Upper Mississippi River Clean Water Act Monitoring Plan

Minnesota-Wisconsin Area Pilot Project

Field Operations Manual

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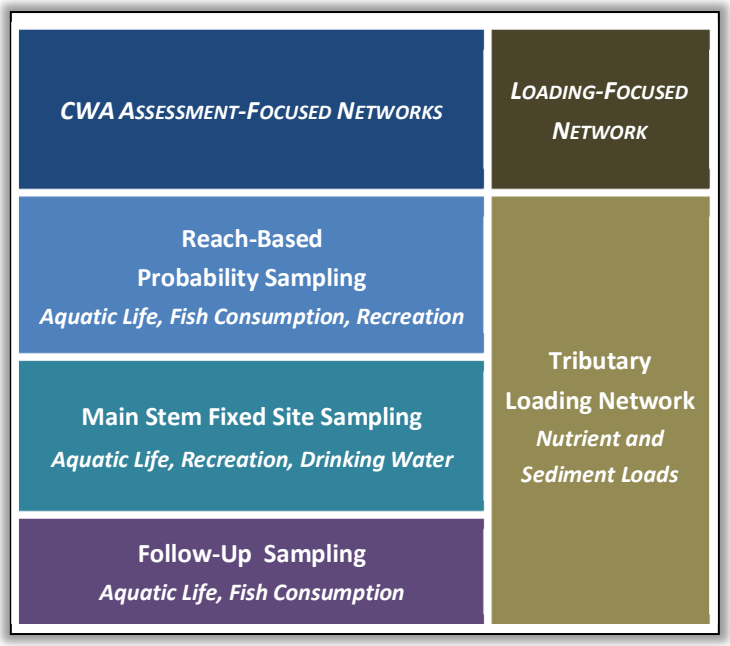
The cover photo is provided courtesy of the Wisconsin Department of Natural Resources.

1. INTRODUCTION

1.1 UMR CWA Monitoring Plan Overview and Rationale

The *Upper Mississippi River Clean Water Act Recommended Monitoring Plan* (*UMR CWA Monitoring Plan*; UMRBA 2014) was developed by the interagency Upper Mississippi River Basin Association Water Quality Task Force to address the lack of a coordinated, comprehensive Clean Water Act (CWA) monitoring approach on the Upper Mississippi River (UMR). The *UMR CWA Monitoring Plan* was adopted by the Upper Mississippi River Basin Association (UMRBA) Board in February 2014 and is structured as a series of networks that uniquely and comprehensively support assessment of aquatic life, fish consumption, recreation, and drinking water use attainment on the UMR (Figure 1); utilizing both newly collected data and existing data sets. This includes both fixed site and probabilistically-selected monitoring locations.

Figure 1: Illustration of UMR CWA Recommended Monitoring Plan, including constituent networks and designated uses which can be assessed utilizing data from these networks



1.2 Strategy Implementation and the Minnesota-Wisconsin Pilot Project

Following the UMRBA Board's approval, the states have moved forward to implement the *UMR CWA Monitoring Plan*. Implementation steps have included compiling existing water quality data in a "virtual pilot" effort, as well as a field pilot to be conducted in the states of Minnesota and Wisconsin on a subset of the UMR's upper reaches, beginning in May 2016.

1.3 Field Operations Manual Scope and Applicability

This *UMR CWA Pilot Monitoring Project Field Operations Manual (Operations Manual)* has been developed to provide the technical and procedural detail necessary for the states of Minnesota and

Wisconsin, as well as other partners, to implement UMR CWA pilot monitoring. While it will inform sampling procedures UMR-wide, the nature of the pilot is such that changes and improvements are expected to be made as a result of this initial effort. Therefore, it is likely that a revised operations manual will follow completion of pilot monitoring.

Further, the pilot monitoring project is reduced in scope from the "full" monitoring program described in the *UMR CWA Monitoring Plan*. Specifically, the following components have been modified for the Minnesota-Wisconsin pilot monitoring project:

- The pilot group has elected to **utilize an artificial substrate sampling approach for macroinvertebrates** during the pilot project. This method is different than the EMAP kick-sampling approach described in the monitoring plan and will require the use of a multimetric index other than the EMAP GRMIn or modified *Ad Hoc* GRMIn (i.e., Wisconsin Large River IBI). Pending the results of an ongoing macroinvertebrate comparison study, EMAP methods may be included in future monitoring.
- **Fish tissue sampling will <u>not</u> be conducted as part of the pilot project.** However, proposed methods are included in Appendix E.
- There are no public water supply intakes on the shared Minnesota-Wisconsin portion of the UMR. As such, **no monitoring will be done for drinking water use-only analytes** (e.g., VOCs, SOCs, phenols, and fluoride).
- **Metals will <u>not</u> be sampled at probabilistic sites** during the pilot project. They will be sampled at fixed sites.
- **Algal toxins (microcystin and cylindrospermopsin) will <u>not</u> be sampled** as part of the pilot project. Note that, as of January 2016, a separate work group has been formed to address the issue of harmful algal blooms on the UMR.
- **Follow-up sampling and monitoring for secondary indicators (e.g., sediment chemistry) is not explicitly addressed in the pilot**, though such monitoring may occur at the discretion of the states.
- **Index site monitoring will <u>not</u> be part of the pilot project**.
- The **tributary loading network will <u>not</u> be sampled** as part of the pilot project.

Therefore, because **this *Operations Manual* is scoped only to describe sampling that will actually occur during the pilot,** it does not necessarily provide all the information needed to implement full monitoring river-wide.

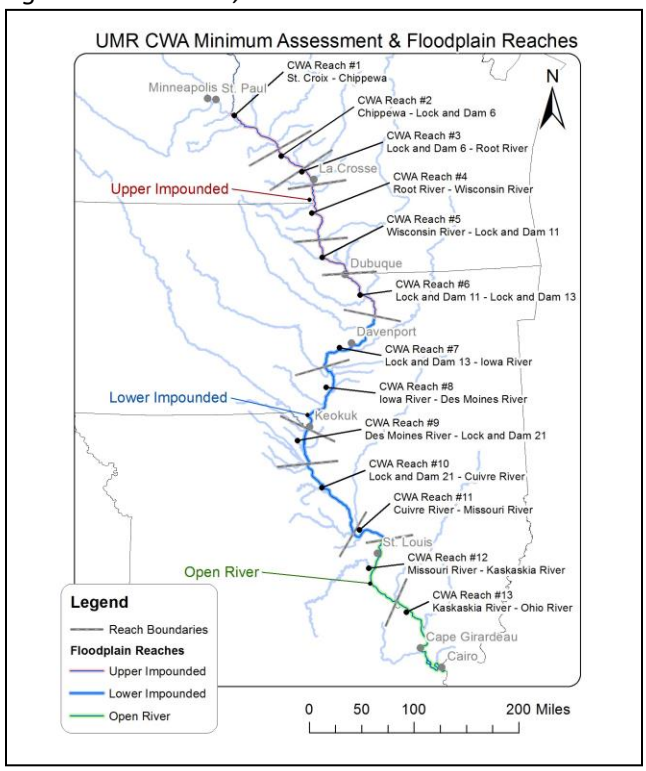
Additionally, this *Operations Manual* is focused on field activities. As such, it does not provide extensive detail on sample site selection, laboratory analytical methods, data management, data analysis/score calculation, or CWA designated use assessment – though some discussion of these functions is included.

2. MONITORING OVERVIEW AND GENERAL PROCEDURES

2.1 Geographic Extent

The *UMR CWA Recommended Monitoring Plan* organizes monitoring around the "minimum CWA assessment reaches" established via an interstate Memorandum of Understanding in 2003. These reaches follow HUC-8 boundaries and are illustrated in Figure 2. An additional reach internal to Minnesota (Reach 0, Twin Cities to the St. Croix River) also falls under the *Plan*.

Figure 2: Minimum, interstate UMR CWA assessment reaches



The geographic extent of the pilot monitoring project, and hence of this *Operations Manual*, is the main stem UMR from Upper St. Anthony Falls (USACE River Mile 854) to the Root River (River Mile 694). This includes the UMR CWA assessment reaches 0 through 3 (Table 1). All sampling takes place in the river's main channel and adjacent shoreline throughout the run of the river (i.e., Lake Pepin and other lake-like areas are included).

Table 1: Geographic extent of pilot monitoring program, UMR assessment reaches 0 through 3

Reach Number	Reach Name (Description/8-digit HUC code)	River Miles	Segment Length (miles)
0	**Assessment Reach 0** (Upper St. Anthony Falls to St. Croix River)	**854-811.5**	**42.5**
1	**Assessment Reach 1 (Rush-Vermillion)** (St. Croix River to Chippewa River/ HUC 07040001)	**811.5-763.4**	**48.1**
2	**Assessment Reach 2 (Buffalo-Whitewater)** (Chippewa River to Lock and Dam 6/ HUC 07040003)	**763.4-714.2**	**49.2**
3	**Assessment Reach 3 (La Crosse-Pine)** (Lock and Dam 6 to Root River/HUC 07040006)	**714.2-693.7**	**20.5**

2.2 Scope of Indicators Monitored

The *UMR CWA Monitoring Plan* specifies sampling across a wide extent of parameters. The pilot monitoring project will include most, but not all, of these parameters, as displayed in Table 2. In brief, the pilot focuses on fixed site and the probabilistic networks, while dropping some indicators (e.g., fish tissue, probabilistic network metals) that are specified in the full *Plan*.

Table 2: Scope of parameters monitored in pilot project, where: ***X****= sampled in pilot, shaded=not sampled in either pilot or full plan, shaded X = sampled in full plan, but not sampled in pilot*

Indicator Group	Indicators	Probabilistic Monitoring (15 sites per reach)	Mainstem Fixed Network (20 sites UMR-wide)	Tributary Loading Network (34 sites)
Biological Communities	Fish	**X**		
	Vegetation	**X** (100 sites per reach)		
	Macroinvertebrates	**X**		
Fish Tissue	Mercury (Hg)	X		
	PCBs	X		
Field	Water Temperature	**X**	**X**	X
	DO (conc.& sat)	**X**	**X**	X
	pH	**X**	**X**	X
	Conductivity	**X**	**X**	X
	Turbidity	**X**	**X**	X
	Secchi Depth	**X**	**X**	
Nutrients	NO3+NO2	**X**	**X**	X
	TN	**X**	**X**	X
	NHx	**X**	**X**	X
	TP	**X**	**X**	X
	DP	**X**	**X**	X
	Chlorophyll a	**X**	**X**	X
Bacteria	*Escherichia coli*	**X**	**X** (April-October)	
Algal Toxins	Microcystin		X	
	Cylindrospermopsin		X	
Miscellaneous	BOD	**X**	**X**	
	Chloride	**X**	**X**	
	Sulfate	**X**	**X**	
	TSS	**X**	**X**	X
	TOC		**X**	
	Hardness (Ca & Mg)	**X**	**X**	X
	Alkalinity	**X**	**X**	
	Fluoride*		X	
Metals	Aluminum (Al)	X	**X**	
	Calcium (Ca)	X	**X**	
	Cadmium (Cd)	X	**X**	
	Chromium (Cr)	X	**X**	
	Copper (Cu)	X	**X**	
	Iron (Fe)	X	**X**	
	Lead (Pb)	X	**X**	
	Magnesium (Mg)	X	**X**	
	Potassium (K)	X	**X**	
	Sodium (Na)	X	**X**	
	Zinc (Zn)	X	**X**	
Other	Arsenic (As)	X	**X**	
	Mercury (Hg)	X	**X**	
	Selenium (Se)	X	**X**	
Organics	VOCs, Pesticides, Other*		X	
	Phenols*		X	
Physical Habitat and Characteristics	Substrate	**X**		
	Depth	**X**		
	Velocity	**X**		
	Discharge**		**X**	X

*Only sampled at fixed sites in proximity to a drinking water intake. **From existing gages near sampling sites, where available.

2.3 Overview of Fixed-Site and Probabilistic Monitoring

As described previously, ***the pilot project focuses on the fixed site and probabilistic network***, which are central to providing a robust characterization of the UMR's condition under the *UMR CWA Monitoring Plan*. A brief description of these networks and the sampling conducted under each of them follows. Further detail is provided under the indicator group-specific procedures later in this manual.

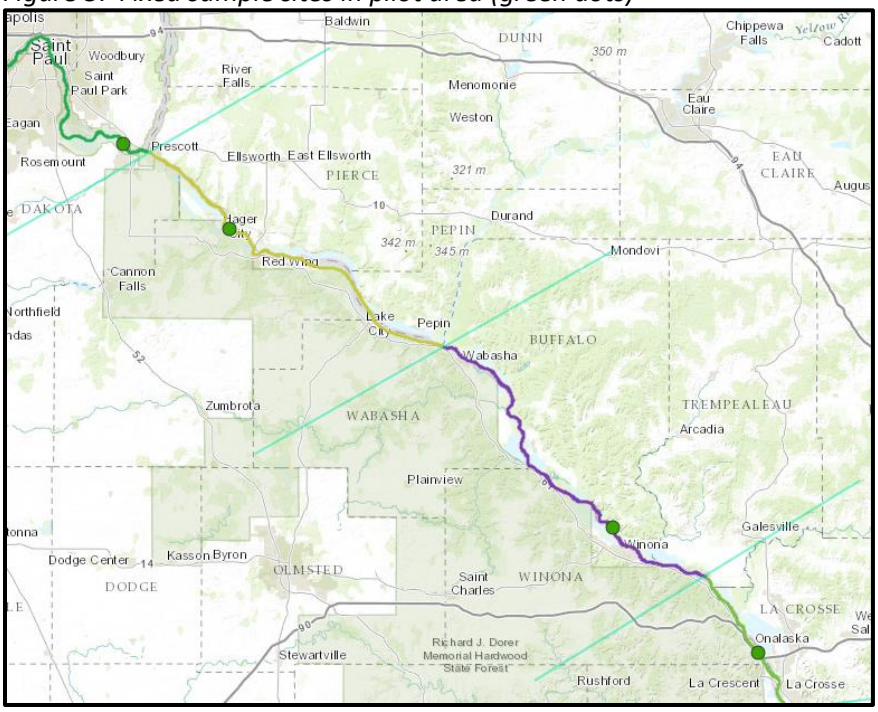
2.3.1 Fixed Site Network

Site Locations: The fixed site network is composed of 20 stations on the UMR, with at least one fixed site placed in each of the CWA assessment reaches and additional sites in reaches where a drinking water intake is present. However, there are no drinking water intakes in the pilot reaches. Therefore, in the area covered by the pilot monitoring project, there are just four fixed sites as shown in Table 3 and Figure 3. Sampling responsibilities for these fixed sites are also indicated in Table 3.

Table 3: Fixed sites in pilot monitoring area.

Pilot Project Site ID	Reach	River Mile	Location Description	Agency Sampling During Pilot	Agency Previously Sampling Location (and site identifier)	Nearby USGS Gage	HUC-8	Latitude	Longitude
UMR 815.6	0	815.3	Lock & Dam 2	MCES	MCES (UMR 815.6)	05344500	7040001	44.76100	-92.86900
UMR 796.9	1	796.9	Lock & Dam 3	MCES	MCES/WI DNR (UM 796.9)	05344980	7040002	44.61200	-92.61000
UMR 728.5	2	728.5	Winona	MPCA	MPCA (S000-096)	05378500	7040003	44.08200	-91.66400
UMR 702.5	3	702.5	Lock & Dam 7	WI DNR	MPCA (S000-067)	05386400	7040006	43.86634	-91.31008

Figure 3: Fixed sample sites in pilot area (green dots)



Indicators: Monitoring at fixed sites is conducted on a monthly basis year-round for field parameters, nutrients, discharge, and other water chemistry. Bacteria *(E. coli)* is sampled monthly during the months of April through October. See Table 2 and Table 4 (below) for details regarding indicators monitored. As there are no drinking water intakes in the pilot reach, drinking-water only contaminants (e.g. VOCs, pesticides, phenols, fluoride) will not be sampled at fixed sites during the pilot.

Table 4: Fixed Site Monitoring Summary

Spatial Design	Index Period	Number of Sites	Media & Frequency (per index period)
Mainstem Fixed Network	Year Round (chemistry, discharge) April to October (bacteria)	20 UMR sites	Water Chemistry: 12x *E. coli*: 7x Discharge: 12x (from existing USGS and USACE gaging stations)

General Sampling Procedures: There are several important distinctions between fixed site and probabilistic site sampling. Perhaps most prominently, there is no biological sampling at fixed sites, so procedures are focused on water chemistry and other physical measurements. Therefore, it is expected that one sampling crew can collect all of the parameters needed at the fixed sites.

Further, fixed sites are typically located at "hard structures" on the river (e.g., bridges, locks and dams) which do not require the use of a boat. Additionally, fixed site monitoring also extends year-round, so it will be necessary to consider any special techniques that apply during winter months at these sites. For these reasons, sampling procedures for fixed sites will differ from those used at probabilistic sites.

