ATTACHMENT C: CATEGORY 1 MACROINVERTIBRATE SAMPLING

St. Louis River AOC R2R Support Projects:

Ecological Monitoring and Assessment (CR#6403):

Benthos Sampling and Identification

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):

Benthos Sampling and Identification

Approval

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A2: TABLE OF CONTENTS

A3: QAPP Distribution List

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A4: Organizational Chart

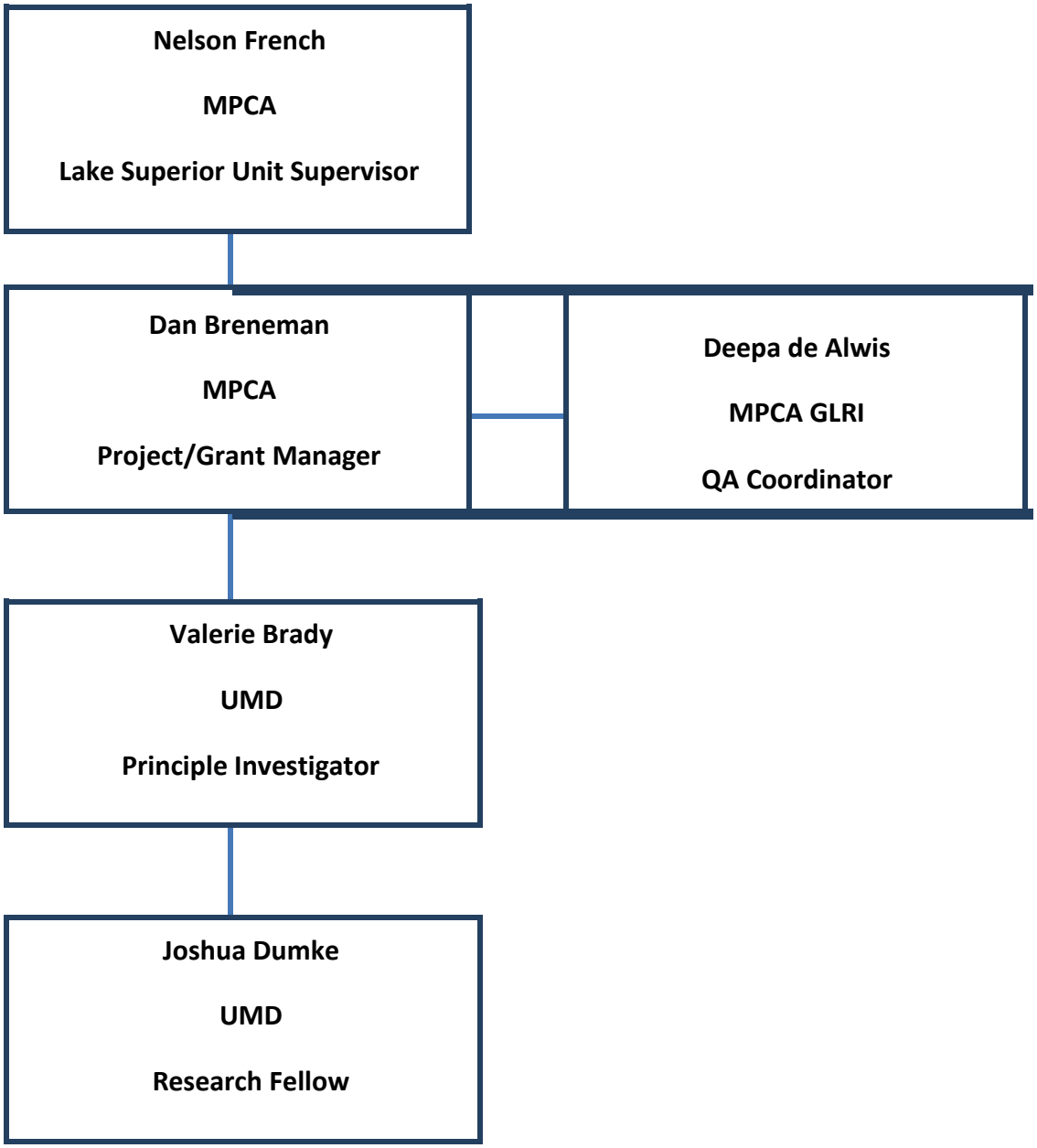

Figure 1. Organizational chart

A4.1: Project Individual Responsibility

The MPCA Project Manager is responsible for project oversight. The principle investigator (PI) and collaborators/employees are responsible for sampling, identification, QC, and production of the dataset. Full responsibilities are listed in Table 1, and a complete chart showing the lines of authority and independent review is shown in Figure 1.

Table 1. Positions and responsibilities

Position	Responsibility
MPCA project manager	Outline objectives, approve work and financial updates, review data upon final delivery
MPCA QA/QC coordinator	Verify adequacy of QAPP and SOPs and ensure that the project is completed according to the QAPP.
PI and collaborators	Establish sampling points and a sampling plan, conduct sampling, process and identify macroinvertebrate samples, enter and QC data, create sample inventory, and document chain-of-custody for all samples
PI	Implement all QA/QC measures, produce reference collection if requested.

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. A major contributor to AOC status is legacy sediment contamination, which is the basis for most resource management decisions when delisting strategies are considered. Sediment characteristics, habitat conditions, and the resulting benthic community provide vital links between AOC status, restoration decisions, and recovered condition. Sediment contamination assessment throughout the AOC is fundamental to prioritizing project areas, establishing objectives, and successfully implementing Remediation-to-Restoration (R2R) project activities. Understanding biotic response relationships between anthropogenic stressors and the benthos is important for determining overall community health, orchestrating restoration opportunities, and ultimately assessing environmental recovery.

