Waukesha Water Utility
SERVING WAUKESHA SINCE 1886

115 DELAFIELD STREET
WAUKESHA, WI 53188-3615

Telephone: (262) 521-5272 • Fax: (262) 521-5265 • E-mail: contactus@waukesha-water.com

DATE: April 14, 2023

TO: Wisconsin Department of Natural Resources

FROM: City of Waukesha Water Utility, on behalf of the City of Waukesha

RE: Diversion Permit Reporting for 2022

The June 21, 2016, Final Decision approving of the Application by the City of Waukesha, Wisconsin for a Diversion of Great Lakes Water from Lake Michigan and an Exception to Allow the Diversion sets forth various conditions of the approval.

Condition J of the Decision requires an annual report be filed that, "documents the daily, monthly and annual amounts of water diverted and returned to the Lake Michigan watershed over the previous calendar year". No water has yet been diverted. It is anticipated that the diversion will commence by September 2023. Thus, in the absence of a diversion, no report is yet required, and there is no diversion of water to report. Although this report is not required, the City of Waukesha Water Utility is providing the following information as a matter of background for the calendar year 2022. The report format follows the requirements of section 12 on page 10 of the DNR approval letter signed on July 29, 2021.

Requirement (a): Compact Principles
The City shall summarize that the diversion was implemented consistent with the requirements of the Council Decision.

Response:
The approved diversion has not commenced to operate during the 2022 calendar year. The City of Waukesha Water Utility continues to construct the infrastructure necessary to deliver water from Lake Michigan which will be returned to the Great Lakes Basin via the Root River in Franklin, Wisconsin.

The anticipated commencement date is before September 2023.

Requirement (b):
The total amount of water purchased daily, monthly, and annually from the City of Milwaukee, including the location(s) of the water meter used to determine the amount of water purchased.

Response:
There was no water purchased from the City of Milwaukee in 2022.

Requirement (c):
The total amount of water sold monthly to each category of customer within the approved diversion area.

Response:
The following table illustrates the gallons sold, by month and customer class, in 2022 from water supplied by existing Waukesha Utility wells:

Water Sold:	2022	Water Sold (gallons)					
Customer Class	**# of Customers**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
RESIDENTIAL	16,748	59,522,300	61,018,500	55,350,700	57,763,700	57,807,700	59,525,100
RES-2 FAMILY	1,285	7,860,200	8,259,800	7,475,100	7,942,100	7,991,200	7,613,200
RES-3 FAMILY	76	482,700	516,900	475,800	481,800	536,900	478,400
MULTI-FAMILY	956	28,509,300	29,845,400	27,406,100	28,574,800	29,331,200	27,994,000
COMMERCIAL -REG	1,273	21,658,100	23,436,300	23,887,900	25,882,000	23,400,600	23,622,800
INDUSTRIAL	142	8,833,700	9,628,600	9,368,600	11,096,600	10,296,700	11,699,700
PUBLIC	119	3,641,200	3,639,100	3,719,100	3,918,900	4,295,800	5,691,800
IRRIGATION	150	2,500	1,400	2,200	3,200	36,800	140,300
TOTAL	**20,749**	**130,510,000**	**136,346,000**	**127,685,500**	**135,663,100**	**133,696,900**	**136,765,300**

	Water Sold (gallons)						
Customer Class	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**
RESIDENTIAL	69,854,100	69,310,900	62,303,100	62,124,152	60,150,503	54,307,700	**729,038,455**
RES-2 FAMILY	8,311,000	8,671,100	7,858,100	8,158,700	8,416,500	7,563,800	**96,120,800**
RES-3 FAMILY	534,300	544,200	525,900	522,500	559,700	504,300	**6,163,400**
MULTI-FAMILY	29,574,900	30,735,800	30,044,700	30,299,200	31,395,600	27,244,000	**350,955,000**
COMMERCIAL -REG	29,984,100	27,810,000	27,445,700	30,935,300	26,620,960	23,055,900	**307,739,660**
INDUSTRIAL	11,885,800	13,000,500	12,238,700	13,192,700	11,881,900	9,839,600	**132,963,100**
PUBLIC	6,206,100	5,516,000	5,155,200	5,220,000	4,023,800	3,573,900	**54,600,900**
IRRIGATION	1,898,100	1,472,800	1,421,500	1,006,000	373,800	126,800	**6,485,400**
TOTAL	**158,248,400**	**157,061,300**	**146,992,900**	**151,458,552**	**143,422,763**	**126,216,000**	**1,684,066,715**

Requirement (d):

The daily, monthly, and annual volume of treated wastewater discharge returned to the Root River and the daily, monthly, and annual volume of treated wastewater discharge returned to the Fox River.

Response:

No diversion occurred in 2022 so no water was returned to the Root River.

Since no diversion took place in 2022, the following table illustrates the gallons of wastewater discharged from the City of Waukesha Clean Water Plant to the Fox River:

2022 Discharge (gallons) from the City of Waukesha Clean Water Plant to the Fox River						
Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	
181,041,000	215,270,000	263,808,000	355,871,000	282,161,000	229,154,000	
Jul	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**
231,417,000	255,910,000	354,312,000	236,378,000	262,143,000	264,446,000	**3,131,911,000**

Requirement (e):

The total consumptive use as defined in Wis. Stat. §281.346(1)(e).

Response:

In 2022, Waukesha Water Utility had thirteen (13) ratepayers that had measured consumptive use, or water used during production. The total water usage associated with production in 2022 was 37,085,500 gallons.

As requested in the responses to the 2021 Report, once the City begins a diversion of Lake Michigan use, DNR requested that consumptive use be calculated using both the Winter Based Method (WBR) plus the water loss (WPSC) + water used by industry (incorporated into their products).

The WBR method primarily focuses on outdoor water use (lawn and landscape watering, car washing, pools) and assumes most of the consumptive use in municipal water supply systems is due to evapotranspiration. Given that the City's water use peaks in summer months, the DNR believes this is

an acceptable method to calculate domestic consumptive use. The “annual withdrawal” would be equal to the amount of water the city purchases from the City of Milwaukee. The sum of winter withdrawals or “winter months” refers to December through February. Withdrawn water that is not returned to the Lake Michigan basin would be due primarily to consumptive use due to evaporative losses (calculated by the WBR method).

The WBR method calculates annual consumptive use according to the following equation:

[(*Sum of all monthly withdrawals* ÷ 12) – (*Sum of winter-month withdrawals* ÷ 3)] ÷ (*Sum of all monthly withdrawals* ÷ 12) × 100

“All months” are January through December, and “winter months” are December through February.

The WBR method calculates summer consumptive use according to the following equation:

Summer consumptive-use coefficient (%) = [(*Sum of* **summer** *monthly withdrawals – Sum of winter monthly withdrawals*) ÷ *Sum of summer monthly withdrawals*] × 100 (5)

“Summer months” are June through August. This basic equation is also used to estimate coefficients for spring (March through May) and fall (September through November).

WBR Method 2022		
Total Annual Withdrawal	0	gallons from Lake Michigan
Sum of Winter Withdrawals	0	gallons from Lake Michigan
Sum of Summer Withdrawals	0	gallons from Lake Michigan
Total of Annual Pumpage	1,881,926,000	gallons from Groundwater
Sum of Winter Pumpage	445,071,000	gallons from Groundwater Dec 2021 thru Feb 2022
% Annual Consumptive-use Coefficient	5.4	using groundwater data
Sum of Summer Pumpage	538,408,000	gallons from Groundwater June 2022 to August 2022
% Summer Consumptive-use Coefficient	17.3	using groundwater data

Per the *Variations in Withdrawal, Return Flow, and Consumptive Use of Water in Ohio and Indiana, with Selected Data From Wisconsin, 1999–2004* by the USGS, page 65, “The public-supply annual average consumptive-use coefficient calculated by use of the WBR method ranged from 6 to 8 percent, and the summer consumptive-use coefficient ranged from 16 to 20 percent for Ohio, Indiana, and Wisconsin.”

However, the city should also include consumptive use from any industry that uses water (food processing, beverage processing) and also add in distribution system losses (such as distribution system water losses reported to the WPCS (e.g. water mail breaks, service leaks, faulty pressure valve).

To summarize, the City’s total consumptive use = consumptive use (WBR) + water loss (WPSC) + water used by industry (water incorporated into product).

City Consumptive Use Percent 2022		
% Annual Consumptive-use Coefficient	5.4	using groundwater data
Total Pumpage	1,881,926,000	gallons from Groundwater
Water Loss (WPSC) in 2022	198,520,045	gallons from Groundwater
Water Used by Industry in 2022	37,085,500	gallons from Groundwater
2022 Consumptive Use	337,247,545	gallons from Groundwater

Lake Michigan Consumptive use:

$$Lake\ Michigan\ Annual\ Consumptive\ Coefficient\ (Percent) = \frac{(Total\ Water\ Purchased\ from\ City\ of\ Milwaukee) - (Total\ Wastewater\ Return\ to\ Root\ River)}{(Total\ Water\ Purchased\ from\ City\ of\ Milwaukee)}\ x\ 100$$

Lake Michigan Consumptive Use for 2022 was 0.

Requirement (f): Water Conservation and Efficiency Plan

A summary of the impact of the implemented Conservation and Efficiency Measures required under Wis. Admin. Code §§ NR 852.04 and NR 852.05, including quantifiable impacts to water use intensity, as defined in Wis. Admin. Code § NR 852.03(29). Water use intensity metric calculation methods as specified by the DNR.

Response:

The following table is a summary of the City's conservation and efficiency measures that occurred in 2022.

Required CEM	2022 Activity
PWS-1 Water Use Audit	Water loss is at 9.7%
PWS-2 Leak Detection and Repair Program	Replaced 9,953 linear feet of mains. Inspected 950 hydrants and repaired leaks.
PWS-3 Information and Education Outreach	Continued education programs and partnerships. Fewer public meetings due to COVID-19 pandemic
PWS-4 Source Measurement	All source water is measured.
PWS-R1 Distribution System Pressure Management	WWU manages system pressure in 10 pressure zones.
PWS-R2 Residential Demand Management Program	48 toilet rebates issued, 1 showerhead rebate issued, 7 rain barrel rebates issued. Sprinkling Ordinance (for all customer classes) was enacted in 2006. Customers are allowed to irrigate twice a week. Street signs and mailers provide information on the sprinkling ordinance. Fines are also in place, 0 violations were reported in 2022. Irrigation Ordinance (for all customer classes) was adopted in 2015 requiring permits for landscape irrigation systems to ensure irrigation systems are efficient. 8 permits were issued in 2022. Audit Program (for residential & non-residential customers) determines high water consumption and sends a postcard to customers that may have a leak. In 2022, 11 residential water audits were conducted; and 22 data logging reports were conducted to evaluate for water leaks.
PWS-R3 Commercial and Industrial Demand Management Program	144 multifamily toilet rebates issued. 1 of the top 15 industrial water users participated in Site-Specific Grant Program. 7 spray rinse valve rebates issued. Audit Program (for residential & non-residential customers), in 2022, 13 data logging reports were conducted for public, commercial, and industrial customers to evaluate for water leaks.
PWS-R4 Water Reuse	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible
Tier 3 Additional CEMs	Instituted monthly billing. Application for a rate increase is pending at PSC. Sewer Ordinance Change (for all customer classes) – In

	2016 Waukesha revised their sewer credit meter ordinance to phase out all sewer credit meters. In 2022, 25 sewer credit meters were retired, 46 sewer credit meters remain.

The testing of the Water Production meters at our wells are tested every two years; with the next test scheduled for Spring 2023. The testing of our meters used for billing occurs in house based on the timing requirements of the Public Service Commission. In 2022, 330 meters were tested.

The 2022 Water Use Intensity Metrics: provide the residential per capita per day water use, calculated by summing the residential, residential – 2 family, residential – 3 family, and residential – multi-family customer categories divided by the estimated population and the number of days in the year.

2022 Calculation of Average Day Water Use per Capita		
Customer Class	**# of Customers**	**Annual Sales (GALS)**
RESIDENTIAL	16,748	729,038,455
RES-2 FAMILY	1,285	96,120,800
RES-3 FAMILY	76	6,163,400
MULTI-FAMILY	956	350,955,000
TOTAL	19,065	1,182,277,655
	DAYS IN 2022	365
	POPUATION IN 2022	71,256
USAGE PER CUSTOMER PER DAY (GPD)		170
USAGE PER CAPITA PER DAY (GPD)		45

Requirement (g): *Additional Conservation and Efficiency Measures*
A description of any additional Conservation and Efficiency Measures implemented.

Response:
Starting in 2006, the City of Waukesha Water Utility ("Utility") implemented a variety of conservation programs. Additionally, the Utility approved a conservation plan in 2012, which was updated in 2022 and is included in Appendix A. An analysis of water savings achieved since the 2012 Plan was implemented demonstrates that by 2021, WWU has exceeded savings goals established for 2030 and 2050. The near-term Program goals (Years 1 to 5) were included as Table 5.3 of the report and future reporting will include status updates of any new or additional measures implemented from this updated report.

Requirement (h):
A statement verifying that no customers outside of the diversion area were sold Lake Michigan water.

Response:
The City of Waukesha Water Utility certifies no Lake Michigan water was diverted in 2022 and therefore no customers inside or outside of the approved diversion area were sold Lake Michigan water.

Requirement (i):
A spatially explicit description of the properties served by the City's water utility, in the manner prescribed by the DNR.

Response:
Please see Attachment B.

Requirement (j): Exis*ting Deep Aquifer Groundwater Wells.*

A report of any City wells filled and sealed or changed to emergency use status in the past year. A description of deep aquifer groundwater wells maintained for emergency use, as allowed under Wis. Admin. Code § NR 810.22, and use of these wells in the previous year.

Response:

The status of City wells will change after the diversion commences, but that has not yet occurred. Please see Attachment C for the 2022 status of the City wells.

Requirement (k): P*harmaceutical and Personal Care Products Recycling and Impacts.*

A summary of the implementation of the pharmaceutical and personal care products recycling and reduction program in the past year.

Response:

The City of Waukesha has a Pharmaceutical and Personal Care Products Reduction Program that incorporated comments from the Wisconsin Department of Natural Resources. The City continues to publicize responsible disposal of pharmaceuticals in our City Newsletters which reach all of the residents of the community. The regular collection point located within the lobby of the City Police Department is back in place following removal during the construction work in 2022. The Waukesha County Sheriff's office, during the two Take Back Days sponsored by the Drug Enforcement Agency (DEA) annually, continues collection efforts. Some pharmacies have also made takeback boxes available to their customer in support of the effort.

Requirement (l): *Monitoring of Root River Flow.*

For at least 10 years after the date the diversion begins, the City shall annually report the results of Root River monitoring to DNR. The report shall include a summary of the monitoring results and a summary of any impacts to the Root River from the City's wastewater discharge.

Response:

The diversion has not yet begun and no return flow to the Root River has commenced, thus there are no monitoring results to report. However, the City of Waukesha Post-Return Flow Root River Monitoring Program was submitted to the Wisconsin Department of Natural Resources for approval on March 27, 2023, a copy is included as Attachment D.

Requirement (m): *Federal and State Permits and Approvals;*

A statement of compliance with all applicable federal and state permits and approvals.

Response:

The City of Waukesha has complied with all applicable federal and state approvals

Waukesha Water Utility 2022
Water Conservation Plan Update

Final
January 2023

GREAT WATER ALLIANCE™

Jacobs

Contents

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Acronyms and Abbreviations

2012 Plan	Waukesha Water Utility Water Conservation Plan (2012)
AWE	Alliance for Water Efficiency
AWE Tool	Alliance for Water Efficiency Water Conservation Tracking Tool
AWWA	American Water Works Association
B:C	benefit to cost
CEM	Conservation and Efficiency Measure
CII	commercial, industrial, and institutional (public)
City	City of Waukesha
Clean Water Plant	City of Waukesha Wastewater Treatment Plant
Compact	Great Lakes – St. Lawrence River Basin Water Resources Compact
gal/day	gallons per day
gpcd	gallons per capita per day
HET	high-efficiency toilet
lf	linear feet
MG	million gallon(s)
mgd	million gallons per day
NR 852	Wisconsin Administrative Code Chapter NR 852
Plan Update	Waukesha Water Utility Water Conservation Plan Update (2022)
PSC	Public Service Commission of Wisconsin
PSC 185	*Wisconsin Administrative Code* Chapter PSC 185
psi	pounds per square inch
Regional Body	Great Lakes – St. Lawrence River Basin Water Resources Regional Body
SEWRPC	Southeastern Wisconsin Regional Planning Commission
WDNR	Wisconsin Department of Natural Resources
WSSA	Water Supply Service Area
WWU	Waukesha Water Utility
USEPA	United States Environmental Protection Agency

1. Introduction

Since 2006, Waukesha Water Utility (WWU) has been a leader in water conservation among Wisconsin water utilities. In 2010, the Wisconsin Department of Natural Resources (WDNR) developed a state rule that establishes mandatory water conservation and efficiency measures (CEMs) for withdrawals in the Great Lakes basin and to promote voluntary water conservation statewide. That rule, *Wisconsin Administrative Code* Chapter Natural Resources (NR) 852 Water Conservation and Water Use Efficiency (NR 852) established a framework for the 2012 WWU Water Conservation Plan (2012 Plan). Since 2012, WWU has invested annually in its water conservation program and tracked water volumes saved through conservation. Details about conservation program implementation and water-savings achievements are documented in annual reports to the Public Service Commission of Wisconsin (PSC) and WDNR in conformance with *Wisconsin Administrative Code* Chapter PSC 185 Standards for Public Utility Service (PSC 185).

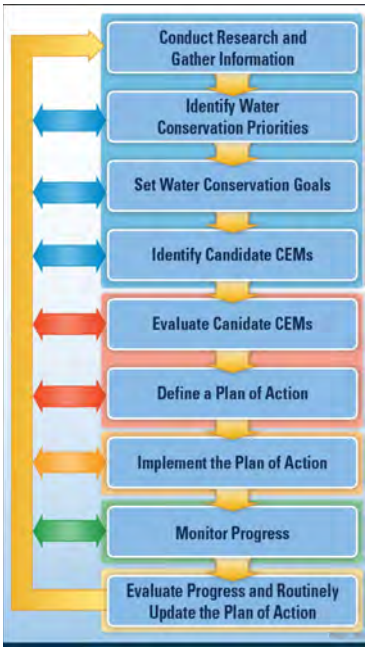
1.1 Purpose

The purpose of the WWU Water Conservation Plan Update (Plan Update) is to continue following the utility's water conservation planning process by completing the following:

- Confirm the water conservation goals.
- Review the conservation program performance over the past 10 years.
- Evaluate the effectiveness of existing CEMs.
- Analyze the benefits and costs of incorporating new CEMs into the program.
- Recommend actions to meet or exceed program water-savings goals.

The Plan Update refines WWU's path forward in customer service-oriented water-use efficiency planning and implementation. It focuses on key strategies for the next 5- and 10-year implementation periods. Because WWU anticipates transitioning to a new Great Lakes water supply within 1 year, the Plan Update will align with the water use and water conservation reporting requirements for the City of Waukesha Great Lakes Diversion, in addition to those of NR 852 and the PSC.

Figure 1-1. Water Conservation Planning Process



1.2 Background

The 2012 Plan was based on a City of Waukesha (City) water supply service area (WSSA) that was delineated by the Southeastern Wisconsin Regional Planning Commission (SEWRPC) in 2009 in conformance with Wisconsin State Statute Chapter 281 Water and Sewage and *Wisconsin Administrative Code* Chapter NR 121 Areawide Water Quality Management Plans. The 2012 Plan included targets for conservation water savings based on WSSA population projections, approved land use plans, and water-demand forecasts.

During review of the City's application for Great Lakes diversion, the City WSSA was modified by the Great Lakes – St. Lawrence River Basin Water Resources Regional Body (Regional Body) to exclude areas located

in the Town of Genesee and the Town of Delafield. The approved diversion service areas as of May 2016 were fixed to the following: [1]

1. *Incorporated land within the boundaries of the City of Waukesha and land outside the City of Waukesha's jurisdictional boundaries that is served with municipal water by the [City of Waukesha] through the WWU as of May 18, 2016. This land is referred to as the "current area served."*

2. *Land lying within the perimeter boundary of the City of Waukesha that is part of the unincorporated land in the Town of Waukesha. These areas are referred to as the "town islands." Town islands are transected or bordered by a WWU water main and are either fully surrounded by territory incorporated in the City of Waukesha or are bordered on one side by a transportation right-of-way and on the remaining sides by territory incorporated by the City of Waukesha.*

When the WSSA was reduced, the service area projected population, water-demand forecast and water-saving goals were also reduced. Table 1-1 compares key criteria that guide the WWU water conservation program. The 2012 Plan was based on the 2012 diversion criteria, and the current WWU Plan Update is based on the 2016 approved diversion criteria.

Table 1-1. Water Conservation Program Key Criteria

WSSA Buildout Condition	2012 Diversion Application	2016 Approved Diversion
Total area served, acres	32,209	28,059
Total population served	97,400[a]	89,000 – 91,290[b]
Average day water demand, mgd	10.1	8.2
Water conservation savings, mgd	1.0	0.8

[a] SEWRPC letter to City of Waukesha March 17, 2009.
[b] Interpolated from SEWRPC estimates.
mgd – million gallons per day

1.3 Water Supply Service Area

Figure 1-2 shows the WSSA. Table 1-2 summarizes the WSSA 2000 land use inventory and 2035 recommended land use plan. Residential is the single largest land use category.

Under current water service rules regulated by the PSC, all customers regardless of location in the service area are subject to the City's conservation measures, including the water rate schedule, outdoor water-use restrictions, financial incentives to install water-saving plumbing fixtures, and conservation educational resources.

[1] Final Decision on the City of Waukesha, Wisconsin's Application for Diversion of Great Lakes Water.