By definition, site location is predetermined for fixed sites, so site selection process is straightforward and sites can be found on the online pilot project viewer. Maps of the sites can be printed out from this viewer as needed. Further, each of the fixed sites in the pilot area corresponds to a location either currently or previously sampled by a state, federal, or local entity. As such, sampling crews may wish to communicate with other these agencies in advance regarding site access and other site considerations. This will also help in identifying any special equipment needed to reach the water surface, to be used in winter, etc. In general, samples should be taken at the "x-point" centerline (i.e., USACE navigation sailing line), but this can be adjusted as needed in light of physical structures, access, etc.

Samples requiring lab analysis (e.g., those for metals, nutrients, miscellaneous, *E. coli,* and other chemistry parameters) will be collected as grab samples using the method (bucket, Van Dorn sampler, etc.) selected by the sampling agency. Sample depth, like that of probabilistic chemistry samples, will be 0.2 meters. Other samples are field-measured at the same depth and results will be recorded on site. Please see the indicator group-specific instructions for details on fixed site sampling.

2.3.2 Probabilistic Network

Site Locations: Fifteen probabilistic sites have been assigned within each CWA assessment reach (i.e., a total of 60 sites in the pilot project area). These sites are allocated along the river's centerline (defined by USACE navigation sailing line) using a site draw conducted by USEPA. An overdraw is part of this allocation, to be used as needed should initially identified sites prove not feasible for sampling. Sample sites are shown in Figure 4. Assignment of monitoring responsibilities for probabilistic sampling is described in Table 5. Note that vegetation requires a different sampling design, see Section 2.3.3.

Figure 4: Probabilistic monitoring locations in pilot area (blue dots)

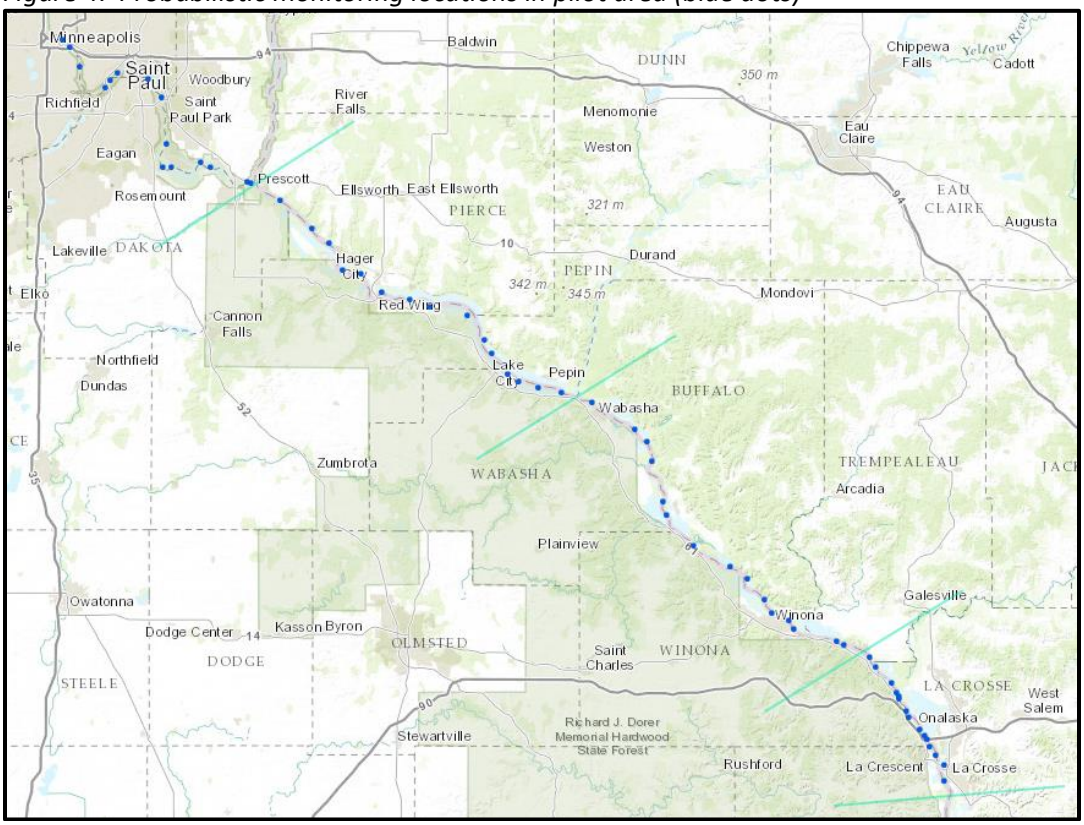


Table 5: Probabilistic Monitoring Responsibilities

Reach	Probabilistic-Chemistry, Fish Tissue, Fish Assemblage, Macroinvertebrates	Probabilistic -Vegetation
0	MPCA	MN DNR
1	MPCA	MN DNR
2	WI DNR	WI DNR
3	WI DNR	WI DNR

Indicators: Biology (fish and macroinvertebrates), water chemistry, field parameters, site characteristics, and discharge are all sampled at the probabilistic locations during a July to September index period. See Table 2 and Table 6 for details. Biological samples are only collected during one sampling event, but water chemistry parameters and *E. coli* are sampled three times during the index period. Vegetation is sampled separately and collected once during the index period.

Table 6: Probabilistic Monitoring Summary

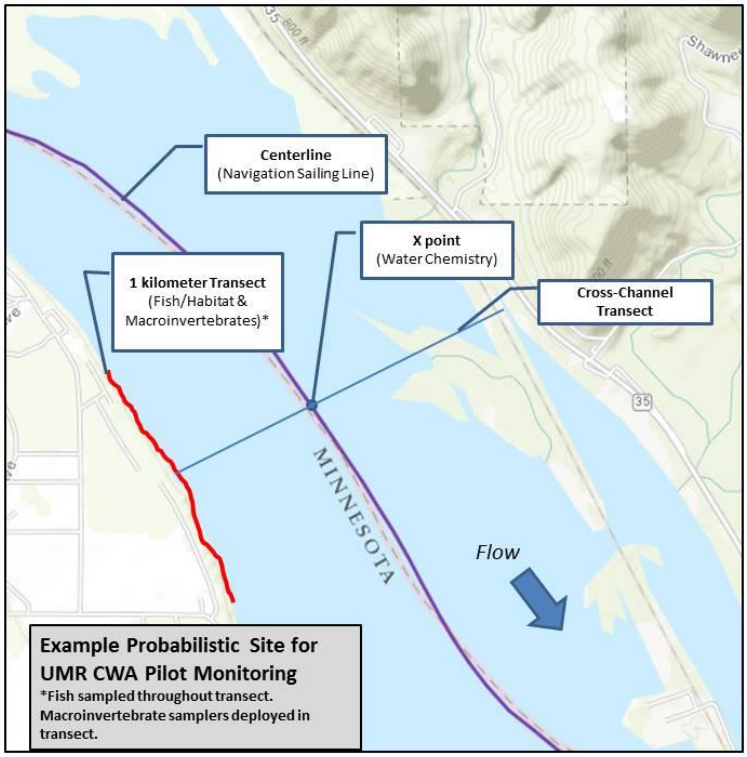
Spatial Design	Index Period	Number of Sites	Media & Frequency (per index period)
Reach-Based Probabilistic	July to September	15 sites per reach	Fish and habitat: 1x Macroinvertebrates: 1x Water Chemistry: 3x *E. coli*: 3x
		100 sites per reach	Submersed Aquatic Vegetation (SAV): 1x

General Sampling Procedures: Unlike fixed sites, probabilistic sites will change in every round of sampling and will not typically fall at hard structures on the river. Additionally, biological sampling takes place at probabilistic sites. As such, probabilistic sampling is boat-based and more equipment-intensive. It will require more than one crew to complete all the sampling types necessary (i.e., separate crews for chemistry, fish, macroinvertebrates, and vegetation). Note that *E. coli* sampling will be done by the chemistry crew.

More detail on indicator group-specific procedures is provided later in this document, but in general for *probabilistic* sampling:

- **Water chemistry sampling will be done via grab samples and take place at the centerline "x point."** Crews will sample at the centerline "x point" identified in the sample draw from USEPA. Water quality samples will be collected at 0.2 meter depth using the method (bucket, Van Dorn sampler, etc.) selected by the sampling agency.
- **Biological (fish and macroinvertebrate) sampling utilizes a 1 kilometer main-channel shoreline (MCS) transect.** Each MCS transect is centered at the intersection of the cross-channel transect and the river right or river left (facing downriver) as pre-designated during sample selection. See Figure 5.
- **If the specified bank cannot be sampled for safety, access, or other reasons, then the opposite bank may be sampled. If the specified site cannot be used (i.e., neither bank can be sampled), a replacement site from the overdraw pool should be used.** These decisions will be driven primarily by fish assemblage sampling. See Section 3.2 for more details.

Figure 5. General Arrangement of Probabilistic Sample Site Including Biology



2.3.3 Probabilistic Network-Vegetation

Site Locations: Submersed aquatic vegetation monitoring is also implemented using a probabilistic design. However, due to the patchiness in vegetation occurrence on the river, a much more intensive spatial design is employed (i.e., 100 sites per reach). Site data are aggregated into an "assessment sample" for calculation of the submersed macrophyte index (SMI; Moore et al. 2012; Table 7). As few as 20 adjacent sites can be aggregated to produce index values that represent smaller areas of the river corridor. See Figure 6 for an example of site location and aggregation.

Table 7. SMI Calculation. SMI is calculated using equations developed by Moore et al. (2012), reproduced here with permission.

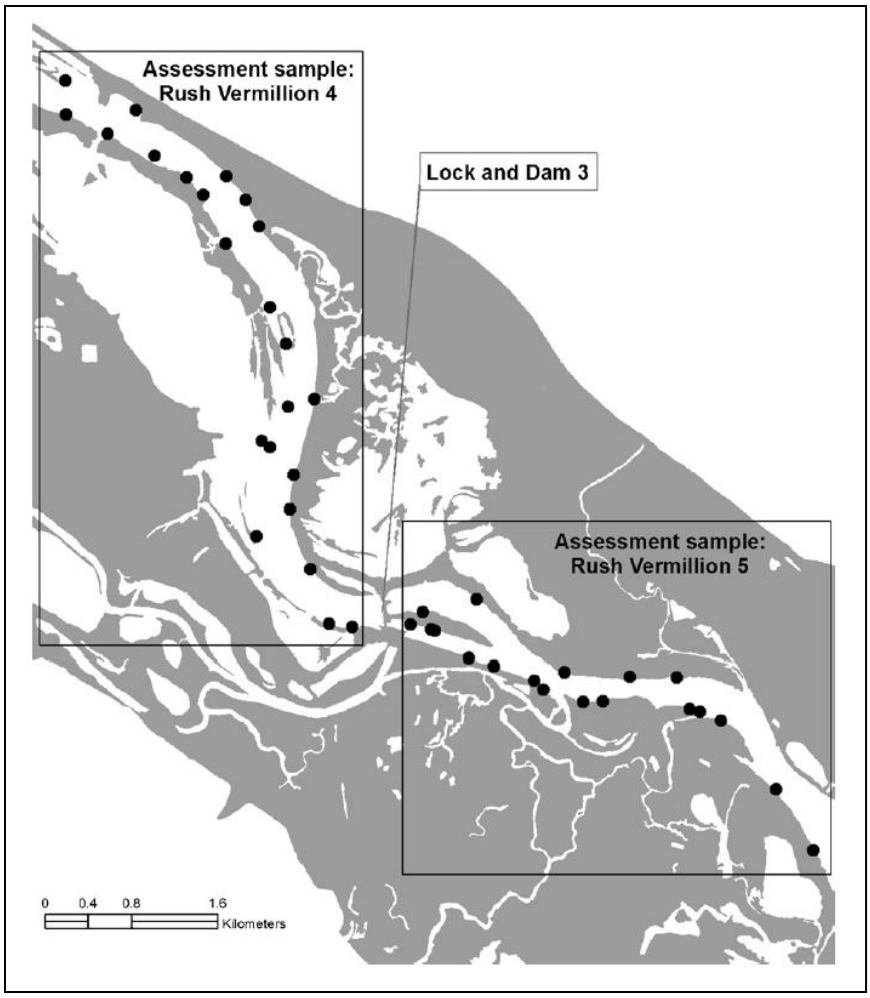
Values derived from continuous scoring ((value − 5th percentile/95th percentile − 5th percentile) × 100) for application in the submersed macrophyte index based on calibration assessment samples.

Metric	**95th Percentile**	**5th Percentile**	**Formula (*x* = value for the sample)**
Percent frequency of submersed macrophytes (number of sample stations where submersed macrophytes occurred divided by the total number of sample stations)	68.1	0	(*x*/68.1) × 100
Rake score of submersed macrophytes (mean of sum of the six sub-station rake scores for sampling stations where submersed macrophytes were present)	7.6	0	(*x*/7.6) × 100
95th percentile of species richness per assessment sample	6.0	0	(*x*/6.0) × 100
95th percentile of depth of submersed macrophyte occurrence (m)	1.9	0	(*x*/1.9) × 100
Final Submersed Macrophyte Index Score (SMI)			Mean of four individual metric scores

Indicators: Submersed aquatic vegetation indicators listed above (Table 7), reflect abundance, richness, and the light environment. Other data captured includes depth, current velocity, and transparency measurements.

General Sampling Procedures: Because of the differing spatial arrangement for vegetation, a separate sampling crew will carry out this monitoring. Vegetation data will be collected in the same July-September index period as other probabilistic samples. See the indicator-specific sampling description later in this *Operations Manual* for more details.

Figure 6: Example allocation of vegetation sites, including aggregation into assessment samples. Reproduced with permission from Moore et al. (2012)



2.4 Site Descriptions/Information

2.4.1 Site Naming

Each site utilized in the pilot project, both fixed site and probabilistic, will have a unique identifier as described in Table 8 below.

Table 8: Site Naming Convention

Site Type	Project ID	Site ID Format	Site ID Example
Fixed	UMRCWA	UMR-XXX.X	UMR-815.6
Probabilistic	UMRCWA	UMR15-XXXX	UMR15-0303*
Vegetation Probabilistic	UMRCWA	UMR15-XXXX	UMR15-3303**

*EPA generated draw number, where 15 indicates the year the sample draw occurred (i.e., 2015).

**Vegetation existing number starting at 3001 (i.e., existing number + 3000). Starting at 3000 gives plenty of space to use numbers 0001 to 2999 for other probabilistic sites, as there are about 400 chemistry/fish/macroinvertebrate sites drawn per cycle.

2.4.2 Site Information

Formal site dossiers will not be prepared for the pilot project. However, site information – for both fixed and probabilistic sites – can be obtained from the online pilot project viewer. Maps of sample sites can be generated directly from the viewer and site attribute information is also available via the viewer. An example map is shown in Figure 7 below.

Figure 7: Example map from online viewer showing a fixed site location (large green dot)

2.4.3 Coordinate System

In order to maintain spatial congruency across multiple sampling groups, a common coordinate system will be used. For the purposes of this field pilot, NAD83 will be the datum and UTM Zone 15N the projected coordinate system as the entire study area lies within this zone. While taking field measurements, please ensure that GPS units are set to this coordinate system.

2.5 Overview of Field Operations

2.5.1 Field Crews

The following field sampling crews will be engaged in pilot monitoring as illustrated in Table 9.

Table 9: Field Crews Engaged in Pilot Monitoring

Network	Reaches	Water Chemistry*	Fish**	Macroinvertebrates	Submersed Vegetation
Fixed Site Monitoring	Reach 0	MCES Chemistry Crew	NA	NA	NA
	Reach 1		NA	NA	NA
	Reach 2	MPCA Chemistry Crew	NA	NA	NA
	Reach 3	WI DNR Chemistry Crew	NA	NA	NA
Probabilistic Monitoring	Reach 0	MPCA Chemistry Crew	MPCA Fish Crew	MPCA Macroinvertebrate Crew	MN DNR Vegetation Crew
	Reach 1				
	Reach 2	WI DNR Chemistry Crew	WI DNR Fish Crew	WI DNR Macroinvertebrate Crew	WI DNR Vegetation Crew
	Reach 3				

*Includes field parameters, nutrients, bacteria, miscellaneous, metals, and other parameters.
**Includes site characteristic (habitat) information.

2.6 Laboratories

Table 10 lists the laboratories involved in analyzing samples from pilot monitoring, along with the agencies utilizing these laboratories.

Table 10: Laboratories Utilized in Pilot Monitoring

Laboratory	Submitting Agencies	Types of Samples Analyzed
Met Council Laboratory	MCES	Water chemistry*, fixed sites.
Minnesota Department of Health	MPCA	Water chemistry*, fixed and probabilistic sites.
Wisconsin State Laboratory of Hygiene	WI DNR	Water chemistry*, fixed and probabilistic sites.
Rithron Labs (Missoula, MT)	MPCA, WI DNR	Macroinvertebrate samples, probabilistic sites.

*Includes field parameters, nutrients, bacteria, miscellaneous, metals, and other parameters.

