

Great Lakes Coastal Wetland Monitoring Program

Standard Operating Procedure

Macroinvertebrate Sampling and Laboratory Processing

Synopsis: A standardized method for collecting and processing macroinvertebrate samples according to Great Lakes Coastal Wetlands Consortium protocols

Last updated April 2019

Contents:

| | |
|---|----|
| 1.0 PURPOSE | 3 |
| 2.0 RESPONSIBILITIES | 3 |
| 3.0 FIELD EQUIPMENT | 3 |
| 4.0 INSTRUMENT CALIBRATIONS AND FIELD PREPARATIONS | 5 |
| 5.0 BRIEF METHOD SUMMARY | 6 |
| 6.0 SAMPLING PROCEDURES | 6 |
| 6.1 Sampling Locations..... | 6 |
| 6.2 Sample Collection..... | 9 |
| 6.3 Additional Supplementary Data..... | 11 |
| 7.0 SAMPLE HANDLING AND CUSTODY | 13 |
| 8.0 LABORATORY PROCESSING OF MACROINVERTEBRATE SAMPLES | 13 |
| 8.1 Laboratory Supplies/Equipment..... | 13 |
| 8.2 Sample Processing..... | 14 |
| 8.3 Laboratory Reference Set..... | 15 |
| 9.0 EQUIPMENT TESTING, INSPECTION, and MAINTENANCE | 16 |
| 10.0 RECORD KEEPING/DATA ENTRY | 16 |
| 11.0 REFERENCES | 17 |
| 12.0 FORMS | 17 |
| 12.1 Field data sheet..... | 17 |

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for sampling and processing macroinvertebrates from Great Lakes coastal wetlands according to protocols developed by the Great Lakes Coastal Wetlands Consortium. The methods described in this SOP describe how to accomplish macroinvertebrate sampling and sample processing by Great Lakes Coastal Wetland Monitoring Program protocols. Index of Biotic Integrity (IBI) scores can be derived from data obtained using these methods.

2.0 RESPONSIBILITIES

Scientists and field teams sampling wetlands as part of a monitoring program following Great Lakes Coastal Wetland Consortium protocols should ensure that all work performed satisfies the specific tasks and requirements outlined in this SOP and the project's quality assurance project plan (QAPP).

All field and laboratory personnel performing macroinvertebrate sampling or laboratory processing as part of a monitoring program following Great Lakes Coastal Wetland Consortium protocols should follow this SOP and the project QAPP.

3.0 FIELD EQUIPMENT

D-frame dip nets – Standard 'D-shaped' macroinvertebrate sampling nets (approximate 26 cm wide) with 500- μ m mesh. D-shaped frame attached to long handle. Each crew should carry at least 2 nets at all times.

Gridded trays – 25-cm x 30-cm x 5-cm deep (approximately) with 5x5-cm gridlines drawn on the inside bottom of the trays. Trays should be light-colored for ease of field-picking invertebrates.

Forceps – various forceps for picking organisms from trays. Include spares since they are frequently lost or damaged in the field. Fine-point forceps are generally preferred.

Stopwatch – at least one waterproof stopwatch per field crew.

Meter stick – at least one meter stick for measuring water depth.

1-m square quadrat – PVC quadrat for estimating plant coverage.

Rod (approx. 2-cm diameter x 1-2 m) – rod is pushed into the sediment to determine organic/soft sediment depth. Plastic or plastic-coated garden stakes work well.

Global Positioning System (GPS) – each field crew will require at least one GPS. Recreational-quality GPS receivers are sufficient. Also carry spare batteries or its charger. Heavily-used GPS's, or those with weak batteries, may require a mid-day recharge. Adapters that connect to 12 v boat batteries are available and may be desired by crews.

Laser range finder – a laser range finder is useful for measuring the distance to shore to estimate site slope, and for estimating the size of large vegetation zones. Spare batteries should also be carried.

Equipment/supply checklist – the checklist should be used before leaving on every field trip.

Field data sheets – pre-printed waterproof data sheets for logging field data.

Printed site map – pre-printed map on waterproof paper.

Field notebooks – waterproof notebooks or extra waterproof paper should be carried by each field crew to note any information not included in field datasheets.

Clipboard with storage compartment – all field sheets should be stored in a closeable clipboard or similar device.

Digital camera – for photo documentation of each site and any anomalies encountered. Spare batteries or the charging unit should also be carried.

Hand tally counters – tally counters, at least one per boat or one for each individual picking invertebrates.

