

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



East Twin River at Krok Road "Ditch" (Station ID 10030630)

## **Introduction**

The purpose for this study was to determine the contemporary status and potential recent changes in water quality, habitat, and the biological communities in the upper reaches of the East Twin River watershed. The streams included in water quality surveys 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).

Previous studies have been conducted in 2001, 2008, 2009, 2011/2012, and 2015 to evaluate conditions of the same streams in the Upper East Twin River watershed. Water chemistry samples and temperature data were only collected in 2011/2012 and 2017. Conclusions from these surveys indicated these streams have impairments from total phosphorus but the physical habitat and biological communities were meeting the designated uses. Assessment data from 2017 were compared to previous years to determine if water resource conditions have changed. An industrial manufacturing facility discharges treated wastewater to the UNT 3000213 to the East Twin River above Cherneyville Road in Kewaunee County. This tributary flows into the East Twin River just downstream of Hwy 29.

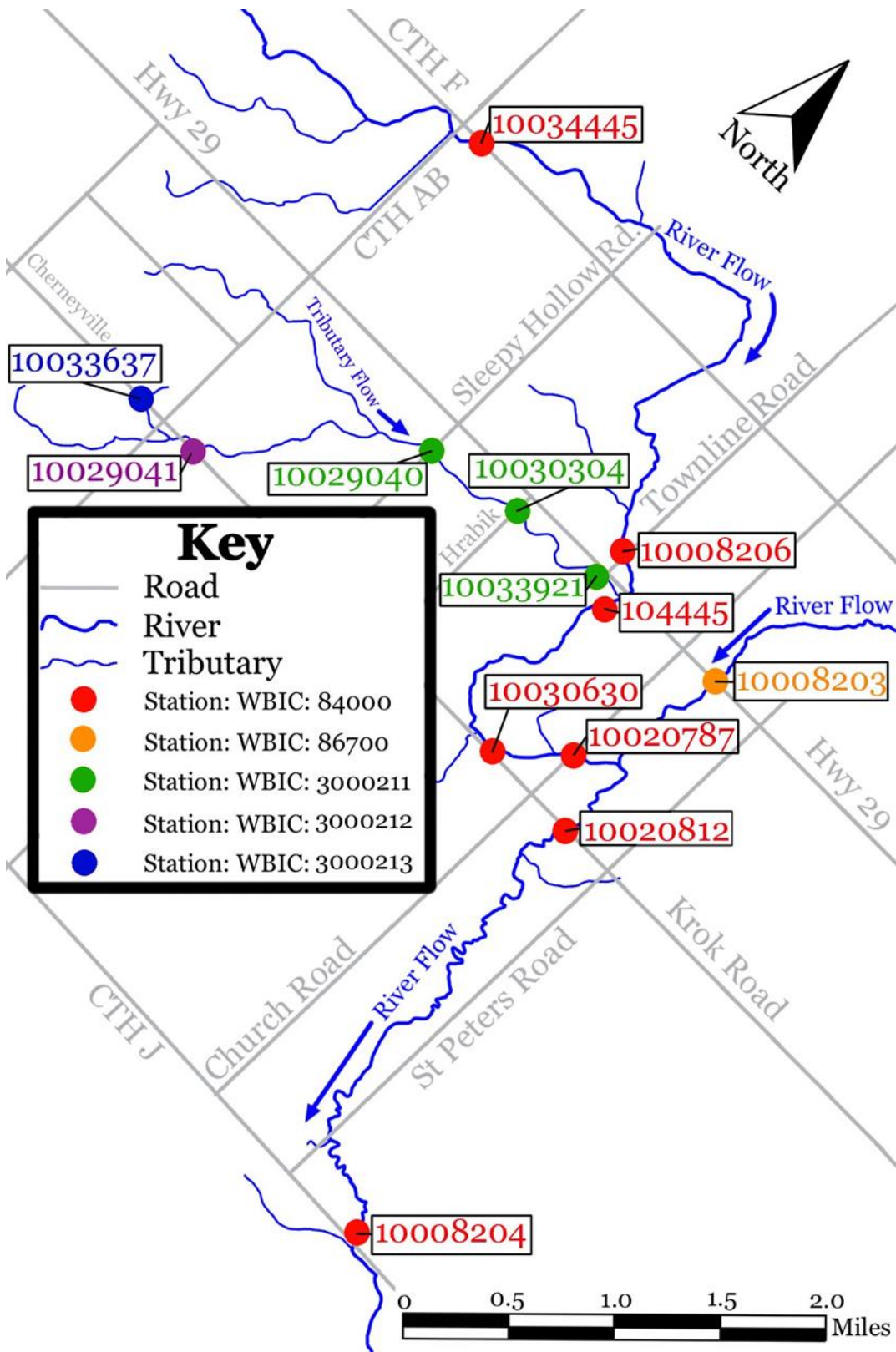


Figure 1. Map of upper East Twin River watershed along with sampling station IDs.

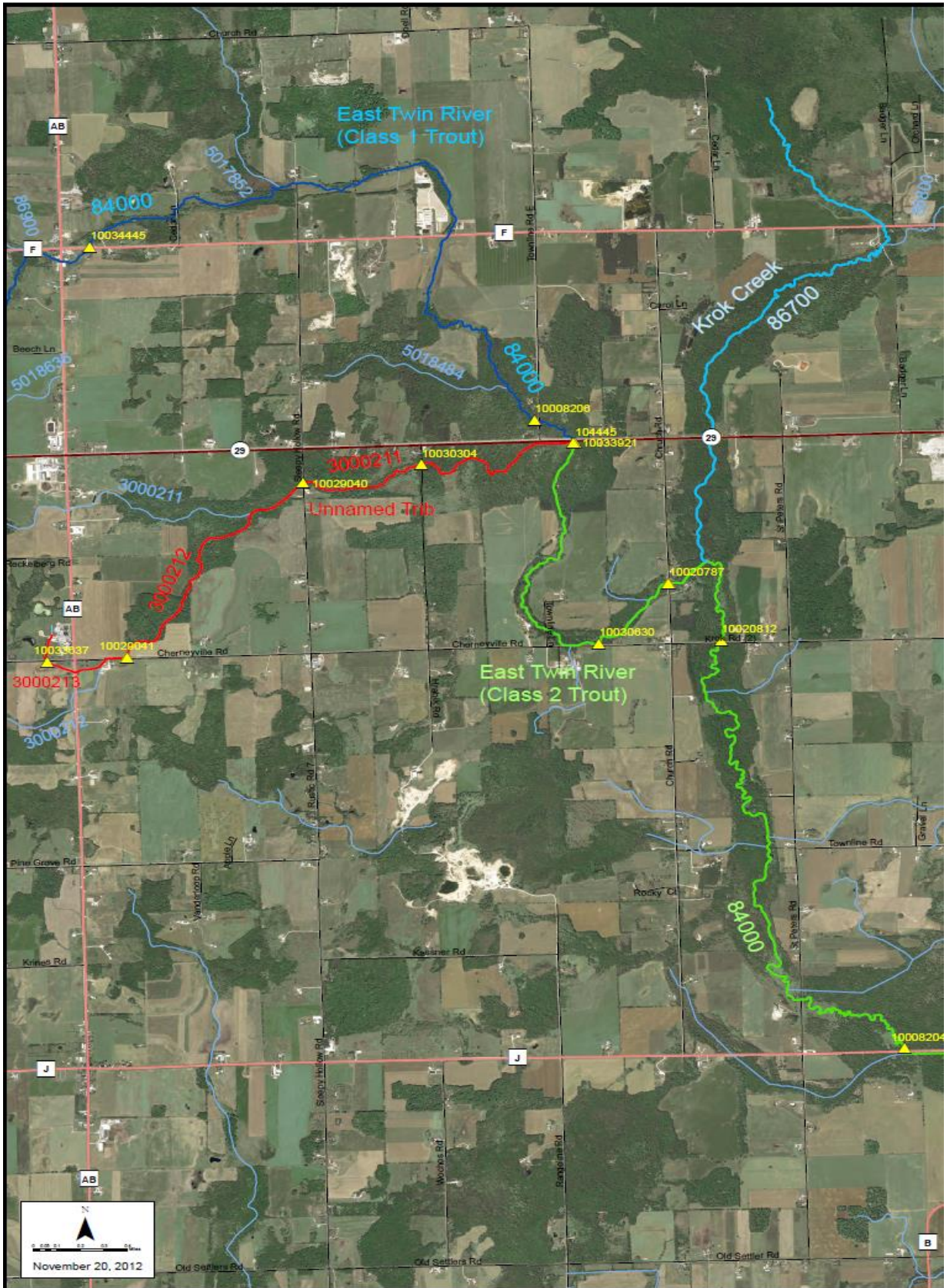


Figure 2. Aerial Map of upper East Twin River watershed.

Along with existing data, current data collected in 2017 were assessed against the *Wisconsin 2018 Consolidated Assessment and Listing Methodology* (WisCALM, 2017) guidance to determine if the streams are meeting water quality standards. The streams surveyed during this project are currently assessed against standards relating to the assigned fish and aquatic life use designations as shown in Table 1. Fish and Aquatic Life use categories evaluated with this project include Cold Water Community (COLD) and Default Fish and Aquatic Life (DFAL). Currently in WisCALM, streams and rivers are being evaluated for placement in a revised aquatic life use classification system, in which the new fish and aquatic life use subclasses are referred to as *Natural Communities*. Natural community classification is important when evaluating the Fish Index of Biotic Integrity (F-IBI). Natural communities in the Upper East Twin River study area include: macroinvertebrate, cool-cold headwater, and cool-warm headwater Streams.