Figure 1-2. Water Supply Service Area

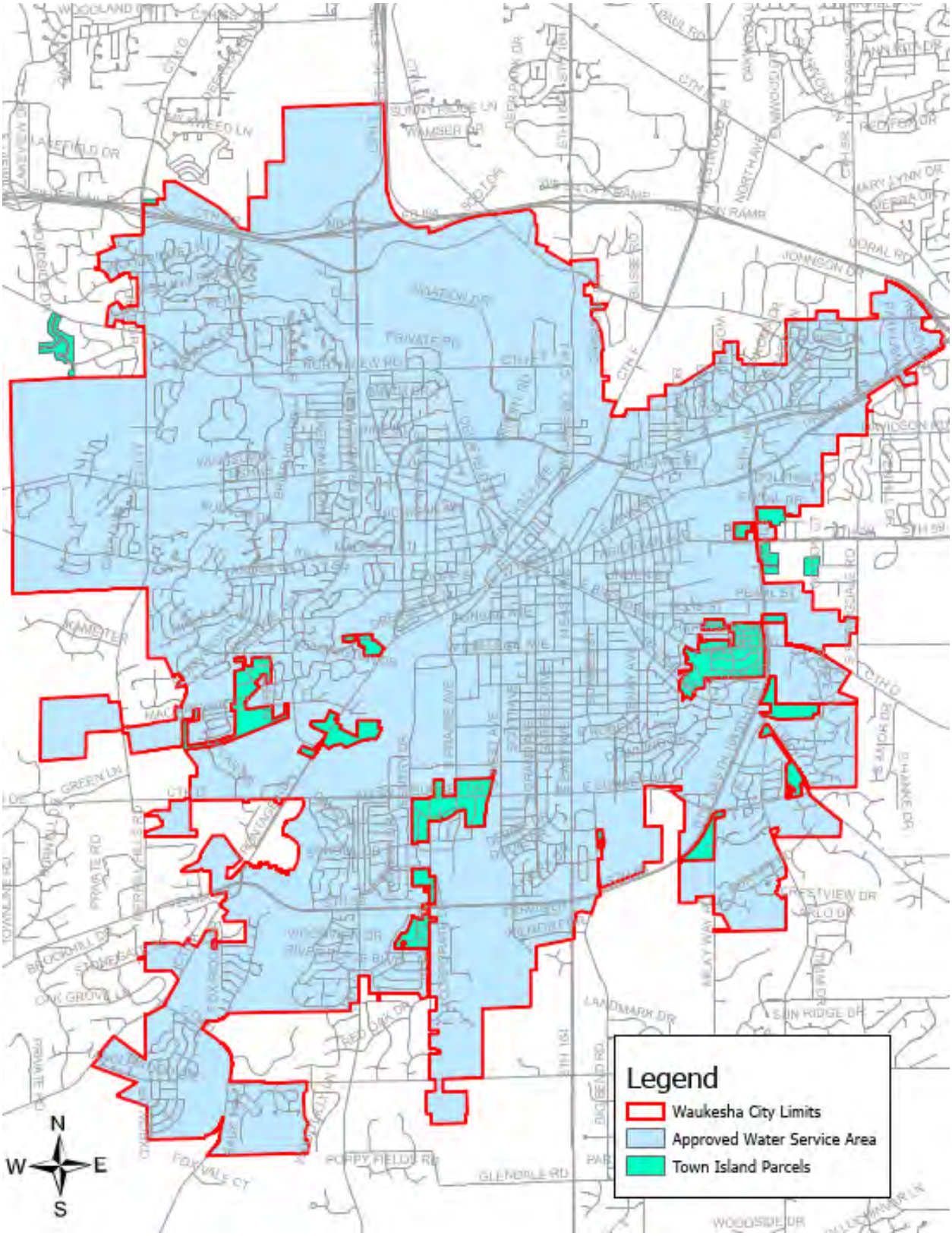


Table 1-2. WSSA Land Use By Civil Division [2]

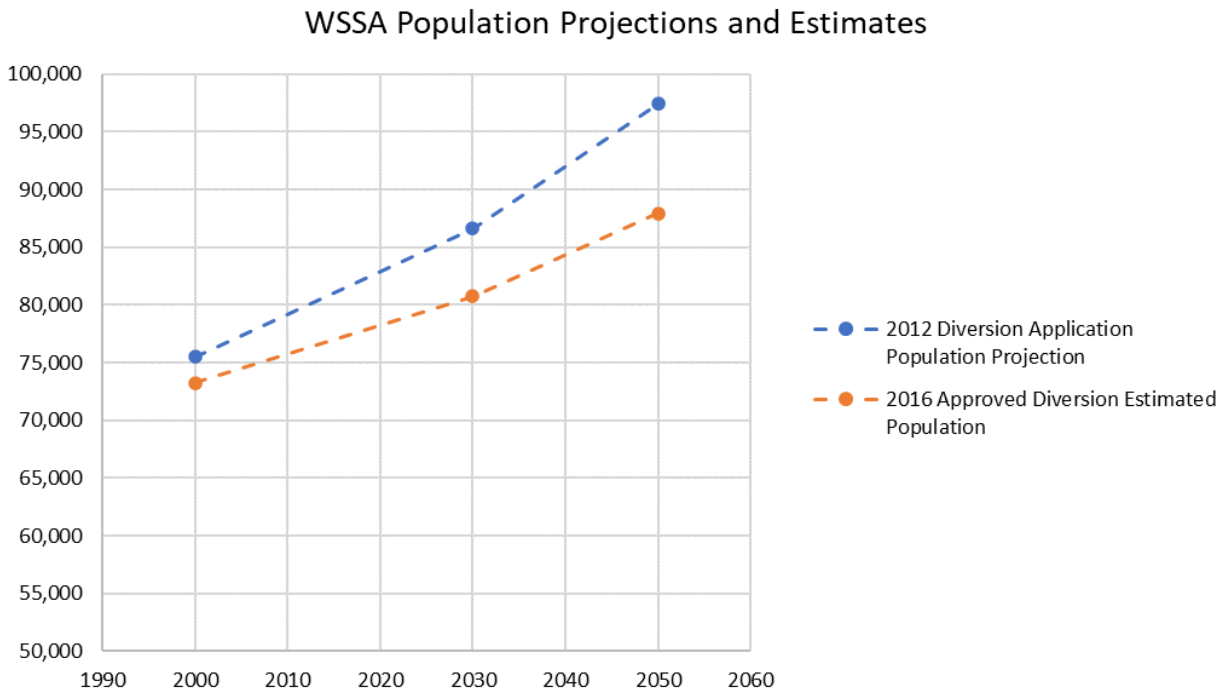
Land Use Categories	City of Pewaukee	City of Waukesha	Town of Waukesha	Grand Total
2000 Land Use Inventory, acres				
Agricultural and Other Open Lands	175	3,460	4,202	7,837
Commercial	0	816	64	880
Environmental Areas and Wetlands	53	1,670	2,711	4,434
Extractive	0	75	0	75
Governmental and Institutional		802	54	856
Industrial	0	987	38	1,025
Multi-family Residential		919	1	920
Recreational	13	500	260	773
Single-Family Residential	208	3,756	3,267	7,231
Surface Water	1	126	33	160
Transportation, Communication, and Utilities	60	2,904	904	3,868
Total	**510**	**16,015**	**11,534**	**28,059**
2035 Recommended Land Use Plan, acres				
Agricultural and Other Open Lands	3	182	808	993
Commercial	0	879	118	997
Environmental Areas and Wetlands	54	1,800	2,868	4,722
Extractive				0
Governmental and Institutional	15	964	162	1,141
Industrial	0	1,639	151	1,790
Multi-family Residential		583	0	583
Recreational	17	641	491	1,149
Single-Family Residential	366	5,999	5,956	12,321
Surface Water	1	114	33	148
Transportation, Communication, and Utilities	55	3,214	946	4,215
Total	**511**	**16,015**	**11,533**	**28,059**

1.4 Service Area Population

The most recent official population projections for the WSSA were prepared by SEWRPC in 2009. The projections were based on municipal estimates from the State of Wisconsin Department of Administration and multiple planning factors, including, but not limited to, land use, household size, demographic trends, and community development plans. Official population projections were not prepared for the smaller, approved Great Lakes diversion service area; however, projected population may be represented by the 2009 estimates less the population associated with Town of Genesee and the Town of Delafield areas included in the 2009 projections. Figure 1-2 depicts these projections and estimates. When 2020 U.S. Census-based population projections are prepared by the State of Wisconsin Department of Administration for Wisconsin municipalities, WWU will work with SEWRPC to prepared updated population projections for the WSSA.

[2] City of Waukesha Application for a Lake Michigan Diversion with Return Flow, April 2013.

Figure 1-3. Service Area Population



1.5 Water System

In 2023, it is anticipated that the WWU water supply will transition from groundwater to surface water.

Table 1-3. WWU Water System Features

Feature	2023	2024 and Beyond
Water supply type	10 groundwater wells	1 surface water pump station
Ground tanks, number	6	4
Elevated tanks, number	5	6
Watermains, miles	334	To be determined
Distribution pressure zones, number	10	10

1.6 Water Conservation Goals and Objectives

The City's water conservation goals include the following:

- Reducing average day demand by 0.4 million gallons per day (mgd) by year 2030 and by 0.8 mgd by year 2050 (the complete development/buildout condition).
- Leveraging lessons learned from implementation of existing City CEMs.
- Using the Alliance for Water Efficiency (AWE) Water Conservation Tracking Tool (AWE Tool) to the extent practical to estimate CEM savings and cost effectiveness.
- Targeting CEMs and customers with the highest potential for cost-effective water savings.

2. Historical Water Use

To evaluate the effectiveness of the water conservation program and make informed recommendations for the Plan Update, WWU water billing, well production, and water-use audit data between 2012 and 2021 were analyzed to review historical water use since the 2012 Plan.

WWU served 20,680 accounts in 2021, a 5% increase in number of accounts compared to 2012. Most WWU customer accounts are residential with some commercial, public, and industrial customers. Over the last 10 years, the number and types of customer accounts have remained consistent with the exception of multi-family housing which were commercial accounts in 2012 and are now residential accounts. WWU also replaced several shared meters at duplexes and triplexes with individual meters, resulting in a small shift of accounts from multi-family to single-family residential. The number of irrigation meters also increased slightly because irrigation rates were implemented in 2017; however, irrigation meters still make up a very small amount of the total accounts. Figure 2-1 shows the distribution of customer accounts in 2012 and 2021, and Table 2-1 shows the number and type of customer accounts in each year.

Figure 2-1. Total Accounts by Customer Class in 2012 and 2021

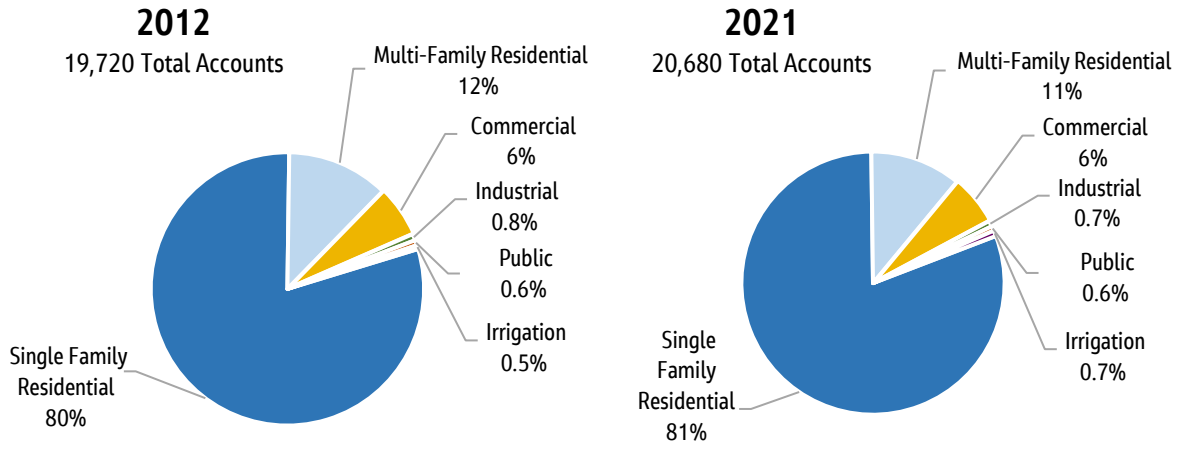


Table 2-1. Total Accounts by Customer Class in 2012–2021

Customer Class	2012	2013	2014	2015	2016	2017
Single-Family Residential	15,764	15,889	15,961	16,051	16,169	16,308
Multi-Family Residential	2,385	2,368	2,358	2,364	2,362	2,358
Commercial	1,208	1,219	1,220	1,230	1,248	1,248
Industrial	151	150	150	150	151	148
Public	122	124	121	119	118	117
Irrigation	90	92	114	118	120	122
Total	**19,720**	**19,842**	**19,924**	**20,032**	**20,168**	**20,301**

Customer Class	2018	2019	2020	2021	Percent Change 2012- 2021
Single-Family Residential	16,414	16,509	16,592	16,675	6%
Multi-Family Residential	2,346	2,336	2,328	2,319	-3%
Commercial	1,259	1,271	1,270	1,277	6%
Industrial	148	148	147	147	-3%
Public	118	118	120	120	-2%
Irrigation	132	137	138	142	58%
Total	**20,417**	**20,519**	**20,595**	**20,680**	**5%**

The total water sold has decreased from 2012 and 2021. Figure 2-2 shows a comparison of the amount of water sold by customer class between 2012 and 2021. A decrease in annual billed consumption from 2,311 million gallons (MG) to 1,805 MG in 2021 represents an overall 22% decrease over the last 10 years. Water use decreased in all customer classes, with the largest percent change in industrial water uses. Table 2-2 includes the annual billed consumption in MG by customer class from 2012 to 2021, including the overall percent change over the last 10 years.

Figure 2-2. Annual Billed Consumption by Customer Class in 2012–2021

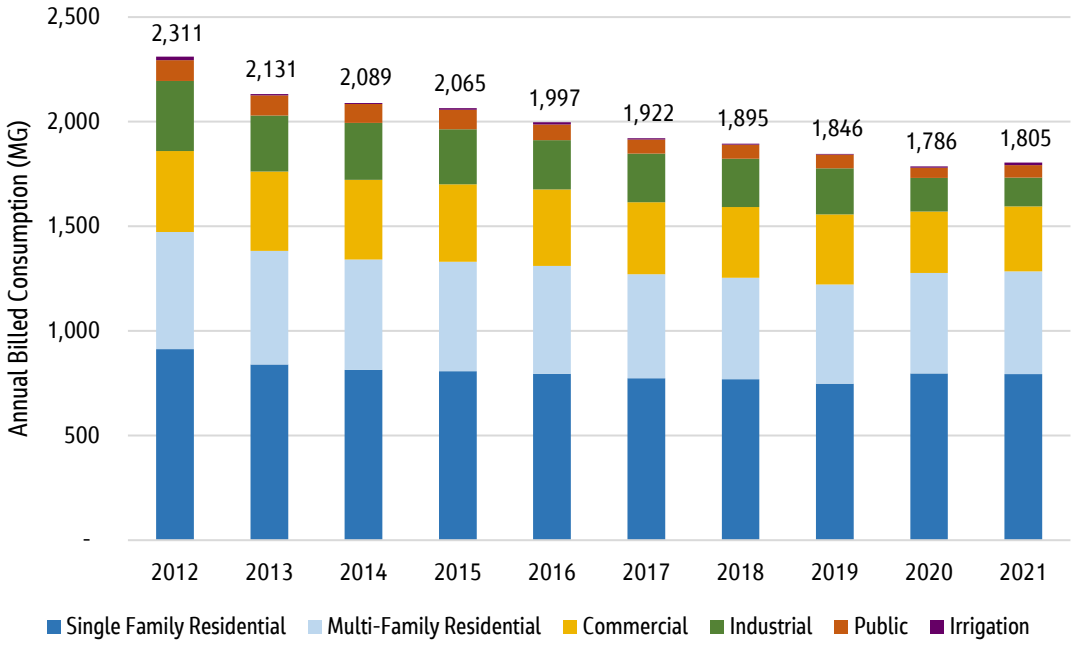


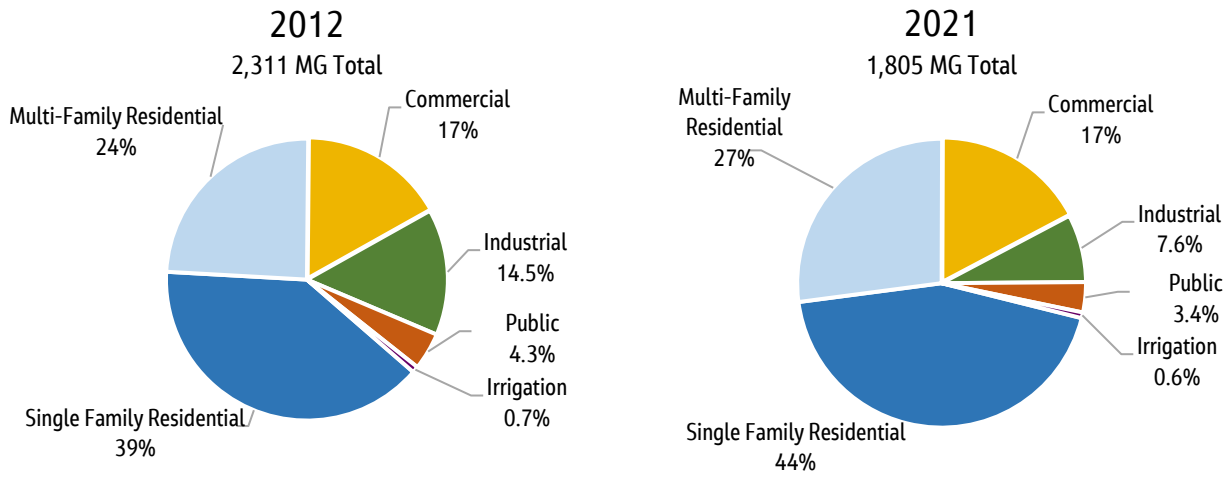
Table 2-2. Annual Billed Consumption (MG) by Customer Class in 2012–2021

Customer Class	2012	2013	2014	2015	2016	2017
Single-Family Residential	913	840	814	807	795	775
Multi-Family Residential	561	542	528	523	515	496
Commercial	387	379	380	371	366	344
Industrial	335	269	272	263	236	233
Public	99	96	91	93	76	67
Irrigation	17	6	4	8	10	7
Total	**2,311**	**2,131**	**2,089**	**2,065**	**1,997**	**1,922**

Customer Class	2018	2019	2020	2021	Percent Change 2012-2021
Single-Family Residential	770	747	797	795	-13%
Multi-Family Residential	484	474	480	490	-13%
Commercial	338	335	293	311	-20%
Industrial	231	220	161	137	-59%
Public	68	65	48	61	-38%
Irrigation	4	3	6	11	-32%
Total	**1,895**	**1,846**	**1,786**	**1,805**	**-22%**

More than 90% of the customer accounts are residential meters (single- and multi-family) and their consumption was approximately 70% of the total water sold in 2021. Commercial and industrial water are the next largest categories of water use. Figure 2-3 shows the percent water sold by customer class in 2012 and 2021. The biggest changes in the composition of water sold is the decrease in percent for industrial water and increase in percent for residential categories (note that total residential water consumption was a net decrease between 2012 and 2021; Table 2-2).

Figure 2-3. Total Billed Consumption by Customer Class in 2012 and 2021



An average demand in gallons per capita day (gpcd) was calculated for residential water by summing all single- and multi-family residential meters and dividing by the service area population. Residential water demand has decreased from approximately 53 gpcd in 2012 to 45 gpcd in 2021; Table 2-3 shows the change in average residential demand from 2012 to 2021.

Table 2-3. Residential Per Capita Water Demand

Year	Per Capita Demand Per Day (gpcd)
2012	53
2013	50
2014	48
2015	48
2016	47
2017	45
2018	44
2019	43
2020	45
2021	45

2.1 Non-Revenue Water

WWU reports revenue and non-revenue water annually to the PSC. Non-revenue water includes apparent losses, unbilled authorized consumption, and reported leakage (real losses). WWU's non-revenue water is typically less than 10%, except 2014 when sustained record low temperatures resulted in an unusually high number of water main and service lateral breaks. Figure 2-4 shows the percentage of non-revenue water from 2012 to 2021, and Figure 2-5 shows the typical breakdown of non-revenue water for 2021.

Figure 2-4. Percent Revenue and Non-Revenue Water from 2012 to 2021

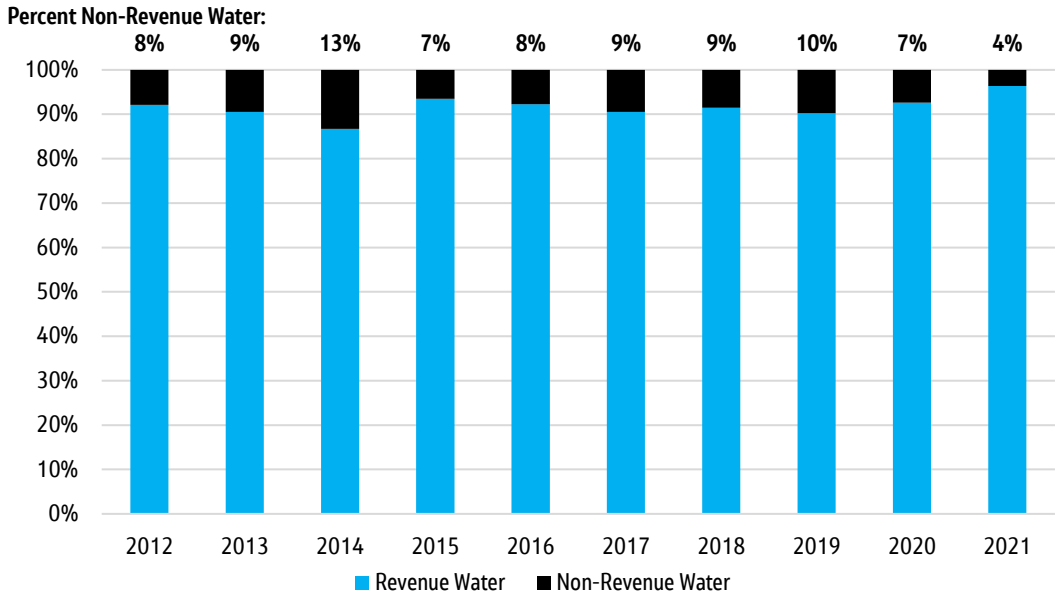
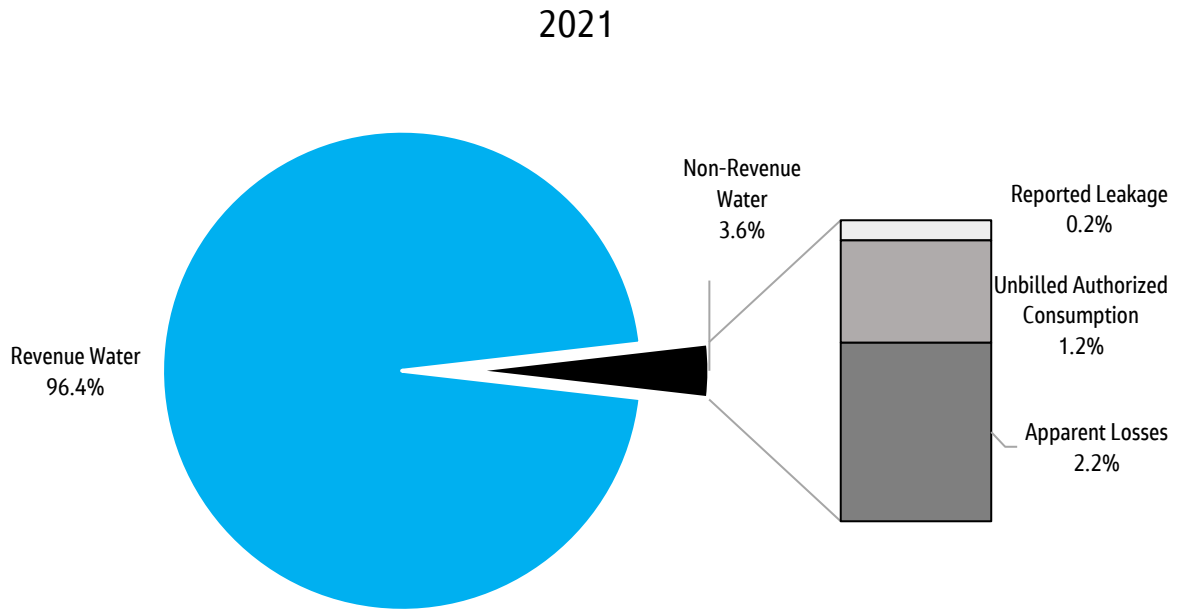


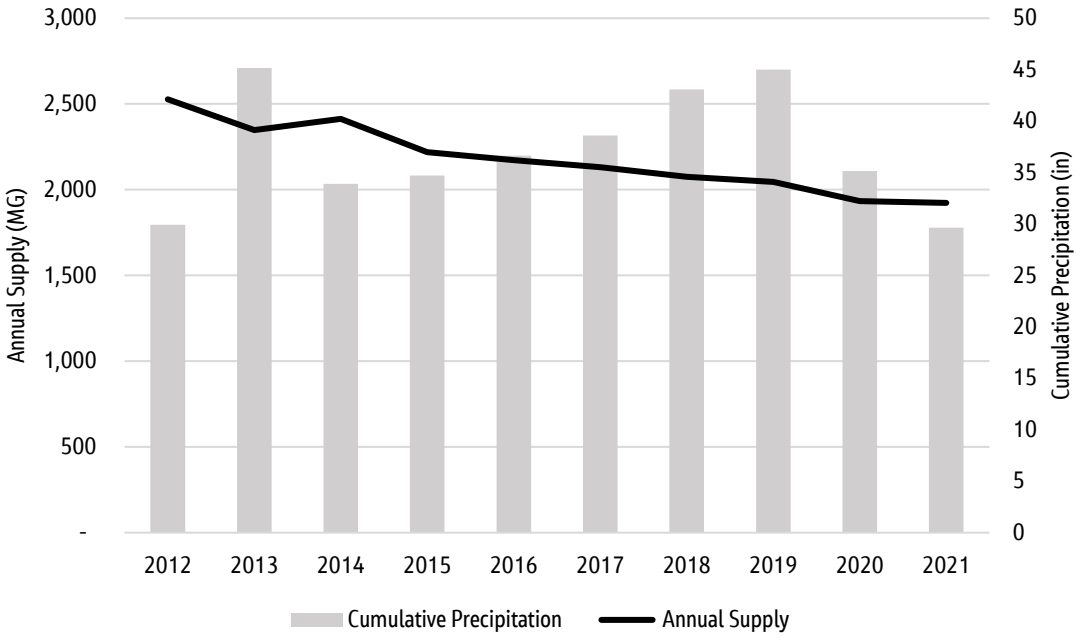
Figure 2-5. Revenue and Non-Revenue Water Losses in 2021



2.2 Seasonal Water Demand

Annual and monthly water supply from pump data at the well locations was compared against cumulative precipitation to observe general trends in different weather years. Figure 2-6 shows the annual supply and cumulative precipitation for 2012 through 2021. Cumulative precipitation increased annually between 2014 and 2019. During this time, there was also an inverse relationship with water demand that would be expected as customer outdoor water use typically declines in years with average-to-above-average precipitation. However, water demand continued to decline in lower precipitation years of 2020 and 2021, which may be attributed to reduced economic activity during COVID-19 and water conservation awareness promoted through the conservation program.

