# St. Louis River AOC R2R Support Projects:

# Ecological Monitoring and Assessment (CR#6403):

## Aquatic Macrophyte Sampling and Analysis

# Quality Assurance Project Plan

Submitted to the Minnesota Pollution Control Agency by:

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# A: PROJECT MANAGEMENT

## A1: Title and Approval Sheet

**St. Louis River AOC R2R Support Projects:**

**Ecological Monitoring and Assessment (CR#6403):**

**Aquatic Macrophyte Sampling and Analysis**

We, the undersigned, have reviewed and support this Quality Assurance document.

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Nelson French, Lake Superior Unit Supervisor, MPCA — Date

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Deepa de Alwis, QA Officer, MPCA — Date

10 Feb 2014

George Host, Principal Investigator,
University of Minnesota Duluth — Date

10 Feb 2014

Carol Reschke, Scientist,
University of Minnesota Duluth — Date

## A2: Table of Contents:

## A3: QAPP Distribution List

**Table 1. QAPP Distribution List**

| | | | |
|---|---|---|---|
| Rajen Patel | US EPA Region 5 | patel.rajen@epa.gov | 312-886-5741 |
| Nelson French | Minnesota Pollution Control Agency | nelson.french@state.mn.us | 218-302-6623 |
| Dan Breneman | Minnesota Pollution Control Agency | Dan.Breneman@state.mn.us | 218-302-6624 |
| Deepa de Alwis | Minnesota Pollution Control Agency | deepa.dealwis@state.mn.us | 651-757-2572 |
| George Host | University of Minnesota Duluth, Natural Resources Research Institute | ghost@d.umn.edu | 218-270-4264 |
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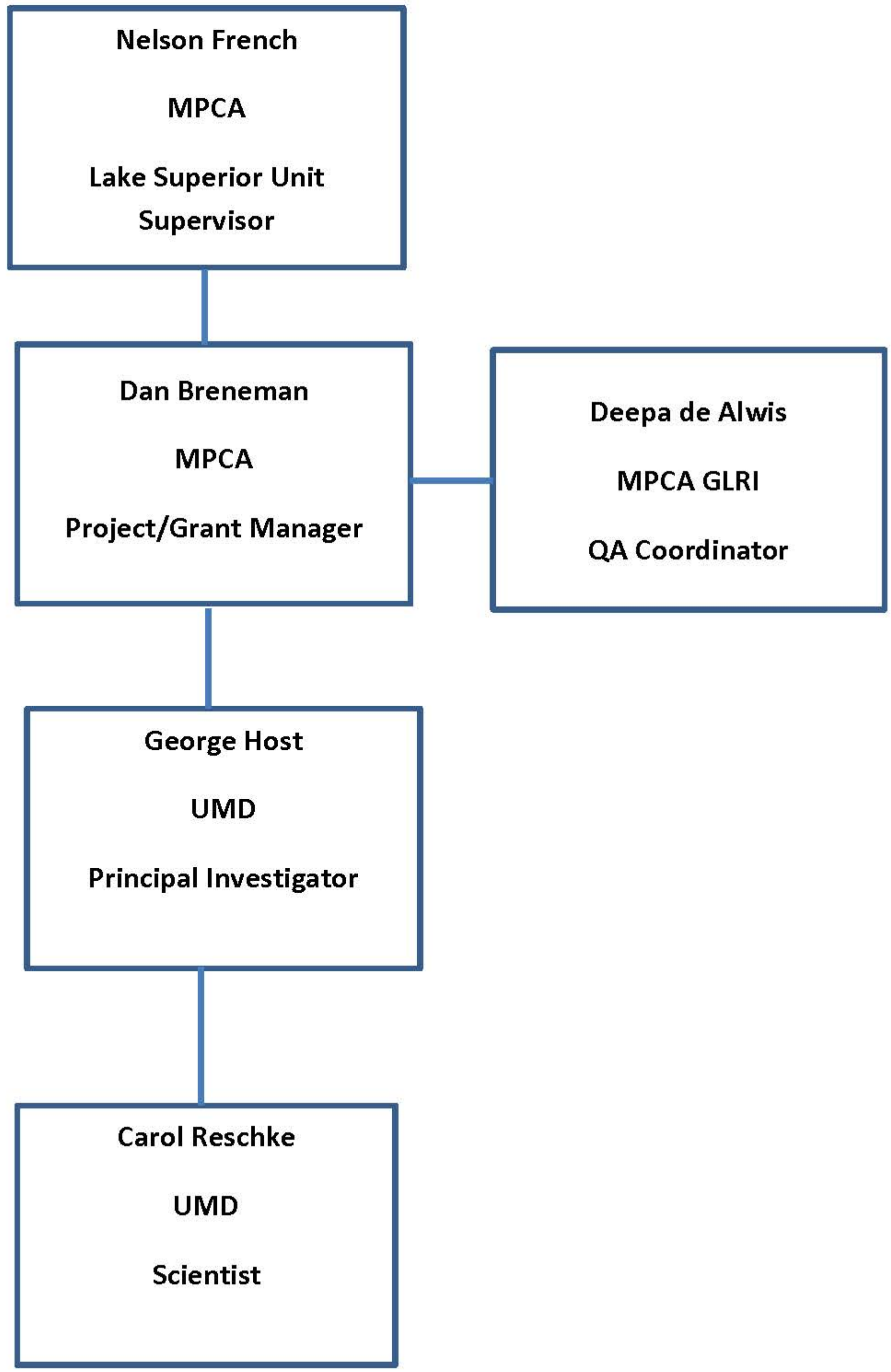
## A4: Organizational Chart



## A4.1: Project Individual Responsibility

**MPCA Project Manager**:

- outline objectives,
- approve work and financial updates, and
- review data upon final delivery

**MPCA Quality Assurance (QA) coordinator:**

- ensure all QA/QC protocols are followed, and
- ensure Quality Assurance Project Plan (QAPP) is reviewed and approved.