**Table 1. Classification of stream communities by river reach in the study watershed.**

| <b>Location</b>   | <b>Classification (according to WI Admin Code NR 102)</b>            |
|---|--|
| 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 DFAL uses are specified in NR 102 and WisCALM as follows:

- Dissolved oxygen: cold water <6 mg/l, DFAL <5 mg/l.
- Water temperature: cold water >73°F, DFAL >86°F.
- Chloride: acute toxicity >757 mg/l, chronic toxicity >395 mg/l.
- Total phosphorus: >0.075 mg/l.

The headwaters of the East Twin River down to CTH B are currently on the Clean Water Act Section 303(d) list of impaired waters for phosphorus. The UNTs 3000212 and 3000213 in their entirety are also on the impaired waters list for phosphorus. UNT 3000211 is on the proposed list for phosphorus waiting EPA approval. UNT 3000213 is on the impaired waters list for water temperature.

## **Methods**

Monitoring was conducted at several locations along the upper reaches of the East Twin River, the UNTs (3000211, 3000212, 3000213), and Krok Creek (Figure 1 and Table 2) following DNR Field Procedures Manuals and guidelines for proper collection and preservation techniques.

**Table 2. Monitoring locations and parameters measured during study**

| Waterbody (WBIC)        | Station ID | Location                        | Parameter       |                  |            |                     |         |      |
|-------------------------|------------|---------------------------------|-----------------|------------------|------------|---------------------|---------|------|
|                         |            |                                 | Water Chemistry | Aquatic Toxicity | Water Temp | Macro invertebrates | Habitat | Fish |
| East Twin River (84000) | 104445     | Below Hwy 29                    | X               | X                |            | X                   | X       |      |
|                         | 10008206   | Townline Road                   | X               | X                | X          | X                   | X       | X    |
|                         | 10008204   | CTH J                           | X               |                  | X          | X                   | X       | X    |
|                         | 10034445   | CTH F, Ellisville               |                 |                  |            | X                   | X       |      |
|                         | 10030630   | Krok Road "Ditch"               | X               |                  | X          | X                   | X       | X    |
|                         | 10020812   | Krok Road Bridge                |                 |                  |            |                     | X       |      |
|                         | 10020787   | Church Road                     |                 |                  |            |                     | X       |      |
| Krok Creek (86700)      | 10008203   | Hwy 29                          | X               |                  | X          | X                   | X       |      |
| Unnamed (3000213)       | 10033637   | Cherneyville Road west crossing | X               | X                | X          |                     | X       |      |
| Unnamed (3000211)       | 10030304   | Hrabik Road                     | X               | X                | X          | X                   | X       |      |
|                         | 10033921   | Stream mouth off Hwy 29         | X               | X                |            |                     | X       |      |
|                         | 10029040   | Sleepy Hollow Road              |                 |                  |            | X                   | X       | X    |
| Unnamed (3000212)       | 10029041   | Cherneyville Road east crossing |                 |                  |            | X                   | X       | X    |

**Note: X means that sampling occurred at the specific station for the indicated parameter**

The objective of choosing these sampling locations was to quantify stream health in the overall watershed and to measure any potential impact to stream health. UNT 3000213 flows into UNT 3000212 then 3000211 and ultimately the East Twin River. Several monitoring stations were selected on the UNTs with the furthest downstream site on UNT 3000211 at its confluence with the East Twin River near Hwy 29. Two stations on the East Twin River (Station IDs 10008206 and 10034445) were selected upstream of this confluence to monitor background reference conditions. In addition, a monitoring site on Krok Creek (Station ID 10008203) provided an additional reference station in a nearby tributary of the East Twin River. Monitoring station ID 104445 on the East Twin River was established downstream of the outlet of UNT 3000211 to detect any possible impacts from the tributary. Finally, a monitoring station about 3.7 miles downstream of the UNT outlet was monitored (Station ID 10008204) quantifying any potential widespread impact of this tributary on the East Twin River.

### **Water Chemistry Sampling**

Grab samples were collected monthly May through October on the East Twin River, UNTs 3000211 and 3000213 and Krok Creek (Table 2, Figure 1). Water sampling dates were selected in the spring for all sampling events to eliminate bias. The samples were shipped to the Wisconsin State Laboratory of Hygiene for analysis for total phosphorus, chloride, and ammonia concentrations. UNT 3000213 was also sampled monthly for chromium (Station ID 10033637).

- [Guidelines and Procedures for Surface Water Grab Sampling \(Dec. 2005 Version 3\)](#)

### **Continuous Temperature**

Water temperature data loggers (HOBO brand) set to record hourly were deployed in the East Twin River, UNTs 3000213 and 3000211, and Krok Creek from May to October 2017 (Table 2, Figure 1).

- [Guidelines and Standard Procedures for Continuous Temperature Monitoring Wisconsin DNR May 2004 \(Version 1\)](#)

### **Ambient toxicity testing**

Grab samples were collected monthly May through October on the East Twin River and UNT (3000211 and 3000213) (Table 2, Figure 1). Water sampling dates were selected in the spring for all sampling events to eliminate bias. The samples were shipped to the Wisconsin State Laboratory of Hygiene to be tested for acute and chronic toxicity using zooplankton, minnows and algae. Specific methods are provided in Appendix A.

- [Aquatic Life Toxicity Testing Methods Manual 2nd Edition 2004](#)

### **Macroinvertebrate Evaluation**

Macroinvertebrate samples were collected in October and obtained by kick-net sampling using a D-frame net. Sampling locations included the East Twin River, UNTs 3000211 and 3000212, and Krok Creek (Table 2, Figure 1). Samples were preserved and sent to University of Wisconsin-Stevens Point Aquatic Biomonitoring Laboratory for identification. Macroinvertebrate Indices of Biological Integrity (M-IBI) were calculated for each sample collected. M-IBI uses several metrics to assess overall stream condition with scores from 0 (poor) to 10 (excellent).

- [Guidelines for Collecting Macroinvertebrate Samples in Wadeable Streams](#)

### **Habitat Assessments**

Aquatic life habitat was evaluated at several locations along the East Twin River, all three UNTs (3000211, 3000212, 3000213) and Krok Creek using the Wadeable Stream Qualitative Fish Habitat Rating protocol. These assessments were completed at all 13 monitoring stations (Table 2, Figure 1). The variables measured included depth and width parameters, bottom characteristics, riffle and bend ratio, abundance of pools, fish cover, streambank erosion and riparian buffer width. Habitat scores can range from 100 (excellent) to 0 (poor).

- [Guidelines for Qualitative Physical Habitat Evaluation of Wadeable Streams](#)
- [Qualitative Habitat Rating less than 10m Form \(3600-532A\) \(R 6/07\)](#)

## Fish Assemblage

Fish surveys were completed in July on the East Twin River and two UNTs (Table 2, Figure 1). Selection of locations was based on past survey locations. A backpack stream shocker was used at four of the sites and a barge stream shocker was used at one site to capture all fish species present. Fish were identified and the number of each species present was tallied. The Index of Biotic Integrity (F-IBI) based on the fish community at each sampling location was calculated. Similar to the M-IBI, the F-IBI relates community structure to community health and water quality. F-IBI scores can range from 100 (excellent) to 0 (poor).

- [Wadeable Stream Fish Community Evaluation Form 3600-230 \(R 7/00\)](#)
- [Guidelines for Assessing Fish Communities of Wadeable Streams in Wisconsin](#)

