Post-Return Flow Root River Monitoring and Quality Assurance Project Plan

Prepared for:

City of Waukesha
115 Delafield Street
Waukesha, WI 53187

Prepared by:

Jacobs

and

SMITHGROUP

November 2025 (updated from July 2023)

Contents

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Summary of Changes

Changes from the prior July 2023 document include:

- Replace the Wisconsin State Laboratory of Hygiene with the Racine Health Department for e-coli laboratory testing.
- Replace fisheries biologist with the U.S. Geological Survey (USGS) for overseeing electrofishing and reporting.
- Update the macroinvertebrate sampling standard operating procedure to include a 250 count sample size to meet Wisconsin Department of Natural Resources guidance.
- Relocation of Root River flow measurement from Site C to Site D.
- Remove redundant flow rate measurement by USGS of return flow at the discharge site.
- Remove Root River water chemistry monitoring for ammonia and nitrate-nitrite.
- Update distribution list and organizational chart.
- Add clarification that statistical comparisons may be used to assess river velocity and stage impacts instead of a hydraulic model because a hydraulic model of sufficient resolution may not be publicly available.
- Miscellaneous minor editing to improve readability and to discuss the City's diversion in past tense.

Acronyms and Abbreviations

CFR	*Code of Federal Regulations*
COC	chain of custody
DO	dissolved oxygen
DMR	discharge monitoring report
EDD	Electronic Data Deliverable
EPA	U.S. Environmental Protection Agency
FTL	Field Team Leader
IBI	Index of Biotic Integrity
LOD	Limit of detection
LOQ	Limit of Quantification
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
SOP	standard operating procedure
SWIMS	Surface Water Integrated Monitoring System
TKN	total Kjeldahl nitrogen
TP	total phosphorus
TSS	total suspended solids
USGS	United States Geological Survey
WDNR	Wisconsin Department of Natural Resources
WPDES	Wisconsin Pollutant Discharge Elimination System

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1. Background and Objectives

The City of Waukesha, Wisconsin, transitioned to a Lake Michigan water supply in November 2023 to replace their groundwater water supply. The City maintains its existing outfall for fully treated wastewater from the Clean Water Plant (CWP) to the Fox River (Mississippi River drainage basin), but also utilizes a new outfall to the Root River (Lake Michigan drainage basin) to return the diverted water to the source watershed. (Figure 1-1).

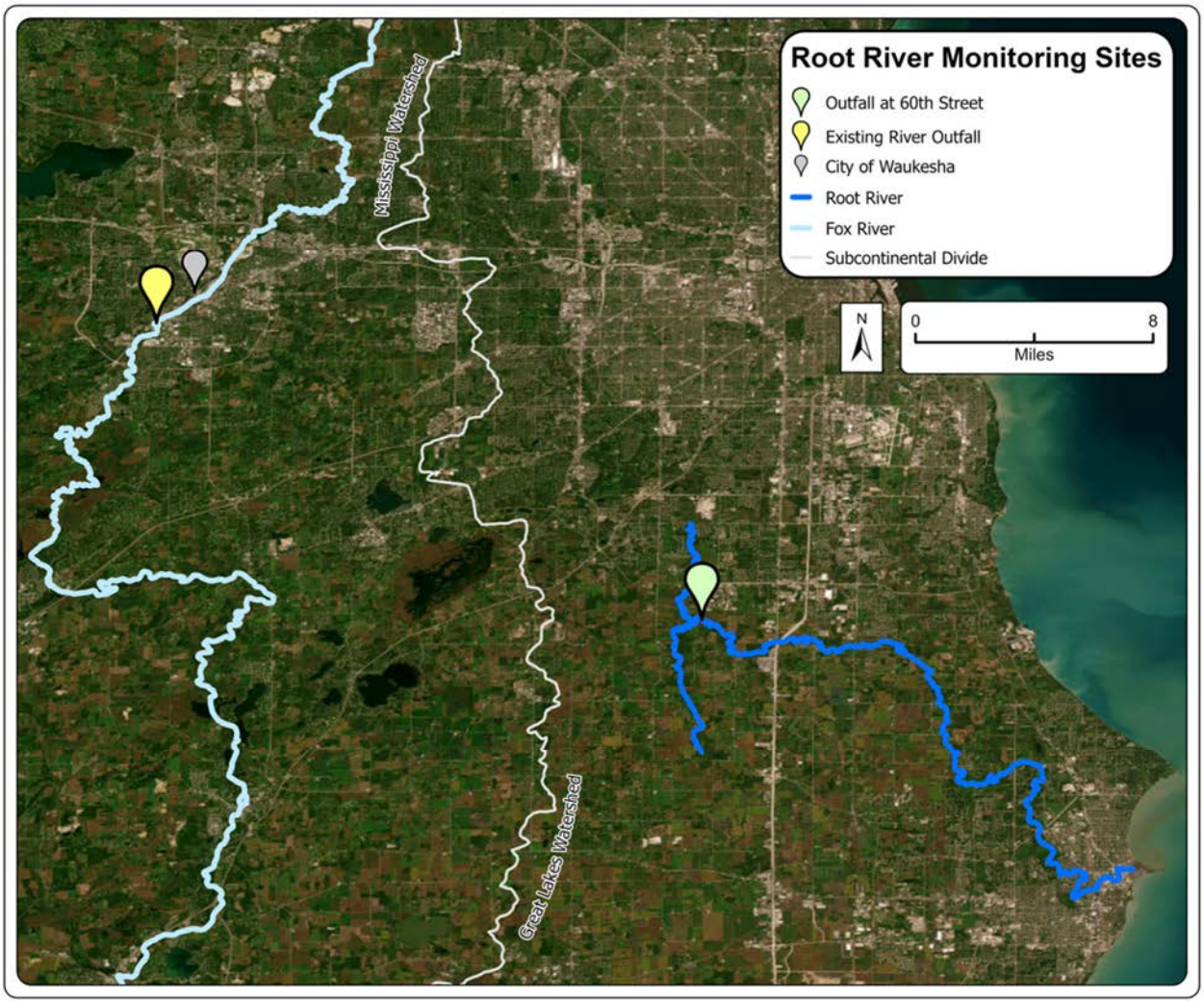

Figure 1-1. Post-Return Flow Site Map

The Root River flows through parts of Milwaukee and Racine counties and into Lake Michigan at Racine, Wisconsin. The river has natural bottom substrate and vegetated riverbanks, and its watershed has a mixture of land uses between its headwaters and Lake Michigan. The headwaters of the Root River are moderately urbanized, the middle has significant agricultural uses, and the lower parts of the watershed near Lake Michigan are heavily urbanized. Return flow enters the Root River in the middle reaches, containing primarily agriculture and lower-density development, downstream of the confluence with the Root River Canal.

This Post-Return Flow Root River Monitoring and Quality Assurance Project Plan (QAPP) summarizes the monitoring plan to collect data and the quality assurance plan for the monitoring activities and data validation. Executing the QAPP will support assessing the impact of return flow on the Root River and allow the City to adaptively manage the return flow and future monitoring efforts. This QAPP is aligned with the U.S. Environmental Protection Agency's (EPA's) Quality Systems Project Level (Level Three) (EPA 2006) to support environmental projects result in high-quality and scientifically based products (EPA 2002).

The QAPP includes flow monitoring, water quality sampling, and ecological assessment methodologies to support data collection at monitoring sites along the Root River. Data collection is anticipated to continue for at least 10 years as required by Condition 11 of the Wisconsin Department of Natural Resources (WDNR) approval of a Lake Michigan water supply with return flow (diversion approval; June 29, 2021).

To meet Condition 11 of the diversion approval, the QAPP will be used to:

> *…monitor the mainstem of the Root River to determine changes that may have resulted from return flow (such as volumes, water temperatures, water quality, and periodicity of discharge) in order to adapt future return flow to minimize potential adverse impacts or maximum potential benefits to water dependent resources of Lake Michigan.*

In preparation for the diversion, the City completed nearly 7 years of voluntary monitoring from 2017 to 2023 to support data collection of water quality, river flow, and biological conditions in the Root River prior to the commencement of return flow. The Pre-Return Flow Root River Data Collection Plan (Jacobs, 2017), sampling activities, and lessons learned were considered in developing this QAPP.

2. Project Organization

The post-return flow monitoring activities involve the coordination of eight organizations including the City of Waukesha (owner), WDNR (state regulating agency), SmithGroup, Inc. (SmithGroup; managing consultant), University of Wisconsin-Parkside (UWP; contracted implementation and monitoring support), Jacobs Engineering Group (Jacobs; data analysis support), the United States Geological Survey (USGS; contracted implementation and monitoring support), the Wisconsin State Laboratory of Hygiene (contracted laboratory support), and the City of Racine Health Department (contracted laboratory support).

Figure 2-1 shows the organization chart for the start of the project, followed by brief descriptions of the roles and responsibilities of each organization.

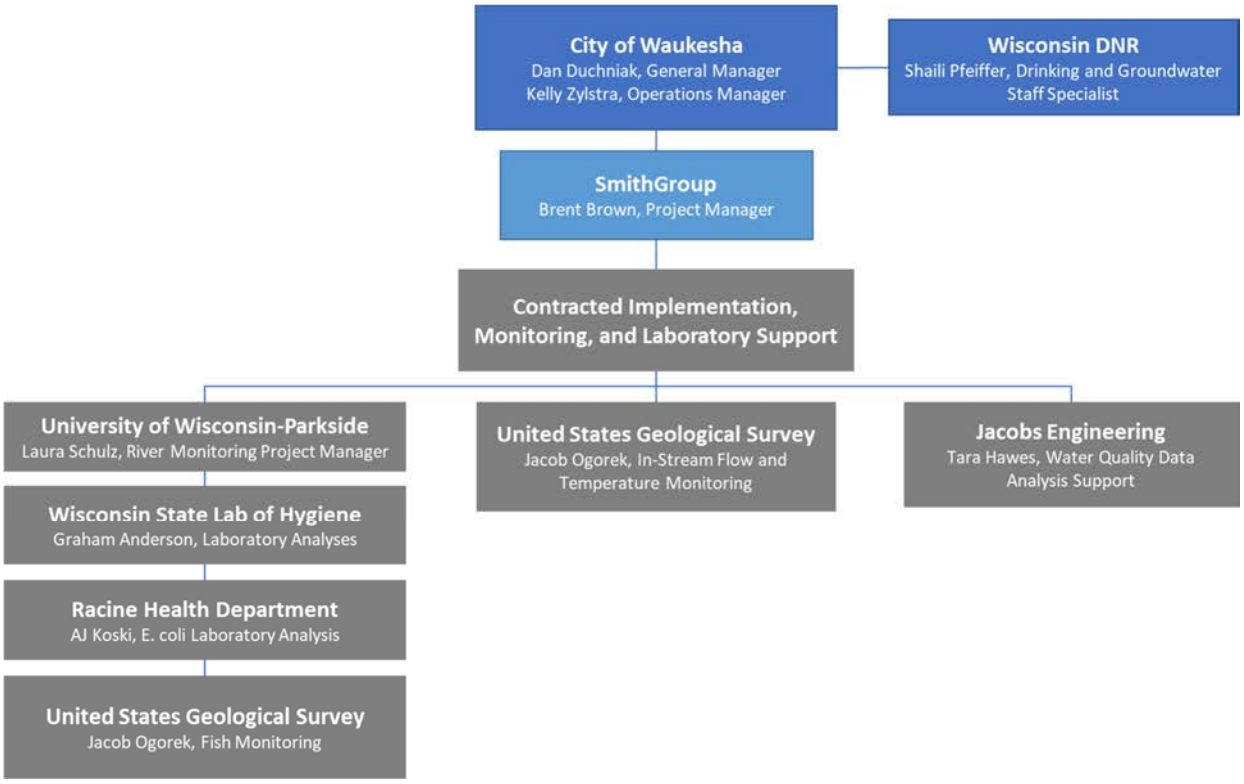

Figure 2-1. Post-Return Flow Root River Monitoring Organization Chart

City of Waukesha, Owner. Oversees project activities and provides strategic direction to the consultant for project execution. Reviews and approves the QAPP. Coordinates with the State Regulatory Agency for required approvals and compliance demonstration.

WDNR, State Regulatory Agency. Reviews the QAPP and coordinates with the Owner and/or Managing Consultant to define project requirements and approve project activities as needed.

SmithGroup, Managing Consultant. Manages project activities and implementation contractors and provides technical and regulatory support for project implementation and data interpretation, analyses, and reporting.

Jacobs Engineering Group, Water Quality Data Analysis Support. Supports Managing Consultant in water quality data management, analysis, and reporting.

University of Wisconsin-Parkside, Contracted Implementation Support. Executes event-based water quality sampling and biological and habitat monitoring activities and subcontracts.

United States Geological Survey, Contracted Monitoring Support. Executes continuous monitoring activities (e.g., flow) and provides technical leadership for fish monitoring.

Wisconsin State Laboratory of Hygiene, Contracted Laboratory. Conducts water quality analyses and submits results according to EPA-approved methods. Identifies and reports any deviations from the standard operating procedures (SOPs).

Racine Health Department, Contracted Laboratory. Conducts E. coli laboratory analysis.

Contracted support may be updated annually, and service providers may change throughout the monitoring program. Changes in contracted entities (e.g., managing consultant, contracted support) will be updated within this QAPP. The updated QAPP will be provided to involved organizations and stored with the project files.

Significant changes in and the removal of sampling locations, significant changes in and the removal of monitoring parameters, as well as reductions in sampling frequency to less than monthly will be updated within this QAPP, provided to involved organizations, and submitted to the WDNR prior to implementing such changes.

2.1 Project Description and Schedule

The post-return flow Root River monitoring activities include routine water quality sampling, continuous water quality and flow monitoring, and biological sampling including fish surveys, macroinvertebrate sampling, and habitat assessments. This may change as the QAPP is implemented and data is analyzed. The following subsections describe the sampling locations, parameters, schedule, and personnel and required resources.

2.1.1 Location

2.1.1.1 In-Stream Root River Monitoring Sites

Five monitoring sites are maintained along the Root River and Root River Canal (Figures 2-2, 2-3, 2-4; Table 2-1). These sites were selected to coordinate with previous and ongoing data collection efforts by the USGS, WDNR, and Southeastern Wisconsin Regional Planning Commission and to collect data that will assist in assessing the potential impact of return flow on the Root River (SEWRPC, 2007). These sites are located upstream and downstream of the return flow outfall, where the outfall is located about 700 feet downstream of Site C. Four of the five sites were monitored as part of the voluntary Pre-Return Flow Root River Data Collection Plan.

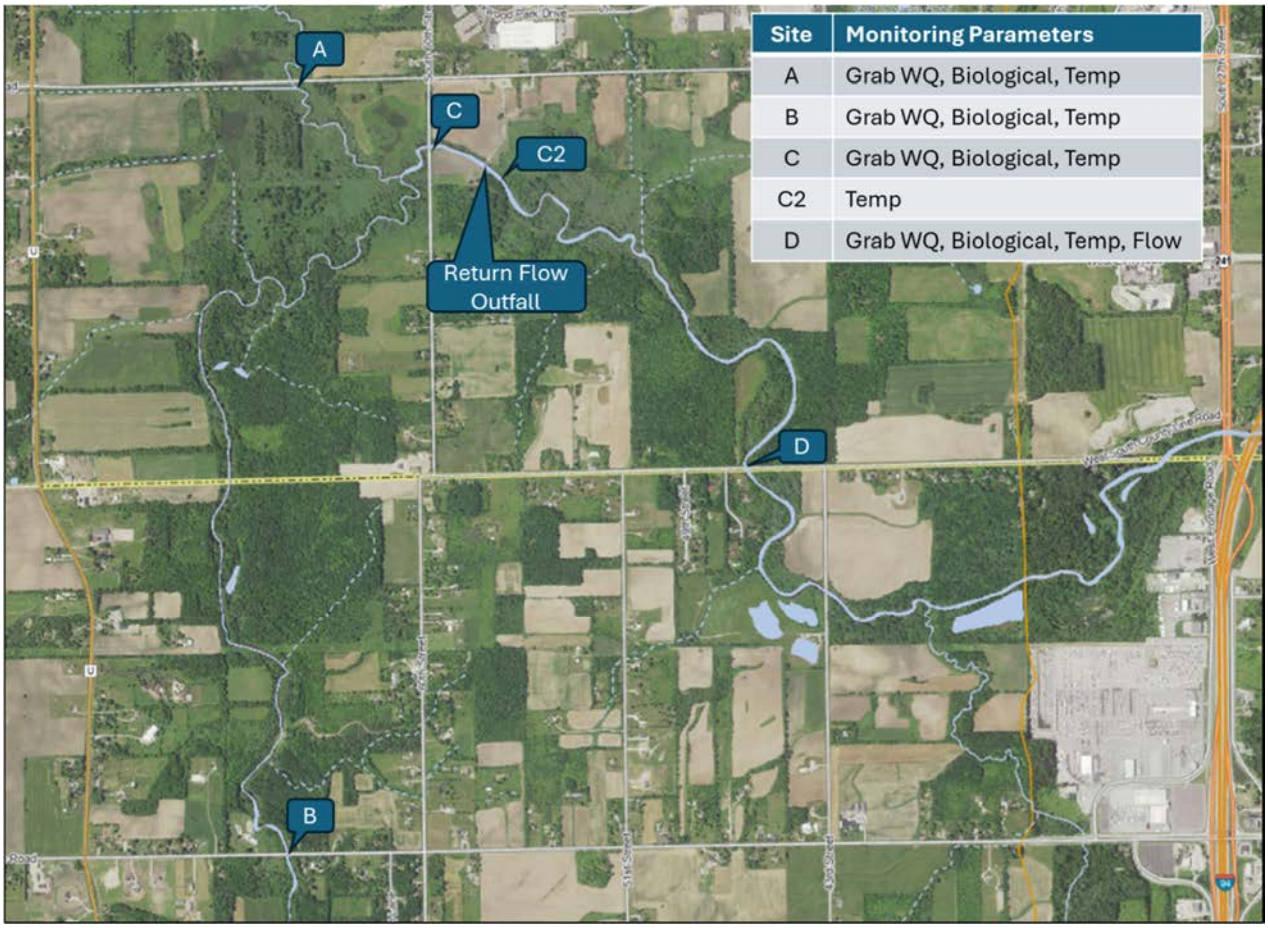

Figure 2-2. Post-Return Flow Root River Monitoring Sites

Figure 2-3 shows the location of the return flow outfall and the surrounding monitoring sites, Site C and Site C2. Downstream of Site C and approximately 300 feet downstream of the return flow outfall, Site C2 provides continuous temperature monitoring at the downstream extent of the City's property boundary. This site was not previously monitored as part of the voluntary Pre-Return Flow Root River Data Collection Plan.

Figure 2-3. Site C, Site C2, and Outfall Location

Table 2-1. Root River In-Stream Data Collection Site Locations

Site ID	Location Description	Comment
A	Root River at Oakwood Rd 42°51'28.60"N 87°59'51.13"W	This data collection site is located on the Root River upstream of the confluence of the Root River and the Root River Canal. Parking is accessible in a slightly steep grassy area on the side of the road. Data collection may be possible from the roadway or through an accessible grassy bank on the river. The river has muddy, soft substrate.
B	Root River Canal at 7 Mile Rd 42°49'48.09"N 87°59'55.92"W	This data collection site has fair accessibility sloping down to the riverbank, but may be more difficult during slippery conditions. Parking is available slightly farther away from the bridge on the south side of the road in the grass. A private residence is north of the bridge, so sampling is recommended on the south side to minimize residential disturbance. The river has rocky, gravel substrate.

Table 2-1. Root River In-Stream Data Collection Site Locations

Site ID	Location Description	Comment
C	Root River downstream of 60th St Bridge; at proposed return flow outfall 42°51'19.83"N 87°59'26.72"W	This data collection site captures data following the confluence of the Root River and the Root River Canal. The City owns the properties on both sides of the river at this location. Parking is accessible in a gravel area on the side of the road. A USGS monitoring shed is located on the east side of the bridge, on the south side of the river, where flow data is collected. There is good access via a path to the USGS gage station on the riverbank. The river has muddy, soft substrate.
C2	Root River downstream of discharge location; at the edge of private property line 42°51'15.48"N 87°59'14.49"W	This data collection site is located approximately 1,000 feet downstream of Site C and 300 feet downstream of the return flow outfall location. It is located at the downstream extent of the property boundary. It may be located further downstream if an access agreement can be obtained from the downstream landowner to allow this site to represent a well-mixed condition between the river and return flow. Historical data has not been collected at this site.
D	Root River at County Line Rd; downstream of proposed return flow outfall 42°50'37.46"N 87°58'32.62"W	This data collection location is at the first downstream road crossing of the return flow discharge location representing fully mixed conditions, with just a small increase in the watershed area. Historical data has been collected at this location. Parking is slightly more difficult but is available on the south side of the road. Access to the riverbank is possible, but difficult, through a steep and rocky section on the east side of the bridge. This may be more difficult to access in the winter months and during slippery conditions. The river has muddy, soft substrate.

Site A - Root River at Oakwood Road

Site B - Root River Canal at 7 Mile Road

Site C - Root River on 60th Street bridge at return flow outfall

Site C2 - Root River downstream of return flow outfall

Site D - Root River at County Line Road

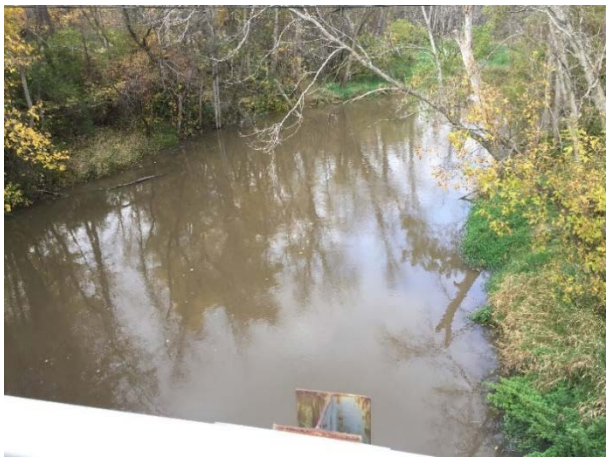

Figure 2-4. Photos of In-Stream Root River Data Collection Site Locations

2.1.1.2 CWP Monitoring Site

In addition to the in-stream Root River monitoring sites, monitoring activities are completed at the CWP and the return flow outfall to meet the City's Wisconsin Pollutant Discharge Elimination System (WPDES) permit requirements. Monitoring sites for WPDES permit required effluent sampling (permit effective date of 11/1/2025) are described in Section 3.1 of the permit (Appendix A).

2.1.2 Sampling Parameters

2.1.2.1 In-Stream Root River Monitoring Parameters

Tables 2-2(a) and 2-2(b) summarize the in-stream Root River event-based sampling and continuous data collection, respectively, including parameters, the method of sample or data collection and parameter measurement, and the frequency and locations of data collection.

Table 2-2(a). Summary of Event-Based Data Collection and Monitoring Activities

Parameter	Frequency	Collection Method	Bottle Material, Size Preservative (if applicable)	Measurement Method	Site Collection Locations[a]
Water Quality Parameters					
Total Nitrogen	Twice per Month (May-Oct), Monthly (Nov-Apr)	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 353.2	A, B, C, D
Chlorophyll A	Twice per Month (May-Oct), Monthly (Nov-Apr)	Grab Sample	Plastic, 1,000 ml None	EPA 445	A, B, C, D
Total Phosphorus (TP)	Twice per Month (May-Oct), Monthly (Nov-Apr)	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 365.1	A, B, C, D
Orthophosphate	Twice per Month (May-Oct), Monthly (Nov-Apr)	Grab Sample	Plastic, 60 ml None	SM4500-PE	A, B, C, D
Total Suspended Solids (TSS)	Twice per Month (May-Oct), Monthly (Nov-Apr)	Grab Sample	Plastic, 1,000 ml None	SM2540D	A, B, C, D
E. coli	Monthly	Grab Sample	Glass, 150 ml None	EPA 9223 B	C, D
Chloride	Monthly	Grab Sample	Plastic, 250 ml None	SM4500-CL^- E	C, D
Dissolved Oxygen (DO)	Twice per Month (May-Oct), Monthly (Nov-Apr)	In Situ Sample	-	Probe	A, B, C, D
Specific Conductance	Twice per Month (May-Oct), Monthly (Nov-Apr)	In Situ Sample	-	Probe	A, B, C, D
pH	Twice per Month (May-Oct), Monthly (Nov-Apr)	In Situ Sample	-	Probe	A, B, C, D

Table 2-2(a). Summary of Event-Based Data Collection and Monitoring Activities

Parameter	Frequency	Collection Method	Bottle Material, Size Preservative (if applicable)	Measurement Method	Site Collection Locations[a]
Temperature	Twice per Month (May-Oct), Monthly (Nov-Apr)	In Situ Sample	-	probe	A, B, C, D
Turbidity	Twice per Month (May-Oct), Monthly (Nov-Apr)	In Situ Sample	-	Probe	A, B, C, D
Biological Parameters					
Macroinvertebrates	Once September-October; November[b]	(WDNR 2000)	-	Refer to SOP (Appendix G)	A, B, C, D[c]
Habitat Assessment	Concurrent with Summer Fish Survey[b]	(WDNR 2002)	-	Refer to SOP (Appendix H)	A, B, C D
Fish	Once June-August; November[b]	(WDNR 2001)	-	Refer to SOP (Appendix I)	A, B, C, D

Notes:

[a] Duplicate samples are collected during water quality sampling events. Duplicate field samples are collected side-by-side to check the homogeneity of the sample matrix, precision of field techniques, and precision of laboratory analysis. Duplicate samples will be collected at the same time as the initial sample. One field duplicate sample will be collected and analyzed at one sampling site for all grab sample parameters during every sampling event.

[b] A macroinvertebrate and fish sampling event in November will be completed during the initial year(s) of the post-return flow monitoring for consistency with the voluntary Pre-Return Flow Root River Data Collection Plan. Biological sampling (macroinvertebrates, fish, and habitat assessments) is anticipated for the first few years of the post-return flow Root River monitoring activities. Sampling may be discontinued if these results indicate that beyond typical environmental variation, there are no measurable impacts resulting from return flow implementation.

[c] Duplicate samples are collected during macroinvertebrate sampling events. Duplicate field samples are collected at the same time and from the same reach as the primary sample. Results are used to evaluate homogeneity of the sample matrix, precision of field techniques, and precision of laboratory analysis. One field duplicate sample will be collected and analyzed at one sampling site during every sampling event.

Table 2-2(b). Summary of In-Stream Root River Continuous Monitoring Activities

Water Quality Parameters	Frequency[a]	Method	Site Collection Locations
Flow	15 Minute	Automated USGS Flow Gage	D[b]
Dissolved Oxygen	Hourly	Continuous Data Sonde	C
Specific Conductance	Hourly	Continuous Data Sonde	C
pH	Hourly	Continuous Data Sonde	C
Temperature	Hourly	Continuous Data Sonde	A, B, C, C2[c], D
Turbidity	Hourly	Continuous Data Sonde	C

Notes:

[a] Frequency of continuous monitoring may be revised at the discretion of USGS protocols and equipment recommendations and availability. The QAPP will be updated accordingly and stored with the project files.

[b] Moved from Site C in January 2025.

[c] The need to continue continuous temperature monitoring at Site C2 will be evaluated annually.

2.1.2.2 CWP and Return Flow Outfall Site Monitoring Parameters

In addition to the water quality parameters, flow monitoring, and biological parameters sampled in the Root River, water quality and flow monitoring are completed at the CWP.

Sampling includes a combination of grab samples and continuous monitoring using data sondes and flow meters. Water quality parameters and flow monitoring completed as part of WPDES permit required effluent sampling are described in Section 3.1 of the permit (Appendix A).

2.1.3 Personnel, Special Training Requirements, or Certifications

2.1.3.1 Personnel and Special Training Requirements

Contracted staff experienced in the collection of water quality samples, fish samples, and habitat assessments in wadable-stream settings will perform the respective sampling activity. Contracted staff will have experience conducting sampling activities under similar conditions to the Root River. Macroinvertebrate laboratory sample preservation and taxonomy will be led by a certified taxonomist for freshwater aquatic insects. Prior to biological sampling of fish and macroinvertebrates, a *Scientific Collectors Permit* will be obtained from WDNR by the sampling leads, in addition to other protocol approvals required within the permit (Appendix B).

All contracted staff will review the QAPP and the relevant SOPs and safety training prior to the onset of sampling or field activities. For continuity with the voluntary pre-return flow data collection activities, the following staff will lead the corresponding sampling activities for at least one calendar year if feasible.

- Dr. John Skalbeck, University of Wisconsin-Parkside, Principal in Charge
- Laura Schulz, University of Wisconsin-Parkside, Field Sampling Project Manager and Water Chemistry Lead
- Dr. Jessica Orlofske, University of Wisconsin-Parkside, Macroinvertebrate and Habitat Assessment Lead
- Ryan Ennis, USGS, Fish Sampling Lead under contract to UWP
- Jake Ogorek, USGS, In-stream Root River Continuous Flow and Temperature Monitoring Lead
- Graham Anderson, Wisconsin State Laboratory of Hygiene, Inorganic Chemistry Supervisor
- AJ Koski, Racine Health Department, Laboratory Coordinator

Personnel from UWP will lead the in-stream Root River sampling events including water chemistry and biological sampling (macroinvertebrates, fish, and habitat assessments). Personnel from USGS will lead the in-stream continuous flow and temperature monitoring and provide technical support to UWP for the electrofishing.

2.1.3.2 Laboratory Certification

Laboratory water quality analyses will be conducted by a WDNR-certified laboratory using EPA-approved analysis methods (40 *Code of Federal Regulations* [CFR] Part 136). For continuity with the voluntary pre-return flow Root River data collection activities, the Wisconsin State Laboratory of Hygiene (Laboratory Certification ID 113133790) will be used for at least one calendar year. If the contracted support team plans to use a different laboratory, it will be coordinated with the City and will include a transition plan. The Racine Public Health Department (Laboratory No 053; License No. 115057) will be used for E. coli testing to reduce the potential for exceeding hold times.

Macroinvertebrate laboratory sample preservation and taxonomy will be led by a taxonomist for freshwater aquatic insects with certification from the Society for Freshwater Science, or similar organization. Preservation and taxonomy may be completed by a certified team member or certified laboratory. For the potential use as an additional resource for taxonomic identification and enumeration support, the following laboratories are state-certified and may be used:

- University of Wisconsin-Steven's Point Aquatic Entomology Laboratory
  Contact: Dr. Jeff Dimick jeff.dimick@uwsp.edu

- University of Wisconsin-Superior's Lake Superior Research Institute
  Contact: Dr. Kurt Schmude kschmude@uwsuper.edu

2.2 Documentation and Records

2.2.1 Field and Laboratory Records

The following types of field and laboratory records are expected as part of this sampling program:

- Completed field data sheets
- Scanned copies of field notebooks
- Computer-based spreadsheet files containing transcribed field data
- Database files containing field data
- Chain-of-custody (COC) forms
- Completed WDNR field forms
- Laboratory data reports
- Laboratory Electronic Data Deliverables (EDD)

Responsible parties for developing, managing, and storing documentation and records will be agreed upon by the City, SmithGroup, Jacobs, and the contracted support teams as needed for monitoring activities.

For all event-based sampling, a field data sheet is completed to document site conditions, equipment calibration procedures and standards, and weather conditions. A blank field data sheet is included in Appendix C.

Water quality sampling data will be entered into an electronic data form (e.g., EQuIS Collect) and stored in an online database accessible to members of the project team. This data will also be recorded in a field notebook. The laboratory conducting water quality analyses will provide EDDs that are compatible with the database. COC forms and laboratory reports will be stored electronically.

Field forms developed by WDNR will be used for habitat assessments (Wadable Stream Quantitative Habitat Evaluation, Form 3600-228) and macroinvertebrate sampling (Macroinvertebrate Field Data Report, Form 3200-081). Scanned field forms, field notes, field data sheets, Microsoft Excel files, laboratory data reports, and COC forms (as applicable) will be stored electronically.

Fish sampling data will be recorded in a field notebook and transcribed to a Microsoft Excel (or similar) electronic file following the sampling event. Scanned field notes, field data sheets, electronic files, laboratory data reports, and COC forms (as applicable) will be stored electronically.

2.2.2 Project Records

Project records including contracts, meeting agendas, meeting minutes, and project planning documentation and correspondence will be stored electronically.

2.2.3 Final Report

Table 2-3 summarizes reports anticipated from the contracted support teams to be produced as part of the post-return flow Root River monitoring activities. Reporting to meet diversion approval requirements is discussed in Section 6.

Table 2-3. Post-Return Flow Root River Monitoring Final Reports

Report	Frequency
Water Quality Report	Annually
Macroinvertebrate Sampling and Habitat Assessment Report	Annually
Fish Sampling Report	Annually

Note: Reporting from USGS is not anticipated because all data will be hosted through the USGS website; the same as other similar USGS gage location data collection and reporting.

2.2.4 Project File Final Disposition

Project records will be stored electronically.

3. Measurement/Data Acquisition

Data collected as part of the post-return flow Root River monitoring activities will be used to determine basic water quality and ecological conditions of the river to meet the requirements of the diversion approval.

The following subsections describe the methodology of flow monitoring, water quality sampling, macroinvertebrate sampling, fish sampling, and habitat assessments. Standard operating procedures and industry guidance are referenced and used as the basis for the described procedures. If service providers or standard operating procedures change throughout the project, the relevant SOPs will be updated accordingly.

3.1 Data Quality Objectives and Criteria

Quantitative and qualitative data quality objectives are defined for the project to support the intended use of the data. Data collection through field sampling and continuous monitoring for water quality and ecological parameters will be used to meet the monitoring requirements of the diversion approval. Representativeness, comparability, completeness, precision, accuracy, sensitivity, and selectivity project data considerations have been addressed through the field sampling and continuous monitoring process design and sampling method requirements including equipment selection; calibration procedures; sample collection, handling, and analysis methodology requirements; and quality assurance (QA)/quality control (QC) procedures.

For event-based water quality in-stream Root River sampling, data quality objectives, including expected ranges for parameters, have been defined and are shown in Table 3-1.

Table 3-1. Water Quality Data Quality Objectives for In-Stream Root River Monitoring

Water Quality Parameter (Laboratory)	Method	LOD and LOQ	Expected Range	Maximum Hold Time
Total Nitrogen	EPA 353.2	0.058 mg/L; 0.192 mg/L	0.01 to 15 mg/L	28 days
Chlorophyll A	EPA 445	0.26 µg/L; 0.86 µg/L	0.01 to 360 µg/L	24 days
Total Phosphorus (TP)	EPA 365.1	0.009 mg/L; 0.030 mg/l	0.01 to 5 mg/L	28 days
Orthophosphate	SM4500-PE	0.004 mg/L; 0.013 mg/L	0.01 to 3 mg/L	3.5 weeks
Total Suspended Solids (TSS)	SM2540D	Varies	1 to 175 mg/L	7 days
E. coli	EPA 9223 B	1 organism/100 mL	n/a[a]	24 hours
Chloride	SM4500-CL- E	1.36 mg/L; 4.55 mg/L	n/a[a]	28 days
Water Quality Parameter (In Situ Measurement)	**Method**	**Range and Resolution**	**Expected Range**	**Maximum Hold Time**
Dissolved Oxygen (DO)	Multi-parameter Probe	0 to 50 mg/L; 0.01 mg/L	0.01 to 15 mg/L	n/a
Specific Conductance	Multi-parameter Probe	0 to 200 mS/cm; 0.1 mS/cm	0.1 to 10 mS/cm	n/a
pH	Multi-parameter Probe	0 to 14; 0.01 pH units	6 to 10	n/a
Temperature	Multi-parameter probe	-5 to 50 deg C; 0.1 deg C	-5 to 35 deg Celsius	n/a
Turbidity	Multi-parameter Probe	0 to 4000 NTU; 0.1 NTU	0.1 to 120 NTU	n/a

Notes:
EPA = Environmental Protection Agency, mg/L = milligrams per liter, µg/L = micrograms per liter,
mS/cm = micro Siemens per centimeter, SM = Standard Method, LOD = Limit of Detection; LOQ = Limit of Quantification,
n/a = not applicable
[a] Parameter not measured as part of pre-return flow monitoring activities. Expected range not defined due to lack of precedent.

3.2 Flow Monitoring

3.2.1 Sample Process Design

Flow monitoring data will be collected in-stream at Site D and at the CWP within the discharge pipeline near the return flow pump station.

Flow monitoring data at Site D has been collected by USGS as an official gaging location using an automated flow gage. Monitoring at Site C was started as part of the voluntary pre-return flow Root River monitoring activities (Table 2-2[b]) but relocated to Site D in January 2025 after the return flow was observed to interfere with water level gaging equipment used to calculate in-stream flow at Site C.

Flow monitoring at the CWP of the return flow volume commenced with the water diversion and is regulated by the WPDES permit.

Analysis of flow monitoring data is discussed in Section 6 (Data Analysis).

3.2.2 Sampling Method Requirements

3.2.2.1 In-Stream Site D

In-stream flow monitoring by USGS at Site D began in January 2025 after relocation from Site C, which had been operating since 2017 using an automated flow gage. In situ flow measurements are recorded by a sensor placed directly at the measurement point with communication cables and a power system to run the data logger. With this site as an official USGS gaging location, the monitoring follows the principles and methods of stream gaging and flow computation practices outlined in *Discharge Measurements at Gaging Stations: U.S. Geological Survey Techniques and Methods* (Turnipseed et al. 2010) and *Measurement and Computation of Streamflow* (Rantz 1982).

Field visits for maintenance of the automated flow gage include the following activities:

- Calibration of the field meter(s)
- Inspection of the site for signs of physical disruption
- Inspection and cleaning of sensor(s) for fouling, corrosion, or damage
- Inspection and cleaning of sensor(s) deployment tube
- Battery (or power) check
- Time check
- Routine sensor cleaning and servicing
- Calibration check (and recalibration, if necessary)
- Data download

3.2.2.2 Return Flow at CWP

The CWP has a mag-meter flow meter located at the CWP along the return flow pipeline immediately downstream of the return flow pump station. This mag-meter is used in controlling the return flow pump station flow to meet the diversion approval requirements for returning the previous year's average daily water demand. Flow monitoring in the discharge pipeline at the return flow pump station is defined by the CWP WPDES Permit for return flow discharge to the Root River (Appendix A Sections 3.2.2 and 6.2.2). Reporting flow is completed on monthly discharge monitoring reports (DMR) (Appendix A Section 6.1).

3.2.3 Sampling Handling and Custody Requirements

Samples are not collected as part of the flow monitoring activities; therefore, there are no defined sample handling and custody requirements.

3.2.4 Analytical Requirements

In-stream flow monitoring data collected by USGS using automated gages will be analyzed according to data processing and qualitative and quantitative evaluation procedures outlined in *Discharge Measurements at Gaging Stations: U.S. Geological Survey Techniques and Methods* (Turnipseed et al. 2010).

Return flow monitoring at the CWP is reported for WPDES permit compliance. This same meter data will be used for reporting under this QAPP.

Section 6 (Data Analysis) discusses analysis of flow monitoring data.

3.2.5 Data Management

Flow monitoring data collected using automated gages and continuous data sondes will be stored in accordance with USGS policy. In-stream flow monitoring data collected by USGS will be published and managed by USGS on the existing *Current Water Data* website (https://waterdata.usgs.gov/wi/nwis/rt). Flow monitoring sites are located near Sites A and B, at Site C between 2017 and 2024, and at Site D starting in January 2025.

3.3 Water Quality Monitoring

3.3.1 Sample Process Design

The post-return flow Root River monitoring activities will include regular water quality sampling and continuous water quality and flow monitoring. Monitoring will occur at five locations along the Root River. Section 2.1 contains the sampling site map, site descriptions, and photos. Tables 2-2(a) and 2-2(b) show water sampling parameters, schedule, and methods.

Grab samples will be collected according to protocols described in the *National Field Manual for the Collection of Water Quality Data* (USGS 2014). Water quality data collection using continuous water quality monitoring will follow the USGS guidelines discussed in *Guidelines and Standard Procedures for Continuous Water-Quality Monitors: Station Operation, Record Computation, and Data Reporting* (USGS 2006).

Water quality monitoring at the CWP and at the return flow outfall site reaeration building is defined by the CWP WPDES permit (Appendix A).

3.3.2 Sampling Method Requirements

3.3.2.1 In-Stream Root River

Water quality samples will be collected in well-mixed stream flow, at approximately mid-depth, where feasible. Seasonal changes in the water level may require the use of a small boat if areas of the stream are determined to be unwadable.

Single grab samples will be collected from bridge access using an isokinetic depth integrating sampler at five equally spaced locations across the river. The grab samples will be combined in a clean bucket, and a composite sample will be placed in the appropriate sample bottles, placed in a cooler, and analyzed by a WDNR state-accredited laboratory using EPA-approved methods.

In situ samples will be measured from bridge access at five equally spaced locations across the river by lowering the multiparameter probe (YSI ProDSS Multiparameter Digital Water Quality Meter or equivalent) into the river such that the probe is placed at approximately mid-depth in the river channel.

Equipment used during regular water quality sampling will be gathered, checked for proper functionality, and loaded into the vehicles prior to each event. Equipment calibrations will take place at the first sampling site. The following equipment will be used:

- USGS WBH-96 (weighted bottle with pin) sampler
- 20-meter calibrated line
- Suspension rope
- WBH-96 1-liter sampler bottle
- Funnel
- 2-gallon composite bucket
- Water chemistry bottles and pre-printed labels for each site plus one field duplicate:
  - 1 1,000-milliliter plastic bottle: TSS and chlorophyll
  - 1 60-milliliter plastic bottle: Orthophosphate
  - 1 250-milliliter plastic bottle: total phosphorus, total nitrogen, chloride, and sulfuric acid ampules (1 ampule for each 250-milliliter bottle),
  - 1 150-milliliter glass bottle: E. coli
- Paper towels
- 2 coolers with ice
- Field notebook and field data sheets (page 8)
- Waterproof and permanent markers
- Deionized water
- Bags for laboratory slips and ice
- Packaging tape
- Custody seals
- Chain of custody sheets
- Measuring tape
- Multi-parameter probe and calibration equipment
- Field safety instructions

Additional details for water sampling methodology are included in the Water Sampling SOP (Appendix D).

For continuously monitored water quality parameters, two types of continuous monitoring configurations will be used:

- Automated Flow Gage—In situ flow measurements recorded by a sensor placed directly at the measurement point with communication cables and power system to run the data logger (see Section 3.2).
- Continuous Data Sonde—Internal-logging combined sensor and recording sonde that is entirely immersed, requires no external power, and stores data within the sonde.

Field visits for maintenance of the data sondes and flow gage will be conducted regularly and will include the following activities:

- Calibration of the field meter(s)
- Inspection of the site for signs of physical disruption
- Inspection and cleaning of sensor(s) for fouling, corrosion, or damage
- Inspection and cleaning of sensor(s) deployment tube
- Battery (or power) check
- Time check
- Routine sensor cleaning and servicing
- Calibration check (and recalibration, if necessary)
- Downloading of data

3.3.2.2 CWP and Return Flow Outfall

Methods for water quality monitoring at the CWP and at the return flow outfall are defined by the CWP WPDES Permit for return flow discharge (Appendix A Sections 3.1 and 3.2). Monitoring parameters between the Fox River and Root River outfalls are the same except for temperature. Return flow

temperature monitoring is required between the outfall and downstream of the reaeration facility located at the return flow discharge site.

3.3.3 Sampling Handling and Custody Requirements

To establish the documentation necessary to trace sample possession from the time of collection, a COC record, which can be obtained from the laboratory, will be completed for every sample event. In order to maintain the COC record, every person who has custody of the sample at any time must sign, date, and note the time on the COC record. When samples are packed for shipping, custody seals will be placed across the latch and across the lid opening of the coolers to confirm that they arrive at the laboratory unopened.

For each water quality sample, the following information shall be clearly marked and labeled on the sample container:

- Water samples: "RR – Sample Site ID (A-D) – MMDDYYYY"
- Field duplicates: "RR – FD – MMDDYYYY"
- Time of sample collection
- Sampled by
- Analyses
- Preservative (if any)

During sampling, filled and labeled containers will be stored in coolers on ice to maintain a temperature of less than or equal to 4 degrees Celsius. The coolers will remain in the custody of the Field Team Leader (FTL) until the end of the sampling event, and the samples will be shipped, transported, or delivered to a laboratory courier in a cooler, on ice, within 24 hours. If used, glass containers will be wrapped in bubble wrap to prevent breakage. Samples will not be collected on Fridays due to sample preservation requirements.

Coolers prepared for shipping will be lined with a cooler liner and packed with ice in double-wrapped resealable plastic bags so that movement of samples is minimized. A COC form will be included in each shipment container describing the following: type of sample, number of containers, type and kind of analysis, and special processing and handling procedures. The FTL will keep the copy of the COC form.

3.3.4 Analytical Requirements

Laboratory water quality analyses will be conducted by a WDNR-certified laboratory using EPA-approved analysis methods (40 CFR 136). Samples will be processed within the recommended hold times as listed in the standard methods, and attention will be given to the varying holding time limits for each parameter. Table 3-1 (see Section 3.1) lists the methods, detection limits, and maximum hold times that will be used for each parameter to document proper quality assurance and quality control (QA/QC).

Additional data qualifiers or comments may be added to laboratory analysis reports to describe results and deviations in the analysis from the standard methods. Data qualifiers and additional comments will be included in the water quality database and reviewed according to QA/QC procedures (refer to Section 3.7, Quality Control Plan).

Appendix E contains laboratory SOPs including QA/QC procedures.

Water quality data collected using automated gages and continuous data sondes will be analyzed according to data processing procedures outlined in *Guidelines and Standard Procedures for Continuous Water-Quality Monitors: Station Operation, Record Computation, and Data Reporting* (USGS 2006). An initial data evaluation will be conducted to verify accurate transfer of data and assess the presence of erroneous data. Data corrections will be applied as needed to adjust for instrument calibration drift, sensor fouling errors, and cross-section variability. Data corrections are based on measurements made during servicing or cross-section surveys and abide by the established "Maximum Allowable Limits" for data correction shown in Table 3-2.

A final data evaluation will be conducted to review the data record and check data corrections. Thereafter, the data will be verified for publication and rated for quality. Data that cannot be verified or are rated as unacceptable will be retained for record checking and review purposes.

Table 3-2. Maximum Allowable Limits for Continuous Water Quality Monitoring Sensors

Water Quality Parameters	Method
Temperature	± 2.0 degrees Celsius
Specific Conductance	± 30 %
Dissolved Oxygen	± 2.0 mg/L or 20%, whichever is greater
pH	± 2 units
Turbidity	± 3.0 turbidity units or ± 30%, whichever is greater

3.3.5 Data Management

Water quality data collected during sampling events will be entered into an electronic data form (e.g., EQuIS Collect) and stored in an online database. The data will also be recorded in a field notebook. The laboratory conducting water quality analyses will provide EDDs that are compatible with the database. COC forms and laboratory reports will be stored electronically.

Water quality data collected at in-stream Root River monitoring sites, including grab samples and in-field measurements, will be reviewed according to quality control procedures described in Section 3.7, and will be uploaded to the Surface Water Integrated Monitoring System (SWIMS) database.

Water quality data collected using automated gages and continuous data sondes will be stored in accordance with USGS policy. In-stream Root River water quality continuous monitoring data collected by USGS will be published and managed by USGS on their streamflow *Current Water Data* website (https://waterdata.usgs.gov/nwis/rt).

Water quality data collected through the CWP WPDES permit will be reported to the WDNR through DMRs, in conformance with the WPDES requirements.

3.4 Macroinvertebrate Sampling

3.4.1 Sample Process Design

The post-return flow Root River monitoring activities includes macroinvertebrate sampling once during September through early October. A sampling event in November will be completed during the initial year(s) of the post-return flow monitoring for consistency with the voluntary Pre-Return Flow Root River Data Collection Plan, however the November sampling will be eliminated if it is not needed to support the WPDES permit. Sampling between locations will occur on consecutive days as weather and site conditions allow.

Macroinvertebrate sampling will be completed consistently with WDNR guidelines, *State of Wisconsin Department of Natural Resources, Guidelines for Collecting Macroinvertebrate Samples from Wadable Streams* (WDNR 2000). Appendix F contains biological data collection reach maps.

Macroinvertebrate sampling is anticipated for the first few years of the post-return flow Root River monitoring activities. Sampling may be discontinued or the frequency of collection reduced if these results indicate that beyond typical environmental variation, there are no measurable impacts resulting from return flow implementation.

3.4.2 Sampling Method Requirements

Macroinvertebrate laboratory sample preservation and taxonomy will be performed by a certified taxonomist for freshwater aquatic insects. Sampling will be conducted using the kick-net method with a 425-micron mesh net. Captured material will be emptied into a clean bucket, filtered through a 425-micron sieve, and then preserved in labeled jars filled with 95% ethanol. Following sampling, hydrologic data will be measured at a minimum of 10 equidistant points perpendicular to the flow of the stream to calculate average depth, velocity, and discharge of the sampling location.

After 24 to 48 hours, macroinvertebrate samples will be drained and replenished with ethanol solution for preservation. For taxonomic identification, a Marchant Box will be used to divide the macroinvertebrate sample into subsamples. At least 250 specimens will be extracted and sorted to be consistent with anticipated 2026 updates to Wisconsin's Consolidated Assessment and Listing Methodology (WisCALM). Sorted specimens will be retained with 70% ethanol at the laboratory.

Additional details for macroinvertebrate sampling methodology are included in the Macroinvertebrate Sampling SOP (Appendix G), including a January 2022 unpublished WDNR memorandum that recommends using a minimum sample size of 250 counts.

3.4.3 Sampling Handling and Custody Requirements

Benthic macroinvertebrate samples will be placed in a tightly sealed plastic or wide-mouth glass jar and preserved with 70 to 75% ethanol after collection at each site. For each benthic macroinvertebrate sample, the following information will be marked on the outside of the jar:

- "RR – Sample Site ID (A-D) – MMDDYYYY"
- Field duplicates: "RR – FD – MMDDYYYY"
- Time of collection
- Sampled by
- Analyses
- Preservative

In addition, a labeled tag will be inserted into the benthic macroinvertebrate sample with the same information. Waterproof paper will be used to prepare the tag, and the labels on both jars and tags will be marked with indelible ink.

Benthic macroinvertebrate samples will be stored in coolers and remain in the custody of the FTL until the cooler is full or ready for shipment or ground transport to the analysis location. Coolers will be packed to minimize movement of samples and will include vermiculite in case of leakage. Samples analyzed at the University of Wisconsin-Parkside by Dr. Jessica Orlofske will be ground transported to the laboratory for analysis.

If samples are to be shipped to an analyzing laboratory for more timely taxonomic identification and enumeration analysis support, each shipping container must contain a COC form, which can be obtained from the laboratory, detailing the samples and direction for analysis. Samples will be preserved prior to shipment. Every person who has custody of the sample at any time must sign, date, and note the time on the COC record. Samples will not be left unattended unless placed in a secured and sealed container with the COC record inside the container. The COC record will include special instructions for the laboratory to follow, which will be consistent with the contract. Samples will be shipped to the laboratory within 24 hours of collection.

3.4.4 Analytical Requirements

Calculation of metrics and indices will align with WDNR protocols and the current peer-reviewed literature (Hilsenhoff 1987; Lillie et al 2003; Merritt et al 2008). Table 3-3 shows metrics and indices to be measured as part of the macroinvertebrate sampling and taxonomic analysis.

Table 3-3. Macroinvertebrate Metrics and Indices

Biotic Indices
Hilsenhoff Biotic Index
Mean Tolerance Values
Macroinvertebrate index of biotic integrity
Taxa Richness
Ephemeroptera-Plecoptera-Trichoptera Generic Richness
Diversity
Shannon's Diversity Index
Trophic Function
Percent Scrapers
Percent Shredders
Percent Gatherers
Depositional Taxa
Proportion of Diptera
Proportion of Chironomidae
Proportion of Isopoda and Amphipoda
Dominance
Evenness

3.4.5 Data Management

Field forms developed by WDNR will be used for macroinvertebrate sampling (Macroinvertebrate Field Data Report, Form 3200-081) as well as field data sheets developed for this project. Scanned field forms and field data sheets, including field notes, macroinvertebrate taxonomic identification data sheets, and COC forms (as applicable) will be stored electronically.

Field and laboratory processing data will be transcribed to a Microsoft Excel spreadsheet and used to develop tables and figures for reporting purposes. Microsoft Excel files will be stored electronically.

3.5 Fish Sampling

3.5.1 Sample Process Design

Fish sampling will be completed once during June through late August. A sampling event in November will be completed during the initial year(s) of the post-return flow monitoring for consistency with the voluntary Pre-Return Flow Root River Data Collection Plan, however the November sampling will be eliminated if it is not needed to support the WPDES permit Sampling between locations will occur on consecutive days as weather and site conditions allow. WDNR staff will be notified prior to the initial fish sampling events to oversee fish taxonomy but will not physically collect samples or direct the processing following collection. WDNR staff will be notified of subsequent sampling events if requested.

Fish sampling will be carried out in alignment with WDNR guidelines, *State of Wisconsin Department of Natural Resources, Guidelines for Assessing Fish Communities of Wadable Streams in Wisconsin* (WDNR 2001).

Fish sampling is anticipated for the first few years of the post-return flow Root River monitoring activities. Sampling may be discontinued or the frequency of collection reduced if these results indicate that beyond typical environmental variation, there are no measurable impacts resulting from return flow implementation.

3.5.2 Sampling Method Requirements

Electrofishing collection will be conducted by at least two or ideally three operators. Due to the dangers inherent to electrofishing, operators will wear proper insulated equipment, and at least one operator must be CPR certified. Additionally, electrofishing will not be performed within 24 hours after a rain event.

Fish sampling reaches will be the same reaches used in the habitat assessments. Similarly, for continuity, reaches established as part of the pre-return flow Root River monitoring activities will be used for this post-return flow Root River monitoring. Revisions to the reaches will be discussed with the City prior to the implementation of new reaches. Appendix F contains biological data collection reach maps.

Electrofishing will begin downstream, moving upstream. Collected fishes will be directly transferred to holding buckets and observed for distress. Fish will be held until the end of the reach (or half the reach length, if the length is greater than 100 meters) and will be identified, counted, and recorded before being released. For game fish, lengths will be recorded.

Unidentifiable species will be recorded with a designation, and a small subset will be euthanized for identification in the laboratory. Protocols for euthanizing and preserving fish samples follow the Animal Care and Use Agreement approved by the University of Wisconsin's Colleges Animal Care and Use Committee (AUCU Protocol# 17-18-02).

Additional details for fish sampling methodology are included in the Fish Sampling SOP (Appendix I).

3.5.3 Sampling Handling and Custody Requirements

If fish samples are taken from the field for laboratory identification, the fish will be euthanized in a strong solution (i.e., 250 mg/L) of MS-222 anesthetic. The fish will be immersed in the solution for 10 minutes, or at least until the fish becomes immobile, stops respiring (as evidenced by a lack of opercular movement), and stiffens its fins. At this point, the fish will be moved into a 10% solution of formalin for fixation and will then be transferred to alcohol for preservation. These activities are covered under an Animal Care and Use Agreement approved by the University of Wisconsin's Colleges Animal Care and Use Committee (AUCU Protocol# 17-18-02). All chemicals used in the field will be transported back to the laboratory for storage and disposal as needed.

For each fish brought back to the laboratory, the following information should be marked on the outside of the jar:

- "RR – Sample Site ID (A-D) – MMDDYYYY"
- Time of collection
- Sampled by
- Preservative

Fish samples shall be stored in coolers while in the field and in a refrigerator while in the laboratory. If samples are to be shipped to an analyzing laboratory, each shipping container must contain a COC form, which can be obtained from the laboratory, detailing the samples and direction for analysis. Every person who has custody of the sample at any time must sign, date, and note the time on the COC record. Samples will not be left unattended unless placed in a secured and sealed container with the COC record inside the container. The COC record will include special instructions for the laboratory to follow, which will be consistent with the contract. Samples will be shipped to the laboratory within 24 hours of collection.

3.5.4 Analytical Requirements

Field results will be transcribed and stored in a Microsoft Excel spreadsheet to calculate fish community diversity indices and Index of Biotic Integrity (IBI) according to *Using the Index of Biotic Integrity (IBI) to Measure Environmental Quality in Warmwater Streams of Wisconsin* (Lyons 1992) and *Development and Validation of Two Fish-Based Indices of Biotic Integrity for Assessing Perennial Coolwater Streams in Wisconsin* (Lyons, 2012). Fish IBI analysis will include 10 metrics and 2 correction factors as shown in Table 3-4.

Table 3-4. Fish Metrics and Indices

Biotic Metrics and Indices
Index of Biotic Integrity (warmwater)
Index of Biotic Integrity (cool-warm transition)
Species Richness and Composition
Total Number of Native Species
Number of Darter Species
Number of Sucker Species
Number of Sunfish Species
Number of Intolerant Species
Percent (by number of individuals) Tolerant Species
Trophic and Reproductive Function
Percent Omnivores
Percent Insectivores
Percent Top Carnivores
Percent Simple Lithophilous Spawners
Fish Abundance and Condition
Number of Individuals per 300 meters Sampled
Percent with Deformities, Eroded Fins, Lesions, or Tumors (DELT)

3.5.5 Data Management

Field data sheets developed for this project and field notebooks will be used for data collection during fish sampling. Scanned field notebooks and field data sheets, including field notes, and COC forms (as applicable) will be stored electronically with the project files.

Field and laboratory processing data will be transcribed to a Microsoft Excel spreadsheet and used to develop tables and figures for reporting purposes. Microsoft Excel files will be stored electronically with the project files.

3.6 Habitat Assessments

3.6.1 Sample Process Design

Habitat assessments will be completed once yearly in concert with summer fish sampling based on WDNR recommendations received as part of the voluntary pre-return flow Root River monitoring activities. Habitat assessments will be performed in alignment with WDNR guidelines, *State of Wisconsin Department of Natural Resources, Guidelines for Evaluating Habitat of Wadable Streams* (WDNR 2002).

Habitat assessments are anticipated for the first few years of the post-return flow Root River monitoring activities. Assessments may be discontinued or the frequency reduced if these results indicate that beyond typical environmental variation, there are no measurable impacts resulting from return flow implementation.

3.6.2 Sampling Method Requirements

For each site, the habitat will be assessed along the entire reach, defined by WDNR as 35 times the average stream width. Therefore, the reach length differs for reach site in proportion to its width. Each reach will be divided into 12 transects and transects will by characterized by various habitat parameters including substrate composition, area of exposed bank, instream habitat for fish, and canopy cover. The extent of major instream habitat types will also be recorded including riffles, runs, pools, and logjams. Appendix F contains biological data collection reach maps.

For continuity, transects established as part of the pre-return flow Root River monitoring activities will be used for the Post-Return Flow Root River Monitoring Plan. Revisions to the transects will be discussed with the City prior to the implementation of new transects. Map data will be used to characterize order and sinuosity and to verify stream bends.

For each site, hydrologic data will be measured in one transect that is free of logjams and debris, if feasible. For subsequent sampling years, the same transect will be used to measure hydrologic data at each site, if possible. Data will be used to calculate depth, velocity, and discharge.

Additional details for habitat assessment methodology are included in the Habitat Assessment SOP (Appendix H).

3.6.3 Sampling Handling and Custody Requirements

During habitat assessments, samples will not be collected for offsite analyses.

3.6.4 Analytical Requirements

Habitat assessments will be performed in alignment with WDNR guidelines, *State of Wisconsin Department of Natural Resources, Guidelines for Evaluating Habitat of Wadable Streams* (WDNR 2002).

3.6.5 Data Management

Field forms developed by WDNR will be used for habitat assessments (Wadable Stream Quantitative Habitat Evaluation, Form 3600-228) as well as field data sheets developed for this project. Scanned field forms, field data sheets, and field notes will be stored electronically with the project files.

Field and laboratory processing data will be transcribed to a Microsoft Excel spreadsheet and used to develop tables and figures for reporting purposes. Microsoft Excel files will be stored electronically with the project files.

3.7 Quality Control Plan

QA/QC is designed to assure the reliability and quality of the analysis and data and to identify any contamination that may result from laboratory methods, equipment, or sample collection. Sample collection, preservation, handling and storage, and analytical procedures will be conducted in accordance with standard methods and practices.

Three types of QA/QC will be performed as part of the Post-Return Flow Root River Monitoring Plan activities. In combination, inclusions of these types of QA/QC procedures align with EPA's Quality System for Environmental Data and Technology "Project" designation.

Type 1 includes regular calibration and operational checks of water quality meters and field equipment and proper documentation of activities and field conditions by the field team members.

Field instruments will be calibrated according to manufacturers' specifications, and these procedures will be documented in a field notebook or field data sheet (included in Appendix C) and submitted following each sampling event. Type 1 activities include documenting other pertinent information and observations concerning the data collection events such as weather conditions, time of data collection, and site conditions that may impact sampling activities and/or results. Type 1 documentation can be summarized as follows:

- Instrument identification
- Calibration information (standards used and results)
- Date and time of calibrations
- Weather conditions and specific location of sample collection
- Site conditions and impacts to sampling activities and results

Type 2 consists of sampling procedures intended to identify the type and estimate the level of contamination. Type 2 QA/QC activities include providing equipment decontamination standards to detail cleaning protocols between collections of water samples. Type 2 activities also include collection of QA/QC duplicate samples and proper labeling of all samples. The Type 2 QA/QC requirements are detailed as follows:

- **Equipment Decontamination Standards.** Equipment decontamination standards will be followed during all sampling events (water quality, macroinvertebrate, fish, habitat) during which water quality samples or measurements are collected. Following water sample collection using the isokinetic depth integrating sampler or the multiparameter probe, sampling equipment is rinsed with deionized water according to the Water Sampling SOP (Appendix D).
- **Field Duplicates.** Duplicate samples will be collected during water quality sampling events and macroinvertebrate sampling events. Duplicate field samples are collected side-by-side to check the homogeneity of the sample matrix, precision of field techniques, and precision of laboratory analysis. Duplicate samples will be collected at the same time as the initial sample. One field duplicate sample will be collected and analyzed at one sampling site during every sampling event. Water field duplicate samples will be measured for all grab sample parameters. Macroinvertebrate field duplicates will be measured for all metrics and indices.

Type 3 provides confirmation of the water quality sampling procedures conducted by the field team and the analytical procedures conducted by the laboratories.

Water quality sampling and measurement of water quality parameters will follow the Water Sampling SOP. Water quality parameters measured with a multiparameter probe are anticipated to fall within expected ranges as defined in Table 3-1. For measurements that fall outside of these ranges, in-field calibration verifications will be conducted to validate the measurement and documented in the field data sheet.

Laboratory water quality analyses will be conducted by a WDNR-certified laboratory using EPA-approved analysis methods (40 CFR Part 136). Plans to use a different laboratory will be coordinated with the City and will include a transition plan.

Samples will be processed within the recommended hold times as listed in the standard methods, and attention will be given to the varying hold time limits for each parameter. Table 3-1 lists the methods and detection limits that will be used for each parameter to document proper QA/QC. Appendix E contains laboratory SOPs including QA/QC procedures.

Additional data qualifiers or comments may be added to laboratory analysis reports to describe results and deviations in the analysis from the standard methods. Data qualifiers and additional comments will be included in the water quality database. Data qualifiers and additional comments may indicate a result that is zero or not detected, greater than zero and less than the limit of detection (LOD), between the LOD and Limit of Quantification (LOQ) or indicate that the sample was analyzed passed the standard hold time. Using best professional judgement, guidance from the implementation and laboratory contractors, and field notes, data in question will be validated with justification, eliminated from the data set, or reconciled using another method (e.g., resampling, data correction, etc.).

4. Assessment/Oversight

Assessment of data quality will be conducted at multiple steps in the project and by multiple project personnel, including contracted implementation, monitoring, and laboratory support teams and consultants such as SmithGroup or Jacobs.

The water quality database will be reviewed quarterly for completeness and accuracy and annually for assessment of water quality outliers or water quality data with analysis qualifiers as determined by the analyzing laboratory. Using best professional judgement, guidance from the implementation and laboratory contractors and field notes, data in question will be validated with justification, eliminated from the data set, or reconciled using another method (e.g., resampling, data correction, etc.).

Upon completion of macroinvertebrate and fish sampling events, as well as habitat assessments, the field sampling team will notify the project team and will provide the field data and summary reports for electronic storage with the project files.

Quality issues, solutions, and updates will be documented as part of the project records.

4.1 Reports to Management

The field sampling team will provide status updates to the project team regarding the status or completion of scheduled field sampling activities and data review activities. Additional status and quality updates may be reported as needed/requested. Status and quality reports will be included in annual reporting as required by the diversion approval.

5. Data Validation and Usability

Data generated for this project as required by the diversion approval will be reviewed using the following checklist. Data review, validation, and reconciliation will be led by the monitoring Project Manager with input from contracted implementation, monitoring, and laboratory support teams.

- **Review for completeness** based on anticipated sampling events, total number of samples, and parameters to be analyzed.
- **Validate generated data** against the project and data quality objectives defined in Section 1 and Section 3.1, respectively.
- **Reconcile data** that does not meet data review and validation criteria. Data requiring reconciliation may include water quality data that fall outside expected ranges (Table 2-1), data collected using methodology that deviates from the SOPs, and data generated following an error in equipment calibration, among others. Using best professional judgement, guidance from the implementation and laboratory contractors, as well as field notes, data in question will be validated with justification, eliminated from the data set, or reconciled using another method (e.g., resampling, data correction, etc.).

6. Data Analysis and Reporting

Data collected as part of the post-return flow Root River monitoring activities will be used to assess changes that may have resulted from return flow (such as volumes, water temperatures, water quality, and periodicity of discharge). This analysis will inform the City to adaptively manage the return flow to minimize potential adverse impacts or provide greater benefits to water dependent resources of the Lake Michigan watershed.

The following subsections include key analysis questions to be answered and discussion of potential analysis methods and principles.

6.1 Velocity and Periodicity of Discharge

- *What impact did return flow have on the flow rate change in the Root River upstream and downstream of return flow?*
- *What impact did the return flow have on the water level?*

Comparison of flow rates and assessment of flow rate change (i.e., periodicity) in the Root River will compare the return flow flow rates with the in-stream flow gaging at Site D. Statistical calculations such as monthly and daily averages, minimum daily flow, maximum daily flow, and rate of change with and without return flow may be used.

To assess the impact of return flow on the water level and velocity, a methodology similar to that used during the City's diversion application may be followed. This could include obtaining the latest publicly available existing conditions hydraulic model (e.g., HEC model) from the Southeastern Wisconsin Regional Planning Commission (SEWRPC)to quantify water elevation and velocity changes resulting from return flow between low and high stream flow, between the outfall and downstream site D. Through recent discussions with SEWRPC, a hydraulic model may not be available for several years and the hydraulic model under development is for flood evaluation purposes and may not be suitable for low-flow evaluations. Consequently, as an alternative to utilizing a hydraulic model, statistical analyses may be used such as comparing flow duration curves or monthly or seasonal high and low flows with and without return flow.

6.2 Water Temperature

- *Has the river temperature changed upstream and downstream of return flow?*
- *Are there seasonal differences to changes in temperature?*
- *If there is a temperature change (i.e., increase) with return flow, what is the spatial extent of the impact caused by return flow?*

Water temperature measurements at in-stream Sites A through D will be compared to determine the extent and magnitude of water temperatures change from upstream locations (A-C) through downstream locations (C2-D). These in-stream temperature readings may be compared to the temperature of the return flow measured at the return flow outfall site reaeration building. The data assessment may use statistical calculations such as monthly and daily averages, and minimum and maximum daily values. Temperature at all locations will be collected throughout the year to allow comparison of statistical calculations for various seasons.

6.3 Water Quality

- *Has the river water quality changed upstream and downstream of return flow with the addition of return flow?*
- *Are there seasonal differences to changes?*
- *Are there other known upstream watershed* impacts that may be influencing these changes?

Water quality grab sample measurements at in-stream Sites A, B, C, and D will be compared to determine the extent and magnitude of water quality changes from upstream locations (A-C) through downstream locations (D). These data will be compared to the water quality of the return flow measured at the return flow outfall site reaeration building or as part of the WPDES reporting. The data assessment may compare event samples and where applicable using statistical calculations such as monthly and seasonal (growing and non-growing) averages.

Through personal knowledge of the team, acquiring knowledge through public sources (e.g., newspaper, WDNR website, Wisconsin Department of Transportation construction website, WDNR permit records, riverkeeper notifications, etc.), or through personal communications (e.g., communication with WDNR, riverkeepers, County, etc.), a qualitative summary of watershed impacts that may affect in-stream water quality will be included in the assessment reporting to provide context or potential explanation of changes in water quality between the sampled sites.

6.4 Water Dependent Resources

- *Is the macroinvertebrate, fish, or diatom (as provided by the Department) community different in the mainstem of the Root River at upstream or downstream locations compared to pre-return flow conditions? What is the spatial extent of the impact caused by return flow?*
- *Are the changes natural variability, an indication of potential upstream watershed impacts, or an indication of changes resulting from return flow?*

Biological sampling at Sites A, B, C, and D will be compared to determine the extent and community differences between pre- and post-return flow conditions. Assessment of biological impacts (either positive or negative) is anticipated to include comparison of both quantitative (e.g., number of fish species, Shannon diversity values, etc.) and qualitative (e.g., site conditions) metrics. The data assessment may compare statistical calculations such as minimum, maximum, and averages of various metrics calculated as part of the biological assessment (e.g., F-IBI, M-IBI, etc.). The spatial impact of the return flow will be assessed using upstream and downstream sites and comparing metric changes between pre- and post-return flow conditions.

Assessment of natural changes is anticipated to be supported by comparing the pre- and post-return flow metrics and statistical calculations and incorporating the assessment of the watershed impacts (refer to Section 6.3). Relevant historical biological surveys such as those included in the pre-return flow biological monitoring reports may also be referenced. If changes are observed that are beyond natural variability, and no watershed impacts are documented, the observed changes (either positive or negative) may be caused by return flow.

6.5 Reporting

As summarized in Section 2.2.3, annual reports are anticipated from the contract support team for event-based water quality monitoring and biological monitoring. Together with the data collected by USGS through in-stream continuous monitoring, these reports will be used to summarize impacts (either positive or negative) to the Root River resulting from return flow [Condition 12(l) of the diversion approval].

The diversion approval requires a report (Condition 12) on the Root River monitoring and an assessment of the return flow impact on the Root River. This diversion approval report is due to the WDNR by March 1 of each year after the diversion commences. The reports and data generated within this QAPP will be used for this diversion approval report requirement. To meet a March 1 deadline, some data may not be available for reporting a complete calendar year. For example, biological data may not be available from the prior year due to the time required to complete the biological monitoring data assessment and reporting from the contracted support. Consequently, available information will be used and prior year biological data may be reported in a subsequent diversion approval report. Data, reports, and analyses completed under this QAPP will be integrated with the City's larger report to the WDNR to meet the diversion approval Condition 12 requirements.

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Appendix A. CWP WPDES Permit

WPDES PERMIT

STATE OF WISCONSIN
DEPARTMENT OF NATURAL RESOURCES

PERMIT TO DISCHARGE UNDER THE WISCONSIN POLLUTANT DISCHARGE ELIMINATION SYSTEM

City of Waukesha

is permitted, under the authority of Chapter 283, Wisconsin Statutes, to discharge from a facility located at
600 Sentry Dr Waukesha WI 53186
To

Fox (IL) River (Upper Fox (IL) River Watershed, Fox (IL) River Basin) in Waukesha County
and
Root River (Root River Watershed, Root-Pike River Basin) in Milwaukee County

in accordance with the effluent limitations, monitoring requirements and other conditions set forth in this permit.

The permittee shall not discharge after the date of expiration. If the permittee wishes to continue to discharge after this expiration date an application shall be filed for reissuance of this permit, according to Chapter NR 200, Wis. Adm. Code, at least 180 days prior to the expiration date given below.

State of Wisconsin Department of Natural Resources
For the Secretary

By _____
Tim Ryan
Field Operations Director

10/31/2025
Date Permit Signed/Issued

PERMIT TERM: EFFECTIVE DATE – November 01, 2025 **EXPIRATION DATE – September 30, 2030**

TABLE OF CONTENTS

1 Influent Requirements

1.1 Sampling Point(s)

Sampling Point Designation	
Sampling Point Number	**Sampling Point Location, Waste Type/Sample Contents and Treatment Description (as applicable)**
702	INFLUENT: 24-hr flow proportional composite samples shall be collected after screening and grit removal and prior to the addition of recycled flows (i.e. filter backwash, sludge centrate water, sludge thickener supernatant and clarifier drains).

1.2 Monitoring Requirements

The permittee shall comply with the following monitoring requirements.

1.2.1 Sampling Point 702 - INFLUENT

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Flow Rate		MGD	Daily	Continuous	
BOD_5, Total		mg/L	Daily	24-Hr Flow Prop Comp	
Suspended Solids, Total		mg/L	Daily	24-Hr Flow Prop Comp	
Mercury, Total Recoverable		ng/L	Monthly	24-Hr Flow Prop Comp	See 'Mercury Monitoring' section.
Cadmium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Chromium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Copper, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	
Lead, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Nickel, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Zinc, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	

1.2.1.1 Sample Analysis

Samples shall be analyzed using a method which provides adequate sensitivity so that results can be quantified at a level of quantitation below the calculated/potential effluent limit, unless not possible using the most sensitive approved method.

1.2.1.2 Total Metals Analyses

Measurements of total metals and total recoverable metals shall be considered as equivalent.

1.2.1.3 Mercury Monitoring

The permittee shall collect and analyze all mercury samples according to the data quality requirements of ss. NR 106.145(9) and (10), Wisconsin Administrative Code. The limit of quantitation (LOQ) used for the effluent and field blank shall be less than 1.3 ng/L, unless the samples are quantified at levels above 1.3 ng/L. The permittee shall collect at least one mercury field blank for each set of mercury samples (a set of samples may include combinations of intake, influent, effluent or other samples all collected on the same day). The permittee shall report results of samples and field blanks to the Department on Discharge Monitoring Reports.