2.7 QA/QC Procedures

QA/QC procedures will be utilized specific to the indicator group. For chemistry, fish, and macroinvertebrate sampling, 10% of samples will be replicated. This translates to two sample sites per reach. Replicate sites will be selected in the order of the site ID assigned during the random site draw by USEPA. See Appendix D for an ordered listing of replicate sites. Vegetation monitoring will not employ resampling. Additionally, for chemistry sampling, a range of 5-10% split samples is recommended. Please see each indicator-specific section for details on QA/QC procedures.

2.8 Data Management/Data Flow

In large part, it is anticipated that data will flow through agencies' "typical" processes – i.e., using agencies' existing lab sheets/data collection platforms, with samples to agencies' labs, and results into

agencies' databases. Data will then be shared both via transmission to other agencies upon request and by compiling in a shared data location via a common spreadsheet/database format. In some cases, such as for macroinvertebrates, a single laboratory will be used. See parameter-specific sections for details on data management/data flow for each indicator group.

3. INDICATOR GROUP-SPECIFIC PROCEDURES

3.1 Water Chemistry and Other Indicators Collected by Chemistry Crew

3.1.1 Indicators

Table 11 summarizes the parameters collected by the chemistry sampling crew. This includes field parameters, nutrients, bacteria (*E. coli*), miscellaneous, metals, and other parameters. Note that the metals, "other" parameters, TOC, and hardness are not collected at probabilistic sites during pilot monitoring.

*Table 11: Water Chemistry and Other Parameters Collected by Chemistry Sampling Crew (where **x**=indicator is sampled at site type)*

Indicator Group	Indicators	Probabilistic Sites 15 sites per reach July to September index period Sampled 3 times in index period	Fixed Sites 1 site per reach Monthly sampling (unless noted otherwise)
Field	Water Temperature	**X**	**X**
	DO (conc.& sat)	**X**	**X**
	pH	**X**	**X**
	Conductivity	**X**	**X**
	Secchi and Water Depth	**X**	
Nutrients	NO3+NO2	**X**	**X**
	TN	**X**	**X**
	NHx	**X**	**X**
	TP	**X**	**X**
	DP	**X**	**X**
	Chlorophyll a	**X**	**X**
Bacteria	*Escherichia coli*	**X**	**X** (April to October)
Miscellaneous	BOD	**X**	**X**
	Chloride	**X**	**X**
	Sulfate	**X**	**X**
	TSS	**X**	**X**
	TOC		**X**
	Hardness (Ca & Mg)		**X**
	Alkalinity	**X**	**X**
Metals	Aluminum (Al)		**X**
	Calcium (Ca)		**X**
	Cadmium (Cd)		**X**
	Chromium (Cr)		**X**
	Copper (Cu)		**X**
	Iron (Fe)		**X**
	Lead (Pb)		**X**
	Magnesium (Mg)		**X**
	Potassium (K)		**X**
	Sodium (Na)		**X**
	Zinc (Zn)		**X**
Other	Arsenic (As)		**X**
	Mercury (Hg)		**X**
	Selenium (Se)		**X**

3.1.2 Equipment

Each sampling agency will utilize its own equipment as needed to complete water chemistry monitoring.

Basic equipment common among sampling entities will include: clipboard, pencil, permanent marker, lab/field sheets, lab bottles, cooler, ice, GPS unit, rain gear, Secchi rod, personal floatation devices, field meter, and preservatives (e.g., acid). Other equipment specific to water collection with vary between fixed and probabilistic locations to reach desired sampling depth.

As *fixed* stations are typically located at hard structures on the river, certain collection equipment provides more efficient means for water collection at desired depth such as weighted bucket, lab line, Van Dorn sampler, automonitor, telescoping rods and longer cable on field meters.

Probabilistic stations will more often be sampled from a motorized watercraft which will allow samplers to collect within closer proximity of the water surface limiting equipment needed. Grab sampling will typically be the most efficient. Agencies may prefer to develop specially-designed equipment to achieve increased efficiency.

3.1.3 Safety

Sampling staff should follow all field safety protocols established by their agency. The following are general safety precautions for chemistry sampling crews.

Sampling crews will use chemicals in the preservation of samples. Safety Data Sheets should be consulted for proper handling of these preservatives (e.g., sulfuric acid, nitric acid, and methanol) to avoid inhalation and eye/skin irritation problems.

Crews should not sample during adverse conditions (presence of lightning, swift current/flooding, gusts/waves greater than the boat can safely navigate). If lightning is present, staff should return to the vehicle (trailer the boat) and wait a minimum of 20 minutes from the last visible lightning flash before returning to the water.

All applicable agency boating safety rules and regulations must be followed. By law, personal flotation devices (PFDs) must be easily accessible (not in storage) when the boat is in operation and /or occupied, including throwable (Type IV) PFDs. Moreover, individual agency policy may require staff to always wear PFDs while on the water. Also, the motor kill switch should be attached to the boat operator (clip to PFD or wrap around wrist) to prevent loss of control should the operator fall out of the boat.

Barge traffic related to transportation and dredging operations is a special circumstance when navigating the Mississippi River. Barge traffic should be given the right-of-way on all occasions when navigating to station locations, special attention should be used during mid-channel sampling procedures to ensure dangerous situations are avoided.

3.1.4 Site Selection

Fixed Sites: Fixed sample sites have been pre-identified in the *UMR CWA Monitoring Plan*. There is one fixed site sample per reach and details regarding these sites can be found on Table 3 and in the pilot project viewer. Since these sites have been successfully sampled in the past and there is no biological

sampling for fixed sites, there should be no site or bank selection issues. As such, there is no need to detail here procedures for switching to other sites, etc. Samples will be collected from as near to the river's "x-point" centerline as possible, recognizing that these sites utilize fixed site infrastructure (e.g., lock and dam) that may dictate specific sampling location.

Probabilistic Sites: Fifteen probabilistic sites have been assigned within each CWA assessment reach. These sites are allocated along the river's centerline (defined by USACE navigation sailing line). Crews will sample at the centerline "x point" identified in the sample draw. Proposed sampling sites should be reviewed in the office (via the pilot project viewer) to identify in advance any potential sampling issues. Since chemistry crew sampling is done at the center line, bank-based issues should not affect chemistry crew sampling – but is it possible these will affect associated biological sampling and as such the chemistry sampling site may need change due to issue with biological sampling. Therefore, chemistry crews will need to coordinate with fish and macroinvertebrate crews.

3.1.5 Frequency/Index Period

Fixed Sites: Water chemistry samples are collected monthly at fixed sites year-round, except that bacteria (*E. coli*) samples are only collected during the months of April through October. Secchi depth is not measured at fixed sites. See Table 4 for details.

Probabilistic Sites: Water chemistry samples are collected three times during the probabilistic monitoring index period of July through September.

3.1.6 Sample Collection

Field Parameters: Temperature, dissolved oxygen (concentration and saturation), pH, and conductivity are all measured by instruments on site at 0.2 meter depth and results recorded to lab sheet or electronic interface per sampling agency processes. Secchi depth is measured using a pole-mounted disk. Water depth may also be measured using the Secchi pole or with hand held sonar.

Nutrients, Miscellaneous, Metals and "Other" Parameters: All of these analytes are collected at a 0.2 meter depth using the equipment and sample bottles specified by the collecting agency. During grab sampling, remove bottle cap and invert bottle lowering into the water until 0.2 meter depth is achieved, then turn bottle upright to begin filling, this will avoid collecting organic debris floating directly on the water surface. Preservation is done according to the protocols of the recipient laboratory. Sample filtration is generally performed at the laboratory, rather than in the field. See Appendix A, which lists preservation and analytical methods for participating laboratories.

Mercury: For *mercury* sample collection, the following Clean Hands/Dirty Hands steps should be used as follows:

1. Designate Clean Hands (CH) and Dirty Hands (DH). CH will wear sterile disposable gloves and will only handle inner bag, bottle and sterile preservative vial. CH should always stand upwind of DH. DH will handle outer bag, and outer preservative carrying container.

2. DH holds outer bag of double bagged gloves and opens outside bag. CH opens inner bag and pulls out 2 gloves and puts them on.

3. DH holds opens outer bag of double bagged bottle. CH opens inner bag and pulls out bottle.

4. CH collects sample in flow which is representative of the stream. Bottle is inverted below water surface.

5. DH opens preservative carrying container and CH takes out a preservative vial and pours it into the sample.

6. CH closes sample bottle and puts it into inner bag of the double bag system which DH is holding.

7. DH closes outer bag, labels outer bag with station number.

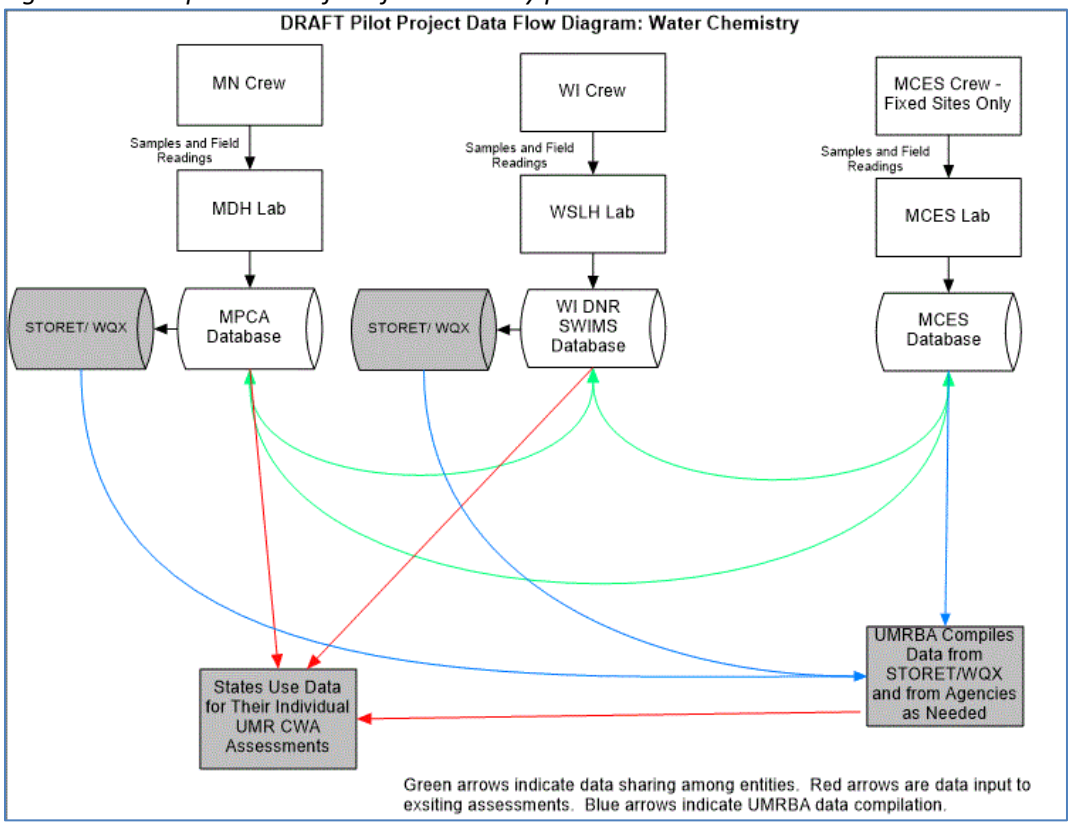
Bacteria (*E.coli*): Bacteria samples will be collected alongside the water chemistry parameters. Due to holding time limitations, crews will need to schedule their field days so that samples can be delivered to laboratories accordingly. Samples must also be held at 4°C until delivery to the lab. Note that samples received after the holding time may still be run and results flagged accordingly.

Note: habitat/site characteristic data (e.g., depth, substrate, velocity) is not collected for chemistry sampling.

3.1.7 Data Flow/Data Management

Chemistry data input and flow will proceed largely according to agencies' existing processes – i.e., using agencies' existing lab sheets/data collection platforms, with samples to agencies' labs, and results into agencies' databases. Data will then be shared both via transmission to other agencies upon request and by compiling in a central data sharing location. Figure 8 illustrates anticipated data flow in the pilot project for chemistry parameters.

Figure 8: Anticipated data flow for chemistry parameters.



3.1.8 Quality Assurance/Quality Control

Quality control considerations are an important aspect for capturing intra-laboratory variability across individual analysis methods. Collecting ten percent replicate samples is a common approach associated with most water quality programs and will be a reasonable method for monitoring laboratory accuracy during this project. Replicate samples should not be biased towards certain locations, time periods or water quality conditions.

Inter-laboratory variability between methods and protocols is an important aspect to explore in a multi laboratory project. Splitting water chemistry collections using precision equipment is an effective approach to capture distinct discrepancies between laboratories before and during sampling index periods. At least one water splitting event should be attempted prior to the beginning of the index period to provide a baseline and allow for modifications if necessary. Within-season splitting events will be necessary to keep a continuous record of comparability between laboratory results.

3.2 Fish Assemblage

3.2.1 Indicators

Fish sampling includes primarily collection of information regarding the fish themselves and also of associated site characteristic (e.g., habitat) data. Tables 12 and 13 summarize fish and site characteristic information to be collected. Fish data are collected in order to support calculation of Great River Fish Index (GRFIn) scores. See Appendix B for a list of metrics included in the GRFIn. Site characteristic data are collected in support of investigating outliers in catch data and to document any unique conditions at the time of sampling.

Table 12: Fish Parameters.

Parameter	Units/Method of Measurement
Species	Name
Deformities, Erosion, Lesions, Tumors (DELTS)	Code, where: D (deformities) = skeletal anomalies of the head, spine or body shape E (erosion) = eroded barbels, fins, or gill covers, substantial fraying or reduction L (lesions) = open sores or exposed tissue, raised warty outgrowths T (tumors) = areas of irregular cell growth which are firm and cannot be broken open easily O (other) = flag and describe in comment Count of fish individuals where these occur, recorded by type and species. If an individual fish has multiple types of DELTS, only one type is recorded.
Count	Number of fish collected, by species.
Fish Length	Fish are optionally measured according to agency protocols. *Note: Fish lengths are not required to compute the GRFIn, and lengths are time-consuming to record, so this is an opportunity to economize sampling time, as needed.*
Weight	Total weight (g) of all fish collected, by species. Can be recorded as batch-weights or individuals recorded separately, at the discretion of crew leaders.

Water quality measurements (see Table 13) are recorded upon arriving at the site, and should be taken approximately 20 m from shore near the midpoint of the sampling reach. If this location is not representative of the sampling reach in general or is unsafe, another location that is representative can be substituted at the discretion of the field crew leader. Specific conductivity, dissolved oxygen, temperature and current velocity should be measured at 0.2 m depth. These measurements can be used to inform crews of physical and chemical conditions that affect sampling efficiency and electric power transfer to the fish, and thus, control box settings.

Water depth is recorded as a general descriptor of the type of habitat found within the electrofishing transect. Water depth should be estimated as the average depth encountered along the length of the transect when the electrofishing boat has disengaged from the shoreline, approximately 20m from the bank. This estimate should be recorded after the transect fishing has been completed, during the recording of other habitat descriptors (see below).

Habitat descriptors are recorded after the electrofishing has been completed, and are intended to reflect significant habitat features within the site. For example, a single stem of submersed vegetation or twig in the water does not constitute a significant habitat feature. However, a snag, vegetation bed, or wingdam that yields numerous fish would constitute a significant habitat feature, and should be recorded as such. Percent vegetation cover should be estimated as the linear percentage of shoreline that is vegetated, as opposed to how far it extends from shore. Overhanging trees may be recorded as

"other" and included in comments if they yield fish from their shade or submerged branches. Boathouses, docks, seawalls, barges, and boat landings are also examples of habitat features that can be recorded as "other" and explained in the comments.

Substrate type is estimated by surveying the dipnetters to determine what type of prevalent bottom type they encountered during the electrofishing process. Dipnetters should occasionally probe the river bottom with their dipnets and evaluate the coarseness of substrates they scrape against, or the contents of the substrate scraped into their nets. The substrate categories are intended to reflect increasingly coarse or harder substrates with higher category values.

Note that the water quality, site characteristic, and habitat parameter data collection described here is what has been agreed to as needed for the interstate sampling effort. States may choose to augment this data collection as necessary to meet their own internal needs.

