Quality Assurance Project Plan

Coastal Wetland Monitoring: Continued Implementation by the GLCWC

Prepared for:
U.S. EPA GLNPO (G-17J) 77 W. Jackson Blvd. Chicago, IL 60604-3590
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A4. Project Organization

This monitoring program will be carried out by many individuals from many organizations located around the Great Lakes basin. Nearly all of the original authors of the GLCWC monitoring plan (GLCWC 2008), including many who contributed to indicator development, and many of the lead scientists from the Great Lakes Environmental Indicators (GLEI) project, are co-principal investigators (co-PIs). The project team (Figure 1) consists of U.S. scientists from Central Michigan University (Donald Uzarski, principal investigator/lead grantee; Thomas Gehring, Dennis Albert), the Natural Resources Research Institute (NRRI) at the University of Minnesota Duluth (Valerie Brady, project coordinator and QA manager, regional team lead; Gerald Niemi, bird and anuran coordinator), the Annis Water Resources Institute (AWRI) at Grand Valley State University (Al Steinman, Carl Ruetz), the University of Notre Dame (Gary Lamberti), Lake Superior State University (Ashley Moerke), State University of New York-Brockport (Douglas Wilcox, regional team lead); the University of Wisconsin (Robert Howe at Green Bay, Nick Danz at Superior, and Joseph Gathman at River Falls), and Northland College (Matt Cooper, assistant QA manager/project coordinator), and two resource management officials: Anne Garwood (Michigan Department of Environmental Quality) and Kurt Kowalski (USGS). Canadian scientists from the University of Windsor (Jan Ciborowski, regional team lead), Environment Canada and Climate Change (Greg Grabas), and Bird Studies Canada (Doug Tozer) are also key participants.

Project coordination and QA management are provided by Don Uzarski (CMU), Valerie Brady (NRRI), and Matthew Cooper (Northland). These individuals will ensure both cost-effective sampling and project oversight, and will be responsible for all reporting to EPA. They will also be responsible for assuring that all teams and participants are properly trained in QA/QC methods and procedures, and will assist with QC evaluations throughout the project. They will schedule regular meetings (once per year face-to-face) and conference calls (bi-monthly) among co-PIs to ensure coordination and foster communication among teams. Regional team leaders will oversee QA/QC of their field teams, ensuring that field crew chiefs understand the importance of QA/QC and understand all methods and procedures. Field crew chiefs will enforce QA/QC with their crews, checking that all crew members can follow all QA methods and pass QC checks on all aspects of their work.

Project Team Background

Dr. Don Uzarski (CMU) is the lead PI on this project. He was a member of the Project Management Team and Chair of the Science Committee that developed the Great Lakes Coastal Wetland Consortium Monitoring Program (GLCWC 2008). His responsibilities included the development of: 1) a stratified-random statistical sampling design; 2) water quality and habitat protocols; and 3) fish and macroinvertebrate sampling protocols and indicators. He was also co-editor of the final report and training manuals. Dr. Uzarski has worked on Great Lakes coastal wetlands since 1997. He has published a number of papers on coastal wetlands in peer-reviewed journals, conference proceedings, and book chapters. He is currently an associate

editor for the international journal *Wetlands* and specializes in manuscripts involving Great Lakes coastal wetlands, invertebrates, and fish.

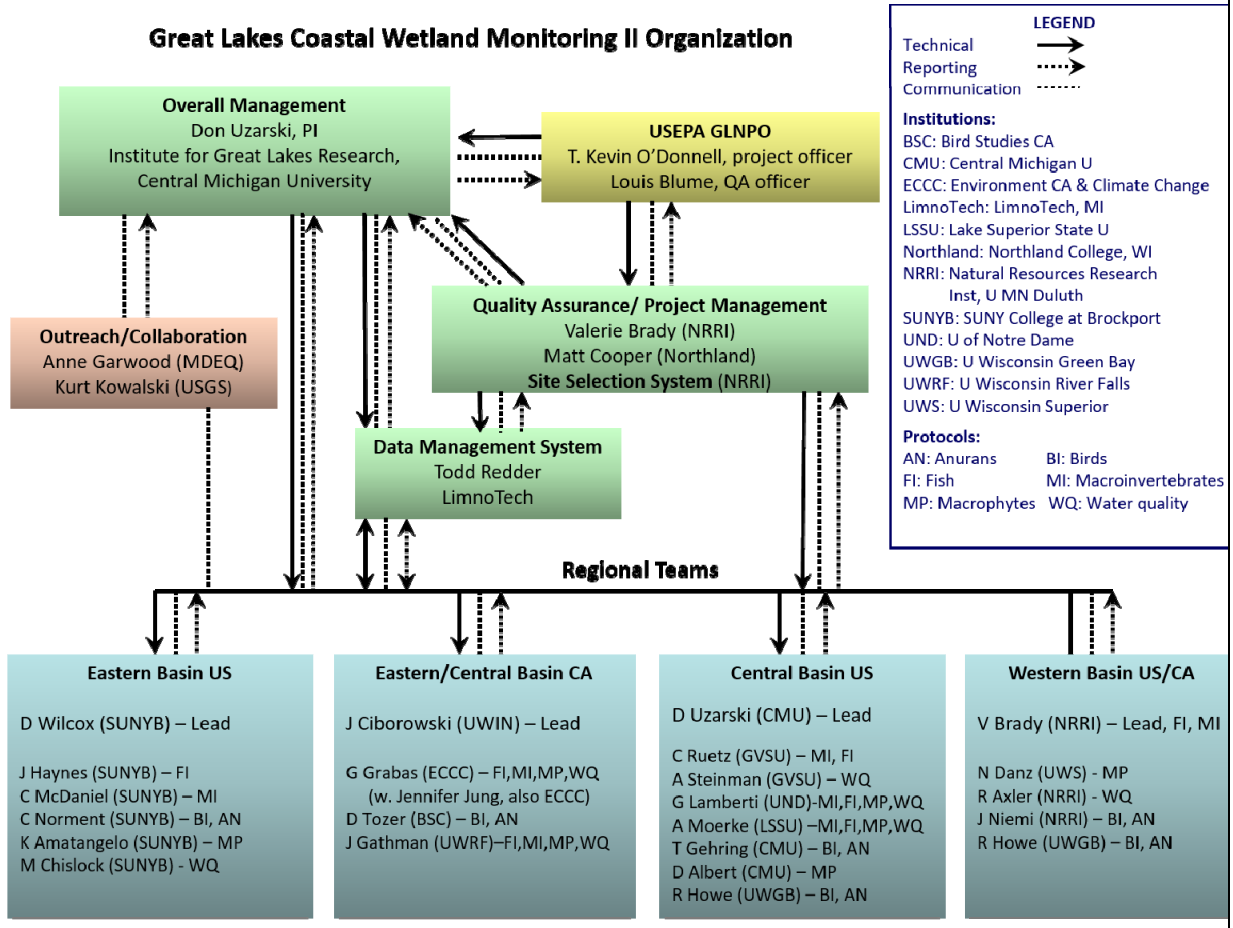

Figure 1. Organizational chart for the project showing lines of technical direction, reporting, and communication separately.

Dr. Valerie Brady (NRRI) was the project coordinator for the Great Lakes Environmental Indicators (GLEI) project, which also worked on development of condition indicators for Great Lakes coastal wetlands. In addition to coordinating the 28 Co-PIs for this project, she also worked on the macroinvertebrate and fish sampling effort, and on data analyses for macroinvertebrate indicators of wetland condition. She helped organize fish and invertebrate field crew training and oversaw QA/QC for this sub-project. She assisted with database development for the entire GLEI project, and helped Co-PIs determine how best to QC their data. She and Dr. Terry Brown (NRRI) completed a data transfer project for US EPA, including methods for assuring that metadata stay associated with datasets after data upload. 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 she has attended all of the GLNPO QA/QC webinars offered recently by US EPA GLNPO. Brady has worked on Great Lakes coastal wetlands since 1989, publishing papers on this topic in peer-reviewed journals and giving numerous presentations at national meetings.

Dr. Dennis Albert (Central Michigan University), Dr. Douglas Wilcox (SUNY Brockport), and Dr. Nick Danz will lead the wetland macrophyte sampling effort. They have conducted extensive research on plants in Great Lakes coastal marshes, with decades of experience in these systems. Dr. Albert led the macrophyte sampling and IBI development effort for GLCWC, and his methods and IBIs will be used in this monitoring effort. Dr. Wilcox was also a PI on the GLCWC project. He will be the regional team lead for the eastern US side of the Great Lakes basin sampling effort.

Dr. Gerald Niemi (NRRI) and Dr. Robert Howe (UWGB) will lead the bird and anuran sampling effort, organizing field crews with collaborators across the basin. Dr. Niemi led the GLEI project, successfully assisting 28 Co-PIs to coordinate sampling efforts, standardize methods, share data, and collaborate on report and manuscript writing. Dr. Howe has led the bird and anuran indicator development for both GLEI and CWM. Both have been working on bird monitoring for several decades, and have published numerous peer-reviewed articles.

Dr. Jan Ciborowski (University of Windsor) will be the regional leader for the central portion of the Great Lakes basin on the Canadian side. He served a similar role in the GLEI project and was a co-PI on a parallel project to define Reference Condition for Great Lakes coastal margin habitats, also greatly assisting with statistical data analysis. His specialty is aquatic macroinvertebrates, and he has decades of experience working on macroinvertebrates in the Great Lakes. He was a coauthor on chapters on macroinvertebrates, fish, water quality, and statistical design in the GLCWC final report. He has published numerous peer-reviewed articles and is also one of the co-coordinators of the Lake Erie Millennium Network.

Roles and Responsibilities:
Dr. Don Uzarski (Central Michigan University):

- Lead PI
- Will partner with Gary Lamberti and Carl Ruetz for fish, macroinvertebrate, and water quality sampling for coastal wetlands across most of the state of Michigan
- Will partner with Dr. Dennis Albert for macrophyte sampling
- Will partner with Thomas Gehring for bird and anuran sampling
- Will coordinate with Dr. Ashley Moerke (Lake Superior State University) for wetland sampling along the Michigan coast of Lake Superior
- Responsible for all reporting to US EPA GLNPO and enforcement of QA/QC requirements

Dr. Valerie Brady (Natural Resources Research Institute, UMD)

- Project coordinator
- Regional team co-leader for fish, macroinvertebrate, and water quality sampling in coastal wetlands in the western portion of the Great Lakes basin, including most of the coast of Lake Superior and the Wisconsin coast of Lake Michigan
- Will partner with Dr. Nick Danz (UW Superior) for macrophyte sampling
- Will be assisted by Dr. Richard Axler (water quality)
- Will work independently as QA manager to oversee QA/QC training and organize QA audits with the assistance of Dr. Matt Cooper (Northland)
- Will manage site selection system

Dr. Gerald Niemi (Natural Resources Research Institute, UMD)

- Will lead the bird and anuran sampling effort, coordinating with all regional team leaders and other bird and anuran specialists across the basin
- Will work with Robert Howe (UW-Green Bay), Thomas Gehring (Central Michigan University), Doug Tozer (Bird Studies Canada), and Greg Grabas and Jennifer Jung (ECCC), and Chris Norment (SUNY-Brockport) or their designees to coordinate and organize training for bird and anuran field crews
- Will review all bird and anuran QA/QC procedures

Dr. Dennis Albert (Central Michigan University)

- Will lead the macrophyte sampling effort for the Uzarski sampling team
- Will coordinate with other macrophyte specialists on other teams to ensure proper training and QA/QC
- Will train other PIs on macrophyte metrics calculations

Dr. Douglas Wilcox (State University of New York at Brockport)

- Regional team leader for fish, macroinvertebrate, macrophyte, and water quality sampling in coastal wetlands of Lake Ontario and eastern Lake Erie
- Will lead the macrophyte sampling effort for the Lake Ontario/eastern Lake Erie sampling team
- Will be assisted by Drs. Chris Norment (birds, anurans), James Haynes (fish), Courtney McDaniel (macroinvertebrates), Michael Chislock (water quality), and Kathyrn Amatangelo (vegetation)

Dr. Jan Ciborowski (University of Windsor)

- Regional team leader for fish, macroinvertebrate, macrophyte, and water quality sampling in coastal wetlands of western US Lake Erie; Canadian shores of lakes Ontario, Erie, and the Huron-Erie connecting channels, and Lake Huron
- Will be assisted by Dr. Joseph Gathman (University of Wisconsin River Falls)

- Will collaborate with Mr. Greg Grabas and Ms. Jennifer Jung (ECCC) and Doug Tozer (Bird Studies Canada)

Dr. Matthew Cooper (Northland College)

- Will assist in overall project management
- Will assist Dr. Valerie Brady with QA/QC
- Will help organize field crew training for all fish and macroinvertebrate field crews

Dr. Carl Ruetz (Grand Valley State University)

- Will organize sampling of eastern Lake Michigan wetlands in collaboration with Dr. Alan Steinman

Anne Garwood (MDEQ)

- Will organize outreach and public information about the CWMP effort
- Will organize informational meetings about the project and its results with resource management agencies around the Great Lakes

A5. Problem Statement

Coastal wetlands are critical components of the Great Lakes ecosystem, have suffered extensive degradation and loss over the past two centuries (Snell 1986, Krieger *et al.* 1992, Schaefer 1994, Environment Canada 2002), and have been greatly affected by land use and pollution (Bedford 1992, Wilcox 1995). Currently, there is no routine monitoring program to determine the status and trends of Great Lakes coastal wetland condition at a basin scale. This project will achieve this goal by assembling a consortium of U.S. and Canadian scientists who will implement the first-ever comprehensive Great Lakes basin-wide coastal wetland monitoring program. The proposed monitoring program relies heavily on methodology previously developed, tested, and scientifically verified by the GLCWC and GLEI projects. Using these scientifically-sound and standardized protocols, we will efficiently and rigorously assess and report the condition of coastal wetlands basin-wide, and provide the baseline for temporal trend monitoring of wetland condition for each of the Great Lakes. Our efforts will provide Great Lakes resource managers and decision-makers with the critical information upon which to base strategic wetland protection and restoration policies that will ultimately improve the health of the Great Lakes ecosystem.

We are using the definition of Great Lakes coastal wetlands as in McKee *et al.* (1992): *"Wetlands may be considered to extend lakeward to the water depth of two meters, using the historic low and high water levels or the greatest extent of wetland vegetation. Hydrologic connections with one of the Great Lakes may extend upstream along rivers since exchanges caused by seiches and longer-period lake-level fluctuations influence riverine wetlands. Wetlands under substantial hydrologic influence from Great Lakes waters may be considered coastal wetlands."* The major types of Great Lakes wetlands that we are sampling are described in Albert *et al.* (2005) as follows: Lacustrine "wetlands are controlled directly by waters of the

Great Lakes and are strongly affected by lake-level fluctuations, nearshore currents, seiches, and ice scour." Riverine wetlands "*occur along and within rivers and creeks that flow into or between the Great Lakes. The water quality, flow rate, and sediment input are controlled in large part by their individual drainages. However, water levels and fluvial processes in these wetlands are directly or indirectly influenced by coastal processes because lake waters flood back into lower portions of the drainage system. Protection from wave attack is provided in the river channels by bars and channel morphology. Riverine wetlands within the Great Lakes also include those wetlands found along large connecting channels between the Great Lakes*…" Finally, barrier-protected wetlands "*originate from either coastal or fluvial processes, but coastal nearshore and onshore processes separated these wetlands from the Great Lakes by creating a barrier beach or other barrier feature. These barriers may be active or part of relict coastal systems abandoned by the lake's margin. These wetlands are protected from wave action but may be connected directly to the lake by a channel crossing the barrier*…"

Recognition and appreciation of the importance of coastal wetlands in the Great Lakes ecosystem has grown markedly in recent decades as numerous important ecosystem functions have been ascribed to these habitats. For example, coastal wetlands provide critical breeding and migratory habitat for wildlife such as birds, mammals, reptiles, and amphibians (Austen *et al*. 1994, Hanowski *et al*. 2007a, Hecnar 2004, Mitsch and Gosselink 1993). These habitats are also critical spawning and nursery areas for many fish species of ecologic and economic importance (Jude *et al*. 2005, Chubb and Liston 1986, Klarer and Millie 1992). Additionally, coastal wetlands trap, process, and remove nutrients and sediment from Great Lakes nearshore waters, and recharge groundwater supplies (Burton 1985, Heath 1992). Accordingly, broad consensus has emerged among scientists, resource managers, and policy-makers on the importance of coastal wetland functions to the entire Great Lakes ecosystem. However, over half of all Great Lakes coastal wetlands have been destroyed by human activities and many remaining coastal wetlands suffer from anthropogenic stressors such as nutrient and sediment loading, fragmentation, invasive species, shoreline alteration, and water level control (Burton 1985, Krieger *et al*. 1992, SOLEC 2007), as documented by a bi-national Great Lakes-wide mapping and attribution project (Albert and Simonson 2004 (Figure 2), Ingram and Potter 2004). Therefore, conservation of remaining coastal wetlands and restoration of previously destroyed wetlands are vital components of restoring the Great Lakes ecosystem. These efforts need to be guided by information on wetland conditions and trends.

A6. Project Description

Our primary objective is to implement a standardized basin-wide coastal wetland monitoring program that will be a powerful tool to inform decision-makers on coastal wetland conservation and restoration priorities throughout the Great Lakes basin. Sampling methodology and indicator calculations will closely follow the protocols contained in the Great Lakes Coastal Wetland Consortium monitoring plan (GLCWC 2008). Tasks include stratified-random site selection of coastal wetlands across the entire Great Lakes basin (Figure 2), sampling of selected wetlands (up to 20% per year for five years), data entry, data quality checks and clean-up, data uploading, and reporting to US EPA GLNPO. Wetland sampling involves collecting indicator data on birds, anurans, fish, macroinvertebrates, macrophyte vegetation, and supporting water quality measurements. All data will be uploaded into a database specifically tailored for these monitoring results.

Regional sampling teams will be led by Uzarski, Albert, Howe, Gehring, Moerke, Lamberti, and Ruetz (U.S. side, central GL basin); Ciborowski, Grabas and Rokitnicki-Wojcik, Gathman, and

Figure 2. Locations of Great Lakes coastal wetlands, based on GIS coverages created by Albert and Simonson (2004) and Ingram and Potter (2004). Map scale causes total wetland area to appear much larger than it actually is.

Tozer (Canadian side, central and eastern GL basin); Brady, Johnson, Niemi, Axler, and Danz (U.S. and Canadian sides, western GL basin); and Wilcox, Norment, Neuderfer, and Haynes (U.S.

eastern GL basin). Bird and anuran crews will sample early in the season during the breeding period (early April - July), while fish, invertebrate, and vegetation crews will sample June – mid September, moving from south to north as the vegetation and invertebrate communities develop. In most instances, the fish, invertebrate, and vegetation crews in each region will travel together for efficiency and safety, sharing boats and other equipment, and providing assistance to each other as needed. Regional site coordinators will keep in close contact with their field crews via cell phone. The typical schedule each year will be for site selection to occur during late winter. Field crews will undergo training in the early summer (early spring for the bird/anuran crews), with field sampling occurring during mid-late summer (spring-early summer for bird/anuran crews). During the late summer, fall, and winter, samples will be processed, data will be entered into the project database, and QA/QC will occur on all data. Data will be provided to EPA GLNPO at the end of the field season (considered to be provisional data, prior to QC validation) and again at the end of winter after QA/QC checks have been completed. Quarterly reports will be submitted to the GLAS system, and semi-annual reports will be sent to GLNPO (see detailed timeline in Table 1 for specifics).

A7. Data Quality Objectives for Measurement Data

The primary DQO for this study is the acquisition of accurate and representative measurements of the biological, habitat, and supplemental water quality parameters for all major Great Lakes coastal wetland complexes, collected in accordance with GLCWC methods. It is very important that the data that we collect are representative of the condition of each wetland, are collected following GLCWC protocols, and are collected similarly by all field crews across the Great Lakes. Sampling methods, protocols, and indicators have already been approved for the GLCWC monitoring program. By following these methods, we will generate representative and reproducible monitoring results.

In addition to all of the instructions throughout this document designed to help insure representative sampling, all field crews need to be aware of how weather events can compromise sampling activities. In particular, riverine systems may experience high flows following storms which would compromise safe, efficient, and representative sampling. Sampling should not be done until streams/rivers return to 150% or less of baseflow conditions. Crews can be assisted with this decision by accessing the nearest USGS gauging station flow records and consulting with their regional team leader. Similarly, high winds and waves can compromise lacustrine sampling and field crew safety. Crews should not attempt to sample during small craft advisories or if they are having difficulty setting fyke nets due to wave action; they should move to sample more protected sites instead until the waves lessen. In all cases, crews should not attempt to sample if there is any concern for their safety.

Table 1. Timeline of tasks and deliverables for the Great Lakes Coastal Wetland Monitoring Project.

Tasks	'15	2016				2017				2018				2019				2020			
	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F
Funding received	X																				
PI meeting		X				X				X				X				X			X
Site selection implemented			X				X				X				X				X		
Sampling permits acquired			X				X				X				X				X		
Data entry system created			X	X																	
Field crew training			X	X			X	X			X	X			X	X			X	X	
Wetland sampling			X	X			X	X			X	X			X	X			X	X	
Mid-season QA/QC evaluations				X				X				X				X				X	
Sample processing & QC					X	X			X	X			X	X			X	X			X
Data QC & provide to GLNPO						X	X			X	X			X	X			X	X		X
Report to GLNPO			X		X		X		X		X		X		X		X		X		X

The co-PIs at each participating institution will be responsible for the QA/QC aspects of the study, with training, oversight, and assistance from Valerie Brady and Matthew Cooper. Co-PIs will conduct mid-season QA checks on their field crews and report the results to Brady and Cooper, who will oversee these checks and other QA audits. 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 she and Cooper have attended all of the GLNPO QA/QC webinars offered by US EPA GLNPO.

Activities to be performed during this project include 1) site selection, 2) sample and data collection of fish, macroinvertebrates, birds, anurans, macrophytes, and supporting water quality and habitat measurements, 3) laboratory identification of macroinvertebrates, 4) laboratory water chemistry measurements, 5) data processing and data entry into the database, 6) data QC, and 7) reporting to EPA GLNPO and the GLAS system. All data collection will follow the methods outlined in the GLCWC sampling manual (GLCWC 2008 and included below). The DQO for data entry is 100% accuracy of data copied from field and laboratory sheets into the database.

For this project, we are collecting monitoring data to be uploaded into a Great Lakes-wide database. Thus, we will be doing limited data analyses and summarization on the collected data. Most data analysis will focus on indicator calculation and indicator refinement. Thus, DQOs focus primarily on proper collection and handling of data and samples, and proper QC of data.

