Water Quality Surveys of the East Twin River (84000), Unnamed Tributaries (3000211, 3000212, & 3000213) to the East Twin River, and Krok Creek (86700) Kewaunee County, WI 2022

East Twin River downstream of Hwy 29

Introduction

The purpose of this study was to supplement previous water quality assessments and determine any possible change in water quality and biological communities within the upper East Twin River and its tributaries. Survey sites included in the present study are the East Twin River (WBIC 84000), Unnamed Tributaries (UNT) to the East Twin River (WBIC 3000211, 3000212, and 3000213), and Krok Creek (WBIC 86700) (Figures 1 & 2).

Evaluations of stream conditions within the upper East Twin River watershed began in 2001 and were subsequently conducted in 2008, 2009, 2011/2012, 2015, and 2017. Results from these monitoring efforts are referenced and included here for comparison to updated water quality results (Gansberg 2017). Water chemistry and temperature data from these assessments indicate impairments from total phosphorus at all sites, and impairment from elevated temperature at UNT 3000213. However, fish and macroinvertebrate communities were meeting designated uses in previous sampling years. An industrial manufacturing facility discharges treated wastewater to UNT 3000213 at Cherneyville Road in Kewaunee County. This

unnamed tributary flows into UNT 3000211 which then confluences with the East Twin River downstream of Hwy 29.

To supplement and reassess the current stream conditions, additional water quality monitoring, continuous temperature monitoring, sediment toxicity testing, and fish community assessments were completed in 2022.

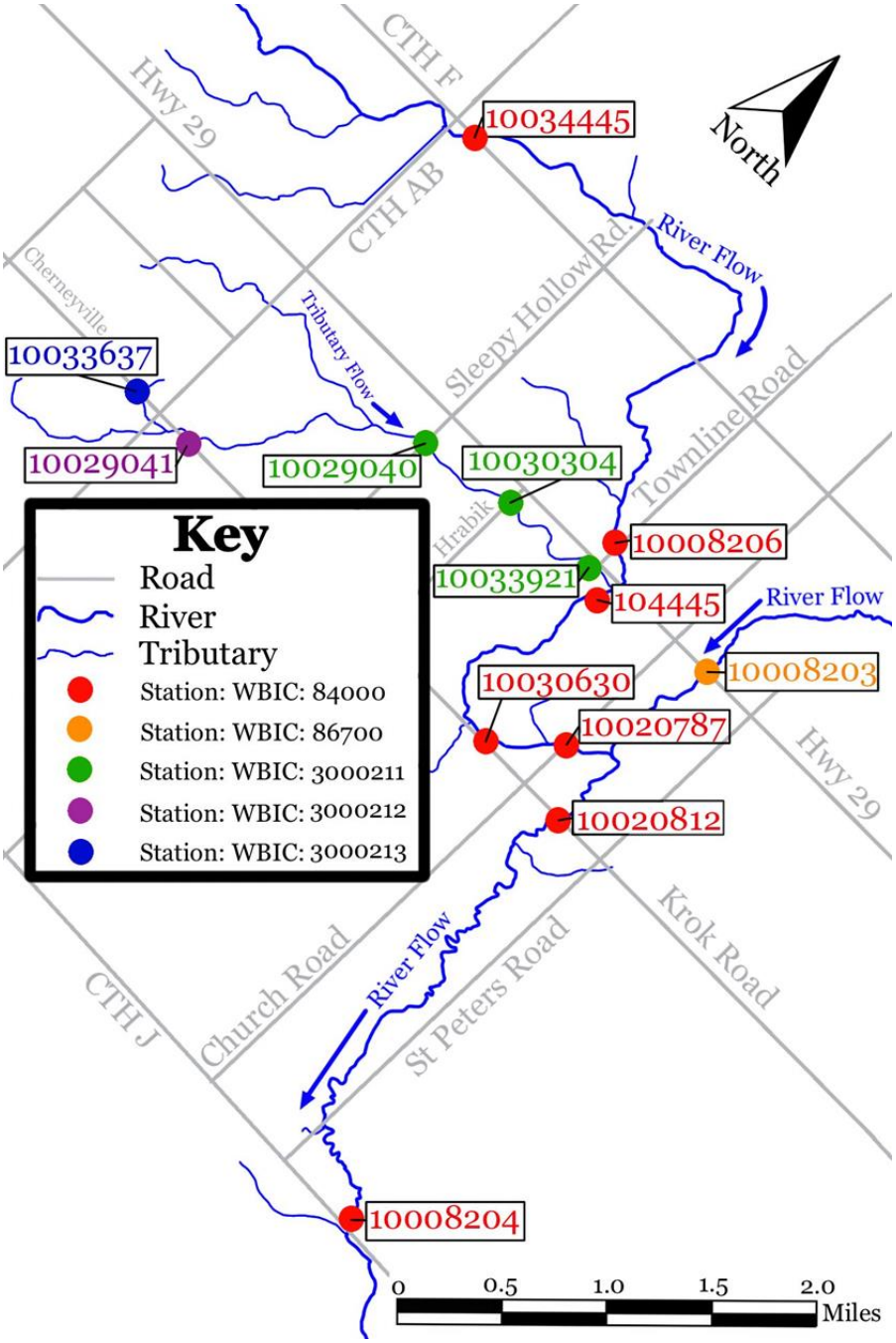

Figure 1. Map of upper East Twin River watershed with locations of current and previous sampling stations.