Table 13: Water Quality, Site Characteristic and Habitat Parameters Affiliated with Fish Sampling. Adapted from Ratcliff et al. (2014)

Variable Name	**Variable Type**	**Units (and Accuracy)**
Transparency tube	Continuous	cm (nearest 1)
Specific conductivity	Continuous	µS/cm (nearest 1)
Water velocity	Continuous	m/s (nearest 0.01)
Water temperature	Continuous	°C (nearest 0.1)
Dissolved oxygen	Continuous	mg/L (nearest 0.1)
Water depth	Continuous	m (nearest 0.1)
Percent vegetation coverage	Categorical: 1: 0% (no aquatic vegetation present 2: 1-19% coverage 3: 20-49% coverage 4: 50% or more coverage	%
Vegetation density	Categorical (sparse or dense)	Scaleless
Substrate	Categorical: 1: Silt (soft and non-gritty) 2: Silt/Clay/Little Sand (soft mixture) 3: Sand/Mostly Sand (firm, coarse) 4: Gravel/Rock/Hard Clay	Descriptive
Structures		
- Woody debris/snags	Binary	Presence or absence
- Tributary mouth	Binary	Presence or absence
- Inlet/outlet channel	Binary	Presence or absence
- Flooded terrestrial	Binary	Presence or absence
- Wing dam/dyke	Binary	Presence or absence
- Revetment (rip-rap)	Binary	Presence or absence
- Low-head dam, closing structure, weir	Binary	Presence or absence
- Other	Binary	Presence or absence

3.2.2 Equipment

While each sampling agency's equipment may vary somewhat, the following are recommended for sampling under the *UMR CWA Monitoring Plan*.

Boat Specifications and Electrode Configuration: The standard large river boom electrofishing boat is a modified 5-6 m aluminum jon boat with an extra-thick puncture-resistant welded-aluminum hull. The boat is equipped with a suitable outboard motor for large river navigation. An auxiliary motor may be

mounted to the transom, and may be used to maneuver the boat during electrofishing; it allows operation at slower speeds and in shallower water than the main motor.

A generator supplies power to a control box, which in turn controls the electrical field configuration. Twin booms extend 2.5 - 3.0 m from the front of the boat, each with an anode dropper array affixed to the front ends. The boat's hull serves as the cathode. There are "kill" switches on board for each member of the crew. There is a kill switch on the control box which shuts off all power coming from the box. There are either positive-pressure kill switches operated by foot pedals mounted on the front deck, or tether kill switches attached to safety belts for the dipnetters, and there is a hand-held switch operated by the driver during operation of the electrofishing equipment. All switches must be "on" in order to activate the electric field. This ensures redundancy within the electrical safety system: the driver and each crew member can kill the electricity from the generator in an unsafe situation.

The control box should be a commercially-produced unit, preferably equipped with adjustable output. Pulsed DC current is used for electrofishing in large rivers because it evokes a galvanotactic (oriented swimming) response in fish that attracts fish to the anodes and allows more opportunity to net them in strong currents. DC current is also much safer for the health of the fish.

Net/Mesh Size: Dip nets will use a ¼ mesh size, consistent with EMAP protocols. The recommended dipnet is a Duraframe, Inc. "Regular D" dipnet with an 8' non-conductive handle, although similar dipnets may be substituted.

Water Quality Equipment: Any water quality meters, probes, sondes, etc. that provide measurements to the specified level of accuracy are suitable.

3.2.3 Safety

Large rivers are often congested by barge and recreational traffic. Extreme precautions should be taken when electrofishing, crossing the navigation channel, and navigating to and from sampling locations. Primary responsibility for safety rests with the crew leader. However, each member of the three-person crew should be alert, aware of safety considerations, trained to recognize safety concerns, and trained in first aid and CPR.

Only a trained pilot should operate the boat, especially when electrofishing. Large rivers have unpredictable and fast currents, and submerged hazards may be present nearly anywhere. Electrofishing is potentially hazardous, and the pilot must be aware of the crew, water conditions, both submerged and above-water hazards, other boaters and people on shore, as well as the control box settings, beginning and ending points of the sampling run, timer, outboard engine conditions, etc. It is highly recommended that agencies require a specific training certification for both large river sampling and electrofishing boat operations.

Table 14. Safety Considerations for Fish Sampling. Adapted from Angradi, et al. (2006)

The electrofishing unit has a high voltage output and is capable of delivering a fatal shock.
Large (>10 kg) silver carp (Hypophthalmichthys molitrix) can jump >2 m out of the water. People have been seriously injured by carp collisions. Silver carp are present in the lower reaches of the UMR. Be alert for jumping fish while running the river and during electrofishing.
Crew members should be able to swim, and should receive CPR, first-aid, and safe boating training.
If the generator is running, do not touch the anode or cathode (if a cathode other than the boat hull is used). Do not touch objects outside the boat. Do not reach into the water. If doing so, make sure all electricity to the water has been turned off by ensuring that all three switches are in the "off" position (unit, pedal, and hand switch).
Do not electrofish in high waves, strong currents, or other conditions that may cause sudden motions of the boat that can cause someone to lose their balance.
Do not fish in the rain due to electrical shock hazards associated with fully wetted equipment.
All members of the electrofishing crew should wear USCG-approved PFDs whenever in the boat.
Good line of sight and communication should be maintained among crew members at all times. The generator is loud and often drowns out verbal communication. Hand signals should be used to communicate boat direction, power on/off, and other vital information.
All crew members should know the location of the nearest hospital.
Use caution around onboard gas tanks. Never refill the generator when it is hot. The generator exhaust gets extremely hot while in use. Caution should be used to ensure that no item is touching the exhaust and that all items near the exhaust are secured so as to ensure they do not shift position while underway and possibly come in contact with the exhaust.
All electrical connections should be checked prior to use to ensure that proper, tight connections are maintained. Loose connections can cause sparking and fire. Prior to each sampling event, all electrical "kill" switches should be checked to ensure they are working properly.
All crew members should know the on-board location of the cell phone, first aid kit, fire extinguisher, and truck keys.

3.2.4 Site Selection

Fish sampling is only done at probabilistic sites. Fifteen probabilistic sites have been assigned within each CWA assessment reach. These sites are allocated along the river's centerline (defined by USACE navigation sailing line). Crews will sample along a bank perpendicular to the centerline "x point" identified in the sample draw and on the assigned bank. See Figure 5.

Proposed sampling sites should be reviewed in the office (via the pilot project viewer) to identify in advance any potential sampling issues. Because the fish sampling is to occur over a 1 km MCS transect, initial determination of site sampling status will be conducted by fish crew leads. Fish crew leads must be in communication with other crew leads (chemistry and macroinvertebrate) to share any issues regarding the sampleability of probabilistic sites. If sites are determined not to be sampleable, they will be replaced with the next overdraw site on the list within the same assessment reach (in order of site ID).

The main-channel bank to be sampled has been randomly determined using the random number generator function in Excel. However, crews have the flexibility to sample the opposite bank if site conditions present safety concerns or is not representative of main-channel border habitat (e.g., due to dams, barge fleeting, etc.). If neither bank can be sampled, the field crew has the discretion to replace the site entirely using an overdraw site. Thorough documentation of conditions resulting in bank or site replacement is required. See further discussion in Section 3.2.5 below.

Note that cases may occur where the randomly selected bank cannot be utilized for macroinvertebrate sampling (due to velocity and/or depth issues) but is suitable for fish assemblage sampling. In such cases, electrofishing will remain on the randomly selected bank even if macroinverbrate sampling is shifted to the opposite bank.

3.2.5 Site Verification

Site verification and sampling status is primarily determined by two factors: 1) whether or not the site is representative of main channel border habitat, and 2) whether or not the site can be safely sampled. In most cases, it is anticipated that this can be determined in the office prior to an actual sampling visit using GIS and staff experience. Questionable sites may need a field visit prior to sampling. Every reasonable attempt should be made by fish crews to determine site sampling status for electrofishing purposes prior to the commencement of any water chemistry sampling or artificial substrate sampler deployment to ensure all indicator crews monitor the same sites and maximize efficiencies.

The 1 km MCS transect is established along a terrestrial shoreline interface and sampling is executed within the near-shore littoral zone. The 1 km MCS transect should be established and sampled along the predetermined random bank unless that bank is not representative of main channel border habitat or is unsafe to sample.

An assigned bank that is not representative of main channel border habitat conditions might include a backwater lake, riparian wetland, constructed marina, or other condition which prohibits sampling within the terrestrial shoreline interface adjacent to the main channel border. Impoundments and Lake Pepin are included within the study design and should be sampled unless other conditions preclude a bank from being sampled. Unsafe conditions could include barge fleeting areas, dams, and lock channels.

A continuous 1 km MCS transect centered on the cross-channel transect (perpendicular line drawn to bank from USACE centerline through x-point) is preferred but some situations may require sliding part of the reach to avoid obstacles, unsafe conditions, tributary confluences, secondary channel openings, etc. The following guidelines apply in evaluation of sample sites:

- Any obstacle or opening <100 m in length should be treated as part of the reach and sampled through or around without further consideration.
- Obstacles or openings 100-500 m in length are avoided and an equal distance is added to the reach where appropriate (upstream and/or downstream end) to ensure 1 km is electrofished. The sampling reach should be adjusted in a manner that results in the most continuous 1 km shoreline transect being sampled as possible.
- If an obstacle or opening >500 m exists along the 1 km MCS transect the opposite bank should be evaluated and sampled if suitable conditions exist.
- If the opposite bank cannot be sampled in accordance with the above rules, then an overdraw site should replace the primary site.

There are many possible combinations of safety hazards and obstacles that may be encountered in the field, not all of which are considered here. The operational goal of fish crew leaders should be to collect a representative fish community sample from main channel border habitat without subjecting the crew to unacceptable risk. Discretion and best professional judgment is granted to deal with sampling irregularities and ensure overall objectives are met. Explain any non-standard methods used or sampling decisions made in the field. Do the best you can and consult the authors of this section or other project partners for additional guidance in special cases that do not seem to fit the circumstances described herein. Each field team should assemble and maintain a file for the sampling sites that

contains supporting information for the decisions that are made with regard to site selection and sampling. This file should document office verification, field visits, decisions regarding sliding and opposite bank sampling, site replacement and overdraw selection, and GPS coordinates for upstream, downstream, and transect midpoint locations, as well as any split transect coordinates. The names of the staff involved in making those decisions should also be recorded within the file. Pictures, maps, and other supporting graphical information are also recommended.

3.2.6 Frequency/Index Period

Fish assemblage sampling is conducted one time during the July to September index period under normal summer baseflow conditions (discharge between the 25th and 75th percentile, preferably near the daily long term median statistic). Whenever possible, crews should make an effort to intersperse sites among reaches and throughout the index period to achieve a representative mixture of conditions. However, cost effectiveness may dictate sampling a cluster of sites within an area over a short span of days. This should not affect the outcome of the study, as the entire index period is thought of as being representative of "summer baseflow" conditions. If unusual hydrological or weather conditions occur during a sampling event, they should be documented, at a minimum, and crew leaders will have discretion as to when sampling should be discontinued for unusual conditions.

3.2.7 Sample Collection

Fish assemblage data are collected by electrofishing with a three-person crew during the day. After electrofishing and processing fish, the crew records fish habitat data (i.e., site characteristics).

Electrofishing Transects: Upon arriving at the site location and completing the pre-sampling water quality data recording, the fish-sampling crew identifies and documents the upstream and downstream boundaries of the electrofishing run using GPS and shoreline features. Fish are sampled by daytime electrofishing along the 1-km shoreline transect. When possible, the sampling run should be centered on the perpendicular intersection of a line through the x-site with shore, although sliding the boundaries of the reach to accommodate island configurations, man-made structures and other obstacles is acceptable. Upstream, downstream, midpoint, and any split transect coordinates are recorded, if different from those recorded in the office site verification process.

The shoreline electrofishing zone extends out from shore to a depth of 6 m (20 ft) or a distance of 30 m (100 ft), whichever is closer to the shore. Electrofishing is conducted for a minimum of 3600 seconds (1h) of total shock time to collect fish from the designated zone. Increased shock time may be necessary to fish shorelines with abundant cover.

Electrofishing: It is recognized that not all makes of electrofishing control boxes allow monitoring of all electrofishing parameters. At a minimum, elapsed sampling time or "on-time" (electrical current applied to water in seconds) will be recorded. As available, standard ancillary electrofishing data will be recorded - volts, amps, frequency (Hz) and duty cycle (%).

For consistency, electrofishing should occur between 1hr after sunrise and 1hr before sunset, so as not to be affected by crepuscular fish activity. Beginning at the upriver end of the transect, the driver maneuvers the boat downriver along the shoreline (Figure 9). The anodes should be positioned to probe into the shoreline interface and visible cover. The two other crew members stand in the bow of the boat and net all fish that are stunned. Stunned fish are placed in an aerated live well. Voltage and

amperage are adjusted to ensure that a stun response by fish is maintained at all times. It may be necessary to adjust power during and between run segments, based on sampling effectiveness and incidental fish mortality. Trained crew members should be able to determine whether insufficient or excessive power is being used.

Electrofishing boat speed should be sufficiently slow to allow the fish netters to recover all stunned fish, including small fish, such as darters, which are difficult to see and do not always rise up off of the bottom when stunned. As much as possible, the anode(s) should be kept perpendicular to the shoreline, and the pilot should ensure that the electrical field is passing over the shallow littoral areas, as well as over the deeper channel margin. The path of the boat (Figure 9) and the field should be analogous to the motion of a person using a metal detector: a side-to-side path with complete lateral coverage and a slow downstream pace.

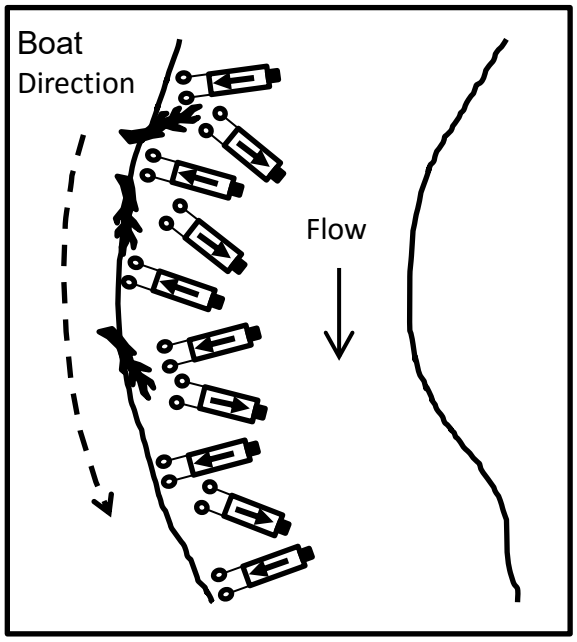


Figure 9: Recommended path of electrofishing boat showing equal coverage of shoreline and channel margins as well as complete application of the field through, over, or around cover objects. The zone should not extend greater than 30 m from shore, or to a depth greater than 6 meters.

Care should be taken to thoroughly work the electric field around objects such as snags, downed trees, piers, boulders, and other potential fish cover until each object yields no more fish. The boat may have to be held over the structure for a few seconds to allow the fish to wriggle out of the cover and up into the field. The minimum electrofishing time for each 1km site is 3600 seconds of shock time. Along shorelines with swift current and/or little cover, it may be necessary to overlap passes along the shore to ensure coverage and achieve the required minimum shock time. There is no upper limit for electrofishing time, although most sites should be able to be sampled within 3600-5400 seconds of shock time. Electrofishing procedures are described in Table 15.

A large live well (> 300 L) should be used to ensure adequate holding capacity that minimizes stress on sampled fish. An aerator should be available to maintain oxygen levels in the tank, and frequent water exchanges or additions are encouraged. If an excessive number of fish are captured, the pilot may choose to mark a landmark or waypoint, and break up the sampling effort to process fish. Fish that appear overly stressed as indicated by failure to regain a righting response after a short time should be processed quickly. Mid-sample processing of fish must be done on the opposite bank or sufficiently far enough downstream that released fish would not be expected to re-enter the sampling site during the remaining electrofishing time. Once the fish from a partial sampling run have been processed, the crew will commence to sample the remainder of the 1km site, beginning at the exact spot where sampling ceased previously. The pilot should use discretion as to how many breaks are needed to process fish during the completion of sampling. Generally, a site should be completed in 2-4 segments. At the completion of a 1km electrofishing run, the crew leader records the end time and the total elapsed shock time, in seconds, on the Fish Sampling Form.

