

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

Final Report for FY07 Wetlands Development Grant

Phase 2b: Development of a Site Level Assessment Method (SLAM) for Forested Wetlands and field validation of the Conservation Assessment and Prioritization System (CAPS)

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Final Report for Phase 2b: Development of a Site Level Assessment Method (SLAM) for Forested Wetlands and Field Validation of the Conservation Assessment and Prioritization System (CAPS)

INTRODUCTION

This report addresses phase 2b of a program to develop a comprehensive state water monitoring and assessment program for wetlands in Massachusetts: development of a Site Level Assessment Method (SLAM) for freshwater forested wetlands and the calibration of metrics used as part of the Conservation Assessment and Prioritization System (CAPS). A preliminary SLAM was developed and implemented in 2008. Field work was conducted from May to September and focused on the assessment of wetland biological community condition in forested wetlands. These data will be used for habitat characterization and development of Indices of Biological Integrity for use in a final SLAM for forested wetlands. Data will also be used to calibrate metrics used in CAPS for landscape-based assessments (Level 1) of ecological integrity.

GEOGRAPHIC SCOPE, SITE SELECTION AND OVERALL STUDY DESIGN

Field work was conducted in freshwater deciduous and mixed deciduous/coniferous forested wetlands that had the hydrogeomorphic (HGM) classification of a slope or flat (hereafter referred to as forested wetlands). Data collected for this phase of the research focused on forested wetland communities in the Chicopee Watershed (figure 1). Sampling sites were selected via a stratified random process. Field data collection involved 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 were conducted in the field by assessing anthropogenic stressors present on the site, algae, macroinvertebrates, vascular plants, bryophytes, epiphytic macrolichens and habitat characterization.

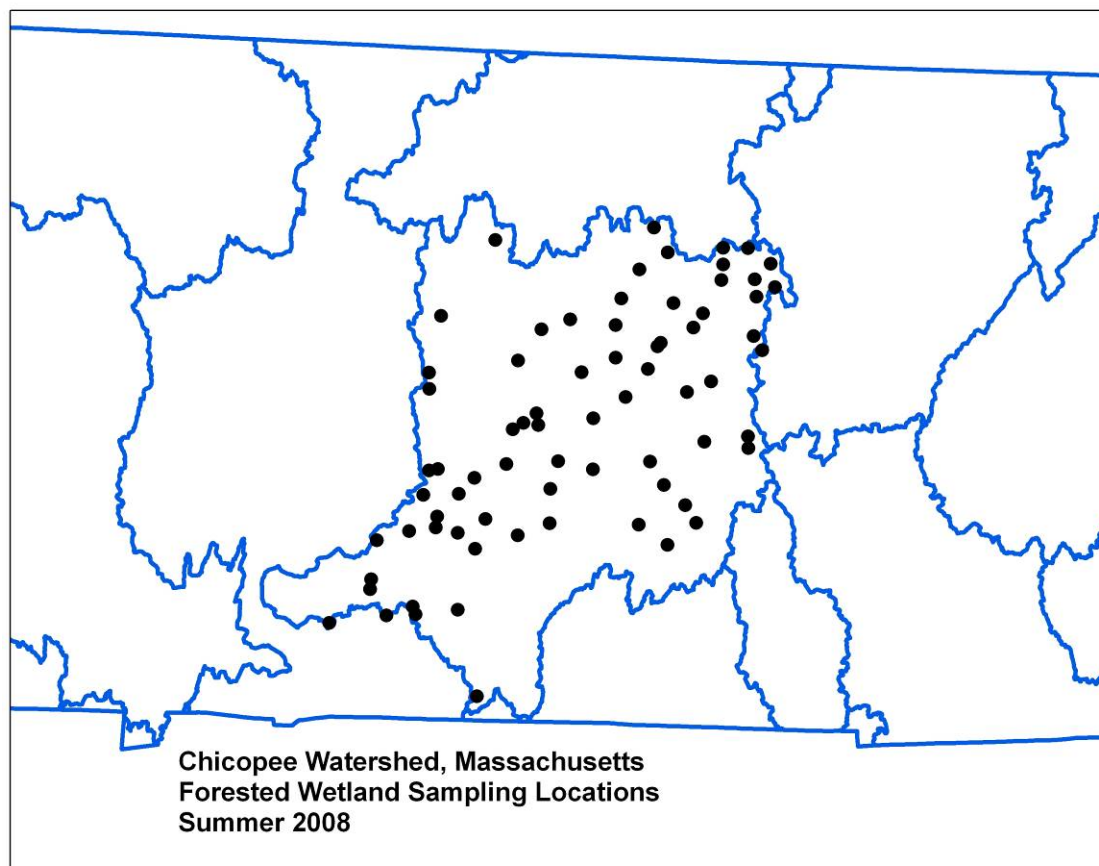


Figure 1: Location of forested wetland sampling points in the Chicopee River Watershed, 2008.

In preparation for the 2008 field season Standard Operating Procedures (SOP) were developed and incorporated into the QAPP which was approved by MassDEP and EPA. The SOP, serving as the draft SLAM, is included as Appendix A to this report.

Sample locations were 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 2008. This created 100 buffer zone insults x IEI bins. Up to five random points that fell within deciduous or mixed forested wetlands (as depicted in MassDEP wetlands; 1:12,000 based on photography from 1993 and 1999) were selected for each bin. Samples within 100 m of a fourth order or larger stream were excluded to avoid areas that might potentially be floodplain forests. All points were separated by at least 500 meters. The 71 sampling plots were selected randomly from among the 100 bins. Within each bin, potential plots were ordered. If a plot needed to be dropped, the next-higher plot in the same bin was used. Note that some bins had fewer than five points or were entirely empty because some combinations of IEI and wetland buffer insults were rare or absent in the landscape.

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

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Plots were 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 fell within 30 m of potential or certified vernal pools, within areas dominated by conifers, or within 30 m of a 3rd order stream or greater were dropped. We dropped areas in close proximity to vernal pools and larger (> 2nd order) streams to avoid sampling invertebrates too close to areas characterized by longer hydroperiods than our target wetland community. Likewise, areas dominated by conifers were avoided because they do not match the target wetland community.

GPS navigation was used to locate each wetland plot. GPS precision was within 10 m or less and the navigator stopped and established the plot once the distance to plot center was 0m. It was not necessary to hit the plot exactly (since it's randomly selected) it just needed to be selected without bias. However, a reasonably precise GPS point was needed of where the plot actually ended up. The strategy was (1) to do the best we could when locating the plot and (2) to take a precise location (precision \leq 10 m RMS) once the plot had been established.

We ended up having to drop five points and will resample the CAPS score for three points using 2009 data. Of the points dropped, two were dropped because on the second visit we discovered beaver activity. The other three were dropped because of landowner issues (e.g. landowner initially agreed but then later rescinded permission; husband granted access but permission was rescinded later by the wife). Three forested wetland points did not correspond to CAPS results for forested wetlands. This is because the sample points were created directly from polygon wetlands, while CAPS results are based on a grid representation of wetlands. In converting polygons to grids, small wetlands and skinny arms of larger wetlands were sometimes lost. The loss of these points is transient, because CAPS has since been modified so that results for the 2009 run include at least 1 cell for each wetland, so we will be able to sample 2009 results for these points.

A histogram of IEI scores for the 68 sites that were used for most of the sampling indicates a good spread (figure 2) and that the sites used will be well suited for our statistical analyses. Scatter plots of IEI scores plotted against Wetland Buffer Insult scores (figures 3-6) indicates that we were successful at implementing an orthogonal sampling design for these two variables. Likewise, scatter plots of IEI versus date (figures 7-11) indicates a favorable distribution that will allow the effects of sampling date to be accounted for in the statistical analyses.

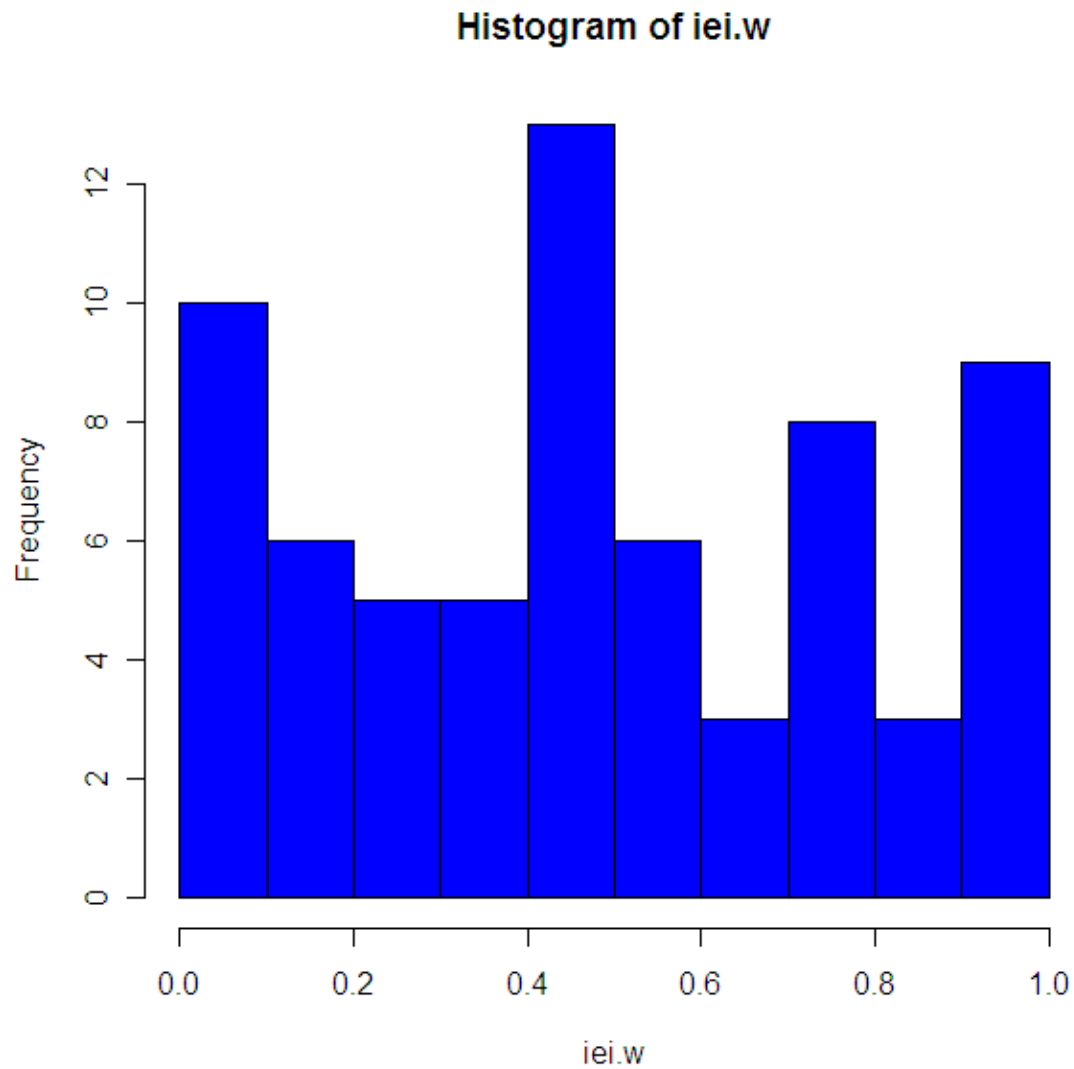


Figure 2. Histogram of IEI scores scaled by watershed for 68 sites sampled in 2008.

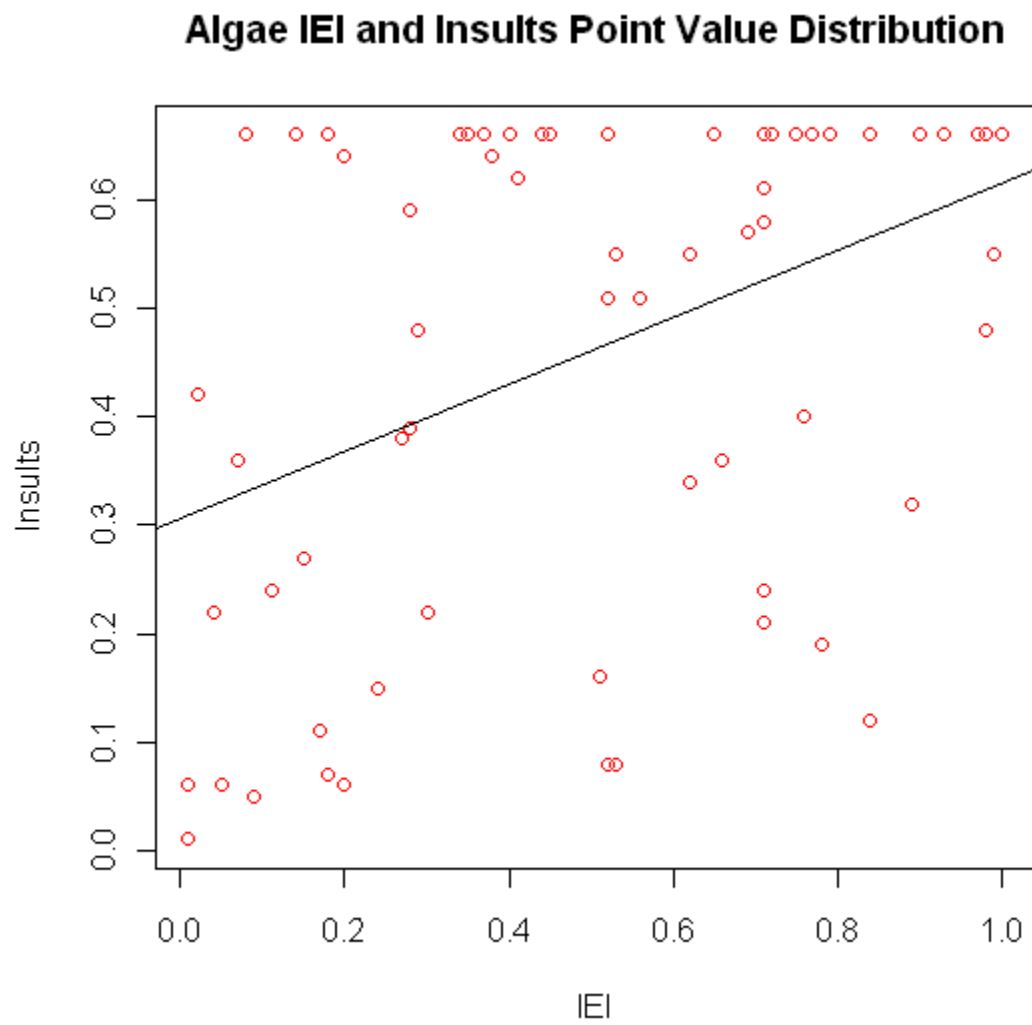


Figure 3. Distribution of Insults and IEI values for each point that was sampled for algae.