Sample containers – 30-ml or larger vials or jars for storing macroinvertebrate samples. Glass screw-top vials (approximately 25-mm diameter x 95-mm tall) with poly-lined caps have been found to work well and are inexpensive; small plastic bottles may also be used. A few additional larger jars should also be carried in the event that large invertebrates are collected.

Internal sample labels – labels with pre-printed fields for the various sample types. Extra waterproof paper should also be carried for spare labels. Many crews prefer to use external labels in addition to internal labels (WQ bottles should ONLY have external labels).

Sample container storage/transport system – a system to ensure sample containers remain organized and protected from breakage during transport.

Sediment coring device – 10-cm deep sediment cores will be collected if sediment %loss on ignition (%LOI) is being determined. This is an optional, though recommended, variable. Coring devices are available for purchase, though a simple coring device can be made by sharpening

the end of a piece of plastic pipe. Core diameter does not matter since samples will be homogenized.

Trowel or similar device for mixing sediment – a garden trowel or large spoon or similar will be used to homogenize sediment if sediment samples are being collected for %LOI analysis.

Plastic bags for storing sediment – Zip top plastic bags should be used for storing sediment if samples are collected. Alternatively, plastic jars may be used.

Plastic pail or tub for homogenizing sediment

Ethanol – 95% ethanol for preserving samples.

Personal equipment – field crews will need waders, appropriate outerwear, sunscreen, insect repellent, etc. for working in coastal wetlands. All personnel should have a personal floatation device.

Binoculars – for navigation and for evaluating shoreline land use.

Boat/motor/trailer with all required safety and maintenance supplies and equipment – many sites will require navigation by boat.

A copy of this SOP – SOP should be printed on waterproof paper.

4.0 INSTRUMENT CALIBRATIONS AND FIELD PREPARATIONS

See the water quality SOP for water quality instrument calibrations.

GPS units: GPS receivers should be tested prior to and after the field season by taking repeated readings at known localities visible on aerial photographs or USGS benchmark locations. Instructions for this are available on the CWMP website. During the field season, field crews should upload GPS readings regularly to their base GIS lab (in this case, the GIS laboratory at the Natural Resources Research Institute). A subset of GPS points uploaded will be displayed on an aerial photograph for crews to confirm upload success. All tests and results should be logged and the logs archived by field crews and with the QA managers.

D-frame nets: D-nets should be inspected for holes before and after each use. All small holes should be repaired with silicone. If nets contain large holes, nets should be swapped out with replacements until they can be patched.

5.0 BRIEF METHOD SUMMARY

Crews should collect macroinvertebrate samples from the major plant zones in each wetland during June through early September, with sampling dates targeting the appropriate phenology for the latitude. Sampling can begin earlier in the season in the most southerly parts of the Great Lakes. Preliminary identification of plant zones can be made from recent aerial photographs of each site (see zone definitions below), with confirmation or re-adjustment made on-site by field crew leaders based on the vegetation actually present at the time of sampling. Samples are collected by sweeping vegetation with D-nets, depositing net contents into gridded light-colored trays, and then picking organisms from the trays. Organisms are preserved in labeled vials of ethanol and later identified to genus (or operational taxonomic unit; see CWMP-specific identification guidelines) in the laboratory. IBI scores can be calculated from these data. Sampling methodology is based on Burton et al. (1999), Uzarski et al. (2004), Cooper et al. (2006), and Cooper et al. (2007).

It is very important that fields on the data sheets are completed appropriately. Blank fields cannot be properly interpreted. If data cannot be collected, this should be explained. Crews should also be aware that there is a large difference between a zero and “no data”. It is especially problematic when no distinction is made between true zeros and no data for water quality parameters because of a problem or just because someone forgot to take the reading.

6.0 SAMPLING PROCEDURES

Sampling dates: Sampling can begin in mid-June in the most southerly regions of the Great Lakes and continue into early September, moving north with the phenology of wetland plant and macroinvertebrate communities.

Crews: In most cases, macroinvertebrates will be sampled during the same sampling trips as fish to maximize efficiency and reduce travel expenses. Therefore, this SOP makes references to the other sampling events to show how sampling is connected/coordinated. Note that there will be some sites or plant zones within a site that cannot be sampled for fish because of insufficient depth, but that will be sampleable for macroinvertebrates and water quality, as well as the other data types.

6.1 Sampling Locations

Site selection: Wetlands should be selected *a priori* using the probabilistic site selection methodology outlined by the Great Lakes Coastal Wetland Consortium and the project QAPP.

Launch location: Before leaving the boat launch, create a waypoint and record its number and lat/long for easy return to the launch and to help future crews locate it quickly. Also, go over the pre-launch checklist (back side of site field sheet) to verify that everything is ready and all needed gear are in the boat.

