Quality Assurance Project Plan

Continuation of the Great Lakes Coastal Wetland Monitoring Program (GLCWMP): 2021-2025

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A3. Distribution List

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A4. Program Organization

This monitoring program is 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 program team (Figure 1) consists of U.S. scientists from Central Michigan University (Donald Uzarski, principal investigator/lead grantee; Matt Cooper, assistant QA manager/program coordinator; Thomas Gehring, Dennis Albert), the Natural Resources Research Institute (NRRI) at the University of Minnesota Duluth (Valerie Brady, program coordinator and QA manager, regional team lead), the Annis Water Resources Institute (AWRI) at Grand Valley State University (Carl Ruetz), the University of Notre Dame (Gary Lamberti), Lake Superior State University (Ashley Moerke), State University of New York-Brockport (Kathryn Amatangelo, regional team lead); and the University of Wisconsin (Robert Howe at Green Bay, Nick Danz at Superior, and Joseph Gathman at River Falls), and two resource management officials: Anne Garwood (Michigan Department of Environment, Great Lakes, and Energy) and Kurt Kowalski (USGS). Canadian scientists from the University of Windsor (Jan Ciborowski, regional team lead), Environment and Climate Change Canada (Giuseppe Fiorino), and Birds Canada (Doug Tozer) are also key participants.

Program coordination and QA management are provided by Don Uzarski (CMU), Valerie Brady (NRRI), and Matthew Cooper (CMU). These individuals ensure both cost-effective sampling and program oversight, and are responsible for all reporting to EPA. They are also responsible for assuring that all teams and participants are properly trained in QA/QC methods and procedures, and assist with QC evaluations throughout the program. They schedule regular meetings (once per year face-to-face) and conference calls/webinars among co-PIs to ensure coordination and foster communication among teams. Regional team leaders 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 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.

Program Team Background and Qualifications
Dr. Don Uzarski (CMU) leads the entire GLCWMP effort. He was a member of the Project Management Team and Chair of the Science Committee that developed what has become the Great Lakes Coastal Wetland Consortium Monitoring Program (GLCWC 2008). As part of the Great Lakes Coastal Wetland Consortium, Dr. Uzarski's 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 GLCWC final report and training manuals. Dr. Uzarski has worked on Great Lakes coastal wetlands since 1997. He has published more than 75 papers on coastal wetlands in peer-reviewed journals, conference proceedings, and book chapters.

Dr. Valerie Brady (NRRI) was the project coordinator for both of the Great Lakes Environmental Indicators (GLEI) projects, which worked on development of condition indicators for Great Lakes coastal wetlands, bays, and high energy coastlines. In addition to coordinating the 28 Co-PIs for the GLEI I and II projects, she also assisted with the macroinvertebrate and fish sampling effort and worked on developing macroinvertebrate indicators of wetland condition. She helped organize GLEI project fish and invertebrate field crew training and oversaw QA/QC. 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 separate 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 Estuarine and Great Lakes (EaGLe) Coastal Indicators Initiative (funded by US EPA STAR), and has attended all of the QA/QC webinars offered 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) and Dr. Nick Danz (University of Wisconsin Superior) 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 the GLCWC effort. Dr. Danz has recently worked closely with Dr. Albert to refine coastal wetland macrophyte indicators.

Dr. Robert Howe (University of Wisconsin Green Bay) leads the bird and anuran sampling effort. Dr. Howe worked on the GLEI I and II projects and led the bird and anuran indicator development for those efforts. He has worked on bird monitoring for several decades and has published numerous peer-reviewed articles.

Dr. Jan Ciborowski (University of Windsor) is 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.

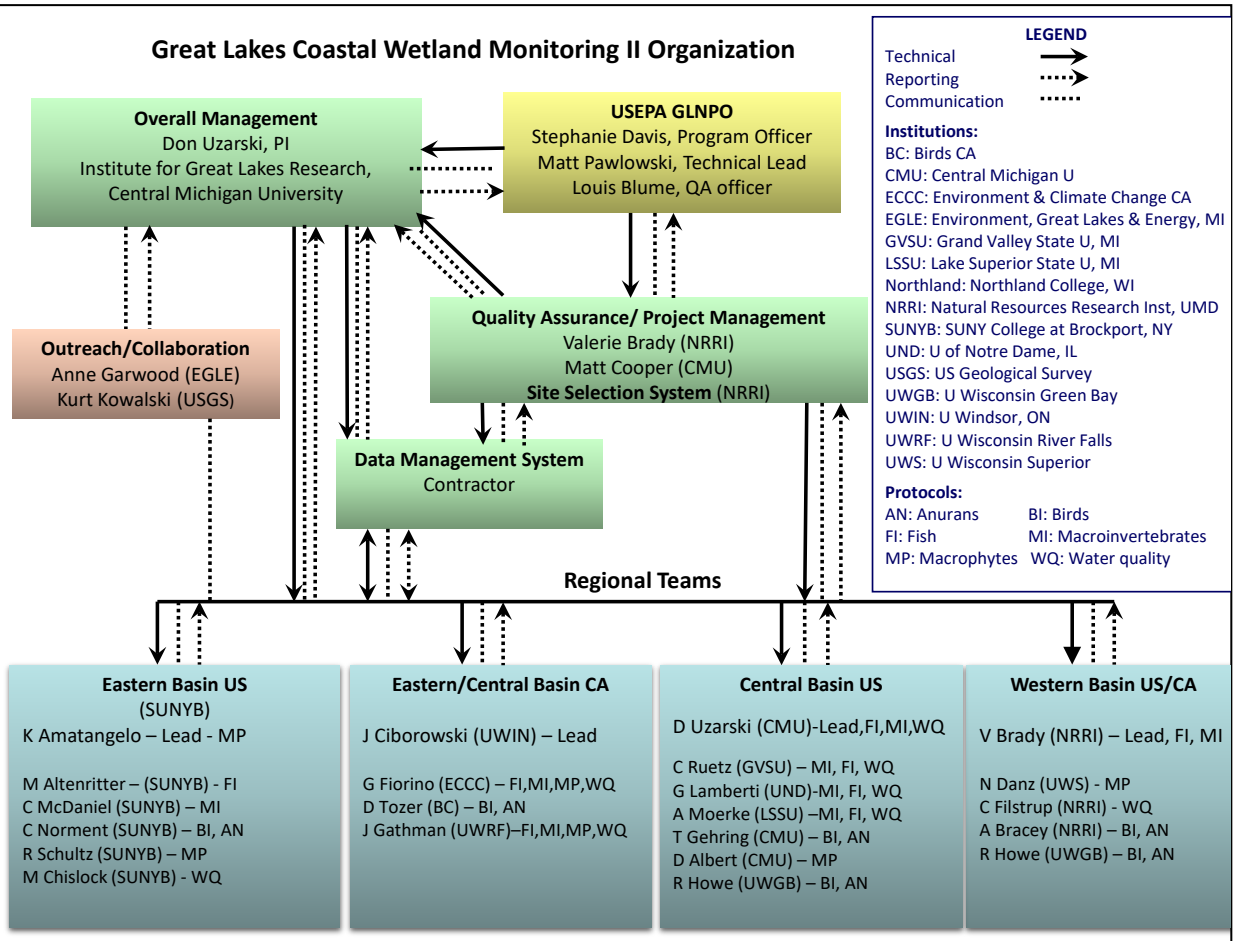

Figure 1. Organizational chart for the program showing lines of technical direction, reporting, and communication separately.

Roles and Responsibilities:

Dr. Don Uzarski (Central Michigan University):

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

Dr. Valerie Brady (Natural Resources Research Institute, University of Minnesota Duluth)

- Program coordinator assisting with all aspects of program management
- 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
- Partners with Dr. Nick Danz (UW Superior) for macrophyte sampling
- Is assisted by Dr. Chris Filstrup (NRRI) for water chemistry analyses
- Partners with Annie Bracey (NRRI) for bird and anuran sampling
- Works independently as QA manager to oversee QA/QC training and organize QA audits with the assistance of Dr. Matt Cooper (CMU)
- Oversees and manages the site selection system database
- Coordinates with the contractor to manage the data entry and data management system and program website

Dr. Robert Howe (University of Wisconsin Green Bay)

- Leads the bird and anuran sampling effort, coordinating with all regional team leaders and other bird and anuran specialists across the basin
- Coordinates with the bird and anuran leaders on other teams to organize training for bird and anuran field crews
- Reviews all bird and anuran QA/QC procedures

Dr. Dennis Albert (Central Michigan University)

- Leads the macrophyte sampling effort for the US Central Basin sampling team
- Coordinates with other macrophyte specialists on other teams to ensure proper training and QA/QC
- Trains other co-PIs on macrophyte metrics calculations

Dr. Kathryn Amatangelo (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
- Leads the macrophyte sampling effort for the Lake Ontario/eastern Lake Erie sampling team
- Is assisted by Greg Lawrence (birds, anurans, invertebrates), Dr. Matt Altenritter (fish), Dr. Michael Chislock (water quality), and Dr. Rachel Schultz (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
- Is assisted by Dr. Joseph Gathman (University of Wisconsin River Falls)

- Collaborates with Giuseppe Fiorino and Ian Smith (Environment and Climate Change Canada) and Dr. Doug Tozer (Birds Canada)

Dr. Matthew Cooper (Central Michigan University)

- Assists in overall program management as part of the leadership team (with Uzarski and Brady)
- Assists Dr. Valerie Brady with QA/QC oversight and management
- Helps organize field crew training for all fish and macroinvertebrate field crews

Dr. Carl Ruetz (Grand Valley State University)

- Responsible for sampling of eastern Lake Michigan wetlands
- Serves as lead fisheries expert for the program

Dr. Kurt Kowalski (US Geological Survey)

- Will coordinate with CWMP crews for collaborative sampling at selected sites
- Will serve as liaison between CWMP and USGS

Anne Garwood (M-EGLE)

- Organizes outreach and public information about the CWMP effort
- Organizes informational meetings about the program 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). Prior to the start of the Great Lakes Coastal Wetland Monitoring project in 2010 there was no routine monitoring program to determine the status and trends of Great Lakes coastal wetland condition at a basin scale. GLCWMP was begun as a project in 2010 to fill that gap and became a full-fledged monitoring program in 2017. To achieve this goal of routinely assessing the condition of Great Lakes coastal wetlands across the entire basin, we assembled a consortium of experienced coastal wetland scientists from the U.S. and Canada. We built this monitoring program on methodology previously developed, tested, and scientifically verified by the GLCWC and GLEI projects. Since 2010 we have efficiently and rigorously assessed and reported on the condition of coastal wetlands basin-wide and have created the baseline for temporal trend monitoring of wetland condition for each of the Great Lakes. Our efforts now provide Great Lakes resource managers and decision-makers the critical information they need for 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, more than 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. Program Description

Our primary objective is to continue and improve a standardized basin-wide coastal wetland monitoring program that has already proven to be a powerful tool for decision-makers for coastal wetland conservation and restoration priorities throughout the Great Lakes basin. Sampling methodology and indicator calculations follow and improve upon those that this team has implemented for the past 10 years of Great Lakes Coastal Wetland Monitoring (https://www.greatlakeswetlands.org/Sampling-protocols). 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 are uploaded into a database specifically tailored for these monitoring results.

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.

Regional sampling teams is led by Uzarski, Albert, Howe, Gehring, Moerke, Lamberti, and Ruetz (U.S. side, central GL basin); Ciborowski and Gathman, Fiorino and Smith, and Tozer (Canadian

side, central and eastern GL basin); Brady, Bracey and Danz (U.S. and Canadian sides, western GL basin); and Amatangelo, Altenritter and Lawrence (U.S. eastern GL basin). Bird and anuran crews sample early in the season during the breeding period (early April - July), while fish, invertebrate, and vegetation crews 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 travel together for efficiency and safety, sharing boats and other equipment and providing assistance to each other as needed. Regional team coordinators keep in close contact with their field crews via cell phone. The typical schedule each year is for site selection to occur during late winter. Field crews 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 are processed, data are entered into the program database, and QA/QC occurs on all data. Provisional data are provided to EPA GLNPO at the end of the field season (considered provisional until all QC validation is complete) and again at the end of winter after QA/QC checks have been completed (final data). Semi-annual reports are sent to GLNPO in the spring and fall (see detailed timeline in Table 1 for specifics).

A7. Data Quality Objectives for Measurement Data

The primary DQO for this program 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 established GLCWMP methods. It is very important that the data that we collect are representative of the condition of each wetland, are collected following GLCWP protocols, and are collected similarly by all field crews across the Great Lakes. Sampling methods, protocols, and indicators have already been approved for the GLCWP monitoring program. By following these methods, we 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 must 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. Crew leaders should determine which sites (from the list of sites assigned to their crew that year, see Section BA: Site selection) they can sample safely on any given day based on wind, weather, and wave conditions. In all cases, crews should not attempt to sample if there is any concern for their safety.

The co-PIs at each participating institution are responsible for the QA/QC aspects of the study, with training, oversight, and assistance from Valerie Brady and Matthew Cooper. Co-PIs conduct mid-season QA checks on their field crews and report the results to Brady and Cooper, who 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 and GLEI II projects and the 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 being performed during this monitoring round 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. All data collection follows the methods outlined in the GLCWMP QAPP and SOPs (https://www.greatlakeswetlands.org/Sampling-protocols). The DQO for data entry is 100% accuracy of data copied from field and laboratory sheets into the database.

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

Tasks	2021				2022				2023				2024				2025			
	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 system updated	X				X				X				X				X			
Site selection for summer		X			X				X				X				X			
Sampling permits acquired		X				X				X				X				X		
Data entry system updated	X				X				X				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 & upload to GLNPO					X	X			X	X			X	X			X	X		X
Report to GLNPO		X		X		X		X		X		X		X		X		X		X

For this monitoring round and sampling program, we are collecting monitoring data to be uploaded into a Great Lakes-wide database. Thus, we are doing limited data analyses and summarization on the collected data. Most data analysis 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 program 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).

A8. Special Training Requirements

All personnel responsible for sampling invertebrates, fish, macrophytes, birds, anurans, and water quality are 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 helps to ensure that rigorous data quality standards are maintained.

A multi-level training and certification program has previously been implemented and is being continued to ensure accuracy of all data collection. A series of training workshops led by regional experts on each respective protocol is held in the 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 includes training on how to meet the data quality objectives for each element of the program; 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 program database; record-keeping and archiving requirements; data auditing procedures; and certification/re-certification exams for each sampling protocol for all program personnel. All program co-PIs, field crew leaders, and as many summer staff as possible participate in these workshops and are certified/re-certified on sampling protocols. 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 are conducted in the field whenever possible, and are supplemented with photographs (for fish, vegetation) or audio recordings (e.g., bird and anuran calls) when necessary. Passing the exams certifies the individual to perform the respective sampling protocol(s). Because not every individual conducts every sampling protocol, participants are tested on the protocols for which they are responsible. The majority of testing and certification takes place during the early-season training workshops, and additional certification is administered by co-PIs as needed. Personnel who are not certified (e.g., part-time technicians, new students, volunteers) are not 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 that are 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 is considered adequate for certification. Lists of who has received which training on what dates are created by each PI or training leader, sent to regional team leaders to compile, and are then provided to lead PI Uzarski and QA officers Brady and Cooper, who store these records electronically.

Note that the training and certification procedures explained here are separate from the QA/QC evaluations explained in section B. However, failure to meet program 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 are trained to consistently locate pre-selected wetlands and sampling locations within each wetland, and are taught strategies to implement when pre-selected wetlands cannot be sampled due to insufficient water depth, unsuitable weather, inaccessibility, or safety concerns. Field crews also receive training in proper GPS procedures, including equipment use and data entry. GPS training includes 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)
- Proper creation of a waypoint (demonstrate proficiency)

Fish: Training for fish sampling is led by the regional team leaders, co-PIs, and fisheries experts on the program. Fish sampling training focuses 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 are trained in proper fish handling procedures that meet both Institutional Animal Care and Use Committee (IACUC) and CWMP protocols. Crews are 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 to set 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 or photography of fish when a positive ID cannot be determined in the field (demonstrate proficiency in preservation and photography; 95% accuracy on determination of when to preserve)

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

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

A crucial element of ensuring accurate invertebrate taxonomy is maintaining open dialogue among program teams. Our past experience 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 use a variety of tools such as regular conference calls or webinars and e-mail sharing of digital photographs to foster this dialogue. Reference collections are maintained at each institution and are used for training staff members. In addition to the open dialogue among team members, individuals participating in macroinvertebrate processing are required to obtain certification before working independently. Trainees must have 100% of their work checked by certified staff until they themselves become certified. Certification for laboratory processing of invertebrate samples is granted by a program PI skilled at working with macroinvertebrates.