ET Samples IEI and Insults Point Value Distribution

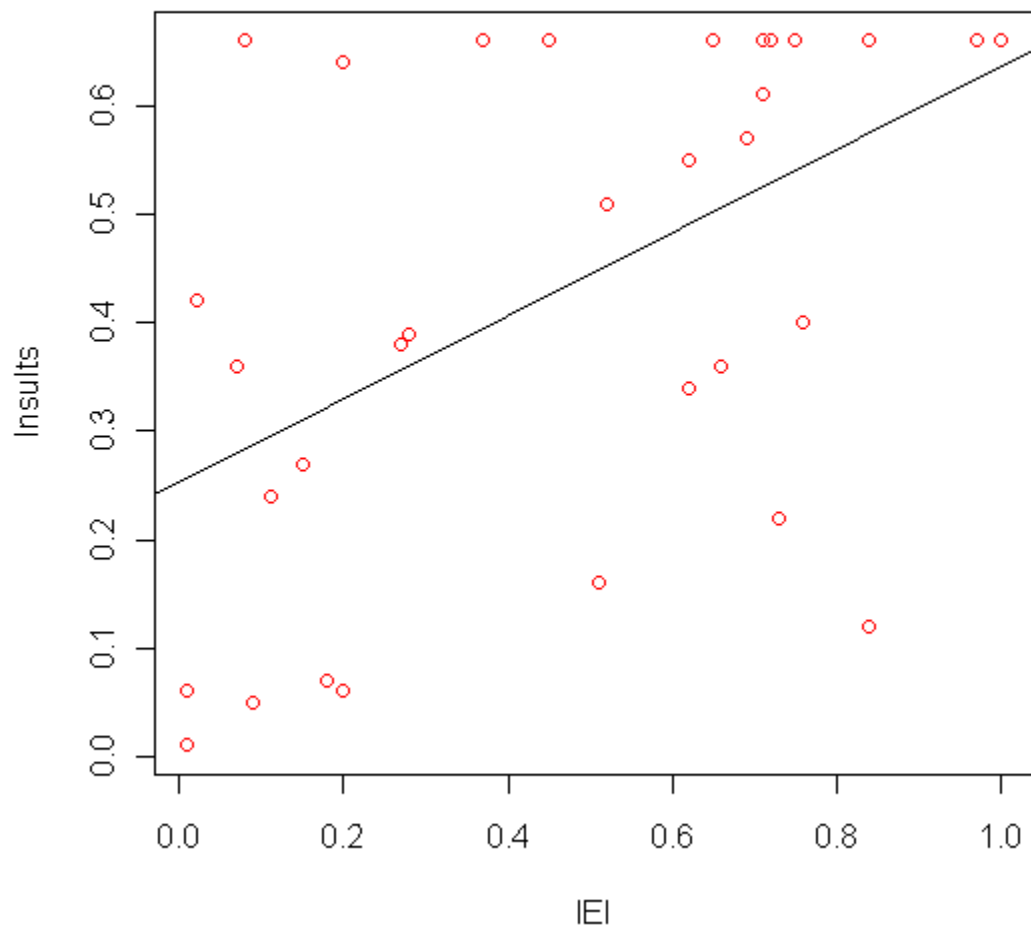


Figure 4. Distribution of Insults and IEI values for each point that was sampled by emergence traps for macroinvertebrates.

PT Samples IEI and Insults Point Value Distribution

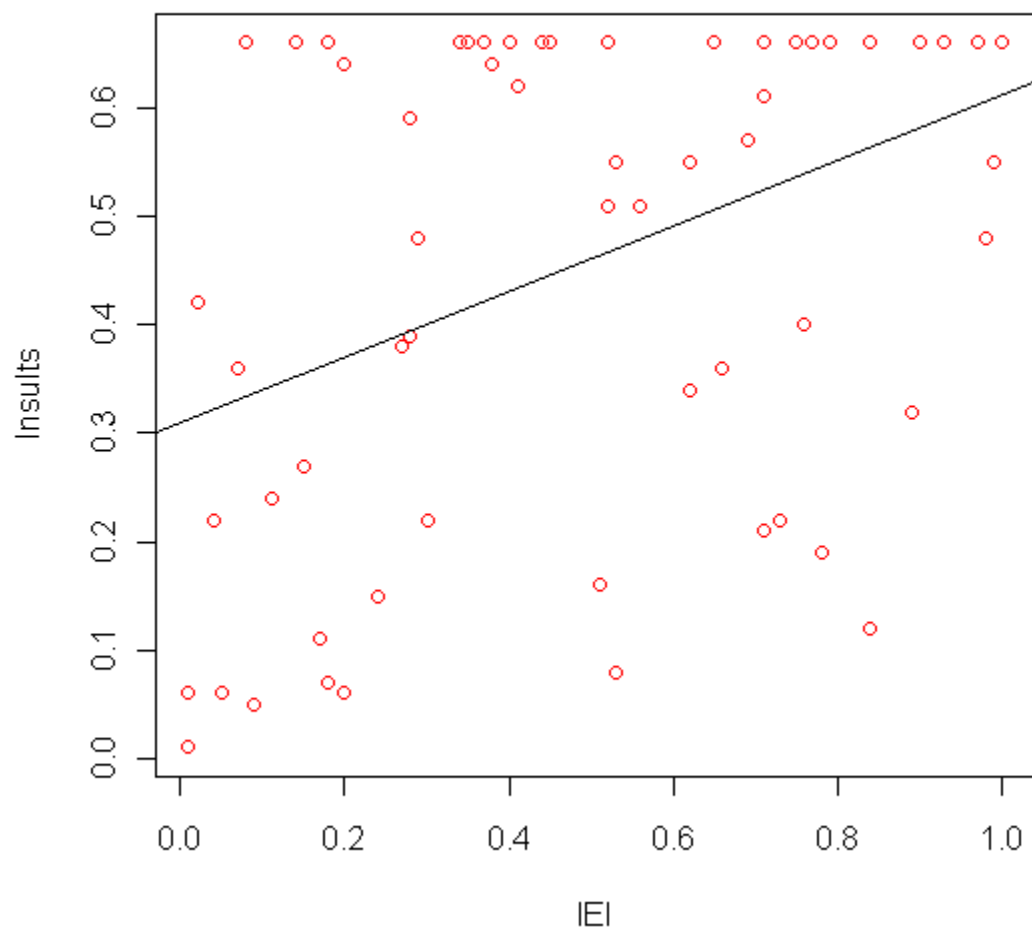


Figure 5. Distribution of Insults and IEI values for each point that was sampled by pit traps for macroinvertebrates.

ST Samples IEI and Insults Point Value Distribution

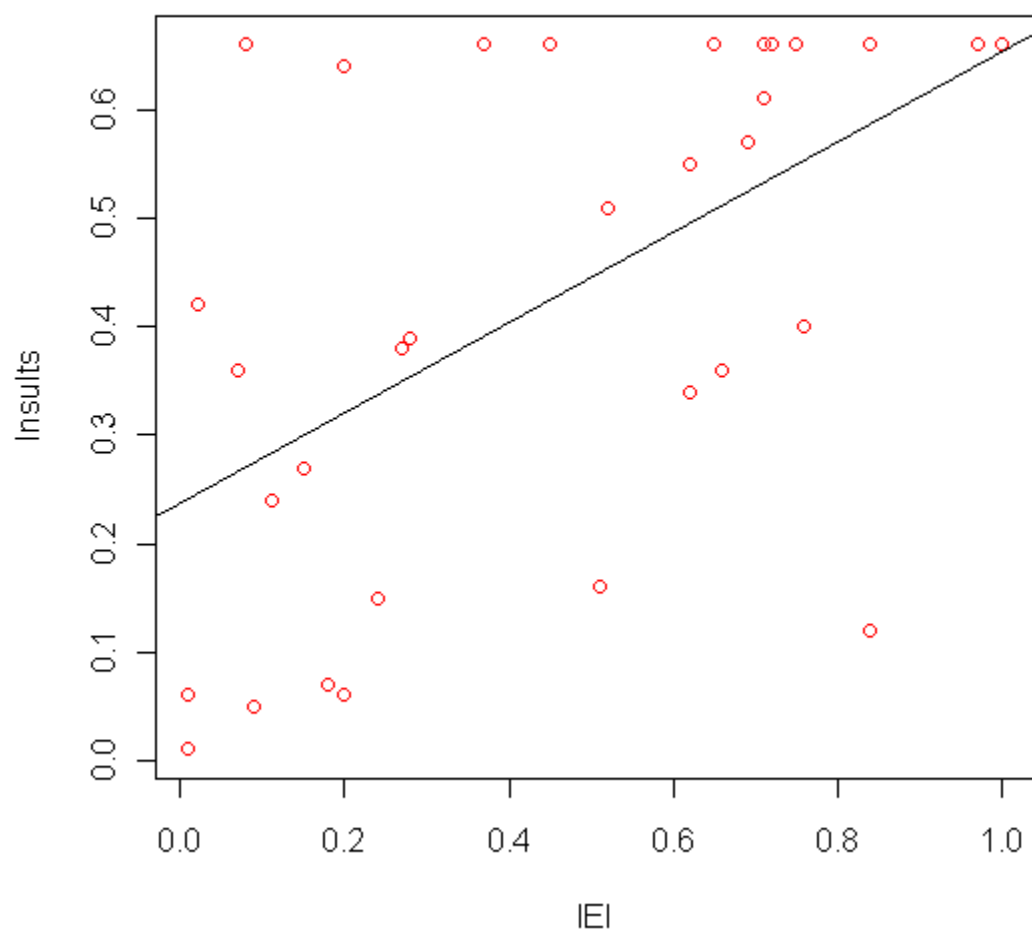


Figure 6. Distribution of Insults and IEI values for each point that was sampled by stovepipe sampling for macroinvertebrates.

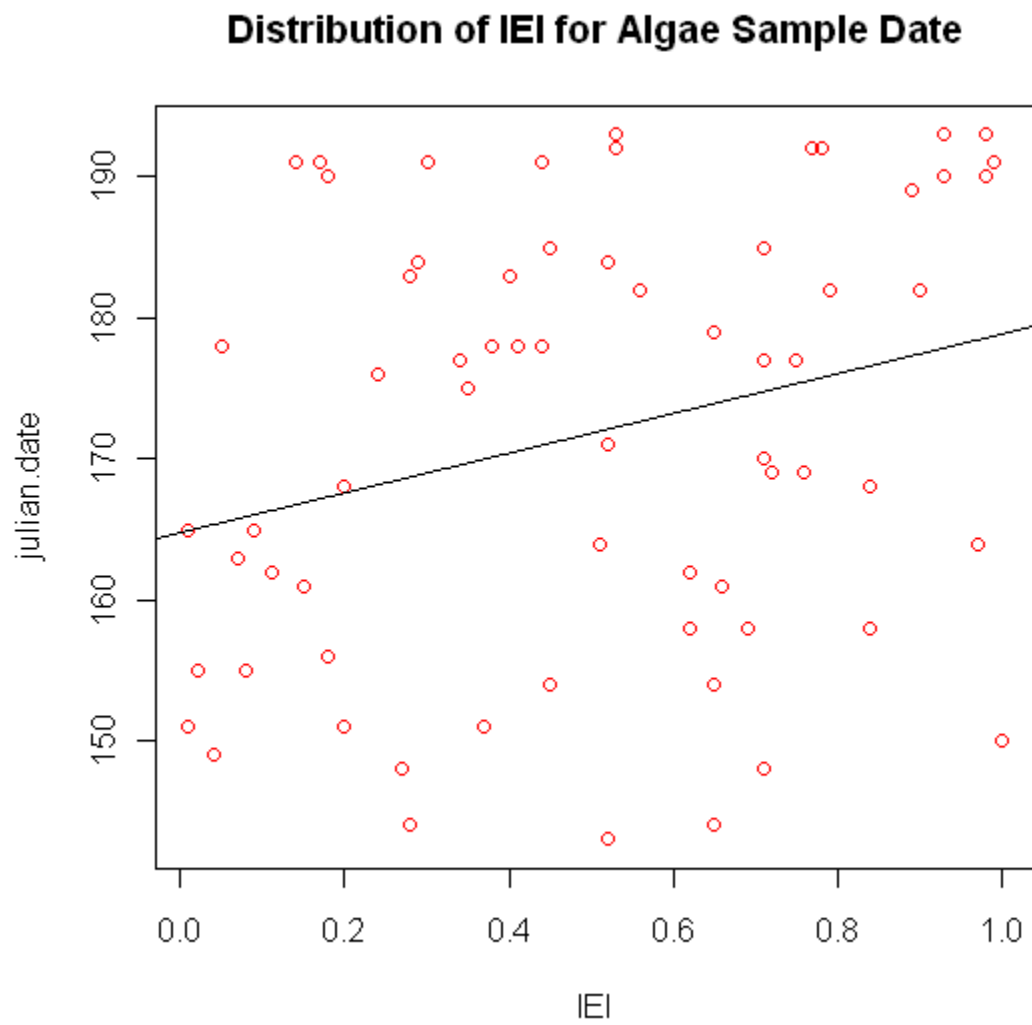


Figure 7. Distribution of IEI values plotted against Julian date for each point that was sampled for algae.