Figure 2-6. Annual Supply and Cumulative Precipitation for 2012 through 2021

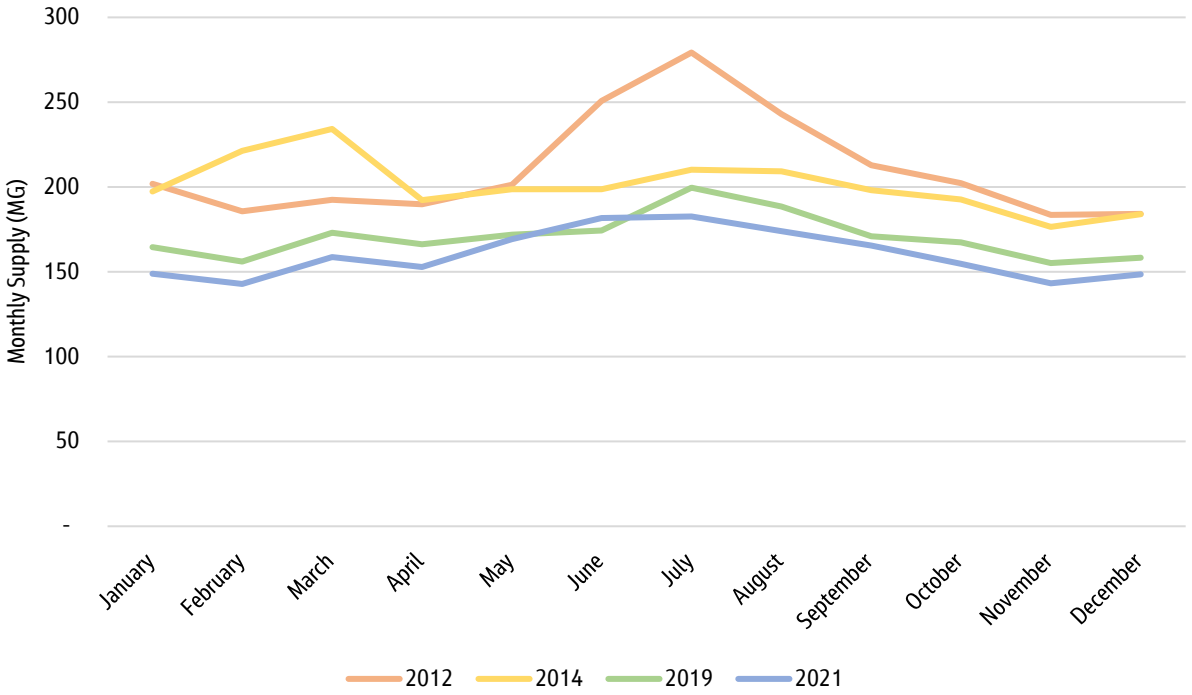


Four years were selected for further analysis of the effects of precipitation on water use: 2012 with historic below-average precipitation during summer months; 2014 with a longer, colder winter and typical precipitation year; 2019 as a year with historic above-average precipitation, and 2021 as the most recent year and with average-to-below-average precipitation. Monthly production data from each year were analyzed for differences in seasonal water demand, shown in Figure 2-7.

Overall trends align with the decrease in annual billed consumption, where monthly water supplied to customers decreased in all months when comparing 2012 to 2021. There is little variation in monthly supply in recent years, indicating that the seasonality of water use is becoming less prominent due to a reduction in outdoor water use. The reduction in outdoor water use during this period is likely due to a combination of water-use efficiency measures and increased precipitation over the last 10 years. The Wisconsin Rainfall Project combines rainfall statistics from the National Oceanic and Atmospheric Atlas 14 and the University of Wisconsin's RainyDay software that downscales global climate models to project precipitation trends at the county level in Wisconsin. Future climate models for Waukesha County indicate that rainfall frequency and quantity will likely continue to increase under all emissions scenarios. [3] This may result in continuing lower demand for outdoor water use during the growing season (May–October), similar to the conditions observed in 2017–2019.

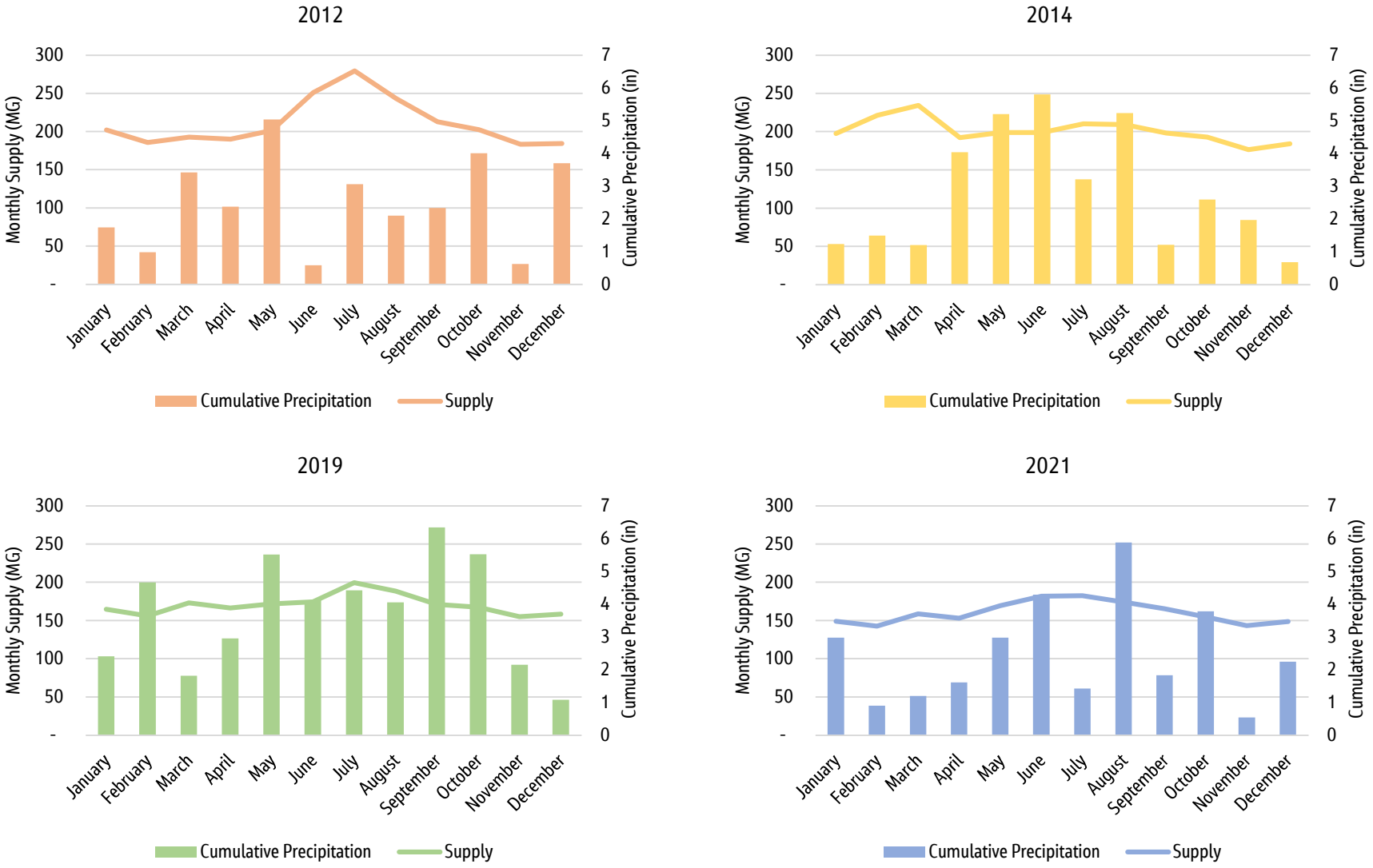
[3] https://her.cee.wisc.edu/the-wisconsin-rainfall-project/

Figure 2-7. Monthly WWU Water Production in 2012, 2014, 2019, and 2021



Cumulative monthly precipitation for each year was analyzed to further interpret possible differences in seasonal water demand. Figure 2-8 shows monthly supply and precipitation data for 2012, 2014, 2019, and 2021. The cumulative precipitation in 2019 was high in most months of the year, particularly during the Wisconsin growing season of May–October. However, decreased precipitation in 2021 resulted in very similar monthly supply trends when comparing 2019 to 2021, indicating that in recent years, the variation in precipitation does not have as much impact to water supply trends as was observed in 2012.

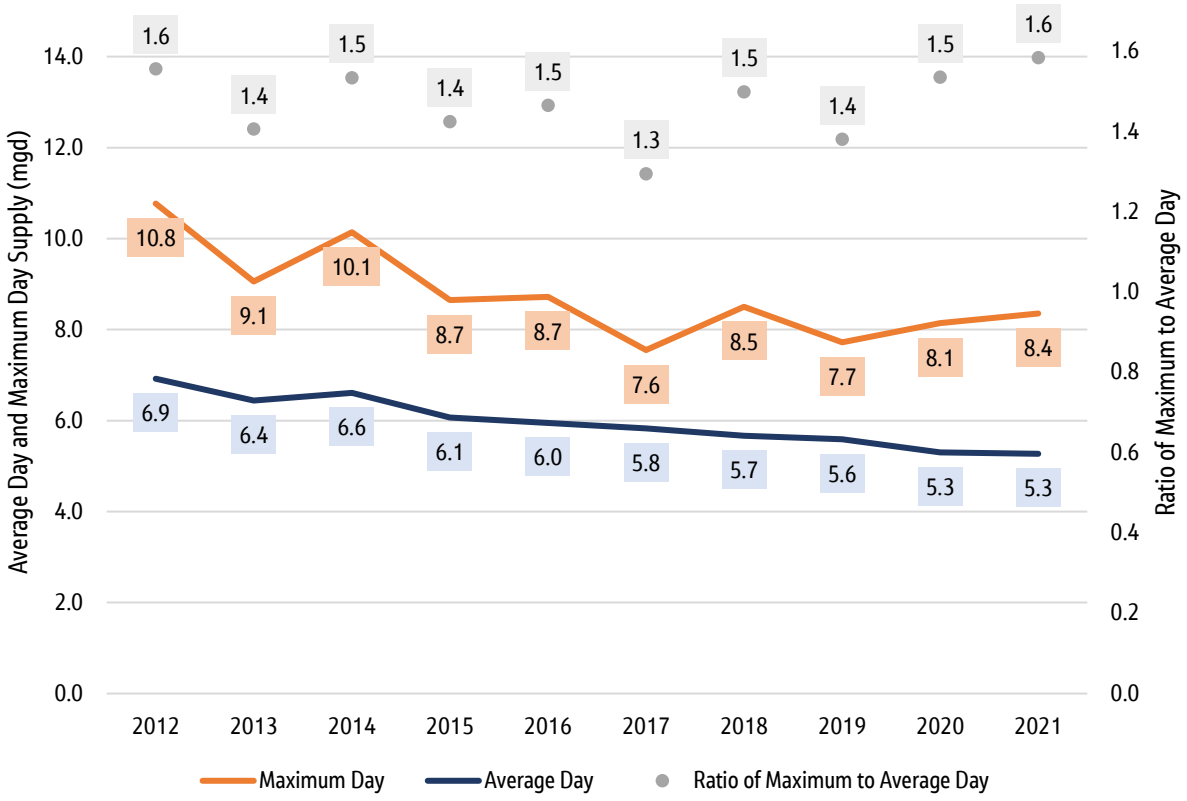
Figure 2-8. Monthly Water Supply and Cumulative Precipitation in 2012, 2014, 2019, and 2021



2.3 Variation in Customer Demand

The annual average and maximum day demand (Figure 2-9) were calculated using the WWU operating data. Both average day and maximum day demands have decreased over the last 10 years. The ratio of maximum to average day remains between 1.3 and 1.6.

Figure 2-9. Annual Average and Maximum Day Water Demand



3. Conservation Program Status: 2012–2021

WWU has long been a leader in water conservation in the state of Wisconsin. The City adopted its 2006 Water Conservation and Protection Plan, which set forth water-savings goals and recommendations for conservation program management and source water protection. Between 2006 and 2012, the City implemented a variety of conservation measures, including the following:

- First in the state to implement residential inclining block water rate structure to encourage conservation
- City ordinance to restrict outdoor irrigation
- High-efficiency toilet rebates
- School and general public information and education campaigns

Figure 3-1. Conservation Program Overview

In 2012, Waukesha adopted the 2012 Plan that established both water-savings goals as described in Section 1 and specific CEMs. These measures include both those required by NR 852 and additional measures to achieve their savings goals. This section summarizes achievements since 2012.

3.1 Summary of 2012 Water Conservation Plan

In the 2012 Plan, the recommended 5-year (2012–2016) implementation plan includes the following elements with projected water savings by CEM listed in Table 3-1 and shown in Figure 3-2:

- New and expanded fixture rebate measures to accelerate replacement of less efficient devices for residential, commercial, industrial, and public customers
- Expanded public education and information
- Additional customer water audits or inspections to design tailored customer demand management strategies
- Increase program data gathering and monitoring to measure program effectiveness

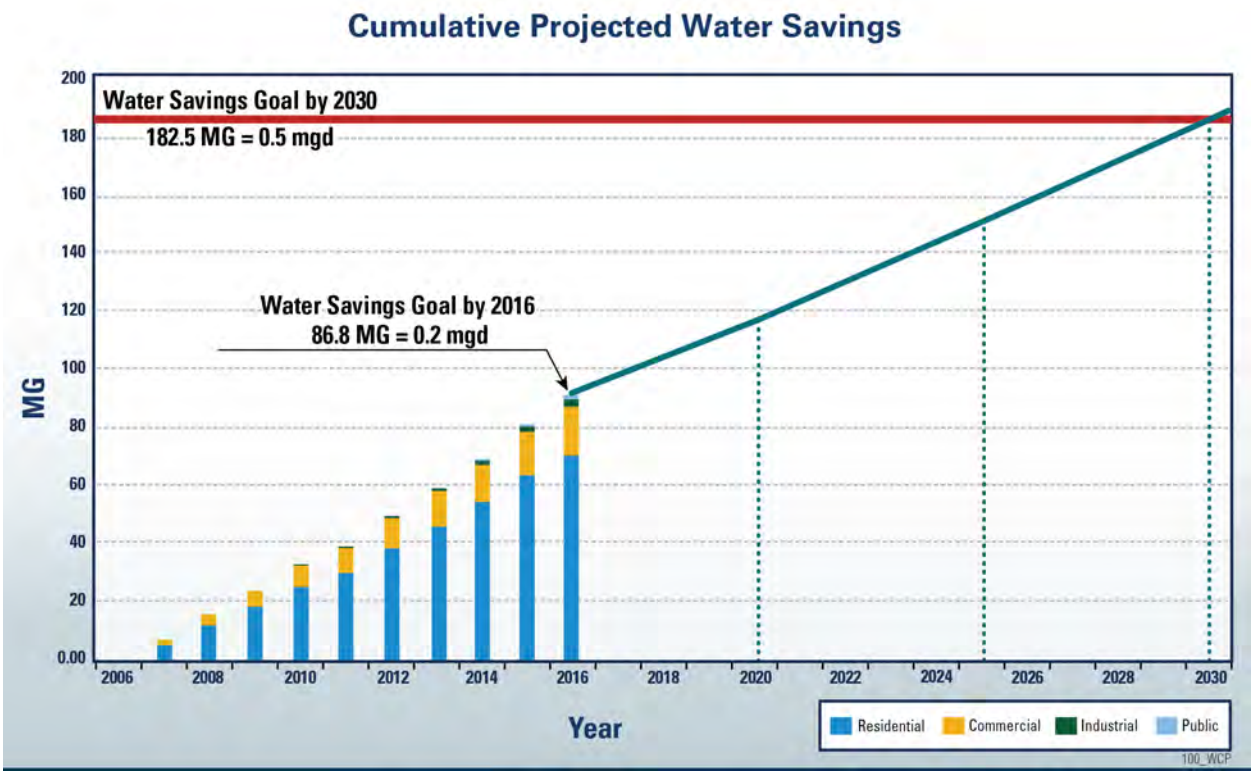
Table 3-1. Projected Water Savings for 2012–2016 in the 2012 Water Conservation Plan

Activity	Projected Water Savings (gallons)
Residential HETs, $100 rebate	7,325,700
Multi-family residential HET direct install, $100 rebate	113,000
Commercial tank-type HET, $100 rebate	34,500
Commercial valve-type HET	57,500
Industrial tank-type HET, $100 rebate	80,400
Industrial valve-type HET, $100 rebate	80,400
Public tank-type HET, $100 rebate	80,400
Public valve-type HET, $100 rebate	80,400
Residential water-efficient showerhead	866,200
Multi-family residential water-efficient showerhead	11,000

Activity	Projected Water Savings (gallons)
Commercial water-efficient showerhead	4,100
Industrial water-efficient showerhead	16,500
Public water-efficient showerhead	15,200
Residential indoor water-use surveys	73,000
Multi-family residential indoor water-use surveys	4,000
Commercial indoor water-use surveys	17,000
Industrial indoor water-use surveys	21,700
Public indoor water-use surveys	21,700
Commercial outdoor water-use surveys	N/A
Public outdoor water-use surveys	N/A
Commercial urinals, $100 rebate	93,100
Industrial urinals, $100 rebate	93,100
Public urinals, $100 rebate	93,100
Commercial spray-rinse valves rebates	1,414,300
Industrial spray-rinse valves rebates	1,414,300
Public spray-rinse valves rebates	1,414,300
Public HE clothes washer rebate	7,000

HET = high-efficiency toilet

Figure 3-2. Water Savings Goal and Projected Water Savings (2012 Plan)



The implementation strategy in 2012 began by building a strong foundation and support for the programs in the early years through public education and incentives for residential, public, and low-income customers and then expanded to include incentives for commercial, large multi-family, and industrial customers. As the program expanded, the City modified the programs to focus on activities with the highest potential for cost-effective water savings. This was done with tailored incentives for commercial and industrial customer accounts and by toilet replacements in large multi-family apartments.

3.2 Conservation Efficiency Measures

NR 852 requires all Public Water Supply (PWS) systems applying for a new or increased Great Lakes water withdrawal, diversion, or water loss to provide documentation showing implementation or completion of specified CEMs. Prior to the submission of its application for a Great Lakes diversion with return flow, the City implemented the CEMs and continued them since adoption of the 2012 Plan. The City will continue the best practices on an ongoing basis into the future. Table 3-2 provides highlights from implementation of the required CEMs from 2013-2021.

