

QUALITY ASSURANCE PROJECT PLAN

St. Louis River AOC Benthic Community Reference Sites

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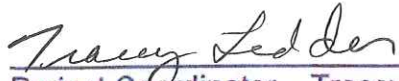
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St. Louis River AOC Benthic Community Reference Sites QAPP Revision 1
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SECTION ONE: PROJECT DESCRIPTION

1.1 PROJECT ORGANIZATION

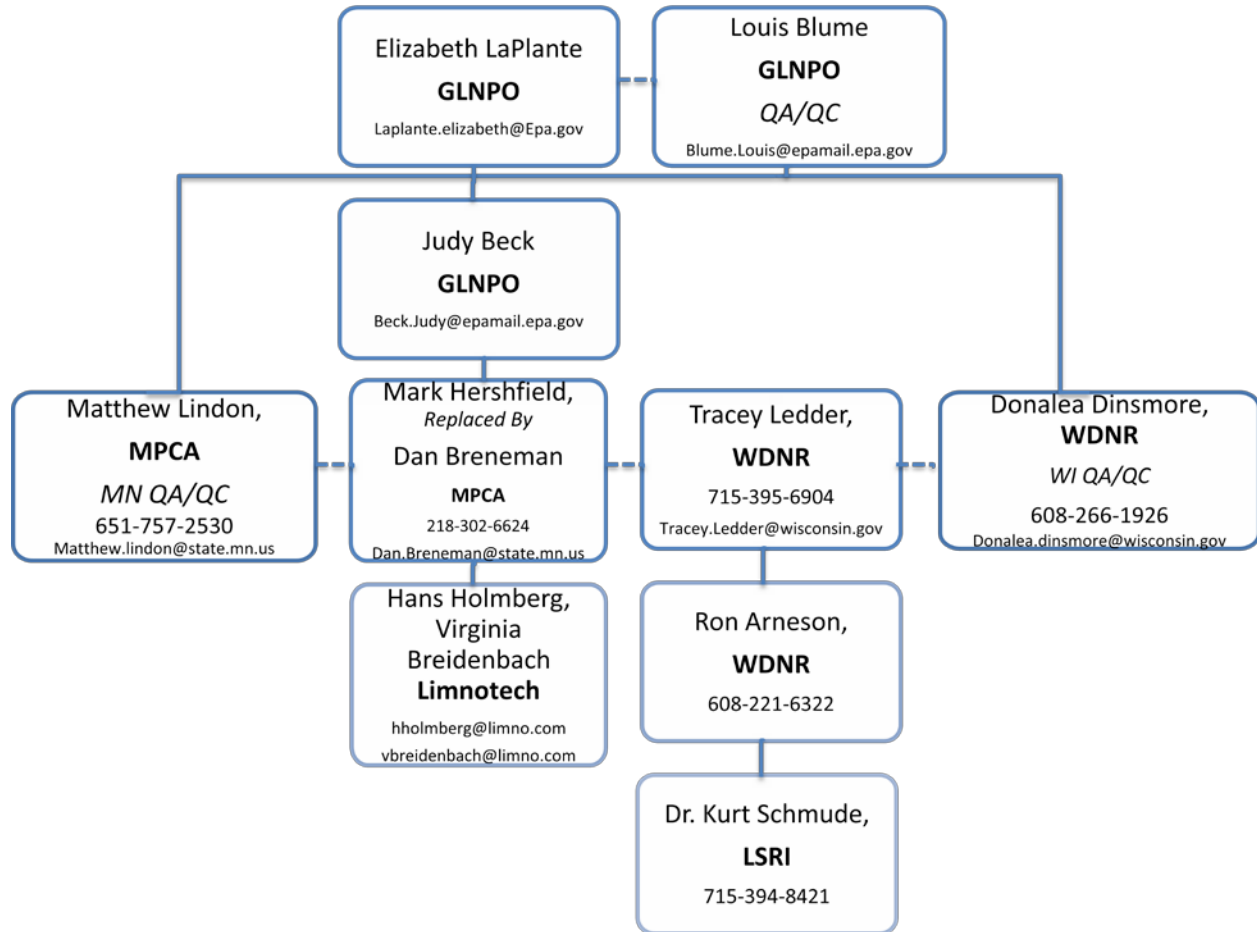
The project is a collaboration between the Wisconsin Department of Natural Resources (WDNR) and Minnesota Pollution Control Agency (MPCA) to collect data on the benthic community in “least impacted” areas of the St. Louis River Area of Concern, in support of the Degradation of Benthos Beneficial Use Impairment (BUI) Target.

Table 1 Project Roles

Name	Responsibility
Tracey Ledder, WDNR Tracey.Ledder@wisconsin.gov 715-395-6904	Prepare QAPP Oversee field sampling Arrange analytical contracting Compile sample results
Mark Hershfield, MPCA Marc.hershfield@state.mn.us 218-302-6633 Replaced by Dan Breneman Dan.Breneman@state.mn.us 218-302-6624	Assist in QAPP development Oversee LimnoTech contract
Hans Holmberg, Virginia Breidenbach, LimnoTech 2217 Vine St., Hudson, WI 54016 hholmberg@limno.com vbreidenbach@limno.com	Assist in QAPP development Carry out field sampling Compile Technical memo
Dr. Kurt Schmude, LSRI 801 N 28 th Street, Superior, WI kschmude@uwsuper.edu 715-394-8421	Identification of macro invertebrates
Ron Arneson, WDNR 2601 Agriculture Dr., Madison, WI Ronald.arneson@wisconsin.gov 608-221-6322	Coordinate analysis of sediment samples with State Lab of Hygiene
Donalea Dinsmore, WDNR Donalea.dinsmore@wisconsin.gov 608-266-1926	QAPP approval and QA oversight
Matthew Lindon, MPCA Matthew.lindon@state.mn.us 651-757-2530	QAPP approval and QA oversight

As a multi-state AOC, this project has been developed collaboratively between the participants to ensure that it meets the needs for both states. Management has directed that the team reach consensus in resolving any disagreements in project design or performance. Ultimately, Minnesota has responsibility for the sampling contract performance and Wisconsin has responsibility for the analytical contract performance.

re 1. Project organization chart



1.2 PROJECT BACKGROUND

The St. Louis River was listed as an Area of Concern (AOC) under the Great Lakes Water Quality Agreement in the late 1980s. The states are responsible for implementing remedial action plans within the AOCs which will lead to the improvement of biological, chemical and physical integrity of these locations and their delisting. Wisconsin and Minnesota have jointly identified cleaning up contaminated sediment and restoring habitat as the top priority issues to address in delisting the St. Louis River Area of Concern (AOC). In some sites in the AOC, habitat restoration is contingent on contaminated sediment remediation. In other sites, habitat is degraded primarily because of physical limitations. In both of these instances, benthic community health is impacted. A healthy benthic community is important to the support of the entire aquatic food web.

Because of the complexity of the AOC's issues, the states recognize the need to work together to plan and implement projects with a consistent approach to information and cross-jurisdictional decision-making. The work conducted in this project will allow evaluation of benthic community health in support of removing the Degradation of Benthos BUI.

One of the beneficial use impairments (BUIs) for which the St. Louis River was listed as an AOC is Degradation of Benthos. The 2008 Delisting Target for this BUI states "The benthic community in historically degraded areas (e.g., chemically, biologically or physically degraded areas) of the AOC does not significantly differ from unimpacted sites of comparable characteristics within the AOC. Benthic communities' characteristics including native species richness, diversity, abundance, and functional groups will be considered when comparing sites". Therefore information is needed on sites considered to be "unimpacted" reference sites within the AOC. Partners in the AOC agree that there are probably no "unimpacted sites" within the AOC, therefore our target will be "least impacted" reference sites. Site physical characteristics, such as water depth, substrate type and vegetation extent, were determined to be important comparable characteristics in previous benthic studies within the AOC.

Several studies on benthos have been carried out since the 1990s. Studies in which benthic community information was collected in a randomized manner were completed in the 1990s. Water depth and physical habitat were determined to be major influences on the benthic community structure. Other studies have been done on the benthic community in specific sites under study for remediation design, and may include a "reference site" for that remediation study. Randomized studies were completed in 2006, with sediment core and other methods used to sample benthic organisms with the goal of locating non-indigenous species. Most of these studies involve similar sampling techniques, the samples were taken by ponar and identified in entirety, although there were different objectives. The data from these studies has not yet been compiled.