Currently, R2R conceptual plans are designed primarily based on sediment contamination recommendations, with little biological survey data available for decision support. There is currently poor understanding of the effects of sediment contaminants and concentrations on benthic macroinvertebrates within the St. Louis River AOC. Establishing relationships between macroinvertebrate taxonomic diversity, density, proportional abundance, sensitive taxa, and behavioral and functional metrics and contamination levels in the AOC will aid restoration decisions and minimize errors when interpreting environmental conditions based solely on contaminant studies.

Thus, MPCA has contracted with the PI to collect and identify aquatic macroinvertebrate samples at R2R and reference locations within the estuary. These data will provide baseline benthos conditions for R2R sites, allowing later sampling to show improvement following R2R work, and will also provide data from least-disturbed locations within similar areas of the estuary as appropriate benthos restoration targets.

The data may also allow establishment of relationships between macroinvertebrate metrics and contaminants.

In addition, MPCA has contracted with the PI to increase the taxonomic resolution of the Diptera family Chironomidae that were collected previously in the USFWS-contracted sampling of 21st Ave West and 40th Ave West, and the Minnesota Land Trust-contracted sampling of Cedar Yard Bay; all previous sampling was done by the PI. The reason for this increase in taxonomic resolution is to better match historic sampling in the harbor in the 1980's and 1990's (see references below), as well as to match taxonomic resolution in the sampling covered by this QAPP.

A5.1: Objective

1. To acquire baseline data on macroinvertebrate richness and abundance that will be used to evaluate the St. Louis River Area of Concern (AOC) "Degradation of Benthos" Beneficial Use Impairment (BUI).

2. To identify to genus the Diptera: Chironomidae collected during the previous sampling efforts at 21st Ave West, 40th Ave West, and Cedar Yard Bay.

A6: Project/Tasks Description and Schedule

The tasks for this project include sampling and identifying aquatic macroinvertebrates from both the current sampling effort and for the Diptera: Chironomidae from 21st and 40th Ave West and Cedar Yard Bay sampling efforts. The PI will be responsible for ensuring that aquatic macroinvertebrates are sampled at each of nine R2R sites and no less than five reference locations that have been matched for habitat similarity and river/estuarine position to R2R sites. Macroinvertebrate sample collection will be by petite ponar dredge and D-frame dip net. Macroinvertebrates will be removed from sample debris, identified, and enumerated. Sampling density at R2R sites will approximate 1 sampling point for every 2-4 acres in area, with lower density at very large sites and higher density at small sites to ensure an adequate number of samples to characterize each site. Sample collection methods will follow previous baseline macroinvertebrate sampling efforts in the St. Louis River estuary, both historical and completed by the PI, including 21st Ave. W, 40th Ave W, and Cedar Yard Bay (cf., NRRI 2010, 2011, 2012).

The PI will ensure that all individuals processed per sample are identified to lowest practical taxonomic unit, typically genus (NRRI 2013). Total Diptera: Chironomidae individuals per sample are also counted, but identifications and counts for genera will be generated from sub-sampling. Total counts of Diptera: Chironomidae genera per sample will be extrapolated based on the slide-mount distribution of taxa (NRRI 2013).

Identifications will be QC'd, data will be entered into a format provided by MPCA to fit the AOC biological database, and all data entry will be QC'd by a second person comparing the paper data sheets to the data entered into the electronic spreadsheet (cf., MPCA 2013, NRRI 2013). At the end of the project, the PI will provide MPCA with the data and a report comparing R2R sites with appropriate reference locations.

Sampling and macroinvertebrate sample processing and identification will be spread across all three years of the project, with approximately 1/3 of the effort accomplished each year. Sampling will take place during August and early September, with sample processing, identifications, data entry, and QC occurring during the fall, winter, and early spring of each year.

DQOs focus primarily on proper collection and handling of data and samples, and proper QC of data.

A7: Data Quality Objective and Criteria for Measurement Data

The primary DQO for this study is the acquisition of accurate and representative samples of the macroinvertebrates within R2R and reference areas in the St. Louis River estuary. It is very important that the data that we collect are representative of each site within the estuary (Figure 2). By following the study design in this document and the methods described here and in our macroinvertebrate SOP (NRRI 2013), we will generate representative and reproducible monitoring results that match sampling events from the 1980's and 1990's. These historic samples provide a historic reference point important for showing how sites have changed through time, and for establishing natural variability at reference locations. In addition, providing increased taxonomic resolution for Diptera: Chironomidae not only matches historic sampling efforts but also greatly increases potential macroinvertebrate diversity at sites, allowing greater ability to compare R2R and reference locations.

In addition to all of the instructions throughout this document designed to help insure representative sampling, we 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 the river returns to 150% or less of baseflow conditions. Similarly, high winds and waves can compromise sampling and field crew safety. Crews will not attempt to sample during small craft advisories or if they are having difficulty holding station points; they should move to sample more protected sites instead until waves and wind lessen. In all cases, crews will not attempt to sample if there is any concern for their safety.