## Results

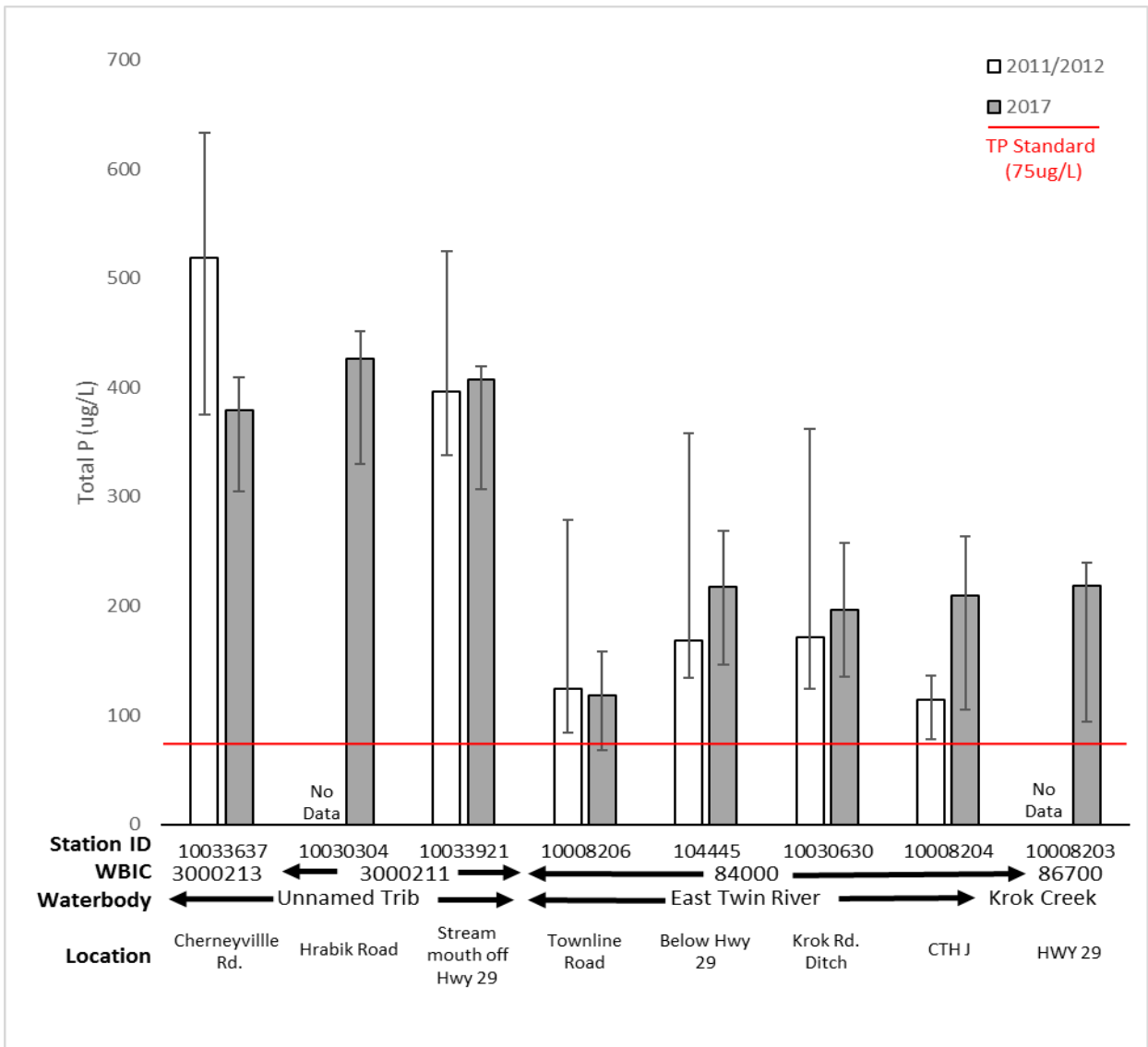
### Water Chemistry

#### ***Total Phosphorus***

As specified in NR 102 of the Wisconsin Administrative Code, the total phosphorus criterion of 0.075 mg/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, 2017) lists waters where the median concentration and 90% confidence interval values exceeds 0.075 mg/l on wadable streams.

As shown in Figure 3, the median and 90% confidence interval values for total phosphorus at all monitoring locations in 2011 clearly exceed the 0.075 mg/l impairment threshold. In 2017, all stations exceeded the criteria except for the East Twin River at Townline Road (Station ID 10008206), where the lower 90% confidence interval was just below 0.075 mg/l.

The East Twin River is currently listed as impaired from its headwaters down to CTH B for total phosphorus. Krok Creek and the UNTs 3000212 and 3000213 are also listed as impaired for phosphorus. UNT 3000211 is proposed for listing.



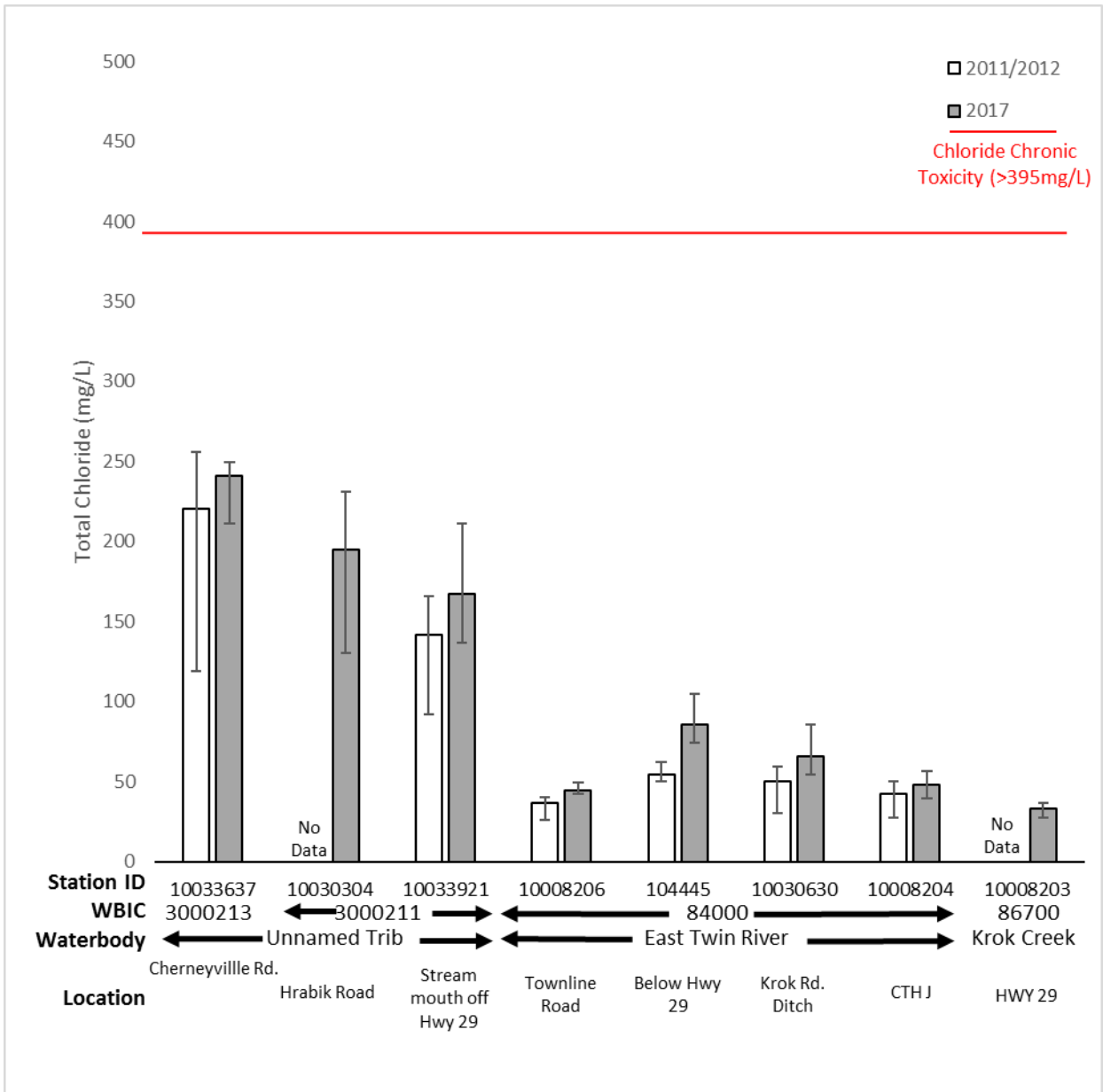
**Figure 3. Total phosphorus data from all stations from sampling taken in 2011/2012 and 2017. Bars indicate median values during each field season, and error bars indicate the 90% confidence interval.**

### ***Chloride***

The protocol for impairment decisions established in 2018 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 it is 757 mg/l.

As shown in Figure 4, none of the chloride samples collected on the UNTs 3000213 and 3000211, the East Twin River or Krok Creek exceeded established thresholds for chronic toxicity in 2011/2012 or 2017. Although they did not exceed any thresholds, it is apparent that higher concentrations of chloride are observed in the UNTs compared to the reference streams.

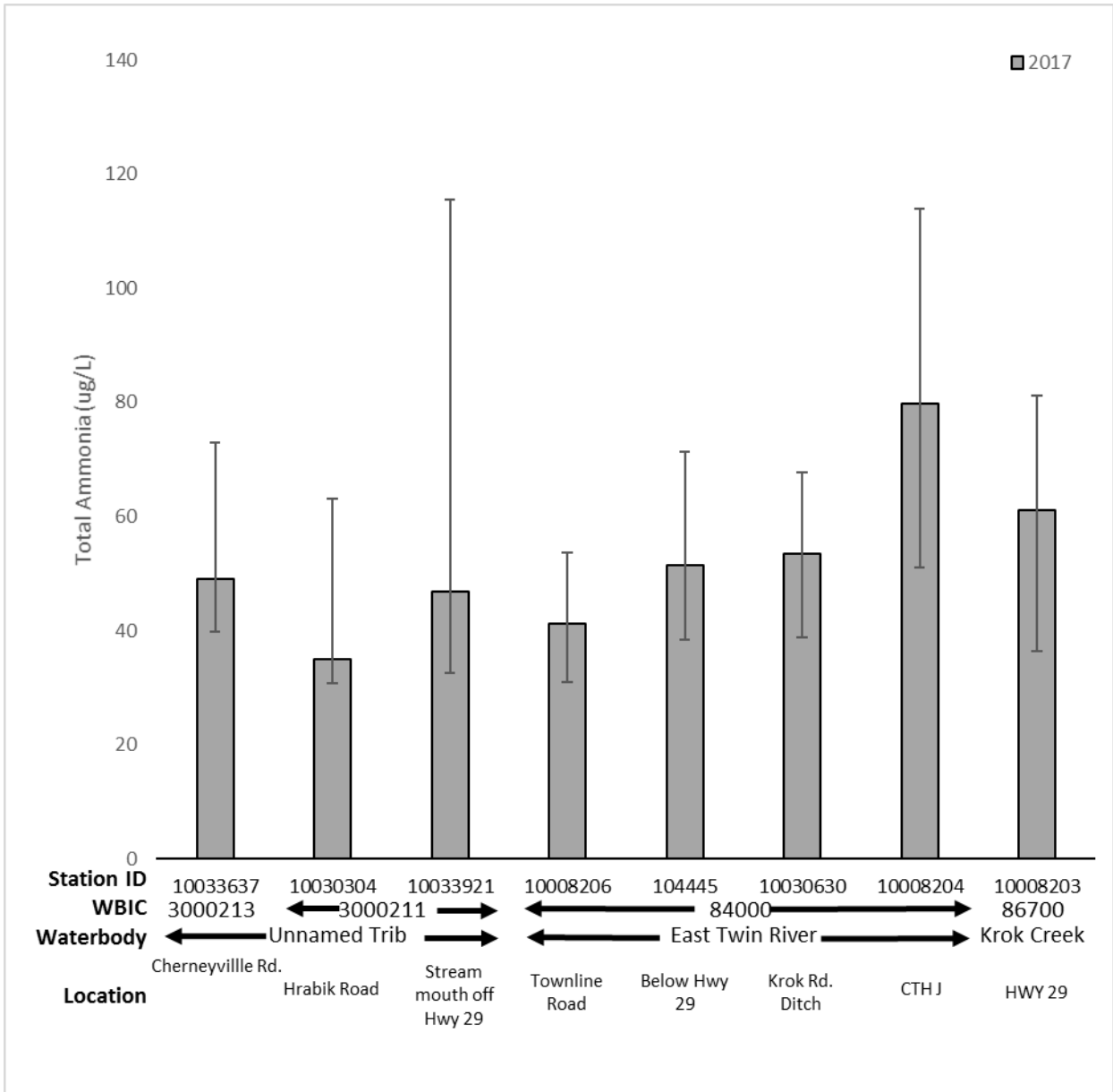




**Figure 4. Chloride from all stations from sampling taken in 2011/2012 and 2017. Bars indicate median values during each field season, and error bars indicate the 90% confidence interval.**