The scope of this project is all of the major coastal wetland complexes along the entire Great Lakes coastline, including Canada. Great Lakes coastal wetlands were mapped into a GIS layer by Albert and Simonson (2004; Figure 2).

A8. Special Training Requirements

All personnel responsible for sampling invertebrates, fish, macrophytes, birds, anurans, and water quality will be trained and certified before sampling begins each year. Several of the regional team leaders (co-PIs) have permanent technicians and staff who have years or decades of experience conducting aquatic sampling, which will help to ensure that rigorous data quality standards are maintained throughout the project.

A multi-level training and certification program will be implemented to ensure accuracy of all data collection. A series of training workshops led by experts on each respective protocol will be held the first spring/early summer before fieldwork begins at several locations across the basin or online (birds and anurans) to ensure good attendance by the majority of field crew staff in each area. The workshop agenda will include training on how to meet the data quality objectives for each element of the project, QAPP review, site verification procedures, safety procedures including safe boating, trailering, and general field safety; hands-on training for each sampling protocol, procedures for entering data into the project database, record-keeping and archiving requirements, data auditing procedures, and certification/re-certification exams for each sampling protocol for all project personnel. All project co-PIs, field crew leaders, and as many summer staff as possible will participate in these workshops and will be certified/re-certified on sampling protocols. Each subsequent spring and as necessary, co-PIs and field crew chiefs will provide training/re-training and certification/re-certification of project staff and field crew members. Outside experts will be brought in for training if there are changes in co-PIs, or as necessary.

To be certified in a given protocol, individuals must pass a practical exam. Training and exams will be conducted in the field whenever possible, and will be supplemented with photographs (for fish, vegetation) or audio recordings (e.g., bird and anuran calls) when necessary. Passing the exams will certify the individual to perform the respective sampling protocol(s). Since not every individual will be conducting every sampling protocol, participants will be tested on the protocols for which they will be responsible. The majority of testing and certification will take place during the early-season training workshops, and additional certification will be administered by co-PIs as needed. Personnel who are not certified (e.g., part-time technicians,

new students, volunteers) will not be allowed to work independently nor to do any identification except under the direct supervision of certified staff members until they can pass the appropriate certification tests. The following paragraphs detail specific items to be covered during the training workshops each year. Preliminary certification criteria (minimum percent correct on certification exams) are also included below. For some criteria, demonstrated proficiency during the field training workshops will be considered adequate for certification. Training and certification records for all participants will be collected by regional team leaders and copied to lead PI Uzarski and QA officers Brady and Cooper. A summary of these records will be included in semi-annual reports to EPA.

Note that the training and certification procedures explained here are separate from the QA/QC evaluations explained in section B. However, failure to meet project QA/QC standards may require participants to be re-trained and re-certified (see section C1 for corrective actions).

Site Selection and GPS Use: Field crews will be trained to consistently locate and select pre-selected wetlands and sampling locations within each wetland, and will be taught strategies to implement when pre-selected wetlands cannot be sampled due to insufficient water depth, unsuitable weather, inaccessibility, or safety concerns. Field crews will also receive training in proper GPS procedures, including equipment use and data entry. GPS training will include extensive instruction on navigating to waypoints, creating waypoints, and determining levels of accuracy available.

Certification Criteria:

- Identify circumstances in which a site can be rejected as unsampleable (90%)
- Identify vegetation zones for stratified sampling (90%)
- Proper use of a GPS to navigate to a waypoint (demonstrate proficiency)
- Determination of GPS accuracy (demonstrate proficiency)

Fish: Training for fish sampling will be led by the regional team leaders, co-PIs, and fisheries experts on the project. Fish sampling training will concentrate on teaching crew members how and where to set fyke nets (and problem-solving with net setting), how to process fish samples to minimize harm to the fish, proper identification of fish, and when and how to collect voucher specimens for ID verification and QA/QC. Field crews will be trained in proper fish care procedures following specific university protocols. Crews will be provided with identification guides and lists of fish species specific to the Great Lakes, including identification criteria for all known invasive species.

Certification Criteria:

- Selecting appropriate locations for fyke nets (demonstrate proficiency)
- Setting of fyke nets (demonstrate proficiency)
- Proper handling of captured fish (demonstrate proficiency)

- Identification of fish species (90% of 20 species, either field-caught or photos, as necessary)
- Proper field data sheet completion (demonstrate proficiency)
- Preservation of fish when a positive ID cannot be determined in the field (demonstrate proficiency in preservation; 95% accuracy on determination of when to preserve)

Macroinvertebrates: Training for macroinvertebrate sampling and processing will be led by regional team leaders, co-PIs, and macroinvertebrate experts on the project. Macroinvertebrate field work training will concentrate on training crew members in the proper selection of invertebrate sampling locations within the wetland, proper use of the D-net to collect macroinvertebrates, field picking organisms, and preserving specimens with proper sample container labeling. Individuals who will be sampling macroinvertebrates will be required to pass a certification evaluation before working independently.

Additional training and certification will be required for macroinvertebrate identification in the laboratory. Preliminary training at the pre-season workshop will focus on acceptable taxonomic reference materials, taxonomic resolution targets for all taxa, sample preservation and archiving, and record keeping. Use of taxonomic keys, etc., will not be covered in the field training because invertebrate taxonomic training will be conducted over the fall and winter by trained taxonomic staff (laboratory staff may not be the same as field crew staff). Temporary workers will be used for sample picking and processing, with QC and oversight by trained staff and laboratory managers. Invertebrate identification will be carried out by trained staff that have done well in appropriate invertebrate identification coursework or similar previous training. All identification work will be overseen by trained taxonomists on staff at each regional laboratory.

A crucial element of ensuring accurate invertebrate taxonomy will be maintaining open dialogue among project teams. Our past experience with similar projects has shown that many QA/QC violations can be avoided when laboratory personal communicate frequently. Such dialogue is an important part of both training and quality assurance. Laboratory staff will use a variety of tools such as regular conference calls, an online forum for taxonomic questions, and e-mail sharing of digital photographs to foster this dialogue. Reference collections will be maintained at each institution and will be used for training staff members. In addition to the open dialogue among team members, individuals participating in macroinvertebrate processing will be required to obtain certification before working independently. Trainees will have 100% of their work checked by certified staff until they themselves become certified. Certification for laboratory processing of invertebrate samples will be granted by a project PI skilled at working with macroinvertebrates.

Certification Criteria—Field Sampling:

- Determining sampling locations within a site (demonstrate proficiency)
- Appropriate D-net sampling technique (demonstrate proficiency)

- Field picking organisms (demonstrate proficiency)
- Preserving, labeling, and storing specimens (demonstrate proficiency)
- Proper field data sheet completion (demonstrate proficiency)

Certification Criteria—Laboratory Processing: (certification will take place as laboratory staff are hired and trained, rather than during field training)

- Sample handling/archiving (demonstrate proficiency)
- Record keeping (demonstrate proficiency)
- Data entry/data backup (demonstrate proficiency)
- QA/QC procedures (demonstrate proficiency)
- Taxonomic Identification (90% of at least 35 taxa)

Water Quality: Training for water quality sampling will be led by the water chemistry experts on the project. Water quality sampling training will focus on proper calibration of water quality meters, proper collection of water samples to minimize contamination, proper field data sheet completion, proper labeling and treatment/preservation of samples, and packaging samples for shipping. Crews will also be trained to recognize when meters are not functioning properly, how to code this on the field data sheets, and basic trouble-shooting techniques. A basic understanding of aquatic ecology/limnology gained either by having taken appropriate undergraduate coursework or equivalent on-the-job training will be a prerequisite for participating in water quality data collection. Water quality analysis in the laboratory will be conducted or supervised by highly-trained individuals with years of experience.

Certification Criteria:

- Calibration of water quality meters (demonstrate proficiency)
- Use of water quality meters (demonstrate proficiency)
- Trouble-shooting of meters (demonstrate proficiency)
- Proper field data sheet completion (demonstrate proficiency)
- Collection and storage of water samples (demonstrate proficiency)

Macrophytes: Macrophyte sampling training will be led by regional team leaders, co-PIs, and aquatic macrophyte experts on the project. Training will include proper transect establishment, location of random sample plots, aquatic vegetation taxonomy, protocols for dealing with problematic identifications, and when to take samples for QA/QC. The collaborators in this project have done extensive plant sampling in Great Lakes coastal wetlands, so their species lists, picture keys, and field data forms include most plants that will be encountered during the project. The species lists also include all of the major invasive plants known from coastal wetlands. Reference materials at university herbaria are available for comparison. Plant materials that cannot be positively identified in the field will be collected for identification that evening or pressed for later identification in the laboratory. Additionally, at QA sites, plants will be collected for QA checks later. One of the most difficult aspects of plant sampling in quadrats

is accurate estimation of the percent coverage for plant species present. Teams will calibrate the estimation of plant coverage as a group during training.

Certification Criteria:

- Transect and plot locations (demonstrate proficiency)
- Taxonomy (75% of 20 species identified in the field; 90% of 20 species, using appropriate identification guides in the laboratory)
- Total percent cover by vegetation within quadrats (sampling team estimate of coverage ±10% of an expert's estimate 90% of the time)
- Determining when to collect voucher specimens for identification in the lab (demonstrate proficiency)
- Proper preservation procedure for specimens (demonstrate proficiency)
- Proper completion of field data sheets (demonstrate proficiency)

Birds and Anurans: Bird and anuran sample testing and training will be provided by regional team leaders, co-PIs, and bird and anuran experts on the project. These individuals have been testing and training students and personnel to conduct bird and anuran surveys for many years. Survey personnel will be evaluated and hired based on their demonstrated proficiency in visual and aural identification of Great Lakes bird and anuran species, and familiarity with the survey protocols. Candidates will be evaluated using audio and visual tests and trained in field-based survey techniques. An objective, secure online testing system (http://www.birdercertification.org) will be modified for target anuran and bird species found in the proposed study areas. The bird certification website was developed by Dr. Howe of UW-Green Bay in response to the need for testing and training individuals in bird identification. Bird and anuran personnel will also be required to demonstrate their knowledge of the survey protocols prior to field activities. Field tools (portable audio players with bird vocalizations, which, when broadcasted into the marsh, elicit calls from secretive species) will be provided to field teams and standardized among survey groups.

Because active ornithological research programs are ongoing at each of the participating institutions, a pool of qualified personnel for bird surveys will be available or recruited for fieldwork. Acceptable candidates will likely have taken a college-level course in ornithology and will have field experience in bird surveys through research projects or volunteer opportunities. Candidates for the fieldwork typically have participated in monitoring programs such as the Marsh Monitoring Program, State or Provincial Breeding Bird Atlases, North American Breeding Bird Survey, or other state or regional monitoring programs.

Skills in anuran identification are more easily acquired than bird identification skills because the diversity of species in the Great Lakes basin is relatively small (Harding 1997). Prior to the training sessions, lead researchers will provide audio CDs or web links of frog and toad vocalizations to trainees so that they can study for tests to be given during field training. During field training, lead researchers will describe known habitat associations of anuran species in the

Great Lakes basin. Bird and anuran personnel will be allowed to retake the test a maximum of 3 times with a minimum of 1 day between subsequent tests.

Certification Criteria:

- Survey plot locations (demonstrate proficiency)
- Visual identification (95% of 20 bird images, including difficult views, immature plumages). This test will concentrate on species likely to be seen rather than heard.
- Aural identification (90% of 30 bird species; 100% for focal species; 15 of 16 calls correctly identified for calling anuran species)
- Proper completion of field data sheets (demonstrate proficiency)
- Appropriate demonstration of how to document calls/species that cannot be readily identified (demonstrate proficiency)
- Audio testing will include a range of species with songs at varying frequencies and volumes to insure adequate hearing by field crews.

Record Keeping, Data Custody, and Data Entry: Brady (NRRI) and Cooper (Northland) will lead training on record keeping, sample chain of custody, data custody, and entry of data into the data management system. This portion of the training will also include data error checking protocols. Metadata training and more complete data QC training will be given to PIs and field crew chiefs by Brady and Cooper in the fall.

Additional Training:
Permanent technicians and staff who are designated as crew leaders will assist regional team leaders (co-PIs) in training temporary summer workers who are not able to attend the field crew training. By providing field crew training at several locations around the basin, most summer workers will be able to attend the training. Uncertified workers will always (100%) work with more experienced personnel until they pass certification and will receive substantial training until they prove themselves competent in field sampling methods.

Regional team leaders (co-PIs) will accompany field crews to 10% of wetland sites each year to ensure that all data collection methods are being done according to established protocols. Brady and Cooper will audit these mid-season QC activities and report the results in the semi-annual report to EPA (and to all co-PIs). They will inform Uzarski and co-PIs of any deficiencies and the required corrections. Follow-up reports from co-PIs demonstrating correction of these deficiencies will be required.

A9. Documentation and Record

We have created a specialized data management system dedicated to GLCWC wetland monitoring data. For each wetland, data include 1) a GIS polygon layer showing wetlands sampled each year; 2) geographic locations of all sampling points within each site by taxa group; 3) macroinvertebrate identifications, counts, and IBI scores; 4) fish identifications, counts, and IBI scores; 5) macrophyte identifications, coverages, and Conservatism values; 6) water quality data; 7) bird identifications and indicator values; and 8) anuran identifications and indicator values. The database is housed at CMU and accessed on-line. Data "uploading" to the data management system is synonymous with data entry. QC of field crew sampling and data collection will take place once per year during the sampling season. QC of laboratory activities and data input will take place each winter by laboratory leaders. All QC records and reports will be maintained with the appropriate datasets, archived, and provided to the QC managers (Brady and Cooper) during audits. Data will be input into the database during the field season and as quickly as possible after the field season ends. Data will be made available to EPA annually after QC checks are completed. This data management system also contains metadata for each data type, including study design, site selection, and reason for rejection of sites that cannot be sampled.

The semi-annual report to US EPA GLNPO will consist of all activities of the previous 6 months, including QC reports and audit results, and brief data summaries. However, the data will be made available in the database, rather than in the report itself.

Records from field and laboratory observations will be archived as hardcopies at CMU, then as electronic spreadsheet versions (for example, *.csv format) following a web-based data entry procedure. All data entry on the web-based system will remain accessible throughout the life of the project. Copies of formal reports and supporting materials (including QC audit reports) will be archived in CMU libraries and identified with CMU technical document coding for future retrieval. Sample sheets completed on-site during field sampling and all field sheets, logs, chain of custody documents, and sample materials will be retained by CMU for a minimum of three years, or made available after that time period.

<u>Deliverables list:</u>

- Semi-annual report to EPA twice per year (including QC reports and QC audit reports)
- Data provided to GLNPO twice per year (see above for data included; includes metadata)
- Indicator values for all sampled wetlands, provided twice per year
- GIS files of site locations and sampling points, provided twice per year
- Final report to EPA
- Final QA/QC report including all difficulties encountered and their solutions

B. DATA ACQUISITION

Each section below deals with one major aspect of wetland monitoring QA.

BA. Site Selection

BA1. Project Design

GLCWC (2008) developed a statistically-sound probabilistic site selection design that allows statistically-valid prediction of overall Great Lakes coastal wetland condition and trends based on a subset (one 'panel') of sites being sampled. The design is similar to the EMAP system for streams and lakes. The NRRI GIS laboratory will implement a framework for probabilistic site selection, with final site selection/rejection being done by regional team leaders over a web-distributed map viewer. Site selection will follow a stratified-random design based on 1) wetland type (riverine, lacustrine [open fringing], barrier-protected; Albert et al. 2006); 2) region (northern and southern, including geopolitical boundaries); and 3) Great Lake. Additional sites outside the probabilistic design will be added to the sampling list each year. These "benchmark" sites include sites being considered for or undergoing restoration and/or protection to assist other agencies in determining on which sites restoration and protection money and efforts should best be spent. Including benchmark sites within a statistically-valid monitoring framework will allow agencies to determine where their sites of interest fall on the condition spectrum of Great Lakes coastal wetlands. Up to 10% of sites sampled in any given year may be benchmark sites that fall outside the random site selection framework.

BA2. Sampling Methods

The site selection process outlined above will result in clusters of sites for each lake (or lake section where lakes cross the regional boundaries); clusters will be defined by the 3 wetland types, 5 lakes, and regions (e.g., northern Lake Michigan riverine wetlands; southern Lake Huron lacustrine wetlands; etc.). A first estimation of the number of wetlands within each cluster is shown in Table BA2-1. For site selection, each regional sampling team leader will use the site selection tool to virtually investigate each site in their region and will deliberately select or reject the site for sampling. Sites can only be rejected for very specific reasons, such as that it is too small, has no access, etc. (see section BA5 for complete list of acceptable site rejection criteria; Brady, Cooper, and Uzarski will verify that regional leads are not inappropriately rejecting sites). After all sites have been selected or rejected, sites will be randomized within each cluster and distributed across five sampling 'frames', each frame representing the sites that will be sampled each of the five years. Each regional sampling team will then begin field sampling by systematically going down their assigned section of the randomly ordered site list for the first year. Teams may still reject sites that fail to meet the minimum criteria once they are visited in the field (for example, it can sometimes be difficult to determine via GIS and aerial photography whether or not barrier-protected wetlands have a surface water connection to the lake).

BA2-1. Approximate number of wetland polygon counts by type and lake section. These are estimates of the number of wetlands and are probably higher than the actual number because of changing numbers of sampleable wetlands each year based on lake levels and human activity.

Lake Section	Lacustrine	Barrier-protected	Riverine	Total
Superior (all)	33	88	77	198
Michigan (N)	71	114	51	236
Michigan (S)	1	21	20	42
Huron(NW)	103	32	60	195
Huron (SW)	31	7	4	42
Huron (CA)	183	94	81	358
Erie (CA)	17	15	32	64
Erie (US)	36	5	43	84
Ontario (CA)	57	53	83	193
Ontario (US)	13	45	59	117
Totals	**545**	**474**	**510**	**1529**
Est. Sampleable (F/I/V)				**1100**

The sites that are actually sampled during year 1 form the basis of a rotating panel design, which will eventually be populated by the sites sampled in each of the five years. This design will ensure that all major wetlands will be sampled over a period of approximately five years, with potential modification of this schedule based on the results of the first year of sampling. Benchmark sites (up to 10% of sites sampled per year) will include sites that are long-term monitoring sites and that sites being considered for or undergoing restoration or protection. This stratified-random site selection process assures that the condition of Great Lakes coastal wetlands basin-wide can be inferred and statistically summarized based on the outcome of a single year's sampling. These predictions can then be re-evaluated with greater precision as additional sites are added to the sample pool in subsequent years.

The pool of wetland sites used for selection will be based on the GLCWC-GLNPO wetland coverage (Albert and Simonson 2004). Wetlands selected for sampling under the random site selection process should meet the following criteria: 1) 4 ha or larger; 2) have a direct, obvious surface water connection to a Great Lake or connecting channel at least every year or so; 3) be close enough to that lake or connecting channel to be influenced by it (e.g., seiches); and 4) contain herbaceous or standing-water wetland zones. Previous basin-wide work by GLCWC and GLEI field crews indicates that smaller wetlands can be either too small to sample, or no longer in existence, and indicators and sampling protocols have not yet been evaluated for use in wooded wetlands. Distance from the lake for lake influence is difficult to quantitatively define. In general, influence of the lake does not transmit more than about 1 km upstream or away from the lake (much less so if the shoreline gradient is steep), so if the wetland is less than this distance from the lake or connecting channel and the vertical relief between the lake level and the wetland is less than 2 m, then the wetland should be selected for sampling. The exceptions tend to occur in drowned river mouths along the eastern coast of Lake Michigan. If water is at the same level all the way across these river-mouth lakes, then the wetlands at the inland end of the lake will still be influenced by the Great Lake. In all cases for riverine wetlands, the most

downstream portion should be sampled, whether this occurs on the Great Lake side or the inland side of the drowned river mouth lake.

During the site selection process, PIs may notice that wetlands have been artificially divided due to human influence or other causes. If the minimum edge-to-edge distance of the wetland polygons in question is 500 m or less and the wetlands are of the same basic type (e.g., lacustrine, barrier-protected, or riverine), these polygons may be identified as needing to be connected in the GIS database to form one wetland complex.

The opposite case may also occur, in which PIs notice during site selection that wetland polygons have been inappropriately lumped together for site selection when they really are separate wetlands because of differences in water flow, lack of connectivity, or incorrect assignment of wetland site type (lacustrine, barrier-protected, riverine). The site selection tool provides an option for PIs to separate these polygons and flag them so that this correction is made in the database.

Support facilities for the site selection task will be provided by the NRRI GIS lab. This facility contains a number of computers running the latest ESRI ArcGIS 10 software. Hardware systems are typically Intel or AMD multi-core processors running at 2.4+ GHz with 0.5+ TB of local storage. Internal networking is mixed 100 and 1000 Mbit Ethernet, with 40 Mbit/s Internet connectivity. The use of ArcMap 10 (Bing maps), QGIS (Google, Yahoo, and OpenStreetMap), and existing collections of Great Lakes coastal imagery developed previously allow interpretation of sites before field work, and verification of GPS information received from the field.

BA3. Sample Handling and Custody

Regional team leaders will receive appropriate materials (wetland maps; GPS coordinates for sampling points, boat access, etc.) in advance of the field season to facilitate sampling and travel logistics and permit preliminary site reconnaissance (the bird and anuran crews may be able to assist with site reconnaissance for the fish/invertebrate/vegetation crews). On-line web-based distribution of maps, sampling points, and transects will allow efficient and rapid distribution of materials to field crews scattered across the basin. Field teams that sample along randomly-located transects within study sites will be given an ordered list of transect start and end points for each wetland. Centralized site selection and map creation will ensure maximum efficiency and a dedicated source of map and logistical assistance. The following tables show the numbers of samples that are expected per site (Table BA3-1) and the numbers of samples that regional teams can expect each year by sample type (Table BA3-2).

BA3-1. Estimated mean number of samples per site by sample type, and the number that require laboratory processing. For fish, vegetation, birds, and anurans, samples requiring processing refers to estimated numbers of unidentifiable taxa or calls that require extra effort after the field sampling in order to be identified.

Sample Type	Samples/site	Composites/sample	Requiring processing
Fish	6	0	1
Invertebrates	6	~10	6
Vegetation	35	0	5
Water quality	2-4	typically 3	2-4 composites
Birds	4	0	0
Anurans	2	0	0

BA3-2. Estimated mean number of samples per year by team and sample type, with samples requiring lab processing shown in parentheses (for fish, vegetation, birds, and anurans, these are the estimated numbers of unidentifiable taxa or calls that are returned to the lab for identification). Numbers were derived by multiplying estimated mean numbers of samples per site by the sampling capacity of teams.