Certification Criteria—Field Sampling:

- Determining sampling locations within a site (demonstrate proficiency)
- Appropriate dip 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 takes 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 is led by the water chemistry experts on the program. Water quality sampling training focuses 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 are also 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 is a prerequisite for leading water quality data collection. Water quality analyses in the laboratory are 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)
- Ability to approach sampling point without disturbing sediment
- Ability to recognize when sampling point sediment has been disturbed at water quality samples cannot be collected from that point on that day
- Collection and storage of water samples (demonstrate proficiency)
- Proper field data sheet completion (demonstrate proficiency)

Macrophytes: Macrophyte sampling training is led by regional team leaders, co-PIs, and aquatic macrophyte experts on the program. Training includes 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 program have done extensive plant sampling in Great Lakes coastal wetlands, so their species lists, picture keys, and field data forms include most plants that are encountered during the program. 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 are collected for identification that evening or pressed for later identification in the laboratory. Additionally, at QC sites, plants are collected for QC 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 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 is provided by regional team leaders, co-PIs, and bird and anuran experts on the program. These individuals have been testing and training students and personnel to conduct bird and anuran surveys for many years. Survey personnel are 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 are evaluated using audio and visual tests and trained in field-based survey techniques. An objective, secure online testing system (http://www.birdercertification.org) has been modified for target anuran and bird species found in the 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 are also 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) are 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 is available or recruited for fieldwork. Acceptable candidates typically have taken a college-level course in ornithology and 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 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 describe known habitat associations of anuran species in the Great Lakes basin. Bird and anuran personnel are allowed to retake the test a maximum of 3 times with a minimum of 1 day between subsequent tests. If they do not pass the test on the third attempt, personnel can only assist bird and anuran team leaders in sampling and cannot collect data on their own. They may re-attempt the test the next season.

Certification Criteria:

- Survey plot locations (demonstrate proficiency)
- Visual identification (95% of 20 bird images, including difficult views, immature plumages). This test focuses 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 includes 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 (CMU) have previously led training on record keeping, sample chain of custody, data custody, and entry of data into the data management system. This portion of the training also included data error checking protocols. These reminders now occur during the annual program meetings and via email reminders and QC checks on data and record keeping.

Additional Training:
Permanent technicians and staff who are designated as crew leaders 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 are able to attend the training. Uncertified workers always (100%) work with more experienced personnel until they pass certification and receive substantial training until they prove themselves competent in field sampling methods.

Regional team leaders (co-PIs) 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 archive the reports of these mid-season QC activities and summarize the results in the semi-annual report to EPA (and to all co-PIs). They inform Uzarski and co-PIs of any deficiencies and the required corrections. Follow-up reports from co-PIs demonstrating correction of these deficiencies are required.

A9. Documentation and Record

We have created a specialized data management system dedicated to GLCWP 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 by PIs occurs once per year during the sampling season. QC of laboratory activities and data input by laboratory leaders occurs each winter. All QC records and reports are maintained with the appropriate datasets, archived, and provided to the QC managers (Brady and Cooper) during audits. Data are put into the database and QC'd as soon as possible after data are collected. Data are 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 consists of all activities of the previous 6 months, including QC reports and audit results, and brief data summaries. However, the data are made available in the database, rather than in the report itself.

Records from field and laboratory observations are archived as hardcopies at each regional lab. All data entry on the web-based system will remain accessible throughout the life of the program. Copies of formal reports and supporting materials are 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 and regional labs for a minimum of three years, or made available after that time period.

Deliverables list:

- Semi-annual report to EPA twice per year (including QC reports)
- Data provided to GLNPO twice per year (see above for data included; includes metadata)
- Indicator values for all sampled wetlands, provided once 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

BA. Site Selection

BA1. Program Design

The project on which this sampling program is based, the Great Lakes Coastal Wetland Consortium (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.

When this sampling program started in 2010/2011, the NRRI GIS laboratory implemented a framework for probabilistic site selection. The initial pool of wetland sites was based on the GLCWC-GLNPO wetland coverage (Albert and Simonson 2004). Wetlands selected for inclusion in our program's sampling framework under the random site selection process needed to meet the following criteria: 1) 4 ha or larger; 2) have a direct, obvious surface water connection to a Great Lake or connecting channel allowing fish passage 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.

Size > 4 ha: Previous basin-wide work by GLCWC and GLEI field crews indicated that smaller wetlands can be either too small to sample, or no longer in existence.

Surface connection required: Fish, aquatic macroinvertebrate, and to some extent vegetation metrics and indicators are influenced by which fish can access the wetland. Our metrics and indicators are calibrated based on fish having access to the wetland at least intermittently.

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 evaluated for potential 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 and are considered Great Lakes coastal wetlands and thus are included in our sample pool. In all cases for riverine wetlands, the most downstream portion possible is sampled, whether this occurs on the Great Lake side or the inland side of the drowned river mouth lake.

Herbaceous vegetation presence: We have not yet developed or evaluated indicators and sampling protocols for use in wooded wetlands. Sites lacking aquatic vegetation do not qualify for sampling in this program.

In 2011 we designed our site select system as 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 may be added to the sampling list each year with approval from EPA GLNPO. These "benchmark" sites include sites being considered for or undergoing restoration and/or protection to assist other agencies in determining which sites should be selected for restoration and protection efforts. Including benchmark sites within a statistically-valid monitoring framework allows 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 can be benchmark sites that fall outside the stratified random site selection framework without invalidating the statistical framework. Benchmark sites are sites of special interest that have been requested for sampling. Many are sites that are undergoing (or have undergone) restoration; some were not even wetlands prior to restoration work. In addition, some sites from the stratified-random pool are sampled more frequently than once every five years because they are long-term monitoring sites (from the 1990's) and the extra data from these sites is used to help evaluate our indicators and metrics, particularly for interannual variability and effects of changing water levels. Criteria for inclusion of benchmark sites are the appropriateness of the request and our sampling methods for assessment of the site; the potential of the site to become a coastal wetland if it is not one already, our ability to sample the number of benchmarks requested, and EPA's approval of the sites proposed for benchmark sampling each year. Sites that are outside the site selection framework will not be used in condition assessment methods.

BA2. Sampling Methods

The site selection process outlined above resulted in clusters of sites for each lake (or lake section where lakes cross the regional boundaries); clusters are defined by the 3 wetland types, 5 lakes, and regions (e.g., northern Lake Michigan riverine wetlands; southern Lake Huron lacustrine wetlands; etc.). An estimation of the number of wetlands within each cluster is shown in Table BA2-1; which of these can actually be sampled depends on lake water levels and safe access by crews and varies from year to year. To set up site selection, in 2011 each regional team leader used the site selection tool to virtually investigate each site in their region via satellite imagery and deliberately selected or rejected the site for sampling. Sites could only be rejected for very specific reasons, such as that it was too small, had no safe access, etc. (see section BA5 for complete list of acceptable site rejection criteria; Brady, Cooper, and Uzarski verified that regional leads did not inappropriately reject sites).

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				**1100**

In addition to assessing which sites fit our sampling criteria, regional team leaders also assessed whether wetlands had been artificially divided into separate sampling polygons due to human influence or other causes. If the minimum edge-to-edge distance of the wetland polygons in question was 500 m or less and the wetlands were of the same basic type (e.g., lacustrine, barrier-protected, or riverine), these polygons were evaluated by Brady and Cooper for dissolving the separate polygons into a single wetland polygon for sampling.

The opposite case could also occur, in which PIs noticed that wetland polygons were inappropriately lumped together when they really were separate wetlands because of differences in water flow, lack of connectivity, or incorrect assignment of wetland site type (lacustrine, barrier-protected, riverine). Brady and Cooper evaluated these cases and created separate polygons for these “lumped” wetlands as appropriate.

The vast majority of such changes to the wetland polygon coverage took place the first two years (2011-2012) of the project and there have been few changes to the polygon coverage since that time except to add additional sites that were missed originally or to add benchmark sites that are undergoing restoration and aspire to become coastal wetlands in the near future.

Following all of the initial (2011) site assessment for appropriateness of sites for our condition indicators, size, connectivity, site access, and appropriateness of site polygons, sites were assigned to sampling panels, one panel for each of the five years of a monitoring round. Sites were randomized within each cluster (again, clusters defined by the 3 wetland types, 5 lakes, and N/S regions) and distributed across the five sampling panels, each panel representing the sites to be sampled in each of the five years in a monitoring round.

The sites in each sampling panel form the basis of a five-year rotating panel design, populated by the sites that have a high probability of being sampleable in any five-year sampling round. This design ensures that all major wetlands are sampled over a period of approximately five years. 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 (benchmark sites that are outside of the sampling design are excluded from these calculations).

Each field season, each regional sampling team systematically examines their assigned set of sites from the randomly ordered site list for that year. Teams may still reject sites that fail to meet the minimum criteria, either by assessment of aerial photos or once the sites are visited in the field. For example, sites that are accessible by boat for fish, macroinvertebrate, vegetation and water quality sampling may not be accessible from the land for bird and anuran crews due to lack of road access; this can be determined via aerial photography. On the other hand, it can sometimes be difficult to determine via aerial photography whether or not barrier-protected wetlands have a surface water connection to the lake and these sites may require in-person assessment by field teams.

Support for site selection is provided by the NRRI GIS lab. This facility contains a number of computers running the latest ESRI ArcGIS software. Hardware systems are typically Intel or AMD multi-core processors running at 2.4+ GHz with 0.5+ TB of local storage. Internal and internet networking is 1000 MB (1 GB) 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 are provided with 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 are often able to assist with site reconnaissance for the fish/invertebrate/vegetation crews). On-line web-based distribution of maps for each site showing site outlines and the location of nearby sites to ensure sampling within targeted site boundaries, pre-determined sampling points (bird/anuran crews) and transects (vegetation crews) allow efficient and rapid distribution of materials to field crews scattered across the basin. SOPs for each team specify how to determine if each crew has arrived at the proper point (bird/anuran crews), transect starting location (veg crews), or overall polygon (fish/invertebrate/water quality crews). Crews are also trained on when and how to move these points or transects should they not be located close enough to the wetland (bird/anuran points) or at an appropriate water depth (vegetation transect start/end points). Crews are also trained to call their team leader for assistance in these instances. Centralized site selection and map creation ensure maximum efficiency and a dedicated source of map and logistical assistance.

For planning purposes, the numbers of samples that could be expected per site is shown in Table BA3-1. These numbers are only estimates because for all sample types except vegetation, the number of samples varies depending on site size (all non-veg samples) and appropriate vegetation types and water depths (fish/invertebrates/water quality). Vegetation crews always sample 45 quadrats per site.

BA3-1. Estimated mean number of samples per site by sample type, and the number that might 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	45	0	5
Water quality	2-4	typically 3	2-4 composites
Birds	4	0	0
Anurans	2	0	0

For planning purposes, the numbers of samples that regional teams can expect each year by sample type is estimated in Table BA3-2. Again, these are only estimates because the number of samples per site varies for all but vegetation crews. Numbers were derived by multiplying estimated mean numbers of samples per site by the site sampling capacity of teams (team sampling capacity includes benchmark sites). Team sampling capacity is static from year to year and is based on budgetary allotment for the crew, which is based on the geographic area that each regional team is responsible for.

BA3-2. Estimated mean number of samples per year by team and sample type for planning purposes, 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 sampling capacity is typically static from year to year.

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, Fiorino *et al*.	210 (35)	210 (210)	1225 (70)	70-140 (70-140)	160 (10)	80 (2)
Amatangelo *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 have been trained on the parameters for site rejection (see section BA5), and the management team provides QC oversight on this aspect of the program. Sites that appear to be inappropriately rejected are the subject of a discussion between the regional team leader and the management team. Target turnaround time for initial site assessment via aerial photography and previous notes about the site is one month prior to the field crew beginning fieldwork to allow for discussion and re-inclusion if sites were inappropriately rejected. Inappropriate site rejections made by field crews on the ground typically cannot be corrected in time for sampling that field season if not discovered in real time. Thus, all field crew leaders are trained to consult with their PI and/or the CWMP leadership team before rejecting a site to prevent inappropriate site rejections.

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 1000 Mbit (1 GB) Ethernet, with 40 Mbit/s Internet connectivity. This applies only to the NRRI computer systems that house the site selection 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 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 can access the site selection system database as long as the computer user has a password and clearance.

BA5. Quality Control Requirements

Regional team leaders 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 for sampling in any given year. 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 navigable by fish at least every other year; 4) there is no safe access for field crews (e.g., no public launch within approximately 7 km); and 5) there is no permitted access due to private property constraints. Most of these conditions do not result in permanent rejection of sites because these conditions are often subject to change based on water levels, crews obtaining better boats or gear, or land ownership changing. Only condition #1 (site is too small) and permanent destruction of a wetland (#2b) result in exclusion of a wetland from consideration for future sampling. Most exclusions in category #1 were made at the start of the program in 2011 and few sites now are excluded due to size. Sites are not permanently excluded from sampling consideration based on vegetation responses to water levels.

Crews have different access issues (#4 and #5 above) based on how and what they sample. Fish/invertebrate and some vegetation crews work from boats and need boat launches within safe boating distance over the open waters of the Great Lakes. Bird/anuran crews, on the other hand, typically hike in from land and need safe road and trail access in the dark and permission

to cross private property. In addition, bird/anuran crews can sample sites more quickly than can fish/invertebrate/vegetation crews, allowing bird/anuran crews to sample more sites than the other crews. Because bird and anuran crews are the first in the field each season, their observations of site access assist fish/invertebrate/vegetation crews.

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

Western Great Lakes	Valerie Brady/ Annie Bracey /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/Annie Bracey
Eastern Great Lakes (US side)	Kathryn Amatangelo/ Greg Lawrence/Matt Altenritter
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Giuseppe Fiorino/Doug Tozer

Site selection QC check:

Because regional team leaders are responsible for accepting/rejecting sites from lists generated by the site selection system, a quality control step has been implemented to ensure that consistent criteria are used by each team to reject sites. The program lead PI (Don Uzarski, Central Michigan University), QA manager (Valerie Brady, NRRI), or assistant QA manager (Matthew Cooper, Central Michigan University) 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. However, at this point in the life of the program, few external datasets are used. If we use an external data source, it will likely 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. All such data will be checked for appropriateness of QC for our program's standards.

When this sampling program started in 2010, the wetland site coverage used was based on the Albert and Simonson (2004) Great Lakes coastal wetland GIS coverage. Use of this database for site selection was mandated in the original RFP and by the necessity of following the original GLCWC sampling protocols. To turn this coverage into sampleable wetland polygons required extensive work and QC in the first year of the project and we maintain and update this layer.

Other data used for site selection included wetland type (Albert *et al*. 2006) and GIS background layers (roads, boat launches, land use, etc.) from state and federal agencies that we use to make maps for crews navigating to site polygons.

Land use data for calculation of the land use and water quality indicator come from the North American Land Change Monitoring System (NALCMS) 2010 land cover of North America at 30 meters (from Landsat imagery) (CEC 2015). The NALCMS is a collaborative initiative by agencies across the U.S., Canada, and Mexico to monitor land cover change and is produced by the multi-national Commission for Environmental Cooperation (see citation for partner organizations). These land cover data are published and provided as a tool for researchers and meet the QC standards of the associated agencies, including the U.S. Geological Survey. Because the NALCMS includes land cover for both the U.S. and Canada, it standardizes the land cover calculations across all CWMP sites.

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

Please refer to CWMP water quality SOPs for greater detail and step-by-step instructions, available on the CWMP website: https://www.greatlakeswetlands.org/Sampling-protocols.

BB1. Program Design

Chemical/physical measurements are made in each vegetation type where fish and macroinvertebrate data are collected, but may also be collected in areas too shallow or deep for fish or macroinvertebrate collection. Fish and macroinvertebrates are collected within vegetation morphotypes (see Sections BC and BD); water quality is collected in association with fyke nets, one water quality sample per vegetation type sampled. In the event that fyke nets are not set due to inappropriate water depth, but invertebrates are collected, then a water quality sample is also collected from that vegetation type. 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). This additional sampling effort is recommended if vegetation patches forming the sampling type are separated rather than contiguous. Water quality data collection is critical at each wetland, but parameters are 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 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: secchi tube water 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

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

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

- Lab: water is collected from each of the fyke net locations within a vegetation morphotype and combined to form a single composite sample, which is 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 disturbance of the area to prevent contamination by suspended sediment. Substrate/sediment may be disturbed by the boat motor; crew exiting the boat; use of poles, paddles, or other items that touch the substrate; and uprooting of vegetation. Crew are trained on approaching each sampling point in a manner that avoids substrate disturbance, as well as how to recognize when substrate disturbance has occurred.