In order to work toward removing the Degradation of Benthos BUI there needs to be information on the benthic community as found in least impacted sites within the AOC that takes into account natural variability related to water depth, substrate type and extent of vegetation. This information is necessary for interpreting the relative condition of the benthic community as sampled in contaminated sites, designing restoration goals, monitoring restoration and ultimately deciding what sites exist in which benthos are degraded for prioritizing future work. The focus at this time is on sediment dwelling

benthos, as the BUI is a contaminated sediment-related one, and legacy sediment contamination is a primary concern within the AOC.

The goal of this project is to collect basic habitat information and benthic community information at reference sites identified by the “Field Truthing and Vegetation Assessment of NRRI-GLEI Reference sites for Near-shore Ecotypes in the SLR AOC (for growing seasons 2007 and 2008)” and other recent studies of wetlands within the AOC. The habitat type Coastal Marsh, as studied in the NRRI-GLEI work, most relates to the Degradation of Benthos BUI for the AOC, as that is the aquatic habitat in which industrially impacted sediments are found. Substrate samples will be taken in shallow, moderate and deeper water habitats in four of these sites. Because physical habitat has been determined to be a strong influence on benthic communities, observational data will also be taken on general substrate type, water depth, vegetation community type and general surrounding landuse, while substrate samples will be taken for analyses of total organic carbon and particle size.

LimnoTech will complete field work and collect field samples under contract with MPCA. The WDNR will fund macroinvertebrate identification at Lake Superior Research Institute (LSRI) and sediment sample analyses at the Wisconsin State Lab of Hygiene (SLH).

As a multi-state AOC, this project has been developed collaboratively between the participants to ensure that it meets the needs for both states. Management has directed that the team reach consensus in resolving any disagreements in project design or performance. Ultimately, Minnesota has responsibility for the sampling contract performance and Wisconsin has responsibility for the analytical contract performance.

1.2.1 Location and General Information

The St. Louis River, the largest tributary to Lake Superior in the United States, drains a 3,879 square-mile watershed in St. Louis, Aitkin, Itasca, Lake and Carlton Counties, Minnesota, and Douglas County, Wisconsin. The upper watershed is largely forested, but does contain areas of industrial mining. The Area of Concern includes the lower 39 river miles of the St. Louis River and that portion of the watershed, the Nemadji River watershed, and a section of western Lake Superior. The Remedial Action Plan, 1992, documents much of the AOC characteristics and history.

The lower river, below the Fond du Lac dam, spreads out to form an approximately 12,000-acre estuary. The lower end of the estuary is the largest industrial harbor on the Great Lakes, while the upper estuary contains large expanses of protected areas.

Figure 2. Benthos Reference Site Location Map



1.2.2 Topography

The St. Louis River's source area lies near Hoyt Lakes, Minnesota, in the Toimi Drumlin field, a predominantly wooded area of boulders, coarse-loamy glacial till and outwash deposits. In the upper part of the St. Louis River, the channel is narrow and deep with a depth ranging from 10 to 30 feet. As the river flows westward across St. Louis County, Minnesota, it passes through forested areas of sand, gravel, and clayey glacial till and outwash deposits. Small tributaries flow through similar wooded landscapes. From the town of Floodwood to Thomson, the river continues to pass through very hilly, wooded glacial moraine. The soils in this area are coarse-loamy fine sands, loamy mantles, sands and gravels, interspersed with some fine loam. Valley slopes increase in size and steepness along the river banks. The Cloquet, another major tributary river, joins just below Brookston. The Cloquet drains predominantly wooded areas of sand and gravel glacial till deposits. Below Thomson, Minnesota, the St. Louis River changes abruptly as it flows through the deep narrow gorge of Thomson slates and greywackes in Jay Cooke State Park. The final reach of the St. Louis drains through the red clay deposits of Glacial Lake Duluth and enters the St. Louis Bay estuary (Remedial Action Plan, 1992).

1.2.3 Site History

Settlement and consequent development of the lower St. Louis River and its watershed followed a pattern similar to that in of other Great Lakes Areas of Concern. The shoreline and open waters of the river's mouth or estuary were transformed from a large, shallow, marshy area, to the slips and waterfront industrial operations which make it one of the largest ports on the Great Lakes today. In this transformation, an estimated 3000 acres of open water and wetlands were filled and another 4000 acres of the harbor or estuary dredged. Rivers of the watershed were also scoured during their use as log flumes by loggers in the early 1900s. Other major hydrologic modifications included the development of 630 miles of drainage ditches in the western and central parts of the St. Louis River Watershed, the creation of a lake reservoir system, and the placement of five hydro-power dams in the river reach from the City of Cloquet, Minnesota to the upper reaches of the estuary. Each of these changes and many others have altered historic hydrologic conditions. Some of the known consequences of these physical alterations are manifested as barriers to fish movement, changes in flow regimes and aquatic habitats, and in the delivery of storm water and its associated pollutants.

The St. Louis River has undergone a long history of degradation resulting from pollution. In 1929, the Minnesota State Board of Health classified the river reach from the City of Cloquet to Lake Superior as "pollutional", oxygen deficiencies and sludge deposits in river bottom areas were commonplace at this time. Complaints of tainted fish flavor and fish kills were also frequent occurrences from the 1940s through the 1970s. Waste water treatment improvements in the early 1980s alleviated most of the problems associated with discharges of conventional pollutants. Issues of toxic substances in the environment have since moved to the forefront of the public agenda. Minnesota and Wisconsin issued fish consumption advisories for the presence of toxic residues in fish in the St. Louis River in 1985.

Historical discharges have left continuing problems: sediments contaminated with mercury, PCBs, dioxins, polynuclear aromatic hydrocarbons (PAHs), and a variety of other metals and organic compounds. Certain areas were identified in the Remedial Action Plan as having particularly elevated levels of sediment contaminants, mostly for PAHs and/or heavy metals such as mercury and lead. Contaminated sediments may act as sources of contaminants to the overall ecosystem of the lower river and estuary. In addition to continuing contributions of contaminants from these sites, other established or potential pollutant sources include continuing industrial and municipal discharges and a significant number of major and minor landfills (Remedial Action Plan, 1992).

1.3 PAST DATA COLLECTION ACTIVITIES

Several studies in which benthic community information was collected in a randomized manner were completed in the 1990s. Analyses of the data from these studies determined water depth and physical habitat to be major influences on the benthic community structure. Other studies have been done on the benthic community in specific sites under study for remediation, and may include a “reference site” for that remediation study. Randomized studies were completed in 2006 (Trebitz, et al, 2010), with petite ponar and other methods used to sample benthic organisms with the goal of locating non-indigenous species. Most of this work does include taxonomic identification to the lowest level practicable. However, this data has not all been compiled for information as relates to the Degradation of Benthos BUI, and much of this sampling has occurred in more industrially impacted areas. Studies, both in the St. Louis River and elsewhere, have documented the importance of physical habitat as a variable affecting a benthic community. Data on least impacted sites is currently lacking in the AOC.

Studies in the St. Louis River have shown that physical habitat features best explained benthic community structure (Breneman, et al, 2000, and others). These variables need to be documented in conjunction with benthic work to account for comparability, and understand variability, both between sites and within sites. Important variables include water depth, whether the sample location is vegetated, substrate type, and potentially turbidity.

1.4 PROJECT DESCRIPTION

This project will sample the benthic community in “least impacted” sites to act as reference for remediation investigations, restoration projects and the overall AOC benthic community documentation. Substrate samples for benthic community analyses will be collected at four of the reference sites established by the “Field Truthing and Vegetation Assessment of NRRI-GLEI Reference sites for Near-shore Ecotypes in the SLR AOC (2007 and 2008 growing season)”. Efforts were made to select appropriate sites on both the Wisconsin and Minnesota side of the river. Substrate samples will be taken in several locations in different microhabitats in the selected sites.