Site verification: When crews arrive at a site they should quickly determine whether or not the site is sampleable for macroinvertebrates. For GLCWC-type monitoring protocols, the criteria are: safe access for the crew, wetland still exists as a wetland with aquatic vegetation, the site has (at least every other year) a surface water connection to a Great Lake or major connecting channel (e.g., St. Mary's River), and there is sampleable vegetation at depths between 5 cm and 1.5 m. If any of these conditions do not exist, then the wetland may not be sampleable for macroinvertebrates. The condition for rejecting a site must be completely explained at the bottom of the site field sheet and documented with photographs. Care should be taken when rejecting due to water depth or vegetation to ensure that there truly are no areas that can be sampled. Even if a site cannot be sampled for macroinvertebrates, if the site is accessible, the first page of the site field sheet should be completed by the crew.

Site description: Upon arrival at the site, while other personnel are collecting water quality samples, one person should begin to complete the front side of the site field form. This describes the site, its surroundings, how it is influenced by the lake, and what human disturbances are potentially affecting the wetland. Site ID comes from the site map and aerial photo provided by the GIS coordination lab. This is VERY important to keep field forms associated with the proper sites and MUST appear on every page of the field sheets. Please also add crew names and identify the crew chief so everyone knows whom to ask if questions arise about a site.

- *Shoreline structure:* estimate the types of shoreline as percents for the site. Should sum to 100%.
- *Nearshore landcover:* estimate landcover that can be seen from your location in the wetland as a percent. Should sum to 100% (note that there is a spot for % that is not visible).
- *Photos:* take photos that illustrate the site and disturbances to it. Pictures of the shoreline can be particularly informative. Record digital photo numbers.
- *Braiding index:* For riverine systems, please select the appropriate braiding index from the list. Choose only one. NOTE: for riverine wetlands, also sketch a cross-section of the wetland and river channel.
- *Hydrologic connection:* choose the description that best fits the wetland. Choose only one.
- *Water level:* select as many from the list as necessary to describe what is influencing the water level of the wetland on the day(s) that you are sampling.
- *Habitat structure:* select all habitats present in the wetland and then choose the most appropriate vegetation zone structure.
- *Disturbances:* circle all disturbances occurring within the site or within 250 m of its boundaries (use binoculars as necessary). Provide additional descriptions as requested on the field form. This information is very useful in interpreting invertebrate IBI scores; please fill it out carefully.
- On the back of the sheet, note *weather conditions* in the appropriate box. Update this as necessary throughout the sampling event.

Zones: Macroinvertebrate sampling is stratified by plant “zone”, which can be thought of as plant morphotypes. Three replicate macroinvertebrate samples should be collected from up to 4 monodominant vegetation zones that are of appropriate depth and meet the size requirements (see below). If there are more than 4 zones, crews should sample the vegetation zones that are the most dominant in the wetland. Crews should draw on the aerial photos of the sites to indicate location and size of vegetation zones, and should also indicate locations of sampling points. Coverage of the dominant emergent or floating-leaved vegetation must be 75% or greater to qualify as a monodominant zone. For example, a zone would be considered a *Typha* zone if the emergent+floating leaved vegetation was dominated (>75%) by *Typha* spp., even if submersed plants were found throughout the *Typha* spp. stand. However, if a zone contains a mix of different emergent species/growth forms or a mix of emergent and floating leaved species/growth forms, it should be avoided, unless it is being sampled as a Mixed Emergent zone. For example, if a stand contains both *Nymphaea* spp. (water lily) and *Schoenoplectus* spp. (bulrush) and neither of these plant types dominates (i.e., neither is >75% of the total emergent+floating leaved community), then the zone should be avoided, unless it is being sampled as a Mixed Emergent zone. Similarly, if a zone contains a mix of *Schoenoplectus* spp. and *Peltandra virginica* and neither plant type dominates the emergent+floating leaved community, then the area should be avoided, unless it is being sampled as a Mixed Emergent zone. A Mixed Emergent zone can be sampled if it represents a significant and conspicuous habitat type within the wetland and there are not 3 other monodominant zones to sample. For SAV zones, there must be very little emergent (< 5 stems per m²) or floating leaved vegetation (< 1 stem per m²) mixed into the SAV for the zone to qualify as an SAV zone. Note that other species or growth forms will likely occur sporadically within a given vegetation zone; however, the dominant plant type will be conspicuous if it exceeds 75% coverage. Also note that zones are not necessarily a monodominant stand of just one species (e.g., *Typha latifolia*), but instead may be a morphotype (see the list of zones below).

