the data set.

12. Waste Minimization and Pollution Prevention

Care will be taken to avoid transport of vegetation and soil to other sites. This will be done by thorough cleaning and inspection of all equipment and clothing prior to

departure from a site. Invasive plant samples will be disposed of in a way to avoid accidental release into the environment.

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