Water Quality Surveys of the East Twin River (84000) and Unnamed Tributary (3000211, 3000212, & 3000213) to the East Twin River, Kewaunee County - 2011 and 2012

By
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1.0 Introduction

The purpose of the study described in this report was to determine the status of the water quality, habitat, and the macroinvertebrate and fisheries communities of the upper reaches of the East Twin River (WBIC 84000) and Unnamed Tributaries to the East Twin River (WBIC 3000211, 3000212, and 3000213) **(Map 1)**. Along with existing data, these new data were assessed against the *Wisconsin 2012 Consolidated Assessment and Listing Methodology* (WisCALM) guidance to determine if the waterbodies are meeting water quality standards.

The headwater of the East Twin River down to State Highway (STH) 29 is classified as Cold Water Community - Class 1 Trout and Exceptional Resource Waters (Wisconsin Administrative Code NR 102). The East Twin River from STH 29 down to CTH J is classified as Cold Water Community – Class II Trout waters. The Unnamed Tributaries to the East Twin River are classified as default Fish and Aquatic Life (DFAL) Communities.

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.
- pH: outside the range of 6-9
- Chloride: acute toxicity >757 mg/l, chronic toxicity >395 mg/l.
- Total phosphorus: >0.075 mg/l.

2.0 Methods

Monitoring was conducted at several locations along the upper reaches of the East Twin River and the Unnamed Tributary to the East Twin River **(Table 1)** following DNR Field Procedures Manuals and guidelines for proper collection and preservation techniques and included the following assessments:

Water chemistry samples – grab samples were collected monthly May through October at four locations on the East Twin River and at three locations on the Unnamed Tributary. The samples were shipped to the Wisconsin State Laboratory of Hygiene (WSLH) for analysis for total phosphorus and chloride concentrations.

Continuous water temperature monitoring – water temperature data loggers (HOBO brand) were placed in the East Twin River at three different locations and in the Unnamed Tributary at two locations from May to October 2011. The loggers recorded water temperature every hour.

Continuous dissolved oxygen/temperature/pH/specific conductivity monitoring – continuous recording meters (Hydrolab brand) were placed in the East Twin River at three different sites to record measurements hourly for 9 to 13 days in mid-summer 2011.

Ambient toxicity testing- grab samples were collected monthly May through October 2011 at two locations on the East Twin River and at three locations on the Unnamed Tributary. The samples were delivered to the WSLH to be tested for acute and chronic toxicity using zooplankton, minnows and algae.

Biological assessments - biological assessment work included collecting stream invertebrates and fish. Macroinvertebrate samples were collected in September, 2011 and were sent to UW-Steven's Point Aquatic Biomonitoring Laboratory for identification. The Hilsenhoff Biotic Index (HBI) and Macroinvertebrate Indices of Biological Integrity (M-IBI) were calculated for each sample collected. HBI is a measure of organic pollution with scores from 0 (excellent) to 10 (very poor). M-IBI uses several metrics to assess overall stream condition with scores from 0 (poor) to 10 (excellent).

Fish surveys were completed in July, 2011. The selection of the fish survey locations were based on past survey locations. A stream shocker was used at all sites using DC current 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 HBI and 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).

Habitat assessments – aquatic life habitat was evaluated at several locations along the East Twin River and the Unnamed Tributary and recorded on the Wadable Stream Qualitative Fish Habitat Rating form. The host of variables measured included depth and width parameters, bottom characteristics, plant growth, percent shading from vegetation, fish cover, streambank erosion and surrounding land use. A habitat score of 100 (excellent) to 0 (poor) was assigned to each location assessed.

3.0 Results

Water chemistry sample results are presented in **Table 2**.

TOTAL PHOSPHORUS (TP)

As specified in NR 102 of the Wisconsin Administrative Code, the total phosphorus criterion of 0.075 mg/l is established for the Unnamed Tributary and the East Twin River. Specific protocols for impairment decisions established in

2012 WisCALM requires six monthly samples to be collected between May and October for assessment purposes.

Based on water samples collected in 2011 and 2012, the Unnamed Tributary at Cherneyville Road (Station ID 10033637) and at the stream mouth just off STH 29 (1033921) exceeds the median threshold for TP impairment. The median TP was 0.523 mg/at Cherneyville Road and 0.397 mg/l at the stream mouth. Both sites clearly exceed the criteria of 0.075 mg/l.

The median TP at all four monitoring locations on the East Twin River clearly exceed the TP impairment threshold. The median concentration at Townline Road (10008206) was 0.132 mg/l; the median just below the confluence with the Unnamed Tributary below STH 29 (104445) was 0.171 mg/l; the median at Krok Road (10030630) was 0.172 mg/l, and the median at CTH J (10008204) was 0.115 mg/l.

CHLORIDE

Specific protocols for impairment decisions established in 2012 WisCALM for chloride requires at least two values within a 3-year period. The acute toxicity threshold is 757 mg/l and the chronic toxicity threshold is 395 mg/l. Six monthly samples were collected at the same locations as the TP samples listed above. Based on water samples collected in 2011 and 2012, none of the chloride samples collected on the Unnamed Tributary or the East Twin River exceeded established thresholds for acute or chronic toxicity. The mean chloride concentration of the samples collected from Cherneyville Road (10033637) was 198.3 mg/l which was considerably higher than the mean concentrations of the samples collected from any of the four locations on the East Twin River which were 34, 56.8, 47.2, and 40.2 mg/l.

Continuous water temperature monitoring

Water temperature data is presented in **Table 3** and graphs in **Figures 1-5**. The warmest water temperature month recorded was July.

Thermistors measured water temperature at Cherneyville Road (10033637) and Hrabik Road (10030304) **(Picture 1)** on the Unnamed Tributary and at Townline Road (10008206) **(Picture 2)**, Krok Road (10030630) **(Picture 3)**, and CTH J (10008204) **(Picture 4)** on the East Twin River from May to October, 2011.

The water temperature of the Unnamed Tributary at Cherneyville Road was very warm reaching a maximum temperature of 38.8°C (101.8°F) in July 2011 with a monthly mean of 29.3°C (84.7°F). However, by the time the water travels downstream and reached Hrabik Road the water cools off significantly and only reached a maximum of 25.6°C (78°F) with a July monthly mean of 20.2°C (68.4°F).

The water temperature of the East Twin River is generally cooler in the uppermost reaches and warms as it travels downstream. The maximum water temperature at Townline Road was 22.1°C (71.8°F) with a July monthly mean of 18.2°C (64.7°F). At Krok Road, the maximum was 23.2°C (73.8°F) and July mean was 18.5°C (65.3°F). At CTH J, the maximum was 26.3°C (79.4°F) and the July mean was 19.1°C (66.3°F).

Specific protocols for impairment decisions established in 2012 WisCALM for water temperature requires at least twenty discrete daily values collected hourly. The impairment threshold for water temperature requires that 10 percent of the mean daily temperature values exceed specific acute maximum values based on stream designated uses. For the East Twin River, that maximum value is >73°F (22.8°C). For the Unnamed Tributary, that maximum value is >86°F (30°). Based on data collected in 2011, both the East Twin River and the Unnamed Tributary are meeting water quality standards and did not exceed the impairment thresholds for water temperature. However, the mean daily water temperature at the Unnamed Tributary at Cherneyville Road did exceed 86°F on 9 out of 139 days monitored between May 31 and October 17, 2011. This equates to 6.5% frequency which is below the 10% threshold.