Activities to be performed during this project include:

1) site selection;
2) sample and data collection of macroinvertebrates and supporting habitat measurements;
3) laboratory identification of macroinvertebrates, including identification to genus of Diptera: Chironomidae collected previously from 21st and 40th Ave West and Cedar Yard Bay;
4) data processing and data entry into database-compatible spreadsheets;
5) data QC; and
6) reporting to MPCA.

The DQO for data entry is 100% accuracy of data copied from field and laboratory sheets into the data entry spreadsheet.

For this project, we will be doing limited data analyses and summarization on the collected data. Most data analysis will focus on comparison of macroinvertebrates between R2R and reference sites. Thus, DQOs focus primarily on proper collection and handling of data and samples, and proper QC of data.

A8: Special Training Requirements

Prior to any work-related effort, all project personnel will be trained in sampling techniques by experienced personnel, and will sample or process samples only under the supervision of personnel experienced in these sampling techniques until they demonstrate proficiency. NRRI taxonomists have been identifying aquatic macroinvertebrates for more than five years each. NRRI's lead taxonomist has had his identifications checked by other, independent taxonomists and is highly accurate in his identifications. All project personnel will also have met laboratory safety training requirements, project and field safety training, and been approved by the department safety officer. For detailed information regarding general laboratory safety and protocols, refer to The University of Minnesota Department of Health and Safety Laboratory Safety Plan (see http://www.nrri.umn.edu/safety/policies.html for documentation).

A9: Documentation and Records

Field datasheets will be created specifically for this project for all site and point data. Locations of sampling points will be acquired via GPS and latitude/longitudes recorded. Data are then entered into database-compatible spreadsheets. A final report comparing baseline data from R2R sites compared to data collected from reference areas will be provided at the end of the project. The data themselves will be provided to MPCA in biological database format in the spring of each year as QC is completed on data entry.

B: DATA GENERATION AND ACQUISITION

B1: Sampling Process and Design

Sampling Points

All sites included in this project (Figure 2, Table 2) will be sampled over two years instead of sampling all points within a site in a single year (with exceptions noted below) to help ameliorate potential weather differences and other chance events. Which R2R sites will be sampled in which years will be decided in collaboration with our MPCA project office based on the schedule of remediation or restoration work. Reference sites will be sampled relatively evenly across all 3 years. Several sites (21st, 40th, and Cedar Yard) have already received one year of sampling; these three sites will be sampled in only one year (2013 or 2015) to increase sample point density at the site beyond that during the original sampling efforts and to provide a second year's worth of data to ameliorate interannual variation. We will use site polygons provided by our MPCA project officer to ensure that all sampling occurs within accurate site boundaries.

Final sampling density within each site will approximate 1 sampling point for every 2-4 acres in site polygon area, with lower density at very large sites and higher density at small sites to ensure adequate

number of samples for site characterization. No site will have less than 4 samples of any sample type collected in any year to ensure adequate samples for statistical analysis (Table 2). For the three sites previously sampled (21st, 40th, Cedar Yard), somewhat lesser numbers of samples will be collected because the sample data can be combined with the data from the previous sampling efforts (Table 2). Point densities are primarily for petite ponar dredge samples, supplemented by D-frame dip net samples at sites expected to have shallow, vegetated habitat. The number of D-net samples collected will depend on the amount of shallow-water emergent or submergent vegetation at the site. Estimates of target numbers of D-net samples at each site are shown in Table 2.

Petite ponar dredge sample points will be either laid out on a grid across each site polygon and/or be placed to match up with recent sampling events (since 2008). In some instances, points will also be placed to intersect relatively recent macroinvertebrate sampling (USEPA 2010 samples; Figure 3). 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 two-thirds of the sample effort at each site. This is important because shallow water macroinvertebrates are different than deeper water invertebrates, and shallow water assemblages are the target for much of the restoration work. How this will work in practice at any given site, for example at a large site, half of the sampling points may be allocated to overlap with points from previous sampling events, with the rest of the points laid out on a grid across the site. 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 have unsampleable substrate (Figure 3). 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.

D-frame dip net sample locations will be allocated at a site by spreading them out across shallow emergent and submergent vegetated areas at each site. Because these vegetation zones cannot be easily seen accurately on aerial photos, target points will be provided by the GIS lab to ensure good point distribution, but crews will be given flexibility to move to the nearest vegetated area if no vegetation occurs at the target point.

For all samples of either type, sample location will be marked using GPS and downloaded each day. Waypoints for each sample point will be recorded on data sheets and provided with the data.