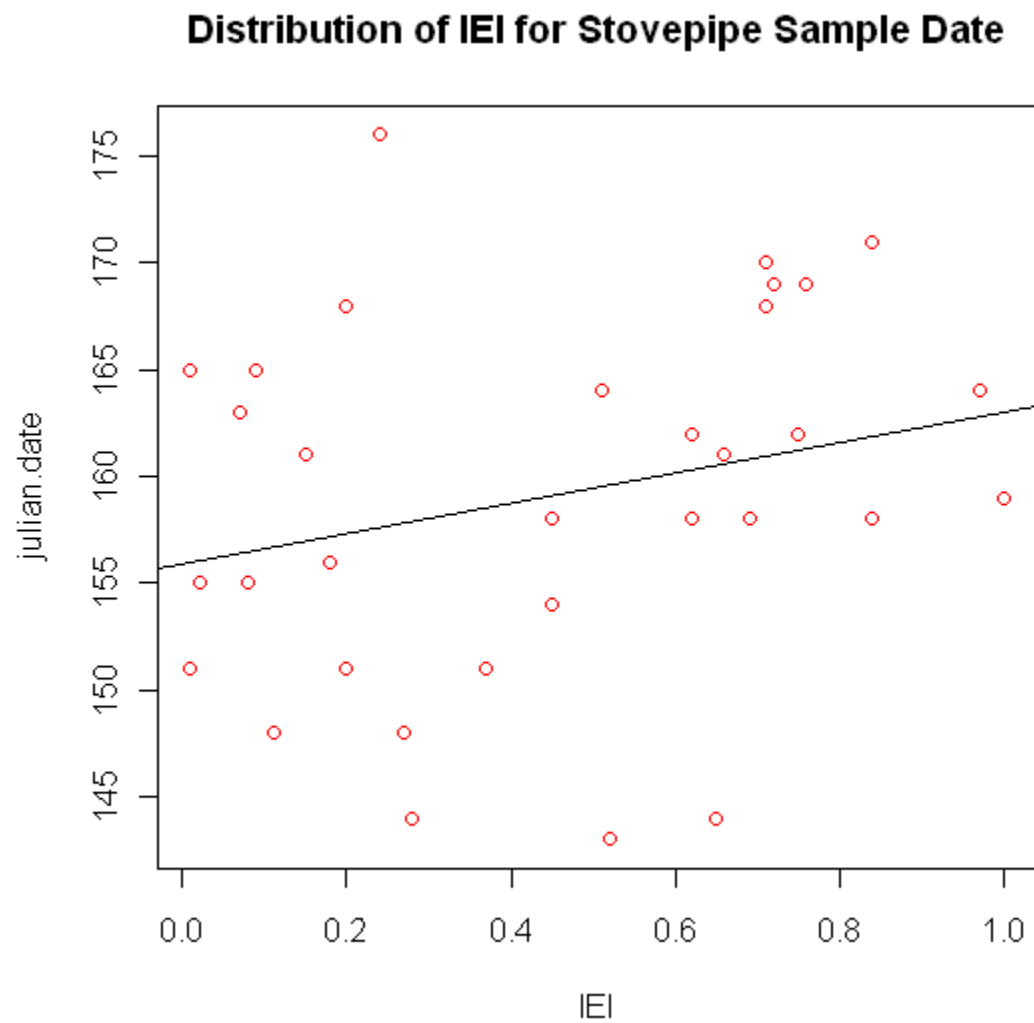


Figure 8. Distribution of IEI values plotted against Julian date for each point that was sampled by stovepipe sampling for macroinvertebrates.

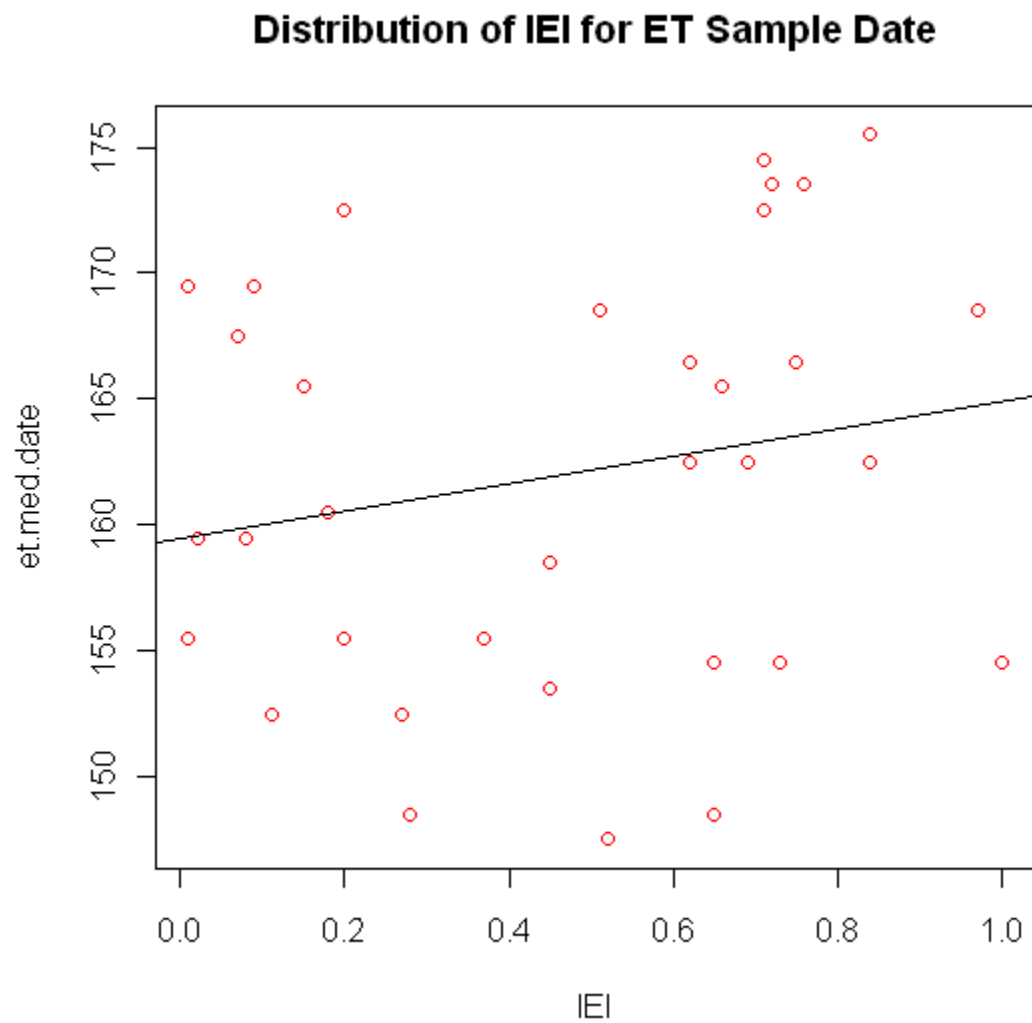


Figure 9. Distribution of IEI values plotted against median Julian date for each point that was sampled by emergence traps for macroinvertebrates.

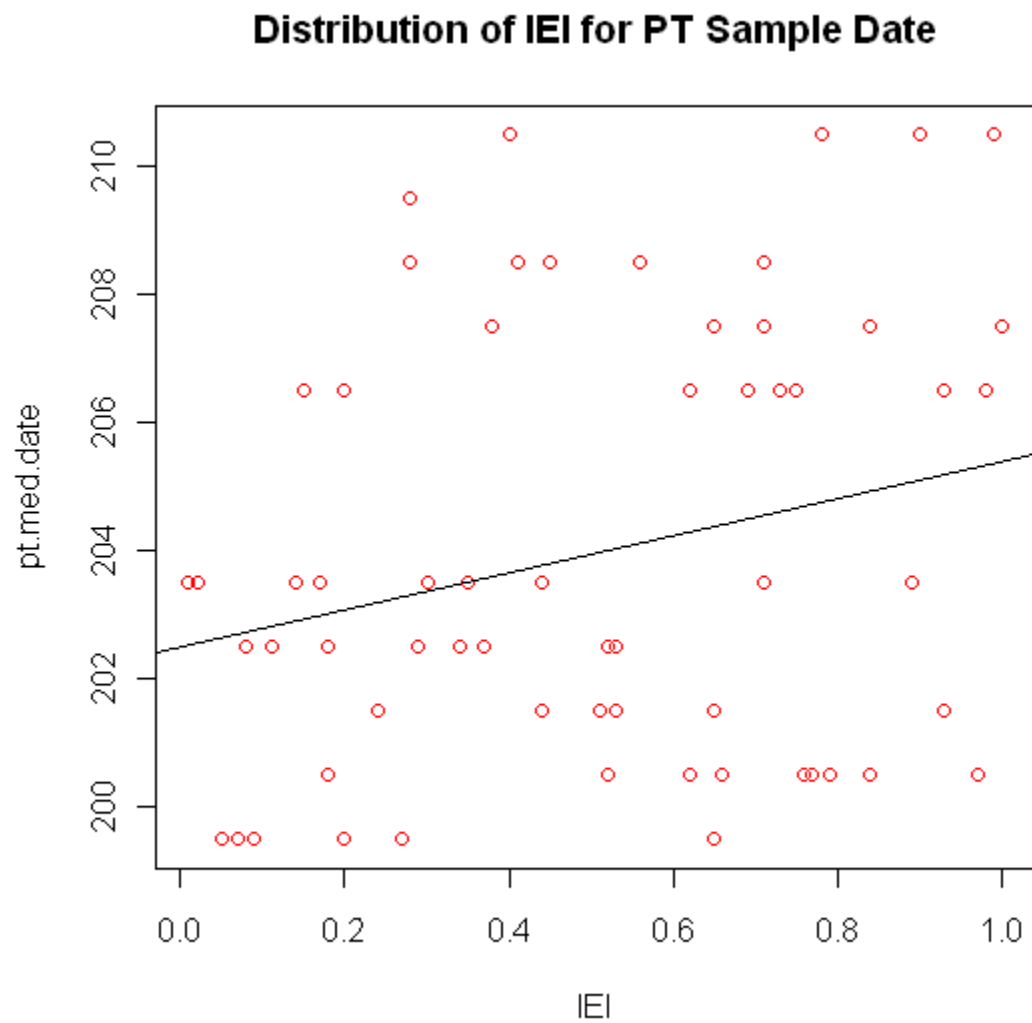


Figure 10. Distribution of IEI values plotted against median Julian date for each point that was sampled by pit traps for macroinvertebrates.

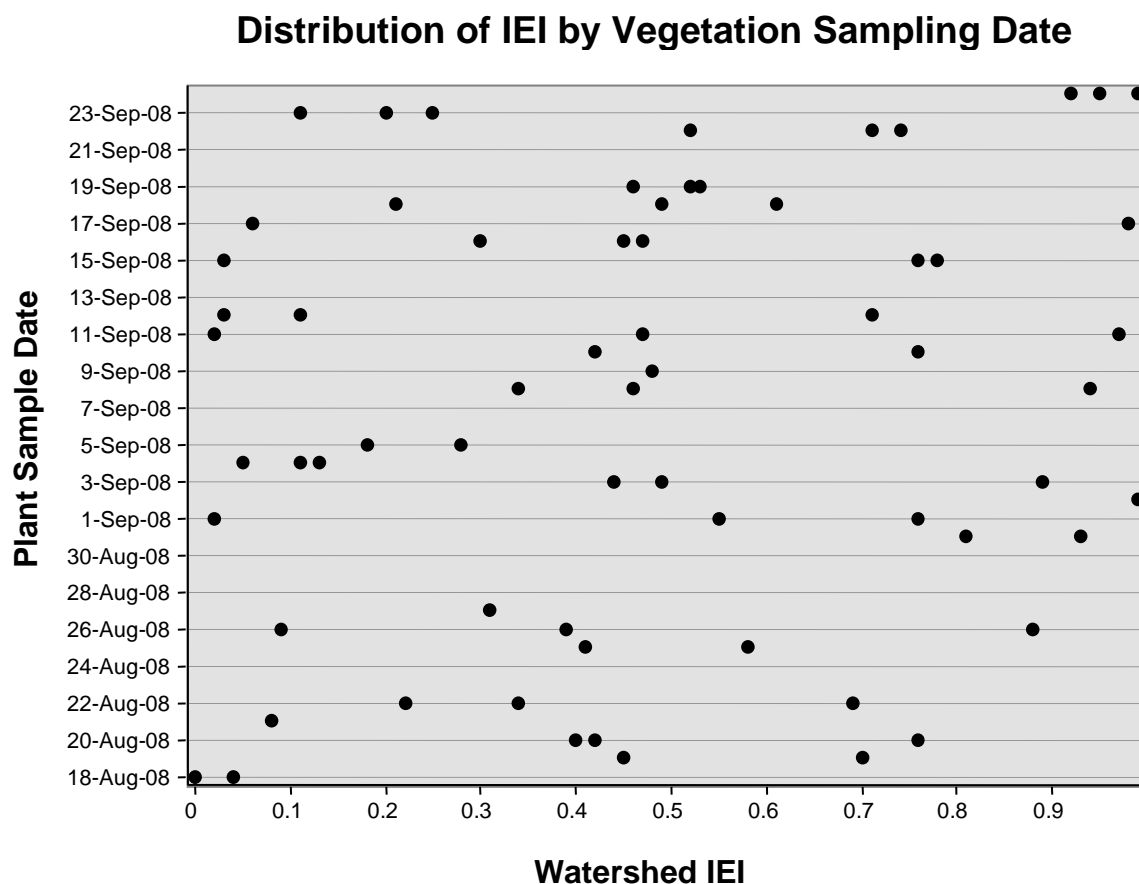


Figure 11. Distribution of IEI values plotted against Julian date for each point that was sampled for vegetation.

SLAM DEVELOPMENT: IMPLEMENTING AND ASSESSING THE DRAFT SLAM

Forested Wetland Biotic Community Assessment

Sampling occurred between May 22 and September 24. Forested wetlands in the Chicopee Watershed were identified using the MassDEP Wetlands Mapping data (1:12,000 based on photography from 1993 and 1999). Approximately 110 sites were visited. Several were dropped due to restricted access or because the wetland point did not meet the necessary conditions (e.g. no beaver influence).

