Quality Assurance Project Plan

Revision 2, May 1, 2012

Benthos & Plankton BUIs Evaluation in Wisconsin's Lake Michigan Areas of Concern

Wisconsin Department of Natural Resources in Cooperation with US Geological Survey

Project Number: GL-00E00876 sub -9KM60

Approval Sheet

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A. Project Description

This section describes the project management, background, problem definition, and project design.

A1. QAPP Distribution

Each of the individuals in table 1 will receive a hard copy of this Quality Assurance Project Plan (QAPP). A copy of the final signed QAPP should be retained by each of these individuals until the completion of laboratory analysis and final acceptance of the data report. All individuals listed below must receive a hard copy of any changes or addendums to the QAPP.

Table 1. QAPP distribution list.

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A2. Project Organization

The Wisconsin Department of Natural Resources (WDNR, hereafter) is the principal grantee for the project. The U.S. Geological Survey (USGS, hereafter) is the principal investigating group for this survey. USGS is responsible for the development, coordination and implementation of the sampling plan and QAPP, and is the principal client for the final data. Benthos and plankton samples will be collected in association with U.S. Environmental Protection Agency (USEPA, hereafter) Great Lakes National Program Office (GLNPO, hereafter) guidelines and by the use of a USGS boat. The University of Wisconsin-Superior, Wisconsin State Laboratory of Hygiene, WDNR, and USGS will provide all analytical services. Figure 1 provides a summary of the project organization for this study. Staff associated with this project and their responsibilities are included in table 2.

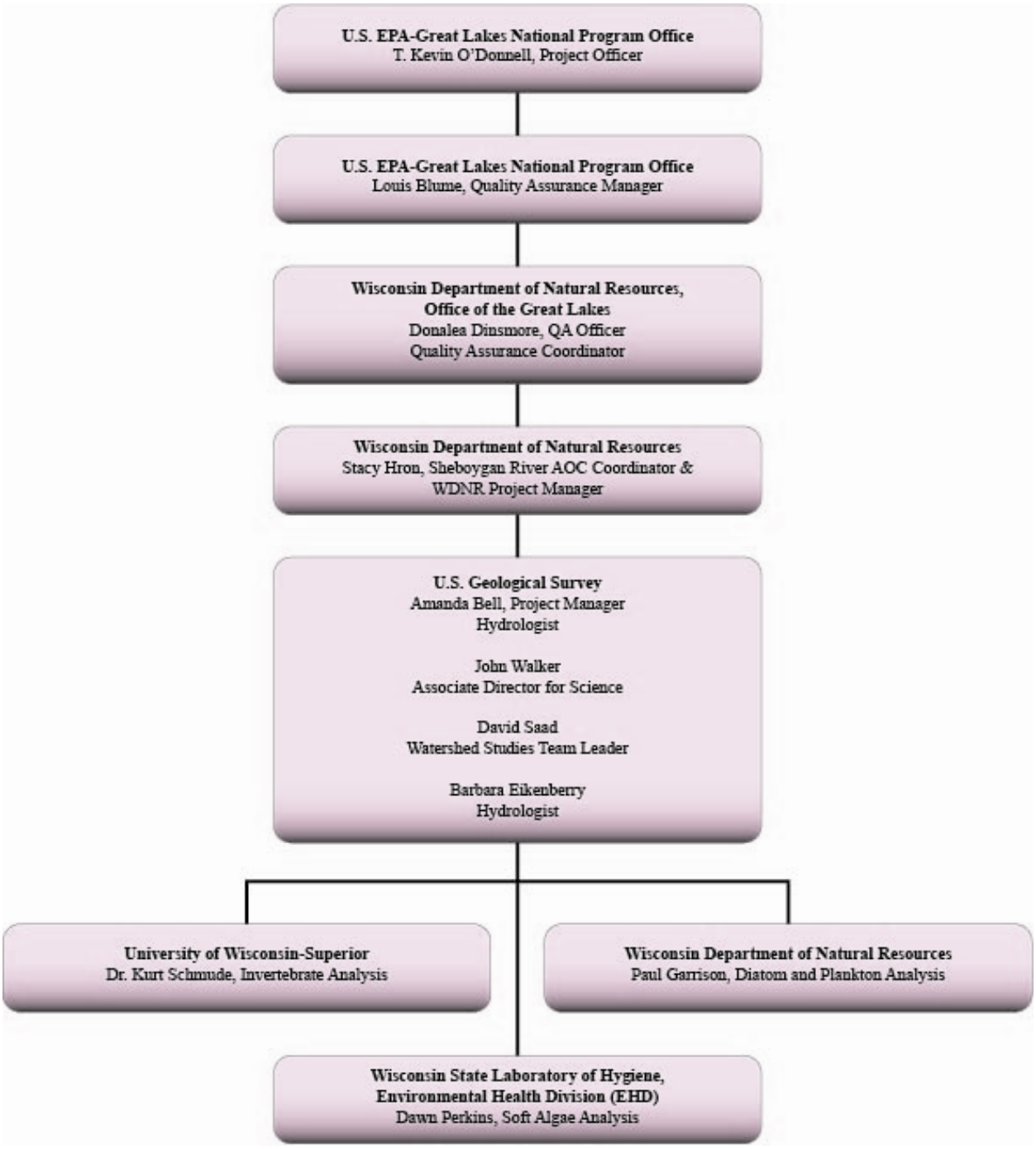

Figure 1. Project Management Organizational Chart

Table 2. Project staff and associated responsibilities.

Person:	Responsibilities:
Amanda Bell U.S. Geological Survey 8505 Research Way Middleton, WI 53562 608-821-3882 ahbell@usgs.gov	USGS Lead Investigator Prepare QAPP Sample collection Analyze loss on ignition of sediment samples
Barbara Eikenberry U.S. Geological Survey 8505 Research Way Middleton, WI 53562 608-821-3882 beikenberry@usgs.gov	Provide technical review and comments on USGS work
Stacy Hron WDNR Project Manager Wisconsin Department of Natural Resources 1155 Pilgrim Road Plymouth WI53073 920-892-8756 X3057 stacy.hron@wisconsin.gov	Provide technical review and comments Project management for WDNR
Donalea Dinsmore WDNR Quality Assurance Coordinator Wisconsin Department of Natural Resources 101 S Webster Street – WT/3 Madison, WI 53703 608-266-1926 donalea.dinsmore@wisconsin.gov	Provide technical review and comments Review and approve QAPP
Dawn Perkins Wisconsin State Laboratory of Hygiene Environmental Health Division (EHD) 2601 Agriculture Drive Madison, WI 53718 608-224-6230 daperkins@mail.slh.wisc.edu	Identify and quantify soft-bodied phytoplankton
Paul Garrison Wisconsin Department of Natural Resources 2801 Progress Road- SS/RC Madison WI53716 608-221-6365 paul.garrison@wisconsin.gov	Identify and quantify zooplankton and diatom phytoplankton
Dr. Kurt Schmude University of Wisconsin-Superior Belknap and Catlin Superior, WI 54880 715-394-8421 kschmude@uwsupe.edu	Identify and quantify benthic invertebrate species

A3. Background

Great Lakes Areas of Concern (AOCs, hereafter; http://www.epa.gov/greatlakes/aoc/) are severely degraded areas within the Great Lakes Basin where beneficial uses of water or biota have been listed as impaired or where environmental criteria are exceeded and impairment is likely. Wisconsin has four AOCs along Lake Michigan's Shoreline: Milwaukee Estuary, Sheboygan River, Lower Green Bay and Fox River, and Menominee River. Table 3 is a list of Wisconsin's four Lake Michigan AOCs.

Table 3. List of Wisconsin's Lake Michigan AOCs with latitude/longitude and drainage area based on USGS gage.

Wisconsin's Lake Michigan Areas of Concern (AOCs)		
Name	**Approximate Decimal Long-Lat Harbor/River mouth**	**Drainage area from USGS gages (square miles)**
Lower Menominee River	-87.592264, 45.093712	3930 (Menominee at McAllister)
Lower Green Bay and Fox River	-88.004528, 44.539139	6330 (Fox River at mouth)
Sheboygan River	-87.703243, 43.748877	418 (Sheboygan at Sheboygan)
Milwaukee Estuary	-87.895958, 43.025215	872 (Milwaukee River at mouth)

Each AOC has a list of beneficial use impairments (BUIs, hereafter) that must be addressed to improve overall water quality. There are a total of 14 BUIs including fish tumors or other deformities, eutrophication or undesirable algae, beach closings, and degradation of benthos (http://www.epa.gov/lakeerie/buia/index.html#What%20Is). Delisting the AOCs for the identified impairments is a high priority for the EPA and WDNR in the four AOCs along Wisconsin's Lake Michigan shoreline. The Sheboygan River is the highest priority given the schedule for completing the removal of contaminated sediment so management has directed an aggressive schedule for addressing its impairments.

Table 4 contains a list of the BUIs specific to each AOC. To address two of the BUIs—degradation of benthos and degradation of phytoplankton and zooplankton populations—the WDNR has entered into a cooperative agreement with the USGS to quantify benthic invertebrate (benthos, hereafter) and phytoplankton/zooplankton (plankton, hereafter) communities of Wisconsin's four Lake Michigan AOCs. It should be noted that the delisting targets for each of the Lake Michigan AOCs includes removal of contaminated sediment as one of the necessary steps to delisting the impairment for degraded benthic and phytoplankton communities. Dredging is expected to occur in each of these AOCs.

To assess whether these communities are degraded in comparison to rivers and harbor areas that are not considered AOCs, six additional river mouths will be sampled (hereafter referred to as non-AOCs) and the communities in these non-AOCs will be compared to the communities in the AOCs. The non-AOC sites were selected by Amanda Bell (hereafter referred to as "the lead") and several other USGS and WDNR personnel based on similar characteristics to the AOCs such as climate, geology, soils, land-use, and geography. The inclusion of non-AOC sites will allow comparison of AOC sites to relatively-unimpacted or less-impacted control sites with natural physical and chemical characteristics that are as close as possible to that of the AOCs. Comparison to less-impacted control sites as site pairs and as a group is consistent with the approaches used by other Great Lakes states, such as Michigan and Ohio (Michigan Department of Environmental Quality, 2008; Ohio EPA, 2008). The selection of the non-AOC sites is detailed in section A5.1.

Table 4. List of beneficial use impairments in the four Wisconsin Lake Michigan Areas of Concern.

Area of Concern	Beneficial use Impairment
Lower Menominee River	Restrictions on fish and wildlife consumption Degradation of fish and wildlife populations Beach closings Degradation of benthos Restriction on dredging activities Loss of fish and wildlife habitat
Lower Green Bay and Fox River	Restrictions on fish and wildlife consumption Tainting of fish and wildlife flavor Degradation of fish and wildlife populations Fish tumors or other deformities Degradation of aesthetics Degradation of benthos Restriction on dredging activities Loss of fish and wildlife habitat Bird or animal deformities or reproductive problems Eutrophication or undesirable algae Restrictions on drinking water consumption, or taste and odor Beach closings Degradation of phytoplankton and zooplankton populations Added cost to agriculture and industry
Sheboygan River	Restrictions on fish and wildlife consumption Eutrophication or undesirable algae Degradation of fish and wildlife populations Fish tumors or other deformities Bird or animal deformities or reproduction problems Degradation of benthos Degradation of phytoplankton and zooplankton populations Restriction on dredging activities Loss of fish and wildlife habitat
Milwaukee Estuary	Restrictions on fish and wildlife consumption Eutrophication or undesirable algae Degradation of fish and wildlife populations Beach closings Fish tumors or other deformities Degradation of aesthetics Bird or animal deformities or reproduction problems Degradation of benthos Degradation of phytoplankton and zooplankton populations Restriction on dredging activities Loss of fish and wildlife habitat

A4. Project Objective and Problem Definition

Null hypothesis for each AOC: The benthos and plankton communities in the AOC are degraded in comparison to non-AOCs.

In order to disprove the null hypothesis for each AOC, the benthos and plankton communities in each of the AOCs must be not statistically different, with 90% confidence, from selected non-AOCs using a weight of evidence approach. Although a 0.5 significance level is often chosen as the desired level of conservatism for statistical decision-making, the project team decided that an alpha of 0.10 maintains statistical integrity while allowing for the natural variability in biotic systems. This balances scientific defensibility with practical achievability.

To test this hypothesis, benthos and plankton samples in the river mouths and harbors of the four Lake Michigan AOCs and six non-AOCs along the western shoreline of Lake Michigan will be collected to provide a community assemblage. Metrics for each site based on the community assemblages will be calculated and used for statistical analyses. These analyses will be used to detect significant differences between paired AOC and non-AOC sites, as well as an overall AOC to non-AOC group comparison.

The Milwaukee Harbor and Green Bay are large and have far more complex systems than any other harbors or rivers along the western Lake Michigan shoreline. Therefore, the plausibility of comparing these sites to the non-AOCs is not feasible. These systems also have on-going contaminant removal projects that will hopefully improve the health of the benthos and plankton communities. The community assessment of these systems will be examined in conjunction with the other locations, but will likely have vastly different community structures. Therefore the information gained from these systems will provide a baseline community assessment for future comparisons within those systems with regards to the BUIs.

The following are the distinct tasks that must be performed to address the hypothesis:

1. Sample the benthos community in the Sheboygan River AOC.
2. Determine if the benthos community in the Sheboygan River AOC is degraded.
3. Sample the plankton community in the Sheboygan River AOC
4. Determine if the plankton community in the Sheboygan River AOC is degraded.
5. Sample the benthos community in the Lower Menominee River AOC.
6. Determine if the benthos community in the Lower Menominee AOC is degraded.
7. Sample the benthos and plankton communities in the Fox River for a baseline assemblage.
8. Sample the plankton community in Lower Green Bay for a baseline assemblage
9. Sample the benthos and plankton communities in the Milwaukee River, Menomonee River, and Milwaukee Inner Harbor for a baseline assemblage

These processes will be followed to complete the tasks described:

1. Gather legacy data
2. Collect community samples
3. Calculate community metrics
4. Analyze community assemblage and metrics
5. Compare AOCs to paired non-AOC sites
6. Compare all AOCs to all non-AOC sites

Currently, there is no consistent legacy data for the benthos and plankton communities to determine if these communities in the four AOCs have improved or degraded since the BUIs were established. Although there are some historical benthos samples collected by WDNR and other researchers, there is little to no data available for the plankton communities. Each of the Stage 2 Remedial Action Plans for the AOCs identified the need to evaluate the communities as a critical first step that must occur before these BUIs can be considered for removal. If future community assessments are required, this project will serve as a template for methods and sampling design, and provide the baseline data necessary for comparisons.