2 In-Plant Requirements

2.1 Sampling Point(s)

Sampling Point Designation	
Sampling Point Number	**Sampling Point Location, Waste Type/Sample Contents and Treatment Description (as applicable)**
101	FIELD BLANK: Collect mercury field blank using standard sample handling procedures.
104	In-Plant Diversion OTHER BYPASS: Sample point for reporting diverted flow which bypasses existing tertiary treatment process of coagulation, flocculation & sedimentation, and granular media filtration prior to ultraviolet disinfection.
105	LAKE MICHIGAN WATER SUPPLY: A grab sample of raw Lake Michigan water shall be collected from the water supply facility, prior to receiving any treatment.

2.2 Monitoring Requirements and Limitations

The permittee shall comply with the following monitoring requirements and limitations.

2.2.1 Sampling Point 101 - FIELD BLANK

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Mercury, Total Recoverable		ng/L	Monthly	Blank	See 'Mercury Monitoring' section.

2.2.1.1 Mercury Monitoring

The permittee shall collect and analyze all mercury samples according to the data quality requirements of ss. NR 106.145(9) and (10), Wisconsin Administrative Code. The limit of quantitation (LOQ) used for the effluent and field blank shall be less than 1.3 ng/L, unless the samples are quantified at levels above 1.3 ng/L. The permittee shall collect at least one mercury field blank for each set of mercury samples (a set of samples may include combinations of intake, influent, effluent or other samples all collected on the same day). The permittee shall report results of samples and field blanks to the Department on Discharge Monitoring Reports.

2.2.2 Sampling Point 104 - InPlant Diversion-Other Bypass

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Flow Rate		MGD	Per Occurrence	Continuous	Start flow measurement at the commencement of bypass operations. Measure flow in daily increments until operation ends and report daily bypass flow on the eDMR. See 'Other

					Bypass Requirements' permit section.
Time		hours	Per Occurrence	Calculated	Report the total duration of 'Other Bypass' within any given day (12:00am - 11:59pm) in which the 'Other Bypass' occurs. See 'Other Bypass Requirements' permit section.

2.2.2.1 Other Bypass Requirements

The Department has determined that an 'other bypass' as defined in s. NR 205.07(1)(u)3., Wis. Adm. Code, may occur at this sewage treatment facility. Furthermore, the Department has previously approved plans in accordance with s. 281.41, Wis. Stats., for the partial bypass around the tertiary treatment process prior to disinfection. A bypass that is defined as a controlled diversion in s. NR 205.07(1)(v), Wis. Adm. Code, is not covered under this sample point. The following requirements shall apply whenever the 'other bypass' operations are in effect:

- The 'other bypass' may only operate during wet weather or other high flow conditions when peak wastewater flow to the sewage treatment facility exceeds the maximum design and operating capacity of the tertiary treatment facilities and when necessary to avoid severe property damage to the sewage treatment facility as described in s. NR 205.07(1)(u)3.a., Wis. Adm. Code. The 'other bypass' may only divert flow around the tertiary treatment process described under the In-Plant Diversion OTHER BYPASS Sample Point description above. In no case shall this include flow diversion which would constitute blending, as defined in s. NR 210.03(2e), Wis. Adm. Code, unless otherwise approved in this permit;
- All flow, inclusive of that wastewater treated or not treated by the tertiary treatment process, shall be disinfected, if required by this permit, prior to discharge, and the flows shall be recombined prior to discharge;
- Effluent from the sewage treatment facility shall be monitored to include all wastewater that is discharged from the facility, including those wastewaters that are diverted around tertiary treatment process and shall meet the effluent limitations for Outfalls 001 and 006 included in this permit;
- Bypassing under this section and the circumstances that lead to the 'other bypass' shall be reported to the Department on the permittee's Discharge Monitoring Report (DMR), and shall include the time, duration, and volume of wastewater routed around the tertiary treatment process.

2.2.3 Sampling Point 105 - Lake Michigan Water Supply

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Flow Rate		MG	Monthly	Calculated	Report the sum of the total monthly intake flows.
Mercury, Total Recoverable		ng/L	Monthly	Grab	See 'Mercury Monitoring' section.
Mercury, Total Recoverable		grams/mo	Monthly	Calculated	See 'Mercury Mass Calculation' section.

Mercury, Total Recoverable		grams/yr	Annual	Calculated	Report the sum of the total monthly intake mass loading for the calendar year on the Annual report.

2.2.3.1 Mercury Monitoring

The permittee shall collect and analyze all mercury samples according to the data quality requirements of ss. NR 106.145(9) and (10), Wisconsin Administrative Code. The limit of quantitation (LOQ) used for the effluent and field blank shall be less than 1.3 ng/L, unless the samples are quantified at levels above 1.3 ng/L. The permittee shall collect at least one mercury field blank for each set of mercury samples (a set of samples may include combinations of intake, influent, effluent or other samples all collected on the same day). The permittee shall report results of samples and field blanks to the Department on Discharge Monitoring Reports.

2.2.3.2 Mercury Mass Calculation

For calculating the monthly mercury mass in grams/month for Outfall 006 the permittee shall multiply the measured mercury concentration in nanograms/liter by the total monthly flow by the conversion factor, 0.00378. The annual mercury mass shall be the summation of the monthly calculated masses over the calendar year and reported on the Annual Report form.

3 Surface Water Requirements

3.1 Sampling Point(s)

Sampling Point Designation	
Sampling Point Number	**Sampling Point Location, Waste Type/Sample Contents and Treatment Description (as applicable)**
001	EFFLUENT: 24-Hr flow proportional composite samples shall be collected from the effluent chamber after the UV disinfection system but before the Parshall flume. Grab samples shall be collected immediately after post aeration and before the Parshall flume.
006	EFFLUENT: Sampling shall be the same as Outfall 001 except monitoring for temperature shall be conducted at the outfall to the Root River after aeration. Flow is monitored at the treatment plant.

3.2 Monitoring Requirements and Effluent Limitations

The permittee shall comply with the following monitoring requirements and limitations.

3.2.1 Sampling Point (Outfall) 001 - EFFLUENT - Fox River

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Flow Rate		MGD	Daily	Calculated	Flow rate calculated by subtracting the flow from 006 meter from the total plant flow meter.
BOD_5, Total	Weekly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November through April each year.
BOD_5, Total	Weekly Avg	7.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May through October each year.
BOD_5, Total	Monthly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November through April each year.
BOD_5, Total	Monthly Avg	7.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May through October each year.
BOD_5, Total	Weekly Avg	310 lbs/day	Daily	Calculated	
Suspended Solids, Total	Weekly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	
Suspended Solids, Total	Monthly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	
pH Field	Daily Max	9.0 su	Daily	Grab	
pH Field	Daily Min	6.0 su	Daily	Grab	
E. coli	Geometric Mean - Monthly	126 #/100 ml	3/Week	Grab	Limit effective May through September each year.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
E. coli	% Exceedance	10 Percent	Monthly	Calculated	Limit effective May through September each year. See the 'E. coli Percent Limit' section in permit below. Enter the result in the DMR on the last day of the month.
Dissolved Oxygen	Daily Min	7.0 mg/L	Daily	Grab	
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	17 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November through April each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective April each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	8.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	5.6 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective June each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	3.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective July each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	4.2 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective August each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	5.8 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective September each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	9.2 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective October each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	12 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November & February each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	11 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective December & January each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	13 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective March each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.6 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective April each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	4.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May & December each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	3.1 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective June each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective July each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.1 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective August each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective September each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	4.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective October each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.1 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November each year.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective January each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.2 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective February each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	6.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective March each year.
Phosphorus, Total	Monthly Avg	0.225 mg/L	Daily	24-Hr Flow Prop Comp	
Phosphorus, Total	6-Month Avg	0.075 mg/L	Daily	24-Hr Flow Prop Comp	
Phosphorus, Total	6-Month Avg	2.94 lbs/day	Daily	Calculated	
Chloride	Weekly Avg	620 mg/L	4/Month	24-Hr Flow Prop Comp	This is an interim limit effective December 2025 through April 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride	Weekly Avg	570 mg/L	4/Month	24-Hr Flow Prop Comp	This is an interim limit effective immediately and through November 2025, and effective again May through October 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride	Weekly Avg	470 mg/L	4/Month	24-Hr Flow Prop Comp	Limit effective beginning November 1, 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride	Monthly Avg	470 mg/L	4/Month	24-Hr Flow Prop Comp	Limit effective beginning November 1, 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride, Variable Limit		lbs/day	4/Month	See Table	Beginning November 1, 2026, look up the chloride mass from the 'Variable Chloride Mass' table and report the variable limit in the 'Chloride Variable Limit' column on the eDMR.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Chloride	Weekly Avg - Variable	lbs/day	4/Month	Calculated	Report the weekly average mass Chloride result in the Chloride column of the eDMR. Limit effective November 1, 2026. See 'Chloride Mass Limit – Non-Wet Weather and Alternative Wet Weather Mass Limit' section in permit below.
Nitrogen, Total Kjeldahl		mg/L	Quarterly	24-Hr Flow Prop Comp	
Nitrogen, Nitrite + Nitrate Total		mg/L	Quarterly	24-Hr Flow Prop Comp	
Nitrogen, Total		mg/L	Quarterly	Calculated	Total Nitrogen shall be calculated as the sum of reported values for Total Kjeldahl Nitrogen and Total Nitrite + Nitrate Nitrogen.
Cadmium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Chromium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Copper, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	
Lead, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Nickel, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Zinc, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	
Mercury, Total Recoverable		ng/L	Monthly	24-Hr Flow Prop Comp	See 'Mercury Monitoring' section in permit.
PFOS		ng/L	Monthly	Grab	See 'PFOS/PFOA Minimization Plan Determination of Need' schedule in permit.
PFOA		ng/L	Monthly	Grab	See 'PFOS/PFOA Minimization Plan Determination of Need' schedule in permit.
Acute WET		TU_a	See Listed Qtr(s)	24-Hr Flow Prop Comp	See WET section below.
Chronic WET	Monthly Avg	1.5 TUc	See Listed Qtr(s)	24-Hr Flow Prop Comp	See WET section below.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Temperature Maximum		deg F	Daily	Continuous	Monitoring in calendar year 2028.

3.2.1.1 Annual Average Design Flow

The annual average design flow of the permittee's wastewater treatment facility is 14.0 MGD. The average annual design flow for the Root River return flow system is (Outfall 006) is 9.3 MGD.

3.2.1.2 *E. coli* Percent Limit

No more than 10 percent of *E. coli* bacteria samples collected in any calendar month may exceed 410 #/100 ml. Bacteria samples may be collected more frequently than required. All samples shall be reported on the monthly discharge monitoring reports (DMRs). The following calculation should be used to calculate percent exceedances.

$$\frac{\#\ of\ Samples\ greater\ than\ 410\ \#/100\ mL}{Total\ \#\ of\ samples} \times 100 = \%\ Exceedance$$

3.2.1.3 Total Metals Analyses

Measurements of total metals and total recoverable metals shall be considered as equivalent.

3.2.1.4 Sample Analysis

Samples shall be analyzed using a method which provides adequate sensitivity so that results can be quantified at a level of quantitation below the calculated/potential effluent limit, unless not possible using the most sensitive approved method.

3.2.1.5 Mercury Monitoring

The permittee shall collect and analyze all mercury samples according to the data quality requirements of ss. NR 106.145(9) and (10), Wis. Adm. Code. The limit of quantitation (LOQ) used for the effluent and field blank shall be less than 1.3 ng/L, unless the samples are quantified at levels above 1.3 ng/L. The permittee shall collect at least one mercury field blank for each set of mercury samples (a set of samples may include combinations of intake, influent, effluent or other samples all collected on the same day). The permittee shall report results of samples and field blanks to the Department on Discharge Monitoring Reports.

3.2.1.6 Effluent Temperature Monitoring

For monitoring temperature continuously, collect measurements in accordance with s. NR 218.04(13). This means that discrete measurements shall be recorded at intervals of not more than 15 minutes during the 24-hour period. In either case, report the maximum temperature measured during the day on the DMR. For seasonal discharges collect measurements either manually or continuously during the period of operation and report the daily maximum effluent temperature on the DMR.

3.2.1.7 PFOS/PFOA Sampling and Reporting Requirements

For grab samples, as defined per s. NR 218.04(10), Wis. Adm. Code, a single sample at a location as defined by the sample point description shall be taken during the time of the day most representative to capture all potential discharges. If extra equipment besides the sample bottle is used to collect the sample, it is recommended that a one-time equipment blank is collected with the first sample. An equipment blank would be collected by passing laboratory-verified PFAS-free water over or through field sampling equipment before the collection of a grab sample to evaluate potential contamination from the equipment used during sample.

If any equipment blanks are performed, these results shall be reported in the comments section of the eDMR and shall also documented in the reports submitted as part of the PFOS/PFOA Minimization Plan Determination of Need schedule of the permit.

3.2.1.8 PFOS/PFOA Minimization Plan Determination of Need

The permittee shall monitor PFOS and PFOA as specified in the table above and report on the effluent concentrations including trends in monthly and annual average PFOS and PFOA concentrations as specified in the PFOS/PFOA Minimization Plan Determination of Need Schedule.

If, after reviewing the data, the Department determines that a minimization plan for PFOS and PFOA is necessary based on the procedures in s. NR 106.98(4), Wis. Adm. Code, the Department will notify the permittee in writing that a PFOS and PFOA minimization plan that satisfies the requirements in s. NR 106.99, Wis. Adm. Code, is required. The permittee shall submit an initial plan for Department approval no later than 90 days after written notification was sent from the Department in accordance with s. NR 106.985(2)(a), Wis. Adm. Code. Pursuant to s. NR 106.985(2)(b), Wis. Adm. Code, as soon as possible after Department approval of the PFOS and PFOA minimization plan, the Department will modify or revoke and reissue the permit in accordance with public notice procedures under ch. 283, Wis. Stats., and ch. NR 203, Wis. Adm. Code, to include the PFOS and PFOA minimization plan and other related terms and condition.

If, however, the Department determines that a PFOS and PFOA minimization plan is unnecessary based on the procedures in s. NR 106.98(4), Wis. Adm. Code, the Department shall notify the permittee that no further action is required. Per s. NR 106.98(3)(a), Wis. Adm. Code, the Department may reduce monitoring frequency to once every 3 months (quarterly) on a case-by-case basis, but only after at least 12 representative results have been generated. If the permittee requests a reduction in monitoring and the Department agrees a reduction would be appropriate, the permit may be modified in accordance with public notice procedures under ch. 283, Wis. Stats., and ch. NR 203, Wis. Adm. Code, to incorporate this change.

3.2.1.9 Chloride Mass Limit – Non-Wet Weather and Alternative Wet Weather Mass Limit

This permit contains a chloride mass limit based on weather conditions. The applicable non-wet weather mass limit is **18,400 pounds/day**. The applicable wet weather mass limit is **75,700 pounds/day**. Report the applicable mass limit on the Discharge Monitoring Report form in the variable limit column. See Standard Requirements for "Applicability of Alternative Wet Weather Mass Limitations" and "Appropriate Formulas for Effluent Calculations".

Variable Chloride Mass Limitation

Parameter	Weekly Average Mass Limitation	Weekly Average Wet Weather Mass Limitation
Chloride	**18,400 lbs/day**	**75,700 lbs/day**

3.2.1.10 Whole Effluent Toxicity (WET) Testing

Primary Control Water: The Fox (IL) River upstream and outside of the mixing zone of the discharge or any other known discharges.

Instream Waste Concentration (IWC): 68%

Dilution Series: At least five effluent concentrations and dual controls must be included in each test.

- **Acute:** 100, 50, 25, 12.5, 6.25% and any additional selected by the permittee.
- **Chronic:** 100, 75, 50, 25, 12.5% and any additional selected by the permittee.

WET Testing Frequency:

Acute tests are required during the following quarters:

- **Acute:** July – September 2026, April – June 2027, October – December 2028, January – March 2029, and April – June 2030

Acute WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in January - March 2031.

Chronic tests are required during the following quarters:

- **Chronic:** July – September 2026, April – June 2027, October – December 2028, January – March 2029, and April – June 2030

Chronic WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in January - March 2031.

Testing: WET testing shall be performed during normal operating conditions. Permittees are not allowed to turn off or otherwise modify treatment systems, production processes, or change other operating or treatment conditions during WET tests.

Reporting: The permittee shall report test results on the Discharge Monitoring Report form, and also complete the "Whole Effluent Toxicity Test Report Form" (Section 6, "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*"), for each test. The original, complete, signed version of the Whole Effluent Toxicity Test Report Form shall be sent to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., P.O. Box 7921, Madison, WI 53707-7921, within 45 days of test completion. The Discharge Monitoring Report (DMR) form shall be submitted electronically by the required deadline.

Determination of Positive Results: An acute toxicity test shall be considered positive if the Toxic Unit - Acute (TU_a) is greater than 1.0 for either species (fathead minnow (Pimephales promelas) and waterflea (Ceriodaphnia dubia)). The TU_a shall be calculated as follows: $TU_a = 100 \div LC_{50}$. A chronic toxicity test shall be considered positive if the Toxic Unit - Chronic (TU_c) is greater than 1.1 for either species. The TU_c shall be calculated as follows: $TU_c = 100 \div IC_{25}$.

Additional Testing Requirements: Within 90 days of a test which showed positive results, the permittee shall submit the results of at least 2 retests to the Biomonitoring Coordinator on "Whole Effluent Toxicity Test Report Forms". The 90-day reporting period shall begin the day after the test which showed a positive result. The retests shall be completed using the same species and test methods specified for the original test (see the Standard Requirements section herein).

3.2.2 Sampling Point (Outfall) 006 - EFFLUENT - Root River

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Flow Rate		MGD	Daily	Continuous	
BOD_5, Total	Weekly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November through April each year.
BOD_5, Total	Weekly Avg	5.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May through October each year.
BOD_5, Total	Monthly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November through April each year.
BOD_5, Total	Monthly Avg	5.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May through October each year.
Suspended Solids, Total	Weekly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Suspended Solids, Total	Monthly Avg	10 mg/L	Daily	24-Hr Flow Prop Comp	
pH Field	Daily Max	9.0 su	Daily	Grab	
pH Field	Daily Min	6.0 su	Daily	Grab	
E. coli	Geometric Mean - Monthly	126 #/100 ml	Weekly	Grab	Limit effective May through September each year. Limit becomes effective year-round per the 'Year-Round Disinfection' schedule in permit.
E. coli	% Exceedance	10 Percent	Monthly	Calculated	Limit effective May through September each year. Limit becomes effective year-round per the 'Year-Round Disinfection' schedule in permit. See the 'E. coli Percent Limit' section in permit below. Enter the result in the DMR on the last day of the month.
Dissolved Oxygen	Daily Min	7.0 mg/L	Daily	Grab	
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	16 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective April each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	14 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	11 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective June & November through February each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	10 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective July each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	9.9 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective August & October each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	9.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective September each year.
Nitrogen, Ammonia (NH_3-N) Total	Daily Max	13 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective March each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	5.8 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective April each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	5.7 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	4.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective June each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	3.3 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective July each year.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	3.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective August each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	4.2 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective September each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	6.7 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective October each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	9.7 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	9.8 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective December each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	11 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective January & February each year.
Nitrogen, Ammonia (NH_3-N) Total	Weekly Avg	13 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective March each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.4 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective April each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective May each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	1.8 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective June & September each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	1.4 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective July each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	1.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective August each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	2.8 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective October each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	4.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective November & December each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.0 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective January each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.1 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective February each year.
Nitrogen, Ammonia (NH_3-N) Total	Monthly Avg	5.5 mg/L	Daily	24-Hr Flow Prop Comp	Limit effective March each year.
Phosphorus, Total	Monthly Avg	0.18 mg/L	Daily	24-Hr Flow Prop Comp	
Phosphorus, Total	6-Month Avg	0.06 mg/L	Daily	24-Hr Flow Prop Comp	
Phosphorus, Total	6-Month Avg	4.65 lbs/day	Daily	Calculated	
Chloride	Weekly Avg	620 mg/L	4/Month	24-Hr Flow Prop Comp	This is an interim limit effective December 2025 through April 2026. See 'Chloride WQBELs Compliance' schedule in permit.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Chloride	Weekly Avg	570 mg/L	4/Month	24-Hr Flow Prop Comp	This is an interim limit effective immediately and through November 2025, and effective again May through October 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride	Weekly Avg	400 mg/L	4/Month	24-Hr Flow Prop Comp	Limit effective beginning November 1, 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride	Monthly Avg	400 mg/L	4/Month	24-Hr Flow Prop Comp	Limit effective beginning November 1, 2026. See 'Chloride WQBELs Compliance' schedule in permit.
Chloride, Variable Limit		lbs/day	4/Month	See Table	Beginning November 1, 2026, look up the chloride mass from the 'Variable Chloride Mass' table and report the variable limit in the 'Chloride Variable Limit' column on the eDMR.
Chloride	Weekly Avg - Variable	lbs/day	4/Month	Calculated	Report the weekly average mass Chloride result in the Chloride column of the eDMR. Limit effective November 1, 2026. See 'Chloride Mass Limit – Non-Wet Weather and Alternative Wet Weather Mass Limit' section in permit below.
Nitrogen, Total Kjeldahl		mg/L	Quarterly	24-Hr Flow Prop Comp	
Nitrogen, Nitrite + Nitrate Total		mg/L	Quarterly	24-Hr Flow Prop Comp	
Nitrogen, Total		mg/L	Quarterly	Calculated	Total Nitrogen shall be calculated as the sum of reported values for Total Kjeldahl Nitrogen and Total Nitrite + Nitrate Nitrogen.

Monitoring Requirements and Effluent Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Cadmium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Chromium, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Copper, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	
Lead, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Nickel, Total Recoverable		µg/L	Quarterly	24-Hr Flow Prop Comp	
Zinc, Total Recoverable		µg/L	Monthly	24-Hr Flow Prop Comp	
Mercury, Total Recoverable		ng/L	Monthly	24-Hr Flow Prop Comp	See 'Mercury Monitoring' section in permit.
Mercury, Total Recoverable		grams/month	Monthly	Calculated	See 'Mercury Mass Calculation' section in permit.
Mercury, Total Recoverable		grams/yr	Annual	Calculated	See 'Mercury Mass Calculation' section in permit.
PFOS		ng/L	Monthly	Grab	Monitoring only. See 'PFOS/PFOA Minimization Plan Determination of Need' schedule in permit.
PFOA		ng/L	Monthly	Grab	Monitoring only. See 'PFOS/PFOA Minimization Plan Determination of Need' schedule in permit.
Acute WET		TU_a	See Listed Qtr(s)	24-Hr Flow Prop Comp	See WET section below.
Chronic WET		TUc	See Listed Qtr(s)	24-Hr Flow Prop Comp	See WET section below.
Temperature Maximum		deg F	Daily	Continuous	Monitoring in calendar year 2028.

3.2.2.1 *E. coli* Percent Limit

No more than 10 percent of *E. coli* bacteria samples collected in any calendar month may exceed 410 #/100 ml. Bacteria samples may be collected more frequently than required. All samples shall be reported on the monthly discharge monitoring reports (DMRs). The following calculation should be used to calculate percent exceedances.

$$\frac{\#\ of\ Samples\ greater\ than\ 410\ \#/100\ mL}{Total\ \#\ of\ samples} \times 100 = \%\ Exceedance$$

3.2.2.2 Total Metals Analyses

Measurements of total metals and total recoverable metals shall be considered as equivalent.

3.2.2.3 Sample Analysis

Samples shall be analyzed using a method which provides adequate sensitivity so that results can be quantified at a level of quantitation below the calculated/potential effluent limit, unless not possible using the most sensitive approved method.

3.2.2.4 Mercury Monitoring

The permittee shall collect and analyze all mercury samples according to the data quality requirements of ss. NR 106.145(9) and (10), Wis. Adm. Code. The limit of quantitation (LOQ) used for the effluent and field blank shall be less than 1.3 ng/L, unless the samples are quantified at levels above 1.3 ng/L. The permittee shall collect at least one mercury field blank for each set of mercury samples (a set of samples may include combinations of intake, influent, effluent or other samples all collected on the same day). The permittee shall report results of samples and field blanks to the Department on Discharge Monitoring Reports.

3.2.2.5 Mercury Mass Calculation

For calculating the monthly mercury mass in grams/month for Outfall 006 the permittee shall multiply the measured mercury concentration in nanograms/liter by the total monthly flow by the conversion factor, 0.00378. The annual mercury mass shall be the summation of the monthly calculated masses over the calendar year and reported on the Annual Report form.

3.2.2.6 Effluent Temperature Monitoring

For monitoring temperature continuously, collect measurements in accordance with s. NR 218.04(13). This means that discrete measurements shall be recorded at intervals of not more than 15 minutes during the 24-hour period. In either case, report the maximum temperature measured during the day on the DMR. For seasonal discharges collect measurements either manually or continuously during the period of operation and report the daily maximum effluent temperature on the DMR.

3.2.2.7 PFOS/PFOA Sampling and Reporting Requirements

For grab samples, as defined per s. NR 218.04(10), Wis. Adm. Code, a single sample at a location as defined by the sample point description shall be taken during the time of the day most representative to capture all potential discharges. If extra equipment besides the sample bottle is used to collect the sample, it is recommended that a one-time equipment blank is collected with the first sample. An equipment blank would be collected by passing laboratory-verified PFAS-free water over or through field sampling equipment before the collection of a grab sample to evaluate potential contamination from the equipment used during sample.

If any equipment blanks are performed, these results shall be reported in the comments section of the eDMR and shall also documented in the reports submitted as part of the PFOS/PFOA Minimization Plan Determination of Need schedule of the permit.

3.2.2.8 PFOS/PFOA Minimization Plan Determination of Need

The permittee shall monitor PFOS and PFOA as specified in the table above and report on the effluent concentrations including trends in monthly and annual average PFOS and PFOA concentrations as specified in the PFOS/PFOA Minimization Plan Determination of Need Schedule.

If, after reviewing the data, the Department determines that a minimization plan for PFOS and PFOA is necessary based on the procedures in s. NR 106.98(4), Wis. Adm. Code, the Department will notify the permittee in writing that a PFOS and PFOA minimization plan that satisfies the requirements in s. NR 106.99, Wis. Adm. Code, is required. The permittee shall submit an initial plan for Department approval no later than 90 days after written notification was sent from the Department in accordance with s. NR 106.985(2)(a), Wis. Adm. Code. Pursuant to s. NR 106.985(2)(b), Wis. Adm. Code, as soon as possible after Department approval of the PFOS and PFOA minimization plan, the

Department will modify or revoke and reissue the permit in accordance with public notice procedures under ch. 283, Wis. Stats., and ch. NR 203, Wis. Adm. Code, to include the PFOS and PFOA minimization plan and other related terms and condition.

If, however, the Department determines that a PFOS and PFOA minimization plan is unnecessary based on the procedures in s. NR 106.98(4), Wis. Adm. Code, the Department shall notify the permittee that no further action is required. Per s. NR 106.98(3)(a), Wis. Adm. Code, the Department may reduce monitoring frequency to once every 3 months (quarterly) on a case-by-case basis, but only after at least 12 representative results have been generated. If the permittee requests a reduction in monitoring and the Department agrees a reduction would be appropriate, the permit may be modified in accordance with public notice procedures under ch. 283, Wis. Stats., and ch. NR 203, Wis. Adm. Code, to incorporate this change.

3.2.2.9 Chloride Mass Limit – Non-Wet Weather and Alternative Wet Weather Mass Limit

This permit contains a chloride mass limit based on weather conditions. The applicable non-wet weather mass limit is **31,000 pounds/day**. The applicable wet weather mass limit is **64,400 pounds/day**. Report the applicable mass limit on the Discharge Monitoring Report form in the variable limit column. See Standard Requirements for "Applicability of Alternative Wet Weather Mass Limitations" and "Appropriate Formulas for Effluent Calculations".

Variable Chloride Mass Limitation

Parameter	Weekly Average Mass Limitation	Weekly Average Wet Weather Mass Limitation
Chloride	**31,000 lbs/day**	**64,400 lbs/day**

3.2.2.10 Whole Effluent Toxicity (WET) Testing

Primary Control Water: The Root River upstream and outside of the mixing zone of the discharge or any other known discharges.

Instream Waste Concentration (IWC): 96%

Dilution Series: At least five effluent concentrations and dual controls must be included in each test.

- **Acute:** 100, 50, 25, 12.5, 6.25% and any additional selected by the permittee.
- **Chronic:** 100, 75, 50, 25, 12.5% and any additional selected by the permittee.

WET Testing Frequency:

Acute tests are required during the following quarters:

- **Acute:** July – September 2026, April – June 2027, October – December 2028, January – March 2029, and April – June 2030

Acute WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in April – June 2031.

Chronic tests are required during the following quarters:

- **Chronic:** July – September 2026, April – June 2027, October – December 2028, January – March 2029, and April – June 2030

Chronic WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in April – June 2031.

Testing: WET testing shall be performed during normal operating conditions. Permittees are not allowed to turn off or otherwise modify treatment systems, production processes, or change other operating or treatment conditions during WET tests.

Reporting: The permittee shall report test results on the Discharge Monitoring Report form, and also complete the "Whole Effluent Toxicity Test Report Form" (Section 6, "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*"), for each test. The original, complete, signed version of the Whole Effluent Toxicity Test Report Form shall be sent to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., P.O. Box 7921, Madison, WI 53707-7921, within 45 days of test completion. The Discharge Monitoring Report (DMR) form shall be submitted electronically by the required deadline.

Determination of Positive Results: An acute toxicity test shall be considered positive if the Toxic Unit - Acute (TU_a) is greater than 1.0 for either species (fathead minnow (Pimephales promelas) and waterflea (Ceriodaphnia dubia)). The TU_a shall be calculated as follows: $TU_a = 100 \div LC_{50}$. A chronic toxicity test shall be considered positive if the Toxic Unit - Chronic (TU_c) is greater than 1.0 for either species. The TU_c shall be calculated as follows: $TU_c = 100 \div IC_{25}$.

Additional Testing Requirements: Within 90 days of a test which showed positive results, the permittee shall submit the results of at least 2 retests to the Biomonitoring Coordinator on "Whole Effluent Toxicity Test Report Forms". The 90-day reporting period shall begin the day after the test which showed a positive result. The retests shall be completed using the same species and test methods specified for the original test (see the Standard Requirements section herein).

4 Land Application Requirements

4.1 Sampling Point(s)

The discharge(s) shall be limited to land application of the waste type(s) designated for the listed sampling point(s) on Department approved land spreading sites or by hauling to another facility.

Sampling Point Designation	
Sampling Point Number	**Sampling Point Location, Waste Type/Sample Contents and Treatment Description (as applicable)**
002	Class B, anaerobically digested, centrifuge thickened, cake sludge. Representative samples shall be collected and composited from the centrifuge and sludge storage bays prior to land application.
005	Class B, anaerobically digested, liquid sludge. Representative samples shall be collected from the sludge storage tank recirculation pump. This outfall is included as an emergency outfall and the facility will need to notify the Department if this outfall needs to be used. Sampling is required annually during the calendar year only if discharge from this outfall occurs.

4.2 Monitoring Requirements and Limitations

The permittee shall comply with the following monitoring requirements and limitations.

4.2.1 Sampling Point (Outfall) 002 - Cake Sludge

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Solids, Total		Percent	Quarterly	Composite	
Arsenic Dry Wt	Ceiling	75 mg/kg	Quarterly	Composite	
Arsenic Dry Wt	High Quality	41 mg/kg	Quarterly	Composite	
Cadmium Dry Wt	Ceiling	85 mg/kg	Quarterly	Composite	
Cadmium Dry Wt	High Quality	39 mg/kg	Quarterly	Composite	
Copper Dry Wt	Ceiling	4,300 mg/kg	Quarterly	Composite	
Copper Dry Wt	High Quality	1,500 mg/kg	Quarterly	Composite	
Lead Dry Wt	Ceiling	840 mg/kg	Quarterly	Composite	
Lead Dry Wt	High Quality	300 mg/kg	Quarterly	Composite	
Mercury Dry Wt	Ceiling	57 mg/kg	Quarterly	Composite	
Mercury Dry Wt	High Quality	17 mg/kg	Quarterly	Composite	
Molybdenum Dry Wt	Ceiling	75 mg/kg	Quarterly	Composite	

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Nickel Dry Wt	Ceiling	420 mg/kg	Quarterly	Composite	
Nickel Dry Wt	High Quality	420 mg/kg	Quarterly	Composite	
Selenium Dry Wt	Ceiling	100 mg/kg	Quarterly	Composite	
Selenium Dry Wt	High Quality	100 mg/kg	Quarterly	Composite	
Zinc Dry Wt	Ceiling	7,500 mg/kg	Quarterly	Composite	
Zinc Dry Wt	High Quality	2,800 mg/kg	Quarterly	Composite	
PCB Total Dry Wt	Ceiling	50 mg/kg	Once	Composite	Monitor once in calendar year 2026.
PCB Total Dry Wt	High Quality	10 mg/kg	Once	Composite	Monitor once in calendar year 2026.
Radium 226 Dry Wt		pCi/g	Quarterly	Composite	
Nitrogen, Total Kjeldahl		Percent	Quarterly	Composite	
Nitrogen, Ammonium (NH_4-N) Total		Percent	Quarterly	Composite	
Phosphorus, Total		Percent	Quarterly	Composite	
Phosphorus, Water Extractable		% of Tot P	Quarterly	Composite	
Potassium, Total Recoverable		Percent	Quarterly	Composite	
PFOA + PFOS		µg/kg	Annual	Calculated	Report the sum of PFOA and PFOS. See PFAS Permit Sections for more information.
PFAS Dry Wt			Annual	Grab	Perfluoroalkyl and Polyfluoroalkyl Substances based on updated DNR PFAS List. See PFAS Permit Sections for more information.

Other Sludge Requirements	
Sludge Requirements	**Sample Frequency**
List 3 Requirements – Pathogen Control: The requirements in List 3 shall be met prior to land application of sludge.	**Quarterly**
List 4 Requirements – Vector Attraction Reduction: The vector attraction reduction shall be satisfied prior to, or at the time of land application as specified in List 4.	**Quarterly**

4.2.1.1 List 2 Analysis

If the monitoring frequency for List 2 parameters is more frequent than "Annual" then the sludge may be analyzed for the List 2 parameters just prior to each land application season rather than at the more frequent interval specified.

4.2.1.2 Changes in Feed Sludge Characteristics

If a change in feed sludge characteristics, treatment process, or operational procedures occurs which may result in a significant shift in sludge characteristics, the permittee shall reanalyze the sludge for List 1, 2, 3 and 4 parameters each time such change occurs.

4.2.1.3 Multiple Sludge Sample Points (Outfalls)

If there are multiple sludge sample points (outfalls), but the sludges are not subject to different sludge treatment processes, then a separate List 2 analysis shall be conducted for each sludge type which is land applied, just prior to land application, and the application rate shall be calculated for each sludge type. In this case, List 1, 3, and 4 and PCBs need only be analyzed on a single sludge type, at the specified frequency. If there are multiple sludge sample points (outfalls), due to multiple treatment processes, List 1, 2, 3 and 4 and PCBs shall be analyzed for each sludge type at the specified frequency.

4.2.1.4 Sludge Which Exceeds the High Quality Limit

Cumulative pollutant loading records shall be kept for all bulk land application of sludge which does not meet the high quality limit for any parameter. This requirement applies for the entire calendar year in which any exceedance of Table 3 of s. NR 204.07(5)(c), Wis. Adm. Code, is experienced. Such loading records shall be kept for all List 1 parameters for each site land applied in that calendar year. The formula to be used for calculating cumulative loading is as follows:

[(Pollutant concentration (mg/kg) x dry tons applied/ac) ÷ 500] + previous loading (lbs/acre) = cumulative lbs pollutant per acre

When a site reaches 90% of the allowable cumulative loading for any metal established in Table 2 of s. NR 204.07(5)(b), Wis. Adm. Code, the Department shall be so notified through letter or in the comment section of the annual land application report (3400-55).

4.2.1.5 Sludge Analysis for PCBs

The permittee shall analyze the sludge for Total PCBs one time during **2026**. The results shall be reported as "PCB Total Dry Wt". Either congener-specific analysis or Aroclor analysis shall be used to determine the PCB concentration. The permittee may determine whether Aroclor or congener specific analysis is performed. Analyses shall be performed in accordance with Table EM in s. NR 219.04, Wis. Adm. Code and the conditions specified in Standard Requirements of this permit. PCB results shall be submitted by January 31, following the specified year of analysis.

4.2.1.6 Lists 1, 2, 3, and 4

List 1 TOTAL SOLIDS AND METALS See the Monitoring Requirements and Limitations table above for monitoring frequency and limitations for the List 1 parameters
Solids, Total (percent)
Arsenic, mg/kg (dry weight)
Cadmium, mg/kg (dry weight)
Copper, mg/kg (dry weight)
Lead, mg/kg (dry weight)
Mercury, mg/kg (dry weight)
Molybdenum, mg/kg (dry weight)
Nickel, mg/kg (dry weight)
Selenium, mg/kg (dry weight)
Zinc, mg/kg (dry weight)
Radium-226, pCi/g (dry weight)

List 2 NUTRIENTS See the Monitoring Requirements and Limitations table above for monitoring frequency for the List 2 parameters
Solids, Total (percent)
Nitrogen Total Kjeldahl (percent)
Nitrogen Ammonium (NH4-N) Total (percent)
Phosphorus Total as P (percent)
Phosphorus, Water Extractable (as percent of Total P)
Potassium Total Recoverable (percent)

List 3
PATHOGEN CONTROL FOR CLASS B SLUDGE

The permittee shall implement pathogen control as listed in List 3. The Department shall be notified of the pathogen control utilized and shall be notified when the permittee decides to utilize alternative pathogen control.

The following requirements shall be met prior to land application of sludge.

Parameter	Unit	Limit
Fecal Coliform*	MPN/gTS or CFU/gTS	2,000,000
OR, ONE OF THE FOLLOWING PROCESS OPTIONS		
Aerobic Digestion	Air Drying	
Anaerobic Digestion	Composting	
Alkaline Stabilization	PSRP Equivalent Process	

***** The Fecal Coliform limit shall be reported as the geometric mean of 7 discrete samples on a dry weight basis.

List 4
VECTOR ATTRACTION REDUCTION

The permittee shall implement any one of the vector attraction reduction options specified in List 4. The Department shall be notified of the option utilized and shall be notified when the permittee decides to utilize an alternative option.

One of the following shall be satisfied prior to, or at the time of land application as specified in List 4.

Option	Limit	Where/When it Shall be Met
Volatile Solids Reduction	≥38%	Across the process
Specific Oxygen Uptake Rate	≤1.5 mg O_2/hr/g TS	On aerobic stabilized sludge
Anaerobic bench-scale test	<17 % VS reduction	On anaerobic digested sludge
Aerobic bench-scale test	<15 % VS reduction	On aerobic digested sludge
Aerobic Process	>14 days, Temp >40°C and Avg. Temp > 45°C	On composted sludge
pH adjustment	>12 S.U. (for 2 hours) and >11.5 (for an additional 22 hours)	During the process
Drying without primary solids	>75 % TS	When applied or bagged
Drying with primary solids	>90 % TS	When applied or bagged
Equivalent Process	Approved by the Department	Varies with process
Injection	-	When applied
Incorporation	-	Within 6 hours of application

4.2.1.7 Daily Land Application Log

Daily Land Application Log

Discharge Monitoring Requirements and Limitations

The permittee shall maintain a daily land application log for biosolids land applied each day when land application occurs. The following minimum records must be kept, in addition to all analytical results for the biosolids land applied. The log book records shall form the basis for the annual land application report requirements.

Parameters	Units	Sample Frequency
DNR Site Number(s)	Number	Daily as used
Outfall number applied	Number	Daily as used
Acres applied	Acres	Daily as used
Amount applied	As appropriate * /day	Daily as used
Application rate per acre	unit */acre	Daily as used
Nitrogen applied per acre	lb/acre	Daily as used
Method of Application	Injection, Incorporation, or surface applied	Daily as used

*gallons, cubic yards, dry US Tons or dry Metric Tons

4.2.1.8 Sludge Monitoring for PFAS

Sampling shall occur for perfluoroalkyl and polyfluoroalkyl compounds (PFAS) listed in the table below and as indicated in sampling point sections above. Monitoring shall occur at each sample point when sludge is generated regardless of the end use (i.e. land applied, hauled to another facility, landfilled).

PERFLUOROALKYLCARBOXILIC Acids (PFCAs)	
PFBA	Perfluorobutanoic acid
PFPeA	Perfluroropentanoic acid
PFHxA	Perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnA	Perfluroroundecanoic acid
PFDoA	Perfluorododecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PERFLUOROALKYLSULFONIC Acids (PFSAs)	
PFBS	Perfluorobutane sulfonic acid
PFPeS	Perfluroropentane sulfonic acid
PFHxS	Perfluorohexane sulfonic acid
PFHpS	Perfluoroheptane sulfonic acid
PFOS	Perfluorooctane sulfonic acid
PFNS	Perfluorononane sulfonic acid
PFDS	Perfluorodecane sulfonic acid
PFDoS	Perfluorododecane sulfonic acid
TELOMER SULFONIC Acids	
4:2FTSA	*1H,1H,2H,2H*-Perfluorohexane sulfonic acid
6:2FTSA	*1H,1H,2H,2H*-Perfluorooctane sulfonic acid
8:2FTSA	*1H,1H,2H,2H*-Perfluorodecane sulfonic acid
PERFLUOROOCTANCESULFONAMIDES (FOSAs)	
PFOSA	Perfluroroctane sulfonamide
NMeFOSA	N-Methyl perfluoroocatane sulfonamide
NEtFOSA	N-Ethyl perfluorooctane sulfonamide
PERFLUOROOCTANCESULFONAMIDOACETIC Acids	
NMeFOSAA	N-Methyl perfluoroocatane sulfonamidoacetic acid
NEtFOSAA	N-Ethyl perfluorooctane sulfonamidoacetic acid
NATIVE PERFLUOROOCTANCESULFONAMIDOETHANOLS (FOSEs)	
NMeFOSE	N-Methyl perfluorooctane sulfonamideoethanol
NEtFOSE	N-Ethyl perfluorooctane sulfonamidoethanol
PERFLUOROALKYLETHERCARBOXYLIC Acids (PFECAs)	
HFPO-DA	Hexafluoropropylene oxide dimer acid

ADONA	4,8-dioxa-3*H*-perfluorononanoic acid
PFMPA	Perfluoro-3-methoxypropanoic acid
PFMBA	Perfluoro-4-methoxybutanoic acid
NFDHA	Nonafluoro-3,6-dioxaheptaoic acid
CHLORO-PERFLUOROALKYLSULFONATE	
9Cl-PF3ONS	9-chloroehexadecafluoro-3-oxanone-1-sulfonic acid
11Cl-PF3OUdS	11-chloroelcosafluoro-3-oxaundecane-1-sulfonic acid
PFEESA	Perfluroro(2-ethoxyethane)sulfonic acid
TELOMER SULFONIC Acids	
3:3FTCA	3-Perfluoropropyl propanoic acid
5:3FTCA	*2H,2H,3H,3H*-Perfluorooctanoic acid
7:3FTCA	3-Perfluoroheptyl propanoic acid

Note: If WDNR Lab Certification removes a particular compound from the reporting list above and upon receiving written communication from the department, reporting for that compound is no longer required.

4.2.1.9 Sampling and Reporting Sludge Samples for PFAS

Representative sludge samples shall be collected at each sample point as listed. At minimum, liquid sludge storage/digesters should be thoroughly mixed prior to sampling. Cake sludge samples should consist of seven equal size discrete samples and be collected from different areas and depths then composited into one sample for laboratory analysis.

Note: If additional equipment is used for collecting sludge samples (i.e., shovels, compositing buckets, bottles, etc.), then a one-time equipment blank is recommended to be collected with the first sample. An equipment blank sample is collected by passing laboratory verified PFAS-free water over or through field sampling equipment before the collection of a representative sludge sample. The equipment blank result shall be reported on the annual Sludge Characteristics Form (3400-049) in the comment section when reporting PFAS concentrations in the sludge.

The permittee shall report each of the PFAS sludge monitoring results on the annual Sludge Characteristics and Monitoring Form (3400-049) as provided by the department. The permittee shall also report the summation of PFOS and PFOA on this same form. All results shall be reported in dry weight. The annual Sludge Characteristics and Monitoring Form (3400-049) are due January 31, of the year following the collection of the sludge samples.

The laboratory performing the analysis on any samples shall be certified for the applicable PFAS compounds in the solids matrix by the Wisconsin Laboratory Certification Program established under s. 299.11, Wis. Stats., and in accordance with s. NR 149.41, Wis. Adm. Code. The department may reject any sample results if results are produced by a laboratory that is not in compliance with certification requirements under ch. NR 149, Wis. Adm. Code.

4.2.1.10 PFAS Land Application Requirements

The department recommends the landspreading and/or land application of sludge be done in a manner consistent with the most recent version of the "Interim Strategy for Land Application of Biosolids and Industrial Sludges containing PFAS".

4.2.2 Sampling Point (Outfall) 005 - Liquid Sludge

Monitoring Requirements and Limitations					
Parameter	**Limit Type**	**Limit and Units**	**Sample Frequency**	**Sample Type**	**Notes**
Solids, Total		Percent	Annual	Composite	
Arsenic Dry Wt	Ceiling	75 mg/kg	Annual	Composite	
Arsenic Dry Wt	High Quality	41 mg/kg	Annual	Composite	
Cadmium Dry Wt	Ceiling	85 mg/kg	Annual	Composite	
Cadmium Dry Wt	High Quality	39 mg/kg	Annual	Composite	
Copper Dry Wt	Ceiling	4,300 mg/kg	Annual	Composite	
Copper Dry Wt	High Quality	1,500 mg/kg	Annual	Composite	
Lead Dry Wt	Ceiling	840 mg/kg	Annual	Composite	
Lead Dry Wt	High Quality	300 mg/kg	Annual	Composite	
Mercury Dry Wt	Ceiling	57 mg/kg	Annual	Composite	
Mercury Dry Wt	High Quality	17 mg/kg	Annual	Composite	
Molybdenum Dry Wt	Ceiling	75 mg/kg	Annual	Composite	
Nickel Dry Wt	Ceiling	420 mg/kg	Annual	Composite	
Nickel Dry Wt	High Quality	420 mg/kg	Annual	Composite	
Selenium Dry Wt	Ceiling	100 mg/kg	Annual	Composite	
Selenium Dry Wt	High Quality	100 mg/kg	Annual	Composite	
Zinc Dry Wt	Ceiling	7,500 mg/kg	Annual	Composite	
Zinc Dry Wt	High Quality	2,800 mg/kg	Annual	Composite	
Nitrogen, Total Kjeldahl		Percent	Annual	Composite	
Nitrogen, Ammonium (NH_4-N) Total		Percent	Annual	Composite	
Phosphorus, Total		Percent	Annual	Composite	
Phosphorus, Water Extractable		% of Tot P	Annual	Composite	
Potassium, Total Recoverable		Percent	Annual	Composite	

Other Sludge Requirements	
Sludge Requirements	**Sample Frequency**
List 3 Requirements – Pathogen Control: The requirements in List 3 shall be met prior to land application of sludge.	**Annual**
List 4 Requirements – Vector Attraction Reduction: The vector attraction reduction shall be satisfied prior to, or at the time of land application as specified in List 4.	**Annual**

4.2.2.1 Sample Frequency and Analytical Requirements when Sludge is Landfilled or Hauled to Another Facility

The permittee is not required to analyze for Total Kjeldahl Nitrogen, Ammonium, Total Phosphorus, Water Extractable Phosphorus, Total Recoverable Potassium, pathogens, and vector attraction parameters unless land application of sludge is initiated. As long as landfilling or hauling to another permitted facility are the sole disposal methods, only List 1 analysis is required prior to disposition. The metals limits in the table above do not apply to landfilled sludge. If sludge is land applied the sample frequency may increase based on the amount of sludge generated in accordance with Table A in s. NR 204.06, Wis. Adm. Code, and all limits and monitoring requirements listed in the table apply.

4.2.2.2 Changes in Feed Sludge Characteristics

If a change in feed sludge characteristics, treatment process, or operational procedures occurs which may result in a significant shift in sludge characteristics, the permittee shall reanalyze the sludge for List 1, 2, 3 and 4 parameters each time such change occurs.

4.2.2.3 Multiple Sludge Sample Points (Outfalls)

If there are multiple sludge sample points (outfalls), but the sludges are not subject to different sludge treatment processes, then a separate List 2 analysis shall be conducted for each sludge type which is land applied, just prior to land application, and the application rate shall be calculated for each sludge type. In this case, List 1, 3, and 4 and PCBs need only be analyzed on a single sludge type, at the specified frequency. If there are multiple sludge sample points (outfalls), due to multiple treatment processes, List 1, 2, 3 and 4 and PCBs shall be analyzed for each sludge type at the specified frequency.

4.2.2.4 Sludge Which Exceeds the High Quality Limit

Cumulative pollutant loading records shall be kept for all bulk land application of sludge which does not meet the high quality limit for any parameter. This requirement applies for the entire calendar year in which any exceedance of Table 3 of s. NR 204.07(5)(c), Wis. Adm. Code, is experienced. Such loading records shall be kept for all List 1 parameters for each site land applied in that calendar year. The formula to be used for calculating cumulative loading is as follows:

[(Pollutant concentration (mg/kg) x dry tons applied/ac) ÷ 500] + previous loading (lbs/acre) = cumulative lbs pollutant per acre

When a site reaches 90% of the allowable cumulative loading for any metal established in Table 2 of s. NR 204.07(5)(b), Wis. Adm. Code, the Department shall be so notified through letter or in the comment section of the annual land application report (3400-55).

4.2.2.5 Lists 1, 2, 3, and 4

List 1 TOTAL SOLIDS AND METALS See the Monitoring Requirements and Limitations table above for monitoring frequency and limitations for the List 1 parameters
Solids, Total (percent)
Arsenic, mg/kg (dry weight)
Cadmium, mg/kg (dry weight)
Copper, mg/kg (dry weight)
Lead, mg/kg (dry weight)
Mercury, mg/kg (dry weight)
Molybdenum, mg/kg (dry weight)
Nickel, mg/kg (dry weight)
Selenium, mg/kg (dry weight)
Zinc, mg/kg (dry weight)
Radium-226, pCi/g (dry weight)

List 2 NUTRIENTS See the Monitoring Requirements and Limitations table above for monitoring frequency for the List 2 parameters
Solids, Total (percent)
Nitrogen Total Kjeldahl (percent)
Nitrogen Ammonium (NH4-N) Total (percent)
Phosphorus Total as P (percent)
Phosphorus, Water Extractable (as percent of Total P)
Potassium Total Recoverable (percent)

List 3
PATHOGEN CONTROL FOR CLASS B SLUDGE

The permittee shall implement pathogen control as listed in List 3. The Department shall be notified of the pathogen control utilized and shall be notified when the permittee decides to utilize alternative pathogen control.

The following requirements shall be met prior to land application of sludge.

Parameter	Unit	Limit
Fecal Coliform*	MPN/gTS or CFU/gTS	2,000,000
OR, ONE OF THE FOLLOWING PROCESS OPTIONS		
Aerobic Digestion	Air Drying	
Anaerobic Digestion	Composting	
Alkaline Stabilization	PSRP Equivalent Process	

* The Fecal Coliform limit shall be reported as the geometric mean of 7 discrete samples on a dry weight basis.

List 4
VECTOR ATTRACTION REDUCTION

The permittee shall implement any one of the vector attraction reduction options specified in List 4. The Department shall be notified of the option utilized and shall be notified when the permittee decides to utilize an alternative option.

One of the following shall be satisfied prior to, or at the time of land application as specified in List 4.

Option	Limit	Where/When it Shall be Met
Volatile Solids Reduction	≥38%	Across the process
Specific Oxygen Uptake Rate	≤1.5 mg O_2/hr/g TS	On aerobic stabilized sludge
Anaerobic bench-scale test	<17 % VS reduction	On anaerobic digested sludge
Aerobic bench-scale test	<15 % VS reduction	On aerobic digested sludge
Aerobic Process	>14 days, Temp >40°C and Avg. Temp > 45°C	On composted sludge
pH adjustment	>12 S.U. (for 2 hours) and >11.5 (for an additional 22 hours)	During the process
Drying without primary solids	>75 % TS	When applied or bagged
Drying with primary solids	>90 % TS	When applied or bagged
Equivalent Process	Approved by the Department	Varies with process
Injection	-	When applied
Incorporation	-	Within 6 hours of application

4.2.2.6 Daily Land Application Log

Daily Land Application Log

Discharge Monitoring Requirements and Limitations

The permittee shall maintain a daily land application log for biosolids land applied each day when land application occurs. The following minimum records must be kept, in addition to all analytical results for the biosolids land applied. The log book records shall form the basis for the annual land application report requirements.

Parameters	Units	Sample Frequency
DNR Site Number(s)	Number	Daily as used
Outfall number applied	Number	Daily as used
Acres applied	Acres	Daily as used
Amount applied	As appropriate * /day	Daily as used
Application rate per acre	unit */acre	Daily as used
Nitrogen applied per acre	lb/acre	Daily as used
Method of Application	Injection, Incorporation, or surface applied	Daily as used

*gallons, cubic yards, dry US Tons or dry Metric Tons

5 Schedules

5.1 Dissipative Cooling Study - Outfall 006

Required Action	Due Date
Submit Dissipative Cooling Study Plan: The permittee shall submit an action plan for a Dissipative Cooling (DC) Study to be completed in the month of November. This plan should ensure that the DC requirements specified in s. NR 106.59, Wis. Adm. Code, are met. See Chapter 11 of the thermal guidance document for additional guidance on the information needed.	03/31/2027
Complete and Submit Dissipative Cooling Study: An updated dissipative cooling study for Outfall 006 meeting the following requirements shall be submitted by the date due for determining the need for sub-lethal effluent limitations at the time of next permit reissuance: 1) A written description of the physical characteristics of the receiving water or outfall that encourage rapid dilution, diffusion, dispersion, or dissipation of heat; 2) A written description of the presence or absence of other thermal loads to the receiving stream; 3) The minimum and maximum effluent temperature for each calendar week monitored. Note: The dissipative cooling study shall be conducted in the month of November. The study shall also include any site-specific information collected as part of the study, including: 1)Information regarding the biological quality of the animal and plant community of the receiving water including, but not limited to, species composition, richness, diversity, density, distribution, age structure, spawning incidence, and presence of any state or federally listed threatened or endangered species; 2) Data concerning the physical characteristics of the receiving water or permitted outfalls that encourage rapid dilution, diffusion, dispersion, and/or dissipation of heat; 3) The minimum and maximum temperature of the receiving water upstream of all permitted outfalls for each calendar month monitored.	03/31/2028

5.2 Water Quality Based Effluent Limits for Chloride - Outfalls 001 and 006

Required Action	Due Date
Chloride Progress Report #1: Submit a chloride progress report. The chloride progress report shall include: the chloride source reduction measures or activities that have been implemented; an analysis of trends in weekly and monthly average chloride concentrations and total mass discharge of chloride based on chloride sampling and flow data; and the actions the permittee plans to take to achieve compliance with the final chloride water quality based effluent limits.	12/31/2025
Achieve Compliance: The permittee shall comply with the final water quality-based chloride effluent limitations: 470 mg/L as both a weekly and monthly average for Outfall 001, and 400 mg/L as both a weekly and monthly average for Outfall 006. Additionally, the permittee shall comply with the non-wet weather and wet weather mass-based limits of 18,400 lbs/day and 75,700 lbs/day for Outfall 001, and 31,000 lbs/day and 64,400 lbs/day for Outfall 006, respectively.	11/01/2026

5.3 Year-Round Disinfection - Outfall 006

Required Action	Due Date
Submit Sampling Plan: Submit a plan for sampling instream E. coli bacteria concentrations of the Root River downstream of Outfall 006 to the Horlick Dam to support the evaluation of need for an extension of effluent disinfection beyond the period of May 1 through September 30 each year to protect recreational uses and human health pursuant to s. NR 210.06(1)(c), Wis. Adm. Code. The sampling plan shall include monitoring points at various locations between Outfall 006 and the Horlick Dam and before and after any other permitted wastewater outfalls and identified recreational sites. The plan shall include but is not limited to a map of proposed sample locations and a timeline for sampling to be conducted from October 1, 2026 through April 30, 2028 along with any other pertinent information.	06/30/2026
Initiate Downstream Sampling and Evaluation: The permittee shall initiate instream sampling in accordance with the approved sampling plan and begin evaluation of the degree and extent of E. coli bacteria present in the receiving water. The permittee shall notify the department when sampling efforts begin.	10/01/2026
Sampling Progress Report #1: Submit a progress report related to the sampling activities conducted from October 1, 2026 through April 30, 2027. Identify any proposed changes to the sampling plan. Include available sample results of samples collected and identify any changes to the sampling plan that were made based on observations that occurred during sampling events.	07/31/2027
Sampling Progress Report #2: Submit a progress report related to the sampling activities conducted from October 1, 2027 through April 30, 2028. Include available sample results of samples collected and identify any changes to the sampling plan that were made based on observations that occurred during sampling events.	07/31/2028
Evaluation Report: The permittee shall submit a final evaluation report that includes the following: 1) The E.coli bacteria levels recorded at the sample points identified in the approved sampling plan along with any additional samples that were collected. 2) An analysis of the dilution and mixing characteristics of the effluent with the receiving water and other wastewater dischargers downstream of Outfall 006 to the Horlick Dam. 3) A conclusion on the likelihood the facility will need to maintain year-round disinfection or an extended disinfection period. The permittee shall submit a request to evaluate the need for an extension of effluent disinfection beyond the period of May 1 through September 30 each year to protect recreational uses and human health pursuant to s. NR 210.06(1)(c), Wis. Adm. Code. If the department determines there is no reasonable cause to extend the disinfection period, permit modification would be required to remove or alter the year-round disinfection requirement and remaining schedule actions.	01/31/2029
Commence Year-Round Disinfection: The year-round disinfection requirement becomes effective, and the permittee shall continue disinfection past September 30th to achieve compliance with the final year-round E. coli limitations.	10/01/2029

5.4 PFOS/PFOA Minimization Plan Determination of Need

Required Action	Due Date

Report on Effluent Discharge: Submit a report on effluent PFOS and PFOA concentrations and include an analysis of trends in monthly and annual average PFOS and PFOA concentrations. This analysis should also include a comparison to the applicable narrative standard in s. NR 102.04(8)(d), Wis. Adm. Code. This report shall include all additional PFOS and PFOA data that may be collected including any influent, intake, in-plant, collection system sampling, and blank sample results.	10/31/2026
Report on Effluent Discharge and Evaluation of Need: Submit a final report on effluent PFOS and PFOA concentrations and include an analysis of trends in monthly and annual average PFOS and PFOA concentrations of data collected over the last 24 months. The report shall also provide a comparison on the likelihood of the facility needing to develop a PFOS/PFOA minimization plan. This report shall include all additional PFOS and PFOA data that may be collected including any influent, intake, in-plant, collection system sampling, and blank sample results. The permittee shall also submit a request to the department to evaluate the need for a PFOS/PFOA minimization plan. If the Department determines a PFOS/PFOA minimization plan is needed based on a reasonable potential evaluation, the permittee will be required to develop a minimization plan for Department approval no later than 90 days after written notification was sent from the Department. The Department will modify or revoke and reissue the permit to include PFOS/PFOA minimization plan reporting requirements along with a schedule of compliance to meet WQBELs. Effluent monitoring of PFOS and PFOA shall continue as specified in the permit until the modified permit is issued. If, however, the Department determines there is no reasonable potential for the facility to discharge PFOS or PFOA above the narrative standard in s. NR 102.04(8)(d), Wis. Adm. Code, no further action is required and effluent monitoring of PFOS and PFOA shall continue as specified in the permit.	10/31/2027

6 Standard Requirements

Chapter NR 205, Wisconsin Administrative Code: The conditions in ss. NR 205.07(1) and NR 205.07(2), Wis. Adm. Code, are included by reference in this permit. The permittee shall comply with all of these requirements. Some of these requirements are outlined in the Standard Requirements section of this permit. Requirements not specifically outlined in the Standard Requirement section of this permit can be found in ss. NR 205.07(1) and NR 205.07(2), Wis. Adm. Code.

6.1 Reporting and Monitoring Requirements

6.1.1 Monitoring Results

Monitoring results obtained during the previous month shall be summarized and reported on a Department Wastewater Discharge Monitoring Report. The report may require reporting of any or all of the information specified below under 'Recording of Results'. This report is to be returned to the Department no later than the date indicated on the form. A copy of the Wastewater Discharge Monitoring Report Form or an electronic file of the report shall be retained by the permittee.

Monitoring results shall be reported on an electronic discharge monitoring report (eDMR). The eDMR shall be certified electronically by a responsible executive or municipal officer, manager, partner or proprietor as specified in s. 283.37(3), Wis. Stats., or a duly authorized representative of the officer, manager, partner or proprietor that has been delegated signature authority pursuant to s. NR 205.07(1)(g)2, Wis. Adm. Code. The 'eReport Certify' page certifies that the electronic report form is true, accurate and complete.

If the permittee monitors any pollutant more frequently than required by this permit, the results of such monitoring shall be included on the Wastewater Discharge Monitoring Report.

The permittee shall comply with all limits for each parameter regardless of monitoring frequency. For example, monthly, weekly, and/or daily limits shall be met even with monthly monitoring. The permittee may monitor more frequently than required for any parameter.

6.1.2 Sampling and Testing Procedures

Sampling and laboratory testing procedures shall be performed in accordance with Chapters NR 218 and NR 219, Wis. Adm. Code, and completed by a laboratory certified or registered in accordance with the requirements of ch. NR 149, Wis. Adm. Code. Groundwater sampling shall be performed in accordance with procedures contained in s. NR 140.16, Wis. Adm. Code, and the WDNR publications, Groundwater Sampling Desk Reference (PUBL-DG-037-96) and Groundwater Sampling Field Manual (PUBL-DG-038-96). The analytical methodologies used shall enable the laboratory to quantitate all substances for which monitoring is required at levels below the effluent limitation and/or groundwater standard. If the required level cannot be met by any of the methods available in ch. NR 219, Wis. Adm. Code, then the method with the lowest limit of detection shall be selected. Additional test procedures may be specified in this permit.

6.1.3 Pretreatment Sampling Requirements

Sampling for pretreatment parameters (cadmium, chromium, copper, lead, nickel, zinc, and mercury) shall be done during a day each month when industrial discharges are occurring at normal to maximum levels. The sampling of the influent and effluent for these parameters shall be coordinated. All 24 hour composite samples shall be flow proportional.

6.1.4 Recording of Results

The permittee shall maintain records which provide the following information for each effluent measurement or sample taken:

- the date, exact place, method and time of sampling or measurements;

- the individual who performed the sampling or measurements;
- the date the analysis was performed;
- the individual who performed the analysis;
- the analytical techniques or methods used; and
- the results of the analysis.

6.1.5 Reporting of Monitoring Results

The permittee shall use the following conventions when reporting effluent monitoring results:

- Pollutant concentrations less than the limit of detection shall be reported as < (less than) the value of the limit of detection. For example, if a substance is not detected at a detection limit of 0.1 mg/L, report the pollutant concentration as < 0.1 mg/L.
- Pollutant concentrations equal to or greater than the limit of detection, but less than the limit of quantitation, shall be reported and the limit of quantitation shall be specified.
- For purposes of calculating fees under ch. NR 101, Wis. Adm. Code, a reporting limit of 2.0 mg/L for BOD_5 and 2.5 mg/L Total Suspended Solids shall be considered to be limits of quantitation.
- For the purposes of reporting a calculated result, average or a mass discharge value, the permittee may substitute a "0" (zero) for any pollutant concentration that is less than the limit of detection. However, if the effluent limitation is less than the limit of detection, the department may substitute a value other than zero for results less than the limit of detection, after considering the number of monitoring results that are greater than the limit of detection and if warranted when applying appropriate statistical techniques.
- If no discharge occurs through an outfall, flow related parameters (e.g. flow rate, hydraulic application rate, volume, etc.) should be reported as "0" (zero) at the required sample frequency specified for the outfall. For example: if the sample frequency is daily, "0" would be reported for any day during the month that no discharge occurred.

6.1.6 Compliance Maintenance Annual Reports

Compliance Maintenance Annual Reports (CMAR) shall be completed using information obtained over each calendar year regarding the wastewater conveyance and treatment system. The CMAR shall be submitted and certified by the permittee in accordance with ch. NR 208, Wis. Adm. Code, by June 30, each year on an electronic report form provided by the Department.

In the case of a publicly owned treatment works, a resolution shall be passed by the governing body and submitted as part of the CMAR, verifying its review of the report and providing responses as required. Private owners of wastewater treatment works are not required to pass a resolution; but they must provide an Owner Statement and responses as required, as part of the CMAR submittal.

The CMAR shall be certified electronically by a responsible executive or municipal officer, manager, partner or proprietor as specified in s. 283.37(3), Wis. Stats., or a duly authorized representative of the officer, manager, partner or proprietor that has been delegated signature authority pursuant to s. NR 205.07(1)(g)2, Wis. Adm. Code. The certification verifies that the electronic report is true, accurate and complete.

6.1.7 Records Retention

The permittee shall retain records of all monitoring information, including all calibration and maintenance records and all original strip chart recordings or electronic data records for continuous monitoring instrumentation, copies of all reports required by the permit, and records of all data used to complete the application for the permit for a period of at least 3 years from the date of the sample, measurement, report or application. All pertinent sludge information, including permit application information and other documents specified in this permit or s. NR 204.06(9), Wis. Adm. Code shall be retained for a minimum of 5 years.

6.1.8 Other Information

Where the permittee becomes aware that it failed to submit any relevant facts in a permit application or submitted incorrect information in a permit application or in any report to the Department, it shall promptly submit such facts or correct information to the Department.

6.1.9 Reporting Requirements – Alterations or Additions

The permittee shall give notice to the Department as soon as possible of any planned physical alterations or additions to the permitted facility. Notice is only required when:

- The alteration or addition to the permitted facility may meet one of the criteria for determining whether a facility is a new source.
- The alteration or addition could significantly change the nature or increase the quantity of pollutants discharged. This notification requirement applies to pollutants which are not subject to effluent limitations in the existing permit.
- The alteration or addition results in a significant change in the permittee's sludge use or disposal practices, and such alteration, addition, or change may justify the application of permit conditions that are different from or absent in the existing permit, including notification of additional use of disposal sites not reported during the permit application process nor reported pursuant to an approved land application plan. Additional sites may not be used for the land application of sludge until department approval is received.

6.2 System Operating Requirements

6.2.1 Noncompliance Reporting

Sanitary sewer overflows and sewage treatment facility overflows shall be reported according to the 'Sanitary Sewer Overflows and Sewage Treatment Facility Overflows' section of this permit.

The permittee shall report the following types of noncompliance by a telephone call to the Department's regional office within 24 hours after becoming aware of the noncompliance:

- any noncompliance which may endanger health or the environment;
- any violation of an effluent limitation resulting from a bypass;
- any violation of an effluent limitation resulting from an upset; and
- any violation of a maximum discharge limitation for any of the pollutants listed by the Department in the permit, either for effluent or sludge.

A written report describing the noncompliance shall also be submitted to the Department's regional office within 5 days after the permittee becomes aware of the noncompliance. On a case-by-case basis, the Department may waive the requirement for submittal of a written report within 5 days and instruct the permittee to submit the written report with the next regularly scheduled monitoring report. In either case, the written report shall contain a description of the noncompliance and its cause; the period of noncompliance, including exact dates and times; the steps taken or planned to reduce, eliminate and prevent reoccurrence of the noncompliance; and if the noncompliance has not been corrected, the length of time it is expected to continue.

A scheduled bypass approved by the Department under the 'Scheduled Bypass' section of this permit shall not be subject to the reporting required under this section.

NOTE: Section 292.11(2)(a), Wisconsin Statutes, requires any person who possesses or controls a hazardous substance or who causes the discharge of a hazardous substance to notify the Department of Natural Resources **immediately** of any discharge not authorized by the permit. **The discharge of a hazardous substance that is not authorized by this permit or that violates this permit may be a hazardous substance spill. To report a hazardous substance spill, call DNR's 24-hour HOTLINE at 1-800-943-0003.**

6.2.2 Flow Meters

Flow meters shall be calibrated annually, as per s. NR 218.06, Wis. Adm. Code.

6.2.3 Raw Grit and Screenings

All raw grit and screenings shall be disposed of at a properly licensed solid waste facility or picked up by a licensed waste hauler. If the facility or hauler are located in Wisconsin, then they shall be licensed under chs. NR 500-555, Wis. Adm. Code.

6.2.4 Sludge Management

All sludge management activities shall be conducted in compliance with ch. NR 204 "Domestic Sewage Sludge Management", Wis. Adm. Code.

6.2.5 Prohibited Wastes

Under no circumstances may the introduction of wastes prohibited by s. NR 211.10, Wis. Adm. Code, be allowed into the waste treatment system. Prohibited wastes include those:

- which create a fire or explosion hazard in the treatment work;
- which will cause corrosive structural damage to the treatment work;
- solid or viscous substances in amounts which cause obstructions to the flow in sewers or interference with the proper operation of the treatment work;
- wastewaters at a flow rate or pollutant loading which are excessive over relatively short time periods so as to cause a loss of treatment efficiency; and
- changes in discharge volume or composition from contributing industries which overload the treatment works or cause a loss of treatment efficiency.

6.2.6 Bypass

This condition applies only to bypassing at a sewage treatment facility that is not a scheduled bypass, approved blending as a specific condition of this permit, a sewage treatment facility overflow or a controlled diversion as provided in the sections titled ‘Scheduled Bypass’, ‘Blending’ (if approved), ‘SSO’s and Sewage Treatment Facility Overflows’ and ‘Controlled Diversions’ of this permit. Any other bypass at the sewage treatment facility is prohibited and the Department may take enforcement action against a permittee for such occurrences under s. 283.89, Wis. Stats. The Department may approve a bypass if the permittee demonstrates all the following conditions apply:

- The bypass was unavoidable to prevent loss of life, personal injury, or severe property damage;
- There were no feasible alternatives to the bypass, such as the use of auxiliary treatment facilities or adequate back-up equipment, retention of untreated wastes, reduction of inflow and infiltration, or maintenance during normal periods of equipment downtime. This condition is not satisfied if adequate back-up equipment should have been installed in the exercise of reasonable engineering judgment to prevent a bypass which occurred during normal periods of equipment downtime or preventive maintenance. When evaluating feasibility of alternatives, the department may consider factors such as technical achievability, costs and affordability of implementation and risks to public health, the environment and, where the permittee is a municipality, the welfare of the community served; and
- The bypass was reported in accordance with the Noncompliance Reporting section of this permit.

6.2.7 Scheduled Bypass

Whenever the permittee anticipates the need to bypass for purposes of efficient operations and maintenance and the permittee may not meet the conditions for controlled diversions in the ‘Controlled Diversions’ section of this permit, the permittee shall obtain prior written approval from the Department for the scheduled bypass. A permittee’s written

request for Department approval of a scheduled bypass shall demonstrate that the conditions for bypassing specified in the above section titled 'Bypass' are met and include the proposed date and reason for the bypass, estimated volume and duration of the bypass, alternatives to bypassing and measures to mitigate environmental harm caused by the bypass. The department may require the permittee to provide public notification for a scheduled bypass if it is determined there is significant public interest in the proposed action and may recommend mitigation measures to minimize the impact of such bypass.

6.2.8 Controlled Diversions

Controlled diversions are allowed only when necessary for essential maintenance to assure efficient operation. Sewage treatment facilities that have multiple treatment units to treat variable or seasonal loading conditions may shut down redundant treatment units when necessary for efficient operation. The following requirements shall be met during controlled diversions:

- Effluent from the sewage treatment facility shall meet the effluent limitations established in the permit. Wastewater that is diverted around a treatment unit or treatment process during a controlled diversion shall be recombined with wastewater that is not diverted prior to the effluent sampling location and prior to effluent discharge;
- A controlled diversion does not include blending as defined in s. NR 210.03(2e), Wis. Adm. Code, and as may only be approved under s. NR 210.12, Wis. Adm. Code. A controlled diversion may not occur during periods of excessive flow or other abnormal wastewater characteristics;
- A controlled diversion may not result in a wastewater treatment facility overflow; and
- All instances of controlled diversions shall be documented in sewage treatment facility records and such records shall be available to the department on request.

6.2.9 Proper Operation and Maintenance

The permittee shall at all times properly operate and maintain all facilities and systems of treatment and control which are installed or used by the permittee to achieve compliance with the conditions of this permit. Proper operation and maintenance includes effective performance, adequate funding, adequate operator staffing and training as required in ch. NR 114, Wis. Adm. Code, and adequate laboratory and process controls, including appropriate quality assurance procedures. This provision requires the operation of back-up or auxiliary facilities or similar systems only when necessary to achieve compliance with the conditions of the permit.

6.2.10 Operator Certification

The wastewater treatment facility shall be under the direct supervision of a state certified operator. In accordance with s. NR 114.53, Wis. Adm. Code, every WPDES permitted treatment plant shall have a designated operator-in-charge holding a current and valid certificate. The designated operator-in-charge shall be certified at the level and in all subclasses of the treatment plant, except laboratory. Treatment plant owners shall notify the department of any changes in the operator-in-charge within 30 days. Note that s. NR 114.52(22), Wis. Adm. Code, lists types of facilities that are excluded from operator certification requirements (i.e. private sewage systems, pretreatment facilities discharging to public sewers, industrial wastewater treatment that consists solely of land disposal, agricultural digesters and concentrated aquatic production facilities with no biological treatment).