Points were sampled for algae, macroinvertebrates, vascular plants, bryophytes and epiphytic macrolichens (Table 1). Not all wetland points were sampled for each biotic community nor for each sampling technique. The stovepipe samples and emergence traps were discontinued after June 28, 2009. There were problems with the collecting jars used for the emergence traps that could not be fixed at the time. We made the decision to stop taking stovepipe samples because of the additional time it took to collect the sample and the amount of material we were collecting.

Because of the limited time available to prepare for the 2008 field season we were contacting landowners and scoping out plots at the same time we were collecting samples. This significantly

slowed us down and decreased the efficiency of sampling. The priority was given to establishing as many plots as possible to increase the number of samples for an analysis of the plant and algae community. This was based on the shorter amount of time needed to survey plants and algae. To address this issue for the 2009 field season we intend to make landowner contacts and establish plots well ahead of the sampling periods.

Table 1 Biotic Community Samples

Biotic Community Sample	Number of Points	Time Period
Algae	71	5/22/08-7/11/08
Stovepipe Sample	35	5/22/08-6/24/08
Emergence Trap Sample	37	5/22/08-6/28/08
Pitfall Trap Sample	68	7/14/08-8/01/08
Plants, Macrophytic Lichens, Bryophytes, Earthworms	68	8/18/08-9/24/08
All Samples Collected	31	5/22/08-9/24/08

Samples were taken within a 30 m radius plot (Figure 12). A detailed description of the plot (includes hydrology, anthropogenic disturbance, etc.) was recorded in a field notebook by each surveyor.

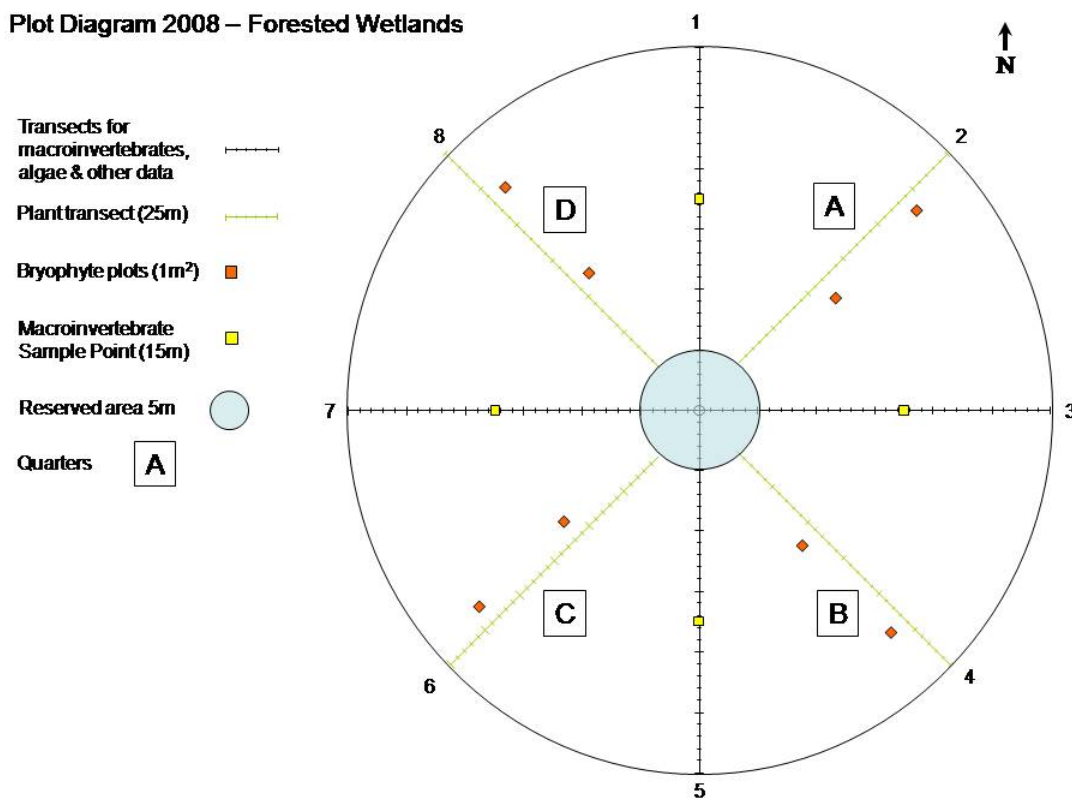


Figure 12. Plot Diagram

Algae

Algae were collected at 71 wetland points (Table 1) (Note: some of the points where algae samples were taken eventually had to be dropped). Three microhabitats were sampled for algae; the water column (less than 0.5m), leaf litter (*Acer rubrum*), and debris and sediment from the surface of the bottom substrate. A water sample was collected first followed by leaf litter and a sample from the surface of the bottom substrate to minimize disturbance. Four samples, each 50 ml, were collected from each microhabitat within the wetland for a total of 12 samples per plot. The depth of the water was recorded for each sample location. Algae samples were preserved in M3 fixative (Potassium Iodide, Iodine (optional), glacial acetic acid, 25% formalin). One ml of M3 was added per 50 ml sample.

Benthic Algae: Leaf Litter

Leaf litter (*A. rubrum*) was collected in areas of standing water closest to the midpoint of odd numbered transects (see Figure 12). If there was no standing water present along a transect we moved in a clockwise direction to find the closest suitable sampling location within the quarter plot. If standing water was lacking within a quarter plot leaves were collected from a wet depression closest to the midpoint of the transect. If there was no suitable location (surface water or wet depressions) present within a quarter, we kept moving through the plot until four samples were collected. The minimum distance samples were spaced was 3 m. A description of the sampling location was recorded for each sample.

At each sampling location we collected *A. rubrum* leaves to cover the bottom of a small bowl (10.5 cm²). We used a metal spoonulet to scrape off the algae on the leaf surfaces. If *A. rubrum* leaves were not available we collected other deciduous leaves of similar size and made a note of the species used. Each leaf was rinsed with DI water after scraping and all scrapings from the small bowl were collected into a 50 ml vile.

Substrate Surface

Debris and sediment was collected from 4 sampling locations within each plot. The same procedure as described previously for locating a suitable leaf litter sample was used to determine the location to take a sample. A large pipette (a turkey baster) was used to collect a 50ml sample of debris and or sediment from the substrate. The sample was collected into a 50ml vile.

Water Sample

A water sample was collected from 4 sampling locations within each plot. The same procedure as described above was used to determine the location for the sample. A 100 ml plastic beaker served as a water sampler. It was dipped three times with sample water before collecting a water sample. We submerged the water sampler to collect the surface water taking care to minimize the collection of organic material. Water samples were not collected in any area where the leaf litter had to be depressed in order to collect a sample. The water grab sample was stored in a 50 ml vile.

Analysis

In total we collected 288 leaf litter subsamples, 228 surface substrate subsamples, and 192 water subsamples (Table 2). Leaf litter was sampled with the same intensity across all points where algae were sampled. This was not the case for the water or surface substrate samples because we could not consistently collect four sub-samples due to the lack of standing water at some wetland points. In the beginning of the field season we were compositing the algae samples. This changed after roughly 20 points were sampled. We decided to keep the samples separate to allow for an analysis of species-area relationships.

Table 2 Algae Subsamples

Microhabitat	# of Subsamples per plot	# of plots
Leaf Litter	4	71
Water Column	4	30
	3	12
	2	11
	1	12
Surface Substrate	4	42
	3	12
	2	9
	1	6
	0	3

We conducted a preliminary diatom analysis to get a general sense of whether diatoms as a group, or certain groups of diatoms, have the potential upon further analysis to yield indices of biological integrity (IBIs) for assessing wetland condition.

The preliminary algae analysis focused on samples collected from leaf litter in the 2008 field season. We focused on leaf litter samples because we appear to have gotten good samples from the field work and have four subplot samples for all points.

We analyzed leaf litter algae samples for ten points, five points with high IEI scores and five points with low IEI scores.

For each site included in the analysis a portion of the leaf litter algae samples (50 ml) for each of the four subplots were composited to create one composite sample for each point. The procedure for compositing the samples were to 1) agitate the samples to re-suspend diatoms, 2) collect 10 ml with a clean pipette (to prevent cross-contamination of samples), 3) combine the 10 ml from each subsample into a vile for a total of 40 ml. The composited samples were sent to an outside expert for analysis. This left us with 40 ml of each of the original subsamples for further analysis.

The samples were sent to Bowling Green State University for diatom community analysis. The analysis was overseen by Rex Lowe. The samples were cleaned by acid digestion to remove excessive organic material and mounted on slides with Naphrax mounting medium. The community analysis was based on a 600 valve (individual diatom) count. The diatom species

identified and valve counts for the 10 samples are listed in Appendix B. A total of 88 species were identified. Although the sample size (10 sites) is too small for any legitimate statistical analysis there are a number of species that are relatively common yet occur predominately in either high or low-IEI sites. Based on these results we concluded that diatoms have strong potential to yield useful IBIs.

Macroinvertebrates

Macroinvertebrates were sampled from May 22, 2008 to August 1, 2008. Several sampling techniques were used to sample the aquatic and terrestrial invertebrate community in forested wetlands. Emergence traps were set at 35 points, stovepipe sampler was used to collect an aquatic macroinvertebrate sample at 34 points, and pitfall traps were set at 68 points (Note: some of the points where emergence trap samples and stovepipe samples were taken eventually had to be dropped). Not all sites were sampled using the same technique due to some problems encountered with the design of the emergence traps, the late start in the season (lack of standing water to collect stovepipe samples), and a few instances where access was denied upon the return visit to a site.

The emergence trap and pitfall samples were initially sorted to Order. No subsampling technique was used. The stovepipe samples have not yet been sorted. We will conduct a subsampling analysis to determine the best procedure to use for the stovepipe samples. This may include fixed counts, large-rare taxa searches, fixed area, or fixed volume approaches.

Insects: Emergence Traps

Four emergence traps were set at 35 plots for 10 days. Emergence traps were set on the water surface or, in the absence of surface water, on the surface of the soil in the wettest depressions. Site selection for trap placement followed the protocol previously described for algae. Traps were placed at least 1 m from the location where algae and stovepipe samples were taken and 3m apart from each other. The subsamples (each trap) were composited upon collection.

We had some difficulty with the design of the collecting jars. Two main problems occurred; the jars were dry upon collection and the jars were leaking when we were setting the traps. We believe this was because the glue used to adhere the funnel to the lid of the jar broke down due to the high concentration of ethanol (95%) being used. We also suspect that in some cases the ethanol simply evaporated. The rest of the trap design worked quite well. They were easy and light to carry long distances and only 4 traps fell over (1 trap fell at 4 different plots). We have rectified the design of the collecting jars and will be using emergence traps again in the 2009 field season.

The total number of specimens collected in the emergence traps for 35 sites was 2,777 (Appendix C). A total of 14 Orders were collected (Collembola was treated as an Order). The most abundant Orders were Diptera (1659), Isoptera (511, note 1 plot contained 382 specimens), Acari (488, note 1 plot contained 479 specimens), Hymenoptera (26), Hemiptera (24), and Araneae (18).

Aquatic Macroinvertebrates: Stovepipe Sampler

Aquatic macroinvertebrates were collected using a stovepipe sampler (5 gallon plastic bucket with the bottom cut off) at 34 sites. Collections were made in two locations dispersed within the plot where surface water and/or wet depressions were present.

Samples were 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 was not present within the plot, we sampled in locations (depressions) with the wettest substrate. We tried to select sampling locations in diagonal quarters of the plot (e.g. quarters 1 & 3 or quarters 2 & 4). If suitable sampling conditions were not present in diagonal quarters we tried to use sampling locations in each of two adjacent quarters. If it was necessary we placed both sampling locations in the same quarter. The minimum distance between samples was 3 m.

To collect an aquatic macroinvertebrate sample the stovepipe sampler was firmly placed into the substrate (few cm deep) and held in place. The water was agitated in the sampler for 10 seconds to dislodge organisms from the substrate and vegetation. If surface water (>1.27 cm) was present we took five sweeps within the sampler with a 500 micron mesh hand net (10.5x12.5 cm). After each sweep, we transferred all material into a 32 oz collecting jar. We inspected the net, removed any clinging organisms and added them to the sample. The jar was only filled halfway with sample material and additional jars were used when necessary. We filled the container with 95% ethanol to preserve the sample. For wet depressions (with little or no standing water) we collected three, one-hand leaf litter grab samples from within the stovepipe. We distributed grabs evenly throughout the stovepipe area. The sample was preserved the same as for the dipnet samples. Samples were rinsed through a 500 micron sieve in the lab to remove excess silt and preserved with 95% ethanol.

Epigeal Macroinvertebrates: Pitfall Traps

Pitfall traps were set in July to collect epigeal macroinvertebrates, although we did not just collect epigeal macroinvertebrates. Traps were made of 16 oz clear plastic cups placed in the ground with the top of the cup flush with the ground surface. Cups were 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) was placed inside the cups to prevent small vertebrates from entering the killing solution. A plastic plate held up with small stakes was placed over the pitfall trap to serve as a roof.

We placed eight pitfall traps, 2 on each transect at 10 and 15m. Traps were placed in areas where the chance of flooding by surface water (avoid pits) was reduced. We collected the contents of pitfall traps after 7 days. If the trap was $>1/2$ full of water it was still collected, but was not sorted and was considered a failure. Each trap was collected separately in a small container. The samples were rinsed with tap water in the lab (to remove the soap) and 95% ethanol was added.

Due to heavy precipitation in July, many traps were flooded. In total 225 individual traps (across all sites) were flooded, 282 traps were in good condition and 22 were partially flooded. The sampling effort varied across the plots due to damage and flooding (Table 3).

Table 3 Pitfall Trap Sampling Effort

Pitfall Traps in Good Condition	
# of sub-samples per plot	# of plots
0	2
1	11
2	12
3	6
4	8
5	4
6	6
7	9
8	10

253 samples have been sorted to Order (Appendix C). Nine classes of invertebrates and 28 Orders (Bivalves have not yet been sorted to Order) were collected. The total number of specimens from the pitfall traps that have been sorted is 20,367. The most abundant Orders were Collembola (10,243), Acari (2,292), Hemiptera (1,286), Hymenoptera (1,273), Diptera (1,741), Araneae (1709), and Coleoptera (1,130).

