Final Report and Implementation Plan for the *E. coli* Monitoring Study of the Black River, Pine Creek and Mill Creek in Berrien and Van Buren Counties, Michigan

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Introduction

The Black River Watershed (BRW) encompasses approximately 183,490 acres, or 287 square miles in Allegan and Van Buren Counties in southwestern Michigan. The BRW includes portions of Casco, Cheshire, Clyde, Ganges and Lee Townships in Allegan County; Arlington, Bangor, Bloomingdale, Columbia, Covert, Geneva, South Haven, and Waverly Townships along with the Villages of Breedsville and Bloomingdale and the Cities of Bangor and South Haven in Van Buren County (Fuller, 2009). According to the Michigan Center for Geographic Information 2002, the 1992 land use/land cover in the Black River Watershed is approximately 58% agriculture, 33% forested upland, 8% water and wetlands, and 1% developed. This project focused mostly on the lower sections of the Black River, from the confluence of Butternut Creek, then flowing west through the City of South Haven where it enters Lake Michigan.

The Paw Paw River Watershed (PPRW) encompasses approximately 446 square miles in Kalamazoo, Van Buren, Berrien and Kalamazoo Counties with the largest portion in Van Buren County (203,720 acres). In the PPRW, there are 39 governmental units including twenty-five townships, four villages, six cities, and one tribe (Pokagon Band of Potawatomi Indians). The watershed contains mostly agricultural (47%) and natural (45%) land cover, along with approximately 7% urban areas (SWMPC, 2008). This project focused on the Pine and Mill Creeks, both coldwater tributaries to the Paw Paw River, located in Berrien and Van Buren Counties. The Pine Creek watershed includes portions of Hartford and Keeler Townships and the City of Hartford. The Mill Creek watershed also includes portions of Hartford and Keeler Townships in Van Buren County, with additional lands in Bainbridge, Coloma, and Watervliet Townships and the City of Watervliet in Berrien County.

The intent of this study was to develop a monitoring program that would help identify sources of *E. coli* within the watersheds and identify appropriate best management practices (BMPs) to address those sources. The results of the monitoring program were expected to be used to focus control measures and target educational materials to specific land users in the watershed to reduce *E. coli* contamination sources, thereby improving water quality within the watersheds.

The goal of this Implementation Plan is to provide strategic direction for planned efforts, milestones, and a timeline to reduce *E. coli* in the Black and Paw Paw River Watersheds. The plan includes recommended and prioritized activities of location-specific BMPs for addressing pathogen sources and to meet the waste load allocations (WLAs) and to improve storm water runoff quality and ultimately reducing beach closings and ensuring rivers, streams and lakes are safe for partial and full body contact.

Threats and Impairments

Pathogens are microorganisms, consisting of bacteria, protozoans, or viruses, whose presence in water bodies can cause disease. Most waterborne pathogens are commonly found in waste from warm-blooded animals, including humans. Illnesses, such as gastroenteritis, are associated with swimming in contaminated water and the severity of illness depends on the amount of exposure and the type of pathogen a swimmer encounters. The U.S. Environmental Protection Agency (USEPA) has determined that *E. coli* and enterococci are appropriate indicators for the presence of waterborne pathogens in fresh water (USEPA, 2011).

Black River - The Black River Watershed Management Plan (BRWMP) indicates that the designated uses for Partial Body Contact and Total Body Contact are both threatened; however, Michigan Department of Environmental Quality's water quality standards for full and partial body contact are currently being met in the Black River. This means that water quality is currently being maintained for activities such as water skiing, canoeing, wading and swimming. Section 6.7.4-Bacteria/Pathogens in the BRWMP indicates that in the last study of fecal coliform bacteria in the Black River Drain (North Branch of the Black River), two sample locations had fecal coliform in excess of water quality standards (WQS).

Michigan's beaches represent an important recreational and economic resource. In a recent study by Michigan State University, Lake Michigan beaches were estimated to attract 13 to 26 million visits per year with an estimated economic impact of at least $1 to $2 billion per year.

Beach closings during the summer season of 2010 prompted additional sampling that revealed exceedances of *E. coli* WQS in Peterson and Phoenix Drains. Follow-up sampling in 2012 again found high levels of *E. coli*, mostly in the Phoenix Drain. The North Beach was closed in August 2013 after a storm event, measured as 3.4 inches in 24 hours in the area.

Pine and Mill Creeks - Section 303(d) of the federal Clean Water Act and the United States Environmental Protection Agency's (USEPA's) Water Quality Planning and Management Regulations (Title 40 of the Code of Federal Regulations, Part 130) requires states to develop Total Maximum Daily Loads (TMDLs) for water bodies that are not meeting water quality standards (WQS) (Alexander, 2009). Since waters in both Pine Creek and Mill Creek were found to be exceeding WQS, MDEQ developed a TMDL in 2009 for Pine and Mill Creeks, establishing a target level of 130 *E. coli* per 100 mL as a 30-day geometric mean and 300 *E. coli* per 100 mL as a daily maximum to protect the total body contact designated use for the TMDL reach from May 1 through October 31, and 1000 *E. coli* per 100 mL as a daily maximum year-round to protect the partial body contact designated use (Alexander, 2009).

Cattle feces are regarded as the most pathogenic to humans, second only to human waste.

Impacted Areas and TMDL Reaches

The South and North Beaches in South Haven have been impacted by high *E. coli* levels, resulting in closed beaches for a day to several weeks in 2010 and closings continue to happen occasionally especially following rain events. These closings have economic, environmental and social implications for the City, which is eager to find the possible sources of *E. coli* and reduce contamination of the beaches.

Studies established the TMDL reach for Pine Creek from the Paw Paw River confluence upstream to 66th Avenue (4.17 miles) and from 66th Avenue upstream to headwaters (5.81 miles) for a total of 9.98 miles. The TMDL reach for Mill Creek is from the Paw Paw River confluence upstream to its origin, for a total of 12.77 miles.

Project Location and Study Design

The Black River originates in Allegan and Van Buren Counties, converging east of the City of South Haven, where it then flows through the City and enters Lake Michigan. The Watershed encompasses approximately 183,490 acres (287 square miles). Pine Creek and Mill Creek are tributaries of the Paw Paw River Watershed, located in Berrien and Van Buren Counties. See Figure 1 for a map of the three watersheds that are subject of this study. (Fuller, 2009).

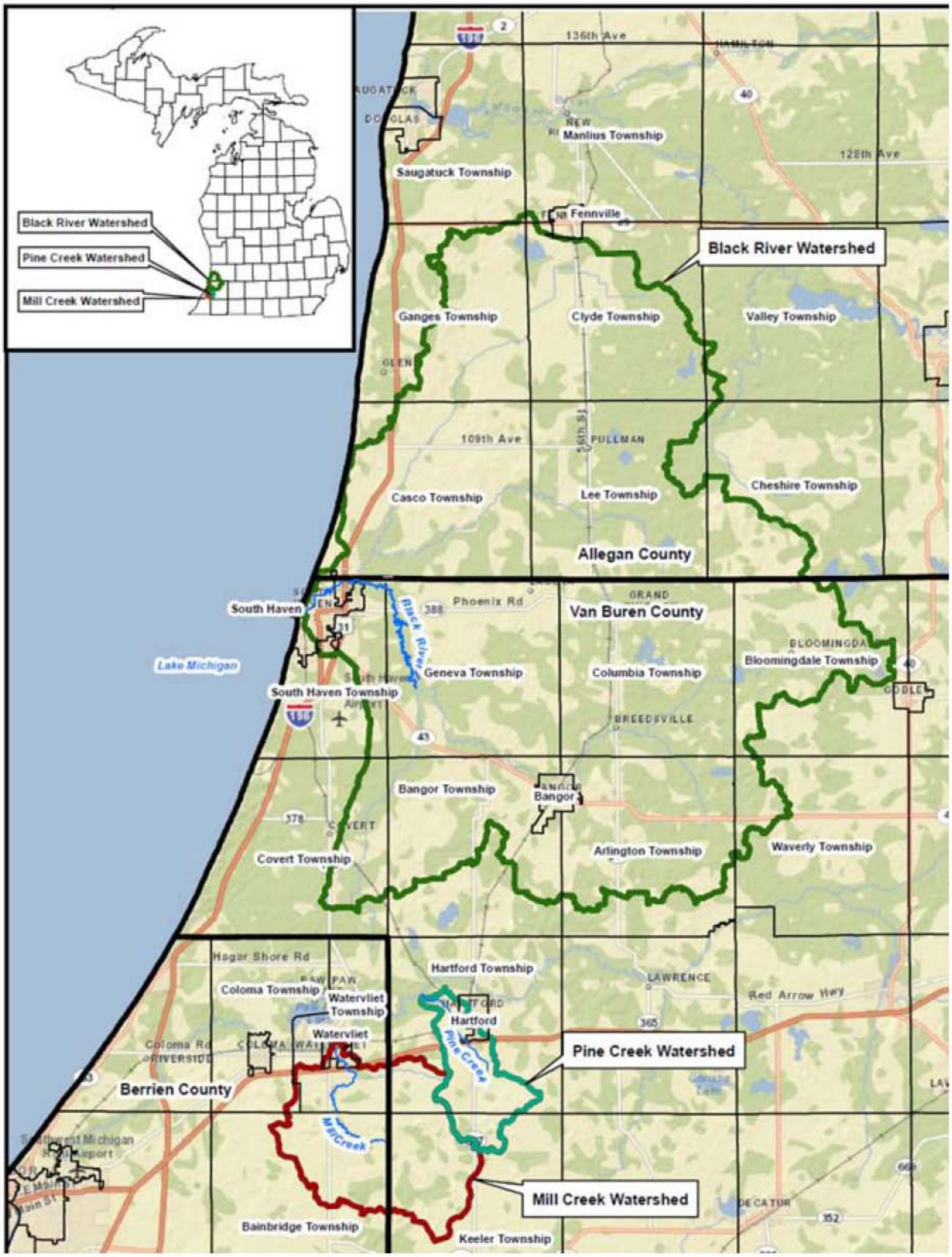


FIGURE 1 – Project Areas

A committee of stakeholders was convened to review existing data, land uses and known and potential sources of *E. coli*. The committee provided input on study objectives and sampling locations based on local knowledge and previous investigations. The committee continued to meet during the project to review results and discuss implementation options. The committee was also instrumental in choosing samples to conduct the Microbial Source Tracking (MST) analysis since with limited funds these could only be done on a limited number of samples collected. Committee members were also instrumental in helping to transport samples to Hope College for the three wet weather sampling events.