The overall question this project aims to answer is: Are the benthos and plankton communities in the four AOCs degraded in comparison to the benthos and plankton communities of the non-AOCs? To answer this we will use multiple lines of evidence gained from this study including an Index of Biotic Integrity (IBI, hereafter), species richness, organism abundance, and species diversity. The similarity of the overall community composition among the sites, as assessed by multivariate analysis, will also be determined. By calculating community-based metrics that can quantify subtle differences between sampled communities, we will be able to determine if the benthos and plankton in the AOCs are significantly different from those in the non-AOCs. If there is no statistically significant difference between the sampled communities from an AOC and a comparable non-AOC site, the data may be used as a benchmark community assemblage that can be used for future assessment.

The WDNR has entered into a cooperative agreement with the USGS to assess benthos and plankton communities in the river mouths of Wisconsin's four Lake Michigan AOCs and six non-AOCs. USGS will act as an impartial research partner to collect and report the community assessment. Recommendations on BUI status will be decided by the WDNR after specific targets for each BUI are met. These recommendations are then submitted to the US Environmental Protection Agency (USEPA, hereafter) for approval.

A5. Project Task Description

To assess the degradation of the communities, multiple sample types will be collected three times in one year (2012). At each sampling location, two types of plankton samples (plankton tow and depth profile) and two types of benthos samples (Ponar dredge and Hester-Dendy samplers) will be collected. The two methods of sample collection for each of the communities are necessary to get the most comprehensive community possible. Final data analysis will pool the enumeration of for each the two communities so that each sampling location has a complete community profile for benthos and plankton. Plankton will move up and down the water column throughout the day based on depth variability of conditions such as water temperature, light penetration, and available nutrients. Vertical plankton tows will collect the zooplankton communities regardless of their depth during sampling. Phytoplankton are too small to be captured by a 63-µm plankton net so whole water samples will be taken using an alpha bottle at depth increments described in the SOP.

Each benthos species has habitat preferences with regards to conditions such as sediment and flow. The two forms of sampling devices that will be used in the study will target benthos that (1) occur naturally in the bed sediments of the rivers by grabbing a surface sediment sample with a Ponar dredge, and (2) require a harder substrate to colonize by deploying Hester-Dendy samplers. All methods for sample collection are based on Standard Operating Procedures (SOPs hereafter) from USEPA's GLNPO for large rivers and lakes (table 5).

Table 5. References for sampling methods.

Sampling Method	Reference
Plankton–tow net	U.S. Environmental Protection Agency, 2010, Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes, EPA Report EPA 905-R-05-001: Great Lakes National Program Office, Chicago, IL. Available at: http://www.epa.gov/greatlakes/monitoring/sop/ Standard Operating Procedure for Zooplankton Sample Collection and Preservation and Secchi Depth Measurement Field Procedures: LG 402
Plankton–depth profile	U.S. Environmental Protection Agency, 2010, Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes, EPA Report EPA 905-R-05-001: Great Lakes National Program Office, Chicago, IL. Available at: http://www.epa.gov/greatlakes/monitoring/sop/. Standard Operating Procedure for Phytoplankton Sample Collection and Preservation Field Procedures: LG 400
Benthos–Ponar dredge	U.S. Environmental Protection Agency, 2010, Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes, EPA Report EPA 905-R-05-001: Great Lakes National Program Office, Chicago, IL. Available at:

	http://www.epa.gov/greatlakes/monitoring/sop/. Standard Operating Procedure for Benthic Invertebrate Field Sampling: LG 406
Benthos– Hester-Dendy sampler	U.S. Environmental Protection Agency, 1994, Assessment and Remediation of Contaminated Sediments (ARCS) Program—Assessment Guidance Document, EPA Report EPA 905-B94-002: Great Lakes National Program Office, Chicago, IL. Available at: http://www.epa.gov/glnpo/arcs/EPA-905-B94-002/B94002-ch7.html Chapter 7. Assessment of Benthic Invertebrate Community Structure

In addition to the in-stream sampling a background literature search will be performed to determine other similar studies that may have occurred historically in these sites or in other areas of the Great Lakes. It is currently not known how much previous sampling of the benthos and plankton has occurred in the sites nor the methods used to collect those samples; therefore, historical data and reports for benthos and plankton communities in the study sites will be investigated to determine if a shift in the communities has occurred from previous sampling efforts. Where historical studies occurred in the selected sites, and the collection methods are comparable to those described here, the data from the reports will be requested from the authors. In order to accept the community data, we will verify the sampling methods, the lab analytical methods, level of taxonomic resolution, and QA/QC characteristics of the overall study to confirm they would be comparable to USGS methods and data. If the methods are similar to those detailed here, and the data will be entered into a Microsoft Access database to be housed at the USGS office in Middleton. The lead will evaluate the data using visual inspection, statistical analyses, and best professional judgment before accepting the data and including it in further data analysis.

A5.1. Site selection and Site-specific Considerations

The sampling will primarily be occurring near the mouth of the rivers with the exception of the plankton sampling in Green Bay (discussed below). The assumption is that the communities at the river mouths will represent community degradation from contaminants introduced both upstream in the watershed and near the sampling location. In order to simplify comparisons between AOCs and non-AOCs, and to minimize the variability associated with benthos in complex river systems, only non-wadeable areas of the sites will be sampled. Data collected will include parameters to characterize the sites and the benthos and plankton communities. Details of all data to be collected and associated methods are discussed below.

The four AOC sites along Wisconsin's Lake Michigan shoreline where chosen for this study to determine the status of the benthos and plankton BUIs. The intention of the data analysis is to look at the

AOCs individually in comparison with sampled non-AOCs to determine whether the benthos and plankton communities are statistically different from the non-AOCs. Ideally, 2 (or more) non-AOCs would be sampled for each AOC to increase the statistical robustness of the design; however, there are not that many rivers of comparable size on the western shoreline of Lake Michigan. Therefore several non-AOCs will be used to compare against more than one AOC. With that in mind, six non-AOC sites were chosen because of characteristics similar to the AOCs including land-use, drainage area, geology, geography, soils, and climate. Although no two river systems are identical, the attempt was made to match the non-AOCs to AOC counterparts as closely as possible based on available environmental data and discussions between USGS and WDNR personnel who are familiar with the individual systems.

A5.1a. Non-AOC Selection

The WDNR and USGS are aware that there are no sites along the western Lake Michigan shoreline that are truly unimpacted; however, the non-AOC sites selected do not have the AOC designation and are therefore assumed to have communities similar to those that would be present in AOCs if the AOCs did not have the specific contamination that was identified during designation. For example, the Manitowoc River (non-AOC) watershed is similar in size and has similar land-use to that of the Sheboygan River (AOC), including agriculture in the headwaters and heavy industry and urban development near the mouth; they are also relatively close in proximity so they have similar soils, climate, and geography. The assumption is that in the absence of the AOC designation, the communities in the Sheboygan River would be similar to that of the Manitowoc River.

Seven non-AOC harbors have been identified as possible comparison sites; however only six of these will be sampled, with the seventh site to be available as an alternative (West Twin River).The feasibility of each site will be determined from local input, site visits, data collection, and professional judgment. One non-AOC is an alternative site to be used if reconnaissance or circumstances during sampling determine that a proposed non-AOC is unsuitable. A map of the approximate sampling locations in each area is available at: http://g.co/maps/gzg4k.

Table 6 lists the rivers that will be sampled and the approximate coordinates of proposed sampling locations.

Table 6. List of AOCs and non-AOCs with latitude/longitude and drainage area based on USGS gage, if available.

Proposed non-AOCs (comparison sites)		
Name	**Approximate Decimal Long-Lat Harbor/River mouth**	**Drainage area from USGS gages (square miles)**
Escanaba River	-87.023391, 45.718166	870 (Escanaba River at Cornell)
Oconto River	-87.830544, 44.894127	966 (Oconto River near Oconto)
Ahnapee River	-87.433056, 44.608866	Not Gaged
Kewaunee River	-87.499389, 44.459425	127 (Kewaunee near Kewaunee)
West Twin River (alternate site)	-87.563848, 44.145584	Not Gaged
Manitowoc River	-87.651565, 44.092347	526 (Manitowoc at Manitowoc)
Root River	-87.779949, 42.732715	190 (Root River at Racine)

Escanaba River

The Escanaba River was selected as a having similar climate, geography and geology as the Menominee River. Both the Menominee and the Escanaba are cold water rivers with relatively high gradients with portions flowing over bedrock. Because of these similarities, although Escanaba is much smaller than the Menominee they are expected to have similar benthic communities. There is historical contamination in the Escanaba from upstream paper companies and water treatment plants, as well as non-point source runoff from urban land uses. Additionally there is a dam within 1 mile of the mouth that restricts fish migration upstream. The proposed sampling location is on the southern bank upstream of the boat launch near the old railroad pilings.

Oconto River

The Oconto River is the second river proposed to be comparable to the Menominee; but may also be used in comparison to the Fox River. Again, the Oconto has a smaller watershed area, but is still considered a cold water stream with similar characteristics. It too has historical contamination from urban runoff, paper mills, water-treatment facilities, and boat-building. The proposed sampling location is on the southern bank upstream of the harbor approximately across from Basin Road.

Ahnapee River

The Ahnapee River is a small river approximately 30 miles from the mouth of the Fox River, and although it drains to Lake Michigan and is much smaller, its proximity to the Fox River lends to a possible comparison. The Ahnapee River is a low gradient stream with a 65-acre impoundment at Forestville. It flows through predominantly agricultural land and wetlands in its 117-square-mile watershed. The Ahnapee River generally has good water quality and supports a healthy warmwater fishery. Other than water-treatment facilities on the river, no industries directly discharge into the river. The proposed sampling location is on the northern bank between the 2nd Street Bridge and 4th Street Bridge.

Kewaunee River

Similarly to the Ahnapee river, the Kewaunee River watershed is predominantly agricultural (79 percent), and has been chosen as a possible comparison to the Fox River. Most of the Kewaunee River supports a warmwater sport fishery and has seasonal runs of salmon and trout from Lake Michigan. Sediment sampling in 1988 revealed levels of oil and grease, total phosphorus, lead and chemical oxygen considered characteristic of moderately polluted sediments. Along with non-point source contaminants from agricultural and urban runoff in the watershed, there are water-treatment facilities, and several industries that may add contaminant to the river. The proposed sampling location is on the southern bank upstream of the boat launch and water treatment outfall.

West Twin River (alternate site)

The West Twin River begins at the confluence of the Neshota River and Devils River and has a combined watershed area of 176 square miles. Land use is largely agricultural but some industries border the river in the city of Two Rivers. Lake Michigan seiche effects extend approximately 1.5 miles upstream of the mouth. There are fish consumption advisories for PCBs. This site is the alternative site if sampling would not be possible in one of the other non-AOC sites.

Manitowoc River

The Manitowoc River is approximately 25 miles north of the Sheboygan River and has been selected as a comparable site. The land-cover is predominantly agriculture with area of protected wetlands

and some urban land cover. There are several municipalities that have water treatment facilities on the river as well as several industries that discharge directly into the river. There are fish consumption advisories for PCBs and heavy metals. There is a USEPA SuperFund site from a Wisconsin Public Service Corp. manufactured gas plant near the river. The proposed sampling location is on the southern bank upstream of the 10th Street Bridge near the corner of Wollmer and 14th Streets.

Root River

The Root River in Racine was selected as a possible comparison to Sheboygan and the Milwaukee Estuary contributing Rivers. The Root River Watershed (approximately 199 square miles) ranges from heavily urbanized at the headwaters and mouth, to agricultural use in the middle drainage area, and back to urban near the City of Kenosha. There are fish consumption advisories for PCBs and heavy metals. There are several water-treatment facilities as well as many industries using the river for discharges. The proposed sampling location is on the east bank upstream of the 6th Street Bridge near Root River Pathway.

A5.2b. Sampling Location Determination and Site-Specific Considerations

Each AOC is unique and site-specific consideration must be accounted for during sampling location selection. This section details the general locations that samples will be collected for the sites and why those locations were chosen. The goal of the sampling location is to obtain a community sample that is representative of the river system upstream; therefore, areas deemed to have high concentrations of specific contaminants such as “hot spots” will be avoided. The exact sampling locations within a site will be finalized by the lead during deployment of the artificial samplers. These must be attached to a permanent structure and will therefore dictate the general location of the other sampling activities. All of the sampling activities will be conducted within ¼ mile upstream or downstream of the artificial samplers. This allows flexibility in depth for the plankton tows, water depth profile, and Ponar dredge samples, while maintaining relatively similar flow and chemical conditions. Every attempt will be made to avoid additional inputs between sampling activities such as tributaries, outflows, or ditches. The

samplers will be deployed in a location that should not impair boat traffic and not be vandalized. The sampling locations were chosen based on several limitations:

1. The sampling locations must be non-wadeable so that there will be ample depth to collect plankton samples.
2. The sampling locations must be within 1 mile of the river mouth so that there will be some mixing with lake water as all of the AOC have some portion of the lakeshore included in the AOC boundaries.
3. The sampling location must not be upstream of an impoundment or other flow barrier that restricts river and lake water mixing, in-stream migration, or creates a scouring area within the channel.
4. The sediments of the sampling locations must not be coarser than sand (such as gravel or bolder), so that the Ponar Dredge is able to retrieve a complete sample.
5. The sampling locations must be near a permanent structure such as a wing-wall, pier, or piling post so that there is a structure to secure the Hester Dendys to during deployment, and so that there is a point of reference for all sampling events.
6. The sampling locations must be located outside of proposed dredging areas so that the samplers are not disturbed.
7. The sampling locations should be in areas of as minimal boat traffic as possible to minimize possible vandalism and disturbance.
8. The sampling area should not be located in the designated navigational channel when possible so that the bed sediment is not scoured by boats with deep drafts.

Dredging activities within the sites are ongoing. These activities are mainly for maintaining the shipping channels and removing contaminated sediment. As part of study design, the dredging plans were evaluated to determine whether these operations would restrict downstream migration of the benthic and plankton communities. Prior to placing samplers, the lead will make every effort to have the most up-to-date and accurate maps of where the dredging activities will be so that the Hester-Dendy's will be sufficiently removed from areas of active dredging. The contacts at WDNR for each system have the most comprehensive knowledge of any dredging activities in the sites and will be contacted frequently for updates on the dredging activities.

The exact location details of each artificial sampler and the extent of the sampling activities will be recorded with a GPS and photographs, so that the sampling crew can be positive of the location and future studies can refer to the location. Each sampling location will be given a STORET number so that the data collected can be loaded into WDNR's Surface Water Integrated Monitoring System database (SWIMS, hereafter). In the case of the sites with replicate samples, each will be given their own numbers so that the data can be analyzed separately.

If after the first sampling event, the lead determines that the sampling location is inappropriate due to dredging activities, vandalism, boat traffic, or other circumstances, the lead reserves the right to move the sampling location upstream or downstream to a more appropriate location.