Field meters:

Water quality measurements using field instruments are made *in situ* at the mid-depth of the water column at a minimum of one location within a vegetation morphotype (i.e., the first fyke net location). Field instrumentation varies 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 (e.g., Yellow Springs Instruments [YSI] 6600 or equivalent instrumentation). *In situ* measurements are 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 it is determined using water collected for other analyses (see below). Supplementary measurements of oxidation-reduction potential (redox) and *in situ* chlorophyll fluorescence depend on the field instrumentation available to field crews. The specifications tabulated below for recommended and supplementary parameters (Table BB4.1) are standardized so that measurements are taken consistently among field crews.

Water sampling:

Water quality samples are taken from a single composite sample collected from the 3 fyke net locations (or 3 macroinvertebrate sample locations) within each vegetation morphotype. From each net location, water is 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 is done 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 are collected from each zone in which 3 fyke nets are set. Care is taken to ensure that no bottom sediment is collected. If bottom sediments are collected, the water is discarded, the carboy and sample bottles are thoroughly rinsed with surface water from the same zone, and the water collection process is started over in an area where the sediment has not been disturbed. In order to avoid potential errors due to larger pieces of detrital debris contaminating water samples, each water sample is poured through an acid-cleaned polypropylene funnel to which a 500 micrometer mesh screen is attached.

Composited water is then mixed by swirling the carboy and dispensed into a 4-L acid-cleaned polyethylene cubitainer for later processing (see below). The remaining water in the carboy is then re-mixed and used to determine secchi tube water clarity. Secchi tube clarity is measured against a light background with the tube shaded from direct sunlight (MPCA 2007, 2006; Anderson and Davic 2004). After this measurement is made, the remaining water is dumped from the carboy and the carboy is rinsed at least 3 times with surface water from the next vegetation morphotype and the water sampling and compositing process is repeated.

Bottles for specific analytes are rinsed with sample water before collection. All samples are 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 are labeled externally with labels that adhere to sample bottles even when wet. They are written on with black fine point Sharpie markers or similar markers that have been tested and proven to be waterproof. Labels consist of site ID, plant morphotype, replicate number, sample date, regional team initials, and crew chief name. Water quality samples in 4-L cubitainers are kept chilled on ice until they can be further processed in the evening. Samples are 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 are filled with raw water; one is frozen as soon as possible for TN and TP (recommended), and the other is frozen as a back-up sample.

- Alkalinity titration: Alkalinity (required) is measured on an unfiltered portion of the sample as soon as possible after collection, but always within 12 hrs. Alkalinity is 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, is 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 is used for chlorophyll-a extraction in the lab. The filter is 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 is stored in a poly bottle and iced (not frozen) for later analysis of chloride or other major ions. (If sample is being 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 is 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 or equivalent setup. The sample is initially iced and then frozen as soon as possible. (Again, if the sample is being shipped to a lab for processing, a frozen duplicate must be maintained by the regional laboratory).

Sample numbers and numbers of sample bottles are noted on field data sheets. Samples 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 accompanies the samples with a copy remaining with the field crew or regional laboratory, whichever initiates the shipment. At the laboratory, all sample codes are entered into the laboratory sample log-in book along with the date received and any notes regarding sample condition. This information is transferred to an electronic archival system maintained by each regional laboratory.

Regional laboratories not processing their own water quality samples 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 is emailed to the receiving lab, which logs in the samples when received, and verifies receipt and condition of samples via email to the shipping lab. Results, including QA/QC data are provided via email to the regional team laboratory. The regional team laboratory retains 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 are inspected and calibrated on the required schedule for each parameter (see section BB7). In case of failure, field crews are trained in the standard procedures to follow that solve many cases of meter failure (see section A8). Crews 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 are used for this program.

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 program have confirmed that they can meet these standards. All parameters are measured within the listed holding times.

Each analytical measurement listed in Table BB4.2 specifies the water chemistry method to be used (with references), which represents the ultimate reference for participating laboratories. All methods and options for arriving at the required measurements and achieving the performance standards listed in Table BB4.1 are included in the references cited. These references are already owned by all of the participating water quality laboratories.

Sample disposal: Disposal of all wastes complies with federal, state, and local regulations governing waste management. The procedures written here meet these regulations. Disposal for samples of raw water (used for TN and TP) and filtered water (used for SRP, Ammonium-N, Nitrate/Nitrite-N, Chloride) is to rinse them down the drain to a wastewater treatment facility.

Raw water that has had its pH reduced, (e.g., for alkalinity titration) is neutralized, diluted and rinsed down the drain to a wastewater treatment facility.

Sample water (filtered or raw) that has been analyzed using a QAPP-approved method for TN, TP, SRP, Ammonium-N, Nitrate/Nitrite-N, or Chloride is collected with other instrument waste in hazardous waste containers which are routinely collected for disposal through each institution’s hazardous waste program.

Table BB4.1. Minimum performance standards for laboratory and field measurements that participating labs must achieve. See footnotes for explanation of measures.

Parameter	Precision*	Accuracy	Range‡	Method Detection Limit§	Limit of Quant.**	Units	Holding times
Ammonium-N	15%	10%†, 20%††	0.003 – 1	0.01	0.03	mg/L	28d
[Nitrate + Nitrite]–N by autoanalyzer or Nitrate-N by IC	15%	10%†, 20%††	0.003 – 1	0.01	0.03	mg/L	28d
Soluble reactive phosphorus (SRP)	15%	10%†, 20%††	0.002 – 1	0.005	0.015	mg/L	28d
Total Phosphorus (TP)	15%	10%†, 20%††	0.002 – 1	0.005	0.015	mg/L	28d
Total Nitrogen (TN)	15%	10%†, 20%††	0.01 – 2	0.01	0.03	mg/L	28d
Chlorophyll-a	15%	10%†	0.5 – 1000	0.5	1.5	µg/L	28d
Alkalinity	10%	10%†	0.5 – 1000	0.5	1.5	mg/L as $CaCO_3$	24h
Turbidity	10%	10%†	0.1 – 4000	0.1	0.3	NTU	2d
Color (dissolved)	10%	10%†	1 – 500	1	3	Color Units	2d
Chloride	10%	10%†, 20%††	0.03 – 1	0.1	0.3	mg/L	28d
Dissolved Oxygen	10%	10%†	0.1 – 20	0.1	0.3	mg/L	x
Specific conductivity (EC25)	10%	10%†	1 – 5000	1	3	µS/cm	x
pH[x]	0.1 units	0.1 units†	4 – 10	NA	NA	Std Units	24h
Temperature	0.1°C	NA	0 – 30	NA	NA	°C	x
Redox (ORP)	10 mV	10 mV†	-2000 to +2000 mV	1	1	mV	x
Secchi tube clarity	10%	NA	0 – 120	1	1	cm	x

*Calculated as Relative Percent Difference (%RPD)
†Calculated as %recovery of a known concentration in Quality Control Check Standards
††Calculated as %recovery of a known concentration in spiked matrix samples
‡ Approximate range of values that analytical method is capable of measuring
§ Calculated annually at each lab following methods in EPA 821-R-16-006
** Limit of Quantification (LOQ): Calculated as 3 * Method Detection Limit

Table BB4.2. Water quality procedures and analytical methods. SM refers to APHA (2005; *Standard Methods*)

Parameter	Procedure	Method Reference
Total Phosphorus (TP)	Persulfate digestion + SRP or Ion Chromatography	EPA 365.3; USGS (2003); Ameel *et al*. (1993, 1998); Dionex Method AN 254 SM 4500-P-J
Soluble Reactive Phosphorus (SRP)	Automated, Ascorbic acid or Ion Chromatography	SM 4500-P-E/F Dionex Method AN 254
Total Nitrogen (TN)	Persulfate digestion and NO_3+NO_2-N	USGS (2003); Ameel *et al.* (2003) SM 4500-P-J
Ammonium-N	Automated phenate (or salicylate) or Ion Chromatography	SM 4500-NH_3-G Or Dionex AN 141
Nitrate-N or Nitrate+Nitrite-N	Automated with cadmium reduction or Ion Chromatography	SM 4500-NO_3-C or SM 4500-NO_3- F
Alkalinity*	Titration (2 pt)	SM 2320 B
Chlorophyll-a	Spectrophotometric or fluorometric	SM 10200 H Axler and Owen (1994)
Chloride	Ion Chromatography	Dionex AN 141
Color (dissolved)	Spectrophotometric	SM 2120 C
Turbidity†	Nephelometric	USGS (2005)

*Usually measured at the motel the evening after sample is collected or in the lab for local sites
†Can be measured *in situ* with a meter or in the lab with a benchtop meter

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

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 are taken at a minimum of 10% of sampled vegetation morphotypes or enough so that each crew generates a minimum of 3 duplicates per year, whichever is greater. Laboratory analytical precision is determined by comparing the results of split samples, duplicate samples, and duplicate spike samples.

Sampling and/or analytical precision are 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.

Accuracy – Accuracy is determined by calculating the recovery of a known addition (or spike) to samples and blanks, as well as recovery of Quality Control Check Standard (QCCS) solutions.

Recovery of known additions involves adding (or spiking) a sample or blank with a known amount of the analyte, and calculating the percent recovery of the spike. Spike concentrations are commonly 1 to 50 times the limit of detection for the analysis and fall within the linear range of the method. Concentrated solutions are used for spikes so that volume change in the sample is negligible. Recovery is expressed as a percentage and is calculated as follows:

$$\%\, recovery = \left[\frac{sample_{spiked} - sample_{unspiked}}{spike}\right] \times 100\%$$

where $sample_{spiked}$ and $sample_{unspiked}$ are the concentrations in the spiked and unspiked sample, respectively, and $spike$ is the known amount of the added spike. Acceptable limits for sample spikes are ± 20% in most cases (Table BB4.1).

Quality Control Check Standard (QCCS) solutions with a certified known concentration are analyzed with each batch of samples. QCCS solutions are analyzed at the beginning and end of each batch to evaluate drift during the analytical run. QCCS solutions are prepared to fall within the linear range of the method. Accuracy is evaluated by calculating the percent recovery as follows:

$$\%\, recovery = \left[\frac{concentration_{measured}}{concentration_{known}}\right] \times 100\%$$

where $concentration_{measured}$ and $concentration_{known}$ are the concentrations of the QCCS solution measured during the run and the certified known concentration, respectively. Acceptable limits for sample spikes are ± 10% in most cases (Table BB4.1).

Matrix spikes and/or QCCS will be analyzed at the rate of one every 20 samples or one per batch, whichever is less.

Bias – Bias is the systematic or persistent distortion of the measurement process so that the value given does not represent the true value. Field bias is assessed by use of field blanks and trip blanks. Adherence to proper sample handling, preservation, and holding time protocols helps minimize field bias. Since the sampling method for all sampling is grab sampling, field blanks (i.e., sampler blanks) are collected at least every 10 vegetation morphotypes sampled (every 3-4 sites). 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 train and work with less experienced personnel. Laboratory bias is determined as part of the lab's internal quality control. Bias effects that fall outside the laboratory's acceptance limits are flagged.

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 vegetation morphotype sampled. Failure to obtain these samples due to meter malfunction, or any other variances from the established sampling protocol, should 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 are routinely screened for representativeness by comparing measured parameter values to historical data from this monitoring program and other sampling efforts and to current and historical data generated by other organizations, if available. In addition, program members 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 program data can be compared to other data. Comparability of data is 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 are 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, sensitivity represents the lowest level of analyte that can be reliably detected by the laboratory analytical method. *Method Detection Limit*s (MDLs) will be calculated once annually according to EPA 821-R-16-006. Note that most labs also conduct ongoing evaluation of the MDL throughout the year by adding data from each successive run to the MDL calculation in order to detect any significant changes to MDLs throughout the year. For the initial annual MDL calculation, a minimum of 7 blanks will be analyzed over the course of 3 days to incorporate any day-to-day variation resulting from lab operations (see EPA 821-R-16-006 and Water Quality SOP for additional details and calculations). The annual MDL will be entered into the project database by each lab for each water quality parameter for which an MDL is required. After the initial MDL study each year, ongoing MDL analysis throughout the year with additional data points should be used by regional labs to detect performance issues that require corrective action. These ongoing MDL studies will not be logged into the project database unless a significant change in workflow has occurred (new instrumentation, etc.).

As the MDL is approached, variance typically increases, which reduces the accuracy of measured values at these low levels. Therefore, a *Limit of Quantification (LOQ)* will also be determined as LOQ = MDL * 3.

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 follow well-established methods (e.g., APHA 2005; USGS 2005). Participating laboratories are 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 limit determination; analysis of field blanks, reagent blanks (or 'method blanks'), laboratory-fortified blanks (or 'matrix spikes'), and laboratory-fortified blank duplicates (or 'matrix spike duplicates'); estimates of precision and accuracy; and documented QC acceptance/rejection criteria. These requirements are satisfied by documentation of laboratory certification (State, Provincial, Federal or international body) as well as having a well-documented laboratory QA/QC program.

Compliance with the QA/QC program is coordinated and monitored by the laboratory's quality assurance officer or manager and must meet the minimum requirements outlined in this QAPP.

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 are conducted by each laboratory in accordance with their standard operating procedures and the individual method requirements. Method-specific data quality objectives (performance standards) are found in Table BB4.1.

Data Qualifiers – Common data qualifiers will be used in this project. Values that are less than the MDL or between the MDL and LOQ will be flagged accordingly. On samples that require dilutions due to matrix interference, the MDL and LOQ will be elevated according to the dilution factor. Data points below LOQ will not be used in the calculation of laboratory precision. MS/MSD samples will be used for this purpose. Samples with values exceeding the upper range of a method on an initial run will be diluted and reanalyzed so they fall within calibration range.

Individuals Responsible For Water Quality QA/QC:

Western Great Lakes	Chris Filstrup/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)	Michael Chislock/Kathryn Amatangelo
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/provincial-certified lab

Mid-Year QA/QC Checks:

Regional team leaders and co-PIs assess water quality sampling methods during each field season. In most cases, this is accomplished by traveling with field crews on at least one occasion per year. The program QA manager and assistant manager provide guidance for 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, informed by field blank assessment
- 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 has a QA/QC program in place for the water quality parameters being measured. Most of the water quality labs in this program are certified (by a state, federal, or internationally recognized accreditation body). Such certification requires audits every 2 years and the routine running of proficiency test (quality control) standards, often multiple times per year, to ensure that machine output is within acceptance criteria (typically +/- 10%, but varies by analyte). Labs that are not state or federally certified at present are implementing yearly internal audits based on those done internally by state-certified labs. Adherence to these plans, which include method blanks, matrix spikes, and duplicates, ensures 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 program laboratories are provided to the program QA managers in mid-winter.

BB6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

All field water quality and laboratory instruments are maintained in accordance with manufacturer's specifications and the requirements of the specific method employed. This maintenance is carried out on a regularly-scheduled basis and is 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 are calibrated in accordance with manufacturer's specifications. Since a number of different instrument brands, models, and configurations are being used, only general calibration methodologies are provided here. Specific electrical conductivity, turbidity, redox (ORP), and pH are calibrated once per field trip (about every 10 days), while dissolved oxygen is calibrated daily. Field crews are required to certify on data entry sheets when meters were calibrated. Maintenance logs, including calibration frequency, are kept at each participating laboratory.

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

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

Redox: Sensors are calibrated using a single-point method with Zobell's solution. The solution supplier's instructions regarding temperature-correction of Zobell's solution are 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 is always used. The sensor is thoroughly rinsed with Zobell's solution prior to calibration. Zobell's solution is allowed to reach room temperature before calibration to reduce the amount of temperature-correction required.

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

pH: Sensors are calibrated using a 3-point method with purchased pH buffers of pH 4, 7, and 10. Buffers are prepared according to supplier's instructions and replaced when listed hold times expire. Sensor manufacturer's instructions are followed to calibrate pH sensors and sensors are rinsed with buffer prior to each calibration point. When possible, the sensor mV reading is 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 are acid-cleaned and DI-water rinsed as per APHA (2005), depending on the analytes being collected. Specifications for each batch of bottles are verified by checking the supplier's certification statement. Standards and reagents are assigned expiration dates based on vendor or method requirements. Materials that exceed the expiration dates are not used. Standards, reagents, and laboratory water is of sufficient purity to meet method and/or instrument manufacturer criteria. Each laboratory, as part of their respective QA program, designates individuals who are responsible for inspecting supplies and consumables, and for providing acceptance criteria. This information is retained in the program files.