***Ammonia***

Figure 5 displays total ammonia results from 2017. Six monthly samples were collected at the same locations and times as the other water samples. Ammonia was not collected in 2011 and 2012. The data suggest none of the ammonia sample results were high enough to cause ammonia toxicity to aquatic life according to the methods of the Environmental Protection Agency (2013) and Wisconsin Administrative Code NR105.



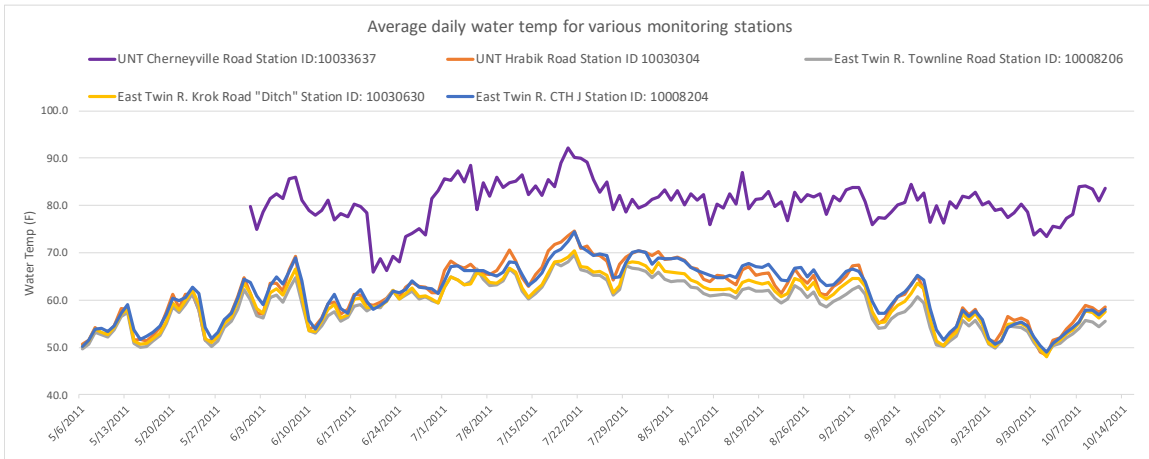
**Figure 5. Total ammonia from all stations in 2017. Bars indicate median values during field season, and error bars indicate the 90% confidence interval.**

***Chromium***

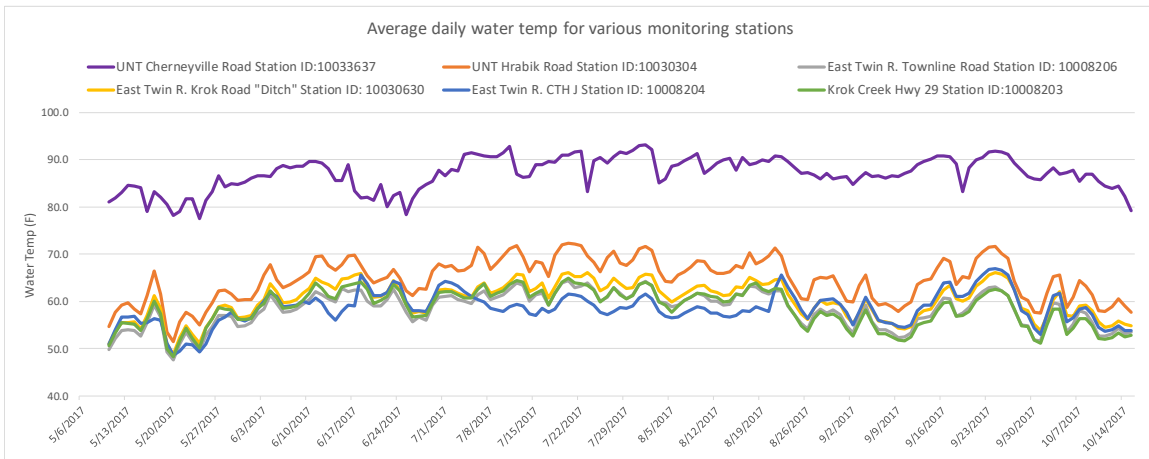
Six monthly water samples were collected at one location on UNT 3000213 (Station ID 10033637). Three sampling events did not detect chromium but the other three samples document levels slightly above the detection limit of 1.0 ug/l (1.48, 1.32, and 1.7 ug/l). As specified in Wisconsin Administrative Code NR 105, the chronic criterion for chromium is 326 ug/l. Thus, the low levels detected would not be considered toxic to aquatic life.

## Continuous Water Temperature

Water temperatures were logged hourly at three stations on the East Twin River and one each on UNTs 3000211 and 3000213 in 2011. The same stations plus the addition of one on Krok Creek were monitored in 2017. Average daily temperature throughout each field season by Station ID are summarized in Figure 6 for 2011 data and in Figure 7 for 2017 data.



**Figure 6. Average daily temperature values at all stations sampled during the 2011.**

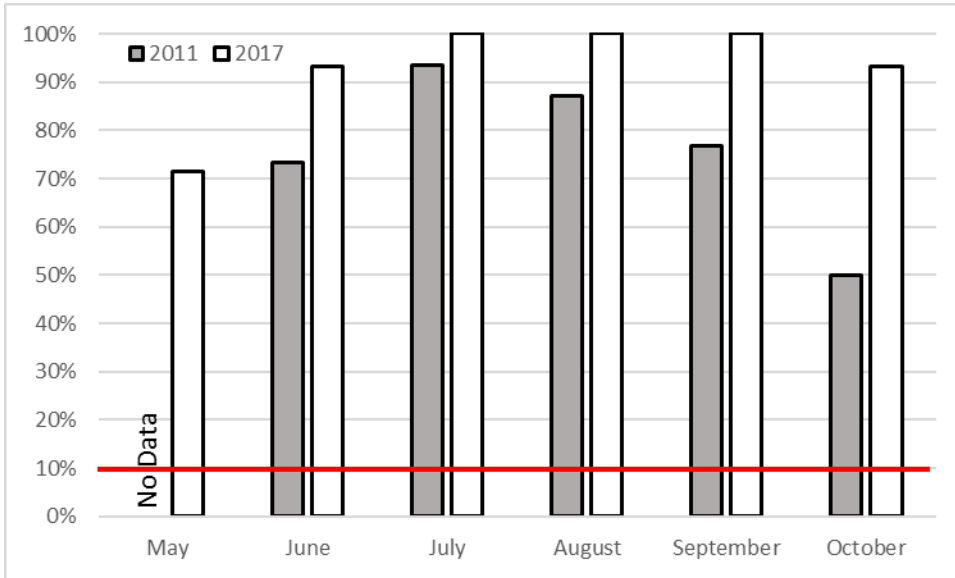


**Figure 7. Average daily temperature values at all stations sampled during the 2017.**

Water temperature of the East Twin River met the cold water quality criteria (maximum summer temperature of 73°F) at all stations except for the furthest downstream station at CTH J (Station ID 10008204). The East Twin River at CTH J exceeded water quality criteria in July and August of 2011, however in 2017, water quality criteria were met in all months.