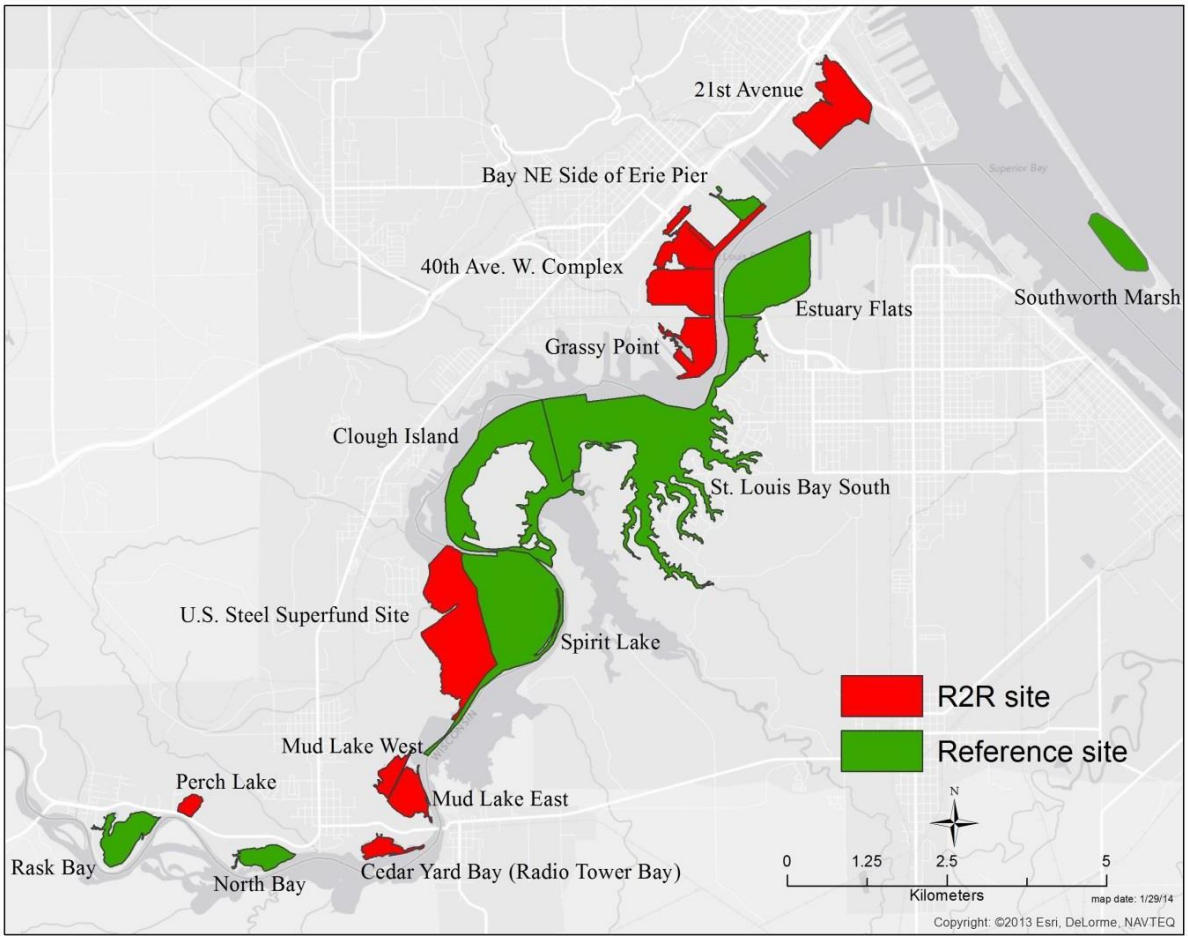

Figure 2. Map of the St. Louis River estuary and site locations. Refer to Table 2 for more information on individual sites.

Table 2. Sites with target numbers of points for each gear type per year and total, and target years to sample sites.

Site Name	Acres	Petite Ponars	D-nets	3 yr total	pts/ac	Target Years
R2R sites						
21st Ave West (complex)	200	50	0	50	0.25*	2013
Grassy Point (complex)	115	28	11	39	0.34	13,14
US Steel (complex)	400	101	29	130	0.33	13,14
Mud Lake East and West (emergent and shallow)	120	30	10	40	0.33	14,15
Cedar Yard Bay (Radio Tower Bay; emergent and shallow)	38	0	7	7	0.18*	2013
Perch Lake (emergent and shallow)	21	8	8	16	0.76	14,15
40th Ave West (complex)	300	50	15	65	0.22*	2015
Reference Sites						
Complex reference (Spirit Lake/Devil's elbow)	450	87	27	114	0.25	14,15
Emergent & shallow reference (Clough Island, Southworth Marsh, Estuary Flats)	500	90	40	130	0.26	13,14
Deep water reference (part of St. Louis Bay South and Spirit Lake)	50	26	0	26	0.52	14,15
Small bay references (North Bay, NE side of Erie Pier)	90	21	22	43	0.48	13,14
Large bay reference (Rask Bay)	106	30	8	38	0.36	14,15
Total	**2389**	**521**	**177**	**698**		

* These sites were previously sampled by NRRI, with 70, 15, and 20 points sampled per site, respectively.