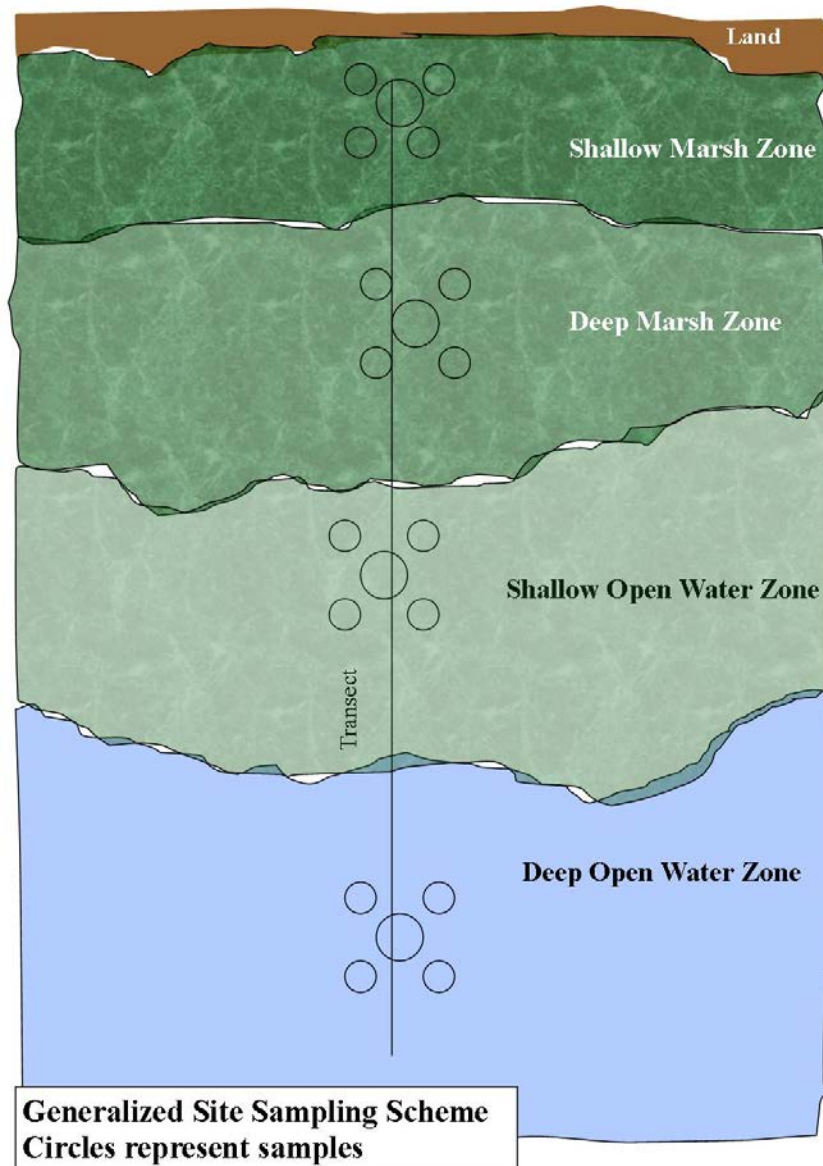
Five substrate samples (replicates) will be taken at each of four sampling locations within each of the four selected reference sites. The sampling locations will be spread along a transect between shallow vegetated and deeper unvegetated microhabitat. See Figure 3. One of the substrate replicates will be sent to the Wisconsin State Lab of Hygiene (SLH) for total organic carbon and particle size analyses. Four of the substrate replicates will be sent to the Lake Superior Research Institute (LSRI) for identification of the macroinvertebrates to the lowest taxa level practicable. The replicates will be treated separately to allow for study of the variability between locations within sites, while also allowing the replicates at each site to be pooled for other comparisons. This sampling will result in 64 petite ponar samples to be analyzed for macroinvertebrates and 16 to be analyzed for total organic carbon and particulate size gradient.

Water quality will be recorded at each location along each transect (secchi depth, dissolved oxygen, pH, specific conductance, turbidity and temperature). Locations will be chosen to represent each of four different microhabitats within each site. A sonar scan will be performed along each transect, which will provide a continuous measurement of water depth, as well as a qualitative measure of bed structure and morphology along the transect. Observational data will be taken on general substrate type, water

depth, general vegetation type and general categories of surrounding landuse. Each sampling location will be geographically located using GPS.

LimnoTech will complete field work and prepare a technical memo of that field work under contract with MPCA. The WDNR will fund macro invertebrate identification at LSRI and sediment sample analysis at SLH.

Figure 3 Generalized Site Sampling Scheme



1.5 QUALITY OBJECTIVES AND CRITERIA

The overall objective for this study is to understand the benthic community structure and variability in the benthic community within a location and among each of four microhabitat types found at coastal marshes identified as least impacted within the St. Louis River Area of Concern for the purpose of providing data to evaluate the Degradation of Benthos Beneficial Use Impairment (BUI). The four microhabitats are Shallow Marsh, Deep Marsh, Shallow Open Water, and Deep Open Water. Qualitative information related to water quality, physical features (substrate, depth, morphology and vegetation type), and land use enhance the understanding of the benthic community and the factors to be considered in comparing this data with impacted sites within the AOC, as specified in the 2008 Delisting Targets.

Data on the types and numbers of different benthic organisms in each location should be of sufficient quality to be used to calculate metrics that allow comparison between sites of comparable characteristics within the AOC. In order to have comparability between least impacted and impaired sites, basic habitat information is also required. The Stage 2 RAP update, 2011, identified the need to document the benthic community in reference sites within the AOC in order to move toward removing the Degradation of Benthos BUI .

Data from this study, by itself, will provide information on the benthic community structure at “least impacted” sites, however data may also be compared to, or pooled with, other study data for other data needs in the future. Requirements for ensuring that data are of suitable quality for their intended use include accuracy, precision, representativeness, comparability and completeness. When these requirements are met, the final project data are considered technically defensible. Data elements for this project are discussed in terms of the appropriate characteristics, defined as:

- Accuracy: The extent of agreement between a measured value and the true value of interest.
- Precision: The extent of mutual agreement among independent, similar or related measurements.
- Representativeness: The extent to which measurements represent true systems.
- Comparability: The extent to which data from one study can be compared to similar studies.
- Completeness: The measure of the amount of data acquired compared to the amount of data planned to fulfill the intended use.

The representativeness and comparability of data generated depend to some extent upon the selection of sampling sites. Sites were chosen based on compilations of existing information that shows the sites to be “least impacted” within the AOC, considering potential historic industrial impacts and current habitat quality. Floristic quality indices (FQIs) were calculated during field investigations for “Field Truthing and Vegetation Assessment of NRRI-GLEI Reference sites for Near-shore Ecotypes in the SLR AOC (2007 and 2008 growing seasons)”. The results of the FQIs show that several areas of the estuary are examples of good regional habitat. Probable Effect Concentration Quotients (PEC-Q) were calculated for sediment samples in similar areas in several studies housed in the GIS-based Sediment Database for the St. Louis River Phase IV. PEC-Q values below 0.1 indicate contaminant concentrations are not likely to cause a toxic effect to benthos, while PEC-Q values above 0.6 indicate contaminant concentrations likely to cause toxicity to benthos. Also, past studies of benthos did include several of the same areas studied in the NRRI-GLEI project and the sediment contamination studies. This information was

tabulated (see Table 3) and the AOC Coordinators for WDNR and MPCA chose least impacted sites for study based on this compiled information.

Secondary sites were also selected to be utilized in the case that field conditions indicate that a selected site may not in fact be “least impacted”. A decision will be made to move to a secondary site if a primary site contains continuous covering of sawmill waste or visible petroleum product.

The goal for completeness is 100% as the sampling was planned to allow statistical comparisons by documenting within-site variability of benthic community structure, however field conditions may affect the success of sampling. No quality control duplicate samples were planned for macroinvertebrate samples as variability is expected, and is itself an object of study. The overall sampling plan is built on the need to understand variability in the benthic community within and among least impacted sites using replicates. A minimum of three replicates per sample location will be required to calculate variability. All four target microhabitats may not be available within each site (see Figure 3). Transects will be located to obtain samples in all four microhabitats whenever possible. If any particular microhabitat is missing in more than one site sampled, secondary sites may be sampled instead. The completeness acceptability level is therefore 75%, less than that and sites with missing replicates or microhabitats will have to be re-sampled for the missing component.

The LSRI laboratory will ensure accurate benthic community identification by following its standard operating procedures by qualified staff, identification review by the senior taxonomist, and confirmation of ambiguous specimens by an outside expert.

Sample location information is needed to document the general location within which the sample was taken. Therefore standard field GPS with 3-meter accuracy is acceptable.

Water quality and substrate analyses for organic carbon and particle size are meant to assist in the description of the habitat type, and are qualitative. There are no environmental criteria associated with these parameters. Data quality objectives are standard field measurement and laboratory requirements as listed in Table 2. Precision and accuracy of laboratory analyses will be documented by the laboratory inclusion of laboratory control samples, calibration checks and blanks according to the laboratory standard operating procedures (SOPs) and current acceptance limits. One sample replicate will be selected for duplicate analyses for total organic carbon and particle size, a double volume sample will be collected from one site and shipped for analyses with the samples.