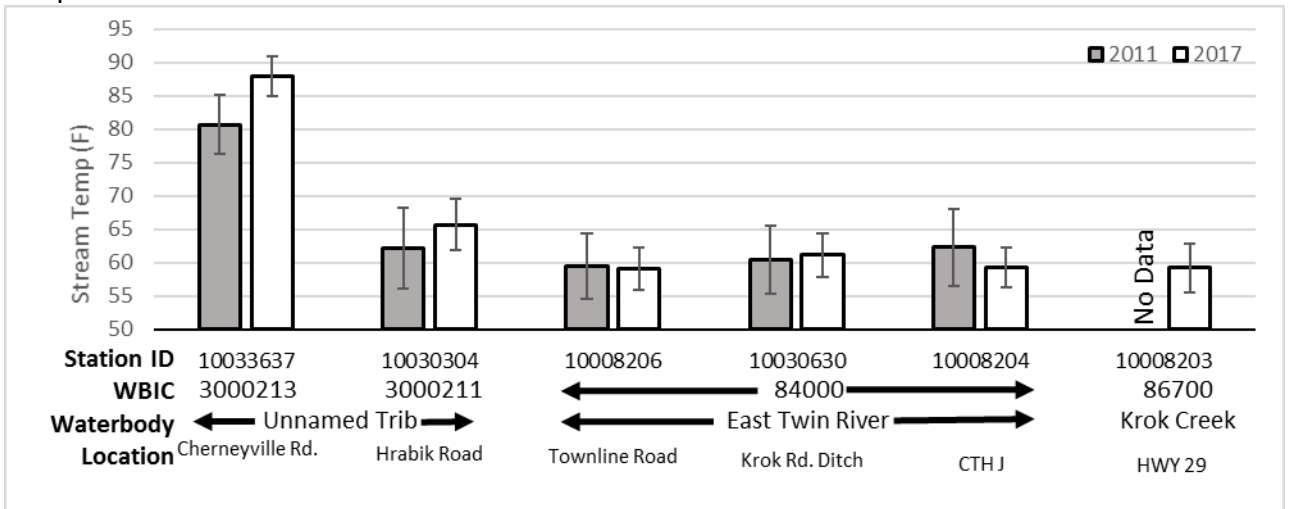
Water temperature in UNT 3000213 at the Cherneyville Road (Station ID 10033637) exceeded the DFAL temperature criteria (maximum summer temperature of 86°F) for all

months monitored in both 2011 and 2017 as shown in Figure 8. Water temperature approximately 2 miles downstream in UNT 3000211 at Hrabik Road (Station ID 10030304) met DFAL temperature criteria for all months monitored.



**Figure 8: Percentage of exceedance values of the Wisconsin Admin. Code for acute temperature criteria for UNT at Cherneyville Road (Station ID 10033637). All values were greater than 10% of daily maximum values, indicating an exceedance of acute criteria for every month of the record.**

Water temperatures were warmer at UNT 3000213 and 3000211 in 2017 than 2011 (Figure 9). Otherwise, temperatures collected in 2017 at all other sites were similar to temperatures in 2011.



**Figure 9: Average of all average daily temperature values for each field station monitored in 2011 and 2017 between June 1 and October 15<sup>th</sup> of each calendar year. Error bars indicate standard deviation of all averaged daily values.**

## Ambient Toxicity Testing

### ***Fathead Minnow (*Pimephales promelas*) Toxicity:***

#### **Acute Toxicity:**

**2011:** No acute toxicity observed at any sampling sites.

**2017:** No acute toxicity observed at any sampling sites.

#### **Chronic Toxicity:**

**2011:** Chronic toxicity was found in UNT 3000213 Cherneyville Road (Station ID 10033637) in May and June of that year.

**2017:** No chronic toxicity as indicated by statistical differences between fish growth in surface water samples and controls, was found at any of the sites. However, on two occasions survival and growth was noticeably lower in the surface water than in control treatments in the chronic fathead minnow toxicity test. In June, survival in the sample collected in the UNT 3000213 (Station ID 10033637) was 15% lower than the control. In September, survival at the same site was 21% lower than the control. Growth was also lower than at any of the other sites on both of these dates, although these differences were not found to be statistically significant.

### ***Water Flea (*Ceriodaphnia dubia*) Toxicity:***

#### **Acute Toxicity:**

**2011:** Acute toxicity was observed in May at UNT 3000213, Cherneyville Road (Station ID 10033637).

**2017:** No acute toxicity observed at any sampling sites.

#### **Chronic Toxicity:**

**2011:** Chronic toxicity was found in UNT 3000213, Cherneyville Road (Station ID 10033637) and in UNT 3000211, Hrabik Road (Station ID 10030304) in May. Chronic toxicity was also found in UNT 3000211 at the stream mouth (Station ID 1033921), and in the East Twin River below this confluence (Station ID 104445), and at the Townline Road site (Station ID 10008206) in July.

**2017:** Chronic toxicity was found in two samples collected during the field season. Reproduction was found to be significantly lower in June at the East Twin River below the confluence of UNT 3000211 (Station ID 104445) and at the UNT 3000213 (Station ID 10033637) in October. The October sample showed both significantly lower reproduction and lowered survival (20% lower than the control).

### ***Green Algae (*Selenastrum capricornutum*) Toxicity:***

#### **Chronic Toxicity:**

**2011:** Chronic toxicity was found at UNT 3000213 (Station ID 10033637) and at the UNT 3000211 at Hrabik Road (Station ID 10030304) in June. Chronic toxicity was also found at the East Twin River below UNT 3000211 at Station ID 104445 in July, however, the reduction in growth of this sample was below the level of concern normally used for algae tests (50% of the control).

**2017:** UNT 3000213 (Station ID 10033637) showed noticeably lower green algae growth in the July and August samples, although only the July sample was found to be statistically different from the control.

Tables associated with toxicity measurements can be found in Appendix B.

### Macroinvertebrates

Macroinvertebrate samples were collected at multiple sites in the project area to calculate a Macroinvertebrate Index of Biological Integrity (M-IBI). The M-IBI is composed of various metrics used to interpret macroinvertebrate data. 2017 M-IBI categorical results for the East Twin River and UNT sites were either the same as 2011 or better as shown in Table 3. The East Twin River at CTH F (Station ID 10034445) went from poor in 2011 to good in 2017. At Krok Road “Ditch” (Station ID 10030630) M-IBI went from fair to good. The other three sites remained the same as either fair or good. Krok Creek (Station ID 10008203) was also assessed in 2017 and received a good M-IBI rating.

UNT 3000212 Cherneyville Road (Station ID 10029041) received poor M-IBI scores in 2008 and 2011 but a fair score in 2017. UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) and Hrabik Road (Station ID 10030304) received a fair M-IBI scores all years assessed.

**Table 3. Macroinvertebrate Indices of Biological Integrity values and condition**

| Waterbody Name (WBIC)   | Station ID | Station Name                    | 2017 M-IBI <sup>1</sup> 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 “Ditch”               | 6.2                               | 2.9                  | --                   |
| Krok Creek (86700)      | 10008203   | Hwy 29                          | 5.0                               | --                   | --                   |
| Unnamed (3000211)       | 10030304   | Hrabik Road                     | 3.3                               | 3.9                  | --                   |
|                         | 10029040   | Sleepy Hollow Road              | 3.2                               | 4.0                  | 4.0                  |
| Unnamed (3000212)       | 10029041   | Cherneyville Road east crossing | 2.7                               | 0                    | 0                    |

<sup>1</sup> M-IBI (Macroinvertebrate Indices of Biological Integrity)

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

Aquatic habitat was evaluated at monitoring stations in the project area as shown in Table 4. Habitat values ranged from poor to good in 2017. In general habitat values in the East Twin River and UNTs were similar or slightly lower in 2017 compared to 2011. The East Twin River at CTH F went from a good rating in 2011 to a poor rating in 2017. Habitat assessments were completed at only two locations on the UNT in 2008 and both received a fair rating.

**Table 4. Habitat Scores and Condition**

| 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 Road                   | 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   | Hwy 29                          | 67                           | --                           | --                           |
| Unnamed (3000213)       | 10033637   | Cherneyville Road west crossing | 45                           | 60                           | --                           |
| Unnamed (3000211)       | 10030304   | Hrabik Road                     | 73                           | 82                           | --                           |
|                         | 10033921   | Stream mouth off Hwy 29         | 55                           | 68                           | --                           |
|                         | 10029040   | Sleepy Hollow Road              | 50                           | 80                           | 45                           |
| Unnamed (3000212)       | 10029041   | Cherneyville Road east crossing | 25                           | --                           | 35                           |

Habitat Score (Based on Simonson et al. (1994))

Score/Condition Category

>75 / Excellent

50-74 / Good

25-49 / Fair

<25 / Poor

-- -- No Data Collected

## Fisheries Assessments

Fish surveys were completed at multiple sites in the project area (Table 5). The Fish Index of Biological Integrity (F-IBI) was calculated and the scores over multiple years are presented in Table 6. In 2017, fish surveys were completed at three locations on the East Twin River and two on the UNTs 3000211 and 3000212.

**Table 5. Fish Inventory of the East Twin and Unnamed Tributaries**

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

Previous fisheries and habitat reports for the East Twin River are available on the WDNR website: <http://dnr.wi.gov/topic/Fishing/reports/index.html>

F-IBI scores rated the East Twin River at Townline Road (Station ID 10008206) as good in 2017. In previous years, scores were fair to excellent. In 2017 F-IBI score in the East Twin River at Krok Road "Ditch" (Station ID 10030630) was excellent. It also received excellent to fair scores in previous surveys. Fish survey was conducted in the East Twin River at Krok Road bridge (Station ID 10020812) received a good rating in 2015. F-IBI scores were excellent all years surveyed at CTH J (Station ID 10008204).

F-IBI scores rated the UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) as good in 2017 and excellent in 2008. Not enough fish were captured at the Cherneyville Road east crossing (Station ID 10029041) to calculate an F-IBI score in 2008 or 2017.



**Table 6: Small Stream Fish IBI Scores for all years of study**

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

<sup>1</sup> F-IBI (Fish Indices of Biological Integrity)

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 all fish survey data available, the modeled and verified natural community (Table 7) of the East Twin River was a cool-cold headwater at both Townline Road (Station ID 10008206) and Krok Road "Ditch" (Station ID 10030630). The modeled natural community at CTH J (Station ID 10008204) was warm headwater but verified as a cool-warm headwater based on the fish assemblage. The modeled natural community at Krok Road bridge (Station ID 10020812) was cool-warm headwater but verified as cool-cold headwater. UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) was modeled as a cool-cold headwater but was verified as a cool-warm headwater based on the fish community. UNT 3000212 at Cherneyville Road (Station ID 10029041) was modeled as a macroinvertebrate stream but due to lack of fish no verification was completed.