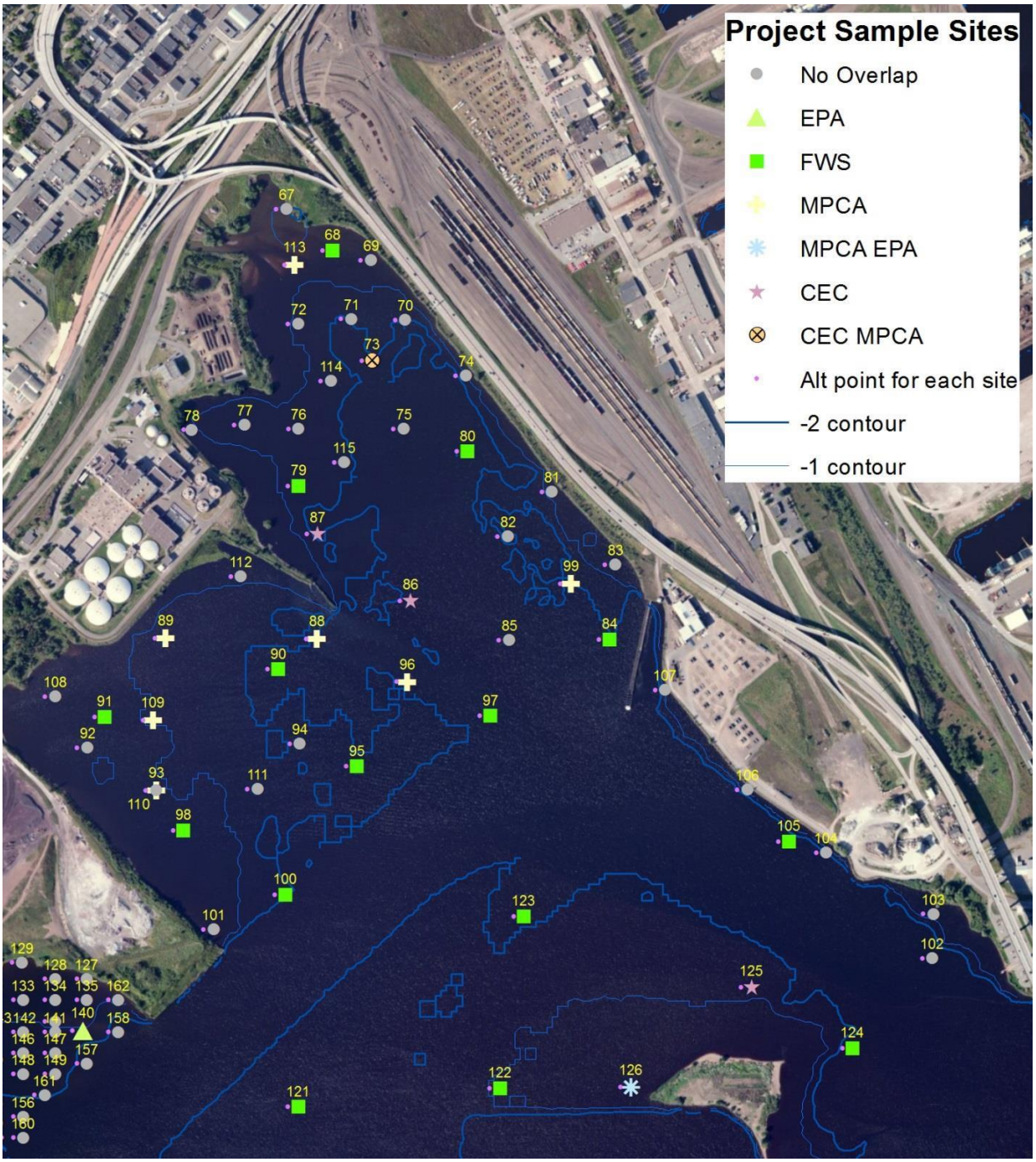

Figure 3. Example of point distribution and overlap of historic sample collections at the 21st Avenue R2R site. Codes are from the MPCA estuary sample database and indicate types of sampling events. 1 m and 2 m depth contours are also shown.

B2: Sampling Methods

Sample Collection

The majority of benthic samples will be collected via petite ponar dredge (0.023 m^2), the most appropriate sampling gear for water depths >1 m and to compare with historic macroinvertebrate records, which were almost all sampled via a dredge. A visual estimate of petite ponar dredge fullness will be recorded to quantify the effectiveness of the petite ponar dredge sample. Fullness estimates less than 25% of petite ponar dredge capacity will be discarded as ineffective sample attempts. Petite ponar dredge samples over 70% full will be retained as successful samples. Up to 3 attempts will be made to get a > 70% full sample at each point. If unsuccessful, the most full petite ponar dredge sample will be kept and its percent fullness recorded on the data sheet. At each site with shallow water vegetation, D-frame dip nets will also be taken to collect macroinvertebrates representative of emergent or submergent vegetative habitat. Dip net samples are taken by bouncing the net off of the bottom substrate and then moving the net quickly up through the water column, brushing and sweeping the net through any vegetation in the process. Each dip/sweep covers approximately 1 m; 10 dips constitute one sample.

All petite ponar dredge samples are washed down through a 250 µm mesh sieve and placed in a sample container with Kahle's preservative. D-nets are outfitted with 500 µm mesh; these samples are also preserved in Kahle's, a combination of ethanol, formaldehyde, and water in a ratio of 15:5:20, respectively. All samples will be carefully labeled with interior and exterior labels that identify: 1) project name, 2) site name or number, 3) sample number and number of containers (i.e. large samples that are placed in multiple containers, A-D), 4) gear type (Dnet or petite ponar dredge), and 5) sample date. Interior labels are written in pencil lead or laser-printed on waterproof paper. Full standard sampling details can be found in the NRRI macroinvertebrate sampling SOP (NRRI 2013).

Supplemental Data

Along with each macroinvertebrate sample collected, supplemental data about the habitat will be collected and recorded on data sheets. Supplemental data include water depth; substrate texture, ascertained by both feel by trained individuals and by comparison to a standard grain-size card (e.g., W. F. McCollough, Beltsville, MD); vegetation presence, type, and percent cover (if visible); and notes about anything unusual (e.g., notable pollution, etc.).

B3: Sample Handling and Custody Requirements

A list of samples, including all label information, will be generated and accompany the samples returning to the laboratory. Chain of custody forms are completed and verified with the field sample list as incoming samples are returned to NRRI. Chain of custody forms and a field sample list are duplicated, a copy filed with field notes or data sheets, and one copy placed in the project log book. Samples are clearly labeled, logged in, and stored in room 126, NRRI. Computerized sample logs track samples as they are processed, identified, and then archived. Archived samples and remaining sample preservatives are deposited in Chemical Storage (Rm. 106, NRRI). Refer to the SOP for further details (NRRI 2013).