Committee/Stakeholders

Name	**Representing**
Nancy Carpenter	Berrien Conservation District
Ken Priest/Debbie Skalecki/Laird Willard	Berrien County Health Department
Rosemary Insidioso/ Jennifer Carpio-Zellar	City of Bangor
Yemi Akinwale	City of Hartford
Roger Huff /Larry Halberstadt/ Steve Oosting/ Paul Vandenbosch /Mayor Burr	City of South Haven
Wendy Ogilvie	FTCH
Wayne Gleiber	Great Lakes Scientific
Ron Sefcik	Hartford Township
Dr. Pickart	Hope College
Chris Bauer/Bruce Washburn	Michigan Department of Environmental Quality
Grant Poole/ Matthew Bussler	Pokagon Band of Potawatomi Indians
David Mulac/ Art Bolt	South Haven resident
Marcy Colclough/Kris Martin	Southwest Michigan Planning Commission
Dave Foerster/Kevin Haight/Bette Pierman	Two Rivers Coalition
Sam Ewbank	Two Rivers Coalition
Matt Meersman	Van Buren Conservation District/Friends of the St. Joe River
Don Hanson	Van Buren County Commissioner
Joe Parman/Peter VanDopp	Van Buren County Drain Commissioner
Mick McGuire/ George Friday	Van Buren/Cass Health Department

*The following entities were invited and sent information, but did not attend meetings: South Haven, Geneva, Keeler, Watervliet, Bainbridge, Bangor Townships, Watervliet City, Berrien County Drain Commissioner, Berrien and Van Buren Farm Bureaus.

Black River Watershed

The study objective for the Black River Watershed was to determine which sites had the highest levels of *E. coli* and what were the sources of *E. coli*. A total of seven sites in the Black River and its tributaries were sampled. The sites included two on the Phoenix Drain, two on tributaries to the Black River within the City of South Haven, one on the North Branch of the Black River, one on the South Branch of the Black River and one on the confluence of Butternut Creek and Tripp Drain. The seven sampling locations in the Black River Watershed are listed below in Table 1 and shown in Figure 2. Wet weather samples were collected during three significant rain events between June 1, 2012 and October 31, 2012. A significant event was considered to be at least 0.25 inches of rainfall within a 24-hour period. Each wet weather sampling event consisted of collecting triplicate samples at each of the seven stations during the rain event. Sampling was done from the most upstream location first and then working downstream to collect subsequent samples. No dry weather sampling was done in the Black River Watershed.

Table 1 - Sample Locations in Black River Watershed

Sample ID	Water Body	Site Description	Latitude, Longitude
BR-01	Unnamed tributary	Kal-Haven Trail State Park	42.41315, -86.25957
BR-02	Phoenix Drain	Outlet	42.40856, -86.27235
BR-03	Unnamed drain	Outlet near Woodland Marina	42.41431, -86.26447
BR-04	North Branch Black River	Baseline Road	42.42095, -86.24369
BR-05	South Branch Black River	71-1/2 Street	42.41699, -86.24003
BR-06	Butternut Creek	Downstream of Creek and Tripp Drain confluence	42.40958, -86.21079
BR-07	Phoenix Drain	Upstream	42.39485, -86.26610

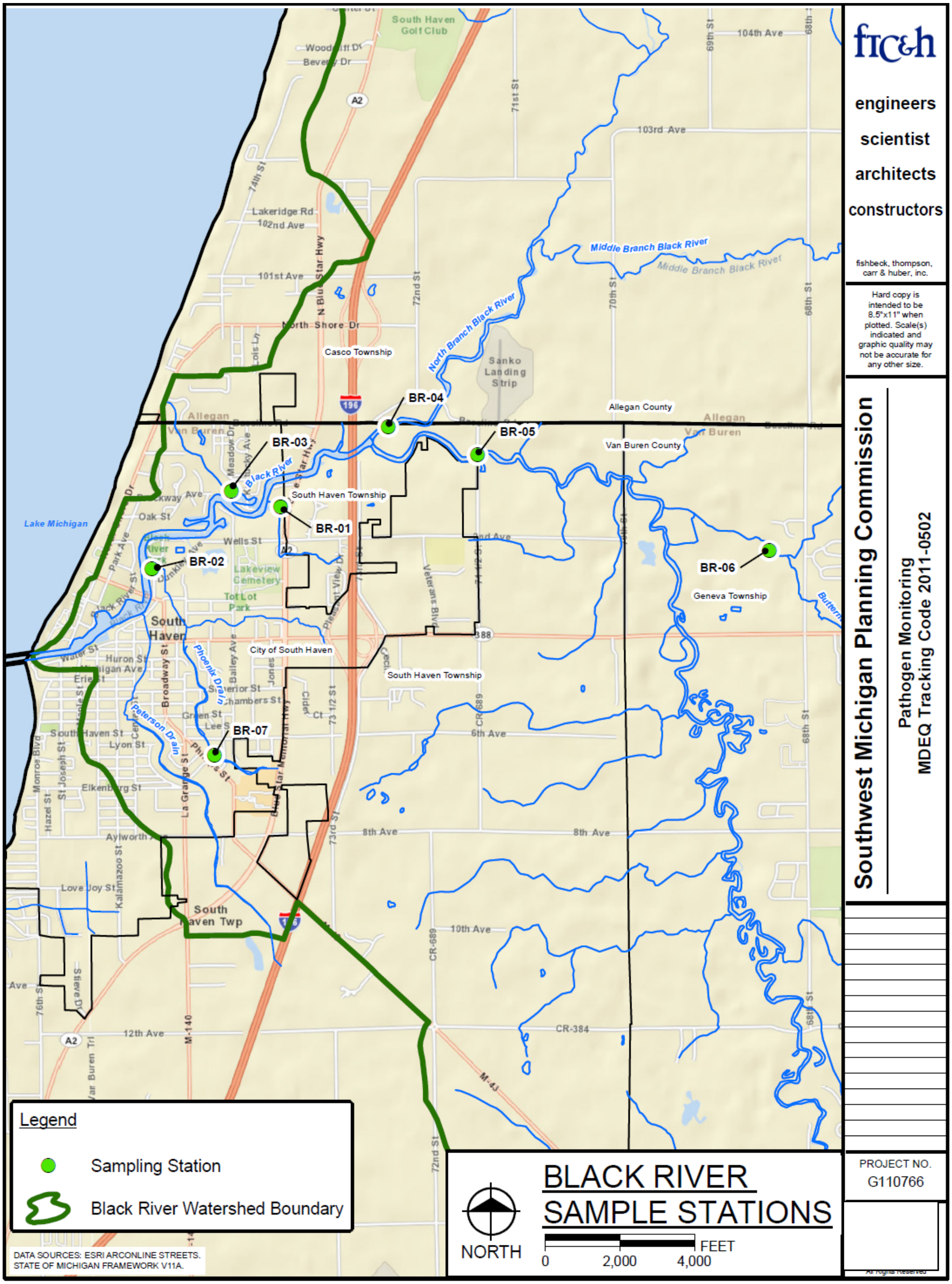


Figure 2: Black River Watershed Sampling Locations

Pine Creek and Mill Creek Watersheds

The study objective for Pine Creek was to determine if the major source of *E. coli* was from the urbanized area or agricultural operations. For Mill Creek, the study objective was to see how *E. coli* responded to flow and how it correlated with the hydrograph. Another objective was to determine if Flaherty Park, where the public has easy access to the creek, was impaired by *E. coli*. The four sampling locations in the Pine Creek Watershed and the one sampling point in the Mill Creek Watershed are listed below in Table 2 and shown in Figure 3. Both dry and wet weather sampling was conducted on Pine Creek at sites PC-01 and PC-02, which are downstream and upstream of the City of Hartford respectively. Only wet weather sampling was conducted for PC-03 and PC-04. For Mill Creek, wet weather sampling was conducted 3 times during a 24 hour storm. Originally it was thought that it would be done at 12 hour intervals, but with scheduling difficulties, samples were collected at 9:30am and around 6:00pm on the first day and between 9:00 and 10:00 am on the next day. Therefore, the samples were approximately collected at hour 0, 9 and 24 instead of 0,12 and 24. For Mill Creek, the correlation of pathogen levels and the hydrograph was not done because the flow monitoring equipment did not record the stream flow data continuously over the study period due to late installation, battery failure, vandalism, debris interference and low velocities (<0.3 f/s). However, water levels were recorded during the *E. coli* sampling, so the recorded water levels were correlated with the *E. coli* results.

Table 2 - Sample Locations in Pine and Mill Creek Watersheds

Sample ID	Water Body	Site Description	Latitude, Longitude
PC-01	Pine Creek	Red Arrow	42.20580, -86.18204
PC-02	Pine Creek	North side of I-94	42.19152, -86.17091
PC-03	Pine Creek	66th Avenue	42.17877, -86.15768
PC-04	Pine Creek	72nd Avenue	42.15709, -86.17353
MC-01	Mill Creek	Flaherty Park	42.19045, -86.25802