Vegetation zones include (see abbreviations on field data sheet):

- *Typha* (cattail)
- *Floating-leaf* (water lilies)
- *Schoenoplectus* (bulrush; if there appear to be stem density differences in thick zones, use the distinctions below)
 - Inner *Schoenoplectus*: relatively-dense inner zones along the shoreline that are protected from wave action by outer zones of vegetation.
 - Outer *Schoenoplectus*: sparse outer zones where stem densities are lower than in inner zones due to wave action.
- *Peltandra-Sagittaria-Pontederia* (arrow-arum-arrowhead-pickerel weed)
- *Sparganium* (bur-reed)
- Wet Meadow (mixed vegetation, typically *Juncus* and *Eleocharis*)
- Mixed Emergent
- *Phragmites*
- Submersed aquatic vegetation (SAV)
- Floating bog mat

- Wild rice (*Zizania*)
- Open water (appropriate only for benchmark sites when a wetland has been degraded to the point that vegetation can no longer persist or if an area is being sampled to provide pre-restoration information or if there truly are no other zones that can be sampled but the site is truly a wetland.)
- Potentially other types if encountered (please contact Valerie Brady or Matt Cooper before creating another zone; the number of zone types needs to be limited).

Zone selection and size: Using a recent aerial photograph of the site, field crews should preliminarily identify vegetation zones. Upon arrival at the site, the crew leader should determine what vegetation zones can actually be sampled based on what is present in nearly monodominant stands with appropriate water depth of 5 cm to 1.5 m. This information should be sketched onto the aerial photo, showing how things have changed since the photograph was taken.

Minimum zone size is approximately 400 m² so that replicates can be separated by at least 15 m. However, in cases where multiple disjointed 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 m² and no patch is smaller than 25 m². If there are enough disjunct patches for a choice to be made by the field crew, the larger patches should be sampled. This strategy will most often be used in riverine wetlands where habitats are heterogeneous and plant patches tend to be small.

A laser range finder should be used to determine the approximate size of the vegetation zone. At least one measurement should be made along the long axis of the zone and at least one distance should be measured along the short axis of each zone. The location of these measurements should be indicated with lines drawn on the aerial photo along with the distances.

Depths: The minimum depth for sampling macroinvertebrates is 5 cm and the maximum depth is 1.5 m.

Replicate placement in zones: Three replicate dip net samples should be collected in each inundated vegetation zone to provide a measure of variance associated with sampling. These replicates should be at least 15 m apart, and should be associated with and adjacent to fish fyke net locations if fish are also being sampled in that zone. Spacing from one replicate sample location to the next (i.e., between replicates 1 & 2 or 2 & 3) should not exceed 250 m.

Select the three sampling points to represent the variability in the vegetation zone. In large vegetation zones, the three sampling points should correspond with different shoreline features if they occur and if this can be done under the spacing constraints mentioned above. For example, in a large lacustrine wetland where the outer *Schoenoplectus* zone extends along

the shoreline lakeward of a cottage, a forested area, and a wet meadow, sampling locations will be chosen to correspond with these different features. However, in many (or most) cases, a given vegetation zone will not cover enough area to be associated with different shoreline features, in which case sample locations should be chosen to represent, to the degree possible, the variability of the zone itself.

6.2 Sample Collection

Avoiding contamination of water quality samples: Since macroinvertebrates, fish (when possible), and water quality will all be sampled in each vegetation zone, field personnel should be very careful not to interfere with one another or compromise the other samples. Every attempt should be made to collect water quality data and water samples first at each location before any movement is made around the point or zone. When traveling to the site by boat, water quality data and water samples should be collected from the boat before anyone enters the water. After water quality sampling, macroinvertebrate sampling and fish net deployment can commence.

Dip-netting macroinvertebrates: Each dip net replicate consists of a composite of sweeps, each one covering the entire water column from the sediment surface up to the water surface while brushing vegetation with the net to incorporate all microhabitats at a given sampling point; this whole effort (bottom to top of water through the vegetation) is counted as a single “sweep”. Care should be taken not to collect excessive amounts of sediment, detritus, or algae, which will make field-picking of organisms difficult. However, many organisms live at the water-sediment interface, so the sediment needs to be gently bumped with the net frame to dislodge organisms into the water column where they can be captured. If large amounts of detritus end up in the net, the net can be partially submerged and the detritus swished vigorously in the water within the net to dislodge invertebrates into the net. The “washed” detritus can then be discarded. Similarly, if large amounts of sediment clog the net, the net can be dipped up and down in the water to get most of the sediment to sieve through without losing invertebrates.