BB9. Data Acquisition Requirements (non-direct measurements)

N/A

BC. Wetland Vegetation Sampling

Please refer to the CWMP vegetation sampling SOP for greater detail and step-by-step instructions, available on the CWMP website: https://www.greatlakeswetlands.org/Sampling-protocols.

BC1. Program Design

Upon arrival at the site, the field crew first determines whether or not the site is sampleable based on the following criteria: safe access, open connection to the lake or connecting channel, site is an emergent wetland, 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 is recorded in the space provided on the field sheet. If sampleable, field crews draw on their aerial image maps of the site to indicate locations of vegetation patches and transects, if they are not using detailed maps provided to them.

The primary data collection at the site is the identification and quantification of all wetland plant species occurring in a specified number of sampling quadrats. Within wetlands, sampling occurs along three transects that run perpendicular to depth contours and that therefore cross the wetland vegetation zones present; the number of vegetation zones varies 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 is also sampled. Note that the vegetation sampling crews identify vegetation zones somewhat differently than do the fish and macroinvertebrate crews, who sample within vegetation morphotypes.

Transects can be no closer than 20 m to each other. In most wetlands, they are much further apart. The starting point of each transect is 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 is $1/6^{th}$ the width of the vegetation zone from the wetland edge. Vegetation is surveyed in 1-m^2 quadrats at regular intervals along transects, for a total of 45 quadrats per wetland (15 quadrats per vegetation zone). All survey quadrats are placed 2 m to the right of the transect line to avoid trampling effects. The length of the transect within a given plant zone is measured, and if the plant zone is greater than 11 m wide, the length of the zone is 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 is used. In this protocol, the field crew locates the midpoint of the narrow zone along the original transect. At this midpoint, an additional transect is placed perpendicular to the original transect. Survey plots along the perpendicular transect in the narrow zone are located at -8, -3, 2, 7, and 12 m

from the zone midpoint along the original transect. Narrow transects are most likely to be needed 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. In these areas, 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 is 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 is calculated from the most recent year's Google Earth 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 is 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, is also 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). New invasives are being added as they invade the Great Lakes. 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 other relevant studies were added to the lists within the Great Lakes Coastal Wetlands Monitoring Plan prior to the initiation of program sampling. Taxonomic descriptions are 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) that are compatible with Michigan's FQA (Herman *et al.* 2001) are 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 are identified to the lowest possible taxonomic unit (typically species). Representative specimens of plants that cannot be identified in the field are 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

are 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 are typically not curated. Almost all invasive exotic species can be recognized, even when sterile, and are included in the analysis. Percent cover for each plant species, total percent vegetation cover, and water depth (cm) is 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 is 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 additionally recommended that supplemental information on depths of organic sediments, water clarity, and underlying mineral soil texture be collected at each vegetation plot. While these data are not required because they cannot be collected from every quadrat, fields to record the data are available on the field data sheets. Depths of organic soils (in centimeters) is 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 is 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 is attached to each unidentified plant and noted on the field form. Each sample is 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 are placed in Ziploc bags for either identification in the laboratory or by herbarium staff. Plants requiring identification by herbarium staff are 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. Plants that resemble rare or endangered species will be photographed only.

Collected plants are placed in a cooler or refrigerator upon return from the wetland, in preparation for pressing within 24 hours. Pressed plants are dried either with heat or with a fan whenever possible. Plant samples are 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 is not being made as part of this program.

BC4. Analytical Methods Requirements

Performance criteria: Most specimens collected in the field are 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.) are identified to the lowest taxonomic levels possible. Specimens of questionable identity are pressed and returned to the laboratory. If fertile, unknown and unusual specimens are sent to appropriate taxonomic experts for confirmation or refinement of taxonomic identity. Sterile or immature plants are identified when possible. Target turnaround time for plant identifications is 3 months after the end of sampling.

Macrophyte data includes 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, indicator calculations use metrics that are not strongly dependent on accurate plant cover estimates, but instead during the final stage of analysis percent cover is converted to broad cover 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 are 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 should borrow a GPS from the fish and invert crew to finish the site and 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 can be marked on printed areal photos of the site that all field crews have. Crew leaders should 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 are 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 of the use of the mean Conservatism scores in the FQI software is that it is 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 mean Conservatism scores.

Recent comparisons of IBI and Mean C scores from lower water conditions (2011- 2013) with recent high water conditions (2016-2019) showed little regional change in scores, although some individual marshes, especially in the highest-energy open lacustrine marshes, had major reductions in scores, but based on long-term observations these marshes will recover when water levels drop. Changes in Great Lakes water levels, as measured by USGS gauging station data, result in reduction of meadow and emergent plant diversity when water levels rise above long-term mean lake levels, often with a corresponding increase in submergent and floating plant diversity, but this does not in itself alter IBI or Mean C scores. The specific location of a given plant species or association of plant species will typically change with either an increase or decrease of water level, although this type of change is less for larger emergent vegetation, such as species of bulrush (*Schoenoplectus*), cattail (*Typha*), common reed (*Phragmites*), spikerush (*Eleocharis*), or bur-reed (*Sparganium*).

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 is assessed during the mid-year QC check (see below, this section).

Bias: Systematic bias by a crew or method. Bias is 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 is calculated for all sites; the target is 90% of target quadrats sampled.

Representativeness: How well sites were sampled is 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 is done for all sites.

Comparability: Data comparability among crews within the program and to other non-project data is 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 depends 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 are most identifiable when field crews are sampling. Uncommon taxa are not particularly detectable because of the small percentage of each site that can be sampled even with 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 program team responsible for vegetation sampling 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 are returned to the laboratory for identification, with assistance from botanical experts when necessary (See section BC4 above). A collection of difficult-to-identify species is maintained to assist with future identifications. This is especially useful for commonly-encountered plants that are often found in non-flowering condition. The vegetation team maintains an ongoing dialogue to ensure accurate and consistent identifications.

Field teams ‘calibrate’ their percent cover estimates with each other during the yearly field training. Field teams seek concurrence 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 do not result in wetland quality ranking errors. Re-measurement of quadrats at a site is 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 do not alter the metrics or the overall site quality ranking. Field team members confer with each other on percent cover

estimates for each quadrat; discrepancies in cover estimates exceeding 10% between individuals in a field team 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)	Kathryn Amatangelo/Rachel Schultz
Eastern Great Lakes (CA side)	Giuseppe Fiorino/Ian Smith

Mid-Year QA/QC Checks:

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

Species Identification: The program macrophyte experts (Dennis Albert, CMU; Nick Danz, UW-Superior; Rachel Schultz, SUNY Brockport) or other individuals whom they designate 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 is 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 is provided to field crews). This QA/QC step is based on a combination of field and laboratory identification. Preserved specimens or digital photographs are used as part of the identification process. This QA/QC evaluation occurs once per year during the sampling period. After verification, the macrophyte experts record the species identified correctly or incorrectly by each participant, which serves as a performance record for each participating individual. The macrophyte experts also distribute a list of particularly difficult taxa that require preservation and lab verification when they are encountered. Corrections are made to the macrophyte database when identification errors are found. Additional corrective actions are explained in section C1.

The program QA manager and assistant manager 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, should be cleaned and inspected annually. Boat motors are also tuned up as necessary for safe operation. Crews carry at least one spare quadrat. Boat repairs are also often necessary. Most towns around the Great Lakes have boat repair shops, which can be used by field crews as necessary. Appropriate spare parts are 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 are 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 are given rigorous disinfection to eliminate the transfer of nonnative and invasive 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. Specific regulations for AIS decontamination of gear for each state are observed.

BC7. Instrument Calibration and Frequency
Recreational GPS accuracy (+/- 10 m) is sufficient for these data. GPS receivers are tested prior to and after the field season by taking repeated readings at a location visible on aerial photos, such as a parking lot with lines. Accuracy is assessed by plotting GPS waypoints on a georeferenced aerial photo of the test location (e.g., the parking lot). During the field season, field crews uploading GPS readings at least weekly. At least once per week, GPS points are plotted on aerial photographs of a sampled site and the image is presented to the field crew to verify that points appear to be within +/- 10 m of the actual sampling locations. All tests and results are 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

Please refer to the CWMP macroinvertebrate sampling SOP for greater detail and step-by-step instructions, available on the CWMP website: https://www.greatlakeswetlands.org/Sampling-protocols.

BD1. Program Design
Upon arrival at the site, the field crew first determines whether or not the site is sampleable based on the following criteria: safe access, open connection to the lake or connecting channel, site is sampleable (e.g., water depth is appropriate), site is an emergent marsh. 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 for sampling, the reason is recorded on the space provided on the field sheet and with pictures.

If the site is sampleable, additional general information about the site is 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 (data fields and lists available on data sheets for crew to use). In addition, field crews draw on their aerial image maps of the site to indicate locations of vegetation morphotypes, any disturbances not visible on the aerial photos, and D-net sampling locations. Crew members for fish and invertebrates collaborate to collect this information and complete the field sheet and map.

Macroinvertebrate samples are collected from the major plant morphotypes in each wetland. Sampling begins in mid-June in the most southerly regions of the Great Lakes and continues into early September, moving north with the phenology of wetland plants and development of macroinvertebrate communities. This is the interval during which emergent plant communities generally achieve maximum annual biomass. In most cases, macroinvertebrates are 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 are some sites or plant morphotypes within a site that cannot be sampled for fish because of inappropriate depth, but that are sampleable for macroinvertebrates and water quality as well as the other data types.

Macroinvertebrate sampling is stratified by plant morphotype. Plant morphotypes 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 morphotype; however, plant morphotypes should be near-monodominant stands that comprise at least 75% of the emergent or floating-leaved plant community. Plant morphotypes include *Typha* (cattail), *Nuphar-Nymphaea* (water lily, combined), *Schoenoplectus* (bulrush, include both dense and sparse areas 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 (not dominated by any one type of emergent vegetation), open water (special cases only, as outlined in section A8), and potentially other types such as floating bog mat. Note that *Schoenoplectus* morphotypes may need to be divided into sparse and dense sampling areas if the *Schoenoplectus* vegetation is more than 50-100 m wide. In these cases the outer (lakeward) edge of this plant stand may only support low stem density while more

shoreward zones are usually sheltered enough from wave action to support denser stands and a different macroinvertebrate community. Mixed emergent morphotypes may be sampled if they are a significant and conspicuous habitat type and there are not three other monodominant morphotypes to sample. Additional morphotypes 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 morphotype is required because GLCWP IBI metrics are formulated for specific plant morphotypes in order to be robust to water level fluctuations. The minimum depth required for sampling macroinvertebrates is 5 cm and the maximum depth is 2 m.

Plant morphotypes are identified by field crew leaders during site reconnaissance when the field crew reaches the site. The minimum area for plant morphotypes to be sampled for macroinvertebrates is 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.

Three replicate field-picked D-frame dip net samples are collected in each inundated plant morphotype to provide a truly representative sample of the macroinvertebrates occurring in this each morphotype. 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 morphotype. 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. The three sampling points are selected to represent the variability in the vegetation morphotype, and thus, likely, the variability in the macroinvertebrates living in this morphotype. In large vegetation stands, the three sampling points 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 sparse *Schoenoplectus* morphotype extends along the shoreline lakeward of a cottage, a forested area, and a wet meadow, sampling locations are chosen to correspond with these different features. However, in many (or most) cases, a given vegetation morphotype does not cover enough area to be associated with different shoreline features, in which case sample locations are chosen to represent, to the degree possible, the variability of the vegetation morphotype itself. It is important to note that replicate sampling locations are being chosen to maximize variability, not minimize it as is often the objective with replicate sampling. The goal here is to capture as much of the variability in macroinvertebrate community structure as possible within the vegetation morphotype while adhering to the constraints of this sampling protocol.

GPS waypoints are made at each sampling point in each plant morphotype. Because macroinvertebrates, fish (when possible), and water quality are all sampled in each plant

morphotype, care must be taken that personnel doing the sampling do not interfere with one another or compromise the other sample types. Water quality data and water samples should be collected first at each location before any movement is made around the point. 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 disturbance of the area to prevent contamination and suspension of sediment. Substrate/sediment may be disturbed by the boat motor; crew exiting the boat; use of poles, paddles, or other items that touch the substrate; and uprooting of vegetation. Crew are trained on approaching each sampling point in a manner that avoids substrate disturbance, as well as how to recognize when substrate disturbance has occurred. After water quality sampling, macroinvertebrate sampling and fish net deployment may commence.

BD2. Sampling Methods

Macroinvertebrate samples are 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 that are used to calculate Index of Biotic Integrity scores. Note that two ways of 'semi-quantifying' sampling effort (counting dip net sweeps and timing of field picking) are 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 single "sweep" commences at the sediment surface, moves the net up through the water column while brushing through and against the vegetation and ends at the water's surface. This is one "sweep". Multiple sweeps are taken at a given point to incorporate all microhabitats and capture a good representative sample of the macroinvertebrates living in the detritus or sediment surface, on the plants, and in the water column. Care is taken not to collect excessive amounts of sediment or detritus, which makes field picking of organisms difficult.

To semi-quantify sampling effort, the number of net sweeps (i.e., 1 to 2-m passes) is 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 are 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 are picked systematically from discrete areas within each pan defined by 5x5-cm grid lines drawn on the inside bottom of sampling pans. This gridding ensures standardization of picking, rather than needing to standardize pan size.

Contents of the dip nets are distributed evenly across the pan prior to picking organisms. The person doing the netting continues to sample and fill the pan(s) until the pan(s) appear to hold a sufficient number of organisms for field picking to begin, at which time the number of net

sweeps is recorded on the data sheet. Though determining when to stop dip net sampling and begin field picking is arbitrary, field crews are trained in this during the pre-season training workshop. The number of dip net sweeps required depends on macroinvertebrate densities in each vegetation patch. For example, in patches 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 patches 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 varies based on characteristics of the site. However, excessive amounts of vegetation and detritus should not be collected because this significantly reduces 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 falls into pans with the net contents. Generally, this amount is a sufficient volume of water (2-5 mm depth in the pan) to facilitate picking of swimming organisms. Too much water in pans greatly reduces picking efficiency and should be avoided.

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 replicate. 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.

The target for each replicate is for 150 macroinvertebrates to be collected. Picking of individual replicates is 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 organisms and the number of person-minutes is recorded on the data sheet. If 150 organisms are not acquired within one-half-person-hour, the timer is stopped, organisms are tallied, and the running timer resumes 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 (and total elapsed time will be recorded). 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 and the number of organisms collected are both recorded.

Macroinvertebrates are picked from sampling pans using forceps and 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 small, 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. 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 requires a substantial effort. When this occurs, the next grid square is picked. If the entire pan is 'picked clean' before the target number of specimens is reached, then timing stops while dip nets are used to refill the pan(s). As noted above, multiple pans may be used to collect a single replicate sample as long as the grid method is used so that collected organisms represent the resident macroinvertebrate community.

Hand tally counters are used to keep track of how many organisms are collected. Specimens are 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.

Additional supplementary data that are collected with each macroinvertebrate replicate sample include (required): latitude/longitude via GPS waypoint, vegetation percent cover by growth form and cover type at both the water surface and sediment surface, organic substrate depth and mineral substrate texture. Note that the supplementary vegetation data are separate from the other vegetation sampling for the program and will be associated with macroinvertebrate and fish data only.

A 1-m^2 quadrat is used to determine surface and subsurface vegetation percent cover and as the location for the substrate assessment. In locations where both fish and macroinvertebrates are being sampled, the quadrat is 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 is used to identify the midpoint of each lead. In locations where fish are not being sampled, quadrats are 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 are thrown over the individual's shoulder to reduce bias in sampling location.

Percent composition by growth form and cover type is estimated visually. Growth forms and cover estimated are the same as those for the vegetation morphotypes listed above. If species (or genera) within each growth form category are known, they are noted on the field data sheet. Although vegetation overlaps, crews are trained to standardize their quadrat percent cover estimates so that percentages sum to 100%; open water (surface) and bare substrate (bottom) are included to ensure that crews have quadrat coverages summing to 100%. Because percent cover can differ substantially from the surface to the bottom of a quadrat, percent cover is estimated separately for the quadrat when looking at just the water surface, and again when looking through the water column to the bottom of the quadrat. A picture is taken of

each vegetation quadrat in case there are questions later about vegetation type or percent cover.