Continuous dissolved oxygen/temperature/pH/specific conductivity monitoring

Continuous recording meters were placed in the East Twin River at three different locations for 9 to 13 days in mid-summer to record hourly water quality. Meters were located at CTH F in Ellisville (10034445), Church Road (10020787), and Krok Road below the confluence with Krok Creek (10020812).

At no time during the sampling period did dissolved oxygen levels fall below the cold water quality standard of 6 mg/l in the East Twin River. Dissolved oxygen levels ranged from 6.1 to 10.4 mg/l.

At no time during the sampling period did water temperature exceed the water quality standard of 22.8°C in the East Twin River. Water temperature ranged from 13-21°C (55.4-69.8°F).

At no time during the sampling period did pH fall outside the acceptable water quality standard range of 6-9 in the East Twin River. pH levels ranged from 7.6 to 8.5.

At no time during the sampling period did specific conductivity levels fall outside an acceptable range in the East Twin River. Wisconsin does not have a water quality standard for specific conductivity at this time. Specific conductivity ranged from 436 to 863 uS/cm.

Ambient toxicity testing

Ambient toxicity test results are presented in **Table 4** and summarized by month below.

Water samples were collected in May and June on the Unnamed Tributary at Cherneyville Road (10033637) and at Hrabik Road (10030304). Samples were collected June, July, August, September, and October at the mouth of the Unnamed Tributary (1033921), and on the East Twin River at Townline Road (10008206) and below the confluence of the Unnamed Tributary (104445). The water samples were sent to the WSLH to be tested for acute and chronic toxicity using *Ceriodaphnia dubia* (water flea), *Pimephales promelas* (fathead minnow), and *Selenastrum capricornutum* (algae).

MAY

Acute Toxicity Tests

Toxicity was observed in the water flea test at Cherneyville Road.

Chronic Toxicity Tests

Toxicity was observed in the fathead minnow test at Cherneyville Road. Toxicity was observed in the water flea test at both Cherneyville and Hrabik Road sites. No toxicity was observed in the algae test.

JUNE

Acute Toxicity Tests

There was no acute toxicity observed in either the water flea or the fathead minnow test at Cherneyville or Hrabik Roads.

Chronic Toxicity Tests

There was no chronic toxicity observed in the water flea test. Toxicity was observed in the fathead minnow test at Cherneyville Road. Toxicity was observed in the algae test at both Cherneyville and Hrabik Road sites.

JULY

Acute Toxicity Tests

There was no acute toxicity observed in either the water flea or the fathead minnow test at the three sites sampled - Unnamed Tributary at the stream mouth, East Twin River below the confluence with the Unnamed Tributary, and Townline Road.

Chronic Toxicity Tests

Toxicity was observed in the water flea test at all three sites. There was no toxicity observed in the fathead minnow test at any of the sites. Toxicity was observed in the algae test on the East Twin River below the confluence with the Unnamed Tributary, however, the reduction in growth was below the level of concern normally used for algae tests (50% of the control).

AUGUST

Acute Toxicity Tests

There was no toxicity observed in either the water flea test or the fathead minnow test at the three sites sampled - Unnamed Tributary at the stream mouth, East Twin River below the confluence with the Unnamed Tributary, and Townline Road.

Chronic Toxicity Tests

There was no toxicity observed in the fathead minnow test or the algae test. The chronic water flea test was invalid due to control organism failure.

SEPTEMBER

Acute Toxicity Tests

There was no toxicity observed in either the water flea test or the fathead minnow test at the three sites sampled - Unnamed Tributary at the stream mouth, East Twin River below the confluence with the Unnamed Tributary, and Townline Road.

Chronic Toxicity Tests

There was no toxicity observed in the water flea test or the algae test. The fathead minnow test resulted in mean weights for the ambient sites that were 20-30% lower than the control, but statistical analysis suggests these were not significantly different. This is likely due to variability between test replicates.

OCTOBER

Acute Toxicity Tests

There was no toxicity observed in either the water flea test or the fathead minnow test at the three sites sampled - Unnamed Tributary at the stream mouth, East Twin River below the confluence with the Unnamed Tributary, and Townline Road.

Chronic Toxicity Tests

There was no toxicity observed in the water flea test, the fathead minnow test, or the algae test.

To summarize, acute and/or chronic toxicity did occur in the Unnamed Tributary in May, June, and July 2011 but not in August, September, or October. Chronic toxicity did occur in the East Twin River in July only both above and below the confluence with the Unnamed Tributary. The aquatic toxicity issue in the Unnamed Tributary is being addressed through the WPDES permitting program.

Biological assessments

Macroinvertebrate, fish and habitat results are presented in **Tables 5, 6, and 7**.

MACROINVERTEBRATES

Aquatic macroinvertebrates were collected on the Unnamed Tributary at Hrabik Road (10030304) and five locations of the East Twin River at CTH F (10034445), Townline Road (10008206), below the confluence with the Unnamed Tributary (104445), Krok Road (10030630), and CTH J (10008204) in October, 2011. Peter Jackson from EPA Region 5 assisted in the sampling activities.

HBI score rated the Unnamed Tributary as "good" water quality based on the degree of organic pollution present. M-IBI rated the stream as "fair" condition. The HBI score was "fair" in the headwaters of the East Twin River at CTH F but considered "poor" condition using the M-IBI. HBI was "very good" at Townline Road, below the confluence of the Unnamed Tributary, and at Krok Road but all three locations rated "fair" using the M-IBI. At the most downstream location at CTH J, the HBI score was "good" but only considered "fair" using the M-IBI.

Macroinvertebrate samples were also collected in 2008 at two locations on the Unnamed Tributary. HBI scores rated the Unnamed Tributary as "very poor" at Cherneyville Road (10029041) with a "poor" M-IBI and "fair" at Sleepy Hollow Road (10029040) for both HBI and M-IBI.

FISH
Fish surveys were completed at three locations on the East Twin River at Townline Road, Krok Road, and CTH J in July, 2011. F-IBI scores rated the stream as "good" at Townline Road and "poor" at both Krok Road and CTH J.

Fish surveys were also completed in 2008 at two locations on the Unnamed Tributary. F-IBI score rated the Unnamed Tributary as "poor" at Sleepy Hollow Road. Only four fish total (all Central Mudminnow) were captured at Cherneyville Road which was not enough to calculate an F-IBI score.

Habitat assessments
Aquatic life habitat was evaluated at four locations on the Unnamed Tributary including Cherneyville Road (10033637), Sleepy Hollow Road (10029040), Hrabik Road (10030304), and at the stream mouth just off STH 29 (10033921). Habitat was evaluated at seven locations on the East Twin River including CTH F (10034445), Townline Road (10008206), below the confluence with the Unnamed Tributary (104445), Krok Road (10030630), Church Road just before the confluence with Krok Creek (10020787), Krok Road below the confluence with Krok Creek (10020812), and CTH J (10008204). Habitat assessments rated both the East Twin River and the Unnamed Tributary as having "good" to "excellent" habitat available for fish and other aquatic life in 2011.