**Table 7. Fish Modeled and Verified Natural Community**

| <b>Waterbody (WBIC)</b> | <b>Station ID</b> | <b>Station name</b>             | <b>Modeled</b>      | <b>Verified Natural Community</b> |
|-------------------------|-------------------|---------------------------------|---------------------|-----------------------------------|
| 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 | Macroinvertebrate   | NA                                |

## **Discussion**

This study assessed the condition of the East Twin River, its unnamed tributaries and Krok Creek for ecological impairment. The overall health of the streams were assessed for chemical, physical and biological conditions. Assessment data from 2017 were compared to prior survey data to determine if water quality conditions have changed and if streams are meeting water quality standards.

Sampling data confirm that the East Twin River, Krok Creek and the UNTs 3000213 and 3000212 clearly exceed water quality standards for phosphorus and should remain on the impaired waters list. The results also support that the UNT 3000211 currently on the proposed impaired waters list is not meeting standards and should be listed.

Chloride, ammonia and chromium concentrations all met water quality standards. Based off the data collected, these water quality constituents do not pose a threat to aquatic life in the streams assessed.

Despite non-point source influences throughout the entire watershed, the phosphorus and chloride concentrations were the highest in UNT 3000213.

Water temperature of the East Twin River at all stations except at CTH J met cold water quality criteria. East Twin River at CTH J (Station ID 10008204) exceeded water quality criteria in July and August of 2011, however in 2017, the water quality criteria were met in all months. During July and August of 2011 when cold water criteria for temperature was not met, the daily maximum temperature was exceeded 12 and 8 days respectively

by 0.7-3.3°C. Taking into consideration the accuracy of +/- 0.53°C of the continuous data collection meters, it can only be assumed that 9 and 2 days respectively did not meet the cold criteria for temperature. This leaves only the month of July in 2011 that did not fully meet cold water criteria for temperature on the East Twin River at CTH J. This single month of failing to meet cold water criteria could be sufficient to propose this segment of the East Twin River to be listed as impaired for temperature. However, this is not recommended. Based on knowledge of this stream and its location at the lower reaches of the East Twin River Class II trout waters, it is likely this segment is naturally on the transition between a cold and warm classification.

Water monitoring data confirm that UNT 3000213 clearly exceed water quality standards for temperature and should remain on the impaired waters list. Temperatures at Cherneyville Road (Station ID 10033637) exceeded the DFAL temperature criteria for all months monitored in both 2011 and 2017. Water temperature approximately 2 miles downstream in UNT 3000211 at Hrabik Road (Station ID 10030304) met DFAL temperature criteria for all months monitored.

Ambient acute toxicity was not observed in 2017 at any station monitored although chronic toxicity was observed in the UNT 3000213 (Station ID 10033637) in July and October and in the East Twin River below Hwy 29 (Station ID 104445) in June.

In comparison, acute toxicity was observed in 2011 in UNT 3000213 (Station ID 10033637) in May. Chronic toxicity was observed in UNT 3000213 in May and June and in the UNT 3000211, Hrabik Road (Station ID 10030304) in May. However, chronic toxicity was also observed in the East Twin River at Townline Road (Station ID 10008206), below Hwy 29 (Station ID 104445), and at the mouth of the UNT 3000211 (Station ID 10033921) in July indicating possible larger-scale watershed problems.

The macroinvertebrate communities indicate that water quality conditions in the East Twin River is in fair to good condition both upstream and downstream from the confluence of UNT 3000211. The macroinvertebrate communities indicate fair to poor water quality condition in the upper reaches of UNT 3000212 at Cherneyville Road (Station ID 10029041) and fair conditions at two sites on UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) and Hrabik Road (Station ID 10030304). Substrate type and watershed-wide non-point sources of nutrient and sediment contributions likely impact the water quality and macroinvertebrate habitat in both the East Twin River and the UNTs.

Blue Iris Environmental, Inc. conducted a cursory evaluation of the stream inhabitants of the East Twin River at three locations on July 1, 2009 (West, 2009). The full survey report is provided in Appendix C. Although a variety of taxa were noted at each of the three locations and ranged from abundant to present, the survey technique used was not meant to create a M-IBI value and therefore it is not possible to rate the overall stream health based on the 2009 results. The taxa Plecoptera (Stonefly) was observed in this survey and

in 2017 by the WDNR at the Krok Road site while Ephemeroptera (mayfly) were only observed in 2009 at that site. Stoneflies are considered intolerant to pollution.

Fish and aquatic life habitat was assessed at multiple locations in the watershed. Qualitative habitat is rated based on riparian buffer width, amount of bank erosion, percent of pools, riffle and runs, substrate type and cover for adult fish. These metrics combined give an overall score for available habitat. In general, habitat in the East Twin River can be considered in good condition. At Church Road (Station ID 10020787) the extensive amount of fine sediment and lack of riffles limit a variety of habitat and therefore it only received a fair score. East Twin River at CTH F (Station ID 10008204) received a poor score since the stream is extremely small and shallow at this location. A previous good score in 2011 showed deeper water and less fine sediment accumulation.

The fish indices of biological integrity (F-IBI) and the macroinvertebrate indices of biological integrity (M-IBI) were used to determine whether current water quality conditions support the Fish and Aquatic Life designated uses.

Natural communities are defined for streams and rivers using model-predicted flow and temperature ranges associated with specific fish and/or macroinvertebrate communities. Segments are initially classified into natural communities based on landscape-scale statistical models that predict long-term flows and temperatures from watershed characteristics such as watershed size, surficial and bedrock geology, topography, climate, and land cover. These predictions represent the realistic potential Natural Community of the segment under current land-cover and climate conditions in the absence of significant site-specific human impacts, such as local riparian degradation. Correct natural community classification is currently a critical step in applying the correct F-IBI to evaluate water quality influences on the fish community and to provide an accurate bio-assessment. Misclassified streams could be assessed with the incorrect IBI, and their environmental condition may be misjudged. The Department has a guidance document that can correct misclassifications (Lyons, 2013). The procedures were followed to correctly assign a natural community at all fish surveys stations so the correct application of the IBI was used.

The East Twin River from its headwaters downstream towards CTH J aligns with a cool-cold headwater stream classification in that it is a small perennial stream with cool to cold summer water temperatures. Coldwater fish range from absent to abundant, transitional fish from common to dominant, and warmwater fish from absent to common. Small-stream fish range from very common to dominant (50-100% of individuals), medium-stream fish from absent to very common (0-50% of individuals), and large-river fish from absent to uncommon (0-10% of individuals).

As the East Twin River approaches CTH J the stream begins a transition from a cool-cold headwater towards a cool-warm headwater stream with cool to warm summer temperatures. Coldwater fish range from absent to common, transitional fish from

common to dominant, and warmwater fish from absent to abundant. Small-stream fish range from very common to dominant, medium-stream fish from absent to very common, and large-river fish from absent to uncommon. The natural community verification of the East Twin River at CTH J can place the stream in either a cool-cold headwater or a cool-warm headwater stream based on the percentage of coldwater and warmwater fish species observed. The percentage catch of coldwater species is outside the range for a cool-warm headwater stream and the percentage catch of warmwater species is outside the range of a cold-cold headwater stream. Based upon available fish data, classification as a Class II trout stream, and continuous water temperature data the East Twin River at CTH J is best represented and is verified as a cool-warm headwater stream.

UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) has a verified natural community classification as a cool-warm headwater stream. The modeled natural community classification as a cool-cold headwater stream is likely inaccurate based on fish surveys conducted in 2008 and 2017. The fish community in this stream aligns with a cool-warm headwater stream. In any given year, the number of coldwater species may range from absent to common in these headwater streams. They are differentiated by the percent of the overall catch consisting of coldwater, transitional, and warmwater species. In 2008, four mottled sculpin, a cold-water intolerant species, were observed and made up 2% of the total catch. In 2017, mottled sculpin were absent. The remainder of the catch in both years was almost entirely transitional species which are common to abundant in both cool-warm and cool-cold headwater streams. It is also important to note that a high percentage of species captured in both years were tolerant of environmental degradation and outside the expected range for either stream community. This is a significant indicator when there is an absence of intolerant species observed in surveys. However, in both years, intolerant species were observed (mottled sculpin or redbreast dace). The capture of these species allows a new natural community classification to be assigned and indicates human-caused impacts are likely not a significant factor in the overall fish community structure.

UNT 3000212 at Cherneyville Road (Station ID 10029041) was classified as a macroinvertebrate stream which is consistent with surveys conducted in 2008 and 2017 in that it had a low abundance of fish. These macroinvertebrate streams are very small, almost always intermittent streams with a wide range of summer temperatures. No or few fish (< 25 per 100 m of wetted length) are present, but a variety of aquatic invertebrates may be common, at least seasonally. M-IBI in 2017 supported this by receiving a fair rating at this location. The low abundance of fish surveyed on this site does not indicate an aquatic life impairment, only that this stream is not conducive to supporting a fish community throughout the year and that a macroinvertebrate stream classification system is justified.

The overall fish community in the Upper East Twin River watershed is in good to excellent condition. Two key metrics that provide insight into the index include number of intolerant species and number of tolerant species. Up to four species intolerant of

environmental degradation have been captured in surveys throughout the years surveyed with no decline in abundance or elimination from sites. The intolerant species observed include brook trout, mottled sculpin, residue dace, and brook lamprey. Of these, both the brook trout and mottled sculpin are considered coldwater species. It is also important to note that throughout all survey stations and years, a degraded tolerant fish community was not observed indicating human based influence of degradation did not significantly alter the fish community assemblage.