Team	Fish	Inv	Veg	WQ	Bird	Anuran
Uzarski *et al*.	240 (40)	240 (240)	1400 (100)	80-160 (80-160)	320 (20)	160 (5)
Brady *et al*.	180 (30)	180 (180)	1050 (150)	60-120 (60-120)	160 (10)	80 (2)
Ciborowski, Grabas *et al*.	210 (35)	210 (210)	1225 (70)	70-140 (70-140)	160 (10)	80 (2)
Wilcox *et al.*	180 (30)	180 (180)	1050 (70)	60-120 (60-120)	160 (10)	80 (2)

BA4. Analytical Methods Requirements

Performance criteria: Regional team leaders will be trained on the parameters for site rejection (see section BA5), and the management team will provide QC oversight on this aspect of the project. Sites that appear to be inappropriately rejected will be the subject of a discussion between the regional team leader and the management team. Target turnaround time for site selection/rejection is one month after the notification that the site selection website is operational.

Database system requirements: Hardware systems are typically Intel or AMD multi-core processors running at 2.4+ GHz with 0.5+ TB of local storage. Internal networking is mixed 100 and 1000 Mbit Ethernet, with dual T1 line Internet connectivity. This applies only to the NRRI computer systems that house the database. Because of the backups provided with this system (see section B10), failure of any component will not result in loss of data. The redundancies built into the system should allow the system to be brought back on-line by NRRI GIS lab and IT staff with a minimum of delay. Any computer with internet access will be able to input data into

the database system (as long as the computer user has the appropriate passwords and clearance).

BA5. Quality Control Requirements

Regional team leaders will inspect aerial photographs and other information (e.g., site access points) for each site as they make the decision whether to accept or reject sites. Sites can only be rejected for specific reasons to ensure that sites are selected randomly rather than deliberately. Reasons for site rejection are limited to: 1) site is too small (< 4 ha); 2) site is known to be dry or to no longer exist as a wetland; 3) site does not have a surface-water connection to the lake; 4) there is no safe access for field crews (e.g., no public launch within approximately 7 km); 5) there is no permitted access due to private property constraints. Based on previous field experience, we expect that the fish/invertebrate/vegetation crews will have a more difficult time getting to sites than will bird/anuran crews. In addition, bird/anuran crews can move faster than fish/invertebrate/vegetation crews, allowing bird/anuran crews to sample more sites than the other crews. Because bird and anuran crews will be the first in the field, their observations of site access will assist fish/invertebrate/vegetation crews.

Individuals primarily responsible for site selection/rejection by regional field team:

Western Great Lakes	Valerie Brady/ Gerald Niemi/Nick Danz
Central Great Lakes (US side)	Don Uzarski/Carl Ruetz/Robert Howe/Tom Gehring
Central Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Doug Tozer/Gerald Niemi
Eastern Great Lakes (US side)	Doug Wilcox/ Greg Lawrence/Chris Norment
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Greg Grabas/Doug Tozer

<u>Site selection QC check:</u>

Because regional team leaders will be responsible for accepting/rejecting sites from lists generated by the site selection process, a quality control step will be implemented to ensure that consistent criteria are used by each team to reject sites. The project lead PI (Don Uzarski, Central Michigan University), QA manager (Valerie Brady, NRRI), and assistant QA manager (Matthew Cooper, Central Michigan University) will examine the list of rejected sites within the site selection system and determine if these rejections are consistent with the criteria above and that the criteria are being applied consistently across the basin. Corrective actions are detailed in section C1.

BA6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

NRRI GIS lab automated backup systems are monitored on a daily basis to ensure immediate repair of any failed components or processes. Combined with the redundancies built into the system, this ensures equipment or system failures will not have an unrecoverable impact on the site selection process.

BA7. Instrument Calibration and Frequency

N/A

BA8. Inspection/Acceptance Requirements for Supplies and Consumables
N/A

BA9. Data Acquisition Requirements (non-direct measurements)
Sources for external datasets of all types will be tracked. Most external data will be from peer-reviewed publications and GIS sources with established metadata lineages; these lineages will be maintained. In rare cases in which unpublished data are used, appropriate metadata will be generated to describe its origin.

Wetland site selection will be based on the Albert and Simonson (2004) Great Lakes coastal wetland GIS coverage. Use of this database for site selection was mandated in the RFP and by the necessity of following the GLCWC sampling protocols. Other data used for site selection will include wetland type (Albert *et al*. 2006), GIS background layers (roads, boat launches, land use, etc.) from state and federal agencies, and the GLEI stressor gradient for some benchmark sites (Danz *et al*. 2005). These datasets will be used for logistical and landscape analysis purposes.

Most of these data have already been published and peer-reviewed. Non-peer-reviewed information, such as the aerial photos, will be visually checked for site location by comparison with the appropriate quad map or Google maps. These photographs will be used as supplemental guidance for field crews rather than to actually generate data. All of the data types mentioned above are in the public domain and are not subject to use restrictions.

BB. Water Quality

BB1. Project Design
Chemical/physical measurements will be made in each vegetation type where fish and macroinvertebrate data are collected. Fish and macroinvertebrates are collected by vegetation zone (see Sections BC and BD); water quality should be collected in association with fyke nets, one water quality sample per vegetation zone. In the event that fyke nets are not set due to very shallow depth, but invertebrates are collected, then a water quality sample should also be collected from that zone. These samples are required. Crews have the option of taking water quality meter samples at each individual fyke net location (or invertebrate dip net replicate point if fyke nets are not set in that zone). This additional sampling effort is recommended if vegetation patches forming the zone are separated rather than contiguous. Water quality data collection is critical at each wetland, but parameters will be classified as critical, recommended, or supplementary on an individual parameter-by-parameter basis in this section. Defining parameters as "critical" does not mean that biological samples should not be taken at a site if water quality parameters cannot be taken because, for example, the DO sensor on the meter is malfunctioning. Every attempt should be made to get critical measurements, including borrowing equipment from a nearby field crew and obtaining a replacement meter as soon as

possible. We also define water quality parameters in terms of 1) field measurements using instruments with sensors used at the site, 2) parameters requiring analysis of a water sample either the evening or the day after the sample was collected, or 3) parameters measured at one of our project water quality laboratories.

Critical:

- Field: temperature, dissolved oxygen, pH, specific conductivity
- Lab: alkalinity, turbidity, soluble reactive phosphorus (SRP), [nitrate+nitrite]-nitrogen, ammonium-nitrogen, chlorophyll-a

Recommended:

- Field: transparency (or secchi) tube clarity
- Lab: total nitrogen (TN), total phosphorus (TP), chloride, color

Supplementary:

- Field: oxidation-reduction potential (redox), in situ chlorophyll fluorescence
- Lab: Sediment percent organic matter

We anticipate that, on average, two or three vegetation zones will be sampled by fish and macroinvertebrate crews in each wetland, although four zones are possible. The basic water quality sampling design is based primarily on the placement of three fyke nets within each vegetation zone at a site. If fyke nets cannot be set in a zone, then macroinvertebrate sampling points will be used instead as water quality sampling locations. Water quality data will be collected from fyke net or D-net locations as follows:

- Field: critical, recommended, and supplementary measurements will be made at the first net set location within each vegetation zone. It is recommended that water quality measurements be made at each net set within the zone, but only one location is required.

- Lab: water will be collected from each of the fyke net locations within a vegetation zone and combined to form a single composite sample, which will be analyzed for critical and recommended water quality parameters.

BB2. Sampling Methods

Past experience sampling water quality in coastal wetlands suggests that, whenever possible, samples and *in situ* measurements should be made off the bow of the boat before any of the field crew enters the water in an effort to prevent contamination and suspension of sediment.

<u>Field meters:</u>

Water quality measurements using field instruments will be made *in situ* at the mid-depth of the water column at a minimum of one location within a vegetation zone (i.e., the first fyke net

location). Field instrumentation may vary among sampling teams, but all groups will collect temperature, dissolved oxygen (concentration and percent saturation), pH, and specific electrical conductivity using sensors with specifications listed in Table BB4.1 below (Yellow Springs Instruments [YSI] 6600 or equivalent instrumentation). *In situ* measurements will be taken at mid-depth with special care that substrates are not disturbed before readings are taken. pH may be measured using a multi-sensor YSI 6600 (or equivalent), or using a portable pH meter in water collected from the same depth for the water chemistry analyses described below. Turbidity may also be directly measured in the field if a field sensor is available, or else it will be determined using water collected for other analyses (see below). Supplementary measurements of oxidation-reduction potential (redox) and *in situ* chlorophyll fluorescence will depend on the field instrumentation available to field crews. The specifications tabulated below for recommended and supplementary parameters (Table BB4.1) are included to set data quality objectives so that these measurements are taken consistently among field crews that are collecting these data.

Water sampling:

Water quality samples will be made on a single composite sample collected from the 3 fyke net locations (or 3 macroinvertebrate sample locations) within each vegetation zone. From each net location, water will be collected by grab-sampling two successive 1-L samples from mid-depth using an acid-washed polyethylene bottle and pouring them into a 10-L polypropylene carboy. This should be done by using a 1-L poly bottle attached to an extension pole to dip water from shallow sites or in dense vegetation. In this manner, 6 L will be collected from a zone in which 3 fyke nets are set. Care will be taken to ensure that no bottom sediment is collected. If bottom sediments are collected, the water will be discarded, the carboy and sample bottles thoroughly rinsed with surface water from the same zone, and the water collection process will be started over. In order to avoid potential errors due to larger pieces of detrital debris contaminating water samples, each water sample will be poured through an acid-cleaned polypropylene funnel to which a 500 micrometer mesh screen is attached.

Composited water will then be vigorously mixed and dispensed into a 4-L acid-cleaned polyethylene Cubitainer for later processing (see below). The remaining water in the carboy can then be re-mixed and used to determine transparency tube water clarity. Transparency tube clarity is measured against a light background and shaded by the body (MPCA 2007, 2006; Anderson and Davic 2004). After this measurement has been made, the remaining water is dumped and the carboy is then rinsed at least 3 times with surface water from the next vegetation zone, where the water compositing process is repeated.

Bottles for specific analytes will be rinsed with sample water before collection. All samples will be stored in the dark, on ice, in the field and then processed further upon return to the lab or to the motel as soon as possible (no more than 12 hrs later). Preservatives should not be used in samples. See sample handling below.

BB3. Sample Handling and Custody

Water quality samples will be labeled externally with labels that will adhere to sample bottles even when wet. They will be written on with black fine point Sharpie markers or similar markers that have been tested and proven to be waterproof. Labels will consist of site ID, plant zone, replicate number, sample date, regional team initials, and crew chief name. Water quality samples in 4-L cubitainers will be kept chilled on ice until they can be further processed in the evening. Samples will be processed in the field or at the motel the same evening as follows, and in every case taking care to vigorously mix water before aliquoting:

- Raw water: 2 x 250 mL poly bottles will be filled with raw water; one will be frozen as soon as possible for TN and TP (recommended), and the other will be frozen as a back-up sample.

- Alkalinity titration: Alkalinity (required) will be measured on an unfiltered portion of the sample as soon as possible after collection, but always within 12 hrs. Alkalinity will be determined by titrating raw water samples with standardized sulfuric acid (APHA 2005, 2 end-point titration).

- Turbidity determination (required if not sent to a lab or measured in the field): two replicate subsamples of raw water are measured as per Table BB4.1 below.

- Filtration for chlorophyll-a: A measured amount of water, approximately 300-1000 mL, will be filtered through a 47 or 42.5 mm Whatman GF/C glass fiber filter into an acid-cleaned, DI-water-rinsed filtration funnel and flask. The filter will be used for chlorophyll-a extraction in the lab. The filter should be carefully folded in half and then in half again with forceps, and then wrapped in aluminum foil with a label identifying it (site, zone, date, field crew, crew chief name, amount of water filtered). All chlorophyll "packets" are subsequently stored in a zip-lock bag that is then stored in a wide-mouth poly bottle that is iced and then frozen as soon as possible. Double-packaging ensures that meltwater does not affect the filters.

- Major ions (recommended): 125-250 mL of the GF/C filtrate will be stored in a poly bottle and iced (not frozen) for later analysis of chloride or other major ions. (If sample will be shipped to a lab for processing, one chilled duplicate should be retained as a back-up).

- Filtration for dissolved nutrients (required; SRP, ammonium-N, and nitrate-nitrite N) and color: Approximately 250 mL of GF/C filtrate from the above filtration will be refiltered through a 0.45 micrometer Millipore (or equivalent) membrane filter directly into an acid-cleaned, DI-rinsed poly bottle via an acid-cleaned, DI-rinsed 60 mL polypropylene syringe fitted to a 24 mm filter holder. The sample will be initially iced and then frozen

as soon as possible. (Again, if the sample will be shipped to a lab for processing, a frozen duplicate must be maintained by the regional laboratory).

Sample numbers and numbers of sample bottles will be noted on field data sheets. Samples will remain in the custody of field crews until they are delivered to the designated water quality laboratory either in person or via courier service. A chain-of-custody form will accompany the samples with a copy remaining with the field crew or regional laboratory, whichever initiates the shipment. At the laboratory, all sample codes will be entered into the laboratory sample log-in book along with the date received and any notes regarding sample condition. This information will be transferred to an electronic sample processing spreadsheet maintained by each regional laboratory. This spreadsheet will be updated as each sample is processed.

Regional laboratories not processing their own water quality samples will ship frozen or appropriately preserved samples via courier, in a cooler filled with dry ice if necessary. A list of samples and date of shipment will be emailed to the receiving lab, which will log in the samples when received, and verify receipt and condition of samples via email to the shipping lab. Results, including QA/QC data will be provided via email to the regional team laboratory. The regional team laboratory will retain frozen duplicates of all water samples as a backup against the loss of samples in shipping.

BB4. Analytical Methods Requirements

Water quality meter measurements (field): Water quality meters will be inspected and calibrated on the required schedule for each parameter (see section BB7). In case of failure, field crews will be trained in the standard procedures to follow that will solve many cases of meter failure (see section A8). Crews will also have the phone numbers of support staff to call for additional guidance and assistance. Crew leaders will explain all equipment failures and the implications of this for the data on the data sheets and transfer this information, along with appropriate error codes, to the database during data input. Table BB4.1 summarizes the field measurement parameter objectives, precision, accuracy, and method detection limits that will be used for this project.

Water chemistry measurements (lab): Table BB4.1 also summarizes the analyte precision, accuracy, and method detection limits for each water chemistry analyte required or recommended for measurement either at the motel (i.e., alkalinity and possibly turbidity), or back at the main water quality laboratories. All laboratories participating in this project have confirmed that they can meet these standards. All parameters will be measured within the listed holding times.

The analytical method listed in Table BB4-2 specifies the water chemistry methods used in the lab for this project and should be used as the ultimate reference by participating laboratories. All methods and options for arriving at the required measurements and achieving the detection limits are carefully and completely spelled out in the references cited. These references are

already owned by, or will be provided to, all of the water quality laboratories participating in this project.

Table BB4.1. Laboratory and field measurement parameter objectives. Precision, accuracy, and method detection limits used for this project.

Parameter	Precision and accuracy (≤ % RPD)	Range	Method detection limit	Reporting limits	Units	Holding times
Ammonium-N	15%	0.002 – 1	<0.002	±0.001	mg/L	28 D
[Nitrate + Nitrite]–N by autoanalyzer or Nitrate-N by IC	15%	0.002 – 1	<0.002	±0.001	mg/L	28 D
Soluble reactive phosphorus (SRP)	15%	0.002 – 1	<0.002	±0.001	mg/L	28 D
Total Phosphorus (TP)	15%	0.002 – 1	<0.002	+0.001	mg/L	28 D
Total Nitrogen (TN)	15%	0.01 – 2	<0.010	+0.005	mg/L	28 D
Chlorophyll-a	10-15%	<1 - 1000	<0.5 ug/L	+1	ug/L	† 28 D
Alkalinity (ANC)	10%	~10-1000	<0.5	±1	mg/L as CaCO3	24 hrs
Turbidity (field and/or lab)	10%	0 – 4,000	0.4	±1	‡NTRU or NTU	2 D
Color (dissolved)	10%	1 – 500	<5	+5	Platinum Color Units	2 D
Dissolved oxygen[x]	±0.3 mg/L	0.1 – >20	<0.3	±0.1	mg/L	[x]
Specific conductivity (EC25)[x]	10%	10 – 2,000	1	±1	µS/cm	[x]
pH[x]	[0.1 Units]	4 – 10	<0.1	±0.1	Std Units	24 hr
Temperature[x]	±0.2 - 0.5	0 – 25	<0.1	±0.1	°C	[x]
Redox (ORP)[x]	±20 mV	-2000 to +2000 mV	10-20 mV	+ 1 mV	mV	[x]
Transparency (or secchi) tube clarity[x]	~ 10%	0 – 120	1-2	±1	cm	[x]

‡Depends upon the optical configuration of the meter;
[x] Field measurement;
† Water may be stored on ice in the dark for up to 48 hrs prior to analysis; otherwise, field- or lab-filtered within 48 hrs and stored frozen at ≤ -20°C for no longer than 28 days prior to analysis; USGS 2005.

Table BB4.2. Water chemistry analytical methods and detection limits. SM refers to APHA (2005; *Standard Methods*)

Parameter	SOP	Analytical method	Detection limit
TP	Persulfate digestion + SRP or Dionex Method An 254	EPA 365.3; USGS (2003); Ameel *et al*. (1993, 1998); Dionex Method AN 254	<0.002 mg P/L
SRP	Automated, Ascorbic acid Dionex Method AN254	SM 4500-P-F Dionex Method AN 254	0.002 mg P/L
TN	Persulfate digestion + NO3/NO2-N	USGS (2003); Ameel *et al.* (2003)	<0.010 mg N/L
Ammonium-N	Automated phenate (or salicylate) or Dionex AN 141	SM 4500-NH_3-G Or Dionex AN 141	0.002mg N/L
Nitrate-N (may be sub-stituted for Nitrate/Nitrite-N)	Ion chromatography	SM 4500-NO_3-C	0.002 mg N/L
Nitrate/Nitrite-N	Automated, cadmium reduction	SM 4500-NO_3- F	0.002 mg N/L
Alkalinity	Titration (2 pt)	SM 2320 B	0.5 mg/L as CaCO3
Chlorophyll-a and Phaeophytin (individually)	Spectrophotometric or fluorometric	SM 10200 H Axler and Owen (1994)	<0.5 ug/L
Color (dissolved)	Spectrophotometric	SM 2120 C	5 Pt-Co color units
Turbidity (field or lab)	Nephelometric	USGS (2005)	0.4 NTU (lab) or 0.4 NTRU (field)

BB5. Quality Control Requirements

Data Quality Objectives

Data quality objectives (DQOs) are qualitative or quantitative statements of:

- Precision (a measure of random error)
- Bias (a measure of systematic error)
- Accuracy
- Representativeness
- Completeness
- Comparability
- Sensitivity

The DQOs must be defined in the context of project requirements and objectives, not the test method capabilities.

Precision – Precision measures how much two or more data values are in agreement with each other. Precision is discussed in the introductory chapter of *Standard Methods for the Examination of Water and Wastewater,* 21st Edition, 2005. Field sampling precision is determined by using field split samples or field duplicate samples. Water quality duplicates will be taken at 10% of sampled vegetation zones. Laboratory analytical precision is determined by comparing the results of split samples, duplicate samples, and duplicate spike samples.

Sampling and/or analytical precision may be determined from split or duplicate samples by calculating the Relative Percent Difference (RPD) as follows:

$$RPD = (A - B) \div ((A + B) / 2) \times 100$$

where A is the larger of the two duplicate sample values and B is the smaller value.

Where three or more replicate samples or measurements have been taken, calculate the Relative Standard Deviation (RSD) instead of the RPD as follows:

$$RSD = (s/\chi) \times 100 \text{ (also called the CV, coefficient of variation)}$$

where **s** is the standard deviation of the replicate values and **χ** is the mean of the replicate values.

Bias – This expresses the degree to which a measured value agrees with or differs from an accepted reference (standard) value due to systematic errors. Field bias should be assessed by use of field blanks and trip blanks. Adherence to proper sample handling, preservation, and holding time protocols will help minimize field bias. Since the sampling method for all sampling will be grab sampling, field blanks (i.e., sampler blanks) will be collected only once per field season. Most of the PIs and many field team leaders have conducted numerous research projects of this kind and their field collection and bottle preparation methods are well tested from previous state and federally (EPA) funded projects. They will train and work with less experienced personnel. It is unlikely that bias that could be corrected by collecting and analyzing field blanks will occur because the sample types we are collecting are, for the most part, difficult to contaminate via field equipment. These sources of error will be eliminated by appropriate training of field crews. However, laboratory bias will be determined as part of its internal quality control. Bias effects that fall outside the laboratory's acceptance limits will be flagged.

Accuracy – This expresses the degree to which an observed (measured) value agrees with an accepted reference standard (certified sample value) or differs from it due to systematic errors. All project laboratories will have analyzed certified, *blind* performance standards for nutrients and other conventional water quality parameters annually as per the conditions established for State, Provincial, or Federal certification programs as well as other EPA-funded projects. All field measurements and laboratory analyses will use USGS, EPA, and APHA standard approved methods. Sensor calibration SOPs will be created for all field meters, and all field personnel using these meters will be trained and required to prove their ability to calibrate field meters.

Completeness – Expressed as the number of valid (usable) data points made to the total number of measurements expected according to the original sampling plan. Percent completeness is determined separately for each parameter and is calculated as follows:

% Completeness = (no. of usable data points ÷ no. of planned data points) x 100

Critical (e.g., required) water quality samples must be taken for a minimum of one suite of samples per fish or invertebrate sampling zone. Failure to obtain these samples due to meter malfunction, or any other variances from the established sampling protocol, will be thoroughly documented on field data sheets. Resulting data will also be qualified in the database to reflect these issues.

Representativeness – The degree to which data accurately and precisely represents parameter variations at a sampling point, or of a process or environmental condition. Representativeness of field data is dependent upon proper sampling program design and is maximized by following the sampling plan, using proper sampling protocols, and observing sample holding times.

Data will be routinely screened for representativeness by comparing measured parameter values to historical data from other sampling efforts and to current and historical data generated by other organizations, if available. However, most data collected will essentially be 'new' for these wetlands, so project staff will rely on their limnological experience with other regional wetlands and streams to identify anomalies, should they occur.