To semi-quantify sampling effort, the number of net sweeps (i.e., 1 to 2-m passes) should be counted and recorded on the sampling data sheet for each replicate sample. After a series of net sweeps is made, the net will contain an assortment of invertebrates, detritus, and vegetation fragments. If nets are not emptied into gridded trays after each sweep, great care must be taken not to wash already-captured invertebrates out of the net when taking subsequent sweeps. Once nets appear to have plenty of invertebrates or vegetation to pick through, net contents can then be emptied into gridded, light-colored picking trays.

Field-picking: If at all possible, samples need to be picked while still at each sample location in case more net sweeps are required to reach the target number of organisms. Net contents should be distributed evenly across each pan prior to picking organisms. The person(s) doing the netting should continue to sample and fill the pan(s) until a sufficient number of organisms has been collected to begin field picking, at which time the number of net sweeps will be

recorded on the data sheet. The number of net sweeps required depends on macroinvertebrate densities in each zone. For example, in zones replete with macroinvertebrates, only 5-10 net sweeps are required to fill pans, while in zones with sparse macroinvertebrate communities, many more sweeps will need to be made before picking can begin.

Avoid adding excessive amounts of vegetation and detritus to pans because this will significantly reduce picking efficiency. A maximum of approximately 75% of the pan surface area should be covered by vegetation and detritus, leaving at least 25% of the pan bottom uncovered in order to see the grid lines. Only a small amount of water (2-5 mm depth) is needed to facilitate picking of swimming organisms.

When possible, multiple individuals should work together to collect each replicate 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 replicate sample. The picking target is 150 macroinvertebrates for each replicate from each zone. Macroinvertebrates should be collected using forceps (no other picking tools are allowed), with individuals working systematically, attempting to pick all specimens from each grid cell before moving on to the next. No other picking tools besides forceps are allowed because this alters the bias of which invertebrates are picked from the pan. Special efforts should be made to ensure that smaller, cryptic, and/or sessile organisms (those resting on or attached to vegetation or debris) are not overlooked within each grid square. Hand-tally counters should be used to keep track of how many organisms are collected.

All aquatic macroinvertebrates should be picked with the exception of microcrustacea (Cladocera, Copepoda, and Ostracoda) since these are too small to be well-represented in our samples. Aerial adults that do not live in the water (flies, dragon and damselflies, etc.) should not be picked even if they appear in the tray. Spiders, aphids, and other invertebrates that live above the water in the emergent vegetation should not be picked even if they are found in the tray. The exception to all this are water striders (Hemiptera: Gerridae, Veliidae, Mesoveliidae, and Microveliidae). These should be captured, if possible.

Picking time: In most cases, 150 organisms per replicate can be obtained within ½-person-hour, the time limit for the first picking. Picking of individual replicates should be timed using a stopwatch. If 150 organisms are picked before ½ person-hour elapses (i.e., two people for 15 minutes; three people for 10 minutes, etc.), then picking stops at 150 and the number of person-minutes is recorded on the data sheet. If 150 organisms are not acquired within ½ person-hour, the clock should be stopped, organisms are tallied, and the running clock will resume as picking continues to the next multiple of 50, regardless of the time required to do so. For example, if after ½ person-hour 115 organisms have been collected, then picking will continue to 150, regardless of how long it takes to do so. Therefore, each replicate sample should contain 50, 100, or 150 organisms. After each replicate sample is picked, the total

number of person-minutes required for collection, as well as the number of organisms collected, should be recorded on the data sheet.

It is important to reduce picking bias. Accordingly, the number of organisms remaining in each of the picked grids should nearly always be exhausted to the point where finding just a few more organisms will require a substantial effort. Only then should the next grid square be picked. If the entire pan is 'picked clean' before the target number of specimens is reached, then timing should stop while dip nets are used to refill the pan(s).

Preservation: Macroinvertebrates should be immediately placed into labeled 30-ml or larger leak-proof vials or bottles containing 95% ethanol. Sample containers should be labeled with both internal and external labels. External labels should only be those that have previously been checked for ability to remain adhered to sample bottles even when wet. They should be written on with black fine point Sharpie markers or similar markers that have been tested and proven to be waterproof. Internal labels should be made from waterproof paper and written on with either waterproof ink or pencil. Labels should include site ID, vegetation zone, replicate number, sample date, and crew chief name (see labeling protocol on field data sheets). If more than one vial is needed for a replicate sample, each should be labeled separately and numbered (e.g., 1 of 2, 2 of 2, etc.). If glass sample vials are used, they must be stored in a manner that prevents breakage during transport.