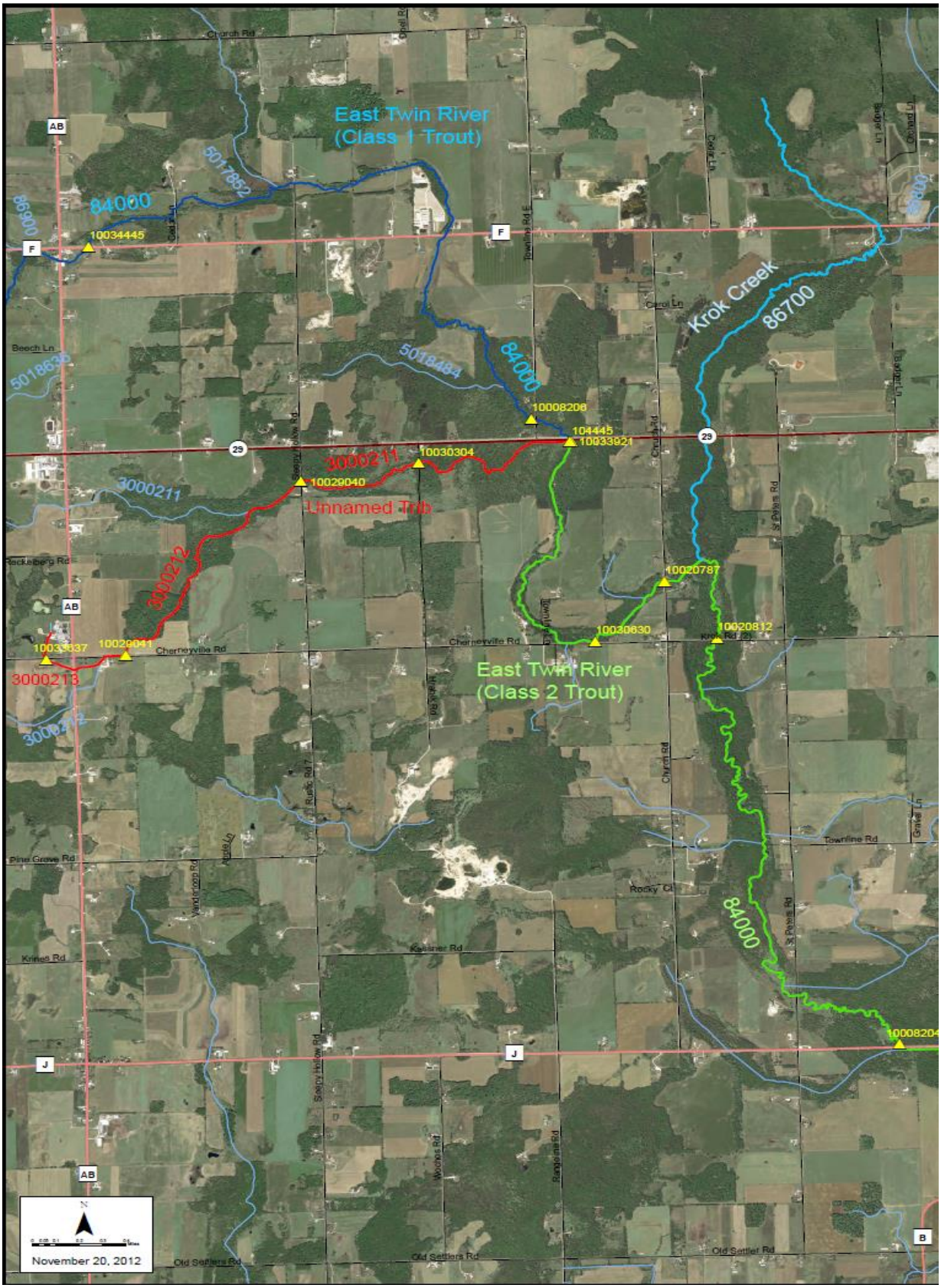

Figure 2. Aerial map of the upper East Twin River watershed and study area.

Water quality data from the 2022 sampling period was assessed against the *Wisconsin 2022 Consolidated Assessment and Listing Methodology* (WisCALM, 2022) to determine if these streams are attaining water quality standards. The streams are evaluated based on their assigned designated uses, shown in Table 1.

Table 1. Classification of stream communities by reach in the study area.

Location	Classification (according to WI Admin Code NR 102)
Headwaters of the East Twin River down to State Highway 29 (Hwy 29)	Cold Water Community – Class 1 Trout and Exceptional Resource Waters
State Highway 29 (Hwy 29) down to CTH B	Cold Water Community – Class 2 Trout Waters
Unnamed Tributaries to the East Twin River	Fish and Aquatic Life (DFAL) Communities
Krok Creek	Cold Water Community

Water quality criteria thresholds for cold water and Default Fish and Aquatic Life (DFAL) uses are specified in NR 102 and WisCALM as follows:

- Dissolved oxygen: cold water <6 mg/L, DFAL <5 mg/L
- Chloride: acute toxicity >757 mg/L, chronic toxicity >395 mg/L
- Total phosphorus: >75 ug/L
- Water temperature: cold water and DFAL criteria provided below in Table 2

Table 2. Temperature criteria in Fahrenheit for cold water and small warmwater streams, from NR 102.25(2) Table 2

Month	Cold	Warm - Small (DFAL)
Jan	68	76
Feb	68	76
Mar	69	77
Apr	70	79
May	72	82
Jun	72	84
Jul	73	85
Aug	73	84
Sep	72	82
Oct	70	80
Nov	69	77
Dec	69	76

The headwaters of the East Twin River downstream to Mishicot are currently on the Clean Water Act Section 303(d) list of impaired waters for phosphorus. The UNTs 3000211, 3000212 and 3000213 in their entirety are also on the impaired waters list for phosphorus. UNT 3000213 is on the impaired waters list for water temperature.

Methods

Monitoring in 2022 was conducted at ten stations within the upper East Twin River watershed (Table 3) following DNR Field Procedures Manuals and guidelines for proper sample collection and preservation. Additional analyses that were not repeated during the 2022 sampling season are described and reported in Appendix A. Sites were selected based on previous water quality monitoring stations, as well as proximity to the wastewater discharge and receiving waters downstream of the discharge. UNT 3000213 flows into UNT 3000212 to UNT 3000211, ultimately flowing into the East Twin River just below Hwy 29. Monitoring stations established along the UNTs and at the confluence with the East Twin River aimed to detect any possible impacts this tributary has on the East Twin River. One station on the East Twin River upstream of the confluence with the UNT (station ID: 10008206) was established to provide background conditions of the East Twin River. An additional monitoring station on nearby Krok Creek provides a separate reference site of conditions within the watershed. Stations further downstream from the confluence of the UNT may detect any broader impacts this tributary has on the East Twin River.

Table 3. Monitoring locations and parameters collected during 2022 sampling

Waterbody (WBIC)	Station ID	Location	Parameter			
			Water Chemistry	Continuous Water Temp	Sediment Toxicity	Fish
East Twin River (84000)	10030630	Krok Road	X	X		X
	104445	Below Hwy 29	X			
	10008206	Townline Rd	X	X		X
	10008204	CTH J		X		
Krok Creek (86700)	10008203	Above Hwy 29	X	X	X	
Unnamed Tributary (3000211)	10030304	Hrabik Rd		X		
	10029040	Sleepy Hollow Rd				X
	10033921	Stream mouth at Hwy 29	X			
Unnamed Tributary (3000212)	10029041	Cherneyville Rd – East crossing				X
Unnamed Tributary (3000213)	10033637	Cherneyville Rd – West crossing	X	X	X	

Water Chemistry Sampling

Monthly grab samples were collected from May through October on three East Twin River stations, UNTs 3000211 and 3000213, and Krok Creek (Table 3). All samples were shipped to the Wisconsin State Laboratory of Hygiene (WSLH) for analysis of total phosphorus, chloride, total suspended solids, ammonia, total nitrogen, and nitrite + nitrate concentrations. In July only, water chemistry analysis also included biochemical oxygen demand (BOD).

Continuous Temperature

Water temperature data loggers (HOBO brand) were placed at 6 locations in the Upper East Twin River Watershed (Table 3). Loggers were set to record water temperature hourly and were deployed from May through October. The same 6 locations were monitored during the 2017 study and all stations except Krok Creek were monitored in 2011. Due to technical malfunctions with the data loggers, only temperature data from 4 out of 6 stations are reported.

Sediment Toxicity

Soft sediment was sampled from two locations – UNT 3000213 and Krok Creek for toxicity testing, with the Krok Creek sample serving as a nonimpacted reference site. A lab-prepared synthetic sediment was used as a lab control. Stream sediment was collected using a shovel to capture the upper-most layer of soft sediment. Approximately 5 gallons was collected from each location and delivered to the WSLH for testing. Two organisms are used to test for sediment toxicity: *Chironomus dilutus* (larval midge) and *Hylalella azteca* (amphipod). These organisms burrow within the sediment and are recommended by USEPA for toxicity testing due to their prolonged exposure to the sediment. Testing occurs by exposing the organisms to the sediment for 10 days. Following the exposure period, organisms were removed from the sediment to determine the number of survivors. Additionally, surviving organisms were dried to measure their dry weight to determine any effects on growth rates. Sediment toxicity is determined based on the difference in growth and survival rates of the test sediments compared to the lab control.

Collection of sediment samples at UNT 3000213 at Cherneyville Rd

Sediment Quality

Additional sediment samples were collected to be tested for sodium, phosphorus, chloride, and nitrate + nitrite. Soft sediment was collected on June 22, 2022 at Krok Creek and UNT 3000213 (Cherneyville Rd) and stored in glass jars, and samples were delivered to the State Lab of Hygiene for analysis. There are no numeric state standards for determining sediment contamination, so the two samples will be compared to assess sediment quality.