The purpose for the side scan sonar is to obtain a qualitative understanding of the bottom and vegetation structures as well as the transitions in habitat type, particularly where this is not visible from the surface. This information will be used to verify that sample locations are positioned well within the targeted habitat type.

Table 2 : Field and Laboratory Measurement Objectives

Parameter	Project Accuracy	Field or Laboratory Method Specifications	
		Resolution	Accuracy
Optical DO % Saturation	±5% of reading	0.1%	± 1% of reading or 1% air saturation*
Optical DO mg/L	±0.5 mg/L	0.1 mg/L	± 0.1 mg/L or 1% or of reading*
Specific Conductivity	±1% of reading	0.001 to 0.1 mS/cm	± 0.5% of reading + 0.001 mS/cm
Temperature	±0.5 °C	0.01 °C	± 0.15 °C
pH	±0.4 units	0.01 unit	± 0.2 unit
Turbidity	±1 NTU	0.1 NTU	± 2% of reading or 0.3 NTU*
Total Organic C	±25%	10 ppm	±25%
Particulate size	±25%	10 %	±25%

*whichever is greater

1.6 TRAINING

LimnoTech field crews collecting samples will have undergone internal training on sampling techniques, sample handling, equipment use, and general field procedures that are based on this QAPP, prior to the first sampling event. Specific emphasis will be placed on QA/QC issues as well as on health and safety. Individuals who operate LimnoTech watercraft are trained in boat operations or have completed a safe boating course and/or have demonstrated their skill and knowledge through previous experience with the LimnoTech boat safety officer.

Health and safety concerns also include the handling of formalin preservative and formazin calibration solutions. Safety goggles and chemical resistant gloves will be used while handling these chemicals. These chemicals will be disposed of in a sink connected to a wastewater treatment plant, flushed with copious amounts of water.

Staff and students involved in the macroinvertebrate sampling and identification at Lake Superior Research Institute will have undergone training as required by LSRI (see LSRI SOP FS/13, Appendix C).

Laboratory analysts at the Wisconsin State Laboratory of Hygiene will have undergone training as specified in their Quality Assurance/Quality Control program.

1.7 DOCUMENTS AND RECORDS

Documents and files related to this project will be kept with the St. Louis River Area of Concern files in the Wisconsin Department of Natural Resources Superior Office, as well as the Wisconsin SWIMS database, and the Minnesota Lake Superior Unit of the Duluth MPCA, and Performance Management and Quality Division, St. Paul. Records include field data sheets, sample shipment documentation, laboratory records of sample handling, electronic files, project records including contracts and final technical memos. Macroinvertebrate sample picking and identification laboratory sheets will be kept in the LSRI project file for five years.

SECTION TWO: DATA GENERATION AND ACQUISITION

2.1 SAMPLING PLAN

“Least impacted” sites were chosen based on the results of the “Field Truthing and Vegetation Assessment of NRRI-GLEI Reference Sites for Nearshore Ecotypes in the SLR AOC (2007 and 2008 growing seasons)”. The near-shore ecotypes were derived from the Lower St. Louis Habitat Plan, 2002. Watershed maps generated by the Natural Resource Research Institute (NRRI) using GIS ArcHydro data were used to determine potential target or reference wetland sites in the St. Louis River Estuary. These maps ranked wetland quality by measuring anthropogenic stress within their respective sub-watersheds. Plant communities were characterized at these sites, photographs were taken and notes were made of the physical condition of the sites, state-listed rare plant species, invasive species and wildlife use. A Floristic Quality Analysis (FQA) was performed on fourteen wetlands on both the Minnesota and Wisconsin sides of the estuary. The ecotypes of primary interest for this study are the Clay Influenced Bay, Sheltered Bay and Estuary Flats as these are ecotypes that in the lower industrial harbor are most likely to need remediation or restoration. Within these ecotypes, the aquatic habitat of Coastal Marsh most correlates to the historically degraded sites within the St. Louis River AOC related to the Degradation of Benthos BUI. Other habitats within the ecotype categories, such as wet meadow, floodplain forest, and terrace forest, were not considered for this project.

See Table 3 for a summary of available information for the sites determined to have been “least impacted” by the NRRI-GLEI report (industrially influenced sites were not considered for this study). Least impacted sites for this project were selected among the eco-types most likely to have industrially influenced counterparts in the AOC, with attempts to sample sites from both Wisconsin and Minnesota.

The least impacted sites chosen for this project were also chosen based on the low likelihood of direct historical industrial impacts. Existing historical sediment assessment data available within the GIS-based St. Louis River Sediment Database Phase IV was also researched for these sites. There is a small amount of sediment chemistry data for most of the NRRI-GLEI identified reference sites. This information was also tabulated for site consideration in Table 3.

One transect will be run within each site. Each transect will begin in shallow vegetated habitat and proceed to deeper, unvegetated habitat. Four sample locations will be determined along each transect within zones of the following microhabitats (Eggers and Reed, 1997; Eggers and Reed, 2011; NRRI, 2009);

- *Shallow Marsh Vegetation*: Water depth approximately 6 inches or more, substrate muck (poorly drained soil with overlying organic layer), emergent vegetation.

- *Deep Marsh Vegetation*: Water depth approximately 6 inches to 3 feet, substrate alluvial, vegetation emergent, floating and floating-leaved and submergent.
- *Shallow, Open Water Vegetation*: Water depth approximately 3 to 6 feet, substrate lacustrine deposits and sediments (fine silt/clay overlying muck), vegetation floating and floating-leaved and submergent.
- *Deeper, Open Water no vegetation*: Water depth approximately 6 feet or more, substrate lacustrine deposits and sediment, no vegetation.

Field reconnaissance will take place prior to the field sample collection. During the reconnaissance expert staff from LimnoTech and the Project Coordinator will visit each identified site to choose sample locations according to the plan. Work includes exploring microhabitat boundaries and setting temporary buoys to identify the transition zones and recording the locational information (coordinates) for the boundary. A sonar scan will be performed along each transect, which will provide a continuous measurement of water depth, as well as a qualitative measure of bed structure and morphology along the transect. Observational data will be taken on general substrate type, water depth, general vegetation type and general categories of surrounding landuse. Each sampling location will be selected to be near the middle of the microhabitat and geographically located using GPS. If a site contains petroleum product, or sawmill waste in quantities that can not be avoided, a secondary site will be sampled instead. In order to avoid zones of transition between the microhabitat types, each sample location will be placed in the middle of the microhabitat zone along the transect.

The LimnoTech sample collection crew will then follow over the next four days, one site per day, and collect samples at the locations designated. Geolocational data and microhabitat observations will be taken at each location. At each location, water quality will be taken first, with time given to allow settling of any disturbed sediments. Water quality to be recorded at each location along the transect includes secchi depth, dissolved oxygen, pH, specific conductance, turbidity and temperature.

Five substrate samples will be taken at each of four sampling locations within each of four reference sites. See Figure 3 for the conceptual design plan. The sampling locations will be spread along a transect within each site, between shallow vegetated and deeper unvegetated microhabitats. The five substrate samples will be taken by petite ponar (or core if ponar is not possible), in the general distribution of the shape of an "X", within approximately one square meter. One of the replicates will be sent to the Wisconsin State Lab of Hygiene for total organic carbon and particle size analyses. Four of the replicates will be sent to the Lake Superior Research Institute for identification of the macroinvertebrates to the lowest taxa level practicable. The replicates will be treated separately to allow for the study of the variability within sites, while also allowing the replicates at each site to be pooled for other comparisons, if necessary. Therefore, 64 macroinvertebrate samples and 16 sediment samples will be analyzed.