The suggested comparable sites may be amended to include or exclude other non-AOCs sampled sites, such as for the Fox River/Green Bay system swapping the Oconto River for Ahnapee River or adding the Oconto River to the comparative. These decisions will be finalized during data analysis by Amanda Bell.

Lower Menominee River

The Menominee River AOC along the Wisconsin/Upper Michigan border is the northernmost AOC in the study and the climate is substantially different than the more southern sites. The sediment in the river is contaminated with arsenic, polycyclic aromatic hydrocarbons (PAHs, or coal tars), paint sludge, and other heavy metals including cadmium, chromium, copper, lead, mercury, nickel, and zinc (Stage 2 Remedial Action Plan for the Lower Menominee River Area of Concern). The area known as the "turning basin," which is just downstream of the Ansul, Inc. and upstream from the former 8th Street Slip, has some of the highest concentrations of arsenic contamination with values of greater than 500 ppm. Aquatic toxicology testing by the WDNR in 1993 with sediments from that area revealed complete mortality. Ansul, Inc. has agreed to remove sediments that are heavily-contaminated with arsenic (50 ppm or greater). This remediation began in 2009, and will continue until the sediment concentration is less than 20 ppm. The sediment removal via dredging will continue in the turning basin during 2012 and 2013. The assumption is that the dredging activities will contain the majority of the arsenic within a "bubble curtain" so that the water soluble portion is removed instead of being transported downstream.

The tentative sampling location for benthos and plankton is downstream of the proposed dredging area in the main channel, slightly upstream of the 6th Street Slip. There is a small island along the southern side of the main channel where old wing-wall pilings are still present which will provide a suitable anchor of sampling devices.

The Menominee watershed typically receives greater amounts of snowfall and generally cooler temperatures than the more southern AOCs; therefore, the Escanaba and Oconto Rivers were selected as comparable river systems due to the climate and proximity to the Menominee River.

Lower Green Bay and Fox River

The Lower Green Bay/Fox River AOC is unique because there is extensive remediation occurring in the river, it is the largest system in this project, and the bay is different from any other system in the Great Lakes. The Fox River has many paper mills on it and historic discharges of contaminants, primarily

polychlorinated biphenyls (PCBs), were noted as the primary cause of AOC designation. Extensive remediation efforts have been on-going in the Fox Basin since the original RAP in 1988 and will be continuing through at least 2017.

Because of the size of the AOC, three separate sampling locations within the area will be sampled: one in Green Bay and two in the lower Fox River. One plankton sample will be collected in the Bay just southeast of Dead Horse Bay near where Green Bay Metropolitan Sewage District (GBMSD, hereafter) has done cyanobacteria sampling. Benthos samples will not be collected in the Bay because those samples require with deep-water sampling techniques which are not being used in this study. Due to the depth and wave action in the Bay, the H-D samplers would be difficult to deploy and insure that the samplers would not tip, become buried with sediment, or migrate without having a stable structure to attach to during colonization. The sample from the Lower Green Bay will be limited to the plankton community only and will be used as a reference point for future plankton community assessment, although the data will be included in data analysis.

For the river locations, one plankton sample and one benthos sample will be collected near another GBMSD sample station (number 7) and one benthos sample will be collected near a WDNR artificial substrate sampling location near the Fox Point boat launch. These sampling locations have historical benthos data and every effort will be made to maintain spatial consistency with the locations sampled by WDNR and GBMSD. The Fox River samples will be comparable in design, depth and method to the other sampling locations where both benthos and plankton communities will be sampled.

The variability of these communities between the locations will be compared using statistical software, but will ultimately remain as separate samples (not pooled during analysis) to increase the robustness of the statistical analysis. Kewaunee and Ahnapee Rivers where chosen as possible comparable sites base on geology, latitude and climate. It is likely that plankton communities in the Bay will be statically different from the river locations within the AOC and the non-AOCs, and may therefore be compared to the historical data collected in the Bay.

Sheboygan River

The Sheboygan River is the smallest of the AOC Rivers. It is also centrally located along Wisconsin's Lake Michigan shoreline, with similar geology, climate, land-use, and other characteristics to several of the non-AOCs. The Sheboygan River has several contaminant concerns: PCBs, PAHS, and volatile organic compounds (VOCs). One primary source of PCBs was an industrial facility operated by Tecumseh Products Company; a primary source of PAHs was a manufactured gas plant (MGP) operated

by Wisconsin Public Service Corporation (WPSC). The Kohler Landfill was historically a source of various pollutants, including VOCs and heavy metals. Extensive efforts have been made since the original RAP in 1993 through several programs such as USEPA's SuperFund, Sheboygan River Priority Watershed Project, Great Lakes Legacy Act, and most recently Great Lakes Restoration Initiative. To avoid the on-going dredging—continuing throughout the 2012 sampling year—the proposed sampling location is downstream of current dredging activities. The approximate location is on the north side of the river at the US Coast Guard Station at the mouth of the river. This is below the 8th Street Bridge and downstream bubble curtain. If the benthos and plankton communities are being affected by the dredging disturbance and contaminants escaping through the bubble curtain, the samples will capture these effects. The non-AOCs sites to be compared with Sheboygan will likely be Manitowoc and Kewaunee Rivers.

Milwaukee Estuary

At the Milwaukee Estuary AOC, three separate and unique river systems converge to create the Milwaukee Inner Harbor. The following information is from The State of the Milwaukee River Basin report (Wisconsin Department of Natural Resources, 2001) and the Stage 2 RAP (Wisconsin Department of Natural Resources, 2011*a*). The Milwaukee River is the largest river in the system with watershed boundaries in seven counties, covering 384 square miles. The upper reaches of the watershed are heavily farmed with a few municipalities; however the lower reaches transition to primary urban land-cover. The Menomonee River Watershed covers 136 square miles in portions of Washington, Waukesha and Milwaukee counties with the majority of the watershed covered by municipalities and urban land (44%). The Kinnickinnic River Watershed is the smallest (33 square miles) and most urban of the Milwaukee River Basin watersheds (87% urban land-cover). The three river systems converge at the Inner Harbor before flowing into Lake Michigan. Because these river systems represent vastly different land-covers and areas, they will be investigated individually for their contributions to the AOC. The original AOC boundary was expanded in 2008 to include upstream reaches that were known sources of contamination such as the Moss American Superfund Site (Little Menominee River), Lincoln Creek, Estabrook Park, and Cedar Creek.

The extent of the AOC upstream in the Kinnickinnic River is small (approximately 2.5 miles) in comparison to the other rivers—each having greater than 10 river miles in the AOC boundary—and did not expand in 2008. Most of the streams within this watershed have been extensively modified through straightening, enclosure or concrete lining. There are ongoing projects to remove concrete lined-sections of the river upstream of Interstate 43 (such as Project Proposal ID: EPAGLNPO-2010-H-2-1054-1043),

just upstream of the AOC boundary. Although there are known sources of contaminants in the Kinnickinnic River, other than non-point contamination for urban land-covers, these contaminants are concentrated in the deeper navigational areas of the river. The median discharge for the other two rivers is at least two orders of magnitude greater than the discharge of the Kinnickinnic (per USGS gages); therefore, it is likely that the water and sediment in the navigational areas of the Kinnickinnic River are more influenced by the mixing of the two larger rivers and the Lake Michigan tidal and seiche effects than by the contributions of the Kinnickinnic River. Consequently, the Kinnickinnic River will not be directly sampled during this project because of its comparably smaller contribution of flow to the system.

Three separate locations in the downstream reaches of this system will be sampled for benthos and plankton communities. One benthos and plankton sample will be collected from the Milwaukee River near the East bank between the Pleasant Street Bridge and Cherry Street Bridge. A sample from the Menomonee River will be collected downstream of Emmber Lane and upstream of the Mitchell Interchange on the southern bank. One final sample will be collected in the Inner Harbor near the Hwy 794 Bridge so that an overall assessment of the communities can be made just before entering the Lake.

These samples will be kept separate during analysis to determine if the benthos and plankton communities in each of these areas are degraded or if a particular system is more degraded and requires more remediation than the other systems for these BUIs. If the Inner harbor site is determined to be more degraded than the other two locations, then sampling in the Kinnickinnic River system would be suggested for future investigations. The Root River in Racine and Manitowoc River in Manitowoc were selected to be similar systems in comparison to the rivers converging in Milwaukee Harbor (Milwaukee River, Menomonee River) based on land-use, latitude, and climate.

A5.2c. Sampling Design Statistical Robustness

A statistical analysis of the study design determined that 6 non-AOC sites would be sufficient to determine differences between AOC and non-AOC sites. Using a nested sequential design of "Location (Site (AOC/non-AOC))," the denominator degrees of freedom were maximized at 30 with the numerator degrees of freedom at 2. For replicates, there was the same statistical power whether the replicate locations were located both in non-AOC or one in a non-AOC and one in an AOC; therefore, it was decided to locate one in a non-AOC and one in an AOC to characterize both.

Table 7 describes what samples will be collected at each location per sampling event.

Table 7. Sample structure for the assessment of benthos and plankton communities. The sites highlighted in blue are AOCs.

Location (Sampling site)		Replicate	Plankton Tow	Plankton Depth Profile	Ponar Dredge	Hester-Dendy
1. Escanaba River			X	X	X	X
2. Lower Menominee River			X	X	X	X
3. Oconto River			X`	X	X	X
4. Lower Green Bay and Fox River	4A. Fox River (near Fox Point)				X	X
	4B. Fox River (near GBMSD site 7)		X	X		
	4C. Lower Green Bay (near GBMSD site 32)		X	X		
5. Ahnapee River			X	X		
6. Kewaunee River			X	X	X	X
7. Manitowoc River		X	X	X	X	X
8. Sheboygan River		X	X	X	X	X
9. Milwaukee Estuary	9A. Milwaukee River		X	X	X	X
	9B. Menomonee River		X	X	X	X
	9C. Inner Harbor		X	X	X	X
10. Root River			X	X	X	X

A5.2 Project Schedule

This section describes the approximate timeline for the project. Each phase is dependent on the earlier phases of the study and all may be occurring simultaneously at later periods in the project.

Phase 1 – Research and Preparation

The project will begin with a data mining effort to determine if historical information is available on benthos and plankton communities in the AOCs, non-AOCs, and other rivers or harbors along the western shore of Lake Michigan with similar characteristics. The research will primarily occur during the months preceding the data collection timeframe, but will continue throughout the project schedule.

This pre-sampling time will also be used to prepare for sampling. Equipment and supplies will be acquired; field assistants will be hired; and notifications for access will be completed.

Phase 2 – Data Collection

Sample collection and data analysis will begin in the early spring of 2012. The artificial samplers will be deployed in mid to late April after the ice is completely melted in the northern sites and daily low temperatures are above 32°C for at least 1 week. Sampling will be conducted three times during the growing season per sampling year: the spring sample will be collected in May/June; the summer sample will be collected in July/August; and the fall sample would be collected in September/October. The sampling events will be separated by at least 4 weeks, but preferably 6 weeks to ensure adequate colonization of the Hester-Dandy samplers. Every attempt will be made to maintain temporal deployment consistency for each site between sampling events.

Phase 3 – Analysis and Report

The final phase will consist of finalizing the data analysis and report writing. A USGS Digital Data Series report will be prepared, and an article detailing the methods, data, and results of this project will be submitted to a peer reviewed journal publication. Progress reports will be prepared and submitted to WDNR and USEPA in January and July for each of the years that the project is continuing once sampling has begun. All reporting required by USEPA will be completed by the USGS and WDNR. Table 8 is the proposed timeline for the Benthos project.

Table 8. Proposed work schedule for Benthos project.

	2012												2013											
Project and Task	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
QAPP writing and approval																								
Data and literature review																								
Site evaluation and selection																								
Collection Device Deployment																								
Sample Collection																								
Sample Analysis																								
Data Analysis																								
Report writing																								
Report review and publication																								

A6. Sampling Design

The goal of the sampling design is to maximize the characterization of sampling locations while minimizing the variability between samples. For the purposes of this project several descriptors will be used to describe the aspects of the sampling scheme:

1 Sites refer to the rivers (or harbor and bay) being sampled, for example the Fox River.
2 Locations refer to the specific area within the sites being sampled, for example there are two sampling locations within the Fox River.
3 A sampling event refers a single spring, summer, or fall sampling trip to collect the samples.
4 Sample types refer to the samples collected from a given sampling device for example the Hester-Dendy samples are from the Hester-Dendy devices.
5 A subsample refers to a given volume of the whole sample that will be analyzed other than for identification, for example a volume of water from the depth profile will be subsampled for Chlorophyll *a*.
6 The analysis is the test preformed on the subsample such as Chlorophyll *a*.

Each community will be examined using multiple sampling techniques to minimize the possible in-stream variation that occurs naturally as micro-habitats. For example, different species have mirco-habitats preferences within a sampling location in a river; these micro-habitats may vary in water depth, amount of light penetration, water velocity, and chemical inputs. There can be very different micro-habitats from one bank of a river to another. By collecting multiple sampling devices (i.e. multiple Ponar dredges) and compositing them into one sample per site per sampling event, the variability within each sampling location can be accounted for.

All sample collections will be performed by boat, so that towing and retrieval speed can be calculated. Coordinates of each sampling location will be recorded on a GPS unit and recorded on the field sheet. Additional water quality measurements to be taken at each sampling event include dissolved oxygen, pH, specific conductance, and water temperature using a water-quality sonde. This information will be used to assess similarities in ambient water quality between the AOCs and non-AOCs. The sonde will be calibrated daily using USGS Standard Solutions for pH and specific conductivity. The personnel using this equipment are evaluated annually for their performance on determining accurate values for these parameters by the USGS.

Plankton sampling

Two types of plankton samples will be collected: one using a tow net and the other water-depth profile sample. The plankton tow sample will use a 63-µm mesh plankton net and will be a vertical tow from 0.5 meters above the bottom of the channel to the surface. This sampling technique is designed to

capture the larger zooplankton which can migrate throughout the water column depending on temperature and light penetration. The other sample is an integrated water-depth profile sample using a Van Dorn-style water sampler to collect a set of whole water samples at 1-meter increments. The sampling will begin at the water surface and continue for a total of 15-liters of water or to 0.5 meters above the bottom of the channel in the sampling location, whichever occurs first.

Benthos Sampling

Two types of benthos samples will be collected: one using a Ponar dredge for natural/in-situ substrates and one using artificial-substrate samplers. A Ponar dredge will be used to collect benthos samples at each site during each sampling event; depending on substrate types three to five subsamples may be collected with the Ponar dredge and composited into a single benthos sample. A small amount of sediment will also be collected from each Ponar dredge subsample to be composited for particle-size analysis and loss-on-ignition, to determine substrate size, type, and organic matter content at each location.