Fish Assemblage

Fish surveys were completed in July on the East Twin River at two stations and the two UNTs (Table 3). A backpack shocker was used at all sites with the goal of capturing all fish present. Fish were identified and the number of each species collected was recorded. This allows for calculation of the Index of Biological Integrity (F-IBI). The F-IBI provides a measure of overall stream health based on fish community structure, with scores ranging from 0 (poor) to 100 (excellent).

East Twin River at Krok Rd

Results

Water Chemistry

Total Phosphorus

As specified in NR 102 of the Wisconsin Administrative Code, the total phosphorus criterion of 75 ug/L is established for the UNTs, East Twin River and Krok Creek. The protocol for impairment decisions requires six monthly samples to be collected between May and October. The department's listing methodology for impaired waters (WDNR, 2022) lists waters where the median concentration and lower 80% confidence interval values exceed 75 ug/L on wadable streams.

As shown in Table 4a, four stations exceed phosphorus criteria on all sampling dates and the remaining two stations exceed phosphorus standards on two or more sampling dates. Median monthly phosphorus concentrations increased from 2017 at the UNT at Cherneyville Rd and decreased from 2011/2012 and 2017 levels at all other sites (Figure 3). Four stations clearly exceed phosphorus criteria, and two sites may meet standards (Table 4b).

The East Twin River is currently listed as impaired from its headwaters downstream to Mishicot for total phosphorus. Krok Creek and the UNTs (3000211, 3000212, and 3000213) are also listed as impaired for phosphorus.

Table 4a. Results of water quality monitoring at 6 sampling stations for total phosphorus.

Date	Total Phosphorus ug/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	**151**	**165**	31.9	**412**	**543**	64.1
6/22/22	**190**	**178**	**79.6**	**274**	**421**	**95.7**
7/20/22	**216**	**244**	63.4	**815**	**535**	**78.7**
8/15/22	**212**	**203**	**149**	**534**	**329**	**288**
9/28/22	**151**	**186**	44.8	**488**	**360**	46.3
10/26/22	**197**	**158**	33.8	**810**	**322**	44.4

***Note: Bolded values indicate the result exceeds the standard of 75 ug/L**

Table 4b. Median values of total phosphorus with upper and lower 80% confidence intervals.

2022 Calculations	Total Phosphorus ug/L; threshold 75 ug/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
Median	193.5	182.0	53.3	510.5	389.3	71.0
LCL	167.2	170.1	40.4	403.4	355.0	53.6
UCL	203.0	205.6	81.9	666.4	470.7	122.9
Relation to Standard	**Clearly Exceeds**	**Clearly Exceeds**	May Meet	**Clearly Exceeds**	**Clearly Exceeds**	May Meet

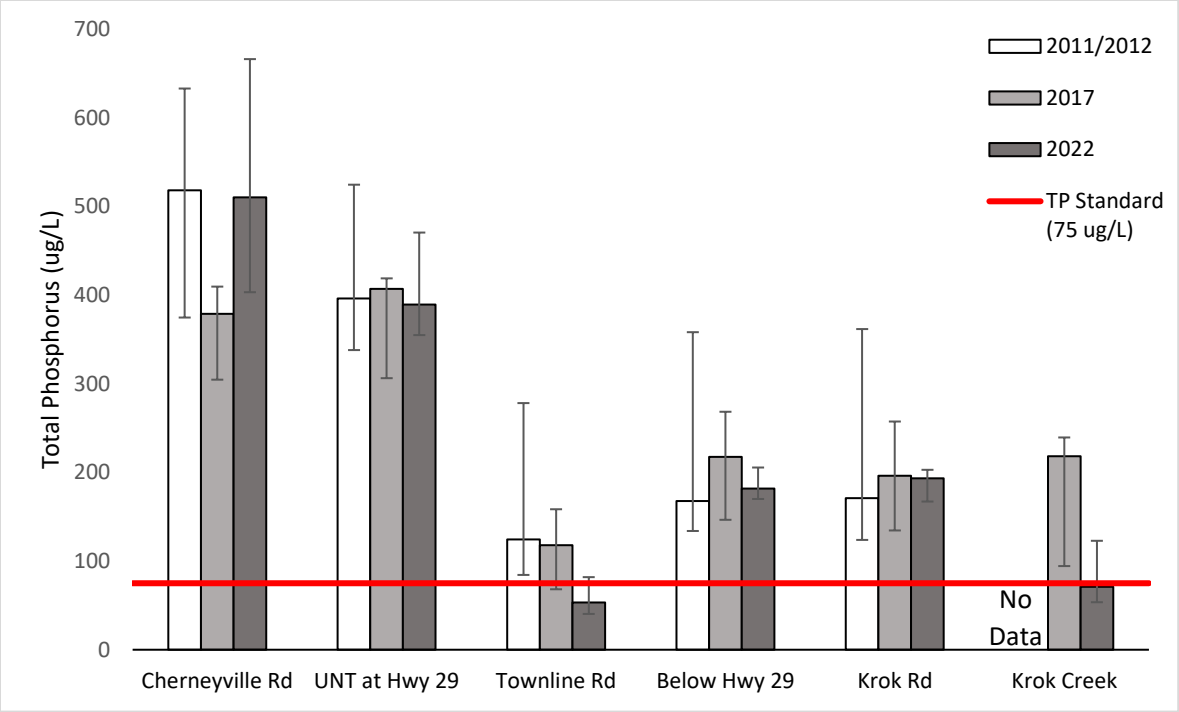

Figure 3. Median total phosphorus results for 6 sampling stations in 2022 compared with the 2017 and 2011/2012 sampling years. Error bars indicate upper and lower 80% confidence interval. Red line indicates the phosphorus limit of 75 ug/L for surface waters.

Chloride

The protocol for impairment decisions established in 2022 WisCALM for chloride requires at least two values within a 3-year period. Six monthly chloride samples were collected at the same time the phosphorus samples were collected. The criterion for chronic toxicity is 395 mg/L and for acute toxicity is 757 mg/L.

As shown in Figure 4 and Table 4c, none of the stations exceeded either chronic or acute toxicity standards for chloride during the entire sampling period. However, there are clearly elevated chloride levels in the UNTs relative to the East Twin River upstream of the confluence with the UNT, and the Krok Creek reference site. The East Twin River at Krok Rd and at Hwy 29 has moderately elevated levels of chloride compared to the two reference sites. In addition, most sites have had increasing chloride levels since 2011.