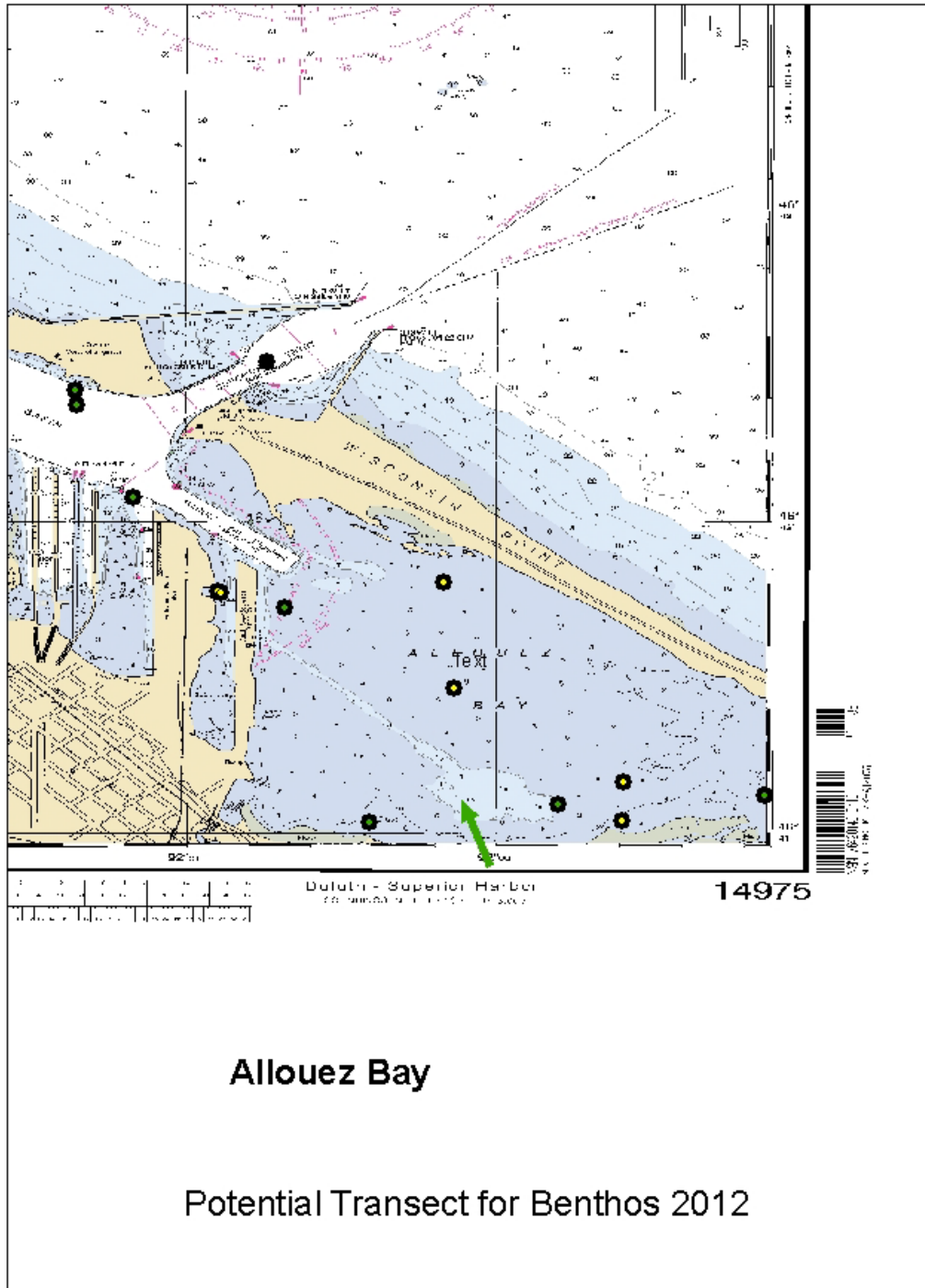
Table 3: Available Information for NRRI, 2009, Reference Sites

Site Type	Site Name	FQI ^a	Water Depth ^a (ft)	Taxa/m ² ^b	Percent non-insect ^b	PEC-Q ^b	%TOC ^b	%Fines ^b
Clay Influenced Bay	Allouez Bay	18.64 Coastal Marsh	3	5 - 10	35 - 54	0 - 0.6	0.1 - 22	0.1-<80
Clay Influenced River Mouth	Pokegama Bay	13.62 Coastal Marsh	3	5 - 10	35 - 54	<0.15	2.8 - 8.1	61 - 80
	Pokegama upstream	No info	No info	7 - 10	54 - 72	<0.1	0.1 - 81	<80
	Little Pokegama Bay	14.7 Fen	No info	13 - 23	0 - 54	0.2	3 - 22	61 - 80
Sheltered Bay	Red River East	20.48 Coastal Marsh	2	7 - 13	36 - 72	<0.16	3 - 8	<80
	<u>Red River West</u>	17.53 Coastal Marsh	2	No info	No info	No info	No info	No info
	Rask Bay	18.73 Coastal Marsh	1	10 - 23	35 - 86	<0.16	3 - 8	20 - 80
	<u>North Bay</u>	19.42 Coastal Marsh	2	No info	No info	<0.2	0.1 - 22	61 - 80
Upper Estuary Flats	Spirit Lake (west bank)	16.96 Coastal Marsh	No info	1 to 10	0 - 54	<5.0 US Steel site influence ?	0.1 - 3	0.1 - <80
	NE Clough Island	No info	No info	No info	No info	No info	No info	No info
	Tallus Island	No info	No info	No info	No info	<0.3	3 - 8	0.1 - <80
	Clough South	12.09 Coastal Marsh	3	7 to 13	0 to 72	<0.13	0.1 - 22	0.1 - <80

Notes: a = NRRI-GLEI Field Truthing report "least impacted" sites
b = St. Louis River Sediment Database Phase IV (rounded values)
bold = Four primary sites selected for this project
underline = two secondary sites chosen in case of need to replace a primary site
Expected FQI is ~ 16 to 17 for this region (NRRI, 2009)

Figure 4. Allouez Bay site

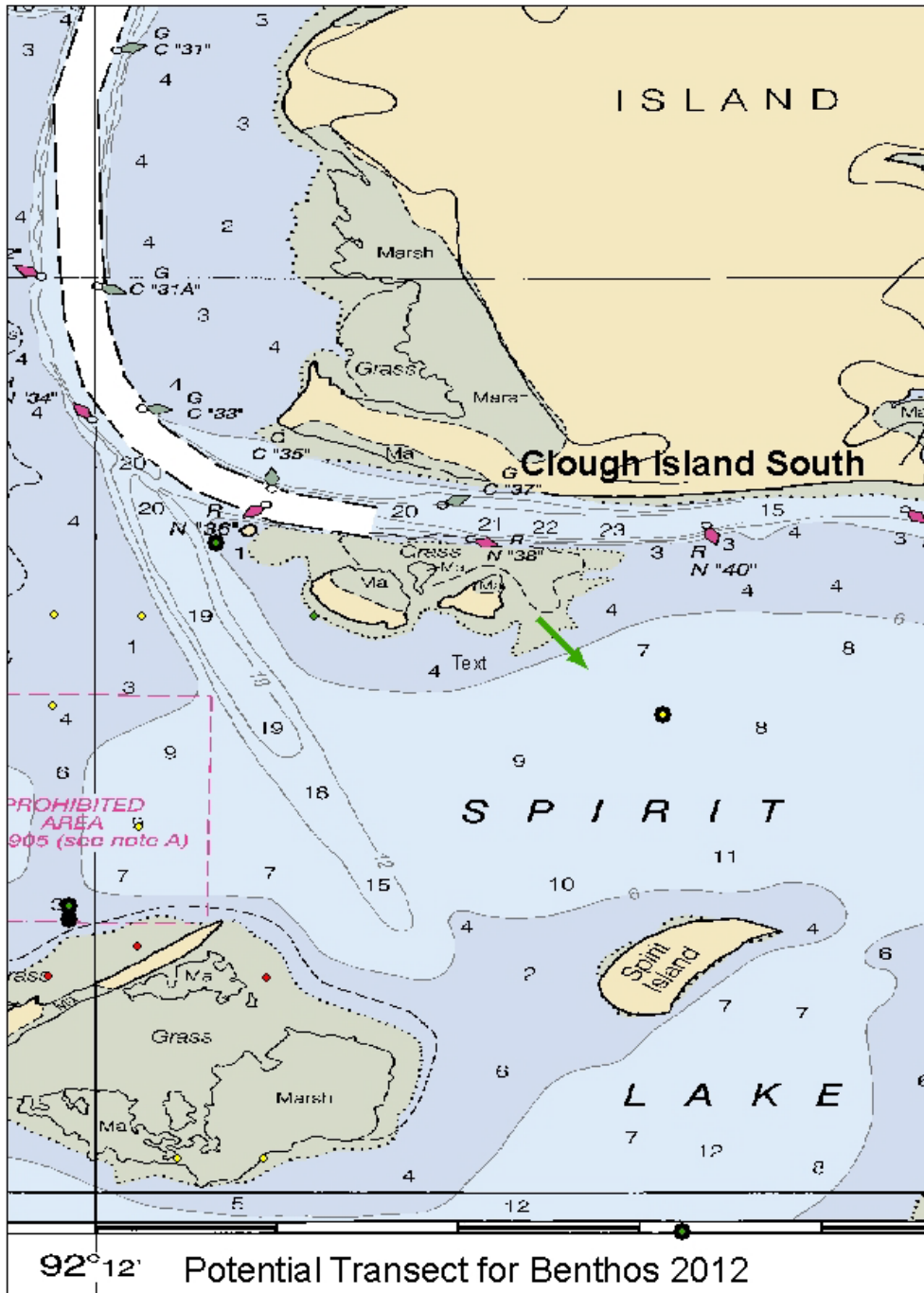
Duluth-Superior Harbor Chart, soundings in feet