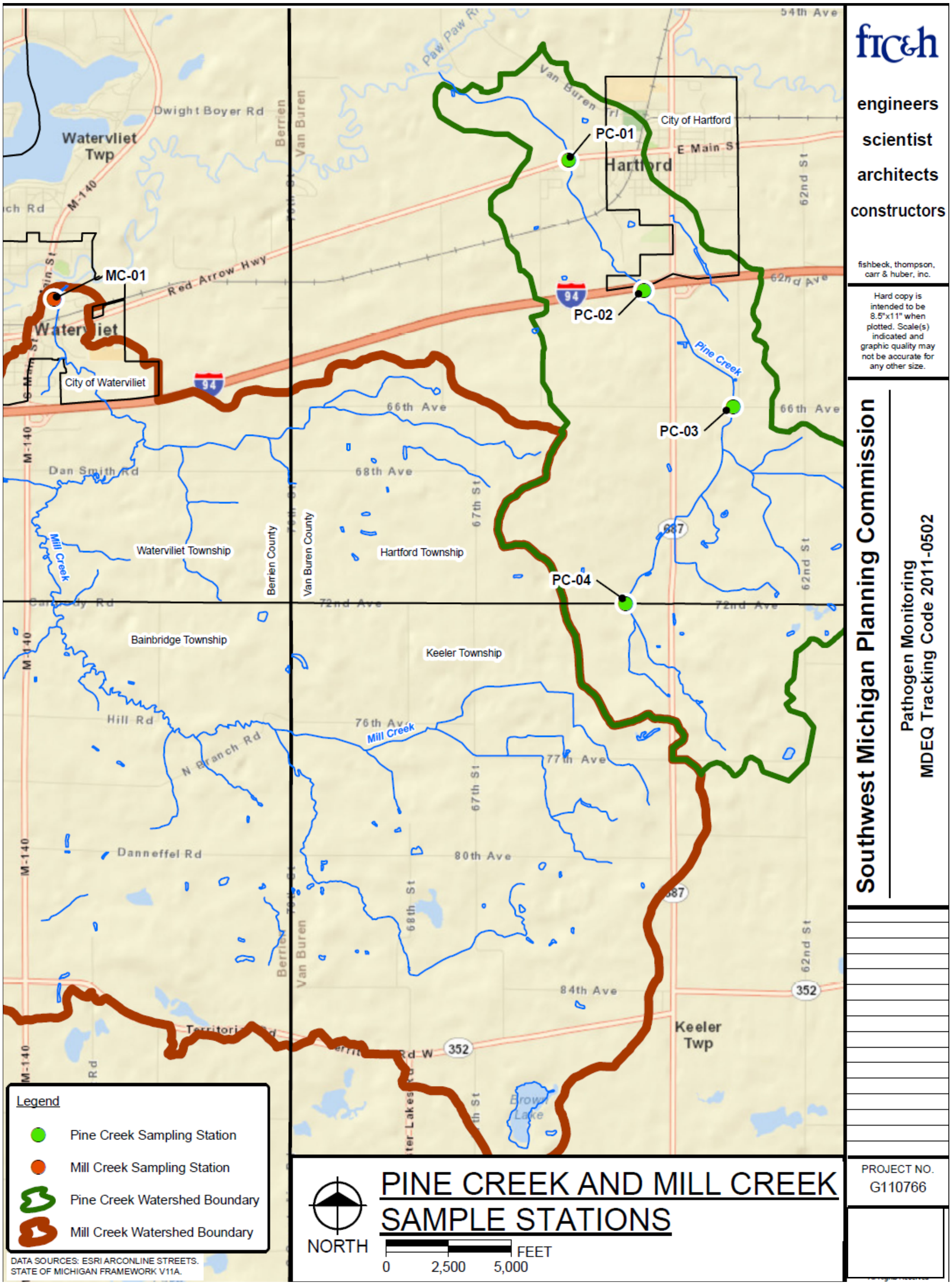


Figure 3: Pine and Mill Creek Sampling Locations

Sampling Results and Suspected Pollutant Sources

Suspected Pollutant Sources from Water Quality Sampling

Although the Black River does not have an established Waste Load Allocation (WLA), all water bodies in the state must meet water quality standards for *E. coli*. The WLA for *E. coli* is always set as the WQS, since *E. coli* is a concentration-based pollutant, as opposed to a loading-based pollutant, such as phosphorus. The Black River WMP identified septic systems, storm water runoff, livestock access, and high waterfowl populations as known sources of pathogens in the watershed.

For Pine and Mill Creeks, possible sources of *E. coli* include runoff from pastureland and land application of manure, failing septic systems, illicit connections to storm sewers and drains, and inputs from wildlife. The WLA for all of these potential sources is the water quality standard for *E. coli (geometric mean of* 300 *E.coli*/100mL).

During this project, samples collected during dry and wet weather were analyzed by using the IDEXX Colilert® reagent method to attempt to classify conditions that would be favorable for runoff from these different sources and determine the most likely sources. High levels (>2,420 *E. coli* per 100 mL) of *E. coli* were detected in all of the samples collected during the July wet weather samples, the Mill Creek wet weather sample in August, and the Pine Creek (PC-03) wet weather sample in October. The results for the July wet weather sampling could actually be much higher, but the lab did not dilute the samples enough to get an accurate reading of the *E. coli* levels. Samples collected in dry weather in Pine Creek at PC-01 and PC-02 were also high. For all the samples taken during 2012, water quality standards for *E. coli* exceeded the 30 day geometric mean 47 out of 51 times (one of the sample months for one location was removed because of quality control issues). There were 20 out of 21 sets of samples that exceeded the 30 day geometric mean in the Black River Watershed, 19 out of 21 in Pine Creek and 8 out of 9 in Mill Creek and they were all in October.

The difference of the results between the wet and dry samples in the Pine Creek Watershed indicates that the sources of *E. coli* are most probably coming from stormwater runoff, carrying pollutants over land and into the streams, rather than persistence sources such as leaking septic tanks, illicit connections or wildlife. Stormwater runoff can be contaminated by manure applied to fields before a rain event or large herds of livestock having direct access to waterways. The results from Pine Creek near Hartford (PC-01 and PC-02) may indicate issues with leaking septic tanks or illicit connections because of the dry weather results. Mill Creek's results indicate that runoff is contributing to *E. coli*, but since no dry weather sampling was conducted for Mill Creek no conclusion can be made regarding the possibility of septic tanks and illicit discharges from municipal sewers being a source. Dry weather testing is needed to better understand all of the potential sources. The correlation of *E. coli* with water levels show that the first flush after the rain events in July and August had the highest levels of *E. coli*. This was not the case in October where overall the levels of *E. coli* were lower than in July and August. Lastly, MDEQ provided manure spreading information for the Pine Creek Watershed (see Appendix). Manure spreading did not occur close to the sampling dates so no conclusion can really be made from the information.

See results in the following tables and graphs and detailed results in the Appendix.

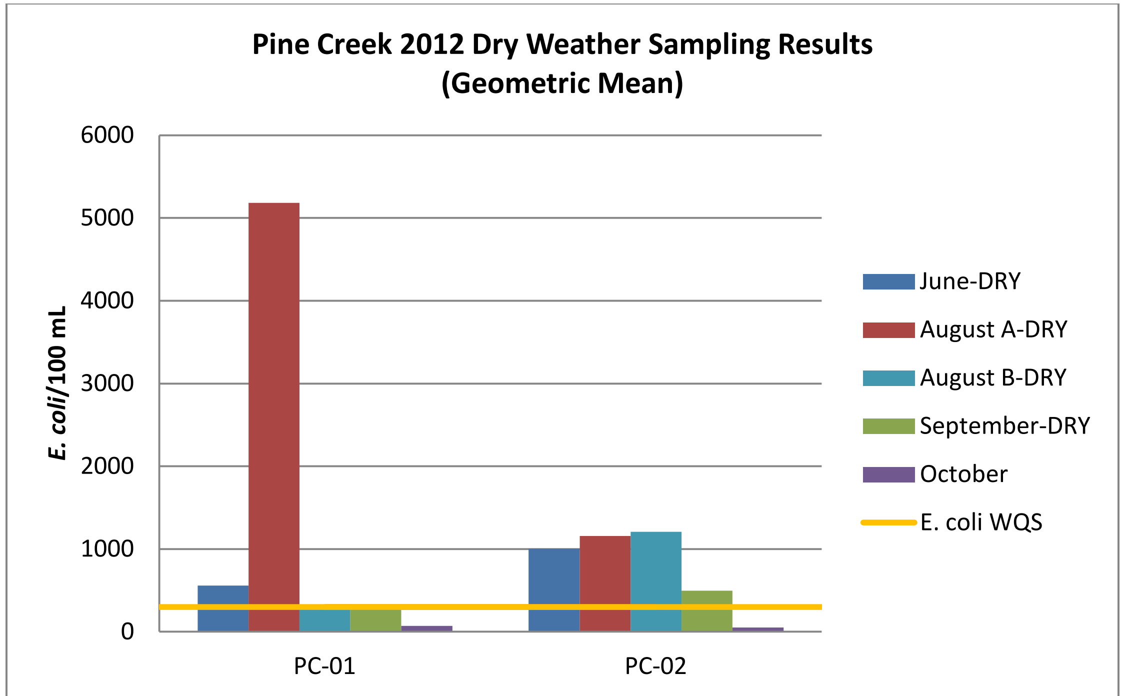
Black River (BC), Pine Creek (PC), and Mill Creek (MC) Wet and Dry Weather Geometric Mean Sampling Results

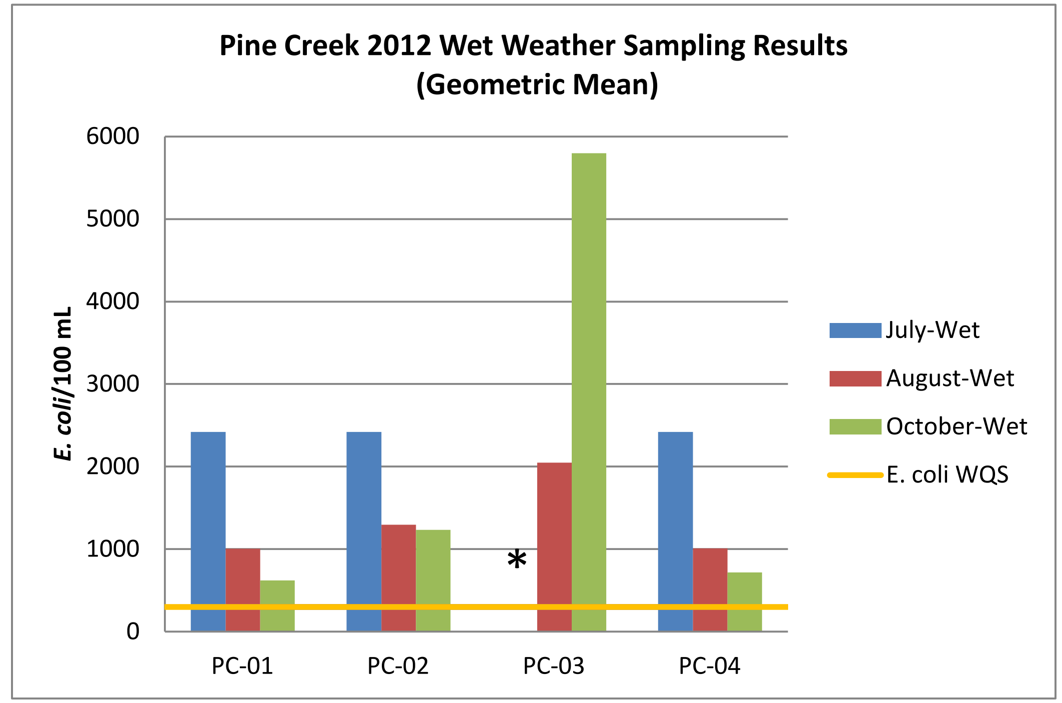
Date	Type of Sampling	BC-01	BC-02	BC-03	BC-04	BC-05	BC-06	BC-07	PC-01	PC-02	PC-03	PC-04	MC-01-0	MC-01-12	MC-01-24
		Sampling Stations													
June-DRY	June-DRY water levels (feet)								1.6	1.0					
	6/28/12 - *E.coli* Results								557.6	1003.7					
July-Wet	July-Wet – water levels (feet)	0.25	1.4	0.125	4	3.8	0.58	0.83	1.42	1.17	0.75	0.25	0.78	0.85	0.65
	July-Wet - *E.coli* Results	2420*	1897.8	2165.1	2420*	2265.7	2420*	2420*	2420*	2420*	NA**	2420*	8653.5	5192.5	3035.6
August A-DRY	August-DRY water levels (feet)								1.33	0.73					
	8/3/12 - *E.coli* Results								5184.2	1156.6					
August B-DRY	August-DRY water levels (feet)								0.8	0.5					
	8/30/12 - *E.coli* Results								331.5	1207.6					
August-Wet	August-Wet – water levels (feet)	0.25	0.58	0.17	3.4	3.2	0.42	0.5	1.17	0.67	1.12	0.17	0.73	0.83	0.71
	August-Wet - *E.coli* Results	322.2	1653.9	1363.7	1689.6	597	523.8	424.4	1006.7	1295.9	2048.7	1008.5	13924.8	5593.4	1724.7
September-DRY	September-DRY water levels (feet)								1	0.5					
	9/25/12 - *E.coli* Results								323.5	498.6					
October-DRY	October-DRY water levels (feet)								1	0.7					
	10/22/12 - *E.coli* Results								72.2	52.5					
October-Wet	October-Wet – water levels (feet)	0.8	0.3	0.8	3	4	0.8	0.8	1.8	1.8	2	0.5	1.04	1.33	1.125
	October-Wet - *E. coli* Results	249.1	709.4	967.6	497.2	562.2	458.3	568.9	620.7	1235.1	5795.8	718.5	773.1	1557.7	234.9