Table 15. Electrofishing Procedures. Adapted from Angradi, et al. (2006)

1	Upon arrival at a sampling site, complete the header information on the Fish Sampling Form including site ID, date, upstream, midpoint, and downstream GPS coordinates of the 1 km sampling site, and target shoreline. If any obstacles or gaps occur in the run that require discontinuous shoreline coverage, document the upstream and downstream extents of these by recording additional GPS coordinates in the comments.
2	Obtain water quality parameter estimates and record. From these measurements, determine control box settings that should be used.
3	Navigate to the upriver end of the electrofishing transect and complete all pre-sampling tasks, including: extend and secure the boom(s), fill the live well and turn the aerator on, don all safety equipment.
4	At a location outside the sampling zone, test the electrofishing unit and kill switches. Using pulsed DC, adjust voltage and amperage to elicit the required galvanotactic fish response. Make voltage and amperage adjustments to ensure that fish are being rolled easily, that smaller fish such as darters are effectively stunned, and that fish are not being injured. Record power output data on the form (volts, watts, amps, pulse rate, pulse width or duty cycle).
5	Record the begin time on the Fish Sampling Form and begin electrofishing. From the top of the zone, proceed slowly downriver, keeping the anode(s) oriented toward shore as much as possible. Use the current to push the boat downstream, and sweep the bow back and forth through each sampling pass. In areas of high current, it may be necessary to overlap passes to ensure adequate coverage and achieve the required minimum shock time (Figure 9). Dipnetters should attempt to net all stunned fish. Avoid netting bias toward larger individuals. Do not attempt to fish in water deeper than 6 m (20 ft). In areas with steep drop-offs, stay along the edge of the drop and close to shore, fishing the shallower margins. If the water is generally shallower than 6 m, the path of the boat should extend out into the channel no more than 30 m (100 ft) from shore. Carefully maneuver the boat around instream cover, fishing slowly to ensure that the cover is yielding no more fish before moving on.
6	Attempt to fish the transect as thoroughly as possible, but do not place the crew in danger in order to fish particular habitats. Safety is the first concern. If part of the transect cannot be fished safely, note this in a comment on the form. Fish processing breaks may be taken at the pilot's discretion, at any point in the transect. Fish processing should occur on the opposite bank of the river or far enough downstream that fish would not be expected to re-enter the sampling site during electrofishing. If processing breaks are taken, electrofishing resumes at the exact spot where it previously ceased.
7	At the end of the sample transect turn off the electrofishing gear and record end time and total shock time.
8	The minimum electrofishing time for each transect is 3600 seconds of shock time. There is no upper limit for electrofishing time, although sites should be able to be sampled generally within 3600-5400 seconds.
9	Processing commences at the end of the run, or at the pilot's discretion, within the transect.
10	Upon completion of fish processing, the habitat information if filled out on the Fish Sampling Form.

Sample Processing: Sample processing includes identifying fish to species, examining them for external anomalies, measuring and weighing, preserving small specimens for later processing, photographing voucher specimens, and selecting specimens to be retained for tissue contaminant analysis. For processing, one crew member records data on the fish sampling form while the other crew members sort, identify, weigh, and measure fish. After they are processed, fish should be promptly released, unless they will be retained for vouchers or tissue analysis. Fish processing procedures are described below and in Table 17.

Fish are recorded by their complete American Fisheries Society (AFS) common name after Page et al. (2013). Abbreviated field data codes may be used to identify fish names, as long as official common names can be linked to these later in the data files.

A total count of individuals for each species collected will ultimately be required in the data file. Crews may opt to process fish in batches by species and record a single count for each species on the Fish Sampling Form, or, if individual fish are recorded, their counts may be summed later, during data analysis. Regardless, a count must be recorded for each fish collected.

Each fish must be examined, both sides, for DELTs (deformities, erosions, lesions, and tumors). Record the presence of DELTS on an individual fish or among fish in a batch using the codes in Table 16. Ensure

that each fish with a DELT is recorded as such. Although individual fish may have multiple DELTs, record only a single (most prevalent) DELT for each fish. Other abnormalities (e.g., blind eyes, pop-eye, fungus) can be recorded using flags.

Table 16. External Anomaly Codes (DELTs).

Category	Code	Description
Deformities	D	Skeletal anomalies of the head, spine or body shape
Erosion	E	Eroded barbels, fins, or gill covers; substantial fraying or reduction
Lesions	L	Open sores or exposed tissue; raised warty outgrowths
Tumors	T	Areas of irregular cell growth which are firm and cannot be broken open easily (masses caused by parasites can be broken open easily)
Other	O	Flag and describe in comments

Fish length data are optional, as the GRFIn does not require length information in its metrics. Crews may choose to record minimum and maximum lengths of each species, or individual lengths, or may choose certain species to measure, depending upon the standard practices of the collecting agencies.

All fished are weighed (g), either as a batch weight, by species, or individually. Care should be taken to have a stable weighing platform where wind and waves do not interfere with accuracy. Crew members should also be vigilant to ensure that excessive water does not accumulate in buckets or trays, and thus, introduce error in the recorded weights. Scales should preferably be electronic, although spring scales may be used. All scales should be calibrated according to instruction manuals and at the proper frequency.

Specimens that cannot be identified with certainty in the field should be preserved in 10% formalin for later processing by the sampling crew. Preserved fish will be processed in the same way as field-processed fish. Crews must label preserved samples with site ID and sample date at a minimum, to identify where and when each sample was collected, and also fulfill any required hazard or biosecurity labeling requirements.

Table 17. Fish Sample Processing Procedures. Adapted from Angradi, et al. (2006)

1	If handling threatened or endangered fishes is permitted under the collecting permit, they should be processed first in order to expedite their return to the water. Otherwise they should be released immediately.
2	For each fish, record the AFS common name (Page et al. 2013), or an abbreviated surrogate code. If a specimen is too small or otherwise cannot be easily identified to species in the field, it should be preserved and retained by the crew for later identification. Do not record field data for fish that are retained as preserved specimens. If processing a partial segment of the site, be sure to release fish in a location where they will not be recollected during remaining sampling within the transect.
3	Record total counts for each species on all batch-weighed fish, or individual counts for individually-weighed fish. It is acceptable to have multiple rows of data for a given species, as long as each fish is only processed and recorded once. For example, if the crew batch-processes a species, and later finds more of that species in the tank, the second batch can be processed and recorded in a new row on the Fish Sampling Form.
4	Examine each fish for DELTs (deformities, erosions, lesions, and tumors). Record the presence of DELTS on an individual fish or among fish in a batch using the codes in Table 16. Other abnormalities (e.g., blind eyes, pop-eye, fungus) can be recorded using flags.
5	Fish lengths are optional, and may or may not be recorded according to standard agency practice.
6	Record the weight of each fish (or batch of fish) in g. For species where the total weight of all individuals in the sample is estimated to be less than 10g, weighing should be done with an electronic scale in a sheltered environment.
7	Preserve unknowns and voucher specimens as needed (Table 18). Note, in a comment, the number of specimens preserved. A digital camera image is usually adequate for larger specimens (e.g., > 150 mm).

Unknowns and voucher specimens: Proof of fish identification capabilities is a necessary QA/QC step for most fisheries programs. Absent this proof, the data and their interpretation are left vulnerable to criticism. However, longstanding methods of preserving and archiving large collections of fish is becoming less common and more difficult, due to increasing regulation of toxic chemicals. Thus, photographic documentation has become more valuable, especially with the availability of high resolution digital cameras.

Each crew member should become familiar enough with the large-river fish assemblages in the region to identify most species. Trained ichthyologists familiar with the fish species of the region should perform final taxonomic identifications of unknowns.

Vouchers, in the form of a photograph or as a preserved specimen, should be retained as a reference for every species encountered each year. Each fish-sampling crew should collect or photograph voucher specimens for each different species encountered at a sampling site. It is strongly recommended that unknown or questionable small fish and all minnows should be preserved at every site for later identification by the fish-sampling crew and/or other trained ichthyologists. Vouchers may be retained from these preserved specimens, as well. Table 18 describes preparation of photo vouchers and preserved specimen vouchers. Large or common species can usually be adequately documented by a photo, as can fish that are readily identified by unique features, such as color, barbels, fin placement, etc.

An effort should be made to document with a photo or by collection any known or suspected non-indigenous exotic or invasive fish species that are captured. The collecting permit may specify that certain species not be returned alive to the water. A spatially-referenced and frequently-updated database of non-indigenous fish species of the U.S. can be searched at http://nas.er.usgs.gov/. Fish sampling crews should be familiar with the potential and reported non-indigenous species in the river and regions they are sampling. Collections of non-indigenous fishes made during the UMRBA-CWA sampling should be submitted by fish-crew leaders to this database via the above web address.

Table 18. Voucher specimens. Adapted from Angradi, et al. (2006)

1	There are two types of voucher samples: 1) preserved individual species vouchers, and 2) photo vouchers. A preserved individual species voucher or photo voucher should be retained for every species captured by every fish-sampling crew each year.
2	Preserved voucher specimens are placed in a leak-proof plastic jar(s) with a screw top with 10% buffered formalin. If possible, 2 representative individuals should be selected. Do not cram fish into jars so that they are fixed in a bent position; they should float freely in the jars. Fish biomass should not exceed 40% of the container contents by weight. Fish >12-cm long should be slit open in the lower right abdomen to promote preservation. Vouchers should be labeled with the site ID and date, at a minimum, as well as required hazardous material and other required safety or biosecurity labeling.
3	Photo vouchers. Place the fish, facing left, on a measuring board (preferably, for scale, or other flat surface). It is recommended to place a paper tag with site ID, date, and species name written on it next to the fish. Use a digital camera to take a high-resolution picture of the fish. Check the quality of the image before releasing the fish. It is recommended to keep a copy of each original image file in a computer folder, as a backup. File names may be edited to identify the fish species subject in each image.

3.2.8 Data Flow/Data Management

Because agencies differ in how they record, process, and store sampling information, this protocol is intentionally general in its requirements. It is presumed that all program participants will exercise care in collecting and recording data, follow good QA/QC protocols in proofing and managing data sets and

data transfer, and will keep appropriate records to document what was done at various steps in the process.

It is acceptable to record field data on paper forms or computer data entry applications. Backup copies of forms and files should be made and stored separately from the originals in case of calamity. Each agency will be responsible for storing and sharing the data it collects, and all parties will agree upon a data processing and analysis plan, including calculation of index scores and interpretation of the results.

3.2.9 Quality Assurance/Quality Control

Crew members should be properly trained in techniques for operating the boat and electrofishing equipment. Proper use of the equipment, including maintaining the electrical field and maneuvering of the boat to optimize capture of fish, is critical to ensuring that a representative sample is collected. QA of fish sample processing depends on correct identification of specimens. Crew members should have sufficient training to identify most fish that are collected. Questionable fish should be retained as preserved specimens, to be identified in the lab or sent out for confirmed identification by a taxonomist. Table 19 provides some QA considerations for fish sampling.

Resampling at a 10% rate is encouraged, in order to best assess method variability. For each reach, a 10% resample rate translates to two replicate sites per reach. Replicate sites were chosen at random as the first two sampleable sites per assessment reach in order of site ID. An ordered table of sample sites to be used for resample site selection is found in Appendix D. Replicate samples should be collected a minimum of one week later than the initial sampling (but within the same index period) and ideally at a similar river stage.

Table 19. QA Considerations for Fish Sampling. Adapted from Angradi, et al. (2006)

Sampling should probably not take place if Secchi depth is < 15 cm (6 inches) or if river stage is elevated > 0.5 m (20 inches) above normal levels. The decision to sample or not is up to the crew leaders.
The transects should be fished before starting habitat data collection so that fish are not spooked from the shoreline.
Electrofishing should take place between 1h after sunrise and 1h before sunset.
Use a digital camera on a high resolution setting for taking photo vouchers and check image quality before releasing fish.
Do not cram fish voucher specimens into jars. They should be free floating so they are not fixed in a bent position.
Netters should wear polarized sunglasses.
When netting shocked fish, avoid size bias.
Avoid shorthand or colloquial common names for fish. Page et al. (2013) is the standard.

3.3 Macroinvertebrates

3.3.1 Indicators

The deployment and collection of artificial substrate samplers for colonization by aquatic macroinvertebrate serves as the primary source of information regarding the health and composition of the macroinvertebrate community. Water quality and site characteristic (e.g., habitat) data are collected to supplement the biological data, and to document conditions at the time of sampling. Macroinvertebrate data are collected in order to support calculation of Wisconsin Large River Invertebrate Index scores. See Appendix C for a list of metrics included in the Wisconsin Large River IBI.

Water quality measurements are recorded at both deployment and retrieval of Hester-Dendy artificial substrate samplers, and should be taken at the location of the sampler after anchoring the boat at each invertebrate site visit. Specific conductivity, dissolved oxygen, pH, and temperature should be measured at 0.2 m depth. Velocity should be measured at the deployment depth of the Hester-Dendy sampler, approximately 1 meter below the surface. These measurements are used to inform crews of physical and chemical conditions present at the time of sampling, and used as a gauge to determine the usefulness of invertebrate samples (e.g., if flows are below threshold levels at time of retrieval, the invertebrate data may not be suitable for assessment).

Habitat descriptors are recorded at the time of Hester-Dendy retrieval, and are intended to reflect significant habitat features within the site. For example, a single stem of submersed vegetation or twig in the water do not constitute significant habitat features. However, a snag, vegetation bed, or riffle that could serve as a source of invertebrate productivity and diversity, should be recorded as such.

Table 20: Site Characteristics/Habitat. Adapted from Ratcliff et al. (2014)

Variable Name	**Variable Type**	**Units (and Accuracy)**
Transparency tube	Continuous	cm (nearest 1)
Specific conductivity	Continuous	µS/cm (nearest 1)
Water velocity	Continuous	m/s (nearest 0.01)
Water temperature	Continuous	°C (nearest 0.1)
Dissolved oxygen	Continuous	mg/L (nearest 0.1)
Water depth	Continuous	m (nearest 0.1)
Percent vegetation coverage	Categorical: 1: 0% (no aquatic vegetation present 2: 1-19% coverage 3: 20-49% coverage 4: 50% or more coverage	%
Vegetation density	Categorical (sparse or dense)	Scaleless
Substrate	Categorical: 1: Silt (soft and non-gritty) 2: Silt/Clay/Little Sand (soft mixture) 3: Sand/Mostly Sand (firm, coarse) 4: Gravel/Rock/Hard Clay	Descriptive
Structures		
- Woody debris/snags	Binary	Presence or absence
- Tributary mouth	Binary	Presence or absence
- Inlet/outlet channel	Binary	Presence or absence
- Flooded terrestrial	Binary	Presence or absence
- Wing dam/dyke	Binary	Presence or absence
- Revetment (rip-rap)	Binary	Presence or absence
- Low-head dam, closing structure, weir	Binary	Presence or absence

- Other	Binary	Presence or absence

3.3.2 Equipment

Invertebrate sampling equipment: The primary sampling gear to be used in the UMR CWA pilot monitoring project for the collection of macroinvertebrates is the modified multi-plate artificial substrate sampler, aka the Hester-Dendy sampler (Hester and Dendy 1962). The Hester-Dendy sampling unit consists of three multiple-plate artificial substrate samplers bound together and suspended under a float (see figure 10). The samplers are constructed of 1/8" hardboard cut into 3" square plates and 1" square spacers. Other items such as plastic washers can also be substituted as spacers. Each sampler consists of 8 square plates arrayed on a three inch long eyebolt with a spacing of 1/8", 1/8", 1/8", 1/4", 1/4", 1/4", and 3/8" which follows the configuration of Ohio EPA (1989) and Weigel and Dimick (2011) (see figure 11). The float is anchored in place using one or more cinder blocks to maintain the samplers in an area with sufficient flow (>0.09 m/s). Rope length equivalent to 4-5 times the depth at which the sampler is deployed should be used when tying the buoy to the anchor.

Figure 10 Sampling rig used for the collection of macroinvertebrates, including 3 Hester-Dendy multi-plate artificial substrate samplers, a float, and concrete block anchor. Image courtesy of Minnesota Pollution Control Agency.