Comparability – The level of confidence with which the project data can be compared to other data. Comparability of data will be achieved by consistently following standard field and laboratory procedures and by using standard measurement units in reporting analytical data. Sample collection and handling techniques, matrix type, and analytical method will be carried out according to standard analytical procedures and field sampling protocols. If quality control criteria (precision, accuracy, and representativeness) are known, data sets can be compared with confidence.

Sensitivity – For laboratory analyses this represents the lowest level of analyte that can be reliably detected by the laboratory analytical method. *Limit of detection (LOD)* is defined as three times the variability (i.e., standard deviation) in the gross signal of the reagent blanks. The LOD is calculated quarterly. As the LOD is approached, the variance typically increases. This requires us to have what is termed the limit of quantification for a method. *Limit of quantification (LOQ)* is defined as the value for an analyte great enough to produce <15% relative standard deviation (RSD) for its replication.

LOQ = 10(S.D.)

where 10(S.D.) is 10 times the standard deviation of the gross blank signal and the standard deviation is measured for a set of two replicates (in most cases).

Lower and upper control limits: To monitor drift in the calibration curves, warning limits are established and plotted. The upper control limit (UCL) is the highest acceptable range of occurrence for a particular QA/QC parameter. The lower control limit (LCL) is the lowest acceptable range of occurrence for a particular QA/QC parameter. UCL and LCL are calculated as:

$UCL = \overline{X} + 3(S.D.)$

$LCL = \overline{X} - 3(S.D.)$

where: $\overline{X}$ = the mean value of the parameter
3(S.D.) = three times the standard deviation of the parameter
Reporting limits (RL) for the majority of analyses are 2 X the LOD. For field measurements, this represents the lowest level of analyte the field analytical method or meter can reliably detect.

QA/QC specifics: Laboratory analytical and QA/QC protocols for determination of SRP, nitrate-N, ammonium-N, alkalinity, turbidity, TN, TP, chlorophyll-a, and color will follow well-established methods (e.g., APHA 2005; USGS 2005). Participating laboratories will be required to adhere to a QA/QC program that includes the following elements for each parameter measured: initial instrument calibration and subsequent calibration verification, initial and ongoing demonstration of analytical capability, method detection level determination, field blanks, reagent blanks (or 'method blanks'), laboratory-fortified blanks (or 'matrix spikes'), laboratory-fortified blank duplicates (or 'matrix spike duplicates'), precision and accuracy estimates, and QC acceptance/rejection criteria. These requirements will be satisfied by documentation of laboratory certification (State, Provincial, or Federal) or by documented laboratory adherence to an equivalent QA/QC program.

Common data qualifiers will be used in this project. Data that are less than the analytical method detection limit will be qualified as < the corresponding value. On samples that require dilutions due to matrix interference, the detection limit will be elevated according to the dilution factor. Data points listed as < will not be used in the calculation of laboratory precision. MS/MSD samples will be used for this purpose. Data points with < values will be converted to zeros for the calculation of accuracy. Data points that exceed the calibration limit of the analytical method will be reported as > in the laboratory reports. Samples with > values will be diluted to be within calibration range and reanalyzed. Data that do not meet laboratory data quality objectives due to matrix interference and instrumentation problems will be qualified as J (estimated positive value), UJ (estimated detection limit) and R (unusable). Data that exceed laboratory control limits by 11-50% will be qualified as J. Data that exceed laboratory control limits by >50% will be qualified as R.

Each water chemistry laboratory has a written QA/QC program that provides rules and guidelines to ensure the reliability and validity of work conducted at the laboratory. Compliance

with the QA/QC program is coordinated and monitored by the laboratory's quality assurance officer or manager.

The stated objectives of the laboratory QA/QC program are to:

- ensure that all procedures are documented, including any changes in administrative and/or technical procedures
- ensure that all analytical procedures are conducted according to sound scientific principles and have been validated
- monitor the performance of the laboratory by a systematic inspection program and provide for corrective action as necessary
- ensure that all data are properly recorded and archived

All laboratory procedures are documented in writing as standard operating procedures (SOPs), which are edited and controlled by the participating laboratory. Internal quality control procedures will be conducted by each laboratory in accordance with their standard operating procedures and the individual method requirements. Method-specific data quality objectives for precision and accuracy are found in Table BB4.1.

Method blanks, matrix spikes, and duplicates will be analyzed at the rate of one every 20 samples or one per batch, whichever is less. Spike recoveries will be used to evaluate analytical accuracy, while relative percent difference between duplicate analyses will be used to assess precision.

Individuals Responsible For Water Quality QA/QC:

Western Great Lakes	Richard Axler/Valerie Brady
Central Great Lakes (US side)	Don Uzarski/Carl Ruetz/Ashley Moerke
Central Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman
Eastern Great Lakes (US side)	Doug Wilcox
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/provincial-certified lab

<u>Mid-Year QA/QC Checks:</u>

Regional team leaders and co-PIs will assess water quality sampling methods during each field season. In most cases, this will be accomplished by traveling with field crews on at least one occasion per year. The project QA manager and assistant manager will provide guidance during the checks, provide oversight on the checks, and receive the QC reports from team leaders/co-PIs.

Performance criteria will include:

- Appropriate sample bottle prep and labeling
- Appropriate contamination avoidance procedures
- Appropriate maintenance and calibration of water quality meters
- Appropriate documentation of meter maintenance and calibration

- Appropriate record keeping for data and samples

Note that separate mid-year QA/QC checks are not required for water quality laboratory analyses because each participating laboratory will have a QA/QC program in place for the water quality parameters being measured. Adherence to these plans, which include method blanks, matrix spikes, and duplicates, will ensure accurate and comparable data among participating institutions. Institutional laboratory QA/QC programs require re-analysis of samples (when possible) in cases when performance standards are not met. QC reports from the project laboratories will be provided to the project QA managers in mid-winter.

BB6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements
All field water quality and laboratory instruments will be maintained in accordance with manufacturer's specifications and the requirements of the specific method employed. This maintenance will be carried out on a regularly-scheduled basis and will be documented in the laboratory instrument service logbook for each instrument. Emergency repair or scheduled manufacturer's maintenance will be provided under a repair and maintenance contract with factory representatives.

BB7. Instrument Calibration and Frequency
Field water quality meters will be calibrated in accordance with manufacturer's specifications. Since a number of different instrument brands, models, and configurations will be used, only general calibration methodologies are provided here. Specific electrical conductivity, turbidity, and redox (ORP) will be calibrated once per field trip (about every 10 days), while dissolved oxygen and pH will be calibrated daily. Field crews will be required to certify on data entry sheets when meters were calibrated. Maintenance logs, including calibration frequency, will be kept at each participating laboratory.

Specific conductivity: Sensors will be calibrated using a 2-point method. Air will be used to establish the zero point; a second point (i.e., the slope) will be established with a purchased conductivity standard, generally 500 µS cm^{-1}. After entering the zero point, the sensor will be rinsed with approximately 100 ml of high standard, the rinse solution will then be discarded, and the probe will be submerged into fresh standard. The instrument will then be allowed to equilibrate before the high standard is entered and the calibration is completed.

Turbidity: Sensors will be calibrated using a 2-point method. DI water will be used as the zero point and a purchased turbidity standard, generally 40 NTU, will be used for the slope. The sensor should be rinsed thoroughly before calibration. Before adding the high standard, the sensor will be rinsed with the high standard solution.

Redox: Sensors will be calibrated using a single-point method with Zobell's solution. The solution supplier's instructions regarding temperature-correction of Zobell's solution will be followed to ensure accurate calibration. There are multiple references that can be used when

calibrating redox sensors and reporting values. The standard hydrogen electrode reference in which Zobell's solution has a redox value of +425 mV at 25°C will always be used. The sensor will be thoroughly rinsed with Zobell's solution prior to calibration. Zobell's solution will be allowed to reach room temperature before calibration to reduce the amount of temperature-correction required.

Dissolved oxygen: Sensors will be calibrated a minimum of once daily using the saturated air method. Sensors will be rinsed with clean water, blotted with a clean paper towel to dry, and placed into a damp calibration chamber. Calibration chambers will not be tightened onto the probe because this may increase the atmospheric pressure within the chamber and affect the calibration. Instruments will be given sufficient time to stabilize and equilibrate before calibration. Manufacturer's instructions will be followed to complete calibration. If barometric pressure is required as a calibration parameter, the pressure will be acquired from a local weather station and entered as 'station pressure' not sea-level pressure.

pH: Sensors will be calibrated using a 3-point method with purchased pH buffers of pH 4, 7, and 10. Buffers will be prepared according to supplier's instructions and replaced when listed hold times expire. Sensor manufacturer's instructions will be followed to calibrate pH sensors and sensors will be rinsed with buffer prior to each calibration point. When possible, the sensor mV reading will be logged in the sensor maintenance log. This raw sensor output can indicate when a sensor needs to be replaced according to manufacturer specifications.

BB8. Inspection/Acceptance Requirements for Supplies and Consumables

All sample containers for chemistry will be purchased new and will be acid-cleaned and DI-water rinsed as per APHA (2005), depending on the analytes to be collected. Specifications for each batch of bottles will be verified by checking the supplier's certification statement. Standards and reagents will all be assigned expiration dates based on vendor or method requirements. Materials that exceed the expiration dates will not be used. Standards, reagents, and laboratory water will be of sufficient purity to meet method and/or instrument manufacturer criteria. Each laboratory, as part of their respective QA program, will designate individuals who will be responsible for inspecting supplies and consumables, and for providing acceptance criteria. This information will be retained in the project files.

BB9. Data Acquisition Requirements (non-direct measurements)

N/A

BC. Wetland Vegetation Sampling

BC1. Project Design

Upon arrival at the site, the field crew will first determine whether or not the site is sampleable based on the following criteria: safe public access, open connection to the lake or connecting channel, site still exists as a wetland and has not been destroyed by human disturbance (sites should still be sampled in this case if it is a benchmark site). If the site is rejected, the reason will be recorded on the space provided on the field sheet. If sampleable, additional general information about the site will be collected by the crew as they conduct their sampling. This information includes information on the wetland shape, hydrologic connection to the lake, shoreline landuse, shoreline type (e.g., sand beach, riprap, etc), and types and amounts of disturbance visible to the crew. (If vegetation crews are traveling with fish and invertebrate crews, only the fish and invertebrate crew will fill out this information on the field sheet). In addition, field crews will draw on their aerial image maps of the site to indicate locations of vegetation patches, any unshown disturbances, and transects.

The primary data collection at the site will be the identification and quantification of all wetland plant species occurring in a specified number of sampling quadrats. Within wetlands, sampling will occur along three transects that run perpendicular to depth contours and that therefore cross the wetland vegetation zones present; the number of vegetation zones will vary depending on each particular wetland. Operationally-defined vegetation zones are wet meadow, emergent vegetation, and submergent vegetation. If a distinct submergent zone is present, it will also be sampled. Note that the vegetation sampling crews will be indentifying vegetation zones somewhat differently than do the fish and macroinvertebrate crews.

Transects will be no closer than 20 m of each other. In most wetlands, they will be much further apart. The starting point of each transect will be randomly-located along the upland or swamp forest edge (or the outer wetland edge if the crew arrives by boat), and the distance from this edge to the first quadrat sampling point will be $1/6^{th}$ the width of the vegetation zone from the wetland edge. Vegetation will be surveyed in 1-m^2 quadrats at regular intervals along transects, for a total of 15-45 quadrats per wetland (15 quadrats per wetland zone). All survey quadrats will be placed 2 m to the right of the transect line to avoid trampling effects. The length of the transect within a given plant zone will be measured, and if the plant zone is greater than 11 m wide, the length of the zone will be divided by 6 to determine the distance between the 5 sampling points in each zone on each transect.

If the vegetation zone is less than 11 m wide, a “narrow” sampling protocol will be used. In this protocol, the field crew will locate the midpoint of the narrow zone along the original transect. At this midpoint, an additional transect will be placed perpendicular to the original transect. Survey plots along the perpendicular transect in the narrow zone will be located at -8, -3, 2, 7,

and 12 m from the zone midpoint along the original transect. Narrow transects are most likely to be encountered in either the wet meadow or submergent marsh zones.

In many coastal wetlands along the southern Great Lakes, invasive *Phragmites australis* has formed a dense monoculture more than 200 m wide. With increased water levels in Lakes Huron and Michigan in 2014 and 2015 [projected], sampling across this entire zone has greatly increased crew effort, reduced efficiency, and increased the likelihood of crew injury. To mitigate these issues without reducing data quality, sampling will be conducted as needed within this zone at 5, 10, 15, 20, and 25 m from the *Phragmites* bed edge (either shoreward or lakeward edge, depending on accessibility), rather than spacing sampling points across the entire width of the zone. The actual width of the zone will be calculated from the most recent year's Google photos. Correlations between Google image interpretation and field surveys are high, and difficulty maintaining a straight transect line in *Phragmites* typically results in reduced accuracy from the field transects. Prior experience and data analysis show virtually no variability in vegetation composition within the *Phragmites* zone, indicating that there will be minimal loss of information by spatially restricting *Phragmites* sampling. When this modified protocol is used, it will be referenced in the comment box and recorded in the database. The direction of entry into the *Phragmites* beds, either from the upland shoreline or from the water, will also be noted.

A list of the most aggressive invasive plants was compiled for the Great Lakes Coastal Wetlands Monitoring Plan (GLCWC 2008), and a list of most upland and wetland invasive species in the Great Lakes region is found in Michigan's Floristic Quality Assessment program (Herman et al. 2001). The Great Lakes Coastal Wetlands Monitoring Plan also contains a thorough list of plants encountered in coastal wetlands of all of the Great Lakes states, as recorded during inventories conducted with USEPA and USCZM funding from 1987 through 2004 (Albert et al. 1987, 1988, 1989; Minc 1997). Species lists from studies by GLEI, Dr. Douglas Wilcox, and other partners will be added to the lists within the Great Lakes Coastal Wetlands Monitoring Plan prior to the initiation of project sampling. Taxonomic descriptions will be cross-walked with the Flora of North America, which is available on-line (http://www.efloras.org/flora_page.aspx?flora_id=1). A new flora, *The Field Manual of Michigan Flora* (Voss and Reznicek 2012) from the University of Michigan Press, incorporates the most recent taxonomic treatments of the Flora of North America. However, local Great Lakes floras (Voss 1972, 1985, 1996), which are compatible with Michigan's FQA (Herman et al. 2001), will be used for field identification to facilitate rapid sampling. Other floras that may prove helpful for identification of difficult wetland plants include Fassett (1957), Crow et al. (2006), and Gleason and Cronquist (1991).

BC2. Sampling Methods

Within each quadrat, all macrophyte species will be identified to lowest possible taxonomic unit (typically species). Representative specimens of plants that cannot be identified in the field will be collected and preserved for identification in the laboratory. Some sterile or immature species, including grasses, sedges, and willows, cannot be identified to species, and while these

will be noted as present, they cannot be used in the FQI analysis. Herbarium staff typically are not willing to identify sterile specimens, and thus sterile species will not typically be curated. Almost all invasive exotic species can be recognized, even when sterile, and will be included in the analysis. Percent cover for each plant species, total percent vegetation cover, and water depth (cm) will be recorded for each quadrat.

Vegetation sampling data are considered critical for the majority of wetland sites (i.e., those not sampled only for birds and anurans). At least 90% of the quadrats must be successfully sampled to consider the site effectively complete and to use the data in subsequent analyses.

Support facilities for vegetation crews include a laboratory or make-shift washing facilities (e.g., tubs, buckets, and hoses in the motel parking lot) and plant presses for preserving plant specimens. The plant curation site will be equipped with dissecting microscopes that magnify to 30x and at least the identification guides by Voss mentioned above.

Supplemental data collection. It is also recommended that percent cover of vegetation detritus or standing dead biomass be recorded for each vegetation quadrat – this is especially important for plots dominated by aggressive invasive plants. It is recommended that supplemental information on depths of organic sediments, water clarity, and underlying mineral soil texture be collected at each vegetation plot. Depths of organic soils (in centimeters) will be measured by forcing a 10 ft (3 m) length of ¾ inch (1.8 cm) aluminum conduit or similar into the substrate until mineral soils are encountered. Water clarity will be simply noted in terms important for vegetation: is the bottom visible or not? In highly turbid waters where the bottom is not visible, submergent and floating plants are typically absent. Mineral substrate is broken into classes that include 1) clay, 2) silt, 3) sand, 4) gravel, 5) pebble, 6) cobble, and 6) boulder, based on rapid field evaluation with the conduit probe and feeling the substrate with the fingers. Presence of two-storied soils, such as a thin veneer of sand over clay can also be noted in the comment field, and can be significant for understanding sediment dynamics within a wetland.

BC3. Sample Handling and Custody

A numbered label will be attached to each unidentified plant and noted on the field form. Each sample will be coded by site location, transect and plot number, and date to facilitate future entering of plant identities on the sample forms and in digital data files. These plants will be placed in Ziploc bags for either identification in the laboratory or by herbarium staff. Plants requiring identification by herbarium staff will be placed in a plant press for drying and storage. There are few difficult-to-identify rare plants in Great Lakes coastal wetlands, so unknown plants can be collected without jeopardizing rare or endangered species.

Collected plants will be placed in a cooler or refrigerator upon return from the wetland, in preparation for pressing within 24 hours. Pressed plants will be dried either with heat or with a fan whenever possible. Plant samples will be destroyed following identification, except for those of interest for the herbarium's collections, or samples kept as short-term identification

aids to assist in training new personnel or as vouchers. A long term voucher collection will not be made as part of this project.

BC4. Analytical Methods Requirements

Performance criteria: Most specimens collected in the field will be identified to the species level during the evening of collection using identification keys and a magnifying glass. Specimens not identifiable to species because of lack of characteristic features (flowers, fruits, etc.) will be identified to the lowest taxonomic levels possible. Specimens of questionable identity will be pressed and returned to the laboratory. If fertile, unknown and unusual specimens will be sent to appropriate taxonomic experts for confirmation or refinement of taxonomic identity. Sterile or immature plants will be identified when possible. Target turnaround time for plant identifications is 3 months after the end of sampling.

Macrophyte data will include a) identifying and quantifying invasive plants that are considered indicators of degraded habitat (Albert and Minc 2004), b) quantifying coverages of submergent and floating-leaved vegetation, and c) comparing local site mean Conservatism (mean C) values to regional mean C values (Herman et al. 2001).

One of the most difficult aspects of plant sampling in quadrats is accurate estimation of the percent cover of plant species present. Thus, during indicator calculations, we will use a protocol that is not strongly dependent on accurate plant cover estimates, but instead during the final stage of analysis converts percent cover to broad coverage classes of 0-25%, 25-50%, 50-75%, or greater than 75% to calculate metrics. For both aggressive invasive species and submergent and floating plants that tolerate or respond to nutrient enrichment or sediment loading, these coarse cover classes are adequate for monitoring wetland quality changes.

Transect start and end points within vegetation zones will be marked using GPS (e.g., waypoints created). This is required. Because quadrats are regularly-spaced within vegetation zones, quadrat points can be interpolated from start and end points. However it is recommended that crews take the time to create waypoints for all quadrats. In case of GPS equipment failure, the vegetation crew will borrow a GPS from the fish and invert crew to finish the site and will inform their regional laboratory of the need for a replacement GPS unit. In cases when no GPS unit is available for replacement of failed equipment, start and end points of transects will be marked with flagging, and these points' GPS coordinates will be recorded at a later date. Crew leaders will explain all equipment failures and the implications of this for the data on the data sheets and transfer this information, along with appropriate error codes, to the database during data input.

Several worksheets developed as part of the Great Lakes Coastal Wetlands Monitoring Plan will be used to calculate macrophyte IBIs. These include 1) a table of wetland quality based on aquatic macrophyte sampling, 2) a flow chart for determining quality rating of submergent marsh zone or submergent component of an emergent marsh zone, 3) a table of species

tolerant of nutrient enrichment, sedimentation, or increased turbidity, and 4) a combined standardized score based on 1-3 above (see Section D for further details on IBI calculations).

Software for the calculation of Conservatism coefficients and associated metrics are contained within the FQI software for Michigan (Herman *et al*. 2001). The Michigan FQI software has been used in prior Great-Lakes-wide coastal wetland plant sampling projects, and has been found to contain almost all wetland plants growing in the Great Lakes. One of the advantages to the use of FQI and mean Conservatism scores is that they are based on the entire flora, not just a few indicator species. For this reason, the lack of a Conservatism score for one or two species at a site does not greatly alter the FQI or mean Conservatism scores.

BC5. Quality Control Requirements

Precision: Precision refers to how similar duplicates or splits are to themselves and is calculated as a % difference: % difference = (A-B)/((A+B)/2) * 100. For vegetation samples, the ability to estimate precision is built into the sampling design with the large number of quadrat samples collected per site.

Accuracy: The systematic difference from a reference standard or an expert. This will be assessed during the mid-year QC check (see below, this section).

Bias: Systematic bias by a crew or method. Bias will be assessed during the mid-year QC check by observing transect and quadrat placement and percent cover estimates, and by cross-validating difficult-to-ID taxa that are preserved. The quadrat method of sampling makes detection of uncommon taxa less likely than some other methods, and may result in lower taxa counts than other methods that cover more of the site (see sensitivity). This is a deliberate trade-off made to sample more sites rather than fewer sites more intensely.

Completeness: Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites.

Representativeness: How well sites were sampled will be determined by plotting all sample points for each site on aerial photographs of the site and by checking the field sheets and database for problems, notes, and flags. This will be done for all sites.

Comparability: Data comparability among crews within the project and to other non-project data will be achieved by using standard, accepted methods; creating metadata explaining the methods; and having strict training and QA/QC for all crews and personnel.

Sensitivity: In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification will depend on the life-stage of the plants and the condition of the plants, which is primarily controlled by the time of year of sampling.

Our sampling is timed so that the most species will be most identifiable when field crews are sampling. Uncommon taxa will not be particularly detectable because of the small percentage of each site that can be sampled even with up to 45 quadrats. Again, this is a deliberate trade-off made to sample more sites rather than fewer sites more intensely.

QA/QC specifics: Members of the project team responsible for vegetation sampling will receive rigorous taxonomic training prior to field sampling. Accurate plant identification is the most important component of vegetation monitoring. During the sampling season, representative specimens that cannot be identified in the field will be returned to the laboratory for identification, with assistance from botanical experts when necessary (See section BC4 above). A collection of difficult-to-identify species will be maintained to assist with future identifications. This can be especially useful for commonly-encountered plants that are often found in non-flowering condition. The project team will maintain an ongoing dialogue to ensure accurate and consistent identifications.