**Principal Investigator (PI):**

- general project and budget oversight,
- review data analysis and summary, and
- review of reports and deliverables.

**UMD Scientist**:

- establish sampling points,
- draft a sampling plan,
- conduct field sampling,
- identify macrophyte specimens,
- entry and quality control of aquatic vegetation and habitat data,
- analysis of data and classification of plant communities,
- summarize composition of classified plant communities,
- description and mapping of plant communities by site,
- verification of plant taxonomic resolution,
- conduct internal QA/QC measures, and
- prepare reports and deliverables.

## A5: Problem Statement and Background

MPCA is currently developing a comprehensive, long term plan to delist the St. Louis River Area of Concern (AOC) under a grant from USEPA and other project partners. A major contributor to AOC status is legacy sediment contamination, resulting in nine Beneficial Use Impairments (BUIs) identified in Remedial Action Plan documents for the AOC (MPCA 2013). The monitoring and assessment of aquatic macrophytes described below will provide biological data pertinent to the delisting of BUI 9: Loss of Fish and Wildlife Habitat. It is necessary to have data regarding the current, degraded status of the AOC in order to determine if restoration efforts would be successful, and to determine if delisting of the AOC is warranted in the future.

Sediment characteristics, habitat conditions, and the resulting aquatic plant communities provide vital links between BUIs, restoration decisions, and recovered condition. Understanding biotic responses to anthropogenic stressors, such as aquatic vegetation response to sediment contamination, is important for determining overall community health, designing restoration plans, and ultimately assessing environmental recovery. Currently, R2R conceptual plans are designed primarily based on sediment contamination recommendations, with limited biological data available for decision support. There is currently inadequate understanding of the effects of sediment contaminants or contaminant concentrations on aquatic macrophytes within the St. Louis River AOC. Establishing relationships between contamination levels in the AOC and vegetation metrics such as macrophyte taxonomic diversity, plant community composition, and lack of vegetation ("emptiness") will aid restoration decisions and minimize errors when interpreting environmental conditions based solely on contaminant studies.

To alleviate this lack of data on biological indicators to assess AOC BUIs, MPCA has contracted with the INVESTIGATORS to sample aquatic macrophyte communities at R2R locations and reference locations within the estuary. These data will provide baseline aquatic vegetation conditions for R2R sites, allowing later sampling to determine if there would be improvement following R2R work, and will also provide data from least-disturbed locations within similar areas of the estuary as appropriate aquatic vegetation restoration targets.

## A5.1 Objectives

1. Quantitatively assess the assemblages of major aquatic macrophyte species within the restoration sites and comparable reference sites.

2. Refine our current plant community classification of aquatic vegetation types in the St. Louis River AOC by combining data sampled for this project with comparable data from recent research efforts in the estuary.

3. Create a map of existing aquatic vegetation in sites sampled using the refined vegetation classification.

4. Determine if there are correlations between aquatic vegetation metrics and sediment quality or water quality in the St. Louis River estuary.

## A6: Project/Tasks Description and Schedule

*Task 1: Sample aquatic macrophytes*

Aquatic macrophytes will be sampled using grid point-intercept methods (MN DNR 2012) at each of seven remediation-to-restoration (R2R) sites within the AOC, as well as at five to eight comparable reference locations that have been matched for habitat similarity. Aquatic macrophyte vegetation data from these samples will be combined with comparable data from other recent research efforts in the St. Louis River estuary (e.g. 21st Ave West and 40th Ave West sampling by NRRI, MN DNR estuary wide sampling, and WI DNR sampling in selected bays and tributaries), which used grid point-intercept sampling methods. The pooled data set will be used to refine a classification of aquatic plant communities in the St. Louis River estuary. The classification will be done using the same methods as in the 21st Ave West and 40th Ave West studies (Host et al. 2012; Brady et al. 2010); however the larger pooled data set is likely to allow a finer resolution to the classification, with vegetation types that more closely relate to fish and macroinvertebrate habitats. The point-intercept data and refined vegetation classification will be used to map existing vegetation in the R2R and reference sites. These baseline data and the refined vegetation classification will provide managers the ability to assess the success of future restoration efforts, allowing future post-restoration sampling to evaluate biotic improvement relative to the baseline samples from restoration sites, and to compare restoration progress with comparable reference sites that serve as models for restoration planning.

Aquatic macrophytes at each of seven R2R sites and up to eight reference locations will be sampled using the point-intercept survey method with sample points evenly spaced in a grid pattern across the sample area. The INVESTIGATORS will be responsible for ensuring that aquatic macrophytes are sampled at each of seven R2R sites and five to eight reference locations that have been matched for habitat similarity and river/estuarine position to R2R sites. Sampling for each site will be repeated in two field seasons to compensate for annual variations in plant abundance and growth. Final sampling density at R2R sites will approximate 1 sampling point for every 2 acres in area, with lower density at very large sites and higher density at small sites to ensure adequate number of samples to characterize each site. Site list and point density are provided in Table 2. Field sampling will be conducted from mid-July until mid-September each year, with approximately 1/3 of the effort accomplished each year.

*Task 2: Laboratory identification of collected aquatic plants*

Aquatic plants collected for laboratory identification will be keyed out and identified to genus or species if possible. Data will be entered into a data format provided by MPCA to fit the AOC biotic data database, followed by QC of all data entered. Laboratory identification will be conducted from mid-July through the fall, winter, and early spring of each year; after each year's specimens are identified, vouchers will be sent to herbaria or experts for QC.

*Task 3: Data entry and analysis*

Field data on aquatic macrophytes and supplemental data on habitat conditions at each sample point will be entered into a database. Data will be analyzed using multivariate statistics software to refine an aquatic vegetation classification. Aquatic vegetation metrics will be summarized from the data set, and vegetation metrics will be correlated with environmental variables, including contaminant concentrations obtained from secondary sources.