6.3 Additional Supplementary Data

Additional data to be collected with each macroinvertebrate replicate sample include: latitude/longitude, vegetation percent cover by growth form, organic substrate depth, mineral substrate texture, and supplementary water quality data (see water quality SOP).

Sampling point locations: All sampling point locations should be recorded and stored using a handheld GPS receiver. In case of GPS equipment failure, the invertebrate crew should seek to borrow a GPS from another crew to finish the site, if possible, and should seek a replacement GPS unit. In the event that this is not possible, crews should endeavor to very accurately mark each sample point on their field map.

Vegetation quadrats: A 1-m² quadrat is used to determine both surface and subsurface vegetation percent coverage at fish and macroinvertebrate sampling points. If both fish and macroinvertebrates are being sampled, see the fish sampling SOP. If only invertebrates are being sampled, quadrats should be placed roughly in the center of the area where dip net sweeps were made, but that has not been trampled and that looks similar to the rest of the zone. To randomize the specific location these quadrats, quadrats may be thrown over the individual's shoulder to reduce bias in sampling location. If the zone is not being sampled for fish, record water depth at the quadrat.

Vegetation percent composition: Percent composition by growth form and cover type should be estimated visually within the quadrat at both the water surface and the sediment surface directly under the quadrat separately. Care should be taken to only include vegetation that is truly within the quadrat and exclude emergent vegetation that has bent in from the sides, etc. Growth forms and cover types to be estimated at the water surface are emergent, submergent vegetation floating at the surface, floating-leaved vegetation, filamentous algae and open water. At the sediment surface growth forms and cover types to be estimated are standing emergent stems (living or dead), submergent vegetation, coarse detritus, filamentous algae and bare sediment. If species (or genera) within each growth form category are known, they should be noted on the field data sheet. This is especially important for Mixed Emergent zones and extra effort should be made to identify as many genera/species as possible in these mixed zones (samples can be collected and placed in labeled zip-top bags in order to seek help from the vegetation crew in identifying submergent vegetation). Although vegetation often overlaps, the percentages should sum to 100%.

Zone/patch size: Crews should estimate the size of zones or the vegetation patches that comprise zones and note these on the data sheet. Range finders may be used to assist with this estimation. At least one measurement should be made along the long axis of the zone and at least one distance should be measured along the short axis of each zone. The location of these measurements should be indicated with lines drawn on the aerial photo along with the distances. Note that range finders can be very inaccurate if there is not a good object to point them at.

Organic substrate depth: Depth of organic substrate should be determined by pushing a 2-cm diameter rod into the substrate until mineral substrates are reached. 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.

Mineral substrate texture: The dominant and subdominant mineral substrate type should be recorded. Mineral substrate texture is determined by feel using the categories and guidelines on the field data forms. This should be done at each D-net replicate area.

Sediment samples: Collection of sediment for percent loss-on-ignition (%LOI) analysis is optional but highly recommended. A 10-cm deep sediment core should be collected using a piston corer or similar coring device. Core diameter does not matter since samples will be homogenized for %LOI. Sediment samples should be associated with each net location if fish are being sampled or the center of dip-netting area if fish are not being sampled. All samples from a given vegetation zone should be homogenized in a pail or tub (this can be done in the laboratory if desired). Approximately 100 ml (e.g., one trowel full) should be placed in a sealed container. Samples may be stored in sealed plastic bags or plastic jars and fully labeled. Samples should be stored on ice and then frozen.

Supplementary water quality data should be collected from each vegetation zone at the beginning of each sampling session. See the water quality SOP for detailed instructions.

Pictures: Crews should take a digital picture of each vegetation zone. For sites, any anomalies encountered or unique anthropogenic impacts should also be photo documented. Digital images should be uploaded to a computer as soon as possible after each sampling trip. Photo file names should include, at a minimum, the site ID and vegetation zone. For photos of anomalies or disturbances, a word of description should be included in the file name. Photos should be organized in directories labeled with site ID and sampling date and uploaded to the data management system during date entry.

Site completion: Before leaving the site, use the checklist on the back side of the site sheet to verify that everything has been completed. The field crew leader must sign this sheet at the bottom to verify that the site has been completely sampled to the best of the crews' ability, and that all information on the field forms is accurate and complete.

7.0 SAMPLE HANDLING AND CUSTODY

Samples should remain in the custody of field crews for the duration of each field trip. At the laboratory, each sample should be logged into the laboratory log book. Entries should include: site ID, plant zone, replicate number, and sample date, along with the date received and any notes about sample condition. This information should be transferred to a sample processing inventory maintained by each laboratory. This inventory should be updated as each sample is processed. Processed samples should remain in the custody of the laboratory. Identified macroinvertebrates should be stored for 5 years after collection. Invertebrate identifications should be compiled in laboratory notebooks, and then entered into the data management system. The hard-copy field data sheets and laboratory notebooks should be stored at each regional laboratory.