Within the quadrat, the depth of organic substrate is determined by pushing a 2-cm diameter rod into the substrate until mineral substrates are reached. Note that this is not refusal depth, which is typically significantly deeper within surficial substrates. Placing the finger at this point of the substrate/water interface allows the rod to be withdrawn and the depth measured with a meter stick. In addition, the dominant and subdominant mineral substrate type is recorded. Mineral substrate texture is determined by feel using the categories and guidelines on the field data forms. Some labs analyze sediment organic matter as percent loss-on-ignition (%LOI). A core of substrate from the quadrat is collected for this purpose if the lab is doing that analysis. A sample size of about 100 mL of substrate is sufficient for analysis. The substrate from each replicate quadrat for a vegetation morphotype is homogenized in a container and only a subsample retained for lab processing (about 100 mL). Substrate samples are kept frozen until analysis. Full details are available in the invertebrate sampling SOP.

The same taxonomic keys are used by all labs for macroinvertebrate identification in the laboratory during the fall and winter. These are specified in the macroinvertebrate SOP and will be updated as new taxonomic keys are published. Each regional team laboratory maintains a reference set of preserved specimens that are used to train new staff and to compare with to ensure consistency in identifications. Standardized record keeping and sample archiving techniques are 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 in the SOP.

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

Samples remain in the custody of field crews for the duration of each field trip. At the laboratory, all sample information is entered in the laboratory log-in form, along with the date

received and any notes about sample condition. This information is transferred to an electronic sample processing spreadsheet maintained by each regional laboratory. This spreadsheet is updated as each sample is processed. Processed samples remain in the custody of the regional team laboratory. Invertebrate identifications are compiled on hard-copy laboratory sheets and then entered into the data management system. The hard-copy laboratory sheets are scanned as a back-up and stored at each regional laboratory.

Identified macroinvertebrates are stored for 5 years after collection, with alcohol preservative levels checked annually. All collections have metadata stored with the collection that decodes each site code into site name, state, county, lake, and latitude and longitude. The metadata also includes information about the program, the PI in charge of the collection, and where to locate more information. These 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 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 PI (Uzarski) and EPA GLNPO, and if no other co-PI is interested in maintaining it. To properly dispose of samples, ethanol should be drained into hazardous waste containers and disposed of following institutional hazardous waste procedures.

BD4. Analytical Methods Requirements

All sampling point locations are 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 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 are 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 has been provided to all identification laboratories. Labs that do not have the expertise to identify particular taxonomic groups are either provided with appropriate training (and certification) or they send those particular taxa to other program labs for identification by qualified individuals. Target turnaround time for macroinvertebrate identifications is 6-7 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 macroinvertebrate samples are collected within each vegetation morphotype. However, our goal with this sampling is to maximize rather than minimize variability to ensure that we are collecting the majority of types of aquatic invertebrates that inhabit each plant morphotype.

What is important for QC standards in this case is the precision with which field crews have captured the target number of macroinvertebrates for each replicate (50, 100, or 150 organisms). Thus, we compare field macroinvertebrate counts of each replicate to the number of invertebrates identified in the laboratory over the winter. If these numbers (number field-picked vs. number identified) differ by more than 25%, field data sheets will be checked for accuracy of field count and identifiers will make notes on probable causes for the discrepancy. If counts differ by >50%, macroinvertebrate PIs will examine these samples and determine whether or not data can be used.

Precision in the field should also pertain to similarity of sample collection by field crew members and similarity in picking of macroinvertebrates. In particular, new crew members must be trained to ignore microcrustacea and to only pick within their grid cell and not be distracted by moving invertebrates in other grid cells.

Accuracy: Accuracy is determined by difference from a reference standard or an expert. This is assessed by exchanging macroinvertebrate samples between adjacent identification laboratories. This QC exchange is done "blinded" so that the laboratory receiving a sample does not know the number or identity of the invertebrates in the sample. All discrepancies in identification are investigated and corrections made to data as appropriate. In addition, all discrepancies trigger retraining of identifiers. The target is at least 95% similarity in identifications across labs. Macroinvertebrate exchanges between adjacent labs are done yearly with this QC overseen by QC managers Brady and Cooper.

Bias: Systematic bias by a crew or method. Bias by crew members is be assessed during the mid-year QC check by observing sample collection and invertebrate picking (see mid-year QC check, below, this section). Systematic bias among crews is assessed during the lab exchange of samples. Because so little of a site can be sampled, only quite common taxa will be collected by this sampling method. 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 is calculated for all plant morphotypes sampled. Each morphotype must have at least 2 replicates for the data from that morphotype to be included in the database.

Representativeness: How well sites were sampled is 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 is done for all sites.

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

samples among adjacent labs to determine accuracy, comparability, and potential bias in identifications (see Accuracy, above).

Sensitivity: In this case sensitivity refers both to the lowest taxonomic levels achievable, and to the ability to detect uncommon taxa. Identification depends 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 are identifiable when field crews are sampling. Crews are trained to minimize damage during preservation of organisms. Uncommon taxa are not likely to be detected because of the small area actually sampled for each site. Detection of uncommon taxa is not the goal of this monitoring program.

QA/QC specifics: All members of the program team responsible for invertebrate sampling and sample processing 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 greatly reduces the need for corrective action. When taxonomic inconsistencies are discovered, team members with expertise are consulted. If they are unable to resolve the taxonomy, then regional or national experts are consulted. Team members working on invertebrate taxonomy use conference calls, webinars, and e-mail to share taxonomic information. After taxonomic issues are resolved, database queries are written to identify other samples that contain the questionable identifications, and those specimens are pulled and re-examined. Additional mid-year QA/QC checks also ensure each laboratory's macroinvertebrate data quality (below).

Individuals Responsible For Macroinvertebrate Sampling and Processing QA/QC:

Western Great Lakes	Valerie Brady/Holly Wellard Kelly
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)	Kathryn Amatangelo/Greg Lawrence
Eastern Great Lakes (CA side)	Jan Ciborowski/Joseph Gathman/Giuseppe Fiorino/Ian Smith

Mid-Year QA/QC Checks:

Macroinvertebrate field sampling: Regional team leaders and co-PIs 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 is assessed for each individual working on macroinvertebrate samples, and notes and scores on their performance rating become part of the permanent record for this program. The program QA manager and assistant manager provide guidance during the checks, provide oversight on the checks, and receive the QC reports from team leaders/co-PIs.

Performance criteria 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 are implemented if performance criteria are not met (see section C1).

Macroinvertebrate laboratory processing: Macroinvertebrate sample processing performance is 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 are accomplished by having laboratories exchange invertebrates from two vegetation zones with each other for blinded re-identification. Distribution of QA/QC samples across laboratories is coordinated by the program QA manager and/or assistant QA manager.

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

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

BD6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements
D-frame nets are inspected before and after sample collection for holes or other damage. Damaged nets are repaired in the field, and replaced at the end of that sampling trip as necessary. Each crew carries one replacement net with them at all times. Crews 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 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 are carried for the GPS units, cameras, and water quality meters.

Water craft, trailers, and sampling gear are subjected to rigorous disinfection to eliminate the transfer of nonnative and invasive 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 one site and the next), or before moving between lakes. Recommended disinfection includes taking the tow vehicle, boat and trailer to a car wash and hosing them and

the sampling equipment with hot soapy water. Use of Vircon Aquatic is also effective at killing many invasive species and is recommended for netting.

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 organisms. Some states (e.g., Minnesota) now require that all boats must be trailered with their drain plugs out. All state laws on AIS decontamination must be observed.

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 are 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 are uploading GPS readings nearly daily. At least once per week, GPS points are plotted on aerial photographs of a sampled site and the image is presented to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season is also checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results are 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

Please refer to CWMP fish sampling SOP for greater detail and step-by-step instructions, available on the CWMP website: https://www.greatlakeswetlands.org/Sampling-protocols.

BE1. Program Design
Upon arrival at the site, the field crew first determines whether or not the site is sampleable based on the following criteria: safe access, open connection to the lake or connecting channel, site is an emergent wetland, 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 is recorded on the space provided on the field sheet and with pictures.

If the site is sampleable, additional general information about the site is 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 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 macroinvertebrates collaborate to collect this information and complete the field sheet and map.

Fish are sampled from the major plant morphotypes in each wetland. Sampling begins 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 development. This is the interval during which emergent plant communities generally achieve maximum annual biomass. In most cases, fish are sampled at the same time that macroinvertebrates are sampled. Therefore, this section duplicates portions of the macroinvertebrate section (BD), especially regarding plant morphotypes and net placement. Note that there are some sites or plant morphotypes within a site that cannot be sampled for fish because of inappropriate depth, but that are sampleable for macroinvertebrates and water quality as well as the other data categories.

Fish sampling is stratified by plant morphotype. Plant morphotypes are patches of vegetation in which a particular plant type or growth form dominates the plant community based on visual coverage estimates. Our sampling design is predicated on our previous data that show that which fish are found in wetland vegetation tends to vary by vegetation morphotype, plant density, and depth. Since depth also affects which plant species grow, this is a secondary consideration to vegetation morphotype. Thus, all fish sampling hinges on the dominant aquatic vegetation morphotypes found in a wetland. Note that other species or growth forms will likely occur within a given plant morphotype; however, the plant type should be near monodominant and comprise at least 75% of the emergent or floating-leaved plant community. Plant morphotypes include *Typha* (cattail), *Nuphar-Nyphaea* (water lily, combined), *Schoenoplectus* (bulrush, include both dense and sparse stands 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 vegetation (no dominant emergent plant type, but a mix of multiple emergent species), open water (special cases only, as outlined in section A8), and potentially other types such as floating bog mat. The mixed emergent plant morphotype may be sampled if it represents a significant and conspicuous habitat type and there are not three other monodominant morphotypes to sample. Additional morphotypes 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 morphotype is required because GLCWP IBI metrics were formulated for specific plant morphotypes.

The original fish IBI of Uzarski *et al*. (2005) relied primarily on bulrush, water lily, and cattail-dominated morphotypes, and these morphotypes are sampled if present. *Schoenoplectus* stands are divided into or categorized as sparse and dense areas when the outer (lakeward) edge of this plant stand supports low stem density and the more shoreward zones, which are sheltered from wave action by the outer zone, support dense *Schoenoplectus*. In high lake-level years, inundated wet meadow 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 1.2 m. Therefore, plant morphotypes with depths ranging from approximately 5 cm to 20 cm are sampled for macroinvertebrates and water quality but not for fish.

Plant morphotypes are identified by field crew leaders once the field crew arrives at the site. The minimum area for plant morphotypes to be sampled is 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.

Three replicate fyke nets are set in each inundated plant morphotype to ensure good representation of fish using this morphotype. Replicates are traditionally taken to provide a measure of variability. But in this case replicates are also used to increase the amount of each plant morphotype's area that is sampled to better capture the diversity of fish species using that morhotype as habitat. Within each plant morphotype, 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 morphotype.

The three fyke net locations are selected to represent, to the degree possible, the variability in the plant zone. In large plant morphotypes, 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 a sparse *Schoenoplectus* stand extends along the shoreline lakeward of a cottage, a forested area, and a wet meadow, sampling locations are chosen to correspond with these different features. However, in many cases, a given vegetation morphotype does not cover enough area to be associated with different shoreline features, in which case sample locations are chosen to represent, to the degree possible, the variability of the morphotype itself, which increases the likelihood of representing variability in the fish assemblage of this habitat. GPS waypoints are created for each fyke net location.

Because macroinvertebrates, fish, and water quality are sampled in each plant morphotype, 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 are collected first at each location before any movement is made around the point. 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 disturbance of the area to prevent contamination and suspension of sediment. Substrate/sediment may be disturbed by the boat motor; crew exiting the boat; use of poles, paddles, or other items that touch the substrate; and uprooting of vegetation. Crew are trained to approach each sampling point in a manner that avoids substrate disturbance, as well as how to recognize when substrate disturbance has occurred. Water quality data and water samples are typically collected from the bow of the boat before anyone enters the water and with the engine off. After water quality sampling has been completed, macroinvertebrate sampling and fish net deployment can commence.

Fish communities are passively sampled using fyke (i.e., trap) nets deployed overnight. At each wetland, three fyke nets are deployed in each fishable plant morphotype. A 'fishable' plant morphotype is one of the plant morphotype or types described above with water depth between 20 and 120 cm. Shallower water cannot be effectively fished with the fyke nets using GLCWP methods.

The fyke nets used by all crews 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 is conducted using three replicate fyke nets in each plant morphotype 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 are 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 – 120 cm. The depth of water in each plant stand dictates net size used since the main difference between large and small nets is height. Nets are placed with the mouth opening perpendicular to the vegetation stand of interest, with leads extending from the center of the mouth of the net into the vegetation. The goal is to catch fishes that are using (inside) the plant morphotype, rather than those moving along the edges of the plant morphotype. Wings should be set at 45° angles to the lead and connected to the outer opening on each side of the net, when possible. In very large plant morphotypes where setting the trap outside of the patch is not possible, nets should be set with the lead pointing toward shore. In dense stands of vegetation where the wings cannot be set at 45 degree angles (e.g., cattail, *Phragmites*), wings should be set at 90 degree angles or so as to block fish that are swimming along the edge of the morphotype from entering the net so that only fish from within the morphotype are captured. 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 are set for one night (at least 12 hr), after which time they are emptied. The trapped fish are identified to species, counted, measured, and released alive. The times of net deployment and recovery are 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]) are measured to the nearest mm (total length). When individuals are counted, separate tallies are made for YOY vs. older individuals of each species. Fish less than 20 mm in length are not counted because the sampling gear does not accurately capture fish this small. All fish are handled following university wildlife use and care guidelines, and an approved program-specific plan is in place at each regional laboratory before sampling begins each year. Appropriate state and provincial permits are also obtained before sampling begins.

All crews 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 (small fish) or high-quality digital photographs (medium size and large fish) are returned to the laboratory for identification, with assistance from experts when necessary. Photographs are used for medium size and large fish or where collection of fish samples is not permitted by law or regulation. When digital images are used, the putative species name (if known), total length, site ID, plant morphotype, date, regional team code, and crew leader's name will be included in the image. Additional photographs of key features are taken to aid identification of unknown specimens. These pictures are uploaded to the database as a record of identification. Each fish sampling crew maintains 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. When species are encountered that are not part of the lab's collection, a voucher specimen should 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. As with the other indicators, ongoing dialogue among program partners ensures consistent taxonomy and minimizes 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 morphotype are determined by the field crew leader not to have representatively sampled the plant morphotype 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 morphotype, 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 3 of each size of the nets specified above, and 6 of each size is recommended. Also required are a boat, trailer, and towing vehicle.

BE3. Sample Handling and Custody

Fish that cannot be identified in the field are preserved or photographed for laboratory identification, depending on fish size and state, provincial, and federal regulations. Fish are humanely euthanized following university animal use protocols for wild animal specimens. Specimens are initially fixed in 10% buffered formalin in plastic containers labeled with both internal and external water- and preservative-proof labels (site ID, plant morphotype, fyke net number, unknown fish ID number, regional team code, and field crew leader name). Whenever unidentified fish specimens are kept or photographed, a note is made on the field data sheets that includes the sample number (if preserved) or photograph number. Samples remain in the custody of field crews for the duration of each field trip. At the laboratory, unidentified fish specimens are 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. Photographs of fish used for identification are also uploaded into the data management system to allow for later verification by other fish experts and provide a record of identification. If a specimen is donated or lent to a museum because of its taxonomic or distributional value, records of its disposition are kept by the regional laboratory and entered into the database. All collections are stored with the collection metadata that decodes each site code into site name, state, county, lake, and latitude and longitude. The metadata also include information about the program, 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 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 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 are saved as waypoints using recreational GPS. In case of GPS equipment failure, the fish crew should borrow a GPS from the vegetation crew to finish the site, if possible, and 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 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 are not 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 are used to try to capture a good representative sample of the species using the plant morphotype and are not expected to be duplicates of each other. It should be noted that variability among samples, even within the same morphotype, is expected to be relatively high due to the high natural variability of fish assemblages using these habitats. However, to maintain continuity of effort, fish net replicates should be set for relatively similar amounts of time. Any nets whose set time (time fished) is more than 50% greater or less than the other nets for that plant morphotype should not be used (i.e., data discarded). In addition, at least 2 replicate nets must be considered to have "fished" for each plant morphotype for the fish data from that morphotype to be considered valid for use and data entry.

Accuracy: The systematic difference from a reference standard or an expert. This refers to both the accuracy of fish identification and the accuracy and correctness of net placements. Both will be assessed during the mid-year QC check (see below, this section). Identification of fish is expected to be 100% for a combination of: 1) identifying commonly-encountered Great Lakes fish old enough to identify and 2) knowing when to preserve or photograph fish whose identity cannot be confirmed in the field. Failure to achieve 100% means that the person cannot identify fish by themselves and must have all of their identifications checked by someone who has passed these tests. Every crew pulling nets must include at least one person who is at this level (most crews have several members at this level). Correctness of net placement is also expected to achieve 100% accuracy. This is a crew test as less experienced members will continually be encountering new situations and will need to rely on the expertise of more experienced crew. Crews that have no experienced members must text photos of the net sets at their first few sites to their team leader or crew chief for a second opinion until they achieve 100% approval on sets in 3 morphotypes.