Habitat assessments were also completed in 2008 at two locations on the Unnamed Tributary. The habitat assessment rated the Unnamed Tributary as 'poor' at Cherneyville Road and "fair" at Sleepy Hollow Road.

4.0 Conclusions and Recommendations

The design of this study was to collect adequate data to fully assess the current condition of the Unnamed Tributary and the East Twin River for impairment determination under the 2012 WisCALM procedures. Data collected in 2011 and 2012 were used to supplement previous data to make that determination.

That objective was fulfilled by the collection of additional water chemistry samples, continuous water temperature monitoring, continuous dissolved oxygen monitoring, ambient toxicity testing, macroinvertebrate, fish, and habitat assessments.

In summary, the water quality of the upper reaches of the East Twin River and Unnamed Tributary are not meeting water quality standards for phosphorus; however, they are meeting standards for water temperature and chloride at all sites monitored. Aquatic toxicity did occur in both the East Twin River and the Unnamed Tributary but was not

found in the last three months sampled. The summer water temperature, aquatic life habitat, and macroinvertebrate community is generally good to fair in the East Twin River but the fish community is poor below STH 29.

Analysis of the water chemistry and fisheries surveys confirm what was suspected previously – the fish and aquatic life use of the upper reaches of the East Twin River and the Unnamed Tributary are impaired and not meeting phosphorus water quality standards.

We recommend that the entire section of the East Twin River that is currently classified as Cold-Class II trout water (starting at State Highway 29 down to CTH B) be included on the 2014 impaired waters list for phosphorus. This listing is supported by "poor" fish F-IBI's in 2011 at two locations. We also recommend additional monitoring between CTH F in Ellisville down to STH 29 to assess possible biological impairment since total phosphorus levels are high at Townline Road.

We also recommend that the entire length of the Unnamed Tributary (starting at the west most crossing on Cherneyville Road (10033637) down to the stream mouth just south of STH 29) which includes WBIC's 3000211, 3000212, and 3000213 be included on the 2014 impaired waters list for phosphorus. This listing is supported by "poor" fish F-IBI's and "poor" macroinvertebrate HBI and M-IBI in 2008.

5.0 References

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Wisconsin Department of Natural Resources. 2000. Guidelines for Collecting Macroinvertebrate Samples from Wadable Streams.

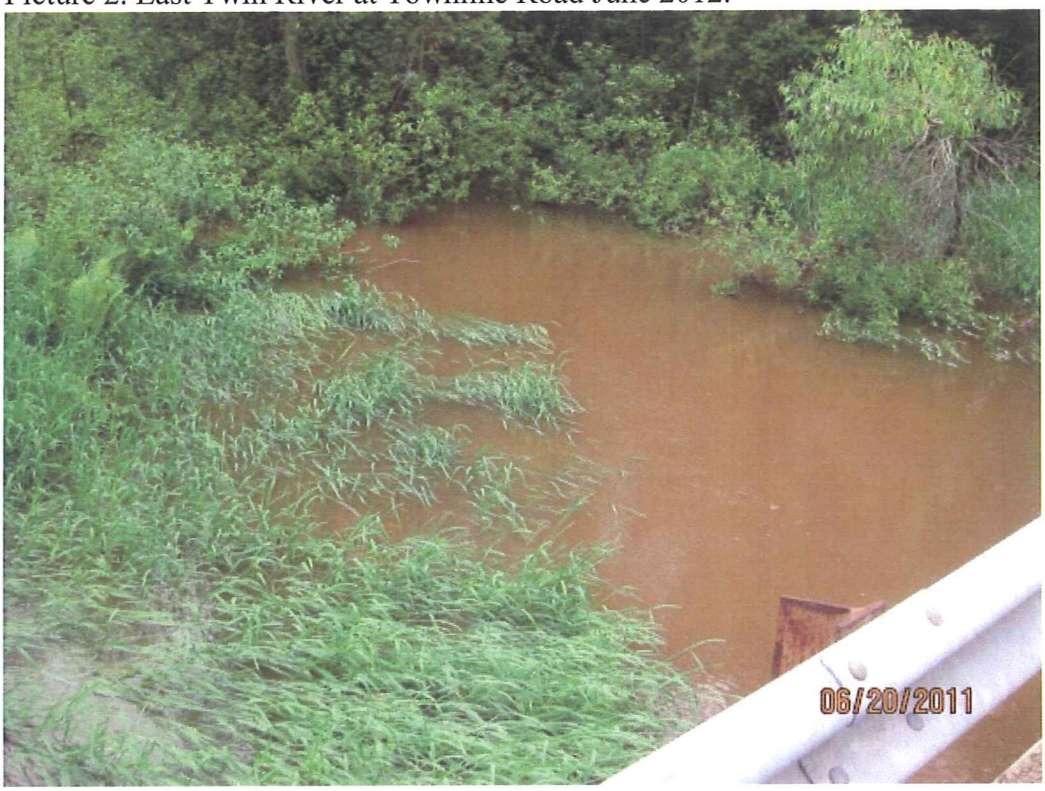
Wisconsin Department of Natural Resources. 2001.Guidelines for Assessing Fish Communities of Wadable Streams in Wisconsin.

Wisconsin Department of Natural Resources. 2005. Surface Water Assessment Team Guidelines and Procedures for Continuous Temperature Monitoring, Version 3.

Wisconsin Department of Natural Resources. 2008. State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition.

Picture 1. Unnamed Tributary at Hrabik Road May, 2011.

Picture 2. East Twin River at Townline Road June 2012.



Picture 3. East Twin River at Krok Road May, 2011.

Picture 4. East Twin River at CTH J May, 2011.