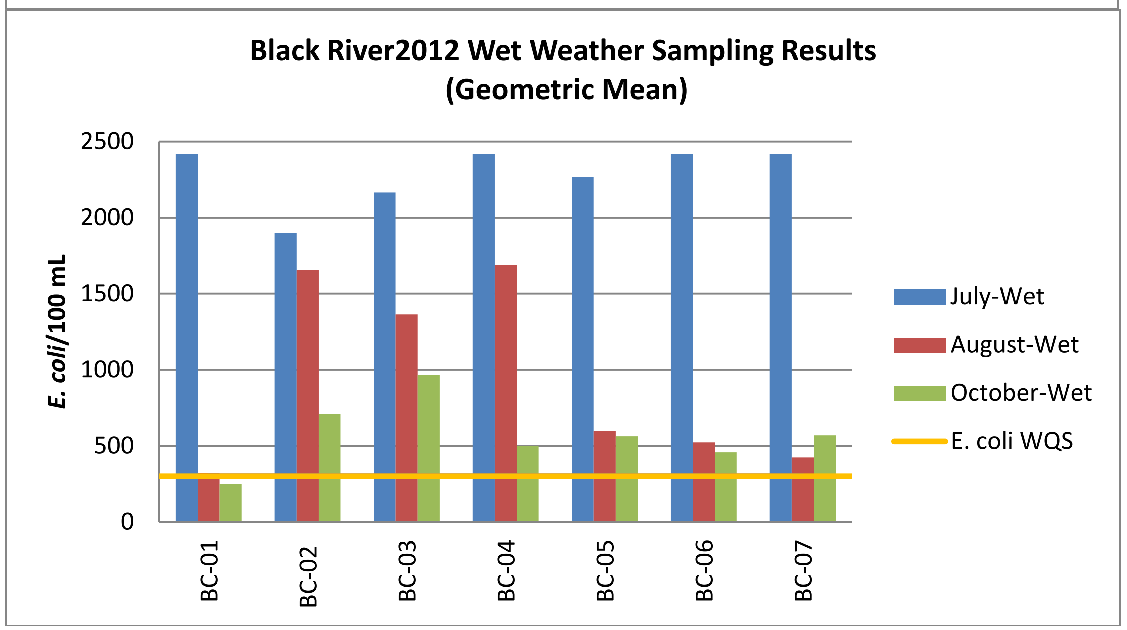
*These samples were not diluted correctly by the lab so the *E.coli* count is > 2420 *E.coli*/100 mL. The actual count may be higher than 2420.

**Two samples were reported with uncharacteristically low MPN values (12-7-19-PC-03-C(I) and 12-7-19-PC-03-R(I) and are probably analysis errors. IDEXX trays for these samples showed unaccountably low number of large cell positives. The two MPN values should be regarded as experimental error and disregarded. Therefore, a geometric mean could not be calculated for PC-03 for the July 19, 2012, wet weather event.

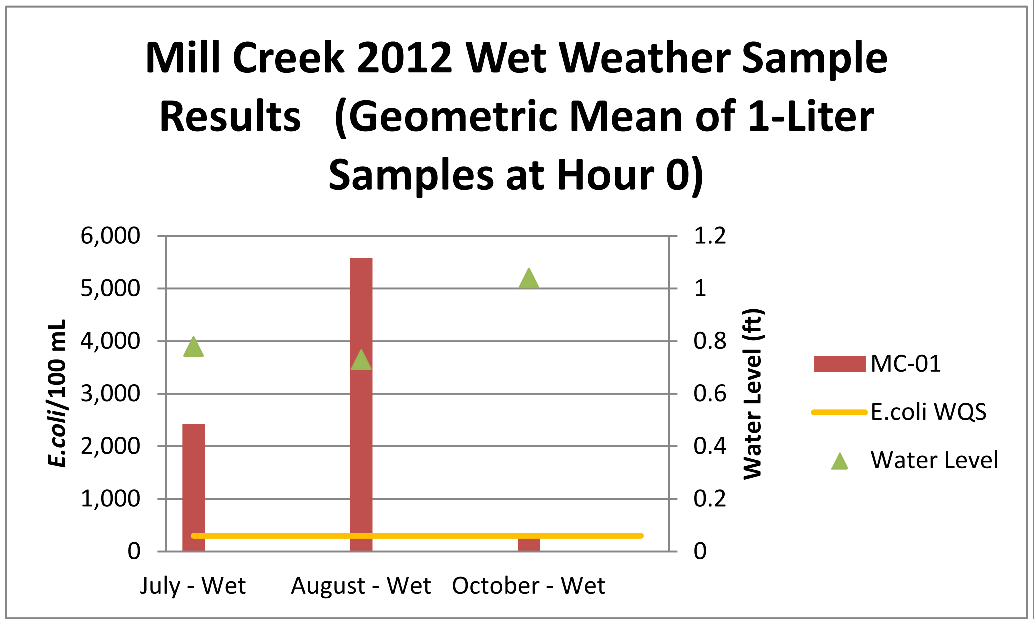
Exceeds *E. coli* Water Quality Standard (300 *E.coli*/100mL) | Does Not Exceed *E. coli* Water Quality Standards

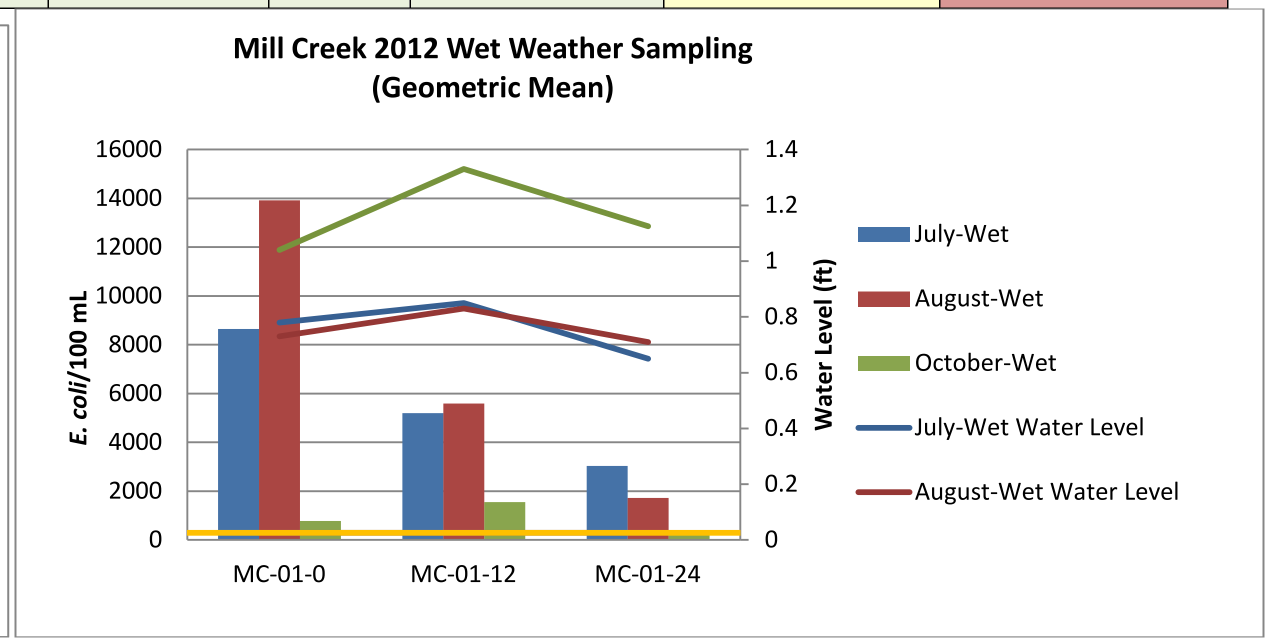

Pine Creek 2012 Dry Weather Sampling Results
(Geometric Mean)
E. coli/100 mL
6000
5000
4000
3000
2000
1000
0
PC-01
PC-02
June-DRY
August A-DRY
August B-DRY
September-DRY
October
E. coli WQS


Pine Creek 2012 Wet Weather Sampling Results
(Geometric Mean)
E. coli/100 mL
6000
5000
4000
3000
2000
1000
0
*
PC-01
PC-02
PC-03
PC-04
July-Wet
August-Wet
October-Wet
E. coli WQS


Black River2012 Wet Weather Sampling Results
(Geometric Mean)
E. coli/100 mL
2500
2000
1500
1000
500
0
BC-01
BC-02
BC-03
BC-04
BC-05
BC-06
BC-07
July-Wet
August-Wet
October-Wet
E. coli WQS

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Average Water Level (ft)	Water Quality Standard (WQS) (*E.coli*/100mL)	Geometric Mean (from *E.coli*/100 mL)	Exceeds WQS
Mill Creek	12-7-19-MC-01-0-L(I)		7/19/2012	9:30 AM	2420		300		
	12-7-19-MC-01-0-C(I)		7/19/2012	9:35 AM	2420		300		
	12-7-19-MC-01-0-R(I)	July - Wet	7/19/2012	9:40 AM	2420	0.78	300	2,420.0	YES
	12-8-17-MC-01-0-R(I)		8/17/2012	9:25 AM	5,794		300		
	12-8-17-MC-01-0-C(I)		8/17/2012	9:27 AM	4,884		300		
	12-8-17-MC-01-0-L(I)	August - Wet	8/17/2012	9:29 AM	6,131	0.73	300	5,577.4	YES
	12-10-18-MC-01-0-L(I)		10/18/2012	9:27 AM	350		300		
	12-10-18-MC-01-0-C(I)		10/18/2012	9:22 AM	285		300		
	12-10-18-MC-01-0-R(I)	October - Wet	10/18/2012	9:25 AM	301	1.04	300	310.8	YES





July samples reported >2420, however number 2420 was used for calculation/charting purposes

Suspected Pollutant Sources from Microbial Source Tracking

An additional analytical method of molecular source tracking was conducted by Hope College to further investigate and identify the sources of *E. coli*. Samples were again measured for viable *E. coli* using the IDEXX Colilert® reagent, which revealed bacterial counts exceeding water quality standards (WQS) for the daily maximum load throughout the watershed and over most of the sampling events. These amounts were necessary in order to conduct the follow up source tracking methods.