In 2006, a significant fish kill of multiple species occurred from a wastewater treatment plant failure at the current location of Agropur, Inc. (Trega Foods) facility. This kill was documented in the East Twin River from the confluence of UNT 3000211 downstream to the confluence of Krok Creek. No pre-fish kill data exists for this stretch of the East Twin River but 84 dead brook trout ranging in size from 4-14" were collected representing 3+ year classes of brook trout. This generally represents a catch per effort mile of 41.4 through this stretch of river. Post fish-kill surveys were conducted at Krok Road "Ditch" (Station ID 10030630) and zero brook trout were captured 2009 and 2011. In 2015, one 8.2" brook trout was captured representing one year class. In 2017, three brook trout ranging in size between 2.5-7.9" were captured representing two year classes. The catch per effort mile for these surveys were 15.3 and 48.3 respectively. The current brook trout size structure and year class distribution may indicate that the population has not fully recovered, however it does appear based on population density that the brook trout population is on a track for recovery. Furthermore, based on the F-IBI ratings of good to excellent, it appears the overall fish community has recovered from the fish kill of 2006. Note that the additional surveys above and beyond the normal DNR fish survey schedule was conducted to assess brook trout population recovery from this incident.

## **Conclusions and Recommendations**

This study assessed the condition of three UNTs, the East Twin River and Krok Creek for ecological impairment. Assessment data from 2017 were compared to prior surveys to determine if water quality conditions have changed and if streams are meeting water quality standards. Overall conclusions and recommendations include:

- All stream locations assessed exceed water quality criteria for phosphorus and should remain on the 303(d) impaired waters list.
- Chloride, ammonia, and chromium levels in the streams are below impaired thresholds.
- Water temperature in UNT 3000213 exceed water quality standards and should remain on the impaired waters list.
- Ambient chronic toxicity was observed in UNT 3000213 on two occasions in 2017 (July and October). Chronic toxicity was observed on the East Twin River below the confluence with the UNT 3000211 (Station ID 104445) in June but is likely unrelated to UNT 3000213 since no toxicity was found in UNT 3000211 in June.

- Macroinvertebrate monitoring confirmed fair to good water quality conditions in the East Twin River and UNTs with a slight improvement over the 2011 assessment.
- Qualitative habitat assessments found mostly good to fair fish and aquatic life habitat available in Krok Creek, the East Twin River and all three UNTs.
- Watershed-wide non-point sources of nutrient and sediment contributions likely impact the water quality and aquatic life habitat in Krok Creek, the East Twin River and all three UNTs and should be controlled to the extent possible.
- The overall fish community in the Upper East Twin River watershed is in good to excellent condition.
- The natural community of the East Twin River at CTH J (Station ID 10008204) should be updated to reflect the current fish community structure of cool-warm headwater.
- The natural community of the East Twin River at Krok Road bridge (Station ID 10020812) should be updated to reflect the current fish community structure of cool-cold headwater.
- The natural community of the UNT 3000211 at Sleepy Hollow Road (Station ID 10029040) should be updated to reflect the current fish community structure of cool-warm headwater.

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## **Appendix A. Whole Effluent Toxicity (WET) Testing methods**

### Ambient Acute *Ceriodaphnia dubia* Toxicity Test

This method is used for measuring the short-term, static-renewal acute toxicity of water samples to the freshwater cladoceran *Ceriodaphnia dubia*. During this test, *C. dubia* that are less than 24 hours old are continuously exposed for 48 hours to 100% samples of East Twin River water as well as a laboratory water control. The lab water control is either moderately hard water (MHW) or dechlorinated Madison tap water depending on which water the *C. dubia* were cultured in. There are 4 replicates set per treatment and each replicate has five *C. dubia*. Therefore, each acute treatment has 20 *C. dubia* exposed in the acute test. The test solutions are renewed after 24 hours. There is no food provided to the *C. dubia* after setting the acute test. Light is provided 16 hours a day and the test is conducted at 20°C. A 2.5% CO<sub>2</sub> treatment is used to control pH drift during the test. Survival is recorded daily. The percent survival per replicate is used to determine statistical difference ( $p \geq 0.05$  is not significant) between the treatments (river samples and the control) using SAS<sup>®</sup>. This method is based on EPA Test Method 2002 and the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Ed. 2.

### Ambient Chronic *Ceriodaphnia dubia* Toxicity Test

This method is used for measuring the short-term, static-renewal chronic toxicity of water samples to the freshwater cladoceran *Ceriodaphnia dubia*. During this test, *C. dubia* that are less than 24 hours old are continuously exposed for seven days to 100% samples of East Twin River water as well as a laboratory water control. The lab water control is either moderately hard water (MHW) or dechlorinated Madison tap water. There are 10 replicates with one female *C. dubia* set for both the control and all the East Twin River samples. The test solutions are renewed daily and each test beaker is supplied with food which includes YFC and algae. Light is provided 16 hours a day and the test is conducted at 25°C. A 2.5% CO<sub>2</sub> treatment is used to control pH drift during the test. Survival and reproduction are recorded daily. The mean neonates per female in 3 broods is used to determine statistical difference ( $p \geq 0.05$  is not significant) between the treatments (all river samples and the control) using SAS<sup>®</sup>. This method is based on EPA Test Method 1002 and the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Edition 2.

### Ambient Acute *Pimephales promelas* Toxicity Test

This method is used for measuring the short-term, static-renewal acute toxicity of water samples to the freshwater minnow *Pimephales promelas* (fathead minnow). During this test, juvenile (4-14 day old) *P. promelas* are continuously exposed for 96 hours to 100% samples of East Twin River water as well as a lab water control. The lab water control for

this test is always dechlorinated Madison tap water which is the water the fish are cultured in. There are 4 replicates of 10 fish each per treatment. Therefore, each acute *P. promelas* treatment has 40 fish that are exposed. Treatments are renewed daily with fresh test solutions for the duration of the test period. Each test beaker is fed brine shrimp on day 2 (after ~48 hours). Light is provided 16 hours a day and the test is conducted at 20°C. A 2.5% CO<sub>2</sub> treatment is used to control pH drift during the test. Survival is monitored each day when the solutions are renewed. The percent survival per replicate is used to determine statistical difference ( $p \geq 0.05$  is not significant) between the treatments (river samples and the control) using SAS®. This method is based on EPA Test Method 2000 and the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Edition 2.

#### Ambient Chronic *Pimephales Promelas* Toxicity Test

This method is used for measuring the short-term, static-renewal chronic toxicity of water samples to the freshwater minnow *Pimephales promelas* (fathead minnow). During this test, larval ( $\leq 24$  hour old) *P. promelas* are continuously exposed for 7 days to 100% samples of East Twin River water as well as a lab water control. The lab water control for this test is always dechlorinated Madison tap water. There are 10 replicates for each treatment and each replicate is set with two fish. Therefore, 20 fish are exposed per chronic treatment. When the test is shut down 2 replicates are combined for weighing purposes so that there are 5 replicate weight results. Treatments are renewed daily with fresh test solution for the duration of the test period. Each test beaker is fed brine shrimp 2-3 times per day. Light is provided 16 hours a day and the test is conducted at 25°C. A 2.5% CO<sub>2</sub> treatment is used to control pH drift during the test. Survival is monitored each day when the solutions are renewed. Survival and growth (dry weight) are measured at the end of the test to determine if there is an effect on the minnows. Statistical significance of mean dry weight per replicate for all treatments (East Twin River water samples and lab control) is determined using SAS® ( $p \geq 0.05$  not significant). This method is based on EPA Test Method 1000 and the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Edition 2.

#### Ambient Chronic *Selenastrum capricornutum* Toxicity Test

During this test, algal cells are continuously exposed for 96 hours to 100% East Twin River water samples and a moderately hard water control. Light and shaking are provided 24 hours a day and the test is conducted at 25°C. River water is filtered through a GF/A filter prior to test preparation to remove indigenous algae. Nutrients are added to all test solutions to stimulate algal growth. Four replicates of each treatment are set on a microplate which is measured using a plate reader at the end of the test. Chlorophyll content (relative fluorescent units) at 96 hours is compared between all treatments to assess the impact on growth. Algal growth (chlorophyll content measured as RFU) is assessed using SAS® ( $p \geq 0.05$  is not significant) to determine statistically significant differences. This method is based on EPA method 1003.0 and the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Edition 2.

## Chemistry

Alkalinity and hardness of East Twin River samples and lab water is measured on samples collected and preserved on the initial set day. East Twin River ammonia levels are also determined using preserved sample from the initial day. All test waters have pH, dissolved oxygen and conductivity measured on fresh test solutions prior to renewal. After renewal, the water is measured for pH and DO.