Analysis

Preliminary analyses of the relationship between IEI or Buffer Insults and macroinvertebrate data at the order level have not yielded significant results. Efforts are underway to identify specimens to family, genus and when possible, species. More rigorous analyses will be conducted once specimen identification work has been completed.

Earthworms

Earthworms were captured on 22 of 68 plots. Eighteen of 68 plots had detectable *Lumbricus terrestris* middens present. Earthworm species identified to date include *Amyntus spp.*, *Eisenia eisenia*, *Apporectodea spp.*, *Lumbricus terrestris*, *Lumbricus rubella*, and *Octolasion tyrraenum*. All are introduced species. Analysis of these data is ongoing.

Vegetation

We sampled plants on 68 plots throughout the Chicopee watershed from mid-August until early October. Results from 64 plots are reported here for vegetation. Four plots were excluded due to errors in the GIS data.

Not surprisingly, forested wetlands in the Chicopee watershed are red maple dominated. Other dominant species include yellow birch, white ash, American elm, red oak, eastern hemlock, white pine, winterberry, highbush blueberry, spicebush, and cinnamon fern. There were 253 unique vascular plant species identified from our plots.

Invasive plants were widespread in forested wetlands across the watershed but not abundant on most plots, a result similar to what we found in forested uplands in the Deerfield Watershed in 2007. Twelve invasive plants were found: Norway maple (*Acer platanoides*), Japanese barberry (*Berberis thunbergii*), Asian bittersweet (*Celastrus orbiculatus*), Winged euonymus (*Euonymus alata*), Glossy buckthorn (*Frangula alnus*), Yellow flag (*Iris pseudacorus*), Morrow's honeysuckle (*Lonicera morrowii*), True forget-me-not (*Myosotis scorpioides*), Reed canary grass (*Phalaris arundinacea*), Creeping buttercup, (*Ranunculus repens*), Common buckthorn (*Rhamnus cathartica*), and Multiflora rose (*Rosa multiflora*).

Sixty percent (38 of 64) of plots had invasive plants on them. *Berberis thunbergii* occurred on 44% of plots, followed by *Rosa multiflora* (34%), *Frangula alnus* and *Celastrus orbiculatus* (20%).

Analysis

We used logistic regression to explore the relationships between IEI and the field-based stressor metric, invasive plants. Logistic regression examines presence-absence data not a particularly powerful test; we are examining presence-absence and a lot of information is lost by not examining the abundance data. However the relationship between IEI and invasive plant cover is fairly strong. As IEI decreased total percent cover of invasives increased ($n=64$, $P=0.0011$, $\rho^2=0.12$; Fig 13). Many low IEI plots were not invaded however nearly all invasives were found on low IEI rated plots. Twelve introduced (but not invasive) species were inventoried but the relationship with IEI was weak.

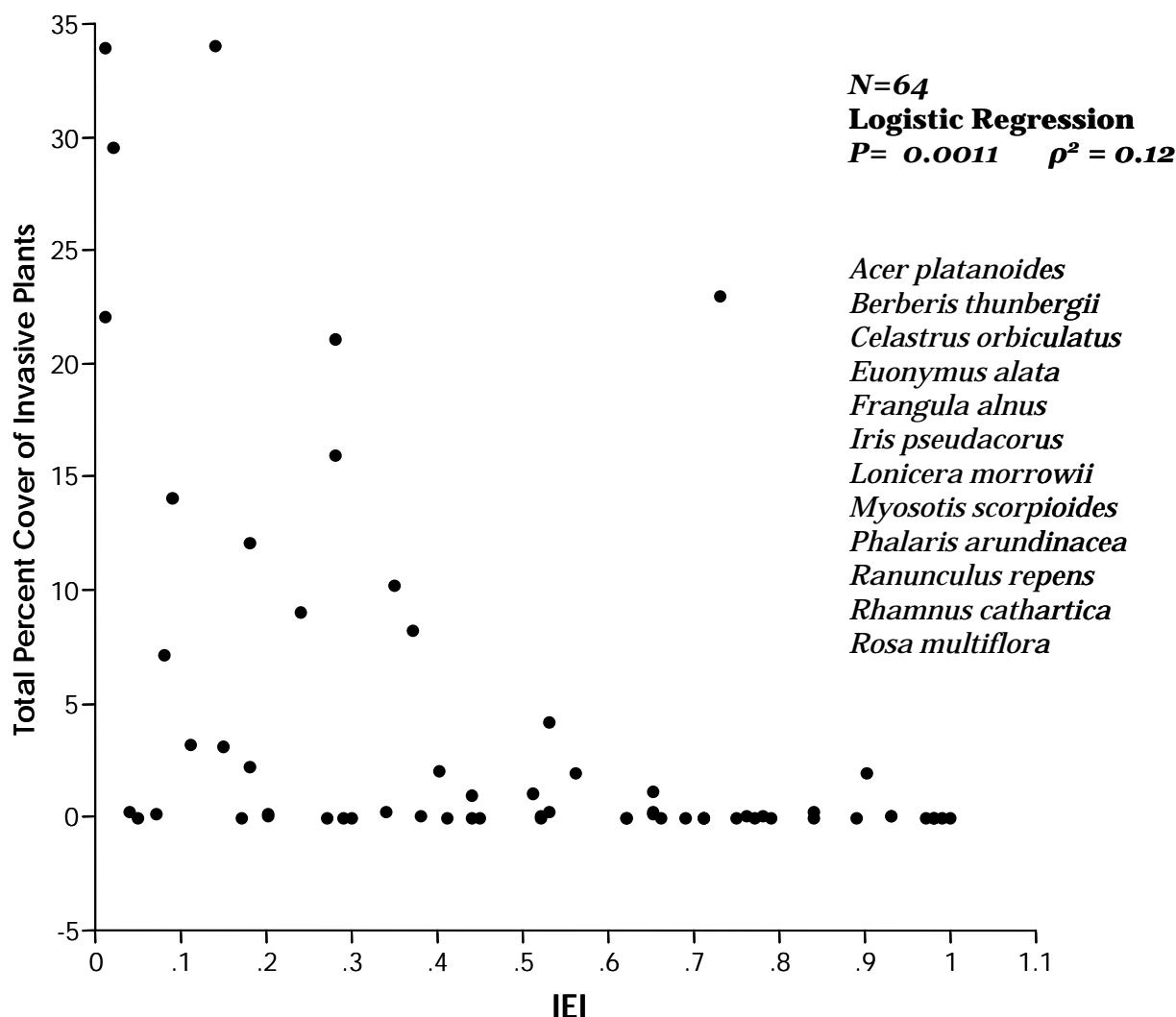


Figure 13. Percent cover of all invasive plants by CAPS IEI. Logistic regression is from presence-absence data.

We are examining advanced techniques more appropriate to these data, ideally to help us identify and interpret ecological community thresholds along multidimensional environmental gradients. Plants will be assigned classifications based on traits, including but not limited to wetland indicator status, nativity, growth form, and life cycle. Other traits we may explore include dispersal type, flowering phenology, and vegetative spread.

Lichen data await analysis. Bryophyte samples are awaiting analysis pending completion of identifications.

Hydrology and Water Chemistry

Temperature loggers (HOBO) were placed at each site in the lowest point. The loggers recorded temperature in 1 hour increments. The purpose of the logger was to try and determine the surface hydrology of the site. We wanted to describe when the wetland was drying or flooding and how flashy the surface water flows were within the wetland. Characterization of the hydrology would

allow us to account for the variability in hydroperiod across wetlands and its effect on the biotic community. We were not able to recover ten of the loggers, probably because they were pulled out or were not adequately staked into the ground. We were able to retrieve loggers for 57 points. There was some difficulty interpreting the temperature readings from the logger. One thing we did not consider was protecting the logger from direct sunlight to reduce high temperature readings. We also did not monitor ambient air temperature which could have been used to assist in determining when the logger was no longer submerged in water. For 2009, we will be covering the loggers to protect them from direct sunlight and installing ambient air temperature loggers (iButton) in conjunction with the HOBO's.

The pH/Conductivity meter was used at each site to take pH and conductivity readings at each location where algae was sampled. If there was no surface water but the ground was saturated we dug a small hole to take readings. These data have not yet been analyzed.

SLAM DEVELOPMENT: REVISION FOR 2009 FIELD SEASON

Much (but not all) of the existing wetlands assessment work done has focused on other types of wetlands. Therefore, we focused our work initially in forested wetlands. Forested wetlands make up the vast majority of wetlands in Massachusetts and are the most difficult to model using aquatic-based metrics (e.g., water quality, aquatic invertebrates). Because they typically lack permanent standing water, forested wetlands are more integrated into the surrounding terrestrial landscape (e.g., forested wetlands can be viewed ecologically as both wetlands and forests). Therefore, it is necessary not only to look at how the surrounding landscape can negatively affect the physical-chemical characteristics of wetlands, but how the landscape can support components of the wetland biota that may be shared between wetland and terrestrial systems.

As we approached the 2008 field season there was a fair amount of uncertainty about what sampling techniques could be effectively and efficiently used in forested wetlands and what taxa (particularly algae and macroinvertebrates) we would be likely to find. A variety of techniques were deployed in 2008 to increase our chances for success and to test the various approaches for eventual inclusion or exclusion from the final SLAM. Experience from the 2008 field season was used to revise the SLAM for 2009 (Appendix D). All of our techniques used in 2008 yielded useful data.

Few changes will be made to the protocol for the 2009 field season other than a greater effort to characterize the hydrology of the wetland point. We will be using shallow groundwater wells to monitor the high water table and will record along transects (using a point intercept method at 5m increments) the presence or absence of surface water. We decided to add these components because of the significance of hydrology and its influence on wetland biotic communities. We also will be using iButton temperature loggers to sample air temperature along with the HOBO data loggers placed in areas of most persistent water to increase our likelihood of modeling hydroperiod for each of our sites in 2009.

The three macroinvertebrate sampling techniques used in 2008 all proved to be effective even as we confronted some logistical challenges in deploying them in the field. We expect to solve the problem of leaky emergence traps by testing and using a different adhesive for connecting the

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funnel to the collecting jar lid. We decided that rather than composite samples from emergence traps as we did in 2008 that all subsamples would be kept separate for 2009. Another small change for 2009 is that emergence traps will be deployed for seven days rather than the ten days used in 2008. This is to accommodate the large number of sites (150) targeted for 2009 field work and to reduce the chance that alcohol in the jars will evaporate prior to collection.

It appears that the three macroinvertebrate techniques sample different organisms. Emergence traps primarily capture adult insects, most of which have aquatic life stages. Stovepipe sampling tends to capture the larval stages of aquatic insects as well as other invertebrates such as clams, snails and crustaceans that are not captured by emergence traps. We won't know for certain until the stovepipe samples have been sorted and the specimens from both stovepipe and emergence traps identified beyond the family level how much overlap there is between these two sampling techniques. However, it is possible that stovepipe sampling may collect insects that would not emerge until after emergence trapping has been completed. Pit trap sampling collects organisms that are not collected by either the emergence traps or stovepipe sampling. For the time being we have decided to include all three techniques in the revised SLAM.

Algae were collected in 2008 using three techniques (leaf litter, water sample and substrate) and stored for future analysis once resources have been acquired for algae identification work. Thus far, we have not been able to determine to what degree these sampling approaches are redundant. However, it is not very time consuming to collect the samples and therefore, we have decided to keep all three methods in the SLAM.

Two changes in our approach to algae sampling were adopted partway through the 2008 field season. We decided that rather than composite the subplot samples for each microhabitat we would keep them separate. This will allow us to statistically adjust for varying sampling effort (number of subplots) among the sites for water and substrate algae samples. For leaf litter samples, we initially selected a set number of deciduous tree leaves for sampling. We eventually decided that it would be more appropriate to line a small bowl with leaves so that we could better standardize each sample by surface area. Both of these changes have been incorporated into the revised SLAM.

In 2008 we used a combination of mustard extractions for all earthworms and quadrat sub-sampling for *L. terrestris* middens. Mustard extractions didn't work as well in wetland soils as they did in upland soils. We concluded that this may not be the best method for sampling earthworms in wetlands. In 2009 we will collect earthworms via soil plugs during excavation of pitfall traps.

We made no changes to the plant sampling protocol or the lichen sampling protocol following the 2008 field season. Bryophyte sampling in 2008 was reduced from 8-1m² subplots to 2-1m² followed by a 15 minute cruise around the plot to collect species not found within quadrats. Sampling intensity was reduced because we didn't have the expertise or funding to identify all samples. In 2009 we will sub-sample bryophytes on 4-0.5m² subplots.

The revised SLAM serves as the 2009 sampling SOP and is included as Appendix D.

IBI DEVELOPMENT AND CALIBRATION OF CAPS METRICS

Specimen Identification

Initial analyses have shown some promise for developing field-based condition metrics (e.g. invasive plants). However, IBI development will depend on more detail identification of specimens collected in 2008 (algae, macroinvertebrates, bryophytes). Additional resources have been acquired to fund some of this identification work. We will begin specimen ID work in the summer of 2009 focusing on the most promising taxa.

The following Macroinvertebrate Orders have been selected to have species level identification work contracted: Araneae, Diptera, Hymenoptera, Hemiptera, Coleoptera, Collembola (Appendix C). These Orders were selected because of their abundance, their relationship to changes in land use and water quality, and resources available for identification work. Individual species will be analyzed to elucidate any dose-dependent relationships that may exist with the stressors modeled in CAPS.

Depending on available resources algae samples will be analyzed to identify diatom species using the 600 valve count method employed for the preliminary analysis. We expect that bryophytes will be identified in the fall of 2009.

Specimen identifications will facilitate development of Indices of Biotic Integrity (IBIs). These IBIs will be incorporated into a Site Level Assessment Method (SLAM) for forested wetlands. The IBIs will also be used to calibrate the CAPS landscape-based models for assessing ecological integrity in wetland and aquatic ecosystems.