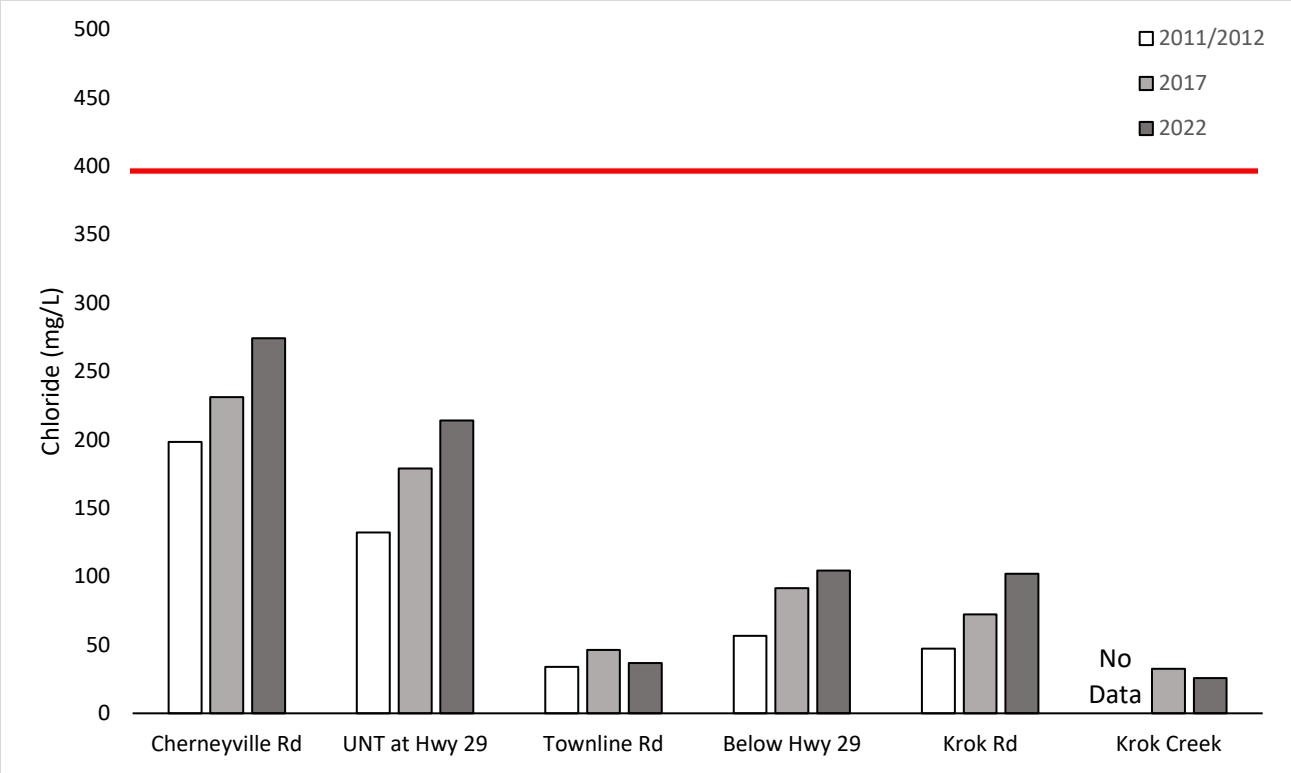

Figure 4. Average monthly chloride at the 6 sample stations in 2022, with comparisons to the 2017 and 2011/2012 sampling years. Red line indicates the chronic toxicity for chloride (>395 mg/L), which was not exceeded at any station during any sampling year.

Table 4c. Results of water quality monitoring at 6 sampling stations for chloride.

Date	Chloride mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	72.7	94.6	37.2	298	221	22.7
6/22/22	99.8	101	35.9	287	265	27.9
7/20/22	125	127	35.9	270	257	31.9
8/15/22	43.2	56.8	35.0	227	103	16.3
9/28/22	124	147	37.9	272	263	29.5
10/26/22	147	99.2	38.6	291	176	27.0

*The following water chemistry parameters do not have a standard for the State of Wisconsin. However, they can be compared against the reference sites to determine if any differences in water quality are documented.

Total Suspended Solids (TSS)

There are no state standards for suspended solids in surface waters. This parameter provides a measure of solid particles that are present within a sample. The Krok Creek reference site had consistent TSS levels of 6.80 mg/L or non-detectable levels. In comparison, the UNT 3000213 at Cherneyville Rd had elevated TSS in July of 26.2 mg/L and the UNT 3000211 at Hwy 29 had a TSS level of 22.0 mg/L in July. The East Twin River at Hwy 29 has higher TSS levels compared with the upstream reference site at Townline Rd.

Table 4d. Results of water quality monitoring at 6 sampling stations for total suspended solids.

Date	Total Suspended Solids mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	3.40	2.00	ND	5.80	4.80	ND
6/22/22	4.40	10.8	6.20	8.80	2.40	6.80
7/20/22	6.60	11.8	2.40	26.2	22.0	6.80
8/15/22	8.40	5.43	5.80	9.60	8.20	6.80
9/28/22	ND	ND	ND	10.2	ND	ND
10/26/22	2.80	4.6	ND	16.4	ND	3.00

Ammonia

There is no state standard for ammonia levels in surface water, though it can potentially be toxic to fish and aquatic life under certain conditions. In non-polluted surface waters, ammonia is typically not detected. Acute ammonia toxicity is calculated based on pH and temperature, and toxicity increases as pH and temperature increase. To determine toxicity, the unionized fraction of ammonia is calculated using temperature, pH, conductivity, and total ammonia (American Fisheries Society). This is then compared to acute ammonia values for waterbodies in Wisconsin based on Wisconsin Administrative Code NR 105.06 Table 2. For all sites monitored in 2022, unionized ammonia was well below values that would be toxic to aquatic life.

Ammonia levels in UNT 3000213 at Cherneyville Rd are elevated by one order of magnitude compared to the other sampling sites. Ammonia levels have decreased since 2017 at all sites except for the Cherneyville Rd station which increased from a monthly average of 0.06075 mg/L in 2017 to 0.184 mg/L of ammonia in 2022, an approximately threefold increase in concentration (Figure 5).

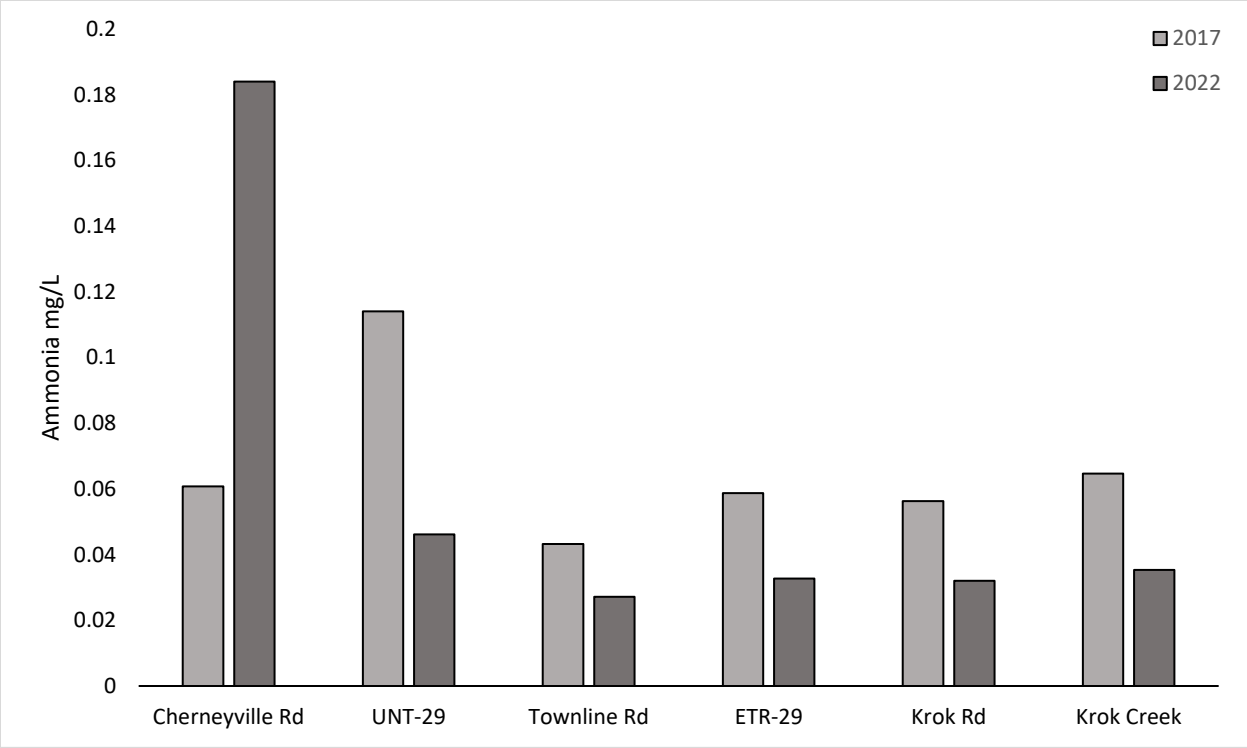

Figure 5. Monthly average ammonia concentrations (mg/L) for the six sampling stations in 2017 and 2022.