Green arrow indicates target transect location

Figure 5. Clough Island South site

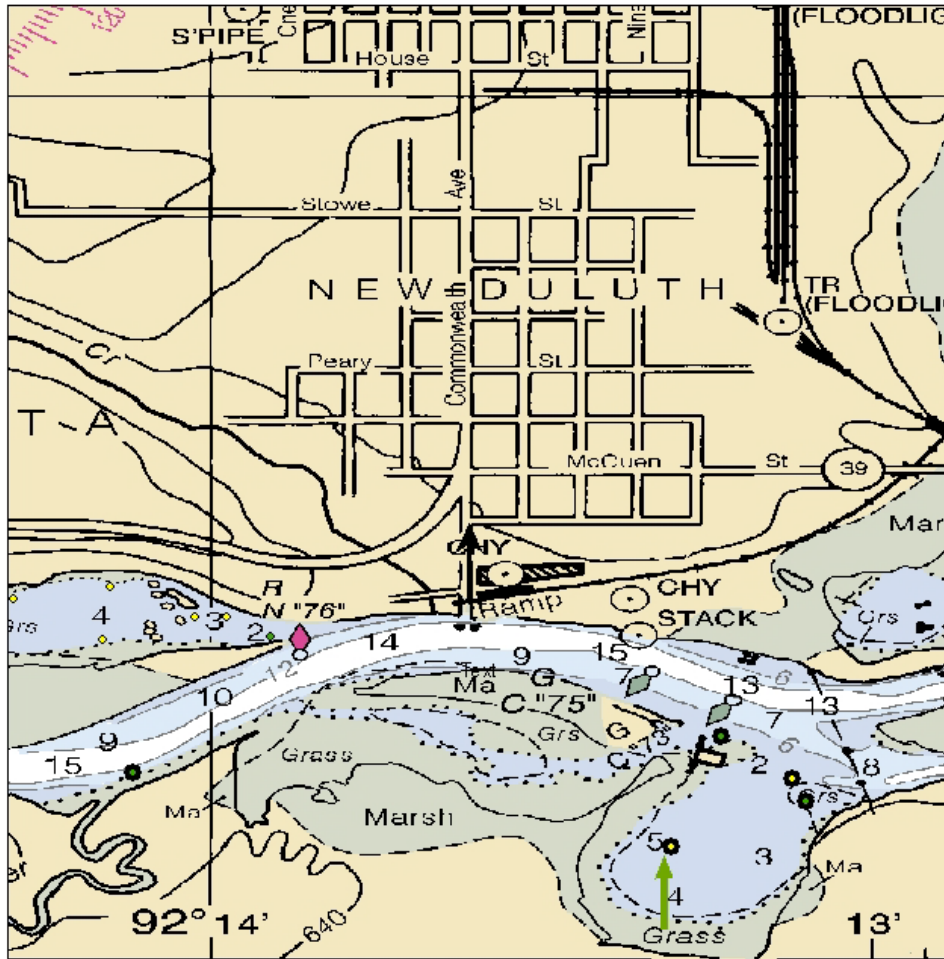
Duluth-Superior Harbor Chart, soundings in feet



Green arrow indicates target transect location

Figure 6. Red River East Site

Duluth-Superior Harbor Chart, soundings in feet



Green arrow indicates target transect location

Red River East

Potential Transect for Benthos 2012

Figure 7. Rask Bay Site



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Rask Bay

Potential Transect for Benthos 2012

2.2 SAMPLING METHODS

The goal for this study is accurate and representative identification of the biological community in sites previously determined to be “least impacted”. Replicate samples will be taken at each location within a site and treated separately to allow study and inclusion of natural variability within locations and within sites. Field data will be entered on a field data sheet (Appendix B). Field reconnaissance will take place prior to the field sample collection to select the sample locations.

2.2.1 Field Method Substrate Sampling

LimnoTech will utilize a 6inch x 6inch petite ponar for sediment sample collection, with a core sampler available in case of substrate sampling difficulty, according to the LimnoTech Sediment Sampling SOP, January 28, 2009 (Appendix A). Deviations from the SOP will be noted, if they occur.

2.2.2 Field Method Bathymetry

Single beam sonar will be used to collect geo-referenced water depths along each site transect where possible. In shallow water depths (<3 ft), data will be collected where vegetation or other objects do not interfere with the sonar beam. There is no limit to the maximum depth for sonar collection within the AOC. The primary purpose of the single beam sonar is to document a bathymetric profile of the reference site. However, the water depth data can be used, along with visual observations of plant types, to guide decisions on sampling locations along each transect in the field as necessary. The single beam sonar data will be mapped in GIS, projected with UTM Zone 15N, NAD83, meters, and provided in the technical memo. A spreadsheet of X-Y coordinates and water depth will be provided electronically.

Sidescan sonar readings will be taken during the reconnaissance work prior to the sampling to better identify sampling locations as well as minimize disturbance while the samples are taken. It will be used to create a visual picture of the bottom surface in a single swath along each transect. The images from side scan sonar will create a visual picture of the substrate variability in each site and the structure associated with this variability (fine vs. coarse material, presence of woody debris, etc). This information is qualitative in nature, but may prove useful in interpreting macroinvertebrate to habitat relationships as well as in guiding site restoration in other areas in the AOC. In shallow water (< 3 ft), sidescan data will be collected where vegetation or other objects do not interfere with the sonar beam. There is no limit to the maximum depth of sonar collection within the AOC. Side scan sonar data will be provided electronically in a video file. Snapshots from the video may be included in the technical memo, as appropriate to describe site conditions.

The LimnoTech standard operating procedure for bathymetry data collection, revision October 2010, is included in Appendix A.

2.2.3 Field Method Water Quality

Water quality measurements of dissolved oxygen, pH, specific conductance, turbidity and temperature will be taken with a YSI multiparameter sonde at each of the sampling locations (6-Series Multiparameter Water Quality Sonde User Manual, Revision H, 2011, <http://www.ysi.com/media/pdfs/069300-YSI-6-Series-Manual-RevH.pdf>). Water quality will be taken once at each sample location along the transect within a site, prior to substrate sample collections. At the shallowest locations, the probe will lay on the bottom surface for recording measurements. For all other locations, water quality measurements will be recorded 6 to 12 inches above the bottom. Manual water depths will be recorded with a weighted tape.

The multiprobe will be calibrated each day prior to deployment. Dissolved oxygen will be calibrated to water saturated air and barometric pressure. Specific conductivity and pH will be calibrated with commercially available standards. Turbidity will be calibrated with a 126 NTU formazin solution. Temperature is checked to an NIST traceable thermometer on an annual basis.

2.2.4 Field Method Secchi Disk

Water clarity measurements will be taken with a standard Secchi Disk according to LimnoTech SOP dated January 28, 2009 (Appendix A).

2.2.5 Field Method Geolocational Data

A handheld GeoXT GPS unit will be used to establish coordinates of the specific sampling locations along each transect. The GeoXT uses Everest multipath rejection technology to provide submeter accuracy with real-time or postprocessed differential correction. A single point will be collected at each substrate location along a transect to represent the center of the "X" of the five replicate sample points. All collected GPS points will be stored in the handheld and downloaded to a secure computer. Locational data will be included in the project technical memo and electronic files. These coordinates will be mapped in GIS and indicated on figures in the LimnoTech technical memo. The UTM Zone 15N, NAD83, meters project will be used. (See the Trimble GeoExplorer 2008 User's Guide at http://trl.trimble.com/docushare/dsweb/Get/Document-414964/GeoExpl2008_100C_%20UserGde_ENG.pdf.)

2.2.6 Sample Containers, Preservation and Holding Times

Containers for substrate samples will be supplied pre-cleaned by the WI SLH. The containers will be quart-size glass jars for TOC, and self-closing bags for particle size analysis. Containers should be filled at least three-quarters full. Sediment samples will be bagged, placed on ice, and shipped overnight to the appropriate laboratory. A Chain-of-Custody form will accompany each cooler shipped.