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). Bias in net placement must also be guarded against and will be checked by the regional PI during the mid-season QC check.

Completeness: Calculated as % complete = (# useable sample pts)/(# planned pts) x 100. Sampling completeness will be calculated for all plant morphotypes sampled. At least 2 of the 3 nets set for each morphotype must be considered to have "fished" (see criteria above) for the data for this morphotype to be considered valid and entered into the database. Some morphotypes contain very few fish because of their water chemistry (low dissolved oxygen) or plant structure. If after two attempts (2 nights of net sets), too few fish have been caught in nets for valid sets, the regional PI must be consulted by the team to determine if the issue is with the sets (the crew) or the type of habitat (in which case, not finding many fish is the true condition of this habitat type).

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 and plant morphotypes.

Comparability: Data comparability among crews within the program 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). The fish IBIs were created to be robust to these 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 program team who are sampling fish are proficient in fish identification. Therefore, all individuals working independently on fish sampling are 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 ensure data quality. Reference collections are maintained by each laboratory that is independently collecting fish data. Voucher specimens for each observed species are preserved in 10% buffered formalin and then transferred to 70% ethanol for the duration of the program. For species in which observed specimens exceed 20 cm, fully documented digital images stored in multiple locations (including the database) are 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)	Matt Altenritter/Kathryn Amatangelo
Eastern Great Lakes (CA side)	Giuseppe Fiorino/Ian Smith

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), but the person doing the QC check will note which crew members are not proficient at identifying fish and confirm with the crew leader that the identifications made by these individuals are always double-checked. The program QA manager and assistant manager 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 nets (satisfactory/unsatisfactory; 100%)
- Correct setting of fyke nets (satisfactory/unsatisfactory; 100%)
- Accuracy of species-level IDs in the field/laboratory (95% for individuals; 100% for team)
- 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 are examined for holes and defects before and after each net set. The occurrence of holes that might have compromised net integrity during a set are noted on field sheets, and if such holes were substantial and affect 2 or more nets per vegetation morphotype, the nets are repaired and re-set. Crews carry net repair kits with them and 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 are used by field crews as necessary. Spare parts carried by crews 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 are carried for the GPS units, cameras, and water quality meters.

To prevent the spread of invasive species, including disease, boats and trailers are drained and inspected upon haul-out while still at the boat launch. Watercraft, trailers, and sampling gear undergo rigorous disinfection to eliminate the transfer of nonnative or invasive 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 one site and the next triggers disinfection protocols), or before moving between 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. Vircon Aquatic is also effective at killing many invasive species and is recommended for netting. No water will be transferred from one Great Lake or 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 organisms. Some states (e.g., Minnesota) now require that all boats must be trailered with their drain plugs out on penalty of fines for violators. All state AIS decontamination requirements must be followed.

BE7. Instrument Calibration and Frequency
Recreational GPS accuracy (+/- 10 m) is sufficient for these data. GPS receivers are tested prior to and after the field season by taking repeated readings at a location visible on aerial photos, such as a parking lot with lines. Accuracy is assessed by plotting GPS waypoints on a georeferenced aerial photo of the test location (e.g., the parking lot). During the field season, field crews upload GPS readings at least weekly. At least once per week, GPS points are plotted on aerial photographs of a sampled site and the image is presented to the field crew to verify that points appear to be within +/- 10 m of the actual sampling locations. All tests and results are 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. Program 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). 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 program has been built on 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 was built on protocols contained in the GLCWC Monitoring Plan (with the exception of certain modifications described in this document), but additional data have been 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) so that all these large datasets are compatible with each other and can be leveraged.

Chin *et al.* (2015) compared the index of ecological condition (IEC), developed by Howe *et al.* (2007) and Gnass Giese *et al.* (2015), with the index of biotic integrity (IBI) developed and used by the GLCWC (2008; Crewe and Timmermans 2005). The analysis used a similar avian data set acquired by the Marsh Monitoring Program (Tozer 2016). Although the two indices (as well as a third index based on a generalist-specialist designation of bird species) were modestly correlated, significant differences were observed in the ranks of individual wetlands across the range of calculated values. Chin *et al.* (2015) concluded that the use of indicator metrics clearly affects the assessment of wetland quality or condition. The IEC approach was sensitive at both the unimpaired as well as the impaired ends of the disturbance spectrum, whereas the IBI approach was sensitive only at the unimpaired end of the spectrum. Since wetland managers and restoration project teams are interested in both highly disturbed and minimally disturbed conditions, the IEC approach is desirable. However, Chin *et al.* (2015) also pointed out that the IBI method of Crewe and Timmermans (2005) has practical advantages because it encourages wetland monitoring of a selected subset of wetland dependent species. This is especially important for community science programs where field data often are collected by non-scientists.

Our current wetland metrics (Tozer *et al.* 2021, Howe *et al.* 2021) for birds and anurans apply lessons from both the IEC and IBI approaches. The analytical framework for calculating indicator metrics continues to follow the IEC approach, which is flexible and capable of employing multiple (and explicit) disturbance gradients. However, we also have incorporated elements of the IBI approach by limiting the analysis to wetland obligate or wetland facultative species, thus reducing the potential for spurious influences that are not strictly related to wetland quality. More recently, we also have demonstrated that geographic variation in bird and anuran distributions can lead to biogeographic biases that are unrelated to wetland quality. We have attempted to resolve this issue by calculating regional IEC values that use only species that are expected for a given lake or combination of lakes. This effort recognizes 4 distinct regions in the

Great Lakes Basin: Lake Superior, northern Lakes Huron and Michigan, Lake Erie and southern Lakes Huron and Michigan, and Lake Ontario.

Continued assessment of the data will be important for refining our indicator metrics given the large degree of variation due to changing water levels, continental changes in bird populations, and other factors.

BF2. Sampling Methods

Breeding anuran and bird populations are sampled in Great Lakes coastal wetlands using GLCWMP protocols that are regularly vetted for efficiency and effectiveness and which are coordinated as much as possible with the Great Lakes Marsh Monitoring Program delivered by Birds Canada.

Anurans

For anurans, unlimited-distance point counts are used to identify presence and calling intensity of species within each wetland. During a 3-minute sampling period all anuran (frog and toad) vocalizations are recorded on datasheets as occurring within or outside a circular sample area centered on the prescribed sampling point (Weeber and Valliantos 2000, GLCWC 2008). Spatially-explicit records are mapped on a standard data form in order to differentiate the direction of calls from the observer. Field samples for each wetland site 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 are located according to the following criteria: 1) points that have been previously sampled within the wetland are selected assuming the locations are consistent with other considerations below, 2) points are 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 are placed in different patches of wetland habitat. Sites are 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 are 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 are dependent on phenology and general weather conditions. In southern regions, anuran counts can generally be initiated in early April, but are later in the northern regions. Some of the survey sites are 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 is recorded on the datasheet 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 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 is set and the surveyors sample for three minutes. Records are 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) is recorded (mapped) on the standard data form. For codes 1 and 2, the number of individuals heard is estimated. Abundance is not recorded for code 3 since there are too many individuals calling to accurately estimate numbers for code 3. Data are entered into the program database after appropriate verification and QA/QC checks.

Birds:
For birds, we 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 sampling points are the same points used for anurans; however, additional points (i.e., 250 m apart) can 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 are sampled. Sample locations are dependent on site accessibility. All species are 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) is recorded within 1-min sub-intervals across the 10-min survey. Focal species are those on which GLCWP 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 is explicitly noted on the survey form.

Tozer *et al.* (2017) estimated the influence of survey duration on detection probabilities of 14 marsh breeding bird species using data from 23,973 point counts conducted throughout the Great Lakes-St. Lawrence region. The analysis was based on data collected during 2008-2016 by the Great Lakes Marsh Monitoring Program as well as the Great Lakes Coastal Wetland Monitoring Program (CWMP). The authors observed small but significant increases in detection for 7 of 14 species based on 15-min compared to 10-min surveys and concluded that 10-min surveys are superior to 15-min surveys because modest gains in detection of some species does not warrant the additional effort. Further analysis of 3,457 CWMP surveys conducted during 2011-2019 showed that 92% of observations used for calculating wetland bird-based indices of ecological condition (IECs) occur during the first 10 min of point count surveys. IECs were identical based on 10-min or 15-min surveys in 78% of cases, and overall, IECs based on 10-min

surveys differed from those based on 15-min surveys with a mean absolute error of 0.4 (SD = 1.3). Based on these results, we shortened the duration of CWMP bird surveys from 15 min down to 10 min starting in 2020. We are investing saved time by surveying more points wherever possible in sampled wetlands, which will increase the accuracy of IECs at the wetland level and the statistical power of analyses at broader scales, such as at the lake or regional level.

Point count surveys are 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 is 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 are surveyed twice per breeding season, with a minimum of 15 days between visits, unless unforeseen circumstances prohibit return visits. One count is in the morning and one count is in the evening.

Bird and anuran data are considered highly desirable at wetland sites that are also sampled for invertebrates, fish, and vegetation. Ideally, data are collected for each of these groups in at least 75% of wetlands surveyed. However, wetland sites that bird and anuran crews cannot access are not dropped from the database.

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

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 are 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 have audio labels at the start and end of each recording and are will be archived in the data management system.

BF4. Analytical Methods Requirements

Performance criteria: All bird and anuran data are recorded on datasheets at the species level. All staff for this group are 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 are reviewed by the regional team leaders and a determination of acceptance is completed within two months after the end of sampling.

All sampling points are saved using recreational GPS. Recreational GPS accuracy is sufficient for these data. GPS receivers are 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 are uploading GPS readings at least weekly. At least once per week, GPS points are plotted on aerial photographs of a sampled site and the image is presented to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season is also checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results are logged and the logs kept with the appropriate GPS units. In case of GPS or recording/playback equipment failure, the crew will notify their regional laboratory of the need for a replacement unit. Crews 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. Two observers per point listening to and observing the same species ensures precision of the data collected. During field training, pairs of observers compare their independent species identifications made while standing at the same point. Acceptance criteria is 95% agreement in species identified and categories of numbers of individuals. Any discrepancies in species identification are discussed with trainers. The same assessment occurs during mid-season QC checks. In addition, all observers have to pass tests to ensure that they can hear the required frequencies of bird and anuran calls.

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) and by having recorded calls at the QC sites verified by experts for 2 sites/yr in each regional area. The performance standard is 95% accuracy on identifications and 100% accuracy on knowing when to have identifications checked and record calls (if possible) for expert assistance.

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 is calculated for all sites and depends on whether the target number of visits was achieved for each point for each taxonomic group (birds or anurans, evaluated separately) during appropriate weather conditions, as well as if the visits were within the breeding time window and collected from appropriate habitat. For each taxonomic group, each site must have the majority of its sampling points sampled correctly (meeting the listed criteria) for the data to be included in the database. Because sites have differing numbers of points (1-6) based on site size, the best criteria that apply to all sites is >50% of sampling points meeting these criteria.

Representativeness: How well sites were sampled is 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, particularly distance between the observation point and the edge of the wetland polygon. How well the sampling dates conform to the breeding window for these taxa is checked. This is done for all sites.

Comparability: Data comparability among crews within the program and to other non-project data is 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 depends on the ability to see/hear species, which is partially weather and surrounding-condition dependent. Crews note on sample sheets conditions that limited their ability to hear taxa at sites (e.g., windy, road noise, etc.). Crew members also have passed identification tests that also test their ability to hear high frequency calls. 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 program 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) to insure that target species are included in the visual and audio tests. All field researchers are required to demonstrate proficiency in visual and audio identification of wetland birds and anurans to insure quality control. Field researchers are required to demonstrate their knowledge of the survey protocols prior to field activities (see section A8). The program 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) are

provided to field teams, and audio recordings are made at selected sites to test for accuracy of the field samples. Use of GIS data sources also provides precise locality information. These aspects of the results (species identifications and locality data) are the major targets of data quality control, plus appropriate attention to weather conditions and phenological timing of optimum counting periods.

The PIs coordinate data validation among the different components of the study.
Quality control of point data includes 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 require 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 has been provided to each field team.

Individuals Responsible for Bird/Anuran Sampling:

Western Great Lakes	Bob Howe/Annie Bracey
Central Great Lakes (US side)	Tom Gehring/Bob Howe
Central Great Lakes (CA side)	Doug Tozer
Eastern Great Lakes (US side)	Greg Lawrence/Kathryn Amatangelo
Eastern Great Lakes (CA side)	Doug Tozer

Mid-Year QA/QC Checks:
Anurans and Birds— Bird and anuran PIs evaluate accuracy and correctness of sampling crews at field sites or by analysis of audio recordings collected from the field, with the program QA manager and assistant manager providing oversight on the results of these checks.

Performance criteria include:
- Correct location of sampling points (100%)
- Accuracy of species-level IDs (95%)
- 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 triggers corrective action (see section C1).

BF6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements
The most important field equipment used by researchers in this program is 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 is tested prior to use to insure the proper functioning of the equipment. For example, multiple tests of GPS waypoints at various locations ensure adequate functioning of the GPS receivers. The database of GPS readings at this standardized point permits quantification of the expected error in locality of data collected during the study. Spare batteries for all units are 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 may be available from the nearest regional team laboratory.

BF7. Instrument Calibration and Frequency
Recreational GPS accuracy is sufficient for these data. GPS receivers are 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 are uploading GPS readings at least weekly. At least once per week, GPS points are plotted on aerial photographs of a sampled site and the image is presented to the field crew to verify that points appear to be reasonably accurate. Accuracy during the field season is also checked *a posteriori* by comparing latitude/longitude at easily recognized localities (e.g., road stream crossings) with GPS readings. All tests and results are logged and the logs kept with the appropriate GPS units.

Broadcast equipment is tested to ensure that 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 are 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 use are the data we used to set up the site selection system and land use measures to inform our stressor gradient for use in 3 of our indicator calculations. Wetland polygons were initially based on the Albert and Simonson (2004) Great Lakes coastal wetland GIS coverage. Use of this database for site selection was mandated in the original RFP setting up the Great Lakes Coastal Wetland Monitoring Program (then project) and by the necessity of following the GLCWC sampling protocols. Other data used for site selection include wetland type (Albert *et al.* 2006), the stressor gradient (Danz *et al.* 2005), and GIS background layers (roads, boat launches, land use, etc.) from state and federal agencies. These datasets are used only 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 are 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.

Land use data for calculation of the land use and water quality indicator and bird and anuran IEC indicators comes from the North American Land Change Monitoring System (NALCMS) 2010 land cover of North America at 30 meters (from Landsat imagery) (CEC 2015). The NALCMS is a collaborative initiative by agencies across the U.S., Canada, and Mexico to monitor land cover change, and it is produced by the multi-national Commission for Environmental Cooperation (see citation for partner organizations). These land cover data are published and provided as a tool for researchers and meet the QC standards of the associated agencies, including the U.S. Geological Survey. Because the NALCMS includes land cover for both the U.S. and Canada, it standardizes the land cover calculations across all CWMP sites.

Sources for external datasets of all types are tracked. Most external data are from peer-reviewed publications and GIS sources with established metadata lineages – these lineages are maintained. In rare cases in which unpublished data are used, appropriate metadata are generated to describe its origin. Any non-peer-reviewed information, such as the aerial photos, used during the program are visually checked for accuracy and proper identification.

B10. Data Management

In the field, data are 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 contain the following basic information: site code, date, start and end time, weather and air temperature, regional team code, all names of sampling crew members, and a signature of the person making the entries. Whenever a sample is collected, a detailed description of the location is recorded. This includes GPS coordinates and waypoint number, sampling equipment used, date, and water depth. Any visual observations are logged along with number of containers and sample identification number for all samples collected. Audio recordings have an audio tag with the same information and the existence of these recordings is noted on field data sheets. Any field duplicates receive an entirely separate identification number. Unknown fish and plants are labeled with a collection number, which is included on the sampling form. All completed data sheets are kept in a secure location by field crews and photocopied and/or scanned at the earliest opportunity. Photocopies are archived in a separate location from the original data sheets.

A secure, password-protected online data management system (DMS) has been developed specifically for this program. 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 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 DMS

can archive all photo records taken by field crews. The DMS is being expanded and updated to archive bird audio recording files.