6.3 Sewage Collection Systems

6.3.1 Sanitary Sewage Overflows and Sewage Treatment Facility Overflows

6.3.1.1 Overflows Prohibited

Any overflow or discharge of wastewater from the sewage collection system or at the sewage treatment facility, other than from permitted outfalls, is prohibited. The permittee shall provide information on whether any of the following conditions existed when an overflow occurred:

- The sanitary sewer overflow or sewage treatment facility overflow was unavoidable to prevent loss of life, personal injury or severe property damage;
- There were no feasible alternatives to the sanitary sewer overflow or sewage treatment facility overflow such as the use of auxiliary treatment facilities or adequate back-up equipment, retention of untreated wastes, reduction of inflow and infiltration, or preventive maintenance activities;
- The sanitary sewer overflow or the sewage treatment facility overflow was caused by unusual or severe weather-related conditions such as large or successive precipitation events, snowmelt, saturated soil conditions, or severe weather occurring in the area served by the sewage collection system or sewage treatment facility; and
- The sanitary sewer overflow or the sewage treatment facility overflow was unintentional, temporary, and caused by an accident or other factors beyond the reasonable control of the permittee.

6.3.1.2 Permittee Response to Overflows

Whenever a sanitary sewer overflow or sewage treatment facility overflow occurs, the permittee shall take all feasible steps to control or limit the volume of untreated or partially treated wastewater discharged, and terminate the discharge as soon as practicable. Remedial actions, including those in s. NR 210.21 (3), Wis. Adm. Code, shall be implemented consistent with an emergency response plan developed under the CMOM program.

6.3.1.3 Permittee Reporting

Permittees shall report all sanitary sewer overflows and sewage treatment overflows as follows:

- The permittee shall notify the department by telephone, fax or email as soon as practicable, but no later than 24 hours from the time the permittee becomes aware of the overflow;
- The permittee shall, no later than five days from the time the permittee becomes aware of the overflow, provide to the department the information identified in this paragraph using department form number 3400-184. If an overflow lasts for more than five days, an initial report shall be submitted within 5 days as required in this paragraph and an updated report submitted following cessation of the overflow. At a minimum, the following information shall be included in the report:
    - The date and location of the overflow;
    - The surface water to which the discharge occurred, if any;
    - The duration of the overflow and an estimate of the volume of the overflow;
    - A description of the sewer system or treatment facility component from which the discharge occurred such as manhole, lift station, constructed overflow pipe, or crack or other opening in a pipe;
    - The estimated date and time when the overflow began and stopped or will be stopped;
    - The cause or suspected cause of the overflow including, if appropriate, precipitation, runoff conditions, areas of flooding, soil moisture and other relevant information;
    - Steps taken or planned to reduce, eliminate and prevent reoccurrence of the overflow and a schedule of major milestones for those steps;
    - A description of the actual or potential for human exposure and contact with the wastewater from the overflow;
    - Steps taken or planned to mitigate the impacts of the overflow and a schedule of major milestones for those steps;
    - To the extent known at the time of reporting, the number and location of building backups caused by excessive flow or other hydraulic constraints in the sewage collection system that occurred concurrently with the sanitary sewer overflow and that were within the same area of the sewage collection system as the sanitary sewer overflow; and

- The reason the overflow occurred or explanation of other contributing circumstances that resulted in the overflow event. This includes any information available including whether the overflow was unavoidable to prevent loss of life, personal injury, or severe property damage and whether there were feasible alternatives to the overflow.

  NOTE: A copy of form 3400-184 for reporting sanitary sewer overflows and sewage treatment facility overflows may be obtained from the department or accessed on the department's web site at http://dnr.wi.gov/topic/wastewater/SSOreport.html. As indicated on the form, additional information may be submitted to supplement the information required by the form.

- The permittee shall identify each specific location and each day on which a sanitary sewer overflow or sewage treatment facility overflow occurs as a discrete sanitary sewer overflow or sewage treatment facility overflow occurrence. An occurrence may be more than one day if the circumstances causing the sanitary sewer overflow or sewage treatment facility overflow results in a discharge duration of greater than 24 hours. If there is a stop and restart of the overflow at the same location within 24 hours and the overflow is caused by the same circumstance, it may be reported as one occurrence. Sanitary sewer overflow occurrences at a specific location that are separated by more than 24 hours shall be reported as separate occurrences; and
- A permittee that is required to submit wastewater discharge monitoring reports under s. NR 205.07 (1) (r), Wis. Adm. Code, shall also report all sanitary sewer overflows and sewage treatment facility overflows on that report.

6.3.1.4 Public Notification

The permittee shall notify the public of any sanitary sewer and sewage treatment facility overflows consistent with its emergency response plan required under the CMOM (Capacity, Management, Operation and Maintenance) section of this permit and s. NR 210.23 (4) (f), Wis. Adm. Code. Such public notification shall occur promptly following any overflow event using the most effective and efficient communications available in the community. At minimum, a daily newspaper of general circulation in the county(s) and municipality whose waters may be affected by the overflow shall be notified by written or electronic communication.

6.3.2 Capacity, Management, Operation and Maintenance (CMOM) Program

- The permittee shall have written documentation of the Capacity, Management, Operation and Maintenance (CMOM) program components in accordance with s. NR 210.23(4), Wis. Adm. Code. Such documentation shall be available for Department review upon request. The Department may request that the permittee provide this documentation or prepare a summary of the permittee's CMOM program at the time of application for reissuance of the WPDES permit.
- The permittee shall implement a CMOM program in accordance with s. NR 210.23, Wis. Adm. Code.
- The permittee shall at least annually conduct a self-audit of activities conducted under the permittee's CMOM program to ensure CMOM components are being implemented as necessary to meet the general standards of s. NR 210.23(3), Wis. Adm. Code.

6.3.3 Sewer Cleaning Debris and Materials

All debris and material removed from cleaning sanitary sewers shall be managed to prevent nuisances, run-off, ground infiltration or prohibited discharges.

- Debris and solid waste shall be dewatered, dried and then disposed of at a licensed solid waste facility.
- Liquid waste from the cleaning and dewatering operations shall be collected and disposed of at a permitted wastewater treatment facility.
- Combination waste including liquid waste along with debris and solid waste may be disposed of at a licensed solid waste facility or wastewater treatment facility willing to accept the waste.

6.4 Surface Water Requirements

6.4.1 Permittee-Determined Limit of Quantitation Incorporated into this Permit

For pollutants with water quality-based effluent limits below the Limit of Quantitation (LOQ) in this permit, the LOQ calculated by the permittee and reported on the Discharge Monitoring Reports (DMRs) is incorporated by reference into this permit. The LOQ shall be reported on the DMRs, shall be the lowest quantifiable level practicable, and shall be no greater than the minimum level (ML) specified in or approved under 40 CFR Part 136 for the pollutant at the time this permit was issued, unless this permit specifies a higher LOQ.

6.4.2 Appropriate Formulas for Effluent Calculations

The permittee shall use the following formulas for calculating effluent results to determine compliance with average concentration limits and mass limits and total load limits:

Weekly/Monthly/Six-Month/Annual Average Concentration = the sum of all daily results for that week/month/six-month/year, divided by the number of results during that time period. [Note: When a six-month average effluent limit is specified for Total Phosphorus the applicable periods are May through October and November through April, except in cases of Water Quality Trading, wherein the applicable periods are January through June and July through December.]

Weekly Average Mass Discharge (lbs/day): Daily mass = daily concentration (mg/L) x daily flow (MGD) x 8.34, then average the daily mass values for the week.

Monthly Average Mass Discharge (lbs/day): Daily mass = daily concentration (mg/L) x daily flow (MGD) x 8.34, then average the daily mass values for the month.

Six-Month Average Mass Discharge (lbs/day): Daily mass = daily concentration (mg/L) x daily flow (MGD) x 8.34, then average the daily mass values for the six-month period. [Note: When a six-month average effluent limit is specified for Total Phosphorus the applicable periods are May through October and November through April.]

Annual Average Mass Discharge (lbs/day): Daily mass = daily concentration (mg/L) x daily flow (MGD) x 8.34, then average the daily mass values for the entire year.

Total Monthly Discharge: = monthly average concentration (mg/L) x total flow for the month (MG/month) x 8.34.

Total Annual Discharge: = sum of total monthly discharges for the calendar year.

12-Month Rolling Sum of Total Monthly Discharge: = the sum of the most recent 12 consecutive months of Total Monthly Discharges.

6.4.3 Effluent Temperature Requirements

Weekly Average Temperature – If temperature limits are included in this permit, Weekly Average Temperature shall be calculated as the sum of all daily maximum results for that week divided by the number of daily maximum results during that time period.

Cold Shock Standard – Water temperatures of the discharge shall be controlled in a manner as to protect fish and aquatic life uses from the deleterious effects of cold shock pursuant to Wis. Adm. Code, s. NR 102.28. 'Cold Shock' means exposure of aquatic organisms to a rapid decrease in temperature and a sustained exposure to low temperature that induces abnormal behavior or physiological performance and may lead to death.

Rate of Temperature Change Standard – Temperature of a water of the state or discharge to a water of the state may not be artificially raised or lowered at such a rate that it causes detrimental health or reproductive effects to fish or aquatic life of the water of the state pursuant to Wis. Adm. Code, s. NR 102.29.

6.4.4 Visible Foam or Floating Solids

There shall be no discharge of floating solids or visible foam in other than trace amounts.

6.4.5 Surface Water Uses and Criteria

In accordance with NR 102.04, Wis. Adm. Code, surface water uses and criteria are established to govern water management decisions. Practices attributable to municipal, industrial, commercial, domestic, agricultural, land development or other activities shall be controlled so that all surface waters including the mixing zone meet the following conditions at all times and under all flow and water level conditions:

a) Substances that will cause objectionable deposits on the shore or in the bed of a body of water, shall not be present in such amounts as to interfere with public rights in waters of the state.

b) Floating or submerged debris, oil, scum or other material shall not be present in such amounts as to interfere with public rights in waters of the state.

c) Materials producing color, odor, taste or unsightliness shall not be present in such amounts as to interfere with public rights in waters of the state.

d) Substances in concentrations or in combinations which are toxic or harmful to humans shall not be present in amounts found to be of public health significance, nor shall substances be present in amounts which are acutely harmful to animal, plant or aquatic life.

6.4.6 Percent Removal

During any 30 consecutive days, the average effluent concentrations of BOD_5 and of total suspended solids shall not exceed 15% of the average influent concentrations, respectively. This requirement does not apply to removal of total suspended solids if the permittee operates a lagoon system and has received a variance for suspended solids granted under NR 210.07(2), Wis. Adm. Code.

6.4.7 *E. coli*

The monthly limit for *E. coli* shall be expressed as a geometric mean. In calculating the geometric mean, a value of 1 is used for any result of 0.

6.4.8 Year-Round Disinfection (*E. coli* Only)

Disinfection shall be provided year-round. Monitoring requirements and the limitations for *E. coli* apply during the period in which disinfection is required. Whenever chlorine is used for disinfection or other effluent uses, the limitations and monitoring requirements for residual chlorine shall apply. A dechlorination process shall be in operation whenever chlorine is used for disinfection or other effluent uses.

6.4.9 Applicability of Alternative Wet Weather Mass Limitations

An alternative wet weather mass limitation applies when:

- The applicable mass limitation (based on annual average design flow) is exceeded; and
- The permittee demonstrates to the satisfaction of the Department that the discharge exceedance is caused by and occurs during a wet weather event. For the purposes of this demonstration, a wet weather event occurs during and immediately following periods of precipitation or snowmelt, including but not limited to rain, sleet, snow, hail or melting snow during which water from the precipitation, snowmelt or elevated groundwater enters the sewerage system through infiltration or inflow, or both. The permittee shall present demonstrations to the Department by attaching them to the Wastewater Discharge Monitoring Report Form(s).

Note: In making this demonstration, the permittee may want to consider presenting a discussion of normal effluent flow rates, the effluent flow rates that resulted in the exceedance and identification of the event, including intensity and duration, which caused the high flow rates. A graph of effluent flow over time may also be helpful.

6.4.10 Whole Effluent Toxicity (WET) Monitoring Requirements

In order to determine the potential impact of the discharge on aquatic organisms, static-renewal toxicity tests shall be performed on the effluent in accordance with the procedures specified in the *"State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition" (PUB-WT-797*, November 2004) as required by NR 219.04, Table A, Wis. Adm. Code). All of the WET tests required in this permit, including any required retests, shall be conducted on the *Ceriodaphnia dubia* and fathead minnow species. Receiving water samples shall not be collected from any point in contact with the permittee's mixing zone and every attempt shall be made to avoid contact with any other discharge's mixing zone.

6.4.11 Whole Effluent Toxicity (WET) Identification and Reduction

Within 60 days of a retest which showed positive results, the permittee shall submit a written report to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., PO Box 7921, Madison, WI 53707-7921, which details the following:

- A description of actions the permittee has taken or will take to remove toxicity and to prevent the recurrence of toxicity;
- A description of toxicity reduction evaluation (TRE) investigations that have been or will be done to identify potential sources of toxicity, including the following actions:
    a) Evaluate the performance of the treatment system to identify deficiencies contributing to effluent toxicity (e.g., operational problems, chemical additives, incomplete treatment)
    b) Identify the compound(s) causing toxicity. Conduct toxicity screening tests on the effluent at a minimum of once per month for six months to determine if toxicity recurs. Screening tests are WET tests using fewer effluent concentrations conducted on the most sensitive species. If any of the screening tests contain toxicity, conduct a toxicity identification evaluation (TIE) to determine the cause. TIE methods are available from USEPA "Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures (EPA/600/6-91/003) and "Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I" (EPA/600/6-91/005F).
    c) Trace the compound(s) causing toxicity to their sources (e.g., industrial, commercial, domestic)
    d) Evaluate, select, and implement methods or technologies to control effluent toxicity (e.g., in-plant or pretreatment controls, source reduction or removal)
- Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented;
- If no actions have been taken, the reason for not taking action.

The permittee may also request approval from the Department to postpone additional retests in order to investigate the source(s) of toxicity. Postponed retests must be completed after toxicity is believed to have been removed.

6.4.12 PFOS and PFOA Requirements

The laboratory performing the analysis on any samples shall be certified for the applicable PFAS compounds in the aqueous matrix by the Wisconsin Laboratory Certification Program established under s. 299.11, Wis. Stats., in accordance with s. NR 149.41, Wis. Adm. Code. All laboratories are required to utilize EPA Method 1633A for sampling PFAS in sludge.

The Department may reject any sample results if results are produced by a laboratory that is not in compliance with certification requirements under ch. NR 149, Wis. Adm. Code.

6.5 Pretreatment Program Requirements

The permittee is required to operate an industrial pretreatment program as described in the program initially approved by the Department of Natural Resources including any subsequent program modifications approved by the Department, and including commitments to program implementation activities provided in the permittee's annual pretreatment program report, and that complies with the requirements set forth in 40 CFR Part 403 and ch. NR 211, Wis. Adm. Code. To ensure that the program is operated in accordance with these requirements, the following general conditions and requirements are hereby established:

6.5.1 Inventories

The permittee shall implement methods to maintain a current inventory of the general character and volume of wastewater that industrial users discharge to the treatment works and shall provide an updated industrial user listing annually and report any changes in the listing to the Department by March 31 of each year as part of the annual pretreatment program report required herein.

6.5.2 Regulation of Industrial Users

6.5.2.1 Limitations for Industrial Users:

The permittee shall develop, maintain, enforce and revise as necessary local limits to implement the general and specific prohibitions of the state and federal General Pretreatment Regulations.

6.5.2.2 Control Documents for Industrial Users (IUs)

The permittee shall control the discharge from each significant industrial user through individual discharge permits as required by s. NR 211.235, Wis. Adm. Code and in accordance with the approved pretreatment program procedures and the permittee's sewer use ordinance. The discharge permits shall be modified in a timely manner during the stated term of the discharge permits according to the sewer use ordinance as conditions warrant. The discharge permits shall include at a minimum the elements found in s. NR 211.235(1), Wis. Adm. Code and references to the approved pretreatment program procedures and the sewer use ordinance.

6.5.2.3 Review of Industrial User Reports, Inspections and Compliance Monitoring

The permittee shall require the submission of, receive, and review self-monitoring reports and other notices from industrial users in accordance with the approved pretreatment program procedures. The permittee shall randomly sample and analyze industrial user discharges and conduct surveillance activities to determine independent of information supplied by the industrial users, whether the industrial users are in compliance with pretreatment standards and requirements. The inspections and monitoring shall also be conducted to maintain accurate knowledge of local industrial processes, including changes in the discharge, pretreatment equipment operation, spill prevention control plans, slug control plans, and implementation of solvent management plans.

The permittee shall inspect and sample the discharge from each significant industrial user as specified in the permittee's approved pretreatment program or as specified in NR 211.235(3). The permittee shall evaluate whether industrial users identified as significant need a slug control plan according to the requirements of NR 211.235(4). If a slug control plan is needed, the plan shall contain at a minimum the elements specified in s. NR 211.235(4)(b), Wis. Adm. Code.

6.5.2.4 Enforcement and Industrial User Compliance Evaluation & Violation Reports

The permittee shall enforce the industrial pretreatment requirements including the industrial user discharge limitations of the permittee's sewer use ordinance. The permittee shall investigate instances of noncompliance by collecting and analyzing samples and collecting other information with sufficient care to produce evidence admissible in enforcement proceedings or in judicial actions. Investigation and response to instances of noncompliance shall be in accordance with the permittee's sewer use ordinance and approved Enforcement Response Plan.

The permittee shall make a semiannual report on forms provided or approved by the Department. The semiannual report shall include an analysis of industrial user significant noncompliance (i.e. the Industrial User Compliance Evaluation, also known as the SNC Analysis) as outlined in s.NR 211.23(1)(j), Wis. Adm. Code, and a summary of the permittee's response to all industrial noncompliance (i.e. the Industrial User Violation Report). The Industrial User Compliance Evaluation Report shall include monitoring results received from industrial users pursuant to s. NR 211.15(1)-(5), Wis. Adm. Code. The Industrial User Violation Report shall include copies of all notices of noncompliance, notices of violation and other enforcement correspondence sent by the permittee to industrial users, together with the industrial user's response. The Industrial User Compliance Evaluation and Violation Reports for the period January through June shall be provided to the Department by September 30 of each year and for the period July through December shall be provided to the Department by March 31 of the succeeding year, unless alternate submittal dates are approved.

6.5.2.5 Publication of Violations

The permittee shall publish a list of industrial users that have significantly violated the municipal sewer use ordinance during the calendar year, in the largest daily newspaper in the area by March 31 of the following year pursuant to s. NR 211.23(1)(j), Wis. Adm. Code. A copy of the newspaper publication shall be provided as part of the annual pretreatment report specified herein.

6.5.2.6 Multijurisdictional Agreements

The permittee shall establish agreements with all contributing jurisdictions as necessary to ensure compliance with pretreatment standards and requirements by all industrial users discharging to the permittee's wastewater treatment system. Any such agreement shall identify who will be responsible for maintaining the industrial user inventory, issuance of industrial user control mechanisms, inspections and sampling, pretreatment program implementation, and enforcement.

6.5.3 Annual Pretreatment Program Report

The permittee shall evaluate the pretreatment program, and submit the Pretreatment Program Report to the Department on forms provided or approved by the Department by March 31 annually, unless an alternate submittal date is approved. The report shall include a brief summary of the work performed during the preceding calendar year, including the numbers of discharge permits issued and in effect, pollution prevention activities, number of inspections and monitoring surveys conducted, budget and personnel assigned to the program, a general discussion of program progress in meeting the objectives of the permittee's pretreatment program together with summary comments and recommendations.

6.5.4 Pretreatment Program Modifications

- Future Modifications: The permittee shall within one year of any revisions to federal or state General Pretreatment Regulations submit an application to the Department in duplicate to modify and update its approved pretreatment program to incorporate such regulatory changes as applicable to the permittee. Additionally, the Department or the permittee may request an application for program modification at any time where necessary to improve program effectiveness based on program experience to date.
- Modifications Subject to Department Approval: The permittee shall submit all proposed pretreatment program modifications to the Department for determination of significance and opportunity for comment in accordance with the requirements and conditions of s. NR 211.27, Wis. Adm. Code. Any substantial proposed program modification shall be subject to Department public noticing and formal approval prior to implementation. A substantial program modification includes, but is not limited to, changes in enabling legal authority to administer and enforce pretreatment conditions and requirements; significant changes in program administrative or operational procedures; significant reductions in monitoring frequencies; significant reductions in program resources including personnel commitments, equipment, and funding levels; changes (including any relaxation) in the local limitations for substances enforced and applied to users of the sewerage treatment works; changes in treatment works sludge disposal or management practices which impact the

pretreatment program; or program modifications which increase pollutant loadings to the treatment works. The Department shall use the procedures outlined in s. NR 211.30, Wis. Adm. Code for review and approval/denial of proposed pretreatment program modifications. The permittee shall comply with local public participation requirements when implementing the pretreatment program.

6.5.5 Program Resources

The permittee shall have sufficient resources and qualified personnel to carry out the pretreatment program responsibilities as listed in ss. NR 211.22 and NR 211.23, Wis. Adm. Code.

6.6 Land Application Requirements

6.6.1 Sludge Management Program Standards And Requirements Based Upon Federally Promulgated Regulations

In the event that new federal sewage sludge standards or regulations are promulgated, the permittee shall comply with the new sewage sludge requirements by the dates established in the regulations, if required by federal law, even if the permit has not yet been modified to incorporate the new federal regulations.

6.6.2 General Sludge Management Information

The General Sludge Management Form 3400-48 shall be completed and submitted prior to any significant sludge management changes.

6.6.3 Sludge Samples

All sludge samples shall be collected at a point and in a manner which will yield sample results which are representative of the sludge being tested, and collected at the time which is appropriate for the specific test.

6.6.4 Land Application Characteristic Report

Each report shall consist of a Characteristic Form 3400-49 and Lab Report. The Characteristic Report Form 3400-49 shall be submitted electronically by January 31 following each year whether or not samples are analyzed. In years in which monitoring does not occur, the report shall be completed by checking on the form that monitoring/land application did not occur.

Following submittal of the electronic Characteristic Report Form 3400-49, this form shall be certified electronically via the 'eReport Certify' page by a responsible executive or municipal officer, manager, partner or proprietor as specified in s. 283.37(3), Wis. Stats., or a duly authorized representative of the officer, manager, partner or proprietor that has been delegated signature authority pursuant to s. NR 205.07(1)(g)2, Wis. Adm. Code. The 'eReport Certify' page certifies that the electronic report is true, accurate and complete. The Lab Report must be sent directly to the facility's DNR sludge representative or basin engineer unless approval for not submitting the lab reports has been given.

The permittee shall use the following convention when reporting sludge monitoring results: Pollutant concentrations less than the limit of detection shall be reported as < (less than) the value of the limit of detection. For example, if a substance is not detected at a detection limit of 1.0 mg/kg, report the pollutant concentration as < 1.0 mg/kg .

All results shall be reported on a dry weight basis.

6.6.5 Calculation of Water Extractable Phosphorus

When sludge analysis for Water Extractable Phosphorus is required by this permit, the permittee shall use the following formula to calculate and report Water Extractable Phosphorus:
Water Extractable Phosphorus (% of Total P) =
[Water Extractable Phosphorus (mg/kg, dry wt) ÷ Total Phosphorus (mg/kg, dry wt)] x 100

6.6.6 Monitoring and Calculating PCB Concentrations in Sludge

When sludge analysis for "PCB, Total Dry Wt" is required by this permit, the PCB concentration in the sludge shall be determined using either congener-specific analysis or Aroclor analysis. The permittee may decide which of these analyses is performed. Analyses shall be performed in accordance with the following provisions and Table EM in s. NR 219.04, Wis. Adm. Code:

- If congener-specific analysis is employed: All PCB congeners shall be delineated. Non-detects shall be treated as zero. The values that are between the limit of detection (LOD) and the limit of quantitation shall be used when calculating the total value of all congeners. All results shall be added together and the total PCB concentration by dry weight reported.
- If Aroclor analysis is employed, reporting protocols, consistent with s. NR 106.07(6)(e), should be as follows: If all Aroclors are less than the LOD, then the Total PCB Dry Wt result should be reported as less than the highest LOD. If a single Aroclor is detected, then that is what should be reported for the Total PCB result. If multiple Aroclors are detected, they should be summed and reported as Total PCBs. If the LOD cannot be achieved after using the appropriate clean up techniques, a reporting limit that is achievable for the Aroclors or each congener for the sample shall be determined. This reporting limit shall be reported and qualified indicating the presence of an interference.

6.6.7 Annual Land Application Report

Land Application Report Form 3400-55 shall be submitted electronically by January 31, each year whether or not non-exceptional quality sludge is land applied. Non-exceptional quality sludge is defined in s. NR 204.07(4), Wis. Adm. Code. Following submittal of the electronic Annual Land Application Report Form 3400-55, this form shall be certified electronically via the 'eReport Certify' page by a responsible executive or municipal officer, manager, partner or proprietor as specified in s. 283.37(3), Wis. Stats., or a duly authorized representative of the officer, manager, partner or proprietor that has been delegated signature authority pursuant to s. NR 205.07(1)(g)2, Wis. Adm. Code. The 'eReport Certify' page certifies that the electronic report form is true, accurate and complete.

6.6.8 Other Methods of Disposal or Distribution Report

The permittee shall submit electronically the Other Methods of Disposal or Distribution Report Form 3400-52 by January 31, each year whether or not sludge is hauled, landfilled, incinerated, or exceptional quality sludge is distributed or land applied. Following submittal of the electronic Report Form 3400-52, this form shall be certified electronically via the 'eReport Certify' page by a responsible executive or municipal officer, manager, partner or proprietor as specified in s. 283.37(3), Wis. Stats., or a duly authorized representative of the officer, manager, partner or proprietor that has been delegated signature authority pursuant to s. NR 205.07(1)(g)2, Wis. Adm. Code. The 'eReport Certify' page certifies that the electronic report form is true, accurate and complete.

6.6.9 Approval to Land Apply

Bulk non-exceptional quality sludge as defined in s. NR 204.07(4), Wis. Adm. Code, may not be applied to land without a written approval letter or Form 3400-122 from the Department unless the Permittee has obtained permission from the Department to self-approve sites in accordance with s. NR 204.06(6), Wis. Adm. Code. Analysis of sludge characteristics is required prior to land application. Application on frozen or snow-covered ground is restricted to the extent specified in s. NR 204.07(3)(l), Wis. Adm. Code.

6.6.10 Soil Analysis Requirements

Each site requested for approval for land application must have the soil tested prior to use. Each approved site used for land application must subsequently be soil tested such that there is at least one valid soil test in the four years prior to land application. All soil sampling and submittal of information to the testing laboratory shall be done in accordance with UW Extension Bulletin A-2100. The testing shall be done by the UW Soils Lab in Madison or Marshfield, WI or at a lab approved by UW. The test results including the crop recommendations shall be submitted

to the DNR contact listed for this permit, as they are available. Application rates shall be determined based on the crop nitrogen recommendations and with consideration for other sources of nitrogen applied to the site.

6.6.11 Land Application Site Evaluation

For non-exceptional quality sludge, as defined in s. NR 204.07(4), Wis. Adm. Code, a Land Application Site Request Form 3400-053 shall be submitted to the Department for the proposed land application site. The Department will evaluate the proposed site for acceptability and will either approve or deny use of the proposed site. The permittee may obtain permission to approve their own sites in accordance with s. NR 204.06(6), Wis. Adm. Code.

6.6.12 Class B Sludge: Fecal Coliform Limitation

Compliance with the fecal coliform limitation for Class B sludge shall be demonstrated by calculating the geometric mean of at least 7 separate samples. (Note that a Total Solids analysis must be done on each sample). The geometric mean shall be less than 2,000,000 MPN or CFU/g TS. Calculation of the geometric mean can be done using one of the following 2 methods.
Method 1:
Geometric Mean = $(X_1 \times X_2 \times X_3 \ldots \times X_n)^{1/n}$
Where X = Coliform Density value of the sludge sample, and where n = number of samples (at least 7)

Method 2:
Geometric Mean = antilog$[(X_1 + X_2 + X_3 \ldots + X_n) \div n]$
Where X = $\log_{10}$ of Coliform Density value of the sludge sample, and where n = number of samples (at least 7)
Example for Method 2

Sample Number	Coliform Density of Sludge Sample	$\log_{10}$
1	6.0×10^5	5.78
2	4.2×10^6	6.62
3	1.6×10^6	6.20
4	9.0×10^5	5.95
5	4.0×10^5	5.60
6	1.0×10^6	6.00
7	5.1×10^5	5.71

The geometric mean for the seven samples is determined by averaging the $\log_{10}$ values of the coliform density and taking the antilog of that value.
$(5.78 + 6.62 + 6.20 + 5.95 + 5.60 + 6.00 + 5.71) \div 7 = 5.98$
The antilog of $5.98 = 9.5 \times 10^5$

6.6.13 Class B Sludge: Anaerobic Digestion

Treat the sludge in the absence of air for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35° C to 55° C and 60 days at 20° C. Straight-line interpolation to calculate mean cell residence time is allowable when the temperature falls between 35° C and 20° C.

6.6.14 Class B Sludge - Vector Control: Incorporation

Class B sludge shall be incorporated within 6 hours of surface application, or as approved by the Department.

6.6.15 Landfilling of Sludge

General: Sewage sludge may not be disposed of in a municipal solid waste landfill unless the landfill meets the requirements of chs. NR 500 to 536, Wis. Adm. Code, and is an approved facility as defined in s. 289.01(3), Wis. Stats. Any facility accepting sewage sludge shall be approved by the Department in writing to accept sewage sludge. Disposal of sewage sludge in a municipal solid waste landfill shall be in accordance with ss. NR 506.13 and 506.14. Sewage sludge may not be disposed of in a surface disposal unit as defined in s. NR 204.03(63).

Approval: The permittee shall obtain approval from the Department prior to the disposal of sludge at a Wisconsin licensed landfill.

6.6.16 Sludge Landfilling Reports

The permittee shall report the volume of sludge disposed of at any landfill facility on Form 3400-52. The permittee shall include the name and address of the landfill, the Department license number or other state's designation or license number for all landfills used during the report period and a letter of acceptability from the landfill owner. In addition, any permittee utilizing landfills as a disposal method shall submit to the Department any test results used to indicate acceptability of the sludge at a landfill. Form 3400-52 shall be submitted annually by January 31, each year whether or not sludge is landfilled.

6.6.17 Sludge Hauling

The permittee is required to submit Form 3400-52 to the Department. If sludge is hauled to another facility, information shall include the quantity of sludge hauled, the name, address, phone number, contact person, and permit number of the receiving facility. Form 3400-52 shall be submitted annually by January 31 each year whether or not sludge is hauled.

6.6.18 Land Application of Sludge Which Contains Elevated Levels of Radium-226

When contributory water supplies exceed 2 pci per liter of Radium 226, monitoring for Radium 226 in sludge is required. Sludge containing Radium 226 shall be land applied in accordance with the requirements in s. NR 204.07(3)(n), Wis. Adm. Code.

7 Summary of Reports Due

FOR INFORMATIONAL PURPOSES ONLY

Description	Date	Page
Dissipative Cooling Study - Outfall 006 -Submit Dissipative Cooling Study Plan	March 31, 2027	31
Dissipative Cooling Study - Outfall 006 -Complete and Submit Dissipative Cooling Study	March 31, 2028	31
Water Quality Based Effluent Limits for Chloride - Outfalls 001 and 006 - Chloride Progress Report #1	December 31, 2025	31
Water Quality Based Effluent Limits for Chloride - Outfalls 001 and 006 - Achieve Compliance	November 1, 2026	31
Year-Round Disinfection - Outfall 006 -Submit Sampling Plan	June 30, 2026	32
Year-Round Disinfection - Outfall 006 -Initiate Downstream Sampling and Evaluation	October 1, 2026	32
Year-Round Disinfection - Outfall 006 -Sampling Progress Report #1	July 31, 2027	32
Year-Round Disinfection - Outfall 006 -Sampling Progress Report #2	July 31, 2028	32
Year-Round Disinfection - Outfall 006 -Evaluation Report	January 31, 2029	32
Year-Round Disinfection - Outfall 006 -Commence Year-Round Disinfection	October 1, 2029	32
PFOS/PFOA Minimization Plan Determination of Need -Report on Effluent Discharge	October 31, 2026	33
PFOS/PFOA Minimization Plan Determination of Need -Report on Effluent Discharge and Evaluation of Need	October 31, 2027	33
Compliance Maintenance Annual Reports (CMAR)	by June 30, each year	35
Industrial User Compliance Evaluation and Violation Reports	Semiannual	45
Pretreatment Program Report	Annually	45
General Sludge Management Form 3400-48	prior to any significant sludge management changes	46
Characteristic Form 3400-49 and Lab Report	by January 31 following each year whether or not samples are analyzed	46
Land Application Report Form 3400-55	by January 31, each year whether or not non-exceptional quality sludge is land applied	47
Other Methods of Disposal or Distribution Report Form 3400-52	by January 31, each year whether or not sludge is hauled, landfilled,	47

	incinerated, or exceptional quality sludge is distributed or land applied	
Wastewater Discharge Monitoring Report	no later than the date indicated on the form	34

Report forms shall be submitted electronically in accordance with the reporting requirements herein. Any facility plans or plans and specifications for municipal, industrial, industrial pretreatment and non industrial wastewater systems shall be submitted to the Bureau of Water Quality, P.O. Box 7921, Madison, WI 53707-7921. All other submittals required by this permit shall be submitted to:
Southeast Region, 1027 W Saint Paul Ave, Milwaukee, WI 53233

Appendix B. Scientific Collectors Permit or Research License

State of Wisconsin
Department of Natural Resources
dnr.wi.gov

Scientific Collectors Permit or Research License Application and Authorization

Form 9400-379 (R 10/16) Page 1 of 4

Notice: Use of this form is required by the department to apply for a scientific collectors permit or research license pursuant to ss. 29.614 and 169.25, Wis. Stats. State Natural Areas require an additional separate permit for research and scientific collection purposes. The license/permit authority may cover migratory birds, nests and parts, but may not be exercised without an appropriate federal permit issued by the U.S. Fish and Wildlife Service. Personal information provided may be used to determine identity of the applicant, participation in natural resources surveys, eligibility for approvals and enforcement purposes. Information may be made available to requesters under Wisconsin's open records law, ss. 19.31 to 19.39, Wis. Stats. A social security number or federal employer identification number is REQUIRED when applying for licenses according to ss. 169.34 and 169.35, Wis. Stats., but the DNR may only disclose it to the Departments of Workforce Development and Revenue.

Mail or deliver this completed form to the appropriate department service center.

Check the one that applies:

☐ **Scientific Collectors Permit** **Fee:** $0
(Used when collecting live fish, nests or the carcasses of wild animals for scientific purposes)

☐ **Scientific Research License** **Fee:** $25.00
+$20.00 late fee if application filed after license expiration date.
(Used when taking and possessing live wild animals [other than fish] from the wild for research purposes.)

Include the required fees and copy of an Institutional Animal Care and Use protocol and approval (9 CFR 2.31) with application.

Applicant Information (please print or type)

Last Name	First	MI	Current License/ Permit No. (if renewal)	DNR Customer ID No.

Agency or Organization	Daytime Telephone Number	Alternate Telephone Number

Street or Route	☐ Social Security **OR** ☐ Federal Employer Identification No.:

City	State	ZIP Code	Date of Birth	Eye Color	Hair Color	Weight	Height	Gender: ☐ Male ☐ Female

Federal Permit No. (if any)	Date Federal Permit Expires	E-Mail Address

Were you at any time during the past year convicted of any violation of the fish or game laws of Wisconsin?

☐ Yes ☐ No If Yes, Explain: _____

Explain Scientific Qualifications of Applicant – Required if applying for scientific research license

Collection Information

Species, Age or Size Class*, and Number of Specimens or Description of Items to be Collected or Possessed

* For game fish and pan fish species list young-of-year separately from larger length ranges

Purpose of Collecting or Possession

Method(s) of Collecting (for Chemical Immobilization, List Agents(s))

Location of Collecting or Possession Site(s) – County for all sites; waters for aquatic collections and civil township for all others

Collection or Possession Period Requested

Scientific Collectors Permit or Research License Application and Authorization
Form 9400-379 (R 10/16) Page 2 of 4

Collection Information (continued)		
Will State Natural Areas Be Used? ☐ Yes ☐ No	If Yes, List Area(s)	Natural Areas Permit Applied For? ☐ Yes ☐ No

Location Where Specimens or Items Will be Kept for Study (Be specific, including name or type of facility and street address.)

Final Disposition of Specimens or Items Will be:

Agents – List names of all agents of the permittee/license holder that are authorized to act under the Scientific Collectors Permit or Scientific Research License

The permittee/licensee is responsible for actions of agents under the scientific collectors permit or research license. Each agent shall comply with all terms and conditions of the permit or license.

Agent Name	Date of Birth	Agent Name	Date of Birth
Agent Name	Date of Birth	Agent Name	Date of Birth
Agent Name	Date of Birth	Agent Name	Date of Birth

Certification

I certify that the information provided on this application is true and correct and that I will comply with the terms and conditions of this permit or license, including special restrictions. I understand that providing incorrect information may result in revocation of my permit or license and possible penalties.

Applicant Signature	Date Signed
If Applicant Less than 18 Years of Age, Signature of Parent or Guardian	Date Signed

Authorization – DNR Use Only			
The license is subject to the following special restrictions and all conditions listed on the back of the license/permit.		License/Permit No.	
		Date Begins	Date Ends**
DNR Personnel Approval (Print Name)	Signature		Date Signed

** *A scientific research license is valid from the date of issuance until the following December 31.*
A scientific collectors permit expires on the date specified on the permit.

Scientific Collectors Permit or Research License Application and Authorization

Form 9400-379 (R 10/16) Page 3 of 4

Section 29.614, Wis., Stats., Scientific Collector Permit

(1) Application for a scientific collector permit shall be submitted to the department. The department may issue a scientific collector permit if the department determines that the applicant is a natural person and is engaged in a bona fide program leading to increased, useful scientific knowledge.
(2) A scientific collector permit shall state the name and address of the permittee, the date of issuance, the purposes for which it is issued, the type, species and number of specimens authorized to be collected or salvaged, the area and period of time in which the specimens may be collected or salvaged, the place where the specimens may be kept and other conditions and limitations that the department requires. A scientific collector permit is not transferable.
(3) A scientific collector permit authorizes the permittee to collect or salvage from the wild, for scientific purposes only, live fish and the nests and carcasses of any wild animals specified in the permit subject to the conditions and limitations specified in the permit and rules of the department. The permittee may use the specimens for the scientific purposes for which collected or salvaged and may transport them or cause them to be transported by common carrier.
Possession of these specimens may not be transferred to any other person, except that these specimens may be exchanged for other specimens for scientific purposes. A scientific collector permit may authorize the use of net guns and tranquilizer guns for activities related to the purpose for which the permit is issued (not sure needed for live fish or dead animals-JAB). Any person who is convicted of violating this chapter shall forfeit the person's permit and the permit is thereby revoked, in addition to all other penalties. Any person so convicted is not eligible for a permit under this section for one year following the conviction.

Section 169.25, Wis., Stats., Scientific Research License

(1) Issuance. (a) The department shall issue a scientific research license to any person who is engaged in a study or in research that the department determines will lead to increased, useful scientific knowledge and who files a proper application and who pays the applicable fee.
(b) The department may also require the person to submit with the license application a copy of any of the following: 1. The person's study plan or research proposal. 2. An approval received by the person under 9 CFR 2.31.
(2) Authorization. A scientific research license authorizes the holder of the license to take from the wild, possess, kill, or propagate the species of native wild animals that the department authorizes under the license.
(3) Scope of license; contents. A scientific research license shall contain the holder's name and address, the date of issuance, and all of the following conditions or limitations: (a) The specific purposes for which it is issued.
(b) The species of wild animals and the number of each species to be studied.
(c) The locations from where the wild animals will be taken.
(d) The locations at which the wild animals will be kept and studied.
(e) The periods of time in which the wild animals may be studied.
(f) Any other conditions or limitations that the department considers reasonable.
(4) Equipment. A scientific research license may authorize the use of net guns, tranquilizer guns and other equipment or supplies for activities related to scientific research or study.
(5) Title to; transfer and disposal of wild animals. (a) A person holding a scientific research license may not transfer and wild animal or its carcass held under the authority of the license unless the purpose of the transfer is to trade the wild animals for other animals for scientific research or classroom demonstrations and the transfer is specifically authorized by the department at the time of the transfer.
(b) A person holding a scientific research license shall release or dispose of a live wild animal possessed under the authority of the license, or its carcass, only in the manner specifically authorized by the department.
(6) Rules. The department may promulgate rules to establish additional standards, limitations, and requirements for scientific research licenses.

Section 169.36, Wis., Stats., Record-keeping and reporting

(5) Scientific Research License. Each person holding a scientific research license shall keep a correct and complete record of all of the following information for each animal:
(a) The disposition of the wild animal, including the date and location of its release into the wild or its transfer to the department.
(b) The cause of death, if known, for a wild animal that dies.

NR 19.11 Scientific collectors permits and scientific research licenses

(1) DEFINITIONS. For the purposes of implementing ss. 29.614 and 169.25, Stats., and within this section, the following definitions apply:
(a) "Qualified natural person" or "person" means any individual complying with s. 29.614, Stats., and this section, not including a corporation, partnership, cooperative, society, association or other organization.
(b) "Bonafide research program" means planned study and investigation undertaken to discover or establish facts or principles leading to increased, useful scientific knowledge.
(c) "Useful scientific knowledge" means new information contributing to the long-term well-being of wild animals and their habitats, or providing educational opportunities in the natural sciences.

(2) APPLICABILITY.
(a) Permits not required. Scientific collectors permits are not required for the collection of wild plants, unprotected wild animals taken legally, or wild animals obtained from licensed game farms or fish hatcheries.
(b) Bird banding. Scientific collectors permits will be required for trapping and banding protected nonmigratory upland game birds.
(c) Licenses. A person is not required to possess a separate hunting, fishing or trapping license while collecting under a scientific collector permit.
(d) Endangered species. Endangered or threatened wild animals may be collected only under authority of endangered species permits issued by the department pursuant to s. 29.604, Stats., and ch. NR 27.
(e) Tagging of fish. Scientific collectors permits are required to capture a wild fish, attach a tag to any part of it, and then to release it back into waters of the state.

(3) PERMIT APPLICATIONS.
(a) Forms. Applications for scientific collectors permits shall be made on application forms provided by the department and include:
1. Name and address of the applicant;
2. Applicant's personal description;
3. Purpose of the request;
4. Species and number of specimens to be collected;
5. Places and times when specimens are to be collected;
6. Method of collecting;
7. Place where collections will be kept; and
8. Such additional information as may be requested by the department.
9. The period of the permit.

(b) Narrative proposal. All permit applications shall be accompanied by a written proposal stating the objectives, justifications, procedures, times and places of collection, application of results and sponsor, if any, of the project described in the application.

(4) PERMIT ISSUANCE.
(a) Issuance. Permits shall be issued in the name of the applicant. All agents of the permittee assisting in the permitted collections will be listed on the permit. Separate copies of permits shall be signed and carried by each person named in the permit when that person is acting under it in the absence of the permittee.

(b) Specimen materials. A permit will be issued for collections yielding preserved specimen materials only when such materials are to be kept in a place and manner where students and the public have access to them. Private collections to be kept in a manner not open to the public will not be approved.

(c) Conditions.
1. 'Contents.' Permits will contain conditions deemed necessary by the department to protect the resources of the state and assure use of specimens taken are in compliance with s. 29.614, Stats.
2. 'Nonresidents.' Permits issued to nonresidents will set forth conditions of removal of specimens from the state.
3. 'Federal permits.'
a. Permits involving the capture, marking, collection, possession or salvage of migratory birds or parts, nests or eggs of migratory birds will not be issued under this section until the applicant possesses a permit issued by the U.S. fish and wildlife service for that activity.
b. Permits under this section are not required for banding or marking capture-and-release activities authorized under a permit issued by the U.S. fish and wildlife service.
4. 'Size of collections.' Permits will not be issued which authorize collections endangering the population of animals the collection would draw from, or exceeding the number of animals required to meet the permittee's objectives.
5. 'Unprotected species.' Permits will not be issued for the collection of protected species if unprotected species can be used to accomplish the same purposes.

(5) PERMIT USAGE.
(a) Disposition of specimens.
1. Living unharmed specimens collected during the course of permitted activities shall be returned to the wild at the point of capture, unless otherwise provided in the permit.
2. Any endangered or threatened species taken unintentionally during the course of permitted activities shall be immediately released if unharmed.
3. Injured or dead wild animal specimens shall be immediately turned over to the department employee named in the permit unless otherwise provided in the permit.
(b) Notification of department. Each permittee shall notify the department employee named in the permit at least 48 hours prior to collecting of the time and place where specimens will be collected.
(c) Marked gear. All traps, nets and any other gear used for capturing wild animals under terms of a permit shall be marked with the permit number, name and address of the permittee.
(d) Trap and net tending. All traps, nets and other capture emptied by the permittee at least once each 24-hour period.
(e) Fishing gear restrictions.
1. 'Gill nets.' Gill nets may not be used in inland waters unless specifically authorized by a permit.
2. 'Buoys.' All buoys and buoy staffs shall be marked and maintained as required by the department. The permit number, name and address of the permittee shall be maintained in plain figures on the bowl of the buoy.
3. 'Sport fishing equipment.' Hook and line fishing equipment and spearing equipment may not be possessed on a boat operating under a permit without prior approval of the department.

(6) RECORDKEEPING AND ANNUAL REPORTS.
(a) Records. Each permittee shall keep current records, in the English language, of all collections under the permit. Records of collections shall be made available to the department during normal business hours, or upon 8 hours notice at other times.
(b) Required reports. Permittees shall supply information requested by the department and annually file a complete and accurate report on forms covering activities conducted under authority of the permit. Unless otherwise provided in the permit, such reports shall be filed using a report form provided by the department not later than January 10 of the year following expiration of the permit.
(c) Content. Annual reports by permittees shall include:
1. The common name, scientific name and number of each species and type of specimen material collected;
2. The date and geographic location of each collection;
3. Disposition of collected specimens; and
4. Any other information requested by the department.

(7) DISPOSITION. Specimens collected under the authority of the scientific collector permit may be transferred to and possessed by an educational institution for exhibition or education purposes upon completion of the project or expiration of the permit. Environmental consulting organizations may retain specimens following permit expiration provided the specimens are marked in a manner prescribed by the department. An educational institution or environmental consulting organization possessing specimens shall possess written proof of source, including the scientific collector permit number of the source and present that proof upon request by the department.

Please Note:
State Natural Areas and **Threatened or Endangered Species**
A separate permit is required for research and scientific collection involving state natural areas or for the collection or possession of threatened or endangered species.
An application can be obtained by writing to or calling:

Department of Natural Resources
Natural Heritage Conservation
Box 7921
Madison, WI 53707
Phone: (608) 261-6449

Federal permits for migratory birds may be obtained from the Special Agent in Charge, U.S. Fish and Wildlife Service, Federal Building, Fort Snelling, Twin Cities, MN 55111.

Notice of Appeal Rights

If you believe that you have a right to challenge this decision, you should know that Wisconsin statutes and administrative rules establish time periods within which requests to review Department decisions must be filed.

For judicial review of a decision pursuant to section 227.52 and 227.53, Wis. Stats., as renumbered by 1985 Wisconsin Act 182, you have 30 days after the decision is mailed, or otherwise served by the Department, to file your petition with the appropriate circuit court and serve the petition on the Department. Such a petition for judicial review shall name the Department of Natural Resources as the respondent.

To request a contested case hearing pursuant to section 227.42, Wis. Stats., as renumbered by 1985 Wisconsin Act 182, you have 30 days after the decision is mailed, or otherwise served by the Department, to serve a petition for hearing on the Secretary of the Department of Natural Resources. The filing of a request for a contested case hearing is not a prerequisite for judicial review and does not extend the 30-day period for filing a petition for judicial review.

This notice is provided pursuant to section 227.48(2), Wis. Stats., as renumbered by the 1985 Wisconsin Act 182.

Appendix C. Blank Field Data Sheet

Root River Data Collection, In Situ Measurements

Air Temp (Degrees F): _____ Personnel: _____

Cloud Coverage: Clear Partial Full Type of Sampling: Water Quality Fish Macroinvertebrates

Precipitation in last 24 hrs (inches): _____ WQ Meter: _____ Calibration Date: _____

Bank (L or R when looking downstream): _____ Meter Calibration: pH: _____ Conductivity (mS/cm): _____

Distance from Bank: _____ DO (mg/L): _____ Turbidity (NTU): _____

_____ Field Duplicate Site: Field Duplicate Date/Time: _____

Site	Date	Time	DO (mg/L)	Conductivity (mS/cm)	pH	Temp (Degrees C)	Turbidity (NTU)	Notes

Appendix D. Water Quality Sampling SOP

Surface Water Chemistry SOP

Written by Laura Schulz, February 2017, updated March 2023

Contents

Purpose

This standard operating procedure (SOP) is applicable to the collection of representative water chemistry samples from surface waters with low velocity. Parameters to be measured from grab samples include: Orthophosphate, Total Phosphorus, Ammonia, Nitrate-N + Nitrite –N, Total Nitrogen, TSS, and Chlorophyll. Parameters to be measured by an YSI multi-parameter probe include: dissolved oxygen, specific conductance, conductivity, pH, temperature, depth, and turbidity.

Equipment and Materials

The following materials are required to undertake this procedure:

- USGS WBH-96 (weighted bottle with pin) sampler
- Suspension rope
- WBH-96 1-Liter Sampler bottle
- Funnel
- 2 gallon composite bucket
- Water Chemistry sample plastic bottles and labels
  - For each site and 1 duplicate:
    - 1 1000 ml sized bottles: TSS and Chlorophyll
    - 1 60 ml bottle: Orthophosphate
    - 1 250 ml bottle: Total Phosphorus, Ammonia, Nitrate-N + Nitrite –N, Total Nitrogen
- Paper towels
- Cooler with ice
- Field notebook and field data sheets (pg. 8)
- Waterproof and permanent markers
- DI water
- Bags for lab slips and ice
- Sulfuric acid ampules – 1 ampule for each 250 ml bottle (4 ml of 24% sulfuric acid)
- Packaging tape
- Test request forms for Wisconsin State Laboratory of Hygiene
- Measuring tape
- YSI multi-parameter probe & calibration equipment
- Nitrile gloves
- Field safety instructions

Collection Procedures and Guidelines

1. Samples cannot be collected on Fridays.
2. A minimum of two personnel with experience or special training in water quality sampling techniques are required to conduct the sample collection.
3. Prior to commencing the sampling event, all field sampling equipment should be decontaminated (see section on field equipment decontamination).
4. Samples shall be collected upstream of road or bridge crossings when feasible. If not feasible, samples shall not be collected directly downstream of the crossing (with the exception of Site C, which is sampled regularly on the downstream side due to the closer proximity to the USGS

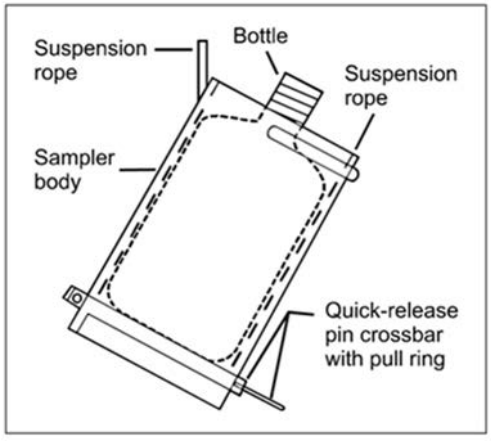

Figure 1: WBH-96 Sampler

sensor). When collecting water samples by wading, the sampling event should always start downstream and work upstream to avoid contaminating un-sampled areas within the water body by disturbing or suspending the sediment.

5. The width of the stream will be divided into five equal segments. One 1 liter sample will be collected from each segment.
6. Water samples will be collected using a WBH-96 sampler (Figure 1)
    a. Assemble the weighted bottle sampler. Secure bottle in sampler with suspension rope.
    b. Lower the sampling device to the predetermined depth or bottom of the river.
    c. When the sampler is at the required depth, allow the bottle to fill completely. (This is usually evidenced by the cessation of air bubbles.) Avoid collecting floating surface debris or disturbed bottom sediment in the water sample.
    d. Retrieve sampler.
    e. Transfer sample into 2 gallon composite bucket.
    f. Repeat as needed to collect necessary volume of water.
7. Gently mix the water in the composite bucket.
8. Using a funnel, pour the water from the bucket into the respective appropriately labeled sample bottles.
    a. 1000 ml bottle for chlorophyll and TSS
    b. 60 ml bottle for orthophosphate
    c. 250 ml bottle for Total Phosphorus, Ammonia, Nitrate-N + Nitrite –N, and Total Nitrogen
9. Repeat 1-8 for duplicate sample.
10. Preserve the 250 ml sample bottle with 1 sulfuric acid ampule (4 ml, 24% sulfuric acid).
11. Cap the sample bottles, place in the ice filled cooler and cool to four degrees Celsius.
12. Rinse sampling equipment including sampler, sampler bottle, funnel, and bucket with DI water.
13. Record all relevant data including weather conditions, personal present, date and time, and any observations in the field notebook.

Justification for Sampling Procedure

During the spring months and high flow, one cannot enter the river to collect samples for safety reasons. Thus, the above sampling protocol was selected to allow for consistency in the sampling method regardless of time and location.

YSI PRODSS Probe Calibration

Conductivity

1. Clean the probe and calibration cup with DI water prior to calibrating.
2. Fill the calibration cup to the second line with the conductivity standard.
3. Carefully immerse the sensors into the solution.
4. Gently rotate and move up and down.
5. Push the calibration key and select conductivity and then select specific conductance.
6. Select calibration value and enter the value of the standard used.
7. Observe the actual measurement reading for stability (white line on graph shows no change for 40 seconds).
8. When stable, select accept calibration.
9. Rinse the bulkhead and sensors in clean water and then dry.

Dissolved oxygen

1. Clean the probe and calibration cup with DI water prior to calibrating.
2. Verify the barometer is reading accurately.
3. Place a small amount of clean water (1/8inch) into the calibration cup.
4. Make sure there are no water droplets on the ODO sensor cap or temperature sensor.
5. Attach the sensor guard to the bulkhead and carefully place the guard/sensor into the calibration cup. Partially tighten the calibration cup to the bulkhead.
6. Turn instrument on and wait 5-15 minutes for the air in the storage container to be completely saturated with water.
7. Push Calibration key and select ODO. Select DO%.
8. Observe the actual measurement reading for stability (white line on graph shows no change for 40 seconds).
9. When stable, select accept calibration.

pH calibration 3 point

1. Clean the probe and calibration cup with DI water prior to calibrating.
2. Fill calibration cup to first line with pH 7 standard.
3. Carefully immerse the probe end of the sensors into the buffer solution.
4. Push the calibration key then select pH.
5. Allow at least one minute to stabilize.
6. Observe the actual measurement readings for stability (white line on graph shows no change for 40 seconds).
7. When stable, select accept calibration.
8. Rinse the sensor 2-3 times with a small amount of pH 4 buffer.
9. Rinse, then fill the calibration cup with pH 4 buffer to the first line.
10. Allow at least on minute to stabilize.
11. Observe the actual measurement readings for stability. (white line on graph shows no change for 40 seconds).
12. When stable, select accept calibration.
13. Repeat steps 6-10 for pH 10 buffer.

Turbidity

1. Clean the probe and calibration cup with DI water prior to calibrating.
2. Fill the calibration cup to the first line with DI water.
3. Immerse the sensors into the water.
4. Push the calibration key, the select turbidity.
5. Select the calibration value and enter 0.00.
6. Observe the data points reading for stability (white line on graph shows no change for 40 seconds).
7. When stable, select accept calibration.
8. Rinse the sensors and calibration cup 2-3 times with small amount of standard.
9. Fill the calibration cup with the standard up to the first line.
10. Immerse the sensors in the second calibration standard.
11. Select calibration value and enter the value of the second calibration standard.
12. Observe the actual measurement readings for stability (white line on graph shows no change for 40 seconds).
13. When stable, select **FINISH** calibration.

YSI 556 Probe Calibration

Occasionally, a YSI 556 multiparameter probe is used when the YSI PRODSS is either not working properly or is out for repairs. Below are the calibration steps for this instrument. Note, the YSI 556 does not have a turbidity sensor.

Conductivity

1. With the probe on press escape to get to the main menu and select calibrate.
2. Select conductivity. Then select specific conductance.
3. Place 55 ml of the conductivity standard into a calibration cup.
4. Put the sensor in the solution, remove bubbles and screw on.
5. Enter the calibration value of the standard.
6. Press enter to see the conductivity calibration screen.
7. Wait at least one minute for sensor to stabilize; there should be no change for 30 seconds. Press enter.
8. Press enter to return to the conductivity calibrate selection screen and escape to return to the main menu.

Dissolved oxygen

1. With the probe on press escape to get to the main menu and select calibrate.
2. Select dissolved oxygen; then select DO%.
3. Place 3 mm of water in the calibration cup. Making sure the DO sensor is dry and not immersed in the water place the sensor in the cup and engage 2 threads of the cup.
4. Use the keypad to enter the current barometric pressure in mmHg. Press enter.
5. Wait 10 minutes for water to become saturated and temperature to equilibrate.
6. Wait for the DO% to stabilize for at least 30 seconds. Then press enter. Press enter again to return to the DO calibration screen.
7. Press escape to return to the main menu.

pH calibration 3 point

1. With the probe on, press escape to get to the main menu. Then select calibrate and then in the calibration screen select pH.
2. Select 3-point option.
3. Put 30 ml of a pH buffer (pH 4, pH 7, or pH 10) in to the calibration cup. Then, put the probe in the calibration cup making sure there are no bubbles on the probe.
4. Screw the calibration cup on. Using the keypad enter the pH of the buffer and press enter.
5. Wait at least one minute for the temperature to equilibrate and for the pH reading to stabilize. Press enter, then press enter again to continue.
6. Rinse the probe with the next standard.
7. Repeat steps 3-6 for the next 2 pH buffer solutions.
8. When done with the 3 calibrations, press enter to return to the pH calibration menu. Press escape to return to the main menu.

YSI PRODSS/YSI 556 Probe Procedures and Guidelines

1. Calibrate probe prior to use. For the YSI 556, make sure the protective cap is placed over the sensors prior to use.
2. Dip probe into the river at each of the five equal-distance increments established for the water chemistry grab samples.
3. Allow readings to stabilize.

4. Record data in field notebook, later transferred to field data sheet (see pages 12 and 13) and/or the EQuIS app.
5. Rinse off probe with DI water.
6. Towel or air dry.

HF – Micro 100 Laboratory Turbidimeter

When the YSI 556 multiparameter probe is used to measure field parameters, one representative water sample is collected at each site and analyzed at a later date using a HF-Micro 1000 Laboratory Turbidimeter. The water sample collected comes from the same 2-gallon composite bucket used for the nutrient sampling. The sample is stored in a refrigerator until analysis, and is labeled "Turbidity Site (A-G) Root River Date". Samples are allowed to warm to room temperature for at least one hour prior to analysis. Below are calibration steps and instructions on how to use the HF-Micro 1000 Laboratory Turbidimeter.

Calibration

1. Press the "cal" key. "Ident" block and the "Cal" block will illuminate on the display screen.
2. The turbidity value in lower row of the display screen will read 1000 NTU. Insert this standard into the sample well and index (see below) to the lowest value and wait for the reading to stabilize.
3. To index a calibration standard, perform the following steps:
    a. Slowly rotate the calibration standard one complete revolution (360◦).
    b. While rotating the standard, locate the position with the lowest turbidity reading.
    c. With the calibration standard positions at this location, install the indexing ring over the black light shield so the pointer of the ring faces forward, towards the operator.
    d. When using the standards in the future, always insert the standard so that the pointer of the indexing ring faces forward. Slowly rotate the standard, back and forth about 5° to find the lowest point. The standard is now indexed and ready for use.
4. Press "enter" when the standard is in the right position. "Store" block will flash and the upper row of the display screen will read 1000 NTU. The lower row will now read 10.0 NTU.
5. Repeat steps 2 and 3 with the 10.0 NTU standard. Lower row will then read 0.02 NTU.
6. Repeat steps 2 and 3 with the 0.02 NTU standard. Once completed, the instrument automatically exits out of calibration mode. Proceed to use the instrument normally.

The following steps describe how to measure the turbidity of a sample using the Micro 100:

1. Turn on the instrument. Allow instrument to warm up for 30 minutes.
2. Rinse the sample cuvette with approximately 20 ml of the sample, capping the cuvette and inverting several times. Discard the used sample and repeat two more times.
3. Completely fill the rinsed cuvette with the sample and cap the cuvette. Ensure the outside of the cuvette is dry and clean from any smudges.
4. Place the cuvette in the Micro 100 and completely rotate the cuvette (360◦). Record the lowest turbidity reading observed.
5. Dispose of the sample.

Field Note Procedures and Guidelines

A field notebook will be maintained by the water chemistry team. The notebook will be water-resistant. All data recorded in the notebook will be transferred to the EQuIS app and/or Field Data Sheet (see pages 12 and 13) upon leaving the field.

Guidelines to follow when recording notes in the field notebook include:

1. Write neatly.
2. Make numbers large.
3. Do not erase or black out a mistake, draw a line through the incorrect value and initial instead.
4. Number pages.
5. Never tear pages out of the notebook.
6. Record everything, never assume you will remember something.

Entering Data into EQuIS

All field data is entered into the EQuIS app on an iPad either during or immediately after the collection day.

1. Open the EQuIS app. Enter your username and password.
2. Select appropriate form from the forms tab or create a new form.
3. Once a sampling event form has been selected, select the ellipses (…) next to the desired site.
4. Select "yes" for ready to sample.
5. Proceed to fill out the required information including field lead name, field crew names, weather, width, YSI calibration, field parameters, date, any notable field notes, and upload a picture of the field notebook sheet(s) associated with the site.
6. Select subforms in the top left corner.
7. Select new. This will automatically create a subform for cross-section L1 (left bank 1). Proceed to enter data for time, DO (%), DO (mg/L), specific conductance (mS/cm), conductivity (mS/cm), pH, water temperature (C◦), turbidity (NTU), and depth (ft).
8. Repeat for cross sections L2 (left bank 2), CB (center bank), R2 (right bank 2), and R1 (right bank 1).
9. Once completed, return to the sites menu by clicking water sample.
10. Repeat steps 3-8 for all sites sampled.
11. Once all the data is entered, select water sample to return to the sites menu. Then select the arrow at the bottom of the screen to upload to the server.
12. Close the application when finished.

For more detailed instructions on how to enter data in the EQuIS app, please refer to the document "EQuIS Collect Mobile Quick Start for Root River Monitoring". For technical support, contact David Linari at David.Linari@jacobs.com and Nora Kodis at Nora.Kodis@jacobs.com .

Laboratory Data from the Wisconsin State Laboratory of Hygiene is also uploaded to the EQuIS database as excel files.

1. Save the excel file with the name wslh_excel_YYYYMMDD.
2. Rename the worksheet within the file as "Root River Lab Format".
3. Insert a # symbol in cell A1 so it reads "#Lab ID".
4. Review columns and update as needed.
5. Open the URL https://na02prod.jacobs.equisonline.com. Enter username and password.
6. Select "Waukesha Water Utility Root River Data Collection Plan" from the Dashboard.

7. Select “WSLH” under the format dropdown. Drag files to the boxed area. Select upload.
8. A green check mark under the EDP EDD status widget should appear if upload was successful.

For more detailed instructions on how to enter data in the EQuIS app, please refer to the document “EQuIS Enterprise Quick Start Root River Monitoring Dashboard”. For technical support, contact David Linari at David.Linari@jacobs.com and Nora Kodis at Nora.Kodis@jacobs.com .

The water quality EQuIS database will be reviewed quarterly for completeness and accuracy and annually for assessment of water quality outliers or water quality data with analysis qualifiers as determined by the analyzing laboratory.

Data Management and Documentation

Scanned copies of the field notebook pages, Field Data Sheets, Wisconsin State Laboratory of Hygiene test request forms, and Wisconsin State Laboratory of Hygiene Reports are stored on a Box cloud folder at UW-Parkside. Files are stored under the master folder “Waukesha”, and then subfolders “Archived Field Notebook pages”, “Archived Field Data Sheets”, “Archived WSLH test request forms”, and “Archived reports from WSLH”.

Scanned copies of the Field notebook pages are labeled as Field_notebook_pages_yearmonthday.
Scanned copies of the archived field data sheets are labeled as Field_data_sheet_yearmonthday.
Scanned copies of the WSLH test request forms are labeled as WSLH_testrequest_yearmonthday.
PDF copies of the reports from the WSLH are labeled as wslh_final_yearmonthday.

Quality Assurance/Quality Control

QA/QC activities include providing instructions for cleaning equipment between collections of water samples, the collection duplicate samples, and proper labeling of all samples.

Equipment Decontamination

All sampling materials, including the WBH-96 sampler, 1-liter sampler bottle, funnel, composite bucket, and YSI probe will be rinsed at least 3 times with DI water between sampling sites. Decontamination between samples at the same site is not necessary.

After each sampling day, sampling equipment will be cleaned using the following protocol:

1. Rinse in DI water at least 3 times
2. Air-dry on laboratory counter.

Field Duplicates

Duplicate field samples are collected side-by-side to check the homogeneity of the sample matrix, precision of field techniques, and precision of laboratory analysis. Duplicate samples are collected at the same time as the initial sample. The initial sample and the duplicate sample for any one parameter shall be taken from the same water dip to compare precision of laboratory analyses. The duplicate sample will be handled in the same manner as the primary sample. The duplicate sample will be stored in an iced cooler, and shipped to the laboratory on the day it is collected. The duplicate sample is analyzed for the same parameters as the primary sample. The duplicate sample shall be labeled with “FD” in place of a Sample Site ID to remove site identification by the laboratory personnel. Sample collectors shall indicate the sample site where the duplicate was collected in the field forms along with the date and time of collection in the field notebook and field data sheet. At a minimum, one duplicate sample should be collected and analyzed at one sampling site for all grab sample parameters during every sampling event.

Water Quality Sample Labeling

For each water quality sample, the following information shall be clearly marked and labeled on the sample container:

To be included on pre-printed labels:

- Water samples: “RR – Sample Site ID (A-F) – MM.DD.YYYY- first or second sampling of the month (1 or 2)”
- Field duplicates: “RR – FD – MM.DD.YYYY – first or second sampling of the month (1 or 2)”
- Preservative (indicated with Yes or No)

To be labeled on the bottles in the field:

- Time of sample collection
- Sampled by (initials)

Test request form/Shipping

To establish the documentation necessary to trace sample possession from the time of collection, a test request form shall be completed for every sample event. There will be one form per sampling location, which will include the sampling time, location, and tests requested. Samples should not be left unattended unless placed in a secured and sealed container with the form inside the container. The test request form (see page 11) shall include special instructions for the laboratory to follow which will be consistent with the contract. Copies of the test request forms are saved on the EQuIS database. If discrepancies are identified, the Field Team Leader (FTL) shall inform the PM/CM team before the samples are analyzed.

Shipping

Coolers prepared for shipping shall be lined with a cooler liner and packed with ice in double-wrapped Ziploc bags so that movement of samples is minimized. Prior to sending or dropping off samples, the lab will be contacted to let them know the quantity and when they can expect the samples. Samples cannot be collected and shipped on Friday. Samples are shipped overnight to ensure the ice does not melt prior to arrival.

All samples will be sent to the Wisconsin State Laboratory of Hygiene at the following address:

WI State Lab of Hygiene
2601 Agriculture Dr
Madison, WI 53718

Safety and Environment

This section describes health, safety and environmental considerations for surface water sampling:

Health and Safety

Field Safety Instructions developed by the contractor for the sampling activities should be followed.

Hazards include, but are not limited to:

- Manual handling injury associated with lifting and moving sampling equipment and samples – to mitigate determine that all loads are an appropriate weight for lifting (<10 kg), use correct lifting posture by bending at the knees, position so that load is balanced and does not cause undue strain, wear sturdy boots and clothing, park field vehicle with equipment close to water body (if possible) to avoid multiple loading and uploading, do not over-pack samples into coolers.
- Injury associated with slips and trips – to mitigate keep a tidy workplace and step carefully around tubing, hosing and other equipment.
- Hit by moving vehicle while sampling – to mitigate sampling team shall wear high visibility clothing, set up traffic controls around sampling area, position site vehicle so that it provides a barrier from potential traffic.
- Sunburn –to mitigate wear suitable clothing (including hat, trousers, long sleeved shirt), apply sunscreen regularly.
- Dehydration and fatigue – to mitigate drink fluids and eat regularly.

- Exposure to water – to mitigate handle water with care minimizing splashing or spills, understand Safety Data Sheet (SDS) for particular parameters of interest, wear appropriate personal protective equipment (PPE) including gloves, waders, escalate PPE requirements if conditions change.
- Exposure to biological hazards (including snakes, ants, mosquitoes, bees, poisonous plants) – to mitigate access sampling points by minimizing exposure to vegetation, plan sampling events at suitable times where risk of biological hazard is reduced, wear appropriate clothing and PPE (long sleeves, long pants, tuck pant legs into socks), make vibrations to alert snakes to your presence. Use insect spray or other insect deterrents.
- Working on or around water courses will require additional PPE which includes (but not limited to) a Type II personal floatation device (PFD) and never working alone. PFDs should be utilized when sampling in deep waters and for all other instances where potential drowning danger exists.

Environment

Sampling contractors will be exposed to environmental waters which may contain contaminants that are hazardous to human health. All personnel participating in field sampling shall be current on Occupational Safety and Health Administration (OSHA) medical screening and surveillance standards. These standards can be found on the OSHA organization web page:

https://www.osha.gov/SLTC/medicalsurveillance/standards.html

Winter Water Quality Sampling

Reasonable efforts shall be implemented to conduct winter water quality sampling for open water or thin ice conditions. Coring through thick ice to retrieve water samples may occur. However, individual sampling sites should be observed and evaluated for the ability to conduct water quality sampling, and sampling shall continue at sites with favorable conditions.

References

HF – Micro 100 Laboratory Turbidimeter Owner's Manual Catalog No. 22155 (5/10) Rev. 3.3

Kempthorne, D; Myers, MD. 2012. A4. Collection of Water Samples. Standard Methods for the Examination of Water and Wastewater, 22 Ed. American Public Health Association; Washington, DC.

ProDSS User Manual Document #626973-01REF

YSI 556 MPS Multi Probe System Operations Manual

Test Request Form for the Wisconsin State Laboratory of Hygiene

State of Wisconsin
Department of Natural Resources
and Laboratory of Hygiene

Test Request – Inorganic Surface Water & Microbiology
Form 4800-024 (R 7/21) Page 1 of 2

Billing and Reporting

Account Number	Field Number (Bottle Label ID)	Report to Address (Non-DNR only)		
350897		900 Wood Rd		
DNR User ID	Report To Name	City	State	ZIP
	UW Parkside GeoScience Dept John Skalbeck	Kenosha	WI	53141
Date Results Needed (mm/dd/yyyy)		Report to Email (Non-DNR only)		
		skalbeck@uwp.edu		

Date and Time of Sample Collection

Date (mm/dd/yyyy)	Time (24-hr clock)	End Date (mm/dd/yyyy)	End Time

Sample Type

Sample Type: (select one)
- (•) SU Surface Water
- ( ) NP Storm Water
- ( ) EF Effluent (Treated Wastewater)
- ( ) IF Influent (Untreated wastewater)
- ( ) D Public Drinking Water
- ( ) MW Monitoring Well
- ( ) PO Private Well
- ( ) SE Sediment
- ( ) SL Sludge
- ( ) SO Soil
- ( ) OW Other Waste
- ( ) _____

Who collected the sample

Collected By (First and Last Name)	Telephone	Email

Where the sample was collected

Station ID (STORET #)	Sample Address or Location Description	
County	Waterbody ID (WBIC)	Point / Outfall (or SWIMS Fieldwork Seq No)

Sample Details

Sample Description / Device Description

Enforcement? ( ) Yes ( ) No
If yes, include chain of custody form.

Is Sample Disinfected? ( ) Yes ( ) No
If yes, how?

If Field QC Sample (select one): ( ) Duplicate ( ) Blank ( )

Grant or Project Number

Depth of Sample: _____ ( ) ft ( ) m ( ) in ( ) cm

Or Top and Bottom of Sample Interval: _____ - _____ ( ) ft ( ) m ( ) in ( ) cm

Analyses Requested

If field filtered, indicate by checking the box on this sheet and noting on the lid of the sample bottle.

Plastic Quart Bottle (No chemical preservation)
☐ Sample field filtered? (Check box if yes)

- ☐ Alkalinity, pH, Conductivity
- ☐ BODs Dissolved
- ☐ BODs Total (900 ml needed)
- ☐ CBODs Total (carbonaceous)
- ☐ Chloride
- ☒ Chlorophyl A (if Field Filtered, give ml _____ filtered)
- ☐ Color
- ☐ Fluoride
- ☐ MBAs Screening
- ☐ pH only (non compliance)
- ☐ Sulfate
- ☐ Turbidity
- ☐ _____

Solids

- ☐ Suspended Sediment
- ☐ Total Dissolved Solids
- ☐ Total Solids
- ☐ % Sand, Silt, Clay
- ☒ Total Suspended Solids (500 ml needed)
- ☐ Total Vol. Susp. Solids (includes Total Susp. Solids)
- ☐ Total Volatile Solids (includes total solids)

60 ml Bottle (No chemical preservation)
☐ Sample field filtered? (Check box if yes)

- ☒ Orthophosphate
- ☐ Silica
- ☐ NO_2+NO_3 as Nitrogen (drinking water)
- ☐ Nitrite (NO_2) as Nitrogen

250 ml Glass Amber

- ☐ TOC (acidified w/Sulfuric Acid)
- ☐ DOC (field filtered and acidified w/Sulfuric Acid)
- ☐ DOC (not field filtered nor acidified)

250 ml Metals Bottle (Acidify w/ Nitric Acid)
☐ Sample field filtered? (Check box if yes)
☐ Low Level Metals. Note: Clean sampling with special bottles
☐ TCLP (Toxicity Characteristic Leaching Procedure - use mason jar)
Total recoverable metals will be run unless otherwise instructed.