Bacteroides organisms do not require oxygen for growth and unlike *E. coli* and other coliforms, they cannot grow in the environment. Testing for Bacteroides in surface water relies not on culture of live organisms, as explained above, but on molecular detection of the bacteria's DNA by isolating the DNA from an environmental sample and testing that DNA by a specific method called quantitative PCR (qPCR). qPCR testing of Bacteroides can distinguish differences in DNA not only between one species and another but, between different sub-strains, or fingerprints, of the bacteria based on the host mammal species. The Bacteroides fingerprint is very short-lived in natural environments (such as a stream or river), unlike *E. coli*, with a half-life of 1-3 days, depending on the temperature of the water. The presence of the Bacteroides fingerprint in a water sample is thought to be a more reliable indicator of a recent and direct fecal discharge into the water than the presence of *E. coli* (Pikaart, 2012).

Four categories of Bacteroides were identified for this study:

1) A general or non-host specific primer set to indicate fecal contamination by any mammal.
2) A human-specific set to indicate a human host
3) A cow-specific set to indicate a bovine host either from cow feces or manure lagoons, with cross-reactivity to other ruminants in many cases.
4) A pig-specific set to indicate swine feces or manure sweepings from a hog farm

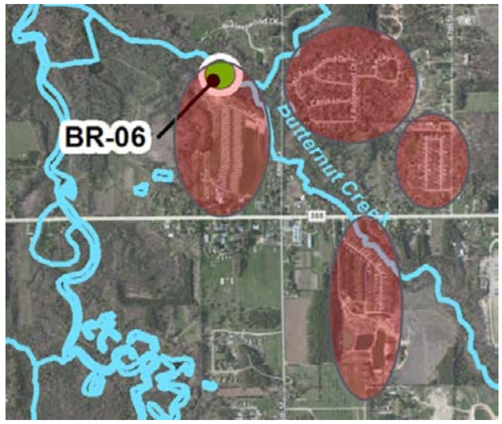
Direct fecal contamination indicated by Bacteroides presence in the Black River and Pine Creek samples was found to be overall quite low. Most samples analyzed fell below the selected "cut-off" limit assumed for meeting the assumed standard, recorded as below detection limit (BDL). Three sites; however, did measure Bacteroides DNA as approaching or exceeding limits relative to the assumed standard, all of which were recorded during the July rain event sampling.

One site on the Black River (BR-06) proved positive in the host-specific assays, matching the human host. Two sites on Pine Creek, PC01 and PC02, proved positive in the host-specific assays, matching the cow host. Additional information about the findings and assumptions can be found in the Appendix. All other sites tested were below the "cut-off" and were considered of minimal risk by direct fecal contamination.

Summary of Pollutant Sources

Pollutant sources in the Black River and Paw Paw River are not obvious and finding the exact sources of *E. coli* will need to continue. As demonstrated in this project, overall *E. coli* contamination was not detected at very high levels, except in the July wet weather event, and a few samples during the other storm events. Bacteroides were not found in a widespread manner across the sampling sites and sampling events.

One site in the Black River (BR-06) matched human-specific material. Potential sources include mobile home sites, residential neighborhood and a campground, as illustrated below.



Bacteroides DNA in two sites in Pine Creek, (PC-02 and PC-04) matched cow (or other ruminant) material as illustrated below. Potential sources include fields adjacent to the stream on which manure is applied, as illustrated below. The green dots represent filed tiles that connect field drainage to the surface waters. DNA matching to Bacteroides from a pig host was not found at any of the sites.

Recommended initiatives to address the highest potential sources based on the results of the study are described later in this report.

Recent research has demonstrated that in stream and near-shore sediment may act as a continuous source of bacterial loading, rather than bacterial loading being solely a temporal, storm-driven phenomenon. A similar study to one described here for nearby Dunes Creek may be useful for the Black River Watershed. A study of Dunes Creek, a small Lake Michigan coastal stream that drains sandy aquifers and wetlands of Indiana Dunes, has shown that sediments play a role in the widespread and consistent occurrence of *E. coli*. Dunes Creek has chronically elevated *Escherichia coli* levels along the bathing beach near its outfall. This study sought to understand the sources of *E. coli* in Dunes Creek's central branch. A systematic survey of random and fixed sampling points of water and sediment was conducted over 3 years. *E. coli* concentrations in Dunes Creek and beach water were significantly correlated. Weekly monitoring at 14 stations during 1999 and 2000 indicated chronic loading of *E. coli* throughout the stream. Significant correlations between *E. coli* numbers in stream water and stream sediment, submerged sediment and margin, and margin and 1 meter from shore were found. Median *E. coli* counts were highest in stream sediments, followed by bank sediments, sediments along spring margins, stream water, and isolated pools; where in forest soils, *E. coli* counts were more variable and relatively lower. Sediment moisture was significantly correlated with *E. coli* counts. Direct fecal input inadequately explains the widespread and consistent occurrence of *E. coli* in the Dunes Creek watershed; long-term survival or multiplication or both seem likely. The authors conclude that (i) *E. coli* is ubiquitous and persistent throughout the Dunes Creek basin, (ii) *E. coli* occurrence and distribution in riparian sediments help account for the continuous loading of the bacteria in Dunes Creek, and (iii) ditching of the stream, increased drainage, and subsequent loss of wetlands may account for the chronically high *E. coli* levels observed. (Byappanahalli, 2003).

Potential Implementation Activities

E. coli poses a public health risk diminishing the value of Southwest Michigan's rivers and beaches. A few specific sites were identified at which practices could be put in place to reduce runoff with *E. coli*. Other management strategies could be implemented throughout the watersheds to minimize potential *E. coli* runoff. Lastly, this report recommends an education and outreach strategy that addresses all of the potential sources and targets information to those interest groups or stakeholders within the watershed.

Water Quality and Land Management Initiatives

Black River Watershed

The suspected sources in the Black River Watershed include failing septic systems, stormwater runoff and land application of manure. Specific recommendations to address these sources include:

Failing Septic Systems

- Identify and repair/replace failing or improperly installed or functioning septic systems, lagoon systems and sanitary pumping stations, using specially trained dogs (http://www.ecsk9s.com/) or other methods to identify human inputs. Focus investigation efforts in the following areas.
  - Single family homes on N. Ridgewood Drive, Allpine Street, Carol Lane, S. Ridgewood Drive, E. Ridgewood Drive
  - Mobile Home Parks north of County Road 388 on 67th Street and south of County Road 388 east of 68th Street.

Stormwater (Polluted) Runoff Reduction

- With new residential, commercial and industrial development encourage or require low impact development techniques (rain gardens, porous parking, green parking lots, infiltration basins, rainwater harvesting) that slow down, spread out and infiltrate stormwater water as much as possible before being released to waterways. (See www.swmpc.org/lid/asp for more information.)
- In already developed areas, install commercial hydrodynamic separation systems (Vortechs or Stormceptor) at stormwater inlets or outfalls to settle and filter out pollutants before discharging to waterways.

- Restore and protect wetlands that rank high for improving water quality (especially *E. coli*) as indicated by the Landscape Level Wetland Function Assessment. (Contact Van Buren Conservation District or Southwest Michigan Planning Commission for more information.)
- Plant native vegetation at outlets of small tributaries to redirect flow and have plants filter out pollutants.
- Investigate possibility of incorporating created wetland or infiltration cells in the landscape to filter water entering beach areas from small pipes or ravines. (See Appendix 2).
- Use altered beach grooming strategies, such as deep beach grooming without leveling to promote drying out of sand.

Farming Practices

- Adjust manure spreading plans to avoid fields adjacent or connected to surface waters in early spring to prevent runoff from large spring rain events.
- Utilize drainage tile management system to control flow through drain tiles and hold runoff from storm events. (See website for more details: https://engineering.purdue.edu/SafeWater/Drainage/ or http://www.nrcs.usda.gov/wps/portal/nrcs/detail/ia/newsroom/releases/?cid=nrcs142p2_008692.)

Partners for implementation include the Van Buren County Health Department, Van Buren Conservation District, Natural Resources Conservation Service (NRCS), Southwest Michigan Planning Commission, City of South Haven, South Haven Township, Geneva Township, Two Rivers Coalition, landowners and Michigan Department of Environmental Quality.

Pine and Mill Creek Watersheds

The suspected sources in the Pine and Mill Creek Watersheds include failing septic systems and land application of manure. Specific recommendations to address these sources include:

Failing Septic System

- Identify and repair/replace failing or improperly installed or functioning septic systems, lagoon systems and sanitary pumping stations, using specially trained dogs (http://www.ecsk9s.com/) or other methods to identify human inputs. Focus investigation efforts in the following areas.
    - First priority is the subdivision on 66th Street, south of Red Arrow highway west of the City of Hartford.
    - Second priority is the American Campground/RV Park on County Road 388 west of 68th Street. (Health Department approved a new septic system within the last 4 years and annual inspections are not indicating any issues).

Farming Practices

- Revise and/or enforce manure spreading plans to limit amount of manure applied, especially in buffer areas adjacent to the stream in which no manure can be applied.
    - Fields surrounding Hartford Dairy in the area of County Road 362 and 64th Street
    - Fields south of the City of Hartford, between 66th Street and 63rd Street, north and south of I-94.

- Utilize drainage tile management system to control flow through drain tiles and hold runoff from storm events. in Hartford Township, Sections 12 and 33. (See website for more details: https://engineering.purdue.edu/SafeWater/Drainage/ or http://www.nrcs.usda.gov/wps/portal/nrcs/detail/ia/newsroom/releases/?cid=nrcs142p2_008692.)
- Ensure producers are inspecting drain tile outlets after rain events to monitor if manure is being discharged to creeks or drains.
- Install buffer strips along all streams to filter runoff
- Restore and protect wetlands that rank high for improving water quality (especially *E. coli*) as indicated by the Landscape Level Wetland Function Assessment. (Contact Van Buren Conservation District or Southwest Michigan Planning Commission for more information.)
- Utilize soil conservation practices that stabilize soil such as cover crops and conservation tillage when possible.

Partners for implementation include the NRCS, Van Buren and Berrien Conservation Districts, Southwest Michigan Planning Commission, Van Buren and Berrien Health Departments, Hartford City and Township, Two Rivers Coalition, Michigan Department of Environmental Quality, homeowners and producers.

Watershed-Wide Collaboration Initiatives (Managerial Strategies)

Managerial strategies are most effective if implemented at a municipal or county level, which often allows enforcement or jurisdictional authority over those practices. Examples of managerial strategies for septic and municipal sanitary systems that could be employed include:

- Adopt a county-wide time-of-sale inspection ordinance for septic systems.
- Adopt and enforce a local ordinance requiring hook up to sanitary system if available within a feasible distance.
- Adopt and enforce a local illicit discharge ordinance that prohibits septic systems from connecting directly to surface water.
- Conduct dye testing in areas suspected to have failed septic systems (this may be difficult because cooperation is needed from homeowners).
- Conduct investigations of storm and sanitary systems to ensure no illicit connections with dye and/or smoke testing or cameras.