Field teams will 'calibrate' their percent cover estimates with each other during the yearly field training. Field teams will concur with each other on percent cover estimates for each quadrat. Plant metrics are designed to be robust so that small errors in percent cover estimates will not result in wetland quality ranking errors. Re-measurement of quadrats at a site will be conducted during training to calibrate individual sampler estimates of vegetation cover. The important test for this re-measurement is not the specific cover value estimates, but the final conversion of the cover values into the site metrics. The metrics are designed to be robust enough that small differences in individual plant cover values will not alter the metrics or the overall site quality ranking. Field team members will correspond with each other on percent cover estimates for each quadrat; discrepancies in cover estimates exceeding 10% between individuals in a field team will trigger a re-sampling of the quadrats in that vegetation zone.

Individuals Responsible For Vegetation QA/QC:

Western Great Lakes	Nick Danz
Central Great Lakes (US side)	Dennis Albert
Central Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman
Eastern Great Lakes (US side)	Doug Wilcox/ Greg Lawrence
Eastern Great Lakes (CA side)	Greg Grabas/ Jennifer Jung

Mid-Year QA/QC Checks:

Coverage estimates: Training and testing/certification for macrophyte cover estimation will be conducted during the early summer training workshop. Additional mid-year QA/QC checks will also be implemented to ensure data quality. The project macrophyte experts (Dennis Albert, CMU; Nick Danz, UW-Superior; Doug Wilcox, SUNY Brockport) or other individuals whom they designate will estimate percent cover in 5% of each participant's plots. Deviations in cover estimates exceeding 10% will trigger re-sampling of the plot and additional corrective action (see section C1).

Species Identification: The project macrophyte experts (Dennis Albert, CMU; Nick Danz, UW-Superior; Doug Wilcox, SUNY Brockport) or other individuals whom they designate will verify the identity of 90% of species (not samples or plots) identified by each participant who is working independently. The performance criteria for this QA/QC step will be 90% accuracy of fertile plants or plants that can typically be identified in sterile condition (a list of these plants that do not fit these criteria will be provided to field crews). This QA/QC step will be based on a combination of field and laboratory identification. Preserved specimens or digital photographs are standardly used as part of the identification process. This QA/QC evaluation will occur once per year during the sampling period. After verification, the macrophyte experts will record the species identified correctly or incorrectly by each participant, which will serve as a performance record for each participating individual. The macrophyte experts will also distribute a list of particularly difficult taxa that require preservation and lab verification when they are encountered. Corrections will be made to the macrophyte database when identification errors are found. Additional corrective actions are explained in section C1.

The project QA manager and assistant manager will provide guidance during the checks, provide oversight on the checks, and receive the QC reports from the macrophyte experts.

BC6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

Dissecting scopes, used for plant identification, will be cleaned and inspected annually. Boat motors will also be tuned up as necessary for safe operation. Crews will carry at least one spare quadrat. Boat repairs are also often necessary. Most towns around the Great Lakes have boat repair shops, which will be used by field crews as necessary. Appropriate spare parts will be carried by crews for boats and trailers, such as spark plugs and appropriate wrenches, tire for the trailer, drain plug, fuel line, and sheer pins (if used). Spare batteries will be carried for the GPS units and cameras. No other equipment used by the vegetation sampling crews requires equipment testing.

Water craft, trailers, and sampling gear will undergo rigorous disinfection to eliminate the transfer of exotic species between sections of the Great Lakes (e.g., eastern vs. western Lake Erie, northern vs. southern Lake Michigan, etc), and before moving between the lakes. Recommended disinfection includes washing the tow vehicle, boat, and trailer at a car wash, and hosing sampling equipment down with hot soapy water. Before leaving each boat ramp,

boats should have their drain plugs removed, drain any water not being used for water quality sampling, and inspect the boat and trailer carefully for vegetation.

BC7. Instrument Calibration and Frequency
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, i.e., benchmarks. During the field season, field crews will be uploading GPS readings nearly daily. At least once per week, GIS personnel will plot GPS points onto aerial photographs of a sampled site and send the image to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season will also be checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results will be logged and the logs kept with the appropriate GPS units.

BC8. Inspection/Acceptance Requirements for Supplies and Consumables
N/A

BC9. Data Acquisition Requirements (non-direct measurements)
N/A

BD. Macroinvertebrate Sampling

BD1. Project Design
Upon arrival at the site, the field crew will first determine whether or not the site is sampleable based on the following criteria: safe public access, open connection to the lake or connecting channel, site is sampleable (e.g., water depth is appropriate). If the site no longer appears to be a wetland, the crew should determine whether or not the site probably was a wetland and has been destroyed by human influences, or if the polygon was actually in error and a wetland did not exist at the site in the recent past. If the site appears to have been a wetland that was destroyed (e.g., water too turbid for vegetation to grow, but substrate is appropriate; evidence of vegetation removal or dredging, etc.), then careful documentation of this wetland loss should be made on the datasheet and with pictures. If the site is rejected, the reason will be recorded on the space provided on the field sheet and with pictures.

If the site is sampleable, additional general information about the site will be collected by the crew as they conduct their sampling. This information includes information on the wetland shape, hydrologic connection to the lake, shoreline landuse, shoreline type (e.g., sand beach, riprap, etc), and types and amounts of disturbance visible to the crew. In addition, field crews will draw on their aerial image maps of the site to indicate locations of vegetation zones, any disturbances not visible on the photos, and D-net sampling locations. Crew members for fish and invertebrates will collaborate to collect this information and complete the field sheet and map.

Macroinvertebrate samples will be collected from the major plant zones in each wetland. Sampling will begin in mid-June in the most southerly regions of the Great Lakes and continue into early September, moving north with the phenology of wetland plant and macroinvertebrate development. This is the interval during which emergent plant communities generally achieve maximum annual biomass. In most cases, macroinvertebrates will be sampled during the same sampling trips as fish. Therefore, this section makes references to the other sampling events to show how sampling is connected/coordinated. Note that there will be some sites or plant zones within a site that cannot be sampled for fish because of insufficient depth, but that will be sampleable for macroinvertebrates and water quality as well as the other data types.

Macroinvertebrate sampling is stratified by plant zone. Plant zones are patches of vegetation in which a particular plant type or growth form dominates the plant community based on visual coverage estimates. Note that other species or growth forms will likely occur within a given plant zone; however, plant types should be near-monodominant stands that comprise at least 75% of the emergent or floating-leaved plant community. Plant zones include *Typha* (cattail), *Nuphar-Nymphaea* (water lily, combined), *Schoenoplectus* (bulrush, include both "inner" and "outer" zones where they occur; see below), *Peltandra-Sagittaria-Pontederia* (arrow-arum-arrowhead-pickerel weed, combined because of similar growth form), *Sparganium* (bur-reed), wet meadow (mixed vegetation, generally containing *Juncus* and *Eleocharis* with enough water to sample with D-nets), submersed aquatic vegetation (SAV), Mixed Emergent, open water (special cases only, as outlined in section A8), and potentially other types such as floating bog mat. Note that *Schoenoplectus* zones will be divided into outer and inner zones in areas where *Schoenoplectus* vegetation is more than 50-100 m wide, since the outer (lakeward) edge of this plant zone may only support low stem density while more shoreward zones are usually sheltered enough from wave action to support a different macroinvertebrate community. Mixed Emergent zones may be sampled if they are a significant and conspicuous habitat type and there are not three other monodominant zones to sample. Additional zones may be identified upon consultation among all macroinvertebrate PIs. In this case, the QAPP, SOPs, and field sheets will be updated accordingly. Stratification by plant zone is required because GLCWC IBI metrics were formulated for specific plant zones in order to be robust to water level fluctuations. The minimum depth required for sampling macroinvertebrates is 5 cm and the maximum depth that will be sampled is 1.5 m.

Plant zones will be preliminarily identified using aerial photographs of each site, with confirmation or re-adjustment made on-site by field crew leaders based on the vegetation actually present at the time of sampling. The minimum area for plant zones to be sampled for macroinvertebrates will be approximately 400 m^2. This minimum patch size is based on keeping the sampling locations for replicate samples separated by at least 15 m. However, in cases where multiple, disjunct, smaller patches of the same vegetation type exist within a wetland, these smaller patches can each be sampled (1 or 2 replicates each) as long as the

combined area exceeds approximately 400 m^2 and no patch is smaller than 25 m^2. If there are enough disjunct patches for a choice to be made by the field crew, the larger patches should be sampled. This strategy will most often be used in riverine wetlands where habitats are heterogeneous and plant zones tend to be small.

Three replicate field-picked D-frame dip net samples should be collected in each inundated plant zone to provide a measure of variance associated with sampling. These replicates should be at least 15 m apart, and should be associated with and adjacent to fish fyke net locations if fish are also being sampled in that zone. Spacing from one replicate sample location to the next (i.e., between sample 1 & 2 or 2 & 3) should not exceed 250 m in cases where the samples are collected in the same patch. Select the three sampling points to represent the variability in the vegetation zone. In large vegetation zones, the three sampling points should correspond with different shoreline features if they occur and if this can be done under the spacing constraints mentioned above. For example, in a large lacustrine wetland where the outer *Schoenoplectus* zone extends along the shoreline lakeward of a cottage, a forested area, and a wet meadow, sampling locations will be chosen to correspond with these different features. However, in many (or most) cases, a given vegetation zone will not cover enough area to be associated with different shoreline features, in which case sample locations should be chosen to represent, to the degree possible, the variability of the zone itself.

GPS waypoints will be made at each sampling point in each plant zone. Since macroinvertebrates, fish (when possible), and water quality will all be sampled in each plant zone, care must be taken so personnel doing the sampling do not interfere with one another or compromise the other samples. Water quality data and water samples should be collected first at each location before any movement is made around the point or zone. When traveling to the site by boat, water quality data and water samples should be collected from the bow of the boat (to avoid sediment disturbance) before anyone enters the water. After water quality sampling, macroinvertebrate sampling and fish net deployment will commence.

BD2. Sampling Methods

Macroinvertebrate samples will be collected with standard 0.5-mm mesh, D-frame dip nets with openings approximately 30 cm wide by 16 cm tall in a D-shape. The sampling protocol detailed here yields qualitative macroinvertebrate samples which will be used to calculate Index of Biotic Integrity metrics. Note that two ways of 'semi-quantifying' sampling effort (counting dip net sweeps and timing of field picking) will be included as meta-data, but are not intended to rigorously quantify sampling effort or macroinvertebrate density.

Each dip net replicate consists of a composite of sweeps, each taken throughout the water column from the sediment surface up to the water surface while brushing vegetation with the net to incorporate all microhabitats at a given sampling point. Care should be taken not to collect excessive amounts of sediment or detritus, which will make field-picking of organisms difficult. To semi-quantify sampling effort, the number of net sweeps (i.e., 1 to 2-m passes) will

be counted and recorded on the sampling data sheet for each replicate sample. Counting of net sweeps is intended to provide a coarse measure of netting effort. After a series of net sweeps is made (and counted), the net will contain an assortment of invertebrates, detritus, and vegetation fragments. Net contents will then be emptied into white pans that are approximately 20 cm wide, 35 cm long and 5 cm deep (a recommended common pan size). This is an approximate size, and pan size can vary among crews because it is not critical to standardization of sampling. Organisms will be picked systematically from discrete areas within each pan from 5x5-cm grid lines drawn on the inside bottom of sampling pans. This gridding will ensure standardization of picking, rather than needing to standardize pan size.

Contents will be distributed evenly across the pan prior to picking organisms. The person doing the netting should continue to sample and fill the pan(s) until a sufficient number of organisms have been collected to begin field picking, at which time the number of net sweeps will be recorded on the data sheet. Though determining when to stop dip net sampling and begin field picking is arbitrary, field crews will be trained in this during the pre-season training workshop. The number of net sweeps required depends on macroinvertebrate densities in each zone. For example, in zones replete with macroinvertebrates, only 5-10 net sweeps are required to fill pans sufficiently to allow the target number of organisms to be picked within the allotted time. In zones with sparse macroinvertebrate communities, many more sweeps will need to be made before picking can begin. The amount of vegetation and detritus deposited in pans will vary based on characteristics of the site. However, excessive amounts of vegetation and detritus should not be collected because this will significantly reduce picking efficiency. In cases where substantial amounts of detritus and vegetation are being collected in nets, a maximum of approximately 75% of the pan surface area should be covered, leaving at least 25% of the pan uncovered (white) in order to see the locations of grid lines. A small amount of water will also fall into pans with the net contents. Generally, this is a sufficient volume of water (2-5 mm depth in the pan) to facilitate picking of swimming organisms. However, water can be added or removed accordingly to make picking as efficient as possible.

When possible, multiple individuals should work together to collect each replicate sample. When multiple individuals are working together on a single sample, they can either work from the same pan or work from separate pans, or a combination (e.g., 2 individuals working from one pan while one individual works from a different pan) and place organisms into a single vial for that sample. The sampling protocol is robust to this type of variation because individuals will systematically focus on one small area at a time within each pan (i.e., a single grid square), which limits interference between individuals that are working from the same pan. One hundred fifty macroinvertebrates should be collected using forceps, working systematically from one end of the pan to the other, attempting to pick all specimens from each square before moving on to the next. Special efforts should be made to ensure that tiny, cryptic and/or sessile organisms (those resting on or attached to vegetation or debris) are not overlooked within each grid square. However, microcrustacea (e.g., rotifers, cladocerans, ostracods, copepods) are not part of this protocol and should be ignored. Hand tally counters will be used to keep track of

how many organisms are collected. In the absence of hand tally counters, individuals working on the same sample will count out loud to ensure that an accurate number of organisms are collected. Specimens will be immediately placed into labeled (site ID, plant zone, replicate number, sample date, regional team initials, and crew chief name) approximately 30-mL leak-proof vials containing 95% ethanol to ensure proper preservation. If glass sample vials are used, they must be stored in a manner that prevents breakage during transport.

For the majority of cases, obtaining 150 organisms per replicate is a relatively easy task. However, in some cases invertebrates are extremely scarce. In these cases, picking time per replicate will be limited; the following is a means of semi-quantification or catch per unit effort. Picking of individual replicates will be timed using a stopwatch. If 150 organisms are picked before one-half-person-hour (i.e. two people for 15 minutes; three people for 10 minutes, etc.) elapses, then picking stops at 150 and the number of person-minutes is recorded on the data sheet. If 150 organisms are not acquired within one-half-person-hour, the clock will be stopped, organisms will be tallied, and the running clock will resume as picking continues to the next multiple of 50, regardless of the time required to do so. For example, if after one-half-person-hour 115 organisms have been collected, picking will continue to 150, regardless of how long it takes to do so. Therefore, each replicate sample should contain 50, 100, or 150 organisms. After each replicate sample is picked, the number of person-minutes required for collection, as well as the number of organisms collected, will be recorded on the data sheet.

Since the goal is for each replicate sample to represent the relative abundances of macroinvertebrate taxa at each sampling location, picking bias must be minimized. Accordingly, the number of organisms remaining in each of the picked grids of the pan should nearly always be exhausted to the point where finding just a few more organisms will require a substantial effort. When this occurs, the next grid square should be picked. If the entire pan is 'picked clean' before the target number of specimens is reached, then timing should stop while dip nets are used to refill the pan(s). As noted above, multiple pans can be used to collect a single replicate sample as long as the grid method is used so that collected organisms represent the resident wetland community.

Additional supplementary data that will be collected with each macroinvertebrate replicate sample include: latitude/longitude via GPS waypoint and vegetation percent cover by growth form and cover type at both the water surface and sediment surface. Note that these supplementary vegetation data are separate from the other vegetation sampling for the project and will be associated with macroinvertebrate and fish data only. A 1-m^2 quadrat will be used to determine surface and subsurface vegetation percent cover. In locations where both fish and macroinvertebrates are being sampled, the quadrat will be placed 1 m to the right of the mid-point of the net lead when looking away from the net box. A ribbon or similar way of marking will be used to identify the midpoint of each lead. In locations where fish are not being sampled, quadrats will be placed roughly in the center of the area where dip net sweeps were made. To semi-randomize the specific location of quadrats when fish are not being sampled,

quadrats will be thrown over the individual's shoulder to reduce bias in sampling location. Percent composition by growth form and cover type will be estimated visually. Growth forms and cover to be estimated are the same as those for the vegetation zones listed above. If species (or genera) within each growth form category are known, they should be noted on the field data sheet. Although vegetation overlaps, crews will be trained to standardize their quadrat percent cover estimates so that percentages sum to 100%; open water (surface) and bare substrate (bottom) will specifically be included to ensure that crews have quadrat coverages summing to 100%.

For laboratory identification, taxonomic keys such as Thorp and Covich (2014) and Merritt *et al.* (2008), along with mainstream literature, will be used for identification. Each regional team laboratory will develop and maintain a reference set of preserved specimens that will be used to train new staff and to compare with to ensure consistency in identifications. Standardized record keeping and sample archiving techniques will be used to facilitate re-evaluation of taxonomy if taxonomic inconsistencies arise. Support facilities include a microscopy laboratory equipped with high-quality dissecting microscopes that magnify to 50x; access to nearby vent hoods for processing preserved samples; appropriate tools for manipulating invertebrates (fine-pointed forceps, needles, etc); and the taxonomic keys listed above.

Macroinvertebrate sampling data are considered critical for the majority of wetland sites (i.e., those not sampled only for birds and anurans) and must be 80% complete for each wetland site (80% of targeted samples are collected).

BD3. Sample Handling and Custody

Macroinvertebrate samples will be labeled with both internal and external labels. External labels will only be those that have previously been checked for ability to remain adhered to sample bottles even when wet. They will be written on with black fine point Sharpie markers or similar markers that have been tested and proven to be water- and ethanol-proof. Internal labels will be made from waterproof paper and written on with either ethanol-proof ink or pencil. Labels will consist of site code, plant zone, replicate number, sample date, regional team code, and crew chief name.

Samples will remain in the custody of field crews for the duration of each field trip. At the laboratory, all sample information will be entered into the laboratory log-in form, along with the date received and any notes about sample condition. This information will be transferred to an electronic sample processing spreadsheet maintained by each regional laboratory. This spreadsheet will be updated as each sample is processed. Processed samples will remain in the custody of the regional team laboratory. Invertebrate identifications will be compiled on hard-copy laboratory sheets, and then entered into the data management system. The hard-copy laboratory sheets will be stored at each regional laboratory.

Identified macroinvertebrates will be stored for 5 years after collection, with alcohol levels checked annually. All collections will have stored with the collection metadata that decodes each site code into site name, state, county, lake, and latitude and longitude. The metadata will also include information about the project, the PI in charge of the collection, and where to locate more information. This metadata must be stored with the invertebrate collection as long as the collection remains in existence. After 5 years, or at any time if the PI or laboratory cannot keep the collection, the lead project PI (Uzarski) and the other invertebrate co-PIs should be notified and offered the collection. Destruction of the collection should only be made with permission from the project PI (Uzarski) and the EPA GLNPO, and if no other co-PI is interested in maintaining it.

BD4. Analytical Methods Requirements

All sampling point locations will be stored using GPS. In case of GPS equipment failure, the invertebrate crew will borrow a GPS from the vegetation crew to finish the site, if possible, and will notify their regional laboratory of the need for a replacement GPS unit. In the event that this is not possible, crews will endeavor to very accurately mark each sample point on their field map. Failures of other equipment are dealt with in section BD6. Crew leaders will explain all equipment failures and the implications of this for the data on the data sheets and transfer this information, along with appropriate error codes, to the database during data input.

Performance criteria: Macroinvertebrates will be identified to the lowest operational taxonomic unit necessary for IBI metric calculations (genus-level in most cases for insect taxa). A list of all macroinvertebrate groups and their targeted identification level will be provided to all identification laboratories before sample identification begins. Labs that do not have the expertise to identify particular groups will either be provided with appropriate training (and certification) or will have those particular taxa identified by qualified individuals. Target turnaround time for macroinvertebrate identifications is 8 months after the end of sampling.

BD5. Quality Control Requirements

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 three replicate samples collected for each vegetation zone will be used to generate the precision estimate.

Accuracy: The systematic difference from a reference standard or an expert. This will be assessed by having a whole-project reference collection verified by aquatic invertebrate experts. This QC will be overseen by Brady and Cooper, the QA managers.

Bias: Systematic bias by a crew or method. Bias will be assessed during the mid-year QC check by observing sample collection and invertebrate picking (see mid-year QC check, below, this section). Because so little of a site can be sampled, only quite common taxa will be collected. This is a known bias with invertebrate sampling and has been written about extensively for stream sampling by Karr and Chu (1999).

Completeness: Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites.

Representativeness: How well sites were sampled will be determined by plotting all sample points for each site on aerial photographs of the site and by checking the field sheets and database for problems notes and flags. This will be done for all sites.

Comparability: Data comparability among crews within the project and to other non-project data will be achieved by using standard, accepted methods; creating metadata explaining the methods; and having strict training and QA/QC for all crews and personnel.

Sensitivity: In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification will depend on the life-stage of invertebrates and their condition after preservation. Life stage is primarily controlled by the time of year of sampling. Our sampling is timed so that the most taxa will be identifiable when field crews are sampling. Crews will be trained to minimize damage during preservation of organisms.

QA/QC specifics: All members of the project team responsible for invertebrate sampling and sample processing will receive training and certification prior to field sampling (see section A8). Proper dip net sampling technique must be learned by all field crew members to prevent sampling bias (see section A8). Open dialogue within and among labs will greatly reduce the need for corrective action. If taxonomic inconsistencies are discovered, team members with expertise will be consulted. If they are unable to resolve the taxonomy, then regional or national experts will be consulted. Project team members working on invertebrate taxonomy will use conference calls, e-mail, and an online forum to share taxonomic information. Once taxonomic issues are resolved, database queries will be written to identify other samples that contain the questionable taxa, and those specimens will be pulled and re-examined. Additional mid-year QA/QC checks will also be implemented to ensure each laboratory's macroinvertebrate data quality (below).

Individuals Responsible For Macroinvertebrate Sampling and Processing QA/QC:

Western Great Lakes	Valerie Brady/Josh Dumke
Central Great Lakes (US side)	Don Uzarski/Carl Ruetz/Ashley Moerke
Central Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman
Eastern Great Lakes (US side)	Doug Wilcox/ Courtney McDaniel
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Greg Grabas/ Jennifer Jung

Mid-Year QA/QC Checks:

Macroinvertebrate field sampling: Regional team leaders and co-PIs will assess macroinvertebrate sampling and picking bias by traveling with regional field crews at least once per field season to observe each crew member collecting and picking samples, and by inspection of the "remains" of each picking pan. Macroinvertebrate sampling performance criteria will be assessed for each individual working on invertebrate samples, and notes and scores on their performance rating will become part of the permanent record for this project. The project QA manager and assistant manager will provide guidance during the checks, provide oversight on the checks, and receive the QC reports from team leaders/co-PIs.