Table 3-2. Implementation Progress: Annual Highlights for Required CEMs

CEM	2013	2014	2015	2016	2017	2018	2019	2020	2021
PWS-1 Water-Use Audit	Water audits of public pools found leaks in the fall and repaired in the spring 2014 Water loss at 8.7%	Water loss at 11.2%	Water loss at 4.1%	Water loss at 5.5%	Water loss at 8.7%	Water loss at 6.3%	Water loss at 8.6%	Water loss at 6.4%	Water loss at 2.4%
PWS-2 Leak Detection and Repair Program	Fix a Leak Week Replaced 17,200 lf of mains	Additional online tools to help customers find leaks Leaks repair at public pools Replaced 11,135 lf of mains	Initiated Hydrant Leak Surveys 2 companies conducted water audits and repaired leaks or changed equipment Replaced 15,582 lf of mains	Replaced 21,830 lf of mains Inspected 2,088 hydrants and repaired leaks	Replaced and insulated laterals to eliminate need to run water during a freeze Replaced 8,156 lf of mains Inspected 1,717 hydrants and repaired 22 leaks	Replaced 10,390 lf of mains Inspected 1,288 hydrants and repaired 16 leaks	Replaced 16,224 lf of mains Inspected 1,933 hydrants and repaired leaks	Replaced 10,551 lf of mains Inspected 1,234 hydrants and repaired leaks	Replaced 8,383 lf of mains Inspected 1,174 hydrants and repaired leaks
PWS-3 Information and Education Outreach	Continued public information messages Held public meetings with giveaways and continued school-age education	Continued education programs and partnerships	Continued education programs and partnerships Launched new website	Continued education programs and partnerships	Continued education programs and partnerships Great Water Alliance held numerous open houses Received certificate from USEPA's Water Sense Program	Continued education programs and partnerships Participated in regional and national association meetings and conferences	Continued education programs and partnerships Great Water Alliance video series and newsletters	Continued education programs and partnerships Fewer public meetings due to COVID-19 pandemic	Continued education programs and partnerships Fewer public meetings due to COVID-19 pandemic
PWS-4 Source Measurement	All source water is measured	All source water is measured	All source water is measured	All source water is measured	All source water is measured	All source water is measured	All source water is measured	All source water is measured	All source water is measured

CEM	2013	2014	2015	2016	2017	2018	2019	2020	2021
PWS-R1 Distribution System Pressure Management	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones	WWU manages system pressure in 10 pressure zones
PWS-R2 Residential Demand Management Program	Increased HET rebate to $100 Number of toilet rebates increased	Toilet rebate program continued	Added a pilot irrigation controller rebate program for a specific newly developed subdivision	Added a showerhead replacement rebate	Initiated the Rain Barrel Rebate Program 101 toilet rebates issued Conducted residential leak audits	92 toilet rebates issued	72 toilet rebates issued	Discontinued the Pilot irrigation controller rebate program for a specific newly developed subdivision due to lack of participation	Fewer rebates likely due to COVID-19 pandemic
PWS-R3 Commercial and Industrial Demand Management Program	Recognition given to large industry for their efficiency success HET toilet rebate program Worked with commercial sites to replace toilets La Casa de Esperanza toilet change outs	Innovative Site-Specific Water-Saving Grant Program Waukesha School District pool valve repair, chiller replacement and replace turf on athletic fields Carroll University and other large water user conservation measures	Added a pilot irrigation controller rebate program Pre-rinse spray valve rebate program started	Added a showerhead replacement rebate 2 multi-family customers replaced toilets 1 Site-Specific Grant awarded Waukesha School District changed on spray valves	229 multi-family toilet rebates issued One of top 15 industrial water users participated in Site-Specific Grant Program	87 multi-family toilet rebates issued	404 multi-family rebates issued	No Site-Specific Grants issued likely due to COVID-19 pandemic	No Site-Specific Grants issued likely due to COVID-19 pandemic

CEM	2013	2014	2015	2016	2017	2018	2019	2020	2021
PWS-R4 Water Reuse	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	Onsite reuse by large industrial customer WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	Onsite reuse by 1 of the top 10 industrial water customers WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible	WWU is required to return 100% of its effluent; therefore, water reuse on a utility scale is not feasible
Tier 3 Additional CEMs	City Municipal Code modified to require efficient irrigation controllers and annual inspections			Sewer Credit Meter Ordinance Adopted	Increase in water rates for irrigation-only meters				Instituted monthly billing Irrigation rates increased

HET = high-efficiency toilet

lf = linear feet

USEPA = United States Environmental Protection Agency

3.3 Conservation Results through 2021

Section 2 provided information on water-use savings between 2012 and 2021 through analysis of production and billing data in a top-down approach. Changes in water use can be influenced by weather, economic conditions, and other factors. A bottom-up approach provides a method of estimating water savings directly attributable to CEMs. WWU uses the AWE Water Conservation Tracking Tool (AWE Tool) to estimate savings resulting from the conservation program. This section summarizes the savings achieved since 2012.

3.3.1 Methodology

Following the publishing of NR 852 in 2011 and prior to the development of the 2012 Plan, the City evaluated numerous CEMs using Version 2.0 of the AWE Tool. The tool is a water conservation calculator that is recommend by the WDNR for estimating water savings and costs associated with CEMs. The initial analysis using the AWE Tool resulted in a short list of candidate CEMs for further evaluation by WWU and stakeholders. Stakeholders were engaged in the water conservation planning process through an online survey, strategic customer interviews, and participation in the water conservation stakeholder committee. Engaging the City's customers and active community members provided valuable insights regarding the level of awareness of the need for conservation and ways to achieve it. The stakeholder committee input helped establish a baseline for the City's approach to future public information and education activities.

Subsequent to the 2012 Plan, and as required by Wisconsin Statutes Chapter PSC 185.97 Standards for Water Public Utility Service, WWU has annually tracked and reported CEM program progress in the AWE Tool for the purpose of reporting annual water savings along with overall cost effectiveness of each CEM.

3.3.1.1 AWE Tool Overview

The AWE Tool is a macros-enabled Microsoft Excel workbook that is navigated via separate worksheets that are grouped into three groups: User Input Sheets, Tracking Tool Output Sheets, and Background Calculation and Data Sheets. The first two categories of worksheets are where the majority of user interaction occurs. Within each User Input worksheet, there are separate modules that guide users through various inputs and calculations. The tool comes pre-loaded with a library of pre-defined conservation measures.

3.3.1.2 Transitioning Between Version 2.0 and Version 4.0

For the 2012 Plan and in subsequent annual water conservation reports, WWU used Version 2.0 of the AWE Tool to estimate conservation water savings. Since 2012, the AWE Tool has been upgraded in collaboration with AWE partners and members, while also incorporating the latest findings from relevant peer reviewed literature. As significant advancements have been made over the last 10 years, it was determined beneficial to the Plan Update to migrate WWU conservation program data to the current AWE Tool Version 4.0. Specific advancements in the tool include:

- **Additional Customer Classes.** With the addition of Multi-Family and Commercial Industrial Institutional (CII) Irrigation Meter, it is easier to track and quantify targeted CEMs.
- **Water Savings for Landscape Conservation.** A module has been added that allows the user to utilize the models' build-in landscape water-use calculator, or the users own estimates can be used.
- **Expanded Pre-Defined Conservation Measures**. There are now 50 pre-defined conservation measures.
- **Updated Standards Modules.** Modules have been updated and expanded throughout to reflect changing codes and standards.
- **New Modules.** Several new modules have been added including a price response module that estimates the effect marginal water-cost changes have on demand, as well as a water loss module.

The most significant changes between the versions of the tool occurred in the user interface, the user inputs, and the tool calculations.

For WWU, utility customer information was aligned with AWE Tool customer classes as shown in Table 3-3.

Table 3-3. AWE Tool and WWU Customer Classes

AWE Tool Customer Classes	WWU Customer Classes
Single-Family	Residential
Multi-Family	Multi-Family Residential 2 Family Residential 3 Family
CII Irrigation Meter	Irrigation
CII Common Meter	Commercial Federal Industrial Municipal Other Public State

3.3.2 Results

Table 3-4 summarizes the CEMs and number of participants entered into the AWE Tool and the model-estimated cumulative savings that have been achieved since inception of the program in 2006.

Table 3-4. Conservation Program Element Participation Rates and Estimated Savings 2006–2021

Activity	Number of Participants	Actual Water Savings (cumulative MG)
Residential HE Toilet, $25 Rebate	89	0.7
Commercial Tank-Type HE Toilet, $25 Rebate	1	0.01
Residential HE Toilet, $100 Rebate	952	8.0
Commercial HE Toilet, Large Multi-family, $100 Rebate	1,377	13.7
CII Grant, Tomorrow's Choice, $5,000	1	0.1
Commercial CII Spray-Rinse Valve Grant, $70	33	0.14
Public CII Spray-Rinse Valve $70 Grant	25	0.10
CII Grant, City Hall Toilet Replacement, $1,000	1	1.4
CII Grant, MetalTek, $1,000	1	4.0
CII Grant, Navistar, $1,000	1	15.0
CII Grant, Golden Guernsey, $1,000	1	1.9
CII Grant, GE Healthcare, $1,000	1	0.3
La Casa Village HE Toilet, $50 Rebate	40	0.4
CII Grant, Horeb Pool Leak Investigation, $1,500	1	0.4
Waukesha South Pool Valve, $0	1	0.3
CII Grant, Waukesha School District Chiller/Condenser Units, $15,000	1	0.6
CII Grant, Carrol Natatorium (Van Male) Upgrades, $2,500	1	0.8
CII Grant, Eaton/Cooper - Recirculating Pump, $10,000	1	3.1
CII Grant, Eaton Lincoln Ave Chiller Unit, $11,000	1	3.4

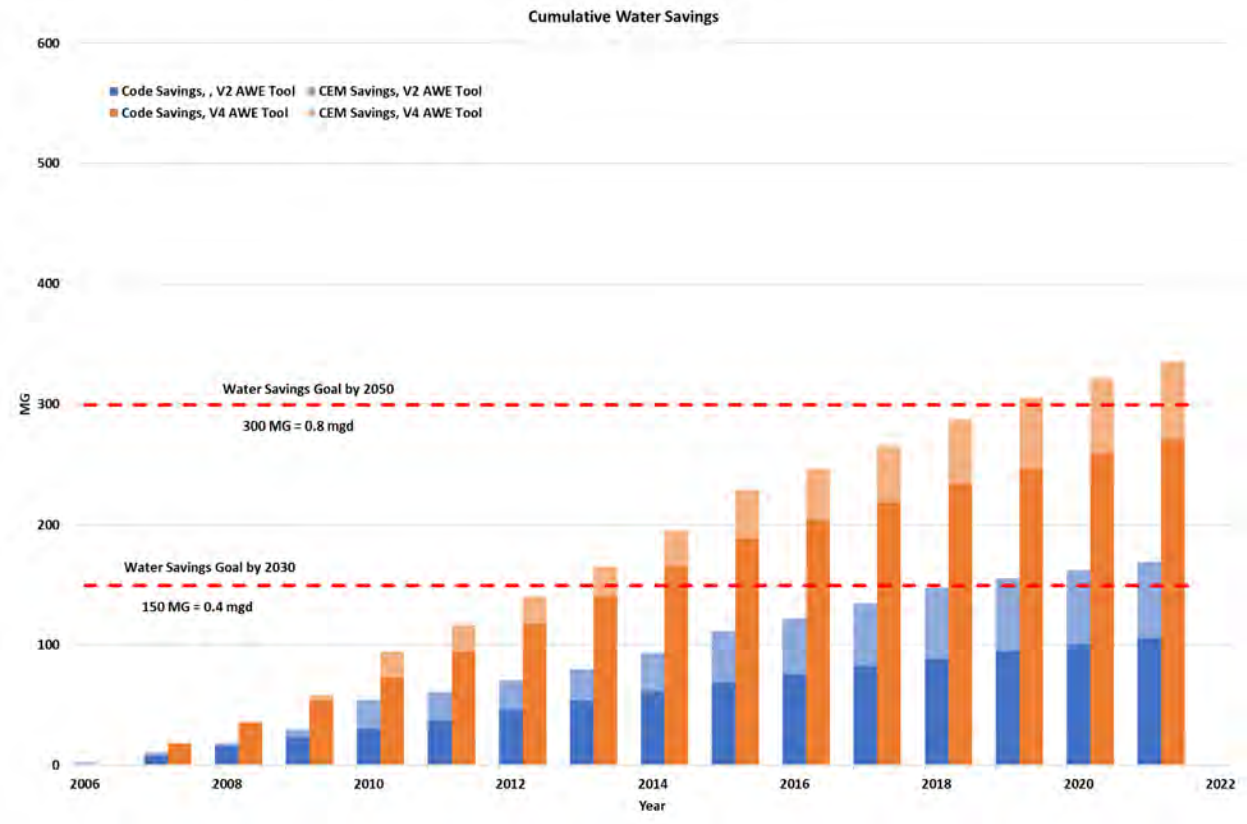
Activity	Number of Participants	Actual Water Savings (cumulative MG)
CII Grant, Waukesha Memorial Hospital Vacuum Pump Replacement, $8,400	1	2.6
CII Grant, Eaton Badger - 2nd Recirculating Pump, $10,000	1	3.1
Waukesha Housing Authority Showerhead Grant	150	0.05
Residential Low Flow Showerhead, $25 Rebate	55	0.02
CII Grant, Alloy Products - Wastewater Recycling System, $1,800	1	1.0
Industrial Tank-Type HE Toilet, $50 Rebate	12	0.2
Residential Rain Barrel, $20 Rebate	51	0.03
CII Grant, Eaton Lincoln Ave Chiller #2 & #3, $15,000	1	4.6
Total	2,801	65.8

HE = high efficiency

As discussed in Section 2, the overall and per capita water use within WWU's service area is trending downward. Using the AWE Tool, it is clear that a significant amount of the savings is the result of the City's three-pronged approach to conservation: incentives, education, and policies.

Figure 3-3 presents cumulative water savings from CEMs and Code Savings (water-use reduction resulting from changes in plumbing fixture standards and codes) through 2021 estimated with Version 2.0 and Version 4.0 the AWE Tool. Also shown are the average day demand water-savings goals for WWU by 2030 and 2050.

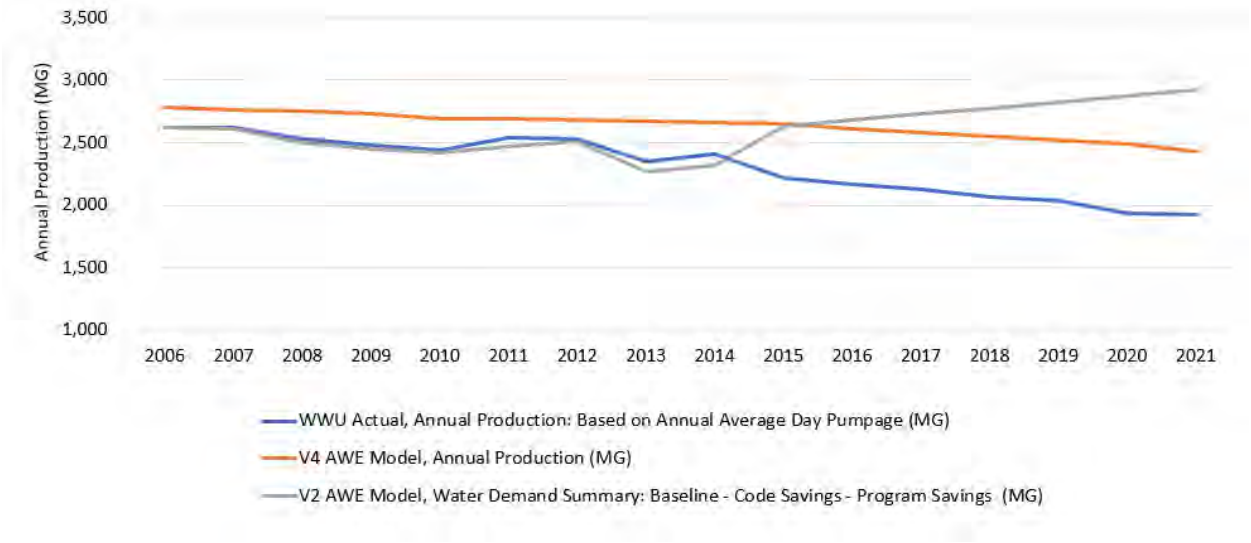
Figure 3-3. Cumulative Water Savings through 2021



The estimated cumulative water savings from CEMs is essentially the same using both versions of the tool; however, the estimated cumulative water savings attributed to Code Savings is significantly greater using Version 4.0 compared to Version 2.0. To conduct a quality control review of the Version 4.0 model-estimated Code Savings, two checks were conducted:

1. AWE Tool technical support services were enlisted to review model input and output.
2. WWU water production data was compared to AWE Tool water production estimates. Version 4.0 output aligns with actual data reasonably well and more closely than Version 2.0 output as shown in Figure 3-4.

Figure 3-4. Actual and AWE Tool Water Production Estimates Comparison



Based on AWE Tool Version 2 water-savings estimates, WWU exceeded the 2030 goal (reduce average day demand by 0.4 mgd) during 2019 and is on track to meet 0.8-mgd savings target by year 2050 (the complete development/buildout condition). Based on the Version 4 water savings estimates, the 2050 goal was achieved during 2019.

3.4 Best Practices and Case Studies

Since beginning its conservation program in 2006, WWU has adopted best practices that are key to the water savings achieved. The following subsections highlight some of the best practices along with case studies that demonstrate those practices in action.

3.4.1 Best Practices

- **Lead by example**—WWU sets an example for its customers by maintaining an efficient water distribution system, fixing leaks, and replacing aging toilets and fixtures with high-efficiency units. The City of Waukesha, Waukesha County, and Waukesha School District have installed water-efficient fixtures in public buildings. Further, the school district replaced athletic field grass with artificial turf.
- **Work with partners**—Collaboration with partners has been a key to WWU's success. The utility works with non-profit organizations locally and statewide, business and trade groups, schools, and others to provide public information, host events, and implement conservation measures.
- **Provide a flexible program with resources for all customers**—WWU's conservation program includes policies, incentives, and educational materials for all of its customers. The program is designed for and managed through continuous performance review, adaptation, and optimization. By implementing a variety of promising CEMs and evaluating measured results, WWU is able to identify ways to meet

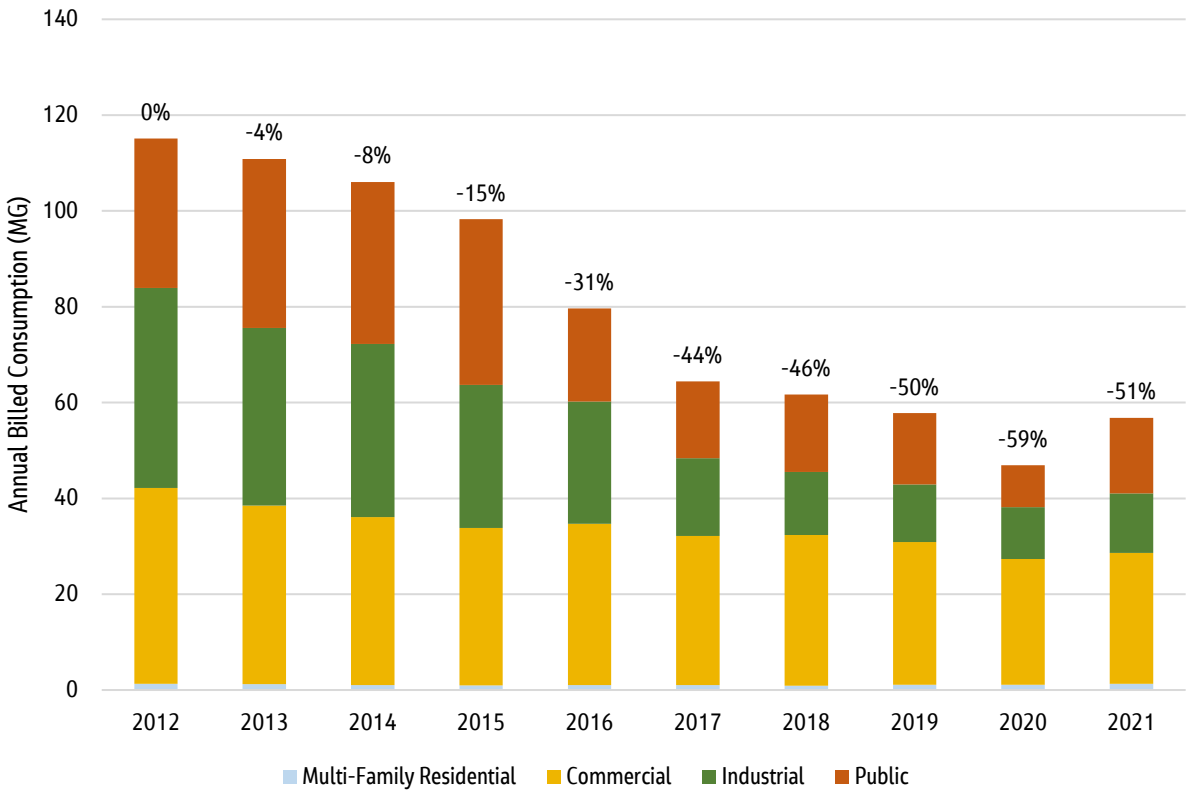
customer needs and cost-effectively save water. If offered incentives do not attract customer participation—for example, the Pilot Irrigation System Controller Rebate offer for a specific new development—the CEM is discontinued. Conversely, successful Innovative Site-Specific Grants that allow non-residential customers to identify unique opportunities to reduce water use and receive financial incentives proportional to measured water savings are strongly supported. WWU uses water billing data to target incentives for highest water users in all customer classes. These approaches accelerate water savings while minimizing program costs.

- **Maintain and increase customer water-use awareness**—In addition to programmatic water conservation education and outreach, WWU enforces its outdoor irrigation restrictions, implemented irrigation water rates in 2017, and converted from quarterly to monthly customer billing in 2021.

3.4.2 Effectiveness of Innovative Site-Specific Grant Program

Since 2012, WWU has issued 19 Innovative Site-Specific Grants. Figure 3-5 presents water-use reductions from customers that have received incentives. While many factors contribute to water savings, there has been an approximate 50% reduction since 2012.

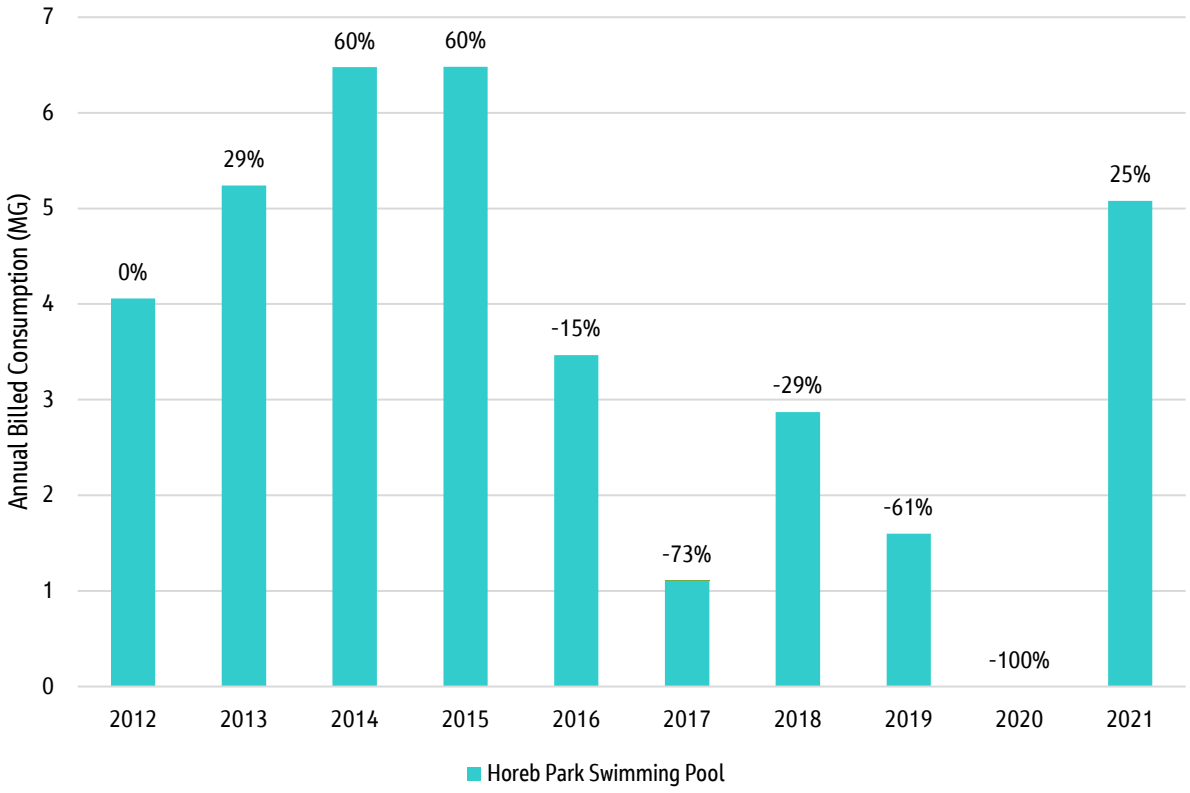
Figure 3-5. Consumption Data for All Accounts that Received Site-Specific Grants



3.4.3 Municipal and Public Case Studies—Swimming Pools

During 2013, WWU worked with the Waukesha School District and the City of Waukesha to perform audits of two public swimming pools—Waukesha South High School pool and the city pool located at Horeb Park. The following year, the District replaced a control valve actuator to prevent the pool from draining during power failures. At the Horeb Park pool, several leaks were identified in the fall of 2013 and repaired in the spring of 2014. Figure 3-6 shows reduced water consumption at the Horeb Park pool following the leak repair. In 2020, the pool was closed due to the COVID pandemic, resulting in no water use that year. The spike in 2021 was attributed to leaks found in the plaster pool liner. In 2022, the City replaced the pool liner and stopped the leaks.