Containers for macroinvertebrate samples will be provided by LSRI. Samples will be sieved in the field to remove large debris, preserved with 10% formalin solution and taken to the laboratory for picking and identification. Samples not picked within one week will be re-preserved with 70-80% ethanol solution.

2.2.7 Boat and Equipment Decontamination Procedures

The boat used for the site sampling will be decontaminated before use in the St. Louis River and after use in this project to prevent the spread of aquatic invasive species from or to other waterbodies. The field crew plans to sample one site per day, the boat will be washed in a car spray-wash port after each site sampling is complete to prevent transport of aquatic invasive species within the estuary. The petite ponar and YSI multiprobe will also be decontaminated after each site is sampled to prevent transport of aquatic invasive species within the estuary.

The petite ponar will be rinsed with site water after each sample within one site to remove all visible sediment and vegetation prior to taking the next sample to avoid sample interference from sample to sample.

2.3 SAMPLE HANDLING AND CUSTODY

2.3.1 Field Handling Procedures

Substrate samples for macroinvertebrate identifications will be handled according to LSRI SOP FS/22, Collecting Benthic Macroinvertebrate Samples Using a Core Sampler, Procedures 8 through 14 (Appendix C). Each ponar grab will be a separate sample. The sample will be sieved in the field with a 250 um mesh sieve and preserved with a 10% formalin solution. To ensure quality benthic data, samples must be handled gently during the sieving process, preserved as quickly as possible to prevent deterioration, and jars must be labeled accurately.

Substrate samples for macroinvertebrates will be labeled both inside and outside of each jar. Labels will include; project name, locality, sample number, replicate number, date and type of preservative. Samples will be identified according to the scheme described in Table 4. Location numbers will begin at the shallow end (L1S#) and increase with habitat depth. Samples will be hand delivered to LSRI within one or two days of sampling completion.

Table 4. Sample numbers

Site Name	Sample Name	Macro Sample #	Sediment Analyses #	NOTE
Allouez Bay	ALLB	L (1-4)S(1-4)	L(1-4)S5	i.e., "ALLBL1S5" is sediment sample
Red River East	REDE	L (1-4)S(1-4)	L(1-4)S5	i.e., "REDEL2S3" is macroinvert
Rask Bay	RASB	L (1-4)S(1-4)	L(1-4)S5	
Clough Island South	CLOS	L (1-4)S(1-4)	L(1-4)S5	Take a sediment duplicate

Sample jars for sediment analyses will be labeled with appropriate identification information (site name, sample identification, date) on labels provided. Sample containers will be placed in plastic bags and stored on ice immediately. Samples will be shipped by overnight courier. A separate Chain of Custody form will be filled out for each laboratory.

2.3.2 Laboratory Handling Procedures

Samples will be logged in and analyzed according to each analytical laboratory's standard operating procedures. Sediment samples should arrive at the laboratory on ice, condition at time of arrival will be noted on the Chain of Custody upon receipt.

2.3.3 Sampling and Field work Oversight

This project was developed by Marc Hershfield and Tracey Ledder with assistance from LimnoTech. MPCA and WDNR jointly discussed and agreed to preliminary sampling site locations according to data available to us at the time. Sampling methods are standard field methods for the water quality field probe utilized and for the petite ponar collection of macroinvertebrates (the same methods as have been utilized in past sampling events in many areas of the St. Louis River). The Project manager Tracey Ledder and Field team lead Doug Bradley, LimnoTech, and Virginia Breidenbach, LimnoTech contract contact will jointly conduct the field reconnaissance prior to the benthic sampling. The LimnoTech staff and Tracey will go over the field procedures and sampling details to insure all aspects of the field work go as planned. During the field work/ sampling Doug and Virginia will insure that field crew adheres to the sampling requirements and communicate any issues to WDNR and MPCA project managers. After the field work is complete LimnoTech staff will go over the event and note any deviation of plans with Project and QA staff, as well as document fieldwork in a Technical Memo.

2.4 ANALYTICAL METHODS

2.4.1 Field Analytical Procedures

The YSI multiprobe will be used to take field measurements of pH, specific conductivity, turbidity and temperature according to standard sonde methods outlined in the User's Manual. Dissolved oxygen will be measured by optical sensor, calibrated to saturated air. The multiprobe is calibrated each day before field work with commercially purchased calibration solutions for standard conductivity, turbidity and pH. Measurements of water quality are taken prior to substrate sampling, the read-outs from the probe are recorded on field sheets.

2.4.2 Laboratory Analytical Procedures

Macroinvertebrate samples will be picked and identified in the LSRI laboratory according to LSRI SOP FS/14 and FS/13. Samples are rinsed with tap water, sieved, and specimens transferred into 3.7 mL scintillation vials. Vials are preserved with 70-80% denatured ethyl alcohol. Depending on the gross number of organisms encountered in each petite ponar, samples may be divided for identification according to LSRI SOP FS/12. Benthic invertebrates will be identified to the lowest taxonomic level practicable, based on current literature, as genus/species identification provides more accurate ecological and environmental information. All student identifications are verified by the Senior Invertebrate Taxonomist to ensure accuracy. See LSRI SOPs in Appendix C.

Sub-sampling of benthos substrate samples will only occur in the case that there are over 500 specimens per sample, otherwise all samples will be picked in entirety (90% picking efficiency as per SOP).

Calculations of taxa per square meter (species richness) and total organisms per square meter (abundance) will be made from the raw count data based on the size of the petite ponar. A square meter has 10,000 cm². The 10,000 cm² will be divided by the length/width size of the sampling device (cm²) to derive the conversion factor for reporting results/m². For example, the number of organisms in a petite ponar sample is multiplied by the conversion factor to be reported as organisms/m² to allow comparison to data from other sampling efforts treated in the same way when the size of the sampling equipment may not be the same.

Sediment will be analyzed for Total Organic Carbon content at the Wisconsin SLH, Madison, according to ESS ORG Method 1560 (SW846 Method 9060 – Revision 0, 1986) (Appendix D). The basis of this method

is the thermal combustion of carbon to carbon dioxide, the carbon dioxide produced is quantified. Acid is first added to the sediment to release inorganic carbon as carbon dioxide. The remaining carbon is the organic portion, which is then analyzed by thermal combustion.

The State Lab of Hygiene will send samples to be analyzed for particle size distribution to the UW-Soil Science Extension laboratory in Madison, WI. (Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of Soils. Agron. J. 54:464-465.) (Appendix D). In this method, the sample is treated with sodium hexametaphosphate to complex Ca^{++} , Al^{3+} , Fe^{3+} , and other cations that bind clay and silt particles into aggregates. Organic matter is suspended in this solution. The density of the soil suspension is determined with a hydrometer calibrated to read in grams of solids per liter after the sand settles out and again after the silt settles. Corrections are made for the density and temperature of the dispersing solution. Particle size is reported as sand (0.05 – 2.0mm), silt (0.002 – 0.05mm) and clay (<0.002mm). A sample of known particle size distribution is analyzed with each batch to check procedural accuracy.

2.5 QUALITY CONTROL

Laboratory-the standard laboratory practices for WSLH are acceptable to DNR and MPCA. The lab QA criteria have been reviewed as part of their WI certification. The sediment analyses are intended to aid interpretation of the data, so the general precision and accuracy is acceptable. Laboratory Control Sample Limits will be used as basis of measuring lab accuracy.

Table 5. Laboratory- QA standards

Total Organic Carbon	Laboratory Control Sample Limits:	25.9%
Total Organic Carbon	Matrix Spike Limits:	76.3% – 148%
Total Organic Carbon	Matrix Spike Duplicate Limits:	6.4%

Multiprobes- will be calibrated daily prior to field work, according to the User’s Manual, with calibration information kept in the field book and calibration logs for each instrument. At the end of the day, each sensor’s calibration will be checked in measurement mode according to the SOP “Calibration of Field Instruments, Revision 2010” (Appendix A). If the calibration of a particular sensor is seen to have drifted beyond acceptance, that day’s data from that sensor (since the last calibration) will be flagged.

Table 6. Calibration check criteria for Multiprobe

Measurement	Post Calibration Criteria
Dissolved Oxygen	+/- 0.5 mg/L of saturation value
Specific Conductance	+/- 5% of standard or +/- 10 us/cm (whichever is greater)
pH	+/- 0.3 pH unit with pH 7 buffer

Turbidity	+/- 5% of standard value
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Macroinvertebrates - Quality control for the macroinvertebrate identification will consist of sample picking, sub-sampling if necessary, and identification as specified in LSRI's SOPs. Each SOP includes checks of accuracy and completeness in sample handling and picking. Identification and enumeration of invertebrates is the responsibility of LSRI's Senior Invertebrate Taxonomist, Kurt Schmude. All identifications made by students and a proportion made by biologists are verified by the senior taxonomist to ensure accuracy.