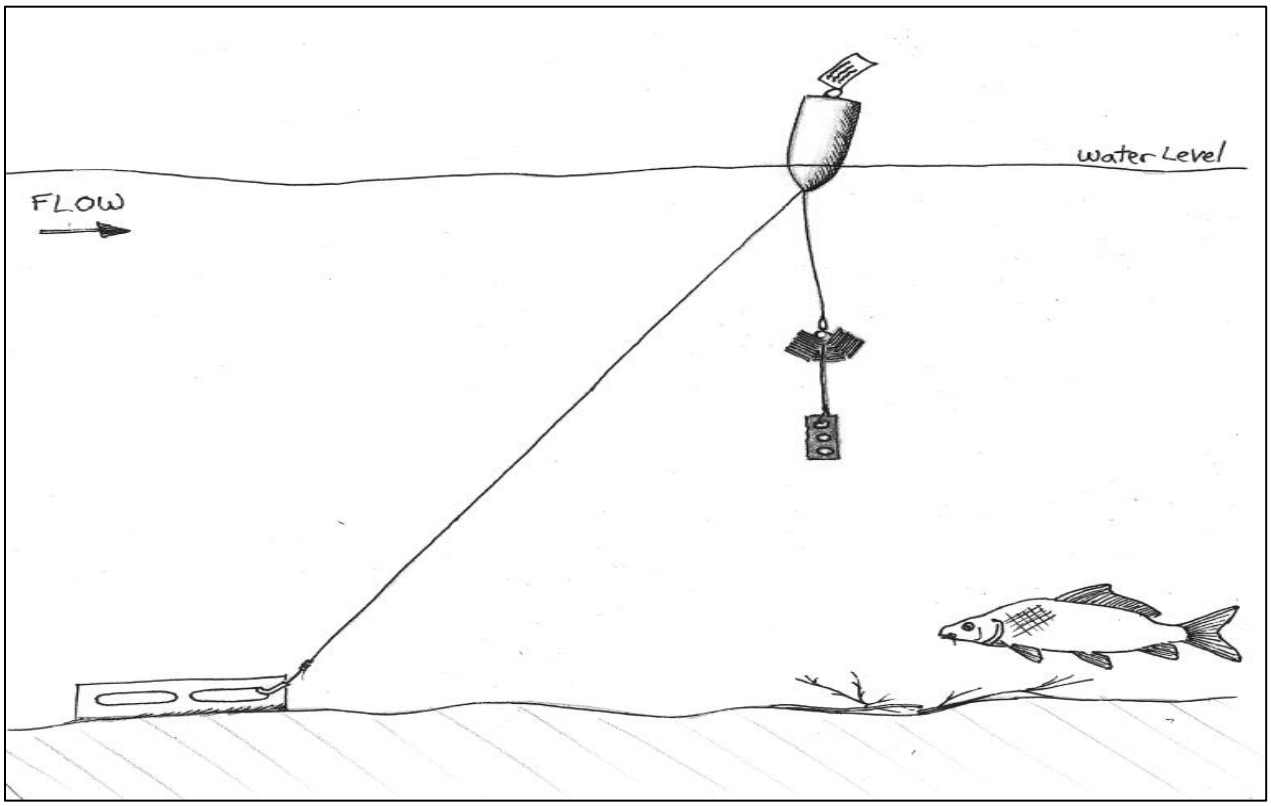
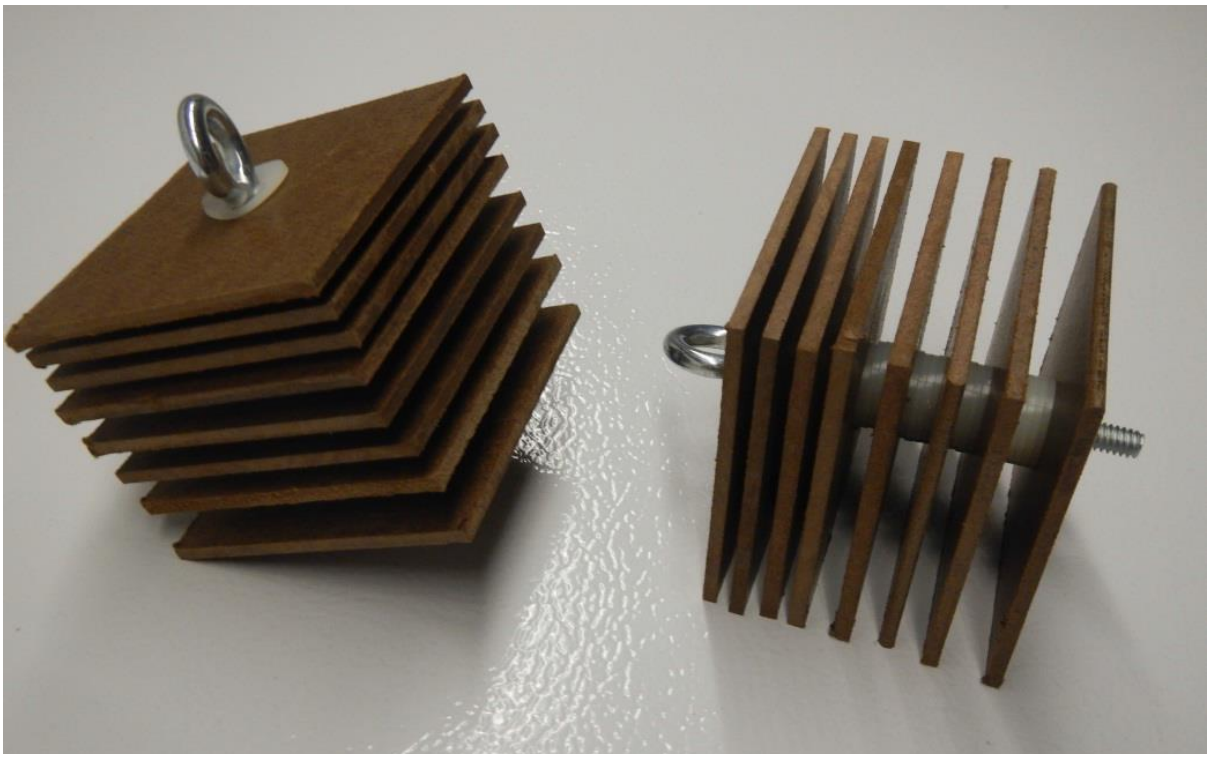


Figure 11 Modified Hester-Dendy multiple-plate artificial substrate sampler used for the collection of a quantitative macroinvertebrate sample. Image courtesy of the Minnesota Pollution Control Agency.



<u>Recommended equipment for multiplate sampler deployment:</u>
Current meter (for measuring flow at HD location)
Rope
Cinder Blocks
Brick (for weighting plate sampler)
Float
Float Label "Scientific Study, Do Not Disturb, Contact # XXXX" (large zip tie for attachment)
Hester-Dendy Plate samplers – 3 samplers zip tied together
Flagging tape
Visit forms
Clip Board
Pencil
Wire cutters/Scissors (for cutting rope and zip ties)
Depth Finder
PFDs
Anchor
Water Quality, multi-parameter probe
Secchi Tube
GPS
Camera

Recommended equipment for multiplate sampler deployment:
Current meter (for measuring flow at HD location)
Whirl Packs or large plastic bags or large jars
#30 Sieve or Sieve Bucket
Bucket
16-oz sample jars for HD samples
Large, pan
Putty knife
Soft brush
Kick Net with 500 µm bag
Squeeze bottles for water the ETOH
95% ETOH
Forceps
Visit form
Pencil
Waterproof label pens
Wire cutters/scissors (for cutting rope or zip ties)
Depth Finder
Internal and external macroinvertebrate sample labels

Boat Specifications: A boat of adequate size, stability and configuration to allow for safe deployment and retrieval of a Hester-Dendy sampling rig will be necessary to conduct the invertebrate sampling portion of the pilot study. There should be adequate space for two people to collect and process samples as well for the storage of multiple sets of sampling equipment. It is recommended that at minimum a 15 foot jon-boat be used, with a suitable outboard motor for large river navigation.

3.3.3 Safety

Large rivers are often congested by barge and recreational traffic. Extreme precautions should be taken when deploying and retrieving artificial substrate samplers, crossing the navigation channel, and navigating to and from sampling locations. Primary responsibility for safety rests with the crew leader. However, each member of the crew should be alert, aware of safety considerations, trained to recognize safety concerns, and trained in first aid and CPR.

Only a trained pilot should operate the boat. Large rivers have unpredictable and fast currents, and submerged hazards may be present nearly anywhere.

Table 21. Safety Considerations for Deployment and Retrieval of Invertebrate Samplers. Adapted from Angradi, et al. (2006)

Crew members should be able to swim, and should receive CPR, first-aid, and safe boating training.
Do not attempt to deploy or retrieve samplers during high winds or in an electrical storm in the rain.
All members of the invertebrate crew should wear USCG-approved PFDs whenever in the boat.
Good line of sight and communication should be maintained among crew members at all times. The outboard motor is loud and can drown out verbal communication. If necessary, hand signals should be used to communicate boat direction, power on/off, and other vital information.
All crew members should know the location of the nearest hospital.
Use caution around onboard gas tanks.
All crew members should know the on-board location of the cell phone, first aid kit, and truck keys.
Large (>10 kg) silver carp (Hypophthalmichthys molitrix) can jump >2 m out of the water. People have been seriously injured by

carp collisions. Silver carp are present in the lower reaches of the UMR. Be alert for jumping fish while running the river and during electrofishing.

3.3.4 Site Selection

Invertebrate samples are only collected at probabilistic sites. Fifteen probabilistic sites have been assigned within each CWA assessment reach. These sites are allocated along the river's centerline (defined by USACE navigation sailing line). Crews will sample along a bank perpendicular to the centerline "x point" identified in the sample draw and on the assigned bank. See Figure 5.

Proposed sampling sites should be reviewed in the office (via the pilot project viewer) to identify in advance any potential sampling issues. Initial determination of site sampling status within the 1 km MCS transect will be conducted by invertebrate and fish crew leads. At the time of sampler deployment, the invertebrate crew leads must be in communication with each other and with those responsible for sampling other parameters (chemistry and fish) to share any issues regarding the sampleability of probabilistic sites. If sites are determined not to be sampleable, they will be replaced with the next overdraw site on the list within the same assessment reach (in order of site ID).

The main-channel bank to be sampled has been randomly determined, however, invertebrate crews have the flexibility to sample the opposite bank if site conditions prevent a representative sample from being collected (e.g., due to dams, barge fleeting, inadequate flow, etc.). If neither bank can be sampled, the field crew has the discretion to replace the site entirely using an overdraw site. Thorough documentation of conditions resulting in bank or site replacement is required. See further discussion in Section 3.3.5 below.

Note that cases may occur where the randomly selected bank cannot be utilized for macroinvertebrate sampling (due to velocity and/or depth issues) but is suitable for fish assemblage sampling. In such cases, electrofishing will remain on the randomly selected bank even if macroinverbrate sampling is shifted to the opposite bank.

3.3.5 Site Verification

Site verification and sampling status is determined by the presence of five factors: 1) the site is representative of main channel border habitat, 2) the sampler can be safely deployed and retrieved, 3) the sampler, once deployed, will be clear of barge traffic and major recreational boat traffic, 4) the location of sampler deployment has adequate depth and flow velocity to maintain minimum depth (1 m between sampler and bottom sediment) and flow requirements (0.09 m/s) throughout the six week sampler deployment, and 5) the location of sampler deployment does not exceed the maximum flow threshold (2 m/s) at the time it is deployed. It is anticipated that these requirements will be present at some point in the 1 km reach and that sites can be verified in the office prior to sampling using GIS and staff experience. Questionable sites may need a field visit prior to sampling. Every reasonable attempt should be made to determine site sampling status prior to the commencement of any water chemistry sampling or artificial substrate sampler deployment to ensure all indicator crews monitor the same sites and maximize efficiencies.

The 1 km MCS transect is established along a terrestrial shoreline interface and samplers are deployed within the near-shore zone, in water that has adequate depth and velocity to meet sampling

requirements. Samplers are to be deployed along the predetermined random bank unless that bank is not representative of main channel border habitat or is unsafe to sample.

In addition to meeting the five requirements above, it is essential that placement of artificial substrate samplers should be reflective of the conditions of the 1 km sampling reach, and not representative of highly localized influences. When selecting sample deployment locations, the following situations should be avoided if possible:

- Samplers should not be placed at or immediately downstream of the outlet of tributary streams. Tributary streams could potentially contribute significant quantities of invertebrates not reflective of large river systems. If possible the samplers should be placed upstream of tributaries, at the furthest point downstream, or on the opposite bank if it is determined that the stream influence cannot be avoided on the randomly chosen bank.
- Samplers should not be placed at or immediately downstream of major point source discharges. If possible, the samplers should be located upstream of the outfall, at the furthest point downstream within the reach, or on the opposite bank if it is determined that the influence of the discharge cannot be avoided.

An assigned bank that is not representative of main channel border habitat conditions might also include a backwater lake, riparian wetland, constructed marina, or other condition which prohibits sampling within the terrestrial shoreline interface adjacent to the main channel border. See the fish assemblage section of this manual for details regarding site evaluation. Please consult these guidelines in cases where the fish sampling crew has not already evaluated a site. Impoundments and Lake Pepin are included within the study design and should be sampled unless other conditions preclude a bank from being sampled. Unsafe conditions could include barge fleeting areas, dams, and lock channels.

There are many possible combinations of safety hazards and obstacles that may be encountered in the field, not all of which are considered here. The operational goal of invertebrate crew leaders should be to deploy and retrieve Hester-Dendy samplers from main channel border habitats without subjecting the crew to unacceptable risk. Discretion and best professional judgment is granted to deal with sampling irregularities and ensure overall objectives are met. Explain any non-standard methods used or sampling decisions made in the field. Do the best you can and consult the authors of this section or other project partners for additional guidance in special cases that do not seem to fit the circumstances described herein.

3.3.6 Frequency/Index Period

Invertebrate artificial substrate samplers are deployed for a six week period during the summer and fall months, ideally in conditions representative of summer baseflow conditions (discharge between the 25th and 75th percentile, preferably near the daily long term median statistic). Samplers should be deployed in the month of July, and retrieved in either August or September, depending on when the samplers where deployed. The six week deployment can be extended by two weeks to accommodate conditions that may be unfavorable for sample retrieval (e.g. high flows, extreme heat, storms). If unusual hydrological or weather conditions are experienced during sample retrieval, they should be documented, and crew leaders will have discretion to discontinue sample retrieval.

3.3.7 Sample Collection

3.3.7.1 Hester-Dendy Deployment

1) Navigate to the sampling reach and begin looking for artificial substrate deployment locations that meet the requirements described above in the site verification section. This can often take time in order to get the right combination of flow conditions, depth, and a safe location outside of major boat traffic areas.
2) Upon locating a candidate sampler rig deployment site, the boat should be anchored to maintain that position.
3) Measure and record flow at 1m depth. Flow should be more than 0.09 m/s and is likely to be maintained over the next 6 weeks. Otherwise locate a different site to deploy the sampler.
4) Measure and record depth. Depth should be at least 2 m over the 6 week deployment period. Otherwise locate a different site to deploy the sampler.
5) Measure and record the locations (UTM NAD83).
6) Make field water quality measurements.
7) Determine the number of cinderblocks required to maintain the sampler rig in position given the flow at the site. Attach a length rope to the cinderblock(s) with sufficient length to maintain the float at the water's surface given the amount of flow at the site and possible increased in water depth over the deployment period.
8) Attach the samplers to the float so that they will be suspended 1 m below the float.
9) If necessary attach flagging to tree or other landmark on shore.
10) Complete invertebrate deployment visit form.

3.3.7.2 Hester-Dendy Retrieval

There are three options for processing the HD samples: field processing, lab processing without preservative, and lab processing with preservative. These are in order of preference and lab processing would only be used due to time constraints.

Field processing (when there is time to process the sample at the station):

1) Locate sampler and anchor boat just downstream of the rig without disturbing the sampler.
2) Measure and record water chemistry, flow and depth at the deployment site.
3) Carefully lift the float from the water and using a 500-µm mesh kick net retrieve the Hester-Dendy rig. Be careful not to include the brick suspended below the HD rig in the net.
4) Place the samplers in a bucket with river water being careful to remove organisms from the kick net bag.
5) Return to the shore and disassemble the HDs in the bucket. Scrape each plate and place the clean plates in a bag.
6) Pour the contents of the bucket through the #30 sieve or sieve bucket. Place organisms and detritus in 16-oz sample jar making sure that no debris or organisms remain in the pan or bucket. Preserve with 95% ETOH. Add an interior locality label and seal. Add an exterior locality label.
7) Complete invertebrate retrieval visit form.

Lab processing <u>without</u> preservative (when the sample cannot be processed at the station but can be processed within 48 hours of collection):

1) Locate sampler and anchor boat just downstream of the rig without disturbing the sampler.
2) Measure and record water chemistry, flow and depth at the deployment site.

3) Carefully lift the float from the water and using a 500-µm mesh kick net retrieve the Hester-Dendy rig. Be careful not to include the brick suspended below the HD rig in the net.
4) Place the samplers in a plastic bag or large plastic jar with river water being careful to remove organisms from the kick net bag.
5) Fill the bag with enough water to cover the samplers and add an interior label. Tightly seal the bag and place in a second bag and seal. Write the station number and date on the bag and place the bag on ice.
6) Complete invertebrate retrieval visit form.
7) Upon returning to the field operations center, place the sample bags into a tray and put in the refrigerator. Samplers need to be processed within 48 hours after collection.
8) When ready to process, remove the sample from the refrigerator and disassemble the samplers and place them in a large pan or bucket with water. Rinse and scrape the material from the samplers and the bag into the pan or bucket.
9) Pour the material from the pan or bucket through a #30 (500 µm) mesh sieve making sure that no debris or organisms remain in the pan or bucket. Rinse the material to a 0.5 L bottle using 95% ethanol in a squeeze bottle. If necessary add additional ethanol to the bottle. Place the interior locality label in the bottle and seal. Add an external label to the bottle.

Lab processing <u>with</u> preservative (when the sample cannot be processed at the station or processed within 48 hours of collection):

1) Locate sampler and anchor boat just downstream of the rig without disturbing the sampler.
2) Measure and record water chemistry, flow and depth at the deployment site.
3) Carefully lift the float from the water and using a 500-µm mesh kick net retrieve the Hester-Dendy rig. Be careful not to include the brick suspended below the HD rig in the net.
4) Place each sampler in a large plastic jar, or large wirl-pak with river water being careful to remove organisms from the kick net bag.
5) Fill each container with enough 95% ETOH to cover the samplers and add an interior label to each jar. Tightly seal each container and add exterior collection labels.
6) Complete invertebrate retrieval visit form
7) When ready to process, remove the samplers from the jars and place in a bucket or large pan. Sieve the remaining ETOH through a #30 (500 µm) mesh sieve. Add water to the bucket and disassemble the samplers. Rinse and scrape the material from the samplers and the bag into the pan or bucket.
8) Pour the material from the pan or bucket through a #30 (500 µm) mesh sieve making sure that no debris or organisms remain in the pan or bucket. Rinse the material to a 16-oz bottle using 95% ethanol in a squeeze bottle. If necessary add additional ethanol to the bottle. Place the interior locality label in the bottle and seal. Add an external label to the bottle.