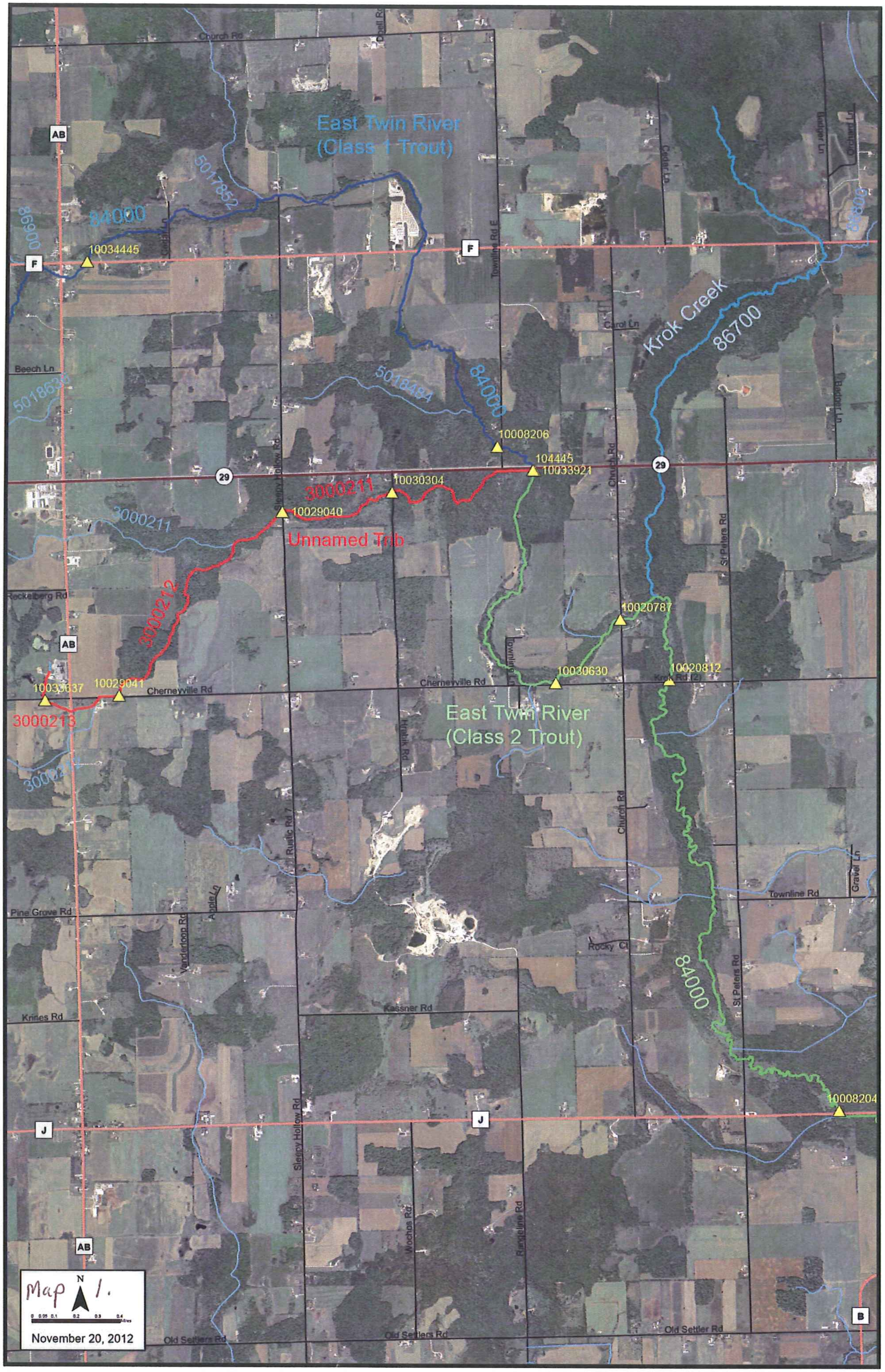

East Twin River
(Class 1 Trout)
84000
5017852
86900
10034445
F
AB
Church Rd
Krok Creek
86700
5018484
5018626
Beech Ln
Carol Ln
Cedar Ln
Badger Ln
Orchard Ln
Townline Rd E
10008206
104445
10033921
29
3000211
10030304
10029040
Unnamed Trib
Sleepy Hollow Rd
Reckelberg Rd
3000212
10033637
10029041
3000213
3000214
Cherneyville Rd
10030630
10020787
10020812
Church Rd
St Peters Rd
East Twin River
(Class 2 Trout)
Hrabik Rd
Townline Rd
Gravel Ln
Pine Grove Rd
Vanderloop Rd
Apple Ln
Rocky Ct
Kassner Rd
Krines Rd
10008204
J
Sleepy Hollow Rd
Wochos Rd
Rangeline Rd
B
Old Settlers Rd
Old Settler Rd
Map 1.
N
0 0.05 0.1 0.2 0.3 0.4 Miles
November 20, 2012

Table 1. Monitoring Locations

Waterbody (WBIC)	Station ID	Location	Parameter						
			Water Chemistry	Aquatic Toxicity	Water Temperature	Dissolved Oxygen	Invertebrate	Habitat	Fish
East Twin River (84000)	10034445	Highway F, Ellisville				X	X	X	
	10008206	Townline Road	X	X	X		X	X	X
	1004445	Below Highway 29	X	X			X	X	
	10030630	Krok Road Ditch	X		X		X	X	X
	10020787	Church Road				X		X	
	10020812	Krok Road Bridge				X		X	
	10008204	CTH J	X		X		X	X	X
Unnamed Tributary (3000211)	10029040	Sleepy Hollow Road					X	X	X
	10030304	Hrabik Road	X	X	X		X	X	
	10033921	Stream mouth off STH 29	X	X				X	
Unnamed Tributary (3000213)	10033637	Cherneyville Road, below Agropur Discharge	X	X	X			X	
Unnamed Tributary (3000212)	10029041	Cherneyville Road, east crossing					X	X	X

Table 2. Results from Water Chemistry Tests
East Twin River and Unnamed Tributary to East Twin River
2011 & 2012

Date	Total Phosphorus (mg/l)**						
	Unnamed Tributary			East Twin River			
	Cherneyville	Hrabik	UT-MO	Townline	Below 29	Krok	CTH J
05/31/11	0.594	0.463	---	0.089	---	0.154	0.115
06/20/11	0.234	0.336	---	0.244	---	0.190	0.135
07/11/11	0.458	---	0.468	0.174	0.204	0.192	0.186
08/22/11	0.850	---	0.389	0.069	0.111	0.106	0.052
09/26/11	0.587	---	0.385	0.830	0.981	1.220	0.114
10/17/11	0.421	---	0.404	0.060	0.138	0.124	0.070
05/09/12	---	---	0.253	---	0.124	---	---
06/18/12	---	---	0.779	---	0.291	---	---
Mean[1]	**0.524**	---	**0.446**	**0.244**	**0.308**	**0.328**	**0.112**
Median[2]	**0.523**		**0.397**	**0.132**	**0.171**	**0.172**	**0.115**

**WisCALM criteria for Total Phosphorus: > 0.075 mg/l.

Date	Chloride (mg/l)*						
	Unnamed Tributary			East Twin River			
	Cherneyville	Hrabik	UT-MO	Townline	Below 29	Krok	CTH J
05/31/11	251	113	---	36.6	---	47.1	37.2
06/20/11	51.8	27	---	16	---	14.3	14.3
07/11/11	194	---	162	31	56.2	43	37.8
08/22/11	277	---	142	37.4	53	52.9	47.8
09/26/11	155	---	105	42.5	51.8	54.6	50
10/17/11	261	---	188	40.7	74.5	71.4	54.1
05/09/12	---	---	63.8	---	42.9	---	---
06/18/12	---	---	---	---	62.2	---	---
Mean[1]	**198.3**	---	---	**34.0**	**56.8**	**47.2**	**40.2**

*WisCALM criteria for Chloride: acute toxicity 757 mg/l; chronic toxicity 395 mg/l.

Sampling sites:

Sample Location	Site Description
Cherneyville	Unnamed Tributary to East Twin River at Cherneyville Rd. Station ID: 10033637
Hrabik	Unnamed Tributary to East Twin River at Hrabik Rd. Station ID: 10030304
UT-MO	Unnamed Tributary to East Twin River at stream mouth. Station ID: 1033921
Townline	East Twin River at Townline Road. Upstream of 29. Station ID: 10008206
Below 29	East Twin River below confluence with Unnamed Tributary. Station ID: 104445
Krok	East Twin River at Krok Road. Between Townline and Church Roads. Station ID: 10030630
CTH J	East Twin River at CTH J. Station ID: 10008204

[1]Mean concentration based on six monthly samples.
[2] Median concentration based on six monthly samples.