Examples of managerial strategies for manure application that could be employed include:

- Investigate if local ordinances can be adopted that require all producers applying manure to have spreading plans.
- Review GAAMPs for timing, amount, and location of allowable manure application.
- Determine if updates are needed to current CAFO permits when they are renewed to address issues that may be leading to *E. coli* reaching waterbodies.

Partners for implementation include the Van Buren and Berrien County Health Department, Van Buren and Berrien Conservation Districts, NRCS, Southwest Michigan Planning Commission, Two Rivers Coalition, Michigan Department of Environmental Quality, City of South Haven, South Haven Township, Geneva Township, Hartford City, Hartford Township, Watervliet City, Watervliet Township, Van Buren and Berrien County Board of Commissioners, homeowners, Realtors, and Chamber of Commerce.

Outreach Initiatives (Public Education and Community Engagement)

Education of residents is often the most important activity to decrease bacteria loads in a watershed. New and existing education and outreach activities are suggested that can be implemented to increase awareness and have stakeholders take personal responsibility for reducing pathogen contamination. The Southwest Michigan Planning Commission has several flyers, articles and a display board that can be utilized by partners. Other recommended educational materials and activities to increase understanding about *E. coli* inputs from septic systems and methods to use for distribution of the materials include:

- "A Homeowner's Guide to Septic Systems" USEPA (#832B-02-006), color brochure, 5 x 5 tri-fold – Distribute through local governments and Health Departments through one-on-one contact and having available in offices.
- "A Homeowner's Guide to Septic Systems" USEPA (#832B-02-005), color booklet, 19 pages - Distribute through one-on-one contact by handing out brochure in office and at educational events and workshops.
- "Do you know where your septic system is?" Michigan Groundwater Stewardship Program, color brochure, 3 x 8 double-fold – Distribute through local government by mailing brochure with tax bill or welcome packet for new homeowners.
- "There's no drama 'til the septic system goes" USEPA, black and white flyer, 3 x 8 single sheet – Distribute through local government by mailing brochure with tax bill.
- "Managing Your Septic System" MSU Extension (#WQ-39 Reprinted September 2005), color booklet, 4 pages - Distribute through local government by posting on website for residential use.
- "Homeowner Septic System Checklist" USEPA (#832-F-03006 Published 2003), color flyer, 1 page - Distribute through local government by posting on website for residential use.
- "Managing Household Wastewater: Septic Systems and Other Treatment Methods" MSU Extension, Home*A*Syst Chapter 11, color booklet, 12 pages – Distribute through MSU Extension by promoting residential assessments using Home*A*Syst evaluations.

Other ideas for messages about septic systems and local ordinances that could be distributed to media outlets, printed in partner newsletters or posted on a website include:

- Create an article about county or local government regulations that require hookup to sanitary system if septic system fails.
- Create an article about need for local ordinance that requires septic inspections every 3-5 years.
- Create an article about Eaton-Barry County and Mid-Michigan Health Department's status of a county wide Time-of-Sale ordinance - Print article in newsletter and post article on website.

Partners include the Van Buren and Berrien County Health Departments, Van Buren and Berrien Conservation Districts, Southwest Michigan Planning Commission, Michigan Department of Environmental Quality and Two Rivers Coalition.

Recommended educational materials and activities to increase understanding about *E. coli* inputs from manure runoff and methods to use for distribution of the materials include:

- "Cows, Streams, and *E. Coli*: What everyone needs to know" Michigan State University Extension E3103 January 2010, bulletin, 4 pages – Distribute through Van Buren and Berrien Conservation Districts and mail to producers in area or provide if conducting site visit.
- Farm*A*Syst; Conservation Reserve Program (CRP); and MAEAP – Disseminate through local governments, Van Buren and Berrien Conservation Districts by posting on website and direct mailings.
- Right-to-Farm Hotline advertised in MSU Extension News article at which to report suspected violations of the GAAMPs – Use local government, Van Buren and Berrien Conservation Districts to post article on website.

Partners include the NRCS, Van Buren and Berrien Conservation Districts, Southwest Michigan Planning Commission, Michigan Department of Environmental Quality and Two Rivers Coalition.

Continued Monitoring

Collection of data in the watershed will provide and increase understanding of bacteria loads in the watershed and will provide knowledge for areas that should be targeted to reduce the risks of bacteria from moving off the land and into surface waters. It should be noted that summer of 2012 was one of the driest on record. It is not known how this may have impacted results.
The sampling program that was done in 2012 could be repeated in the coming year to collect additional data and again in future years when management strategies have been implemented to measure levels of *E. coli* contamination to see if any improvements can be reported. If possible future sampling could be done in early spring when the heaviest land applications of manure seem to be occurring and the spring rains produce runoff. The sampling results can then be compared to the spreading activities and adjustments to spreading plans can be recommended. The labs conducting the analysis also need to ensure that the appropriate dilutions take place to get accurate counts of the *E. coli.*

A partnership with the Pokagon Band of Potawatomi could be established to coordinate additional water sampling in the Pine/Mill Creek area to help further guide and monitor the effectiveness of implementation efforts.

The stakeholders also thought that utilizing canines might also be helpful to pin down the exact locations/sources of human *E. coli* in the Black River, Pine Creek and possibly Mill Creek. Scent trained canines provide rapid screening source tracking for human fecal contamination in stormwater, lakes, rivers, and streams. Environmental Canine Services provides this service and it has been effective in other watersheds in Michigan. For more information on this approach visit http://www.ecsk9s.com/. The other benefit about using the dogs is the media attention it usually draws, so it can double as an education/outreach opportunity also.

Along with further Microbial Source Tracking in targeted areas, an alternative to determine if human waste is present is to test for caffeine. Reasonably priced and easy to use test kits are now commercially available so this might be a good opportunity for a volunteer monitoring activity.

Conclusions

E. coli contamination of surface waters is a watershed-wide issue, and not confined to one source or one area of the watershed. Although the results do not point to a specific discharge as the ultimate source of contamination, implementation of the recommendations will work toward reducing potential sources and eventually reduce the overall levels of *E. coli* in the watersheds.

This project has provided a greater understanding of the sources of the *E. coli* and how *E. coli* levels relate to activities in the watershed. As technology advances in the understanding of how to identify sources of *E. coli*, more progress can be made in addressing those sources directly and effectively.

The partners involved in this project have made progress and will continue to collaborate on this issue. The partners will begin to implement the affordable BMPs as soon as possible and will continue to look for additional funding to continue the momentum of implementing BMPs to reduce *E. coli.*

Field investigation have proven to be the most cost effective method of identifying sources, and partners will continue to work with alternative methods of investigation to be as efficient and accurate as possible.

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Appendix 1: Detailed *E. coli* Sampling Results

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
Black River	12-7-19-BC-01-L(I)	July - Wet	7/19/2012	2:30 PM	2420	0.25	300			
	12-7-19-BC-01-L(D)	July - Wet	7/19/2012	2:30 PM	2420	0.25	300			
	12-7-19-BC-01-C(I)	July - Wet	7/19/2012	2:32 PM	2420	0.25	300			
	12-7-19-BC-01-C(D)	July - Wet	7/19/2012	2:32 PM	2420	0.25	300			
	12-7-19-BC-01-R(I)	July - Wet	7/19/2012	2:35 PM	2420	0.25	300			
	12-7-19-BC-01-R(D)	July - Wet	7/19/2012	2:35 PM	2420	0.25	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-7-19-BC-02-L(I)	July - Wet	7/19/2012	10:55 AM	1986	1.4	300			
	12-7-19-BC-02-L(D)	July - Wet	7/19/2012	10:55 AM	1553	1.4	300			
	12-7-19-BC-02-C(I)	July - Wet	7/19/2012	11:00 AM	1733	1.4	300			
	12-7-19-BC-02-C(D)	July - Wet	7/19/2012	11:00 AM	1733	1.4	300			
	12-7-19-BC-02-R(I)	July - Wet	7/19/2012	11:05 AM	1986	1.4	300			
	12-7-19-BC-02-R(D)	July - Wet	7/19/2012	11:05 AM	1733	1.4	300	1,897.8	YES	
	12-7-19-BC-03-L(I)	July - Wet	7/19/2012	3:00 PM	2420	0.125	300			
	12-7-19-BC-03-L(D)	July - Wet	7/19/2012	3:00 PM	2420	0.125	300			
	12-7-19-BC-03-C(I)	July - Wet	7/19/2012	3:03 PM	2420	0.125	300			
	12-7-19-BC-03-C(D)	July - Wet	7/19/2012	3:03 PM	2420	0.125	300			
	12-7-19-BC-03-R(I)	July - Wet	7/19/2012	3:05 PM	1733	0.125	300			
	12-7-19-BC-03-R(D)	July - Wet	7/19/2012	3:05 PM	2420	0.125	300			
	12-7-19-BC-03(B)	July - Wet	7/19/2012	2:57 PM	0	0.125	300	2,165.1	YES	Samples reported >2420, however number 2420 used for calculation/charting purposes
	12-7-19-BC-04-L(I)	July - Wet	7/19/2012	1:45 PM	2420	4	300			
	12-7-19-BC-04-L(D)	July - Wet	7/19/2012	1:45 PM	41	4	300			
	12-7-19-BC-04-C(I)	July - Wet	7/19/2012	2:00 PM	2420	4	300			
	12-7-19-BC-04-C(D)	July - Wet	7/19/2012	2:00 PM	2420	4	300			
	12-7-19-BC-04-R(I)	July - Wet	7/19/2012	2:05 PM	2420	4	300			
	12-7-19-BC-04-R(D)	July - Wet	7/19/2012	2:05 PM	2420	4	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-7-19-BC-05-L(I)	July - Wet	7/19/2012	12:49 PM	2420	3.8	300			
	12-7-19-BC-05-L(D)	July - Wet	7/19/2012	12:49 PM	1986	3.8	300			
	12-7-19-BC-05-C(I)	July - Wet	7/19/2012	1:02 PM	2420	3.8	300			
	12-7-19-BC-05-C(D)	July - Wet	7/19/2012	1:02 PM	2420	3.8	300			
	12-7-19-BC-05-R(I)	July - Wet	7/19/2012	1:15 PM	1986	3.8	300			
	12-7-19-BC-05-R(D)	July - Wet	7/19/2012	1:15 PM	2420	3.8	300	2,265.7	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-7-19-BC-06-L(I)	July - Wet	7/19/2012	11:35 AM	2420	0.58	300			
	12-7-19-BC-06-C(I)	July - Wet	7/19/2012	11:37 AM	2420	0.58	300			
	12-7-19-BC-06-R(I)	July - Wet	7/19/2012	11:40 AM	2420	0.58	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-7-19-BC-07-L(I)	July - Wet	7/19/2012	10:40 AM	2420	0.83	300			
	12-7-19-BC-07-C(I)	July - Wet	7/19/2012	10:42 AM	2420	0.83	300			
	12-7-19-BC-07-R(I)	July - Wet	7/19/2012	10:44 AM	2420	0.83	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-8-17-BC-01-L(I)	August - Wet	8/17/2012	10:57 AM	355	0.25	300			
	12-8-17-BC-01-C(I)	August - Wet	8/17/2012	10:59 AM	301	0.25	300			
	12-8-17-BC-01-R(I)	August - Wet	8/17/2012	11:01 AM	313	0.25	300	322.2	YES	
	12-8-17-BC-02-L(I)	August - Wet	8/17/2012	9:21 AM	1,664	0.58	300			
	12-8-17-BC-02-C(I)	August - Wet	8/17/2012	9:23 AM	2,098	0.58	300			
	12-8-17-BC-02-R(I)	August - Wet	8/17/2012	9:25 AM	1,296	0.58	300	1,653.9	YES	
	12-8-17-BC-03-L(I)	August - Wet	8/17/2012	11:15 AM	1,670	0.17	300			
	12-8-17-BC-03-C(I)	August - Wet	8/17/2012	11:17 AM	754	0.17	300			
	12-8-17-BC-03-R(I)	August - Wet	8/17/2012	11:20 AM	2,014	0.17	300			
	12-8-17-BC-03-C(D)	August - Wet	8/17/2012	11:17 AM	1,850	0.17	300	1,363.7	YES	
	12-8-17-BC-04-L(I)	August - Wet	8/17/2012	10:31 AM	1,467	3.4	300			
	12-8-17-BC-04-C(I)	August - Wet	8/17/2012	10:33 AM	1,842	3.4	300			
	12-8-17-BC-04-R(I)	August - Wet	8/17/2012	10:35 AM	1,785	3.4	300	1,689.6	YES	