☐ Aluminum	☐ Copper	☐ Selenium
☐ Antimony	☐ Hardness-as $CaCO_3$	☐ Silver
☐ Arsenic	☐ Iron	☐ Sodium
☐ Barium	☐ Lead	☐ Strontium
☐ Beryllium	☐ Magnesium	☐ Thallium
☐ Boron	☐ Manganese	☐ Titanium
☐ Cadmium	☐ Mercury	☐ Vanadium
☐ Calcium	☐ Molybdenum	☐ Zinc
☐ Chromium, Total	☐ Nickel	☐ _____
☐ Cobalt	☐ Potassium	☐ _____

250 ml Nutrients Bottle (Acidify w/ Sulfuric Acid)
☐ Sample field filtered? (Check box if yes)

☒ Tot.-Phosphorus	☒ NO_2 + NO_3 as Nitrogen	☐ Total Kjeldahl-N
☒ Ammonia-N	☐ COD	☒ Total Nitrogen

- ☐ Tot. Dis. Phosphorus (filter, then acid preserve in 60 ml bottle)
- ☐ Low Level Total Phosphorus (special bottles needed)

250 ml Round Bacteria Bottle

- ☐ E. coli by MPN, non-potable
- ☐ Enterococci by MPN, non-potable

For lab use:
Sample Temp _____ °C
☐ Iced

Please enclose this form in the mailer along with the sample and send to the State Lab of Hygiene.

Root River Data Collection Summary, In-Situ Measurements

Air Temp (Degrees F): _____ Personnel: _____

Cloud Coverage: Clear Partial Full Type of Sampling: Water Quality Fish Macroinvertebrates

Precipitation in last 24 hrs (inches) _____ WQ Meter: _____ Calibration Date: _____

_____ Meter Calibration: pH: _____ Conductivity (mS/cm): _____

_____ DO (%): _____ Turbidity (NTU): _____

Date _____ Field Duplicate Site: Field Duplicate Date/Time: _____

Site	Date	Time	DO (%)	DO (mg/L)	Specific conductance (ms/cm)	Conductivity (mS/cm)	pH	Temp (Degrees C)	Turbidity (NTU)	Depth (ft)	Barometric Pressure (mmHg)	Notes
A												
B												
C												
D												
F												
G												

Root River Data Collection Sample Location, In-Situ Measurements

Air Temp (Degrees F): _____ Personnel: _____

Cloud Coverage: Clear Partial Full Type of Sampling: Water Quality Fish Macroinvertebrates

Precipitation in last 24 hrs (inches) _____ WQ Meter: _____ Calibration Date: _____

Width of stream (ft): _____ Meter Calibration: pH: _____ Conductivity (mS/cm): _____

Width of interval (ft): _____ DO (%): _____ Turbidity (FNU): _____

DATE: _____ Field Duplicate Site (Y/N): Field Duplicate Time: _____

Site: _____

Cross section	Date	Time	DO (%)	DO (mg/L)	Specific Conductance (mS/cm)	Conductivity (mS/cm)	pH	Temp (Degrees C)	Turbidity (NTU)	Depth (ft)	Notes
LB1											
LB2											
CB											
RB2											
RB1											
Average											

River cross section →

LB1 LB2 CB RB2 RB1

Downstream

Appendix E. Wisconsin State Lab of Hygiene SOPs

Wisconsin State Laboratory of Hygiene
Environmental Health Division
Water Microbiology Section

EHD MICRO METHOD 300

Total Coliform/*E.coli* Enzymatic Substrate

Colilert®, Colisure®, Colilert-18® in Presence Absence and Quanti-Tray® Formats
SM9223B

1.0 Scope and Application

1.1 The Safe Drinking Water Act and the Groundwater Rule require that all potable water be free of total coliform and *E.coli.*

1.2 The Beach Act requires recreational samples to be tested for either enterococci or *E.coli*. Wisconsin has adopted the *E.coli* standard and the Colilert® and Colilert-18® MPN methods are approved for this testing.

1.3 The method describes identifying total coliform/*E.coli* using the presence/absence and quantitative formats.

1.4 This procedure outlines the steps to simultaneously detect total coliform and *E.coli* in potable water, source water, recreational water, surface water and wastewater.

1.5 The Colisure® method can only be used for drinking water samples.

1.6 When the unit uses Colilert-18® for presence/absence tests, the unit uses a Quanti-Tray®, but records presence/absence. A pre-warming step is not required for the use of Colilert-18® for quantitative analysis in Quanti-Trays® or for the use of Colilert® or Colisure®.

2.0 Summary of Method

2.1 Chlorine-free samples are individually poured off to 100mL.

2.2 The reagent is added to 100 ml of the sample.

2.3 The sample is then incubated for a specified time at 35º C ± 0.5º C.

2.4 A color change (from clear to yellow with Colilert® and Colilert-18® and from yellow to magenta for Colisure®) indicates the presence of total coliform bacteria in the sample and is interpreted as “Total Coliform Present” for potable waters. If there is no color change, the sample is interpreted as “Total Coliform Absent”.

2.5 All Total Coliform Present samples are checked for the presence of fluorescence using a long wavelength UV light (366 nm). The presence of *E.coli* is indicated by a sky-blue fluorescence. If there is no fluorescence, the sample is absent for *E.coli.*

2.6 From a Quanti-Tray ®, the number of total coliform positive wells and/or the number of fluorescence wells (*E.coli)* are counted. Quanti-Tray® results are reported as a most

probable number (MPN) according to a statistically derived number using the manufacturer's provided chart or software.

3.0 Regulatory Deviations

3.1 Regulatory deviations are discussed in each specific method SOP.

4.0 Definitions

4.1 Total Coliform is defined with this method as ortho-nitrophenyl-ß-D-galactopyranoside (ONPG) or chlorophenol red-ß-D-galactopyranoside (CPRG) being hydrolyzed by the ß-D-galactosidase enzyme which is produced by total coliform and creates a color change in the sample.

4.2 *E.coli* is defined with this method as 4-methylumbelliferyl-ß-D-glucuronide (MUG) hydrolyzed by ß-glucoronidase which is produced by *E.coli* and produces a fluorescent blue that can be view with a long wavelength (UV) light.

4.3 MERI – Madison Energy Recovery, Inc.

4.4 QT- Quanti-Tray

4.5 MPN-Most Probable Number

4.6 DPD-N, N-diethyl-p-phenylenediamine

5.0 Interferences

5.1 Samples that are extremely turbid or contain high iron content could interfere with the color change for Colilert® and Colilert-18®. These samples will be tested with Colisure®.

5.2 The test will not be performed if chlorine is present in the sample. The suspect sample will be shaken 25 times and approximately 5mL of the sample is poured into a clean vial. The presence of chlorine is checked by adding a small amount (about 0.1 g) of DPD to the 5mL of sample. The development of a pink color indicates the presence of chlorine.

5.3 Samples with a heterotrophic plate count of more than 20,000/1 mL before reagent is added may cause a false-positive test.

5.4 Samples that result in colors other than method-specific color change will be rejected and a new sample will be requested from the utility or source.

6.0 Safety, Waste Management and Pollution Prevention

6.1 All samples and cultures may contain potentially harmful pathogenic organisms. Care must be taken not to contaminate work area, other staff or one self. All spills must be decontaminated with disinfectant solution using the following procedure:

6.1.1 Place a paper towel over the spill.

6.1.2 Pour disinfectant solution over the entire spill without excessive splashing.

6.1.3 Let the disinfectant solution sit on the spill for at least 5 minutes before wiping up and/or sweeping up the spill.

6.1.4 If broken glass is involved, sweep up with a broom and discard in the red sharps container.

6.1.5 While wearing gloves, wipe up the liquid with paper toweling and discard in the MERI barrel.

6.2 Dispose of any cultures or media containing cultures in the MERI Barrel or dish pans to be autoclaved before disposal.

6.3 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

6.4 Solutions and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be discarded.

6.5 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive facility (ref.16.5).

6.6 All laboratory waste, excess reagents and samples must be disposed of in a manner consistent with applicable rules and regulations.

6.7 Waste disposal guidelines are described in the University of Wisconsin “Laboratory Safety Guide” (ref.16.6). Specific waste disposal guidelines are detailed in the Environmental Health Division’s “Waste Management” SOP (ref. 16.7).

7.0 Equipment and Supplies

7.1 35º C incubator

7.2 160 mL clear bottles with or without sodium thiosulfate

7.3 Quanti-Tray/2000® vessels for MPNs

7.4 6 watt long wavelength (366 nm) UV light

7.5 Laboratory Information Management System (LIMS) capable of performing IDEXX MPN calculations, IDEXX MPN chart, or IDEXX software (IDEXX MPN 3.1)

7.6 Quanti-Tray™ Sealer PLUS

7.7 Pipettes if dilutions are need

7.8 Paper towels (e.g. Wipe-all 10s)

8.0 Reagents and Standards

8.1 Colilert®, Colilert-18®, Colisure® stored at room temperature, stored away from light, and used before manufacturer's expiration date

8.2 Colilert®/Colilert-18® comparator

8.3 99 mL deionized water blanks if dilutions are needed.

8.4 Anti-foam

8.5 Ethanol solution (e.g. 70%)

9.0 Sample Collection, Preservation, Shipping, Handling and Storage

9.1 Samples are shipped at ambient temperatures for potable drinking water samples.

9.2 Water samples submitted from public water systems have a maximum 30 hour holding time.

9.3 Water samples submitted by Indian Tribes (IT) have a maximum 30 hour holding time.

9.4 There is no 30 hour hold time regulation for private homeowner samples. Private water samples referenced under NR812 can be tested up to 48 hours past collection time. The samples referenced under NR812 are: newly constructed wells, pump work, maintenance, well construction/redevelopment, and property transfer (i.e., real estate transaction) water samples collected by a licensed plumber or well driller (ref. 16.18).

9.5 Water samples from private wells that are not Indian Tribes (IT) nor referenced under NR812 have a holding time of two calendar days.

9.6 Pool water samples must be checked for the presence of chlorine before testing. Pool water samples must also be checked to verify that the sample was collected in a bottle containing sodium thiosulfate before testing. If the sample contains chlorine and/or is collected in a bottle that does not contain sodium thiosulfate, the sample is invalidated and not tested.

9.7 "No test" situations for drinking water samples:

9.7.1 Public water system and Indian Tribe (IT) samples are not analyzed >30 hours after sample collection date/time (ref. 16.1). Samples referenced under NR812 are not analyzed >48 hours after collection (ref. 16.18). Water samples from private wells that are not Indian Tribes (IT) or referenced under NR812 are not analyzed two calendar days after collection.

9.7.1.1 NR812 samples will be rejected if:

9.7.1.1.1. The sample bottle contains sodium thiosulfate

9.7.1.1.2. The sample bottle does not contain adequate volume to test for free chlorine (approximately 5 mL) and total coliform-*E.coli* (100 mL).

9.7.1.1.3. The samples contains free chlorine above 0.1 mg/L.

9.7.1.1.4. The sample is received frozen.

9.7.1.1.5. The sample is received by analysts after 48 hours of sample collection.

9.7.2 Drinking water samples received frozen or partially frozen are not tested.

9.7.3 Samples found to contain chlorine are not tested. Samples received in plain bottles and have a chlorine residual written on the sample collection form are not tested.

9.7.4 Pool water samples that are received in bottles that do not contain sodium thiosulfate are not tested. Pool water samples found to contain chlorine are not tested.

9.7.5 Sample volumes less than 100 mL are not tested.

9.8 Samples for surface or recreational waters and wastewater are shipped on ice (ref. 16.3). A temperature is taken and recorded on the sample collection form upon receipt. If the temperature of the sample is greater than 10°C upon receipt, the sample is tested and a reportable comment that reads "WATER MICROBIOLOGY SAMPLE RECEIVED WARM. RESULTS UNCERTAIN" is added to the sample data in LIMS.

9.9 If the sample was collected the same day the lab receives it, the lab accepts the sample and a WARM comment data flag will not be added if the temperature of the sample is greater than 10°C and received on ice. The explanation comment "WATER MICROBIOLOGY SAMPLE RECEIVED WARM, COLLECTED SAME DAY. RESULTS VALID" is added. If the sample is received without ice, there is no flag reported with the results if the sample is analyzed within 2 hours of collection. After 2 hours, the sample results are flagged with "WATER MICROBIOLOGY SAMPLE RECEIVED WARM. RESULTS UNCERTAIN".

9.10 The holding time for surface or recreational waters and wastewater is 8 hours from the time of collection until the sample is put in the incubator. Since this is not possible in most cases, the data is flagged when the sample is tested after 8 hours from collection.

9.11 "No test situations" for surface or recreational waters and wastewater:

9.11.1 Frozen or partially frozen samples may not be tested unless ice was collected.

9.11.2 Sample volumes less than 100 mL.

9.11.3 Samples older than 1 day are not tested unless the sampler requests the sample still be tested. USGS requests samples to be tested no matter how old.

9.11.4 Chlorine present.

10.0 Quality Control

10.1 Please refer to the Environmental Health Division Quality Assurance Manual (ref. 16.4) for general information on quality control procedures.

10.2 Each new lot of reagent is QC'd when received (ref. 16.8)

10.3 Each new box of bottles and Quanti-Trays® are checked for sterility, volume and fluorescence when received (ref. 16.9, 16.10).

10.4 The Quanti-Tray sealer is checked for proper sealing monthly (ref. 16.11).

10.5 Reagent is stored away from light and kept at 4-30ºC.

10.5.1 If any reagent packet is discolored, discard the packet and do no use (ref. 16.4).

10.6 Each lot of reagent is tested with positive and negative cultures when received and on a monthly basis. If correct reactions are not observed, the associated lots are not used for analysis. (ref. 16.8)

10.7 Stock cultures are checked for purity and performance every 4 weeks. (ref.16.13)

10.8 If dilutions are required for this method, 1 mL of sample is put into a 99 mL sterile polished water dilution blank for a two log reduction and if further reduction is required, additional dilutions maybe performed until a correct dilution is obtained.

10.8.1 The laboratory water used for dilutions must be monitored for conductivity (ref. 16.15) and pH (ref. 16.16) each time dilutions are made, according to EPA Standards (ref. 16.2).

10.8.2 Laboratory water, Type 1 polished water, is tested for:

10.8.2.1 Monthly: pH, conductivity, heterotrophic plate count (for sterility), nitrate, nitrite, ammonia, total organic carbon

10.8.2.2 Quarterly: bacterial inhibition via heterotrophic plate count

10.8.2.3 Annually: silica and trace metals

11.0 Method Calibration and Standardization

11.1 Incubator temperatures are recorded twice daily during business days and once on Saturdays to insure temperatures are within limits. If no one works on Sundays, thermometers with min/max readings are used to ensure temperatures do not exceed operating limits on Sundays and other days that temperatures are not recorded (e.g., holidays).

11.2 Thermometers are calibrated each calendar year with a NIST thermometer or NIST traceable thermometer. No mercury thermometers are used.

12.0 Procedure

12.1 Samples that are delivered to room 203 need to be transferred to room 203 in Horizon before any subsequent processing.

12.2 Before processing samples, spray bench top work area with an ethanol solution and wipe away ethanol with a paper towel.

12.3 Ensure there is at least one inch of headspace (open air space) within the sample bottle, then thoroughly mix sample by shaking vigorously 25 times.

12.3.1 If the sample bottle contains less than one inch of headspace, pour the sample into a larger volume sterile secondary bottle. Shake the sample vigorously 25 times, perform a chlorine test according to SOP ESS MICRO QA 266 (ref.16.14), and pour 100 ml of sample back into its original sample bottle.

12.3.2 When batching the sample(s) in Horizon, indicate the secondary bottle lot used and associated sample number(s) as MICP prep level comment.

12.3.3 Proceed to step 12.5.

12.4 Perform a chlorine test on the sample according to SOP ESS MICRO QA 266 (ref.16.14) under section 12.2.

12.5 Pour off sample to the 100 mL line on the original sampling bottle.

12.5.1 Alternatively, if the sample arrives in a bottle that requires a transfer into a secondary bottle (i.e., the original bottle doesn't contain a 100 mL line, a miscellaneous bottle lot that has not be tested, or a bottle lot that has failed volume calibration according to SOP ESS MICRO QA 212 (ref. 16.9)), aseptically pour 100 mL of the sample into a sterile secondary bottle that has a 100 mL calibrated line.

12.5.2 Transfer sample container labels from the original container to the secondary bottle immediately after the pour has been completed.

12.5.3 When batching the sample(s) in Horizon, indicate the bottle lot used for transfer and the sample number(s) that were transferred as MICP prep level comment.

12.6 If using Colilert-18® for presence/absence testing, the sample is still required to be put in a Quanti-Tray. This pre-warming step is not required if using Colilert-18® for quantitative analysis or Colilert® or Colisure®.

12.7 Aseptically transfer contents of reagent into bottle.

12.8 Close cap tightly and shake to dissolve reagent.

12.9 When all samples are processed, spray area with ethanol solution and wipe away ethanol and possible debris with a paper towel.

12.10 If identification of total coliform is requested, place a label on the sample bottle (or Quanti-Tray, if used) and on the test request form that states, "ID If Unsafe".

12.11 If a client requests "numbers" or "counts" use the Quanti-Tray/2000® (QT) method:

12.11.1 Label the Quanti-Tray/2000® with the sample number

12.11.2 Pour off sample to 100 mL and add reagent, wait for reagent to dissolve.

12.11.3 Shake sample 25 times, add antifoam if necessary, and aseptically add sample to QT. Run the QT through the QT sealer according to manufacturer instructions.

12.11.4 100 mL and 1.0 mL of sample are performed on surface water samples unless requiring more dilutions and 100 mL only are performed on beach and drinking water samples.

12.11.5 If a client requests "ID if unsafe", place a blue "ID if unsafe" sticker on both the paperwork and the Quanti-Tray® to indicate that the sample will undergo identification if it is total coliform positive.

12.12 For both presence/absence and quantitative samples you must create and clone a batch for sample data management into the Chemware/Horizon system and print a label with the HBN #, HBN barcode, analyst initials and date/time. Place the label on the first bottle in the batch. See ref.16.12.

12.13 Place samples into 35ºC incubator and incubate according to the chart below (Table 1-section 17.1)

12.14 Record sample numbers, time and analyst's initials in logbook of the 35ºC incubator. This process is used for analyst ease in finding samples and logging samples out since data is currently managed by the Chemware Horizon system.

12.15 Place a sticker with the starting incubation time on each batch. For Colilert-18®, instead indicate the appropriate readout window and indicate "C18" on the sticker. Place racks on shelves corresponding to day of week, sample type, and starting incubation time or readout window.

12.16 Results are read out after specified incubation times using the following criteria in Table 2. See "Allowable Read-Out Times" table for specific set-up read-out times. (Table 2-section 17.2)

12.17 Record the sample on ESS MICRO FORM 147 Incubator Sample Login Form, located in a binder in the walk-in incubator, with the time and initials when samples are read in, out, and sample source (ref. 16.17).

12.18 A color change equal to or greater than the comparator for Colilert® and Colilert-18® (from clear to yellow with Colilert® and Colilert-18®) indicates the presence of total coliform bacteria in the sample. If colors are border-line, the sample must be incubated for up to 28 hours for Colilert® and 22 hours for Colilert-18®. If a sample exhibits a color greater than the comparator at any point between 24-28 hours, it is reported as present. If color is still lighter than the comparator after additional incubation, the samples are reported as total coliform absent. If the color change is indeterminate, invalidate the sample at no charge to the customer and ask them to resample, if possible, and ask them to retest requesting Colisure.

12.19 There is no comparator commercially available for Colisure®. A color change from yellow to magenta indicates the presence of total coliform bacteria in the sample. If a magenta color or an intermediate color does not appear between 24-48 hours, the samples are reported as total coliform absent. If colors are intermediate, the sample must be incubated for up to 48 hours. If a sample appears magenta at any point between 24-48 hours, it is reported as present for total coliforms. If the color change is indeterminate and the sample is nearing 48 hours or at a time when it is not feasible to continue incubation up to 48 hours, invalidate the sample at no charge to the customer and ask them to resample, if possible.

12.20 All total coliform positive samples are screened for the presence of *E.coli* by turning the incubator light off and placing the sample approximately 5 inches from a 366 nm, long wavelength UV light in the darkened room. The presence of blue fluorescence indicates the presence of *E.coli*. For Colilert® and Colilert-18® samples a comparator is used. If a sample exhibits fluorescence greater than the comparator at any point between 24-28 hours, it is reported as present for *E.coli*. If the fluorescence is still lighter than the comparator after additional incubation, the samples are reported as *E.coli* absent. If the fluorescence change is indeterminate, invalidate the sample at no charge to the customer and ask them to resample, if possible.

12.21 There is no comparator commercially available for Colisure®. All total coliform positive samples are screened for the presence of *E.coli* by turning the incubator light off and placing the sample approximately 5 inches from a 366 nm, long wavelength UV light in the darkened room. The presence of blue fluorescence indicates the presence of *E.coli.* If the fluorescence change is indeterminate, the sample must be incubated for up to 48 hours. If a sample exhibits fluorescence at any point between 24-48 hours, it is reported as present for *E.coli*. If the fluorescence is indeterminate and the sample is nearing 48 hours or at a time when it is not feasible to continue incubation up to 48 hours, invalidate the sample at no charge to the customer and ask them to resample, if possible.

12.22 The sample results are recorded using the analytical batch in the Chemware/Horizon software as total coliform present or absent, with *E.coli* present or absent (ref.16.12). When there is a total coliform positive or total coliform and *E. coli* positive sample, it is entered into the analytical batch and the data review must be performed by a second analyst. If all sample results are negative for total coliform and *E. coli*, the data review can be performed by the same initial analyst.

12.23 For quantitative analyses, the number of chromogenic/fluorescent large and small wells is counted and recorded in the analytical batch. Chemware/Horizon will calculate the MPN according to the IDEXX™ chart provided or software. If the result is associated with a dilution of the sample either change the initial volume or the dilution factor before recording large and small wells. Once the worklist is posted, ensure the dilution is correct by performing a Quality Control Batch Review. Results of each dilution not used for the final result for total coliform and *E. coli* are recorded in the "comment" section of the analytical batch. The purpose is to maintain an electronic record that the analysis had been conducted.

12.24 Chemware/Horizon will not report an MPN of <1 when no wells are positive. If no wells are positive the analyst must enter zero (0) large wells, enter zero (0) small wells, save the results, and then type "<1" into the text box next to the result field. If this is not done, no result will report. The analyst that performs the data review must verify that this is done when necessary.

12.25 Chemware/Horizon will not report an MPN when 49 positive large wells and 48 positive small wells are entered for results. When a 49/48 (every well is positive) result is entered and saved, the analyst must type the IDEXX Quanti-Tray MPN Table result ">2419.6"

into the text box next to the result field. If this is not done, no result will report. The analyst that performs the data review must verify that this is done when necessary.

12.26 After the results are posted the Quality Control Report is generated and the analyst reviews the results that will be reported to the client. Corrections to results are made before the data review step is performed. A second analyst must perform the data review step for samples in Quanti-Trays® regardless if results are positive or negative. It is not necessary for a second analyst to perform the data review step on weekends.

12.27 After the results are accepted, the samples are transferred to the MERI in Horizon and thrown in the MERI barrel for disposal.

13.0 Data Analysis and Calculations

13.1 The manufacturer-defined detection limit is one total coliform/*E.coli* per 100 mL.

13.2 Presence/absence is reported for most drinking water samples.

13.3 For all Quanti-Tray methods if 100 mL of sample is used, the MPN (most probable number) is generated by Chemware/Horizon from the IDEXX chart or software. The MPN calculation is checked periodically to assure the correct number is generated.

13.4 If a dilution needs to be entered into Horizon use the following equation:

13.4.1 Dilution = (100 ml / volume per mL analyzed)

Example:

Volume analyzed = 30 ml
Dilution = (100 ml / 30 ml)
Enter a dilution of 3.3 into Horizon

13.5 If the dilution is manually determined the MPN is determined by:

13.5.1 Total coliform or *E.coli* / 100 mL = MPN from chart or software X (100 mL / volume per mL analyzed).

Example:

MPN = 24
Volume analyzed = 0.01 mL
Total coliform or *E.coli* / 100 mL = 24 X (100 mL / 0.01 mL) = 240,000
Total coliform or *E.coli* MPN/ 100 mL = 240,000

13.5.2 Alternative determination is by logs per 100 mL:

- The MPN is determined by IDEXX software or chart and Table 3.
- The number of zeros is added based on the reverse of the log of ten per 100 mL.

Example:

MPN = 24

The volume used is 0.01 which is minus two logs per 1 ml or minus 4 logs per 100 mL.
So 4 zero's will be added to the result.
24 + 0000
240,000

14.0 Method Performance

14.1 Initial demonstration of capability data (IDOCs) and on-going annual demonstrations of capability (ODOCs) are performed by each analyst and must fall within defined acceptance criteria. DOC data is stored here: M:\EHD\ESS(4900)\ESS Micro(4920)\QAQC\DOC documentation.

14.2 Proficiency test samples are performed once annually. PT results are stored here: M:\EHD\ESS(4900)\ESS Micro(4920)\QAQC\Proficiency Testing.

14.3 Internal quality control is performed on a regular basis, including all enzymatic reagents to ensure acceptable performance (ref 16.9). Quality control data is stored electronically at M:\EHD\ESS(4900)\ESS Micro(4920)\QAQC and in hardcopy in the laboratory QC binder.

15.0 Data Assessment and Management

15.1 Samples must be incubated within the stated time parameters.

15.1.1 Contingency: If a sample is incubated longer than stated time parameters and the sample is negative, the sample may be reported out as total coliform absent with a disclaimer on the report stating the sample was not incubated within time constraints.

15.1.2 Contingency: If samples are positive when incubated over the time parameters, the results are reported as lab accidents.

15.2 Other than for incubation warm up, samples where temperatures were not within limits, the results are flagged with a disclaimer and reported out of range (±0.5ºC) or the samples are reported as lab accidents.

15.3 Any other data that doesn't meet quality control standards during the testing process will be reported and flagged or the results invalidated.

16.0 Related Documents

16.1 Federal Register, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical & Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule, 40 CFR parts 141 and 143, Vol. 64, No 230

16.2 EPA Manual for the Certification of Laboratories Analyzing Drinking Water Criteria and Procedures Quality Assurance 5th Edition

16.3 APHA, 2005. *Standard Methods for the Examination of Water and Wastewater,* 21st Edition.

16.4 Environmental Health Division Quality Assurance Manual, Wisconsin State Laboratory of Hygiene.

16.5 Wisconsin State Laboratory of Hygiene, AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, State Laboratory of Hygiene.

16.6 University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, https://ehs.wisc.edu/laboratory-safety-guide/

16.7 EHD GENOP 038, "Waste Management," Environmental Health Division, Wisconsin State Laboratory of Hygiene."

16.8 ESS MICRO QA 202, "Colilert®, Colilert-18 ®, Colisure ®, Enterolert, Pseudalert, Colitag ™ and Readycult® Quality," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.9 ESS MICRO QA 212, "Sample Bottle Sterility/Calibration/Fluorescence," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.10 ESS MICRO QA 214, "Quanti-Tray ® Sterility Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.11 ESS MICRO QA 218, "Quanti-Tray ® Sealer Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.12 ESS MICRO GENOP 411, "Cheware/Horizon Process for Analytical Testing," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.13 ESS MICRO QA 206, "Maintenance of Stock Cultures for Quality Control," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.14 ESS MICRO QA 266, "Free Chlorine Testing of Drinking Water Samples," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.15 ESS MICRO IOP 504, "Orion Star A212 Conductivity Meter Calibration and Measurement," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.16 EHD GLASSWARE MEDIA IOP 301, "Meridian MR-10 pH Meter ," EHD Glassware Media Dept., Wisconsin State Laboratory of Hygiene.

16.17 ESS MICRO FORM 147 "Incubator Sample Login Form", EHD Glassware Media Dept., Wisconsin State Laboratory of Hygiene.

16.18 Wisconsin Statutes by the Legislative Reference Bureau. Department of Natural Resources. Chapter NR 812, "Well Construction and Pump Installation". https://docs.legis.wisconsin.gov/code/admin_code/nr/800/812.pdf

17.0 Tables and figures

17.1 Table 1—Color Change:

Reagent	Incubation time	Total Coliform Absent	Total Coliform Present
Colilert®	24-28 hrs	Clear	Yellow*
Colilert-18®	18-22 hrs	Clear	Yellow*
Colisure®	24-48 hrs	Yellow	Magenta

*Color must be equal to or greater than the comparator for Colilert® and Colilert-18®. If colors are border-line, the sample may be incubated for up to 28 hours for Colilert® and 22 hours for Colilert-18®. If color is still lighter than the comparator after additional incubation, the samples are reported as total coliform absent. If the color change is indeterminate, invalidate the sample for any of the methods.

17.2 Table 2 – Readout times

Setup Time Military Time	Colilert® read time - Next day: 24-28 hrs	Colilert-18® read time – Next day: 18 – 22 hrs	Colisure® read time – Next day to following day: 24-28 hrs
0700	0700 - 1100	0100 - 0500	0700 – 0700 next day
0800	0800 - 1200	0200 - 0600	0800 – 0800 next day
0900	0900 - 1300	0300 - 0700	0900 – 0900 next day
1000	1000 - 1400	0400 - 0800	1000 – 1000 next day
1100	1100 – 1500	0500 - 0900	1100 – 1100 next day
1200	1200 - 1600	0600 - 1000	1200 – 1200 next day
1300	1300 – 1700	0700 - 1100	1300 – 1300 next day
1400	1400 – 1800	0800 - 1200	1400 – 1400 next day
1500	1500 - 1900	0900 - 1300	1500 – 1500 next day
1600	1600 - 2000	1000 - 1400	1600 – 1600 next day
1700	1700 - 2100	1100 - 1500	1700 – 1700 next day

17.3 Table 3 – Zero's added per 100 mL

Volume used	Log	Zero added to result
100 mL (0)	10^2	0
1 mL (2)	10^0	2
0.01 (4)	10^{-2}	4
0.0001 (6)	10^{-4}	6

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18.0 Version Tracking Table

Version number	Version date	Changes Made	Version author
	12/01/2009	Add the recording of Total Coliform and *E.coli* per Groundwater Rule Changed Format to Current WSLH format for SOPs Added Table 3 to SOP	
	1/19/2009	Added action under "Interferences" regarding sample rejection due to atypical l results	
6	12/17/2012	In section 9.2.1—changed 48 hrs to 30 hrs for age of public water system samples not to be tested. In section 10.6—added testing media when received and on a monthly basis. In section 12—added info about using the Chemware/Horizon system Re-formatted	J. Olstadt
7	3/28/2016	Added NR812 information Changed receipt time from 6 hours to 8 hours for surface water and wastewater samples. Clarified the icing information.	J. Allan
8	6/6/18	Changed volume sampled from 98-102 to 100 ml.	A. Beck
9	1/30/2019	Elaborated on incubation process and reading out of samples.	P. Mullen
9	2/4/2019	Sections 6, 10, and 12: Fixed the Referenced Documents mentioned in the sections to match the correct reference numbers in the Referenced Documents Section (#16)	O.Feider
10	3/18/2019	Included monitoring of conductivity and pH of laboratory water used for dilutions and added corresponding references.	P. Mullen
10	4/30/2019	Changed "safe" terminology to "total coliform absent" or "*E.coli* absent"	M.Collins

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11	5/13/2019	Revised Method Performance section	M. Collins
11	6/14/2019	Revised Section 13, Data Analysis and Calculations, to include an example of how to enter a dilution directly into Horizon. Revised Section 12, Procedure, to include instructions on an alternative method to pour off a sample into a sterile secondary container for analysis. Revised Section 12, Procedure, to included instructions on how to ensure there is at least one inch of head space in sample bottles, as well as, instructions to handle samples with less than one inch of headspace.	Z. Zopp
11	7/19/2019 10/1/2019	Revised warm comments in sections 9.8 and 9.9 to reflect updates in Horizon. Added that reagent is stored away from light in section 8.1 Changed location of HBN sticker in section 12.10 Added Form 147 to section 12.15 and what to record on Form 147.	S. Johnson-Windsor
12	11/12/2019 2/17/2021	In Section 15, changed the temperature range from ± 2°C to ± 0.5°C to match temperature range for the method. In Section 12: added a step to disinfect area with ethanol before and after testing, changed "> 2420" to "> 2419.6" to reflect actual number used. Section 10 added to discard any discolored packets. Added ethanol and paper towels to Reagents and Supplies. Clarified language in section 13 for calculations.	S. Johnson-Windsor
12	10/9/2020	Changed frequency of PTs from twice annually to once annually; removed NELAC reference	M. Collins

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12	6/4/2021 7/13/2021	Replaced EHD Media IOP 300 with IOP 301 Added Wisconsin Legislation reference for NR812. Added details for “no test” situations under NR812 legislation and definitions Updated references within method text.	S. Johnson-Windsor
12	01/25/2022	Updated reference to related documents within text.	A. Cooke
OB 1	03/24/2022	Document transitioned to OnBase. In Header: SOP ID updated to EHD from ESS, Water Microbiology Department updated to ‘Section’. Footer updated to include transition to OnBase. Table of contents removed, hyperlinks removed. Language referring to revision updated to ‘version’.	S. Johnson-Windsor

EHD INORG METHOD 151.1
Chlorophyll *a*, Fluorescence
(EPA 445.0, rev. 1.2, Sept. 1997 and Welschmeyer, 1994)

1. Scope and Application

1.1. Chlorophyll *a*, a characteristic algal pigment, constitutes approximately 1 to 2% (dry weight) of planktonic algal biomass. This feature makes chlorophyll *a* a convenient indicator of algal biomass.

1.2. This method is applicable to the analysis of chlorophyll *a* in surface waters.

1.3. The Limit of Detection was determined according to ESS INO QA 116. The detection limit is dependent on sample volume filtered and fluorescence intensity. The detection limit for the instrument is 4 µg/L in the extract, which is always 13 mL. Based on a filtered volume of 200 mL, the sample limit of detection (LOD) is 0.26 µg/L, ((4 µg/L x 0.013L)/0.2 L) and the limit of quantification (LOQ) is 0.87 µg/L. Applicable concentration range for samples is dependent on volume filtered. The instrument is calibrated to approximately 800 µg/L.

2. Summary of Method

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

2.2. This method deviates from EPA 445.0 in the following ways (note that WDNR has approved these method modifications—see ref. 15.20):

2.2.1. Millipore type SM, 47 mm 5.0 µm membrane filters are used instead of the glass fiber filters recommended in the method to provide continuity with Wisconsin's historical chlorophyll data (WIDNR long-term trend monitoring data).

2.2.2. A Branson Ultrasonic Cleaner (Bath type sonication) is used to aid in extracting the chlorophyll from the algal cells instead of a tissue grinder. Bath type sonication is more efficient and is comparable to tissue grinding under most circumstances (see ref. 15.3, 15.4, and 15.5).

2.2.3 The instrument is calibrated every day of analysis. The instrument software uses linear regression rather than response factors for calibration.

2.2.4 A quality control sample (QCS) is run every day of analysis prior to sample analysis.

2.2.5 All sample results are determined "uncorrected", with no acidification for pheophytin correction according to Welschmeyer (15.1) and EPA 445.0, rev 1.2,

1997) (15.2). Because the fluorescence spectrometer used for this test is a higher resolution instrument, pheophytin correction is unnecessary (see reference 15.14).

2.2.6 Thirteen (13) mL of 90% acetone is used for extraction.

3. Safety, Waste Management, & Pollution Prevention

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, and the University of Wisconsin Laboratory Safety Guide (see ref. 15.6- 15.7).

3.2 All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (ref. 15.7).

3.3 Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (ref. 15.7).

4. Sample Preservation and Preparation

4.1 Samples for chlorophyll *a* analysis filtered in the field must be folded and put into a 15 mL polypropylene centrifuge tube, labeled with the sample volume filtered, wrapped in foil, and promptly shipped to the lab on ice.

4.2 Samples for chlorophyll *a* analysis to be filtered in the lab must be collected in a plastic quart bottle and be packed with ice in a dark cooler at the time of collection. These samples must be kept in the dark before filtering and filtering must occur within 48 hours of receipt. For these samples, filter no more than 200 mL of sample through a 47 mm, 5 µm pore size membrane filter and applying vacuum. Vacuum should not exceed 6 inches of mercury (20kPa). Less volume should be filtered if the chlorophyll concentration is expected to be high or filtering takes longer than 10 minutes. The filtration process must be performed in subdued light. For detailed filtering instructions please see appendix 2 at the end of this SOP. Refer to EHD INORG GENOP 151 (15.18) to process these samples through HORIZON.

4.2.1 Fold the filter into quarters, insert into a graduated 15 mL polypropylene conical centrifuge tube with a screw cap, and store in a dark freezer (instrument #45 at < -20°C). Be sure to record the appropriate information, including volume filtered, on the lab filtered chlorophyll log sheet.

4.3 Store field filtered samples in freezer upon arrival at the lab. Insert filters into graduated 15 mL polypropylene conical centrifuge tubes with screw caps when necessary.

4.4 Thirteen mL of 90% aqueous acetone solution is added to all samples prior to sonification.

4.5 Samples may be held at -20˚C for up to 3½ weeks after filtering. Although there is no mandated holding time for chlorophyll, the laboratory strives to complete analyses with the recommended 3½ week holding time.

4.6 Periphyton samples collected on filters will be handled in exactly the same manner as

field filtered chlorophylls with any deviations mentioned in EHD INORG GENOP 151 (15.18).

4.7 Periphyton samples collected on glass slides will be prepared according to Appendix 4 and processed through HORIZON according to EHD INORG GENOP 151 (15.18).

5. Interferences and Comments

5.1. Any substance that fluoresces at 680 nm may interfere in the accurate measurement of chlorophyll *a*. Using the narrow slit width (3.0 nm) eliminates most common interferences.

5.2. Handle samples in subdued light to prevent photochemical breakdown of the chlorophyll.

5.3. Handle filters with forceps to prevent breakdown of chlorophyll from hand contact.

5.4. Protect the acetone extract from more than momentary exposure to light.

6. Reagents and Standards

6.1 Aqueous acetone solution (90%): Mix 90 parts (by volume) reagent grade acetone with 10 parts reagent water (by volume). This solution has an expiration date of one year from date prepared. Record appropriate information in logbook #14 (under the 90% Acetone tab). The reagent code will also be written on the bench records, the bottle itself, and the repipettor. This logbook is located in room 119.

6.2 Reagent water, ASTM Type I: Prepare by passing R.O. water through a U.S. Filter Pure-Plus Water System.

6.3. Chlorophyll *a* Standard stock: Obtained from Sigma Chemical (St. Louis, MO.) in dry form and diluted with aqueous 90% acetone (6.1). Sigma #C-6144, (chlorophyll *a* from *Anacystis nidulans* algae) 1 mg size.

6.3.1. In subdued light, quantitatively transfer the entire contents of the vial to a 100 mL volumetric flask using 90% acetone to rinse all material from the vial. Dilute to the mark with additional 90% acetone and mix thoroughly. The nominal concentration is about 10 mg/L. The actual chlorophyll *a* concentration can be determined by averaging four replicate readings using the spectrophotometric method (uncorrected) described in EHD INORG IOP 151.1 (see appendix 1), although this is not required. All pertinent information, including the stock standard code, manufacturer, lot#, date received, concentration, date prepared, analyst's initials and expiration date must be recorded in the standards logbook #ESS810, located in the Wet Chemistry area. The stock standard must be stored in a light tight box in the -20ºC freezer, located in the alcove in room 119. The expiration date is one year from the date prepared.

6.3.2. Working standards: Prepare the following working standards after making stock standard as in 6.3.1. All standards are diluted to volume with aqueous 90% acetone (6.1). Please note that the below working standards are nominal concentrations. The exact concentration will vary from lot to lot. All concentrations for the working standards need to be determined by averaging three replicate readings using the spectrophotometric method (uncorrected) described in EHD INORG IOP 151.1 (see appendix 1). All pertinent

information (as in 6.3.1) must be recorded in the standards logbook #ESS475, located in the Wet Chemistry area, room 119. Transfer working standards to screw capped amber bottles and label. The working standards must be stored in a light tight box in the -20ºC freezer, located in the alcove in room 119. The expiration date is six months from the date prepared.

Volume of stock (6.3.1) standard (mL)	Diluted to volume (mL)	Nominal concentration mg/L
0.30*	250	0.012
2.5	500	0.050†
5	500	0.100†
10	500	0.200†
25	500	0.500†
20	250	0.800

*Use an electronic, variable volume Rainin pipette to prepare this standard. Class A volumetric pipettes may used for the rest.

† These standards are rotated as the IPC

6.4. Quality control sample (QCS): Prepare from a different Sigma lot than the stock standard. Sigma catalog # C5753 (chlorophyll *a* from spinach)

6.4.1. Prepare a 10 mg/L (nominal concentration) stock QCS. The actual concentration can be determined as in 6.3.1 (average of four replicate readings). Transfer to a screw capped amber bottle, and label. All pertinent information must be recorded in the standards logbook #ESS810, located in the Wet Chemistry area, room 119. The stock QCS must be stored in a light tight box in the -20ºC freezer, located in the alcove in room 119. The expiration date should be one year from the date prepared.

6.4.2. Prepare a 200 µg/L (nominal concentration) QCS (to be used with every analytical run) and determine actual concentration as in 6.3.2 (average of three replicate readings). Transfer to a screw capped amber bottle, and label. All pertinent information must be recorded in the standards logbook #ESS475, located in the Wet Chemistry area. The QCS must be stored in a light tight box in the -20ºC freezer, located in the alcove in room 119. The expiration date is six months from the date prepared.

7. Apparatus

7.1 Standard laboratory glassware including membrane filtration apparatus.

7.2 Millipore Type SM, 47 mm, 5.0 µm pore size membrane filters.

7.3 Calibrated 15 mL polypropylene centrifuge tubes with screw caps.

7.4 Vacuum source with an adjustable vacuum gauge.

7.5 Light-tight box capable of holding a 40-tube test tube rack.

7.6 Branson Model 5210 MT Ultrasonic Cleaner for cell disruption.

7.7 International Equipment Company Model K centrifuge, capable of attaining 675XG.

7.7.1 The centrifuge will be verified annually with a NIST traceable laser tachometer to ensure that it can attain the 675XG force requirement. See 11.5 for how to calculate g force.

7.8 Perkin-Elmer fluorescence spectrometer, model LS – 55.

7.9 Re-pipet dispenser, 25 mL capacity.

7.10 Rainin variable volume electronic pipettes, Eppendorf mechanical air displacement pipettes and standard class A volumetric pipettes.

7.11 Digital laser tachometer (Fisher catalog number 13-245-278), traceable to NIST

8. Quality Control Types, Acceptance Criteria, & Corrective Actions.

8.1 Please refer to the Environmental Health Division Quality Assurance Manual (15.10) for general information on quality control procedures. Important specifics include:

8.1.1 Accuracy and precision calculations.

8.1.2 Corrective action and result qualification procedures (including documentation requirements) for instrument problems or analytical problems.

8.2 A Laboratory Reagent Blank (LRB) will be analyzed with every analytical run. This is made by taking a membrane filter (7.2), placing it in a 15mL polypropylene centrifuge tube (7.3), adding 13mL of 90% acetone (6.1), and carrying it through the entire preparation procedure. This will be analyzed at the beginning of the analytical run, and after every 20 samples and must be within ±0.26 µg/L, the LOD based on filtered volume of 200 mL. If the LRB fails it should be re-analyzed. If it still fails the analyst should evaluate if recalibration would improve the blank reading. If recalibration is done the samples back to the last good LRB and IPC must be re-analyzed. If recalibration does not cause the blank to be acceptable, the 20 samples associated with that LRB must be qualified with a comment stating that the LRB exceeded acceptable limits.

8.3 A working QCS (see section 6.4.2) is run at the beginning of every analytical run. The observed concentration of the QCS must be within ± 10% of the true value (6.4.2) before proceeding with analysis. Re-prepare the QCS if prep error is suspected and reanalyze. If QCS still fails, re-calibrate and try again. If subsequent attempts fail and samples cannot be stored, proceed with the analyses and qualify all results.

8.4 The Limit of Detection (LOD, the concentration at which the result is definitely distinguishable from a blank) must be verified annually, or after any significant work is done on the instrument. For more information on LOD protocol, see EHD QA 116 (15.13).

8.5 At least 10% of lab filtered chlorophyll samples are analyzed in duplicate. The difference between the duplicate measurements must be within control limits before

sample results are considered acceptable. Samples that fail to meet QC limits will be qualified. Since the majority of samples are field filtered and planktonic material tends to be heterogenous in nature, little corrective action can be taken to improve precision. Visual examination of the extract, documentation and notification of data users through qualifiers is about all that can be done. Consequently, entire batches of data are not qualified based on duplicate QC failures.

8.5.1 The QC limits for duplicate analyses can be found in HORIZON (15% RD) or the duplicate must be within ± the LOD of the original result.

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

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

8.8 An Instrument Performance Check (IPC) (see section 6.3.1) is run every 10 samples. The IPC must be within ± 10% of the true value. If it deviates from this acceptable limit, the analyst will attempt to determine whether the cell has become dirty, the instrument has drifted, or the IPC is contaminated. If the problem can be identified, it is corrected, the instrument re-calibrated and all samples back to the last valid IPC will be reanalyzed.

8.9 Dilutions are typically made by adding 1mL of sample to 4mL of 90% acetone solution using mechanical air displacement pipettes (7.10). Dilute high samples, add the sample numbers to analytical run list, change the dilution factor to reflect the 5x dilution, and analyze along with an IPC and CB every ten samples and at end of the run of diluted samples. Dilution concentrations should be within 90%-110% of the original concentration. If dilutions do not agree with the initial concentration, another different dilution should be performed to verify. If two serial dilutions do not agree (90%-110%), the sample result must be qualified.

8.10 An initial demonstration of capability (DOC) and annual continued proficiency checks will be performed according to reference 15.12.

8.11 Linear Dynamic Range (LDR) - determined when the instrument is set-up, when a new method is being developed, or when, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate it be redetermined. Calibrate the instrument. Standards at continually higher concentrations than the top standard are analyzed until the percent recovery exceeds 10%.

8.12 Record date, analyst, intensities of top standard and QCS, standards and QCS codes, HORIZON batch number, and any applicable comments in instrument logbook #ESS798 for instrument IC133 (LS-55 [7.8]).

9. Method Calibration

9.1 The calibration curve is constructed using a blank and six (6) standards of increasing concentration of chlorophyll *a* from approximately the limit of quantification (LOQ = 12 µg/L) up to approximately 800 µg/L (see section 6.3.2). These concentrations are for chlorophyll *a* in the acetone solution extract.

9.2 The working calibration standards (6.3.2) are set out on the counter and allowed to warm to room temperature in the dark and used to calibrate the instrument each analysis day. The stock working calibration standards are discarded after six (6) months.

9.3 The sample chlorophyll concentrations are calculated directly within the instrument software using a linear regression. The standards are entered in the instrument sequence in mg/L (ppm) even though the samples are reported out in µg/L (ppb) chlorophyll *a*. This is done due to limitations of the software correction factor field. Please refer to software printout in Figure 1 for further explanation. Since all standards and samples must have the same concentration units in the software, the reporting units for the reference samples must be changed manually after every analytical run. This is done by making a single line through the (ppb) above the sample results, and initial and date the correction.

9.4 The calibration curve must have a correlation coefficient r ≥0.999. The curve is printed out for visual verification. If unable to achieve the ≥0.999 r coefficient, visually check for standards that are obviously bad, re-make standards as needed, and recalibrate. **DO NOT** proceed with the analysis until the problem is resolved.

9.5 The 2016 TNI Standard (15.9) requires the % Relative Error (%RE) to be calculated and evaluated for each the low and midpoint standards and must pass acceptance criteria before continuing analysis. The acceptable %RE for the low and midpoint standards is ±50% and ±30%, respectively. These values will be manually entered into HORIZON into the %RELOW and %REMID standards, located at the end of the batch. Due to HORIZON limitations, only recovery will be calculated. Since %RE = Recovery – 100%, the acceptable limits for the low and midpoint standards in HORIZON become 50-150% and 70-130%, respectively.

10. Procedure

10.1 Refer to EHD INORG GENOP 151 (15.18) to determine procedures necessary to process samples through HORIZON.

10.2 All tubes must be unwrapped, or unpackaged as necessary, being sure to place the barcode label (with the lab slip number) on the tube. Place any filters received in foil, or in a miscellaneous container, in a graduated 15 mL polypropylene conical centrifuge (7.3) tube with screw cap and be sure to transfer barcode label to tube. All tubes are placed in racks of 36 (due to max sample places in centrifuge), in the order of the worklist (field filtered first, then lab filtered).

10.2.1 Barcode label should be placed on the tube so that the barcode is in line with the tube. If the barcode is the other direction it will not be able to be scanned.

10.3 Add 13 mL of aqueous 90% acetone (section 6.1), using a repipette dispenser, to each sample tube. Shake vigorously for 20 seconds to break up filter. Place tubes in the light-

tight box when not being processed.

10.4 Suspend rack with tubes in the ultrasonic bath with water one inch from the top. Cover (to exclude light) and sonicate for 25 minutes. Shake tubes vigorously for 20 seconds and return the rack to the light-tight box.

10.5 Place the light-tight box in the < 4°C cold room and allow the extract to steep for at least 2 hours but not to exceed 24 hours (overnight).

10.6 Shake sample tubes vigorously after steeping overnight. Clarify the extract by centrifuging the tubes for 15 minutes at approximately 675XG (setting of 35-40 on International Equipment Company Model K centrifuge).

10.7 Put the pump tubing on the LS-55 fluorescence spectrometer (7.8) into the pump roller and adjust tension to obtain smooth flow. Rinse the fluorescence cell with 90% acetone (6.1).

10.8 Open FLWinlab software and select "chlorophyll.mth" under the applications listed.

10.9 Under the "Setup parameters" tab change the destination filename to: U<fiscal yr. Date>.rpt

10.10 Under the "References" tab change standard concentrations to 3 decimal places. The software automatically changes concentrations to 2 decimal places when closed.

10.11 Under "Samples" tab, enter QCS (Horizon sample number), IPC, (both corr. fact. = 1000), CB (corr.fact. = 65), the sample lab numbers and correction factors ((13/vol filtered) x 1000). Sample numbers may be entered using the barcode scanner attached to the instrument and the lab worklist which has all the barcodes printed out (and the volumes to calculate the correction factors).

10.12 Place aspiration tubing in blank 90% acetone and click on "Measure background" button, (value should be near 0.000). Always set the background to 0.000 regardless of what was measured. Calibrate the instrument by running a blank and subsequent standards in increasing concentration order (linear, with calculated intercept). The correlation coefficient (corr) must be ≥ 0.999 before samples can be analyzed.

10.13 After calibration, evaluate and verify the calibration process (QCS, IPC, and CB.) before beginning analysis of samples. Analyze the IPC and CB every ten samples and at the end of the run, and a LRB at the beginning and after every 20 samples. Take the appropriate corrective action described in the Quality Control Section (8) if any IPC, CB or LRB exceeds limits.

10.13.1 Record the fluorescence intensity of the QCS and the top calibration standard in the instrument logbook #ESS798 along with the standard and QCS codes and HORIZON batch number.

10.14 Remove one tube at a time from the light-tight box and using the sipper system, aspirate sample into the instrument. The intensity will be measured and the concentration will be automatically calculated. Both values are recorded in the .rpt data file. Return the sample to the light tight box in case reanalysis is required. At end of run click on "Save Results" button.

10.15 If the fluorescence intensity of a sample is greater than the top standard intensity, return

the sample tube to the light-tight box so it can be diluted and re-analyzed at the end of the analytical run by making a new "Samples" list, with a correction factor that reflects the proper dilution.

10.16 When all samples have been analyzed, print the calibration and sample files using the printer icon. Data are saved on the network in G:/Flwinlab/Data/filename.rpt.

10.17 Transfer the data according to EHD INORG GENOP 151 (ref. 15.18).

11. Calculations.

11.1 The sample intensities are converted to concentration by the software based on a linear regression calibration curve. A correction factor is applied to convert the concentration from the regression to the sample concentration in µg/L.

11.2 The general equation for determining the chlorophyll is as follows:

11.2.1 mg/L from regression*13 mL (extract volume)*1/mL sample filtered * 1000 µg/mg = chlorophyll a in µg/L.

11.3 The correction factor is used to convert the concentration of chlorophyll *a* in the extract to the concentration of chlorophyll *a* in the sample based on the extract volume and the volume of sample filtered. This process is accomplished using a correction factor.

11.3.1 The Perkin-Elmer instrument software has a limit of 2 decimal places. Consequently, we cannot calibrate in units of µg/L because the correction factor (see 11.2.1) has too few decimal places and it would be have to be rounded. For example, if calibrating in µg/L, the correction factor would be 0.065. However, the software would round that factor to 0.06, which would bias test results. To get around this problem, we calibrate in mg/L and add a multiplication factor so we can report in units of µg/L. Details of the correction factor follow.

11.3.2 Correction Factor = (13 mL of sample in extract / mL of sample filtered)*1000. For most samples, the factor is: (13mL / 200 mL)*1000=65.

11.3.3 For dilutions (section 8.9) the correction factor needs to reflect the dilution (multiplied by 5 for a fivefold dilution). For example, a sample diluted 1 to 5 that has 13 mL of extract and 200 mL of sample filtered would need a correction factor of 325 (65*5=325). This ensures that the result is properly calculated by the software. For the correct way to enter dilutions into the software see ESS INO GENOP 151 (15.19).

11.4 Duplicates and spikes are calculated as shown in the EHD QA manual (15.10).

11.5 For converting RPM to Relative Centrifugal Force (G-force) the following equations are used:

$$g = N^2 \times 1.118 \times 10^{-5} \times r$$

$$N = \sqrt{\left[\frac{g}{(1.118 \times 10^{-5} \times r)}\right]}$$

Where: g = G-force or relative centrifugal force (RCF)

N = revolutions per minute (RPM)
r = radius of rotor (cm)

12. Data Management

12.1 QC data will be evaluated in the HORIZON operating system.

12.2 The entire analytical run is passed on to another chemist for QC audit. An analytical run will include: cover sheet with queue, batch number, and HBN, a batch worklist for each of the prep batches and any and all analytical batches, and all raw data.

12.3 Once the QC audit has been completed the entire run is stapled together and filed with the other chlorophyll runs.

13. Definitions

13.1 Definitions of terms in this SOP may be found in section 3.0 of Method 445.0 (see ref. 15.2).

13.2 General definitions of other terms that may be used in this method are found in the EHD Quality Assurance Manual (see ref. 15.10).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (LODs and DOCs) were generated in compliance with the reference method and the standard operating procedures: EHD QA 115 (see ref. 15.12), and EHD QA 116 (see ref. 15.13). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement, and will be retained according to the applicable records disposition authorization (RDA).

15. References

15.1 Welschmeyer, 1994 Fluorometric analysis of chlorophyll *a* in the presence chlorophyll b and pheopigments. Lmnol. Oceanogr. 39(8), pp. 1985-1992, (1994)

15.2 Environmental Protection Agency (EPA) Method 445.0 rev 1.2 (September 1997).

15.3 Garrison, P., Comparison of Grinding samples vs. Sonicating, Memorandum, (1990).

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15.5 Nelson, D.H. Improved Chlorophyll Extraction Method, Science, 132, p. 351, (1960).

15.6 AG DR SAFETY GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan, Wisconsin State Laboratory of Hygiene.

15.7 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.8 Axler, R.P, and C.J. Owen. Measuring Chlorophyll and Pheaophytin: Whom Should you Believe? Lake and Reserv. Manage. 8(2): pp. 143-151. (1994).

15.9 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.10 Quality Assurance Manual, Environmental Health Division, Wisconsin State Laboratory of Hygiene.

15.11 ESS INO METHOD 150.1, "Chlorophyll, Spectrophotometric, Trichromatic and Monochromatic Methods." Archived.

15.12 EHD QA 115, "Initial and Ongoing DOC Procedures," Environmental Health Division, Wisconsin State Laboratory of Hygiene.

15.13 EHD QA 116, "LOD Procedures," Environmental Health Division, Wisconsin State Laboratory of Hygiene.

15.14 Kennedy-Parker, D., G. Krinke, G. Bowman, P. Rasmussen, and R. Arneson, "Maintaining Continuity in Chlorophyll Trend Data While Improving the Analytical Method," poster presentation, Wisconsin State Laboratory of Hygiene, and Wisconsin Department of Natural Resources, Feb., 2003.

15.15 LS 55 Luminescence spectrometer User's Guide, PerkinElmer Ltd., part # 09934436, release A, Aug. 2000.

15.16 FL WinLab Software User's Guide, PerkinElmer Ltd., part # 09934434, release A, Aug. 2000.

15.17 Fl WinLab Software disk, V4.00.02, L225-8001 Issue B, June 2001.

15.18 EHD INORG GENOP 151, "Chlorophyll HORIZON Procedure."

15.19 Environmental Protection Agency (EPA) Method 446.0 rev 1.2 (September 1997)

15.20 WDNR approval for method modifications, Zana Sijan, 07/16/2021 e-mail: O:\SOP\EHD\ESS\Inorganic\Final\ESS INO METHOD 151.1 BHCR3-Chlorophyll Method Deviations-DNR approval 07-16-2021.pdf

15.21 Wisconsin Administrative Code NR149, Laboratory Accreditation, Wisconsin Department of Natural Resources, 06/29/2021.

16. Version Tracking

Ver. Date	Ver #	Revised by	Changes Made
July, 2011	4.0	D. Kennedy-Parker	Some formatting changes, updated section 6 to reflect new standards prep, using 1mg stock instead of 5mg stock for Sigma. Removed confusing language about minimum volume in section 1. Added corrective language about the LRB to section 8.3. Removed LDR definition since it is not acceptable for this method.
Jan. 2012	5.0	S. Hill/B. Clary	Added outside document references to section 15. Added appendix 3, sample volume correction factor table. Added section 8.4, LOD information.

March 2014	6.0	B. Clary	Updated sections 8.5.1, 10, and 12 and also Appendix 2 for HORIZON. Added to sample handling, section 4. Added appendix 4 for periphytons. Corrected some procedural issues. Added reference to HORIZON GENOP 151 (15.19). Corrected logbook references in Appendix 1 and section 6. Updated figure 1 to reflect actual run.
April 2017	7.0	B. Clary	Updated location of logbook in 6.1. Removed language in 6.3.1, 6.3.2, and 6.4.1 about checking the concentration of the stock standards. Changed concentration of WQCS in 6.4.2 to 200ug/L to reflect current practice. Added section 10.2.1. Changed reference 15.12 to current; removed 15.14. Updated references throughout.
02/07/2019	7.0	B. Clary	6.3.1 & 6.4.1 & 10.13.1—updated logbooks. 8.5.1—added duplicate limit of 15% relative difference. 8.11—removed language about instrument 96 as it is no longer in use 9.3—corrected mistake originally claiming we corrected the samples to ppb when in fact we correct the reference samples to ppm. 10.11—changed to refer to Horizon sample number, not just "QCS" 10.12—added language to set background to 0.000 15.19—added reference EPA 446.0 15—updated some of the references Appendix 1 number 7—added calculation
3/22/2021	8.0	B. Clary	Sect. 3—added pollution prevention info Inserted 8.11 to add LDR. Added section 9.5 about % relative error. Added timeframe to 10.5. Added temp and instrument to 4.2.1. Added instrument number to IOP section 7. Also updated spreadsheet in IOP section 7. Updated references
10/14//21	9	B. Clary	Updated 7.7 from 500XG to 675XG as stated in the EPA method. Added 7.7.1 requiring annual verification of centrifuge in response to NELAC audit deficiency BH2. 10.6: updated centrifugation from 30 min at 500XG to 15 min at 675XG Added 11.5, relative centrifugal force equation 7.11 added digital laser tachometer Added reference 15.20—DNR approval of method modifications Added reference 15.21, NR149
11/30/21	OB1	S.D. Hill	Transition to OnBase Updated references

Appendix 1
EHD INORG IOP 151.1
Determination of Chlorophyll *a* Standards and Quality Control Sample Concentrations

The actual concentration of the stocks, standards, and the 2nd source quality control sample (QCS) used to calibrate and verify the Perkin-Elmer LS-55 fluorescence spectrometer, must be determined by spectrophotometric means, prior to analysis of samples for chlorophyll *a*. The following process must be used for those determinations.

1. Prepare the standards and QCS as in EHD INORG METHOD 151.1 section 6.3 and section 6.4.
2. Turn on the Beckman DU-650 spectrophotometer and click on the VIS lamp to ON. Allow the instrument to warm up for one hour. The instrument will go through an automatic system check which includes wavelength calibration check, stray light and lamp intensity verification. DO NOT proceed if any error messages are displayed during the start-up sequence. Call for Beckman service if start-up problems cannot be corrected by the analyst. Install the cell holder in place on beam track. Use the 1mm cell holder for stock solutions, and the 5mm cell holder for working standards and QCS.
3. Select **fixed wavelength, Method, A:\ Unchloro, Exit**. The method and wavelengths are now programmed. The analysis should be performed in dim light.
4. Fill the 5mm cell with 90% acetone and click on **Blank** at the bottom of the screen. This will zero the instrument on all wavelengths. Click on number **1** type in "CB" using keyboard, or screen keys, and right click on mouse to read. The intensities should be near zero.
5. Empty cell and fill with appropriate solution, select next number, type in nominal concentration and right click to read. Empty cell and repeat for each replicate. Do four replicates for stock solutions, and three replicates for working solutions.
6. End with a blank (CB) and print results.
7. Enter values into M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\Chlorophyll\Standards\Chla Std determination Template.xlt. This will calculate the actual chlorophyll *a* concentrations according to the following equations from Method 446 Section 12 (15.19):

$$C_E = 11.85*(Abs_{644} - Abs_{750}) - 1.54*(Abs_{647} - Abs_{750}) - 0.08*(Abs_{630} - Abs_{750})$$
$$C_S = C_E * V_E * DF / (V_S * L_C)$$

Where:

C_E = Concentration (mg/L) of chlorophyll a in the extraction solution analyzed
Abs_{644} = Absorbance value measured at 664nm
Abs_{750} = Absorbance value measured at 750nm
Abs_{647} = Absorbance value measured at 647nm
Abs_{630} = Absorbance value measured at 630nm
C_S = Concentration of the whole sample
V_E = Extract volume (13mL)
DF = Dilution Factor (1 for these standards)
V_S = Sample Volume (13mL)
L_C = Cell length (5cm)

Calculate average of replicates and record in logbook #ESS475, located in the General Chemistry area. Label amber solution bottles with reagent code, preparation date, analyst, and expiration date. Stock standards expire in one year, and working solutions expire in six months. Store all in chlorophyll freezer (Instrument #45) at -20ºC.

Appendix 2
Filtering Samples for Chlorophyll *a* Analysis

1. Refer to EHD INORG GENOP 151 (15.18) to determine the procedures necessary to process these samples through HORIZON.
2. Adjust vacuum gauge to approximately -6 inches Hg. Nearly all the way out. Make this adjustment with your finger completely covering the end of the vacuum jet.
3. Connect filtering flask to vacuum jet.
4. Insert bottom half of filtering funnel into flask.
5. Place 5.0 µm filter on fritted portion of filtering funnel.
6. Clamp upper portion of filtering funnel over filter.
7. Rinse entire apparatus with reagent water.
8. Shake sample container well and pour into graduated cylinder (max. volume 200 mL).
9. Immediately pour graduated cylinder contents into filtering funnel. Rinse the graduated cylinder into the filtering funnel with reagent water.
10. As liquid level reaches filter, rinse the sides of the filtering funnel and close the vacuum jet.
11. Remove filter and place into 15 mL capped tube. Put lab number on tube and record the sample volume filtered on the batch worklist.
12. Place in light tight box, until all filtering is complete, then transfer to light tight box in -20º C freezer.
13. Rinse all parts of filtering apparatus with reagent water and re-assemble with new filter.
14. When all samples are filtered, rinse apparatus, disassemble and store below counter.

Appendix 3

Chlorophyll a Sample Volume Correction Factor for PE LS-55

VOLUME	C.F.x1000	VOLUME	C.F.x1000
10	1300.00	700	18.57
25	520.00	750	17.33
30	433.33	800	16.25
50	260.00	850	15.29
60	216.67	900	14.44
100	130.00	950	13.68
150	86.67	1000	13.00
200	65.00	1050	12.38
250	52.00	1100	11.82
300	43.33	1150	11.30
350	37.14	1200	10.83
400	32.50	1250	10.40
450	28.89	1300	10.00
500	26.00	1350	9.63
550	23.64	1400	9.29
600	21.67	1450	8.97
650	20.00	1500	8.67

Appendix 4
Procedure for Periphyton Samples Collected on Glass Slides

1. Periphyton samples will arrive on a number of glass slides. For the procedure for processing these through HORIZON see EHD INORG GENOP 151 (15.18). All samples will be held in the freezer in the solids room in room 119 until preparation day. Make sure to follow proper safety procedures; wear gloves for this operation.
2. Label a 15mL centrifuge tube (7.3) for each periphyton sample and place all tubes in a green rack.
3. Rinse the plastic funnel into the waste bucket with the squeeze bottle of 90% Acetone (6.1). Place the funnel into the centrifuge tube.
4. Remove the first glass slide from the sample. Use a razorblade to scrape each side of the slide into the funnel. Rinse the razorblade into the funnel with the squeeze bottle. Make sure to use the squeeze bottle sparingly as the total volume in the tube will be 13mL.
5. After scraping both sides of the slide and rinsing the razorblade, rinse both sides of the slide with the acetone solution.
6. Repeat steps 4 and 5 for each slide in the sample. Make sure to record the number of slides in order to correctly compute the periphyton concentration and adjust the LODs in HORIZON according to EHD INORG GENOP 151 (15.18).
7. After completing the process for each slide in the sample rinse the funnel with the acetone solution into the tube to make sure all the material makes it into the tube. Dilute the tube up to the 13mL mark with the acetone solution.
8. If over-dilution has occurred make a note of this and adjust the LOD in HORIZON accordingly.
9. Take these samples through the remaining preparation process as normal from this point.
10. Analyze the samples as any other chlorophyll samples with a correction factor of 1.
11. Hand-calculate the periphyton result per area using the following equation:

    (Chlorophyll a result in µg/L*V)/(S*0.0038m^2) = Periphyton concentration in µg/m^2

    Where V is the volume of extract in L (usually 0.013L) and S is the number of slides. This is the value that will be entered into HORIZON according to ESS INO GENOP 151 (15.18).

Figure 1

```
Quantitation results file:C:\FLWINLAB\DATA\UY1108.rpt
Generated on :11-08-2013, at time:11:40:44
**************************************************************
Measurement conditions
Method:                 C:\FLWINLAB\METHODS\Chorophyll.mth
Analyst:                BAC*1022
Comments:               ESS INO METHOD 151.1 rev.5 January, 2012
(EPA 445.0 rev.1.2 and Welschmeyer,1994)
Chlorophyll 0.0- approx. 550ppb
EM slit 3 nm EX slit 5 nm
Std conc. entered as mg/L
Sample results for Chlorophyll a reported as ug/L

--------------------------------------------------------------
Ex. wavelength (nm):    436
Em. wavelength (nm):    680
Ex. slit (nm):          2.5
Em. slit (nm):          3.0
Integration time (s):   1.00
Em. filter:             open

Sipper parameters:
Pump time(s):   10.0
Delay time(s):  0.0
Purge time(s):  0.0
Purge direction backwards
**************************************************************
Reference sample results
--------------------------------------------------------------
Std#    Conc*Fact          Intens.          BG     Factor
           (ppb)
--------------------------------------------------------------
Cal BLK    0.000           0.000           0.000           1.00
Std 0.0089          0.009           0.625           0.000           1.00  1
Std 0.0429          0.043           4.065           0.000           1.00  1 Standards are entered as mg/L in second column
Std 0.0915          0.091           9.409           0.000           1.00  1
Std 0.1840          0.184          18.478           0.000           1.00  1
Std 0.4637          0.464          47.589           0.000           1.00  1
Std 0.7386          0.739          75.166           0.000           1.00  1 Any sample that has intensity >top std is diluted
--------------------------------------------------------------
Fit equation:
Y = 102.139 x + -0.134                                          Regression equation used for calculations
Correlation     1.0000                                          Correlation Coefficient (r)
**************************************************************
Unknown sample results
--------------------------------------------------------------
Std#            Conc*Fact  Intens.       BG      Factor       Info   Conc*fact column converts sample concentration to ug/L
                (ppb)                                                using ((13mL extraction vol/vol filtered in mL)*1000)
--------------------------------------------------------------
16206           200.994   20.395        0.000    1000.00             HORIZON number for QCS, factor of 1000. Generally first.
IPC 42.9ppb      43.610    4.320        0.000    1000.00             Factor of 1000 for all IPCs
CB <0.26ppb       0.085    0.000        0.000      65.00             Factor of 65 for all CBs
16207             0.085    0.000        0.000      65.00             HORIZON number for first LRB, factor of 65
103994001         2.335   18.212        0.000      13.00
104456001         3.499    5.364        0.000      65.00             65 represents 200mL filtered, basis for LOD.
104456002         7.456   11.582        0.000      65.00
104456003         8.098   12.591        0.000      65.00
104456004         5.662    8.762        0.000      65.00       X5    Info column contains dilution
104458001         2.368    3.586        0.000      65.00
104458002        11.374   17.738        0.000      65.00
104458003        20.094   31.440        0.000      65.00
104458004         4.121    6.341        0.000      65.00
104458005         7.183   11.153        0.000      65.00
IPC 91.5ppb      88.500    8.905        0.000    1000.00
CB <0.26ppb       0.085    0.000        0.000      65.00
```

EHD INORG METHOD 220.3

Ammonia Nitrogen and Nitrate+Nitrite Nitrogen (EPA Methods 350.1 and 353.2)

1. Scope and Application

1.1 This method is applicable to the simultaneous determination of ammonia (NH_3-N) and nitrate+nitrite (NO_3+NO_2-N) in surface, drinking and ground waters, and domestic and industrial waste samples which have been preserved with sulfuric acid (H_2SO_4). The range for the ammonia method is 0.012 to 1.0 mg NH_3-N/L. The range for the nitrate method is 0.055 to 3.0 mg NO_3+NO_2-N/L.

1.2 The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for ammonia are 0.012 and 0.039 mg/L NH_3-N, respectively.

1.3 The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for nitrate are 0.055 and 0.184 mg/L NO_3+NO_2-N, respectively.

2. Summary of Method

2.1 NH_3-N: Alkaline phenol and sodium hypochlorite react with ammonia to form a blue indophenol compound that is proportional to the concentration of ammonia. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The color is intensified by adding sodium nitroprusside. The resulting water-soluble, colored dye is measured colorimetrically at 630 nm.

2.2 NO_3+NO_2-N: The sample is passed through a copperized cadmium column that reduces nitrate (NO_3) quantitatively to nitrite (NO_2). The total nitrite (NO_2) (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble, magenta colored dye is measured colorimetrically at 520 nm.

2.3 The determinative steps in this method are identical to EPA 350.1 and 353.2 (15.1). However, because the EPA method is written specifically for air-segmented continuous flow technology that is no longer available, the specific “plumbing” scheme (i.e., pump tubes and reagent proportions, etc.) are adapted to match the Lachat QuikChem Methods 10-107-06-1-J and 10-107-04-1-J (15.2).

2.4 Federal regulations (40 CFR 136) prohibit the direct measurement of ammonia unless comparability data are on file that show preliminary distillation is not required. The WSLH evaluated the necessity of preliminary distillation to confirm the validity of direct automated measurement. This study can be found at M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\Nutrients\ammonia distillation study.

3. Safety, Waste Management, and Pollution Prevention

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (15.3).

3.2 All laboratory waste, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (15.4).

3.3 Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (15.4).

4. Sample Handling and Preservation

4.1 Samples are collected in Wisconsin State Lab of Hygiene (WSLH) 250 mL plastic bottles. Bottle quality is verified prior to use (15.11).

4.2 Samples are preserved in the field by the addition of 1 mL of 25% H_2SO_4 per 250 mL sample. They are stored at ≤6°C (but not frozen) until analysis is performed.

4.3 Maximum holding time (after sample acidification) is 28 days from date of collection.

5. Interferences

5.1 Calcium, magnesium, iron and copper ions, or other metals may precipitate if present in sufficient concentration. Ethylenediamine-tetra acetic acid (EDTA) is added to the sample to prevent this problem.

5.2 Color, turbidity, and certain organic species may interfere.

5.2.1 Estimating a correction for sample color may be calculated by running the samples through the manifold with *all* reagents pumping *except* hypochlorite, which is replaced by ASTM Type-I water. The resulting absorbance readings are then subtracted from those obtained for samples determined with all reagents resulting in complete color formation.

5.2.2 Turbidity is removed by manual filtration. The presence of suspended matter may restrict flow through the reduction column.

5.2.3 Samples that contain large concentrations of oil and grease may coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

5.3 Screen for and, if present, remove residual chlorine by pretreatment of the sample with ascorbic acid prior to analysis for both Drinking Water and Effluent matrices (15.19).

6. Reagents and Standards

Ammonia

6.1 **Reagent Water (ASTM Type-I water):** All reagents and standards must be made with ASTM Type I water.

6.2 **Carrier:** Add 1 mL H_2SO_4 to 1 L ASTM Type-I water. Prepare fresh for each day of analysis. Two liters can be made at one time. Degas with helium.

6.3 **Alkaline Phenol:** Dissolve 88 mL liquid phenol (88%) in a 1 L flask containing about 500 mL ASTM Type-I water. While stirring, slowly add 32 g NaOH. Note; if the percent phenol changes with a new bottle of phenol, the amount of phenol to be added to the 1 L flask must be recalculated. Two liters can be made at one time. Cool, dilute to 1 L, mix, and filter through a Millipore 0.45 µm filter. Store in a dark bottle. The expiration date is 6 months.

6.4 **Sodium Hypochlorite Solution:** Dilute 500 mL of commercial bleach containing 5.25% available chlorine (e.g. Clorox®) to 1 L with ASTM Type-I water. Two liters can be made at one time. Because Clorox® is a proprietary product its formula is subject to change and adjustments in volume may be necessary. Ultra Clorox® contains 6 % available chlorine, therefore 438 mL (of Ultra Clorox®) should be used per 1 L and diluted with ASTM Type-I water. Other sodium hypochlorite solutions may also be used. The expiration date is 6 months.