Three tandem Hester-Dendy (H-D) artificial-substrate samplers will be deployed at each site, attached to a concrete block and anchored to an immobile structure. These artificial substrate samplers will be deployed six weeks prior to the first sampling event to allow adequate time for colonization. The invertebrates that have colonized the samplers will be scraped into sample bottles and will be re-deployed for the next sampling event.

The final assessment for each community at each location will pool assemblages from sample types during analysis; however, the sample from different seasons will remain separate. For example the plankton community for the spring sample at the Menominee River will include all plankton identified in the plankton tow samples and the water depth profile sample from the first sampling event, including soft algae, diatoms, large-cell zooplankton, and small-cell zooplankton. This will result in a total of 6 assemblages for each location.

All methods for sample collection are based on reports published or used by the USEPA for large rivers and lakes, or are detailed in peer-reviewed papers publically available (detailed in section B2). Every laboratory used by this project has standard operating procedures in place for sample analysis and quality assurance practices.

A7. Quality Objectives and Criteria

The main goal of the sampling crew to ensure all data collected is of the utmost quality. The lead will be responsible for overseeing all sampling practices, data received, data analysis and report writing. It is imperative that all aspects of this study are completely transparent and replicable. Natural environmental variability may influence precision and accuracy of the data but this is taken into account by compositing multiple samples for each sampling event and each site. For example, we will be deploying three Hester-Dendy samplers at each site so that they can be composited into one sample, providing a larger surface area for benthos colonization and minimizing microhabitat preferences. The sampling strategy is ambitious and thorough; however it is the opinion of the lead that the data gathered by this project will provide a complete description of the benthos and plankton communities in the sampled sites.

A7.1 Sampling efficiency

Missing a sampling event is unacceptable due to the limited samples that are being collected at each site. This section details the effort that will be made in the case that the sampling event must be delayed.

Sampling Team

To minimize sampling variability, all efforts will be made to ensure that the lead will be present for all sampling activities including sampler deployment, sample collection, and sample processing. A minimum of one additional USGS-employed sampling assistant must be present during all sampling activities and will be working in tandem with the lead during the sampling activities. Ideally the same assistant would be present for all activities; however, it is the discretion of the lead to choose the assistant based on personnel availability, competence, training, and funding. If additional persons from the WDNR, USEPA, universities, local stakeholders, or other organizations, would like to observe or assist in the sampling activities it is at the discretion of the lead to accept or deny their request based on the personal safety of all involved, spatial availability in the sampling location, potential effects on the integrity of the sampling activities, and other factors.

If extenuating circumstances, such as illness or family emergency arises for the lead or selected assistant, the sampling activities will be postponed for no more than one week. If the personal emergency

lasts longer than one week, a capable substitute will be designated by the lead to replace the person that is unavailable.

Sampling Activities

Replicate plankton and benthos samples will be collected at two locations (Sheboygan and Manitowoc River) for each sampling event. It is not the intention of these replicate samples to produce exact duplicates but to measure sampling and natural variability within a system. These co-located replicate samples will be collected within a 100-m^2 area at each station. Although the statistical similarity of the replicate samples will be determined, these samples will be used individually during data analysis to increase robustness of the sampling design.

To minimize disturbance of the different sampling substrates, samples will be collected in the following order: water quality data, plankton samples, and benthos samples (Ponar grab samples and deployment or retrieval of artificial samplers). Because no other water or sediment samples are included in this proposal, the samples for this proposal will be collected without regard to other samples.

If an artificial sampler(s) is lost due to vandalism, high flows, or some other unforeseen circumstances, a new sampler(s) will be deployed so that there is a complete set for the next retrieval. The remaining samplers will be analyzed for community composition with comments regarding the number of available samplers. If all of the samplers at a site were lost, two complete sets of new samplers will be deployed in a more secure location; one set of the samplers will be allowed to colonize for 4 weeks and retrieved for analysis with comments regarding the shortened length of deployment. The second set will be retrieved during the next complete sampling event and analyzed with the other samples as scheduled.

Sampling Delays due to Uncontrollable Events

Every attempt will be made to maintain temporal consistency for each site between sampling events; however, personal safety of the sampling crew will be the first priority of the lead. Uncontrollable events will be dealt with as needed. In cases where extreme weather occurs, including storms, floods, droughts, tornados, etc., sampling maybe postponed until the system has had adequate time to recover. For example, in cases of extreme high flows such as when the stream flow is greater than the 75th percentile (considered *above normal* by the USGS), the lead will evaluate the sites for safety based on knowledge from USGS and WDNR personnel familiar with each site. In some instances, these high flows may scour the streambed and detach benthic invertebrates, or may flush the ambient plankton communities downstream. Ideally, the six-week colonization period of the samplers and system will allow

the proceeding sampling events to recover fully from the high flows. If the system has not had adequate time to recover when the 6-week sample collection is scheduled, sampling maybe postponed for up to 2 weeks to allow additional recovery time. If upon sample collection, the lead determines that the high flows have affected the communities for a given time period, the sample will still be collected and analyzed with comments detailing the scouring event(s).

A8. Special Training/Certifications

All USGS field personnel will have current CPR, AED, and First Aid certifications. Anyone operating a USGS vessel will have successfully completed US Department of Interior's Motorboat Operator Certification Course within the previous five years. USGS personnel who will be interpreting biological data also have specialized training in taxonomy, ecology, biological assessment of streams, and methods of statistical analysis. USGS personnel have additional training in water-quality assessment methods and participate in annual USGS quality assurance testing. All staff assigned to sample collection activities have received proper training in sample collection and field analysis and have demonstrated their ability to perform these duties.

A9. Documentation and Records

A9.1 QAPP Control, Distribution and Updates

The original approved QAPP will be retained by Donalea Dinsmore, WDNR QA Coordinator for the WDNR in the Madison, Wisconsin office. A copy of the original approved QAPP will be distributed to individuals identified in Section A.3 Distribution List of this document. In the event that Project Members identified in Section A.4 Project/Task Organization wish to modify or append to the QAPP and supporting protocols, Amanda Bell will forward the request to Project Members for their review and consideration. Acceptance with conditions or denial of proposed revisions will be the responsibility of the Donalea Dinsmore of the WDNR, and T. Kevin O'Donnell and Louis Blume of USEPA-GLNPO. Approved modifications or addendums to the QAPP and supporting protocols will be made to the original QAPP and distributed to Project Members. The updated QAPP cover page will clearly identify the appropriate date and version. Field forms and resulting database entries will include the date and version for the revised QAPP.

A9.2 Reports, Data and Field Records

The follow information will be recorded on every field sheet and label produced for this study: site name, location, date, and beginning time. Additionally the following information will be recorded on the field sheets: crew members, description of photographs taken, notes regarding site conditions, sampling locations, among other information.

1. Sample type specific information:
2. Hester-Dendy: deployment depth, GPS location, permanent structure device is attached to
3. Ponar dredge: number of dredges, depth of each dredge, GPS location of each dredge

Field sheets to be used during sampling will be produced using the Surface Water Integrated Monitoring System (SWIMS) Database controlled by the WDNR, and stand water quality and stream gaging field sheets created by the USGS. Labels will be printed on waterproof paper and in the format requested by each particular laboratory. Field sheets and sample labels will be reviewed for completeness by the lead within 24-hours of sampling. All field sheets and field notes will be scanned and electronic copies will be retained along with the Access database, housed on a secure USGS computer. The original field sheets will be kept at the USGS office in Middleton, WI for a minimum of 5 years.

Data Management

The data, where appropriate, will be entered into the SWIMS database, the USGS National Water Information System (NWIS), and/or an Access database to be house on a secure USGS computer system. Each laboratory distributes their data differently. The Wisconsin State Laboratory of Hygiene (WSLH, hereafter) directly loads their data into the SWIMS or NWIS system as appropriate. UW Superior and Paul Garrison have indicated they will provide an electronic Excel file with the community data. This will be loaded in the Access database and SWIMS were possible.

All biological assessment results (reports, data, and field records) will be proofed, electronically scanned if necessary, and electronically stored in the USGS computers. The water quality field data from the YSI multi-probe water-quality sonde is recorded onto a field form. All water quality data results will be proofed and electronically transferred and stored in the USGS computers. Data reduction and analysis will utilize MS Excel, Access, and Primer-E software.

For the conclusion of this project, a final USGS Data Series report will be prepared and an article detailing the methods, data, and results of this project will be submitted to a peer reviewed journal publication. Progress reports will be prepared and submitted to WDNR and USEPA in January and July

for each of the years that the project is continuing. All reporting required by USEPA will be completed by the USGS and WDNR.

Bi-annual and final reports will be prepared by USGS and WDNR, and submitted to the USEPA in MS WORD and Excel software applications. All field forms will be scanned as Acrobat .PDF images. The project will include frequent, high-quality, digital-photographic documentation of sampling efforts and locations. Photographs will be uploaded to a computer as .JPG format. Following peer and project member reviews and completion of the final report, the report will be linked to publically available WDNR, USGS and USEPA web sites, as requested and appropriate. All original monitoring interim and final reports, field forms, project correspondence and photographs will be retained by the USGS for the biological assessments and water quality monitoring. Identical copies of both will be provided for WDNR and USEPA records.

B. Data Generation and Acquisition

This section describes the steps required to assess the benthos and plankton communities in the AOCs and non-AOC's along Lake Michigan's western shoreline.

B1. Outside data and literature acquisition

Several known plankton and benthos community studies have been completed by federal, state or university personnel acting under strict guidance procedures; however, it is not known how much additional previous sampling of the benthos and plankton has occurred in the sites selected for this project, nor the methods used to collect those samples. Several persons, such as professors, federal/state/ local biologists, and private consultants who are familiar with the sampling locations of known studies have volunteered to provide historical data that they have acquired throughout their tenure.

A background literature search will be performed to determine other similar studies that may have occurred historically in these sites or in other areas of the Great Lakes. These papers must be from a peer-reviewed source including journal articles, publically available reports from state or federal agencies, or published masters and doctorate theses. We will be requesting information from universities; local, state, and federal agencies; and private firms that are known to take aquatic samples in the sites. Boolean searches will include terms such as: Lake Michigan, benthic or benthos, invertebrates, plankton, Wisconsin, estuaries, river, mouth, harbors, Great Lakes, Green Bay, Milwaukee, Menomonee, Sheboygan, Menominee, and Marinette, among others. The reports will be used to build knowledge of historical benthic and plankton communities, and the citations for these reports will be entered into an EndNote citation database to be housed at the USGS office in Middleton.

All available historical data and reports for benthos and plankton communities in the study sites will be evaluated to determine if the data can be compared to the current study to assess whether a shift in the communities has occurred. Where the historical studies occurred in the selected sites, and the collection methods are comparable to those described here, the data from the reports will be requested from the authors. In order to accept the community data, we will verify the sampling methods and QA procedures of the sampling efforts to confirm they meet GLNPO standards and would be comparable to USGS methods and data. If the methods are similar to those detailed here, and the data will be entered into an Access database to be housed at the USGS office in Middleton. A preliminary community

assessment using selected metrics such as regional IBI scores, taxonomic richness, diversity indices (for example, Shannon-Weaver diversity index) and non-parametric ordination scores will be calculated. These assemblage data and these metrics will be compared the data collected by this project to determine temporal changes in the communities. These findings will be presented as complimentary analyses to the analyses completed with the data collected for this project. It is at the discretion of WDNR whether to consider the findings gathered from the data mining effort of the project in determining the status of the particular BUI.

B2. Experimental Design

Four separate sample types will be collected for the sites: a plankton tow sample, a depth profile plankton sample, a Ponar grab sample, and a Hester-Dendy sample. The 63µm plankton tow will be analyzed for large-cell zooplankton identification and enumeration only. The depth profile plankton sample will be analyzed for small-cell (<63µm) zooplankton identification and enumeration, soft algae phytoplankton identification and enumeration, diatom phytoplankton identification and enumeration, chlorophyll *a* concentration, and ash-free dry mass. The Ponar grab sediment sample will be analyzed for benthos identification and enumeration, sediment particle size, and loss-on-ignition (organic matter content). The artificial substrate benthos sample will be analyzed for benthos identification and enumeration only. Table 9 summarizes the sample types and analyses. Additionally, for each location, the following non-biotic information will be collected: field-measured water-quality data (temperature, dissolved oxygen, specific conductance, and pH), GIS location, photographs and possible videos.

Table 9. Sample types and analyses that will be performed for each.

Sample Type	Analysis
Plankton Tow	Large cell (<63µm) zooplankton identification
Depth Profile Plankton	Small cell (<63µm) zooplankton identification Soft algae identification Diatom phytoplankton identification Chlorophyll *a* Ash free dry mass
Ponar grab (sediment)	Benthos identification Particle size Loss-on-ignition (organic matter)
Hester Dendy artificial substrate	Benthos identification

B3. Sampling Methods

All sample collections will be performed by boat, so that towing and retrieval speed can be calculated. Coordinates of each sampling location will be recorded on a GPS unit (eTrex 10 by Garmin) and those coordinates will be recorded on field sheets, uploaded to a USGS computer and incorporated in the WDNR's SWIMS database. Photographs will be taken during sampling to document sampling methods and site characteristics. Images will be uploaded to a USGS computer and maybe used in the final report. Additional field measurements to be taken at each sampling event include dissolved oxygen, pH, specific conductance, and temperature using an YSI multi-probe water-quality sonde (model 6920V2).

All methods for sample collection are based on reports published or used by the USEPA for large rivers and lakes, or are detailed in peer-reviewed papers publically available (table 5). The text that is specific to this project is included below. Every laboratory has standard operating procedures in place for sample analysis and quality assurance practices. These documents are in the attachments.

Plankton Collection

The methods for zooplankton collection are based on the USEPA's Standard Operating Procedures (SOPs) for zooplankton sample collection and preservation GLNPO Water Quality Survey (WQS) (LG402, Revision 10, March 2005); however, because the samples will be performed in the harbors, bays and rivers, the deeper water sample will not be collected:

> *"One sampling tow is performed at each station from 20 meters below the water surface to the surface using a 63µm net. If the station depth is less than the specified depth, the tow is taken from about 0.5 meters above the bottom to the surface. The tow net, with a screened sample bucket attached at the bottom, is lowered to the desired depth, and raised at 0.5 meters/second to collect zooplankton from the water column. After lifting the net from the water it is sprayed with a garden hose to wash the organisms down into the bucket. The sample is concentrated into the sample bucket and is transferred to a sample storage bottle."*

The net to be used is composed of a 20 inch net ring and bridle (part number 7-E50), 63 µm Nitex 1:3 plankton net (part number 30-E28) with a 3 ½ inch by 89 mm adaptor (part number 48-D80), and 500 ml dolphin bucket (part number 47-E28) available from WildCo. The total length of the tow net is 1.8 meters. A minimum of one tow will be conducted at each location. If the depth of the water column is less

than 6.8 meters, additional tows will be performed so that a minimum of 5 meters of water depth are sampled, not including the length of the tow net. This sampling protocol is expected to result in a sufficient number of organisms based on past experience; however each sample will be inspected visually to confirm its adequacy. The 63µm tow sample will be preserved with glutaraldehyde (25% in water, at 1 mL per 100 mL sample), and sent to Paul Garrison at the WDNR for zooplankton identification and enumeration in accordance with GLNPO SOP LG 403, Zooplankton Analysis (table 6).