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-8-17-BC-05-L(I)	August - Wet	8/17/2012	10:10 AM	638	3.2	300			
	12-8-17-BC-05-C(I)	August - Wet	8/17/2012	10:12 AM	836	3.2	300			
	12-8-17-BC-05-R(I)	August - Wet	8/17/2012	10:15 AM	399	3.2	300			
	12-8-17-BC-05-C(D)	August - Wet	8/17/2012	10:12 AM	565	3.2	300			
	12-8-17-BC-05-B	August - Wet	8/17/2012	10:17 AM	0	3.2	300	597.0	YES	
	12-8-17-BC-06-L(I)	August - Wet	8/17/2012	9:42 AM	538	0.42	300			
	12-8-17-BC-06-C(I)	August - Wet	8/17/2012	9:44 AM	504	0.42	300			
	12-8-17-BC-06-R(I)	August - Wet	8/17/2012	9:45 AM	530	0.42	300	523.8	YES	
	12-8-17-BC-07-L(I)	August - Wet	8/17/2012	9:12 AM	379	0.5	300			
	12-8-17-BC-07-C(I)	August - Wet	8/17/2012	9:14 AM	364	0.5	300			
	12-8-17-BC-07-R(I)	August - Wet	8/17/2012	9:15 AM	554	0.5	300	424.4	YES	
	12-10-18-BC-01-L(I)	October - Wet	10/18/2012	12:55 PM	279		300			
	12-10-18-BC-01-C(I)	October - Wet	10/18/2012	1:00 PM	228		300			
	12-10-18-BC-01-R(I)	October - Wet	10/18/2012	12:58 PM	243		300	249.1	NO	
	12-10-18-BC-02-L(I)	October - Wet	10/18/2012	11:35 AM	663		300			
	12-10-18-BC-02-C(I)	October - Wet	10/18/2012	11:37 AM	714		300			
	12-10-18-BC-02-R(I)	October - Wet	10/18/2012	11:40 AM	754		300	709.4	YES	
	12-10-18-BC-03-L(I)	October - Wet	10/18/2012	1:15 PM	884		300			
	12-10-18-BC-03-L(D)	October - Wet	10/18/2012	1:15 PM	933		300			
	12-10-18-BC-03-C(I)	October - Wet	10/18/2012	1:17 PM	882		300			
	12-10-18-BC-03-R(I)	October - Wet	10/18/2012	1:20 PM	1,162		300			
	12-10-18-BC-03-B	October - Wet	10/18/2012	1:25 PM	0		300	967.6	YES	
	12-10-18-BC-04-L(I)	October - Wet	10/18/2012	12:30 PM	397		300			
	12-10-18-BC-04-C(I)	October - Wet	10/18/2012	12:32 PM	556		300			
	12-10-18-BC-04-R(I)	October - Wet	10/18/2012	12:35 PM	557		300	497.2	YES	

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-10-18-BC-05-L(I)	October - Wet	10/18/2012	12:15 PM	576		300			
	12-10-18-BC-05-C(I)	October - Wet	10/18/2012	12:17 PM	565		300			
	12-10-18-BC-05-C(D)	October - Wet	10/18/2012	12:17 PM	428		300			
	12-10-18-BC-05-R(I)	October - Wet	10/18/2012	12:20 PM	546		300	562.2	YES	
	12-10-18-BC-06-L(I)	October - Wet	10/18/2012	12:00 PM	605		300			
	12-10-18-BC-06-C(I)	October - Wet	10/18/2012	12:02 PM	437		300			
	12-10-18-BC-06-R(I)	October - Wet	10/18/2012	12:05 PM	364		300	458.3	YES	
	12-10-18-BC-07-L(I)	October - Wet	10/18/2012	11:25 AM	420		300			
	12-10-18-BC-07-C(I)	October - Wet	10/18/2012	11:27 AM	738		300			
	12-10-18-BC-07-R(I)	October - Wet	10/18/2012	11:30 AM	594		300	568.9	YES	
Pine Creek	12-06-PC-01-L(I)	June	6/28/2012	10:50 AM	579.4	1.75	300			
	12-06-PC-01-C(I)	June	6/28/2012	10:52 AM	648.8	1.33	300			
	12-06-PC-01-R(I)	June	6/28/2012	10:55 AM	461.1	1.67	300	557.6	YES	
	12-06-PC-02(B)	June	6/28/2012	9:52 AM	0		300			
	12-06-PC-02-L(I)	June	6/28/2012	10:01 AM	1119.9	0.25	300			
	12-06-PC-02-C(I)	June	6/28/2012	10:05 AM	980.4	1	300			
	12-06-PC-02-C(D)	June	6/28/2012	10:05 AM	1046.2	1	300			
	12-06-PC-02-R(I)	June	6/28/2012	10:08 AM	920.8	1.21	300	1,003.7	YES	
	12-7-19-PC-01-L(I)	July - Wet	7/19/2012	9:42 AM	2420	1.42	300			
	12-7-19-PC-01-C(I)	July - Wet	7/19/2012	9:45 AM	2420	1.42	300			
	12-7-19-PC-01-R(I)	July - Wet	7/19/2012	9:47 AM	2420	1.42	300			
	12-7-19-PC-01-B	July - Wet	7/19/2012	9:37 AM	0	1.42	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-7-19-PC-02-L(I)	July - Wet	7/19/2012	9:12 AM	2420	1.17	300			
	12-7-19-PC-02-C(I)	July - Wet	7/19/2012	9:15 AM	2420	1.17	300			
	12-7-19-PC-02-R(I)	July - Wet	7/19/2012	9:17 AM	2,420	1.17	300	2,420.0	YES	Samples reported >2420, however number 2420 used for calculation/ charting purposes

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-7-19-PC-03-L(I)	July - Wet	7/19/2012	8:45 AM	2,420	0.75	300			Two samples were reported with uncharacteristically low MPN values (12-7-19-PC-03-C(I) and 12-7-19-PC-03-R(I) and are probably analysis errors. IDEXX trays for these samples showed unaccountably low number of large cell positives. The two MPN values should be regarded as experimental error and disregarded. Therefore, a geometric mean could not be calculated for PC-03 for the July 19, 2012, wet weather event.
	12-7-19-PC-03-C(I)	July - Wet	7/19/2012	8:48 AM	214	0.75	300			
	12-7-19-PC-03-R(I)	July - Wet	7/19/2012	8:50 AM	231	0.75	300	NA	NA	
	12-7-19-PC-04-L(I)	July - Wet	7/19/2012	8:25 AM	2,420	0.25	300			Samples reported >2420, however number 2420 used for calculation/ charting purposes
	12-7-19-PC-04-C(I)	July - Wet	7/19/2012	8:28 AM	2,420	0.25	300			
	12-7-19-PC-04-R(I)	July - Wet	7/19/2012	8:30 AM	2,420	0.25	300	2,420.0	YES	
	12-07-PC-01-L(I)	August	8/3/2012	11:20 AM	9,800	1.33	300			
	12-07-PC-01-C(I)	August	8/3/2012	11:22 AM	5,170	1.33	300			
	12-07-PC-01-R(I)	August	8/3/2012	11:23 AM	2,750	1.33	300			
	12-07-PC-01-L(D)	August	8/3/2012	11:21 AM	723	1.33	300	5,184.2	YES	
	12-07-PC-02-L(I)	August	8/3/2012	10:50 AM	422	0.73	300			
	12-07-PC-02-C(I)	August	8/3/2012	10:51 AM	6,490	0.73	300			
	12-07-PC-02-R(I)	August	8/3/2012	10:52 AM	565	0.73	300			
	12-07-PC-02(B)	August	8/3/2012	10:53 AM	0	0.73	300	1,156.6	YES	
	12-8-17-PC-01-L(I)	August - Wet	8/17/2012	8:33 AM	959	1.17	300			
	12-8-17-PC-01-C(I)	August - Wet	8/17/2012	8:35 AM	1,017	1.17	300			
	12-8-17-PC-01-R(I)	August - Wet	8/17/2012	8:37 AM	1,046	1.17	300			
	12-8-17-PC-01-C(D)	August - Wet	8/17/2012	8:35 AM	717	1.17	300	1,006.7	YES	