6.5 **Buffer:** Dissolve 50 g disodium ethylenediamine-tetraacetate (Na_2EDTA) and approximately 3.0 g NaOH (use 3-5 grams NaOH if color formation appears on the ammonia manifold mixing coil, EPA Method 350.1) in 900 mL of ASTM Type-I water. Two liters can be made at one time. Cool, dilute to 1 L, mix, and filter through a Millipore 0.45 µm filter. The expiration date is 6 months. Degas with helium.

6.6 **Sodium Nitroprusside:** Dissolve 3.5 g of $Na_2Fe(CN)_5NO{\cdot}2H_2O$ (alternate name: sodium nitroferricyanide) in 900 mL of ASTM Type-I water and dilute to 1 L. Two liters can be made at one time. Filter through a Millipore 0.45 µm filter. Reagent is light sensitive, store in dark container. The expiration date is 6 months. Degas with helium.

6.7 **Ammonia Stock Standard (1000 mg NH_3-N/L):** A pre-made stock solution may be obtained from an approved UW vendor, e.g. RICCA or ERA. Stock is refrigerated at ≤6°C. Expiration date is 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.7.1 Alternatively, dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl), dried at 105°C for 1 hour, in 900 mL ASTM Type-I water. Add 1 mL concentrated H_2SO_4 and dilute to 1 L (1.0 mL = 1.0 mg NH_3-N). Stock is refrigerated at 4°C. The expiration date is 6 months.

Nitrate

6.8 **Reagent Water (ASTM Type-I water):** All reagents and standards must be made with ASTM Type I water.

6.9 **Carrier:** Add 1 mL H_2SO_4 to 1 L ASTM Type-I water. Two liters can be made at one time. Degas with helium. The expiration date is 1 week.

6.10 **Ammonium Chloride-EDTA buffer, pH 9.1:** In a fume hood, to approximately 1000 mL of ASTM Type-I water in a 2L volumetric flask, add 210 mL concentrated hydrochloric acid (HCl), 190 mL concentrated ammonium hydroxide (NH_4OH), and 2.0 g disodium EDTA. Dissolve and dilute almost to volume. Allow to cool. Adjust the pH to 9.1 ± 0.1 with approximately 45 mLof NaOH solution. Dilute to volume. The expiration date is 6 months.

6.10.1 7.5 N Sodium hydroxide: In a 500 mL volumetric flask slowly add 150 g NaOH to approximately 250 mL of ASTM Type-I water. Caution: the solution will get very hot. Dissolve the NaOH, let the solution cool and dilute to volume. The expiration date is 6 months.

6.11 **Sulfanilamide Color Reagent:** Combine approximately 500 mL of ASTM Type-I water, 100 mL 85% phosphoric acid (H_3PO_4), 40 g sulfanilamide ($C_6H_8N_2O_2S$), and 1.0 g N-(1-naphthyl) ethylenediamine dihydrochloride ($C_{12}H_{14}N_2{\cdot}2HCl$). Dissolve and dilute to 1 L. Two liters can be made at one time. Filter through a Millipore 0.45 µm filter. Reagent is light sensitive, store in dark container. Refrigerate at 4° C. The expiration date is 1 month.

6.12 **Nitrate Stock Standard (1000 mg NO_3-N/L):** A pre-made stock solution may be obtained from an approved UW vendor, e.g. RICCA or ERA. Stock is refrigerated at ≤6°C. Expiration date is 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.12.1 Alternatively, dissolve 7.218 g potassium nitrate (KNO_3), in 900 mL ASTM Type-I water. Preserve with 2 mL chloroform and dilute to 1 L (1.0 mL = 1.0 mg NO_3-N). Refrigerate at 4°C. The expiration date is 6 months.

6.13 **Nitrite Stock Standard (250 mg NO_2-N/L):** Dissolve 1.518 g potassium nitrite (KNO_2) or 1.232 g of sodium nitrite ($NaNO_2$) in 900 mL ASTM Type-I water. Preserve with 2 mL of chloroform and dilute to 1 L (1.0 mL = 0.25 mg NO_2-N). Refrigerate at 4°C. The expiration date is 6 months.

Standards

6.14 **Quality Control Standard (QCS):** The stock solution(s) used to prepare the QCS must originate from a source different from that used for the calibration standards. Both are purchased at a concentration of 1000 mg NO_3-N/L and 1000 mg NH_3-N/L. Pre-made stock solutions are obtained from approved UW vendors such as RICCA or ERA. Stocks are refrigerated at ≤6°C and expire 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.14.1 Quality Control Working Standard (QCS): Dilute 1.5 mL of Nitrate standard (6.14) (1 mL = 1 mg N) and 0.6 mL of Ammonia standard (6.14) (1 mL = 1 mg N = 1.22 mg NH_3) to 1000 mL (this makes a 1.5 and 0.6 mg/L solution of Nitrate-N and Ammonia-N respectively). Add 1.0 mL of concentrated H_2SO_4 per 1000 mL before diluting to the mark. Refrigerate at 4°C. The expiration date is 28 days.

6.15 **Working Standard Solution:** Prepare the following standards by diluting suitable volumes of standard solution (6.7) for Ammonia and (6.12) for Nitrate to ASTM Type-I water. Add 1.0 mL of concentrated H_2SO_4 per 1000 mL before diluting to the mark. Working standards are refrigerated at 4°C. The expiration date is 28 days.

6.15.1 Note: All working, stock, and QCS standards must be entered into the Standards Log located in the Wet Chemistry Laboratory. All working and QCS standards must be entered in Horizon.

Concentration of Standard mg NH_3-N/L	Concentration of Standard mg NO_3-N/L	Volume of NH_3 Standard Solution (6.7) mL	Volume of NO_3 Standard Solution (6.12) mL
1.0	3.0	1.0 mL (1 L)	3.0 mL (1 L)
0.75	2.0	0.75 mL (1 L)	2.0 mL (1 L)
0.50 (IPC)	1.0 (IPC)	0.5 mL (1 L)	1.0 mL (1 L)
0.30	0.75	0.30 mL (1 L)	0.75 mL (1 L)
0.20	0.50	0.20 mL (1 L)	0.50 mL (1 L)
0.10	0.25	0.20 mL (2 L)	0.50 mL (2 L)
0.03	0.10	0.06 mL (2 L)	0.20 mL (2 L)
0.0 (Reagent Blank)	0.0 (Reagent Blank)	ASTM Type-I water	ASTM Type-I water
25 spike solution	100 spike solution	2.5 mL (100 mL)	10 mL (100 mL)

7. Apparatus

7.1 Filter tubes: 20 x 150 mm, disposable borosilicate glass.

7.2 Lachat QuikChem 8500 Series 2 Automated Flow Injection Analyzer consisting of:

7.2.1 XYZ Sampler

7.2.2 Peristaltic pump

7.2.3 Colorimetric detector

7.2.4 Colorimeter equipped with 10 mm path length flow cell and 630 ηm interference filter for Ammonia. Colorimeter equipped with 1 mm path length flow cell and 520 ηm interference filter for Nitrate.

7.2.5 Reaction Module 10-107-06-1-J with heating unit set at 60°C with 650 cm length tubing.

7.2.6 Reaction Module 10-107-04-1-J with cadmium column

7.2.7 Data System

7.3 Motorized pipettes: 10 mL, 1.0 mL, and 100 µL, calibrated according to EHD INORG GENOP 200 (15.10)

7.4 Disposal Culture tubes: 13 x 100 mm, disposable borosilicate glass

8. Quality Control Types, Acceptance Criteria, & Corrective Action

8.1 Please refer to the Environmental Health Division Quality Assurance Manual (15.6) for general information on quality control procedures. Important specifics include:

8.1.1 Accuracy and precision calculations.

8.1.2 Corrective action and result qualification procedures (including documentation requirements) for instrument problems or analytical problems.

8.2 **An instrument logbook** is maintained for each instrument. Maintenance, performance problems, date calibrated, analyst, and other pertinent information are documented in the logbook.

8.3 **The Correlation Coefficient (*r* value)** is the measure of the linearity of the standard curve and must be ≥0.995 to be acceptable and before proceeding with sample analysis. The 2016 TNI Standard (15.7) requires the % Relative Error (%RE) to be calculated and evaluated for each the low and midpoint standards and must pass acceptance criteria before continuing analysis. The acceptable %RE for the low and midpoint standards is ±50% and ±30%, respectively. These values will be manually entered into HORIZON into the %RELOW and %REMID standards, located at the end of the batch. Due to HORIZON limitations, only recovery will be calculated. Since %RE = Recovery – 100%, the acceptable limits for the low and midpoint standards in HORIZON become 50-150% and 70-130%, respectively.

8.4 **A Quality Control Standard (QCS)** is analyzed at the beginning of each run. The analytical result must be within ± 10% of the true value to continue the analysis. If the recommended limits are exceeded, corrective action includes reanalyzing the QCS or the analyst may recalibrate if necessary.

8.5 **A Laboratory Reagent Blank (LRB)** is prepared (6.15) and analyzed at the beginning of each run. **A Method Blank (MB)** is prepared by filtering ASTM Type-I water and adding sulfuric acid according to ESS INO METHOD 100.2 Filtering Procedure (15.15) and must meet the same criteria as a LRB, or associated samples will need to be re-filtered or qualified (15.15). The LRB blank must meet one of the following criteria listed in the Wisconsin Laboratory Certification Manual (15.5): 1) Lab Reagent Blank must be less than the detection limit of the method; 2) Lab Reagent Blank <5% of sample concentration; 3) Lab Reagent Blank <5% of the regulatory limit. If it does not meet one of these criteria, the recommended corrective action to take may include reanalyzing the LRB, qualifying the samples or the analyst may choose to recalibrate, if necessary. In general, a lab reagent blank is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, and improper Y-intercept, poor source of material for reagent blank, etc.), then the blank may be accepted as "zero" providing that the logic supporting this decision is well documented. The LRB is equivalent to the CB for this method.

8.6 **One Laboratory Fortified Blank (LFB)** is prepared and analyzed at the beginning of each run. The spike recovery for the LFB must be within ±10% of the true value. If LFB exceeds acceptance criteria, corrective action will include reanalyzing the LFB, preparing a new LFB, qualifying the data or recalibrating if necessary. Prepare the LFB using LRB/CB and spiking solution (6.15), where the spike solution is equal to 1.0% of the total volume -- e.g., add 0.06 mL of spike solution to 5.94 mL of LRB/CB.

8.6.1 **Lab Fortified Method Blanks (LFMB)** are prepared and analyzed at a minimum of 5% of lab filtered samples. LMFBs will be prepared the same as LFBs, using the MBs from the lab filtered prep batches in place of LRB/CB. The MBs used for LFMBs are the 2nd, 3rd, and all subsequent MBs from a prep filter batch. A LFMB using the end MB from the prep filter batch is optional if and only if the previous LFMBs meet the 5% minimum. The spike recovery criteria and corrective action for LFMBs are equivalent to the LFB (8.6).

8.7 **Matrix Spikes and Duplicates:** Prepare **a minimum of 10%** of the samples, per matrix, as duplicates and spikes. Spikes are prepared in the same manner as the LFB, substituting the sample in place of the LRB/CB. If the duplicate (precision QA) is not met (Horizon limits are 10% relative difference), the matrix group (including spike and duplicate) must be reanalyzed. If limits are exceeded a second time, the samples from this matrix group must be re-filtered and reanalyzed. If the spike recovery (accuracy QA) does not fall within the specified control limits (Horizon limits are 90-110% recovery), corrective action requires reanalysis of the matrix group (including the spike and duplicate) on the same run. If limits are exceeded a second time, qualify the matrix group (15.6).

8.8 **An Instrument Performance Check (IPC)** and **Check Blank (CB)** must be analyzed after every 10 cups. The IPC must be within ±10% of true value. Choose a standard with a concentration near the middle of the calibration range. The CB must be less than the LOD. In general, a CB is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, and improper y intercept, poor source of material for reagent blank, etc.), then the blank may be accepted as "zero" providing that the logic supporting this decision is well documented. All data must be bracketed by acceptable IPCs and CBs. If an IPC or CB fails, corrective action requires that all previous samples back to the last acceptable IPC and CB be reanalyzed. The CB is equivalent to the LRB for this method.

8.9 **Demonstration of Capability (DOC):** An initial DOC and annual DOC proficiency checks are performed according to EHD QA 115 (15.8). The QCS (6.14) may be used for the annual DOC.

8.10 **Limit of Detection (LOD):** The LOD must be determined or verified every 13 months or whenever there is a change in the method. Use the procedure outlined in EHD QA 116 (15.9).

8.11 **Linear Calibration Range (LCR):** The LCR must be verified every six months or whenever there is a change in the method. The initial demonstration of linearity must use a sufficient number of standards to insure that the curve is linear. The verification of linearity must include a minimum of one blank and 3 standards. If any verification data exceeds the actual values by ±10%, linearity must be re-established. If any portion of the range is shown to be nonlinear, a sufficient number of standards must be analyzed to clearly define the nonlinear portion.

8.12 **A Nitrite Column Efficiency standard (CEFF)** is prepared and analyzed at the beginning of each run to check the efficiency of the cadmium column. Dilute 1.5 mL NO_2-N standard (6.13) to 250 mL with ASTM Type I water. The NO_2-N column efficiency standard is run through the column. The efficiency is determined by the equation:

$$\frac{\text{Measured Concentration of the } NO_3\text{-N QCS Standard [6.14]}}{\text{Measured Concentration of the } NO_2\text{-N Column Efficiency Standard [8.12]}} \times 100\%$$

The calculated efficiency must be within 100±10% to proceed. If the column efficiency fails, corrective action requires reanalyzing the CEFF, preparation of a fresh CEFF, or the analyst may choose to recalibrate with a new cadmium column if necessary. Prepare a fresh CEFF daily.

8.13 **Sample Dilution:** If the estimated concentration of analyte in a sample exceeds the highest calibration standard, a bench dilution should be performed. Dilutions at the bench are typically performed by diluting an appropriate volume of sample with the carrier (6.2). Motorized pipettes may be used to deliver/dilute volumes up to 10 mL. For volumes greater than 10 mL, Type-A glass, volumetric pipettes should be used. Diluted samples should be mixed thoroughly prior to analysis. See Appendix 1 for recommended dilutions.

8.14 **Dilution Verification:** When a dilution is made, the dilution must be verified by comparing the diluted result with the original result. The comparison is done by dividing the diluted result by the original result and expressing the calculation as a percent. The acceptable range is 90% to 110%. If the dilution verification is not within the acceptable range, a different dilution must be made. This second dilution would then be verified against the first dilution. If a group of samples is diluted identically, at least 10% of the dilutions must be verified.

9. Method Calibration

9.1 Refer to section 6 for making standards and reagents.

9.2 Calibration curve is a linear, 1st order polynomial curve.

9.3 Open the method template and set the data system parameters and operating conditions for the Lachat 8500 with the *Omnion* software (15.12).

9.3.1 Set up manifolds as shown in Figure 1 and 2.

9.3.2 Pump ASTM Type-I water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline has been obtained (about five minutes).

9.3.3 Engage the cadmium column on the nitrate manifold by turning the cadmium column bypass switch to the "on" position after the reagents have passed through the second mixing coil. To disengage the cadmium column turn the bypass to the "off" position. Always disengage the cadmium column from reagent flow before changing over to water at the end of the analytical batch.

9.4 Place standards in the appropriate rack on the autosampler and calibrate the instrument by injecting the standards. The data system will then correlate the concentrations with the instrument responses for each standard.

9.4.1 After the calibration passes (8.3) the samples may be analyzed. If the calibration is not satisfactory use an appropriate corrective action to diagnose possible causes and recalibrate.

10. Procedure

10.1 See also Instrument Operating Procedure (15.12).

10.2 Samples should be filtered prior to analysis (15.15).

10.3 Import the sample identification numbers from Horizon into the Tray Table of the Lachat Omnion software. This will include:

10.3.1 A duplicate and spike for every 10 samples in a matrix group.

10.3.2 A Lab Reagent Blank per run.

10.3.3 One Lab Fortified Blank per run.

10.3.4 One Lab Fortified Method Blank for every 20 lab filtered samples.

10.3.5 One Quality Control Standard per run.

10.3.6 One Column Efficiency Sample per run for Nitrate.

10.4 Analyze standards and Quality Control samples (i.e., IPC, CB, LRB, LFB, QCS, and CEFF). If QC samples pass acceptance criteria, the batch may continue with sample analysis.

10.5 Shutdown procedure:

10.5.1 When the analytical batch is complete, the cadmium column should be immediately disengaged from reagent flow to prevent introduction of air or water. Transfer reagent lines to ASTM Type-I water to rinse followed by 10% HCl for five minutes. Flush the reagent lines and the manifolds with ASTM Type-I water after cleaning with acid solution.

10.5.2 Remove reagent lines from ASTM Type-I water and pump air through in order to dry. Release pump-tubes from cartridges and turn off instrument.

10.5.3 Waste disposal: The waste can be poured down the sink with water. See the University of Wisconsin Laboratory Safety Guide (15.4).

11. Calculations

11.1 Changes in absorbance due to color change are directly related to the amount of analyte present in each standard or sample. The absorbance signal creates a change in the voltage output which in turn, is converted to a digital format as the peak appears on the computer screen. The response variable, *peak area*, is converted to a digital signal by the software and regressed vs. concentration with a 1st order polynomial regression formula. Concentration of analyte in the unknown sample is estimated by the software, based on the standard calibration curve.

11.2 If the estimated concentration of ammonia or nitrate exceeds the highest calibration standard, a manual dilution should be performed and documented on the benchsheet and in the Lachat run. The final result will be verified when the batch is checked for quality control. For dilution verification instructions, see section 8.13.

12. Data Management

12.1 The analytical run, the *Run Time Report*, and the QC Parameters section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced chemist who did not perform the original analysis (15.13). The reviewer must initial and date the analytical run.

12.2 Export results from Omnion to Horizon (see EHD INORG GENOP 113, Horizon Procedures 15.14).

12.2.1 Review results by selecting Edit Results under Batches.

12.2.2 Review QC Results by selecting the QC display in Edit Results.

13. Definitions

13.1 Definitions of terms in this SOP may be found in the reference method (15.1). General definitions of other terms that may be used in this method are found in the WSLH Quality Assurance Manual (15.6).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (LODs and DOCs) were generated in compliance with the reference method and the Inorganic Chemistry Department's standard operation procedures: EHD QA 115 (15.8) and EHD QA 116 (15.9). Supporting data will be retained according to the applicable Records Disposition Authorization (RDA). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement.

15. References

15.1 United States Environmental Protection Agency. 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100, Method 353.2 (Nitrate + Nitrite) and Method 350.1 (Ammonia), edition 2.0, 1993.

15.2 Zellweger Analytics, Lachat Instruments Division. Determination of Ammonia (Phenolate) by Flow Injection Analysis Colorimetry (Method 10-107-06-1-J, June 1990). Determination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis (Method 10-107-04-1-J, December 1998).

15.3 Wisconsin State Laboratory of Hygiene. AD Safety GENOP 102, Chemical Hygiene Plan for the Agriculture Drive Facility.

15.4 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.5 Wisconsin Department of Natural Resources Lab Certification Program, 06/29/2021, Wis. Administrative Code Chapter NR 149.

15.6 Wisconsin State Laboratory of Hygiene. Quality Assurance Manual.

15.7 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.8 Wisconsin State Laboratory of Hygiene. EHD QA 115, Initial and Ongoing DOC Procedures.

15.9 Wisconsin State Laboratory of Hygiene. EHD QA 116, LOD Procedures.

15.10 Wisconsin State Laboratory of Hygiene. EHD INORG GENOP 200, Pipette Performance Checks.

15.11 Wisconsin State Laboratory of Hygiene. EHD INORG QA 101, Bottle Check Procedure.

15.12 Wisconsin State Laboratory of Hygiene. EHD INORG IOP 105, Instrument Operating Procedure for QuikChem 8500, Automated Ion Analyzer and Computer Protocol.

15.13 Wisconsin State Laboratory of Hygiene. EHD INORG QA 107, Q.C. Audits of Analytical Runs for ESS Wet Chemistry Area.

15.14 Wisconsin State Laboratory of Hygiene. EHD INORG GENOP 113, HORIZON Procedures for EHD Inorganic Chemistry.

15.15 Wisconsin State Laboratory of Hygiene. EHD INORG METHOD 100.2, Filtering

Procedure.

15.16 QuikChem 8500 Series II, Operations Manual, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.17 Omnion 3.0 Software User Manual, 09/2007, Edition 4. / Omnion 4.0 Software User Manual, Lachat Instruments, Hach Co., 04/2015, Edition 1.

15.18 QuikChem 8500 Series II, Maintenance and Troubleshooting, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.19 Wisconsin State Laboratory of Hygiene. EHD INORG IOP 220, Chlorine Neutralization in Drinking Water Samples.

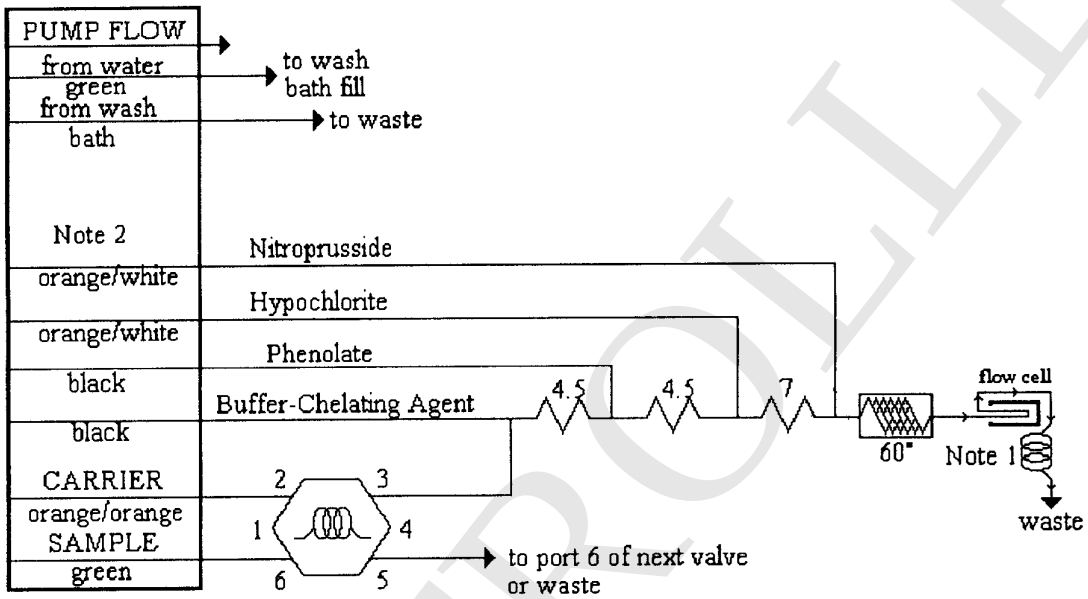
16. Revision Tracking Table:

Rev. #	Rev. date	Changes Made	Revision author
9	04/12/17	3. Added "Pollution Prevention" to section title 8. Deleted redundant QA Manual paragraph 8.12, 11.2 Added dilution verification information. 8.8, 14.1, & 15.8 Updated ESS INO QA 115 to EHD QA 115—DOC Procedures 8.5, 8.6 changed "should" to "must"	G. Anderson
10	04/08/19	1.3—changed nitrate LOD from 0.019 to 0.036 and LOQ from 0.061 to 0.10 mg/L. 7.2.4—changed 1.0 cm cell to 1.0 mm cell for nitrate. 8.6—added QC limits (in response to EPA July, 2018 audit, def. # 21.b)	J.S. Thorngate
11	02/03/20	1.1—changed nitrate range from 3.0 to 6.0 mg/L 1.2—changed ammonia LOD from 0.015 to 0.017 and LOQ from 0.048 to 0.058 mg/L (effective 1/15/2020 in Horizon)—to match LOD study done 2/09/18 and verified on 8/14/19, but not previously changed in Horizon. 1.3—changed nitrate LOD from 0.036 to 0.016 and LOQ from 0.10 to 0.054 mg/L (effective 1/15/2020 in Horizon). 6.15.1—changed nitrate standard concentrations and volumes from 3.0 to 6.0 mg/L, 2.0 to 3.0 mg/L, and 0.10 to 0.05 mg/L. Section 3: added pollution prevention information 6.10—changed recipe for ammonium chloride reagent. 6.10.1—added information on sodium hydroxide reagent used to adjust pH of ammonium chloride reagent. Sections 9 and 10: Updated for standardization of SOPs. Figures 1 and 2, Sections 15.17 and 15.18: Updated to QC 8500.	L. Klicko
12	04/16/20	1.3—changed nitrate LOD from 0.016 to 0.040 mg/L and LOQ from 0.054 to 0.132 mg/L (effective in Horizon 02/25/2020) 6.15.1—changed nitrate standard concentrations and volumes from 6.0 to 3.0, 3.0 to 2.0, and 0.05 to 0.10 mg/L. Section 12: Updated to reflect Horizon 12.	L. Klicko
13	03/11/21	1.1-1.3 Updated Ammonia LOD from 0.017 to 0.012 mg/L and LOQ from 0.058 to 0.039 mg/L. Updated Nitrate LOD from 0.040 to 0.055 mg/L and LOQ from 0.132 to 0.184 mg/L. Both due to new Initial LOD study using MB and filtered LODSP (effective in Horizon 03/05/2021). 6.7, 6.12 Added alternative method for either preparing or ordering standard depending on laboratory needs.	R. Riessen

		8.3 Added definition of Correlation Coefficient along with %RE requirement. 9.4.1 Deleted required r-value for calibration, replaced with cross-reference to section 8.3, which includes r and %RE criteria. 15.4 Updated UW Safety reference 15.7 Updated from NELAC 2009 to 2016. SOP reformatted for consistency with other SOPs.	
14	11/05/21	5.3, 15.19 Added chlorine interference for ammonia analysis and reference procedure for dechlorinating (in response to May 2021 DNR audit, def. 1B). 6.9 Expiration date of carrier changed from one day to one week. 8.5 Added information/criteria for Method Blanks (in response to April 2021 NELAC audit, BLO1). 8.6 One LFB must be run per the DNR NR149 update. 8.6.1, 10.3.4 Added LFMB per DNR NR 149 update 8.10 Following all accreditor suggestions, LODs will now be calculated/verified every 13 months instead of six. Appendix 1 added to aide analyst in diluting over range samples.	R. Riessen / L. Klicko
OB1	11/30/21	Transition to OnBase Updated references	S.D. Hill

Figure 1

AMMONIA MANIFOLD DIAGRAM



QC8500 Sample Loop: 125 cm x 0.022" i.d.

Interference Filter: 630 nm

Manifold Tubing: 0.5 mm (0.022" i.d.) This is 2.5 uL/cm.

4.5: 70 cm of tubing on a 4.5 cm coil support

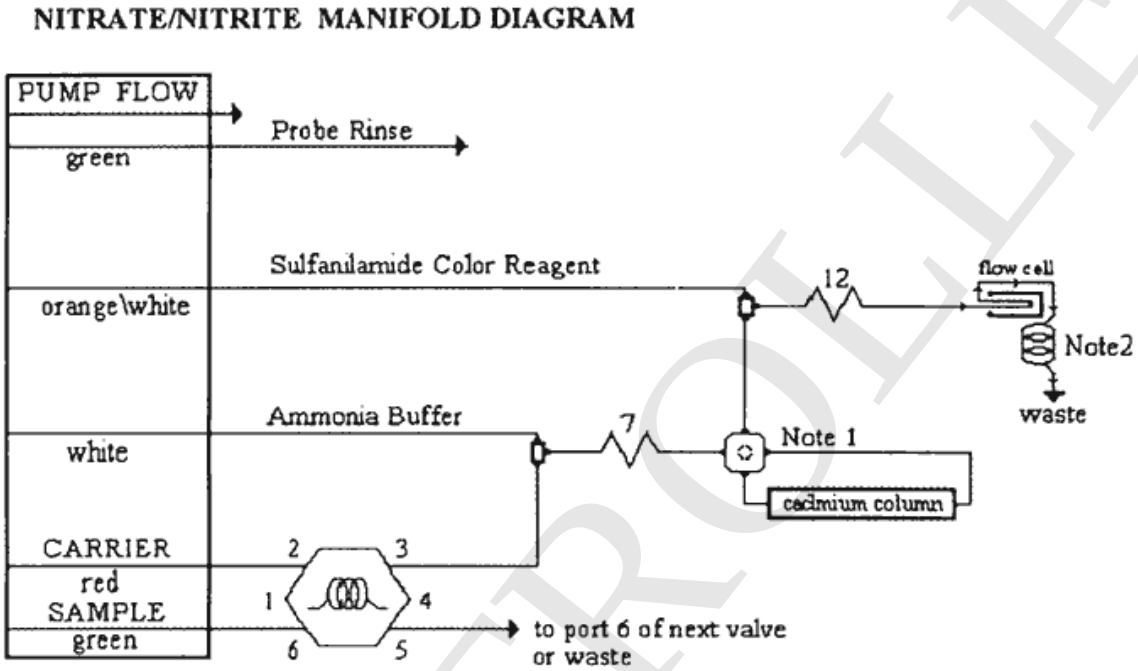
7: 135 cm of tubing on a 7 cm coil support

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The 60° coil represents 650 cm of 0.8 mm (0.032" i.d.) tubing wrapped around the heater block at the specified temperature.

Note 1: This is a 200 cm backpressure loop of 0.022" i.d. tubing.

Note 2: Tygon pump tubes must be used for this method.

Figure 2



QC8500 Sample Loop: Microloop

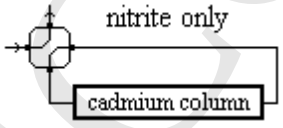
Interference Filter: 520 nm

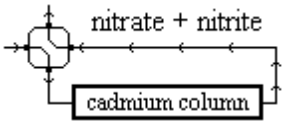
Manifold tubing: 0.5 mm (0.022 in) i.d. This is 2.5 uL/cm.

7: 135 cm of tubing on a 7 cm coil support

Apparatus: An injection valve, a 1.0 mm path length flow cell, and a colorimetric detector module is required.

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold. Use collar PN 50007 for the sample lines.





Note 2: This is a 100 cm backpressure loop of 0.022” i.d. tubing.

Appendix 1.

Dilution Guide

Ammonia:

Initial Instrument Response (mg/L)	Approximate Dilution	Approximate Final Value (mg/L)
2.5	X4 – X5	3
5	X8 – X10	7
6	X10 – X15	9
7	X20	15
8	X25 - X50	23
9	X50 – X100	37
10	X100 – X200	90
11	X500 – X1000	>250

EHD INORG METHOD 240.0
Total Nitrogen Persulfate Digestion
(EPA Method 353.2)

1. Scope and Application

1.1 This method is applicable to the determination of Total Nitrogen (TN) in drinking, ground, surface, domestic, and industrial waste samples which have been preserved with sulfuric acid (H_2SO_4). The range for the total nitrogen method is 0.058 to 10.0 mg N/L.

1.2 The method limit of detection (LOD) = 0.058 mg/L

1.3 The method limit of quantification (LOQ) = 0.192 mg/L

2. Summary of Method

2.1 Total Nitrogen is the sum of nitrate (NO_3-N), nitrite (NO_2-N), ammonia (NH_3-N), and organic nitrogen compounds. Samples are digested in an autoclave for 30 minutes at 121°C and 15-20 psi with potassium persulfate, boric acid, and sodium hydroxide to convert all forms of nitrogen to nitrate. The digested sample is passed through a copperized cadmium column that reduces nitrate quantitatively to nitrite. The total nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble, magenta colored dye is measured colorimetrically at 520 nm.

2.2 The determinative steps in this method are referenced from EPA 353.2 (15.1). However, because the EPA method is written specifically for air-segmented continuous flow technology that is no longer available, the specific "plumbing" scheme (i.e., pump tubes and reagent proportions, etc.) are adapted to match the Lachat QuikChem Method 10-107-04-4-A (15.2). The specific flow scheme used in this SOP is from Lachat Method 10-107-04-4-A (15.2) and USGS Report 03-4174 (15.3). Three variations from these methods that we implemented are: we do not use any blank correction, we digest all standards, blanks and QCS with the samples, and we recrystallize the potassium persulfate.

3. Safety, Waste Management, & Pollution Prevention

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility (15.4).

3.2 All laboratory waste, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (15.5).

3.3 Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (15.5).

4. Sample Handling and Preservation

4.1 Samples are collected in a Wisconsin State Lab of Hygiene (WSLH) 250 mL plastic bottle. Bottle quality is verified prior to use (15.12).

4.2 Samples are preserved in the field by the addition of 1 mL of 25% H_2SO_4 per 250 mL sample. They are stored at ≤6°C (but not frozen) until analysis is performed.

4.3 Maximum holding time (after sample preservation) is 28 days from date of collection.

5. Interferences

5.1 Calcium, magnesium, iron and copper ions, or other metals may precipitate if present in sufficient concentration. Ethylenediamine-tetra acetic acid (EDTA) is added to the buffer to prevent this problem.

5.2 Color, turbidity, and certain organic species may interfere.

5.2.1 Estimating a correction for sample color may be calculated by running the samples through the manifold with *all* reagents pumping *except* sulfanilamide, which is replaced by ASTM Type-I water. The resulting absorbance readings are then subtracted from those obtained for samples determined with all reagents resulting in complete color formation.

5.2.2 Turbidity can be removed by manual filtration through a 0.45µm filter prior to analysis. The presence of suspended matter may restrict flow through the reduction column and manifold tubing.

5.2.3 Samples that contain large concentrations of oil and grease may coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

6. Reagents and Standards

6.1 **ASTM Type-I Water**: All reagents and standards must be made with ASTM Type-I water.

6.2 **Carrier solution**: In a 1 L volumetric flask, add 500 mL ASTM Type-I Water and 1.0 mL concentrated H_2SO_4. Dilute to the mark and invert to mix. Use this reagent to perform any dilutions at the instrument. Degas with helium prior to use. Expiration date is 7 days.

6.3 **Ammonium Chloride Buffer, pH 8.5**: In a hood, to approximately 1000 mL of ASTM Type-I water in a 2L volumetric flask, add 210 mL concentrated hydrochloric acid (HCl), 190 mL concentrated ammonium hydroxide (NH_4OH), and 2.0 g disodium EDTA. Dissolve and dilute almost to volume. Allow to cool. Adjust the pH to 8.5 ± 0.1 with HCl or NH_4OH solution. Dilute to volume. Filter through a 0.45µm filter. Store at room temperature. The expiration date is 6 months.

6.4 **Sulfanilamide Color Reagent**: To approximately 1500 mL in a 2L volumetric flask of ASTM Type-I water, add 200 mL 85% phosphoric acid, (H_3PO_4), 80 g sulfanilamide ($C_6H_8N_2O_2S$), and 2.0 g N-(1-naphthyl)ethylenediamine dihydrochloride ($C_{12}H_{14}N_4 \cdot 2HCl$). Dissolve and dilute almost to volume. Allow to cool. Dilute to volume. Filter through a 0.45 µm filter. Store in a dark brown bottle and keep in a cool, dark place. The expiration date is 1 month.

6.5 **Digestion Solution**: Dissolve 40 g of potassium persulfate ($K_2S_2O_8$), 18 g boric acid (H_3BO_3), and 9 g sodium hydroxide (NaOH) in 500 mL of ASTM Type-I water. Dissolve and dilute to 1 L.

6.5.1 A pre-made low-nitrogen potassium persulfate reagent may be obtained from an approved UW vendor, e.g. Fischer.

6.5.2 Alternatively, potassium persulfate may be recrystallized to remove nitrogen contamination using the following procedure.

Potassium Persulfate Recrystallization: Potassium persulfate ($K_2S_2O_8$) is used for the digestion solution, but needs to be recrystallized twice to remove nitrogen contamination from the reagent.

1. Add 100 g of potassium persulfate to approximately 600 mL of ASTM Type-I water in a 1L Erlenmeyer flask. Dissolve the potassium persulfate in the flask using a medium sized stir bar while heating to 60°C.
2. Vacuum filter the 60°C solution through a porcelain Buchner funnel using Whatman 40 filter paper.
3. Cool solution to about 4°C by placing the flask in an ice water bath. Swirl the flask occasionally to prevent the solution from freezing. Cool a squirt bottle of ASTM Type-I water to about 4°C for rinsing.
4. Vacuum filter the 4°C solution using the Buchner funnel and a new Whatman 40 filter. Rinse the flask with cold ASTM Type-I Water. Save the white potassium persulfate crystals on the filter.
5. Discard the filtrate from the 1L flask.
6. Repeat steps 1 through 5 a second time using the crystals from the filter and a clean 1L flask.
7. Keep the crystals on the filter and vacuum dry. Alternatively, put the crystals on a pie plate and leave in a desiccator to dry. Yield is about 80%.
8. Store at room temperature in a dry location.

6.6 **Nitrate Stock Standard (1000 mg NO_3-N/L)**: Dissolve 7.218 g potassium nitrate (KNO_3) (dried at 105°C for 1 hr and cooled in a desiccator) in 900 mL ASTM Type-I water. Preserve with 2 mL chloroform and dilute to 1 L (1.0 mL = 1.0 mg NO_3-N). Refrigerate at 4°C. The expiration date is 6 months.

6.7 **Nitrite Stock Standard (250 mg NO_2-N/L)**: Dissolve 1.518 g potassium nitrite (KNO_2) or 1.232 g of sodium nitrite ($NaNO_2$) (dried at 105°C for 1 hr and cooled in a desiccator) in 900 mL ASTM Type-I water. Preserve with 2 mL of chloroform and dilute to 1 L (1.0 mL = 0.25 mg NO_2-N). Refrigerate at 4°C. The expiration date is 6 months.

6.8 **Quality Control Standard (QCS)**: The stock solution used to prepare the QCS must originate from a source different from that used for the calibration standards. A stock standard with the concentration of 1000 mg NO_3-N/L is purchased. Pre-made stock solutions are obtained from vendors such as LabChem, VWR, or ERA. The stock standard is refrigerated at 4°C and expires 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.8.1 Quality Control Working Standard (QCS): Dilute 2.5 mL of Nitrate standard (6.8) (1 mL = 1 mg N) to 500 mL (this makes a 5.0 mg NO_3-N/L solution). Preserve with 0.5 mL of concentrated H_2SO_4 before diluting to the mark. The expiration date is 28 days. Refrigerate at 4°C.

6.9 **Working Standard Solutions**: Prepare the following standards by diluting suitable volumes of standard solution (6.6) for Nitrate to ASTM Type-I water (add 1.0 mL of concentrated H_2SO_4 per 1000 mL before diluting to the mark). Working standards are refrigerated at 4°C. The expiration date is 28 days.

6.9.1 Note: All stock, QCS, and working standards must be entered into Horizon and the Standards Logbook located in the Wet Chemistry Laboratory.

6.9.2

Concentration of Standard mg NO_3-N/L	Volume of NO_3 Standard Solution (6.6) mL
10.0	10.0 mL (1 L)
7.5	7.5 mL (1 L)
5.0 (IPC)	5.0 mL (1 L)
3.0	3.0 mL (1 L)
1.0	1.0 mL (1 L)
0.5	0.50 mL (1 L)
0.10	0.10 mL (1 L)
0.0 (Reagent Blank)	ASTM Type-I water
200 spike solution	20 mL (100 mL)

7. Apparatus

7.1 Digestion tubes, 16 x 125 mm, disposable borosilicate glass.

7.2 Autoclave.

7.3 Lachat 8500 Automated Flow Injection Ion Analyzer consisting of:

7.3.1 XYZ Sampler.

7.3.2 Peristaltic Pump.

7.3.3 Colorimetric detector

7.3.4 Colorimeter equipped with 10 mm path length flow cell, and 520 ηm interference filter.

7.3.5 Reaction unit or manifold with cadmium column (Figure 1)

7.3.6 Data System

7.4 Motorized pipettes: 10 mL, 5.0 mL, 1.0 mL, and 100 µL (15.11).

7.5 Polypropylene caps for disposable digeston tubes: 16 mm.

7.6 Vortex mixer

8. Quality Control Types, Acceptance Criteria, and Corrective Actions

8.1 Please refer to the Environmental Health Division Quality Assurance Manual (15.7) for general information on quality control procedures. Important specifics include:

8.1.1 Accuracy and precision calculations.

8.1.2 Corrective action procedures (including documentation requirements) for instrument problems or analytical problems.

8.2 **An instrument logbook** is maintained for each instrument. Maintenance, performance problems, date calibrated, analyst, and other pertinent information are documented in the logbook.

8.3 **A Quality Control Standard (QCS)** is analyzed at the beginning of each run. The analytical result must be within ± 10% of the true value to continue the analysis. If the

recommended limits are exceeded, corrective action includes reanalyzing the QCS, or the analyst may recalibrate if necessary.

8.4 **A Laboratory Reagent Blank (LRB)** is digested and analyzed initially for the first 20 samples and every 20 samples thereafter. The LRB must meet one of the three criteria listed in the Wisconsin Laboratory Certification Manual (15.6): 1) LRB must be less than the detection limit of the method; 2) LRB <5% of sample concentration; 3) LRB <5% of the regulatory limit. If it does not meet one of these criteria, the recommended corrective action may include reanalyzing the LRB, qualifying the samples, or recalibrating. In general, a LRB is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, and improper Y-intercept, poor source of material for reagent blank, etc.), then the blank may be accepted as "zero" providing that the logic supporting this decision is well documented. The LRB is equivalent to the CB for this method.

8.5 **One Laboratory Fortified Blank (LFB)** is digested and analyzed initially for the first 20 samples and every 20 samples thereafter. The spike recovery for the LFB must be within ±10% of the true value to proceed. If LFB exceeds acceptance criteria, corrective action will include reanalyzing the LFB, qualifying the data or recalibrating if necessary. Prepare the LFB using LRB/CB and spiking solution (6.9), where the spike solution is equal to 1.0% of the total volume -- e.g., add 0.05 mL of spike solution to 4.95 mL of LRB/CB.

8.6 **Matrix Spikes and Duplicates:** Prepare **a minimum of 10%** of the samples, per matrix, as duplicates and spikes. Duplicate (precision) limits are 10% RD. If these limits are exceeded, the matrix group (including spike and duplicate) must be reanalyzed. If limits are exceeded a second time, the samples from this matrix group will be reanalyzed. Spikes are prepared in the same manner as the LFB, substituting the sample in place of the LRB/CB. Spike (accuracy) limits are 90-110% Rec. If these limits are not met, corrective action requires reanalysis of the matrix group (including the spike and duplicate) on the same run. If limits are exceeded a second time, qualify the matrix group (15.7).

8.7 **An Instrument Performance Check (IPC)** and a **Check Blank (CB)** will be analyzed after every 10 cups. The IPC must be within ±10% of true value. Choose a standard with a concentration near the middle of the calibration range. The CB must be less than the LOD. In general, a CB is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, and improper y intercept, poor source of material for reagent blank, etc.), then the blank may be accepted as "zero" providing that the logic supporting this decision is well documented. All data must be bracketed by acceptable IPCs and CBs. If an IPC or CB fails, corrective action requires that all previous samples back to the last acceptable IPC and CB be reanalyzed. The CB is equivalent to the LRB for this method.

8.8 **Demonstration of Capability (DOC):** Initial DOCs and ongoing DOCs are performed according to EHD QA 115 (15.9).

8.9 **Limit of Detection (LOD):** This must be determined or verified every 13 months. Determine the method LOD using the procedure outlined in EHD QA 116 (15.10).

8.10 **Linear Calibration Range (LCR):** The LCR must be verified every six months or whenever there is a change in the method. The initial demonstration of linearity must use a sufficient number of standards to insure that the curve is linear. The verification of linearity must include a minimum of one blank and three standards. If any verification data exceeds the actual values by ±10%, linearity must be re-established. If any portion of the range is shown to be nonlinear, a sufficient number of standards must be analyzed to clearly define the nonlinear portion.

8.11 **A Nitrite Column Efficiency sample (COLEFF)** is digested and analyzed at the beginning of each run to check the efficiency of the cadmium column. Dilute 5.0 mL NO_2-N standard (6.7) to 250 mL with ASTM Type-I water (this makes a 5.0 mg NO_2-N/L solution). This sample must be entered into Horizon and the Standards Logbook located in the Wet Chemistry Laboratory. This is the same concentration as the NO_3-N QCS (6.8). The efficiency is determined by the equation:

$$\frac{\text{Measured concentration of the } NO_3\text{-N QCS standard [6.8.1]}}{\text{Measured concentration of the } NO_2\text{-N column efficiency sample [8.11]}} X\ 100\%$$

The calculated efficiency must be within 100±10% to proceed. If the column efficiency fails, corrective action requires reanalyzing the COLEFF, or the analyst may choose to recalibrate with a new cadmium column if necessary.

8.12 **Sample Dilution:** If the estimated concentration of analyte in a sample exceeds the highest calibration standard, a pre-digestion dilution should be performed and noted on the bench sheet. This dilution factor will be entered into Horizon when finalizing the prep batch (15.15). Motorized pipettes may be used to deliver/dilute volumes up to 10 mL. For volumes greater than 10 mL, use Class A glass volumetric pipettes. Dilutions that are done at the bench during analysis are typically performed by diluting an appropriate volume of sample with the carrier solution (6.2). Diluted samples are mixed thoroughly prior to analysis. When a bench dilution is made, the dilution must be verified by comparing the diluted result with the original result. The comparison is done by dividing the diluted result by the original result and expressing the calculation as a percent. The acceptable range is 90% to 110%. If the dilution verification is not within the acceptable range, a different dilution must be made. This second dilution would then be verified against the first dilution. If a pre-digestion dilution is done, a second dilution, at a different dilution factor, must be done and compared to the first dilution. The acceptable range is 90% to 110%. If a group of samples is diluted identically, at least 10% of the dilutions must be verified.

8.12.1 Samples diluted prior to digestion should be edited in the prep batch in Horizon. Enter the initial and final volumes of the dilution OR enter the dilution factor in the Dilution column after selecting the Results display in Edit Result under Batches.

- Ex: 0.5 mL in initial volume and 5 mL in final volume for a 10 dilution factor

9. Method Calibration

9.1 Refer to section 6 for making standards and reagents.

9.1.1 Working standards (6.9) are digested along with the samples as described in Appendix 1.

9.2 Calibration curve is a linear, 1st order polynomial curve.

9.3 Set the data system parameters and operating conditions for the Lachat 8500 with the *Omnion* software (15.13)

9.3.1 Set up manifold as shown in Figure 1.

9.3.2 Pump ASTM Type-I water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline has been obtained (about five minutes).

9.3.3 Engage the cadmium column on the manifold by turning the cadmium column bypass switch to the "on" position after the reagents have passed through the second mixing coil. To disengage the cadmium column turn the bypass to the "off" position. Always disengage the cadmium column from reagent flow before changing over to water at the end of the analytical batch.

9.4 Place standards in the appropriate rack on the autosampler and calibrate the instrument by injecting the standards. The data system will then correlate the concentrations with the instrument responses for each standard.

9.4.1 After the calibration passes (a minimum Correlation Coefficient, $r \geq 0.995$ is required to proceed) the samples may be analyzed. If the calibration is not satisfactory use an appropriate corrective action to diagnose possible causes and recalibrate.

10. Procedure

10.1 See also Instrument Operating Procedure (15.13).

10.2 See Appendix 1 for standard/sample digestion procedure.

10.3 Create a worklist using HORIZON as explained in the Horizon Procedures (15.13).

10.4 Import the sample identification numbers from Horizon into the **Tray Table** of the Lachat *Omnion* software. This will include:

10.4.1 One Lab Reagent Blank for every 20 samples.

10.4.2 One Lab Fortified Blank for every 20 samples.

10.4.3 One Quality Control Standard per digestion batch.

10.4.4 One COLEFF Standard per digestion batch.

10.4.5 A duplicate and spike for every 10 samples in a matrix group.

10.5 Analyze standards and Quality Control samples (i.e., IPC, CB, LRB, LFB, QCS, and COLEFF). If QC samples pass acceptance criteria, the batch may continue with sample analysis.

10.6 Shutdown procedure:

10.6.1 When the analytical batch is complete, the cadmium column is immediately disengaged from reagent flow to prevent introduction of air or water into the column.

10.6.2 Transfer reagent lines to ASTM Type-I water to rinse followed by 10% HCl for about 10 minutes. Flush the pump tubing and manifold with ASTM Type-I water after cleaning with acid solution. Pump dry after rinsing. Release pump tubing from cartridges and turn off instrument.

10.6.3 Waste disposal: The waste and samples will be acidic and will need to be neutralized according to the Laboratory Safety Guide (15.5).

11. Calculations

11.1 Changes in absorbance due to color change are directly related to the amount of analyte present in each standard or sample. The absorbance signal creates a change in the voltage output which in turn, is converted to a digital format as the peak appears on the computer screen. The response variable, *peak area*, is converted to a digital signal by the software and regressed vs. concentration with a 1st order polynomial regression formula. Concentration of analyte in the unknown sample is calculated by the software, based on the standard calibration curve.

11.2 If the concentration of nitrate exceeds the highest calibration standard, a manual dilution must be performed and documented on the bench sheet. The dilution correction will be documented in Horizon.

12. Data Management

12.1 The analytical run, the *Run Time Report*, and the QC Parameters section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced chemist who did not perform the original analysis (15.14). The reviewer must initial and date the analytical run.

12.2 Export results from Omnion to Horizon (see EHD INORG GENOP 113, Horizon Procedures 15.15).

12.2.1 Review results by selecting Edit Results under Batches.

12.2.2 Review QC Results by selecting the QC display in Edit Results.

13. Definitions

13.1 Definitions of terms in this SOP may be found in the reference method (15.1). General definitions of other terms that may be used in this method are found in the EHD Quality Assurance Manual (15.7).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (DOCs and LODs) were generated in compliance with the reference method and the standard operating procedures: EHD QA 115 (15.9) and EHD QA 116 (15.10). Supporting data will be retained according to the applicable Records Disposition Authorization (RDA). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement.

15. References

15.1 United States Environmental Protection Agency. 1993. *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA/600/R-93/100, Method 353.2, Rev. 2.

15.2 HACH Analytics, Lachat Instruments Division. *Determination Total Nitrogen in Manual Persulfate Digests* (Method 10-107-04-4-A) Revised December 2010.

15.3 Methods of Analysis by the U.S. Geological Survey. Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen in Water. Water-Resources Investigation Report 03-4174.

15.4 Wisconsin State Laboratory of Hygiene. AD Safety GENOP 102. *Chemical Hygiene Plan & General Laboratory Safety Plan for the Agriculture Drive Facility*.

15.5 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.6 Wisconsin Department of Natural Resources Lab Certification Program, 06/29/2021, Wisconsin Administrative Code Chapter NR149.

15.7 Wisconsin State Laboratory of Hygiene, Environmental Health Division, *Quality Assurance Manual.*

15.8 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.9 Wisconsin State Laboratory of Hygiene. EHD QA 115, *Initial and Ongoing DOC Procedures*.

15.10 Wisconsin State Laboratory of Hygiene. EHD QA 116, *LOD Procedures*

15.11 Wisconsin State Laboratory of Hygiene. EHD INORG GENOP 200, *Pipette Performance Checks*.

15.12 Wisconsin State Laboratory of Hygiene. EHD INORG QA 101, *Bottle Check Procedure*.

15.13 Wisconsin State Laboratory of Hygiene. EHD INORG IOP 105, *Instrument Operating Procedure for QuikChem Automated Ion Analyzer*.

15.14 Wisconsin State Laboratory of Hygiene. EHD INORG QA 107, *Q.C. Audits of Analytical Runs for ESS Wet Chemistry Area*.

15.15 Wisconsin State Laboratory of Hygiene. EHD INORG GENOP 113, *HORIZON Procedures* for EHD Inorganic Chemistry.

15.16 QuikChem 8500 Series 2, FIA Automated Ion Analyzer, User Manual, Lachat Instruments, Hach Company, Edition 4, June, 2008.

15.17 Software User Manual, Omnion 3.0 Software, Lachat Instruments, Hach Co., Edition 4, Sept. 2007.

15.18 Software Manual, Omnion 4.0, Hach, Edition 1, April, 2015.

15.19 QuikChem 8500 Series Automated Ion Analyzer Training Manual, Lachat Instruments, Hach Co., Edition 4, May, 2008.

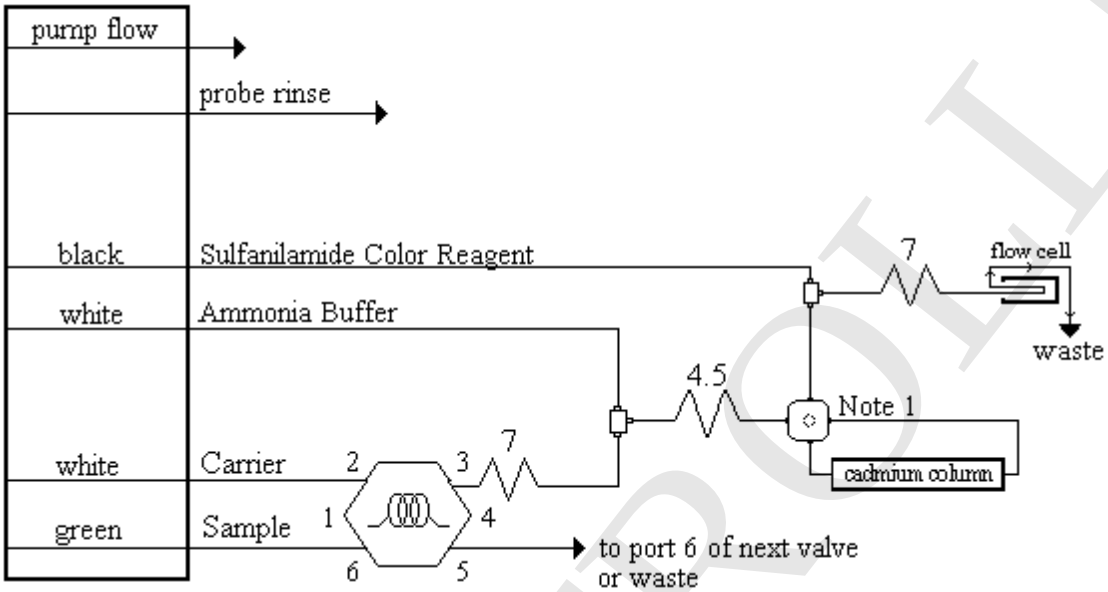
16. Revision Tracking Table:

Rev. #	Rev. date	Changes Made	Rev. author
1	6/27/16	Updated for Horizon	Wes Kotila
2	12/03/2019	Updated LOD and LOQ Updated range of concentration Updated standards Changed recipe for Ammonium Chloride from 1L to 2L Changed recipe for Sulfanilamide from 1L to 2L Changed Lachat references from 8000 to 8500 Updated DOC LOD to EHD QA 115 Section 3.3—added specific section regarding pollution prevention. Updated link to UW Safety Added dilution verification Many additional small wording changes throughout SOP Section 8.6—added QC limits for MS and Dups. Section 8—changed “should” to “must” or “will”	Anthony Plourde
3	07/24/2020	Section 1.1: Included all matrices listed in the reference method. Section 1.2: Updated LOD from 0.024 to 0.058 mg/L and LOQ from 0.080 to 0.192 mg/L (effective in Horizon 07/16/2020). Section 6.9: Updated low standard from 0.25 to 0.10 mg/L to meet TNI requirement. Sections 9 and 10. Updated to standardize SOPs and to consolidate QC requirements. Section 12: Updated to reflect Horizon 12. Section 15.8: updated TNI reference. Appendix 1: Added digestion procedure to its own section for ease of use. Updated 15.13, 15.16-15.19 for Lachat QuikChem 8500 Series 2.	Royce Riessen
4	11/05/21	Section 1: Updated formatting for consistency with other SOP’s. Section 2.1: Added information about TN components and digestion procedure.	L. Klicko / R. Riessen

		Section 6.2: Reformatted carrier information to match other SOP's. Section 6.5: Added option for purchasing low-nitrogen potassium persulfate. Included using cool rinsing water for recrystallization procedure. Section 6.8.1 and 8.11: Added final concentration to working standards. 7.1, 7.5: Updated disposable glass tubes from 20 x 150 mm/13 x 100 mm to 16 x 125 mm. 8.5 For consistency with accredited tests, one LFB will now be analyzed for every 20 samples per the DNR NR149 update. 8.9 Following all accreditor suggestions, LODs will now be calculated/verified every 13 months instead of six. Appendix 1: Updated volume of sample/digestion acid to reflect using 5 mL.	
OB1	11/30/21	Transition to OnBase References updated	S.D. Hill

Figure 1

TOTAL NITROGEN MANIFOLD DIAGRAM



Manifold Tubing: 0.5 mm (0.022 in) i.d. This is 2.54 µL/cm.

QC8000 Sample Loop: Microloop (16cm) 0.3 mm i.d.

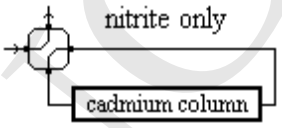
Interference Filter: 520 nm

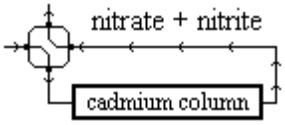
Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

4.5: 70 cm of tubing on 4.5 cm coil support

7: 135 cm of tubing on a 7 cm coil support

Note 1: This is a two state switching valve used to place the cadmium in-line with the manifold.





APPENDIX 1:

Total Nitrogen Digestion Procedure

1. Load the test tube racks with disposable digestion tubes (7.1) so you have enough for your samples, standards, LRBs, LFBs, QCS, COLEFF, duplicates, and spikes according to the analytical run.

1.1. Label the first and last tube of every row (i.e. 1, 10, 11, 20, etc.).

2. Transfer 5 mL* of each sample to a digestion tube with a motorized pipette.

2.1. If concentration of a sample is believed to be over range, dilutions may be made using 0.00 standard (6.9) and sample as long as total volume in tube is equal to 5 mL.

2.2. The LFB and spiked samples should be prepared according to 8.5 and 8.6.

3. The Standards rack may be set up as shown below.

		5.0	**5.0**					**0**	**0**
10	**7.5**	**5.0**	**5.0**	**3.0**	**1.0**	**0.5**	**0.10**	**0**	**0**
10	**7.5**	**5.0**	**5.0**	**3.0**	**1.0**	**0.5**	**0.10**	**0**	**0**
10	**7.5**	**5.0**	**5.0**	**3.0**	**1.0**	**0.5**	**0.10**	**0**	**0**

4. All digestion tubes must have 5 mL of liquid before the addition of digestion acid. Add 2.5 mL of working digestion acid solution (6.5) to each tube, vortex, and cover with caps (7.5) (do not press caps down).

5. Autoclave the digestion tubes for 30 minutes at 121°C, 15-20 psi.

6. Remove the tubes from the autoclave, press caps down securely, and allow them to cool.

7. Allow any particulate matter to settle.

8. Analyze with the colorimetric method (10).

* Note: Other volumes may be used as long as the ratio of sample/standards/QC to digestion acid remains the same.

EHD INORG METHOD 310.2
Phosphorus, Total, Persulfate Digestion
(EPA 365.1)

1. Scope and Application

1.1. This method is applicable to the determination of total phosphorus in drinking, ground and surface waters and domestic and industrial wastes in the range of 0.009 to 1.0 mg P/L.

1.2. The method limit of detection (LOD) = 0.009 mg/L

1.3. The method limit of quantification (LOQ) = 0.030 mg/L

2. Summary of Method

2.1. Samples are digested in an autoclave for 30 minutes at 121°C and 15-20 psi with ammonium persulfate and sulfuric acid to convert all phosphorus to orthophosphate. The orthophosphate ion $(PO_4)^{3-}$ reacts with ammonium molybdate and antimony potassium tartrate, under acidic, conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of the orthophosphate in the sample.

2.2. The determinative steps in this method are identical to EPA method 365.1 (15.1). However, because the EPA method is written specifically for air-segmented continuous flow technology that is no longer available, the specific "plumbing" scheme (pump tubes and reagent proportions, etc.) used are adapted to match the Lachat flow injection instrumentation. The specific flow scheme used in this SOP is from Lachat method 10-115-01-1-F (15.2).

3. Safety, Waste Management, & Pollution Prevention

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (15.3).

3.2. All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (15.4).

3.3. Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (15.4).

4. Sample Handling and Preservation

4.1. Samples are collected in a State Lab of Hygiene (SLH) 250 mL plastic bottle. Bottle quality is verified following the procedure outlined in reference (15.11).

4.2. Samples are preserved in the field by the addition of 1 mL of 25% H_2SO_4 per 250 mL sample to a pH of less than 2. They are cooled to ≤6°C, but not frozen, until analysis is performed.

4.3. Maximum holding time (after sample acidification) is 28 days from date of collection.

5. Interferences

5.1. Concentrations of ferric iron $(Fe)^{3-}$ greater than 50 mg/L will cause a negative error due to precipitation of, and subsequent loss, of orthophosphate. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

5.2. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg/L positive error in orthophosphate.

5.3. A list of interferences is documented in Method 365.1, section 4 of EPA Methods for Chemical Analysis of Water and Wastes (1993) (15.1).

6. Reagents and Standards

6.1. **Reagent water (ASTM Type I water):** All reagents and standards must be made with ASTM Type I water (U.S. Filter Corp., Lowell, MA).

6.2. **Stock acid solution, 5.6M Sulfuric Acid (H_2SO_4):** Dilute 310 mL of concentrated H_2SO_4 to 1 L with ASTM Type I water (Caution: solution will get hot). Store in a glass container. Expiration date is 6 months.

6.3. **Working digestion acid solution:** Dissolve 12.8 g ammonium persulfate ($(NH_4)_2S_2O_8$) and 32 mL of 5.6M H_2SO_4 (6.2) in a 100 mL volumetric flask. Dilute to mark with ASTM Type I water. Prepare daily.

6.4. **Stock Ammonium Molybdate Solution:** In a 1 L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_20]$ in approximately 800 mL of ASTM Type I water. Dilute to the mark and invert to mix. Store in plastic and refrigerate at ≤6°C. Expiration date is 6 months.

6.5. **Stock Antimony Potassium Tartrate Solution:** In a 1 L volumetric flask dissolve 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 3H_2O$) in approximately 800 mL of ASTM Type I water. Dilute to the mark and invert to mix. Store in a dark bottle and refrigerate at ≤6°C. Expiration date is 6 months.

6.6. **Molybdate Color Reagent:** (Rinse down sides of flask and mix between each reagent). To a 1 L volumetric flask add approximately 500 mL ASTM Type I water and then add 21.0 mL concentrated H_2SO_4 (Caution: solution will get hot). When the flask can be comfortably handled, add 72.0 mL Stock Antimony Potassium Tartrate Solution (6.5) and 213 mL Ammonium Molybdate Solution (6.4). Dilute to mark. Store in glass jar and refrigerate at ≤6°C. To prevent bubble formation, degassing with helium at 140 kPa (20 lb/in^2) through a helium degassing tube may be done for one minute prior to use. Expiration Date is 7 days.

6.7. **Ascorbic Acid Reducing Solution (0.33M):** In a 1 L volumetric flask dissolve 60.0 g ascorbic acid in about 700 mL of ASTM Type I water. Add 1.0 g dodecyl sulfate

($CH_3(CH_2)_{11}OSO_3Na$) (wetting reagent). Dilute to the mark and invert to mix. Filter through a 0.45 µm filter and refrigerate at ≤6°C. Discard if the solution becomes yellow. Expiration date is 7 days.

6.8. **Carrier:** Sulfuric Acid 0.16 M. In a 2 L volumetric flask add 1800 mL ASTM Type I water and 18.0 mL concentrated H_2SO_4. Dilute to the mark and invert to mix. Use this reagent to perform any dilutions at the instrument. If needed, degas with helium prior to use. Expiration date is 7 days.

6.9. **NaOH-EDTA Cleaning Solution:** In a 1 L volumetric flask dissolve 65 g of NaOH and 6.0 g disodium EDTA in about 500 mL of ASTM Type I water. Dilute to mark and invert to mix. Store in a dark plastic bottle. Expiration date is N/A.

6.10. **Stock phosphorus standard (100 mg P/L):** A pre-made stock solution may be obtained from an approved UW vendor, e.g. RICCA or ERA. Stock is refrigerated at ≤6°C. Expiration date is 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.10.1. Alternatively, Dissolve 0.4393 g of potassium phosphate monobasic (KH_2PO_4) (dried at 105°C for 1 h) in 900 mL ASTM Type I water. Add 1.0 mL of concentrated H_2SO_4 and dilute to 1 L: 1.0 mL = 0.100 mg P (100 mg P/L) and refrigerate at ≤6°C. Expiration date is 6 months.

6.11. **Working standard solutions:** Prepare the following standards by diluting suitable volumes of standard solution (6.10) to 1 L with ASTM Type I water. Preserve standards with 1 mL/L concentrated H_2SO_4 before diluting to the mark and refrigerate at ≤6°C. Expiration date is 28 days.

Concentration of Standard (mg P/L)	Volume of stock standard 6.10 (mL)
0.00 (and Reagent Blank)	0.0 (1 L)
0.016	0.16 (1 L)
0.050	0.50 (1 L)
0.250	2.5 (1 L)
0.500	5.0 (1 L)
1.00	10.0 (1 L)
30 Spike Solution	30.0 (100 mL)

Note:
1) Digest extra tubes (depending on length of run) of each the zero (i.e. reagent blank) and 0.5 mg/L standards because they are used for the CBs and IPCs (8.6).
2) All working, stock, and QCS standards must be entered into the Standards Log located in the Wet Chemistry Laboratory.
3) All Stock and QCS standards must be entered into the Standards Log located in Horizon (15.17).

6.12 **Quality Control Stock Standard:** The stock solution used to prepare the QCS must originate from a different source than the calibration standards. A pre-made 50 mg/L stock solution may be obtained from vendors such as LabChem, VWR, or ERA. Stocks are refrigerated at ≤6°C and expire 6 months after opening or on the manufacturer's expiration

date, whichever is sooner.

6.13 **Quality Control Working Standard (QCS):** Dilute 4.0 mL of 50 mg/L QCS Stock Standard (6.12) and 0.5 mL of concentrated H_2SO_4 to 500 mL with Type I water. 1.0 mL = 0.0004 mg P (0.4 mg P/L). Expiration date is 28 days.

6.14 **NA_2ATP (adenosine5-triphosphate, disodium salt hydrate):** Acros Organics 102800100. This standard is an organic bound form of phosphorus in a dry chemical form and requires refrigeration. Expiration date is 10 years from receipt.

6.15 **Laboratory Control Sample (LCS) Stock (500 mg/L):** Weigh 0.2966 g Adenosine 5'-triphosphate disodium salt (NA_2ATP) (6.14) that has been dried at 103ºC for 1 hour and cooled in a desiccator. Dissolve the salt in Type I water in a 100 mL volumetric flask. Add 0.1 mL concentrated H_2SO_4 and dilute to volume with Type I water. 1 mL =0.5 mg P (500 mg p/L). Expiration date is 6 months.

6.16 **Laboratory Control Sample (LCS):** Dilute 0.6 mL of LCS stock (6.15) and 0.5 mL concentrated H_2SO_4 to 500 mL with Type I water. Concentration = 0.6 mg P/L. Expiration date is 28 days.

7. Apparatus

7.1. Digestion tubes, 16 x 125 mm and 20 x 150 mm, disposable borosilicate glass.

7.2. Autoclave.

7.3. Lachat 8500 Series II System.

7.3.1. Multichannel proportioning pump

7.3.2. Injection module with a 150 cm x 0.7 mm i.d. sample loop.

7.3.3. Reaction unit or manifold (Figure 1)

7.3.4. Colorimetric detector

7.3.5. Colorimeter equipped with 10 mm path length flow cell and 880 nm interference filter.

7.3.6. Data system

7.3.7. Heating unit: 37°C; use 175 cm length tubing.

7.4. Motorized pipettes: 10 mL, 5 mL, 1.0 mL, and 0.1 mL (15.10).

7.5. Polypropylene caps for disposable digestion tubes: 16 mm and 20 mm.

7.6. Vortex mixer.

7.7. Autosampler.

8. Quality Control Types, Acceptance Criteria, & Corrective Actions

8.1. Please refer to the **Environmental Health Division Quality Assurance Manual** (15.6) for general information on Quality Control Procedures. Important specifics include:

8.1.1. Accuracy and precision calculations.

8.1.2. Corrective action and result qualification procedures (including documentation requirements) for instrument problems or analytical problems.

8.2. **An instrument logbook** is maintained for each instrument. Maintenance, performance problems, date calibrated, analyst, and other pertinent information are documented in the logbook.

8.3. **The Correlation Coefficient (*r* value)** is the measure of the linearity of the standard curve and must be ≥0.995 to be acceptable and before proceeding with sample analysis. The 2016 TNI Standard (15.7) requires the % Relative Error (%RE) to be calculated and evaluated for each the low and midpoint standards and must pass acceptance criteria before continuing analysis. The acceptable %RE for the low and midpoint standards is ±50% and ±30%, respectively. These values will be manually entered into HORIZON into the %RELOW and %REMID standards, located at the end of the batch. Due to HORIZON limitations, only recovery will be calculated. Since %RE = Recovery – 100%, the acceptable limits for the low and midpoint standards in HORIZON become 50-150% and 70-130%, respectively.

8.4. **A Quality Control Standard (QCS)** is digested with each run (6.13). The analytical result must be within ±10% of the true value to continue the analysis. If the QCS exceeds the recommended recovery limits, corrective action includes reanalyzing the QCS, recalibrating, or redigesting and reanalyzing the run.

8.5. **A Laboratory Control Standard (LCS)** is digested with each run (6.16). This standard is an organic bound form of phosphorus that evaluates digestion efficiency. The analytical result must be within +/- 10% of the true value to continue the analysis. If the limits for the LCS are exceeded, corrective action includes reanalyzing the LCS or recalibrating and reanalyzing the LCS. If the LCS still exceeds the limits, the run must be reset or the samples qualified.

8.6. **A Laboratory Reagent Blank (LRB)** is digested and analyzed initially for the first 20 samples and for every 20 samples thereafter. The LRB must meet one of three criteria listed in the Wisconsin Department of Natural Resources Lab Certification Program (15.5). 1) Lab Reagent Blank must be less than the detection limit of the method. 2) Lab Reagent Blank must be <5% of sample concentration. 3) Reagent Blank must be <5% of the regulatory limit. If the LRB does not meet one or more of these criteria, the recommended corrective action is re-digestion of the samples associated with the LRB in question. If the measured concentration of the LRB is more negative in magnitude than -LOD and there is no apparent source causing the problem (e.g., baseline drift, improper y-intercept, poor source material used to prepare the LRB, etc.) then the LRB may be accepted as having an estimated concentration of "zero" providing the logic supporting this decision is well documented.

8.7. **A Laboratory Fortified Blank (LFB)** is digested and analyzed initially for the first 20 samples and for every 20 samples thereafter. The spike recovery must be within ±10% of the true value to proceed. If the LFB exceeds the recommended recovery limits, corrective action includes reanalyzing the LFB, recalibrating, or redigesting and reanalyzing the run. Prepare the LFB using LRB/CB and spiking solution (6.11), where the spike solution is equal to 1.0% of the total volume -- e.g., add 0.05 mL of spike

solution to 4.95 mL of LRB/CB.

8.8. **Matrix Spikes (MS) and Laboratory Duplicates (LD):** Prepare a **minimum of 10%** of the samples, per matrix, with duplicates and spikes. Spikes are prepared in the same manner as the LFB, substituting the sample in place of the LRB/CB. If the duplicate acceptance criteria (precision QA) are not met (10% relative difference), the matrix group (including spike and duplicate) must be redigested and reanalyzed with the next analytical batch. If the duplicate limits are exceeded a second time, qualify all results within the matrix group. If the spike recovery (accuracy QA) does not fall within the specified control limits (90-110% recovery), the matrix group (including spike and duplicate) must be redigested and reanalyzed with the next analytical batch. If it fails a second time, qualify all results within the matrix group.

8.9. **An Instrument Performance Check (IPC)** and **Calibration Blank (CB)** must be analyzed immediately after calibration and then after every 10 cups. The IPC must be within ±10% of true value. Choose a standard with a concentration near the middle of the calibration range to use as the IPC. The CB must be less than the LOD. In general, a CB is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the CB is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, improper-Y intercept, poor source material used to prepare the CB, etc.), then the CB may be accepted as "zero" providing the logic supporting this decision is well documented. All data reported from each analytical batch must be bracketed by acceptable IPCs and CBs. If an IPC or CB fails, corrective action is to reanalyze all samples back to the last acceptable IPC and CB.

8.10. **Demonstration of Capability (DOC)**: An Initial DOC and annual continued proficiency checks are performed according to EHD QA 115 (15.8).

8.11. **Limit of Detection (LOD)**: The LOD must be verified every 13 months or reestablished whenever there is a significant change in the method or instrumentation. Verify or establish the method LOD using the procedure outlined in EHD QA 116 (15.9).

8.12. **Linear Calibration Range (LCR)**: The LCR must be verified every six months or whenever there is a significant change in the method or instrumentation. The initial demonstration of linearity must use sufficient standards to insure that the curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by more than ±10%, linearity must be re-established. If any portion of the range is shown to be nonlinear, a sufficient number of standards must be used to clearly define the nonlinear portion.

8.13. **Sample Dilution**: If the estimated concentration of analyte in a sample exceeds the highest calibration standard a bench dilution must be performed. Dilutions at the bench are typically performed by diluting an appropriate volume of sample with the digested reagent blank. Motorized pipettes may be used to deliver/dilute volumes up to 10 mL. For volumes greater than 10 mL, Class A glass, volumetric pipettes should be used. Diluted samples should be mixed thoroughly prior to analysis. Samples may also be diluted prior to digestion. Dilution Verification: When a dilution is made, the dilution must be verified by comparing the diluted result with the original result. The comparison is done by

dividing the diluted result by the original result and expressing the calculation as a percent. The acceptable range is 90% to 110%. If the dilution verification is not within the acceptable range, a different dilution must be made. This second dilution would then be verified against the first dilution. If a group of samples is diluted identically, at least 10% of the dilutions must be verified.