In addition to the 63µm sample, 1 liter of water from each meter of depth will be collected using Van Dorn style sampler device for a maximum of 15 liters of water based on EPA-GLNPO SOP LG400. The Van Dorn style sampler will collect a water sample from a selected depth when a messenger is deployed to release the closures. The sampling will begin at the water surface and continue for a total of 10-liters of water or to 0.5 meters above the bottom of the channel in the sampling location, whichever occurs first. If the sampling location is less than 2 meters deep, additional depth profile samples will be collected to obtain a minimum of 5 liters of water which would provide enough water to split for the various samples. The sampler will be attached to a cable marked at 1-meter increments to ensure accurate depth samples.

Using a standard water splitter, several subsamples for separate analyses will be taken from this integrated depth profile sample. One approximately 250-mL aliquot will then be placed in 250-mL plastic bottle, preserved with glutaraldehyde (25% in water, at 1 mL per 100 mL sample), and sent to Dawn Perkins at WSLH (Schedule F2034A1) for soft algae phytoplankton identification and enumeration in accordance with ESS BIO METHOD 2035 (see attachment). One1-L aliquot will then be placed in 1-L plastic bottle, preserved with glutaraldehyde (25% in water, at 1 mL per 100 mL sample), and sent to Paul Garrison at the WDNR for diatom phytoplankton identification and enumeration in accordance with GLNPO SOP LG 404, Phytoplankton Analysis (see attachment). One 5-15 mL aliquot of this water sample will be subsampled and filtered for chlorophyll *a* using Millipore Mixed Cellulose Ester Membranes (Millipore Brand SMWP04700), placed in analysis vials, and preserving on dry ice until delivering to WSLH for analysis according to ESS BIO METHOD 151.1 (see Attachments, Schedule I251UNF) (table 6). One 5-15 mL aliquot will be subsampled and prepared in the field for ash-free dry mass by filtering through a glass fiber filter (Whatman™, 934-AH™), wrapping the filter in aluminum foil, placing in a Petri dish, and preserving on dry ice until delivering to WSLH for analysis using schedules I650JLT and I650JVL (USEPA methods 160.2 and SM 2540E-17thedition).

Table 10. Plankton sample laboratory disposition.

Sample type	Disposition	Information gained	Sample Container
63µm plankton tow	WDNR	Community assessment of zooplankton	1-Liter Plastic Bottle
Depth profile sample	WSLH	Community assessment of soft algae phytoplankton	250-mililiter plastic bottle
	WDNR	Community assessment of diatom phytoplankton	1-Liter Plastic Bottle
	WSLH	Chlorophyll a concentration	Glass Fiber Filter wrapped in foil
	WSLH	Ash free dry mass	Glass Fiber Filter wrapped in foil

Benthos Collection

The two methods for benthos collection are based on the USEPA Assessment and Remediation of Contaminated Sediments (ARCS) Program—Assessment Guidance Document, Chapter 7: Assessment of Benthos Community Structure (EPA 905-B94-002) and Weigel and Dimick (2011).These methods are comparable to current methods used by the WDNR and other researchers such that the data collected here may be compared to other studies within and around Wisconsin.

The first method involves a grab sample of the bottom sediment using a Ponar dredge (part number 1725-F50, available from WildCo). The Ponar dredge sampler grabs a 9" by 9" area of sediment with an approximate sample volume of 8,200mL. A Ponar dredge will be used to collect benthos samples at each site during each sampling event; depending on substrate types three to five subsamples may be collected with the Ponar dredge and composited into a single benthos sample. Ideally five subsamples will be collected; however, substrates that are thick, heavy loams and clay, or those with a large amount of organic debris may not filter well and may overwhelm the benthos sample. Therefore, in those locations the lead will determine if fewer subsamples will be sufficient as discussed with Dr. Kurt Schmude (personal communication, September 30, 2011). The number of dredges will be indicated on field forms and sample labels. To minimize costs of analyzing multiple benthos samples for each location, multiple times per year, compositing the dredge samples into a single sample will produce a more comprehensive taxa list for the locations and will then be more comparable between sites. Although USEPA's ARCS does not require more than one sample per location, the investigators feel that a composite sample will more accurately reflect the communities with the AOCs and non-AOCS (see http://www.epa.gov/reg3hscd/risk/eco/faqs/composite.htm for more information). The material from each dredge will be placed in a separate bucket for transport back to the boat launch for further processing.

A small amount of sediment from each grab sample will be collected, composited and homogenized after removing large debris such as sticks and trash. From this, samples for particle-size and loss-on-ignition will be collected to determine substrate size and organic matter content. These subsamples will be placed into separate double Ziploc freezer-weight baggies and stored on iced until analysis. The particle-size samples will be sent to WSLOH (schedule I495ELT) and loss-on-ignition (volatile-on-ignition) will be conducted by Amanda Bell at the USGS (page 451, I-5753-85, *in* Fishman and Friedman, 1989) (table 7). Each grab sample will then be elutriated to remove debris, larger sand and inorganic particles and rinsed with native site water to remove finer sediment through a 500 μm wash frame (part number 188-E50 available from WildCo). Any mussels found in the sample will be checked for other attached benthic invertebrates (which will be added to the main sample) and placed in a separate sampling container with preservative so that they do not crush the smaller organisms during shipping. The individual Ponar samples will then be composited into one sample, transferred into a collection bottle, stained with Rose Bengal stain, and preserved with 10% formalin solution before submission to Dr. Kurt Schmude at the University of Wisconsin-Superior for identification and enumeration in accordance with GLNPO SOP LG 407, Invertebrate Analysis (table 11).

The second benthos sample is collected using Hester-Dendy artificial samplers. At each location, 3 samplers will be attached to a concrete block to anchor the samplers to the bottom of the channel. Each H-D samplers consists of eight 3" diameter plates of hardened Masonite that are separated by spacers to create 3 single spaces (1/8"), 3 double spaces (1/4"), and 1 triple space (3/8"), totaling 120 sq. inches of surface area (0.09 sq. meters). The total surface area of the three H-D is 360 sq. inches. The concrete block will then be attached to a stable or permanent structure (such as wing wall or pier piling) using a wire rope. After allowing 6 weeks for colonization, the samplers will be retrieved during the collection of the other samples. The samplers will be disassembled in the field, the invertebrates scraped into a sample container with a leak-proof screw-top lid, reassembled, and then redeployed for the next sampling effort. The benthos samples will be preserved with Lugol's solution by adding 0.3 mL Lugol's solution to 100 mL sample and stored in the dark (Standard Methods for the Examination of Water and Wastewater, 2005).The samples will then be sent to Dr. Schmude at the University of Wisconsin-Superior for identification and enumeration in accordance with GLNPO SOP LG 407, Invertebrate Analysis (table 11).

Table 11. Laboratory disposition for benthos samples.

Sample type	Disposition	Information gained	Sample Container
Ponar grab	University of Wisconsin–Superior	Community assessment of benthos	1-Liter Plastic Bottle
	USGS	Loss on ignition/organic matter content	Double Ziploc baggie
	WSLOH	Sediment particle size distribution	Double Ziploc baggie
Hester-Dendy sampler	University of Wisconsin–Superior	Community assessment of benthos	1-gallon leak-proof pail

Additional Measures

An YSI Sonde will be used to gather in-situ readings at each location. An YSI sonde will be calibrated daily according to manufacturer recommendations using calibration standards from the USGS National Water Quality Laboratory. The YSI manual is included in the attachments. These calibration readings are entered into the Calibration Log for the sonde. The mutliprobe end of the sonde is then placed into the water near the Hester-Dendy sampling location at approximate the mid-channel depth and allowed to equilibrate before the variable readings are recorded on the field sheets. While readings are being taken, trained and experienced personnel can examine readings on the output screen and if they notice any anomalies, take corrective actions as necessary. Examples of corrective actions include abnormal site readings that may require inspection of sensor, check of electronic connections, use of a backup sensor to collect readings, etc. Field personnel on survey are responsible for ensuring field sheets are complete and that the data collected are accurate. The lead is responsible for ensuring the overall integrity of sample collection and field sheets. Any problems encountered in sampling that could affect the data are documented on field sheets. The parameters collected and sensitivity of the parameters are summarized in table 12.

Table 12. YSI Sonde Parameters and sensitivities.

Parameter	Sensitivity
pH	0.01 units
Dissolved oxygen	0.01 mg/L
Water temperature	0.01 °C
Specific conductivity	1 µS/cm

The coordinates of each Hester-Dendy location will be determined using a hand-held GPS. The model that selected for this project is a Garmin e-Trex® 10 because of its ruggedness and waterproof design. All locations will be stored as Waypoints in the handheld and those locations will be downloaded to a secure USGS computer at the conclusion of each sampling event. To increase the accuracy of the readings "Waypoint Averaging" will be used. Preferred accuracy is within 10 feet.

B3. Sampling Handling and Custody

All containers will be supplied by the WDNR or WSLH. After collection, all samples will be placed into the appropriate containers as summarized in tables 10 and 11. Samples will be preserved or stored as indicated in section B3. Field sheets developed from WDNR's SWIM database, USGS standard techniques, and labels requested by the laboratories will be completed for each location summarizing field parameters and sample information. Below is an example of what each sample label will contain at a minimum:

Site name:_____ Date:_____
Location: _____ Begin Time:_____
Analysis to be performed:_____
Contact: Amanda Bell, ahbell@usgs.gov 608-821-3882

Prior to laboratory custody the samples will remain in the custody of the USGS. The benthic samples will be shipped via FedEx with tracking numbers to Dr. Schmude at UW Superior. The samples for Paul Garrison will be delivered by USGS personnel to his laboratory. The samples to be analyzed by the State Laboratory of Hygiene will also be delivered by USGS personnel. The samples to be analyzed for loss on ignition will remain in the USGS facility in Middleton until analysis. All samples will be shipped or delivered within one week of completion of the sampling event.

B4. Analytical Methods

All analytical methods are detailed in the attachments and are in accordance with approved USEPA, GLNPO, or USGS standards. All biological communities will be identified to the lowest taxonomic level possible based on current literature. The lead prefer genus/species level identification to achieve the most accurate community data for metrics such as the Index of Biotic Integrity (IBI,

hereafter); however, due to time constraints, family-level identification for individuals that are damaged, immature, missing sexual organs, or otherwise would greatly increase identification time, is acceptable. Genus/species identification provides more accurate ecological and environmental information, but family-level identification provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. Hilsenhoff's family biotic index, for example, usually indicates greater pollution than the species/genus level Hilsenhoff Biotic Index (HBI, hereafter) for "unpolluted" or "slightly polluted" streams (Hilsenhoff, 1988). Regardless of the taxonomic level of identification, only those taxonomic keys that are peer-reviewed and available publically (i.e., published) should be used.

Zooplankton

The 63µm plankton-tow sample will be analyzed for zooplankton identification and enumeration by Paul Garrison at the WDNR in accordance with GLNPO SOP LG 403, Zooplankton Analysis. In summary, the samples are rinsed from the sample bottle through a 63-µm mesh sieve with DI water to remove the formalin. The sample is then washed into a glass jar, shaken to break up clumps, and poured into the Folsom plankton splitter. The sample is split immediately by rotating the splitter before the organisms can settle. One subsample from the splitter is placed in a labeled jar with the fraction of the original volume (1/2). The second subsample is then split again, with one subsample of that saved in a labeled jar with the fraction of the original volume (1/4). This process is repeated until the last two subsamples contain 200-400 microcrustaceans. The samples are placed in separate jars and labeled accordingly. Four subsamples, including the final two subsamples are examined and enumerated under a dissecting microscope. The samples are concentrated and examined according to protocol with some organisms needing greater magnification under a compound microscope. Rotifers and nauplii identification and enumeration are completed separately due to their smaller size. Subsamples are taken from the samples split previously. The subsamples are placed in a Sedgwick-Rafter cell and covered with a glass cover slip. The organisms are then identified and enumerated under a compound microscope at 100x magnification.

Soft Algae Identification

The soft algae identification will be performed by Dawn Perkins at the WSLH for schedule F2034A1 based on the methods detailed in ESS Bio Method 2035 (see attachments). In summary, the presence-absence screen procedure uses a Palmer-Maloney nannoplankton chamber and compound

microscope to identify phytoplankton present. To quantify the phytoplankton the number of cells of each taxon is counted within the 5-50 non-overlapping nannoplankton chamber or field areas and then those counts are used to calculate the abundance in the entire sample. The alternative identification and quantification technique uses an inverted microscope with Utermöhl sedimentation chambers & towers. The chambers and towers are prepared, the sample is homogenized, and a predetermined volume of sample is carefully pipetted into the tower. The tower is allowed to settle for a set time depending on the depth in the tower. The tower is then drained into the Utermöhl sedimentation chamber and the sample is viewed on the inverted microscope. The number of cells is enumerated at the lowest taxonomic unit possible (i.e. genus, species, etc.) found in 5-100 random non-overlapping microscope fields at 500x or greater magnification. Those counts are used to calculate the abundance in the entire sample.

Diatom Identification

Paul Garrison at the WDNR will analyze diatom phytoplankton identification and enumeration for the depth profile sample in accordance with GLNPO SOP LG 401, Phytoplankton Analysis. The diatoms are cleaned using Hydrogen peroxide followed by potassium dichromate (based on van der Werff, 1956) unless large amount of organic matter are present in which case the cases are cleaned using boiling nitric acid. In summary, an allotment of the plankton sample, based on the abundance of diatoms in the sample, is concentrated by centrifugation, and placed in a 1000 mL beaker. Approximately 5 mL of H2O2 is added to the sample and allowed to react for several minutes before a partial spoonful of potassium dichromate is added to the sample. The mixture is rinsed from the sides of the beaker using deionized water (DIW) during the reaction. Once the reaction if complete the sample is poured into a 50 mL centrifuge tube and spun for 10 minutes at a setting of 80. After the 10 minutes, the liquid is pipetted off and the sample is rinsed again with DIW. This washing process is repeated until the liquid has no color, usually 4 rinses, and is then centrifuged one more time. The final pellet is placed into a 50 mL beaker and the volume is brought to 20 mL with washing from the final centrifuge tube; 2 drops are added to the solution to minimize diatom clumping.