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 helps ensure the system's usefulness to future researchers, managers, and the public. To accommodate requests for raw data, the DMS can export the data in Microsoft Excel spreadsheet (*.xlsx) and Microsoft Access database (*.mdb) formats. Export files are generated by the DMS each night for all key datasets, and users can also request customized versions of the spreadsheets and databases (e.g., including filters for site and year). Finally, on a semi-annual basis, a comprehensive Access database version of the master database is generated and delivered to EPA and its subcontractor. These exported files give the CWMP dataset an indefinite digital shelf life without any dependency on the infrastructure used for this particular program.

The DMS uses 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, and full and incremental snapshots are taken and stored on a local backup server at CMU. Incremental backups are performed nightly and stored at secure locations (on premise and offsite). End-to-end encryption is used for any data that are copied or moved from the backup server to an off-site location. Nightly backup email reports are generated and sent to appropriate CMU IT staff for monitoring purposes. Full system or volume restores can be performed via the previous full snapshot as required. Incremental backups are maintained in the time intervals between full snapshots, which allows for the immediate recovery of folders and individual files in the case of accidental deletion or hardware failure. Additional backup measures for individual components of the DMS are managed by LimnoTech and include: 1) a full nightly SQL backup of the PostgreSQL database and upload to a secure cloud storage service (Sharefile), and 2) storage and source code version control for the CWMP web application in a GitHub repository.

Specifics:
The DMS, including the web application, database, and a geospatial server, are currently hosted on a Windows Server 2019 virtual machine managed by CMU's IT staff. The hardware specifications of the server include an Intel® Xeon® E5-266 v3 @ 2.30 GHz CPU, 8 GB of RAM, and 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 is provided through a Microsoft Active Server Pages .NET (ASP.NET) web application that 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 requires 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., has been instituted. Corrective actions include replacing lost data whenever feasible (e.g., field crews are still on-site or in the vicinity), particularly if the problem would result in a large loss of data for the site or omitting from the database any suspect data that cannot be replaced. Conference calls or webinars with PIs are 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 have been 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 is available at all times when sampling crews are in the field to help deal with questions and problems as they arise. Our experience 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 are 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 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 triggers 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 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.
	Collection equipment rinsing protocol performed properly	Additional training/re-certification of field crew Rinsing protocol re-performed before samples collected. If samples were collected, samples are discarded. Samples re-collected appropriately if crew is still on-site or in the vicinity.
	Samples collected from appropriate locations within a site	Additional training/re-certification of field crew Re-collection of samples if crew is still on-site or in the vicinity.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
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.
		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.
Invertebrates	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 or samples re-collected correctly if crew is still on-site or in the vicinity.
	Habitats are correctly and thoroughly sampled	Additional training/re-certification of relevant field crew members
		Field forms flagged for under- or over-sampled sites or samples re-collected correctly if crew is still on-site or in the vicinity.
	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 or samples re-collected correctly if crew is still on-site or in the vicinity.
	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; removal of data from the database where labeling confusion cannot be fixed.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
Fish	Plant morphotypes 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. Leadership determination of whether data must be excluded from database.
	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. . Leadership determination of whether data must be excluded from database.
	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. Exclusion of fish data where IDs cannot be confirmed or corrected.
	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 Exclusion of fish data where IDs cannot be confirmed or corrected.

Table C1.1. (continued).

Protocol	Performance standard	Corrective actions
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
		Data sheets are corrected, if possible, or flagged.
		Data from inappropriate sampling locations is removed from database or quarantined for research use only.
	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. Data are removed from database in severe cases.
	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. Data are removed from database in severe cases.
	100% of field survey forms are completed correctly	Additional training/re-certification of field crew members

Any corrective actions affecting data integrity are noted and included in our semi-annual reports to GLNPO.

C2. Reports to Management

Annual program PI meetings cover the following general topics: overall program 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 meetings and numerous email communications from the management team keep co-PIs working in close collaboration and solving any problems as they become known.

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

the QA managers are responsible for notifying regional team leaders in the event that reports are overdue. During these checks, when PIs are out in the field with their teams, crew leaders and members are encouraged to discuss how the sampling season is progressing and trouble-shoot any methods or techniques that are creating difficulty for the crew. This is particularly important during times of very high or very low water levels as crews adjust to these situations.

The semi-annual report to EPA is written by regional team and taxonomic team leaders in collaboration with their co-PIs. The report is compiled and edited by the management team, and is submitted by the program leader (Uzarski). The report includes a program status update, a discussion of QC problems and their solutions and corrective actions, results of QC checks, and a summary of results to date. Reports are distributed to everyone involved with the program and are posted on the program website. Any issue that may have a major impact on program outputs will be reported to the EPA program officer immediately. The program officer is also invited to all annual PI meetings to help ensure close communication with EPA. Numerous additional webinars and conference calls take place on an *ad hoc* basis as PIs and teams communicate and coordinate with each other.

QC audits on field season activities take place at the end of each field season for field data collection activities and are conducted by the PI or field crew chief, if the crew chief has several years of experience with the program. The field season audit covers completeness of sampling activities at each site for all program elements. During the field season, while crews are sampling, crews are directed to check data sheets before they leave a site to ensure all data have been collected and crew leaders audit the data sheets at the end of each day to see if anything was missed while the crew may still be close enough to the site to go back and collect the data. This end-of-season audit is one more check on data completeness and allows PIs to determine how to deal with sites for which some data are missing.

Other elements of the field season audit include sampling gear status and summarization of field crew debriefings (crew leader and crew members). Field crew debriefings at the end of the field season reflect on the season’s sampling, what went well and what did not, where improvements could be made, and where safety could be improved. Field data QC includes 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. PIs inform the QA managers of data and QC issues that cannot be resolved and/or that affect site data integrity (e.g. sites with incomplete data). QA managers ensure that data anomalies are flagged or removed from the data management system.

QC audits on data entry and data management occur in the early spring after most data have been processed over the winter and entered into the database system. Data audits assess completeness of checks for data entry errors; investigations into data 'flags' in the database, how these are being resolved, and identification of data that 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. QA managers work with the database manager on these audits and resolving issues, with inclusion of co-PIs as necessary. Issues that cannot be fixed or resolved are flagged, quarantined or completely removed from the database, depending on the severity of the issue. EPA is notified of flagged issues when they are provided with the data to ensure they watch for the data flags.

A summarization of the results will be developed by collaboration among all investigators involved with this monitoring program in conjunction with the US EPA program 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 PDF (or other suitable format) within 90 days of the end of the contract period. This report will include an 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. The report will be distributed to everyone who is part of this program and will be made publicly available after review and approval by GLNPO.

D. DATA VALIDATION AND USABILITY

D1. Data Review, Validation, and Verification Requirements

All data entry is subject to a 100% QC check, either by a second person (fish, macroinvertebrate, vegetation, habitat and water chemistry data) or through dual entry with a 100% match validation comparison (bird and anuran data). The review and validation steps below are over and above this 100% QC of data entry of field and lab data.

Quantitative data: After data are entered into the data management system and QC'd for data entry errors (100% QC), the data are checked for out-of-range values. In other words, the data are 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 are double-checked for data entry errors. Checks 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 include confirmation 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 are flagged and notes included on the data checks that have been done. These data flags allow researchers and others using the data 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 the results.

Qualitative data: Review of the data is the responsibility of the principal investigators, who devote at least a portion of the post-field season to this effort. Information from existing data

sources as well as experience of the principal investigators is used to 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 nearby sites (from this sampling program or from prior datasets) or from knowledge of the natural history of the taxa. Background knowledge about these taxa play an important role in data review, although unexpected but potentially significant observations are not dismissed without follow-up discussion. The lead PIs for each taxonomic group check the taxa lists for uncommon taxa and those that are rarely found in the Great Lakes region. These identifications are verified, if possible, or flagged as suspicious, if verification is not possible.

Indicator calculations: Below we describe how all metrics and indicators are calculated. Metric and indicator calculation is the end goal for all of this data collection. Please note, reference sites are not required when calculating IBI scores. Sites classified as reference and impacted sites were used in the original development of our IBIs (e.g., Cooper et al. 2018, Uzarski et al. 2004). At that time, these sites were selected based on water quality, surrounding land use, and other known anthropogenic disturbances. During original development, the individual IBI metrics were selected and calibrated based on these gradients of human disturbance. Now, IBI scores for fish and macroinvertebrates are calculated without the need to have sites classified as reference or impacted *a priori*. Note also that neither the vegetation IBI nor the anuran or bird IECs are based on the need to have sites classified as reference or impacted.

Water Quality and Land Use:

The Water Quality and Land Use Indicator (hereafter referred to as the Water Quality Indicator for brevity) is calculated using the metrics below (Table D1.1) to compare water quality related disturbance across coastal wetland sites. This index includes ten *in situ* water quality variables, eight land cover variables (as proportions within 1 km and 20 km buffers from the wetlands), and one Principal Component Analysis (PCA). For a water quality and land cover dataset, values within each variable are rank-transformed such that the rank order is proportionate to the inferred degree of anthropogenic disturbance. Variables with high values indicative of low disturbance are ranked positively, with high ranks given to high values. Variables with high values indicative of high disturbance are ranked negatively, such that higher values receive lower ranks. For variables in which extreme (low or high) values indicate disturbance, values are ranked by their distance from the median value. The PCA is calculated from raw values of all water quality variables and land cover proportions and then scaled so that higher PC1 values indicate higher ranked values. Ranks from all 19 variables are then summed and each summed index value is scaled from 0 to 100 (Table D1.2). More details on the calculations can be found in Harrison *et al*. 2019.

Table D1.1. Water Quality and Land Use Indicator variables and the direction of ranking associated with disturbance (positive, negative, or distance from the median).

Indicator Variables	**Rank Direction**	**Variable type**
Water Clarity	Positive	Water Quality
Specific Conductance	Negative	Water Quality
Total Nitrogen	Negative	Water Quality
Nitrite + Nitrate	Median centered	Water Quality
Ammonium	Negative	Water Quality
Total Phosphorus	Negative	Water Quality
Soluble Reactive Phosphorus	Negative	Water Quality
Chlorophyll *a*	Negative	Water Quality
Dissolved Oxygen	Median centered	Water Quality
pH	Median centered	Water Quality
Agriculture – 20 km	Negative	Land Cover
Agriculture – 1 km	Negative	Land Cover
Developed – 20 km	Negative	Land Cover
Developed – 1 km	Negative	Land Cover
Natural Vegetation – 20 km	Positive	Land Cover
Natural Vegetation – 1 km	Positive	Land Cover
Wetlands – 20 km	Positive	Land Cover
Wetlands – 1 km	Positive	Land Cover
PC1	Positive/Negative	PCA

Land use data for calculation of the land use and water quality indicator comes from the North American Land Change Monitoring System (NALCMS) 2010 land cover of North America at 30 meters (from Landsat imagery) (CEC 2015). The NALCMS is a collaborative initiative by agencies across the U.S., Canada, and Mexico to monitor land cover change, and it is produced by the multi-national Commission for Environmental Cooperation (see citation for partner organizations). These land cover data are published and provided as a tool for researchers and meet the QC standards of the associated agencies, including the U.S. Geological Survey. Because the NALCMS includes land cover for both the U.S. and Canada, it standardizes the land cover calculations across all CWMP sites.

Table D1.2. Example of 4 of the 19 variables included in SumRank for six sites in the CWMP dataset, including their raw values, median-centered value (for NO_3^-), ranked values, summed rank (summed total across all 19 variables), and the scaled rank (from 0 to 100).

	Raw values					Variable Ranks				Summed Ranks	
Site	Clarity (cm)	NO_3^- (mg/L)	NO_3^- Center	SpCond. (µS/cm)	Natural Veg 20 km	Clarity Rank	NO_3^- Rank	SpCond Rank	Nat Veg Rank	Summed Rank	Scaled Rank
8	100	0.005	0.019	492	0.259	309	355	69	127	2436	29.1
10	99	1.408	1.384	599	0.231	306	12	41	110	2082	22.6
15	100	0.005	0.019	278	0.300	309	355	240	143	2436	29.1
20	94	0.005	0.019	708	0.292	283	355	21	139	1822	17.9
63	56	0.125	0.101	422	0.347	99	114	94	182	2994	39.2
118	100	0.050	0.026	90	0.582	309	355	21	374	5933	68.4

Interpretation of Water Quality and Land Use Indicator scores

Higher scores are indicative of sites with low disturbance, whereas lower scores are indicative of sites with high disturbance, based on surrounding land cover and *in situ* water chemistry monitoring. Scores for each site are relative to other sites within the dataset used for the calculations; thus the disturbance gradient is dependent on the sites included in the calculations for any given year or among-year comparison.

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 D1.3).

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 D1.3).

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 D1.3). 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 D1.3).

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 D1.3).

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 D1.3).

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 D1.3).

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 D1.3).

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 D1.3).

Table D1.3. Macrophyte metrics calculated from quadrat data. Scores will be summed to get an overall IBI score for the site, ranging from 0 to 50.

	Wetland Quality			
Metric	**HIGH (5)**	**MEDIUM (3)**	**LOW (1)**	**VERY LOW (0)**
A: INVASIVE COVER (entire site)[1]	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 -0.95	0.6-0.79	< 0.6
D: INVASIVE COVER (wet meadow and dry emergent zones)[2]	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 -0.95	0.6-0.79	< 0.6
G: INVASIVE COVER (flooded emergent and submergent zone)[3]	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 -0.95	0.6-0.79	< 0.6

[1]Invasive species of entire site to include in analysis: *Acorus calamus* (sweet flag), *Agrostis gigantea*, *Butomus umbellatus* (flowering rush), *Centaurea stoebe* (spotted knapweed), *Cirsium arvense* (Canadian thistle), *Cirsium palustre* (marsh thistle), *Cirsium vulgare* (bull thistle), *Glyceria maxima* (tall manna grass), *Hydrocharis morsus-ranae* (European frog's-bit), *Impatiens glandulifera* (touch-me-not), *Iris pseudacorus* (yellow flag), *Lythrum salicaria* (purple loosestrife), *Myriophyllum spicatum* (Eurasian water milfoil), *Nitellopsis obtusa* (starry stonewort), *Phalaris* arundinacea (reed canary grass), *Phragmites australis* (tall reed), *Persicaria lapathifolium* (nodding smartweed), *Potamogeton crispus* (curly pondweed), *Rhamnus cathartica* (common buckthorn), *Rumex crispus* (curly dock), *Solanum dulcamera* (bittersweet nightshade), *Stachys palustris* (hedge-nettle), *Trapa natans* (water chestnut), *Typha angustifolia* (narrow-leaved cattail), *Typha* X *glauca* (hybrid cattail).

[2]Invasive species of wet meadow and dry emergent marsh: *Acorus calamus* (sweet flag), *Agrostis gigantea*, *Centaurea stoebe* (spotted knapweed), *Cirsium arvense*, *Cirsium palustre*, *Cirsium vulgare*, *Glyceria maxima* (tall manna grass), *Impatiens glandulifera, Iris pseudoacorus*, *Lythrum salicaria*, *Phalaris arundinacea*, *Phragmites australis*, *Persicaria lapathifolium*, *Rhamnus cathartica* (common

buckthorn), *Rumex crispus*, *Solanum dulcamera* (bittersweet nightshade), *Stachys palustris* (hedge-nettle), *Typha angustifolia*, *Typha* X *glauca*.

[3]Invasive species of flooded emergent and submergent zone to include in analysis: *Butomus umbellatus*, *Hydrocharis morsus-ranae*, *Lythrum salicaria*, *Myriophyllum spicatum*, *Nitellopsis obtusa* (starry stonewort), *Phalaris arundinacea*, *Phragmites australis*, *Potamogeton crispus*, *Trapa natans* (water chestnut), *Typha angustifolia*, *Typha* X *glauca*.

10. Evaluating wetland quality using submergent and floating plant species:

Evaluating the quality of the portion of a wetland dominated by submergent or floating plants requires a multi-step process (Table D1.4) because several factors can influence the presence and density of these plants. Table D1.4 summarizes the ranks for submergent or emergent zones using submergent and floating plants. It is common for submergent plants to cover only a portion of the bottom substrate in a marsh, so sparse submergent or floating vegetation does not necessarily indicate degraded conditions. High coverage (>75%) of submergent or floating vegetation, with a predominance (>50%) of nutrient-enrichment or sediment-and-increased-turbidity tolerant species (Table D1.5) typically indicates that either agriculture or urban development has resulted in increased nutrient, sediment, or turbidity in the water (Index score = 1), but not to a level that would result in complete elimination of submergent or floating vegetation (Index score = 0). Under such conditions, other submergent and floating plants can be more common, in which case the wetland is considered less degraded (Index score = 3). Submergent and floating vegetation cover ranging from 25-75% is the typical condition for most emergent and submergent wetlands, and Index scores of 3 or 5 indicate this increased quality. Coverage values of less than 25% indicate degraded conditions if only nutrient-enrichment or sediment-and-increased-turbidity tolerant species are present, but are typical for other submergent or floating plant coverage values in many marshes (Index score = 5).