## **Appendix B. Toxicity Test Results Shown in Tables.**

### **Fathead Minnow Acute Tests – Survival %**

| SITE DESCRIPTION                           | STATION NO. | 05/09/2017 | 06/20/17 | 07/25/17 | 08/22/17 | 09/19/17 | 10/17/17 |
|--|-------------|------------|----------|----------|----------|----------|----------|
| Lab Water Control                          | NA          | 100.0      | 100.0    | 100.0    | 100.0    | 100.0    | 100.0    |
| ETR-3 East Twin River below Highway 29     | 104445      | 100.0      | 100.0    | 100.0    | 100.0    | 97.5     | 100.0    |
| ETR-5 East Twin River at Townline Road     | 10008206    | 100.0      | 100.0    | 100.0    | 100.0    | 100.0    | 97.5     |
| UT-4 Unnamed tributary at stream mouth     | 10033921    | 100.0      | 100.0    | 100.0    | 100.0    | 100.0    | 100.0    |
| UT-7 Unnamed tributary at Hrabik Road      | 10030304    | 97.5       | 100.0    | 97.5     | 100.0    | 100.0    | 100.0    |
| UT-8 Unnamed tributary at Cherneyville Rd. | 10033637    | 100.0      | 100.0    | 100.0    | 100.0    | 100.0    | 100.0    |

### ***Ceriodaphnia dubia* (waterflea) Acute Tests – Survival %**

| SITE DESCRIPTION                           | STATION NO. | 05/09/2017 | 06/20/17 | 07/25/17 | 08/22/17 | 09/19/17 | 10/17/17 |
|--|-------------|------------|----------|----------|----------|----------|----------|
| Lab Water Control                          | NA          | 95.0       | 35.0     | 100.0    | 100.0    | 100.0    | 95.0     |
| ETR-3 East Twin River below Highway 29     | 104445      | 100.0      | 95.0     | 100.0    | 100.0    | 95.0     | 100.0    |
| ETR-5 East Twin River at Townline Road     | 10008206    | 95.0       | 100.0    | 100.0    | 100.0    | 100.0    | 100.0    |
| UT-4 Unnamed Tributary at stream mouth     | 10033921    | 95.0       | 95.0     | 100.0    | 100.0    | 100.0    | 100.0    |
| UT-7 Unnamed Tributary at Hrabik Road      | 10030304    | 88.8*      | 95.0     | 100.0    | 100.0    | 100.0    | 100.0    |
| UT-8 Unnamed Tributary at Cherneyville Rd. | 10033637    | 90.0       | 100.0    | 100.0    | 100.0    | 100.0    | 100.0    |

\* Replicate was set with 4 organisms instead of 5 due to lab accident. 1 mortality in that replicate = 75% survival, which resulted in a lower overall mean.

**Fathead minnow Chronic Tests – Growth (mean mg/fish)**

| SITE DESCRIPTION                           | STATION NO. | 05/09/2017 | 06/20/17 | 07/25/17 | 08/22/17 | 09/19/17 | 10/17/17 |
|--|-------------|------------|----------|----------|----------|----------|----------|
| Lab Water Control                          | NA          | 0.441      | 0.435    | 0.301    | 0.335    | 0.384    | 0.330    |
| ETR-3 East Twin River below Highway 29     | 104445      | 0.511      | 0.444    | 0.354    | 0.462    | 0.410    | 0.399    |
| ETR-5 East Twin River at Townline Road     | 10008206    | 0.439      | 0.437    | 0.353    | 0.453    | 0.398    | 0.389    |
| UT-4 Unnamed Tributary at stream mouth     | 10033921    | 0.503      | 0.453    | 0.343    | 0.446    | 0.454    | 0.389    |
| UT-7 Unnamed Tributary at Hrabik Road      | 10030304    | 0.527      | 0.427    | 0.394    | 0.464    | 0.444    | 0.436    |
| UT-8 Unnamed Tributary at Cherneyville Rd. | 10033637    | 0.506      | *0.422   | 0.357    | 0.454    | **0.372  | 0.434    |

\*Survival = 85% (control 100%)

\*\*Survival = 74% (control 95%)

***Ceriodaphnia dubia* (waterflea) Chronic Tests – Reproduction (mean neonates/female)**

| SITE DESCRIPTION                           | STATION NO. | 05/09/2017 | 06/20/17 | 07/25/17 | 08/22/17 | 09/19/17 | 10/17/17 |
|--|-------------|------------|----------|----------|----------|----------|----------|
| Lab Water Control                          | NA          | 25         | 28       | 27       | 23       | 16       | 33       |
| ETR-3 East Twin River below Highway 29     | 104445      | 28         | 18       | 32       | 26       | 21       | 39       |
| ETR-5 East Twin River at Townline Road     | 10008206    | 32         | 27       | 24       | 26       | 18       | 43       |
| UT-4 Unnamed tributary at stream mouth     | 10033921    | 24         | 36       | 37       | 21       | 18       | 37       |
| UT-7 Unnamed tributary at Hrabik Road      | 10030304    | 31         | 33       | 39       | 23       | 20       | 35       |
| UT-8 Unnamed tributary at Cherneyville Rd. | 10033637    | 32         | 31       | 27       | 22       | 19       | 26*      |

\*Survival = 80% (control 100%)

***Selenastrum capricornutum* (green algae) Chronic Test – Mean Growth (fluorescence)**

| SITE DESCRIPTION                           | STATION NO. | 05/09/2017 | 06/20/17 | 07/25/17 | 08/22/17 | 09/19/17 | 10/17/17 |
|--|-------------|------------|----------|----------|----------|----------|----------|
| Lab Water Control                          | NA          | 698        | 687      | 658      | 652      | 624      | 657      |
| ETR-3 East Twin River below Highway 29     | 104445      | 779        | 763      | 701      | 668      | 708      | 687      |
| ETR-5 East Twin River at Townline Road     | 10008206    | 774        | 732      | 698      | 643      | 678      | 689      |
| UT-4 Unnamed Tributary at stream mouth     | 10033921    | 811        | 815      | 717      | 635      | 666      | 696      |
| UT-7 Unnamed Tributary at Hrabik Road      | 10030304    | 806        | 836      | 733      | 634      | 657      | 694      |
| UT-8 Unnamed Tributary at Cherneyville Rd. | 10033637    | 821        | 777      | 631      | 619      | 615      | 665      |

## Appendix C. Blue Iris Environmental Report of Findings, Biological Survey of East Twin River, Kewaunee County 2009.

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*Blue Iris Environmental, Inc.*

June 20, 2009

Joe Musial  
E4640 Pheasant Road  
Algoma, WI 54201

Dear Mr. Musial

RE: Report of Findings  
Biological Survey of East Twin River, Kewaunee County, WI

At your request, Blue Iris Environmental, Inc. (Blue Iris) has conducted a biological assessment of macroinvertebrates (and fish if observed) inhabiting portions of East Twin River in Kewaunee County, WI. Blue Iris conducted the survey on July 1, 2009 at three locations along East Twin River. The survey was not meant to be either qualitative or quantitative merely a cursory evaluation of stream inhabitants. This evaluation might be used to provide a preliminary assessment of water quality and/or establish a basis for more in depth evaluations. Blue Iris did not retain any specimens but did record observations which are presented herein.

Observations of instream macroinvertebrates were taken at the following locations:

- Location A - East Twin River – north of Hwy 29 on Town Line Road
- Location B - East Twin River – along Church Road
- Location C - East Twin River – along Kroc Road

At each station, Blue Iris made observation upstream of the culvert and traversed an area approximately 50 to 100 yards upstream. Observations of inhabitants were recorded in a one hour effort per site. Observations included searches of bottom sediments, undercut banks, overhanging vegetation, submerged logs, as well as near shore vegetation (search for emergence of dragonfly and damselfly).

Stream temperatures ranged from 54°F at Location A to 56°F at the other two locations. Substrate at Location A included a bottom with finely washed gravel in the center with soft sediment along the sides. Substrate at Location B had slightly more soft sediment throughout the stretch with numerous sticks and woody deposits on the bottom. Substrate at Location C was nearly all sand bank to bank with some rock and scant aquatic vegetation. All sites were canopied with scrubs (alder) and hardwood over story.

### Summary of Findings

The following chart is a summary of findings.

| Taxa                     | Location A | Location B | Location C |
|--------------------------|------------|------------|------------|
| Annelida                 |            |            |            |
| Hirudinea (leech)        | C2sp       |            |            |
| Crustacea                |            |            |            |
| Amphipoda (scuds)        | A          | C          | A          |
| Decapoda (crayfish)      | C          | C          | C          |
| Isopoda                  | C          |            |            |
| Mollusca                 |            |            |            |
| Gastropoda (snails)      | A          | P          | P          |
| Pelecypoda (clams)       |            | P          |            |
| Insecta                  |            |            | P2sp       |
| Ephemeroptera (mayflies) |            |            |            |
| Odonata                  |            | P          | C3sp       |
| Anisoptera (dragonfly)   | C          |            |            |
| Zygoptera (damselfly)    | P          |            | P          |
| Plecoptera (stonefly)    |            |            | P          |
| Hemiptera (true bugs)    | P          | P          | P          |
| Coleoptera (beetles)     |            |            | C          |
| Trichoptera (caddisfly)  | A3sp*      | C2sp       |            |
| Diptera (fly)            |            |            |            |
| Tabanus                  | P          |            | P          |
| Chironomids              | C          |            |            |
| Fish                     |            |            |            |
| Mud minnow               |            | 2          |            |
| Stickleback              | 1          | 1          |            |

A = Abundant

C = Common

P = Present

\*A3sp = abundant, 3 species noted

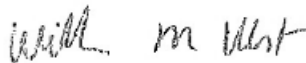
## Discussion

Of the three sites, Location A and C had the most biological organisms per unit effort. Location A had probably the most individuals of the two most likely because it had better substrate (finely washed gravel) which is conducive to macroinvertebrate populations. While Location C had fewer organisms per unit effort, this site was the only one which contained individuals from the stonefly and mayfly groups (highly desirable). All three sites possessed a preponderance of Amphipoda (number one organism identified) and Trichoptera. While Location B had good habitat, several of the taxa noted were a single individual (noted as present). Blue Iris noted that Location B had a very sparse population of macroinvertebrates - even species tolerant of poor water quality were low in numbers. While this was a very cursory evaluation, the differences between the sites should be noted as substantial even to the casual observer.

If you have questions regarding this report, please do not hesitate to contact me at 920-730-0684 or my cell at 920-450-4641.

SINCERELY,

BLUE IRIS ENVIRONMENTAL, INC.



William M. West, *President*