Performance criteria will include:
- Correct D-net sweep procedure (satisfactory/unsatisfactory)
- Thoroughness of sampling of habitats (satisfactory/unsatisfactory)
- Efficiency and accuracy of picking (satisfactory/unsatisfactory)
- Accuracy of picking 'mobile' taxa (satisfactory/unsatisfactory)

'Mobile' taxa (e.g., amphipods) are those that are highly mobile and difficult to sample/capture. Corrective actions at the field crew-level will be implemented if performance criteria are not met (see section C1).

Macroinvertebrate laboratory processing: Macroinvertebrate sample processing performance will be assessed at the lab/institution level because past experience on similar projects has shown that invertebrate identification is most effectively accomplished using a team approach in which individuals specialize in certain taxonomic groups or specific elements of sample processing (initial sorting, family-level ID, genus-level ID). Mid-year QA/QC evaluations will be accomplished by having laboratories exchange invertebrates from two vegetation zones with each other for blinded re-identification. Distribution of QA/QC samples across laboratories will be done by the project QA manager and/or assistant QA manager.

Performance criteria will include:
- Percentage of taxa correctly identified to appropriate taxonomic level (95%)
- Proper sample preservation and labeling (100%)

Corrective action at the laboratory-level will be implemented if performance criteria are not met (see section C1).

BD6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

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. Crews will also carry extra forceps. Boat and motor repairs are also often necessary. Most towns around the Great Lakes have boat repair shops, which will be used by field crews as necessary. Spare

parts carried by crews will include spark plugs and appropriate wrenches, a spare tire for the trailer, drain plug, fuel line, shear pins (if used), and a spare propeller shared among several crews working in the same region. Spare batteries will be carried for the GPS units, cameras, and water quality meters.

Water craft, trailers, and sampling gear will undergo rigorous disinfection to eliminate the transfer of exotic species between sections of the Great Lakes (e.g., eastern vs. western Lake Erie, northern vs. southern Lake Michigan, etc. Rule of thumb = more than 150 miles between 1 site and the next), or before moving between the 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.

Most US states now require that, before leaving each boat ramp, boats should have their drain plugs removed, drain any water not being used for water quality sampling, and inspect the boat and trailer carefully for vegetation and other “passengers”. Some states (e.g., Minnesota) now require that all boats must be trailered with their drain plugs out.

BD7. Instrument Calibration and Frequency
Microscopes are typically calibrated and aligned every 2-4 years, depending on scope type and manufacturers recommendations. Recreational GPS accuracy is sufficient for these data. GPS receivers will be tested prior to and after the field season by taking repeated readings at known localities visible on aerial photographs. During the field season, field crews will be uploading GPS readings nearly daily. At least once per week, GIS personnel will plot GPS points onto aerial photographs of a sampled site and send the image to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season will also be checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results will be logged and the logs kept with the appropriate GPS units.

BD8. Inspection/Acceptance Requirements for Supplies and Consumables
N/A

BD9. Data Acquisition Requirements (non-direct measurements)
N/A

BE. Fish Sampling

BE1. Project Design
Upon arrival at the site, the field crew will first determine whether or not the site is sampleable based on the following criteria: safe public access, open connection to the lake or connecting channel, site is sampleable (e.g., water depth is appropriate). If the site no longer appears to be

a wetland, the crew should determine whether or not the site probably was a wetland and has been destroyed by human influences, or if the polygon was actually in error and a wetland did not exist at the site in the recent past. If the site appears to have been a wetland that was destroyed (e.g., water too turbid for vegetation to grow, but substrate is appropriate; evidence of vegetation removal or dredging, etc.), then careful documentation of this wetland loss should be made on the datasheet and with pictures. If the site is rejected, the reason will be recorded on the space provided on the field sheet and with pictures.

If the site is sampleable, additional general information about the site will be collected by the crew as they conduct their sampling. This information includes information on the wetland shape, hydrologic connection to the lake, shoreline landuse, shoreline type (e.g., sand beach, riprap, etc.), and types and amounts of disturbance visible to the crew. In addition, field crews will draw on their aerial image maps of the site to indicate locations of vegetation zones, any unshown disturbances, and fyke net set locations. Crew members for fish and invertebrates will collaborate to collect this information and complete the field sheet and map.

Fish will be sampled from the major plant zones in each wetland. Sampling will begin in mid-June in the most southerly regions of the Great Lakes and continue into early September, moving north with the phenology of wetland plant and macroinvertebrate development. This is the interval during which emergent plant communities generally achieve maximum annual biomass. In most cases, fish will be sampled during the same sampling trips as macroinvertebrates. Therefore, this section duplicates the text with portions of the macroinvertebrate section (BD), especially regarding plant zones and net placement. Note that there will be some sites or plant zones within a site that cannot be sampled for fish because of insufficient depth, but that will be sampleable for macroinvertebrates and water quality as well as the other data categories.

Fish sampling is stratified by plant zone. Plant zones are patches of vegetation in which a particular plant type or growth form dominates the plant community based on visual coverage estimates. Note that other species or growth forms will likely occur within a given plant zone; however, the plant type should be near monodominant and comprise at least 75% of the emergent or floating-leaved plant community. Plant zones include *Typha* (cattail), *Nuphar-Nyphaea* (water lily, combined), *Schoenoplectus* (bulrush, include both “inner” and “outer” where they occur), *Peltandra-Sagittaria-Pontederia* (arrow-arum-arrowhead-pickerel weed, combined because of similar growth form), *Sparganium* (bur-reed), wet meadow (mixed vegetation, generally containing *Juncus* and *Eleocharis* if there is enough water to sample with fyke nets), submersed aquatic vegetation (SAV), Mixed Emergent, open water (special cases only, as outlined in section A8), and potentially other types such as floating bog mat. Mixed Emergent zones may be sampled if they represent a significant and conspicuous habitat type and there are not three other monodominant zones to sample. Additional zones may be identified upon consultation among all fish PIs. In this case, the QAPP, SOPs, and field sheets will be updated accordingly. Stratification by plant zone is required because GLCWC IBI metrics

were formulated for specific plant zones in order to be robust to water-level fluctuations. The IBI of Uzarski *et al*. (2005), which is being used for this monitoring program, relied primarily on bulrush, water lily, and cattail-dominated zones, and these zones should be sampled if present. *Schoenoplectus* zones will be divided into outer and inner zones in areas where *Schoenoplectus* vegetation is more than 50-100 m wide because the outer (lakeward) edge of this plant zone may only support low stem density while more shoreward zones are usually sheltered enough from wave action to support different fish species. In high lake-level years, inundated wet meadow zones may also be deep enough to sample with fyke nets. The minimum depth required for sampling fish is 20 cm and the maximum depth that will be sampled is 1 m. Therefore, plant zones with depths ranging from approximately 5 cm to 20 cm will be sampled for macroinvertebrates and water quality but not for fish.

Plant zones will be preliminarily identified using aerial photographs of each site, with confirmation or re-adjustment made on-site by field crew leaders based on the vegetation actually present at the time of sampling. The minimum area for plant zones to be sampled will be approximately 400 m^2. This minimum patch size criterion is designed to ensure that replicate nets are set no less than 20 m apart. However, in cases where multiple, disjunct, smaller patches of the same vegetation type exist within a wetland, these smaller patches can each be sampled (1 or 2 nets each) as long as the combined area exceeds approximately 400 m^2 and no patch is smaller than 100 m^2. This strategy will most often be used in riverine wetlands where habitats are heterogeneous and plant zones tend to be small.

Three replicate fyke nets are set in each inundated plant zone to provide a measure of variance associated with sampling. Within each plant zone, each of the three replicate nets should be located at least 20 m from any other net to prevent the nets from interfering with one another. Spacing from one net to the next (i.e., between net 1 & 2 or 2 & 3) should not exceed 150 m in cases where the nets are set in the same patch. Note that macroinvertebrate sampling locations are associated with and adjacent to fyke net locations. Water quality is also sampled in each plant zone.

Select the three fyke net locations to represent, to the degree possible, the variability in the plant zone. In large plant zones, the three sampling points should correspond with different shoreline features if they occur and if this can be done under the spacing constraints mentioned above. For example, in a large fringing wetland where the outer *Schoenoplectus* zone extends along the shoreline lakeward of a cottage, a forested area, and a wet meadow, sampling locations will be chosen to correspond with these different features. However, in many cases, a given vegetation zone will not cover enough area to be associated with different shoreline features, in which case sample locations should be chosen to represent, to the degree possible, the variability of the zone itself. GPS waypoints should be created for each fyke net location.

Since macroinvertebrates, fish, and water quality will all be sampled in each plant zone, care must be taken so that activities of personnel engaged in one type of sampling do not interfere with one another or compromise the other samples. Water quality data and water samples should be collected first at each location before any movement is made around the point or zone. When traveling to the site by boat, water quality data and water samples should be collected from the bow of the boat before anyone enters the water (to avoid sediment disturbance). After water quality sampling has been completed, macroinvertebrate sampling and fish net deployment will commence.

Fish communities will be passively sampled using fyke (i.e., trap) nets deployed overnight. At each wetland, three fyke nets will be deployed in each fishable plant zone. A 'fishable' plant zone is one of the plant zones or types described above with water depth between 20 and 100 cm. Shallower or deeper water cannot be effectively fished with the fyke nets using GLCWC methods.

Fyke nets should conform to these specifications:

Large nets

Lead:

-25' x 3' (± 1 ft x 3 inches)
-3/16" mesh (exact match required)
-weighted line on bottom
-floats on top
-loops on top and bottom of each end for poles or similar strategy

Trap:

-4' x 3' frames (2 frames, size ± 6 inches x 3 inches, approximately 3' apart)
-3/16" mesh (exact match required)
-1st hoop approximately 3' from second box
-hoops approximately 1.5' apart
-hoops diameter 30" (± 3 inches)
-5 hoops
-funnels on 1st and 3rd hoops
-funnel hole inside diameter 6-1/2" (± 1 inch)
-wings 6' long, 3/16" mesh (exact match required on mesh size)

Small nets

Lead

-25' x 1.5' (± 1 ft x 3 inches)
-3/16" mesh (exact match required)
- weighted line on bottom
-floats on top
-loops on top and bottom of each end for poles (or similar strategy)

Trap:

- 3' x 18" (2 frames, size ± 4 inches x 2 inches, approximately 18" apart)

-3/16” mesh (exact match required)
-1st hoop approximately 18” from back box
-hoops approximately 12” apart
-hoop diameter 12” (± 3 inches)
-5 hoops - 2 funnels
-funnels on 1st and 3rd hoops
-funnel hole inside diameter 4” (± 1 inch)
-wings 6’ long, 3/16” mesh (exact match required on mesh size)

BE2. Sampling Methods

Fish sampling should be conducted using three replicate fyke nets in each plant zone for one net-night (Uzarski *et al*. 2005, Brady *et al.* 2007). The timing of sampling should correspond to the phenology and relative maturity of the vegetation in each system (crews will work from south to north). The method of determining locations at which to set nets is described above, and locations will correspond to places where macroinvertebrates and water quality are sampled and water is deep enough. Sampling will be completed by mid-September as seasonal movements of fish to winter locations may bias estimates of community composition.

Two sizes of fyke nets will be used (see required and recommended specifications above). Smaller nets should be set in water approximately 20 - 50 cm deep; larger nets are set in water depths of 50 – 100 cm. The depth of water in each plant zone will dictate net size used since the main difference between large and small nets is height. Nets should be placed with the mouth opening perpendicular to the vegetation zone of interest, with leads extending from the center of the mouth of the net into the vegetation. Therefore, fishes in the plant zone or moving along the edge of plant zone are likely to be caught. Wings should be set at 45° angles to the lead and connected to the outer opening on each side of the net. In very large plant zones where setting the trap outside of the patch is not possible, nets should be set with the lead pointing toward shore. Nets should be set so that the top of the cod end is above the water surface to prevent turtles and other air-breathing vertebrates from drowning.

Nets will be set for one night (at least 12 hr), after which time they will be emptied. The trapped fish will be identified to species, counted, measured, and released alive. The times of net deployment and recovery will be recorded on field data sheets. A minimum of 25 individuals per species and age category (2 categories: young-of-year [YOY] vs. older [i.e., not YOY]) will be measured to the nearest mm (total length). When individuals are counted, separate tallies will be made for YOY vs. older individuals of each species. Fish less than 20 mm will not be counted because the sampling gear does not accurately capture fish this small. All fish will be handled following university wildlife use and care guidelines, and an approved project-specific plan will be in place at each regional laboratory before sampling begins. Appropriate state and provincial permits will also be obtained before sampling begins.

All crews will have photo keys of Great Lakes fish species, including their key taxonomic characteristics, developed previously by Uzarski *et al.* (for GLCWC) and Brady *et al.* (for GLEI). If a fish cannot be identified in the field, representative specimens or high-quality digital photographs will be returned to the laboratory for identification, with assistance from experts when necessary. Photographs will be used for large fish or where collection of fish samples is not permitted by law or regulation. Each fish sampling crew will maintain a reference set of preserved specimens (fixed in 10% buffered formalin and then transferred to 70% ethanol for storage) in their laboratory if allowed by state, provincial, or federal regulations. Many regional laboratories already have reference collections of preserved specimens which can be used for this project. However, if species are encountered that are not part of the lab's collection, a voucher specimen will be collected and preserved, if allowed. For large specimens (>20 cm), or where collection of fish specimens is not allowed, fully documented digital photographs will be collected instead of specimens. When digital images are used, the putative species name (if known), total length, site ID, plant zone, date, regional team code, and crew leader's name will be included in the image. Additional photographs of key features should be taken to aid identification of unknown specimens. As with the other indicators, ongoing dialogue among project partners will ensure consistent taxonomy and minimize the need for corrective actions.

Fish samples are considered critical for targeted wetland sites (i.e., those not selected for bird and anuran sampling only). However, occasionally, nets are knocked down by waves, vandalized, or damaged by turtles or mammals. If 2 of the 3 nets in a plant zone are determined by the field crew leader not to have representatively sampled the plant zone for these or other reasons, the nets will be re-set for a second night. The senior field crew leader and/or the regional team leader should be consulted on this decision. If the fishing integrity of nets is not maintained for at least 2 of the 3 nets for a second night, the regional team leader must be consulted about whether to reset nets or move to the next site. In addition, if fewer than 10 fish are collected in total from all 3 nets within a plant zone, the nets will be re-set for an additional night. Prior to deployment, all nets must be inspected for holes or other damage and repaired as necessary. Significant net repairs may need to be done by a net company.

Fish sampling crews need relatively little in the way of support facilities. Needs include a laboratory with nearby vent hoods for handling preservative. The laboratory should be equipped with dissecting microscopes that magnify to 20x, and appropriate guides for Great Lakes fish (e.g., Bailey *et al.* 2004, Hubbs *et al.* 2004, Corkum 2010). Fish crews need a minimum of 5 of each size of the nets specified above, and 6 of each size are recommended. Also required are a boat, trailer, and towing vehicle.

BE3. Sample Handling and Custody

Fish that cannot be identified in the field will be preserved or photographed for laboratory identification, depending on fish size and state, provincial, and federal regulations. Fish will be humanely euthanized following university animal use protocols for wild animal specimens. Specimens will be initially fixed in 10% buffered formalin in plastic containers labeled with both

internal and external water- and preservative-proof labels (site ID, zone, fyke net number, unknown fish ID number, regional team code, and field crew leader name). Whenever unidentified fish specimens are kept, a note will be made on the field data sheets. Samples will remain in the custody of field crews for the duration of each field trip. At the laboratory, unidentified fish specimens will be entered into the laboratory log-in sheet, along with the date received and any notes about sample condition. At this point, fish can be placed in 70% ethanol as long as they have been fixed in formalin for at least 2 days.

Preserved fish or their photographs will be stored for up to 5 years after collection in case there are further questions about proper identification. If a specimen is donated or lent to a museum because of its taxonomic or distributional value, records of its disposition will be kept by the regional laboratory and entered into the database. All collections will have stored with the collection metadata that decodes each site code into site name, state, county, lake, and latitude and longitude. The metadata will also include information about the project, the PI in charge of the collection, and where to locate more information. This metadata must be stored with the fish collection as long as the collection remains in existence. After 5 years, or at any time if the PI or laboratory cannot keep the collection, the lead project PI (Uzarski) and the other fish co-PIs should be notified and offered the collection. Destruction of the collection should only be made with permission from the project PI (Uzarski) and the EPA GLNPO, and if no other co-PI is interested in maintaining it.

BE4. Analytical Methods Requirements

All fish net sample locations will be saved as waypoints using recreational GPS. In case of GPS equipment failure, the fish crew will borrow a GPS from the vegetation crew to finish the site, if possible, and will inform their regional laboratory of the need for a replacement GPS unit. If this is not possible, crews will carefully mark the location of fyke net sets on their paper field maps. Other equipment failures are dealt with in section BE6. Crew leaders will explain all equipment failures and the implications of this for the data on the data sheets and transfer this information, along with appropriate error codes, to the database during data input.

Performance criteria: Fish are identified to species, although it may not be possible to identify some very young specimens. Fish smaller than 20 mm total length will not be counted or identified because fish this small are not accurately sampled by nets of our mesh size. All regional laboratories have qualified personnel who can identify Great Lakes fish to species. Target turnaround time on preserved difficult identifications is two months after the end of sampling.

BE5. Quality Control Requirements

Precision: Precision refers to how similar duplicates or splits are to themselves and is calculated as a % difference: % difference = (A-B)/((A+B)/2) * 100. The three nets set per vegetation zone at each site will be used to estimate precision of fish sampling.

Accuracy: The systematic difference from a reference standard or an expert. This will be assessed during the mid-year QC check (see below, this section).

Bias: Systematic bias by a crew or method. Bias will be assessed during the mid-year QC check by observing net placement and fish species ID (see mid-year QC check, below, this section). Fish are mobile, which means that a few nets can sample more of a site. However, fish can also detect the nets and may avoid entering them. This is a known bias incorporated into the protocol (all gear types have biases for or against various types of fish), and has been studied fairly well (Murphy and Willis 1996; Ruetz *et al.* 2007).

Completeness: Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites.

Representativeness: How well sites were sampled will be determined by plotting all sample points for each site on aerial photographs of the site and by checking the field sheets and database for problems notes and flags. The number of nets that "fished" (stayed upright and intact) will be calculated. All these will be done for all sites.

Comparability: Data comparability among crews within the project and to other non-project data will be achieved by using standard, accepted methods; creating metadata explaining the methods; and having strict training and QA/QC for all crews and personnel.

Sensitivity: In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification will depend on the particular taxa and age of fish. Taxa types that are known to be difficult to identify (e.g., shiners) will be recommended for routine preservation. Uncommon taxa may not be detectable because of the low percentage of each site that can be sampled. In addition, some fish will avoid the nets (see bias). This protocol is robust to these known issues.

QA/QC specifics: Damaged nets or sets determined to be tampered with or unproductive by field personnel (for example, due to a collapse or substantial holes) should be re-set for a second night (see above). Because fishes are primarily identified in the field, it is essential that all members of the project team who will be sampling fish are proficient in fish taxonomy. Therefore, all individuals working independently on fish sampling will be trained, tested, and certified prior to sampling (see Section A8). Additional QA/QC checks as well as laboratory reference collections of preserved specimens or photographs will ensure data quality. Reference collections will be made and maintained by each laboratory that is independently collecting fish data. Voucher specimens for each observed species should be preserved in 10% buffered formalin and then transferred to 70% ethanol for the duration of the project. For species in which observed specimens exceed 20 cm, fully documented digital images stored in multiple locations will be sufficient to verify identifications. When digital images are used to

verify species identity, the species name, location, date, regional team code, and field crew leader's name must be written on a white background and made clearly visible in the image.

Individuals Responsible For Fish Sampling QA/QC:

Western Great Lakes	Josh Dumke/Valerie Brady
Central Great Lakes (US side)	Don Uzarski/Carl Ruetz/Ashley Moerke
Central Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman
Eastern Great Lakes (US side)	Doug Wilcox/James Haynes
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Greg Grabas/Jennifer Jung

Mid-Year QA/QC Checks:

Regional team leaders or co-PIs will annually assess fish data quality for crews collecting fish data. Whenever possible, evaluations will be made both in the field and on the laboratory's reference collection. Mid-year field QA/QC evaluations will be conducted at the crew-level since fish identification is generally done as a team (individuals only identify species about which they are confident and either pass the fish to another team member or preserve/photograph the specimen for laboratory identification). The project QA manager and assistant manager will provide guidance during the checks, provide oversight on the checks, and receive the QC reports from team leaders/co-PIs.

Performance criteria will include:
- Correct identification of plant zones for setting fyke nets (satisfactory/unsatisfactory)
- Correct setting of fyke nets (satisfactory/unsatisfactory)
- Accuracy of species-level IDs in the field/laboratory (95%)
- Proper handling of live fish (100%)
- Determination of when to retain specimens for laboratory ID (100%)

Failure to meet performance criteria will trigger corrective action (see section C1).

BE6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

Fyke nets will be examined for holes and defects before and after each net set. The occurrence of holes that might have compromised net integrity during a set will be noted on field sheets, and if such holes were substantial and affect 2 or more nets per zone, the nets will be repaired and re-set. Crews will carry net repair kits with them and will repair nets in the field whenever possible. Major repairs may require the assistance of a net-making company. Boat or motor repairs are also often necessary. Most towns around the Great Lakes have boat repair shops, which will be used by field crews as necessary. Spare parts carried by crews will include spark plugs and appropriate wrenches, a spare tire for the trailer, drain plug, fuel line, sheer pins (if used), and a spare propeller shared among several crews working in the same region. Spare batteries will be carried for the GPS units, cameras, and water quality meters.

To prevent the spread of invasive species, including disease, boats and trailers will be drained and inspected upon haul-out while still at the boat launch. Water craft, trailers, and sampling gear will undergo rigorous disinfection to eliminate the transfer of exotic species between sections of the Great Lakes (e.g., eastern vs. western Lake Erie, northern vs. southern Lake Michigan, etc. Rule of thumb = more than 150 miles between 1 site and the next), or before moving between the 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. No water will be transferred from one section of a Great Lake to another section or to an inland lake or water body.

Most US Great Lakes states now require that, before leaving each boat ramp, boats should have their drain plugs removed, drain any water not being used for water quality sampling, and inspect the boat and trailer carefully for vegetation and other "passengers". Some states (e.g., Minnesota) now require that all boats must be trailered with their drain plugs out.