Sediment - Quality control in the State Lab of Hygiene and Soil Science Lab will be carried out according to the laboratory Quality Assurance Management Plan and method SOPs. A method blank, laboratory control and matrix spike/matrix spike duplicate is run with each batch or every 20 samples. Relative percent difference and percent recovery are calculated to document precision and accuracy, respectively.

2.6 EQUIPMENT MAINTENANCE

2.6.1 Field Instrument Preventative Maintenance

Multiprobe maintenance involves making sure each sensor is in good condition and clean of fouling. The sensors should be stored with a small amount of water in the cap provided with the sensor to maintain humidity. Care, Maintenance and Storage instructions are included in Section 2.10 of the Users Manual.

2.6.2 Laboratory Instrument Preventative Maintenance

Each laboratory maintains equipment according to their Standard Operating Procedures and Quality Assurance Management Plans.

2.7 INSTRUMENT CALIBRATION

2.7.1 Field Instrument Calibration

Multiprobe calibration is carried out according to the YSI User's Manual and Calibration SOP. See Appendices A and B. The multiprobe must be warmed up prior to calibration, and sensors should be allowed to stabilize before the actual calibration step is finalized. The temperature sensor does not require field calibration, accuracy should be checked against another reliable thermometer at least on a yearly basis.

The pH sensor is calibrated with two buffer solutions that bracket the expected pH of the water to be monitored. The specific conductance calibration only requires a one-point standard solution calibration. The turbidity meter will be calibrated to a 126 NTU formazin solution. The dissolved oxygen sensor is calibrated to water-saturated air and the current barometric pressure.

2.7.2 Laboratory Instrument Calibration

Each laboratory calibrates its instruments according to the SOPs. The TOC method utilizes a 5-point calibration for quantification purposes. The calibration is good for one week, initial calibration check standards and a second source standard is run with every batch.

2.8 INSPECTION OF SUPPLIES

Sample containers will be supplied by the appropriate analytical laboratory. Sample containers will be inspected for condition upon arrival and the method appropriate preservative. If there is a problem with containers or preservative upon receipt, the appropriate laboratory will be contacted immediately so that the correct container and/or preservative can be obtained.

2.9 NON-DIRECT MEASUREMENTS

Historical data was utilized in the selection of sites for this study. The NRRI report "Field Truthing and Vegetation Assessment of NRRI-GLEI Reference Sites for Near-shore Ecotypes in the SLR-AOC" was funded in part by MPCA and the USEPA. That project was peer reviewed by the St. Louis River Alliance Habitat Work Group. The available sediment chemistry information for each site was found in the GIS-

Based St. Louis River Sediment Database Phase IV. The historical studies in this database were done under different funding sources, and for differing objectives, however, quality control information was documented in the database. The database itself was built in several phases by consultants for MPCA, under several Quality Assurance Project Plans.

2.10 DATA MANAGEMENT

Field day information including unique occurrences affecting sampling, time on and off each site, photo documentation and instrument calibrations will be kept in a field book. Field measurements and observations will be written into field data forms. LimnoTech will provide a technical memo of the fieldwork for this project.

Project data will be sent from the SLH directly to the Wisconsin SWIMS database, and a file will be sent to the Minnesota Performance Management and Quality Division database. LSRI will provide a file to be added into the Wisconsin SWIMS database and the Minnesota Performance Management and Quality Division database. An update to the Phase IV database is currently under development by a joint WI/MN team with assistance from AOC partners. This project data will eventually be added to the AOC-wide benthos data in conjunction with the AOC Database system.

The Wisconsin SWIMS database will calculate 15 different indices on the macroinvertebrate data. These indices include an Index of Biotic Integrity, Hilsenhoff's Biotic Index, Family Biotic Index, taxa richness, abundance and other common indices including feeding guild indices. Metrics such as Hilsenhoff's Biotic Index that apply to habitat types not sampled in this project are automatically calculated by SWIMS but will not be utilized for this project.

SECTION THREE: ASSESSMENT AND OVERSIGHT

3.1 ASSESSMENTS AND RESPONSE ACTION

LimnoTech field staff will carry out the field sampling according to this QAPP. LimnoTech will prepare a checklist of required supplies and equipment for use in field sampling preparation (see Appendix B). At least one State project lead will be present during the reconnaissance and field sampling to assure that sample locations are selected according to the project specifications and the sampling is done according to plan. Any problems or issues encountered in the field will be resolved immediately (during the field event). See section 2.3.3.

Field conditions at the time of sampling may require adjustments in sampling method or location. Weather conditions may require a delay in sampling (i.e., flood events) or other site conditions may require adjustment of sampling locations (i.e., if large amounts of sawmill debris are encountered, or if contamination is encountered). Two secondary sites have been identified for sampling in the case that one of the primary sites is judged to be inappropriate upon investigation. In the event that adjustments are considered needed, the Project Coordinator will be contacted to discuss modifications to the QAPP prior to implementation and the agreed upon changes will be documented.

3.2 REPORTS TO MANAGEMENT

LimnoTech will provide a final technical memorandum documenting field sampling procedures and results by June 29, 2012.

Lake Superior Research Institute will provide a final report including sample identification information and documentation of problems encountered by January 31, 2012. Data supplied will include raw data and calculated totals such as taxa/m² and organism/m².

Each analytical laboratory will provide a results package for the sediment analyses. Data will be provided directly to SWIMS.

The Project Coordinator will report to the Office of Great Lakes on fieldwork accomplished and analyses accomplished on a semi-annual schedule. A summary report of findings will also be prepared for the final report. The summary report will include an interpretation of the range of variability in benthic communities within and between the least impacted sites.

SECTION FOUR: DATA VALIDATION AND USE

4.1 DATA REVIEW

Macroinvertebrate identifications will undergo review according to LSRI's SOPs. The Senior Invertebrate Taxonomist checks all identifications.

Each analytical laboratory will review their analyses and flag any quality control issues according to their SOPs. This information will be included in the final data package.

The Project Coordinator will review deliverables to ensure that all results have been received and are useable. Benthic macroinvertebrate data will be reviewed and basic analyses, such as taxa per meter-squared and abundance per meter-squared, will be utilized to characterize the study sites. If a site appears to be other than a "least impacted" site, that site may be re-sampled or a secondary site may be sampled.

4.2 VERIFICATION AND VALIDATION METHODS

Project personnel will review project data and resulting quality control information for the sediment sample results. There are no environmental criteria associated with the parameters of total organic carbon and particle size. Accuracy of the TOC will be assessed using the Laboratory Control Sample performance. It is the intent of this project that laboratory analytical issues be identified early at the laboratory and that the laboratory staff communicate with the Project Coordinator so that necessary corrective actions can be taken.

4.3 DATA USE

Macroinvertebrate community data from this project will provide a better understanding of the benthic community structure in least impacted sites within the Area of Concern. Data from this study will be utilized to understand the range of variability within and among habitat types at least impacted sites. Variance components analysis for the transect, habitat type within transect, and replicate habitat type as factors for each response variable will be calculated. Metrics most often used as a response variable in benthic studies in the AOC include taxa/m² and abundance/m², but include others such as non-insect taxa, depositional zone taxa, feeding guilds. A study of metrics in the future may identify other metrics that are useful in the St. Louis River estuary. Metrics that apply to specific habitats not sampled here, for example Hilsenhoff's Biotic Index, will not be utilized in site comparisons. The understanding of variability is critical as a foundation for understanding and quantifying targets for the Degradation of Benthos BUI as well as for remediation and restoration project long-term monitoring purposes.

Variability in a benthic community must be understood in order to compare sites for interpretation of degradation.

Total organic carbon, particle size distribution, water depth, and vegetative habitat type are important parameters to be examined in site comparisons to understand the limits and associations of the benthic community within the AOC. This project's data will be used in the future for purposes of remedial decisions, restoration documentation and AOC-wide determinations as regards the removal of the Degradation of Benthos BUI.

The GIS-based St. Louis River Sediment Quality Database Phase IV includes some benthic community data, however several studies have been completed since that time frame included in the database. It is the intent of partners in the AOC to compile all benthic data for an update to the Phase IV database that will be used in future AOC project prioritization and documentation of the status of the benthic community for purposes of the removal of the Degradation of Benthos BUI.

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