8.0 LABORATORY PROCESSING OF MACROINVERTEBRATE SAMPLES

8.1 Laboratory Supplies/Equipment

Sample log-in notebook – a notebook or spreadsheet to log samples into the laboratory.

Sample inventory sheet – a sheet that identifies the exact stage of processing for every sample housed in the laboratory and identifies the individual working on the sample at that stage.

Identification sheets –laboratory notebooks or data sheets for keeping records of macroinvertebrate specimen IDs and counts.

Forceps – various forceps for manipulating organisms under magnification.

Petri dishes, watch glasses or similar containers – small clear dishes for processing invertebrate samples under microscopes and for temporarily storing samples while processing.

Ethanol – 95% ethanol for preserving samples.

Dissecting microscopes – stereo microscopes (at least some of which are capable of 50x or greater magnification) with appropriate light source for identifying macroinvertebrates in the laboratory.

Small storage vials – 5-15 ml (approximate) storage vials for long term storage of macroinvertebrate samples. Vials must have appropriate caps to prevent ethanol from evaporating over time. Poly-lined caps have been found to work well.

Vial storage system – A storage system such as vial trays stored in a cabinet is necessary to keep archived samples organized.

Taxonomic keys – an appropriate set of taxonomic keys for identifying macroinvertebrates to genus level (see CWMP macroinvertebrate identification guide: Levels of Identification and Other Taxa Notes”).

8.2 Sample Processing

Strict inventory control of macroinvertebrate samples must be maintained since each laboratory will likely have multiple individuals working on various aspects of sample processing. Sample inventory sheets will be used to track every aspect of sample processing. Fields within inventory sheets will contain actions such as ‘Sample Received by Laboratory,’ ‘Initial Picking,’ ‘Invertebrates identified,’ ‘All Data Entered Into Database,’ ‘XX invertebrates sent to specialist,’ etc. Inventory sheets will be designed to track who is working on each action listed. At a minimum, a quality control auditor should be able to easily determine the location and progress of any macroinvertebrate sample in the custody of the regional laboratory.

For macroinvertebrate identification, taxonomic keys listed in the “Levels of Identification and Other Taxa Notes” working document should be used (see attached). This document also shows the target lowest operational taxonomic unit for each group. This is typically genus for insects. However, chironomid larvae need only be identified to tribe or subfamily (e.g., Chironomini, Tanytarsini, Tanypodini, etc.) for the GLCWC IBI calculations. The taxa notes document goes into great detail for specimen identification and should be studied carefully by all identifiers. Specimen IDs and counts should be recorded along with the following information: date(s) that the sample is being worked on, site ID, vegetation zone, replicate number, and sample processor’s name. Any difficulties encountered during identification (e.g., missing gills) should

also be noted. Taxa identification and specimen counts are considered a 'laboratory record' so identification sheets should be archived accordingly.

In most cases, identified organisms will be placed into smaller vials (e.g., 5-15 ml) for archiving. Each laboratory must develop a system for archiving samples that allows later taxonomic verification and QA/QC. For taxonomic groups with many individuals, vials for archiving samples may contain specimens at the family (i.e., one family per vial) or order level. Discretion must be used when determining the taxonomic level at which samples are stored. Some regional laboratories may choose to archive samples at either a higher taxonomic level (e.g., class) or store entire processed replicate samples together.

Vials should be filled with 70-95% ethanol for long-term storage. Labels containing the site ID, vegetation zone, replicate number, taxonomic identification, and sample processor's initials should be included within each vial. Vials should be organized in containers such as plastic or cardboard trays labeled with all pertinent sample information and stored in a secure location. Ethanol levels should be inspected periodically to prevent dessication of archived samples.

Active communication among teams is essential to consistent taxonomy. Individuals who are processing macroinvertebrate samples should use a variety of techniques to communicate with other teams while working through particularly difficult identifications. E-mail and phone calls as well as an online forum where photos can be posted will all be used to aid macroinvertebrate processing and ensure accuracy. As new technicians are trained in laboratory processing of macroinvertebrates, they will also receive training on how to best communicate with other project participants working on macroinvertebrate processing.