Table 4e. Results of water quality monitoring at 6 sampling stations for ammonia.

Date	Ammonia mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	0.0367	0.0338	0.0184	0.108	0.0647	0.0312
6/22/22	0.0439	0.0376	0.0498	0.0995	0.0640	0.0533
7/20/22	0.0382	0.0298	0.0151	0.297	0.0458	0.0284
8/15/22	0.0283	0.0297	0.0254	0.289	0.0410	0.0287
9/28/22	ND	ND	ND	0.127	ND	ND
10/26/22	0.0133	ND	ND	0.184	0.0152	ND

Total Nitrogen

There are currently no standards for total nitrogen in waters classified for fish and aquatic life. Total nitrogen can be used to determine if an organic discharge is high in nitrogen. Total nitrogen should be assessed in conjunction with ammonia and nitrate + nitrite to evaluate the overall impact.

In 2022, total nitrogen levels at the two reference sites (Townline Rd and Krok Creek) ranged from 2.69 mg/L-7.32 mg/L. The East Twin River at Krok Rd and Hwy 29 had levels higher than the reference sites, ranging from 5.63-20.5 mg/L. The UNT 3000213 at Cherneyville had the highest total nitrogen, ranging from 19.1-38.9 mg/L and UNT 3000211 at Hwy 29 ranged from

15.0-35.3 mg/L. Overall, September had the highest levels of total nitrogen at all sites (Table 4f).

Table 4f. Results of water quality monitoring at 6 sampling stations for total nitrogen.

Date	Total Nitrogen mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	8.06	9.03	5.62	26.3	18.4	3.14
6/22/22	12.4	12.4	6.42	31.4	27.7	5.14
7/20/22	10.1	10.6	6.41	19.1	17.1	6.27
8/15/22	5.63	6.93	4.29	29.0	13.2	2.69
9/28/22	17.8	20.5	7.32	38.9	35.3	5.54
10/26/22	13.8	9.33	5.13	34.0	15.0	3.07

Nitrate + Nitrite

Nitrate + nitrite is analyzed alongside ammonia and total nitrogen and provides a measure of inorganic nitrogen. There is no state standard for nitrate + nitrite. Groundwater in agricultural areas has been observed to have higher background levels of nitrate + nitrite, which can often cause elevated levels in surface waters, especially in stream systems dominated by groundwater influence.

A similar pattern to total nitrogen is observed among the 6 sites sampled in 2022. The Krok Creek and Townline Rd reference sites have the lowest levels of nitrate + nitrite (1.03-5.50 mg/L), the East Twin River at Krok Rd and Hwy 29 have increased levels compared to the reference sites (4.17-19.7 mg/L), and the two UNTs at Cherneyville Rd and Hwy 29 have the highest levels of nitrate + nitrite (11.5-36.3 mg/L) (Table 4g).

Table 4g. Results of water quality monitoring at 6 sampling stations for nitrate + nitrite.

Date	Nitrate + Nitrite mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
5/23/22	6.92	7.90	4.85	23.7	16.14	2.14
6/22/22	11.6	10.9	5.84	30.4	26.1	4.18
7/20/22	8.97	9.01	6.00	14.5	14.3	5.50
8/15/22	4.17	5.56	3.16	26.3	11.5	1.03
9/28/22	16.8	19.7	6.95	36.3	34.4	5.06
10/26/22	12.8	8.50	4.77	32.5	13.9	2.46

Biochemical Oxygen Demand (BOD)

There is no state standard for BOD, however, typical levels for non-polluted environments are around 2 mg/L. BOD concentrations of 10 mg/L is indicative of some organic pollution but is of minimal concern to fish. For reference, BOD of untreated municipal wastewater ranges from 100-300 mg/L and 20-30 mg/L following treatment. High levels of BOD in streams can decrease

dissolved oxygen levels through the breakdown of organic materials by bacteria that consume oxygen.

During the 2022 sampling season, BOD samples were taken once in July. Only 2 of the 6 sample sites contained detectable levels of BOD. The UNT 3000213 at Cherneyville Rd had a BOD result of 10.1 mg/L, compared to the UNT 3000211 at Hwy 29 of 2.69 mg/L. At the two reference sites, Krok Creek and Townline Rd, BOD was not detected in the sample (Table 4h).

Table 4h. Results of water quality monitoring at 6 sampling sites for BOD.

Date	BOD mg/L					
	East Twin River			Unnamed Tributary		
	Krok Rd	Hwy 29	Townline Rd	Cherneyville Rd	UNT-Hwy 29	Krok Creek
7/20/22	ND	ND	ND	10.1	2.69	ND

Continuous Water Temperature

Hourly water temperature data is summarized in Figures 6a-6c below. Monitoring in 2011 did not include the Krok Creek station, but all other sites were monitored in 2011, 2017, and 2022. Due to technical difficulties, data was not recovered from two sites in 2022: East Twin River at Krok Road (Station ID: 10030630) and Krok Creek (Station ID: 10008203).