8.13.1. Samples diluted prior to digestion should be edited in the prep batch in Horizon. Enter the initial and final volumes of the dilution OR enter the dilution factor in the Dilution column after selecting the Results display in Edit Result under Batches.

- Ex: 0.5 mL in initial volume and 5 mL in final volume for a 10 dilution factor

9. Method Calibration

9.1. Refer to section 6 for making standards and reagents.

9.1.1. Working standards (6.11) are digested along with the samples as described in Appendix 1.

9.2. Calibration curve is a linear, 1st-order polynomial curve.

9.3. Open the method template and set the data system parameters and operating conditions for the Lachat 8500 with the *Omnion* software (15.12).

9.3.1. Set up manifold as shown in Figure 1.

9.3.2. Pump ASTM Type-I water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline has been obtained (about five minutes).

9.4. Place standards in the appropriate rack on the autosampler and calibrate the instrument by injecting the standards. The data system will then correlate the concentrations with the instrument responses for each standard.

9.4.1. After the calibration passes (8.3) the samples may be analyzed. If the calibration is not satisfactory use an appropriate corrective action to diagnose possible causes and recalibrate.

10. Procedure

10.1. See also Instrument Operating Procedure (15.12).

10.2. See Appendix 1 for standard/sample digestion procedure.

10.3. Create a worklist using Horizon as explained in the Horizon Procedures (15.17).

10.4. Import the sample identification numbers from Horizon into the Tray Table of the Lachat *Omnion* software. This will include:

10.4.1. One Lab Reagent Blank for every 20 samples.

10.4.2. One Lab Fortified Blank for every 20 samples.

10.4.3. One Quality Control Standard per digestion batch.

10.4.4. One Laboratory Control Standard per digestion batch.

10.4.5. A duplicate and spike for every 10 samples in a matrix group.

10.5. Analyze standards and Quality Control samples (i.e., IPC, CB, LRB, LFB, QCS, and LCS). If QC samples pass acceptance criteria, the batch may continue with sample analysis.

10.6. Shutdown procedure:

10.6.1. After the run is complete, switch reagent lines to the NaOH-EDTA solution (6.9) for approximately five minutes, then rinse with ASTM Type I water for five minutes.

10.6.2. Remove reagent lines from ASTM Type I water and pump air through in order to dry. Release pump-tubes from cartridges and turn off instrument.

10.6.3. Waste disposal: The waste will be acidic and will need to be neutralized according to the Laboratory Safety Guide (15.4).

11. Calculations

11.1 The phosphorus concentration in the unknown samples is calculated by the instrument software based on the standard calibration curve. The phosphorus concentration result is obtained by transferring the data from the Lachat instrument to Horizon (12.2) and can also be obtained directly from the *Run Time Report*, which should be printed for a hard copy.

11.2 If the estimated concentration of phosphorus exceeds the highest calibration standard, a manual dilution (8.13) must be performed and documented on the bench sheet. For dilution verification instructions, see section 8.13. The Lachat 8500 software does not incorporate the dilution correction into the result. The dilution correction will be calculated by the Horizon program. The final result will be verified mathematically, by an experienced chemist who did not perform the original analysis, when the batch is checked for quality control (15.13).

12. Data Management

12.1. The analytical run, the *Run Time Report*, and the QC Parameters section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced chemist who did not perform the original analysis (15.13). The reviewer must initial and date the analytical run.

12.2. Export results from Omnion to Horizon (see EHD INORG GENOP 113, Horizon Procedures 15.17).

12.2.1. Review results by selecting Edit Results under Batches.

12.2.2. Review QC Results by selecting the QC display in Edit Results.

13. Definitions

13.1 Definitions of terms in this SOP may be found in the reference method (EPA Method 365.1). General definitions of other terms that may be used in this method are found in the EHD Quality Assurance Manual (15.6).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (LODs and DOCs) were generated in compliance with the reference method and the Inorganic Chemistry Department's standard operation procedures: EHD QA 115 (15.8) and EHD QA 116 (15.9). Supporting data will be retained according to the applicable Records Disposition Authorization (RDA). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement.

15. References

15.1 U.S. Environmental Protection Agency, Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100, Rev. 2, August 1993, Method 365.1

15.2 Lachat Instruments, Determination of Total Phosphorus by Flow Injection Analysis Colorimetry (Acid Persulfate Digestion Method), QuikChem Method 10-115-01-1-F Revised October 1994.

15.3 Wisconsin State Laboratory of Hygiene, AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility.

15.4 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.5 Wisconsin Administrative Code NR149, Department of Natural Resources Lab Certification Program, effective June 29, 2021.

15.6 Quality Assurance Manual, Environmental Health Division, Wisconsin State Laboratory of Hygiene.

15.7 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.8 Wisconsin State Laboratory of Hygiene, EHD QA 115, Initial and Ongoing DOC Procedures.

15.9 Wisconsin State Laboratory of Hygiene, EHD QA 116, LOD Procedures.

15.10 Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 200, Pipette Performance Checks.

15.11 Wisconsin State Laboratory of Hygiene, EHD INORG QA 101, Bottle Check Procedure.

15.12 Wisconsin State Laboratory of Hygiene, EHD INORG IOP 105, Instrument Operating Procedure for QuikChem Automated Ion Analyzer.

15.13 Wisconsin State Laboratory of Hygiene, EHD INORG QA 107, Q.C. Audits of Analytical Runs for ESS Wet Chemistry Area.

15.14 QuikChem 8500 Series II, Operations Manual, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.15 Omnion 3.0 Software User Manual, 09/2007, Edition 4. / Omnion 4.0 Software User Manual, Lachat Instruments, Hach Co., 04/2015, Edition 1.

15.16 QuikChem 8500 Series II, Maintenance and Troubleshooting, Lachat Instruments, Hach Co., 01/2016, Edition 2.

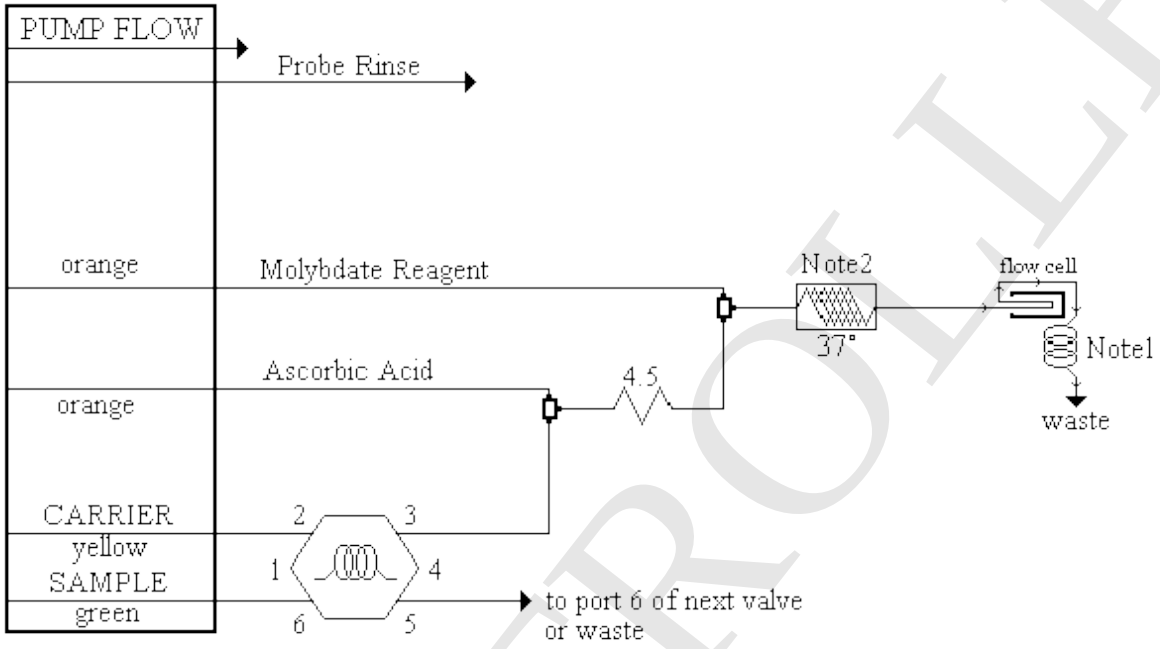
15.17 Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 113, HORIZON Procedures for EHD Inorganic Chemistry

16. Revision Tracking Table:

Rev. #	Rev. date	Changes Made	Rev. author
4	Sept. 2014	Updated for Horizon	W. Kotila
5	04/12/2017	8.11 Added dilution verification information 8.6 Changed "should" to "must" 8.8, 14.1, 15.8 Updated DOC procedure reference	Anthony Plourde
6	10/09/17	6.13, 6.14, 6.15 Added new standard LCS 8.4 Added new standard LCS	Graham Anderson
7	3/5/2019	1.1, 1.2, 1.3 Updated LOD from 0.005 mg/L to 0.008 mg/L and the LOQ from 0.016 mg/L to 0.027 mg/L Updated references to Lachat 8500 Series 2 Updated references for LOD Procedures 8.7 added QC limits (in response to EPA July, 2018 audit, def. # 21.b) 7.3.2: changed manifold tubing from 0.8 to 0.7 mm.	Jennifer Thorngate
8	01/21/2020	1.3 Changed LOQ from 0.027 to 0.028 mg/L to agree with LOD studies from 04/23/2019 and 08/29/2019. Horizon was updated on 1/15/2020. Section 3: added pollution prevention information as required by NH NELAP. Section 8 caption: added extra wording as required by NH NELAP. Sections 9 and 10. Updated to standardize SOPs and to consolidate QC requirements. Section 12: Updated to reflect Horizon 12. Appendix 1: Added digestion procedure to its own section for ease of use.	Royce Riessen
9	03/11/2021	1.1, 1.2, 1.3 Changed LOD from 0.008 to 0.012 mg/L and LOQ from 0.028 to 0.040 mg/L to agree with LOD study from 02/16/2021. Horizon was updated on 03/08/2021. 6.10 Added alternative method for either preparing or ordering standard depending on laboratory needs. 6.14 Added expiration date for ATP. 8.3 Added definition of Correlation Coefficient along with %RE requirement.	Royce Riessen

		8.13.1 Updated to reflect Horizon 12 9.4.1 Deleted required r-value for calibration, replaced with cross-reference to section 8.3, which includes r and %RE criteria. Added 11.2, procedure on over-range results. 15.4 Updated UW Safety reference 15.7 Updated from NELAC 2009 to 2016. SOP reformatted for consistency with other SOPs.	
10	11/05/2021	7.1, 7.5: Updated disposable glass tubes from only 20 x 150 mm to include 16 x 125 mm. 8.7 One LFB must be analyzed for every 20 samples without exception per the DNR NR149 update. 8.11 Following all accreditor suggestions, LODs will now be calculated/verified every 13 months instead of six. Appendix 1: Updated volume of sample/digestion acid to reflect using 5 mL. LOD was recalculated using the 99th percentile blank calculation to meet DNR data needs. New LOD = 0.009 mg/L, LOQ = 0.03 mg/L (effective in Horizon 06/17/2021).	R. Riessen
OB1	11/30/21	Transition to OnBase References updated	S.D. Hill

Figure 1: PHOSPHORUS MANIFOLD DIAGRAM



Carrier: 0.16M sulfuric acid (6.8)
Manifold Tubing: 0.7 mm (0.028 in) i.d.
Sample Loop: 150 cm
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

4.5: 70 cm of tubing on a 4.5 cm coil support

Note 1: 2 m back pressure loop, 0.52 mm (0.22 in.) i.d.
Note 2: 175 cm of tubing on the heater.

APPENDIX 1:

Total Phosphorus Digestion Procedure

1. Load the test tube racks with 16 x 125 mm disposable digestion tubes (7.1) so you have enough for your samples, LRBs, LFBs, QCS, LCS, duplicates, and spikes according to the analytical run.

1.1. Label the first and last tube of every row (i.e. 1, 10, 11, 20, etc.).

2. Transfer 5 mL* of each sample to a digestion tube with a motorized pipette.

2.1. If concentration of a sample is believed to be over range, dilutions may be made using 0.00 standard (6.11) and sample as long as total volume in tube is equal to 5 mL.

2.2. The LFB and spiked samples should be prepared according to 8.7 and 8.8.

3. The standards rack should be set up as shown below using a volume of 10 mL in each tube and 20 x 150 mm disposable digestion tubes (7.1):

	0.5	**0.5**	**0.5**				**0**	**0**	**0**
1.0	**0.5**	**0.5**	**0.5**	**0.25**	**0.05**	**0.016**	**0**	**0**	**0**
1.0	**0.5**	**0.5**	**0.5**	**0.25**	**0.05**	**0.016**	**0**	**0**	**0**
1.0	**0.5**	**0.5**	**0.5**	**0.25**	**0.05**	**0.016**	**0**	**0**	**0**

4. All **sample** digestion tubes must have 5 mL of liquid before the addition of digestion acid. Add 0.31 mL (0.62 mL for standards rack) of working digestion acid solution (6.3) to each tube, vortex, and cover with caps (7.6). Do not press caps down.

5. Autoclave the digestion tubes for 30 minutes at 121°C, 15-20 psi.

6. Remove the tubes from the autoclave, press caps down securely, and allow them to cool.

7. Allow any particulate matter to settle.

8. Analyze with the colorimetric method (10).

* Note: Other volumes may be used as long as the ratio of sample/standards/QC to digestion acid remains the same.

EHD INORG METHOD 310.6
Automated Phosphorus, Dissolved Orthophosphate
(Automated, EPA 365.1)

1. Scope and Application

1.1. This method is applicable to the determination of orthophosphate phosphorus in drinking, ground and surface waters, and domestic and industrial wastes in the range of 0.004 to 1.00 mg P/L.

1.2. The method limit of detection (LOD) = 0.004 mg/L

1.3. The method limit of quantification (LOQ) = 0.013 mg/L

2. Summary of Method

2.1. Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of orthophosphate phosphorus (PO_4-P) to form an antimony-phospho-molybdate complex. This complex is reduced to a blue-colored complex by ascorbic acid which absorbs light at 880 nm. The color is proportional to the orthophosphate phosphorus (PO_4-P) concentration.

2.2. The determinative steps in this method follow EPA 365.1 (15.1) except that the phenolphthalein indicator is not added (as in EPA 365.1, section 11.3.1) because it will cause an interference with the low limit of detection, and it is only important for samples with a pH over 8.3. For Safe Drinking Water Act (SDWA) compliance samples, we will check the pH prior to testing and ensure that it is less than 8.3.

3. Safety, Waste Management, & Pollution Prevention

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (15.3).

3.2. All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (15.4).

3.3. Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see the University of Wisconsin Laboratory Safety Guide, chapter 6 (15.4).

4. Sample Handling and Preservation

4.1. Samples are collected in 60 mL polyethylene bottles. Bottle quality is verified prior to use (15.11).

4.2. Samples must be filtered within 15 minutes of collection through a 0.45 μm filter, stored at ≤6°C, but not frozen, and analyzed within 48 hours from time collected. Samples not field filtered will be filtered following the *Filtering Procedure* (15.14) and a qualifier (flag) in the Horizon result comment field will be added. The qualifier will say "Orthophosphate sample not filtered within 15 minutes of sample collection."

NOTE: If analyzing for orthophosphate in drinking water samples, samples shall not be filtered (NR 809.113 Table A, Parameter 20, Footnote 12).

4.3. Maximum holding time is 48 hours from date of collection.

5. Interferences

5.1. Concentrations of ferric iron (Fe^{3+}) greater than 50 mg/L will cause a negative error due to precipitation of, and subsequent loss, of orthophosphate. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

5.2. Silica forms a pale blue complex that also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg/L positive error in orthophosphate.

5.3. Arsenate is determined similarly to orthophosphate and should be considered when present in concentrations higher than orthophosphate.

6. Reagents and Standards

6.1. **Reagent water (ASTM Type-I water)/Carrier:** All reagents and standards must be made with ASTM Type-I water. To prevent bubble formation in the carrier, carrier may be degassed with helium at 140 kPa (20 lb/in^2) through a helium degassing tube for one to three minutes prior to use.

6.2. **Stock Ammonium Molybdate Solution:** In a 1 L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_20$] in approximately 800 mL of ASTM Type-I water. Dilute to the mark and invert to mix. Store in plastic and refrigerate at ≤6°C. Expiration date is 6 months.

6.3. **Stock Antimony Potassium Tartrate Solution:** In a 1 L volumetric flask dissolve 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$) or 3.22 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) in approximately 800 mL of ASTM Type-I water. Dilute to the mark and invert to mix. Store in a dark bottle and refrigerate at ≤6°C. Expiration date is 6 months.

6.4. **Molybdate Color Reagent:** (Rinse down sides of flask and mix between adding each reagent). In a 500 mL volumetric flask add approximately 250 mL ASTM Type-I water and 17.5 mL concentrated H_2SO_4 (Caution: solution will get hot). When the flask can be comfortably handled, add 36 mL Stock Antimony Potassium Tartrate Solution (6.3) and 106.5 mL Ammonium Molybdate Solution (6.2). Dilute to mark and store at ≤6°C. As long as proportions remain the same, other final volumes of reagent may be made. To prevent bubble formation, reagent may be degassed with helium at 140 kPa (20 lb/in^2) through a helium degassing tube for one to three minutes prior to use. Expiration date is 2 weeks.

6.5. **Ascorbic Acid Reducing Solution (0.33M):** In a 500 mL volumetric flask dissolve 30.0 g ascorbic acid in approximately 300 mL of ASTM Type-I water. Add 0.5 g dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$) (wetting reagent). Dilute to the mark and invert to mix. Refrigerate at ≤6°C. As long as proportions remain the same, other final volumes of reagent may be made. Filter the reagent through a 0.45 µm filter if air bubbles persist throughout a run. Discard if the solution becomes yellow. Expiration date is 2 weeks.

6.6. **NaOH-EDTA Cleaning Solution:** In a 1 L volumetric flask dissolve 65 g of NaOH and 6.0 g disodium EDTA in about 500 mL of ASTM Type-I water. Dilute to mark and invert to mix. Store in a dark plastic bottle. There is no expiration date for this cleaning reagent.

6.7. **Stock orthophosphate standard:** A pre-made 100 mg P/L stock solution may be obtained from an approved UW vendor, e.g. RICCA or ERA. Stock is refrigerated at ≤6°C. Expiration date is 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.7.1. Alternatively, dissolve 0.4393 g of potassium phosphate monobasic (KH_2PO_4) (dried at 105˚C for 1 h) in 900 mL ASTM Type-I water. Add 1.0 mL of concentrated H_2SO_4 and dilute to 1 L: 1.0 mL = 0.100 mg P (100 mg P/L) and refrigerate at ≤6°C. Expiration date is 6 months.

6.8. **Working standard solutions:**

6.8.1. Prepare the following standards by diluting suitable volumes of standard solution to the final volumes found in Table 1 with ASTM Type-I water. Store at ≤6°C. Expiration date is 2 weeks.

Table 1.

Concentration of Standard (mg P/L)	Volume of stock standard 6.7 (mL)
0.00 (and Lab Reagent Blank)	0.0 (500 mL)
0.005	0.10 (2 L)
0.010	0.20 (2L)
0.025	0.50 (2 L)
0.050	0.50 (1 L)
0.250	1.25 (500 mL)
0.500	2.50 (500 mL)
1.00	5.00 (500 mL)
30.0 Spike Solution	15.0 (50 mL)

Note:
1) All working and stock standards must be entered into the Standards Logbook located in the Wet Chemistry Laboratory.
2) All Working Standards and QCS working standards must be entered into the Standards Log located in Horizon (15.18).

6.9 **Quality Control Stock Standard:** The stock solution used to prepare the QCS must originate from a different source than the calibration standards. A pre-made 50 mg/L stock solution may be obtained from an approved UW vendor, e.g. RICCA or ERA. Stock is refrigerated at ≤6°C. Expiration date is 6 months after opening or on the manufacturer's expiration date, whichever is sooner.

6.9.1 Alternatively, dissolve 0.4393 g of potassium phosphate monobasic (KH_2PO_4) (dried at 105˚C for 1 h) in 1800 mL ASTM Type-I water. Add 2.0 mL of concentrated H_2SO_4 and dilute to 2 L: 1.0 mL = 0.050 mg P (50 mg P/L) and refrigerate at ≤6°C. Expiration date is 6 months.

6.10 **Quality Control Working Standard (QCS):** Dilute 2.50 mL of 50 mg/L QCS Stock Standard (6.9) to 250 mL with ASTM Type-I water. 1.0 mL = 0.00050 mg P (0.50 mg P/L). Refrigerate at ≤6°C. Expiration date is 2 weeks.

7. Apparatus

7.1. Lachat 8500 System Series II.

7.1.1. Multichannel proportioning pump.

7.1.2. Injection module with a 75 cm x 0.7 mm i.d. sample loop.

7.1.3. Reaction unit or manifold (Figure 1).

7.1.4. Colorimetric detector.

7.1.5. Colorimeter equipped with 10 mm path length flow cell and 880 nm interference filter.

7.1.6. Data system.

7.1.7. Heating unit: 37°C; using the 175 cm length of tubing.

7.2. Motorized pipette: 10 mL, 1.0 mL, and 0.1 mL (15.10).

7.3. Disposable culture tubes: 13 x 100 mm disposable glass.

7.4. Autosampler.

8. Quality Control Types, Acceptance Criteria, & Corrective Actions

8.1. Please refer to the Environmental Health Division Quality Assurance Manual (15.6) for general information on Quality Control Procedures. Important specifics include:

8.1.1. Accuracy and precision calculations.

8.1.2. Corrective action procedures (including documentation requirements) for instrument problems or analytical problems.

8.2. **An instrument logbook** is maintained for each Lachat. Maintenance, performance problems, date calibrated, analyst, and other pertinent information are documented in the logbook.

8.3. **The Correlation Coefficient (*r* value)** is the measure of the linearity of the standard curve and must be ≥0.995 to be acceptable and before proceeding with sample analysis. The 2016 TNI Standard (15.7) requires the % Relative Error (%RE) to be calculated and evaluated for each the low and midpoint standards and must pass acceptance criteria before continuing analysis. The acceptable %RE for the low and midpoint standards is ±50% and ±30%, respectively. These values will be manually entered into HORIZON into the %RELOW and %REMID standards, located at the end of the batch. Due to HORIZON limitations, only recovery will be calculated. Since %RE = Recovery – 100%, the acceptable limits for the low and midpoint standards in HORIZON become 50-150% and 70-130%, respectively.

8.4. **A Quality Control Standard (QCS)** is analyzed with each run. The analytical result must be within ±10% of the true value to continue the analysis. If the QCS exceeds the recommended recovery limits, corrective action includes reanalyzing the QCS, or recalibrating and reanalyzing the run.

8.5. **A Laboratory Reagent Blank (LRB)** is prepared (6.8) and analyzed at the beginning of each run. **A Method Blank (MB)** is prepared by filtering ASTM Type-I water according to EHD INORG METHOD 100.2 Filtering Procedure (15.14) and must meet the same criteria as the LRB, or associated samples will need to be re-filtered or qualified (15.14). The LRB must be less than the LOD of the method (see section 8.8 regarding negative values). If it does not meet this criteria, recalibrate and analyze the LRB again. The LRB is equivalent to the CB for this method.

8.6. **A Laboratory Fortified Blank (LFB)** is prepared and analyzed at the beginning of each run. The spike recovery must be within ±10% of the true value. If the LFB exceeds the recommended recovery limits, corrective action includes reanalyzing the LFB or recalibrating and reanalyzing the run. Prepare the first LFB of the run using LRB/CB and spiking solution (6.8), where the spike solution is equal to 1.0% of the total volume -- e.g., add 0.06 mL of spike solution to 5.94 mL of LRB/CB.

8.6.1. **Lab Fortified Method Blanks (LFMB)** are prepared and analyzed at a minimum of 5% of lab filtered samples. LMFBs will be prepared the same as LFBs, using the MBs from the lab filtered prep batches in place of LRB/CB. The MBs used for LFMBs are the 2nd, 3rd, and all subsequent MBs from a prep filter batch. A LFMB using the end MB from the prep filter batch is optional if and only if the previous LFMBs meet the 5% minimum. The spike recovery criteria and corrective action for LFMBs are equivalent to the LFB (8.6).

8.7. **Matrix Spikes (MS) and Laboratory Duplicates (LD):** Prepare a **minimum of 10%** of the samples, per matrix, with duplicates and spikes. Spikes are prepared in the same manner as the LFB, substituting the sample in place of the LRB/CB. If the duplicate acceptance criteria (precision QA) are not met (10% relative difference), the matrix group (including spike and duplicate) must be reanalyzed. If the duplicate limits are exceeded a second time, qualify all results within the matrix group. If the spike recovery (accuracy QA) does not fall within the specified control limits (90 – 110% recovery), the matrix group (including spike and duplicate) must be reanalyzed with the next analytical batch. If it fails a second time, qualify all results within the matrix group.

8.8. **An Instrument Performance Check (IPC)** and **Calibration Blank (CB)** must be inserted at the beginning of the run and after every 10 cups. The IPC must be within ± 10% of true value. The **CB** must be less than the LOD. For negative values, a method blank (reagent blank, CCB, etc.) is within acceptable QC limits if the observed concentration is greater than the negative LOD (<LOD and >-LOD). If the observed concentration of the CB is between the negative LOD and negative LOQ (<-LOD and ≥-LOQ) the data will be evaluated to determine a cause for the problem (e.g., baseline drift, improper y intercept, poor source of material for reagent blank, etc.) and may require corrective action (e.g. recalibrating and/or making new standards/reagents). However, if there is no apparent source causing the problem, then the blank may be acceptable providing that the logic supporting this decision is well documented. The lowest acceptable value for a CB is negative the LOQ. All data reported must be “bracketed” by acceptable IPCs and CBs. If an IPC or CB exceeds limits, corrective action requires that all previous samples back to the last acceptable IPC and CB be reanalyzed. The CB is equivalent to the LRB for this method.

8.9. **Demonstration of Capability (DOC)**: An Initial DOC and annual continued proficiency checks are performed according to EHD DIV-WIDE QA 115 (15.8).

8.10. **Limit of Detection (LOD)**: The LOD must be verified every 13 months or reestablished whenever there is a significant change in the method or instrumentation. Verify or establish the method LOD using the procedure outlined in EHD DIV-WIDE QA 116 (15.9).

8.11. **Linear Calibration Range (LCR)**: The LCR must be verified every 6 months or whenever there is a significant change in the method or instrumentation. The initial demonstration of linearity must use sufficient standards to insure that the curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by more than ±10%, linearity must be re-established. If any portion of the range is shown to be nonlinear, a sufficient number of standards must be used to clearly define the nonlinear portion.

8.12. **Sample Dilution**: If the estimated concentration of analyte in a sample exceeds the highest calibration standard a bench dilution must be performed. Dilutions at the bench are typically performed by diluting an appropriate volume of sample with reagent blank. Motorized pipettes may be used to deliver/dilute volumes up to 10 mL. For volumes greater than 10 mL, Class A glass, volumetric pipettes should be used. Diluted samples should be mixed thoroughly prior to analysis. Dilution Verification: When a dilution is made, the dilution must be verified by comparing the diluted result with the original result. The comparison is done by dividing the diluted result by the original result and expressing the calculation as a percent. The acceptable range is 90% to 110%. If the dilution verification is not within the acceptable range, a different dilution must be made. This second dilution would then be verified against the first dilution. If a group of samples is diluted identically, at least 10% of the dilutions must be verified.

8.12.1. Samples diluted at the bench will be documented/calculated by entering the sample number followed by an X and the dilution factor into the Lachat **Tray Table** (e.g. 123456789 X5).

9. Method Calibration

9.1. Refer to section 6 for making standards and reagents.

9.2. Calibration curve is a linear, 1st-order polynomial curve, with a weighting method of 1/x.

9.3. Set the data system parameters and operating conditions for the Lachat 8500 with the Omnion software (15.12).

9.3.1 Set up manifold as shown in Figure 1.

9.3.2 Pump ASTM Type-I water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline has been obtained (about five minutes).

9.4. Place standards in the appropriate rack on the autosampler and calibrate the instrument by injecting the standards. The data system will then correlate the concentrations with the instrument responses for each standard.

9.4.1. After the calibration passes (8.3) the samples may be analyzed. If the calibration is not satisfactory, use an appropriate corrective action to diagnose possible causes and recalibrate.

10. Procedure

10.1. See also Instrument Operating Procedure (15.12).

10.2. Create a worklist using Horizon as explained in the Horizon Procedures (15.18).

10.3. Import the sample identification numbers from Horizon into the **Tray Table** of the Lachat *Omnion* software. This will include:

10.3.1. A duplicate and spike for every 10 samples in a matrix group.

10.3.2. One Lab Reagent Blank per run.

10.3.3. One Lab Fortified Blank per run.

10.3.4. One Lab Fortified Method Blank for every 20 lab filtered samples.

10.3.5. One Quality Control Standard per run.

10.4. Analyze standards and Quality Control samples (i.e., IPC, CB, LRB, LFB, and QCS). If QC samples pass acceptance criteria, the batch may continue with sample analysis.

10.5. Shutdown procedure:

10.5.1. After the run is complete, rinse with ASTM Type-I water for five minutes.

10.5.1.1. As needed, switch reagent lines to the NaOH-EDTA solution (6.6) for approximately five minutes followed by an additional ASTM Type-I water rinse for five minutes.

10.5.1.2. If the baseline drifts and/or peaks are not coming back down to the baseline and cleaning the system with NaOH-EDTA does not help, the tubing around the heater may need to be changed.

10.5.2. Remove reagent lines from ASTM Type-I water and pump air through in order to dry. Release pump-tubes from cartridges and turn off instrument.

10.5.3. Waste disposal: The waste will be acidic and will need to be neutralized according to the Laboratory Safety Guide (15.4).

11. Calculations

11.1. The orthophosphate concentration in the unknown samples is calculated by the instrument software based on the standard calibration curve. The orthophosphate concentration result is obtained by transferring the data from Omnion to Horizon (12.2) and can also be obtained directly from the *Run Time Report*, which should be printed for a hard copy.

12. Data Management

12.1. The analytical run, the *Run Time Report*, and the QC Parameters section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced

chemist who did not perform the original analysis (15.13). The reviewer must initial and date the analytical run.

12.2. Export results from Omnion to Horizon (see EHD INORG GENOP 113, Horizon Procedures, 15.18).

12.2.1. Review results by selecting Edit Results under Batches.

12.2.2. Review QC Results by selecting the QC display in Edit Results.

13. Definitions

13.1 Definitions of terms in this SOP may be found in the reference method (EPA 365.1 and/or QuikChem Method 10-115-01-1-A). General definitions of other terms that may be used in this method are found in the EHD Quality Assurance Manual (15.6).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (MDLs and IDOCs) are generated in compliance with the reference method and the Inorganic Chemistry Department's standard operating procedures: EHD DIV-WIDE QA 115 (15.8) and EHD DIV-WIDE QA 116 (15.9). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement.

15. References

15.1. U.S. Environmental Protection Agency, Methods for the Determination of Inorganic Substances in Environmental Samples, EPA-600/R-93/100, August 1993, Method 365.1, rev. 2, (TNI method code 10070005).

15.2. QuikChem Method 10-115-01-1-A Determination of Ortho Phosphate in Waters by Flow Injection Analysis Colorimetry, Revised 29 November, 2007.

15.3. LABWIDE SAFETY 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, State Laboratory of Hygiene.

15.4. UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.5. Wisconsin Administrative Code NR149, Department of Natural Resources Lab Certification Program, 06/29/2021.

15.6. Wisconsin State Laboratory of Hygiene, Environmental Health Division, EHD DIV-WIDE PLAN 001, *Quality Assurance Manual--General*, and EHD INORG PLAN 001, *Inorganic, TECL, & Metals Supplement*, .

15.7. 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.8. Wisconsin State Laboratory of Hygiene, EHD DIV-WIDE QA 115, *Initial and Ongoing DOC Procedures*.

15.9. Wisconsin State Laboratory of Hygiene, EHD DIV-WIDE QA 116, *LOD Procedures*.

15.10. Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 200, *Pipette Performance Checks*.

15.11. Wisconsin State Laboratory of Hygiene, EHD INORG QA 101, *Bottle Check Procedure*.

15.12. Wisconsin State Laboratory of Hygiene, EHD INORG IOP 105, *Instrument Operating Procedure for QuikChem Automated Ion Analyzer*.

15.13. Wisconsin State Laboratory of Hygiene, EHD INORG QA 107, *Q.C. Audits of Analytical Runs for ESS Wet Chemistry Area*.

15.14. Wisconsin State Laboratory of Hygiene, EHD INORG METHOD 100.2, *Filtering Procedure.*

15.15. QuikChem 8500 Series II, Operations Manual, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.16. Omnion 3.0 Software User Manual, 09/2007, Edition 4. / Omnion 4.0 Software User Manual, Lachat Instruments, Hach Co., 04/2015, Edition 1.

15.17. QuikChem 8500 Series II, Maintenance and Troubleshooting, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.18. Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 113, *HORIZON Procedures for EHD Inorganic Chemistry*

15.19. Orthophosphate Standards Study, M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\Nutrients\Lachat\Ortho P Standards Study\Ortho P Study.xlsx

16. Revision Tracking Table:

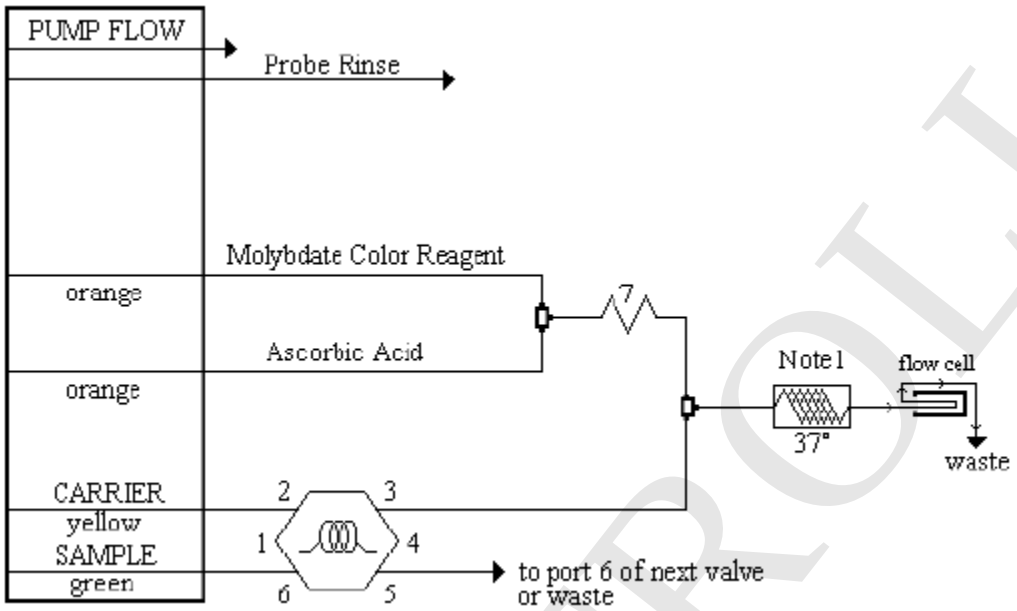
Rev. #	Rev. date	Changes Made	Rev. author
1	09/03/19	1.1, 1.2 updated LOD/LOQ using MB in addition to LRB. 2.2 Amended wording about phenolphthalein indicator and checking pH on SDWA samples. Section 3: added pollution prevention paragraph. 6.4, 6.5 Increased prepared volume of reagents. 6.8.1 Added 0.10 std into curve for added accuracy near low end of curve. Section 8 title updated to QC Types, Acceptance Criteria, & Corrective Actions 8.4 Changed procedure so that corrective action will be performed when a LRB exceeds the LOD (in response to July, 2018 EPA DW audit def. #14.b) 8.5, 8.6 changed spiking volume for consistency with other Lachat analyses. 8.9, 8.10 changed LOD, LCR verifications from every six months to annually.	R. Riessen
2	12/19/19	Updated reference method from SM 4500-P F to EPA 365.1 per EPA requirement. 8.9, 8.10 changed LOD, LCR verifications from annually to every six months per EPA method.	R. Riessen
3	05/14/20	1.1, 1.2, 1.3 Changed LOD from 0.0034 to 0.005 mg/L and LOQ from 0.011 to 0.015 mg/L to agree with LOD study from 04/24/2020. Horizon was updated on 5/11/2020. Sections 9 and 10. Updated to standardize SOPs and to consolidate QC requirements. Section 12: Updated to reflect Horizon 12. Section 15: updated some references	R. Riessen
4	02/05/21	1.1-1.3: Change in method required a new initial LOD study. LOD was changed from 0.005 to 0.0023 and LOQ was changed from 0.015 to 0.008 (effective 02/04/2021). 4.2 Added note concerning total orthophosphate. 6.4, 6.5 Increased prepared volume of reagents. 6.7, 6.9 Added alternative method for either preparing or ordering standards and QCS depending on laboratory needs. 6.8.1 Adjusted spike to 30 mg/L to match Total Phosphorus. 8.3 Added definition of Correlation Coefficient along with %RE requirement. 13.1, 15.2 Changed reference method from QuikChem Method 10-115-01-1-V to QuikChem Method 10-115-01-1-A to aid in detection limit and QC recovery issues with LFB/MS.	R. Riessen

		Updated ref. 15.4 15.19 Orthophosphorus Standard Study for 2 week expiration date added to references. Figure 1: Updated to reflect QuikChem Method 10-115-01-1-A.	
5	11/05/21	1.1-1.3: LOD study was done due to increased sample load (adding more variation) and new analysts running analysis. LOD was changed from 0.0023 to 0.003 and LOQ was changed from 0.008 to 0.01 (effective 07/09/2021). 4.2 Added field filtering requirement and qualifier for non-field filtered samples (in response to May 2021 DNR audit, def. 6C). 8.5 Added information/criteria for Method Blanks (in response to April 2021 NELAC audit, BLO1). 8.6, 10.3.3 LFB will be run at the beginning of the run only and no longer with every 20 samples as the acceptability requirements for LFB and MS are now the same (effective 05/12/2021). 8.6 One LFB must be run per the DNR NR149 update. 8.6.1, 10.3.4 Added LFMB per DNR NR 149 update. 8.7 Tightened MS criteria from 85-115% to 90-110% per EPA method requirements (effective 05/12/2021). 8.10 Following all accreditor suggestions, LODs will now be calculated/verified every 13 months instead of six. 9.4.1 Deleted required r-value for calibration, replaced with cross-reference to section 8.3, which includes r and %RE criteria. 15.5.1.2 Added suggestion to change tubing around heater.	R. Riessen
OB1	11/30/21	6.5 Optional filtering if air bubble formation persists. Transition to OnBase References updated	S.D. Hill
OB2	05/26/22	1.1, 1.2, 1.3 Changed LOD from 0.003 to 0.004 mg/L and LOQ from 0.010 to 0.013 mg/L to agree with LOD study from 2/1/2022. Horizon was updated on 2/1/2022. 4.2 Note: Updated to clarify requirement as stated in Laboratory Accreditation Program Bulletin - April 2022 from the WDNR. References updated	J. Thorngate

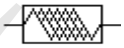
Ver. #	**Changes Made**	**Author**

OB2	8.5 - Removed reference to WI NR 149.48 for blank acceptability. Method requires blank < LOD (in response to April 2022 EPA audit, def. 5.1.3.1). 8.8 Added requirement for LRB/CB to be ≥-LOQ (in response to an April 2022 EPA audit recommendation). Updated references Deleted date column from revision tracking table Deleted replaces in the header	R. Riessen

FIGURE 1: ORTHOPHOSPHATE MANIFOLD DIAGRAM



Carrier:	Reagent water (ASTM Type-I water)
Manifold Tubing:	0.7 mm (0.028 in) i.d.
8500 Sample Loop:	75 cm x 0.7 mm i.d.
Interference Filter:	880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module are required. The  shows 175 cm of tubing wrapped around the heater block at 37°C.

7: 135 cm of tubing on a 7 cm coil support

Note 1: 175 cm of tubing on the heater.

***** If air spikes occur, add a 200 cm back pressure loop, 0.5 mm (0.022 in.) i.d.

EHD INORG METHOD 340.1

Total Suspended Solids (Dried at 103-105°C) (SM 2540 D) Volatile Suspended Solids (Ignited at 550 ± 50°C) (SM 2540 E)

1. Scope and Application

1.1. This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.

1.2. The Reporting Limit is 2 mg/L both Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) (based on a 500 mL sample volume and a 1 mg capture weight).

2. Summary of Method

2.1. Total Suspended Solids are defined as those solids which are retained by a glass fiber filter (particle retention size of 1.5 µm) and dried at 103-105°C.

2.2. The residue obtained from the TSS determination is ignited at 550 ± 50 °C in a muffle furnace. The loss of weight on ignition is defined as the VSS.

2.3. An aliquot of well mixed sample is filtered through a glass fiber filter and the residue retained on the filter is dried at 103-105°C (TSS). The filter, containing the dried residue, is placed in a muffle furnace and ignited at 550 ± 50 °C for 30 minutes, desiccated and weighed. The weight lost on ignition is the VSS.

2.4. The analytical balance used for the TSS test is interfaced directly with a Personal Computer (PC). All weights are captured directly on the PC and calculations are made with a spreadsheet template.

2.5. A deviation from SM 2540 D & E is that samples are not brought to constant weight. Samples are dried overnight, for a minimum of 8 hours with supporting documentation, of the date/time, in and out of the oven (10.9). The Method Blank must be less than 2 mg/L (10.5). This process is approved by the Wisconsin Laboratory Certification Program (16.3).

2.6. A deviation from SM 2540D is that a maximum sample volume of 500 mL rather than 1000 mL is filtered; a minimum yield of 1.0 mg rather than 2.5 mg of dried residue is obtained. The resulting reporting limit is 2 mg/L rather than 2.5 mg/L. This reporting limit is required by Wisconsin Administrative code NR 149.48(4)(c)—see ref. 16.14 This reporting limit has been used in Wisconsin since 2000 (see ref. 16.16 and 16.17).

3. Safety, Waste Management, & Pollution Prevention

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (16.4).

3.2 All laboratory waste, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (16.5).

3.3 Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (16.5).

4. Sample Handling and Preservation

4.1 Samples must be preserved by icing immediately after collection and stored at ≤ 6°C upon receipt by the laboratory.

4.2 Samples are collected in Wisconsin State Lab of Hygiene (WSLH) glass or plastic quart bottles. Bottle quality is verified prior to use (16.6).

4.3 Analysis must be started within 7 days after sample collection.

5. Interferences

5.1 Filtration apparatus filter material, pre-washing, post-washing, and drying temperatures are specified because these variables have been shown to affect results.

5.2 Samples high in dissolved solids, such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.1) minimizes this potential interference.

5.3 Non-representative particulates, such as leaves, sticks, fish and lumps of other matter, should be excluded from the sample if it is determined that their inclusion is not desired in the final result. Sample results must be flagged appropriately if materials are excluded during the analysis process.

6. Reagents and Standards

6.1 **ASTM Type-I Water:** The Method Blank and all rinsing must be done with ASTM Type-I Water.

6.2 **Quality Control Stock Standard:** Used for Suspended Solids (7,000 mg/L), Total Solids (27,000 mg/L) and Total Dissolved Solids (20,000 mg/L). In a 2-L, volumetric flask, dissolve 40 g NaCl and 14 g Infusorial Earth (Fisher I22-3, Diatomaceous Earth) in 500 mL ASTM Type-I water. Dilute to volume, place a stir bar in the solution, and mix on a stir plate. Use the Proficiency Testing dispenser to dispense 5 mL of stock solution into each container and seal. Record in logbook. The containers are kept in a drawer and expire in two years.

6.3 **Quality Control Working Standard (35 mg/L):** Open a container (6.2) and pour the contents (5 mL) into a 1 L**,** volumetric flask. Rinse the container several times into the volumetric flask and dilute to 1 L**.** Store at ≤6°C. Record in the standards logbook and update the standard in Horizon (See *Solids Transfer*, EHD INORG IOP 300 (16.10)). Expiration date is 7 days.

7. Apparatus

7.1 Glass micro-fiber filters, 5.5 cm, without organic binder, Whatman Type 934-AH. (1.5 µm particle retention size).

7.2 Filtration apparatus with reservoir and a coarse (40-60 micron) fritted disc as a filter support.

7.3 Suction flask: 1000 mL, 2000 mL.

7.4 Drying oven for operation at 103-105°C.

7.5 Muffle furnace for operation at 550 ± 50 °C.

7.6 Desiccator

7.7 Analytical balance (e.g., Mettler AT200), capable of weighing to 0.1 mg, an RS-232C interface and a personal computer with spreadsheet software.

7.8 Disposable aluminum drying pans, 60 mm.

7.9 ASTM Type-I Reagent Grade Water. Prepared from U.S. Filter PURELAB PLUS UV/UF System.

7.10 Manufactured to be wide-bore, volumetric pipettes: 10 mL - 100 mL. Available from Fisher Scientific and NCL of Wisconsin. Pipettes are verified prior to first use as detailed in EHD INORG GENOP 200, *Pipette Performance Checks* (16.21).

7.11 Class-A graduated cylinders; 250 mL, 500 mL.

NOTE: If using non-glass graduated cylinders perform quarterly verification of cylinders as found in Appendix 1.

8. Quality Control Types, Acceptance Criteria, & Corrective Actions

8.1 A **Laboratory Duplicate** (**LD**) is analyzed for every ten samples for every matrix type. If a duplicate analysis exceeds the QC limits (RD = 15%), all samples in that specific matrix group will be reanalyzed or qualified if not enough sample is available to repeat the analyses or if there are holding time issues. If the subsequent QC limit is still exceeded, the most recent results will be reported and the results qualified. Refer to the QA Manual (16.7).

8.2 A **Method Blank (MB)** is analyzed initially for the first 20 samples and for every 20 samples thereafter. The MB is prepared by transferring 500 mL of ASTM Type-I water onto a pre-washed filter. If the MB is greater than the reporting limit (2 mg/L) the samples associated with that blank will be re-dried, desiccated and re-weighed. If the MB still exceeds the reporting limit, all samples associated with that blank will be reanalyzed or qualified if there is not enough sample to repeat the test or if holding time becomes an issue.

8.3 **Demonstration of Capability TSS (DOC):** This must be completed initially and then annually by anyone who performs the test. Analyze four repetitions of a QCS and calculate the mean, standard deviation and the % Bias. The % Bias must be ±15% and the Relative Standard Deviation (RSD) must be within 10% to demonstrate that the analyst can perform the test (16.8). Note: If the reporting limit changes, then the Demonstration of Capability must be performed again.

8.4 **Demonstration of Capability TVSS (DOC):** This must be completed initially and then annually by anyone who performs the test. Analyze four replicates of a completed influent sample that has been homogenized and calculate the mean and standard deviation. The Relative Standard Deviation (RSD) must be within 10% to demonstrate that the analyst can perform the test. Note: If the reporting limit changes, then the Demonstration of Capability must be performed again.

8.5 A **Quality Control Standard TSS (QCS)** (6.3) is analyzed with each batch. A pipette is used to transfer **200 mL** of the control standard. The Analytical result must be within ±15% of the true value to accept the data. If the QCS exceeds the limits, reset the samples. If there is insufficient sample to reset, qualify the results.

8.6 Please refer to the Environmental Health Division Quality Assurance Manual (16.7) for general information on quality control procedures. Important specifics include:

8.6.1 Accuracy and precision calculations.

8.6.2 Corrective action and result qualification procedures (including documentation requirements) for instrument problems or analytical problems.

9. Method Calibration

9.1 Each day the balance is used the calibration is verified (16.9).

10. Procedure for Total Suspended Solids

10.1 Preparation of the glass fiber filter: Place the filter *(with the grid side of the filter facing down)* on the base of the filtering apparatus and assemble the funnel. While vacuum is applied, wash the filter with three successive 20 mL volumes of ASTM Type-I water. Remove all traces of water by continuing to apply vacuum after the water has passed through. Remove filter from apparatus and place in a small aluminum pan and ignite at 550 ± 50 °C for 30 minutes in case volatile suspended solids may be required. Document filter preparation in the solids filter prep log book. Pre-washed filters are stored in an oven (103-105 °C) or desiccator prior to use. When they are stored in an oven and needed, place filters in a desiccator to cool for 90 minutes.

10.1.1 Alternatively, pre-weighed (i.e. pre-washed) filters may be purchased from a UW approved vendor.

10.2 Set up the PC by opening BalLink12. Click on Settings and scroll down to Data Settings. Change the Horizontal movement to 0 and the Vertical to 1. Download the sample worklist containing the samples to be tested and copy and paste onto the template (16.10).

10.3 Record the tare weight of the aluminum pan + filter on the bench sheet.

10.4 If a sample has been field filtered, place the field filtered filter in a pre-weighed pan + filter. Add the collector-provided weight of the field filter to the weight of the pan + filter from 10.1.

10.5 Select a sample volume (maximum volume of 500 mL) that will yield no less than 1.0 mg and no more than 200 mg of suspended solids. If the mass of dried residue is more than 200 mg, reset the sample with less volume; if greater than 1 mg of capture weight report the result as is. In the event the mass of dried residue is <1 mg, the results should be qualified if the test cannot be performed with a larger volume. If qualifying the result, estimate the reporting limit based upon the sample volume as follows:

$$\text{Reporting Limit } \left({}^{\text{mg}}/_{\text{L}}\right) = \frac{1 \text{ mg}}{\text{Sample Vol. (mL)}} \text{ x } 1000 \left({}^{\text{mL}}/_{\text{L}}\right)$$

Example: If 200 mL of sample yielded <1 mg of capture weight, qualify the result as *"Low Sample Volume"* and report the result as "*< *5 mg/L*".

10.6 Place a pre-washed filter on the filtering apparatus and apply vacuum. Wet the filter with a small volume of ASTM Type-I water to seat it against the fritted support.

10.7 Shake the sample vigorously and quantitatively transfer the sample to the filter with a large orifice, volumetric pipette for volumes up to 200 mL, or graduated cylinder for volumes up to 500 mL. If the TSS is high, making sub-sampling difficult, pipette the desired volume while stirring the sample with a magnetic stirrer, and quickly transfer it to the filtering apparatus. Remove all traces of water by continuing to apply vacuum after sample has passed through. If the sample takes longer than 10 minutes to filter, discard the filter and reanalyze using a smaller sample volume.

10.8 Rinse the pipette or graduated cylinder onto the filter with a small amount of ASTM Type-I water. Rinse retained solids on filter with three successive aliquots of about 10

mL of ASTM Type-I water (Note: Samples with high dissolved solids may require additional rinses). Remove all traces of water by continuing to apply vacuum after the water has passed through the filter.

10.9 Carefully remove the filter from the filter support and place back in the aluminum drying pan. Dry overnight, for a minimum of 8 hours, at 103-105°C.

10.10 Cool in a desiccator for about 90 minutes and weigh the sample. The gain in weight of the tared dish is a measure of the solids of the sample.

11. Procedure for Volatile Suspended Solids

11.1 After determining the final weight in the TSS analysis (10.10) place the filter with sample in the muffle furnace and ignite at 550 ± 50 °C for 30 minutes.

11.2 Desiccate for about 90 minutes and weigh.

11.3 If the blank result is greater than 2 mg/L, repeat the igniting, desiccating and weighing cycle for all samples associated with that blank until a constant weight is obtained (i.e. weight change is < 0.5 mg).

12. Calculations

12.1 Perform all calculations in a spreadsheet template (16.10). The spreadsheet should be set up to give results for TSS and VSS with the following calculations:

12.2 Total Suspended Solids:

$$\text{Total Suspended Solids } \left({}^{\text{mg}}/_{\text{L}}\right) = \frac{(\text{A} - \text{B}) \text{ x } 1000}{\text{C}}$$

A = weight of filter + dried residue in mg
B = weight of filter in mg
C = volume of sample filtered in mL

12.3 Volatile Suspended Solids:

$$\text{Total Volatile Suspended Solids } \left({}^{\text{mg}}/_{\text{L}}\right) = \frac{(\text{A} - \text{D}) \text{ x } 1000}{\text{C}}$$

A = weight of residue + filter in mg from Total Suspended Solids analysis (12.2)
D = weight of residue + filter in mg after ignition (11.2)
C = volume of sample filtered in mL

13. Data Management

13.1 The analytical run, including raw data, and the QC Display section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced chemist who did not perform the original analysis (16.11). The reviewer must initial and date the analytical run.

13.2 Export results from the excel spreadsheet to Horizon (see EHD INORG IOP 300, *Solids Transfer* 16.10).

13.2.1 Review results by selecting Edit Results under Batches.

13.2.2 Review QC Results by selecting the QC display in Edit Results.

13.2.2.1 If all samples in a batch request only TSS, either cancel (CA) the VSS analyte for the MBs in Edit Results or add the qualifying comment "Exclude VSS; TSS only." in the comment section for all MBs in QC.

14. Definitions

14.1 Definitions of terms in this SOP may be found in the reference methods (16.1, 16.2). General definitions of other terms that may be used in this method are found in the WSLH Quality Assurance Manual (16.7).

15. Method Performance

15.1 Where applicable, the laboratory's initial accuracy and precision data (LOD, DOC) were generated in compliance with the reference method and the Environmental Health Division's standard operating procedures (16.8, 16.12). Data generated within the last two years will be kept on file within the Inorganic Chemistry Department. Data older than two years may be archived in the basement. Data will be retained according to the applicable records disposition authorization (O:\RDA's\Final RDA\EHD_2017_WSLH.pdf)

16. References

16.1 American Public Health Association, American Water Works Association, and Water Environment Federation. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st edition, (Methods 2540D and 2540E).

16.2 United States Environmental Protection Agency. 1983. *Methods for Chemical Analysis of Water and Wastes* (EPA-600/4-79-020, Method 160.4).

16.3 LabNotes, Laboratory Certification Program, Wisconsin DNR, winter 2013.

16.4 Wisconsin State Laboratory of Hygiene. LABWIDE SAFETY 102. *Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility*.

16.5 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

16.6 Wisconsin State Laboratory of Hygiene. EHD INORG QA 101. *Bottle Check Procedure*.

16.7 Wisconsin State Laboratory of Hygiene. *Quality Assurance Manual, Environmental Health Division.*

16.8 Wisconsin State Laboratory of Hygiene. EHD DIV-WIDE QA 115. *Initial and Ongoing DOC Procedures*.

16.9 Wisconsin State Laboratory of Hygiene. EHD INORG GENOP 202. *Calibration, Maintenance, and Accuracy Verification for Balances*.

16.10 Wisconsin State Laboratory of Hygiene. EHD INORG IOP 300. *Solids Transfer*.

16.11 Wisconsin State Laboratory of Hygiene. EHD INORG QA 107. *QC Audits of Analytical Runs for ESS Wet Chemistry Area*.

16.12 Wisconsin State Laboratory of Hygiene. EHD DIV-WIDE QA 116. *LOD/LOQ Procedures*.

16.13 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

16.14 Wisconsin Administrative Code NR 149, "Laboratory Certification and Registration," June 29, 2021.

16.15 Operating Instructions, Mettler AT balances, Mettler-Toledo AG, 1990.

16.16 Wisconsin Department of Natural Resources, "Recommendations of the BOD LOD Technical Group," (includes TSS recommendations), 07/12/1999. O:\SOP\EHD\ESS\Inorganic\Final\ESS INO METHOD 340.1_TSS Reporting recommendations DNR 12-17-1999.pdf

16.17 Wisconsin Department of Natural Resources, "When to Report *Less Thans* for BOD and Suspended Solids", Memo, 2003. O:\SOP\EHD\ESS\Inorganic\Final\ESS INO METHOD 340.1_TSS Reporting Summary DNR 2003.pdf

16.18 ASTM International, "Standard Practice for Calibration of Laboratory Volumetric Apparatus", Reapproved 2012. M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\SOLIDS\Graduated Cylinder Quarterly Verification\Reference Information\E542.9221 ASTM Calibration of Volumetric.pdf

16.19 ASTM International, "Standard Specification for Laboratory Glass Graduated Cylinders", Reapproved 2019. M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\SOLIDS\Graduated Cylinder Quarterly Verification\Reference Information\E1272.8321 ASTM Graduated Cylinders.pdf

16.20 Calculation Verification of Quarterly Graduated Cylinders Template. M:\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\SOLIDS\Graduated Cylinder Quarterly Verification\Reference Information\Quarterly Verification - Calculations.pdf

16.21 Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 200, *Pipette Performance Checks*.

17. Revision Tracking Table

Rev. #	Rev. Date	Changes Made	Rev. Author
11	March, 2014	Revision 10 referred to the old DNR solids letter of May 8, 2001, which is now obsolete. The DNR no longer requires a quarterly verification of constant weight for drying samples overnight. Dry time must be at least 8 hours. Updated the DNR reference for this requirement (15.4) Added "Pollution Prevention" to the title of section 3. In the Sample Handling & Preservation section changed storage temperature from < 4 °C to ≤ 6 °C to meet specific requirements from state regulations (NR 219, NR 809, NR 149.46(4)(e)) Changed all ESS LIMS and qawrksht instructions to Horizon instructions. Added revision tracking table	J. Thorngate
12	Aug. 9, 2017	Dropped reference method EPA 160.4 because SM 2540E is now an approved method under WI NR219. In section 10.1 added information on the new logbook for documenting dates/times in/out of oven for washing, cleaning, and prepping filters (response to April, 2017 NELAC audit def. # 9) In section 2.6 added information regarding maximum volume filtered and minimum yield of residue (response to April, 2017 NELAC audit def. #14I) Added sections 16.16, 16.17: WDNR guidance documents regarding filtering up to 500 mL of sample to obtain at least 1 mg of residue, which results in a reporting limit of 2 mg/L (response to April, 2017 NELAC audit def. #14I). Section 16.8: updated DOC procedure reference	G. Anderson/S. Hill
13	5/11/2020	Section 3: updated with additional information Section 8 caption: added **Types, Acceptance Criteria, & Corrective Actions** 8.6.2: added "and result qualification" Section 12: updated instructions to Horizon 12 15.1: added "Data will be retained according to the applicable records disposition authorization." 16.5 updated ref. for Lab Safety Guide 16.13 updated TNI reference	L. Klicko

14	11/17/2020	7.11, 16.18, 16.19, 16.20, and Appendix 1: added information and procedure regarding the use of non-glass Class-A graduated cylinders.	R. Riessen
15	10/15/21	1.2 Verbiage changed from LOD to Reporting Limit to reflect WDNR Guidance: Recommendations of the BOD LOD Technical Group. 7.10 Added new pipette criteria for updated NR149. 8.1 Results will no longer be averaged after 2nd QC failure. Most recent results will be reported (in response to April 2021 NELAC audit, BHC/R1). 8.5 Tightened QC criteria for QCS from ±25% to ±15%. 10.1.1 Added purchased alternative to washing filters in-house. 13.2.2.1 Added TSS only comment/procedure for MBs. 16.21 Added reference for pipette performance checks.	R. Riessen
OB1	11/30/21	Transition to OnBase Updated references	S.D. Hill
OB2	06/01/22	2.5, 6.1, 8.2 Updated LRB to MB to match Horizon naming. 2.6, 8.2-8.4 Changed remaining LOD references to reporting limit. 6.3 Changed from 4 °C and ≤6 °C	R. Riessen/ L. Klicko

APPENDIX 1:

Gravimetric Verification of non-glass Class-A Graduated Cylinders

1. Each graduated cylinder being verified must have a unique ID. This list can be found at:: O:\SOP\EHD\ESS\Inorganic\Draft\Inorg pipettes.xls
2. Open Excel Worksheet. The worksheet is found at:

   \\slhfile\grp\EHD\ESS(4900)\ESS Inorg(4910)\General Chemistry\SOLIDS\Graduated Cylinder Quarterly Verification\Verification Template.xltm
3. Enter the date, the temperature of the reagent water to be used. The spreadsheet will automatically find the correct Z-Factor. Enter the analyst initials and the balance instrument #.
   a. Enter the graduated cylinder ID.
   b. Enter the max volume of the cylinder volume and the volumes to validate will populate.
   c. Record weights in the column **rep #1 (g)** if balance is located near computer

      OR

   d. If no computer is available, print off sheet and write in the weights for later entry into the template.
4. Place a clean, dry graduated cylinder on the balance and tare.
5. Fill a clean squirt bottle with ASTM Type-I water for volume verification.
6. Aliquot the volume indicated in the **Vol (mL)** column into the graduated cylinder, ensuring the meniscus of the water rests on the corresponding graduation.
7. Once the weight has stabilized, record the weight on the worksheet.
8. Continue adding water for each required volume.
9. Discard the water and repeat steps 5-7 for **rep #2 (g)**. If a mistake was made during one replicate, add/remove water and record new weight.
10. Check if results fall within the Acceptance Criteria.
    a. All replicates must “pass” for quarterly verification.
11. If the graduated cylinder fails the criteria, take corrective action and perform the analysis again, recording the results in a new portion of the same spreadsheet. Record the corrective action taken next to the failed analysis on the hard copy when it has been printed.
12. If the criteria have been met, print the worksheet and file the hard copy in the Pipette Performance logbook for reference.
13. Save copy of the worksheet, including the year and quarter in the name, e.g. 2020Q1 Verification.

EHD INORG METHOD 141.0
Chloride
(SM 4500-Cl^- E)

1. Scope and Application

1.1 This method covers the determination of chloride in drinking, ground, and surface waters; and domestic and industrial wastewaters.

1.2 The applicable range is 1.36 to 100 mg/L. This range may be extended by diluting the sample prior to analysis.

1.3 The method limit of detection (LOD) = 1.36 mg/L.

1.4 The method limit of quantification (LOQ) = 4.55 mg/L.

2. Summary of Method

2.1 Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms the highly colored ferric thiocyanate, of which the absorbance is proportional to the chloride concentration. Ferric thiocyanate absorbs strongly at 480 ηm. The calibration curve is non-linear.

2.2 The reaction may be written as follows:

$$Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + 2(SCN)^-$$

$$(SCN)^- + Fe^3 \rightarrow (Fe[SCN]) + 2$$

2.3 The determinative steps in this method are identical to SM 4500-Cl^- E (15.1). However, because the reference method is written specifically for air-segmented continuous flow technology that is no longer available, the specific "plumbing" scheme (pump tubes and reagent proportions, etc.) used are adapted to match the Lachat flow injection instrumentation. The specific flow scheme used in this SOP is from Lachat QuikChem Method 10-117-07-1-B (15.2).

3. Safety, Waste Management, & Pollution Prevention

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive (15.3).

3.2 All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide, chapter 7 (15.4).

3.3 Pollution prevention is practiced through source reduction, minimizing waste, chemical substitution, recycling, and other means. For details see University of Wisconsin Laboratory Safety Guide, chapter 6 (15.4).

3.4 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely

hazardous materials. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the Material Safety Data Sheets.

3.4.1 Mercuric Thiocyanate

3.4.2 Nitric acid

3.4.3 Ferric Nitrate

3.5 Due to the presence of mercuric thiocyanate, dispose of waste through UW Safety. Attach SDS sheet for color reagent to UW Safety disposal form; 30% of the instrument waste is color reagent, the remainder is water. Note: Do not label the bottle as "waste."

4. Sample Handling and Preservation

4.1 Samples should be collected in approved plastic or glass bottles.

4.2 Maximum holding time is 28 days from date of collection.

4.3 Samples are stored at 4º C in a walk-in cooler until analysis.

5. Interferences

5.1 Halides which also form strong complexes with mercuric ion (e.g., Br^-, I^-) give a positive interference. If suspected, samples will be analyzed by Ion Chromatography.

5.2 Substances which reduce iron (III) to iron (II) and mercury (III) to mercury (II) (e.g., sulfite, thiosulfate). If suspected, samples will be analyzed by Ion Chromatography.

5.3 Samples with high turbidity may cause interference. Turbidity can be removed by filtration through a 0.45 µm filter prior to analysis (15.13).

6. Reagents and Standards

6.1 ASTM Type I water: All reagents and standards must be made with ASTM Type I water. (U.S. Filter Corp., Lowell, MA).

6.2 Carrier Reagent is ASTM Type I water.

6.3 Color reagent – Mercuric Thiocyanate 0.06% solution (order from RICCA or GFS Chemicals). The color reagent must be filtered. Use a Millipore 0.45µm filter. Store the filtered portion in a bottle, in a dark place. Follow the manufacturers recommended expiration date.

6.4 Stock Chloride Standard (1000 mg Cl/L): In a 105°C oven, dry 3.0 g primary standard grade sodium chloride (NaCl) for one hour. Cool in a desiccator. In a 1 L volumetric flask, dissolve 1.648 g sodium chloride in about 500 ml ASTM Type I water. Dilute to the mark and invert to mix. Expiration Date is 6 months.

6.5 Spike Solution (2500.0 mg Cl/L): In a 105°C oven, dry 5.0 g primary standard grade sodium chloride (NaCl) for one hour. Cool in a desiccator. In a 500 ml volumetric flask dissolve 2.06 g sodium chloride in about 250 ml ASTM Type I water. Dilute to the mark and invert to mix. Expiration Date is 6 months.

6.6 Working standard solutions: Prepare the following standards by diluting suitable volumes of standard solution (6.4) to specified volumetric flasks.

mg Cl/L	mL of standard solution (6.4)/100 mL
0.0 (also Lab Reagent Blank)	0.0 mL
2.0	0.2 mL
5.0	0.50 mL
10.0	1.0 mL
25.0	2.5 mL
50.0	10. mL (200 mL)
75.0	7.5 mL
100.0	10 mL
2500.0 (Spike Solution)	See 6.5

6.7 Quality Control Standard (QCS): This stock standard comes from a different source than what the calibration standards are made from. Typically order a pre-made stock solution from vendors like LabChem, RICCA, or VWR. The expiration date is 6 months or manufacturer's expiration date, whichever comes first.

6.8 Quality Control Standard (QCS): Use the 1000 mg/L stock (6.7) solution to make the 3 QCS's.

mg Cl/L	mL of QCS stock solution/100 mL
9.0	0.9 mL
45.0	4.5 mL
75.0	7.5 mL

Note:

1) All working, stock and QCS standards must be entered into the standard log book.
2) The 0.0 mg/L standard is used for the Lab Reagent Blank (LRB), the Calibration Blank (CB), and to prepare the Lab Fortified Blank (LFB).
3) The holding time for the working and QCS standards is 28 days.

7. Apparatus

7.1 Autosampler.

7.2 Lachat 8500 System.

7.2.1 Multichannel proportioning pump.

7.2.2 Injection module with an 18.5 cm x 0.8 mm i.d. sample loop.

7.2.3 Reaction unit or manifold (see figure 1 for diagram)

7.2.4 Colorimetric detector

7.2.5 Colorimeter equipped with 10 mm path length flow cell and 480 nm interference filter.

7.2.6 Data system

7.2.7 10 mL, 1.0 mL, and 0.1 mL motorized pipette.

7.3 Culture tubes: 13 x 100 mm, disposable, glass.

8. Quality Control Types, Acceptance Criteria, & Corrective Actions

8.1 Please refer to the **Environmental Health Division Quality Assurance Manual** (15.6) for accuracy and precision calculations and other general information on quality control procedures. Important specifics include:

8.1.1 Accuracy and precision calculations.

8.1.2 Corrective action and result qualification procedures (including documentation requirements) for instrument problems or analytical problems.

8.2 **An instrument logbook** is maintained for each flow injection instrument. Maintenance, performance problems, date calibrated, analyst, and other pertinent information are documented in the logbook.

8.3 **The Correlation Coefficient (*r* value)** is the measure of the linearity of the standard curve and must be >0.995 to be acceptable and before proceeding with sample analysis. The 2016 TNI Standard (15.7) requires the % Relative Error (%RE) to be calculated and evaluated for each the low and midpoint standards and must pass acceptance criteria before continuing analysis. The acceptable %RE for the low and midpoint standards is ±50% and ±30%, respectively. These values will be manually entered into HORIZON into the %RELOW and %REMID standards, located at the end of the batch. Due to HORIZON limitations, only recovery will be calculated. Since %RE = Recovery – 100%, the acceptable limits for the low and midpoint standards in HORIZON become 50-150% and 70-130%, respectively.

8.4 **Three Quality Control Standards (QCS)** are analyzed with each calibration. The analytical result must be within ± 10% of the true value to continue the analysis. If QCS fails, recalibrate and analyze again.

8.5 **A Laboratory Reagent Blank (LRB)**, aka “Check Blank (CB)” is analyzed with each calibration and must meet 1 of 3 criteria listed in the Wisconsin Department of Natural Resources Lab Certification Manual (15.5). 1) Reagent blank must be less than the detection limit of the method. 2) Reagent blank <5% of sample concentration. 3) Reagent blank <5% of regulatory limit. If it does not meet one of these criteria, recalibrate and analyze the blank again. In general, a method blank (reagent blank, CCB, etc.) is within acceptable QC limits when the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source of error, and the calibration intercept is satisfactory, then the blank may be accepted as “zero” providing that the logic supporting this decision is well documented. The LRB is equivalent to the CB of this method.

8.6 **One Laboratory Fortified Blank (LFB)** is required in each analytical batch. The LFB spike recovery must be within ± 15% (85-115%) of the true value to proceed. If the LFB exceeds acceptance criteria, corrective action will include attempting to identify the source of the problem, reanalyzing the LFB, preparing a new LFB, qualifying the data or recalibrating if necessary. Prepare the LFB using LRB/CB and spiking solution (6.5), where the spike solution is equal to 1.0% of the total volume -- e.g., add 0.06 mL of spike solution to 5.94 mL of LRB/CB.

8.7 **Matrix Spikes and Duplicates:** Prepare a **minimum of 10%** of the samples per matrix with duplicates and spikes. Spikes are prepared in the same manner as the LFB, substituting the sample in place of the LRB/CB. Spike limits are 85-115% recovery. Duplicate limits are 10% relative difference. If spike recovery or duplicates do not fall within the specified control limits, the matrix group (including spike and duplicate) must be reanalyzed on the same run after trying to identify the source of the problem (e.g., sample characteristics including color, sample homogeneity, turbidity, etc.). If it fails again, qualify that matrix group.

8.8 **An Instrument Performance Check (IPC or CCV, "Continuous Calibration Check")** and **Check Blank (CB or LRB),** must be analyzed every 10 samples. The IPC must be within ±10% of true value. Choose a standard with a concentration near the middle of the calibration range. The CB must be less than the LOD. In general, a method blank (reagent blank, CCB, etc.) is within acceptable QC limits if the observed concentration is less than the LOD, but greater than the negative LOD (<LOD and >-LOD). However, if the measured concentration of the blank is less than the negative LOD (<-LOD) and there is no apparent source causing the problem (e.g., baseline drift, and improper y intercept, poor source of material for reagent blank, etc.), then the blank may be accepted as "zero" providing that the logic supporting this decision is well documented. All data reported must be "bracketed" by acceptable IPC's and CB's. If an IPC or CB fails, corrective action requires that all previous samples back to the last acceptable IPC and CB be reanalyzed. The CB is equivalent to the LRB for this method.

8.9 **Demonstration of Capability (DOC):** This must be completed initially and then annually by anyone who performs the test. Run 4 repetitions of a QCS and follow the guidelines given in the EHD DIV-WIDE QA 115 (15.11).

8.10 **Limit of Detection (LOD):** This must be determined every 13 months or if there is a significant change to the method of analysis. Determine the method LOD using the procedure outlined in EHD DIV-WIDE QA 116, "LOD Procedures" (15.12).

8.11 **Linear Calibration Range (LCR):** The LCR must be determined every 6 months or whenever there is a change in the method. The initial demonstration of linearity must use sufficient standards to insure that the curve is linear. Verify the linearity by analyzing a minimum of a blank and 3 standards spread over the expected linear range. If any verification data exceeds the initial values by ±10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

8.12 **Sample Dilution**: If the estimated concentration of analyte in a sample exceeds the highest calibration standard, a bench dilution must be performed. Dilutions at the bench are typically performed by diluting an appropriate volume of sample with the reagent

blank. Motorized pipettes may be used to deliver/dilute volumes up to 10 mL. For volumes greater than 10 mL, Type A glass, volumetric pipettes must be used. Diluted samples should be mixed thoroughly prior to analysis. See appendix 1 for recommended dilutions.

8.13 **Dilution Verification:** When a dilution is made, the dilution must be verified by comparing the diluted result with the original result. The comparison is done by dividing the diluted result by the original result and expressing the calculation as a percent. The acceptable range is 90% to 110%. If the dilution verification is not within the acceptable range, a different dilution must be made. This second dilution would then be verified against the first dilution. If a group of samples is diluted identically, at least 10% of the dilutions must be verified.

9. Method Calibration

9.1 Refer to section 6 for making standards and reagents.

9.2 Calibration curve is a not linear; a 2nd order polynomial curve is calibrated.

9.2.1 Working calibration standards are analyzed in duplicate and Omnion models/equations are then calculated as two-dimensional averages of the measurements (15.15).

9.3 Open the method template and set the data system parameters and operating conditions for the Lachat 8500 with the *Omnion* software (15.9).

9.3.1 Set up manifold as shown in Figure 1.

9.3.2 Pump ASTM Type-I water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline has been obtained (about five minutes).

9.4 Place standards in the appropriate rack on the autosampler and calibrate the instrument by injecting the standards. The data system will then correlate the concentrations with the instrument responses for each standard.

9.4.1 After the calibration passes (8.3) the samples may be analyzed. If the calibration is not satisfactory use an appropriate corrective action to diagnose possible causes and recalibrate.

10. Procedure

10.1 See also Instrument Operating Procedure (15.9).

10.2 Import the sample identification numbers from Horizon (15.8) into the Tray Table of the Lachat Omnion software. This will include:

10.2.1 A duplicate and spike for every 10 samples in a matrix group.

10.2.2 One Lab Reagent Blank.

10.2.3 One Lab Fortified Blank.

10.2.4 Three Quality Control Standards (one for each segment of the curve).

10.2.5 A Calibration Blank (CB/LRB) and Instrument Performance Check (IPC) should be analyzed after the curve and after every 10 cups and at the end of each run.

10.3 Analyze standards and Quality Control samples (i.e., IPC, CB, LRB, LFB, and QCS). If QC samples pass acceptance criteria, the batch may continue with sample analysis.