B4: Analytical Requirements

Macroinvertebrate Laboratory Sample Processing

Preserved samples are drained through a 250 um sieve under a ventilation hood to remove the Kahle's preservative prior to processing. Samples are then rinsed under running tap water before processing, and re-preserved in 70% ethanol. Discarded preservative is stored in containers labeled with appropriate hazardous waste information and transferred to Hazardous Chemical Storage (Rm 138a, NRRI).

Samples ready for processing are signed out of the project log book by lab personnel. Samples may be in multiple containers, so all containers for that sample are concurrently processed. All sample information contained inside the sample container is verified with outside labels and project log book information.

Each sample is washed through a sieve series in preparation for removing invertebrates from the sediment and detritus. For this project, two sieve sizes will be used: 4 mm and 0.25 mm. Sample material is rinsed through stacked 4 and 0.25 mm sieves, and the material captured by the 4 mm sieve is sorted in a tray or sorting dish. Sample material is spread evenly throughout the tray and invertebrates are removed under a 2X magnification lens. Material collected in the 4 mm sieve is always sorted in its entirety (whole picked). Material caught in the 0.25 mm sieve may be completely sorted or may be subsampled (see next paragraph). Material passing through the 0.25 mm sieve (and thus that is < 0.25 mm in size) is deposited in a waste receptacle and does not go down the sanitary sewer system.

Samples should take a maximum of 3-4 hours to process (i.e., remove the invertebrates from the sediment, vegetation, or detritus in the sample). Due to the amount of material contained in a sample, it may be necessary to sub-sample the portion caught in the 0.25 mm sieve by splitting the material into halves or quarters. Samples are split when laboratory workers estimate that the volume and/or material type will not allow the entire sample to be processed within the 3-4 hour predetermined time period. Because of the volume of sediment in petite ponar dredge samples, the portion in the 0.25 mm sieve is usually split into quarters, or at least halves, unless the sample volume is very small and composed of a material easy to pick through (e.g. 200 mL of sand). If one-quarter of a sample requires less than 1 hour to process, then the entire sample will be processed (e.g., total processing time of 4 hrs). Similarly, if one-quarter of a sample requires 2 hours to complete, then a second quarter can be processed within the 4 hr time limit, resulting in one-half of the sample being processed. Samples that contain very large amounts of small detritus may take 4 hrs to process only one-quarter of the sample.

Sub-sampling itself is accomplished with a sample splitter, sectioned tray, sieve template, or partitioned basin. Which sub-sampling device is used will depend on sample volume and type. The standard lab sample splitter, which splits each sample into halves each time, works for most types of substrate. However, matted plant materials may have to be split using a partitioned basin or sectioned tray. Sub-sampled portions not being processed are returned to the container with 70% ethanol and labeled with appropriate sample information in addition to the fraction remaining. Full details about sub-sampling can be obtained by in the SOP (NRRI 2013).

Organisms are removed from detritus using forceps under 2X to 7X magnification, depending on the size fraction being processed (higher magnification for smaller size fractions). Invertebrates are placed in vials labeled with the same information as on the container labels as well as with the amount of sample processed (i.e., 1/4, 1/2, or whole), a vial number, the total number of vials for that particular sample (e.g., 1 of 3), and initials of lab personnel. The number of vials accompanying each sample will depend on the abundance of organisms, but one vial will be designated solely for any Diptera: Chironomidae larvae that may be found in a sample. This facilitates the later sub-sampling and slide-mounting of these larvae for identification to genus (see *Identification of Diptera: Chironomidae to Genus* below).

Samples that have been processed are subject to quality assurance/ quality control (QA/QC) guidelines, which specify that 10% of samples are checked for accurate removal of organisms. Accurate sample processing is defined as removal of at least 95% of all organisms. If a sample fails QC, it is repicked and other samples processed by that technician are checked until all pass QC. Each completed sample and accompanying vials are designated "complete" by placing an additional label on the container indicating its status (i.e., picked) and date completed. Sample remnants that pass QA/QC inspection may be discarded. Vials containing sorted organisms preserved in 70% ETOH are logged into a vial chain of custody form. No samples will remain outside of the appropriate sample jar when the technician responsible for a particular sample has left the laboratory.

Sample Identification

For identification, macroinvertebrate vials are signed out for identification using the vial chain of custody form. Organisms are identified to the lowest taxonomic level using appropriate keys (Brinkhurst 1986, Wiggins 1996, Merritt et al. 2008, Thorp and Covich 2010), enumerated, and recorded on an identification data sheet. A reference collection can be made for the project, if requested. QC for macroinvertebrate identifications will consist of sending 10% of samples to Dr. Kurt Schmude, University of Wisconsin Superior's Lake Superior Research Institute, who is a regional expert on aquatic macroinvertebrates. Based on the QC results, taxa will be re-identified as necessary to correct identification data.

Identification of Diptera: Chironomidae to genus

Midge larvae (Diptera in the family Chironomidae) from both the samples collected in this project and from the samples previously collected at 21st and 40th Ave West and Cedar Yard Bay will be permanently mounted on microscope slides to allow for higher magnification for further taxonomic identification. Identification to genus, as requested by MPCA, requires head capsule removal to ensure ventral viewing of individual mouth parts. Organisms are soaked in 95% ETOH, preserved in CMC-10 mounting medium (Masters Company, Inc., Wood Dale, IL), and placed ventral side up under a cover slip. Generally, Diptera: Chironomidae are sub-sampled in a watch glass, and 8 randomly-selected individuals are placed on a slide. For this project, three slides will be created per sample, yielding 24 Diptera: Chironomidae to represent the sample. Individual placement on the slide and label information will follow the standard template.