To prepare the identification an enumeration slides, no more than 12 drops of sample and DIW are added to a cover slip and allowed to air dry. The cover slips are warmed according to protocol and a slide is prepared. A drop of Naphrax is added to the slide and the cover slip is place face down on the drop. The slide is then heated, cover slip secured, and placed under a microscope for examination. The slides are labeled and stored with the archived pellet. Additional slides are prepared in the same fashion as need with a minimum of two slides per sample. The number of cells is enumerated and identified at the

lowest taxonomic unit possible (i.e. genus, species, etc.) along a transect across the slide. Valves are counted if more than half the valve is in the field of view.

Chlorophyll a

The filtering for chlorophyll a and the ash-free dry mass will occur in the field using a predetermined volume of water from the depth profile sample. The chlorophyll a sample will be sent to WSLH for analysis according to ESS BIO METHOD 151.1 (Schedule I251UNF).

> *"Algal cells are concentrated by filtering a known volume of water through a membrane filter (47 mm, 5.0 µm poresize). The pigments are extracted from the concentrated algal sample in a solution of aqueous 90% acetone aided by bath type sonication. The chlorophyll a concentration is determined by fluorescence. The excitation wavelength is 436 ηm with a slit width of 5.0 ηm. The fluorescence is measured at a wavelength of 680 ηm and a slit width of 3.0 ηm. The fluorescence spectrophotometer is calibrated with pure chlorophyll a standards of a known concentration. The resulting calibration curve is used to determine the chlorophyll a concentration in the sample extracts. The concentration of the chlorophyll a in the natural water sample is reported in µg/L."*

Ash-Free Dry Mass

WSLH will also analyze the ash-free dry mass sample using schedules I650JLT and I650JVL (USEPA methods 160.2 and SM 2540E-17TH ED). For biological purposes the difference between Standard Methods2540 D. Total Suspended Solids Dried at 103–105°C, and 2540 E: Fixed and Volatile Solids Ignited at 550°C, approximate the ash-free dry mass of the suspended plankton biomass.

> *"A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids."*

And

> *"The residue from Method B, C, or D is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.*

Benthos Identification

The benthos identification and enumeration for both the Ponar and Hester-Dendy samples will be performed by Dr. Kurt Schmude's Taxonomy Laboratory at the Lake Superior Research Institute's (LSRI), UW Superior, based on the methods detailed in FS/14 (Picking Benthic Invertebrates from

Samples)and FS/13 (Identification of Benthic Invertebrates) in accordance with GLNPO SOP LG 407, Invertebrate Analysis. The sample from the Ponar dredges and Hester-Dendy will have been prepared in the field according to Section B2.

To prepare the samples for identification:

"Samples are rinsed through a sieve to remove the preservative, and debris and organisms retained by the sieve are transferred back into a sample jar. Small portions of the sample are placed into a gridded Petri dish for picking, and water is added to dilute the sample. Organisms are removed from each subsample using forceps while viewing through a dissecting microscope. The animals are separated into taxonomic groups (depending on the project requirements) and placed into vials containing ethyl alcohol."

For identification and enumeration:

"Benthic invertebrates are identified and enumerated separately by taxonomic group while viewing through a compound microscope (e.g., Oligochaeta or larvae of Chironomidae), or dissecting microscope (e.g., all other invertebrates) using fine-tipped forceps. Only one sample should be opened and processed at a single work station at a time; this will avoid mixing specimens among samples.
Taxonomic identification level depends on the specimen. Benthic invertebrates are identified to the following taxonomic levels (unless otherwise specified by project requirements): 1) Oligochaeta are identified to lowest taxonomic level possible, usually species. All other specimens are identified as pieces (without heads), immature tubificids (without chaetae), immature tubificids without hair chaetae, or immature tubificids with hair chaetae. 2) Larvae and pupae of Chironomidae are identified to subfamily or tribe (very immature or damaged specimens), genus, species group, or species. 3) Other macroinvertebrates are identified to the following taxonomic levels: insects to genus or species; Mollusca to family, genus, or species; Crustacea to genus or species; Hirudinea to genus or species; Nematoda to phylum; and Cnidaria to genus."

Sediment Particle Size

The sediment particle size samples will be sent to WSLH (schedule I495ELT), which is ultimate analyzed by the University of Wisconsin Extension (UWEX) Soils Laboratory. The methods are based on the hydrometer procedure by Bouyoucos (1962). In summary:

"The percentage of sand, silt and clay in the inorganic fraction of soil is measured in this procedure. The method is based on Stoke's law governing the rate of sedimentation of particles suspended in water. The sample is treated with sodium hexametaphosphate to complex Ca++, Al3+, Fe3+, and other cations that bind clay and silt particles into aggregates. Organic matter is suspended in this solution. The density of the soil suspension is determined with a hydrometer calibrated to read in grams of solids per liter after the sand settles out and again after the silt settles. Corrections are made for the density and temperature of the dispersing solution."

Loss on Ignition

The loss on ignition will be performed in the USGS facility in Middleton, WI. The methods are described in Fishman and Friedman, 1989 (page 451, code 00496). In summary:

> *"A portion of well-mixed sample is dried at 105°C. A portion of dry, well-mixed sample is carefully weighed and then ignited at 550°C. The loss of weight on ignition represents the amount of volatile solids in the sample."*

B5. Quality Control

Field Quality Assurance

To minimize disturbance of the different sampling substrates, samples will be collected in the following order: water quality data, plankton tows, Ponar grab samples and deployment or retrieval of artificial samplers. The lead will be present for all sampling activities to minimize sampling variability. Replicate zooplankton tows and benthos samples will be collected at two locations (Manitowoc River and Sheboygan River) for each sampling event. These co-located replicate samples will be collected within a 100-m2 area at each station. The community data from replicate samples will be compared to each other to determine site variability. If the statistical similarity of the samples during data analysis is within 95% confidence limits, the data will be used individually during data analysis to increase robustness of the sampling design. If these data are significantly different, the lead will investigate the reason for the variance, whether it is environmental variability or sampling error. If it is determined to be environmental variability, the data will be included in further analyses. If it is determined to be sampling error, data analyses will be conducted without the sample for abundance analysis, but may be included for richness and/or presence/absence analysis because these analyses do not require counted individuals in the samples, just presence of the taxon.

Analytical Quality Control

Each laboratory has their own QA/QC methods and checks that must comply with USEPA standards. The SOPs for each specific analysis contain details regarding the quality control audits and methods. In general the SOPs call for analysis duplicate for 10% of samples.

Zooplankton

Requirements for GLNPO's Zooplankton analysis quality control are as follows:

"In general, ten percent of all samples analyzed are analyzed in duplicate by a second analyst. If a data set has less than 10 samples, at least one sample from that data set should also be analyzed in duplicate. Samples are counted by the second analyst while still in the plankton wheel (or other counting chamber) or Sedgewick Rafter cell, so that only interanalyst variation is quantified, and not variation associated with sub-sampling.
Results from the second analyst are reported under the same sample number as the original sample, with the exception that the seventh character is replaced by a "Q".
Percent similarity will be calculated for the samples analyzed in duplicate by two analysts, according to the following formula:

$$PSC = 1 - 0.5 \sum_{i=1}^{K} |a - b|$$

Where:
a and b are, for a given species, the relative proportions of the total samples A and B, respectively, which that species represents.
It is expected that the two counts should have a similarity of 90%. If not, the reasons for the discrepancies between analysts should be discussed. If a major difference is found in how the two analysts have been identifying organisms, the last batch of samples that have been counted by the analyst under review may have to be recounted."

Soft Algae Identification and Diatom Identification

The soft algae and diatom identification quality assurance procedures are both based on GLNPO's SOP LG401 Phytoplankton Analysis:
Ten percent of all samples collected are analyzed by a second analyst. At least 1 duplicate count is done per data set if the data set contains less than 10 samples. This includes identification, and tabulation of data. Duplicate counts and measurements by two analysts should be done for both Utermohl samples and diatom slide counts. Utermohl samples are counted by the second analyst while still in the counting chamber so that only interanalyst variation is quantified, and not variation associated with sub-sampling. Results from the second analyst are reported under the same sample number as the original sample, with the exception that the seventh character is replaced by a "Q." The Bray-Curtis Index is to be used as a quantitative method of species-level comparison for both enumerations and calculated biovolumes produced by the two analysts. The Bray-Curtis measure is calculated as follows:

$$PS_{jk} = 200 \frac{\sum_{i-1}^{i} (min\, A_{ij} A_{jk})}{\sum_{i-1}^{i} A_{ij} + A_{jk}}$$

Where:
PSjk = percentage similarity between samples j and k,
Aij = abundance of taxon i in sample j, and
Ajk = abundance of taxon i in sample k.
An interim minimum acceptance value of 60 is currently being used until enough data accumulate to determine a more appropriate value. The two taxonomists will discuss the results from all samples which fail to meet this criteria. If a major difference is found in how the two analysts have been identifying or measuring organisms, the last batch of samples that have been counted by the analyst under review will be recounted or measured."

Chlorophyll *a*

The quality assurance techniques for chlorophyll *a* are based on ESS BIO METHOD 151.1 (Schedule I251UNF):

"A Laboratory Reagent Blank (LRB) will be analyzed with every analytical run. This is made by taking a membrane filter, placing it in a 15mL polypropylene centrifuge tube, adding 13mL of 90% acetone, and carrying it through the entire preparation procedure. This will be analyzed at the beginning of the analytical run, and after every 20 samples and must be within ±0.26 µg/L, the LOD based on filtered volume of 200mL. If the LRB fails it should be re-analyzed. If it still fails the analyst should evaluate if recalibration would improve the blank reading. If recalibration is done the samples back to the last good LRB and IPC must be re-analyzed. If recalibration does not cause the blank to be acceptable, the 20 samples associated with that LRB must be qualified with a comment stating that the LRB exceeded acceptable limits.

A working QCS is run at the beginning of every analytical run. The observed concentration of the QCS must be within ± 10% of the true value before proceeding with analysis. Re-prepare the QCS if prep error is suspected and reanalyze. If QCS still fails, re-calibrate and try again. If subsequent attempts fail and samples cannot be stored, proceed with the analyses and qualify all results.

At least 10% of lab filtered chlorophyll samples are analyzed in duplicate. The difference between the duplicate measurements must be within control limits before sample results are considered acceptable. Samples that fail to meet QC limits will be qualified. Since the majority of samples are field filtered and planktonic material tends to be heterogeneous in nature, little corrective action can be taken to improve precision. Visual examination of the extract, documentation and notification of data users through qualifiers is about all that can be done. Consequently, entire batches of data are not qualified based on duplicate QC failures. The QC limits for duplicate analyses can be found in the LIMS QL data set.

Field duplicate analyses are only analyzed when our clients provide us with duplicate filters. Therefore, separate QC limits have not been developed for these tests.

A 90% acetone blank (Calibration Blank—CB) is run at the beginning of each analytical run, every ten samples, and at the end of each analytical run. The blank must be < 0.26 µg/L based on a 200 mL volume (sample LOD). If the initial blank exceeds the LOD, the intercept from the calibration is examined to determine whether there was a problem at calibration, the initial blank is contaminated or if the fluorescence cell is dirty. If the intercept is high or the cell dirty, it is cleaned and the instrument re-calibrated. The initial blank and QCS must be acceptable before proceeding with analysis.

An Instrument Performance Check (IPC) is run every 10 samples. The IPC must be within ± 10% of the true value. If it deviates from this acceptable limit, the analyst will attempt to determine whether the cell has become dirty, the instrument has drifted, or the IPC is contaminated. If the problem can be identified, it is corrected, the instrument re-calibrated and all samples back to the last valid IPC will be reanalyzed.

Dilutions are typically made by adding 1mL of sample to 4mL of 90% acetone solution using mechanical air displacement pipettes. Dilute high samples, add the sample numbers to analytical run list, change the dilution factor to reflect the 5x dilution, and analyze along with an IPC and CB at beginning, every ten samples, and at end of the run of diluted samples. Dilution concentrations should be within 90%-110% of the original concentration. If dilutions do not

agree with the initial concentration, another different dilution should be performed to verify. If two serial dilutions do not agree (90%-110%), the sample result must be qualified.

Ash-Free Dry Mass

For the analysis of Total Suspended Solids Dried at 103–105°C: "Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. If volatile solids are to be determined, treat the residue according to 2540E."

For the analysis of Fixed and Volatile Solids Ignited at 550°C: "Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. Weight loss of the blank filter is an indication of unsuitability of a particular brand or type of filter for this analysis."

Benthos Identification

The laboratory will pick the invertebrates contained within the sample according to the SOP. The laboratory at UW Superior has participated in many USEPA. Because the sampling is quantitative all organisms in the sample will be enumerated unless subsampling is necessary, in which all organisms in the subsample will be enumerated. Quality control procedures for the benthic invertebrate picking are as follows:

"Benthic invertebrate picking should only be conducted by personnel who have read and understood this SOP, who have been properly trained, and who have demonstrated competency in following this procedure. All procedures outlined in this SOP should be followed exactly; any deviations from this SOP should be approved (prior to sample picking) by a supervisor or project principal investigator.
Record data on pre-printed datasheets and/or in project-specific laboratory notebooks, following the documentation procedures outlined in the LSRI Quality Management Plan. Data storage time is project-specific, but typically does not exceed five years from the date the project is completed (i.e., final report is signed) or terminated.
A quality control (QC) check must be performed by qualified personnel who are experienced in sorting and picking benthic invertebrate samples. All QC checks must be performed immediately following picking of the sample. A QC check should be conducted on 10% (1 out of 10, randomly selected) of an individual's picked samples for each project. The individual performing the QC check must go through the "Sorted Debris" container for the randomly chosen sample and count the number of benthic invertebrates found in the debris. Calculate the percent picking efficiency for each sample using the following calculation:

$$\text{Percent Picking Efficiency} = \left(\frac{A}{A+B}\right) * 100\%$$

Where:
A = the number of organisms found by the primary picker

B = the number of organisms missed by the primary picker and found during the QC check
Ensure that a >90% picking efficiency is achieved. If an individual fails to achieve a >90% picking efficiency on a QC check, then QC checks should be performed on that individual's next five consecutive samples until a >90% efficiency is achieved. If an individual fails to meet the >90% picking efficiency on all five consecutive samples, corrective actions should be taken, such as re-training the individual.
Allow a reduced accuracy (i.e., lower percent picking efficiency) in the following two situations (and based on the project objectives): 1) When a sample contains a low density of benthic invertebrates; low numbers of organisms can produce artificially high percentages of error. For example, if three organisms were found during the first pick of a sample, and two additional specimens were found during the QC check, then 40% of the organisms were missed during the first pick. However, only two specimens were missed overall. 2) When the percent picking efficiency does not have any effect on the interpretation of the data samples do not need to be repicked.
For identification:

"Identification and enumeration of invertebrates will be the responsibility of LSRI's Senior Invertebrate Taxonomist, Dr. Kurt Schmude. All identifications made by students and a proportion made by the biologists are verified by the senior taxonomist for accuracy. All identifications will be based on current taxonomic literature. Confirmation by outside expert taxonomists will be obtained if deemed necessary. All invertebrates will be housed and maintained at LSRI upon completion of the project, or returned to the granting agency if required."