Table 3. Results from Continuous Water Temperature Monitoring
East Twin River and Unnamed Tributary to East Twin River
2011

	Unnamed Tributary		**East Twin River**		
	Cherneyville Road (10033637)	Hrabik Road (10030304)	Townline Road (10008206)	Krok Road (10030630)	CTH J (10008204)
June[1]	77.3	60.6	58.9	59.6	57.8
July	84.7	68.4	64.7	65.3	66.3
August	81.1	65.5	62.0	63.5	67.7
September	79.9	57.5	55.3	56.6	57.8
Maximum[2]	**101.8**	**78.0**	**71.8**	**73.8**	**79.4**
% of mean daily values exceed impairment threshold[3]	6.5%	0%	0%	0%	0.6%

[1] Monthly mean water temperature in Degrees F.
[2] Maximum water temperature recorded.
[3] WisCALM acute threshold >86°F for Unnamed Tributary and >73°F for the East Twin River.

Table 4. Results from Ambient Toxicity Tests
East Twin River and Unnamed Tributary to East Twin River
Summer & Fall 2011

Date	Acute									
	Water Flea					Fathead Minnow				
	UT-1	UT-2	UT-MO	Below	T-3	UT-1	UT-2	UT-MO	Below	T-3
05/31/11	**Fail**	Pass	----	----	----	Pass	Pass	----	----	----
06/20/11	Pass	Pass	----	----	----	Pass	Pass	----	----	----
07/11/11	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass
08/22/11	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass
09/26/11	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass
10/17/11	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass

Date	Chronic														
	Water Flea					Fathead Minnow					Algae				
	UT-1	UT-2	UT-MO	Below	T-3	UT-1	UT-2	UT-MO	Below	T-3	UT-1	UT-2	UT-MO	Below	T-3
05/31/11	**Fail**	**Fail**	----	----	----	**Fail**	Pass	----	----	----	Pass	Pass	----	----	----
06/20/11	Pass	Pass	----	----	----	**Fail**	Pass	----	----	----	**Fail**	**Fail**	----	----	----
07/11/11	----	----	**Fail**	**Fail**	**Fail**	----	----	Pass	Pass	Pass	----	----	Pass	**Fail**	Pass
08/22/11	----	----	[1]	[1]	[1]	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass
09/26/11	----	----	Pass	Pass	Pass	----	----	**Fail**[2]	**Fail**[2]	**Fail**[2]	----	----	Pass	Pass	Pass
10/17/11	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass	----	----	Pass	Pass	Pass

Sampling sites:

Sample Number	Site Description
UT-1	Directly below Agropur discharge. Unnamed Tributary to East Twin River at Cherneyville Rd. Station ID 10033637
UT-2	Unnamed Tributary to East Twin River at Hrabik Rd., downstream approximately 2 miles. Station ID 10030304
UT-MO	Unnamed Tributary to East Twin River at stream mouth. Station ID 1033921
Below	East Twin River below confluence with Unnamed Tributary. Station ID 104445
T-3	East Twin River at Townline Rd. Station ID 10008206

[1]Water Flea chronic test was invalid due to control organism failure and poor performance throughout treatments. There was not enough sample left to reset the test.

[2] There was a notable difference between ambient sites and the control (a reduction in mean weight of 23% for T-3, 20% for Below, and 32% for UT-MO), although statistical analyses suggests they were not significantly different.

Table 5. Results from Macroinvertebrate, Habitat, and Fish Assessments East Twin River and Unnamed Tributaries to East Twin River 2011

	Location	Station ID	Macroinvertebrate HBI		Macroinvertebrate IBI		Habitat		Fish	
			Hilsenhoff Biotic Index[1]	Water Quality	Indices of Biological Integrity[2]	Condition Category	Habitat Score[3]	Habitat Quality	Index of Biotic Integrity[4]	Fish Quality
East Twin River	CTH F - Ellisville	10034445	5.636	Fair	1.6	Poor	72	Good	---	---
	Townline Road	10008206	4.484	Very Good	4.3	Fair	80	Excellent	70	Good
	Below confluence w/Unnamed Trib. below STH 29	104445	4.459	Very Good	4.5	Fair	77	Excellent	---	---
	Krok Road	10030630	4.324	Very Good	2.9	Fair	58	Good	10	Poor
	Church Road before confluence w/ Krok Creek	10020787	---	---			67	Good	---	---
	Krok Road below confluence w/ Krok Creek	10020812	---	---			67	Good	---	---
	CTH J	10008204	4.714	Good	3.3	Good	52	Good	20	Poor
Unnamed Tributary 3000213	Cherneyville Road	10033637	---	---			60	Good	---	---
Unnamed Tributary 3000211	Sleepy Hollow Road	10029040	---	---			80	Excellent	---	---
	Hrabik Road	10030304	4.803	Good	3.9	Fair	82	Excellent	---	---
	Stream mouth, off STH 29	10033921	---	---			68	Good	---	---

Table 6. Results from Macroinvertebrate, Habitat, and Fish Assessments Unnamed Tributaries to East Twin River 2008

	Location	Station ID	Macroinvertebrate		Macroinvertebrate IBI		Habitat		Fish	
			Hilsenhoff Biotic Index[1]	Water Quality	Indices of Biological Integrity	Condition Category	Habitat Score[2]	Habitat Quality	Index of Biotic Integrity[3]	Fish Quality
Unnamed Tributary 3000212	Cherneyville Road	10029041	9.948	Very poor	0	Poor	---	Poor	Not enough fish to run IBI	---
Unnamed Tributary 3000211	Sleepy Hollow Road	10029040	6.325	Fair	4.0	Fair	---	Fair	14	Poor

[1]Hilsenhoff Biotic Index, 1987

Biotic Index	Water Quality Rating	Degree of Organic Pollution
0.00–3.50	Excellent	No apparent organic pollution
3.51–4.50	Very good	Possible slight organic pollution
4.51–5.50	Good	Some organic pollution
5.51–6.50	Fair	Fairly significant organic pollution
6.51–7.50	Fairly poor	Significant organic pollution
7.51–8.50	Poor	Very significant organic pollution
8.51–10.0	Very poor	Severe organic pollution

[2] Macroinvertebrate Indices of Biological Integrity (M-IBI)

M-IBI	Condition Category
7.5-10	Excellent
5.0-7.4	Good
2.6-4.9	Fair
0-2.5	Poor

[3]Habitat Assessment

Habitat Score	Habitat Rating
>75	Excellent
50-74	Good
25-49	Fair
<25	Poor

[4] Fish Index of Biotic Integrity (F-IBI)

Fish IBI Score	Fish Integrity Rating
100-90	Excellent
80-60	Good
50-30	Fair
20-10	Poor
0 or no score	Very Poor

Table 7. Upper East Twin River Fish Survey Results 2001-2011.