*Task 4: Create aquatic vegetation maps of reference and restoration sites*

The refined aquatic vegetation classification will be used to create maps of aquatic vegetation types at each site sampled. Mapping will be done using GIS software.

## A7: Data Quality Objective (DQO) and Criteria for Measurement Data

The primary DQO for this study is the acquisition of accurate and representative measurements of the biological, habitat, and environmental parameters for the study sites in the St. Louis River Estuary, collected in a manner consistent with Minnesota's Sensitive Lakeshore Identification Manual (MN DNR 2012). By following these methods, we will generate representative and reproducible monitoring results.

In addition to using the DNR Manual (MN DNR 2012), which is designed to insure representative sampling, our field crew staff are aware of how weather events can compromise sampling activities. In particular, riverine systems may experience high flows following storms which would compromise safe, efficient, and representative sampling. Sampling should not be done until streams/rivers return to 150% or less of baseflow conditions. Likewise if extreme drought occurs, sampling should not be done until streams/rivers return to 50% or more of baseflow conditions. Crews can be assisted with this decision by accessing the nearest USGS gauging station flow records and consulting with colleagues working in the AOC.

### Accuracy

Accuracy is the closeness of a measured value to its true value, and there are several components of accuracy in this study. One is in species identification. The project macrophyte expert (Carol Reschke, University of Minnesota Duluth, NRRI) is already expert at identifying about 65 to 70% of the aquatic plant species in the estuary. The remaining plants include narrow-leaf species of *Potamogeton*, as well as some grasses, sedges, rushes, and composites.

Any plants not easily keyed to species will be verified during the course of the project by consulting with herbarium staff at one of four herbaria: University of Minnesota Duluth (Olga Lakela Herbarium), University of Minnesota (Bell Museum), University of Wisconsin Superior, or University of Wisconsin Stevens Point (Freckmann Herbarium). A set of voucher specimens will also be compiled during the project, with at least one voucher specimen included for at least 90% of all plant taxa in the final dataset. These voucher specimens will be shown to aquatic macrophyte experts at one of the four herbaria, and specimens will be offered for deposition at the herbarium in exchange for confirming the taxonomic identity. The performance criteria for this QA/QC step will be 90% accuracy of fertile plants or plants that can typically be identified in sterile condition. This QA/QC step will be based on a combination of field and laboratory identification. Preserved specimens or digital photographs are routinely used as part of the identification process.

**Precision**

For vegetation samples, the ability to estimate precision (the closeness of repeated measurements of the same quantity) is built into the sampling design with a large number of points sampled per site, and the sample number split between two sample years. A comparison of similarity of species composition in a site between sample years can be used to quantify precision using a simple distance measure such as 1 - (2w/a+b), where w is the number of species in common between sample years, a is the number of species recorded in year 1 and b is the number of species recorded in year 2. A lower value of the distance measure is interpreted as greater similarity between sample years; however it is understood that year to year variability is characteristic of many plant communities.

**Representativeness**

This refers to how well the sampling represents vegetation of the site at large. The grid point-intercept sampling design ensures that the major habitats within each site will be sampled proportionally to their area, while additional targeted sample points will ensure sampling of less common habitats. To verify this, we will plot all points sampled in each site on aerial photographs of the site in GIS, and compare sample data to the GIS datasets used to select sample points.

**Comparability**

Data comparability among crews within the project and to other non-project data will be achieved by using standard, accepted point-intercept sampling methods; creating metadata explaining the methods; and having consistent training and QA/QC for all field staff.

**Completeness**

Completeness is calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites. Also a species/area curve for sample points will be created to access adequacy of the sample.

**Bias**

This refers to systematic bias by a crew or sampling method. Bias will be minimized by the consistency of field crew; investigator C. Reschke will be primarily responsible for the sampling, and she will train and oversee any other samplers. The grid point-intercept sampling method itself reduces bias by recording only presence/absence data rather than requiring an estimate of percent cover, a common source of sampling bias among investigators, especially in sampling submerged aquatic vegetation.

**Sensitivity**
In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification will depend on the life-stage of the plants and the condition of the plants, which is primarily controlled by the time of year of sampling. Our sampling is timed so that the most species will be most identifiable when field crews are sampling. Constructing a species/area curve will help assess how well uncommon species were sampled.

## A8: Special Training Requirements

### Sampling and Plant Identification
Aquatic macrophyte sampling will be led by Carol Reschke (NRRI), who has years of experience with aquatic plant identification and wetland vegetation sampling. Her field assistants/boat operators have field sampling experience using the grid point-intercept methods described below. Field training will consist of a review of proper field sampling methods at the beginning of each field season. Training will include review of site maps, review of equipment needed, location and efficient navigation to the grid of sample points, protocols for collecting and labeling plant specimens, and protocols for when to take samples for QA/QC. In addition, we will ensure that all field personnel are up to date on the suite of relevant safety training required by the University of Minnesota.

The collaborators in this project have conducted extensive plant sampling in the St. Louis River estuary, so species lists, picture keys, and field data forms include most plants that will be encountered during the project. The species lists also include all of the major invasive plants known from estuary wetlands.

### Field Crew Safety
All field crews are trained on water safety, especially regarding the safety risks involving high winds. Waves during high winds can compromise field crew safety. The field crew will not be allowed to sample during small craft advisories or if they are having difficulty navigating to sample points due to wave action. If possible they should move to sample more protected sites, or discontinue field sampling until conditions are favorable. In all cases, field crews should not attempt to sample if there is any concern for their safety.

## A9: Documentation and Records

We will create a customized Access database to store vegetation, environment, and other relevant GIS data from the St. Louis River estuary. The database will be formatted so that it is compatible with the AOC biotic data database being developed for the MPCA. The database will allow compilation of new data collected during this project, as well as recently sampled data from other projects' point-intercept sampling of aquatic vegetation in the estuary.