10.4 Shutdown procedure:

10.4.1 When the analytical batch is complete, transfer reagent lines to ASTM Type-I water to rinse for five minutes.

10.4.2 Remove reagent lines from ASTM Type I water and pump air through in order to dry. Release pump-tubes from cartridges and turn off instrument.

10.4.3 Waste disposal: The waste must be collected and disposed of according to the University of Wisconsin Laboratory Safety Guide (15.4).

11. Calculations

11.1 The chloride concentration is obtained directly from the *Run Time Report* and must be printed for a hard copy.

11.2 If the estimated chloride concentration exceeds the highest calibration standard, a manual dilution will be performed and documented on the benchsheet. The Lachat 8500 software does not incorporate the dilution correction into the result. The dilution correction will be calculated by the Horizon program (15.8). See section 8.12 for dilution verification instructions. The final result will be verified mathematically, by an experienced chemist who did not perform the original analysis, when the batch is checked for quality control (15.10).

12. Data Management

12.1. The analytical run, the *Run Time Report*, and the QC Parameters section in Horizon, where all quality control is calculated for pass/fail criteria, will be reviewed for quality control prior to accepting results (see section 8). The reviewer must be an experienced chemist who did not perform the original analysis (15.10). The reviewer must initial and date the analytical run.

12.2. Export results from Omnion to Horizon (see EHD INORG GENOP 113, Horizon Procedures, (15.8).

12.2.1. Review results by selecting Edit Results under Batches.

12.2.2. Review QC Results by selecting the QC display in Edit Results.

13. Definitions

13.1 Definitions of terms in this SOP may be found in the reference method (15.1).

13.2 For general definitions, please see the Quality Assurance Manual, (15.6).

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (LODs and DOCs) were generated in compliance with the reference method and the standard operating

procedures: EHD DIV-WIDE QA 115, Initial and Ongoing DOC Procedures (15.11), and EHD DIV-WIDE QA 116, LOD Procedures (15.12). Supporting data will be retained according to the applicable Records Disposition Authority (RDA). Data generated within the last two years will be kept on file within the Inorganic Chemistry Dept. Data older than two years may be archived in the basement.

15. References

15.1 American Public Health Association, American Water Works Association, and Water Environment Federation. (2005). Standard Methods for the Examination of Water and Wastewater. Method 4500-Cl^- E-1997, 21st edition.

15.2 Zellweger Analytics, Lachat Instruments Division. *Determination of Chloride by Flow Injection Analysis Colorimetry*, (QuikChem Method 10-117-07-1-B, March 23, 2001).

15.3 Wisconsin State Laboratory of Hygiene, LABWIDE SAFETY 102, *Chemical Hygiene Plan & General Laboratory Safety Plan for the Agriculture Drive Facility*, State Laboratory of Hygiene.

15.4 UW-Madison policy UW-6066, Chemical Hygiene Plan and Policy: https://policy.wisc.edu/library/UW-6066, including the "Chemical Safety Guide," https://ehs.wisc.edu/labs-research/chemical-safety/chemical-safety-guide/. Previous: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/LabSafetyGuide_Full.pdf

15.5 Wisconsin Administrative Code NR 149, "Laboratory Certification and Registration," June 29, 2021.

15.6 Wisconsin State Laboratory of Hygiene, Environmental Health Division, EHD DIV-WIDE PLAN 001, *Quality Assurance Manual-General*. EHD INORG PLAN 001, *QA Manual-Inorganic Chemistry/TECL/Metals Supplement.*

15.7 2016 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2016.

15.8 Wisconsin State Laboratory of Hygiene; EHD INORG GENOP 113, *"HORIZON Procedures for EHD Inorganic Chemistry".*

15.9 Wisconsin State Laboratory of Hygiene; EHD INORG IOP 105, "*Instrument Operating Procedure for QuikChem Automated Ion Analyzer"*.

15.10 Wisconsin State Laboratory of Hygiene; EHD INORG QA 107*, "Q.C. Audits of Analytical Runs for ESS Wet Chemistry Area"*.

15.11 Wisconsin State Laboratory of Hygiene; EHD DIV-WIDE QA 115, "*Initial and Ongoing DOC Procedures*."

15.12 Wisconsin State Laboratory of Hygiene; EHD DIV-WIDE QA 116, "*LOD Procedures*"

15.13 Wisconsin State Laboratory of Hygiene; EHD INORG METHOD 100.2, *"Filtering Procedure"*

15.14 QuikChem 8500 Series II, Operations Manual, Lachat Instruments, Hach Co., 01/2016, Edition 2.

15.15 Omnion 4.0 Software User Manual, Lachat Instruments, Hach Co., 04/2015, Edition 1.

15.16 QuikChem 8500 Series II, Maintenance and Troubleshooting, Lachat Instruments, Hach Co., 01/2016, Edition 2.

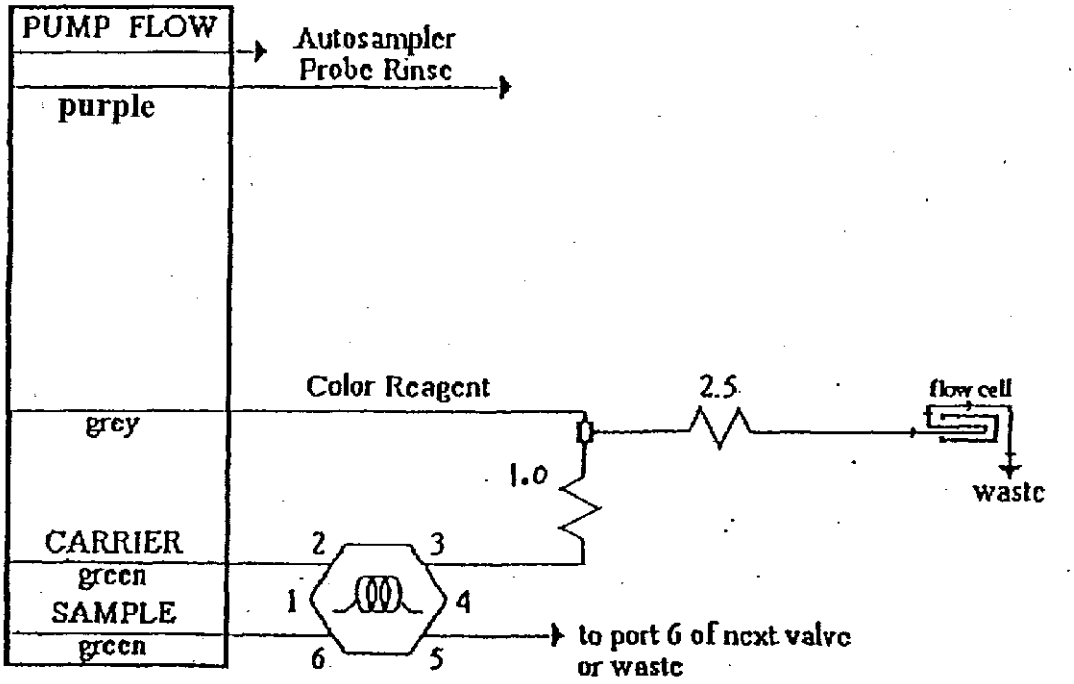
16. Version Tracking Table:

Rev. #	Rev. date	Changes Made	Rev. author
6	Feb. 2014	Updated SOP for the new Horizon LIMS	C. Thielmann
7	04/12/2017	8.12, 11.2 Dilution verification information added 8.9, 14.1, 15.11 Updated DOC procedure reference. 8.7, 8.12 Changed "should" to "must"	Anthony Plourde
8	02/07/20	Section 1: changed LOD from 1.0 to 0.93 mg/L, changed LOQ from 3.2 to 3.09 mg/L (both effective in Horizon on 12/18/19. Added Pollution Prevention information to section 3 (in response to NH NELAC audit of May, 2019) 8.7 added SP and Dup QC limits (in response to July, 2018 EPA DW audit, def. #21b) 8.10: updated to EHD QA 116 reference Sections 9 and 10. Updated to standardize SOPs and to consolidate QC requirements. Section 11: deleted accuracy and precision calculations, because they are in the QA Manual. References updated to new Omnion and instrument manuals.	G. Anderson/S. Hill
9	07/24/20	Section 1: changed LOD from 0.93 to 1.36 mg/L, changed LOQ from 3.09 to 4.55 mg/L (both effective in Horizon on 06/08/20. 6.6: Added 75 mg/L standard to help with curve/recoveries. Updated some references	R. Riessen
10	09/10/21	3.5 Added information on disposal and percentage of color reagent in the instrument waste. 8.3 Added definition of Correlation Coefficient along with %RE requirement. 8.10 Following all accreditor suggestions, LODs will now be calculated/verified every 13 months instead of six. 9.2.1 Calibration information added for clarity. 9.4.1 Deleted required r-value for calibration, replaced with cross-reference to section 8.3, which includes r and %RE criteria. 15.9—updated UW Safety reference Appendix 1 added to aide analyst in diluting over range samples.	R. Riessen / L. Klicko

Ver. #	Changes Made	Ver. Author
OB1	8.6, 10.2.3 Only one LFB will be analyzed per calibration according to DNR 149 update. 8.4, 8.6, 8.7 QCS, LFB, and MS limits updated in Horizon from 90-110% to 85-115% on 11/24/2021 after annual QC limit review. Transition to OnBase Updated References	S.D. Hill
OB2	8.4 QCS limits updated to 90-110% from 85-115% as a result of internal audit performed 1/19/23; updated in Horizon 3/10/23. Updated header, updated references, removed date from version tracking table	J. Thorngate

Figure 1

CHLORIDE MANIFOLD DIAGRAM



CARRIER is ASTM Type 1 Water.
All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
1" is 70.0 cm of tubing on a 1 inch coil support.
2.5" is 168 cm of tubing on a 2.5 inch coil support.
APPARATUS: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module are required.
Sample Loop: 18.5 cm.
Filter: 480 nm.

Appendix 1:

Dilution Guide

Initial Instrument Response (mg/L)	Approximate Dilution	Approximate Final Value (mg/L)
175	X2; X3	200
200	X3	240
250	X4	320
300	X5; X6	450
400	X10	800
500	X15	1250
600	X25	2100
700	X50	3600
850	X100	7700

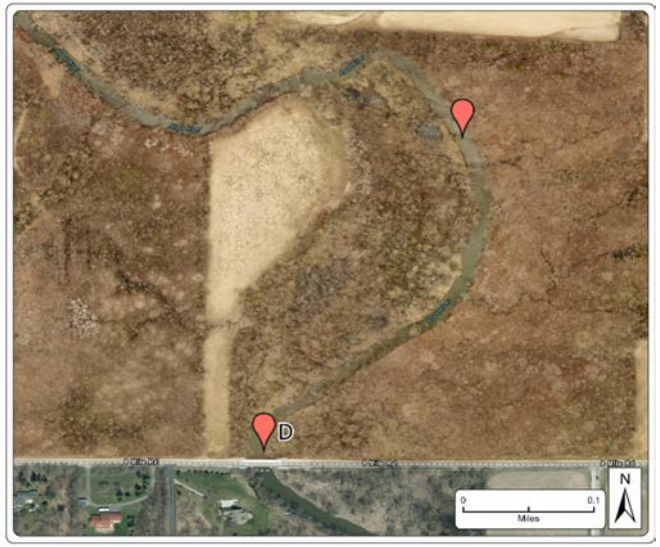
Appendix F. Biological Data Collection Reach Maps

(A) Root River at Oakwood Road – Biological and habitat sampling reach shall be 35 times the stream width, or approximately 1,225-foot reach.

(B) Root River Canal at 7 Mile Road – Biological and habitat sampling reach shall be 35 times the stream width, or approximately 1,050 feet.

(C) Root River on 60th Street bridge, at return flow outfall – Biological and habitat sampling reach shall be 35 times the stream width, or approximately 1,995 feet. This reach crosses temperature sampling Site C2 and the return flow outfall.

(D) Root River at County Line Road – Biological and habitat sampling reach shall be 35 times the stream width, or approximately 1,470 feet.



Appendix G. Macroinvertebrate Sampling SOP

Benthic Macroinvertebrate Field Sample Collection

Contents

Purpose

This standard operating procedure (SOP) is applicable to the collection of benthic macroinvertebrate samples from wadable streams. This document also describes the collection of physical and chemical parameters that support the diagnostic application of benthic macroinvertebrates for aquatic biomonitoring.

Equipment and Materials

The following materials are required to undertake this procedure:

- Field sheets
- Clipboard
- Pencils
- Permanent markers
- Waterproof labels
- Labeling tape

- Secure closure (zip-top) bags
- Basic tool kit and duct tape
- GPS unit
- Digital Camera (waterproof preferred)
- Velocity meter (OTT Hydromet MF Pro electromagnetic flow meter)
- Measuring Tape
- Ruler
- Multiparameter Probe, YSI ProDSS multi-parameter meter, able to assess the following parameters: water temperature, pH, dissolved oxygen, conductivity, and turbidity, and calibration equipment
- Cooler
- Sample bottles (water)
- Ice packs
- Extra batteries or batter chargers
- Kicknet mesh size 425 µm
- Stopwatch
- Wash bottles
- Plastic spoons
- Tweezers
- Bucket(s)
- Sieve(s) mesh size 425 µm
- White trays
- Sample jars (BMIs)
- ETOH
- Life – jackets (PFDs)
- First aid kits (field and vehicle)
- Cell phones
- Throw bags
- Waders
- Boots
- Raingear
- Gloves (rubber, neoprene)
- Safety goggles
- Sunscreen
- Hat
- Sunglasses
- Insect Repellant

Collection Procedures and Guidelines

Pre-departure

1. Field team members are contacted, and a field sampling date as well as back-up field sampling date are scheduled, typically no less than two-weeks in advance. A calendar invitation is submitted to relevant parties indicating when field activities will take place.
2. Collect all the necessary equipment, ensure that all equipment is calibrated prior to departure and in working order.
3. Ensure that the weather conditions and flow levels are appropriate for sampling prior to departure.

Arrival

4. Ensure that all travel safety guidance is followed (General Field Safety Instructions SOP).
5. Assess the safety of the site. All members of the field crew should be aware of potential dangers and knowledgeable of safety precautions. A thorough inspection is important for preventing accidents. Proceed with collection only when conditions are appropriate.

Primary Site Data

6. Complete the station summary on the Macroinvertebrate Field Data Report form (Appendix 1)
7. Complete the Sample and Site descriptors section of the Macroinvertebrate Field Data Report form (Appendix 1), except for channel and flow characteristics (stream width, depth, velocity, and discharge), because doing so would disturb the substrate and bias the invertebrate sample.
8. Follow the same procedure for water quality assessment used specified in the Surface Water Chemistry SOP.

Invertebrate Collection

9. Define the kick area and path in the erosional zone (riffle) of the sampling reach before entering the stream. Inform field team members so that this area is not disturbed.
10. At the downstream end of the kick area, place the kick net downstream of the sampler, flat side of the triangle resting on the substrate of the stream.
11. Walk backward in an upstream zigzag direction, dragging the net along the bottom of the stream while walking.
12. Kick the substrate to disturb it to a depth of ~5-10 cm if possible. For large cobble, turn over and rub your foot over the surface to dislodge macroinvertebrates clinging to the interstitial spaces. Brush the surface of large boulders with your hand or foot.
13. The net should always be held close to the area that is being disturbed to ensure that most of the disturbed substrate and organisms are swept into the net by the current.
14. Continuously zigzag over the stream bottom from bank to bank in an upstream direction for a period of 3 minutes.
15. If the sampler needs to stop to get around an obstruction, take a rest, or remove large cobbles from net, the timer pauses the stopwatch while the sampler lifts the mouth of the net from the water. The stopwatch is then restarted when the sampler is ready to continue sampling by placing the net back in the stream.
16. The timers spots the sampler and alerts them of any upcoming obstructions while the sampler is traveling backwards because they may not be able to detect all hazards.
17. Record all relevant data including weather conditions, personnel present, date and time, and any observations in the field notebook.

Sample Transfer

18. Splash the side of the net in the river to transfer all material to the collection cup at the end of the net (ensure that the mouth of the net is out of the water).
19. Remove the collection cup attached to the end of the net and empty the contents directly into a wide-mouth plastic sample jar, pail or sieve. Always work over a pail or tray in case of an accidental spill.
20. Wash any material remaining in the cup/net into the sample jar/pail/sieve using a squeeze bottle and forceps to remove any clinging animals.
21. Carefully rinse and discard any stones and large green leaves that have freshly fallen into river and are not invertebrate habitat.
22. Transfer sample from pail/sieve (if using) to sample jar. Check pail/sieve to ensure that no organisms remain.
23. Leave room in the sample jars for ETOH. Use extra jars if needed. Maintain appropriate ratio of sample to ETOH to appropriately preserve the organisms in the sample.
24. Double check the net/cup/pail/sieve for remaining macroinvertebrates.
25. Label the inside, outside and top of jar. The inside label should be written on waterproof paper marked by soft pencil. The outside of the jar should be in waterproof pen. All labels should have the following information: RR – Sample Site ID (A-D) – MMDDYYYY or Field duplicates: RR – FD – MMDDYYYY – Analysis, preservative, time of

sample collection, sample collector's initials, and sample jar number (e.g., 1 of 2, 2 of 2). If the amount of sand and gravel in your net is extensive and will likely require the use of many sample jars.

Sample Preservation

26. Wear protective gloves and goggles
27. Add 80% ETOH into jar until the sample is completely covered.
28. Optional: Wrap top of jar with parafilm and seal with the lid. Parafilm helps to prevent leaks and reduces fumes.
29. Cap jar, gently swirl the sample to distribute the ETOH. DO NOT shake the jar as large gravel and rocks in the sample will damage the organisms.
30. Transport samples in a leak-proof container, such as a cooler or plastic tote. Use packing material to support sample jars, if necessary.

Channel Characteristics

31. Establish a transect perpendicular to the flow; in or near the benthic macroinvertebrate sampling area.
32. Measure the wetted width (the current water level width) of the channel.
33. Record measurements on the field sheet and indicate whether the measurements were taken upstream or downstream of the kicknet sampling area.
34. Divide the wetted width into a minimum of 5 intervals, for larger stream widths (>10 m) use 10 intervals. Use the velocity meter to record the water depth and velocity (at 60% of the depth) at each point. If available calculate the discharge using the velocity meter. Record the measurements on the field sheet. Note any unusual readings or errors in the field notebook.

Return

1. Following field activities, all field team members assist with the transport and inspection of all field equipment and supplies.
2. Upon return from the field all equipment is inspected for damage, cleaned, dried, and stored. All samples are inspected to ensure that labels are legible.
3. Appropriate parties are notified by email that field sampling has been completed.

Field Note/Sheet Procedures and Guidelines

The field sheets and field notebook will be maintained by the benthic macroinvertebrate team. The notebook will be water-resistant and the field sheets will be printed on water-resistant paper.

Guidelines to follow when recording notes in the field notebook/field sheets include:

1. Write neatly.
2. Make numbers large.
3. Do not erase or black out a mistake, draw a line through the incorrect value and initial instead.
4. Number pages.

5. Never tear pages out of the notebook.
6. Record everything, never assume you will remember something.

Quality Assurance/Quality Control

Quality Assurance and Control (QA/QC) is an ongoing process. Its goal is the prevention, early detection and correction of field and analytical data collection errors.

1. All members of the field crew must ensure that all data sheets are filled in correctly and completely before leaving the site.
2. All members of the field crew must determine if the data are reasonable before they leave the field, and if not, the measurements should be repeated before leaving. This may require taking a calculator to determine if some measurements seem reasonable.

Field Duplicate

One sampling location per early fall sampling event (Sept/Oct) will be randomly selected for the collection of a field duplicate. A field duplicate benthic macroinvertebrate sample will be collected using identical procedures from a comparable location in the same reach immediately following the collection of the primary site sample. If possible, the duplicate location will be located upstream to avoid contamination and disturbance from the collection of the primary benthic macroinvertebrate sample. The same personnel will collect both the primary sample and the field duplicate sample. The sample label will distinguish the field duplicate from the primary site sample (see sample transfer section above). The field duplicate will be treated using the same procedures and processes used for the primary site samples (Benthic Macroinvertebrate Sample Processing SOP).

Chain of Custody/Shipping

All samples will be transported immediately to UW-Parkside where they remain in the custody of Dr. Jessica Orlofske.

Data Management and Documentation

The original and a physical copy of the field sheets are stored in secure locations on the UW-Parkside premise. Scans of the field sheets accompany the annual report.

Field sheets are converted to a digital format using Microsoft Excel or equivalent. All Root River benthic macroinvertebrate field data are combined into single file for a calendar year (Root River BMI Field YEAR).

Scanned data sheets and data files are stored locally with back-ups to campus OneDrive and iCloud. Cloud and local file organization are identical with a Root River Project parent folder and all files pertaining to a calendar year located with a Root River Report YEAR folder.

Safety and Environment

This section describes health, safety and environmental considerations for benthic macroinvertebrate sampling:

Health and Safety

Field Safety Instructions developed by the contractor for the sampling activities should be followed. Please refer to the General Field Safety Instructions SOP and the Task Hazard Analysis for more details. Some hazards are included here:

Hazards include, but are not limited to:

- Manual handling injury associated with lifting and moving sampling equipment and samples – to mitigate determine that all loads are an appropriate weight for lifting (<10 kg), use correct lifting posture by bending at the knees, position so that load is balanced and does not cause undue strain, wear sturdy boots and clothing, park field vehicle with equipment close to water body (if possible) to avoid multiple loading and uploading, do not over-pack samples into coolers.
- Injury associated with slips and trips – to mitigate keep a tidy workplace and step carefully around tubing, hosing and other equipment.
- Hit by moving vehicle while sampling – to mitigate sampling team shall wear high visibility clothing, set up traffic controls around sampling area, position site vehicle so that it provides a barrier from potential traffic.
- Sunburn –to mitigate wear suitable clothing (including hat, trousers, long sleeved shirt), apply sunscreen regularly.
- Dehydration and fatigue – to mitigate drink fluids and eat regularly.
- Exposure to water – to mitigate handle water with care minimizing splashing or spills, understand Safety Data Sheet (SDS) for particular parameters of interest, wear appropriate personal protective equipment (PPE) including gloves, waders, escalate PPE requirements if conditions change.
- Exposure to biological hazards (including snakes, ants, mosquitoes, bees, poisonous plants) – to mitigate access sampling points by minimizing exposure to vegetation, plan sampling events at suitable times where risk of biological hazard is reduced, wear appropriate clothing and PPE (long sleeves, long pants, tuck pant legs into socks), make vibrations to alert snakes to your presence. Use insect spray or other insect deterrents.
- Working on or around water courses will require additional PPE which includes (but not limited to) a Type II personal floatation device (PFD) and never working alone. PFDs should be utilized when sampling in deep waters and for all other instances where potential drowning danger exists.

Environment

Sampling contractors will be exposed to environmental waters which may contain contaminants that are hazardous to human health. All personnel participating in field sampling shall be current on Occupational Safety and Health Administration (OSHA) medical screening and surveillance standards. These standards can be found on the OSHA organization web page:

https://www.osha.gov/SLTC/medicalsurveillance/standards.html

References

Wisconsin Department of Natural Resources. 2000. *Guidelines for Collecting Macroinvertebrates from Wadable streams.*

Appendix 1

State of Wisconsin
Department of Natural Resources

Macroinvertebrate Field Data Report

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Instructions: **Bold** fields must be completed.

Station Summary

Waterbody Name	**Waterbody ID Code**	Site Mile	Station No.	**Sample ID (YYYYMMDD-CY-FD)**

Starting Location	Township	Range	Section	¼ - ¼	¼

Latitude - Longitude Determination Method Used	**Datum Used**

Start Latitude	**Start Longitude**	End Latitude	End Longitude	7.5" Quad Map Name

Basin Name	**Watershed Name**	**County**

Sample and Site Descriptors

Sample Collector (Last Name, First)	**Project Name**

Sampling Device

☐ Kick Net ☐ Surber Sampler ☐ Eckman
☐ Ponar ☐ Artificial Substrate ☐ Hess Sampler ☐ Other: _____

Habitat Sampled

☐ Riffle ☐ Run ☐ Pool
☐ Microhabitat ☐ Shoreline Composite ☐ Proportionally-Sampled Habitat
☐ Littoral Zone ☐ Profundal Zone ☐ Wetland

Total Sampling Time (min)	Estimated Area Sampled (m²)	Number of Samples in Composite	**Replicate No. _____ of _____**

Reason for Sampling

☐ Least Impacted Reference ☐ Baseline ☐ Impact / Treatment Site
☐ Control Site ☐ Trend ☐ Other: _____

Water Color ☐ Clear ☐ Turbid ☐ Stained	**Water Temp. (C)**	D.O. (mg/l)	D.O. (% sat.)	pH (su)	Turbidity (NTUs)	TDS (mg/l)

Conductivity (umhos/cm)	Stream Order	Stream Gradient (m/km)	**Estimated Stream Velocity (mps)** ☐ Slow ☐ Moderate ☐ Fast

Measured Velocity (mps)	**Average Stream Depth (m)**	**Average Stream Width (m)**

Composition of Substrate Sampled (Percent):

Bedrock: _____ Boulders (261 mm - 4.1 m dia.): _____ Rubble (65 - 260 mm dia.): _____ Gravel (2 - 64 mm dia.): _____

Sand: _____ Clay: _____ Silt: _____ Muck: _____ Overhanging Vegetation: _____

Aquatic Macrophytes: _____ Leaf Snags: _____ Course Woody Debris: _____ Other (_____): _____

Embeddedness of Substrate at Sample Site (%)	**Canopy Cover at Sample Site (%)**

Macroinvertebrate Field Data Report

Form 3200-081 (R 5/00) Page 2 of 2

Stream and Watershed Descriptors

N = Not a problem
U = Present, but uncertain as to degree of impact
P = Present, and probably creating a problem
Blank = Uncertain

Factors that may be Influencing Water Resource Integrity	Local	Water-shed	Factors that may be Influencing Water Resource Integrity	Local	Water-shed
Biological			**Chemical**		
Macrophytes			Chlorine		
Filamentous Algae			Organic Toxics		
Planktonic Algae			Inorganic Toxics		
Diatoms / Periphyton			Nutrients		
Slimes			Dissolved Oxygen		
Iron Bacteria			Other - Specify:		
Exotics - Specify:			**Sources of Stream Impacts**		
Other - Specify:			Urban NPS		
Physical			Construction Erosion		
Sludge			Point Source - Specify:		
Thermal			Cropland Erosion		
Turbidity			Pasturing		
Sedimentation / Channel Aggradation			Bank Erosion		
Hydraulic Scour / Channel Incision			Barnyard Run-Off		
Bank Erosion			Tile Drainage - Organic Soils		
Upstream Channelization			Tile Drainage - Mineral Soils		
Local Channelization			Septic Systems		
Low Flow			Tributary(s)		
Upstream Impoundment			Springs		
Downstream Impoundment			Wetland Drainage		
Other - Specify:			Other - Specify:		

Comments

Special Instructions for Laboratory

For Lab Use Only		
Sample Sorter	Taxonomist	Estimated Percent of Sample Sorted
Date Processed	Specimens Saved	

Benthic Macroinvertebrate Sample Processing

Contents

Purpose

This standard operating procedure (SOP) describes the method used for identification and enumeration of benthic invertebrates from freshwater samples. Samples are collected and then transported to the laboratory for processing, subsampling, and/or picking (extraction). Detailed sampling instructions are described in the Benthic Macroinvertebrate Field Sample Collection SOP.

Benthic invertebrates are identified to the lowest taxonomic level possible based on current literature, or they are identified to the taxonomic level required by the project. Genus/species identification provides more accurate ecological and environmental information, but family-level identification provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. Regardless of the taxonomic level of identification, only those taxonomic keys that are peer-reviewed and available publicly (i.e., published) should be used (Barbour et al., 1999).

Equipment and Materials

The following materials are required to undertake this procedure:

- Data Sheets
- Taxonomic References
- Microcentrifuge tubes
- Microcentrifuge tube tray
- Fine-Tipped Forceps
- Blunt-Tipped Forceps
- Feather-tipped Forceps
- Permanent Marking Pen
- Dissecting and Compound microscopes with light sources
- Waterproof label paper

- Pencils
- Petri dishes or gridded sorting trays
- Tally Counters
- 70% Ethanol
- Plastic spoons
- Plastic disposable pipettes
- Sieves
- Optional Equipment for subsampling:
  - Marchant Box
  - Vacuum pump
  - Large Volumetric Flasks
  - Plastic tubing
  - Rubber stoppers
  - Small plastic or glass jars

Procedures and Guidelines

Sample handling

1. Carefully rinse samples and replace 70% ethanol upon receipt at the laboratory, because ethanol used to preserve samples in the field may have evaporated or diluted, leaving an unknown concentration of preservative which can lead to decomposition of the sample. A sieve with a mesh size of 400 µm or less must be used to ensure that all organisms collected in the field sample are retained in the laboratory sample.

Subsampling

Samples may be subsampled if required by program or project objectives. If not required, proceed to the section on sorting.

2. Wash the sample into a sieve to remove preservative.

3. Wash large material, rocks, twigs, macrophytes gently and thoroughly over the sieve. Return washed material to the sorted residue container or discard; do not add washed material to the Marchant box.

4. Transfer the entire sample into the Marchant box.

5. Add a sufficient amount of water to the cells so the cells are between ½ and ¾ full. **Do not overfill the** cells. The water level should be **below** the top of each cell.

6. Secure the lid to the Marchant box so that it is water tight.

7. Flip the Marchant box over (180 degrees, top to bottom).

8. Gently agitate the sample in the open space of the lid to equally distribute the sample.

9. Quickly flip the box back over (180 degrees, bottom to top) so the sample is evenly distributed in each of the 100 cells. Note: This step takes practice; several attempts may be required to achieve an even distribution.

10. Repeat steps 6 to 8 if the sample is not evenly distributed. **TIP:** Be sure to flip the box quickly so that the majority of the sample does not settle into the first couple of rows.

11. Randomly select a cell using a ten-sided die or a random number generator.

12. Extract the subsample from the cell using a vacuum pump or suction device, and transfer into petri dish, sorting tray, or small labeled jar with 70% ETOH.

13. Extract the following sample portions in separate, labeled jars with 70% ETOH: first 5 cells (1% of the sample each), cells 6 to 10 (5%), 11 to 15 (5%), 16-20 (5%), 21-30 (10)%, 31-40 (10%), 41-50 (10%), remaining cells (50%).

Sorting

14. Record the sample label information on the project-specific data sheet.

15. Place approximately 1 teaspoon of material into a petri dish or gridded sorting tray and place under a dissecting microscope at low magnification to maximize field of view. Use higher magnification as appropriate to ensure all organisms are removed.

16. Remove specimens and separate into coarse taxonomic groupings. Use microcentrifuge tubes organized into the microcentrifuge tray to separate and label invertebrates. Use tally counters to track the number of each taxonomic group and the total number of specimens for the entire sample or by subsample (if appropriate). Record values for each portion of the sample if subsampling is used.

17. Resuspend debris by gently shaking the petri dish or sorting tray and examine the contents of the tray a second time to ensure no organisms were missed. Repeat a third time, if necessary.

18. Continue processing the sample or the subsamples until the desired number of organisms is reached. If subsampling, complete the portion of the sample that was required to achieve the desired count. If subsampling is not used, complete the tray that was required to achieve the desired count.

19. Record the percent of the sample that was required to achieve the desired count.

20. Ensure all vials and sorted debris (extracted cells) are labeled, preserved and retained for quality control (QC) audits of sorting efficiency. Do not recombine the sorted debris with the original sample.

21. Preserve, label and retain unsorted debris.

Identifications

Benthic invertebrates are identified and enumerated separately by taxonomic group while viewing through a compound microscope (e.g., Oligochaeta or larvae of Chironomidae), or dissecting microscope (e.g., all other invertebrates) using fine-tipped forceps. Only one sample should be opened and processed at a single work station at a time; this will avoid mixing specimens among samples.

22. Pour the specimens from the vial into a small petri dish. Rinse the vial into the dish using 70-80% ethanol in a wash bottle. Add enough ethanol to the watch glass to cover the specimens.

23. Examine the vial label, vial, and its lid under a compound microscope for attached specimens.

24. Examine the specimens under the compound or dissecting microscope and use taxonomic keys and other supportive taxonomic literature to identify the specimens and verify the counts using a tally counter. Note that only entire specimens or heads should be counted to avoid double counting of specimens.

25. Taxonomic identification level depends on the specimen. Benthic invertebrates are identified to the following taxonomic levels (unless otherwise specified by project requirements):

- Insects to genus or species, except Chironomidae to family or subfamily; Mollusca to family; Crustacea to family, Hirudinea to genus; Oligochaeta to family, Nematoda to phylum.

26. Return each specimen to labeled taxon-specific microcentrifuge tubes with 70%-80% ETOH. Place all specimens from a single taxon from one sample into a single vial, depending on the objectives of the study.

27. Immediately record the following information on a project-specific datasheet: family, genus, or species; counts of larvae, pupae, and adults as appropriate for the taxonomic group; and any comments.

28. Store all microcentrifuge tubes submerged in the ethanol in the sample jar with the sorted debris. This reduces jar space, minimizes the loss of ethanol from the microcentrifuge tubes and keeps all the material from a sample together. Submit completed sample to certified taxonomist for QA/QC.

Quality Assurance/Quality Control

This procedure must only be conducted by taxonomists who have the appropriate training and experience in the identification of freshwater benthic invertebrates. Identifications are made by the certified taxonomist, trained biologist(s), or trained biology student(s). All identifications made by students are verified by the certified taxonomist (Dr. Jessica Orlofske) for accuracy.

Sorting precision/efficiency

29. The debris from the first three samples for any student or staff will be re-sorted by the certified taxonomist and every third sample thereafter. Based on the resorting the percent sorting efficiency will be calculated according to the following formula:

$$\%SE = \left(1 - \frac{\#\ Organisms\ \ Missed}{Total\ \ Organisms\ Found}\right) * 100$$

30. The average percent sorting efficiency must be greater than or equal to 95%. If the average percent sorting efficiency is less than 95% all the samples processed by that student or staff member must be resorted. Furthermore, the student or staff member will receive guidance and further training as necessary.

Identification Audit

31. An identification audit is a complete re-identification and enumeration of the specimens obtained from an individual sample. An identification audit is performed on every sample, unless the sample was identified and enumerated by the certified taxonomist.

32. Four types of errors are resolved by the identification audit: misidentifications, incorrect enumerations, insufficient taxonomic identification, or questionable taxonomic resolution. Each of these errors are tallied for each sample and corrective training is provided to students or staff unable to maintain an identification error rate less than 5% as determined by the following formula:

$$\frac{\#\,Incorrect\ \ Identifications}{Total\ \ Organisms\ \ Found\ \ in\ \ Audit} * 100 = \%\ \ Identification\ \ Error$$

33. Confirmation by outside expert taxonomists will be obtained if deemed necessary.

34. All invertebrates will be housed and maintained in the laboratory upon completion of the project, or returned to the agency if required.

Reporting

Once processing and enumeration has been completed and QA/QC conducted data sheets are scanned and photocopied. The original and a physical copy of the data sheets are stored in secure locations on the UW-Parkside premise. Scans of the data sheets accompany the annual report.

Data Management and Documentation

Data sheets are converted to a digital format using Microsoft Excel or equivalent. All Root River benthic macroinvertebrate data are combined into a single file for a calendar year (Root River BMI Analysis YEAR).

Scanned data sheets and data files are stored locally with back-ups to campus OneDrive and iCloud. Cloud and local file organization are identical with a Root River Project parent folder and all files pertaining to a calendar year located with a Root River Report YEAR folder.

Safety and Environment

This section describes health, safety and environmental considerations for the processing of benthic macroinvertebrate samples.

Health and Safety

Laboratory Safety Instructions developed by the contractor for the sampling activities should be followed.

Hazards include, but are not limited to:

- Use of hazardous chemicals - limit exposure to ethanol and fumes by using personal protective equipment and working in a well-ventilated area.
- Repetitive motion fatigue – take breaks and switch tasks to avoid stiffness and strain.

References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002.

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Marchant, R. 1989. A subsampler for samples of benthic invertebrates. Bulletin of the Australian Society for Limnology 12, 49-52.

State of Wisconsin
Department of Natural Resources
PO Box 7921, Madison WI 53707-7921
dnr.wi.gov

Wadeable Macroinvertebrate Field Data Report

Form 3200-081 (R 08/14)

Instructions: **Bold** fields must be completed.

Station Summary

Waterbody Name	**Waterbody ID Code**	**Sample ID (YYYYMMDD-CY-FD)**

Sampling Location

SWIMS Station ID	**SWIMS Station Name**	**Database Key**

Latitude	**Longitude**	**Lat/Long Determination method (circle)**	**Datum Used if using GPS**
		SWIMS SWDV GPS	**NAD 27** or **NAD83**

Basin (WMU)	**Watershed Name**	**County**

Sample and Site Descriptors

Sample Collector (Last Name, First)	**Project Name**

Sampling Device

☐ Kick Net ☐ Surber Sampler ☐ Eckman
☐ Ponar ☐ Artificial Substrate ☐ Hess Sampler ☐ Other: _____

Habitat Sampled

☐ Riffle ☐ Run ☐ Pool
☐ Other ☐ Shoreline Composite ☐ Proportionally-Sampled Habitat
☐ Littoral Zone ☐ Profundal Zone ☐ Wetland

Total Sampling Time (min)	**Estimated Area Sampled (m²)**	**Number of Samples in Composite**	**Replicate No.** _____ **of** _____

Reason for Sampling

☐ Least Impacted Reference ☐ Baseline ☐ Impact / Treatment Site
☐ Control Site ☐ Trend ☐ Other: _____

Water Temp. (C)	**D.O. (mg/l)**	**D.O. (% sat.)**	**pH (su)**	**Conductivity (umhos/cm)**	**Transparency (cm)**

Water Color

☐ Clear ☐ Turbid ☐ Stained

Estimated Stream Velocity (m/s)

☐ Slow (< 0.15 m/s) ☐ Moderate (0.15 m/s - 0.5 m/s) ☐ Fast (>0.5 m/s)

Measured Velocity circle units mps **or** cfs	**Average Stream Depth of reach (m)**	**Average Stream Width of reach (m)**

Composition of Substrate Sampled (Percent):

Bedrock:_____ Boulders (basketball or larger):_____ Rubble (tennisball or basketball):_____ Gravel (ladybug to tennisball.):_____

Sand: _____ Clay:_____ Silt/Muck:_____ Overhanging Vegetation:_____

Aquatic Macrophytes:_____ Leaf Snags:_____ Course Woody Debris: _____ Other (_____):_____

Embeddedness of Substrate at Sample Site (%) _____ **Canopy Cover at Sample Site (%)** _____

Stream and Watershed Descriptors

N = Not a problem
U = Uncertain
PL= Present, Low Impact
PH= Present, High Impact

Factors that may be Influencing Water Resource Integrity	Local	Water-shed	Factors that may be Influencing Water Resource Integrity	Local	Water-shed
Biological			**Chemical**		
Algae: - Diatoms / Periphyton			Chlorine		
- Filamentous Algae			Dissolved Oxygen		
- Planktonic Algae			Nutrients (P, N....)		
Other -Specify:			Toxics: - Inorganic (Metals)		
Iron Bacteria			- Organic (PCBs, pesticides ...)		
Macrophytes			Other - Specify:		
Slimes			**Sources of Stream Impacts**		
Other - Specify:			Bank Erosion		
Physical			Point Source - Specify:		
Bank Erosion			Pasturing of Livestock		
Channelization - Upstream			Runoff: - Barnyard		
- Downstream			- Construction		
Hydraulic Scour / Channel Incision			- Cropland		
Impoundment: - Upstream			- Urban		
- Downstream			Septic Systems		
Low Flow			Tile Drainage - Organic Soils		
Sedimentation			- Minerals soils		
Sludge			Springs		
Thermal			Tributary(s)		
Turbidity			Wetland		
Other - Specify:			Other - Specify:		

Comments

Special Instructions for Laboratory

For Lab Use Only		
Sample Sorter	Taxonomist	Estimated Percent of Sample Sorted
Date Processed	Specimens Saved	

Assessment of Increased Sample Counts on the WDNR Macroinvertebrate IBI
Prepared by Mike Shupryt
January 2022

WDNR is reviewing its current bioassessment tools as part of process to eventually codify biocriteria. A review of the macroinvertebrate Index of Biotic Integrity (mIBI) by USEPA & Tetra Tech revealed that the mIBI worked well, but improvements could be explored. The first recommendation was to further refine WDNR's demonstration that the mIBI was predictably responding to disturbance. That lead to the creation of the new WDNR Stream Disturbance Model (Tetra Tech 2021). The model is used in this report to identify "reference" sites, but further refinements related to mIBI response to disturbance is not discussed here. Secondly, it was identified that WDNR sample counts (i.e. number of individuals identified in each mIBI sample) was on the very low end of other agency methods. Smaller sample counts had been commonplace in bioassessments, but contemporary bioassessments typically use much larger values (Carter and Resch 2013). Here I analyze the relationship of macroinvertebrate metrics used in the WDNR's macroinvertebrate Index of Biotic Integrity (mIBI) to increased sample counts. I propose correction factors that can be applied to specific metrics so they become bias-resistant to increased sample counts and make recommendations on future samples sizes for mIBI bioassessments.

The WDNR mIBI was developed by Weigel (2003) and has been used for waterbody condition assessments for years at WDNR. The macroinvertebrate collection methods used in the Weigel (2003) study were based on Hilsenhoff's (1987) standardized collection, identification and enumeration procedures. This includes a sample count that is ≥125 individuals, typically identified to species. The Hilsenhoff method includes a random grid subsetting procedure that identifies and enumerates all the individuals in a randomly selected grid. If the total number of individuals is <125, then another grid is selected and identified in its entirety, and so-on until ≥125 individuals are identified. This results in sample sizes that are nearly always greater than 125 individuals. For instance, at all the Wadeable Trends Stream Network (WSTN) samples collected since 2010 (44 sites collected once annually), the median sample size is ~175 (±66 SD).

As bioassessment programs have progressed through the years, many agencies have settled on a sample size much greater than 125 individuals, typically 300 or some even 500 individuals (Carter and Resch 2013). For comparison of EPA Region 5 states, Minnesota PCA, Michigan EGLE, Illinois EPA each use a ≥300 individual count for their assessment programs. (MPCA 2014, MDEQ 2014, IEPA 2008). Ohio EPA uses a Hester-Deny method that relies on standardized surface area and not subsetting procedure to standardize level of effort (OEPA 2015). Indiana DEM is currently updating their macroinvertebrate procedures. In addition to Region 5 state agencies, larger sample sizes for macroinvertebrate bioassessments are well supported in the scientific literature (Doberstein et al. 2008, Nichols et al. 2006, Vlek, et al. 2006, Ostermiller and Hawkins 2004). While nationally many states use ≥ or = 300 individual sample counts, some of these sates have only adopted procedures for increased sample sizes in the last 10 years. Carter and Resch (2013) speculate this is due to State's attempt to standardize to practices used by the EPA's National Aquatic Resource Surveys (https://www.epa.gov/national-aquatic-resource-surveys).

I conducted an analysis of the effects of sample counts (i.e. number of individuals identified per sample) on the mIBI and its component metrics to determine the implications of increasing sample size. Second, for any metrics that were correlated to sample size I developed a correction factor to adjust the metric for larger sample sizes. Lastly, I tested the new, corrected metrics in the original mIBI scoring framework to verify the corrections have reduced the correlation of mIBI to sample size.

I downloaded all of the macroinvertebrate data from SWIMS from Jan 1st 2000 to Dec 31st 2020 as possible samples for analysis. First, I eliminated all samples that were obviously large river Hester-Dendy samples, as those are a different field/laboratory method and IBI calculation. Some samples were unclear if they were large river or wadeable, so I opted to be more restrictive and have a smaller and more accurate dataset. I excluded any samples from a "Large River" Natural Community, any sample from Strahler stream order >5 and any sample remaining with greater than 600 individuals to ensure none were Hester-Dendy samples (min. count 500). Any sample containing fewer than 125 individuals was also eliminated. 125 is the minimum number for calculation for the mIBI, and while very low sample counts may represent degradation, low counts may also be due to inadequate sample collections. So that any further analysis of the metrics was truly looking at the effect of sample size, and not confounded by environmental disturbance, I selected only reference sites for further analysis. This was defined as stream reaches classified as Best Reference, Reference or Sub Reference by the WDNR's stream disturbance model. Lastly all remaining sites with >400 individuals were hand-checked assure they were not from large rivers. The final dataset resulted in 2,048 samples from 1,329 unique locations.

The resulting dataset consisted of many samples that were >300 individuals (Fig 1). These were from earlier experimental designs looking at the effect of sample size on the mIBI (~2003, although no changes to mIBI procedures implemented) or from other samples where the subsetting procedures randomly resulted in extraordinarily large sample counts, or other experimental designs not well documented in SWIMS. The distribution of sample counts is obviously heavily weighted to those with fewer than 200 individuals (~70%). However, there was also 411 samples with counts >225 allowing for a robust assessment of sample size without over-weighting the influence of a few large count samples.

Table 1. Macroinvertebrate metrics that have the strongest correlation to sample count.

DNR Parameter	Metric	Pearson's *r*
80104	Num. EPT Individuals	0.45
80129	Species Richness	0.19
80034	Genera Richness	0.17
80119	Num. Insect Taxa	0.13
80101	Num. Deposition Tolerant Genera	0.11

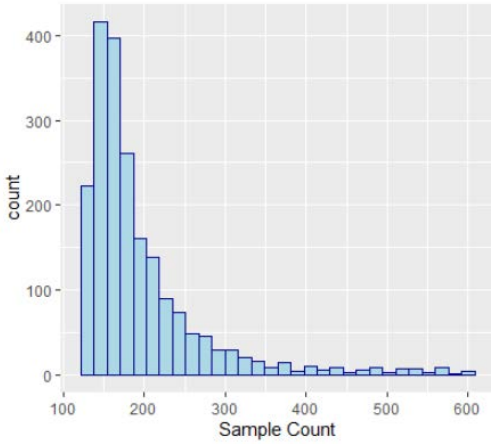

Figure 1. Histogram of sample counts from 2,048 reference site mIBI samples.

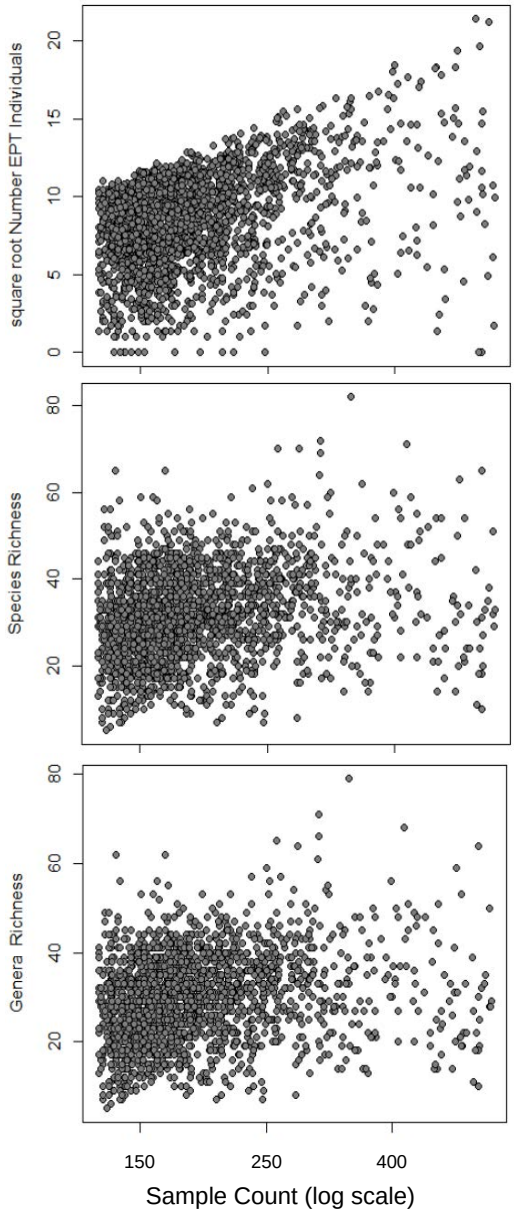

Figure 2. Scatterplot of the three metrics that most strongly correlated to sample counts.

To narrow down the list of potential metrics that would need adjustment with larger sample counts I calculated Pearson correlation among all mIBI metrics and sample count. Any metric that resulted in correlation of absolute value $r \geq 0.1$ was considered possibly correlated and required further examination (Table 1). The Number of EPT Individuals was clearly correlated ($r = 0.45$) to sample counts and needed to be adjusted, all other metrics were below $r = 0.2$. From visual examination of the relationship I selected $r = 0.15$ as a cutoff for metric adjustments, which is fairly inclusive as this degree of correlation is minor (for example, $r = 0.15$ means that ~2% of the variation in the metric is explained by sample count). With the remaining three metrics I then conducted linear regressions (Figure 2). The General Richness metric was dropped from further analysis because sample count explained < 5% of the total variation (R^2 ~= 0.03) while the Number of EPT Individuals and Species Richness had 4% of the variation explained by sample counts ($R^2 = 0.13$ and 0.04, respectively).

To remove the positive bias of Number EPT Individuals and Species Richness to sample size I constructed a straightforward correction factor. I used the regression slope between each metric and sample count to correct the metrics when the sample count was greater than 175. I selected 175 as a baseline as this was roughly the median sample count from all mIBI samples in this dataset (actual median = 172). For example, the (non-transformed) slope for Number EPT Individuals was ~ 0.33. Meaning that, for every three individuals identified one was an EPT taxa. A simple equation can then standardize the metric to 175 taxa by subtracting the expected increase in metric value solely due to sample counts greater than 175 individuals, but maintaining relative variability (i.e. not fixing y-intercept):

$$Number\ EPT\ Individuals\_Corrected = (sqrt(Number\ EPT\ Individuals) - (sample\ count - 175) \times 0.018)^2$$

$$Species\ Richness\_Corrected = Species\ Richness - (sample\ count - 175) \times 0.025$$

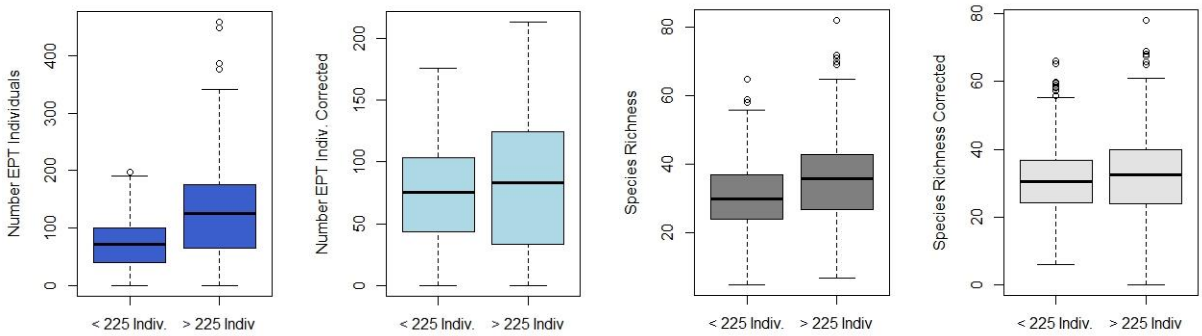

Figure 3. Boxplot of Number of EPT Individuals and Species Richness at low and high-count samples (above/below 225 individuals) before and after sample size correction factors. After correction metrics are more bias-resistant to sample counts.

The correction factors were able to adequately remove the relationship between sample counts and the candidate metrics. The Number of EPT Individuals Corrected now had a very weak relationship with sample counts (R^2 = 0.003). Before correction, there was a significant difference between the Number of EPT Individuals and sample counts < and > 225 (student's t-test, p<0.001, Fig 3 left two panels), where these same groups were no longer different after correction (p=0.54). Correction of the Species Richness metric had similar success with reducing the relationship with sample counts (R^2 <0.001) and the significant difference among groups was eliminated after correction (p<0.001 before, p=0.23 after, Fig 3 right two panels).

While testing the metrics provides insight into the inner workings of the mIBI, most users are interested on the implications to the mIBI score itself. I tested the influence of the corrected metrics on the mIBI by comparing the original mIBI and Corrected mIBI on the total set of 7,259 samples that passed the screening phase (2,048 reference samples plus all other samples). Overall, changes to the mIBI were usually less than 1 point on a 10-ish point scale (Fig 4 left panel). Unsurprisingly, the largest corrections happened among sites with the largest sample counts (Fig 4 right panel). At sample counts between 225 and 400 individuals the mean difference in Corrected mIBI was 0.37 (±0.48 SD) points. This correction is <5% of the total range of observed mIBI scores.

I conclude from this analysis that sample sizes can be increased for standard mIBI collections. With only minor correction factors applied to two of the metrics any bias form increasing sample sizes was easily mitigated. Furthermore, because the correction factors standardize the current metrics to the sample size that was used in mIBI creation, no additional adjustments need to be made to the mIBI scoring. Additionally, because the corrected metrics are scaled to 175 individuals, the median sample count used under the ≥125 count method, will still be valid once a larger sample count method is adopted. While the scores are comparable, the larger sample count method contains all the benefits of increasing sample size such as increased accuracy and precision, more robust to rare taxa and decreased interannual variability from sample collection artifacts (Doberstein et al. 2008, Vlek, et al. 2006, Cao and Hawkins, 2005, Ostermiller and Hawkins 2004).

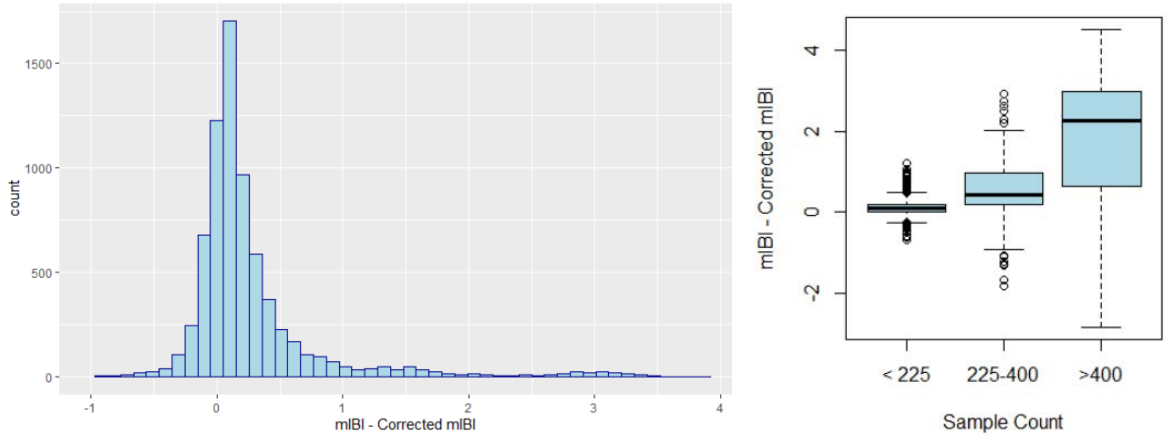

Figure 4. Histogram of the difference in Macroinvertebrate IBI minus Corrected mIBI (left panel) showing that most differences are small (-1 to 1 point) while some differences are larger, >2 points. The sites with the largest differences are extremely high sample counts (>400) shown by the boxplot of differences by sample counts (right panel).

Two important steps remain in updating the mIBI. One is to code these changes into SWIMS, which should be fairly easy given the straightforward equations. I recommend a new DNR Parameter Code be created to save both the corrected and uncorrected metrics in SWIMS for posterity. The second is to determine a minimum sample count for future mIBI samples. If set at ≥250, the subsetting procedure will yield a larger number and perhaps, result in sample counts very close to 300. If set at ≥300 Wisconsin will be more in line with other Region 5 states, but perhaps be sampling more than necessary and running into lab capacity in the future. In exploratory conversations with the UW-Stevens Point Aquatic Biomonitoring Laboratory (ABL) they are open to the ≥250 sample size method. If a sample count of ≥250 is chosen, per sample cost would be ~2x current level ($160 per sample) and ≥300 sample count ~2.4x ($194 per sample, J. Dimick personal communication February 2nd, 2022). However, this would lower the capacity of total number of samples the ABL could analyze per year. The WDNR has been well below the maximum cap for 3-4 years in a row and ≥250 would only have a marginal effect on actual number of samples collected.

I recommend the 250 minimum sample count method. This will more closely align Wisconsin with Region 5 states, the current state of the science and the increased certainty that comes with larger sample sizes as WDNR moves toward formalizing biocriteria in rule and for use in other rules (site-specific criteria for TP). Since the minimum sample count method results in ~50 more individuals sampled than the target, on average, the average sample count will be ~300 individuals. Although the per sample cost will increase to $160, the number of wadeable stream mIBI samples submitted it has been declining for many years. This increase in per sample cost can be absorbed under current monitoring budgets and UW-SP ABL capacity without severely limiting the number of sites sampled each year. Examining just the WSTN samples over the last six years only 6 out 250 samples (~2.4%) fewer than 250 total individuals in the sample. This was estimated by multiply the total sample count by 100/percent of sample sorted (percent sample sorted is only recently reported in SWIMS). That means 97% of samples likely had enough sample material to identify 250 individuals. WDNR would not need to

alter any field collection protocols, beyond perhaps spending another 60 seconds collecting each sample to ensure enough individuals are captured. No adjustments need to be made to field, laboratory or analytical methods or Impaired Waters Assessments (i.e. WisCLAM methodology) besides those described in this report.

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Appendix H. Habitat Assessment SOP

State of Wisconsin
Department of Natural Resources

Guidelines for Evaluating Habitat of Wadable Streams

Revised June 2002

Bureau of Fisheries Management and Habitat Protection
Monitoring and Data Assessment Section
101 S. Webster St.
Madison, WI 53707

(Modified from Simonson, et al. 1994.
Guidelines for Evaluating Fish Habitat in Wisconsin Streams
USDA Forest Service General Technical Report NC-164)

OBJECTIVES OF BASELINE MONITORING OF WADABLE STREAMS

The overall objective of baseline monitoring of streams is to gather information for science-based assessment and management of stream resources. Habitat, macroinvertebrate, and fish community data collected using standardized field protocols, provides objective physical and biological criteria with which to evaluate the condition of stream resources. Baseline information gathered from "least-impacted" reference streams will provide "reference conditions" i.e. the best attainable conditions for streams of similar type (class), information that can be used to objectively determine whether a stream is meeting its potential. Baseline information will allow resource managers to:

1. Classify streams according to their aquatic life potential, and determine whether streams are meeting their potential.
2. Help determine why some streams are not meeting their use potential.
3. Document the status and trends of the physical and biological integrity of stream resources over time and space.
4. Quantify and rank existing and emerging land and water use factors impacting streams.
5. Direct and evaluate Department land and water resource management activities, based on objective, quantifiable, physical and biological information.

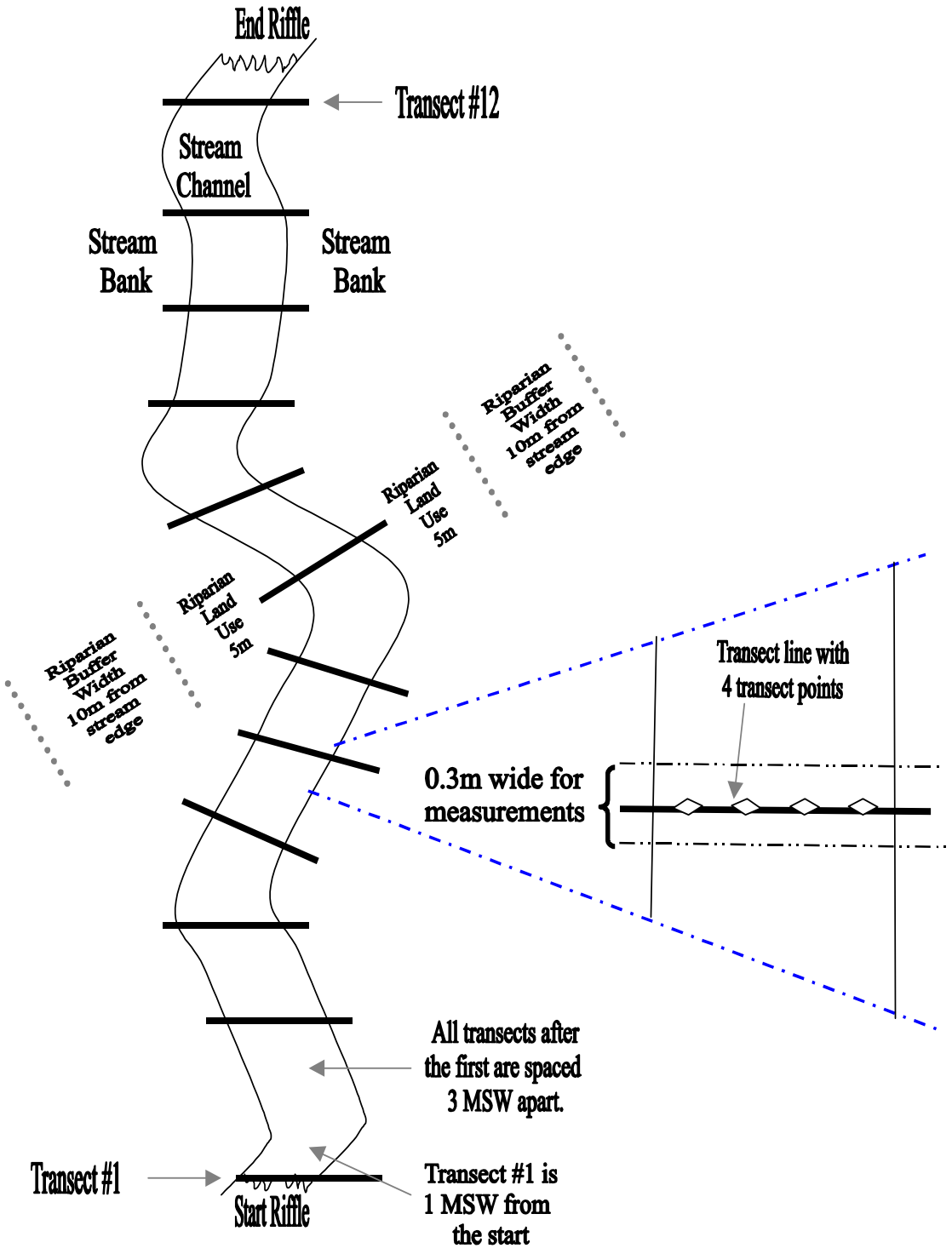
General Sampling Procedures:

Mean stream width (MSW) is an important characteristic of each stream assessment station (reach), and is used to define the length of the station and the spacing of habitat measurements (i.e., distances between transects) for most wadable streams. The MSW is based on the mean of **10** preliminary measurements of stream width from throughout the station (within approximate station boundaries), including all types of macro-habitats. If the stream width does not vary significantly throughout the approximate station length, the 10 width measurements can be taken closer to the start of the station to save time, instead of walking the entire approximate station length. **Station length should be 35 times the MSW for streams between 2.9 m and 23 m MSW.** For streams with a MSW less than 2.9 m, a 100 m long station should be sampled, and streams greater than 23 m MSW an 800 m long station is assessed. If the water level appears to be substantially (**> 0.15 m**) above normal, sampling should not occur (see **Station Summary** for determination of water levels). Once the MSW for a station has been determined, this value is used for **all** future habitat sampling, including future years when changes in riparian land use or instream habitat improvements may have caused a change in the actual stream width.

If a stream has well-developed pool-riffle structure, then each station should start and end at the downstream end of a riffle (Figure 1), even if this requires that stations be somewhat more or slightly less than 35 times the MSW in length (the distance between the second last and the last transect being greater or less than 3 MSW). Fish community data is collected within the same stream reach in which the habitat is assessed, and beginning and ending the station at the downstream end of riffles helps facilitate fish capture. Ideally, stations should not contain permanent tributaries or hydraulic controls (e.g., dams, old bridge abutments); and the beginning and end of the station should be some distance away from bridges to avoid the influence of stream ponding or scour on the fish and benthic invertebrate community by bridges or old bridge abutments.

Habitat within a station is quantified using the transect method (Figure 1). Sampling of stations proceeds in an upstream direction and a variety of channel, substrate, and bank characteristics are measured or visually estimated along transects. A minimum of 12 transects are sampled within each station to provide an overall assessment of stream habitat. **Parameters unlikely to vary substantially within a station (flow, water chemistry parameters) are measured only once for each station**.

FIGURE 1. Station and Transect description. Station length is 35 x MSW.



Data Collection:

Four data sheets are used in the stream habitat evaluation: **Station Summary**, **Flow Data**, **Map Data**, and **Transect Data**. Clean and completed data sheets are attached. The first three sheets apply to the whole station, and there is typically only one of each of these sheets filled out per station (a second Station Map may be needed for stations with diverse habitat). The Transect Data sheet applies to data collected along transect lines across the stream, and 12 or more sheets are filled out per station. Guidelines for filling out each data sheet are given on the following pages. A list of equipment used for stream habitat evaluations and suppliers is presented in Table 1.

STATION SUMMARY DATA SHEET

This sheet summarizes location, water characteristics, and large-scale channel and basin characteristics for the entire station. Much of the data on this form are derived from U. S. Geological Survey (USGS) maps or from the other data sheets. The parameters on this sheet are as follows:

Location ----------

Stream Name The name of the stream as shown on the most recent USGS 7.5' topographic map. USGS maps can be accessed on the WDNR Intranet. The stream name used here should be identical to that used on all other data sheets, and to that used for all other stations on the same stream. Make sure the spelling of the name is accurate and includes all parts of the stream name (e.g., "West Branch", "Middle Fork", "River", "Creek", "Brook", "Run", etc.) to avoid confusion. Other commonly used names for the stream can be written here in parentheses.

Waterbody ID Code A unique seven-digit number identifies each stream (all streams, rivers, and lakes in Wisconsin). All waterbodies have or should have an assigned number. These numbers are available on the WDNR Intranet, under the listing for “SWIS Tabular Database Access System” for the WDNR Register of Waterbodies (ROW).
(http://dnrnweb.dnr.state.wi.us:8890/dnr/pk_swis_web_row.row_search)
As with Stream Name, Waterbody ID Code should be the same for all stations on a stream.

Site Mile The reporting of this parameter is optional. The distance along the stream channel from the mouth of the stream to the downstream end of the station. This distance is a useful shorthand for indicating and identifying the location of the station. Site mile should be measured on the most recent USGS 7.5' topographic map to the nearest 0.1 mile using a map measurer (map wheel).

Station No. If a stream has two or more stations, the downstream station is number 1, the next upstream is number 2, and so on. If there is only one station, the number is 1. Always assign a station number.

Date Fill in the date when the habitat data were collected for the station, use the YYYYMMDD format (e.g., 20000607 equals June 7, 2000).

Starting Location A precise narrative description of the point on the stream where the habitat survey began (i.e., the downstream edge of the station). The description should include the exact distance and direction of the start from a "permanent" landmark such as a bridge, building, road marker, rock formation, etc. When referring to roads or bridges include the complete name of the road. **Avoid using landmarks that might be lost in future years** (e.g., don't use tree or fence lines). Make the description as specific and precise as possible so that someone visiting the station for the first time can easily find the starting point.

Township, Range, Section, ¼ - ¼ Section, 1/4 Section Legal description for the Starting Location of the station within the Public Lands System. These can be determined from recent USGS 7.5' topographic maps, a detailed county map, or a county Plat book. On a topographic map, a "land locator" template is useful for determining the ¼ - ¼ and 1/4 Sections, which are indicated by a compass direction (NW, NE, SW, or SE). Note that in Wisconsin, all Townships are "N" (north), but Range can be either "E" or "W" (east or west). Make sure the appropriate letter is included for both Township and Range.

Latitude and Longitude It is important that geographic coordinates of the **start** of the station are recorded, along with the Method Used to determine latitude and longitude (e.g. USGS map, mapping software, global positioning system (GPS) units). The geodetic Datum Used upon which the coordinates of the map or GPS coordinates are based (e.g. North American Datum 1983 (NAD 83)) should also be recorded. Datum for USGS topo maps are shown on the map legend. Latitude and longitude units eventually need to be converted into decimal degrees. This can be done in the office after the field data is collected. To convert a GPS reading from degrees, minutes, seconds into decimal degrees: divide the seconds by 60 and add to the minutes, then divide the minutes by 60 and add to the degrees. To convert degrees, minutes, into decimal degrees: divide minutes by 60 and add to degrees.

7.5' Quad Map Name The name of the USGS 7.5' topographic map on which the station is found.

Basin Name and Watershed Name The name of the DNR Basin and watershed in which the stream is located. These are listed in the Basins and Watersheds of Wisconsin table found on the Wisconsin Department of Natural Resources internet web site at:
http://www.dnr.state.wi.us/org/gmu/sidebar/watersheds.html

County The name of the county in which the station is located.

***Water Characteristics*----------**

All water characteristics should be measured in water of moderate current at least 0.15 m above the bottom and 0.15 m below the surface (if possible).

Time The time (in "military" format; i.e., 9:30 AM is 0930 hours and 9:30 PM is 2130 hours) at which measurements of water characteristics are made.

Air Temperature If possible, measure air temperature during the warmest part of the day to estimate maximum values. Take the air temperature in the shade with a **dry** thermometer; evaporation from a wet thermometer will lead to a measured air temperature lower than the true value. Measure to the nearest 1 degree Celsius.

Water Temperature Take the water temperature in mid-channel, during the warmest part of the day to estimate maximum values, if possible. Measure water temperature away from any large objects that project above the surface. Such objects may act to efficiently transmit heat and influence local water temperature. Avoid areas of the stream where subsurface or bank springs may be present. Measure to the nearest 1 degree Celsius.

Conductivity The reporting of this parameter is optional. If reported, measure with a high-quality

electronic meter. Most conductivity meters have built-in automatic temperature compensation to 25 °C (77 °F), but this should be confirmed before using the meter. On some older meters the temperature compensation must be set by hand, and on others, there is no compensation. For the latter meters, conductivity at 25 °C can be calculated using procedures outlined in "Standard Methods for the Analysis of Water and Wastewater", a book available at many WDNR offices. Whatever meter is used, it should be calibrated before every use. Measure conductivity in umhos/cm.

Turbidity The reporting of this parameter is optional. If reported, measure with a high-quality electronic meter, which should be calibrated before every use. Measure and report conductivity in nephelometric turbidity units (NTUs).

Total Dissolved Solids The reporting of this parameter is optional. If reported, measure with a high-quality meter, which should be calibrated routinely. Follow the manufacturer's instructions for use and maintenance (e.g., the membrane and the electrolyte for the probe should be replaced frequently during the field season). Report total dissolved solids in milligrams per liter (parts per million).

Dissolved Oxygen (DO) The reporting of this parameter is optional. If reported, measure with a high-quality meter, which should be air-calibrated before every use. Follow meter manufacturer's instructions for use and maintenance (e.g., the membrane and electrolyte for the probe should be replaced frequently during field season, or immediately if there is an air bubble in the probe electrolyte solution). Report DO in milligrams per liter (parts per million).

Dissolved Oxygen % Saturation The reporting of this parameter is optional. If reported, measure with a high-quality dissolved oxygen meter, which should be air-calibrated before every use.

pH The reporting of this parameter is optional. If reported, measure with a high-quality meter, which should be calibrated routinely. Follow manufacturer's instructions for use and maintenance (e.g., the membrane and electrolyte for the probe should be replaced frequently during field season). Report pH to 0.1 units.

Flow Taken from the **Flow Data** sheet. Flow data can be calculated in cubic feet or meters per second, but should be reported on the **Station Summary** data sheet in cubic meters per second (CMS). To convert CFS to CMS, multiply the CFS value by 0.0283. To convert CMS to CFS, divide CMS by .0283. There is also a web site that can do many of these conversions for you: http://www.sciencemadesimple.net/conversions.html

Water Level An estimate of the level of the stream at the station. Check the appropriate category, and measure the vertical distance (nearest 0.01 m) if "Above" or "Below" normal. If there are areas of stream bed that are dry but look as if they would normally be underwater, then the water level is "Below"; measure the vertical distance between the current water level and the "Normal" water level. If the stream is flowing over or through areas that have terrestrial vegetation (e.g., grasses, forbs, willows, but not bulrushes and cattails) then the water level is "Above"; measure the vertical depth of water above the normal water line. Otherwise, the water level is "Normal" (at or near baseflow). **Sampling should not occur if the water level appears to be substantially (0.15 m) above normal.** Note: Channel characteristics rather than the amount of precipitation in the recent past should be used to determine water level. Streams with a high proportion of ground water input may retain normal flows well into drought periods. Conversely, such streams may show little response to heavy rains, particularly if the local water table has been greatly lowered by prolonged drought. On the other hand, streams that are runoff dominated may fluctuate greatly in water level in response to short-term wet and dry periods.

Water Clarity Record whether the water is Clear, Turbid from suspended sediment, or Stained due to dissolved organic compounds.

Channel and Basin Characteristics ----------

Stream Widths These 10 spaces are provided for the determination of Mean Stream Width. Ten preliminary measurements of stream width (nearest 0.1 m) throughout the approximate station length should be made to determine the MSW. These measurements should be taken at different points to incorporate the variation of pools, riffles, and runs. If it appears that the width of the stream is relatively uniform throughout the approximate station length, all stream width measurements can be taken closer to the start of the station to save time.

Mean Stream Width This space is provided for the average (nearest 1 m) of the above Stream Width measurements. This value is used to determine the length of stream to sample (Station Length) and the distance between transects. The Mean Stream Width value can be rounded up to make easier to determine Station Length and Transect Spacing. For further explanation see page 3, **General Sampling Procedures**.

Transect Spacing Record the distance (nearest 1 m) between transects. For streams between 2.9 m and 23 m wide, start the first transect at **1 times** the Mean Stream Width from the downstream end of the station, and the rest of the transects are spaced **3 times** the Mean Stream Width from each other. If the stream is less than 2.9 m Mean Stream Width, twelve transects are also established, but the first transect is 4m upstream of the start of the downstream end of the station, and each subsequent transect is spaced 8 m apart. On streams greater than 23 m Mean Stream Width, the number of transects is increased to 20; with all transects spaced 40 m apart.