Sediment Particle Size

The quality control procedure for sediment size analysis is, "A standard soil of known particle size content is analyzed with each batch of samples to check for instrument calibration and procedural accuracy."

Loss on Ignition

At least 10% of all samples will be analyzed in duplicate. Duplicate determinations should agree within 5% of their average weight.

Laboratory quality assurance checks

Each analysis has specific standard operating procedures that direct analyses, and quality assurance checks. The SOPs and USEPA methods for the analyses are available in the attachments.

Data Verification

To ensure accurate data, within one week of receipt of any laboratory data, the project manager will review data for any missing data, outliers and questionable data. If any data seem to be inaccurate the project manager will contact the laboratory and discuss the questionable data. If the data are verified to be correct, the data will be retained in the data set and will be used in further data analysis. If the data is deemed incorrect, it will be removed from the data set and will not be included in further analysis. The reasoning for removing the data will be included in notations and comments of the data base.

This data check will also be used to ensure proficient sampling procedures. If it is deemed that the data are invalid due to sampling procedures, the project manager will consult with the WDNR Quality Assurance Coordinator, and WDNR Project Manager to adjust the procedures to ensure valid sampling methods are conducted during the future sampling events.

B6. Instrument/Equipment Testing, Inspection and Maintenance

All field equipment will be inspected for proper operation prior to sampling to ensure the collection of quality data. For example, the artificial samplers and plankton nets will be inspected for tears or other damage and repaired or replaced as needed. Field instruments will be maintained according to USGS National Field Manual (NFM) guidance and manufacturer's instructions. YSI multi-probe water quality sonde will be calibrated daily for each measured parameter using calibration standards.

Between each site, all gear will be disinfected according to the "Boat and Gear Disinfection Protocol" established by WDNR. The gear includes, but is not limited to: boats, trailers, waders, Ponar Dredge, plankton net, buckets, nets, sieves, and forceps. There will not be ample drying time between sites therefore a chemical disinfection process will be completed between sites. All gear will be sprayed with a 200 ppm solution of chlorine bleach, with a 10-minute contact minimum. Because chlorine beach is corrosive to metal and toxic to aquatic life at this concentration, an 800 ppm sodium thiosulfate solution will be sprayed on the gear to neutralize the bleach after the disinfection period. This disinfection process will be performed on a fairly level surface, preferably vegetated and away from street drains to prevent the disinfection solutions from enter surface waters directly. The gear will then be rinsed with native water at each site prior to sampling. At the conclusion of each sampling event, all gear will be disinfected and then allowed to dry for 5 days before packing away for storage.

The boat and trailer will be visually inspected prior to launching and after loading for aquatic vegetation. Any visible debris will be removed before leaving the boat ramp. The drain pug will be

removed and the boat will be drained before leaving the launch. The trailer and inside and outside of the boat will be disinfected with the bleach solution along with the other equipment. At the conclusion of each sampling event the boat and trailer will be powerwashed to remove any remaining disinfection solutions and in preparation for storage.

Each laboratory is responsible for maintenance of equipment necessary to perform the analyses requested. Laboratory instruments should be maintained according to manufacturers' instructions and criteria defined in standard operating procedures for each laboratory. Properly trained personnel will perform all operation, maintenance, and calibration procedures.

B7. Instrument/Equipment Calibration and Frequency

The only instrument or equipment necessary for biological sample collection that will need calibration is the YSI multi-probe water-quality sonde for selected water-quality measurements. The multi-probe sonde used to measure temperature, dissolved oxygen, pH, and specific conductance in the field is calibrated according to the USGS NFM and results of calibrations are recorded in a log book kept with the instrument. Temperature is calibrated every three to four months on a five-point scale. All routine and special maintenance and calibration information for laboratories will be recorded in appropriate logbooks and files. Laboratory instrument calibration requirements for all analyses will be met prior to use of any instrument for sample analysis.

B8. Inspection/Acceptance of Supplies and Consumables

All sample containers and preservation consumables will be ordered new from respected vendors. Because the analyses for this project do not include trace metal or other contaminant-sensitive analyses, the containers do not need to be pre-cleaned, but will be rinsed three times with native water from the sampling site. All containers will be visually inspected for possible leaks prior to use, and then sealed and bagged to prevent any sample loss.

B9. Data Management

Field sheets to be used during sampling will be produced using the SWIMS Database controlled by the WDNR, and stand water quality and stream gaging field sheets created by the USGS. All field sheets will be reviewed for completeness before leaving the sample collection site. All documents will be

scanned electronically to a secure USGS computer and hard copies and compact discs with the data will be archived at the USGS WWSC for a minimum of five years.

Each laboratory distributes their data differently. Laboratory analytical results from WSLH will be entered into the Laboratory Information Management System (LIMS), electronically loaded into the SWIMS or NWIS systems as appropriate, loaded into the Access database, and stored on a secure USGS computer for data analysis. UW Superior and Paul Garrison have indicated they will provide an electronic Excel file with the community data. This will be loaded in the Access database and SWIMS, where possible.

Biological community data for benthos, zooplankton, and diatoms will be entered into Excel spreadsheets, e-mailed to the lead, and stored on USGS computers. Results of analyses for algal identification and counting are entered into the WSLH LIMS within 6 months of sample receipt. Important electronic communication will be printed and stored with the field notes and raw data at the USGS WWSC.

Data analysis will be performed using Microsoft Excel, Microsoft Access, Primer-E statistical software, and SPlus+. Each program has unique features that will be used to organize, compute, analyze, or store the data on USGS computers. USGS has current licenses for each of these programs. Progress reports and the final report will be written in Microsoft Word and transmitted to WDNR and USEPA using the USGS email system. No other specialized programs are planned for data storage, retrieval, or analysis of data.

C. Assessment and Oversight

C1. Assessments and Response Actions

The data provided by this study will provide the data necessary to complete the project objectives using multiple approaches. The data gathered from the physical samples will provide the information to gauge the current status of the benthos and plankton communities; whereas the literature search and data mining will allow the lead to evaluate historical changes in the sites.

Data gathered from this project will provide an assessment of the community structure of benthos and plankton present in the AOCs and non-AOCs sampled. The communities will be compared seasonally within each site and seasonally between sites to determine similarities and differences of the communities between AOCs and non-AOCs. The non-AOCs will provide a reference comparison as the communities in those locations have not been identified as having Beneficial Use Impairments. The AOCs will be

compared to non-AOCs while limiting the statistical influence of characteristics such as watershed size, geography, substrate, temperature, and water quality among others.

The Milwaukee Harbor and Green Bay are large and have far more complex systems than any other harbors or rivers along the western Lake Michigan shoreline. Therefore, the plausibility of comparing these sites to the non-AOCs is not feasible. These systems also have on-going contaminant removal projects that will hopefully improve the health of the benthos and plankton communities. The community assessment of these systems will be examined in conjunction with the other locations, but will likely have vastly different community structures. Therefore the information gained from these systems will provide a baseline community assessment for future comparisons within those systems with regards to the BUIs.

Although final decisions on the degradation of the communities may not be based on the historical data gathered during the literature search part of the project, these data will be compared to current community assemblages to determine if the communities have changed over time. If the communities are statically different, further investigation into what changes are occurring within the communities will be conducted. This information may be useful in setting future goals for species richness, abundance, or presence/absence of certain taxa.

Throughout the duration of this project USGS and WDNR will maintain regular (quarterly, at minimum) verbal and electronic communication. If any situation arises that requires revision to the project design, USGS and WDNR would discuss these modifications; both parties must agree to any suggested design revisions or the revisions will not be made. Short-term revisions (e.g., day-to-day) to scheduled sample events due to inclement weather conditions or high river discharge events would not be expected to significantly impact project data quality, quantity, results and conclusions. Under these circumstances, there is no need to formally contact other members of the project group. Longer-term revisions (e.g., week-to-week as a result of watershed wide flood event) could significantly impact project data quality, quantity, results and conclusions. Under these circumstances, a more formal notification and modification to the projects study design, sampling numbers, and sample protocols could be warranted. The decision to significantly modify project sample frequency, number of sample events, sample protocol and techniques would be made by Amanda Bell, USGS, and Stacy Hron, WDNR, following consultation with other members of the project team including those listed in section A4.

C1.1. Field Readiness

Prior to field sampling the sampling crew will organize and inspect the supplies and equipment. If any supplies are needed, they will be ordered immediately to ensure delivery before sampling. Field sheets and labels will be printed and organized by sites. Table 13 is a checklist for loading the equipment in preparation for sampling.

Table 13. Equipment and supplies checklist.

Equipment	Number needed per site	Total number needed for trip	Have	Loaded	Need to order
Plankton net	1	1			
Plankton dolphin bucket	1	1			
Plankton ring	1	1			
Buckets	8	8			
Hester-Dendys	3	45			
500 mL bottles	4	60			
Ponar Dredge	1	1			
GPS	1	1			
Camera	1	1			
Wire Cable	1	1			
Wire Cutters	1	1			
Concrete blocks	2	30			
Alpha bottle	1	1			
Field Sheets	1	15			
Label sheets	1	15			
Clear tape to affix labels	1	1			
FedEx labels	1	15			
Coolers	1	5			
Dry Ice	10 pounds	150 pounds			
Formalin	100 mL	2 L			
Rose Bengal Stain	1 mL	1 bottle			
Ponar Sieve	1	1			
Ziplock baggies	10	150			
YSI Sonde	1	1			
pH calibration standards	1 of each (3)	1 gallon of each (3)			
Specific conductivity standards	1 of each (3)	4 bottles of each (3)			

C1.2. Data Assessment

To ensure accurate and complete data, the lead will review data for any missing data, outliers and questionable data within one week of receipt of any laboratory data. If any data seem to be inaccurate, the lead will contact the laboratory and discuss the questionable data. If the data are verified to be correct, the data will be retained in the data set and will be used in further data analysis. If the data is deemed incorrect, it will be removed from the data set and will not be included in further analysis. The reasoning for removing the data will be included in notations and comments in the database.

This data check will also be used to ensure proficient sampling procedures. If it is deemed that the data are invalid due to sampling procedures which may be found to be inadequate given the site characteristics, the lead will consult with the WDNR Quality Assurance Coordinator and WDNR Project Manager to adjust the procedures to ensure valid sampling methods and/or site locations are conducted during the future sampling events.

C2. Reports to Management

Quality assessments will be completed after each sampling event by reviewing biological and water quality sample protocols and schedules. The need for and recommendations for adjustments to the sample design and protocol will be made, as appropriate, and only after consideration by members of the project review group identified in table 1.

Biannual progress reports will be prepared by USGS and submitted to Donalea Dinsmore of WNDR for review before forwarding to USEPA Project Manager in June 2012, December 2012, and June 2013. A final data report will be completed by USGS, reviewed by WDNR, submitted to USEPA, and published as a Digital Data Series in the USGS publications network with full public access. An interpretive report of the findings of the study will be written by USGS, reviewed by WDNR, reviewed by USEPA, and submitted to a peer-review journal for final publication.

C3. Technical Team Review

Once the data analysis is complete the lead will review the finding with a technical team including those that know about the systems and the biological communities to review the assumptions made previously about the comparisons between and among sites. The decision criteria for accepting or rejecting the null hypothesis may be modified at that time based on the results of the analyses.

D. Data Validation, Analysis, and Usability

D1. Data Review, Verification, and Validation

Data Analysis

The assessment for each community at each location will pool the community assemblages (i.e. species list) during analysis; however, the samples from different seasons will remain separate. For example the plankton community for the spring sample at the Menominee River will include all plankton identified in the plankton tow samples and the water depth profile sample from the first sampling event, including soft algae, diatoms, large-cell zooplankton, and small-cell zooplankton. This will result in a total of 6 assemblages for each sampling location.

Because of the complexity of the systems and the technical team's concerns about the comparability of Lower Green Bay and Fox River AOC and the Milwaukee Estuary AOC to the non-AOC selected sites, it's unlikely a decision will be made as to whether the communities in these systems are degraded based on this study. The site-to-site comparisons will be performed on them; however, the team recognizes that significant remediation work remains in these AOCs and additional technical discussions are necessary before a decision can be made on whether this study's approach is fully applicable to these systems. Additional data collection and analyses may be necessary. The overall AOC to non-AOC comparisons may provide insight into specific goals for these two AOCs such as having populations of specific species, or attaining a certain species richness level.

Site-To-Site Comparisons for Benthos

The first level of data analysis will be a site-to-site comparison between the AOCs and selected non-AOCs. Regional Index of Biological Integrity (IBI) scores (Weigel and Dimick, 2011) will be calculated for the benthic invertebrate samples. The methods being used are similar to those used by WDNR and the regional-based IBI scores are helpful in comparing the collected data for this project to similar studies along the lake shore. It is assumed that the IBI score for these samples will be collectively lower than scores from other large rivers around Wisconsin due to their slow-flow, lacustrine characteristic, so the categorical designations (i.e. good, fair, and poor) will not be included in during analyses.

The IBI scores for each AOC and non-AOC will be calculated and the collective non-AOC IBI scores will be used to calculate a standard deviation that will serve as a measure as to the similarity between sites. Natural temporal variability of IBI scores within a single site may be as large as a 9% deviation from the mean (Andrew Fayram, personal communication, 14 March 2012). Therefore, if the standard deviation of the collective non-AOC sites is less than 10 (on a scale of 100) then that will be the value that will be used to determine whether the IBI of the AOC is less than that of the non-AOCs. The value of ten was chosen instead of 9 because the IBI scores are in increments of 5. One standard deviation has been chosen as the cutoff for comparison because if the variation is high, then the standard deviation will be also; however, if the non-AOC sites have little variability in their IBI scores, then the scores of the AOCs should be within that range if the community is not degraded. If the AOC scores are greater than one standard deviation (or 10%, whichever is greater) below the non-AOCs, then the community is still degraded. For the rest of the section when the term standard deviation is used, it is referring to either the standard deviation or the 10%, whichever is greater.

There will be a multi-tiered analysis to determine benthos degradation; if two or more of the analyses determine the site to be degraded, then the overall AOC will be determined to have a degraded benthos community. First, the range of scores for the non-AOC sites will be used as a rapid comparison tool to assess whether each AOC is within or above that range. If the seasonal IBI score for a particular AOC is one standard deviation below the non-AOC IBI range, then the seasonal sample will be deemed to have a benthos community that is more degraded than the collective non-AOCs. The range will include the lowest IBI scores for the non-AOC sites; therefore, if the IBI scores of any AOC are greater than one standard deviation below the range, the AOC score will be below any single comparison non-AOC sites.