Figure 3-6. Consumption Data for Horeb Park Swimming Pool Before and After Site-Specific Grant

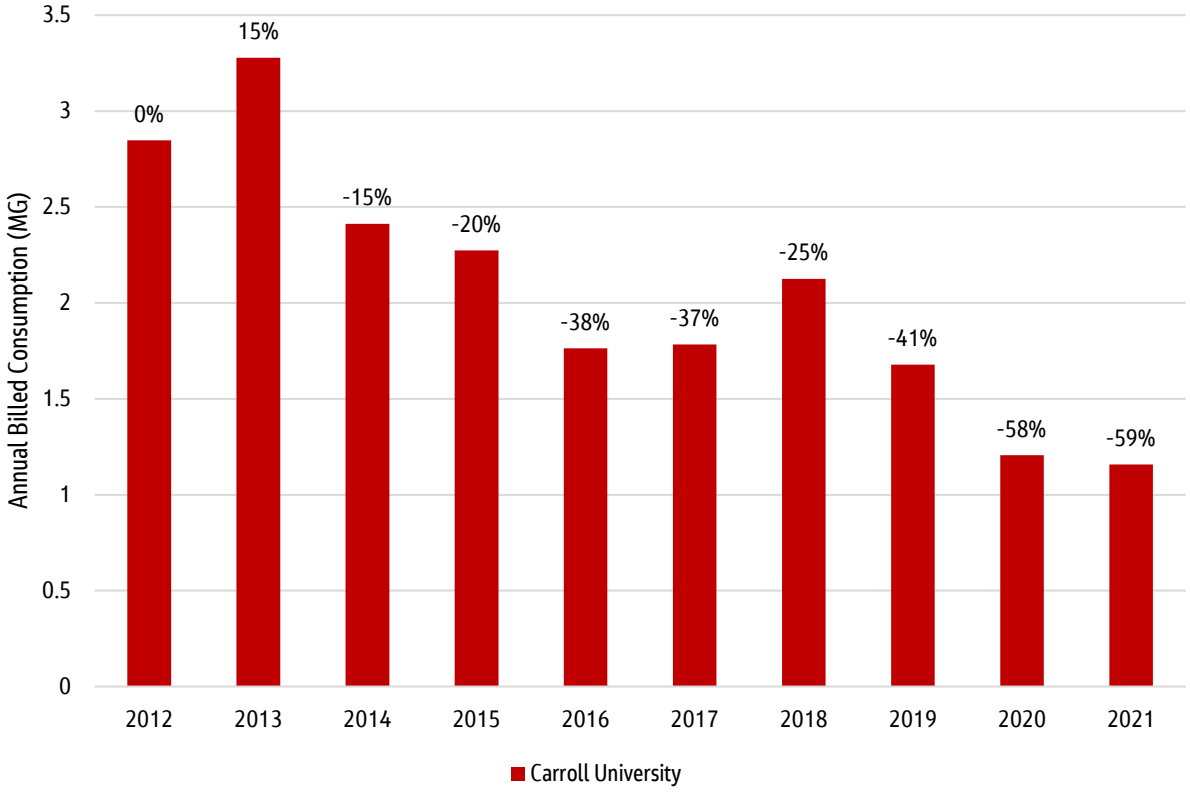


3.4.4 Commercial Case Study—Carroll University

Carroll University reduced its water use over the years through a series of conservation measures and active awareness campaigns. The university also partnered with the Waukesha School District and Waukesha County with input from the utility to develop local and regional environmental educational materials including water conservation.

In 2014, Carroll University installed domestic water heaters, thereby eliminating the need to import water through a lateral from a distant building and replaced water softeners, clothes washers, toilets, sinks, urinals, shower heads, and pre-rinse spray valves with high-efficiency models. Figure 3-7 shows a downward trend in water consumption since the university implemented conservation measures.

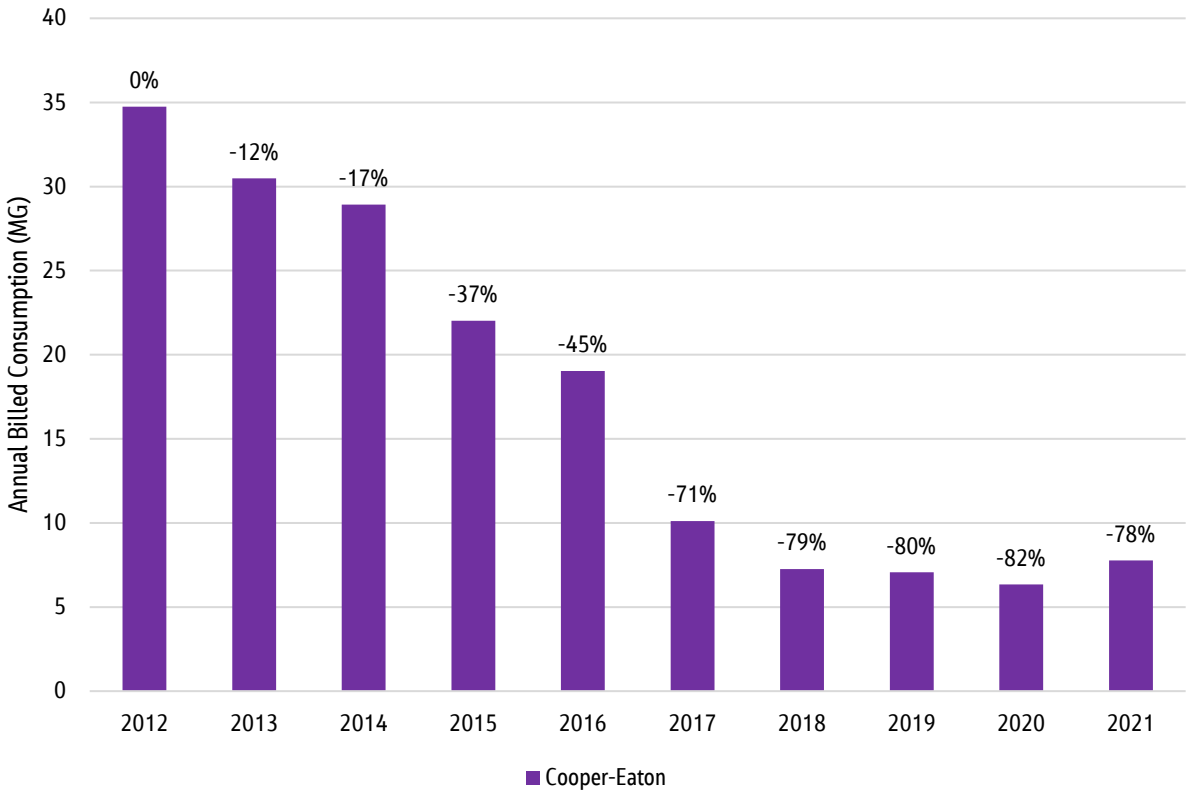
Figure 3-7. Consumption Data for Carroll University Before and After Site-Specific Grant



3.4.5 Industrial Case Study—Process Equipment and Onsite Reuse

Eaton-Cooper produces 3-phase transformers, which is a fairly water-intensive manufacturing process. In 2015, they replaced a once-through cooling system with two large water chiller recirculation systems with multiple cooling loops at one of their locations. This innovation is estimated to save 6.2 MG per year. At a second location, they installed three recirculating water chillers—one in 2015 and two in 2018. Figure 3-8 shows a significant reduction in their water use when the water-conserving equipment was installed.

Figure 3-8. Water-Use Data for Cooper-Eaton Before and After Site-Specific Grants



4. Potential Conservation Efficiency Measures Evaluation

In addition to infrastructure management measures, successful water conservation programs across the country incorporate a combination of public information, incentives, and regulations to achieve efficient water use across their service area. CEMs are focused on operating a water-tight water treatment and distribution system, public and school-age education, and a portfolio of measures to address water used by utility customers. To increase the effectiveness of water conservation programs, utilities generally select a small number of CEMs for implementation initially and modify the program as needed over time given to regulatory requirements, budget and staffing constraints, detailed water-use analysis, and customer input.

4.1.1 Infrastructure Management

Infrastructure management CEMs to be implemented during the planning horizon include the following activities:

- Continue use of the City's hydraulic distribution system model to evaluate and further optimize pressure and customer demand changes; conduct a formal system pressure management evaluation after converting to Lake Michigan water and modifying the system hydraulic grade line.
- Maintain implementation of the present leak mitigation measures including water main replacement, rapid repair of identified leaks, and the Hydrant Leak Survey program.
- Continue to measure source water.
- Continue to individually meter and bill customers on a monthly basis.
- Continue to conduct water-use inspections and audits.

4.1.2 Public Information and Education and School Education

No conservation program can be successful without the informed participation of its customers. Therefore, the City will continue to gather data and work closely with customers so that it can measure the water saved from changed water-use behaviors and their associated costs. Specific outreach activities the City is considering in the near future include the following:

- Refresh its website's online library of resources including conservation tips, online water-use calculators, leak detection guidance, information on the sprinkling ordinance and other policies, and conservation incentives. The Water Conservation Resource Library will include information for all customer classes and content on commercial dishwashers, ice makers, steamers, car washes, and food services; cooling technologies; water use audits; irrigation systems; water-efficient landscaping; and the benefits of using Waster-Sense labeled products.
- Maintain and elevate customers' water-use awareness through enforcement of the sprinkler ordinance and information about available irrigation water rates and discretionary water-use.
- Continue to provide presentations to a broad cross-section of customers, community groups, service organizations, and business groups.
- Continue school-age education and engagement.
- Continue partnerships locally, regionally, and statewide.

4.2 Conservation Program Measures to Continue

As detailed in previous sections, actual water-use savings have exceeded the goals established in the 2012 Plan. A combination of public information, incentives, and policies provide a strategic balance that WWU will continue during the 5-year planning horizon. In addition to maintaining an efficient treatment and distribution system as noted previously, Table 4-1 summarizes programs that will be extended.

Table 4-1. Conservation Program Measures to be Extended

Program	Implementation Methods
Public Information and Education	▪ WWU website ▪ Great Water Alliance website and information hub ▪ Newsletters, bill stuffers and bill messages (WWU and City of Waukesha) ▪ Newspaper articles, public service announcements ▪ Social media ▪ Brochures and advertisement content for City Parks and Recreation Department Activity Guide ▪ Videos ▪ Public outreach and community meetings ▪ School program ▪ Street signs (sprinkler ordinance requirements) ▪ Yard signs (Brown Lawn Campaign or similar campaigns) ▪ Giveaways ▪ Customer water-use audits and leak alerts
Incentives	▪ Toilet Rebate Program ▪ Shower Head Rebate Program ▪ Rain Barrel Rebate Program ▪ Innovative Site-Specific Water-Saving Measures Grant Program – This program has been highly successful in saving large volumes of water and achieving a high-benefit-cost ratio ▪ Implementation focus will be on the top water users in various customer categories
Policies	▪ Sprinkling Ordinance and Enforcement ▪ Efficient Irrigation Standards ▪ Increasing block rate for residential users ▪ Monthly billing

4.3 Potential New Program Measures Considered

In addition to the conservation programs recommended for continuation, a number of new measures were considered during the planning process as summarized in Table 4-2.

Table 4-2. Potential New Conservation Measures

Program	Implementation Considerations
Enhanced customer leak detection through data collectors	Installing data collectors (antennas) on water towers throughout the distribution system would provide WWU the ability to identify customer leaks in "real time." The program would need to be phased in given the cost of the equipment. Use of software to provide alerts when unusual spikes in water use indicating potential leaks occur would provide real-time customer awareness of leaks.
Reuse	As noted in Section 3, WWU must return all effluent resulting from Lake Michigan diversion, so municipal reuse is not a viable option. Through the Innovative Site-Specific Grant Program, however, industrial onsite reuse has proven effective. Additional focus on this may be considered.

Program	Implementation Considerations
Water softener disconnection / elimination	The USEPA estimates that, on average, residential water softeners use 25 gallons of water or more per day, or up to 10,000 gallons per year. Due to the chemistry of Waukesha's current groundwater source, most residential customers use water softeners. An estimated 13,000 residential softeners are being used today. Once the surface water source is online, water softeners will not be needed. A program to encourage disconnection and abandonment has the potential for significant water savings. Implementation could include information on why softeners won't be needed, a rebate, or other incentive such a haul-away program.
Ice-Maker Replacement	Encouraging ice-maker replacement could be a standalone rebate program or part of the Site-Specific Grant Program.
Rain / Freeze Sensor	Rain and freeze sensors work by turning off automatic irrigation systems based on soil moisture, low temperatures, and other conditions. Materials developed for the now discontinued Pilot Irrigation Control Rebate program could be used to jump start a rebate program.
Washing Machine Rebate	Clothes washers are becoming increasingly efficient. A program to encourage their replacement could increase savings – especially in commercial facilities or a multi-family units or universities with communal laundry rooms. Encouraging their replacement could be a standalone rebate program or part of the Site-Specific Grant Program.
Low-income plumbing assistance program	Plumbing assistance programs are designed to provide plumbing contractors for low-income customers that cannot afford to repair leaks or replace inefficient fixtures. Often, a utility will contract with plumbing contractors who are assigned to repair leaks to qualifying customers. Income qualification is often determined in partnership with public assistance programs such as County Health Departments or similar governmental units.
Public / low-income housing retrofit program	This program would focus on installing high-efficiency fixtures into housing units. Often rebates are not effective since residents may not be able to invest in fixture and wait for the rebate. A program could be designed to include bulk purchases of fixtures or contracting with plumbers to install the fixtures. Other programs provide vouchers that can be redeemed at home improvement stores or plumbing houses.
Commercial sector community challenge	Some utilities have organized highly publicized challenges for restaurants, hotels, or other commercial sectors. The incentive for some of these programs are recognition, media coverage, and related publicity rather than financial incentives or rebates; however, the Site-Specific Grant Program could be used.

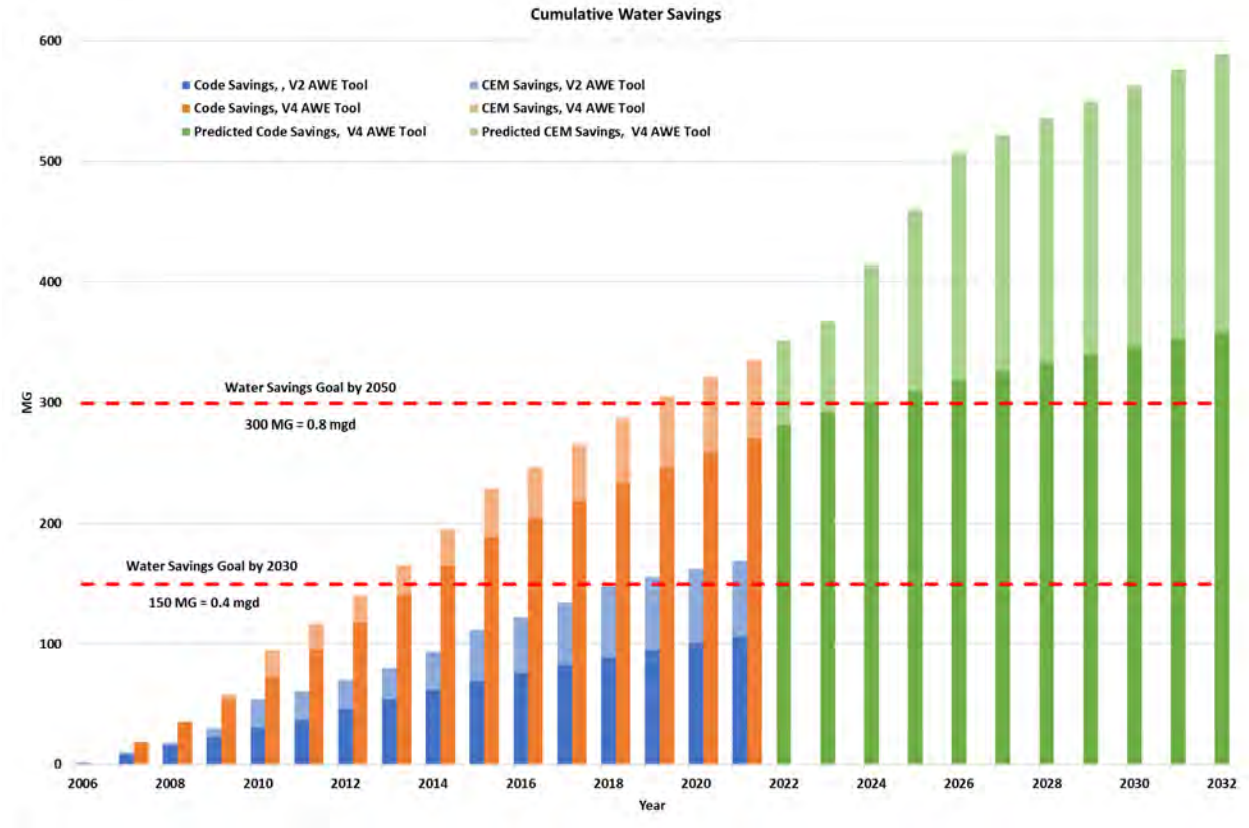
4.3.1 AWE Tool Results

Projected savings from several of the programs identified in Table 4-2 can be evaluated using the AWE Tool and assumptions such as participation levels, existing water use, and related factors. Other measures such as public information, water challenges, and similar measures are not quantifiable. Generally, programs with a benefit-cost ratio of one or greater are considered cost-effective. Table 4-3 presents results from evaluating considered conservation programs, and Figure 4-1 is a graph combining savings to date with potential total savings from potential measures presented in Table 4-3 as well as passive savings resulting from technology standards and code changes (code savings).

Table 4-3. Forecast Outcomes Attributable to Potential New Conservation Measures

Program	Water Savings, 2022 through 2035 (MG)	Utility Benefit-Cost Ratio	Society Benefit-Cost Ratio
Enhanced Customer Leak Detection; Data Collectors	0.53	0.01	0.01
SF Continuation of $100 HET Rebate	10.17	2.7	1.1
SF Continuation of $25 Showerhead Rebate	0.03	0.1	0.5
SF Continuation of $20 Rain Barrel Rebate	0.02	0.1	0.0
MF Continuation of $100 HET Rebate	22.60	3.0	1.5
MF Public/Low-Income Retrofit Program	5.25	2.0	2.0
MFR Shared Washer Rebate (WF <=4)	3.38	0.7	1.3
CII Continuation of $100 HET Rebate	1.98	3.8	1.9
CII Washing Machine Rebate	3.38	1.0	0.8
CII Continuation of $35 Spray-Rinse Valve Rebate	0.38	1.2	5.4
SF Low-Income Plumbing Assistance Program	0.21	0.1	0.3
CII Continuation of Large Landscape Water Audit	1.10	0.5	0.5
CII Continuation of Innovative Site-Specific Grant Program	43.95	4.7	4.7
SF Water Softener Disconnection	94.91	22.5	22.5
CII Ice-maker Replacement	4.38	3.9	0.2
SF Landscaping Rain/Freeze Sensor	2.86	0.2	0.0
Forecast Total	195	14.5	14.3

Figure 4-1. Combined Actual and Proposed Program Estimated Water Savings



The estimated water savings shown in Figure 4-1 do not include estimated savings in response to increased price. WWU has significant water rate increases scheduled in the next few years. When historical and future rates are input to the AWE Tool, additional cumulative water savings are estimated. These price increase response water savings are anticipated to occur but are not included in the projected cumulated water savings at this time. It is recommended that these estimated water savings be reviewed and potentially included in the cumulative water savings total in five years, after the rate increases have gone into effect.

Further, the estimated savings present in 4-1 demonstrate that not all of the conservation measures evaluated in Table 4-3 are needed to achieve the 2030 and 2050 savings goals.

5. Recommended Implementation Plan

With progress made to date, and anticipated water savings in response to rate increases and discontinued use of water softeners, water conservation program recommendations for the next 10 years focus on maintaining – rather than significantly expanding – program activities. Projected water savings, benefits, costs, recommended program budget, and a proposed implementation schedule are provided through 2027. With the significant changes to WWU operations and regulatory compliance reporting anticipated with the transition to a Lake Michigan water supply in 2023, a review of the conservation program is recommended in 2027.

5.1 Projected Water Savings

As noted, WWU achieved its 2030 savings goal during 2019 based on savings estimated using Version 2.0 of the AWE Tool and achieved its 2050 savings goal during that same year using Version 4.0 of the AWE Tool with the revised estimates of Code Savings driving most of the difference in results. Additionally, the utility is forecasting steep increases in water rates as the costs of securing the City's water future accrue; conservation is a service to help customers by reducing their usage and monthly bills. The AWE Tool was used to estimate the projected water savings from conservation program measures and from passive savings that are the result of plumbing code changes that require water-efficient fixtures. Table 5-1 and Figure 5-1 summarize the estimated water savings since 2006 and the projected water savings from the recommended CEMs through 2027. The result is over 200 MG expected to be cumulatively saved through 2027, which indicates the City will be in a strong position to achieve its water-savings goal of 0.8 mgd by 2050.

While the conservation program gradually expanded from 2006 through 2021, it is now recommended the program focus on maintaining the conservation measures with the highest potential for cost-effective water savings within all customer classes and concentrate efforts on the City's top water users. The actions will ensure a strong return on the City's investment while maintaining customer satisfaction and utility service standards.

Table 5-1. Estimated Cumulative Conservation Program Water Savings by Customer Class, 2006–2027

Customer Class	Conservation Program Water Savings (MG)				
	2023	2024	2025	2026	2027
Single-Family	10	11	11	12	12
Multi-Family	17	19	20	22	24
CII Irrigation Meter	0	0	0	0	0
CII Common Meter	50	53	56	60	64
Total (MG)	**77**	**82**	**87**	**93**	**99**
Total (mgd)	0.21	0.22	0.24	0.26	0.27

5.2 Other Projected Benefits

Water conservation provides other benefits to the City and its customers, including the following:

- Reduced wastewater pumping and treatment costs
- Reduced water pumping and treatment costs
- Reduced volume of water needed to meet projected future water demands
- Fewer greenhouse gas emissions from water and wastewater treatment and pumping

Table 5-2 summarizes estimated projected savings resulting from the implementation of water-saving CEMs.

Table 5-2. Estimated Savings from Utility-Avoided Costs

Avoided Cost Type	2023	2024	2025	2026	2027
Water Supply	$5,309	$5,654	$5,999	$6,412	$6,826
Water Distribution	$20,573	$21,909	$23,245	$24,848	$26,451
Wastewater Collection and Treatment	$21,538	$22,937	$24,336	$26,014	$27,692
Total	$47,420	$50,500	$53,579	$57,274	$60,969

5.3 Projected Program Costs

Annually, WWU funds the water conservation program with about $62,000 from water utility revenues and $30,000 from the Clean Water Plant (City of Waukesha Wastewater Treatment Plant). Program funding is used for rebates, customer water-use audits, public education and outreach, program administration, and performance auditing, customer service, annual reporting, and plan updates. It is anticipated that program costs will remain approximately the same during this planning horizon with some adjustments for inflation and related factors.