Species	Townline Road			Krok Road		CTH J		
	2001	2009	2011	2009	2011	2001	2009	2011
Brook Trout	3	20	18					
Central Mudminnow	8	42	14	78	55	21	25	105
Brook Lamprey	2	7	3			2	4	3
Mottled Sculpin	41	78	39	7	3	11	27	29
White Sucker	1	1		13	9	44	31	31
Creek Chub		1		20	6	48	111	61
Pearl Dace	10	13		42	1	5	15	3
Common Shiner				1		29	68	5
Hornyhead Chub						13	34	24
Brook Stickleback	11					2	3	26
Southern Redbelly Dace						18	28	12
Redside Dace				1			3	7
Johhny Darter	1					18	3	6
Blacknose Dace		1		1		5	7	3
Rainbow Trout							1	
Longnose Dace	1					1	1	
Brown Trout						2		
Bluntnose Minnow						4		
Total	78	163	74	163	74	223	361	315
IBI[1]	70 good	50 poor	70 good	20 poor	10 poor	20 poor	20 poor	20 poor

[1]Fish Index of Biotic Integrity

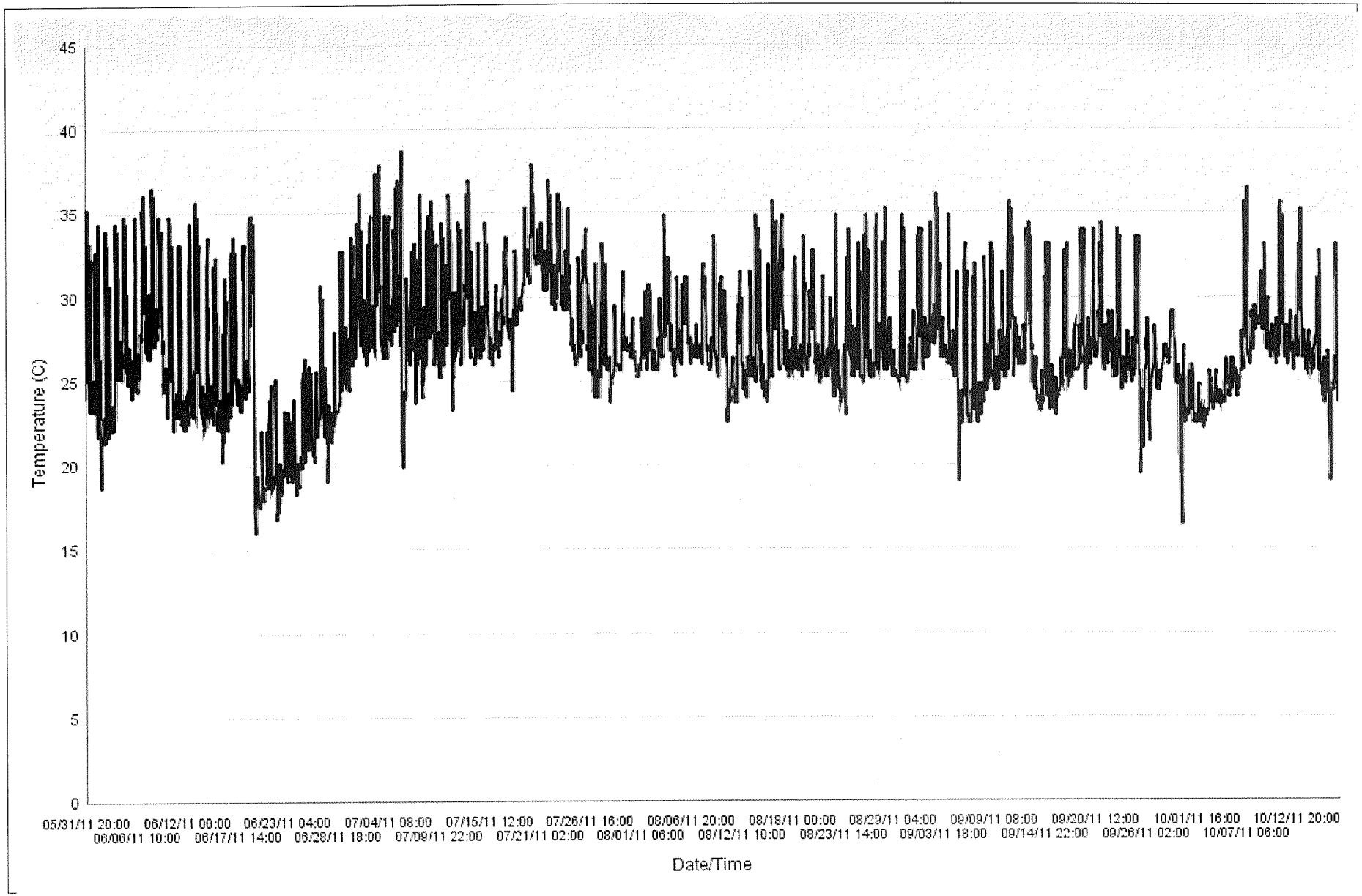
Figure 1.

Station ID: 10033637

Station Name: Unnamed Trib to East Twin River at Cherneyville Rd

Fieldwork Event Start: 05/31/2011 00:00

Fieldwork Event End: 10/17/2011 00:00



SWIMS: Temperature for a Selected Fieldwork Event

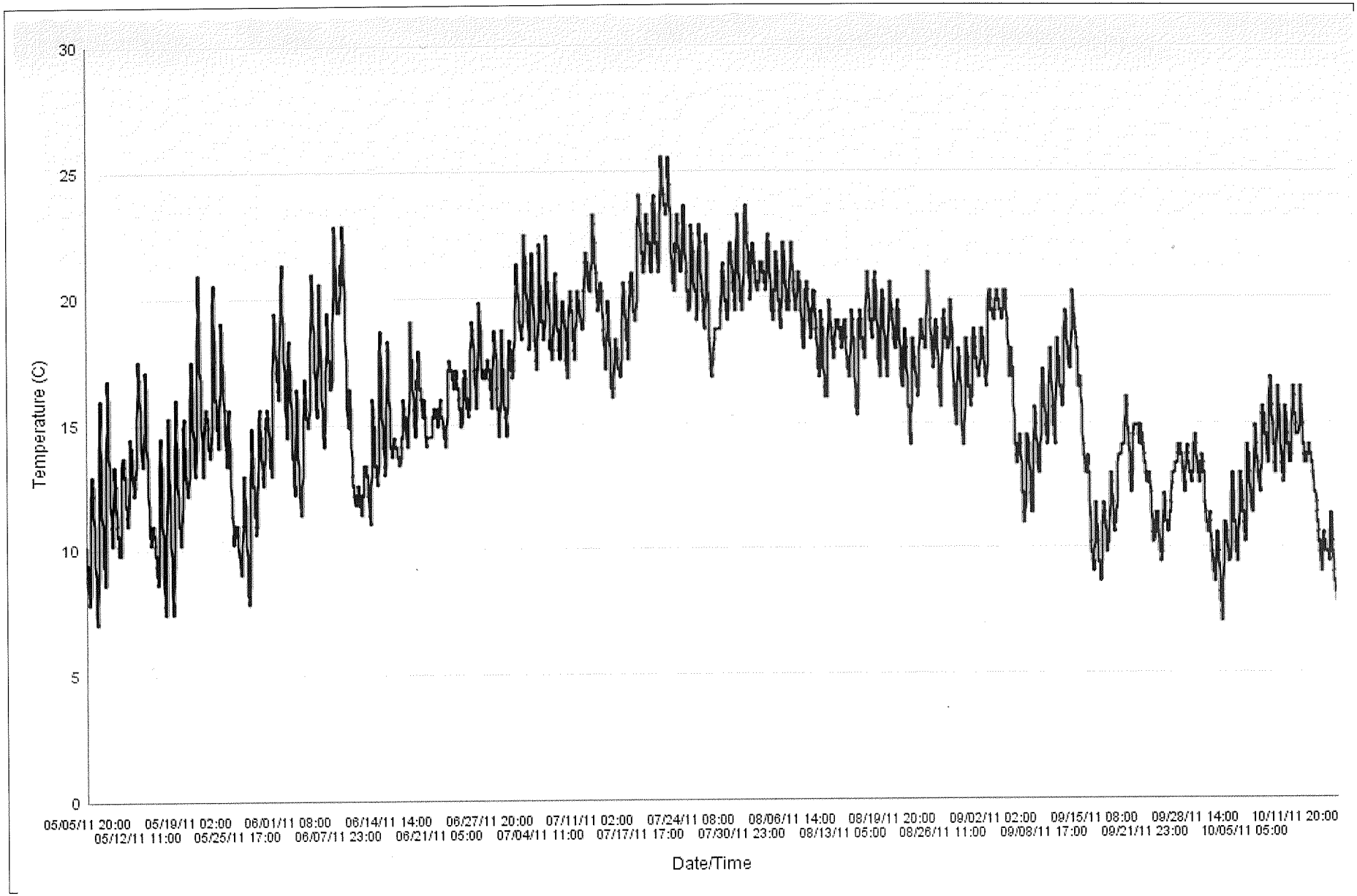
Figure 2.