3.3.8 Data Flow/Data Management

Because agencies differ in how they record, process, and store sampling information, this protocol is intentionally general in its requirements. It is presumed that all program participants will exercise care in collecting and recording data, follow good QA/QC protocols in proofing and managing data sets and data transfer, and will keep appropriate records to document what was done at various steps in the process.

It is acceptable to record field data on paper forms or computer data entry applications. Backup copies of forms and files should be made and stored separately from the originals in case of calamity. Each agency will be responsible for storing and sharing the data it collects, and all parties will agree upon a data processing and analysis plan, including calculation of index scores and interpretation of the results.

3.3.9 Quality Assurance/Quality Control

Crew members should be properly trained in techniques for evaluating riverine habitat, handling invertebrate samples, and operating the boat. Proper deployment of sampling gear is critical to ensuring that a representative sample is collected. In order to determine the repeatability of the sampling technique, two sites per reach will have duplicate Hester-Dendy samplers deployed. See Appendix D. Sampler should be in similar flow and depth conditions, within close proximity to each other. Duplicate samplers should not be immediately up/downstream of each other. They should side by side, or up/downstream and offset so they are not receiving the same flow, and drift. Duplicate sights should be determined randomly, prior to sample deployment.

3.4 Submersed Aquatic Vegetation

3.4.1 Indicators

Submersed aquatic vegetation sampling includes collection of information regarding species abundance and richness, depth of occurrence, and substrate type, as well as select water quality parameters, water velocity and transparency. Vegetation data is collected in order to support the calculation of a Submersed Macrophyte Index (SMI) scores. See Table 7 for a summary of SMI calculation.

3.4.2 Equipment

Standard equipment used in LTRM and in development of the SMI will be employed. This includes an extendable, two-headed rake made by welding two 14-tine steel garden-rake heads back-to-back, a transparency (Secchi) tube, and a velocity meter. Data will be recorded on a field laptop computer, using an MS Access database.

3.4.3 Safety

Sites within 100 m of navigation dams will be replaced with alternate sites. Standard safety protocols used in LTRM data collection will be followed, including use of personal floatation, and adherence to hazardous weather guidelines. All personnel are certified by state and organizational authority to operate watercraft.

3.4.4 Site Selection

Probabilistic Site Assignment: Vegetation is sampled using a spatially intensive probabilistic design of 100 sites per reach, which has been developed independently of the probabilistic design used for water chemistry, fish, and macroinvertebrate sampling. Sites should be reviewed in the office prior to field work to identify any safety or access concerns, and replacements utilized as needed. Sites are initially assigned on the river's centerline and then further refined in the field.

Figure 12: Submersed aquatic vegetation sampling site layout

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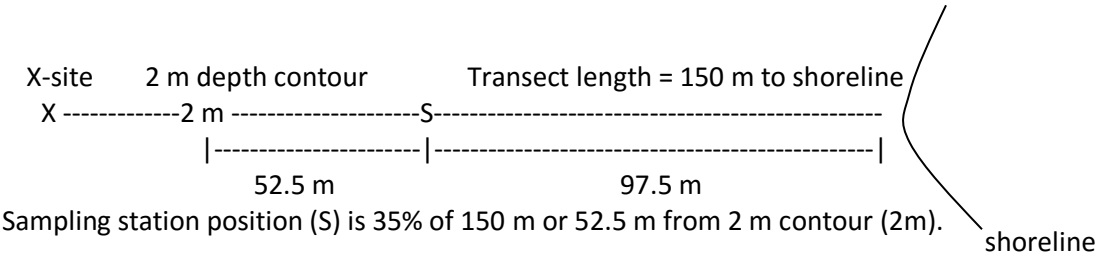
Site Layout (Example – Station position=35%, Transect Length = 150 m)



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Positioning the Location of the Sampling Station Relative to the X-site: To position the sampling station relative to the X-site:

a) As described in Figure 12, the sampling station is located in >0.2 m of water between the X-site and sample bank at a random distance from the 2.0 m contour. This situation assumes the sampling frame accurately depicts the secondary channels and the channel depths rise regularly from mid-channel to shore. Where these assumptions do not hold, the following rules should be followed to position the sampling station.

b) If the X-site is < 2.0 m deep AND the channel still qualifies as a secondary-channel, then the X-site becomes the sampling station. The rationale is that the design has successfully placed the station in the zone where vegetation may be present.

c) If the channel no longer qualifies as a secondary channel, the site is "non-target" and is replaced.

d) If the calculated sampling station is >2.0 m deep (e.g., the Station Position puts the presumed Station in a deeper channel), then the sampling station is moved to the closest point that is <2.0 m deep. The crew should first search along the line between the presumed sampling station and sample bank. If the boat ends up perpendicular to shore with the front end touching shore and the back of the boat in greater than 2 meters of water, the crew should search along the transect from the presumed sampling station through the X-site and toward the opposite shore. If <2.0 m not found, search in channel area up to 100 m up and downstream of X-site. If <2.0 m depth not found, site is "non-target" because the channel does not have zone where vegetation is expected to be present. The unusual situation would only apply if the entire channel was >2.0 m deep and had such extremely steep bank angles as to preclude sampling. In most cases, if the 2.0 m contour is adjacent to the sample bank, then sample with the boat as close to shore as practical.

e) If the sampling station is <0.2 m deep, then move back towards X-site until reach >0.2 meters and sample there.

f) If there are hazards (or areas <0.2 m deep) between the X-site and sampling station, but the station is expected to be between 0.2 and 2.0 m deep, than navigate around hazards, return to the transect line, and proceed to sampling station. If station turns out to be <0.2 m or >2.0 m deep, refer to above rules.

3.4.5 Frequency/Index Period

Vegetation is sampled once per year in a late July/early August time frame. Index period is the same as that for water chemistry, fish, and macroinvertebrates.

3.4.6 Sample Collection

Vegetation Sampling: Specifics of vegetation sampling are as follows:

a) The sampling area is a 2 m ring around a standard 16 ft jon boat. Sampling follows Long Term Resource Monitoring procedures (Yin and Langrehr 2000) as detailed below.

b) Search sampling area for any visual species and record on data form.

c) For non-rooted floating (NRF), rooted floating (RF), and emergent (EM) species, assign a cover of 1-5 based on the visual percent of the 2m ring occupied by each lifeform.

d) For non-rooted floating (NRF), rooted floating (RF), and emergent (EM) species, assign a cover of 1-5 based on the visual percent of the 2 m ring occupied by each individual species.

e) Plants are collected from six sub-sampling areas - four off the corners of the boat and two off either side of mid-boat.

f) At each sub-sampling area:
   (1) Visually search for any species within the sub-sampling area and record
   (2) Extend rake out 1.5 m and lower to the sediment then drag along bottom back to the boat.
   (3) Read and record the depth on the rake.
   (4) Twist the rake 180 degrees and lift out of water.
   (5) Record a 1 on the data form for any RF or EM species on the rake.
   (6) Gently remove floating or emergent species while retaining submersed species and flip any submersed species touching the metal of the rake back onto the tines.
   (7) Sweep rake through the water once to compact submersed vegetation
   (8) Assign submersed vegetation a 1 to 5 based on amount on the rake and record on the data form.
   (9) Identify individual species and assign a 1 to 5 based on amount on the rake and record on the data form.

   Repeat steps 1-9 above for each sub-sampling area.

Water Transparency Measurement: Determine water clarity with transparency tube. A transparency tube, also known as turbidity or Secchi tube, is used to measure water clarity or provide estimates of turbidity and suspended particulate matter concentrations in water. The transparency tube is a clear, graduated, plastic tube (4.5 cm diameter * 120 or 60 cm long) that has a Secchi disc image and drain tube at the bottom. A representative water sample is collected and poured into or drained from the tube slowly until the Secchi image disappears (filling) or appears (draining). The height of water above the disk at this point is recorded. The process is repeated and the average of the two readings is calculated. Make sure that the sample is sufficiently mixed and that the reading is taken as quickly as possible in order to prevent sediment from accumulating on the Secchi disk image.

Water Velocity Measurement: Determine water velocity per standard Long Term Resource Monitoring protocols (Soballe and Fischer 2004) and recorded on data forms. Use a compass to determine and record the direction of the current.

3.4.7 Data Flow/Data Management

Data from all field teams will be combined at the end of the field season and each member will have a complete data set for their records. A copy of the data will also be distributed to MPCA.

3.4.8 Quality Assurance/Quality Control

After data from the field teams are combined a QAQC of the data will be conducted using SAS. All flagged data will be reviewed and corrected if necessary.

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5. APPENDICES

Appendix A: Water Chemistry Methods Comparison Tables

Table 1: Nutrient-Related Parameters

Analyte Name (per plan)	Agency	Agency Analyte Name	Lab Method	Method Detection Limit	Reporting Limit	Units	Filtration (Y/N and size/type)*	Preservation	Hold time
NO3+NO2	MN (MDH)	Nitrate + Nitrite Nitrogen, Total	EPA 353.2	0.005	0.050	mg/L	N	H2SO4	28 days
	WI (SLH)	NITROGEN NO3+NO2 DISS (AS N)	EPA 353.2	0.019	0.061	mg/L	Y, Millipore, cat# HAWG047S6, 0.45 µm pore size, 47mm diameter	H2SO4 cool to 6C	28 days
	MCES	Nitrate-Nitrogen (NO3)	SMEWW 4500-NO3 H-2000	0.010	0.050	mg/L	N	Chloroform, cooled to 4C	28 days
	MCES	Nitrite-Nitrogen (NO2)	SMEWW 4500-NO3 H-2000	0.003	0.030	mg/L	N	Chloroform, cooled to 4C	28 days
Total Nitrogen (TN)	MN (MDH)	Calculated as (TKN + NO2-NO3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	WI (SLH)	NITROGEN TOTAL	USGS Rept 03-4174	0.030	0.096	mg/L	N	H2SO4 cool to 6C	28 days
	MCES	Calculated as (TKN + NO2-NO3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NHx	MN (MDH)	Ammonia Nitrogen, Total	ASTM D3731-87	0.003	0.050	mg/L	N	H2SO4	28 days
	WI (SLH)	NITROGEN NH3-N DISS	EPA 350.1	0.015	0.048	mg/L	Y, Millipore, cat# HAWG047S6, 0.45 µm pore size, 47mm diameter	H2SO4 cool to 6C	28 days
	MCES	Ammonia Nitrogen (NHx)	EPA 350.1	0.005	0.060	mg/L	N	H2SO4, cooled to 4C	28 days
Total Phosphorus (TP)	MN (MDH)	Phosphorus, total	SM 4500PI (drinking water) EPA 365.1 (nonpotable water)	0.0024206 (drinking water) 0.000857 (nonpotable water)	0.003	mg/L	N	H2SO4	28 days
	WI (SLH)	PHOSPHORUS TOTAL	EPA 365.1	0.005	0.016	mg/L	N	H2SO4 cool to 6C	28 days
	MCES	Total Phosphorus (TP)	EPA 365.4	0.030	0.010	mg/L	N	H2SO4, cooled to 4C	28 days
DP	MN (MDH)	Phosphorus, dissolved	SM 4500-PI(F)	0.000725 (DW) 0.000857 (NP)	0.003	mg/L	Y	H2SO4	28 days
	WI (SLH)	PHOSPHORUS TOTAL DISS	EPA 365.1	0.005	0.016	mg/L	Y, Sterivex-HV Filter, 0.45 µm, PVDF	H2SO4 cool to 6C	28 days
	MCES	Dissolved Phosphorus (DP)	EPA 365.4	0..03	0..01	mg/L	Y, 0.45u Membrane Filter	H2SO4, cooled to 4C	28 days

Chlorophyll a	MN (MDH)	Chlorophyll-A	SM 10200 H	NA	1.000	ug/L	Y glass fiber, 1.0 um 47 mm filter (field filtered)	< 4 deg C	28 days
	WI (SLH)	CHLOROPHYLL A, FLUORESCENCE (WELSCHMAYER 1994)	EPA 445	1.300	4.350	ug/L	Y, Millipore Type SM, 47 mm, 5.0 um SMWP, membrane filter	cool to 6C and store in dark	30 days
	MCES	Chlorophyll-a (Trichromatic, Uncorrected)	ASTM D3731-87	NA	1.000	ug/L	Glass Fiber Filter	Preserved with 1 mL $MgCO_3$. Stored in the dark and cooled to -20^{o}C	30 days
	MCES	Chlorophyll-a (Pheophytin-corrected)	ASTM D3731-87	NA	1.000	ug/L	Glass Fiber Filter	Preserved with 1 mL MgCO3. Stored in the dark and cooled to -20oC	30 days

Table 2: Miscellaneous Parameters

Analyte (per plan)	Agency	Agency Analyte Name	Lab Method	Method Detection Limit	Reporting Limit	Units	Filtration (Y/N and size/type)*	Preservation	Hold time
BOD	MN (MDH)	Biochemical Oxygen Demand	Hach 10360	NA	0.500	mg/L	N	< 4 deg C	2 days
	WI (SLH)	BOD, 5 Day	SM 5210B	NA	2.000	mg/L	N	Cooled to ≤ 6°C but not frozen	48 hours
	MCES	Total Biochemical Oxygen Demand (5-Day) (TBOD5)	SMEWW 5210 B-01	NA	0.200	mg/L	N	Cooled to ≤ 6°C but not frozen	48 hours
Chloride	MN (MDH)	Chloride	EPA 300.1 (Total, not dissolved)	0.027	0.500	mg/L	N	Cooled to 4°C.	28 days
	WI (SLH)	CHLORIDE DISS	EPA 300.0	0.031	0.100	mg/L	Y Fisherbrand, cat# 09-719-008, 0.45 µm (micron) pore size, 33mm diameter	Cooled to ≤ 6°C	28 days
	MCES	Chloride (Cl)	SMEWW 4500-Cl-E	0.500	2.000	mg/L	0.45u Membrane Filter	Cooled to 4°C.	28 days
Sulfate	MN (MDH)	Sulfate	EPA 300.1 (Total, not dissolved)	0.202	0.500	mg/L	N	< 4 deg C	28 days
	WI (SLH)	SULFATE DISS	EPA 300.0	0.160	0.500	mg/L	Y Fisherbrand, cat# 09-719-008, 0.45 µm (micron) pore size, 33mm diameter	Cooled to ≤ 6°C	28 days
	MCES	Sulfate (SO4)	EPA 300.0	0.200	0.500	mg/L	0.45u Membrane Filter	Cooled to 4°C.	28 days
TSS	MN (MDH)	(TSS) Solids, Suspended	SM 2540 D-1997	NA	1.000	mg/L	Pall Gelman A/E 1 micron 47 mm glass fiber filter	< 4 deg C	7 days
	WI (SLH)	RESIDUE TOTAL NFLT (TOTAL SUSPENDED SOLIDS)	SM2540D	2.000	2.000	mg/L	Glass micro-fiber filters, 5.5 cm, without organic binder, Whatman Type 934-AH. (1.5 µm size).	Cooled to ≤ 6°C	7 days
	MCES	Total Suspended Solids (TSS)	SMEWW 2540 E	1.000	3.000	mg/L	Glass Fiber Filter (1.5 µm size)	Cooled to 4°C.	7 days
TOC	MN (MDH)	Total Organic Carbon	SM 5310 C-2000	0.258	1.000	mg/L	N	H2SO4	28 days
	WI (SLH)	CARBON TOTAL ORGANIC	Standard Methods 5310C	0.300	1.000	ppm C	N	H2SO4	28 days
	MCES	Total Organic Carbon (TOC)	SMEWW 5310 A/C	0.050	1.000	mg/L	None	Preserved with H_2SO_4 to a pH <2 and cooled to 4°C	28 days

Hardness (Ca & Mg)	MN (MDH)	Hardness (as CaCO3)	SM 2340 B-1997	NA	10.000	mg/L	N	HNO3	180 days
	WI (SLH)	HARDNESS TOTAL CACO3	EPA 200.7 + calc	0.560	1.760	mg/L	N	HNO3	6 months
	MCES	Hardness (HRD)	SMEWW 2340 C (Titration Method)	NA	5.000	mg/L	None	Cooled to 4°C.	28 days
Alkalinity	MN (MDH)	Alkalinity (Total as CACO3)	SM 2320 B 21st ED	NA	10.000	mg/L	N	< 4 deg C	14 days
	WI (SLH)	ALKALINITY TOTAL CACO3	SM2320B	2.550	2.550	mg/L	N	Cooled to ≤ 6°C	14 days
	MCES	Alkalinity, Bicarbonate (ALK)	EPA 310.2	3.000	10.000	mg/L	None	Cooled to 4°C.	14 days