Permanent slide mounts will be identified to the lowest taxonomic level under a compound microscope (Merritt et al. 2008, Wiederholm 1983). Each individual will be assigned a particular slide number, position, and side, so a separate reference collection for these organisms is not necessary because the location of individual organisms can be easily determined. Diptera: Chironomidae identification will be conducted by our most experienced aquatic macroinvertebrate taxonomist, who has worked in the NRRI taxonomy laboratory for 10 years. For added QC, these identifications will undergo 10% QC by Dr. Kurt Schmude at the University of Wisconsin Superior, a recognized regional expert.

B5: Quality Control

PI Brady will ensure that all SOP and QC procedures are followed, that samples are tracked via chain-of-custody, and that all deliverables are prepared and transferred to MPCA. These include verification of sample point location appropriateness; verification of sample processing and QC; verification of macroinvertebrate taxonomic identification and QC; production of a reference collection, if desired by MPCA; and verification of data entry and QC. Dr. Brady received QA/QC training as a post-doctoral associate at the US EPA Mid-Continent Ecology Division between 1997 and 2000. She subsequently worked on QA/QC for the GLEI-I project and Estuarine and Great Lakes (EaGLe) Coastal Indicators Initiative (funded by US EPA STAR), and is currently the QC officer for the GLEI-II and Great Lakes Coastal Wetland Monitoring projects (funded by GLRI through EPA GLNPO). She has attended all of the GLNPO QA/QC webinars offered recently by US EPA GLNPO (approximately 12 hours of training).

Accuracy

The systematic difference from a reference standard or an expert.

The PI will ensure accurate macroinvertebrate identification by ensuring employees follow standard operating procedures (NRRI 2013, also attached), use the appropriate standard identification guides (Simpson and Bode 1980; Oliver and Roussel 1983; Wiederholm 1983; Wiggins 1996; Merritt and Cummins 2008; Thorp and Covich 2010), that identification is done by experienced taxonomists (NRRI employees Robert Hell (10 yrs experience) and Joshua Dumke (2 yrs experience)), and that there is 10% external QC of samples (NRRI 2013) by regionally recognized aquatic macroinvertebrate expert Dr. Kurt Schmude of the Lake Superior Research Institute at University of Wisconsin Superior.

Internal quality control procedures for initial sample processing and subsampling will involve sorting efficiency. These checks will be conducted on 10% of the samples by independent observers who re-examine 100% of sorted substrate from each randomly-selected sample. Quality control calculations are as follows:

$$\left(\text{———}\right)$$

- SE is the sorting efficiency, expressed as a percentage,
- n_1 is the total number of specimens in the first sort,
- n_2 is the total number of specimens in the second sort.

If SE is < 95%, then that technician will be re-trained and all of the samples that they processed will be re-processed by another employee.

Internal quality control procedures for taxonomic determinations of invertebrates will involve checking accuracy and precision of macroinvertebrate identifications between NRRI taxonomists. Ten percent of samples will be randomly selected and the macroinvertebrates from those samples will be re-identified by an independent taxonomist (Dr. Kurt Schmude, University of Wisconsin Superior's Lake Superior Research Institute, a recognized regional aquatic macroinvertebrate expert). Ten percent of Diptera: Chironomidae samples will also be sent to Dr. Schmude for quality control.

Precision

Precision refers to how similar duplicates or splits compare to themselves and is calculated as a % difference: % difference = (A-B)/((A+B)/2) * 100. The high number of samples collected at each site can be used to generate a precision estimate by using the 5 closest samples to any given sample of interest (that is, precision is calculated for each sample A by comparison with each of the 5 nearest samples (B1-B5); these percent differences can then be averaged). Note that precision is often quite low for benthos samples because natural environmental variability is very high.

Representativeness

Representativeness refers to how well sites were sampled. Sample density at sites is generally one point for every 2-4 acres, with a minimum of 4 samples of each sample type for small sites. Cedar Yard Bay, 21st Avenue West, and 40th Avenue West have already been sampled by NRRI under previous contracts (NRRI 2010, 2011, and 2012). The number of samples already acquired from these sites is not counted in the points-per-acre calculations in Table 2, which makes these sites appear under-sampled, when in fact they are not. Refer to Table 2 for further details.

In addition, samples are also spread over all areas of each site polygon using GIS to ensure that all parts of each site will be sampled. This sampling grid is stratified by depth to ensure that adequate effort is expended in shallower areas that should have higher macaroinvertebrate density and diversity. MPCA is also interested in better representing macroinvertebrates living within vegetated areas; thus areas that should have aquatic vegetation are deliberately targeted with at least 10% of sampling points within each site polygon.

Comparability

Comparability is being assured by adhering to standard benthos sampling procedures used in previous benthos sampling efforts in the estuary (NRRI 2010, 2011, and 2012). These include using 250 um operative mesh size and collecting most samples using a petite ponar dredge, as well as identifying Diptera: Chironomidae to genus.