Station ID: 10030304

Station Name: Unnamed Tributary to East Twin River at Hrabik Road

Fieldwork Event Start: 05/05/2011 18:00

Fieldwork Event End: 10/17/2011 10:00



SWIMS: Temperature for a Selected Fieldwork Event

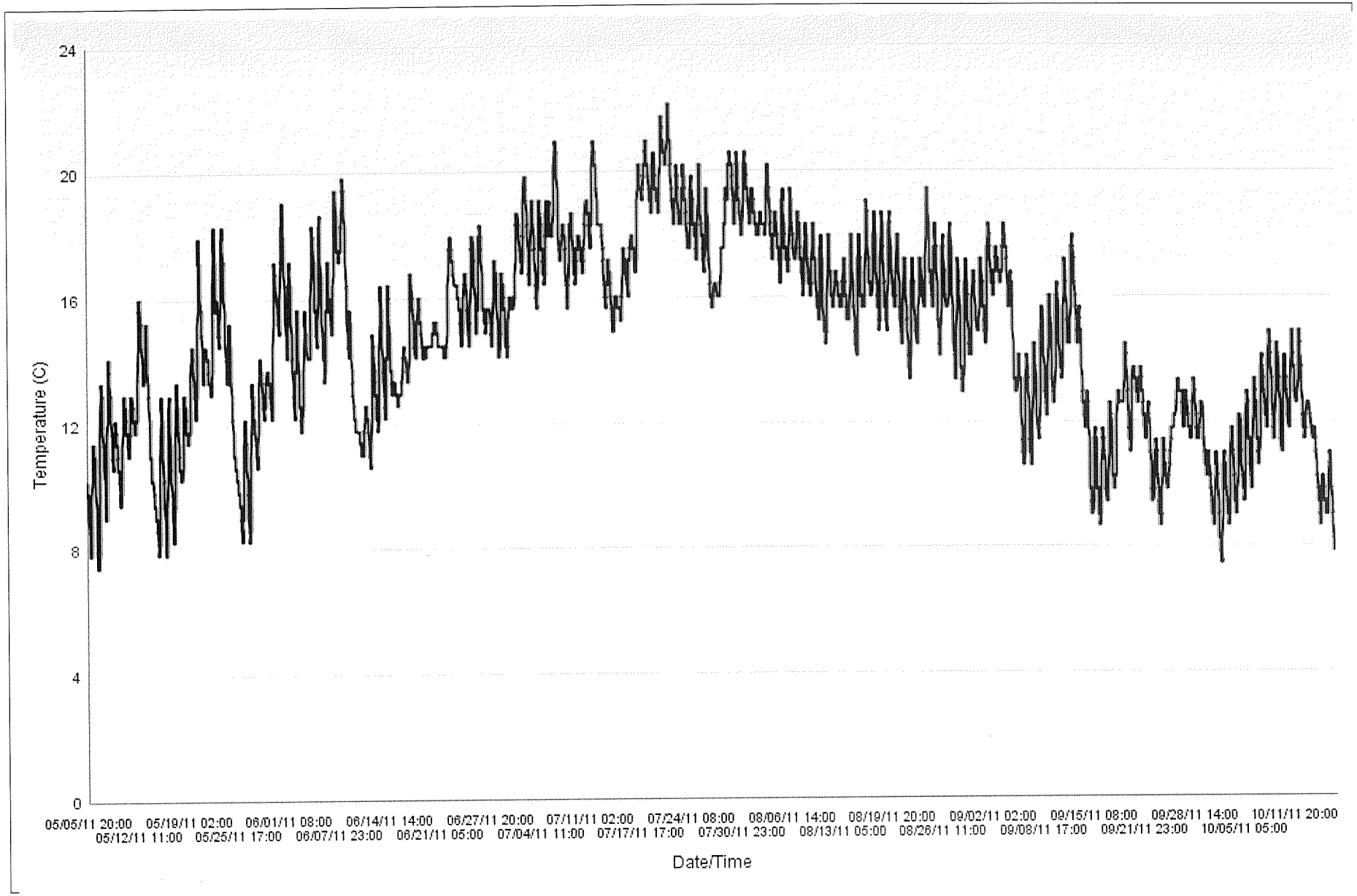
Figure 3.