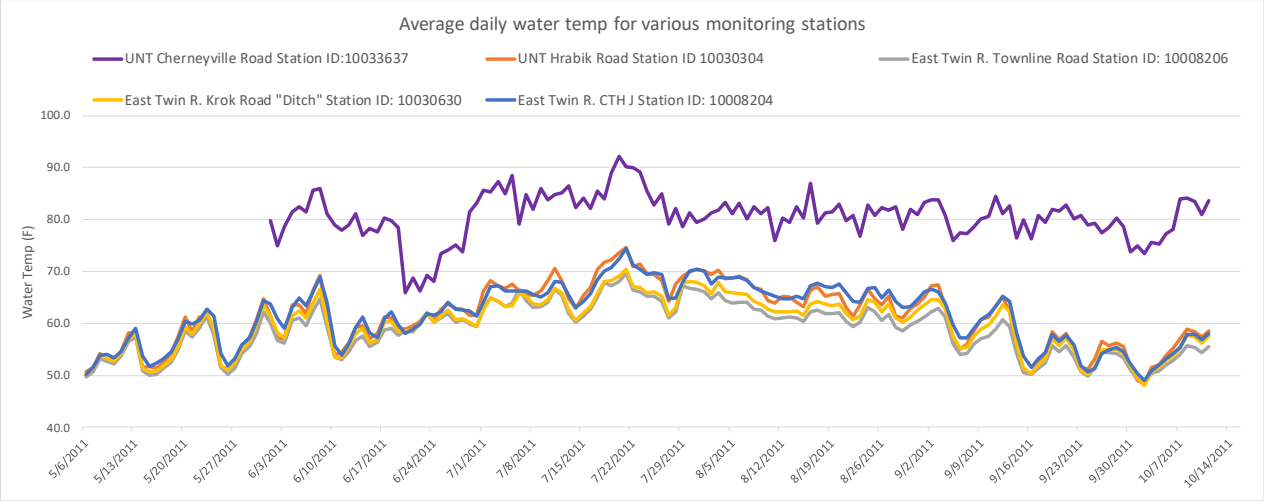

Figure 6a. Average daily temperature at all stations sampled during 2011

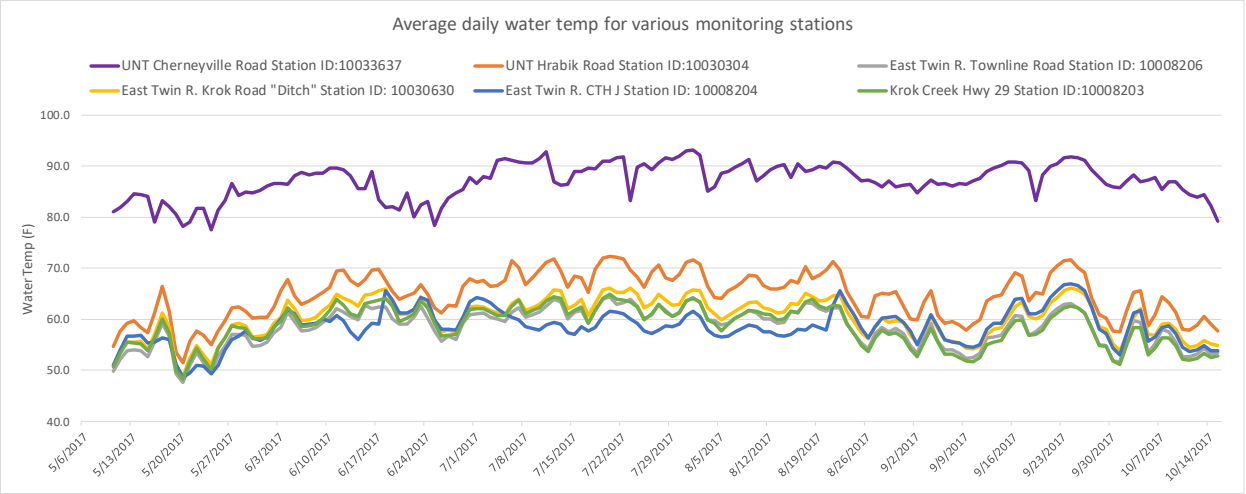

Figure 6b. Average daily temperature at all stations sampled during 2017.

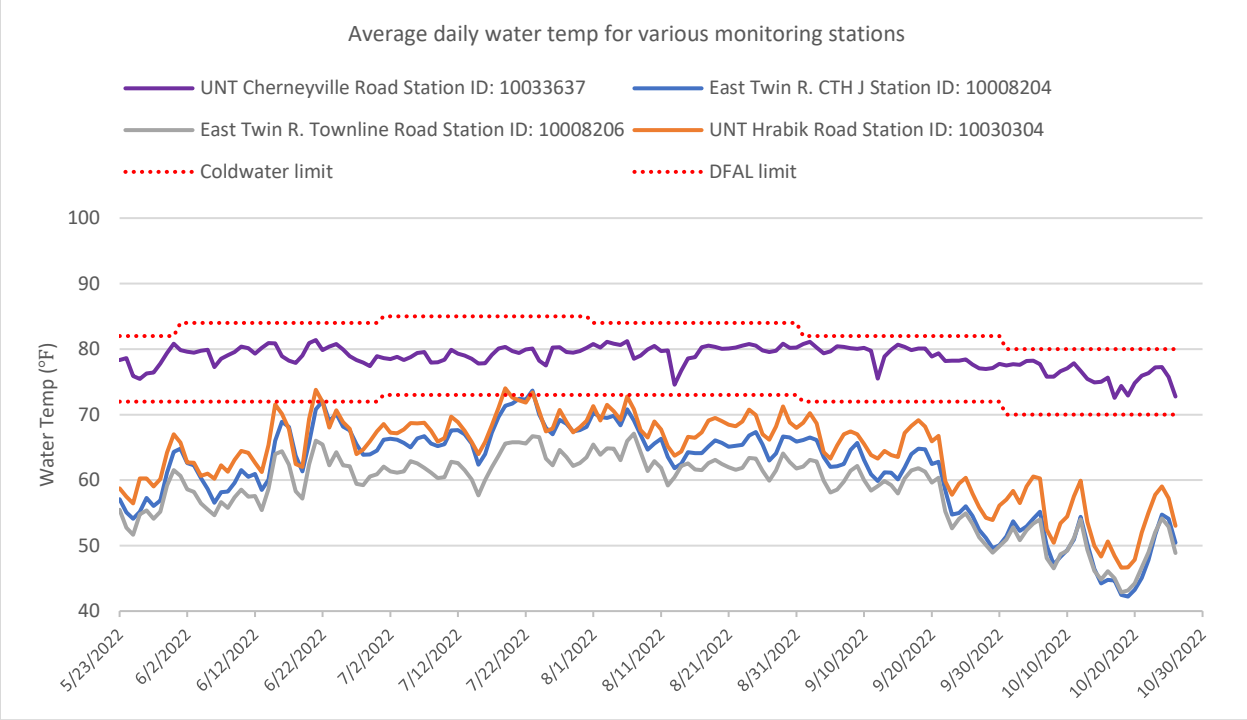

Figure 6c. Average daily temperature at all stations sampled during 2022. Lower dashed red line indicates the coldwater temperature criteria and upper dashed red line indicates fish and aquatic life criteria; temperature values for each category are found in Table 1.

During the 2022 sampling period, water temperature in the East Twin River met the coldwater criteria at the Townline Rd station. The East Twin River at CTH J exceeded the coldwater limit multiple days in June, July, and August. Both UNTs were below the temperature limits for fish and aquatic life for the entire sampling period.

The 2022 temperature data indicates an improvement in meeting water temperature criteria for the UNT 3000213 at Cherneyville Rd. The station had exceeded DFAL temperature criteria for all months monitored in 2011 and 2017 but was below the DFAL threshold in all sampling months in 2022 and will be recommended for removal from the impaired waters list for temperature. The UNT 3000211 at Hrabik Rd continued to meet the DFAL water temperature criteria for all sampling months in 2022.

Sediment Toxicity Testing

Chironomus dilutus

Survival: The survival of *C. dilutus* ranged from 50%-100% with mean site survival ranging between 88.8%-92.5% survival. There was no significant difference between the test sites and lab control.

Growth**:** Growth was not significantly different between the lab control and study sites, though slightly less in the lab control.

Hyalella azteca

Survival: Overall survival of *H. azteca* was 50%-100% and mean survival ranged from 77.5%-95% per site. Survival was significantly higher in the test sites compared to the lab control. There was no significant difference between the two study sites.

Growth: Growth was not significantly different between the lab control and study sites, though slightly less in the lab control.

Overall, toxicity testing indicates that the two test organisms are not impacted by sediment contamination at the sampling sites. Survival and growth rates of the test organisms reveal no significant differences between the reference site (Krok Creek) and the test site (UNT 3000213). Comparison against the lab control also indicate no significant difference between the two sample sites. Complete survival and growth data are presented in Appendix B.

Sediment Quality

Sediment results are reported in Table 5 below for Krok Creek and UNT 3000213 at Cherneyville Rd. Both samples did not have detectable levels of nitrate + nitrite. Chloride, sodium, and phosphorus levels were all substantially higher at the UNT compared with Krok Creek. Phosphorus levels were especially elevated at the UNT, about 7.5 times higher than at Krok Creek. There are no state standards for sediment chemistry, but this analysis indicates that levels of these selected parameters are elevated at UNT 3000213 compared with the Krok Creek reference site.