Completeness

Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites based on the number of targeted points for each site polygon versus the number of points that were actually sampled with either a petite ponar dredge or a D-frame dip net. Some

points may not be sampled due to substrate that cannot be penetrated by the petite ponar dredge (e.g., packed sand, gravel, or other rocks; wood waste; etc.).

Bias

Systematic bias by a crew or method. Bias in benthos sampling is a known problem because specific sampling methods select for or against various taxa. In this instance bias has been determined to be preferable to loss of comparability. Thus, the PI has been requested to use the most common methods (e.g., petite ponar dredge and D-frame dip net) that have been previously used to sample the estuary to ensure comparability with previous sampling (cf., NRRI 2010, 2011, 2012). Because the method biases are known and have been documented in the published literature, they can be explained in final reports and metadata. These methods are the methods described here in this QAPP and in the NRRI macroinvertebrate sampling SOP (NRRI 2013).

Sensitivity

In this case sensitivity refers both to the most-specific taxonomic levels achievable and to the ability to detect uncommon taxa. Identification will depend on the life stage of invertebrates and their condition after preservation. Life stage is primarily controlled by the time of year of sampling. Sampling is timed (August and early September) so that the most taxa will be identifiable when field crews are sampling. Crews are trained to minimize damage during preservation of organisms. Detecting uncommon taxa is a function of sampling effort, which is a cost trade-off. A sampling density of 0.25 - 0.5 points/acre has been determined to be both affordable and a high enough intensity to sample all but uncommon taxa that live in the habitats being sampled and will be present at the time of sampling in an aquatic life stage.

B6: Instrument/Equipment Testing, Inspection and Maintenance

The petite ponar dredge will be examined before each sampling day to ensure that it is functioning properly. D-frame nets will be inspected before and after each set of sample collections for holes or other damage. Damaged nets will be repaired in the field, and replaced at the end of that sampling trip as necessary. Each crew will have one replacement net with them at all times. Spare batteries will be carried for the GPS units and cameras.

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 invasive species hitchhikers.

B7: Instrument/Equipment Calibration & 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. All tests and results will be logged and the logs kept with the appropriate GPS units.

B8: Inspection/Acceptance of Supplies and Consumables

N/A

B9: Non-direct Measurements

N/A

B10: Data Management

Raw QC'd data are merged with a taxonomic database maintained by NRRI to check for errors and provide full taxonomic hierarchy for all individuals identified. Taxa not listed in the database are checked for correctness of identification and/or the current information and taxonomic name are compared to the Integrated Taxonomic Information System (ITIS) database for confirmation (see http://www.itis.usda.gov).

Data from field and laboratory sheets will be entered into an Excel spreadsheet in a format that allows for easy importation into the MPCA biological database. Counts will be corrected for subsampling and slide mounting. Data entry is subjected to 100% QC to ensure that all data are correct before being provided to MPCA. Data QC is accomplished by having a second person compare the data sheets with the data entered into the electronic spreadsheet. MPCA Project Management is responsible reviewing data upon final delivery.

C: ASSESSMENT AND OVERSIGHT

C1: Assessment and Response Actions

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

C2: Reports to MPCA

The PI 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 REPORTING, DATA REDUCTION, AND DATA VALIDATION

D1: Data Reporting Requirements

The required final data format is out lined in the following tables.

Table 3. Minimum fields of record for benthos data

Field Name	Definition
STATIONID	Station ID code
LongDD_83	Geographical coordinates (decimal degrees; NAD83 datum).
LatDD_83	Geographical coordinates (decimal degrees; NAD83 datum).
STUDYNAME	Study name.
CONTACT	Contact person/agency.
SAMPLEID	Sample ID code.
FIELDREP	Identifies field replicate samples (samples collected in close proximity).
SAMPDATE	Sample date (YYYYMMDD).
Collection_Gear	Type of collection gear used to obtain macroinvertebrate sample.
Lowest_ITIS_TSN	ITIS Taxonomic Serial Number for the lowest level taxa assigned
Count	Count of identified taxa

Table 4. Additional fields or information provided in an accompanying dataset

Field Name	Definition
STATIONID	Station ID code
SAMPLEID	Sample ID code.
FIELDREP	Identifies field replicate samples (samples collected in close proximity).
SAMPDATE	Sample date (YYYYMMDD).
SAMPTIME	Sample time.
Collection_Gear	Type of collection gear used to obtain macroinvertebrate sample
Lowest_ITIS_TSN	ITIS Taxonomic Serial Number for the lowest level taxa assigned
Lowest_Taxa	Laboratory naming scheme (assigned to ITIS number recommended)
Taxonomic Level	Hierarchical (variable number of categories- Class, Order, Family, etc.)
Count	Count of identified taxa
No./Area	Individual taxa by surface area (number of individuals/meter^2)
Notes	Additional notes about the result

D2: Data Review, Validation and Verification

Processing is subject to protocol review, and data will undergo standard laboratory QA/QC procedures.

Taxonomic data and counts will be entered into the PI's laboratory database, and provided to MPCA as electronic spreadsheet files that include the information in tables 3 and 4 as well as metadata and sample point supplemental data (water depth, substrate type). Data will be verified and approved by the MPCA QA officer. A technical summary document describing methods and quality assurance procedures and results will be included as part of the final report.

D3: Verification and Validation Methods

Described above.

D4: Reconciliation with User Requirements and Project deliverables

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.

- Dataset including verified data and metadata in Excel in a format compatible with MPCA biological database.
- Final report on results including taxonomic narrative, QC summary, and data comparison between R2R and most appropriate reference sites.

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