# B: MEASUREMENT/DATA ACQUISITION

## B1/B2: Sampling Design and Sampling Methods

The grid point-intercept sampling method (Madsen 1999) records frequency of occurrence (presence/absence) as the measure to estimate plant abundance and individual species abundance. We use this method to document any aquatic macrophytes of any growth form present at each sampling point, which is defined by the method as a 1 $m^2$ area. Estimates of overall vegetation frequency are later calculated by determining the proportion of survey points that "hit" or intercept vegetation. Frequencies of individual species can also be calculated by determining the proportion of survey points that "hit" or intercept an individual plant species (MN DNR 2012).

The grid point-intercept vegetation survey methodology has been extensively used in Minnesota by the lead aquatic plant ecologist (Donna Perleberg) of the Minnesota DNR Wildlife Shallow Lakes Program (MN DNR 2012), and has been adopted by the Wisconsin DNR as their standard lake vegetation survey method. In comparisons of several boat-based aquatic vegetation survey methods, the grid point-intercept method was found to provide the most rapid, repeatable, GIS-based method to assess lake-wide plant species abundance and associated depth data (MN DNR 2012). Williams et al. (2008) recommended the point-intercept survey for whole- lake assessments where statistical comparisons are needed. Furthermore, because the grid point-intercept method collects only frequency-of-occurrence data, other advantages include consistency in data collection between different surveyors, ability to monitor a variety of plant growth forms, opportunity to monitor at flexible times throughout the growing season, and uncomplicated data analysis (Nichols 1984, Elzinga et al. 2001). In addition, frequency data are recommended as an appropriate abundance estimate when studying long-term changes in communities (Nichols 1999). It may not be appropriate to estimate areal coverage from these data because accuracy would be dependent on the resolution (spacing of points) of the survey (Williams et al. 2008).

In the grid point-intercept method, survey points are established throughout the littoral (or the vegetated) zone on a grid derived by establishing points at a fixed spacing using a computerized geographic information system (GIS). A systematic placement of survey points is appropriate because vegetation mapping of project areas is a primary objective. Although we have recent maps of depth contours in the estuary, flooding in June 2012 likely changed many depths. Therefore the exact extent of the vegetated littoral zone is unknown and it is easier to establish sample points regularly across entire bays. Overall we plan to sample approximately 265 to 270 points each year for a project total of at least 800 sample points. Points will be distributed among sites according to size of each site (Table 2), with no fewer than 10 total points per site. Sampling for most sites will be repeated in two field seasons to compensate for annual variations in plant abundance and growth. The exceptions are the R2R sites at 21st Ave West and 40th Ave West, which were previously sampled by NRRI in 2011 and 2010, respectively, and therefore these two sites will be sampled only one summer during this project.

Final sampling density at R2R sites will approximate 1 sample point for every 2 acres in area, with somewhat lower densities at very large sites and higher densities at small sites to ensure adequate number of samples to characterize each site. Locations of R2R and Reference sites are shown in Figure 1; and a site list, with approximate sample point density, and sample year is provided in Table 2.

Aquatic macrophytes will be sampled in the R2R and reference sites using a variation of the grid point-intercept sampling method described in Chapter 2 of the Minnesota's Sensitive Lakeshore Identification Manual for sampling lake-wide vegetation (MN DNR 2012). The MN DNR methods are designed for sampling entire lakes; we have modified the method to sample bays or portions of the St. Louis River estuary. Each sample site as defined by site boundaries provided by MPCA will be sampled as if it were an entire small lake. Field survey methods for sampling aquatic vegetation at each sample point will be similar to the aquatic vegetation sampling protocol used in 2010-2011 within the 21st Ave West and 40th Avenue West Project Areas (Host et al. 2012; Brady et al. 2010), and in 2010 aquatic vegetation sampling completed by John Lindgren of MN DNR Fisheries throughout the estuary (MN DNR unpublished data; Reschke et al. 2011).