5.4 Recommended Conservation Program Elements

The recommended program elements over the 10-year planning period are designed to continue the momentum of WWU's conservation success and maintain the program to prevent erosion in water savings over time. Table 5-3 summarizes recommended program elements for the first 5 years. The implementation strategy is designed to maintain strong community support through public education and incentives for residential water users. Voluntary conservation would be expected to lead to the greatest savings, particularly for existing homes, businesses, industries, and institutions. Throughout the planning period, measures would be emphasized within various customer "markets" to affect the greatest savings and the lowest costs.

5.4.1 Conservation Program Recommendations: 2023-2027

Administrative needs over the 5-year implementation phase for the plan includes additional customer service representative training and reporting activities to effectively communicate and manage the conservation incentive programs. The tasks and related budget requirements are shown in the proposed budget described earlier in this section. The administrative requirements could include contracts for purchasing or installation of conservation fixtures, an efficient rebate tracking and accounting method that would apply credits to customer accounts, and similar activities. Data management efforts are anticipated to increase over time as the conservation program is expanded.

5.4.2 Conservation Program Recommendations: 2028-2032

Given the significant changes anticipated for WWU over the next 5 years, including water conveyance of Lake Michigan water, changes in system operating pressures, water rate increases, and other factors, it is recommended that WWU conduct an informal refresh of its conservation program after 5 years (in 2028). This refresh would incorporate an update to system-wide production data, utility costs, water-use savings, and program participation. The water softener discontinuation program is expected to result in not only substantial water-use savings, but also improvements to effluent discharge water quality, which may result in additional cost avoidance not included in this update's utility-savings estimates presented in previous sections.

Table 5-3. Near-Term Program Elements (Years 1 to 5)

Program Element	Actions
Municipal Infrastructure	▪ Continue hydrant surveys, leak audits, large meter calibration and main replacement, pressure management, and other distribution system measures. ▪ Work with City, County, School District, and other governmental entities to identify potential public facility retrofit opportunities. ▪ Identify top 1 to 5 parks with high outdoor water use and estimate retrofit costs. ▪ Prepare for data collector installation program to provide real-time customer monitoring. Procurement and installation are estimated to be a 6- to 10-year program. Consideration may be given to using a software program to "read" data and send leak alerts.
Public and School Education and Information	▪ Continue school programs and tours. ▪ Continue to collaborate with the county and other groups for speaker series on water conservation. ▪ Continue partnerships to spread conservation messages and events. ▪ Update website with additional conservation resources.
Rebates and Incentives: Residential	▪ Continue $100 HET rebate and publicize program. ▪ Continue showerhead rebate program. ▪ Continue leak notifications and water audits. ▪ Consider holding a HET distribution event to distribute a target number of toilets in 1 day. ▪ Continue to work with Waukesha Housing Authority and non-profits on retrofit program as part of the HET and showerhead rebate program. ▪ Consider a pilot program with Waukesha Housing Authority for minor plumbing and leak repair (combined with a fixture replacement). ▪ Consider a washing machine rebate for shared laundry facilities in multi-family housing or as a Site-Specific incentive. ▪ Begin planning/implement a water softener discontinuation program with the Clean Water Plant.
Rebates and Incentives: Commercial, Industrial and Institutional (Public)	▪ Continue HET rebates. ▪ Continue leak notifications and data logs. ▪ Continue spray-rinse valve program. ▪ Continue innovative site-specific incentives. ▪ Consider a washing machine rebate for commercial laundromats or as a site-specific incentive.
Policies, Regulations, and Enforcement	▪ Continue to administer and publicize sprinkling ordinance. ▪ Continue to publicize irrigation ordinance.
Reporting, Monitoring, and Plan Updates	▪ Streamline databases to facilitate auditing and reporting. ▪ CEM effectiveness audit/monitoring. ▪ Prepare and submit annual report to PSC.

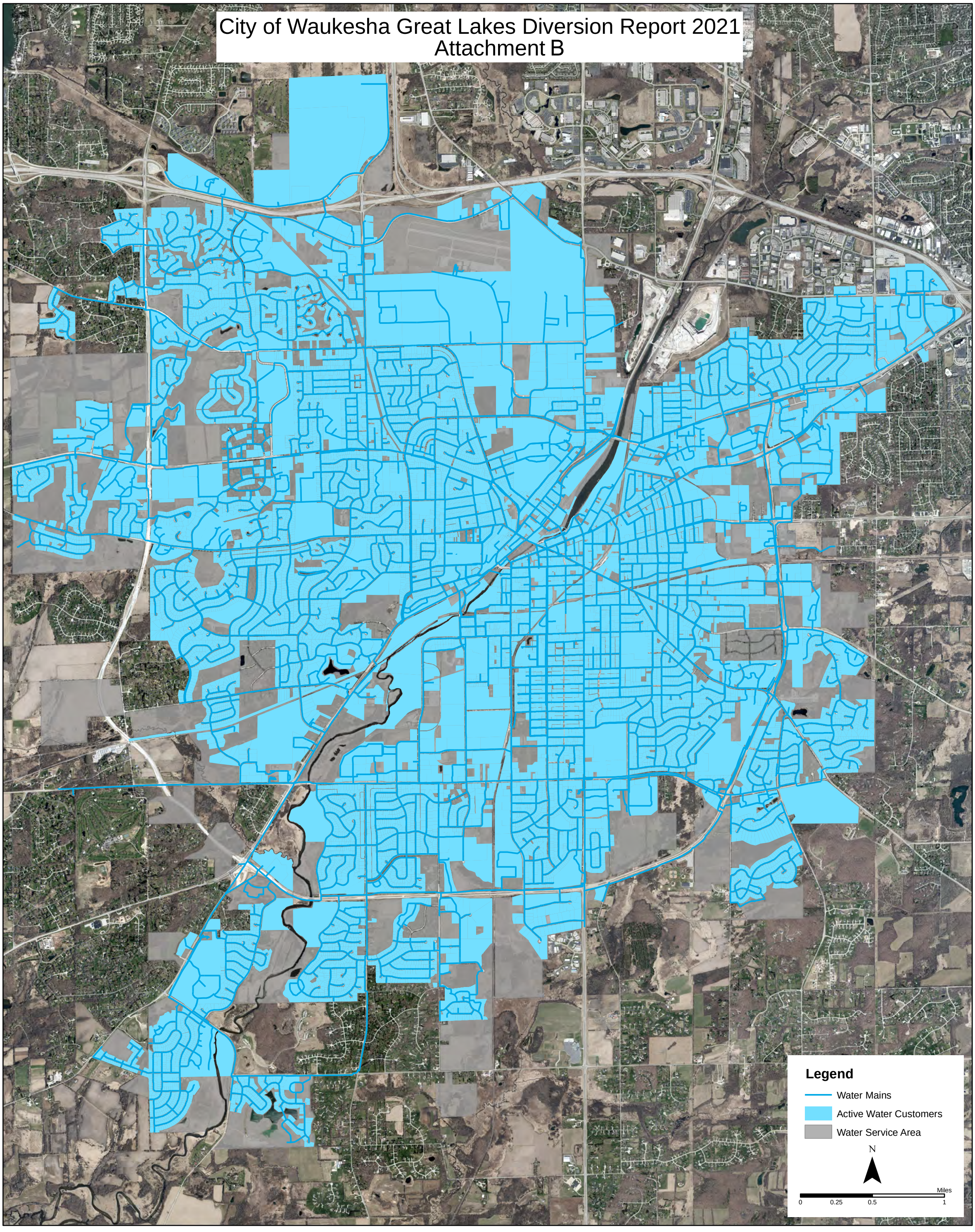
6. Summary

WWU has a cost-effective water conservation program that meets the regulatory requirements of NR 852 and PSC 185 administered by WDNR and the PSC, respectively. An analysis of water savings achieved since the 2012 Plan was implemented demonstrates that by 2021, WWU has exceeded savings goals established for 2030 and 2050. Because water conservation savings can erode as water-using fixtures and equipment age and if customers' behaviors change over time, it is recommended that WWU continue to maintain its conservation program for residential, commercial, industrial, and public customers.

Additionally, in light of anticipated changes over the past several years, including introduction of Lake Michigan water and associated reduction in water softener use for most customers, as well as planned water rate increases, water-use patterns are expected to change. It is recommended that the conservation plan and program be reviewed in about 5 years (2027–2028).

The Plan Update was unanimously approved by the WWU Commission on January 19, 2023.

City of Waukesha Great Lakes Diversion Report 2021
Attachment B



Station	2022 Status	Aquifer Used	2022 Hours Run Time [1]	2022 Total Output (mg)[2]	Planned Status for 2023	Planned Status After Transition
Well 2	Water Level Monitoring by USGS only	Deep Sandstone	0	0	No change	Unknown
Well 3	Used daily with HMO	Deep Sandstone	6,140	261,168	No change	Maintain for Emergency Use
Well 5	Non-Compliant	Deep Sandstone	423	28,827	No change	Permanently Abandon
Well 6	Non-Compliant	Deep Sandstone	431	55,895	No change	Permanently Abandon
Well 7	Non-Compliant	Deep Sandstone	2,717	113,865	No change	Maintain for Emergency Use
Well 8	Used daily with HMO and blending with 11 & 12	Deep Sandstone	4,842	543,202	No change	Maintain for Emergency Use
Well 9	Non-Compliant	Deep Sandstone	110	8,821	No change	Maintain for Emergency Use
Well 10	Used daily with HMO	Deep Sandstone	5,570	560,963	No change	Unknown
Well 11	Used daily	Sand and Gravel	3205	28,651	No change	Permanently Abandon
Well 12	Used daily	Sand and Gravel	3,475	96,682	No change	Permanently Abandon
Well 13	Used daily	Sand and Gravel	5,869	182,968	No change	Permanently Abandon

[1] Per requirements of the Stipulation Order, non-compliant wells ca be operated a maximum of 2 days per month per well for sampling and maintenance.

[2] As reported in the Annual Withdrawal Report to DNR.

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

Prepared by:

Jacobs

1610 N 2nd Street, Suite 201
Milwaukee, WI 53212

For:

City of Waukesha

City of Waukesha
115 Delafield Street
Waukesha, WI 53187

March 2023

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Acronyms and Abbreviations

CFR	*Code of Federal Regulations*
COC	chain of custody
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
TSS	total suspended solids
USGS	United States Geological Survey
WDNR	Wisconsin Department of Natural Resources

Distribution List

City of Waukesha

Dan Duchniak, General Manager, dduchniak@waukesha-water.com

Wisconsin Department of Natural Resources

Shaili Pfeiffer, NR Staff Specialist, shaili.pfeiffer@wisconsin.gov

Jacobs

Brent Brown, Project Manager, brent.brown@jacobs.com

University of Wisconsin–Parkside

John Skalbeck, Implementation Support Project Manager, skalbeck@uwp.edu

United States Geological Survey

Rob Waschbusch, Upper Midwest Water Science Center Central Data Chief (Wisconsin), rjwaschb@usgs.gov

Wisconsin State Laboratory of Hygiene

Graham Anderson, Inorganic Chemistry Supervisor, graham.anderson@slh.wisc.edu

1. Background and Objectives

The City of Waukesha, Wisconsin, will obtain a new Lake Michigan water supply by July/August 2023 to replace the current groundwater water supply. The City will maintain its existing outfall for fully treated wastewater from the Clean Water Plant to the Fox River (Mississippi River drainage basin), but will also utilize a new outfall to the Root River (Lake Michigan drainage basin) to return the diverted water to the source watershed. (Figure 1-1).

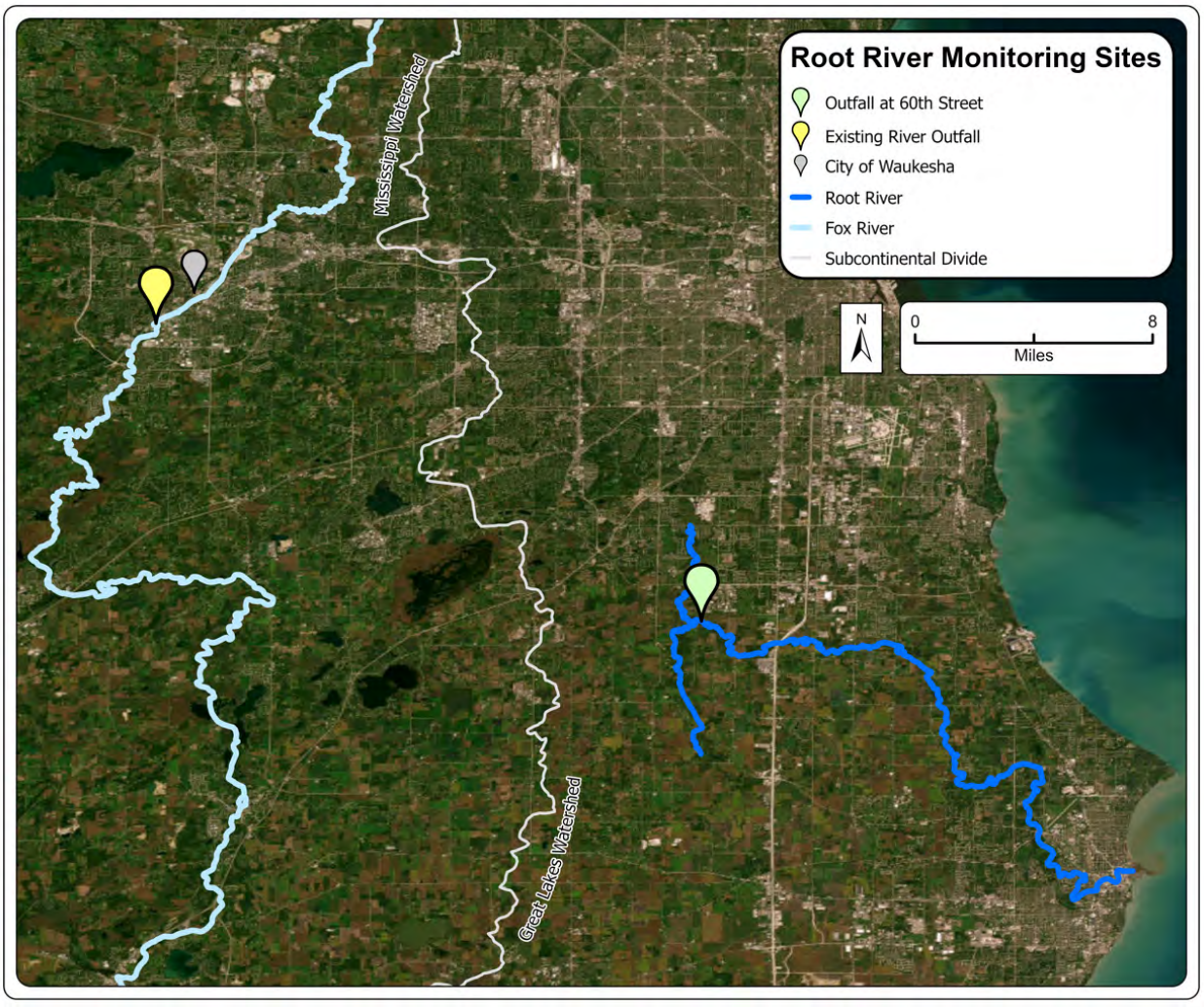

Figure 1-1. Post-Return Flow Site Map

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 will enter 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 1) monitoring plan to collect data and 2) the quality assurance plan for the monitoring activities. Executing the QAPP will support assessing the impact of return flow on the Root River and allow the City to adaptively management the return flow and future QAPP efforts. This QAPP is aligned with the U.S. Environmental Protection Agency's (EPA's) Quality Systems Project Level (Level Three) (EPA 2006) to ensure environmental projects result in high-quality and scientifically based products (EPA 2002).

The QAPP includes water quality sampling and ecological assessment methodology to support data collection at monitoring sites along the Root River. The 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:

> *…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 pre-return flow monitoring from 2017 to 2023 to support data collection of water quality, 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 serve as the basis for this QAPP.

2. Project Organization

The post-return flow monitoring activities will involve the coordination of six key organizations including the City of Waukesha (Project Owner), WDNR (State Regulating Agency), Jacobs Engineering Group (Jacobs; Managing Consultant), University of Wisconsin-Parkside (Contracted Implementation Support), the United States Geological Survey (USGS) (Contracted Monitoring Support), and the Wisconsin State Laboratory of Hygiene (Contracted Laboratory). Figure 2-1 shows the organization chart for the project, followed by brief descriptions of the roles and responsibilities of each organization.

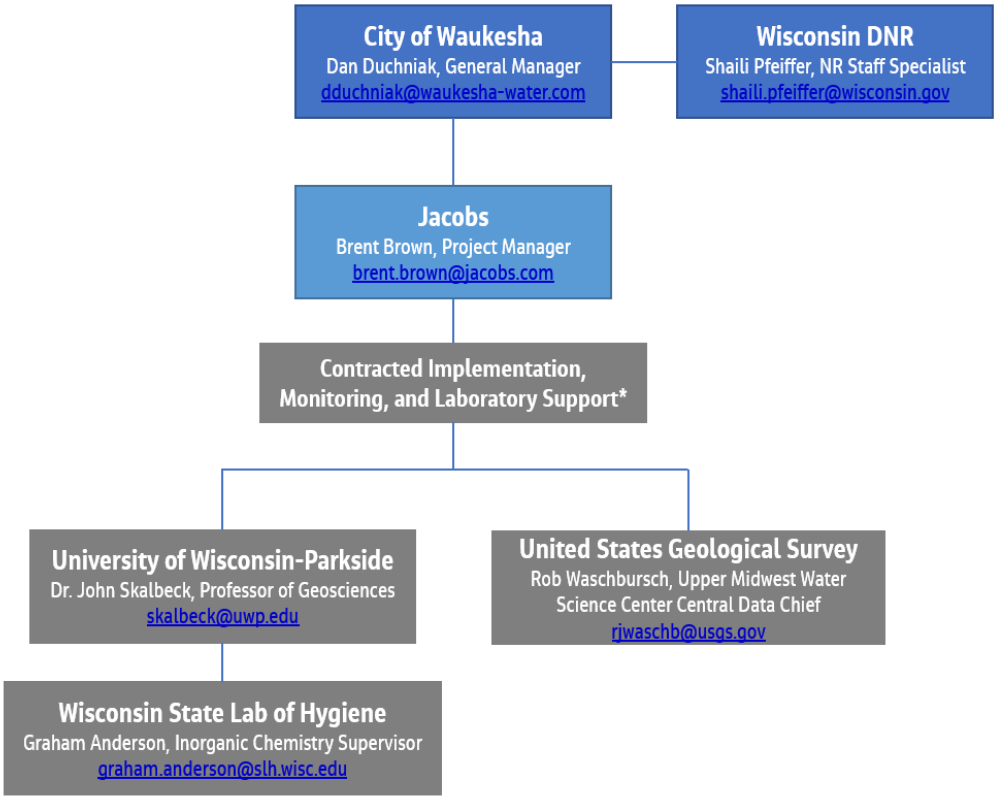

Figure 2-1. Post-Return Flow Root River Monitoring Organization Chart

**Note: Implementation support, monitoring, and laboratory contracts will be renewed annually; therefore, service providers may change.*

City of Waukesha, Project 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 Project Owner or Managing Consultant to define project requirements and approve project activities as needed.

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

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

United States Geological Survey, Contracted Monitoring Support. Executes continuous monitoring activities (e.g., flow) as defined by the QAPP.

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).

2.1 Project Description and Schedule

The post-return flow Root River monitoring activities will include routine water quality sampling, continuous water quality and flow monitoring, and biological sampling including fish surveys, macroinvertebrate sampling, and habitat assessments. The following subsections describe the sampling locations, parameters, schedule, and personnel and required resources.

2.1.1 Location

Six monitoring sites were identified as potential data-collection locations (Figures 2-2 and 2-3; Table 2-1) along the Root River and Root River Canal. 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, which is located about 300 ft downstream of Site C. These sites were also monitored as part of the voluntary Pre-Return Flow Root River Data Collection Plan.

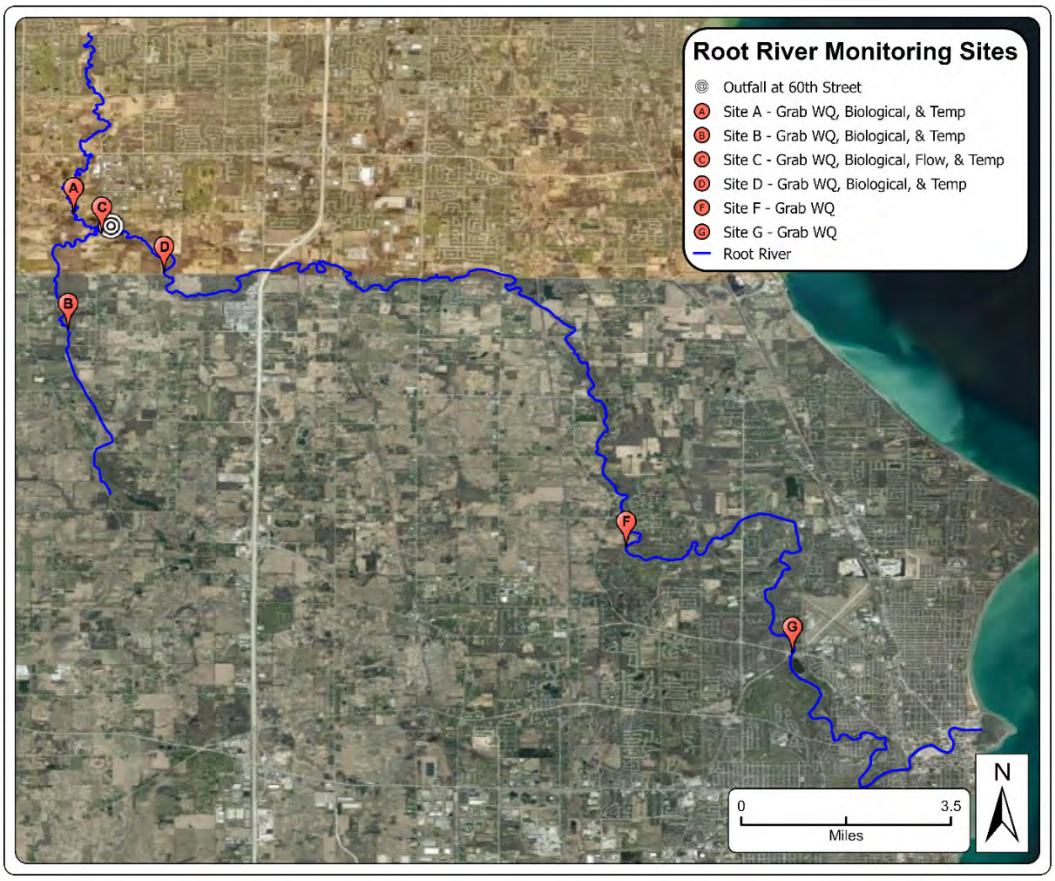

Figure 2-2. Post-Return Flow Root River Monitoring Sites

Table 2-1. 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.
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.
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.
F	Root River at Johnson Park 42°46'38.82"N 87°51'51.17"W	This data collection site is located off of a green steel bridge in the Johnson Park/Golf Course near the maintenance building. The site has good accessibility on the north side of the river, and a small parking area is available near the Golf Course dumpsters. Historical data has been collected at this location. Permission for site access will be needed by from the Golf Course. The substrate is gravely at this site.
G	Root River downstream of Horlick Dam 42°45'6.96"N 87°49'26.65"W	This data collection site is located on the main stem of the Root River immediately below the Horlick Dam. Parking is available at Water's Edge, and from here the railing of a retaining wall at the dam can be seen. Along the railing is a steep but accessible pathway to the base of the dam. Historical data has been collected at this location, and river flow is measured by USGS. The river substrate is mostly rock.