Table 5. Results of sediment analysis at Krok Creek and UNT 3000213

Location	Parameter			
	Chloride (mg/kg)	Sodium (mg/kg)	Phosphorus (mg/kg)	Nitrate + Nitrite (mg/kg)
Krok Creek	88.7	98.3	379	ND
UNT 3000213	424	892	2800	ND

Fisheries Assessment

Fish surveys were repeated in 2022 at four sites (Table 3), and fish inventory from all sampling dates is reported below (Table 6). High conductivity at all sites during July resulted in a decrease in efficacy of electroshocking equipment and ultimately low catch rates of the fish community. The low fish numbers are more likely a function of equipment issues than an actual decrease in fish populations.

The Fish Index of Biological Integrity (F-IBI) from previous sampling years is reported in Table 6. No F-IBI scores were calculated from the 2022 fish surveys due to insufficient population sizes. In previous sampling years with calculated F-IBI scores, fish communities ranged from excellent to good. In 2011 the East Twin River at Krok Rd and Townline Rd both had fair F-IBI scores.

Table 6. Fish Inventory of the East Twin River and Unnamed Tributaries

Waterbody (WBIC)	East Twin River (84000)																Krok Creek (867000)	Unnamed Tributary (3000212)			Unnamed Tributary (3000211)		
Station ID	10008206						10030630					10008204					10020812	10029041			10029040		
Location	Townline Rd						Krok Rd					CTH J					Krok Creek	Cherneyville Rd east crossing			Sleepy Hollow Rd		
Species	2001	2009	2011	2015	2017	2022	2009	2011	2015	2017	2022	2001	2009	2011	2015	2017	2015	2008	2017	2022	2008	2017	2022
Blacknose Dace		1					1					5	7	3		4				1	49	14	13
Bluntnose Minnow												4											
Brook Lamprey	2	7	3		1							2	4	3									
Brook Trout	3	20	18	16	5				1	3							2						
Brook Stickleback	11			2						1		2	3	26	2						6		
Brown Trout												2			9	6							
Central Mudminnow	8	42	14	8	22		78	55	30	28		21		105	70	53	54	4			3	2	
Common Shiner							1		29			29	68	5	29	68	7				1		
Creek Chub		1		1	5		20	6	34	9		48	111	61	56	74	3		11	1	175	89	3
Finescale Dace				2																			
Green Sunfish					1					1					2	5						1	
Hornyhead Chub										1		13	34	24	27	39							
Johnny Darter	1				2							18	3	6	1	6	1						
Longnose Dace	1										1	1	1			2							
Mottled Sculpin	41	78	39	12	68		7	3	16	8		11	27	29	18	37	11				4		2
Pearl Dace	10	13		3	3		42	1	68	5		5	15	3	10	8	62				9	1	
Pumpkinseed Sunfish																					1		
Rainbow Trout										1			1		2	4							
Redside Dace							1		7	1			3	7	1	1						1	
Southern Redbelly Dace												18	28	12	16	12					1		
White Sucker	1	1					13	9	9	6		44	34	31	21	62					4		
Yellow Perch															1								
Total	78	163	74	44	107	0	163	74	194	64	1	223	339	315	265	381	140	4	11	2	253	108	18

Table 7. Small Stream Fish IBI scores for all study years

Waterbody (WBIC)	Station ID	Station Name	2022 F-IBI	2017 F-IBI	2015 F-IBI	2011 F-IBI	2009 F-IBI	2008 F-IBI	2001 F-IBI
East Twin River (84000)	10008206	Townline Rd	NA	80	90	40	90	--	100
	10030630	Krok Rd	NA	100	100	50	100	--	--
	10020812	Krok Rd Bridge	--	--	90	--	--	--	--
	10008204	CTH J	--	100	100	100	100	--	100
Unnamed Tributary (3000211)	10029040	Sleepy Hollow Rd	NA	80	--	--	--	100	--
Unnamed Tributary (3000212)	10029041	Cherneyville Rd east crossing	NA	NA	--	--	--	NA	--

Score/Condition Category

91 -100 / Excellent
61 - 90 / Good
31 - 60 / Fair
0 - 30 / Poor
-- – No Data Collected
NA – Not enough fish to calculate IBI

Based on fish surveys from 2017, the modeled natural communities for each station were verified or adjusted. Results from that analysis are presented below (Table 8) and no changes to the natural community verification have been made since 2017 due to insufficient fish catch during the 2022 sampling period.

Table 8. Modeled and Verified Natural Community based on 2017 fish surveys.

Waterbody (WBIC)	Station ID	Station name	Modeled	Verified Natural Community
East Twin River (84000)	10008206	Townline Road	Cool-Cold Headwater	Cool-Cold Headwater
	10008204	CTH J	Warm Headwater	Cool-Warm Headwater
	10030630	Krok Road "Ditch"	Cool-Cold Headwater	Cool-Cold Headwater
	10020812	Krok Road Bridge	Cool-Warm Headwater	Cool-Cold Headwater
Unnamed (3000211)	10029040	Sleepy Hollow Road	Cool-Cold Headwater	Cool-Warm Headwater
Unnamed (3000212)	10029041	Cherneyville Road east crossing	Macroinver-tebrate	NA

Conclusion and Recommendations

This study assessed the condition of the three UNT's, the East Twin River and Krok Creek for ecological impairment. Assessment data from 2022 were compared to prior surveys to determine if water quality conditions have changed and if the streams are meeting water quality standards. Overall conclusions and recommendations include:

- The UNTs contribute significant phosphorus to the East Twin River. Efforts to reduce phosphorus loading to these streams should be a priority.
- The UNTs have overall higher concentrations of total phosphorus, chloride, total suspended solids, ammonia, total nitrogen, nitrate + nitrite, and biochemical oxygen demand than the East Twin River or Krok Creek.
- None of the stations monitored exceeded chloride toxicity standards. However, there are clearly elevated chloride levels in the UNTs relative to the East Twin River upstream of the confluence with the UNT, and the Krok Creek reference site. Long-term trend monitoring on the Kewaunee River indicates average chloride levels around 40 mg/L, consistent with the patterns shown at the Townline Rd and Krok Creek sampling sites. Chloride levels in UNTs continue to increase from 2011, with levels approximately six times the background chloride levels at reference sites, around 200-250 mg/L.
- The 2022 temperature data indicates an improvement in meeting water temperature criteria for the UNT 3000213 at Cherneyville Rd. The station had exceeded DFAL temperature criteria for all months monitored in 2011 and 2017 but was below the DFAL threshold in all sampling months in 2022. For all three years, average daily air temperatures fall within the expected range, based on historical variation, indicating that extreme weather did not influence stream temperature trends. This stream segment will be recommended for removal from the Unknown Pollutant/Elevated Temperature impaired waters list in 2024.
- Water temperature in the East Twin River at CTH J exceeded the cold water temperature criteria in June, July, and August and will be reviewed. However, this segment of stream is likely a natural transition zone from cold water to warmwater and may not reflect an impairment. Additional fish surveys should be conducted in the East Twin River upstream and downstream of CTH J to confirm the stream natural community in this cold to warm water transition zone.
- Sediment toxicity testing indicates that the two test organisms (larval midge and amphipod) are not impacted by sediment contamination below the wastewater discharge to the UNT.
- Soft sediment below the wastewater discharge has considerably higher concentrations of sodium, phosphorus and chlorides compared to the Krok Creek reference site. Additional monitoring for heavy metals should be conducted.
- High stream conductivity prevented adequate fish surveys in 2022. Additional surveys should be completed in the future to reassess the fish community in the East Twin River and both UNT's.