Data Analysis

The overarching goal of the data analysis is to determine whether CAPS IEI and the component ecological integrity metrics (e.g., habitat loss, connectedness, etc.) are related to observed ecological conditions, and to further quantify the magnitude and nature of those relationships. To accomplish this goal, we will use a variety of statistical methods including principally quantile regression (Cade et al. 1999) and a custom analytical method based on the method of indicator species analysis (Dufrene and Legendre 1997). The data input for both analytical methods will be a list of the sample points and the corresponding values for each of the CAPS metrics and a suite of variables representing the presence or standardized abundance of each species or group of species and/or one or more derived biotic indices (e.g., Simpson's diversity index).

Quantile regression is used to estimate functional relations near the boundaries of data distributions and for analyzing effects of ecological limiting factors, where the relevant rates of change estimated are near the extremes of distributions. We will use linear and nonlinear quantile regression, as appropriate, to examine the relationship between each CAPS metric and the extremes of each of the biotic response variables. Based on our preliminary analysis, we expect the upper extremes of abundance of some taxa to be strongly related to the ecological integrity gradient, even though the mean shows no relationship, indicating that perhaps ecological integrity as measured primarily affects the ability of some species to achieve high levels of abundance.

Indicator species analysis is typically used to identify species that are significant indicators of discrete habitat types or conditions based on their relative abundance across habitat types and their ubiquity of occurrence across samples within each habitat type. Here, we will develop a custom application of this basic method based on a similar method being developed by Dr. Mathew Baker at the University of Maryland, Baltimore, called Taxa Indicator Threshold Analysis (TITAN). Briefly, our approach involves subdividing samples into low versus high integrity plots based on a sequentially advancing threshold in values of the ecological integrity metric under consideration (e.g., IEI) and computing indicator species values for each species or species' group. Through a combination of bootstrap and Monte Carlo randomization procedures, we will identify which species are significant indicators of the ecological integrity gradient, the threshold in ecological integrity value that leads to the greatest indication of the gradient, and the level of uncertainty in the threshold delineation (both in terms of the magnitude of the indication and the location of the threshold).

Data analysis will occur late in 2009 and will be finished in early 2010.

REFERENCES

- Cade BS, Terrell JW, and Schroeder RL. 1999. Estimating effects of limiting factors with regression quantiles. *Ecology* 80: 311-323.
- Dufrene M and Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67:345-366.

Appendix A

Draft Site Level Assessment Method (SLAM) for Forested Wetlands

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 1 will focus on forested wetland communities in the Chicopee Watershed, 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

4. Sample Collection, Preservation, and Handling

Macroinvertebrates will be collected and preserved in 95% 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.

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 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%)
- Field notebook
- Flagging
- Forceps
- GPS (Global Positioning System)
- Hand lens
- Hip chain

HOBO Pendant Temperature/Light Data Logger
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 computer
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)
Rite-in-rain paper and pen
Scissors or jack knife
Shovel
Stakes
String
SOP
Spoonulet
Squirt bottle
Standard solutions for calibration of pH/Conductivity/Temp meter
Stovepipe sampler
Tap water
Tooth brush
Trowel or bulb planter
Turkey baster (large Pipette)
Water/detergent solution
White pan

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 *

Standard solutions for calibration of pH/Conductivity/Temp meter

Tap water

Water/detergent solution

* M3 solution

7. Calibration & Training

Equipment calibration procedures for the GPS units, pH/CON 10 pH/Conductivity/C° Meter 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 19 and September 30, to ensure adequate assessment of the targeted wetland biotic communities. Forested wetlands in the Chicopee Watershed 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 2008. 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 72 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 \leq 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 Diagram form. 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.)

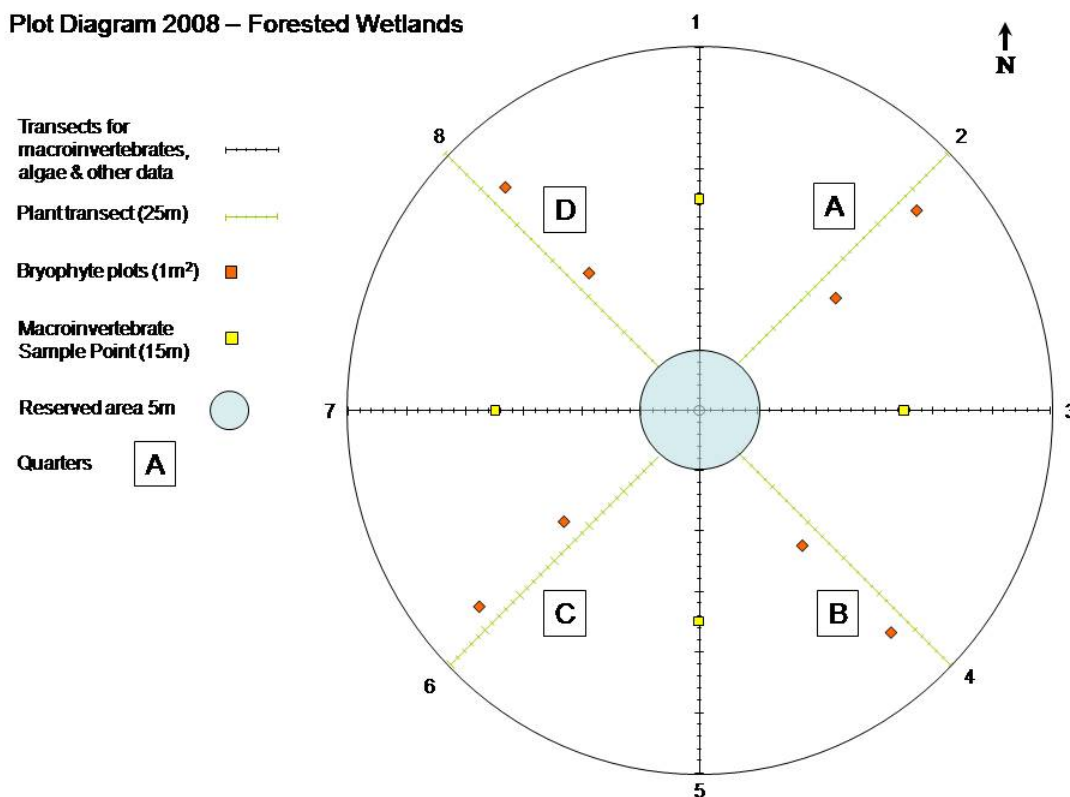


Figure 1.

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.

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. Depressions will only be counted if they are sufficiently obvious that they could be recognized even if groundcover vegetation is dense. Topographic complexity will be expressed as number of micro-topographic depressions per 100 m of transect length.

(b) Hydrology

Characterization of the hydrology of the wetland will be recorded in a field notebook. Surveyors will include the following information:

Hydroperiod

A HOBO Pendant temperature/light data logger will be placed in the water and left for the duration of the study period (about 4 months) to determine the relative hydroperiod of the wetland.

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. Attach flagging or string to the data logger and record the placement location on the plot diagram. The data logger will record temperature and light every 2 hours. Collect data loggers upon the completion of the biotic community assessment.

Data will be uploaded and analyzed to determine the relative hydroperiod (hydroperiod during the survey period) of the wetland based on the temperature data. Procedures for uploading data and setting recording intervals will be followed according the manufacturer’s instructions (See QAPP Appendix J).

Water depth

Indicate whether the assessment area is inundated, has surface water, is moist or is dry. Walk through the assessment area and based on field indicators of surface water, visually estimate the percent of the assessment area inundated during the most recent high water period as well as the average and maximum depth of the inundated portion of the assessment area.

Record water depth at the time of sampling for each biotic sampling area.

(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. 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. Note on the Plot Diagram form the transects and/or quarters from which readings were taken.

(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 implemented other elements of the SOP.

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 from May-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). 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. Samples will be stored in amber colored viles to reduce the transmission of light. 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 Diagram form the transects and/or quarters from which samples were taken and a description of the sampling location.

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. Rinse each leaf with DI water after scraping. Collect all scrapings from the small bowl into a 50 ml vile. Keep rinsing the pan with DI water until there is 50ml in the vile. Add 1ml of M3 per 50ml of benthic leaf scrapings for preservation.

Thoroughly clean the pan and spoonula 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 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 Diagram from the transects and/or quarters from which samples were taken.

Rinse a 100 ml plastic beaker which will serve as a water sampler three times with sample water before collecting a water 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. The water grab sample will be collected in a 50 ml vile. Add 1ml of M3 per 50ml of the water sample for preservation. Repeat for each transect.

Clean the water grab sampler after sampling.

(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 vile. Add 1ml of M3 per 50ml of the water sample for preservation. Repeat for each transect.

8.2.2.2 Macroinvertebrates

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

(a) Earthworms

Four 20 cm x 20 cm x 20 cm soil pits will be dug and hand-sorted for earthworms in May and June. Soil pits will be dug in areas that are not inundated with water nearest the midpoint of each odd numbered transect. If the assessment area is entirely inundated with water then earthworms will not be sampled.

Excavate a soil pit 20 cm by 20 cm wide and 20 cm deep. Hand sort the soil and detritus and capture all earthworms encountered. Rinse the earthworms with water in a shallow pan then placed them in 70% isopropyl alcohol. At the end of each field day transfer samples to 10% formalin solution for storage. Label Sample vials with plot ID, date, and surveyor ID. Earthworm species identifications will follow Schwert (1990) and Reynolds (1977).

(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) 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 Diagram 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.

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. Label containers with site ID, date of collection, surveyor ID, and description of microhabitat. Containers will be stored in the lab until they are processed.

(c) Insects: Emergence Traps

Four emergence traps per plot will be set and collected after ~10 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 in areas that were not 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 Diagram form the transects and/or quarters where emergence traps are set.

Fill a jar (with funnel top) with 95% 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 occurs. Upon collection of the traps replace the jar lids with fully enclosed lids and add ethanol as needed. Label jars with site ID, start and end date of collection, surveyor ID, and description of microhabitat. Jars will be stored in the lab until processed.

(d) Epigeal macroinvertebrates

Pitfall traps will be set out in June 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 2 cm of water and dishwashing soap 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 10 m apart along transects (2 on each transect) within 5-10 m of the transect line. Place traps in areas where the chance of flooding by surface water (avoid pits) is reduced. Collect the contents of pitfall traps after ~48 hrs. Combine the contents of the traps from one site into a plastic 32 oz or 16 oz container and add 95% ethanol. Label jars with site ID, start and end date of collection, and surveyor ID. Samples will be stored 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, Macgee & 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 macro-lichens 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 8-1 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. 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).

8.6 Protocol for Decontamination of Field Equipment

Inspect all equipment for debris and removed 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 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.

13. References

Anderson, L.E., H.A. Crum, and W.R. Buck. 1990. List of the mosses of North America north of Mexico. *The Bryologist* 93:448-499.

- Anderson, L.E. 1990. A checklist of Sphagnum in North America north of Mexico. *The Bryologist* 93:500-501.
- Brinson, M. M. 1993. "A hydrogeomorphic classification for wetlands," Technical Report WRP-DE-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. NTIS No. AD A270 053.
- Cobb, B., E. Farnsworth, and C. Lowe. 2005. *A Field Guide to the Ferns and their Related Families*. Houghton Mifflin Co., Boston, MA. 281 + xviii pp.
- Connors, B. 2006. *Protocols for Decontaminating Biomonitoring Sampling Equipment*. ME Department of Environmental Protection DEPLW0641.
- Cowardin, L.M., V. Carter, F.C. Golet, and E.T. LaRoe. 1979. *Classification of wetlands and deepwater habitats of the United States*. FWS/OBS-79/31. U.S.D.I. Fish and Wildlife Service, Washington D.C. http://wetlands.fws.gov/Pubs/Reports/Class_Manual/class_titlepg.htm
- Danielson, T. 2004. *Protocols for Collecting Water Grab Samples in Rivers, Streams, and Freshwater Wetlands*. ME Department of Environmental Protection DEPLW0637.
- Danielson, T. 2006. *Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands*. ME Department of Environmental Protection DEPLW0634
- DiFranco, J. 2006. *Protocols for Sampling Aquatic Macroinvertebrates in Freshwater Wetlands*. ME Department of Environmental Protection DEPLW0640.
- Esslinger, T.L. 2007. A cumulative checklist for the lichen-forming fungi, lichenicolous, and allied fungi of the continental United States and Canada. North Dakota State University: <http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm>.
- Gleason, H.A. 1952. *The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada*. Hafner Press, NY, NY. 3 volumes, 1,732 + lxxv pp.
- Gleason, H.E. and A. Cronquist. 1991. *Manual of the Vascular Plants of Northeastern United States and Adjacent Canada*. 2nd edition. New York Botanical Garden Press.
- Jackson, Scott. 1995. *Delineating Bordering Vegetated Wetlands Under the Massachusetts Wetlands Protection Act: A Handbook*. MA Department of Environmental Protection. Boston, MA. 89 + 5 pp.
- Magee, D.W. and H.E. Ahles. 1999. *Flora of the northeast: A Manual of the Vascular Flora of New England and Adjacent New York*. 1213 pages. University of Massachusetts Press, Amherst, MA.
- McGarigal, K., B.W. Compton, S.D. Jackson, K. Rolih, and E. Ene. 2005. *Conservation Assessment Prioritization System (CAPS) Highland Communities Initiative PHASE 1. Final Report*. Landscape Ecology Program, Department of Natural Resources Conservation, University of Massachusetts, Amherst (URL: http://www.umass.edu/landeco/research/caps/reports/caps_reports.html)

- Merrit, R.W., K.W. Cummins (editors). 1996. An introduction to the aquatic insects of North America, Third Edition. Dubuque, IA: Kendall/Hunt Publishing Company. 862 pp.
- Newcomb, L. 1977. Newcomb's Wildflower Guide. Little, Brown & Co., Boston, MA. 490 + xxii pp.
- Petrides, G.A. 1972. A Field Guide to Trees and Shrubs. Houghton Mifflin Co., Boston, MA. 428 + xxxii pp.
- Reynolds, J. W. 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum Miscellaneous Publication, Toronto, Ontario, Canada.
- Scanlon, J. 2006. MassWildlife Landcover Mapping Decision Rules. Internal Document. Massachusetts Division of Fisheries and Wildlife, Westborough, MA.
- Schauff, M.E. 1986. Collecting and Preserving Insects and Mites: Techniques and Tools. Systematic Entomology Laboratory, USDA, Washington, D.C.
- Somers, P. K. Lombard, and R. Kramer. 2006. A Guide to Invasive Plants in Massachusetts. Massachusetts Division of Fisheries & Wildlife, Natural Heritage & Endangered Species Program, Rabbit Hill Road, Westborough, MA 01581.
- Schuster, R.M. 1974. The Hepaticae and Anthrocerotae of North America: East of the Hundredth Meridian Volume 3. Columbia University Press, New York, NY, USA and London, UK.
- Schwert, D. P. 1990. Oligochaeta: Lumbricidae. Pages 341–356 in D. L. Dindal, editor. Soil biology guide. John Wiley and Sons, New York, New York, USA.
- Stevenson, R.J. 2001. Using Algae to Assess Wetlands in Multivariate Statistics, Multimetric Indices, and Ecological Risk Assessment Framework. pages 113-140 in Bader, R.B., Batzer, D.P. and Wissinger, S.A. (eds.), Bioassessment and Management of North American Freshwater Wetlands. John Wiley & Sons, NY, NY.
- Swain, P.C. and J.B. Kearsley. 2001. Classification of the Natural Communities of Massachusetts DRAFT - 2001- (version 1.3) Reprinted 2004. Massachusetts Division of Fisheries and Wildlife, Westborough, MA.
- U.S. EPA, 2002. *Methods for Evaluating Wetland Condition: Developing an Invertebrate Index of Biological Integrity for Wetlands*. Office of Water, U.S. Environmental Protection Agency, Washington , DC. EPA-822-R-02-019
- U.S. EPA. 2002 *Methods for Evaluating Wetland Condition: Using Algae to Assess Environmental Conditions in Wetlands*. Office of Water, U.S. Environmental Protection Agency, Washington , DC. EPA-822-R-02-021

Appendix B. Diatom Species Analysis from 10 Leaf Litter Samples.