Station Length The length of the station, following the center of the stream channel. Measure, using a tape measure, to the nearest 1 m. For streams less than 2.9 m Mean Stream Width, the Station Length is 100 m. Streams with Mean Stream Width greater than 2.9 m but less than 23 m the Station Length is equal to 35 times the Mean Stream Width. For streams greater than 23 m Mean Stream Width, the Station Length is 800 m.

Channel Condition A qualitative assessment of whether or not the station has been channelized or ditched (straightened and dredged to create a channel with few bends and generally uniform widths and depths). If the station shows no evidence of channelization, check "Natural". If the station appears to have been channelized many years before, but seems to be returning to a more natural morphology (beginnings of stream meanders or pool-riffle formation evident), check "Old Channelization". If the station appears to have been channelized within the last few years, or there is little evidence of meander or pool-riffle formation, check "Recent Channelization". If the station has been channelized, and is a straight, uniform channel kept in place over long distances by concrete stream banks and/or a concrete bed (or is kept in place by other artificial means, such as metal bulkheads or brick retaining walls), check "Concrete Channel".

Percent Channelization An estimate of the proportion of the **assessed stream reach** that is channelized.

Sinuosity The length of the meandering stream channel measured over a 1 km straight line distance within which the stream assessment reach is located. Measure with a map wheel on a USGS 7.5' topographic map. This can be done in the office before or after sampling.

Gradient The overall decrease in elevation (on a per kilometer basis) of the stream over the entire station (= elevation drop / distance). Determine from USGS 7.5' topographic maps, using a map wheel. First, find the downstream and upstream ends of the station on the map. Then find the first contour line that **crosses** the stream upstream of the station and the first contour line that **crosses** the stream downstream of the station. For low gradient streams this may require going to additional maps, covering many miles of stream, and possibly including other streams. With the map wheel, determine the distance along the stream channel between these two contour lines. Then determine the elevation drop between these two lines. To determine elevation drop, count these two contour lines plus each additional contour line that crosses the stream between these two points (sometimes there are no contour lines crossing the stream between these two points). Subtract one of these lines, and multiply the remaining number of contour lines by the difference between two lines. (The difference between two lines is usually either 10 ft or 20 ft. Most topographic maps have 10 ft contours, but some have 20 ft contours; check the legend at the bottom of the map.) For example, if there are 2 contour lines that cross the stream in the station, plus the first line above and below the station, there are 4 lines. The difference in elevation between these = (4 – 1) multiplied by either 10 ft or 20 ft. Divide the elevation drop by the distance measured by the map wheel. This is the gradient for the station. Convert feet/mile to m/km by dividing by 5.3.

Stream Order A qualitative measure of stream size, based on the amount of branching of the watershed upstream from the station, using Strahler's modification of Horton's original system. Generally, the higher the order, the larger the stream. Determine from USGS 7.5' topographic maps; usually requires multiple maps because the entire stream network upstream from the station must be examined. In making determinations, all "blue lines" (streams) on the maps, including intermittent streams, are included. The order system is as follows: All streams (including intermittent streams) from their source downstream to their first tributary are **First** order (stream order is "1" on data sheet). When two first order streams meet, the stream below this confluence is **Second** order (stream order is "2"). When two second order streams meet, the stream below this confluence is **Third** order (stream order is "3"), and so on. When two streams of unequal order meet, the stream order below this confluence is equal to the higher of the two orders. For example, if a first and a third order stream meet, the stream below this confluence is third order. Stream order increases only when two streams of equal order meet. Wadable streams are typically first through fourth order.

Basin Area The reporting of this parameter is optional. The basin area equals the surface area of the entire watershed upstream from the downstream end of the station. Basin area can often be determined from the book "Drainage Area Data for Wisconsin Streams" (U.S. Geological Survey Open-File Report 83-933), which is available at most WDNR offices. This book gives the drainage area in square miles (multiply square miles by 2.590 to get square kilometers for this data sheet) for many locations on many different streams. If the exact location (within 0.25 miles) of the station is not given in the book, but basin areas for locations downstream **and** upstream of the station are given, then the basin area for the station can be determined by linear interpolation (use the Site Mile for the station and stream miles for the downstream and upstream locations with known basin areas to interpolate). If no data from upstream or downstream locations are available, basin area can be determined by using a planimeter, or by digitizing the area within watershed boundary on USGS 7.5' topographic map(s).

Mean Distance Between Bends Taken from the DISTANCE SUMMARY of the **Station Map** data sheet.

Mean Distance Between Riffles Taken from the DISTANCE SUMMARY of the **Station Map** data sheet.

Total (Sum) Length of All: Riffles Pools Runs Taken from the DISTANCE SUMMARY of the **Station Map** data sheet.

Mean Length of Individual: Riffles Pools Runs Taken from the DISTANCE SUMMARY off of the **Station Map** data sheet.

Photographic Documentation **(optional) ----------**

An accurate time-series of photographs (digital or 35mm slides) of the station may be important for documenting changes in habitat that occur over the duration of habitat management projects, or changes in stream habitat associated with changes in watershed land use. Photographs should be taken from the same point in the stream each time the station is sampled. The first photograph taken at each station should be on the **Station Summary** data sheet, so that subsequent photographs can later be identified as to location. The frame numbers of photographs taken at set locations in the station should be recorded on the **Station Summary** data sheet. Some convenient locations, such as looking upstream at the station from the downstream end of the station and looking downstream from the upstream end of the station, are listed on the data sheet. Additional locations, looking upstream from the upstream end of the station and looking downstream from the downstream end of the station, are included on the data sheet and can be used to document conditions upstream and downstream from the station. Film should be developed promptly and slides should be immediately labeled with Stream Name, Date, Station Number, location within the station (e.g., looking upstream from the upstream end), and any other pertinent information.

Person(s) Who Collected Habitat Data The **full** names of the person(s) who actually measured or estimated the habitat parameters (water level, substrate coverage, bank vegetation/land use, etc.) during the habitat survey. All field crew members should participate in all aspects of the habitat survey both collecting and recording information.

COMMENTS / NOTES Any and all information that seems to be relevant to the habitat survey but is not recorded anywhere else on the data sheets. This information could include weather conditions (especially regarding the last significant precipitation in the watershed), notes on habitat features that were unusual or difficult to interpret, problems with equipment or measurements, and observations on biotic characteristics of the stream and riparian zone. Note model number and serial number (or some other unique identifier) for each of the meters used to determine WATER CHARACTERISTICS.

STATION MAP DATA SHEET

This data sheet provides a quantitative and visual description of the length and position of the major macro-habitat features of the station (Bends, Pools, Runs, Riffles, Islands, Log jams, Beaver dams). On the MAP DATA sheet (page 3), record the length of the feature and its distance from the downstream edge of the station (measure to the nearest 1 m with a tape measure). Include the downstream and upstream boundaries of the station and the distances to the nearest fixed reference point in the station (e.g., USGS benchmark, bridge, rock formation, etc.). Record all bends and riffles within and immediately upstream and downstream of the station (if within 35 stream widths of the station), and any islands, logjams, or beaver dams within the station. Also include any other specific habitat or environmental problems within or adjacent to the station. The back of the MAP DATA sheet (page 4) is used for an optional hand-drawn map of the station. The hand-drawn map will not be captured in the electronic statewide stream database, and should only be draw if of value to local resource managers. At stations with high macro-habitat heterogeneity, more than one Station Map data sheet may be required. The variables on the data sheet are as follows:

Stream Same as for Stream Name on the **Station Summary** data sheet.

Waterbody ID Code Same as for **Station Summary** data sheet.

Site Mile Same as for **Station Summary** data sheet.

Station No. Same as for **Station Summary** data sheet.

Date Same as for **Station Summary** data sheet.

Distance From Start (column) The distance, following the center of the channel, from the downstream end of the station to the downstream end (or middle, in the case of bends) of each Stream Feature that is encountered (measure to the nearest 1 m with a tape measure). It may be helpful to measure from permanent features downstream of the start of the station as indicated by a minus sign "-" to the start of the station (start of the station = distance "0"). This helps to identify the start of the station. All Stream Feature measurements are measured from the start of the station, and the last value should equal the Station Length (from the Station Summary data sheet).

Stream Feature (column) Record the stream macro-habitats encountered while moving upstream from the downstream end of the station. Macro-habitats include bends, riffles, runs, pools, islands, dams, and logjams, and are defined as:

Bends: Curves in the channel where the channel changes from its prevailing direction by at least **60 degrees**. Distances should be measured to and from the center of the bend. Bend angles can be measured with a compass by subtracting the headings of the channel upstream and downstream from the bend.

Riffles: Areas of the stream characterized by shallower than average maximum depths and obvious surface turbulence. Water velocity is faster than average. In large streams and rivers, deep, fast riffles are called rapids. During high flows some riffles may become runs.

Runs: Areas of the stream with average maximum depths and little or no surface turbulence. Water velocities may be fast or slow, but the water surface appears generally smooth. Runs with slow velocities are sometimes called glides. During droughts, many shallow runs may become riffles.

Pools: Areas of the stream with deeper than average maximum depths, with no obvious surface turbulence or broken water. Water velocities are always slow. The longitudinal profile of the streambed in a pool is often bowl shaped. "Pocket water" refers to groups of small pools located behind boulders or other obstructions to flow, often in areas of otherwise fast or turbulent flow.

Islands: Areas of land between the stream banks that are surrounded on all sides by a substantial portion of the stream's water. Areas with nearly all of the stream's flow on one side and minimal flow on the other are not considered islands. The number, position, size, and shape of islands may vary with water level. Islands contain soil or numerous rocks; exposed sand or gravel/cobble bars are considered islands, but boulders that project above the water surface are not.

Dams: Intentional structures (constructed by either humans or beavers) that, when in good repair, completely cross the stream channel and block flow. Usually, dams pool water behind them, and there is a sharp drop in water surface elevation at the dam.

Log Jam: A group of three or more large diameter (> 0.20 m) intermingled logs partially or completely submerged in the channel that substantially alter flow and sedimentation patterns. When large and dense, logjams may be similar to dams in their appearance and impact on the stream.

Distance Summary ----------

Distance Summary measurements can be obtained from the Distance From Start and Stream Feature columns on the Map data sheet.

Distances Between Bends The distance between the middle of one bend and the middle of the next bend upstream. Measure and record only those bends with a change in direction of at least 60 degrees (can be determined with a compass). Record the distances between bends within and adjacent to the station. The first row is the distance between the first bend within the station and the first bend downstream outside of the station, if there is a bend within a distance of 35 times the mean stream width (MSW) from the downstream end of the station. The second row (1st - 2nd) is the distance between the first and second bends upstream from the start of the station; the third row (2nd - 3rd) is the distance between the second and third bends upstream, and so forth. The last row "- Upstream" is the distance between the most upstream bend within the station and the first upstream bend outside of the station, if there is a bend within a distance of 35 times the MSW from the upstream end of the station. The "sum" and "mean" rows summarize all the distances between bends.

Distances Between Riffles The distance between the upstream end of one riffle and the start of the next upstream riffle. The actual length of each riffle is **not** included in this distance. Fill in each row following the same protocol as for Distances Between Bends.

***Length of Individual Riffles, Pools, and Runs*----------**

Riffles, Pools, and Runs The length of each riffle, pool, and run within the station, starting with the downstream-most one of each type and working upstream to the upstream end of the station. These columns can be filled out using the information in the Stream Feature column.

STATION FLOW DATA SHEET

This data sheet is used when calculating instantaneous flow rate, also known as discharge. The data on this sheet are from one stream location within the station that ideally meets the following criteria. The location should be in an area of smoothly flowing water with no obvious turbulence (i.e., a run). The channel should be free of obstructions to the flow of water, and flow should be in a uniform downstream direction (i.e., no eddies). Banks should not be undercut, the bottom should be relatively smooth, and depths should change gradually across the stream.

Discharge is measured using a transect technique, with depths and water velocities measured at set intervals across the width of the stream. Once a suitable location has been chosen, a tape measure is used to determine the actual stream width and to provide a guideline for depth and velocity measurements. Depth and velocity should be measured at a **minimum** of **10 points** along the transect, and all measurements must be very precise. Stream discharge is the sum of the products of depth, velocity, and width interval for each measurement point. The parameters on this data sheet are as follows:

Stream Name Same as for Stream Name on the **Station Summary** data sheet.

Waterbody ID Code Same as for **Station Summary** data sheet.

Site Mile Same as for **Station Summary** data sheet.

Station No. Same as for **Station Summary** data sheet.

Date Same as for **Station Summary** data sheet.

Stream Width The actual width (nearest 0.1 m) of the stream (wetted portion of channel) along the transect.

Distance from Left Bank The distance (nearest 0.01 m) along the transect line, perpendicular to the direction of flow, from the left bank (looking upstream) at which depth and velocity measurements are made. In streams narrower than 3 m, measurements should be taken at evenly spaced intervals that are narrow enough to allow for as least **10 separate measurements**. For example, if a stream is 2.1m wide, then depth and velocity measurements should be taken every 0.2 m. In streams greater than 3 m but less than 10 m in width, depth and velocity measurements should be taken **every 0.3 m**. In streams wider than 10 m, depth and velocity measurements should be taken **every 0.5 m**.

Depth The depth (nearest 0.01 m or ft) of the stream at that point. This should be determined with a calibrated wading staff, such as the one used for making velocity measurements.

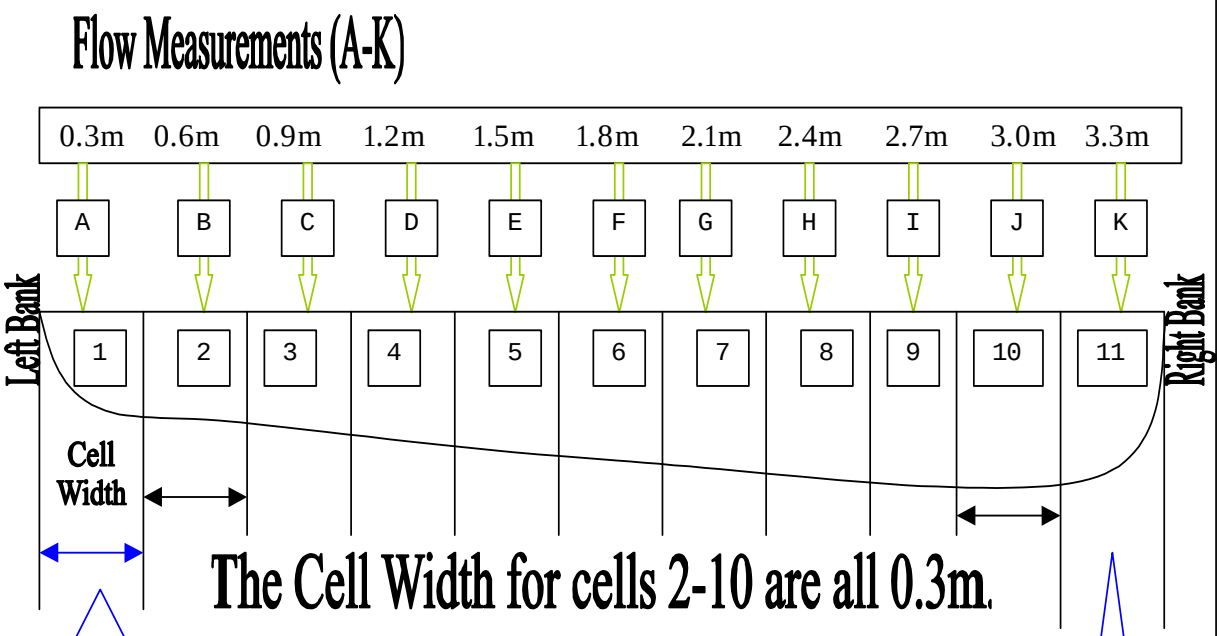
Velocity The velocity (nearest 0.01 m/second or ft/second) of water at that point on the transect. Velocity should be determined with a high quality current (flow) meter, either an electronic or rotating-cup meter, attached to a calibrated, top-setting, wading staff for accurate and precise placement in the water column. In water **shallower than 0.8 m**, a single velocity measurement is made at a depth of **60%** of the distance between the water surface and the bottom of the stream. For example, if the water depth is 0.19 m, then velocity is measured 0.11 m below the water surface. In water **deeper than 0.8 m**, two velocity measurements are made one at **20%** and the other at **80%** of the distance between the

water surface and the bottom of the stream. For example, if the depth is 1.1 m, then velocity measurements are made 0.22 m and 0.9 m below the water surface. The mean of these two measurements is then used in calculations.

Cell Width In most instances, cell width is equal to the interval (nearest 0.01 m) between the points where velocity and depth are measured. For all but the first and last points on the transect, the cell width for a particular point is equal to one half the distance between it and the previous point plus one half the distance between it and the next point. If points are evenly spaced (e.g., every 0.3 m), this is equivalent to the distance between two points (e.g., 0.3 m). For the first and last points, the cell widths are somewhat different. For the first point (nearest the left bank), the cell width is equal to the distance between the left bank and the first point plus one half the distance between the first and second points. Thus, if the first point is 0.3 m from the left bank, then the cell width for this point is 0.45 m. For the last point (furthest from left bank and closest to right bank), the cell width is equal to the distance between the right bank and the last point plus one half the distance between the last and next-to-last point. Thus, if the last point is 3.3 m from the left bank, the stream width is 3.5 m, and the interval between points is 0.3 m, then the cell width for this point is 0.35 m (Figure 2).

Product Depth times velocity times cell width (**make sure units are all in meters**). Values in the Product column are summed to give the discharge for the station in cubic meters per second, but can also be recorded on this sheet in cubic feet per second; however, on the **Station Summary Data** sheet it must be recorded in CMS. See Flow on the **Station Summary Data** sheet (Page 7) for more information about conversion values.

FIGURE 2: Calculation of Cell Width for a 3.5m wide stream.



To calculate the cell width for cell #1, first measure the distance from the left bank to point A. Second add 1/2 the distance from point A to point B. In this case the distance from the left bank is 0.3m plus 0.15m (1/2 distance between A and B). Thus, the cell width is 0.45m.

To calculate the cell width for cell #11 first measure the distance from the right bank to point K. Second add 1/2 the distance from point K to point J. In this case the distance from the right bank is 0.2m plus 0.15m (1/2 distance between K and J). Thus, the cell width is 0.35m.

TRANSECT DATA SHEET

This data sheet is used for recording information on the physical characteristics of stream and riparian habitat along a minimum of 12 transects within the station. One data sheet is filled out for each transect. On streams between 2.9 m and 23 m MSW, the first transect is located a distance of one MSW upstream from the downstream end of the station. Subsequent transects are spaced three MSWs apart**. If possible the start (first transect on the downstream end) of the station and the end of the station (last transect) should end on a riffle or in a shallow run even if this increases the distance between the last and second to last transect**. For streams less than 2.9 MSW the station length is 100 m, the first transect is located 4 m upstream from the start of the station, and subsequent transects are spaced 8 meters apart. On streams greater than 23 m MSW the station length is 800 m, the number of transects is increased to 20, and all transects are spaced 40 m apart. For baseline monitoring, the fish community is assessed within the habitat station; starting and ending the station in a shallow run or riffle will help insure that all fish can be captured within the station since blocknets are not used. Each transect consists of several measurements or visual estimates made within 0.3 x 0.3 m quadrate centered around each of the **4** equally-spaced **transect points**, along the **transect line** that is perpendicular to the flow of water (Figure 1). The number of transects, and hence the number of **Transect Data Sheets**, depends on the length of the station, but is always **a minimum of 12**.

Stream Name Same as for Stream Name on the **Station Summary** data sheet.

Waterbody ID Code Same as for **Station Summary** data sheet.

Site Mile Same as for **Station Summary** data sheet.

Station No. Same as for **Station Summary** data sheet.

Date Same as for **Station Summary** data sheet.

Transect No. The transect at the downstream end of the station is number 1, the next one upstream is 2, the next one upstream from that is 3, and so on. Thus, each transect data sheet for a station should have a different Transect number.

Distance from Start The distance, following the stream channel, from the downstream end of the station ("Start") to the current transect. This should be measured to the nearest 1 m with a tape measure. If all transects are positioned three MSWs apart, and Transect No. 1 is located 1 MSW from the downstream end of the station, then Distance from Start should equal [(Transect No. - 1) x (3 x MSW)] + MSW.

Stream Width Stream width measurements are taken with a tape measure to the nearest 0.1 m, along the transect line. Stream width is the actual wetted width of the channel along the transect. Islands, isolated pools, backwaters not in contact with the stream at the transect, and wetlands or swamps along the stream are not included in the measurement.

Habitat Type Check the habitat type that exists at the transect line. Check only the predominant type, even if more than one type is present. See the definitions for riffle, pool, and run in the station **Map Data** sheet (pages 10-12).

Bankfull Depth The reporting of this parameter is optional. Bankfull is the volume of water that fills

the stream channel to the top of its banks but does not overflow onto the flood plain, and on average occurs every 1.5 years. The tops of point bars or central bars within the active stream channel, the height above the stream that exposed plant roots below an intact layer of soil are visible, and the base of mature alder (Alnus spp.), are good indicators of stream water elevation at bankfull. One stream bank is usually lower than the other, and the **lowest stream bank** is the one that should be used to determine bankfull depth. Bankfull depth is measured to the nearest .01 m from the stream bottom at the deepest point (thalweg) in the stream. If there is no obvious stream bank, or the stream edges are marsh-like with emergent vegetation, it may not be possible to measure bankfull depth. In these situations, draw a line through the bankfull depth space on the data sheet.

Bankfull Width The reporting of this parameter is optional. Bankfull width is the maximum width the stream could reach before overflowing the bank. Measure to the nearest .01 m.

***Channel Position Measurements*----------**

Several characteristics, including Depth, Embeddedness, Substrate, Algae Abundance, Macrophyte Abundance, and Canopy/Shading are each measured at four evenly spaced positions along the transect line. To determine these four positions divide the Current Stream Width along the transect line into fifths (5 equal segments). Starting from the left bank (facing upstream), measurements are made at each of the four boundaries between segments; i.e., at 1/5 the distance between the left and right banks, at 2/5 the distance, 3/5 the distance, and 4/5 the distance. Each measurement is entered in the appropriate column on the form. For example, if the stream is 2.7 m wide, each segment is 0.54 m, and depth measurements are taken along the transect at 0.54, 1.08, 1.62, and 2.16 m from the left bank. An additional Water Depth measurement is made at the deepest point (the thalweg) along the transect line, if the deepest point is not located at one of the four evenly spaced points. In the event that the deepest point occurs at one of the transect point, record the depth measurement in the Channel Position (Fifths of Current Stream Width) column **and** Deepest Point column.

Water Depth The depth of the stream at each transect point. This should be measured to the nearest 0.01 m with a meter stick or calibrated wading staff, such as the one used for making velocity measurements. Make sure the measuring device (meter stick or wading staff) is not sticking into the sediment, so that only the actual water depth is measured. In water depths greater than one meter, use another measuring device or stack one meter stick on top of the other to get the actual water depth. **If the water is too deep to wade, then estimate Water Depth.** If a boulder is directly on the transect point, measure the depth next to the boulder.

Depth of Fines & Water The total depth of the water **plus** the depth of sand, silt, or other fine sediments (< 2 mm in diameter) that overlay or comprise the streambed. Measure to the nearest 0.01 m by pushing a meter stick down into the sediment. **Do not push the meterstick down into hard clay or gravel – only measure the amount of fine sediment lying on top of the bottom substrates (ie. fine sediment may be lying on top of a layer of clay or gravel. If there is a lot of resistance when pushing the meterstick into the sediment, you are probably measuring the clay or gravel in addition to the fine sediment lying on top.) If the bottom substrate is gravel and there is not a layer of fine sediment covering it (feel the stream bottom with your hand to determine if there is any fine sediment over the gravel), do not measure the depth of the gravel.** The combined measurement of Depth of Fines & Water is later converted to depth of fines by subtracting the Water Depth (measured above).

Embeddedness of Coarse Gravel and Rubble/Cobble Embeddedness is the degree to which coarse **gravel and rubble/cobble (rocks 16 - 260 mm in diameter**) are surrounded by or covered with sand, silt, and other fine substrates < 2 mm in diameter. Visually estimate (to the nearest 10%) the average

amount of embeddedness within a 0.3 m x 0.3 m quadrate on the stream bottom centered on the transect point. As a guide for estimation, if embeddedness is 100%, then rocks are completely buried by fine sediments. If embeddedness is 75%, then rocks are completely surrounded and half-covered by fine sediment. If embeddedness is 50%, then rocks are surrounded by sediment but their top surfaces are clean. If embeddedness is 25%, then rocks are half surrounded by fine sediment and their top surfaces are clean. If embeddedness is 0%, then there is essentially no fine sediment surrounding or covering rocks. Do not confuse attached algae on rocks with fine sediment. Embeddedness values are for all areas of the quadrate with coarse gravel or rubble/cobble substrates; if these two substrate types are absent then put a dash on the data sheet; embeddedness cannot be estimated. In some instances (e.g. turbid or deep water) it may be difficult to see or feel the streambed, in this case one should use their feet and feel the substrate to estimate Embeddedness**.**

***Percent of the Stream Bottom Covered*----------**

A description of the materials that make up the streambed, within the area that is covered by water. With your hand feel the substrate composition and visually estimate the percent composition of the stream bottom within a **0.3 m x 0.3 m quadrate centered** on the transect line. If turbid or deep water make it difficult to see the stream bottom, use your feet to feel the substrate and estimate substrate composition. **The sum of the values for all substrate categories must equal 100%**. Estimate each category to the nearest 5%; if a category listed on the sheet is not present in the quadrate, enter a zero for that category. If a bottom type that is not listed on the sheet is present, identify the category and record the percentage next to "Other". When the surface of the bottom is a mixture of substrate types (e.g., a sand-fine gravel mixture), or a mosaic of types (e.g., a patch of pure sand in one area and a patch of pure fine gravel in an adjacent area), make an estimate of the percent substrate composition of the surface of the stream bed. The substrate categories are as follows:

Bedrock: Solid, uniform rock bottom.

Boulder: Rocks with a maximum length of 261 mm - 4.1 m.

Rubble/ Cobble: Rocks with a maximum length of 65 mm - 260 mm.

Gravel: Rocks with a maximum length of 2 mm - 64 mm.

Sand: Inorganic material smaller than fine gravel but coarser than silt. The material found on a beach. Maximum length of 0.062 mm - 1.9 mm.

Silt: Fine inorganic material, typically dark brown in color. Feels greasy and muddy in hands. Loose; does not retain shape when compacted into a ball. Will not support a person's weight when it makes up the stream bottom. Maximum diameter of 0.004 - 0.061 mm.

Clay: Very fine inorganic material; individual particles barely or not visible to the naked eye. Either dark brown or gray in color. Feels gummy and sticky in hands; slippery when underfoot. Retains shape when compacted, and partially or completely supports a person's weight when it makes up the stream bottom. Maximum diameter of 0.00024 - 0.0005 mm.

Detritus: Partially decayed organic matter such as leaves, sticks, dead macrophytes, etc. When very fine, may appear similar to silt.

ALGAE (%) A visual estimate (**nearest 10%**) of attached and filamentous algae within each quadrate. Filamentous Algae is algae attached to the bottom or banks that forms long filaments, and Attached Algae is algae attached to the bottom or banks that forms a mat or crust, but does not form long filaments.

MACROPHYTES (%) A visual estimate (**nearest 10%**) of submergent and emergent plants within each quadrate. Submergent and emergent macrophytes are defined Cover for Fish, below.

CANOPY / SHADING (%) The degree to which canopy vegetation intercepts sunlight to the stream channel. Estimate to the **nearest 10%** at each channel transect position using a concave Forest Densiometer (if available). The densiometer should be held at elbow height and read facing upstream. If a Densiometer is not available, circle shading on the data sheet and make a visual estimate of the percent shading over the entire stream-reach surface within the 35 MSW station.

Cover for Fish Measure the length (m) of cover for fish along a 0.3 m band centered along the transect line. Fish Cover is defined as any objects, channel features, or bank features that provide complete shelter from the current, or provide visual isolation for a fish that is at least 0.20 m in total length. W**ater must be at least 0.20 m deep for cover to exist**. Measure (to the nearest 0.01 m) the length of each cover type along (parallel to) the transect line within the 0.3 m wide band centered on the transect. If the cover for fish (e.g., a submerged log crosses the transect line at an angle, only the length of the cover that crosses the transect is measured and recorded. If a cover type is absent, enter a zero on the datasheet. Cover types present but not listed on the sheet should be specified and recorded in the column listed "Other". Habitat improvement devices that provide cover are listed under "Other". The actual lengths (m) of each cover type along the transect line are later used to determine the percentage (length of cover divided by stream width at the transect, times 100) of the transect with cover.

Undercut Banks:	Banks that overhang the water by at least **0.20 m** at a point where the water is at least **0.20 m** deep. To be considered cover for adult gamefish, the bottom of the undercut bank must be no more than 0.10 m above the water surface.
Overhanging Vegetation:	Thick vegetation overhanging the water that meets the same criteria for cover as Undercut Banks.
Woody Debris:	Large pieces or aggregations of smaller pieces of wood (e.g., logs, large tree branches, root tangles) located in or in contact with water at least **0.20 m** deep.
Other Debris:	Pieces of human-made debris found in or in contact with water at least **0.20 m** deep, that provide shelter or visual isolation for fish. Examples include old tires, abandoned farm implements, and discarded home appliances.
Boulders:	Rocks at least the size of small boulders (**> 0.26 m**; see Stream Bottom Types) that are located in or in contact with water at least **0.20 m** deep. Large pieces of concrete and other artificial rocky aggregates also belong in this category.

Submerged Macrophytes: Vascular plants that normally have all or nearly all of their biomass below the surface of the water. Examples include Potamogeton, Vallisneria, Elodea, Ceratophyllum, and Myriophyllum. To count as cover, submerged macrophytes must be rooted in water at least **0.20 m** deep and must be dense enough to provide shelter or visual isolation for fish.

Emergent Macrophytes: Vascular plants that normally have a significant portion of their biomass above the surface of the water. Examples include bulrushes, sedges, cattails, and water lilies. To count as cover, emergent macrophytes must be rooted in water at least **0.20 m** deep and must be dense enough to provide shelter or visual isolation for fish.

Bank Erosion The degree to which each stream bank is susceptible to loss of material when inundated by water (either from precipitation or from stream flow during floods) or subject to heavy winds. More simply, the amount of the bank that is exposed soil. For the right and left bank along the transect-line, measure the length (nearest 0.01 m) of contiguous bare soil within 1 m of the stream edge (Figure 3). The stream edge is the edge of the wetted stream channel under "normal" flow conditions. If the flow is above or below normal, estimate where the wetted stream channel edge would be under normal flow conditions. Record the length of bare soil for each bank separately. **Patchy clumps of vegetation or other bank features (e.g., exposed rock) must be > 0.5 m long or they are counted in the measurement of bare soil**. If the length of bare soil is > 1 m from the stream, record > 1; if there is no contiguous bare soil, record 0. Also, visually estimate the Percent, to the nearest 10%, of the surface area (essentially the length) of each bank that is bare soil. The percent bare soil estimate requires that the crest of the bank is visually determined, and then the area of bare soil in a line from the stream edge to the crest of the bank is visually estimated. If the bank crest is not easily discernible, estimate the bank erosion within **5 m** of the stream edge. It may help to measure the length of the entire Bank (from stream edge to the crest of the bank), if easily discernible, and then divide into the length of bare soil to obtain the percent bank erosion.

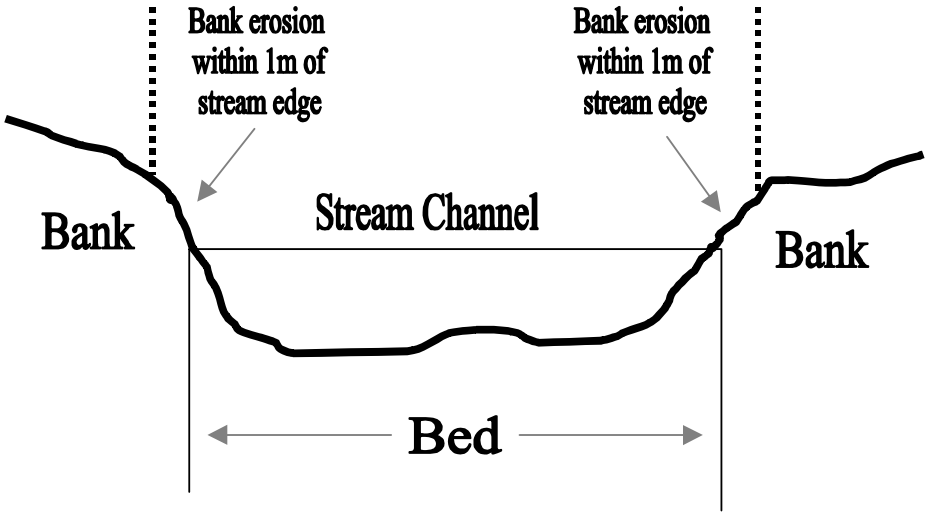


FIGURE 3. Bank Erosion description.

Riparian Land Use The amount of various land uses on both banks. In baseline habitat evaluations "banks" are defined as the land from the edge of the stream at normal water level to a point **5 m inland**, following the contours of the land (Table 2). This definition avoids confusion in identifying the actual banks. There are two major types of land uses within the riparian zone. **Disturbed Land Uses** are unnatural, human-related uses, while **Undisturbed Land Uses** are characterized by relatively unaltered natural vegetation and soils. Visually estimate each category listed below to the nearest 10% for both banks combined. **The sum of estimates must equal 100 %.** The land use must be > 1 m wide (along the transect- line) to count. If a category listed on the sheet is not present along the transect, enter a zero for that category. If a category that is not listed on the sheet is present, specify the identity of that category and list the percentage next to "Other". The listed categories are as follows:

Disturbed Land Uses

Cropland: Land that is plowed and planted with crops on a yearly basis or is regularly mowed for hay.

Pasture: Land that is regularly grazed by livestock.

Barnyard: Land that is used to confine and feed high densities of livestock. Also known as feedlots, this land often contains little vegetation and large volumes of manure and mud. Usually associated with farmsteads.

Developed: (Commercial/Residential/Urban): Includes lands that have been modified for human use. Buildings used for commerce or industry, plus residential buildings. Includes all roads (paved and unimproved), railroads, paths > 2 m wide, parking lots, and yards, etc. Also, parks, playgrounds, golf courses, ball fields, and associated roads, parking lots, etc.

Undisturbed Land Uses

Meadow: Land dominated by grasses and forbs with few woody plants, which is not subject to regular mowing or grazing by livestock.

Shrub: Land dominated by small (< 3 m high) woody plants, such as alders, honeysuckle. or juvenile box elders and willows.

Woodland: Land dominated by trees (either coniferous or deciduous), most of which are taller than 3 m.

Wetland: Low-lying land that is covered with standing water for much of the year.

Exposed Rock: Land covered by exposed bedrock outcrops, boulders, riprap, gabions, or other natural materials along the banks.

Slumping or "cut" banks with little vegetation and exposed soil eroding into the stream are not considered a separate category but are included with the land use found at the top of the bank. For example, an eroding, bare bank in an otherwise wooded area would be include as **Woodland** land use, while a severely eroding bank in a pasture would be included as **Pasture** land use. If a cut bank with a narrow band (1 m wide) of undisturbed land use (e.g., **Meadow**) at the top of the bank is followed by a disturbed land use (e.g., **Pasture**), the cut bank is included as **Meadow**.

Riparian Buffer Width Measure the width of contiguous Undisturbed Land Uses (above) from the streams edge out **10 m** along the transect-line, following the contours of the land, for both banks (Table 2). If no undisturbed land uses are directly adjacent to the stream, then the riparian buffer width is 0 m; if undisturbed land uses are present from the stream edge to a point > 10 m, then the riparian buffer width is recorded as > 10 m. Riparian buffer widths 10 m from the stream should be measured to the nearest 1 m.

Table 1: Gear used to sample stream habitat, and the postal and email addresses, and telephone numbers of the suppliers are listed at the end of the table.

Item	Supplier
Measuring Tapes Used for measuring short distances	
Keson - Model OTR - 10m – 165m	Forestry Suppliers, Inc. Stock #39972, 165 ft., 50 meters
Flagging Tape Used for marking habitat stations and fish sampling areas	
Biodegradable (lasts for one year) or Vinyl (for a more permanent mark)	Forestry Suppliers, Inc.
Map Gear Used for determining gradient, sinuosity, and legal description of areas sampled	
Land Locating Map Template - 40 Acre	Forestry Suppliers, Inc. Stock #45660 - Type A
Map Measurer - PECO Swivel Handle #45240 Digital Map Measurer #45251	Forestry Suppliers, Inc.
Clipboard Used for recording and storing data in the field	
Cruiser Mate Sheet Holder	Forestry Suppliers, Inc. Stock #53282
Meter Sticks Used for measuring depths and other short (< 1 m) distances	
Maple - Meter stick - with metal ends	Fischer Scientific* Stock # S32052
Paper	
"Rite in the Rain" Water-proof paper 208511 Bulk Cut Sheets (500) $27.25 8511 Copier Sheets (200) $21.25	J. L. Darling Corp. 8 1/2" X 11" - White
Camera Digital, or conventional film, used for documentation photographs of habitat before and after improvement.	
Film - Color Slide (Ektachrome - ASA 100, 200 or Kodachrome ASA 64)	
Forest Densiometer Used to estimate overstory canopy density; more objective and precise than visual estimation of stream shading.	
Spherical Crown Densiometer (Concave)	Forestry Suppliers, Inc. Stock # 43888

Table 1: (continued).

Item	Supplier
Topographic Maps Used for locating sampling sites and determination of station characteristics such as gradient, sinuosity, etc.	
7.5' or 15' Topographic Maps	WI Geological & Natural History Survey
Aerial Photographs	County Land Conservation Department WDNR Bureau of Forestry
County Plat Books Used to identify landowners when seeking permission to access streams	
	Most county extension offices
	Milwaukee Map Service, Inc.
	Rockford Map Publishers, Inc.

Addresses ----------

Forestry Suppliers Inc.
205 West Rankin St.
P.O.B. 8397
Jackson, MS 39204
1 - 800 / 647 - 5368
http://www.forestry-suppliers.com

J. L. Darling Corp.
2212 Port of Tacoma Rd.
Tacoma, WA 98421
206 / 383 - 1714
http://www.riteintherain.com

Milwaukee Map Services Inc.
959 Mayfair Rd.
Milwaukee, WI 53226
414 / 774 - 1300
1 - 800 / 525-3822
http://www.mapservice.com

Rockford Map Publishers
P.O.B. 6126
Rockford, IL 61125
1 - 800 / 447 - 2222
http://www.rockfordmap.com

Ben Meadows Co.
P.O.B. 80549,
Atlanta, GA 30366
1 - 800 / 241 - 6401 (order)
http://www.benmeadows.com

Fischer Scientific*
4500 Turnberry Dr.
Hanover Park, IL 60103
1 - 800 / 766 - 7000 (order)
630 / 259 - 1200
http://www.fischersci.com

Wisconsin Geological and Natural History Survey
3817 Mineral Point Rd.
Madison, WI 53705
608 / 262 - 1705
http://www.uwex.edu/wgnhs/intro.htm

VWR Scientific Products
Chicago Regional Distribution Center
800 East Fabyan Parkway
Batavia, IL 60510
1 - 800 / 932 - 5000 (order)
630 / 879 – 0600
http://www.vwr.com

*The State of Wisconsin has a contract with Fischer Scientific and other vendors for substantial discounts on equipment and supplies purchases. To receive these discounts Regional WDNR staff should set-up an account with Fischer or other vendors by contacting their Regional purchasing agent. Along with a discount on equipment and supplies, there are no shipping charges on regular or hazardous materials.

Evaluation of benthic algae by viewing bucket

Contents

Purpose

This standard operating procedure (SOP) establishes a standardized method for performing semi-quantitative field measurements of benthic algae and non-vascular plant coverage in wadable streams using a viewing bucket.

Definitions

ALGAE (%) A visual estimate (nearest 10%) of attached and filamentous algae along a sampling transect (WDNR 2002). Filamentous algae is algae attached to the bottom or banks that forms long filaments (WDNR 2002). Attached algae is algae attached to the bottom or banks that forms a mat or crust, but does not form long filaments (WDNR 2002).

Equipment and Materials

The following materials are required to undertake this procedure:

- Viewing bucket (for example, Wildco Fieldmaster View Bucket 77212)
- Datasheets or field book
- Pencils
- Waders, hip, or knee boots
- GPS unit
- Digital Camera (waterproof preferred)

- Safety gear
  - Life – jackets (PFDs)
  - First aid kits (field and vehicle)
  - Throw bags
- Crew member gear
  - Cell phones
  - Raingear
  - Sunscreen
  - Hat
  - Sunglasses
  - Insect Repellant

Collection Procedures and Guidelines

Observations of benthic algae are combined with habitat evaluation surveys. Once field sampling dates have been determined, a calendar invitation is submitted to relevant parties indicating when and where field activities will take place.

Pre-departure

1. Collect all the necessary equipment, including the viewing bucket.
2. Ensure that the weather conditions and flow levels are appropriate for sampling prior to departure.

Arrival

3. Ensure that all travel safety guidance is followed (General Field Safety Instructions SOP).
4. Assess the safety of the site. All members of the field crew should be aware of potential dangers and knowledgeable of safety precautions. A thorough inspection is important for preventing accidents. Proceed with collection only when conditions are appropriate.

Algae estimation procedure

5. Establish the sampling transects following the procedure outlined in the Wisconsin Department of Natural Resources (2002) *Guidelines for Evaluating Habitat of Wadable Streams.*
6. Upon arrival to the sampling transect, ensure that algae estimation is performed at the appropriate time in the sequence of field tasks. Specifically, viewing bucket measurements should be completed before any sampling procedure or activity that may disturb bottom sediments to avoid increasing turbidity at the location. The field crew should note any disturbance to the bottom sediments on the field sheets or in the field book.
7. Divide each transect into four evenly spaced positions by dividing the wetted width by five. Indicate the distances in the appropriate columns of the field form or in a field notebook.
8. Starting from the left bank (facing upstream), carefully immerse the viewing bucket into the stream so that approximately 4 inches of the bottom of the bucket is underwater. The field crew member should be downstream of the viewing bucket to minimize sediment disruption obscuring visibility of the bottom. The field crew member should bend over or squat in the water to view the bottom of the stream without interference. If glare or floating is a problem, a small amount of water can be added to the viewing bucket. Visually estimate the percent of stream bottom covered to the nearest 10%. Record the measurement in the appropriate column of the form or in the field notebook.

9. Repeat the procedure (step 8) at the remaining segment boundaries (i.e., at 2/5th the distance between the left and right banks, etc.).
10. Complete any other required field measurements at each transect before proceeding to the next transect.

Return

1. Following field activities, all field team members assist with the transport and inspection of all field equipment and supplies.
2. Upon return from the field, all equipment is inspected for damage, cleaned, dried, and stored appropriately.
3. Appropriate parties are notified by email that field sampling has been completed.

Field Note/Sheet Procedures and Guidelines

The field sheets and field notebook will be maintained by the habitat evaluation team. The notebook will be water-resistant and the field sheets will be printed on water-resistant paper.

Guidelines to follow when recording notes in the field notebook/field sheets include:

1. Write neatly.
2. Make numbers large.
3. Do not erase or black out a mistake, draw a line through the incorrect value and initial instead.
4. Number pages.
5. Never tear pages out of the notebook.
6. Record everything, never assume you will remember something.

Quality Assurance/Quality Control

Quality Assurance and Control (QA/QC) is an ongoing process. Its goal is the prevention, early detection and correction of field and analytical data collection errors.

1. All members of the field crew must ensure that all data sheets are filled in correctly and completely before leaving the site.
2. All members of the field crew must determine if the data are reasonable before they leave the field, and if not, the measurements should be repeated before leaving. This may require taking a calculator to determine if some measurements seem reasonable.

Data Management and Documentation

The original and a physical copy of the field sheets are stored in secure locations on the UW-Parkside premise. Scans of the field sheets accompany the annual report.

Field sheets are converted to a digital format using Microsoft Excel or equivalent. All habitat evaluation data are combined into single file for a calendar year (Root River Habitat YEAR).

Scanned data sheets and data files are stored locally with back-ups to campus OneDrive and iCloud. Cloud and local file organization are identical with a Root River Project parent folder and all files pertaining to a calendar year located with a Root River Report YEAR folder.

Safety and Environment

This section describes health, safety and environmental considerations for algae evaluation conducted during wadable stream habitat evaluation:

Health and Safety

Field Safety Instructions developed by the contractor for the sampling activities should be followed. Please refer to the General Field Safety Instructions SOP and the Task Hazard Analysis for more details. Some hazards are included here:

Hazards include, but are not limited to:

- Manual handling injury associated with lifting and moving sampling equipment and samples – to mitigate determine that all loads are an appropriate weight for lifting (<10 kg), use correct lifting posture by bending at the knees, position so that load is balanced and does not cause undue strain, wear sturdy boots and clothing, park field vehicle with equipment close to water body (if possible) to avoid multiple loading and uploading, do not over-pack samples into coolers.
- Injury associated with slips and trips – to mitigate keep a tidy workplace and step carefully around tubing, hosing and other equipment.
- Hit by moving vehicle while sampling – to mitigate sampling team shall wear high visibility clothing, set up traffic controls around sampling area, position site vehicle so that it provides a barrier from potential traffic.
- Sunburn –to mitigate wear suitable clothing (including hat, trousers, long sleeved shirt), apply sunscreen regularly.
- Dehydration and fatigue – to mitigate drink fluids and eat regularly.
- Exposure to water – to mitigate handle water with care minimizing splashing or spills, understand Safety Data Sheet (SDS) for particular parameters of interest, wear appropriate personal protective equipment (PPE) including gloves, waders, escalate PPE requirements if conditions change.
- Exposure to biological hazards (including snakes, ants, mosquitoes, bees, poisonous plants) – to mitigate access sampling points by minimizing exposure to vegetation, plan sampling events at suitable times where risk of biological hazard is reduced, wear appropriate clothing and PPE (long sleeves, long pants, tuck pant legs into socks), make vibrations to alert snakes to your presence. Use insect spray or other insect deterrents.
- Working on or around water courses will require additional PPE which includes (but not limited to) a Type II personal floatation device (PFD) and never working alone. PFDs should be utilized when sampling in deep waters and for all other instances where potential drowning danger exists.

Environment

Sampling contractors will be exposed to environmental waters which may contain contaminants that are hazardous to human health. All personnel participating in field sampling shall be current on Occupational Safety and Health Administration (OSHA) medical screening and surveillance standards. These standards can be found on the OSHA organization web page:

https://www.osha.gov/SLTC/medicalsurveillance/standards.html

References

Wisconsin Department of Natural Resources. 2002. *Guidelines for Evaluating Habitat of Wadable Stream*

Appendix I. Fish Sampling SOP

Standard Operating Procedure for the Collection, Identification, and Enumeration of Fishes
By Mike Pauers, February 2017
Edited and updated by Laura Schulz, March 2023

Purpose/Introduction

This standard operating procedure document details the equipment and methods to be used for the assessment of the fish community of the Root River. Briefly, fishes will be collected via electrofishing, identified, counted, and recorded in the field, and released back to the Root River. Back in the laboratory, the data will be transferred to a spreadsheet that will calculate the diversity (e.g., Shannon) and Index of Biotic Integrity (IBI; warmwater) for the community at each sampling location. Due to the use of electrofishing gear, safety is of utmost importance, and procedures for safe electrofishing are included as well.

This protocol, while following standard and accepted practices, is specific to the Post-Return Flow Root River Monitoring Plan, and should not be duplicated, without proper modification, for other projects.

Locations

The first sampling location occurs near the intersection of 60th Street and Oakwood Rd in Franklin, WI (Site C). Three additional target, wadable locations were identified for sampling immediately downstream (Site D), and immediately upstream (Site A) from Site C on the main channel and the Root River Canal (Site B). See Figure 1 for more detail.

Electrofishing Safety

Human Safety

Electrofishing is inherently dangerous. That having been said, it is entirely possible to do safely, for both collectors and fishes. Electrofishing should be performed by no fewer than two operators (one electrofisher and one netter), although three (electrofisher, netter, netter/bucketer) is ideal. At least one operator must be certified to perform cardiopulmonary resuscitation (CPR). All operators, and any and all observers who wish to enter the water must be wearing neoprene, non-breathable waders to insulate them against the electrical current produced by the electrofishing unit; breathable waders do not provide enough insulation to protect operators and observers from shock. Additionally, operators must wear heavy rubber, 'lineman'-type gloves; observers, as long as they do not handle the electrofishing equipment, need not wear such gloves.

When in operation, electrofishing units typically produce an alarm to alert everyone in the vicinity that the unit is on and introducing electrical current to the water; all operators and observers must listen for this sound and know what it means. All operators and observers must be aware of the long, tail-like cathode extending from the electrofishing unit; accidentally stepping on this could cause the electrofisher to lose their footing.

Fish Safety

To make the electrofishing experience as safe as possible for the fishes, several factors must be considered. First, electrofishing should not be performed during the 24 hours following a

rainstorm. Rain can change the chemical properties of water in numerous ways, which will influence the conductivity of the water, hampering effective electrofishing. Further, rain events will change the behavior of the fishes, making them much less likely to be caught.

The electrofishing unit has switches that can adjust the output wattage and electrical waveform of the current. These should be set to the recommendations of the manufacturer, considering the depth, temperature, and conductivity of the water. An output wattage that is too high can kill the fish, and certain waveforms will be more disturbing and damaging than others.

After capture, the fishes should be placed into large buckets or tubs filled with water from the river. The fishes can then be observed to ensure proper recovery from the electricity. The orientation of the body, respiration rate, and recovery time of all fishes should be carefully observed by the operators; any unusual behaviors should be noted, as they can be indicative of an improperly-set electrofishing unit. Ideally, the fishes will be held in this bucket until the reach is completed; if the reach is long, then periodic breaks should be taken to identify, count, and release the fishes before the end of the reach is reached. Upon release, a healthy fish should begin swimming away from the operators almost immediately; any fish that struggle or do not swim after being released should be euthanized if necessary.

Equipment

The following equipment should be used while electrofishing:

- Neoprene chest waders and rubber 'lineman'-type gloves for all operators
- DC Pulse, backpack-mounted electrofishing unit
- Spare, fully-charged battery for the electrofisher
- Small-mesh (~1/4 - 1/2 inch), electrofishing-safe landing nets (at least 2, if not 3)
- Large buckets for holding captured fishes (1 or 2)
- Small bucket for anaesthetizing injured or unidentifiable fish (1)
- Measuring board for recording lengths of game fishes
- MS-222 (tricane methane sulfonate) for anaesthetizing injured or unidentifiable fish
- 10% formalin, 70% ethyl alcohol, and assorted jars for bringing euthanized or unidentified specimens back to the lab
- General first-aid kit
-YSI PRODSS or alternative multiparameter probe for routine water analysis prior to sampling

Notifying the Project Team

Team members at UW-Parkside and Jacobs are notified of sampling dates via email and outlook calendar invite prior to the sampling event from the UW-Parkside fish sampling team lead. If sampling dates and/or details change, the UW-Parkside and Jacobs teams are notified via text or email asap. An email is also sent from the UW-Parkside team lead to the WI DNR inviting them to the sampling event.

The UW-Parkside team is notified via text from the team lead and/or the UW-Parkside student workers when the sampling crew has finished and returned to campus. The Jacobs Team is notified via email asap upon completion of each sampling day from the UW-Parkside team.

Departure and Arrival Procedures

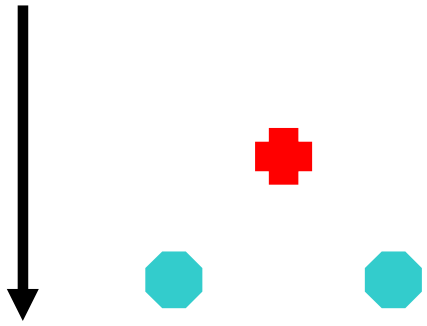
The UW-Parkside team gathers all of the equipment at UW-Parkside either the evening before or the morning of the sampling event. Weather and river depth is assessed the prior evening by the UW-Parkside team lead using both current weather predictions and the USGS monitoring gauges on the Root River (near Site A , Site B and Site C). Sampling will not occur if rain is in the forecast and/or the river is too deep (generally greater than 3.5 feet) and/or the river depth is actively rising. The UW-Parkside team lead will also visually inspect the site upon arrival to ensure current river and weather conditions are save for electrofishing. Site conditions are recorded in the field by the UW-Parkside team lead or a UW-Parkside student on water-proof paper and later saved to a Box cloud folder at UW-Parkside and included in the fish reports.

Collection Procedures

Determining Reach Length
Following standard EPA guidelines, the electrofishing reach length should be 35 times the mean width of the stream. Using the habitat data collected by the Habitat Team, the length of the reach will be determined by calculating a mean wetted width for each site, and multiplying that by 35. At the field site, the beginning and end of a reach of appropriate length will be marked with flagging tape. The flagging tape will be tied to trees at a height of about 1.25 m above the ground, and will be in place for the sampling season; if needed, it will be replaced annually. The starting point will be downstream of the end; electrofishing is performed while moving upstream, against the current.

Setting the Electrofisher
Using the most recent available measurements of conductivity and temperature, or after measuring them in the field, the electrofisher should be set according to the specifications of the manufacturer. At this time, all connections and cables should be checked for fit and tightness.

Obtaining Fishes
All operators should put on their waders and gloves and enter the water safely; the other operators should especially watch and help the electrofisher operator as necessary. When all operators have entered the water, the holding buckets should be filled with water, and the electrofishing unit should be turned on. The electrofisher operator should be in the front-center of the operator group, flanked by the netters/bucketers, as follows:



(Arrow: direction of current; Cross: electrofisher operator; Octagons: netters/bucketer)

As the operators commence sampling, the netters/bucketers should remain behind and to the sides of the electrofisher, so as to maximize the width of stream covered by the nets. The netters/bucketers should be careful not to step on the cathode tail of the electrofishing unit; standing to either side of the electrofisher should avoid this.

Once electrofishing commences, and fishes have begun to be captured, fishes should be transferred immediately to the holding buckets and observed for signs of distress. Fishes should be held in the buckets until the end of the reach is reached, unless the reach is particularly long (≥ 100 m); in case of a long reach, the operators should stop about halfway along the reach to identify, count, and release the captured fishes. If any of the operators experiences any "tingling" or mild shocks from the electrofishing unit, they should inform the electrofisher operator immediately, and an alternate plan (i.e., using a smaller crew, getting a different pair of waders, etc.) should be devised.

Electrofishing should continue until the entire reach is completed. Upon reaching the end of the reach, all fishes will be identified, counted, and released to the river.

YSI PRODSS Probe Procedures and Guidelines

Prior to the start of the electrofishing event at each site, surface water chemistry data is collected. Readings are measured via a bridge crossing at all sites using a YSI PRODSS multiparameter probe. Measured parameters include DO (% and mg/L), specific conductance (mS/cm), conductivity (mS/cm), temperature (C◦), turbidity (NTU), pH and depth (feet). The width of the stream will be divided into five equal segments. Steps include:

1. Calibrate YSI PRODSS at UW-Parkside prior to heading to the sampling sites (refer to the Surface Water Chemistry SOP).
2. Dip probe into the river at each of the five equal-distance increments.
3. Allow readings to stabilize.
4. Record data on field data sheet. Scanned copies of the field data sheets (see Water Chemistry SOP) are stored in a Box cloud folder at UW-Parkside and included in the final fish report.
5. Rinse off probe with DI water.
6. Towel or air dry.

For more details on surface water chemistry sampling, refer to the Water Chemistry SOP.

When the YSI PRODSS is unavailable, acceptable alternatives include a YSI 556 and a HF – Micro 100 Laboratory Turbidimeter. Refer to the Water Chemistry SOP for further details.

Field Note and Record Procedures

Once the reach is complete, all captured fishes will be identified, counted, and released. All fishes should be identified to species in the field. The identities and numbers of each species should be recorded in a waterproof notebook by the operators. Further, if any game fishes (bluegills, basses, pikes) are captured, they should be measured before being released, and their lengths recorded along with the other data.

If a positive identification of a particular species is not possible (e.g., members of the Family Cyprinidae are notoriously difficult to identify), the unidentifiable fishes should be sorted and counted, and a designation (e.g., 'Unidentified Cyprinid A' or 'Unidentified Species A') recorded in the field notes. A small subsample (2-5 individuals) of these fishes should then be euthanized, preserved, and taken back to the laboratory for positive identification.

If necessary, the fish will be euthanized in a strong solution (i.e., 250 mg/L) of MS-222 anesthetic. The fish will be immersed in the solution for a period of 10 minutes, or at least until the fish becomes immobile, stops respiring (as evidenced by a lack of opercular movement), and stiffens its fins. At this point, the fish will be moved into a 10% solution of formalin for fixation, and will then be transferred to alcohol for preservation. These activities are covered under an Animal Care and Use Agreement approved by the UW Colleges Animal Care and Use Committee (AUCU Protocol# 17-18-02). Also, any and all chemicals used in the field will be transported back to the laboratory for storage and disposal as needed.

For each fish brought back to the laboratory, the following information should be marked on the outside of the jar:

- "RR – Sample Site ID (A-D) – MMDDYYYY"
- Time of collection
- Sampled by
- Preservative

Fish samples shall be stored in coolers while in the field, and will be transported to a refrigerator back at UW-Parkside or UW-Waukesha.

Once all species have been properly identified, all data should be transferred to an Excel spreadsheet. Using this spreadsheet, various indices of fish community diversity (e.g., Shannon) and quality (e.g., Index of Biotic Integrity) should be calculated according to methods described in Barbour et al. (1999) and Lyons (1992).

General Safety

All electrofishing safety practices, as detailed above, shall be followed at all times.

In addition to these problems, other safety concerns may present themselves during fieldwork. These may include, but are not limited to:
- Lifting and moving heavy objects (don't overpack cases, boxes, or buckets; lift and move heavy objects as a team as necessary)
- Injuries due to slipping, tripping, or falling (walk slowly and carefully, especially over unstable or slippery terrain)
- Sunburn or heat-related fatigue (wear lightweight, long-sleeved/legged clothing; hats, sunglasses, and sunscreen may also be helpful)
- Dehydration (drink water regularly, and safe drinking water should be available to the team)
- Fatigue (good sleep the night before fieldwork, and regular meals before and during fieldwork can prevent this)
- Biological hazards (biting/stinging insects, poisonous plants; operators should remain observant and aware of all such hazards, and warn each other as necessary)
- Water-related hazards (moving slowly and carefully while in the water, especially in deep and/or fast sections of the stream; helping each other as necessary)

Data Management and Documentation

Scanned field notes, water chemistry field data sheets (see Water Chemistry SOP), and excel files containing the fish data and biotic index calculations are stored on a Box cloud folder at UW-Parkside. The master folder is labeled "Waukesha". Within the master folder, files are stored under the folder labeled "Fish reports" and then subfolders including "scanned field notes" and "excel files". The "scanned field notes" subfolder is further divided into "fish counts and habitat conditions" and "water data".

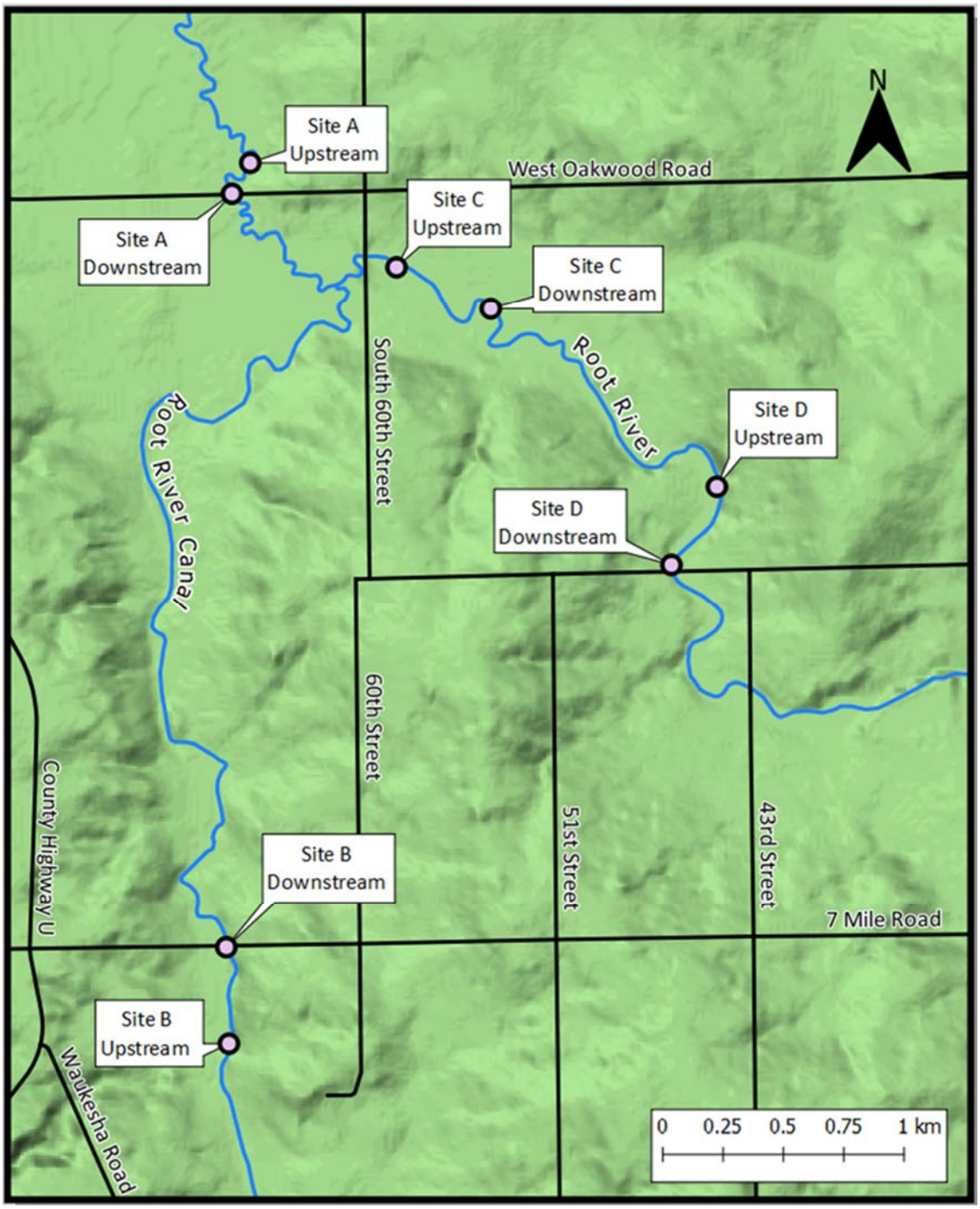
Scanned field notes files are labeled as fieldnotesfish _yearmonth.
Scanned water chemistry field data sheets are labeled as waterdatafish_yearmonth.
Excel files are labeled as fishdata_yearmonth.

References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. Second Edition. Document No. 841-B-99-002. Environmental Protection Agency, Washington, DC, USA.

Lyons, J.D. 1992. Using the Index of Biotic Integrity (IBI) to Measure Environmental Quality in Warmwater Streams of Wisconsin. General Technical Report NC-149. United States Department of Agriculture – US Forest Service, St. Paul, MN, USA.

Figure 1: Map of upstream and downstream extent of sampling locations along the Root River Canal and Root River.



Appendix J. General Field Safety SOP

General Field Safety Instructions

Contents

Purpose

This standard operating procedure (SOP) describes the general safety guidelines that must be used during field studies.

Field studies may involve long hikes through dense vegetation; work in wet environments where terrain is slippery or unstable; work in very remote locations where emergency services or cell phone service may not be available; and/or work in lakes and streams where swift, turbid, or deep water, along with slippery surfaces, can present safety challenges. Unpredictable and changing weather, heat, humidity, stinging insects, and/or poisonous plants can also present safety hazards. For these reasons, student research assistants may not sample alone; at least one other student research assistant or faculty/staff member must be present. It is recommended that faculty/staff not sample alone, however, if sampling alone is necessary the staff member must have a cell phone for emergency contact. It is the responsibility of the faculty member to determine if there are hazards associated with sampling that would require sampling in teams (e.g., long treks, uncertain conditions, fast moving water, enclosed spaces, and/or steep or rugged terrain). Sampling from a boat, including a canoe, must be conducted with at least two students and/or staff members present. Field work must never be conducted during thunderstorm/lightning conditions.

This SOP is intended to raise awareness about the natural hazards that can be found while working in the field and to provide a guide for protecting personal health and safety while conducting field studies. Faculty/staff and student research assistants must take responsibility for their own safety and comfort by preparing properly for the work to be conducted during the field day. Contact your supervisor or the project's Principal Investigator if you have any concerns about your ability to prepare properly for field studies.

Field sampling/research activities must only be conducted by individuals who have read this SOP, have had proper training, and have demonstrated competency on the safe and accurate performance their assigned activities in the field. All steps in the safety and procedural training must be clearly documented in writing with trainee's and trainer's signatures and maintained on file.

Equipment and Materials

The following materials are required to undertake this procedure:

- Appropriate clothing for environment and weather conditions (e.g., hat, rain gear, long sleeves, long pants, chest waders, etc.)
- Appropriate footwear (e.g., water-proof boots, hiking shoes, boat shoes, etc.)
- Backpack (to carry field gear)
- Cell phone
- Compass or GPS Unit
- Emergency contact numbers for all faculty and students
- Prescription medication (for emergency use with individuals having insect or environmental allergies)
- Drinking water
- First Aid Kit including (at minimum):
    - Antihistamine (e.g., Benadryl)
    - Antiseptic Wipes or Rubbing Alcohol
    - Band-Aids
    - Fine-Tipped Tweezers
    - Hydrocortisone Cream
    - Tape
- Insect repellent
- Hand Sanitizer
- Personal Flotation Device (PFD, when sampling from a boat or at sites with high current velocity and/or adjacent deep water; US Coast Guard Approved Type I, II, II, or V; one for each person)
- Safety Line
- Food (depending on length of field day)
- Sunscreen
- Sunglasses and/or Hat (optional)
- Waterproof Bag (to carry cell phone, radio, wallet, keys, datasheets, etc.)
- Work Gloves (waterproof, heavy protection, laboratory, etc.)
- Traffic cones
- Reflective vests
- Waterproof jacket and pants

Procedures and Guidelines

Preparing for Field Work

1. Read the assigned project SOPs, Quality Assurance Project Plan, or other project planning documentation and understand the field activities to be conducted, including the health and safety hazards they present.
2. Complete and document all safety training, as well as any and all hands-on procedural training.
3. Follow all safety requirements in this SOP and any other applicable project-specific SOPs.
4. Workers are advised to see their supervisor immediately if there is any question about this SOP or about project-specific field safety procedures. Workers must inform their supervisor if they have a known allergy or a limiting medical condition prior to conducting research activities in the field.
5. Determine and prepare the appropriate clothing and equipment:

5.1. Consult with the project supervisor or field leader about what type of field conditions can be expected for the field study. This may change from day to day.
5.2. Review the weather forecast for the area where the field study will be conducted prior to going into the field.
5.3. Wear light-colored clothing and dress in layers to avoid biological reactions and interactions (e.g., poison ivy or wood ticks).

6. Consult a map of the field site and become familiar with the location and route.
7. Notify the field contact (trip log) of where you are going for the day and estimate when you will return. Provide the field contact with a telephone number where you can be reached, and the make/model of the vehicle you will be driving (if known).
8. Review the map of the field site(s) and look for the safest route to your sampling location(s).
   8.1. Note the starting location/parking location (i.e., bridge/intersection/parking lot)
      8.1.1. If parking is available, park the vehicle away from the road, in a secure area.
      8.1.2. If parking is unavailable at the site, pull out of traffic on stable ground. In high traffic areas or when working on or near roadways, deploy traffic cones to notify other vehicles of your presence. Wear reflective clothing or vests when on or near roadways.
      8.1.3. In the case of a vehicle emergency, first contact emergency services, then contact your supervisor. Field work should only resume when it is safe to do so.
9. Assess the conditions and safety gear in your backpack and remember to take the backpack into the field. Ensure the backpack is not prohibitively heavy and can be easily carried into the field.
10. Apply sunscreen and/or insect repellent if needed.
11. Always bring extra fluid (e.g., drinking water or a drink with electrolytes).
12. Always bring a snack for energy and a lunch for a full day in the field.
13. After sampling, wash hands or use hand sanitizer before eating or touching your face.
14. Be aware of the terrain to avoid accidents.
15. Do not handle poison ivy, poison sumac; water hemlock (bulbet or common); or wild parsnip.
16. Do not go out if thunderstorms are predicted. If a thunderstorm approaches (e.g., you see lightning or hear thunder) while you are working seek shelter immediately. For personal safety: avoid water, high ground, and open spaces. Avoid all metal objects including electric wires, fences, machinery, motors, power tools, etc. Unsafe places include underneath canopies, small picnic or rain shelters, or near trees. Where possible, find shelter in a substantial building or in a fully enclosed vehicle with the windows completely shut.
    16.1. If lightning is striking nearby when you are outside, you should: crouch down with feet together and place hands over ears to minimize hearing damage from thunder. Avoid proximity (minimum of 15 ft.) to other people.
    16.2. SUSPEND ACTIVITIES for 30 minutes after the last observed lightning or thunder.
17. Contact the field contact promptly when sampling is completed to inform him/her of a safe return.
18. Return all samples and equipment to the appropriate laboratory spaces.

Sampling in Wadable Streams

1. Wear chest waders.
2. Look for the safest point to enter the stream channel.
3. Be aware of stream velocity, water depth, and bottom conditions. Conditions can change significantly over the season.
4. A wearable personal flotation device (PFD) is recommended for sites with high current velocity, adjacent deep water (i.e., >2 feet deep), and/or unknown depth.
   4.1. Evaluate the site for safety before entering the stream. If unsure of the stream velocity, depth or bottom conditions, wear a PFD, use a safety line or station a coworker on the shore with a throwable safety line, and

use a sturdy stick or wading rod to test the bottom and/or proceed with caution as you access the stream. Do not enter the stream if the water velocity appears fast enough to knock a person down.

5. Water appearance and/or odor can indicate water pollution or a safety hazard. Do not enter the water if there are highly unusual water characteristics. If these conditions exist:
   5.1. Collect the sample from the stream bank if possible.
   5.2. Record the unusual characteristics and report the incident to your supervisor for reporting to the Wisconsin Department of Natural Resources.
   5.3. Take a photograph of any unusual visual characteristics.
6. Use caution around unstable soils and "quicksand." When water pressure is high enough and friction between sand particles is low, stream beds can lose the ability to bear a load. Sand bars and shifting sands along rivers and streams can become very unstable, like "quicksand."

Procedure for Removal of Ticks

1. After the field day, examine gear and remove ticks.
2. If you find a tick attached to your skin, use fine-tipped tweezers to grasp the tick as close to the skin surface as possible. Pull upward with steady, even pressure. Don't twist or jerk the tick; this can cause the mouth-parts to break off and remain in the skin. If this happens, remove the mouth-parts with tweezers. If you are unable to remove the mouth easily with clean tweezers, leave it alone and let the skin heal.
3. Thoroughly clean the bite area and your hands with antiseptic wipes or soap and water.
4. If desired, secure ticks to a piece of tape for later identification.
5. Record the date of the bite, location on the body and the location of the field trip.
6. See a doctor, if a rash or fever develops within several weeks of removing a tick. Inform the health care professional that the rash or fever may be the result of a tick bite.

Procedure for Prevention and Treatment of Insect Stings

1. Avoid using perfumed soaps, shampoos, and deodorants. Do not wear cologne or perfume.
2. Remain calm and still if a single stinging insect is flying around. If attacked by several stinging insects at once, run to get away from them (bees release a chemical when they sting, which may attract other bees).
   2.1. A shaded area is better than an open, sunny area to get away from the insects.
   2.2. Avoid jumping into water. Some insects are known to hover above the water and may continue to sting as you surface for air.
3. Workers with a history of severe allergic reactions to insect bites or stings should consider carrying an epinephrine auto injector (e.g., EpiPen) and make their coworkers aware of their allergy or should wear a medical identification bracelet or necklace stating their allergy. All of the coworkers should be trained by the individual having the allergy to recognize the symptoms and to administer the appropriate first aid to assist the victim.
4. A letter of permission should be written by the individual having the allergy in order for coworkers to be able to administer appropriate first aid should the individual become unconscious.
5. If a sting occurs, stay with the sting victim while they rest and in case there is an allergic reaction.
   5.1. Remove the stinger using gauze wiped over the area or by scraping a fingernail over the area.
   5.2. Never use tweezers or squeeze the stinger.
   5.3. Wash the site with an antiseptic wipe or soap and water.
   5.4. When available, apply ice to reduce swelling.
   5.5. Do not scratch the sting site.
   5.6. Call 911 and proceed to a hospital emergency room if the person is suffering a severe allergic reaction, such as swelling or difficulty breathing, or has had a severe reaction in the past. If possible meet the emergency medical team enroute for immediate medical attention.

Procedure for Prevention and Treatment of Contact with Poisonous Plants

1. Learn to identify poisonous plants and avoid contact with them.
2. If you come into contact with a poisonous plant, immediately rinse skin with rubbing alcohol, then soap, and finally lots of COLD water. Hot water will open skin pores. Do not wait for symptoms to appear. Symptoms may occur immediately or within a few days of contact and may include a red rash, raised bumps, patches, streaking, weeping blisters, swelling, or itching.
3. Rinse the contact site with cold water frequently to avoid further spread the plant allergen.
4. Scrub under nails.
5. Apply wet compresses, calamine lotion, or hydrocortisone cream to the skin to reduce itching and blistering. Follow package directions.
6. An antihistamine such as Benadryl can be taken to help relieve itching. Follow directions on the package. Drowsiness may occur.
7. Call 911 and proceed to a hospital emergency room if the person is suffering a severe allergic reaction, such as swelling or difficulty breathing, or has had a severe reaction in the past. If possible meet the emergency medical team enroute for immediate medical attention.