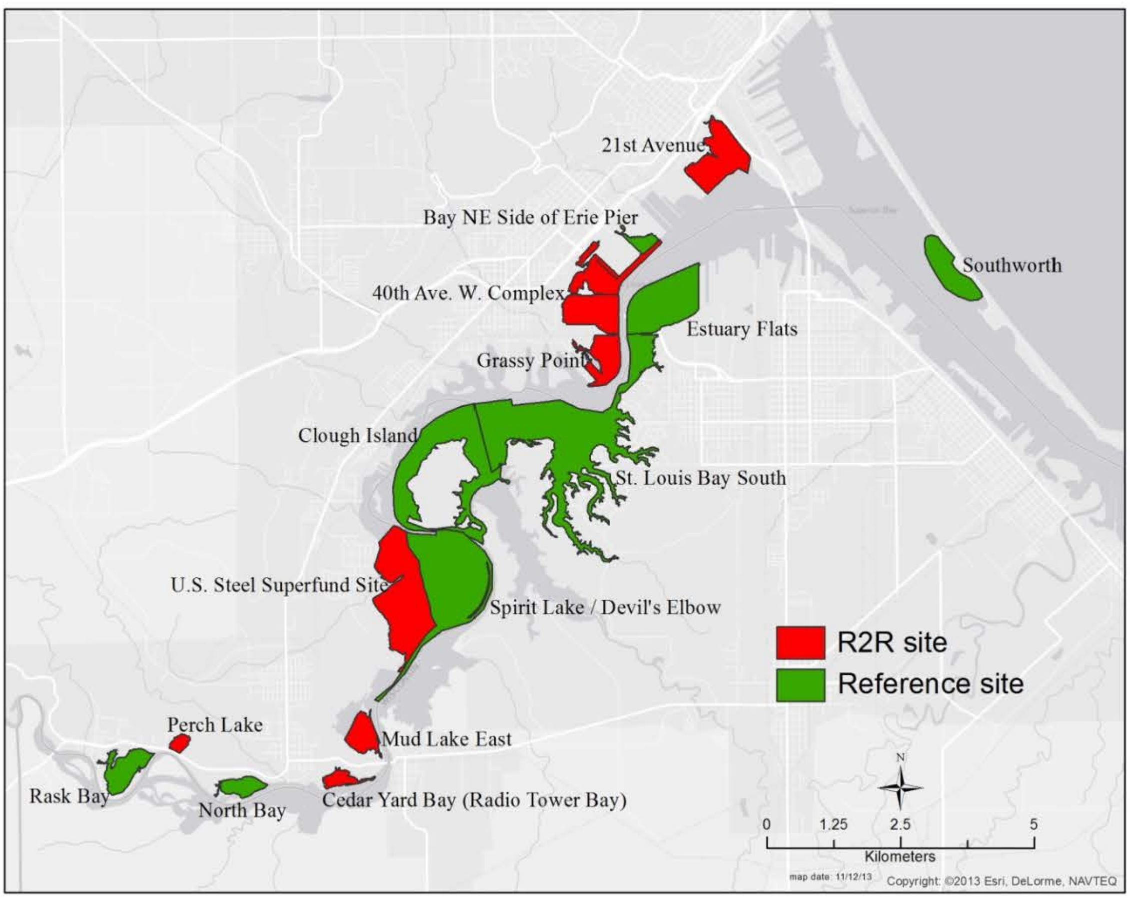



**Figure 1. Map of the St. Louis River estuary and site locations. Refer to Table 2 for more information on individual sites.**

**Table 2. Pre-Restoration and Reference Sampling Sites, 2013 - 2015**

| Restoration Sites | Acres | pts/yr | # yrs | Total pts | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|
| 21st Ave West Complex | 201 | 92 | 1 | 92 | 92 | | |
| Grassy Point Complex | 115 | 30 | 2 | 60 | 30 | 30 | |
| US Steel | 250 | 63 | 2 | 126 | | 63 | 63 |
| Mud Lake East | 80 | 20 | 2 | 40 | | 20 | 20 |
| Cedar Yard/Radio Tower Bay | 38 | 19 | 1 | 19 | 19 | | |
| Perch Lake | 21 | 6 | 2 | 12 | | 6 | 6 |
| 40th Ave West Complex | 325 | 82 | 1 | 82 | | 82 | |
| **Reference Sites** | **Acres** | **pts/yr** | **# yrs** | **Total pts** | **2013** | **2014** | **2015** |
| Spirit Lake / Kilchlis Meadow | 160 | 40 | 2 | 80 | 80 | | 80 |
| Clough Island - shallows | 82 | 21 | 2 | 42 | | 21 | 21 |
| Estuary Flats - shallows | unk | 10 | 2 | 20 | 5 | 5 | |
| Southworth Marsh - shallows | unk | 10 | 2 | 20 | 5 | | 5 |
| Bay by Erie Pier (40$^{th}$ Ave West) - shallows | unk | 5 | 2 | 10 | 5 | 5 | |
| North Bay - shallows | 60 | 15 | 2 | 30 | 15 | | 15 |
| Rask Bay - shallows | 98 | 25 | 2 | 50 | | 25 | 25 |
| St. Louis Bay South - deepwater | unk | 6 | 2 | 12 | 6 | 6 | |
| **Project Total** | | | | 695 | 257 | 263 | 235 |

*Sample Point Selection in GIS*

In previous studies of the St. Louis River estuary, we identified water depth, fetch, and sediment texture as the three most important physical variables influencing aquatic vegetation in the estuary (Brady et al. 2010; Host et al. 2012). We will use these features in combination with aerial imagery to ensure that sample points selected are representative of the range of community types present in each sample site. ArcMap Geographic Information Systems (GIS) will be used to superimpose grids of evenly spaced sample points for comparison with other GIS data layers. Within each site to be sampled, we will adjust the spacing of the grid (density of sample points) to allow the area to be sampled at a final density of approximately 1 sample point for every 2 acres in area, while adequately representing the range of habitats present. Within each site we will allocate about 90% of the sample points to water depths less than 3 m deep, since we have found that aquatic vegetation in the estuary occurs primarily at water depths less than 2.7 m (Brady et al. 2010). Having some sample points at depths from 3 to 9 m allows us to confirm our current estimates of maximum water depths that allow growth of submerged aquatic vegetation. Limiting samples to depths no greater than 9m (ca 30 ft) is consistent with recommendations of the Minnesota Sensitive Lakeshore Identification Manual (MN DNR 2012).

Sample points will be selected from a grid across each site polygon as well as be placed to match up with recent sediment contaminant sampling (since 2008, as provided by MPCA). Grid points will be stratified by depth (0-1 m, 1-2 m, and > 2 m) to ensure that shallower depths (< 2 m) receive at least 2/3 of the sample effort at each site. In some instances, points will also be placed to coincide with relatively recent macroinvertebrate sampling (USEPA 2010 samples). The NRGIS lab will assist with point allocation on a grid system for each site, and will provide target points, along with alternate points for each target point in case the target point proves to be on dry land or is inaccessible. Target points and alternates will be uploaded to a GPS unit and used by field crews to navigate to the appropriate locations for each sample attempt.

After we select the final sets of points to be sampled in each restoration and reference area, we will evenly divide those points between sample years so that sites are sampled over two field seasons; with the exception that 40th Ave West and 21st Ave West sites will only be sampled in one field season, and data from that one field season will be combined with data collected in 2010 and 2011 from those two sites.

Data layers available in GIS that will be used for sample point selection include 1) bathymetric and sediment texture data produced during recent surveys by the Fond du Lac Natural Resources staff using a hydroacoustic sensor, 2) fetch data produced by GIS models, 3) locations of USGS CEC sample points, locations of MPCA contaminant sample points, and maps of SAV based on hydroacoustic sensor data from EPA (Angradi et al, 2013). Fond du Lac Natural Resources used a BioSonics Digital Echosounder System in combination with BioSonics VBT (Visual Bottom Typer) data processing software to provide bathymetric measurements and sediment textures. Ted Angradi of EPA used a pair of Biosonics transducers (420 and 120 Khz) DT-X Surface Unit to produce point maps of SAV, and then points were interpolated to produce a shapefile of polygons where SAV was present in portions of the estuary in 2011 and 2012.