BE7. Instrument Calibration and Frequency

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 large features that can be seen on aerial photographs to check GPS accuracy. During the field season, field crews will be uploading GPS readings nearly daily. At least once per week, GIS personnel will plot GPS points onto aerial photographs of a sampled site and send the image to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season will also be checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results will be logged and the logs kept with the appropriate GPS units.

BE8. Inspection/Acceptance Requirements for Supplies and Consumables

N/A

BE9. Data Acquisition Requirements (non-direct measurements)

N/A

BF. Bird and Anuran Sampling

BF1. Project Design

Over the past thirty years considerable field data have been gathered and analyzed to develop anuran and bird monitoring protocols in the Great Lakes region, especially in wetlands (Niemi 1980; Hanowski *et al.* 1990, 2007a,b; Gibbs and Melvin 1993; Howe *et al.* 1998, 2007; Weeber and Valliantos 2000; Price *et al.* 2004, 2007; Meyer 2006; Crewe and Timmermans 2005; Etterson *et al.* 2009). [Note that the terminology of "anuran" replaced "amphibian" in 2017 to more accurately reflect that we are primarily sampling frogs and toads that vocalize. Other amphibians such as salamanders are not being sampled.] This project will build on this existing work by 1) establishing a strategic baseline of site-specific data and 2) articulating and validating a clear, scientifically rigorous plan for long term monitoring of bird and anuran populations in Great Lakes coastal wetlands. The field component of this monitoring program will use the protocols contained in the GLCWC Monitoring Plan (with the exception of certain modifications described in this document), but additional data will be collected to improve the protocols, if necessary, and to insure compatibility with the existing volunteer Marsh Monitoring Program (Weeber and Valliantos 2000, Meyer 2006) and with the standardized protocol prepared by US Fish and Wildlife Service for landbird monitoring (Knutson *et al.* 2008).

The index of biotic integrity developed and used by GLCWC (2008; Crewe and Timmermans 2005) will be compared to the probability-based indicator of ecological condition developed by the GLEI project (Howe *et al.* 2007). Both methods are compatible with the field data collection methods described below. Indicators should be easy to understand and calculate, and effective at describing the ecological health of Great Lakes coastal wetlands.

We will sample breeding anuran and bird populations using the GLCWC protocols (below) in approximately 200 wetland sites per year over a five-year period. Data collection will be coordinated as much as possible with the annual volunteer Marsh Monitoring Program. We also will critically examine a subset of the Great Lakes wetland sites (approximately 50) to potentially improve the biological, logistical, statistical, and monetary efficiency of the GLCWC monitoring protocols. Results from these surveys, coupled with results of the Marsh Monitoring Program and other studies, will document a comprehensive baseline condition and will provide an assessment of short-term trends and inter-annual variation of bird and anuran communities in Great Lakes coastal wetlands.

BF2. Sampling Methods

Anurans

For anurans, unlimited-distance point counts will be used to identify presence and calling intensity of species within each wetland. During a 3-minute sampling period all anuran (frog and toad) vocalizations will be recorded as occurring within or outside a circular sample area

centered on the prescribed sampling point (Weeber and Valliantos 2000, GLCWC 2008). Spatially-explicit records will be mapped on a standard data form in order to differentiate the direction of calls from the observer. Field samples for each wetland site will consist of 1 – 6 survey points spaced systematically at least 500 m apart in or around the wetland, with the number of points based on wetland size. Point-count samples within each site will be located according to the following criteria: 1) points that have been previously sampled within the wetland will be selected assuming the locations are consistent with other considerations below, 2) points will be located near the most convenient access point(s) to the wetland (in order to encourage long-term monitoring), and 3) to the extent possible, points will be placed in different patches of wetland habitat. Sites will be visited up to three times per breeding season during peak vocalization periods, with a minimum of 15 days between visits (unless inclement weather or other unforeseen circumstances intervene). Surveys will be conducted from one-half hr after sunset to four and one-half hrs after sunset and only during acceptable weather conditions (GLCWC 2008). Dates of sampling will be dependent on phenology and general weather conditions. In southern regions, anuran counts can generally be initiated in early April, but will be later in the northern regions. Some of the survey sites will be sampled by observers with digital audio recorders over an extended point count duration to assess the accuracy of 3-minute point samples.

Calling intensity for each detected species individual or grouping will be recorded using a 3-level calling code index:

Code 1: Calling individuals can be counted and calls are not simultaneous. Exact counts of the number of calling individuals are made in this case.
Code 2: Calls of individuals can be distinguished but some calling is simultaneous. Estimated counts of the number of calling individuals are made in this case.
Code 3: A full calling chorus with calls continuous and overlapping. Reliable counts and even estimates are unrealistic at this level of calling intensity and no counts are required.

At each station, surveyors will wait quietly for at least one minute to allow anurans to start calling again after potentially being disturbed by the surveyor's presence. After this initial settle-down period, a timer will be set and the surveyor will sample for three minutes. Records will be made of all species heard calling at an unlimited distance in all directions around the surveyor. Using the appropriate four-letter species code, the relative position of each individual or chorus (codes 1 and 2) will be recorded (mapped) on the standard data form. For codes 1 and 2, the number of individuals heard will be estimated. Abundance does not need to be recorded for code 3 since there are too many individuals calling to accurately estimate numbers for code 3.

Data will be entered into the project database after appropriate verification and QA/QC checks. Indicator evaluation, refinement and further development will be conducted on the verified database.

Birds:
For birds, we will use fixed-distance and (simultaneously) unlimited-distance counts at points located at least 250 m apart within each wetland habitat, with the number of points dependent on wetland size. Bird points to be sampled will be the same points used for anurans; however, additional points (i.e., 250 m apart) may be included depending on the configuration and size of the wetland. To get a good representation of the bird community, both shoreline (i.e., approximate upland/wetland interface) and wetland interior stations will be sampled. Sample locations will be dependent on site accessibility. All species will be recorded as within or outside of a 100-m radius circular sample area centered on the sample point. Presence of a pre-designated set of "focal" species (GLCWC 2008) will be recorded within 1-min sub-intervals across the 10-min survey. Focal species are those on which GLCWC indicators are based (Tozer et al. 2017). Non-focal species are recorded to the minute interval of first detection only and are not tracked across sub-intervals of time. Known or suspected cases of double-counting individuals beyond 100 m between adjacent points will be explicitly noted on the survey form.

Note that in 2019 the duration of the point count was reduced from a 15-min count to a 10-min count. This decision was based on the following rationale. 1) A peer-reviewed paper by Tozer *et al.* (2017) that analyzed 23,973 15-min point counts gathered from 2008 to 2016 many of which were gathered during previous years of this study. The study compared 10-min to 15-min counts and concluded, depending on the species, that 10-min counts were superior to 15-min counts because modest gains in some species did not warrant the additional effort. 2) Reducing the time to 10-min will allow an increase in the number of points that could be sampled in a season (on average one additional point per day per survey crew) or potentially reduce the cost to gather the same number of samples in a season. 3) The 10-min count is more consistent with national protocols for bird counts (Conway 2011, Matsuoka *et al*. 2014). And 4) The 10-min point count will be compatible with the Great Lakes Marsh Monitoring Program, which is also switching to 10-min counts.

Point count surveys will be conducted either from one-half hr before sunrise to 4 hr after sunrise or 4 hr before sunset. The number of birds seen or heard will be recorded during 10-minute observation periods (5 min of passive observation followed by 5 min of broadcast calling and passive observation) at each point count station (GLCWC 2008). Wetlands will be surveyed twice per breeding season, with a minimum of 15 days between visits, unless unforeseen circumstances prohibit return visits. One count will be in the morning and one count in the evening. We are interested in understanding how the number of samples within a wetland, number of visits to a wetland, detectability of birds, utility of audio playbacks, and distance detection. Influences metric calculations.

Bird and anuran data are considered highly desirable at wetland sites that are also sampled for invertebrates, fish, and vegetation. Ideally, data will be collected for each of these groups in at

least 75% of wetlands surveyed. However, wetland sites that bird and anuran crews cannot access will not be dropped from the database for incompleteness.

Bird and anuran crews will need little in the way of support facilities beyond that provided by the NRRI GIS laboratory that will assist them in verifying the accuracy of their GIS equipment, assisting with site selection, and providing site maps. Audio playback units will be standardized by quality of equipment used and checked with decibel meters to insure proper audio levels. Field survey data sheets will include a check that these audio levels are adequate each day. The individuals gathering data on birds and frogs will double-enter all data. LimnoTech will provide the necessary support on data management.

BF3. Sample Handling and Custody

Sampling of all bird and anuran populations is completed without handling any biota; it is instead completely based on visual observations or aural detections. Therefore, there is no protocol for handling organisms or a chain of custody for samples. However, data sheets will be carefully maintained by field crews, scanned and/or photocopied at the end of each field trip to provide back-up copies, and archived at the regional institution for each field team after the data have been entered into the electronic database. QA/QC recordings made in the field will have audio labels at the start and end of each recording.

BF4. Analytical Methods Requirements

Performance criteria: All bird and anuran data will be recorded at the species level. All staff for this group will be certified to be able to identify birds and anurans to species. Identification of rare species such as black rail, king rail, or other rare species should include: 1) when possible, a recording or photo of the species identified, 2) detailed notes of the observation, 3) verification by another individual, presumably one familiar with the species, or 4) with follow-up visits to the site. Rare-species observations will be reviewed by the regional team leaders and a determination of acceptance completed within two months after the end of sampling.

All sampling points will be saved using recreational GPS, which is sufficient for this project. In case of GPS or recording/playback equipment failure, the crew will notify their regional laboratory of the need for a replacement unit. Crews will note any equipment failures on their field sheets and in the database when they input data.

BF5. Quality Control Requirements

Precision: Precision here refers to how similar duplicates or splits are to themselves and is calculated as a % difference: % difference = (A-B)/((A+B)/2) * 100. The sampling of several points per wetland will provide the precision estimate for anuran and bird surveys.

Accuracy: The systematic difference from a reference standard or an expert. This will be during the mid-year QC check (see below, this section) and by having recorded calls at the QC sites verified by experts for 2 sites/yr in each regional area.

Bias: Systematic bias by a crew or method. Bias will be assessed during the mid-year QC check by observing sample point location at sites and playback call solicitation. The methods used here have known bias against cryptic/secretive species, which will cause systematic differences with other methods which do not have this bias. Detectability analysis has the ability to assess the severity of bias for common vs. secretive species.

Completeness: Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all sites.

Representativeness: How well sites were sampled will be determined by plotting all sample points for each site on aerial photographs of the site and by checking the field sheets and database for problem notes and flags. This will be done for all sites.

Comparability: Data comparability among crews within the project and to other non-project data will be achieved by using standard, accepted methods; creating metadata explaining the methods; and having strict training and QA/QC for all crews and personnel.

Sensitivity: In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification will depend on the ability to see/hear species, which is partially weather and surrounding-condition dependent. Crews will note on sample sheets conditions that limited their ability to hear taxa at sites (e.g., windy, road noise, etc). In general, all species of birds and anurans can be identified to species. In some cases, it may be difficult to differentiate American coot from common moorhen. In these cases, the generic code (MOOT) will be used to record the detections. Very uncommon taxa may not be detectable because the entire site cannot be sampled; however, at most sites most calling taxa should be detectable by this method. The exceptions are secretive species (see bias).

QA/QC specifics: Because this investigation encompasses such a large area, unavoidable sources of variation are inherent in any sampling plan; however, our sampling methodology attempts to minimize this variation (e.g., sampling during optimal time-of-day and weather conditions). An objective online testing system is already in place (http://www.birdercertification.org) and will be modified to insure that target species are included in the visual and audio tests. All field researchers will be required to demonstrate proficiency in visual and audio identification of wetland birds and anurans to insure quality control. Field researchers will be required to demonstrate their knowledge of the survey protocols prior to field activities (see section A8). The project team includes biologists who have been instrumental in developing bird and anuran monitoring protocols and who have many years of experience in training field workers. Field tools (portable audio players with bird vocalizations, which, when broadcasted into the marsh, elicit calls from secretive species) will be provided to field teams, and audio recordings will be made at selected sites to test for accuracy of the field samples. Use of GIS data sources also will provide precise locality

information. These aspects of the results (species identifications and locality data) will be the major targets of data quality control, plus appropriate attention to weather conditions and phenological timing of optimum counting periods.

The PIs will coordinate data validation among the different components of the study. Quality control of point data will include inspection of results in the context of site history, extent of available habitat (as determined from satellite images or other sources), knowledge of the identification skills of contributors, and information about surveys (e.g., survey time, weather conditions, and unusual abundance values). Records with the following attributes will demand explicit documentation or follow-up field investigation: 1) rare species recorded during only one year and not during subsequent years, 2) rare species observed by a field worker but not known to occur in specific sites by the principal investigators, and 3) rare species recorded in areas where little or no appropriate breeding habitat is present. A list of rare species that require further documentation for the region will be provided to each field team.

Individuals Responsible for Bird/Anuran Sampling:

Western Great Lakes	Gerald Niemi/Bob Howe
Central Great Lakes (US side)	Tom Gehring/Bob Howe
Central Great Lakes (CA side)	Doug Tozer
Eastern Great Lakes (US side)	Doug Wilcox/Chris Norment
Eastern Great Lakes (CA side)	Doug Tozer

Mid-Year QA/QC Checks:

Anurans and Birds— Bird and anuran PIs will evaluate accuracy and correctness of sampling crews at field sites or by analysis of audio recordings collected from the field, with the project QA manager and assistant manager providing oversight on the results of these checks.

Performance criteria will include:
- Correct location of sampling points (95%)
- Accuracy of species-level IDs (98%)
- Accuracy of abundance category estimates (90%)
- Correct criteria and techniques for the identification of rare species (100%)
- Correct use of field survey forms (100%)

Failure to meet performance criteria will trigger corrective action (see section C1).

BF6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

The most important field equipment used by researchers in this project will be hand-held recreational-grade geo-positioning systems (GPS), various playback recorders (e.g. MP3 players, CD players and speakers), binoculars, spotting scopes, and digital audio recorders (e.g. Sony PCM-D50 digital recorders). All equipment will be tested prior to use to insure the proper functioning of the equipment. For example, multiple tests of GPS waypoints at various locations

will ensure adequate functioning of the GPS receivers. The database of GPS readings at this standardized point will permit us to quantify the expected error in locality of data collected during the study. Spare batteries for all units will be carried by all crews, along with spare memory chips for the recorders. It is unlikely that any of this equipment can be repaired by field crews in the event of breakage; spare equipment will be available from the nearest regional team laboratory.

BF7. Instrument Calibration and Frequency
Recreational GPS accuracy is sufficient for these data. As described above, GPS receivers will be tested prior to and after the field season by taking repeated readings at known localities, i.e., benchmarks. During the field season, field crews will upload GPS readings nearly daily. In order to verify that recorded points have acceptable accuracy, GIS personnel will plot GPS points onto aerial photographs of a sampled site and send the image to the field crew, or field crews will plot and evaluate points on digital base maps (e.g., ArcGIS layers, Google Earth) that are available in the field. Whenever necessary, new site coordinates will be determined to accurately reflect sampling localities. Accuracy during the field season will also be checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. Broadcast equipment will be tested to ensure they emit species calls at the minimum required volume (80 dB at 1 m in front of the speakers). All of these accuracy checks and tests will be logged on field sheets at the time of sampling.

BF8. Inspection/Acceptance Requirements for Supplies and Consumables
N/A

B9. Data Acquisition Requirements (non-direct measurements)

The only non-direct measurement data we plan to use are the data used to create the site selection system. Wetland site selection will be based on the Albert and Simonson (2004) Great Lakes coastal wetland GIS coverage. Use of this database for site selection was mandated in the RFP and by the necessity of following the GLCWC sampling protocols. Other data used for site selection will include wetland type (Albert *et al.* 2006), the GLEI stressor gradient (Danz *et al.* 2005), and GIS background layers (roads, boat launches, land use, etc.) from state and federal agencies. These datasets will be used for logistical and landscape analysis purposes. Most of these data have already been published and peer-reviewed. Non-peer-reviewed information, such as the aerial photos, will be visually checked for correct site location by comparison with the appropriate quad map or Google maps. These photographs will be used as supplemental guidance for field crews, rather than to actually generate data. All of the data types mentioned above are in the public domain and are not subject to use restrictions.

Sources for external datasets of all types will be tracked. Most external data will be from peer-reviewed publications and GIS sources with established metadata lineages – these lineages will be maintained. In rare cases in which unpublished data is used, appropriate metadata will be

generated to describe its origin. Any non-peer-reviewed information, such as the aerial photos, used during the project will be visually checked for accuracy and proper identification.

B10. Data Management

In the field, data will be recorded on water-proof paper (except bird and anuran crews, who do not work in the rain or the water and can use regular paper), using pencils, water-proof pens, or permanent markers. Site datasheets for all field crews will contain the following basic information: site code, date, start and end time, weather and air temperature, regional team code, all names of sampling crewmembers, and a signature of the person making the entries. Whenever a sample is collected, a detailed description of the location will be recorded. This will include GPS coordinates and waypoint number, sampling equipment used, date, and water depth. Any visual observations will be logged along with sample volumes, number of containers, and sample identification number for all samples collected. Audio recordings will have an audio tag with the same information and the existence of these recordings will be noted on field data sheets. Any field duplicates will receive an entirely separate identification number. Unknown fish and plants will be labeled with a collection number, which will also be included on the sampling form. All completed data sheets will be kept in a secure location by field crews and photocopied at the earliest opportunity. Photocopies will be archived in a separate location from the original data sheets.

A secure, password-protected online data management system (DMS) has been developed by LimnoTech Inc. specifically for this project. The system includes data entry forms designed around the layout of field data sheets, which allows field crews to enter data quickly and efficiently, reducing entry errors. Another feature of the data entry system is the use of drop-down menus, which greatly reduces the frequency of data entry errors, particularly for scientific names of taxa. Field crews will double-check 100% of the entered data against the field sheets, switching personnel so that a different person checks the data than the person who completed data entry. The sample-type-specific data structures and site-level metadata defined in the GLCWC Monitoring Plan (2008) as well as the previous NRRI-developed data management system were used as a basis for developing this new system.

The DMS also handles the metadata that accompanies the data (e.g., methods, study design, field data error codes, description of indicator calculations, etc.), which will help ensure the system's usefulness to future researchers, managers, and the public. To accommodate requests for raw data, the DMS can export the data as 'flat files' that can be imported into standard statistical, spreadsheet, and database programs for analysis. These exported files will give the dataset an indefinite digital shelf life without any dependency on the infrastructure used for this particular project.

The DMS uses the PostgreSQL Relational Database Management System (RDMS) with PostGIS spatial extensions. We use continuous (WAL) archiving on the live database with forced

snapshots every three hours between 6 am and 6 pm. The server that houses the DMS has been configured to use CMU's Veeam Backup Solution. This backup solution provides end-to-end encryption including data at rest. Incremental backups will be performed nightly and stored at secure locations (on premise and offsite). Nightly backup email reports will be generated and sent to appropriate CMU IT staff for monitoring purposes. Incremental backups are kept indefinitely and restores can be performed for whole systems, volumes, folders and individual files upon request.

Specifics:
Hardware systems are typically Intel or AMD multi-core processors running at 2.4+ GHz with 0.25+ TB of local storage. Internal networking is mixed 100/1000 Mbps and 10 Gbps Ethernet, with two 10 Gbps Internet connections.

The interface to the data will be through a Microsoft Active Server Pages .NET (ASP.NET) server system which provides typical user and group level authentication and access control. For example, field crews may have access to enter data, but adding new species to drop-down taxa lists may require membership in an 'administrator' group to ensure consistent methodology.

C. ASSESSMENT AND OVERSIGHT

C1. Response/Corrective Actions

Real-Time Remediation of Problems:
A system of immediately reporting any field or laboratory failures, mistakes in sampling protocols, etc., will be instituted. Corrective actions will include replacing lost data if possible and omitting any suspect data that cannot be replaced by taking additional samples. The monthly conference calls for all PIs will be used in part to discuss and deal with any problems that arise in training, site selection, sampling, sample processing, data entry, or QA/QC. All co-PIs will be advised to contact the management team (Uzarski, Brady, Cooper) immediately if major problems arise that require immediate attention. At least one person on the management team will be available at all times when sampling crews are in the field to help deal with questions and problems as they arise. Our experience with past projects suggests that an environment of open communication greatly reduces the need for corrective actions later. Cell phone availability by the management team also helps provide a second layer of safety for field crews.

Field crew chiefs will be responsible for assessing sampling activities and water quality meter readings, and noting, and when possible, correcting any problems, errors, or values that seem unlikely. Field crew chiefs will receive assistance on problem-solving and error-correction via cell phone support from their regional team leader or a management team member.

Failure to meet mid-year QA/QC objectives will trigger corrective actions. In most cases, mid-year QA/QC checks are conducted at the laboratory or institution-level even though training and certification (section A8) takes place at the individual level. Table C1.1 lists corrective actions to be taken when QA/QC performance criteria are not met.

Table C1.1. Quality assurance/quality control performance standards and corrective actions when performance standards are not met during mid-year evaluations.