Station ID: 10008206

Station Name: East Twin River - East Twin River - Townline Road

Fieldwork Event Start: 05/05/2011 18:00

Fieldwork Event End: 10/17/2011 10:00



SWIMS: Temperature for a Selected Fieldwork Event

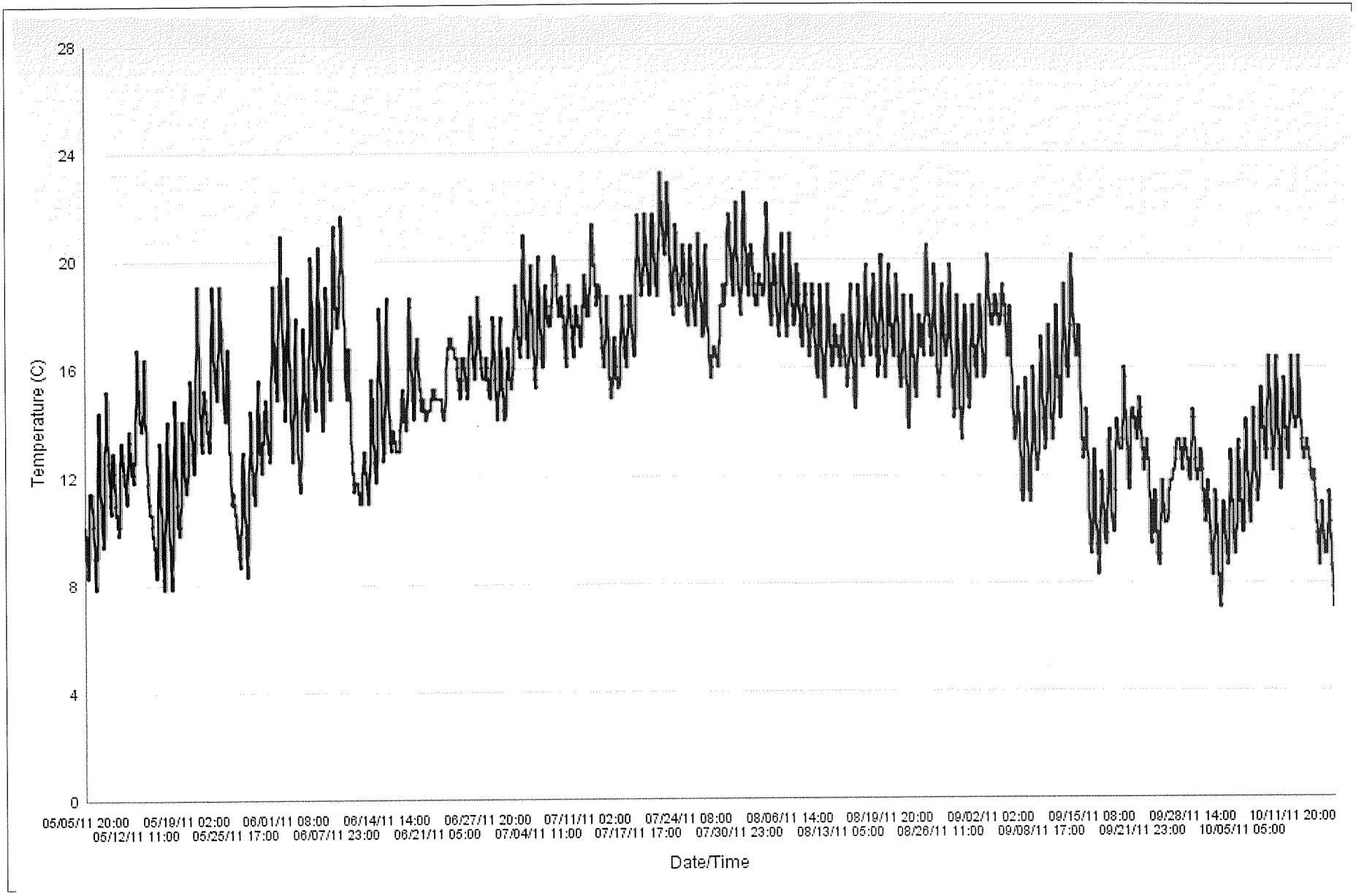
Figure 4.

Station ID: 10030630

Station Name: East Twin River at Krok Road

Fieldwork Event Start: 05/05/2011 18:00

Fieldwork Event End: 10/17/2011 18:00



SWIMS: Temperature for a Selected Fieldwork Event

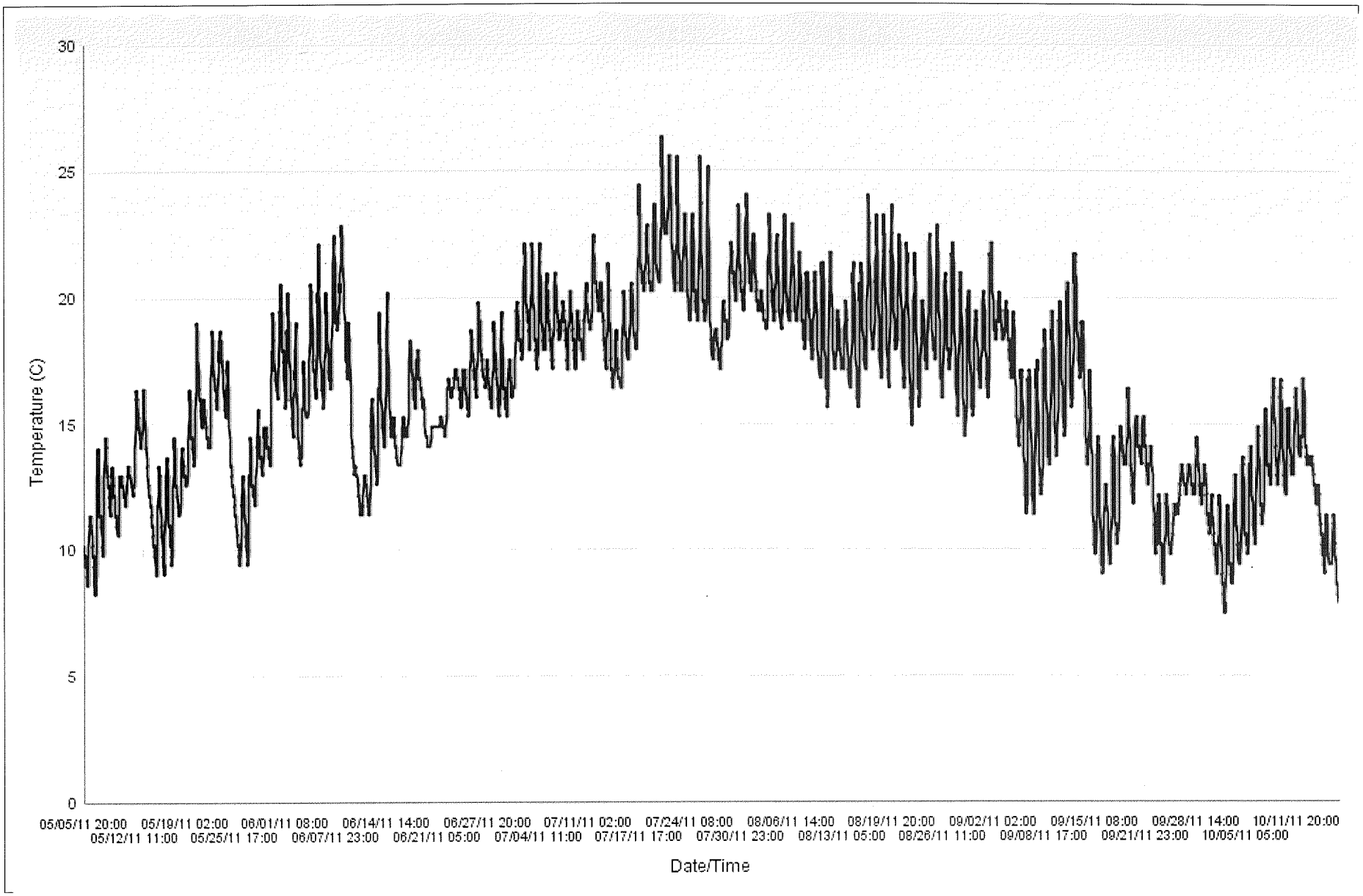
Figure 5.

Station ID: 10008204

Station Name: East Twin River - Hwy J

Fieldwork Event Start: 05/05/2011 18:00

Fieldwork Event End: 10/17/2011 10:00



SWIMS: Temperature for a Selected Fieldwork Event