*Preparation for Field Surveys*

We will create field sample forms similar to those used in 2010-2011 that include a list of the most common aquatic plant species in the estuary, as well as blanks for filling in additional species. The field form provides a checklist for documenting which species are present at each sample point, with room for recording seven sample points on each sheet. Maps of field sites showing distribution of the grid of sample points will be prepared to assist with navigation to points and keeping track of which points have been sampled. GIS coordinates for all sample points will be loaded onto two GPS units for navigation to each sample point. Field equipment will be checked prior to each day's fieldwork to confirm that everything is in good working order.

*Field Survey Methods*

Vegetation sampling will be conducted from mid-July until mid-September. Using a fishing boat or airboat, we will navigate the boat to each sampling point using GPS (accuracy typically within 5-6 m), and drop an anchor. At each sampling point we will first record any floating-leaf or emergent plants visible within a circular 1 $m^2$ sample area; then submerged aquatic plants in the same 1 $m^2$ area will be gathered by use of a double-headed rake attached to a rope. A circular sampling hoop will be available for estimating the 1 $m^2$ sample area for points sampled from the boat. The rake will be tossed into the water and allowed to sink down until it hits bottom; the rake will then be pulled a short distance along the bottom within the sample area, and then pulled by rope to the surface. Any plants gathered by the rake are then removed from the rake and placed into a plastic tub, and plant species are sorted, identified, and recorded on field forms. The rake will be tossed two times at each sample point. Some shallow water sample points may be too shallow for boat access; these sample points will be sampled by wading to the sample point, and the circular sampling hoop will be used to delineate the sample areas in shallow water. A similar procedure will be followed, except that instead of tossing a double-headed rake, the field crew will use a smaller hand-held rake (a 3-pronged hand cultivator) to sweep the sample area for submerged aquatic plants.

All plants will be identified to the level of genus and species when practical. For plants such as narrow-leaved pondweeds, which are difficult to identify to species, genus-level categories may be used and specimens collected for laboratory identification, if possible. Any plants that cannot be easily identified to species in the field will be collected in sealed plastic bags (e.g. Figure 2) and carefully labeled with 1) site code, 2) sample point code, 3) collection date, and 4) initial estimate of species or genus name. These specimens will then be examined with a dissecting microscope and keyed to either species or genus if possible. Since field specimens from sample points are not always in good condition for identification (e.g. often lacking flowers or fruits), there are likely to be some macrophyte specimens that cannot be identified; these will be recorded in the database as unknowns. Plant nomenclature will follow the MN DNR County Checklist (MN DNR 2010). Any algae collected by the rake will be noted; specimens of very common or abundant algae such as *Cladophora* spp. will be collected for identification to genus.

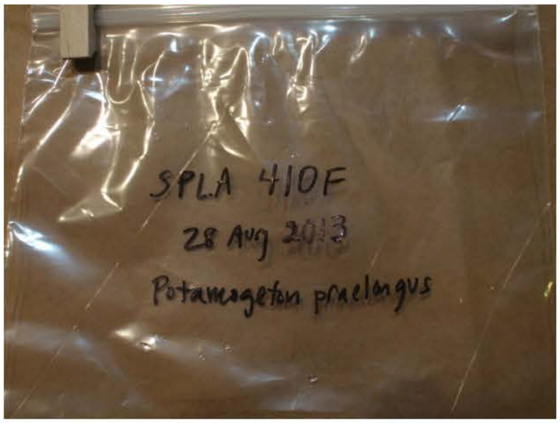



**Figure 2: Example of a labeled plastic bag from field collection, for illustration only.**
SPLA is the site code for Spirit Lake, 410F is the sample point location recorded with our GPS unit. The bag is a temporary collection bag that is discarded after the fresh plant is either identified or pressed for later identification.

*Supplemental Data*

In addition to plant species identification, we will also record the following environmental conditions at each sampling point: (1) water depth, (2) water clarity as measured by Secchi disk depth or turbidity tube depth, and (3) substrate type (muck, detritus, silt, sand, clay, gravel, rubble, boulder). Water depth will be measured either with a weighted cord marked in 1 dm increments, or in shallower water with a pole marked in 1 dm increments. Secchi disk depth will be measured with a standard Secchi disk lowered on a cord marked in either metric or English units; the person measuring Secchi depth will remove sunglasses before determination of Secchi depth. If the bottom is visible, then a turbidity tube marked in centimeters will be used as an indicator of water clarity; the person using the turbidity tube will remove sunglasses before determination of water clarity in the turbidity tube. Bulk samples for substrate characterization will be collected from the upper 8 cm of the bottom. A combination of up to three substrate types will be reported to describe the conditions at each sampling point, with one type rated as dominant and the others as second or third most prominent. Before leaving each sample point, a GPS waypoint will be taken to record the specific sampling location, to compare with the target location.

## B3: Sample Handling and Custody Requirements

When necessary for identification, plant specimens will be collected in zip-lock plastic bags for either fresh identification with hand lens or dissecting microscope, or specimens will be pressed for later identification in the laboratory or by herbarium staff. Specimens of submerged aquatic plants will have estuary water added to plastic storage bags. NRRI will obtain the necessary permits to appropriate and transport infested waters, and for collection of aquatic plants from the MN DNR and WI DNR. Estuary water collected with plant specimens will be disposed into a septic system.