Protocol	Performance standard	Corrective actions
Site Selection	100% of sites that are rejected by regional teams meet appropriate rejection criteria	Additional training/re-certification of regional team leader (co-PI) and field crew chiefs Sample sites that were erroneously rejected
Water Quality	Appropriate preparation of 100% of sample containers	Additional training/re-certification of regional team leader (co-PI) and field crew chiefs If contamination confirmed/suspected, data will be quarantined from analyses.
	Appropriate maintenance and calibration of water quality meters	Additional training/re-certification of field crew If meter errors confirmed/suspected, data will be quarantined from analyses.
	Appropriate documentation of meter maintenance and calibration	Additional training/re-certification of field crew members Verification of meter performance
	Appropriate labeling and record keeping for data and samples	Additional training/re-certification of field crew Correction of record-keeping and labeling errors when possible. Quarantine of samples that have irreconcilable labeling errors. Flagging of record errors that cannot be corrected.
Macro-phytes	Total percent cover estimates within 10% of expert's estimates	Additional training/re-certification of vegetation crew members Correction of coverage estimates on data sheets for affected data.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
	90% accuracy of species-level identification by crews using field guides/ microscope.	Additional training/re-certification of vegetation crew members
		Correction of mis-identified plants on datasheets and in database
	100% accuracy completing field data sheets	Correction of field data sheets and training of crew members.
Inverte-brates	D-net sweep sampling methods are performed correctly	Additional training/re-certification of relevant field crew members
		Field forms flagged for inappropriately-sampled zones or sites
	Habitats are correctly and thoroughly sampled	Additional training/re-certification of relevant field crew members
		Field forms flagged for under- or over-sampled sites
	Samples are picked with few invertebrates and mobile taxa missed.	Additional training/re-certification of relevant field crew members
		Field forms flagged for under-picked samples, and those under-represented for mobile taxa
	95% of taxa are correctly identified	Additional training of macroinvertebrate laboratory staff
		Re-identification of taxa in question; correction of laboratory forms and database
	100% of samples are properly preserved and labeled	Additional training/re-certification of field crew chiefs and crew members
		Immediate re-preservation of all affected samples. Corrections on labeling wherever possible; flags on all samples where labeling cannot be corrected.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
Fish	Plant zones are correctly identified for setting fyke nets	Additional training/re-certification of field crew chiefs
		Consult with management team about whether or not error is serious enough to trigger re-sampling of sites
		Flag datasheets for sites where net setting errors may have occurred but sites will not be re-sampled.
	Fyke nets are set correctly	Additional training/re-certification of field crew chiefs
		Consult with management team about whether or not error is serious enough to trigger re-sampling of sites
		Flag datasheets for sites where net setting errors may have occurred but sites will not be re-sampled
	95% of species are correctly identified in the field/laboratory	Additional training/re-certification of field crew members
		Correct errors on field sheets and in database
	100% of fish are handled appropriately	Additional training/re-certification of field crew members
	Unidentifiable and difficult specimens are retained for laboratory ID 100% of the time	Additional training/re-certification of field crew members
		Flag questionable identifications on data sheets and in database
Birds & anurans	95% of sampling locations are located correctly	Additional training/re-certification of field crew members
		Data sheets are flagged so GPS sample locations can be checked
	98% of calls are accurately identified	Additional training/re-certification of field crew members
		Data sheets are corrected, if possible, or flagged.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
	90% of abundance category estimates agree with those of experts	Additional training/re-certification of field crew members Data sheets are corrected, if possible, or flagged
	100% of rare species identification attempts are done correctly	Additional training/re-certification of field crew members Data sheets are corrected, if possible, or flagged
	100% of field survey forms are completed correctly	Additional training/re-certification of field crew members

Any corrective actions will be noted and included in our semi-annual reports to GLNPO.

C2. Reports to Management

PI conference calls will cover the following general topics: overall project status, field crew and field work status, lab work status, data entry status, recent problems and solutions, QA/QC reports and analysis, and other topics as needed. These calls will keep co-PIs working in close collaboration and solving any problems as they become known. Notes from all conference calls and meetings will be distributed to all co-PIs and team leaders within two weeks.

Regional team leaders, co-PIs, and resident experts conducting mid-season QC checks on field crews will write up the results of these QC checks and provide them to their regional team leader, the QA managers, the project leader (Uzarski), and team/crew members who were reviewed. Regional team leaders will be responsible for ensuring that these reports are provided on time, and the QA managers will be responsible for notifying regional team leaders in the event that reports are overdue.

The semi-annual report to EPA will be written by taxonomic leaders in collaboration with their co-PIs (Uzarski, Brady, and Cooper for fish and invertebrates, Niemi and Howe for birds and anurans, Albert, Danz and Wilcox for vegetation; Axler will assist with supporting water quality data). The report will be compiled and edited by the management team, and will be submitted by the project leader (Uzarski). The report will include a project status update, a discussion of QC problems and their solutions and corrective actions, results of QC checks, results of QC audits (written by the QA managers), and a summary of results to date. Reports will be distributed to everyone involved with the project. Serious issues that have a major impact on the project will be reported to the EPA project officer immediately. The project officer will also

be invited to all annual PI meetings to help ensure close communication with EPA. Numerous additional meetings and conference calls will take place on an *ad hoc* basis during this project.

QC audits will take place at the end of each field season for field data collection activities. The field season audit will cover completeness of sampling activities at each site for all project elements. Sites with incomplete data will be flagged and reported to all PIs. Other elements of the field season audit include sampling gear status, data flags on data sheets and how these are being investigated, completeness and location of all samples awaiting processing (and checking of expiration dates on samples), and summarization of field crew leader debriefings. Reports will be written by the QA manager and assistant manager and will be provided within 30 days to all co-PIs and will be included in the winter semi-annual report.

QC audits on data entry and data management will occur in the early spring after most data have been processed over the winter and entered into the database system. Data audits will assess completeness of checks for data entry errors; investigations into data 'flags' in the database, how these are being resolved, and which data are too problematic to be trusted; 'mapping' of all GPS points with double-checks on location validity by field crew chiefs; and audits of how biotic identifications are being verified. Reports on these audits will be written by the QA manager and assistant manager and provided within 30 days to all co-PIs and included in the summer semi-annual report.

A summarization of the results will be developed by collaboration among all investigators involved with this monitoring proposal in conjunction with the US EPA project officer. A final report from this work will be written in a similar fashion as the semi-annual reports (see above, this section) and submitted to GLNPO in MS Word format or other suitable format at the end of the proposed project period between September 2015 and November 2015. This report will include a compilation of scientific manuscripts, each following the standard format of published scientific articles: 1) introduction, 2) description of methods and study area, 3) presentation of results, 4) discussion of the relevance of findings, and 5) a bibliography of pertinent literature. A detailed account of field results, additional descriptions of statistical methods, and results of QC audits will be provided as appendices. The report will be distributed to everyone who is part of this project and will be made publicly available after review and approval by GLNPO.

D. DATA VALIDATION AND USABILITY

D1. Data Review, Validation, and Verification Requirements

Quantitative data: After data are entered into the data management system, the data will be checked for out-of-range values. In other words, the data will be checked for values that are unlikely to occur and thus may be the result of some sort of error (e.g., very high or low pH or dissolved oxygen values, etc). Additionally, the highest and lowest values in all datasets will be double-checked for data entry errors. Checks will consist of reviewing original field data sheets for data entry errors or notes that may provide evidence of a cause for a suspicious value (e.g., 'DO meter would not calibrate correctly'). Checks will also confirm that appropriate instrument calibration and maintenance were being done by field crews. Impossible values that remain (e.g., pH > 14) will be deleted. Unlikely values will be flagged and notes included on the data checks that have been done. These data flags will allow PIs to check these data values during statistical analyses to determine whether or not they are true statistical outliers and to investigate how much they are influencing statistical results.

Qualitative data: Review of the data will be the responsibility of the principal investigators, who will devote at least a portion of the post-field season meetings and conference calls to this subject. Information from existing data sources as well as experience of the principal investigators will help identify data records that need to be validated. In many cases, the taxonomic identification errors or transient bird taxa can be detected by evaluating results from multiple surveys at the same site or from knowledge of the natural history of the taxa. Background knowledge about the taxa will play an important role in data review, although unexpected but potentially significant observations will not be dismissed without follow-up information. The lead PIs for each taxonomic group will check the taxa lists for uncommon taxa and those that are rarely found in the Great Lakes region. These identifications will be verified, if possible, or flagged as suspicious, if verification is not possible.

Indicator calculations: Metrics will be calculated for each taxonomic group according to GLCWC formulae which are detailed below.

Vegetation:

1. Compute overall INVASIVE COVER for the **entire site** by summing the coverage values for all invasive plants and dividing by the number of quadrats. This is the INVASIVE COVER score for the entire site (Table D1.1).

2. Compute overall INVASIVE FREQUENCY for the **entire site** by summing the number of quadrats containing invasive species and dividing by the total number of quadrats (Table D1.1).

3. Compute the MEAN CONSERVATISM INDEX for the **entire site** by totaling the conservatism score for each species and dividing by the number of species. This can be rapidly computed

using the Michigan FQA software (Herman *et al.* 2001). The mean conservatism index for all species at a site (total) is then divided by the mean conservatism index for native species (native) and this ratio is used in the macrophyte IBI (Table D1.1). Low scores (0.79 or lower) reflect large numbers of exotic species and degraded conditions.

4. Compute overall INVASIVE COVER for the **wet meadow and dry emergent zone** by summing the cover values for all INVASIVE plants in these zones and dividing by the number of quadrats in these zones. This is the INVASIVE COVER score for the wet meadow and dry emergent zone and can be used to estimate the zone quality (Table D1.1).

5. Compute overall INVASIVE FREQUENCY for the **wet meadow and dry emergent zone** by summing the number of quadrats (in these zones) containing INVASIVE species and dividing by the total number of quadrats in the wet meadow and dry emergent zones (Table D1.1).

6. Compute the MEAN CONSERVATISM INDEX for the **wet meadow and dry emergent zone** by totaling the conservatism score for each species in these zones and dividing by the number of species. This can be rapidly computed using the Michigan FQA software (Herman *et al.* 2001). The mean conservatism index for all species (total) in the **wet meadow and dry emergent zone** is divided by the mean conservatism index for native species (native) and this ratio is used in the macrophyte IBI (Table D1.1).

7. Compute overall INVASIVE COVER for the **flooded emergent and submergent zone** by summing the cover values for all invasive plants in these zones and dividing by the number of quadrats in these zones. This is the INVASIVE COVER score for the **flooded emergent and submergent zone** and can be used to estimate the zone quality (Table D1.1).

8. Compute overall INVASIVE FREQUENCY for the **flooded emergent and submergent zone** by dividing the number of quadrats (in these zones) containing invasive species and dividing by the total number of quadrats in the **flooded emergent and submergent zone** (Table D1.1).

9. Compute the MEAN CONSERVATISM INDEX for the **flooded emergent and submergent zone** by totaling the conservatism score for each species in these zones and dividing by the number of species. This can be rapidly computed using the Michigan FQA software (Herman *et al.* 2001). The mean conservatism index for all species (total) in the **flooded emergent and submergent zone** is divided by the conservatism index for native species (native) and the ratio is used in the macrophyte IBI (Table D1.1).

Table D1.1. Macrophyte metrics calculated from quadrat data. Scores will be summed to get an overall IBI score for the site.

	Wetland Quality			
Metric	**HIGH (5)**	**MEDIUM (3)**	**LOW (1)**	**VERY LOW (0)**
A: INVASIVE COVER (entire site)	Absent	<25 %	25-50%	>50%
B: INVASIVE FREQ. (entire site)	Absent	<25 %	25-50%	>50%
C: Mean conservatism of entire site (native/total)	>0.95	0.8 -0.94	0.6-0.79	< 0.6
D: INVASIVE COVER (wet meadow and dry emergent zones)	Absent	<25 %	25-50%	>50%
E: INVASIVE FREQ. (wet meadow and dry emergent zones)	Absent	<25 %	25-50%	>50%
F: Mean conservatism score of wet meadow and dry portion of emergent zones (native/total)	>0.95	0.8 -0.94	0.6-0.79	< 0.6
G: INVASIVE COVER (flooded emergent and submergent zone)	Absent	<25 %	25-50%	>50%
H: INVASIVE FREQUENCY (flooded emergent and submergent zone)	Absent	<25 %	25-50%	>50%
I: Mean conservatism of flooded emergent and submergent zones (native/total)	>0.95	0.8 -0.94	0.6-0.79	< 0.6

Macroinvertebrates:

IBI scores will be calculated according to the metrics below. These metrics have been tested extensively for bulrush-dominated fringing wetlands, and will be validated and modified for other plant zones and wetland types as the project progresses.

Table D1.2. Macroinvertebrate IBI for the wet meadow zone: Dominated by *Carex* and *Calamagrostis*

Metric	Score=1	Score=3	Score=5
Odonata richness (genera)	0	>0 to 3	>3
Relative abundance Odonata (%)	0 to <1	>1 to 5	>5
Crustacea plus Mollusca richness (genera)	<2	2 to 6	>6
Total richness (genera)	<10	10 to 18	>18
Relative abundance Gastropoda (%)	0 to 1	>1 to 25	>25
Relative abundance Sphaeriidae (%)	0	>0 to 3	>3
Evenness	0 to 0.4	>0.4 to 0.7	>0.7
Shannon diversity index	0 to 0.4	>0.4 to 0.9	>0.9
Simpson index	>0.3	>0.15 to 0.3	0 to 0.15

Table D1.3. Macroinvertebrate IBI for the *Schoenoplectus* plant morphotype: Often dense *Schoenoplectus* mixed with *Pontedaria* and submergents, protected from wave action.

Metric	Score=0	Score=1	Score=3	Score=5	Score=7
Odonata richness (genera)		0	>0 to 1	>1 to 2	>2
Relative abundance Odonata (%)		0	>0 to <2	2 to 7	>7
Crustacea + Mollusca richness (genera)		0 to 2	>2 to 4	>4 to 6	>6
Total richness (genera)		<10	10 to 14	>14 to 18	>18
Relative abundance Gastropoda (%)		0	>0 to 2	>2 to 4	>4
Relative abundance Sphaeriidae (%)		0	>0 to 0.05	>0.05	
Ephemeroptera + Trichoptera richness (genera)		0	>0 to 3	>3	
Relative abundance Crustacea + Mollusca (%)		<8	8 to 30	>30	
Relative abundance Isopoda (%)	0	>0 to 1	>1 to 10	>10 to 20	>20
Evenness		0 to 0.4	>0.4 to 0.7	>0.7	
Shannon diversity index		0 to 0.4	>0.4 to 0.9	>0.9	
Simpson index		>0.3	>0.15 to 0.3	0 to 0.15	
Relative abundance Amphipoda (%):					

If 40 to 60 and total score from Inner *Schoenoplectus* zone is 41 or greater, subtract 5;

If 40 to 60 and total score from Inner *Schoenoplectus* zone is >41, then add 5.

Table D1.4. Macroinvertebrate IBI for the outer *Schoenoplectus* morphotype: Relatively sparse, usually monodominant stands, subject to direct wave action.

Metric	Score=0	Score=1	Score=3	Score=5	Score=7
Odonata richness (genera)		0	>0 to <1	1 to 2	>2
Relative abundance Odonata (%)		0	>0 to <1	1 to 2	>2
Crustacea + Mollusca richness (genera)		0 to 2	>2 to 4	>4 to 5	>5
Total richness (genera)		<8	8 to 13	>13 to 17	>17
Relative abundance Gastropoda (%)		0	>0 to 3	>3 to 5	>5
Relative abundance Sphaeriidae (%)		0	>0 to 0.05	>0.05	
Total number of families		0 to 7	>7 to 12	>12	
Relative abundance Crustacea + Mollusca (%)		<8	8 to 30	>30	
Evenness		0 to 0.4	>0.4 to 0.7	>0.7	
Shannon diversity index		0 to 0.4	>0.4 to 0.9	>0.9	
Simpson index		>0.3	>0.15 to 0.3	0 to 0.15	

Interpretation of macroinvertebrate IBI scores

All values should be based on the median of at least three replicates taken from each zone. When all vegetation zones are present, wetlands are scored as follows: A total score of 31 to 53 (0% to 15% of possible score) = “Extremely Degraded”, or “in comparison to other Great Lakes wetlands, this wetland is amongst the most impacted”; total score of >53 to 76 (>15% to 30% of possible score) = “Degraded” or “the wetland shows obvious signs of anthropogenic disturbance”; total score of >76 to 106 (>30% to 50% of possible score) = “Moderately Degraded” or “the wetland shows many obvious signs indicative of anthropogenic disturbance;” total score of >106 to 136 (>50% to 70% of possible score) = “Moderately Impacted” or “the wetland shows few, but obvious, signs of anthropogenic disturbance;” total score of >136 to 159 (>70% to 85% of possible score) = “Mildly Impacted” or “the wetland is beginning to show signs indicative of anthropogenic disturbance”; total score of > 159 to 182 (>85% to 100% of possible score) = “Reference Conditions” or “the wetland is among the most pristine.”

When only a subset of vegetation zones are present, category scores are adjusted as follows:

Wet Meadow only = 9 to 14; >14 to 19; >19 to 27; >27 to 34; >34 to 39; >39 to 45
Inner *Schoenoplectus* only = 11 to 19; >19 to 29; >29 to 41; >41 to 53; >53 to 62; >62 to 72
Outer *Schoenoplectus* only = 11 to 18; >18 to 26; >26 to 37; >37 to 48; >48 to 56; >56 to 65
Wet Meadow + Inner *Schoeno.*= 20 to 33; >33 to 47; >47 to 66; >66 to 84; >84 to 99; >99 to 113
Wet Meadow + Outer *Schoeno.*= 20 to 32; >32 to 46; >46 to 64; >64 to 82; >82 to 96; >96 to 110
Inner and Outer *Schoeno.*= 22 to 38; >38 to 55; >55 to 79; >79 to 102; >102 to 119; >119 to 137

Fish:
Final IBI metrics and scoring thresholds for the four vegetation zone types. Catch per unit effort (CPUE) was catch net^{-1} $night^{-1}$. Final zone scores are calculated by re-scaling the sum of all metrics for a vegetation zone to a 100-point scale. Specifics and trait information can be found in Cooper *et al.* 2018.

Table D1.5. Fish IBI calculations for several wetland vegetation morphotypes. See Cooper *et al.* 2018 for full details and trait information.

	Scoring		
Bulrush (*Schoenoplectus* spp.)	0	1	2
Evenness	0-0.4	>0.4-0.8	>0.8
Non-native species richness	≥2	1	0
Native Cyprinidae CPUE	0	>0-50	>50
Smallmouth bass CPUE	<2	2-5	>5
% Black+brown bullhead	0	>0-25	>25
Johnny darter CPUE	0	>0-0.34	>0.34
Common carp CPUE	>2	>0-2	0
% Carnivore (invertivore+piscivore+zooplanktivore)	>90	40-90	<40
% Richness of high and extra-high temperature spawners	100	>82-100	0-82
% Richness short-lived species	<20	20-60	>60
% Richness species particularly sensitive to environmental degradation	0	>0-15	>15
Final score for zone = (sum of metrics / 22) * 100			
Cattail (*Typha* spp.)			
% Richness native species	<60	60-<100	100
Non-native species richness	>2	1-2	0
% Native Cyprinidae	0-20	>20-50	>50
Rock bass CPUE	0	>0-3	>3
% Black+brown bullhead	0	>0-25	>25
% Richness benthic habitat species	>75	30-75	<30
% Richness nest spawners	0	>0-70	>70
% Richness of high and extra-high temperature spawners	100	60-<100	<60
% Richness large and extra-large species	>40	20-40	<20
% Richness species particularly sensitive to environmental degradation	0	>0-8	>8
Final score for zone = (sum of metrics / 20) * 100			

	Scoring		
	0	1	2
Water lily (*Nuphar advena* sp., *Nymphaea odorata* sp.)			
Evenness	<0.5	0.5-0.75	>0.75
Non-native species richness	>2	>0-2	0
Rock bass CPUE	<2	2-6	>6
Smallmouth bass CPUE	0	>0-3	>3
% Black+brown bullhead	<5	5-30	>30
Yellow perch CPUE	0	>0-10	>10
% Common carp	>3	>0-3	0
% Richness carnivore species (invertivore+piscivore+zooplanktivore)	<50	50-75	>75
% Richness vegetation spawners	<15	15-40	>40
% Richness species particularly sensitive to environmental degradation	0	>0-10	>10
Final score for zone = (sum of metrics / 20) * 100			
Submersed Aquatic Vegetation			
Evenness	<0.2	0.2-0.80	>0.80
Non-native species richness	>3	1-3	0
% Richness native species	<75	75-95	>95
% Native Cyprinidae	<20	20-60	>60
Johnny darter CPUE	0	>0-2	>2
Rock bass CPUE	0	>0-5	>5
% Common carp	>5	>0-5	0
% Richness carnivore species (invertivore+piscivore+zooplanktivore)	<50	50-80	>80
% Richness large and extra-large species	>40	>8-40	0-8
% Richness short-lived species	<20	20-70	>70
% Richness species particularly sensitive to environmental degradation	<5	5-20	>20
Final score for zone = (sum of metrics / 22) * 100			

Birds and Anurans:
Bird and anuran indicators are a matter of active refinement by researchers.

D2. Validation and Verification Methods

All field data entered into the data management system will be double-checked against the original field data sheets by a second person from the same field crew, or will have double-entry of data (e.g., bird and anuran data). We have found that both of these methods are effective at catching data entry errors.

Data that are flagged as suspicious will first be checked against field data sheets by the appropriate field crew chiefs; if the data are not typos, then they will be brought to the attention of the regional team leader or appropriate co-PI, who will apply the appropriate

cautionary code flag or remove the data from the database, depending on the data type and error (see D1).

Field data sheets will be photocopied or scanned to PDF files as soon as crews return from the field. Copies will be mailed or emailed to the Uzarski lab at Central Michigan University at the end of each field season for archiving. Original data sheets will be used for data entry and data verification in the data management system, and thus will remain in the custody of the regional team leader. After this is complete, the regional team leader will archive the original data sheets.

The data management system will be maintained by NRRI. Each year at the end of the field season the preliminary data will be provided to GLNPO. In addition, after QC is complete, that year's data will again be provided to GLNPO to replace the earlier, un-QC'd data. All flags on data that have not been resolved will remain in the DMS and be provided to GLNPO. The metadata accompanying the data in the DMS will explain the various types of flags. In most cases, notes within the DMS will explain the cause of flags in more detail. Data users will have to be pro-active in searching for flags, their cause, and explanation within datasets that they download for use.

D3. Reconciliation with Data Quality Objectives

The primary DQO for this study is accurate and representative measurements of the biological indicators for all major Great Lakes coastal wetland complexes, with supporting physical and chemical parameters, all collected in accordance with the GLCWC (2008) monitoring program. To evaluate how well we have met this DQO, we will assess how many of the major coastal wetland complexes we were able to sample, how representative that selection was based on our site selection process, and how well each wetland was sampled (see representativeness under each B5 section). Wetland sampling assessment will consist of a visual examination of sampling points for each wetland plotted on an aerial photo to determine whether all major habitats were sampled, and an examination of the field sheets for notes on unresolved sampling problems at any site. Problems we have previously encountered include fyke nets being washed ashore by storms, fyke net damage by muskrats and turtles, and sampler and meter malfunctions. If these issues cannot be resolved in the field, wetlands that have incomplete sampling for any parameter listed as critical will be considered for re-sampling. All such information will be included in the data management system and in reports.

A secondary DQO is providing the data, site indicators, and general information about the project to managers, management agencies, other researchers, and other interested parties. MDEQ will be assisting with this outreach part of the project. QA for this objective will consist of ensuring that MDEQ staff are included in PI meetings so that they understand the project and are up-to-date, reviewing the lists of contacts, etc, with MDEQ to ensure that major stakeholders are not missed, and reviewing all products produced by MDEQ to ensure accuracy.

Our intention is that only 100% accurate products created using QC'd data be available for use by non-project members.

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