Index of Ecological Integrity	1	0.99	0.98	0.98	0.97	0.05	0.04	0.02	0.01	0.01
Diatom Flora										
(cf before a species name indicates a resemblance)	281	442	602	372	741	861	993	351	611	701
<i>Achnantheidium minutissimum</i> (Kütz.) Czarn.								99		
<i>Achnantheidium</i> sp.		2				6				2
<i>Aulacoseira crenulata</i> (Ehrenberg) Thwaites					345		76			
<i>Cocconeis placentula</i> Ehr.				2		1				
<i>Cyclotella ocellata</i> Pant.						18				
<i>Cymbella hauckii</i> Van Heurck										7
<i>Cymbella naviculaformis</i> Auersw. ex Heribaud									39	
<i>Cymbella tumidula</i> Grun.						2				
<i>Diadesmis paracontenta</i> Lange-Bertalot and Werum		1								
<i>Decussata placenta</i> (Ehr.) Lange-Bertalot & Mezeltin		1			1		2		2	
<i>Encyonema minutum</i> (Hilse in Rabenhorst) D.G. Mann				2	2					
<i>Eunotia bilunaris</i> Ehr. Mills.			9		10	27	175		42	48
<i>Eunotia carolina</i> Patrick		111	38	2	2	2				
<i>Eunotia curvata</i> (Kütz.) Lagerst										18
<i>Eunotia curvata</i> f. <i>bergii</i> Woodhead & Tweed	1	5	6	9					6	7
<i>Eunotia elegans</i> Østrup	7	70	82	11	5					
<i>Eunotia exigua</i> (Breb. Ex Kütz.) Rabenh.		3		2	10	106		2		148
<i>Eunotia fallax</i> A. Cleve		4	5							
<i>Eunotia flexuosa</i> Bréb. ex Kütz.			2		23					2
<i>Eunotia</i> girdle view 12-23 µm			60		4	26	57		8	8
<i>Eunotia</i> girdle view 30-45 µm		36		15						
<i>Eunotia incisa</i> W. Sm. ex Greg,		20								
<i>Eunotia meisteri</i> Boyer	1		20	2						
<i>Eunotia monodon</i> Ehr.							3			

Appendix B: Diatom Species Analysis from 10 Leaf Litter Samples

<i>Eunotia paludosa</i> v. <i>trinacria</i> (Krasske) Norpel	545	126	61	277	11	3	1		
<i>Eunotia pectinalis</i> (O.F. Müller) Rabenhorst					3	12	11	20	9
<i>Eunotia perpusilla</i> Grun.						8			
<i>Eunotia</i> cf. <i>praerupta</i> Ehr.				1					
<i>Eunotia rhomboidea</i> Hust.			2						
<i>Eunotia septentrionalis</i> Østrup						7			17
<i>Eunotia serra</i> (Ralfs) Ehr.			4						
<i>Eunotia sudetica</i> O.F. Muller		3		5			1		
<i>Eunotia tautoniensis</i> Hust. Ex Patrick	8	19	141	36					
<i>Fragilaria neoproducta</i> Lange-Bertalot					2				
<i>Fragilaria vaucheria</i> (Kütz.) Peters.							1		
<i>Fragilariaforma viriscens</i>						171	39		
<i>Frustulia krammeri</i> Lange-Bertalot & Metzeltin				1					
<i>Frustulia pseudomagaliessmontana</i> Camburn & Charles				1					
<i>Frustulia saxonica</i> Rabh	27	1	71	133					
<i>Frustulia vulgaris</i> (Thwaites) DeToni							3		
<i>Gomphonema angustatum</i> (Kütz.) Rabenh.							22	42	5
<i>Gomphonema gracile</i> Ehr.							4		12
<i>Gomphonema parvulum</i> (Kütz.) Kütz.							39	19	119
<i>Gomphonema</i> sp. (girdle views)					12	4	17	25	26
<i>Hantzschia amphioxys</i> (Ehr.) Grunow									5
<i>Luticola cohnii</i> (Hilse) D.G. Mann									4
<i>Luticola mutica</i> (Kütz.) DG Mann									2
<i>Meridion allensmithii</i> Brandt					10				
<i>Meridion circulare</i> (Greville) Agardh					64		12		228
<i>Navicula asellus</i> Weinhold ex Hustedt									1
<i>Navicula cocconeiformis</i> Greg. ex Greville								2	
<i>Navicula cryptocephala</i> Kütz								58	177

Appendix B: Diatom Species Analysis from 10 Leaf Litter Samples

<i>Navicula exigua</i> (W. Gregory) O. Müller						2			
<i>Navicula minima</i> Grunow in Van Heurck							2		
<i>Navicula notha</i> Wallace						2			
<i>Navicula</i> cf. <i>tantula</i> Hust.						4			
<i>Navicula</i> sp.						2			
<i>Neidium affine</i> v. <i>amphirhynchus</i> (Ehr.) Cleve						2			
<i>Neidium alpinum</i> Hust.						2			
<i>Neidium bisucatum</i> (Lagerst.) Cl.		16	2						
<i>Neidium</i> sp.						2			
<i>Nitzschia dissipata</i> v. <i>media</i> (Hantzsch) Grunow									2
<i>Nitzschia frustulum</i> (Kütz.) Grun				4		9	12		
<i>Nitzschia palea</i> (Kütz.) W. Smith						2			
<i>Nitzschia</i> cf. <i>palustris</i> Hust.						2			2
<i>Nitzschia</i> cf. <i>recta</i> Hantz.									18
<i>Nitzschia</i> cf. <i>vermicularis</i> (Kütz.) Hantz.								2	
<i>Nitzschia</i> sp.				31		10	62	6	13
<i>Pinnularia abaujensis</i> v. <i>lacustris</i> Camburn & Charles				2					
<i>Pinnularia abaujensis</i> v. <i>linearis</i> (Hust.) Patr.		7	10	5					
<i>Pinnularia abaujensis</i> v. <i>rostrata</i> Patr.		8							
<i>Pinnularia abaujensis</i> v. <i>subundulata</i> (Mayer) Patrick		12		1					
<i>Pinnularia acrosphaeria</i> Rabh.				4					
<i>Pinnularia biceps</i> W. Greg.								2	
<i>Pinnularia brebissonii</i> (Kütz.) Rabh.						22			
<i>Pinnularia</i> girdle view		96		18	24	8	15	26	10
<i>Pinnularia hilseana</i> Janisch ex Rabh.	8	59	58	74	2	13	8		
<i>Pinnularia legumen</i> (Ehr.) Ehr.				4				2	
<i>Pinnularia maior</i> (Kütz.) Cleve	1		7	2	3	1			
<i>Pinnularia microstauron</i> v. <i>adarondakensis</i> Camburn & Charles					2				

Appendix B: Diatom Species Analysis from 10 Leaf Litter Samples

<i>Pinnularia nodosa</i> (Ehr.) W. Sm.					4		9			
<i>Pinnularia obscura</i> Krasske								2		
<i>Pinnularia rupestris</i> Hantzsch	2		22	1	10		2			2
<i>Placoneis elginensis</i> (Greg.) E. J. Cox									2	2
<i>Placoneis neglecta</i> (Krasske) Lowe									2	
<i>Planothidium lanceolatum</i> (Bréb. ex (Kütz.) Round & Bukhtiyarova							4	201	2	
<i>Planothidium</i> sp.									2	
<i>Pseudostaurosira brevistriata</i> (Grunow) Williams & Round									2	
<i>Sellaphora pupula</i> (Kütz.) Mereschk.							2	4	21	
<i>Stauroneis anceps</i> Ehr.							2		53	
<i>Stauroneis</i> cf. <i>kriegeri</i> Patr.					2		12		2	6
<i>Stauroneis phoenicentron</i> (Nitz.) Ehr.							8		6	2
<i>Staurosira construens</i> Ehr.							8		1	
<i>Staurosira construens</i> v. <i>venter</i> (Ehr.) Hamilton							4	14		
<i>Staurosirella leptostauron</i> (Ehr.) D.M. Williams et Round							2			
<i>Synedra acus</i> v. <i>radians</i> (Kütz.) Hust.										13
<i>Synedra rumpens</i> (Kütz.)								9		
<i>Synedra rumpens</i> v. <i>fragilarioides</i> Grun.							103			
<i>Synedra</i> sp.						2		62		
<i>Tabellaria flocculosa</i> (Roth) Kütz						2	90			5
<i>Ulnaria ulna</i> (Nitz.) Compere								4		
	600	600	600	600	600	600	600	600	600	600

Appendix C. Macroinvertebrate Orders

Emergence traps: 35 sites/composite samples		
Class	Total	Notes
Insecta	10	
Arachnida	3	
Entognatha	1	Collembola has recently been updated to class but we treated it as an Order, Enognatha was the previous class that Collembola was placed
Order	Total	Notes
diptera	1659	selected to contract for species ID
isoptera	511	*1 plot contained 382 isopterans that were covered in acari
acari	488	subclass/ *1 plot contained 479
hymenoptera	26	selected to contract for species ID
hemiptera	24	selected to contract for species ID
araneae	18	selected to contract for species ID
collembola	14	selected to contract for species ID
coleoptera	13	selected to contract for species ID
ephemeroptera	7	
opiliones	7	
trichoptera	5	
lepidoptera	3	
thysanoptera	1	
psocoptera	1	
Total number	2777	

Appendix C: Macroinvertebrate Orders

Pitfall traps	253 samples	*not complete, 46 more samples to process
Class	Total	
Insecta	13	
Arachnida	4	
Diplopoda	3	
Gastropoda	1	
Chilopoda	2	
Malacostraca	2	
Bivalvia	1	not classified to order
Maxillopoda	1	1 subclass
Entognatha	1	
Orders	Total	
collembola	10243	selected to contract for species ID
diptera	1741	selected to contract for species ID
coleoptera	1130	selected to contract for species ID
hymenoptera	1273	selected to contract for species ID
hemiptera	1286	selected to contract for species ID
isoptera	2	
trichoptera	11	
lepidoptera	40	
ephemeroptera	0	
thysanoptera	43	
psocoptera	47	
orthoptera	134	
mecoptera	3	
plecoptera	1	
pseudoscorpiones	14	
opiliones	25	
araneae	1709	selected to contract for species ID
acari	2292	selected to contract for species ID, but have not identified a taxonomist
isopoda	46	
pulmonata	84	
copepoda	11	
amphipoda	1	
bivalvia*	4	not sorted to Order
julida	142	
polydesmida	35	
chordeumatida	1	
lithobiomorpha	4	
geophilomorpha	1	
unknown	44	
Total number	20367	

Appendix D

Revised Site Level Assessment Method (SLAM) for Forested Wetlands

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 SuAsCo 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
- 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 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 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
- iButton
- 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 ½" 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)
- 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 Miller's and SuAsCo 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.