Note: Data collection at Site E was discontinued during pre-return flow monitoring, but site nomenclature was maintained for consistency between the pre- and post-return flow plans.

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 D - Root River at County Line Road

Site F - Root River at Johnson Park

Site G - Root River downstream of Horlick Dam

Figure 2-3. Photos of Data Collection Site Locations

2.1.2 Sampling Parameters

Tables 2-2(a) and 2-2(b) summarize the 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	Events per Year	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)	18	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 353.2	A, B, C, D, F, G
Nitrate-Nitrite (NO_3- NO_2)	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 353.2	A, B, C, D, F, G
Chlorophyll A	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 1,000 ml None	EPA 445	A, B, C, D, F, G
Total Phosphorus (TP)	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 365.1	A, B, C, D, F, G
Orthophosphate	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 60 ml None	SM4500-PE	A, B, C, D, F, G
Ammonia-Nitrogen	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 250 ml 24% Sulfuric Acid	EPA 350.1	A, B, C, D, F, G
Total Suspended Solids (TSS)	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	Grab Sample	Plastic, 1,000 ml None	SM2540D	A, B, C, D, F, G
Total Kjeldahl Nitrogen (TKN)	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	-	n/a	Calculation	A, B, C, D, F, G
Organic Nitrogen	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	-	n/a	Calculation	A, B, C, D, F, G
Dissolved Oxygen (DO)	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	In Situ Sample	n/a	Multi-parameter Probe	A, B, C, D, F, G
Specific Conductance	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	In Situ Sample	n/a	Multi-parameter Probe	A, B, C, D, F, G

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

Parameter	Frequency	Events per Year	Collection Method	Bottle Material, Size Preservative (if applicable)	Measurement Method	Site Collection Locations[a]
pH	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	In Situ Sample	n/a	Multi-parameter Probe	A, B, C, D, F, G
Temperature	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	In Situ Sample	n/a	Multi-parameter probe	A, B, C, D, F, G
Turbidity	Twice per Month (May-Oct), Monthly (Nov-Apr)	18	In Situ Sample	n/a	Multi-parameter Probe	A, B, C, D, F, G
Biological Parameters						
Macroinvertebrates	September-October; November[b]	1[b]	(WDNR 2000)	n/a	Refer to SOP (Appendix E)	A-D[c]
Habitat Assessment	Concurrent with Summer Fish Surveys[b]	1[b]	(WDNR 2002)	n/a	Refer to SOP (Appendix G)	A-D
Fish	June-August; November[b]	1[b]	(WDNR 2001)	n/a	Refer to SOP (Appendix F)	A-D

Note:

[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 shall 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 also be completed during the first year 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 three 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 shall be collected and analyzed at one sampling site during every sampling event.

Table 2-2(b). Summary of Continuous Data Collection

Water Quality Parameters	Frequency	Method	Site Collection Locations
Flow	15 Minute	Automated Flow Gage	C
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-D
Turbidity	Hourly	Continuous Data Sonde	C

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 be selected to perform the respective sampling activity. Contracted staff will have numerous years of 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 any other protocol approvals required within the permit (Appendix A)

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. Plans to use different leads will be coordinated with the City of Waukesha.

- Dr. John Skalbeck, University of Wisconsin-Parkside, Field Sampling Project Manager
- Laura Schulz, University of Wisconsin-Parkside, Water Chemistry Lead
- Dr. Jessica Orlofske, University of Wisconsin-Parkside, Macroinvertebrate and Habitat Assessment Lead
- Dr. Mike Pauers, University of Wisconsin-Parkside, Fish Sampling Lead
- Robert Waschbusch, USGS, Flow Monitoring Lead
- Graham Anderson, Wisconsin State Laboratory of Hygiene, Inorganic Chemistry Supervisor

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 and Expiration Date August 31, 2023) will be used for at least one calendar year. If the contracted implementation support team plans to use a different laboratory, it will be coordinated with the City of Waukesha and will include a transition plan.

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

- 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. Kent 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, Jacobs, and the contracted implementation, monitoring, and laboratory 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 B.

Using a tablet, water quality sampling data will be entered into an EQuIS Collect data form and stored in an online database accessible to members of the project team as needed. This data will also be recorded in a field notebook. The laboratory conducting water quality analyses will provide EDDs that are compatible with the EQuIS Collect 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 file following the sampling event. Scanned field notes, field data sheets, Microsoft Excel 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 shows reports anticipated to be produced as part of the post-return flow Root River monitoring activities.

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

2.2.4 Project File Final Disposition

Project records will be stored electronically.

3. Measurement/Data Acquisition

3.1 Data Quality Objectives and Criteria

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.

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 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

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
Nitrate-Nitrite (NO_3-NO_2)	EPA 353.2	0.055 mg/L; 0.184 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
Ammonia-Nitrogen	EPA 350.1	0.012 mg/L; 0.039 mg/L	0.01 to 3 mg/L	28 days
Total Suspended Solids (TSS)	SM2540D	Varies	1 to 175 mg/L	7 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

3.2 Water Quality Sampling

3.2.1 Sample Process Design

The post-return flow Root River monitoring activities will include regular water quality sampling and continuous water quality and flow monitoring. Water sampling will take place at six 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).

3.2.2 Sampling Method Requirements

Water quality samples will be collected in well-mixed stream flow, at approximately mid-depth, where feasible. Samples will be collected upstream of road or bridge crossings when 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 should 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, ammonia, nitrate-n + nitrite –n, total nitrogen (and sulfuric acid ampules – 1 ampule for each 250-milliliter bottle)
- 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 C).

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.
- 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

3.2.3 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-H) – 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.

3.2.4 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 3.6, Quality Control Plan).

Appendix D 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

3.2.5 Data Management

Using a tablet, water quality data collected during sampling events will be entered into an EQuIS Collect data form 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 EQuIS Collect database. COC forms and laboratory reports will be stored electronically.

Water quality data collected using automated gages and continuous data sondes will be stored in accordance with USGS policy.

3.3 Macroinvertebrate Sampling

3.3.1 Sample Process Design

The post-return flow Root River monitoring activities will include macroinvertebrate sampling once during September through early October. A sampling event in November will also be completed during the first year of the post-return flow monitoring for consistency with the voluntary Pre-Return Flow Root River Data Collection Plan. Sampling between locations will occur on consecutive days as weather and site conditions allow.

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

Macroinvertebrate sampling is anticipated for the first 3 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.

3.3.2 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 100 specimens will be extracted and sorted. 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 F).

3.3.3 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-H) – 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.

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 should 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.

3.3.4 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
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

3.3.5 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.

3.4 Fish Sampling

3.4.1 Sample Process Design

Fish sampling will be completed once during June through late August. A sampling event in November will also be completed during the first year of the post-return flow monitoring for consistency with the voluntary Pre-Return Flow Root River Data Collection Plan. Sampling between locations will occur on consecutive days as weather and site conditions allow. WDNR will be notified prior to each fish sampling event to oversee fish taxonomy but will not physically collect samples or direct the processing following collection.

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 3 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.

3.4.2 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 transects will be the same transects used in the habitat assessments. Similarly, for continuity, transects 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 transects will be discussed with the City prior to the implementation of new transects. Appendix E 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 transect (or half the transect length, if the length is greater than 100 meters) and will be identified, counted, and recorded before being released. For game fish, lengths will also 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 G).

3.4.3 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 should 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

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). Fish IBI analysis will include 10 metrics and 2 correction factors as shown in Table 3-4.

Table 3-4. Fish Metrics and Indices

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)

3.4.5 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.

3.5 Habitat Assessments

3.5.1 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 3 years of the post-return flow Root River monitoring activities. Assessments may be discontinued 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

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 E 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).

3.5.3 Sampling Handling and Custody Requirements

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

3.5.4 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).

3.5.5 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.

3.6 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 B) 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 C).
- **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). For continuity with the voluntary pre-return flow Root River monitoring activities, the Wisconsin State Laboratory of Hygiene will be used for at least one calendar year. 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 D 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 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 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 per 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 Jacobs 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 2.1, 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.).

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Appendix A. 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
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Collection Information (continued)

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

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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.

Scientific Collectors Permit or Research License Application and Authorization

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(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 B. 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 C. 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 (4ml 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

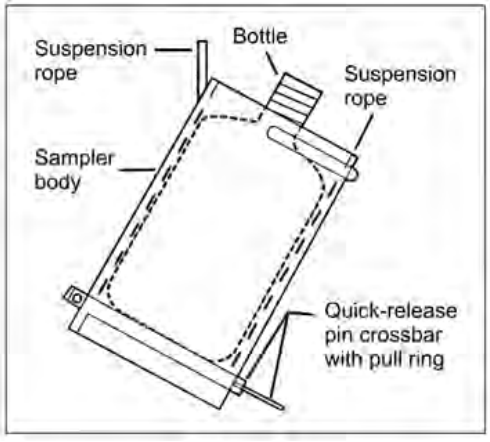


Figure 1: WBH-96 Sampler

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

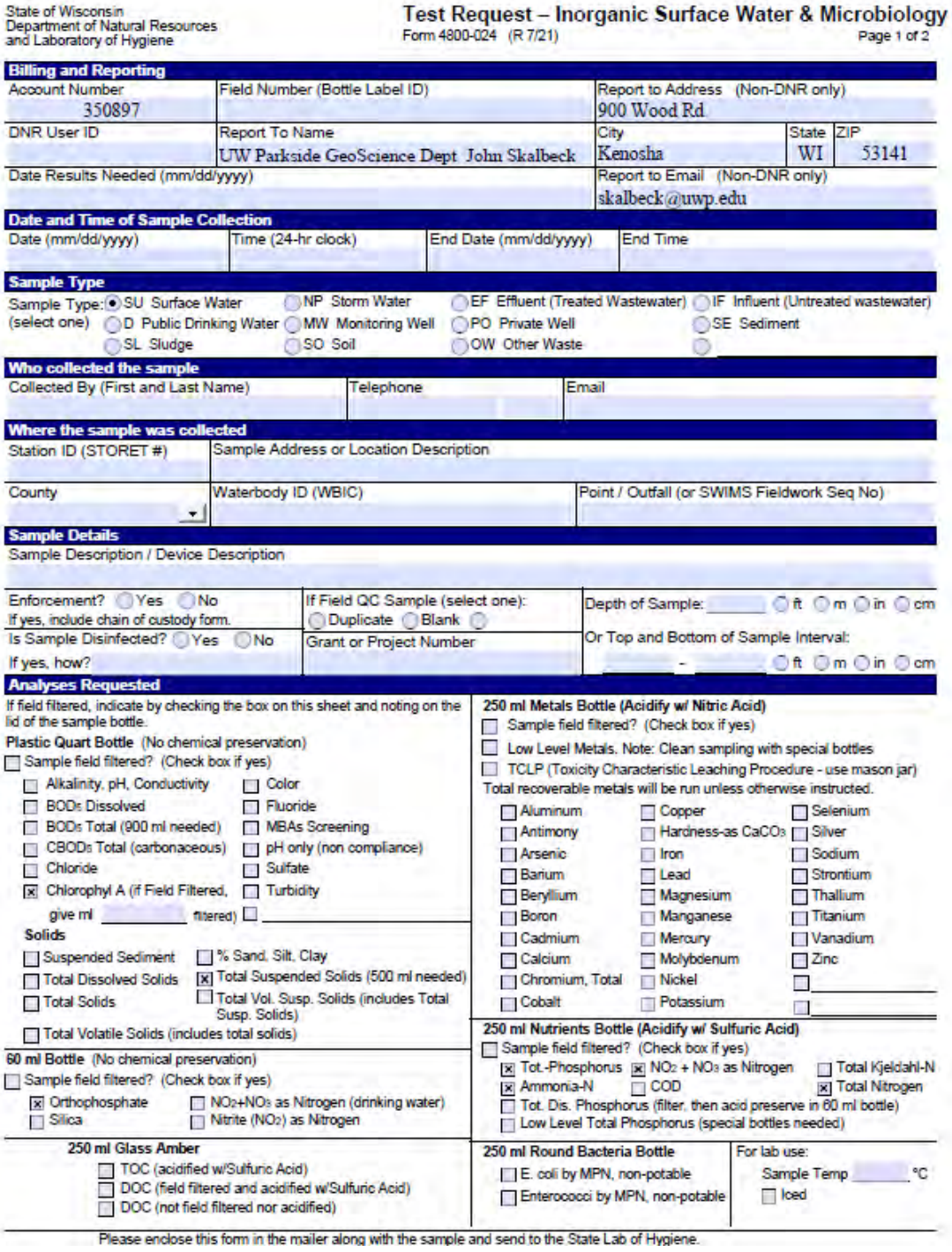
HF – Micro 100 Laboratory Turbidimeter Owner's Manual Catalog No. 22155 (5/10) Rev. 3.3

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

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

- ☒ Tot.-Phosphorus
- ☒ Ammonia-N
- ☒ NO_2 + NO_3 as Nitrogen
- ☐ COD
- ☐ Total Kjeldahl-N
- ☒ 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 D. Wisconsin State Lab of Hygiene SOPs

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).

15.4 Bowman, G. and Easterday, P. Proposed improvements in chlorophyll testing at the State Laboratory of Hygiene, Memorandum, (1995).

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.

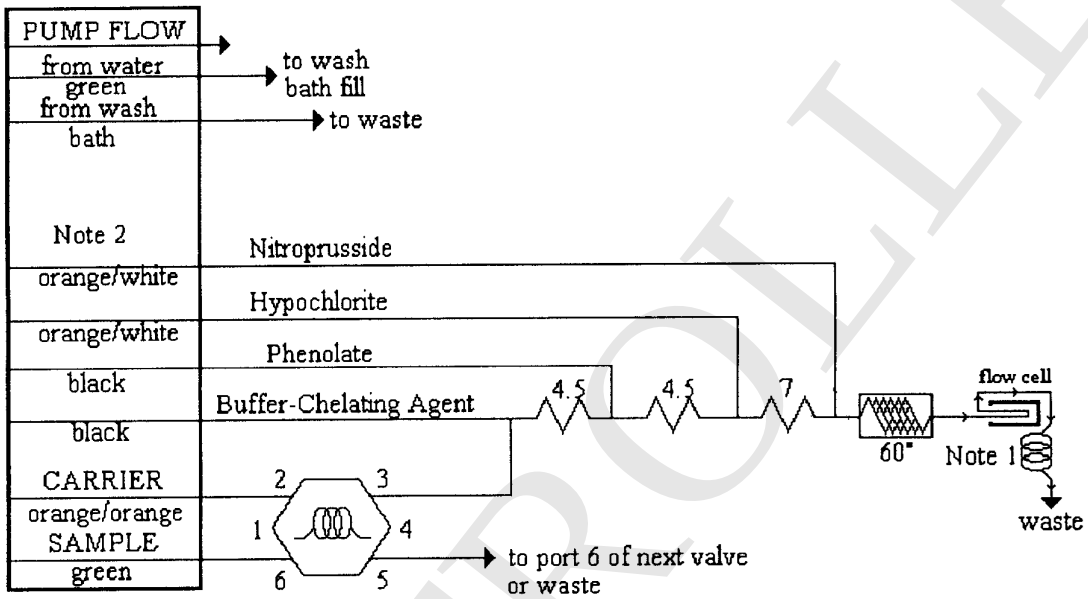
16. Revision Tracking Table:

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

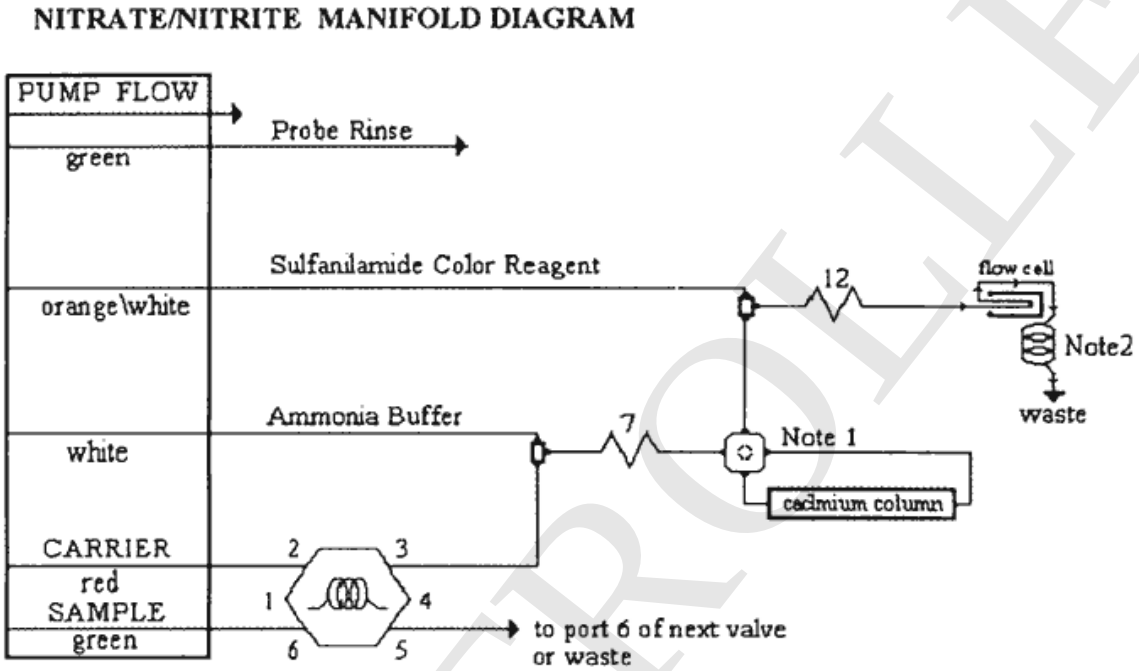
7: 135 cm of tubing on a 7 cm coil support

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

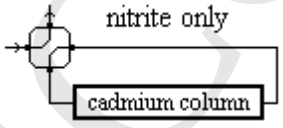
Interference Filter: 520 nm

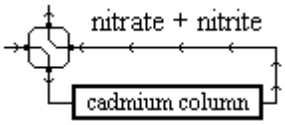
Manifold tubing: 0.5 mm (0.022 in) i.d. This is 2.5 uL/cm.

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.

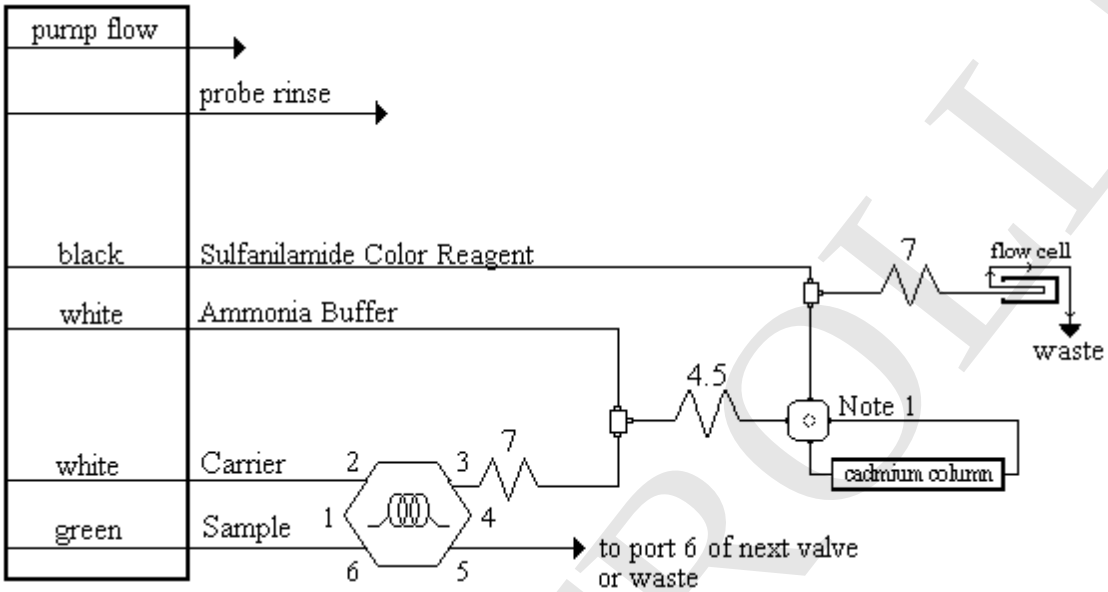
16. Revision Tracking Table:

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.

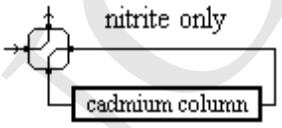
Interference Filter: 520 nm

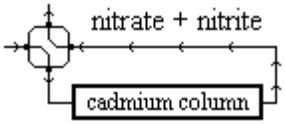
Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

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.