Table 3: Metals

Analyte (per plan)	Agency	Agency Analyte Name	Lab Method	Method Detection Limit	Reporting Limit	Units	Filtration (Y/N and size/type)*	Preservation	Hold time
Aluminum	MN (MDH)	Aluminum	EPA 200.8	0.048	5 (Drinking Water), 20 (Nonpotable Water)	ug/L	N	HNO3	180 days
	WI (SLH)	Aluminum, Total Recoverable	EPA 200.7	10.000	30.000	ug/L	N	HNO3	6 months
	MCES	Aluminum (Al)	EPA 200.8	2.000	5.000	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Calcium	MN (MDH)	Calcium	EPA 200.7	0.067	2.00	mg/L	N	HNO3	180 days
	WI (SLH)	Calcium, Total Recoverable	EPA 200.7	0.060	0.200	mg/L	N	HNO3	6 months
	MCES	Calcium (Ca)	EPA 200.8	0.020	1.000	mg/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Cadmium	MN (MDH)	Cadmium	EPA 200.8	0.016	0.10	ug/L	N	HNO3	180 days
	WI (SLH)	Cadmium, Total Recoverable	EPA 1638	0.011	0.037	ug/L	N	HNO3 after receipt	6 months
	MCES	Cadmium (Cd)	EPA 200.8	0.200	0.500	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Chromium	MN (MDH)	Chromium	EPA 200.8	0.019	10 (Drinking Water) 1 (Nonpotable Water)	ug/L	N	HNO3	180 days
	WI (SLH)	Chromium, Total Recoverable	EPA 1638	0.057	0.190	ug/L	N	HNO3 after receipt	6 months
	MCES	Chromium (Cr)	EPA 200.8	0.080	0.160	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Copper	MN (MDH)	Copper	EPA 200.8	0.038	10.0	ug/L	N	HNO3	180 days
	WI (SLH)	Copper, Total Recoverable	EPA 1638	0.030	0.100	ug/L	N	HNO3 after receipt	6 months
	MCES	Copper (Cu)	EPA 200.8	0.300	0.600	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days

Iron	MN (MDH)	Iron	EPA 200.7	6.421	0.020	mg/l	N	HNO3	180 days
	WI (SLH)	Iron, Total Recoverable	EPA 200.7	0.100	0.010	mg/L	N	HNO3	6 months
	MCES	Iron (Fe)	EPA 200.8	0.020	1.000	mg/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Lead	MN (MDH)	Lead	EPA 200.8	0.007	1.00	ug/L	N	HNO3	180 days
	WI (SLH)	Lead, Total Recoverable	EPA 1638	0.004	0.014	ug/L	N	HNO3 after receipt	6 months
	MCES	Lead (Pb)	EPA 200.8	0.100	0.500	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Magnesium	MN (MDH)	Magnesium	EPA 200.7	0.054	2.00	mg/L	N	HNO3	180 days
	WI (SLH)	Magnesium, Total Recoverable	EPA 200.7	0.100	0.300	mg/L	N	HNO3	6 months
	MCES	Magnesium (Mg)	EPA 200.8	0.006	1.000	mg/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Potassium	MN (MDH)	Potassium	EPA 200.7	0.167	0.50	mg/L	N	HNO3	180 days
	WI (SLH)	Potassium, Total Recoverable	EPA 200.7	0.100	0.300	mg/L	N	HNO3	6 months
	MCES	Potassium (K)	EPA 200.8	0.030	1.000	mg/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Sodium	MN (MDH)	Sodium	EPA 200.7	0.017	0.50	mg/L	N	HNO3	180 days
	WI (SLH)	Sodium, Total Recoverable	EPA 200.7	0.100	0.300	mg/L	N	HNO3	6 months
	MCES	Sodium (Na)	EPA 200.8	0.020	1.000	mg/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Zinc	MN (MDH)	Zinc	EPA 200.8	0.230	20 (Drinking Water) 10 (Nonpotable Water)	ug/L	N	HNO3	180 days
	WI (SLH)	Zinc, Total Recoverable	EPA 1638	0.130	0.430	ug/L	N	HNO3 after receipt	6 months
	MCES	Zinc (Zn)	EPA 200.8	0.800	1.600	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days

Table 4: Other Parameters

Analyte (per plan)	Agency	Agency Analyte Name	Lab Method	Method Detection Limit	Reporting Limit	Units	Filtration (Y/N and size/type)*	Preservation	Hold time
Arsenic	MN (MDH)	Arsenic	EPA 200.8	0.038	1.00	ug/L	N	HNO3	180 days
	WI (SLH)	Arsenic, Total Recoverable	EPA 1638	0.028	0.094	ug/L	N	HNO3 after receipt	6 months
	MCES	Arsenic (As)	EPA 200.8	1.000	1.000	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days
Mercury	MN (MDH)	Mercury	EPA 1631E	0.150	10.000	ng/l	N	HCL or bromine monochloride	90 days
	WI (SLH)	Mercury, Total Recoverable	EPA 1631	0.140	0.450	ng/l	N	HCl	90 days
	MCES	Mercury (Hg)	EPA 245.7, Revision 2.0	0.200	0.500	ng/l	None	Preserved with Optima HCL to a pH of <2 and cooled to 4° C.	28 days
Selenium	MN (MDH)	Selenium	EPA 200.8	0.140	5 (Drinking Water) 1 (Nonpotable Water)	ug/L		HNO3	
	WI (SLH)	Selenium, Total Recoverable	EPA 1638	0.300	1.100	ug/L	N	HNO3 after receipt	6 months
	MCES	Selenium (Se)	EPA 200.8	0.800	1.000	ug/L	None	Preserved with HNO_3 to a pH <2 and cooled to 4°C.	180 days

Table 5: Bacteria

Analyte (per plan)	Agency	Agency Analyte Name	Lab Method	Method Detection Limit	Reporting Limit	Units	Filtration (Y/N and size/type)*	Preservation	Hold time
Bacteria	MN (MDH)	*Escherichia coli*	SM 9223 B	NA	1	MPN/100 mL	None	Cooled to 4°C.	24 hours
	WI (SLH)	*Escherichia coli, MPN*	COLILERT QUANTITRAY MPN	NA	1	MPN/100 mL	None	Cooled to 4°C.	6 hours
	MCES	*Escherichia coli*	IDEXX 2000	NA	1	MPN/100 mL	None	Cooled to 4°C.	6 hours

Appendix B: Great River Fish Index (GRFIn) Metrics. Adapted from Pearson, et al. (2011)

Fish Assemblage Metric	Metric Class
Proportion of invertivore individuals	Trophic
Proportion of non-indigenous individuals	Composition
Proportion of individuals with DELTS	Fish Health
Proportion of detritivore Individuals	Trophic
Proportion of native individuals	Composition
Total deep-bodied sucker biomass (kg)	Biomass
Total number of fish species (exclusive)	Richness
Number of darter species	Richness
Catch per unit effort of native species	Relative Abundance
Number of minnow species	Richness

Appendix C: Wisconsin Large River Macroinvertebrate Index Metrics. Adapted from Weigel and Dimick (2011)

Metric Category	Metric *(Abbreviation)*
Taxon richness and composition	Number of insect taxa *(Insect-T)*
	% insect individuals *(Insect-%I)*
	Number of EPT taxa (EPT-T)
	% individuals in the top 3 taxa *(Dom_3-%I)*
Tolerance and composition	Mean pollution tolerance value *(MPTV)*
	% intolerant EPT individuals with maximum tolerance = 2 *($IntolEPT_2$-%I)*
	% tolerant chironomid individuals with minimum tolerance value = 8 *($TolChir_8$-%I)*
Ecology	Number of unique combinations of the 4 ecology trait niches (rheophily, thermal preference, habitat, and trophic status) *(EcoFTN)*
	% gathering insects *(Gath-%I)*
	% scraper insects *(Scr-%I)*

Appendix D: Ordered List of Probabilistic Sites per Reach for Replicate Sampling Purposes

Below is listed the resampling order for all probabilistic sites in the pilot reaches. It is anticipated that the first two listed sites per reach (highlighted) will be utilized for replicate sampling. However, in the event that a sample site is dropped from among the first two, then the third would be included as reach sample and so on through the order as necessary.

Replicate Order (within reach)	Reach 0	Reach 1	Reach 2	Reach 3
1	UMR15-0361	UMR15-0301	UMR15-0061	UMR15-0241
2	UMR15-0362	UMR15-0303	UMR15-0062	UMR15-0242
3	UMR15-0363	UMR15-0304	UMR15-0063	UMR15-0243
4	UMR15-0364	UMR15-0305	UMR15-0064	UMR15-0244
5	UMR15-0365	UMR15-0306	UMR15-0065	UMR15-0245
6	UMR15-0366	UMR15-0307	UMR15-0066	UMR15-0246
7	UMR15-0367	UMR15-0308	UMR15-0067	UMR15-0247
8	UMR15-0368	UMR15-0309	UMR15-0068	UMR15-0248
9	UMR15-0369	UMR15-0310	UMR15-0069	UMR15-0249
10	UMR15-0370	UMR15-0311	UMR15-0070	UMR15-0250
11	UMR15-0371	UMR15-0312	UMR15-0071	UMR15-0251
12	UMR15-0372	UMR15-0313	UMR15-0072	UMR15-0252
13	UMR15-0373	UMR15-0314	UMR15-0073	UMR15-0253
14	UMR15-0374	UMR15-0315	UMR15-0074	UMR15-0254
15	UMR15-0375	UMR15-0316	UMR15-0075	UMR15-0255

Appendix E: Fish Tissue Contaminant Methods

In May 2016, the pilot monitoring group determined that fish tissue monitoring would be dropped from the pilot project. As methods had already been developed at that point, the group elected to keep them in the field operations manual as an Appendix and available as a reference for future monitoring efforts.

Fish Tissue Contaminants

Indicators

Fish contaminant concentrations are a co-indicator for the fishable-swimmable goals of the Clean Water Act. For the 2016 pilot monitoring, mercury and PCBs are the contaminants of concern that will be used for 305(b)-type assessments. This information is intended to complement sport fish advisory efforts and is not intended to replace what each state has historically done or would plan to do in the future. Reaches with unusually high levels may indicate that there are still some active sources that can be investigated later.

Mercury and PCB tissue concentrations will be measured in fillets from target species groups and size classes. These species and sizes of fish will be analyzed individually by each state's sport fish advisory labs. Fish of the same species and approximate age have had a similar length of exposure to contaminants in the river. This allows reaches to be compared to each other. The following fish are sought from each reach:

- Ten 15-17" Black Basses: Smallmouth or Largemouth bass (mixed is ok)
- Ten 18-21" Common Carp

The ranges listed above are preferred size ranges. The primary purpose of the size classes is to obtain fish of roughly the same age. However, if fish specifically in the size range cannot be collected, it is better to have some fish for a reach than none at all. As such, a variation in size of up to 1" from the above guidelines is acceptable is needed.

Table E1: Summary of fish tissue sampling

Sampling Type	Index Period	Number of Sites per Reach	Fish Species Group	Size Class	Number of Fish Retained per Reach	Total Samples per Reach	Sample Type	Analytes
Baseline: Reach-Based Probabilistic	July to September	15	Top Predator (Black Basses)	15-17" (4-6 yrs)	10	20	Skin-on fillet	Mercury PCBs
			Bottom-Feeder (Common Carp)	18-21" (4-6 yrs)	10			

Equipment

Specimens for tissue analysis are collected as part of fish assemblage monitoring via electroshocking. In addition to the materials listed under fish assemblage monitoring (Section 3.2.2), the following

equipment is, in general, needed for fish tissue sample collection: cooler, ice, aluminum foil, and labeling materials. As each state will submit its fish samples per its typical process, the specific equipment used by a crew may vary somewhat among states.

Safety

Tissue samples are collected as part of fish assemblage monitoring via electroshocking. As such, please see safety procedures under fish assemblage monitoring (Section 3.2.3).

Site Selection

Fish tissue samples are collected from probabilistic sites, as part of fish assemblage monitoring (see Section 3.2.4).

Frequency/Index Period

Fish tissue samples are collected one time during the July to September index period, as part of fish assemblage monitoring (see Section 3.2.5).

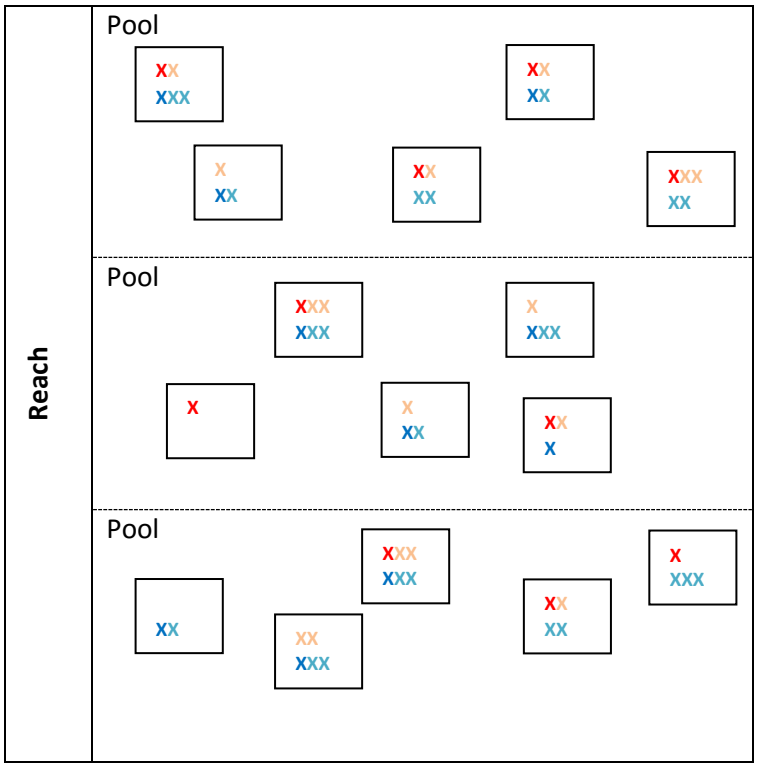
Sample Collection

Number of Sites/Number of Samples: Fish for tissue analysis will be collected as part of the random site fish electroshocking work. Under the probabilistic design, 15 sites will be sampled for a community assessment in each reach. Across these fifteen sites, 10 fish per species group per reach will be retained for tissue analysis (i.e., 20 total fish per reach as there are two species groups).

Discretion will be given to samplers to spread these samples to the greatest extent possible across the reach and across pools in the reach. Samplers should try to spread samples across the whole reach while still getting a full sample of 10 specimens in each reach.

The availability of each species in these size classes is likely not equally distributed across the random sites. To address this issue, samplers should keep all fish meeting the size and species requirements as they start electroshocking in each reach until reaching the goal of 10 of each species. If the goal is not met in the whole reach, some limited targeted fishing is an elective of the sampling crew to complete the sample target. If more than one fish of each species is collected from any one site, substitute fish meeting the objectives as available in subsequent stations. In short, keep the first 10 fish of each species substituting in others as they are collected. Figure 10 provides a stylized example of this approach.

*Figure E1: Example of fish tissue sampling/retention, where each box represents a sample site and **X**=retained top predator species fish, X = not retained and X= retained bottom feeder species fish, X = not retained*



In instances where there is known and significant variation in tissue concentrations between pools within a reach (e.g., between Pools 1 and 2, and between Pools 5 and 5A), a greater number of samples may be collected if desired by the sampling entity. It is especially important to get 3-5 Common Carp in Pool 2 and in Pool 5A if possible as previous data show levels lower than in surrounding pools.

Fish Species and Sizes Preferred: Fish will be sampled from a top predator group and a bottom-feeder group. In general, fish will be of a size that is representative of the "middle" of the age distribution for a particular species. The top predator species group to be utilized for purposes of the pilot is the Black Basses (Smallmouth and Largemouth Bass), in the size range of 15-17" (4-6 years old). It is possible that different predator species groups will need to be utilized in areas of the river beyond the pilot project, as Black Basses will not likely be available river-wide. For the bottom-feeder fish group, Common Carp will be sampled river-wide, with a size target of 18-21" (4-6 years old). As noted previously, some variation from the size class (within 1") is acceptable if needed to meet sample number target.

Sample Preparation/Type**:** For the purposes of the pilot project, it is assumed that whole fish will be submitted for analysis, with skin-on fillets then prepared at the laboratory. Field crews should follow their own state's processes for specific sample preparation procedures. Generally, each specimen should be wrapped in aluminum foil shiny side out. They should be labeled with the date, station id,

reach number, species and length. Specimens should be placed on ice and kept frozen until delivery to the laboratory for processing. Skin-on fillets from individual fish will then be prepared.

Data Flow/Data Management

Samples will be processed through states' own laboratories and data will flow through the typical processes utilized by the state for other fish tissue results.

Quality Assurance/Quality Control

Crews will follow QA/QC protocols established by their states for fish tissue sampling. Replicate sampling is not expected to be part of fish tissue monitoring.