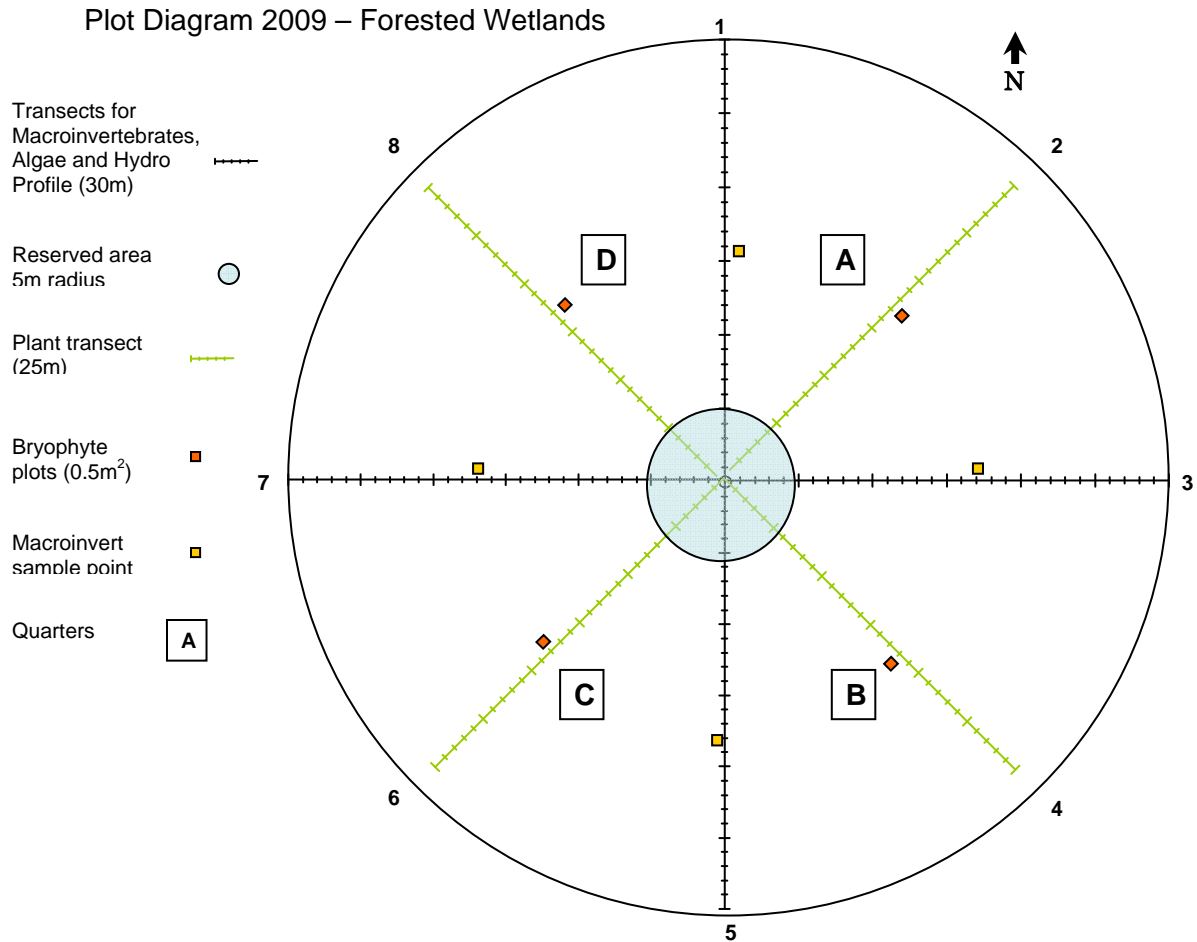


Diagram of sampling area. Eight 25 m transects run at 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° compass bearings. The location for all samples (algae, water chemistry, etc.) will be noted on the plot diagram.

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 ≥ 15 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.

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. The water level where the HOBO is placed will be recorded at each plot visit 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.

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.

Data will be uploaded and analyzed to determine the relative hydroperiod (hydroperiod during the survey period) of the wetland based on the temperature data. Procedures for uploading data and setting recording intervals will be followed according to the manufacturer's instructions (See QAPP Appendix J).

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. 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.

(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.

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 vile. Keep rinsing the pan with DI water until there is 50ml in the vile. Add 1ml of M3 per 50ml of benthic leaf scrapings for preservation.

Clean the pan and spoonula 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

Rinse a 100 ml plastic beaker which will serve as a water sampler three times with sample water before collecting a water 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. The water grab sample will be collected in a 50 ml vile. Add 1ml of M3 per 50ml of the water sample for preservation. Repeat for each transect.

Clean the water grab sampler after sampling.

(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 vile. 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 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 during excavation of pitfall traps and via midden counts (Lawrence and Bowers 2002, Hale et al 2005):

Collect earthworms from the soil at 4 pitfall trap excavations at 15m (see below).

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.

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

(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. Containers will be stored in the lab 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 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 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 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.

- g. 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.

- h. 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.
- i. 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.
- j. 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).
- k. 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.
- l. Refer to resources on regional flora if necessary (Gleason & Cronquist 1991, Macgee & 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. 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).

8.6 Sampling Intensity

For each watershed 3 to 8 sites will be selected to conduct a sampling intensity analysis to determine species area relationships for invertebrates collected with emergence traps and the stovepipe sampler, and algae. The number of samples will be doubled for each site (e.g. 4 emergence traps increased to 8). Sites will be selected randomly along a hydrologic gradient. The sites will be categorized grossly to very wet and dry based on the first site visit in May. Rarefaction will be used to analyze species richness across the range of sample sizes to determine the number of samples required to adequately represent species diversity.

8.7 Protocol for Decontamination of Field Equipment

Inspect all equipment for debris and removed 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 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.

13. References

Anderson, L.E., H.A. Crum, and W.R. Buck. 1990. List of the mosses of North America north of Mexico. *The Bryologist* 93:448-499.

Anderson, L.E. 1990. A checklist of *Sphagnum* in North America north of Mexico. *The Bryologist* 93:500-501.

Brinson, M. M. 1993. "A hydrogeomorphic classification for wetlands," Technical Report WRP-DE-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. NTIS No. AD A270 053.

Cobb, B., E. Farnsworth, and C. Lowe. 2005. *A Field Guide to the Ferns and their Related Families*. Houghton Mifflin Co., Boston, MA. 281 + xviii pp.

Connors, B. 2006. Protocols for Decontaminating Biomonitoring Sampling Equipment. ME Department of Environmental Protection DEPLW0641.

Cowardin, L.M., V. Carter, F.C. Golet, and E.T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. FWS/OBS-79/31. U.S.D.I. Fish and Wildlife Service, Washington D.C.

http://wetlands.fws.gov/Pubs_Reports/Class_Manual/class_titlepg.htm

Danielson, T. 2004. Protocols for Collecting Water Grab Samples in Rivers, Streams, and Freshwater Wetlands. ME Department of Environmental Protection DEPLW0637.

- Danielson, T. 2006. Protocols for Sampling Algae in Wadeable Rivers, Streams, and Freshwater Wetlands. ME Department of Environmental Protection DEPLW0634
- DiFranco, J. 2006. Protocols for Sampling Aquatic Macroinvertebrates in Freshwater Wetlands. ME Department of Environmental Protection DEPLW0640.
- Esslinger, T.L. 2007. A cumulative checklist for the lichen-forming fungi, lichenicolous, and allied fungi of the continental United States and Canada. North Dakota State University: <http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm>.
- Gleason, H.A. 1952. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. Hafner Press, NY, NY. 3 volumes, 1,732 + lxxv pp.
- Gleason, H.E. and A. Cronquist. 1991. Manual of the Vascular Plants of Northeastern United States and Adjacent Canada. 2nd edition. New York Botanical Garden Press.
- Jackson, Scott. 1995. Delineating Bordering Vegetated Wetlands Under the Massachusetts Wetlands Protection Act: A Handbook. MA Department of Environmental Protection. Boston, MA. 89 + 5 pp.
- Magee, D.W. and H.E. Ahles. 1999. Flora of the northeast: A Manual of the Vascular Flora of New England and Adjacent New York. 1213 pages. University of Massachusetts Press, Amherst, MA.
- McGarigal, K., B.W. Compton, S.D. Jackson, K. Rolih, and E. Ene. 2005. Conservation Assessment Prioritization System (CAPS) Highland Communities Initiative PHASE 1. Final Report. Landscape Ecology Program, Department of Natural Resources Conservation, University of Massachusetts, Amherst (URL: http://www.umass.edu/landeco/research/caps/reports/caps_reports.html)
- Merritt, R.W., K.W. Cummins (editors). 1996. An introduction to the aquatic insects of North America, Third Edition. Dubuque, IA: Kendall/Hunt Publishing Company. 862 pp.
- Newcomb, L. 1977. Newcomb's Wildflower Guide. Little, Brown & Co., Boston, MA. 490 + xxii pp.
- Petrides, G.A. 1972. A Field Guide to Trees and Shrubs. Houghton Mifflin Co., Boston, MA. 428 + xxxii pp.
- Reynolds, J. W. 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum Miscellaneous Publication, Toronto, Ontario, Canada.
- Scanlon, J. 2006. MassWildlife Landcover Mapping Decision Rules. Internal Document. Massachusetts Division of Fisheries and Wildlife, Westborough, MA.
- Schauff, M.E. 1986. Collecting and Preserving Insects and Mites: Techniques and Tools. Systematic Entomology Laboratory, USDA, Washington, D.C.

Somers, P. K. Lombard, and R. Kramer. 2006. A Guide to Invasive Plants in Massachusetts. Massachusetts Division of Fisheries & Wildlife, Natural Heritage & Endangered Species Program, Rabbit Hill Road, Westborough, MA 01581.

Schuster, R.M. 1974. The Hepaticae and Anthocerotae of North America: East of the Hundredth Meridian Volume 3. Columbia University Press, New York, NY, USA and London, UK.

Schwert, D. P. 1990. Oligochaeta: Lumbricidae. Pages 341–356 in D. L. Dindal, editor. Soil biology guide. John Wiley and Sons, New York, New York, USA.

Stevenson, R.J. 2001. Using Algae to Assess Wetlands in Multivariate Statistics, Multimetric Indices, and Ecological Risk Assessment Framework. pages 113-140 in Bader, R.B., Batzer, D.P. and Wissinger, S.A. (eds.), Bioassessment and Management of North American Freshwater Wetlands. John Wiley & Sons, NY, NY.

Swain, P.C. and J.B. Kearsley. 2001. Classification of the Natural Communities of Massachusetts DRAFT - 2001- (version 1.3) Reprinted 2004. Massachusetts Division of Fisheries and Wildlife, Westborough, MA.

U.S. EPA, 2002. *Methods for Evaluating Wetland Condition: Developing an Invertebrate Index of Biological Integrity for Wetlands*. Office of Water, U.S. Environmental Protection Agency, Washington , DC. EPA-822-R-02-019

U.S. EPA. 2002 *Methods for Evaluating Wetland Condition: Using Algae to Assess Environmental Conditions in Wetlands*. Office of Water, U.S. Environmental Protection Agency, Washington , DC. EPA-822-R-02-021

Appendix E: Field Data Forms Used in 2008

FORM General Site Information	PointID:	Pg of
Surveyors:	Date:	

Water geochemistry			
	pH	Conductivity	Temperature
1			
2			
3			
4			

Water depth					
Transect	5m	10m	15m	20m	25m
1					
2					
3					
4					

Topographic Complexity

Comments:

Include the following info: Number of transects, transect lengths, transect azimuth, location of emergence traps (ET), pitfall traps (PT), where stovepipe and algae samples were taken, location of data logger and denote vascular plant transects, and bryophyte plots. Add comments below.

Comments:

[illegible]

FORM Bryophytes	PointID:	Pg	of
Surveyors:	Date:		
(D)=Dead (V)=Voucher	Start Time:		

[illegible]

Cover class + = <1%, 1=1-5%, 2=6-25%, 3=26-50%, 4=50-75%, 5=>75%

Cover class += <1%, 1=1-5%, 2=6-25%, 3=26-50%, 4=50-75%, 5=>75%

Notes:

Herbarium of the University of Massachusetts, Amherst
BRYOPHYTES or LICHENS of The Chicopee Watershed

Taxon: _____

PlotID: _____ **Subplot/Transect#** _____

State: _____ **County:** _____ **Town:** _____

UTM e: _____ **UTM n:** _____ **Zone:** _____ **Datum:** NAD 83

Substrate & Site Characteristics (circle all that apply):

Soil: mineral soil, gravel, sand, loam, silt, clay, litter, duff, humus, peat, moss, or litter-fall

Rock type: granitic, serpentine, metamorphic, sedimentary, volcanic, or calcareous

Rock feature: outcrop, boulder, cliff, crevice, ledge, talus, or under-hang

Tree or Shrub: species: _____ **location:** base, trunk, branch, root, stump, snag, recently fallen tree, rotten log (decay class: _____), bark, wood, or tree root-wad

Light: full sun, partial shade, full shade **Elevation:** _____ **ft.** **Slope** _____ **% Aspect:** _____°

Habitat: bog/fen, dense/open/cut forest, lake/pond, meadow, seep, spring, swamp, waterfall stream/creek/river (intermittent), wetland, seasonally wet area, splash zone, or submerged

Site Moisture Regime: dry, mesic, moist, or wet

Collector: _____ **Coll. No.** _____ **Date:** _____

Verified by: _____ **Date:** _____ **Notes:** _____

Appendix E: Field Data Forms used in 2008

Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Appendix E: Field Data Forms used in 2008

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Benthic Algae Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Surface Area:	Amt. of M3 added:
Comments:	

Appendix E: Field Data Forms used in 2008

Phytoplankton Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Amt. of M3 added:	
Comments:	

Phytoplankton Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Amt. of M3 added:	
Comments:	

Phytoplankton Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Amt. of M3 added:	
Comments:	

Phytoplankton Sample	
Date:	Plot ID:
Sample ID:	Collector ID:
Amt. of M3 added:	
Comments:	

Appendix E: Field Data Forms used in 2008

Emergence Trap Sample		Emergence Trap Sample	
Date Set:	Date Collected:	Date Set:	Date Collected:
Plot ID:	Sample ID:	Plot ID:	Sample ID:
Collector ID:		Collector ID:	
Comments:		Comments:	

Emergence Trap Sample		Emergence Trap Sample	
Date Set:	Date Collected:	Date Set:	Date Collected:
Plot ID:	Sample ID:	Plot ID:	Sample ID:
Collector ID:		Collector ID:	
Comments:		Comments:	

Emergence Trap Sample		Emergence Trap Sample	
Date Set:	Date Collected:	Date Set:	Date Collected:
Plot ID:	Sample ID:	Plot ID:	Sample ID:
Collector ID:		Collector ID:	
Comments:		Comments:	

Emergence Trap Sample		Emergence Trap Sample	
Date Set:	Date Collected:	Date Set:	Date Collected:
Plot ID:	Sample ID:	Plot ID:	Sample ID:
Collector ID:		Collector ID:	
Comments:		Comments:	

Appendix E: Field Data Forms used in 2008

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Macroinvertebrate Sample (PT)	
Date Set:	Date Collected:
Plot ID:	Sample ID:
Surveyor ID:	
Comments:	

Appendix E: Field Data Forms used in 2008

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	

Macroinvertebrate Sample (ST)	
Date:	Plot ID:
Sample ID:	Collector ID:
Comments:	