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Appendix A. Additional Water Quality Analyses

Ambient Toxicity Testing

Grab samples were collected during monthly sampling to be tested for ambient toxicity. Samples were sent to the State Lab of Hygiene and analyzed for acute and chronic toxicity using algae, zooplankton, and minnows. Results from 2011 and 2017 are summarized below for the three different test organisms.

Fathead minnow (Pimphales promelas) toxicity

No acute toxicity was observed at any of the sampling sites in 2011 or 2017. In 2011, chronic toxicity was reported at UNT 3000213 (Cherneyville Rd) in May and June. In 2017, no chronic toxicity was indicated by statistical differences at any site. However, some observational differences in growth and survival occurred among UNT 3000213 and the control. In June, survival was 15% lower than the control and in September, survival was 21% lower than the control. However, these differences are not statistically significant.

Water flea (Ceriodaphnia dubia) toxicity

Acute toxicity was reported in May 2011 at UNT 3000213 (Cherneyville Rd). No acute toxicity was reported at any site in 2017. Chronic toxicity was found in 2011 at UNT 3000213 and UNT 3000211 (Hrabik Rd) in May. Additionally, chronic toxicity was observed in July in UNT 3000211 at the stream mouth (Below Hwy 29), in the East Twin River below the confluence, and at the Townline Rd station. In 2017, chronic toxicity was reported at two sites: in the East Twin River below the confluence of UNT 3000211 in June and at UNT 3000213 in October.

Green algae (Selenastrum capricornutum) toxicity

Chronic toxicity was found in 2011 at UNT 3000213 and 3000211 in June and at the East Twin River below Hwy 29 in July. In 2017, green algae growth was much lower in UNT 3000213 in July and August compared to the control, but only the July sample was significantly different from the control to indicate chronic toxicity.

Macroinvertebrate Evaluation

Macroinvertebrate samples were collected in October by kick-net sampling using a D-frame net. Collections were made in 2008, 2011, and 2017. Samples were preserved in ethanol and sent to the Aquatic Biomonitoring Laboratory at the University of Wisconsin-Stevens Point for identification. Macroinvertebrate Indices of Biological Integrity (M-IBI) were calculated for each sample. M-IBI scores range from 0 (poor) to 10 (excellent) and provide a measure of overall stream condition in relation to the macroinvertebrate life found in the stream. Comparative M-IBI scores are shown below.

The 2017 M-IBI scores were either the same as 2011 or better. The East Twin River at CTH F went from poor to good and Krok Road went from fair to good. Krok Creek was evaluated only in 2017 and was rated as good. UNT 3000212 at Cherneyville Road improved in 2017 by receiving a fair score, compared with poor M-IBI scores in 2008 and 2011. UNT 3000211 at both stations (Sleepy Hollow Rd and Hrabik Rd) had a fair M-IBI score in all years sampled.

Table A1. Macroinvertebrate Index of Biological Integrity (M-IBI) values and condition categories

Waterbody Name (WBIC)	Station ID	Station Name	2017 M-IBI Condition	2011 M-IBI Condition	2008 M-IBI Condition
East Twin River (84000)	104445	Below Hwy 29	4.6	4.5	--
	10008206	Townline Road	3.8	4.3	--
	10008204	CTH J	6.9	3.3	--
	10034445	CTH F, Ellisville	7.1	1.6	--
	10030630	Krok Road	6.2	2.9	--
Krok Creek (86700)	10008203	Above Hwy 29	5.0	--	--

Unnamed Tributary (3000211)	10030304	Hrabik Road	3.3	3.9	--
	10029040	Sleepy Hollow Road	3.2	4.0	4.0
Unnamed Tributary (3000212)	10029041	Cherneyville Road east crossing	2.7	0	0

Score/Condition Category

7.5 – 10 / Excellent
5.0 – 7.49 / Good
2.51 – 4.99 / Fair
0 – 2.5 / Poor
-- – No Data Collected

Habitat Assessments

Habitat for aquatic life was evaluated in 2017 and 2011, and in 2008 at Cherneyville Road only using the Wadable Stream Qualitative Fish Habitat Rating protocol. Variables measured include the abundance of pools, depth and width, substrate characteristics, streambank erosion and riparian buffer width, available cover for fish. Scores range excellent (75) to poor (<25) and scores are compared below in Table A1. Overall, habitat ranged from good to poor in 2017 and were similar or slightly lower than in 2011.

Table A2. Habitat Scores and Conditions

Waterbody Name (WBIC)	Station ID	Station Name	2017 Habitat Condition Score	2011 Habitat Condition Score	2008 Habitat Condition Score
East Twin River (84000)	104445	Below Hwy 29	67	77	--
	10008206	Townline Rd	62	80	--
	10008204	CTH J	62	52	--
	10034445	CTH F Ellisville	18	72	--
	10030630	Krok Road "Ditch"	62	58	--
	10020812	Krok Road Bridge	50	67	--
	10020787	Church Road	43	67	--
Krok Creek (86700)	10008203	Above Hwy 29	67	--	--
Unnamed Tributary (3000213)	10033637	Cherneyville Rd west crossing	45	60	--
Unnamed Tributary (3000211)	10030304	Hrabik Rd	73	82	--
	10033921	Stream mouth below Hwy 29	55	68	--
	10029040	Sleepy Hollow Rd	50	80	45

Unnamed Tributary (3000212)	10029041	Cherneyville Rd east crossing	25	--	35

Score/Condition Category

>75 / Excellent
50-74 / Good
25-49 / Fair
<25 / Poor
-- – No Data Collected

Appendix B. Sediment Toxicity Test Results

Chironomus dilutus – Survival

Percent Survival per Replicate

Field ID	1	2	3	4	5	6	7	8	Mean	Std Error
Lab Control	100	100	90	100	100	100	90	50	91.3	6.5
UNT	70	90	100	90	100	100	100	90	92.5	3.9
Krok	80	90	90	100	80	80	100	90	88.8	3.2

Chironomus dilutus – Growth (Ash Free Dry Weight)

mg per surviving *C. dilutus*

Field ID	1	2	3	4	5	6	7	8	Mean	Std Error
Lab Control	1.63	2.06	0.99	0.95	1.38	1.58	1.09	2.02	1.46	0.17
UNT	1.95	2.33	1.19	1.95	1.75	1.92	1.42	1.64	1.77	0.13
Krok	2.24	1.72	1.21	1.41	1.92	2.48	1.09	1.87	1.74	0.18

Hyalella azteca – Survival

Percent Survival per Replicate

Field ID	1	2	3	4	5	6	7	8	Mean	Std Error
Lab Control	80	100	80	50	70	80	80	80	77.5	5.2
UNT	90	90	80	100	100	90	100	100	93.8	2.8
Krok	90	100	80	90	100	100	100	100	95.0	2.9

Hyalella azteca – Growth (Ash Free Dry Weight)

mg per surviving *H. azteca*

Field ID	1	2	3	4	5	6	7	8	Mean	Std Error
Lab Control	0.16	0.12	0.13	0.35	0.09	0.07	0.13	0.15	0.15	0.03
UNT	0.20	0.17	0.22	0.10	0.12	0.12	0.27	0.31	0.19	0.03
Krok	0.18	0.23	0.25	0.09	0.12	0.20	0.20	0.27	0.19	0.02