8.3 Laboratory Reference Set

Each laboratory should develop and maintain a reference set of preserved specimens that will include at least 3 individuals (when possible) of each taxon. If laboratories have reference sets that they are maintaining for multiple projects, these master reference sets can be used for this project as well. The reference set can be used to train new individuals and for cross-laboratory taxonomic verification. Specimens that are removed from their respective sample vials to be included in the reference set will be replaced with a label indicating that the specimen(s) was moved to the reference set; this should also be noted on the data sheet. Labels within the vials in the reference set should also note where the specimen came from (site ID, vegetation zone, replicate number, taxonomic identification, and sample processor's initials).

9.0 EQUIPMENT TESTING, INSPECTION, and MAINTENANCE

D-frame nets will be inspected before and after each set of sample collections for holes or other damage. Damaged nets will be repaired in the field, and replaced at the end of that sampling trip. Each crew will have one replacement net with them at all times. Crews should also carry

extra forceps and hand tally counters. Boat repairs are also often necessary. Most towns around the Great Lakes have boat repair shops, which will be used by field crews as necessary. Spare parts carried by crews will include spark plugs and appropriate wrenches, tire for the trailer, drain plug, fuel line, sheer pins (if used), and a spare propeller. Spare batteries will be carried for the GPS units, cameras, and water quality meters.

All units that store files electronically should be backed up nightly to a laptop. This includes water quality meters, GPS units, cameras, and any other such equipment. This will help protect against massive data loss should equipment malfunction or be damaged or lost.

Boats, trailers, and sampling gear must undergo rigorous disinfection to eliminate the transfer of exotic species between sections of the Great Lakes (e.g., eastern vs. western Lake Erie, northern vs. southern Lake Michigan, etc), before moving between the lakes, or before moving more than 100 miles between sites. This means that all boats, trailers, and gear need to be washed well, preferably through a car wash, before moving the gear between sections of the Great Lakes. See the Fish Sampling SOP for more detail on gear disinfection and the use of Virkon Aquatic.

10.0 RECORD KEEPING/DATA ENTRY

Each field crew should carry with them maps and recent aerial photos of the sites to be visited, maps showing locations of the nearest boat launches, field data sheets for each scheduled site as well as spare datasheets, and a field notebook to document any additional information not contained on the field data sheet. Each field data sheet should be initialed by the crew chief once a site is completed, and page 2 of the site-level sheet requires the field crew chief's signature. Sample locations should be noted on the site map in the event that the GPS receiver is not functional. Additional notes on anomalies or anthropogenic disturbances can also be made on the site map.

Documentation in the laboratory will include sample log-in, sample inventory sheets, and identification sheets on which macroinvertebrate processing and identification records are kept. When multiple individuals are working on the same sample, data should be compiled into a single record for the sample. A supervising individual should initial the record prior to the data being entered into the data management system (DMS).

A separate identification record should be kept for the macroinvertebrate reference set that includes the taxonomic ID, site ID, vegetation zone, replicate number, sample date, and the individual who identified the organism. This is the same information to be included on the internal labels for each vial in the reference set.

All records should be entered into the DMS as soon as possible. Data collected in the field should be entered into the DMS as soon as possible after returning from each field trip. All data

will be verified by a second person after it is entered into the DMS. Field notes explaining anomalies, disturbances, etc. will also be entered into the DMS. After completing laboratory processing of each replicate invertebrate sample, data will be entered into the DMS and verified by a second individual. All hard copy records (field data sheets, field notebooks, site maps with hand-written notes, sample inventories, laboratory notebooks with macroinvertebrate IDs, etc.) should be scanned so that an electronic copy is stored as a backup; paper records should be compiled and stored in a secure location at each laboratory.

11.0 REFERENCES

- Burton, T.M., D.G. Uzarski, J.P. Gathman, J.A. Genet, B.E. Keas, and C.A. Stricker. 1999. Development of a preliminary invertebrate index of biotic integrity for Lake Huron coastal wetlands. *Wetlands* 19:869-882.
- Cooper, M.J., D.G. Uzarski, and T.M. Burton. 2007. Macroinvertebrate community composition in relation to anthropogenic disturbance, vegetation, and organic sediment depth in four Lake Michigan drowned river-mouth wetlands. *Wetlands* 27(4): 894-903.
- Cooper, M.J., D.G. Uzarski, T.M. Burton, and R.R. Rediske. 2006. Macroinvertebrate community composition relative to chemical/physical variables, land use and cover, and vegetation types within a Lake Michigan drowned river mouth wetland. *Aquatic Ecosystem Health and Management* 9(4):463-479.
- Uzarski, D.G., T.M. Burton and J.A. Genet. 2004. Validation and performance of an invertebrate index of biotic integrity for Lakes Huron and Michigan fringing wetlands during a period of lake level decline. *Aquatic Ecosystem Health and Management* 7:269-288.

12.0 FORMS

12.1 Field Data Sheets