Plastic collection bags will be marked with site code, sample point number, date, and a preliminary plant name (e.g. Figure 2) to facilitate later entering of plant identities on the field forms or into the digital database. Plant specimens will be stored in a field cooler or refrigerator until they can be identified or pressed. Plant specimens will be identified or pressed as soon as possible after sampling. Fragile aquatic plants will be floated in water in a shallow tub over herbarium mounting sheets, and the mounting sheets will be lifted out of the water so that aquatic plants are properly spread out and mounted on sheets before being dried in a plant press. Pressed plants will be dried either with heat or with a fan whenever possible. Reference materials at university herbaria are available for comparison with collected plant specimens. Plant samples will be destroyed following identification, except for those of interest for herbaria collections, or samples kept as short-term identification aids to assist in training new personnel or as vouchers. A voucher collection consisting of at least one specimen of 90% of the taxa in the final dataset will be compiled for confirmation by herbarium staff.

*Laboratory Identification of Aquatic Plants*

**Performance criteria**: Most specimens collected in the field will be identified to the species level as soon as possible after collection using identification keys and a hand lens or dissecting microscope. Specimens not identifiable to species because of lack of characteristic features (flowers, fruits, etc.) will be identified to the most precise taxonomic level possible. Specimens of questionable identity will be pressed. Unknown and unusual specimens that have flowers or fruits present will be sent to appropriate taxonomic experts for confirmation or refinement of taxonomic identity. Sterile or immature plants will be identified when possible; however, it may not be possible to identify all specimens. Target turnaround time for plant identifications is six months after the end of field sampling.

## B4: Analytical Methods

Field data will be entered into computer databases after the majority of plants have been identified. Aquatic plant data and associated environmental data will be analyzed using cluster analysis and ordination procedures with PC-ORD (version 6) software for multivariate analysis of ecological data (McCune and Grace 2002). Plant communities will be classified using multivariate cluster analysis routines, and then classes identified by cluster analyses will be evaluated in ordinations to determine the classification that is best correlated with environmental features. The final plant community classes will be assigned to each sample point and those classes will be used to prepare maps showing current vegetation of each R2R and reference site. Vegetation maps of reference sites will be useful models for designing restoration plans for R2R sites.

## B5: Quality Control

The investigators will ensure that sampling methods and QC procedures are followed, and that all deliverables are prepared and transferred to MPCA. QC includes verification of sample point location appropriateness; verification/QC of plant identification; production of a voucher collection; and verification of data entry and data entry QC. Data entry done by investigators will be compared with field forms during the data entry process to double-check accuracy of data entry.

## B6: Instrument/Equipment Testing, Inspection, and Maintenance

The sampling rake, GPS units, and other sampling equipment will be examined before each sampling day to ensure that everything is in working order. Spare batteries will be carried for the GPS units and camera.

Water craft, trailers, and sampling gear will undergo rigorous disinfection to eliminate the transfer of exotic species when moving from the estuary to other lakes. Recommended disinfection includes taking the tow vehicle, boat, and trailer to a car wash, and hosing them and the sampling equipment down with hot soapy water.

In compliance with Minnesota law, before leaving each boat ramp, boats will have their drain plugs removed, drain any water not being used for water quality sampling, and inspect the boat and trailer carefully for vegetation and other "passengers".

## B7: Instrument/Equipment Calibration and Frequency

Microscopes are typically calibrated and aligned every 2-4 years, depending on scope type and manufacturers recommendations. Recreational GPS accuracy is sufficient for these data. GPS receivers will be tested prior to and after the field season by taking repeated readings at known localities visible on aerial photographs.

## B8: Inspection/Acceptance of Supplies and Consumables

Before setting out field crew will ensure an adequate supply of pencils, pens, data sheets, batteries, and other field equipment, and make sure all needed supplies are available on the boat.

## B9: Non-direct Measurements

### B9.1: Sources of Secondary Data

Secondary data on contaminants in the St. Louis River AOC will be obtained from two databases: the MPCA's St. Louis River Area of Concern Sediment Database (SLRAOCDB) and a recent publication by the USGS on chemicals of emerging concern (CEC) in water and sediment samples from the St. Louis River AOC (Christensen et al. 2012). Concentrations of contaminants at MPCA and USGS contaminant sample points will be correlated with vegetation metrics derived from sets of two to five vegetation sample points located near contaminant sample points. We will use non-parametric correlation coefficients in our statistical analyses. The purpose of these correlations is to identify contaminants that may be influencing vegetation patterns or may be limiting growth of aquatic vegetation in sparsely vegetated areas of the AOC.

### B9.2: Rationale for Selecting Sources

The MPCA and USGS datasets represent relevant sediment and water contaminant data which may assist with interpretation of vegetation patterns in the St. Louis River AOC. One of the important questions that needs to be addressed for BUI delisting efforts is what is limiting or preventing growth of aquatic vegetation in the 21st Ave West site. The data in the SLRAOCDB and the USGS CEC dataset are of known data quality and meet the requirements of the project.

### B9.3: Quality Requirements for Secondary Sources

The correlations between aquatic vegetation metrics and contaminant concentrations will only use data meeting the following criterion:

- Data are included in the current SLR AOC DB (Phase 7), or the 2012 USGS CEC online publication

The quality of SLR AOC data base and the associated calculation is addressed in the St. Louis River Area of Concern Data System Improvement Project Quality Assurance Project Plan (Lindon 2013, Crane 2001, 2004 and 2005). The quality of the USGS CEC dataset is addressed in Christensen et al. (2012).

## B10: Data Management

In the field, data will be recorded on water-resistant paper, using pencils, water-proof pens, or permanent markers. Site datasheets for all field crews will contain the following basic information: site name, date, and names of sampling crew members. Data recorded at sample points will include point number, GPS waypoint number, habitat data listed previously, and plant data. Unknown plants will be labeled with a sample number. All completed data sheets will be kept in a secure location by field crews and scanned or photocopied at the earliest opportunity. Scans or photocopies will be archived in a separate location from the original data sheets. GPS units will be downloaded every day or two, and the data files backed up at NRRI.

Data will be entered in either an Excel spreadsheet or an Access database designed to match the format needed for the MPCA's biotic data database (currently being developed) for St. Louis River estuary AOC. Electronic files will be backed-up on a second computer or external hard drive. Data spreadsheets will be prepared for running data in PC-ORD for classification and ordination analyses.