Secondly, the seasonal IBI score of an AOC is below one standard deviation of both of the seasonal IBI scores of the selected non-AOC comparison sites, then the benthos community in that AOC is considered more degraded than the selected non-AOC sites. Figure 2 is a simplified decision tree for benthos community degradation. If two or more of the seasonal samples are determined to be degraded then the benthos for that site are deemed to be degraded.

Finally, the median IBI score for each of the AOC seasonal samples will be calculated and compared to the median values for the comparison non-AOCs. If the median values are one standard

deviation below of both of the median IBI scores of the selected non-AOC comparison sites, then the benthos community in that AOC is considered more degraded than the selected non-AOC sites. Figure 2 is a simplified decision tree for the benthos community degradation. Note that the findings of this study will be provided to WDNR, who has responsibility for determining the status of the impairment and whether the targets have been met for delisting the AOC.

Site-To-Site Comparisons for Plankton

An IBI has not been calculated for river-mouth plankton communities in this region; therefore a Shannon Diversity Index (SDI) will be used to describe the entropy of the communities. This is a common index in ecological data analysis that incorporates the species richness and abundance in a community and can be compared on a one-to-one basis. The approach used to compare the SDI between AOC and non-AOCs will be similar to that of the benthos IBI comparison. The SDI values of the non-AOC site will be used to calculate a standard deviation. If the standard deviation is less than 10% of the range, then the 10% value will be used as the cutoff. First, if any AOC has a SDI for a seasonal sample one standard deviation below the range of the non-AOCs, then the plankton community at that site is deemed to be degraded for that sample. Secondly, the seasonal SDIs for each AOC and the selected non-AOCs will be compared, and if the AOC value is greater than one standard deviation below both of the non-AOCs, then the plankton is deemed to be degraded. Finally, the median Shannon Diversity Index for the AOC will then be compared to that of the selected non-AOC sites. If the median index for the AOC is below one standard deviation of both of the median indices of the selected non-AOC comparison sites, then the plankton community in that AOC is considered more degraded than the selected non-AOC sites. Figure 2 is a simplified decision tree for the benthos community degradation. If two or more of the analyses determine the site to be degraded, then the overall AOC will be determined to have a degraded plankton community. As with the benthos results, the findings of this study will be provided to WDNR, who has responsibility for determining the status of the impairment and whether the targets have been met for delisting the AOC

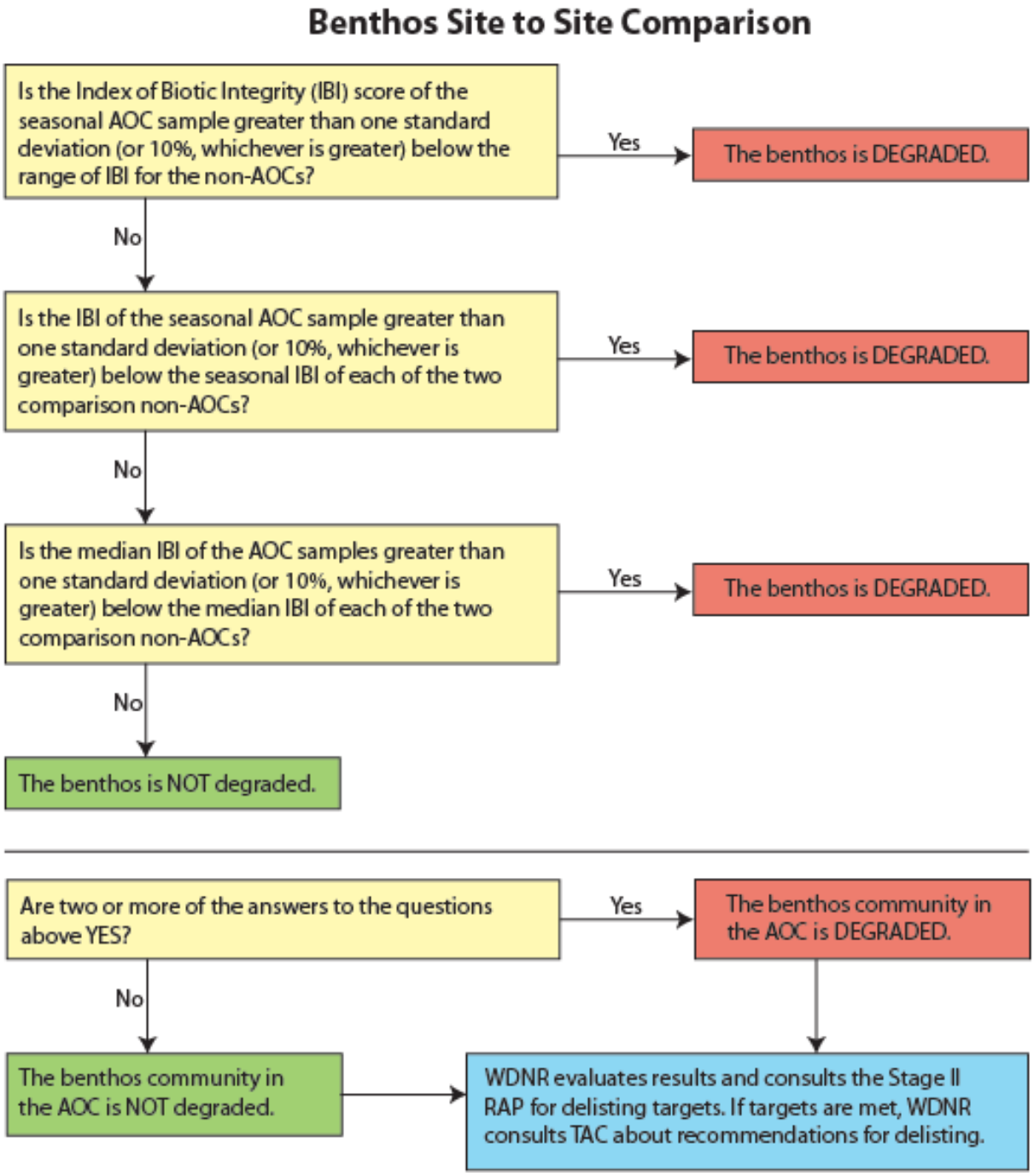

Figure 2. Benthos community degradation decision tree. This decision diagram will be used for each AOC for each seasonal sample. If two or more seasonal samples for an AOC are determined to be degraded then the overall AOC is determined to be degraded.

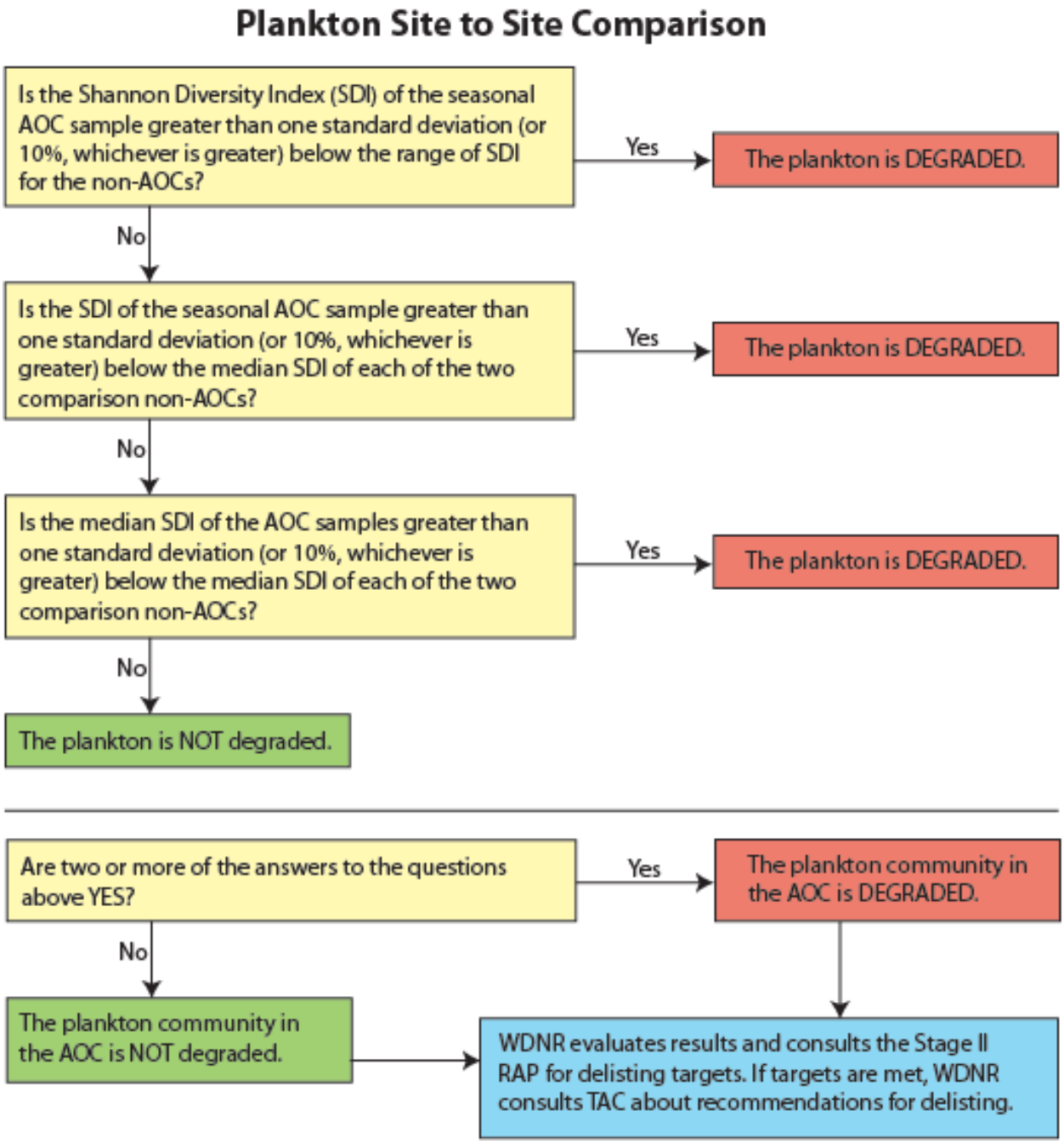

Figure 3. Plankton community degradation decision tree. This decision diagram will be used for each AOC for each seasonal sample. If two or more seasonal samples for an AOC are determined to be degraded then the overall AOC is determined to be degraded.

Overall AOC to Non-AOC Comparison

Multivariate, multi-metric, and correlation methods will also be used to analyze the data. The goal of these analyses is to provide community diversity characteristics for AOCs that are considered degraded. For example if the non-AOC sites all have a certain species that are absent from a given AOC, these analyses will provide a list of these species. These analyses take into account natural difference between the sites including geographical, climatic, and substrate.

Software designed to incorporate the non-normality of ecological data will be used to analyze variability in the biological community data from the sampled AOCs and non-AOCs. Using non-parametric multivariate statistical analyses in the Primer statistical program (Clarke and Gorley, 2006), specifically the distance-based techniques (Anderson, 2006), and observed-over-expected methods such as those used by Meador, *et al*. (2008) and Carlisle and Hawkins (2008), the community data will be compared amongst the sites and differences between taxa richness, composition, and abundance will be determined for benthos and plankton communities. Routines to be used in PRIMER will likely include (but not limited to) nMDS (non-metric Multi-Dimensional Scaling) to derive plankton and benthos community site scores; PCA (Principal Components Analysis) to derive environmental site scores; and ANOSIM (Analysis Of SIMilarity) to determine the extent plankton and benthos communities vary across sites. Probability values are based on 1,000 random permutations that are used to develop a nonparametric probability distribution. Site-specific scores based on similarities between communities will be used to determine whether a given site is statistically different from the others. Location specific differences such as drainage area, substrate, soil type, latitude/longitude, land cover, and climate will be incorporated as well. These analyses will provide similarity correlations and a significance value (p-value). If the p-value is less than or equal to 10% the site grouping are deemed statically different.

Final iterative analyses to determine the level of similarity required to determine differences will be conducted using a 90% confidence cut off. Additional analyses may also include determining the species that are contributing the most significant differences to the grouping for AOCs and non-AOCs.

D2. Verification and Validation Methods

Data flagged as proofed and complete is public record. Those data are readily available to the public upon request in paper or electronic format. Similarly, final project reports will also be available to the public in electronic format.

D3. Reconciliation with User Requirements

As discussed earlier, the goal of this project is to quantify benthos (benthic invertebrate) and plankton (phytoplankton/zooplankton) communities in Wisconsin's four Lake Michigan AOCs (Menominee River, Lower Green Bay and Fox River, Sheboygan River, and Milwaukee Estuary) and six non-AOCs. The inclusion of non-AOC sites allows comparison of AOC sites to relatively-unimpacted or less-impacted control sites with physical and chemical characteristics that are as close as possible to that of the AOCs. The community data within and between the AOCs and non-AOCs will be analyzed, and the differences and similarities will assist in determining the status of the communities and, when appropriate, may support removal of the "Degradation of Benthos" and "Degradation of phytoplankton and zooplankton populations" BUIs in each AOC.

The assumptions described above and proposed sampling plan are reasonable and technically sound. While a more extensive spatial and temporal plan and effort could provide for a more statistically robust design, it would require significantly more effort in terms of time, materials and costs than is currently feasible. The additional effort may not provide any more information relative to the project's primary objectives of providing community assessment of benthos and plankton in Wisconsin's Lake Michigan AOCs and determining the status as Beneficial Use Impairments. This project will provide statistically defensible assessment of the current state of the four Wisconsin Lake Michigan AOCs in comparison to non-AOCs.

E. Supporting Documents

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E2. Attachments

7 Laboratory Forms
- Algae ID Labslip 2010 Non-DNR Non-Private Fillable.pdf
- Inorganic Labslip_4800-015_fill1.pdf

8 Laboratory SOPs
- Phytoplankton ID & Enumeration WSLH ESS Bio Method 2035.pdf
- FS.14v3_Invertebrate Picking.pdf
- FS.13v2_Invertebrate Identificationl.pdf
- FS.12v2_Benthic Invertebrate Subsampling.pdf
- ESS INO METHOD 151_1 rev 4_Chlorophyll a.doc
- SOP Phytoplankton Analysis_LG401.pdf
- SOP Zooplankton analyses_LG403.pdf
- SOP Solids.pdf

9 Field Collection SOPs
- FS.16v3_Hester Dendy Processing.pdf
- SOP Zooplankton Collection_LG402.pdf
- SOP Phytoplankton collection_LG400.pdf
- SOP Benthos Ponar collection_LG406.pdf
- SOP Chlorophyll a Sampling_LG404.pdf

10 Equipment User Manuals
- YSI-6-Series-Manual-RevF.pdf