Complete absences of submergent or floating plants can indicate several conditions. In lower stream reaches (drowned river mouths, connecting rivers, or deltas), this absence can indicate that the stream velocity is too high for these plants to persist. Emergent plants may, however, be able to persist in these higher velocity regions of a stream. However, in protected bays or in slow-flowing lower reaches of streams, lack of submergent and floating vegetation typically indicates that sedimentation or turbidity is preventing plant establishment or persistence. When conditions are windy or when turbidity is the result of fine mineral or organic sediments, turbidity is often evident and can be directly linked to lack of wetland vegetation. However, when conditions are calm, surface waters can be clear, but thick, loose sediments will often be evident and easily stirred up during plant sampling. Another complication can be that strong winds may stir up sediment even though conditions are adequate for submergent and floating plants to occupy the wetland. In this case, the wetland should be judged on the basis of the vegetation present, not on the basis of the short-term turbidity.

Table D1.4. Flow chart for determining quality rating of submergent marsh zone or submergent component of an emergent marsh zone.

	Plant Coverage	**Type of submergent plants present**	**Index Score**
Submergent or Floating Vascular Plant Species Present	>75%	>50% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species[4]	1 LOW
		<50% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species	3 MODERATE
	25-75%	>50% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species	3 MODERATE
		<50% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species	5 HIGH
	<25%	>75% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species	1 LOW
		<75% nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species	5 HIGH
Submergent or Floating Plant Species Absent	0%	Clear water in rapidly flowing streams or where bottom consists of cobbles or rock	? REQUIRES FURTHER ANALYSIS
		Highly turbid at time of survey, loose bottom sediments	0 VERY LOW
		Clear water, but thick loose bottom sediments	0 VERY LOW
Only Algae Present			0 VERY LOW

[4] Nutrient-enrichment tolerant species or sediment-and-increased-turbidity tolerant species are listed in Table D1.5.

Table D1.5. Species tolerant of nutrient enrichment, sedimentation, or increased turbidity.

Stress	Species
Nutrient Enrichment	*Ceratophyllum demersum*
	Elodea canadensis
	Lemna minor
	Myriophyllum spicatum
	Potamogeton crispus
	Stuckenia pectinata
	Algae, including *Cyanophyta* sp. but not *Chara* sp.
Sedimentation and Increased Turbidity	*Butomus umbellatus*
	Ceratophyllum demersum
	Elodea canadensis
	Heteranthera dubia
	Myriophyllum spicatum
	Potamogeton crispus
	Potamogeton foliosus
	Potamogeton pusillus
	Ranunculus longirostris
	Stuckenia pectinata

Combined standardized score: A combined standardized score is calculated by adding the wetland quality scores from Table D1.3 (A through I) and Table D1.4. Each of these ten numeric scores ranges from zero to five, with a maximum total score of 50 and a minimum score of zero. The Combined numeric quality scores, which can range from 0 to 50, and their equivalent descriptive quality scores are shown in Table D1.6.

Table D1.6. Combined standardized score from Table D1.3 and Table D1.4.

Combined Numeric Score	Combined Descriptive Scores
0-7	Extremely Degraded
8-16	Degraded
17-25	Moderately Degraded
26-33	Moderately Impacted
34-41	Mildly Impacted
42-50	Reference

Macroinvertebrates:

Aquatic macroinvertebrate IBI scores are calculated based on the metrics below. These metrics have been tested extensively for bulrush-dominated fringing wetlands, and will be modified and validated for other plant morphotypes and wetland types as the program accumulates enough data for these analyses.

Table D1.7. Macroinvertebrate IBI for the wet meadow zone: Typically 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 D1.8. Macroinvertebrate IBI for the dense *Schoenoplectus* plant morphotype: 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 Dense *Schoenoplectus* morphotype is 41 or greater, subtract 5;
If 40 to 60 and total score from Dense *Schoenoplectus* morphotype is >41, then add 5.

Table D1.9. Macroinvertebrate IBI for the sparse *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	
Rel. 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 plant morphoptype. When all vegetation morphotypes 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”;
- A total score of >53 to 76 (>15% to 30% of possible score) = “Degraded” or “the wetland shows obvious signs of anthropogenic disturbance”;
- A 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;”
- A 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;”

- A 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”;
- A 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 morphotpyes 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
- Dense *Schoenoplectus* only = 11 to 19; >19 to 29; >29 to 41; >41 to 53; >53 to 62; >62 to 72
- Sparse *Schoenoplectus* only = 11 to 18; >18 to 26; >26 to 37; >37 to 48; >48 to 56; >56 to 65
- Wet Meadow + Dense *Schoenoplectus* = 20 to 33; >33 to 47; >47 to 66; >66 to 84; >84 to 99; >99 to 113
- Wet Meadow + Sparse *Schoenoplectus* = 20 to 32; >32 to 46; >46 to 64; >64 to 82; >82 to 96; >96 to 110
- Dense and Sparse *Schoenoplectus* = 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 morphotypes are shown in Table D1.10. Catch per unit effort (CPUE) is catch per net per night; since nets should only be set for a single night for this program, no adjustment is needed to catch per net numbers. Final morphotype scores are calculated by re-scaling the sum of all metrics for a vegetation morphotype to a 100-point scale. Specifics and trait information can be found in Cooper *et al.* 2018.

Table D1.10. Fish IBI calculations for several wetland vegetation morphotypes. See Cooper *et al.* 2018 for full details and trait information.

	Scoring		
Sparse or Dense 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 morphotype = (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 morphotype = (sum of metrics / 20) * 100			

Table D1.10. Continued.

	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 morphotype = (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 morphotype = (sum of metrics / 22) * 100			

Birds:

Bird indicators are based on occurrences of targeted wetland species (Table D1.11) during standardized 10 minute point counts (Uzarski *et al*. 2017). The calculations lead to a likelihood-based INDEX OF ECOLOGICAL CONDITION (IEC) based on the methods described by Howe *et al*. (2007a, 2007b), Gnass Giese *et al*. (2015), Jung *et al*. (2020), and Howe *et al*. (2021). In all cases, IEC values range from 0 (most disturbed or lowest possible quality) to 10 (least disturbed or highest quality.

1. Determine PRESENCE OR ABSENCE (1 or 0) of 15 prescribed wetland bird species or species groups (Table D1.11) in either of two standardized 10 minute point counts during the breeding season. A species needs to be present during only one of the two counts to yield a count of 1.

1a. Alternatively, calculate the PROBABILITY of each bird species or species group at multiple points in a wetland, where probability is simply the number of points (each sampled twice) where the species was present divided by the total number of points sampled. This probability method is more robust than the presence or absence method.

2. Obtain parameters for a set of BIOTIC RESPONSE (BR) FUNCTIONS representing each of the targeted wetland species or species groups. These previously derived BR functions are based on a specific environmental gradient. Different sets of functions can be evaluated; results will yield indicator metrics representing the different gradients. Standard BR functions are available showing species' responses to a multivariate gradient representing the "human footprint" impacts on the wetland (Elliott 2018). Variables used to derive the "human footprint" include wetland size, developed land and roads within 2 km of the wetland's center, agricultural land within 2 km, developed/agricultural land in the watershed flowing into the wetland, and human population within the watershed. Note: The BR functions are equivalent to pre-determined COEFFIENTS OF CONSERVATISM often used for plant species. In this case, however, the standards are functional responses rather than single values for each species.

3. Use the field data (PRESENCE OR ABSENCE or PROBABILITY values) and the selected BR FUNCTIONS as input for a computer algorithm in the free, public domain R statistical computing environment (R Core Team 2020). The R code is available with instructions from the Great Lakes Coastal Wetland Monitoring Program (GLCWMP). An Excel spreadsheet for calculating IEC values also is available online. These tools use maximum likelihood estimation to calculate the IEC value, which ranges from 0-10 for a site of interest.

4. IEC values can be calculated for the entire Great Lakes Basin or for one of 4 Great Lakes REGIONS: LO = Lake Ontario; LEsHM = Lake Erie and southern Lakes Huron and Michigan; nLHM = northern Lakes Huron and Michigan; and LS = Lake Superior. Regional calculations use a subset of species (excluding species that are rare or absent in the area) and region-specific BR functions.

Table D1.11. Bird species and species groups used for calculation of Index of Ecological Condition (IEC) metrics.

#	Taxon	Species
1	BITTERN	American Bittern (*Botaurus lentiginosus*) and Least Bittern (*Ixobrychus exilis*)
2	TERNS	Black Tern (*Chlidonias niger*), Common Tern (*Sterna hirundo*), and Forster's Tern (*Sterna forsteri*)
3	COYE	Common Yellowthroat (*Geothlypis trichas*)
4	DABxMAL	Dabbling (marsh) ducks (*Anas* spp., *Mareca* spp., *Aix sponsa*), excluding Mallard (*Anas platyrhynchos*)
5	EAOS	Bald Eagle (*Haliaeetus leucocephalus*) and Osprey (*Pandion haliaetus*)
6	EUST	European Starling (*Sturnus vulgaris*)
7	GBH_GE	Great Blue Heron (*Ardea herodias*) and Great Egret (*Ardea alba*)
8	WREN	Marsh Wren (*Cistothorus palustris*) and Sedge Wren (*Cistothorus stellaris*)
9	MOOT	Common Gallinule (*Gallinula galeata*) and American Coot (*Fulica americana*)
10	PBGR	Pied-billed Grebe (*Podilymbus podiceps*)
11	RWBL	Red-winged Blackbird (*Agelaius phoeniceus*)
12	SACR	Sandhill Crane (*Grus canadensis*)
13	RAIL	Sora (*Porzana carolina*), Virginia Rail (*Rallus limicola*) , King Rail (*Rallus elegans*), and Yellow Rail (*Coturnicops noveboracensis*)
14	SWSP	Swamp Sparrow (*Melospiza georgiana*)
15	RARE	Rare/seldom recorded marsh obligates: Wilson's Snipe (*Gallinago delicata*), Northern Harrier (*Circus hudsonius*), Black-crowned Night Heron (*Nycticorax nycticorax*)

Anurans:

Anuran (frog and toad) indicators are based on occurrences of targeted wetland species (Table D1.12) during standardized samples at a given point during a given year, where a standard sample consist of 3 visits during early spring, late spring, and early summer (Uzarski *et al*. 2017). The timing of these 3 visits depends on thresholds of water and air temperature and therefore the sampling schedules vary among geographic regions and years. Presence/absence (1 or 0) for a species at a given site during a given year is determined by the occurrence (value = 1) or absence (value = 0) during any of the 3 counts. In other words, the value for a given species will be 0 if the species was absent during all 3 seasonal samples.

Values assigned for each species at a given site are used to calculate a likelihood-based INDEX OF ECOLOGICAL CONDITION (IEC) based on the methods described by Howe *et al*. (2007a, 2007b), Gnass Giese *et al*. (2015), Jung *et al*. (2020), and Howe *et al*. (2021). In all cases, IEC

values range from 0 (most disturbed or lowest possible quality) to 10 (least disturbed or highest quality. Details of the method are described below.

1. Determine PRESENCE OR ABSENCE (1 or 0) of 10 prescribed wetland anuran species or species groups (Table D1.12) in any of 3 standardized 3 minute point counts during spring and early summer. A species needs to be present during only one of the 3 counts to yield a count of 1.

1a. Alternatively, calculate the PROBABILITY of each bird species or species group at multiple points in a wetland, where probability is simply the number of points (each sampled 3 times) where the species was present divided by the total number of wetland points sampled. This probability method is more robust than the presence or absence method, but it requires greater sampling effort at each wetland.

2. Obtain parameters for a set of BIOTIC RESPONSE (BR) FUNCTIONS representing each of the targeted wetland species or species groups. These previously derived BR functions are based on a specific environmental gradient. Different sets of functions can be evaluated; results will yield indicator metrics representing the different gradients. Standard BR functions are available showing species' responses to a multivariate gradient representing the "human footprint" impacts on the wetland Elliott (2018). Variables used to derive the "human footprint" include wetland size, developed land and roads within 2 km of the wetland's center, agricultural land within 2 km, developed/agricultural land in the watershed flowing into the wetland, and human population within the watershed. Note: The BR functions are equivalent to pre-determined COEFFIENTS OF CONSERVATISM often used for plant species. In this case, however, the standards are functional responses rather than single values for each species.

3. Use the field data (PRESENCE OR ABSENCE or PROBABILITY values) and the selected BR FUNCTIONS as input for a computer algorithm in the free, public domain R statistical computing environment (R Core Team 2020). The R code is available with instructions from the Great Lakes Coastal Wetland Monitoring Program (GLCWMP). An Excel spreadsheet for calculating IEC values also is available online. These tools use maximum likelihood estimation to calculate the IEC value, which ranges from 0-10 for a site of interest.

4. IEC values can be calculated for the entire Great Lakes Basin or for one of 4 Great Lakes REGIONS: LO = Lake Ontario; LEsHM = Lake Erie and southern Lakes Huron and Michigan; nLHM = northern Lakes Huron and Michigan; and LS = Lake Superior. Regional calculations use a subset of species (excluding species that are rare or absent in the area) and region-specific BR functions.

Table D1.12. Anuran species and species groups used for calculation of Index of Ecological Condition (IEC) metrics.

#	Taxon	Species
1	AMTO	American toad (*Anaxyrus americanus*) or Fowler's toad (*Anaxyrus fowleri*)
2	BULL	American bullfrog (*Lithobates catesbeianus*)
3	GRTR	Eastern gray treefrog (*Dryophytes versicolor*) or Cope's gray treefrog (*Dryophytes chrysoscelis*)
4	CHFR	boreal chorus frog (*Pseudacris maculata*) or western chorus frog (*Pseudacris triseriata*)
5	GRFR	green frog (*Lithobates clamitans*)
6	NLFR	northern leopard frog (*Lithobates pipiens*)
7	SPPE	spring peeper (*Pseudacris crucifer*)
8	WOFO	wood frog (*Lithobates sylvaticus*)
9	OTHE	Other species: pickerel frog (*Lithobates palustris*) or mink frog (*Lithobates septentrionalis*)

D2. Validation and Verification Methods

All field data entered into the data management system are double-checked against the original field data sheets by a second person from the same field crew (100% QC), or have double-entry of data (e.g., bird and anuran data), again 100% QC. We have found that both of these methods are effective at catching data entry errors.

Data that are flagged as suspicious are first checked against field data sheets by the appropriate field crew chiefs; if the data are not typos, then they are brought to the attention of the regional team leader or appropriate co-PI, who applies the appropriate cautionary code flag or removes the data from the database, depending on the data type and error (see D1).

Field data sheets are photocopied or scanned to PDF files as soon as crews return from the field. Copies are archived at each regional lab separately from the original data sheets; scans are archived in a Google folder or on a system that is backed up routinely. Original data sheets are used for data entry and data verification in the data management system. After this is complete, the regional team leader archives the original data sheets.

The data management system is hosted and maintained by CMU with the assistance of a contractor. Each year at the end of the field season the preliminary data is provided to GLNPO. In addition, after QC is complete, that year's data is again be provided to GLNPO to replace the earlier, un-QC'd data. This data delivery occurs in conjunction with the spring semi-annual report to EPA. All flags on data that have not been resolved remain in the DMS and are

provided to GLNPO. The metadata accompanying the data in the DMS explains the various types of flags. In most cases, notes within the DMS explain the cause of flags in more detail. Data users must 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 biological indicators for all major Great Lakes coastal wetland complexes, with supporting physical and chemical parameters, all collected in accordance with the Great Lakes Coastal Wetland Monitoring Program. To evaluate how well we have met this DQO, we assess how many of the major coastal wetland complexes we are able to sample, how representative that selection is based on our site selection process, and how well each wetland is sampled (see representativeness under each B5 section). 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 are considered for re-sampling. All such information is included in the data management system.

A secondary DQO is providing the data, site indicators, and general information about the program to managers, management agencies, other researchers, and other interested parties. Michigan EGLE is assisting with the outreach part of the program. QA for this objective consists of ensuring that EGLE staff are included in PI meetings so that they understand the program and are up-to-date, reviewing the lists of contacts, etc, with EGLE to ensure that major stakeholders are not missed, and reviewing all products produced by EGLE to ensure accuracy. Our intention is that only 100% accurate products created using QC’d data be available for use by non-program members.

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