Vegetation metrics will be derived from data spreadsheets; these measures are the basic descriptive data we use to summarize plant community characteristics for comparison with environmental measurements such as water depth, wind fetch, or contaminant concentrations.

*Vegetation metrics to be calculated:*

1. Compute overall RELATIVE FREQUENCY for each plant taxon in the estuary by summing the number of occurrences for each taxon in the whole dataset, and dividing that sum by the total number of points sampled. This metric provides information on how common or rare each plant taxon is within the estuary.

2. Compute RELATIVE FREQUENCY for each plant taxon in each plant community type identified in the vegetation analyses, by summing the number of occurrences for each taxon in a plant community type, and dividing that sum by the total number of points sampled in that plant community type. Summaries of plant community composition will be presented with plant taxa sorted by their relative frequencies.

3. Compute TAXA RICHNESS for each sample point, by summing the number of plant species or taxa recorded as present at each sample point. This is a measure of plant diversity that we use for correlations with environmental conditions, and for evaluating success of restoration efforts.

4. Compute INVASIVE FREQUENCY for each site by summing the number of sample points with invasive species present, and dividing that sum by the total number of sample points at that site. This measure will help us evaluate the presence of invasive plant species at R2R and Reference sites.

5. Compute INVASIVE FREQUENCY for each plant community in the estuary by summing the number of sample points per community with invasive species present, and dividing that sum by the total number of sample points at that plant community. This measure will help us describe the presence of invasive plant species within each plant community type.

6. Compute EMPTINESS for each site by summing the number of sample points that lack vegetation, and dividing that sum by the total number of sample points at that site. This measure is used in correlations with contaminant data to evaluate influence of contaminants on aquatic vegetation.

# C: ASSESSMENT AND OVERSIGHT

## C1: Project management and assessment

MPCA Project Management is responsible for overall project oversight and will continually work with the investigators and MPCA's GLRI QA coordinator on project status and quality issues.

## C2: Reports to MPCA

The INVESTIGATORS will provide annual data transfers to MPCA and informal reports on project status as requested. MPCA Project Management will report on project status via the following

- GLRI's GLAS project tracking system
- GLRI's Quarterly project report

# D: DATA VALIDATION AND USABILITY

## D1/D2: Data Review, Verification, Validation, and Validation of Methods

The required final data format is outlined in Tables 3 and 4 below.

**Table 3. Minimum fields of record for aquatic plant community data at each sample point**

| Field Name | Definition |
|---|---|
| STATIONID | Station ID code for site where samples were collected |
| LongDD_83 | Longitude geographical coordinates (decimal degrees; NAD83 datum) |
| LatDD_83 | Latitude geographical coordinates (decimal degrees; NAD83 datum) |
| STUDYNAME | Study name |
| CONTACT | Contact person/agency |
| SAMPLEID | Sample Point ID code |
| FIELDREP | Identifies field replicate samples (samples collected in close proximity) |
| SAMPDATE | Sample date (YYYYMMDD) |
| Plant Community | Community type to which sample point is assigned in classification results |
| Taxa richness | Number of plant taxa present at a sample point; a measure of diversity |
| Empty | Identifies lack of vegetation at a sample point (1 = empty; 0 = vegetated) |
| Water_depth | Water depth in meters at sample point |
| Secchi_depth | Secchi depth in meters at sample point |
| Sediment_texture | Dominant sediment texture at sample point |
| Notes | |

**Table 4. Additional fields for aquatic macrophyte composition at each sample point**

| Field Name | Definition |
|---|---|
| STATIONID | Station ID code |
| SAMPLEID | Sample Point ID code |
| FIELDREP | Identifies field replicate samples (samples collected in close proximity) |
| SAMPDATE | Sample date (YYYYMMDD) |
| Lowest_ITIS_TSN | ITIS Taxonomic Serial Number for the lowest level taxa assigned |
| Taxon_code | Eight character code for plant taxon used in data analyses |
| Taxon_ID | Scientific name for plant taxon |
| Taxonomic_level | Hierarchical level at which taxon is identified (e.g. family, genus, species) |
| Presence/Absence | Presence of taxon at each sample point (present = 1; absent = 0) |
| Empty | Identifies lack of vegetation at a sample point (1 = empty; 0 = vegetated) |
| Notes | |

**Quantitative data**: Data will undergo standard laboratory QA/QC procedures. After data are entered into the data management system, the data will be checked for out-of-range values. In other words, the data will be checked for values that are unlikely to occur and thus may be the result of some sort of error (e.g., very high water or Secchi depths, etc).

**Qualitative data**: Background knowledge about the taxa will play an important role in data review, although unexpected but potentially significant observations will not be dismissed without follow-up information. Investigator C. Reschke will check the taxa lists for uncommon taxa and those that are rarely found in the St. Louis River estuary. These identifications will be verified, if possible, or flagged as suspicious, if verification is not possible.

## D3: Reconciliation with User Requirements

Routinely, discrepancies between the original identifications and the QC identifications are discussed among the taxonomists, and necessary rectifications to the data are made. Discrepancies that cannot be rectified by discussions are routinely sent out to taxonomic specialists for identification.

The INVESTIGATORS will discuss with MPCA the usability of the data and information gathered for removing the Beneficial Use Impairment: degradation of fish and wildlife habitat (BUI 9). If the data/information is deemed to be unusable, then remedies will be discussed with the MPCA.

### D3.1: Project deliverables

- Verified aquatic macrophyte dataset and metadata in Excel in a format compatible with MPCA's AOC biotic-data database.
- GIS layer of all points sampled for all biotic types, coded by biota and date sampled, with FGDC-compliant metadata.
- Final report comparing R2R sites to Reference locations for all vegetation sampled, including a refined aquatic plant community classification, and an analysis of vegetation patterns relative to contaminant concentrations.

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