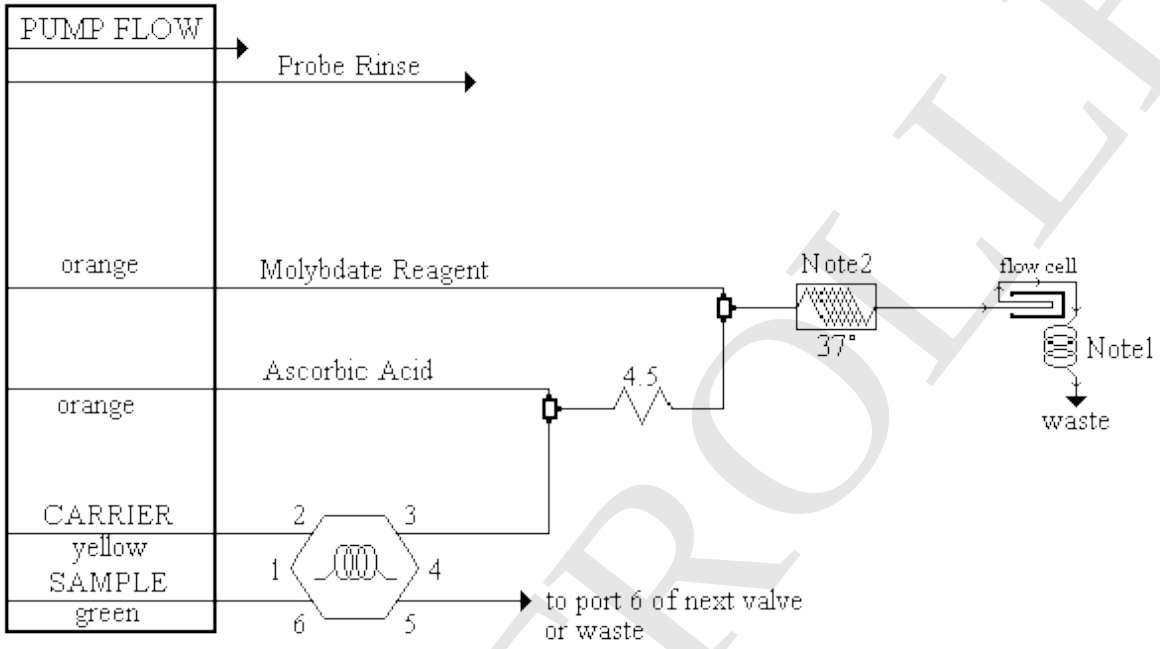
15.17 Wisconsin State Laboratory of Hygiene, EHD INORG GENOP 113, HORIZON Procedures for EHD Inorganic Chemistry

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:

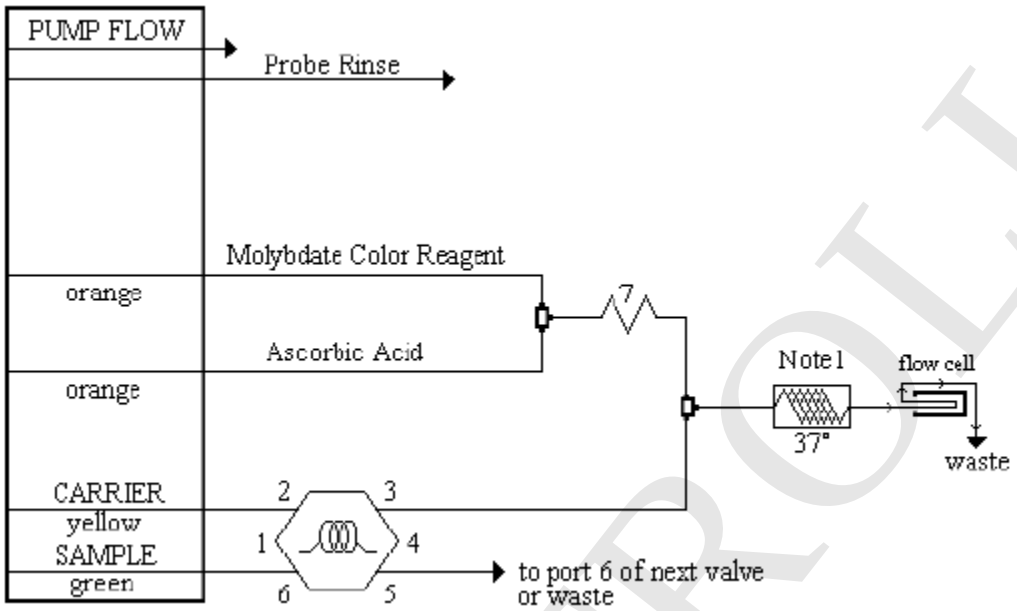
Rev. #	Rev. date	Changes Made	Rev. author
1	09/03/19	1.1, 1.2 updated LOD/LOQ using MB in addition to LRB. 2.2 Amended wording about phenolphthalein indicator and checking pH on SDWA samples. Section 3: added pollution prevention paragraph. 6.4, 6.5 Increased prepared volume of reagents. 6.8.1 Added 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

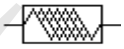
Ver. #	**Changes Made**	**Author**

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.

Appendix E. 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 ft. 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 ft.

(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 ft.

(D) Root River at County Line Road – Biological and habitat sampling reach shall be 35 times the stream width, or approximately 1,470 ft.

Appendix F. Macroinvertebrate Sample 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

Form 3200-081 (R 6/00) Page 1 of 2

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:

$$\%SE = \left(1 - \frac{\#\ Organisms\quad Missed}{Total\quad Organisms\ Found}\right) * 100$$

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. U.S. Environmental Protection Agency, Office of Water, Washington, DC. http://www.epa.gov/owow/monitoring/rbp/

Environment Canada (2012) Canadian Aquatic Biomonitoring Network Laboratory Methods: Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples. Environment Canada, Ottawa, ON. 52 pp.

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 (m^2)**	**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	

Appendix G. 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.

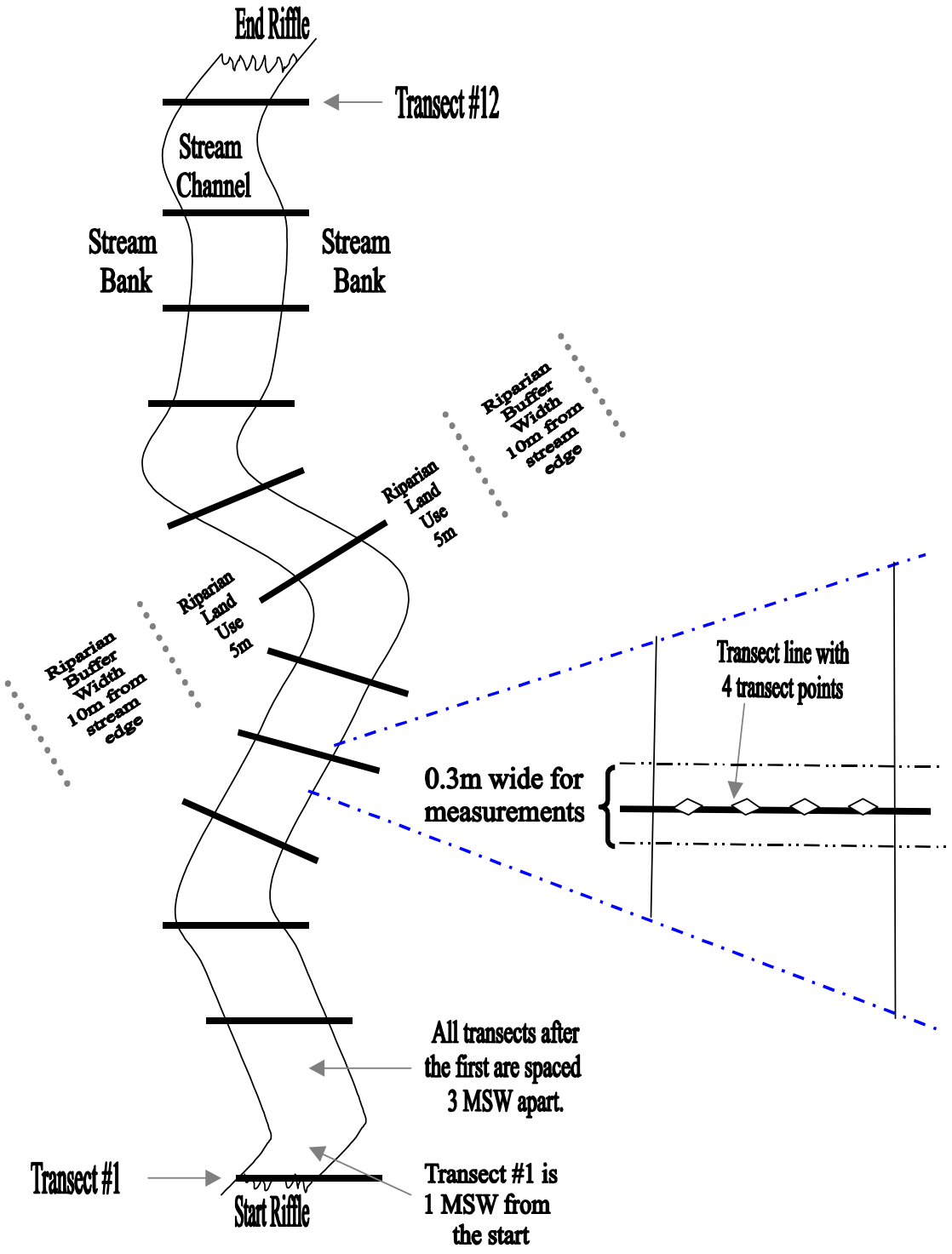
General Sampling Procedures:

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.

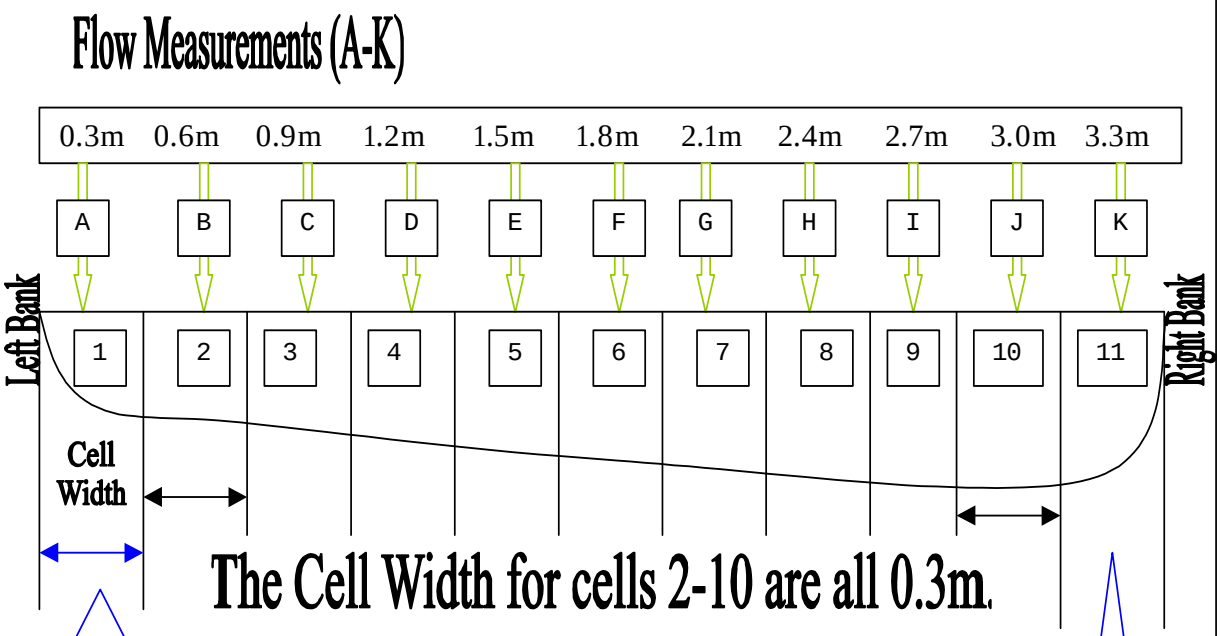
Velocity The velocity (nearest 0.01 m/second or ft/second) of water at that point on the transect. Velocity should be determined with a high quality current (flow) meter, either an electronic or rotating-cup meter, attached to a calibrated, top-setting, wading staff for accurate and precise placement 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.

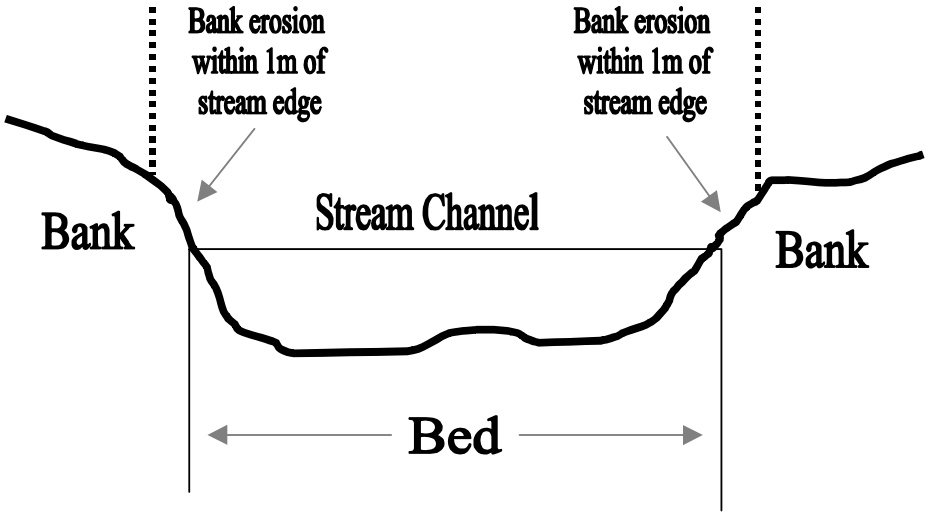


FIGURE 3. Bank Erosion description.

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.

State of Wisconsin
Department of Natural Resources

Wadable Stream Quantitative Habitat Evaluation

Form 3600-228 (R 6/07)

Instructions: Bold fields must be completed. Record all measurements in metric units.

Station Summary

Stream Name	**Waterbody ID Code**	**SWIMS Station ID**	FH Database ID

Date (MMDDYYYY)	**Station Name**

Latitude - Longitude Determination Method Used	**Datum Used**

Start Latitude	**Start Longitude**	End Latitude	End Longitude	**County**

Water Characteristics

Time (24-hr clock)	Air Temperature (C)	Water Temperature (C)	Conductivity (µs/cm)	Transparency (cm)

Dissolved Oxygen (mg/l)	Dissolved Oxygen % Saturation	pH

Flow (m^3/sec) (from Flow Data sheet)	**Water Level (check one - measure distance if Above or Below Normal):**	**Water Clarity:**
	☐ Normal ☐ Below: _____ (m) ☐ Above: _____ (m)	☐ Clear ☐ Turbid ☐ Stained

Channel and Basin Characteristics

Stream Widths (m)

Mean Stream Width (m)	**Transect Spacing (m)**	**Station Length (m)**

Channel Condition: (check one) ☐ Natural ☐ > 20-year-old Channelization ☐ 10- to 20-year-old Channelization ☐ < 10-year-old Channelization ☐ Concrete Channel

Percent Channelization	**Sinuosity**	**Gradient (m/km)**	**Stream Order**	Basin Area (km^2)

(from Map Data Sheet)

Mean Distance (m) Between: **Bends:** _____ **Riffles:** _____

Total (Sum) Length (m) of All: **Riffles:** _____ **Pools:** _____ **Runs:** _____

Mean Length (m) of Individual: **Riffles:** _____ **Pools:** _____ **Runs:** _____

Person(s) Who Collected Data (Full Names)

Comments / Notes

Flow Data

Instructions: * 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.

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.

Stream Name	Waterbody ID Code	**Date (MMDDYYYY)**	**Stream Width (ft or m)**

Distance from Left Bank (ft or m)	Depth (ft or m)	Velocity * (ft/sec or m/sec)	Cell Width (ft or m)	Product (Depth x Velocity x Cell Width)

Flow = Σ Products = ______________ ft^3/sec / m^3/sec

If ft^3/sec, then divide by 35.3 for m^3/sec

Report Value to Station Summary ______________ m^3/sec

Map Data

Stream Name	Waterbody ID Code	**Date (MMDDYYYY)**

Dist. from Start (m)	**Stream Feature** (Bend, Riffle, Pool, Run, Log Jam, etc.)

Draw map of station on back of this sheet (optional)

Distance Summary

Distance Between Bends (m)
Measured from Center of Bend

Downstream - 1st:	
1st - 2nd:	
2nd - 3rd:	
3rd - 4th:	
4th - 5th:	
5th - 6th:	
6th - 7th:	
7th - 8th:	
8th - 9th:	
9th - 10th:	
10th - 11th:	
11th - Upstream:	
Sum:	
Mean:	

Distance Between Riffles (m)
Measurements from the Upstream end of one Riffle to the Downstream end of the next Riffle Upstream

Downstream - 1st:	
1st - 2nd:	
2nd - 3rd:	
3rd - 4th:	
4th - 5th:	
5th - 6th:	
6th - 7th:	
7th - 8th:	
8th - 9th:	
9th - 10th:	
10th - 11th:	
11th - Upstream:	
Sum:	
Mean:	

Length (m) of Individual Riffles, Pools and Runs

1st Riffle:	1st Pool:	1st Run:
2nd Riffle:	2nd Pool:	2nd Run:
3rd Riffle:	3rd Pool:	3rd Run:
4th Riffle:	4th Pool:	4th Run:
5th Riffle:	5th Pool:	5th Run:
6th Riffle:	6th Pool:	6th Run:
7th Riffle:	7th Pool:	7th Run:
8th Riffle:	8th Pool:	8th Run:
9th Riffle:	9th Pool:	9th Run:
10th Riffle:	10th Pool:	10th Run:
11th Riffle:	11th Pool:	11th Run:
Sum:	Sum:	Sum:
Mean:	Mean:	Mean:

N

Transect Data

Stream Name	Waterbody ID Code	**Date (MMDDYYYY)**	**Transect No.**

Distance from Start (m)	**Stream Width (m)**	**Habitat Type:** ☐ Riffle ☐ Pool ☐ Run	Bankfull Depth (m) (optional)	Bankfull Width (m) (optional)

	Deepest Point	Channel Position (Fifths of Current Stream Width) 1/5	2/5	3/5	4/5
Water Depth (m)					
Depth of Fines and Water (m)					
Embeddedness (nearest 10%) of Course Gravel and Rubble/Cobble					

Percent (nearest 5%) of Stream Bottom Covered				**Section Total Must = 100%**
Bedrock (solid slab)				
Boulder (261 mm - 4.1 m)				
Rubble / Cobble (65 - 260 mm)				
Gravel (2 - 64 mm)				
Sand (0.062 - 1.9 mm)				
Silt (0.004 - 0.061 mm)				
Clay				
Detritus				
Other - Specify:_____				

Percent (nearest 10%) of Stream Bottom Covered				
Algae (attached & fila.)				
Macrophytes				
Canopy / Shading (circle one)				

Cover for Adult Gamefish: Length (nearest 0.01 m) of transect within 0.15 m upstream or downstream in water at least 0.20 m in depth

Undercut Banks	Overhanging Vegetation at least 0.20 m overhang	Woody Debris	Other Debris	Boulder	Submerged Macrophytes	Emergent Macrophytes at least 0.20 m deep	Other - Specify:

Bank Erosion: Length of Continuous Bare Soil (nearest 0.01 m) within 1 m of stream

Left:_____ (m) Right: _____ (m)

% of Eroded Bank to the crest or within 5 m of stream edge

Left:_____ (%) Right: _____ (%)

Riparian Land Use: Percent (nearest 10%) of Bank within 5 m of stream edge, along transect **Section Total Must = 100%**

Cropland	Pasture	Barnyard	Developed	Meadow	Shrubs	Woodland	Wetland	Exposed Rock	Other - Specify:

Riparian Buffer Width: Length (nearest 1.0 m) of Undisturbed Land Uses along transect, within 10 m of stream

Left:_____ (m) Right: _____ (m)

Appendix H. 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 transect is completed; if the transect is long, then periodic breaks should be taken to identify, count, and release the fishes before the end of the transect 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

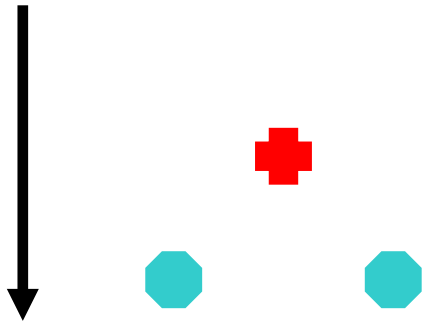
The UW-Parkside team gathers all of the equipment at UW-Parkside either the evening before or the morning of the sampling event. Weather and river depth is assessed the prior evening by the UW-Parkside team lead using both current weather predictions and the USGS monitoring gauges on the Root River (near 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 Transect Length
Following standard EPA guidelines, the electrofishing transect length should be 35 times the mean width of the stream. Using the habitat data collected by the Habitat Team, the length of the transect 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 transect 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 transect is reached, unless the transect is particularly long (≥ 100 m); in case of a long transect, the operators should stop about halfway along the transect 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 transect is completed. Upon reaching the end of the transect, 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 transect 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".

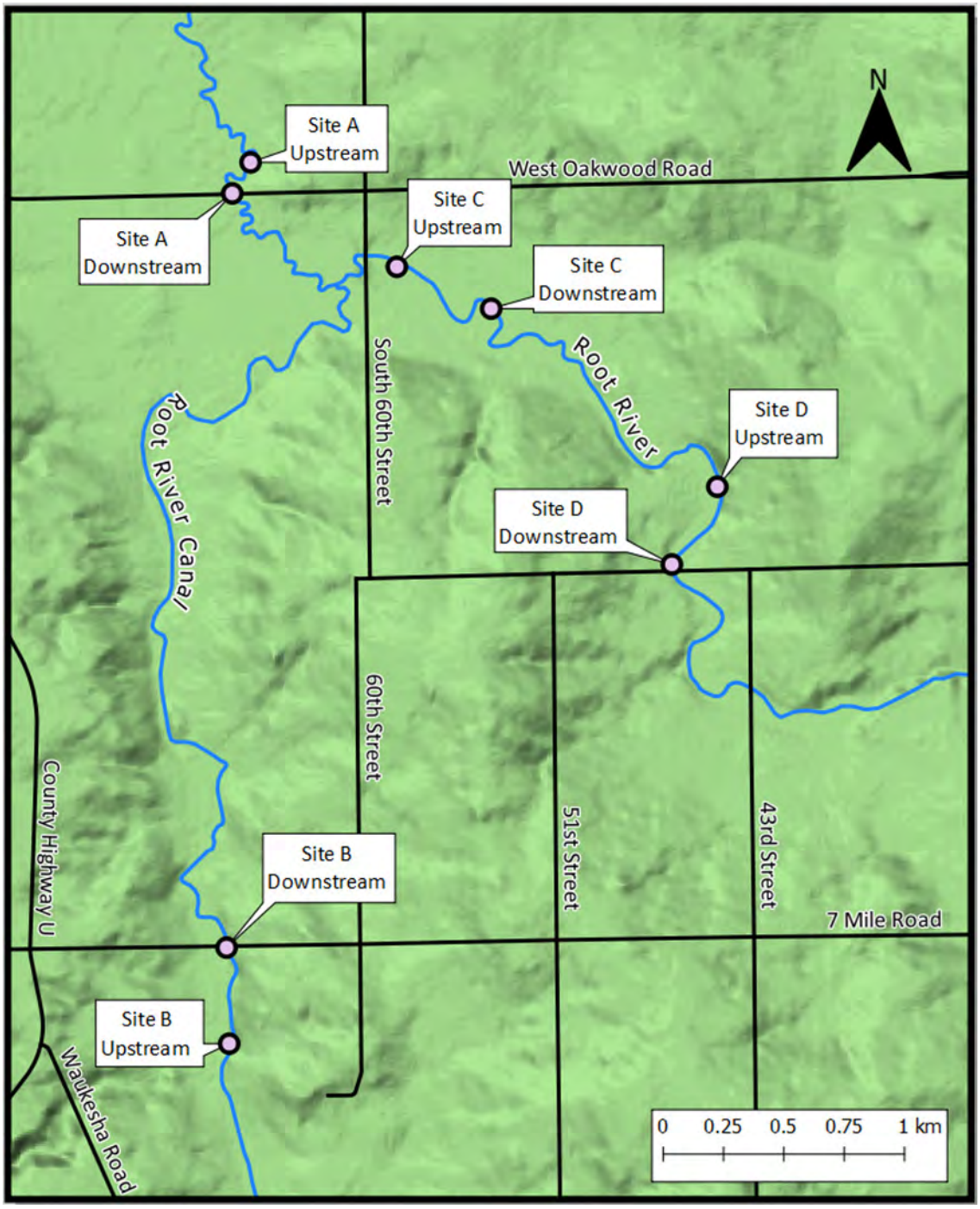
Scanned field notes files are labeled as fieldnotesfish _yearmonth.
Scanned water chemistry field data sheets are labeled as waterdatafish_yearmonth.
Excel files are labeled as fishdata_yearmonth.

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 I. 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.