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-8-17-PC-02-L(I)	August - Wet	8/17/2012	8:13 AM	1,334	0.67	300			
	12-8-17-PC-02-C(I)	August - Wet	8/17/2012	8:15 AM	1,112	0.67	300			
	12-8-17-PC-02-R(I)	August - Wet	8/17/2012	8:17 AM	1,467	0.67	300	1,295.9	Y ES	
	12-8-17-PC-03-L(I)	August - Wet	8/17/2012	7:58 AM	2,187	1.12	300			
	12-8-17-PC-03-C(I)	August - Wet	8/17/2012	8:00 AM	2,282	1.12	300			
	12-8-17-PC-03-R(I)	August - Wet	8/17/2012	8:02 AM	1,723	1.12	300	2,048.7	YES	
	12-8-17-PC-04-L(I)	August - Wet	8/17/2012	7:43 AM	1,017	0.17	300			
	12-8-17-PC-04-C(I)	August - Wet	8/17/2012	7:45 AM	933	0.17	300			
	12-8-17-PC-04-R(I)	August - Wet	8/17/2012	7:47 AM	1,081	0.17	300			
	12-8-17-PC-04-R(D)	August - Wet	8/17/2012	7:47 AM	1,076	0.17	300			
	12-8-17-PC-04-B	August - Wet	8/17/2012	7:40 AM	0	0.17	300	1,008.5	YES	
	12-08-PC-01-L(I)	August	8/30/2012	9:40 AM	420	0.8	300			
	12-08-PC-01-C(I)	August	8/30/2012	9:45 AM	262	0.8	300			
	12-08-PC-01-R(I)	August	8/30/2012	9:50 AM	331	0.8	300	331.5	YES	
	12-08-PC-02-L(I)	August	8/30/2012	9:08 AM	1,153	0.5	300			
	12-08-PC-02-C(I)	August	8/30/2012	9:10 AM	1,334	0.5	300			
	12-08-PC-02-R(I)	August	8/30/2012	9:12 AM	1,145	0.5	300			
	12-08-PC-02-L(D)	August	8/30/2012	9:08 AM	1,153	0.5	300			
	12-08-PC-02(B)	August	8/30/2012	9:15 AM	0	0.5	300	1,207.6	YES	
	12-09-PC-01-L(I)	September	9/25/2012	8:50 AM	364	1	300			
	12-09-PC-01-C(I)	September	9/25/2012	8:55 AM	323	1	300			
	12-09-PC-01-R(I)	September	9/25/2012	9:00 AM	288	1	300			
	12-09-PC-01-R(D)	September	9/25/2012	9:00 AM	350	1	300			
	12-09-PC-01(B)	September	9/25/2012	8:40 AM	0	1	300	323.5	YES	

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	12-09-PC-02-L(I)	September	9/25/2012	8:00 AM	538	0.5	300			
	12-09-PC-02-C(I)	September	9/25/2012	8:05 AM	457	0.5	300			
	12-09-PC-02-R(I)	September	9/25/2012	8:10 AM	504	0.5	300	498.6	YES	
	12-10-18-PC-01-L(I)	October - Wet	10/18/2012	10:50 AM	512		300			
	12-10-18-PC-01-C(I)	October - Wet	10/18/2012	10:52 AM	670		300			
	12-10-18-PC-01-R(I)	October - Wet	10/18/2012	10:55 AM	697		300			
	12-10-18-PC-01-R(D)	October - Wet	10/18/2012	10:55 AM	404		300			
	12-10-18-PC-01-B	October - Wet	10/18/2012	11:00 AM	0		300	620.7	YES	
	12-10-18-PC-02-L(I)	October - Wet	10/18/2012	10:30 AM	1,081		300			
	12-10-18-PC-02-C(I)	October - Wet	10/18/2012	10:32 AM	1,198		300			
	12-10-18-PC-02-R(I)	October - Wet	10/18/2012	10:35 AM	1,455		300	1,235.1	YES	
	12-10-18-PC-03-L(I)	October - Wet	10/18/2012	10:20 AM	7,701		300			
	12-10-18-PC-03-C(I)	October - Wet	10/18/2012	10:22 AM	5,172		300			
	12-10-18-PC-03-R(I)	October - Wet	10/18/2012	10:25 AM	4,888		300	5,795.8	YES	
	12-10-18-PC-04-L(I)	October - Wet	10/18/2012	10:00 AM	789		300			
	12-10-18-PC-04-C(I)	October - Wet	10/18/2012	10:03 AM	767		300			
	12-10-18-PC-04-R(I)	October - Wet	10/18/2012	10:05 AM	613		300			
	12-10-18-PC-04-R(D)	October - Wet	10/18/2012	10:05 AM	471		300	718.5	YES	
	12-10-PC-01-L(I)	October	10/22/2012	10:10 AM	146		300			
	12-10-PC-01-C(I)	October	10/22/2012	10:12 AM	41		300			
	12-10-PC-01-R(I)	October	10/22/2012	10:15 AM	63		300	72.2	NO	
	12-10-PC-02-L(I)	October	10/22/2012	9:50 AM	74		300			
	12-10-PC-02-C(I)	October	10/22/2012	9:52 AM	63		300			
	12-10-PC-02-R(I)	October	10/22/2012	9:55 AM	31		300			
	12-10-PC-02-R(D)	October	10/22/2012	9:55 AM	41		300			
	12-10-PC-02(B)	October	10/22/2012	9:58 AM	0		300	52.5	NO	

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
Mill Creek	MC-01-0-R (I)	July-Wet	7/19/2012	9:30 AM	9,000	0.83	300			
	MC-01-0-R (D)	July-Wet	7/19/2012	9:30 AM	10,000	0.83	300			
	MC-01-0-C (I)	July-Wet	7/19/2012	9:35 AM	8,000	0.83	300			
	MC-01-0-C (D)	July-Wet	7/19/2012	9:35 AM	8,000	0.83	300			
	MC-01-0-L (I)	July-Wet	7/19/2012	9:40 AM	9,000	0.83	300			
	MC-01-0-L (D)	July-Wet	7/19/2012	9:40 AM	8,000	0.83	300	8,653.5	YES	Info from GLS Lab report
	MC-01-12-R (I)	July - Wet	7/19/2012	6:20 PM	4,000	0.83	300			
	MC-01-12-R (D)	July - Wet	7/19/2012	6:20 PM	7,000	0.85	300			
	MC-01-12-C (I)	July - Wet	7/19/2012	6:23 PM	5,000	0.85	300			
	MC-01-12-C (D)	July - Wet	7/19/2012	6:23 PM	5,000	0.85	300			
	MC-01-12-L (I)	July - Wet	7/19/2012	6:26 PM	7,000	0.85	300			
	MC-01-12-L (D)	July - Wet	7/19/2012	6:26 PM	6,000	0.85	300			
	MC-01-12 (B)	July - Wet	7/19/2012	6:29 PM	5	0.85	300	5,192.5	YES	Info from GLS Lab report. Sample @ 6:29 was indicated as <10 - so value of 5 used.
	MC-01-24-R (I)	July - Wet	7/20/2012	9:18 AM	2,100	0.65	300			
	MC-01-24-R (D)	July - Wet	7/20/2012	9:18 AM	3,400	0.65	300			
	MC-01-24-C (I)	July - Wet	7/20/2012	9:20 AM	3,700	0.65	300			
	MC-01-24-C (D)	July - Wet	7/20/2012	9:20 AM	3,400	0.65	300			
	MC-01-24-L (I)	July - Wet	7/20/2012	9:22 AM	3,600	0.65	300			
	MC-01-24-L (D)	July - Wet	7/20/2012	9:22 AM	2,000	0.65	300	3,035.6	YES	Info from GLS Lab report
	MC-01-0-R (I)	August - Wet	8/17/2012	9:25 AM	12,000	0.73	300			
	MC-01-0-C (I)	August - Wet	8/17/2012	9:27 AM	15,000	0.73	300			
	MC-01-0-L (I)	August - Wet	8/17/2012	9:29 AM	15,000	0.73	300	13,924.8	YES	Info from GLS Lab report
	MC-01-12-R (I)	August - Wet	8/17/2012	6:10 PM	5,000	0.83	300			
	MC-01-12-C (I)	August - Wet	8/17/2012	6:12 PM	5,000	0.83	300			
	MC-01-12-C (D)	August - Wet	8/17/2012	6:12 PM	8,000	0.83	300			
	MC-01-12-L (I)	August - Wet	8/17/2012	6:14 PM	7,000	0.83	300	5,593.4	YES	Info from GLS Lab report

Watershed	Sampling Locations	Month	Date	Time	*E. coli*/100 mL	Water Level (feet)	Water Quality Standard (WQS)	Geometric Mean (from E.coli/100 mL)	Exceed WQS	Notes
	MC-01-24 B	August - Wet	8/18/2012	8:52 AM	50	0.71	300			
	MC-01-24-R (I)	August - Wet	8/18/2012	8:55 AM	1,500	0.71	300			
	MC-01-24-C (I)	August - Wet	8/18/2012	8:57 AM	1,800	0.71	300			
	MC-01-24-L (I)	August - Wet	8/18/2012	8:59 AM	1,900	0.71	300	1,724.7	YES	Info from GLS Lab report. Sample @ 8:52 was indicated as <100 - so value of 50 used.
	MC-01-0-C (D)	October-Wet	10/18/2012	9:20 AM	600	1.04	300			
	MC-01-0-C (I)	October-Wet	10/18/2012	9:21 AM	1,100	1.04	300			
	MC-01-0-R (I)	October-Wet	10/18/2012	9:24 AM	600	1.04	300			
	MC-01-0-L (I)	October-Wet	10/18/2012	9:26 AM	700	1.04	300	773.1	YES	Info from GLS Lab report
	MC-01-12-R (I)	October-Wet	10/18/2012	6:00 PM	1,400	1.33	300			
	MC-01-12-L (I)	October-Wet	10/18/2012	6:01 PM	1,500	1.33	300			
	MC-01-12 B	October-Wet	10/18/2012	6:02 PM	5	1.33	300			
	MC-01-12-C (I)	October-Wet	10/18/2012	6:04 PM	1,800	1.33	300	1,557.7	YES	Info from GLS Lab report. Sample @ 6:02 was indicated as <10 - so value of 5 used.
	MC-01-24-C (I)	October-Wet	10/19/2012	10:05 AM	220	1.125	300			
	MC-01-24-L (I)	October-Wet	10/19/2012	10:06 AM	190	1.125	300			
	MC-01-24-R (I)	October-Wet	10/19/2012	10:07 AM	310	1.125	300	234.9	NO	Info from GLS Lab report

Appendix 2: Manure Spreading in the Pine Creek Watershed

Red Arrow Dairy Manure Spreading (Pine Creek Watershed) (source MDEQ)

Field	Subfield	Township	Section	Application Date	Application Rate (gallon/acre)	Downstream Sampling Point	Tile outlet
Rudel	E	Hartford	21	4/12/2012	10,000	PC-02	no tile outlets, just upstream
Rudel	N	Hartford	21	4/13/2012	10,000	PC-02	no tile outlets
Rudel	W	Hartford	21	5/16/2012	10,000	PC-02	no tile outlets
Rudel	W	Hartford	21	5/17/2012	10,000	PC-03	no tile outlets
22	2	Hartford	28	3/17/2012	3,000	PC-02	No tile outlets, no direct drainage to creek
22	4	Hartford	28	3/19/2012	3,000	PC-02	No tile outlets, no direct drainage to creek
22	5	Hartford	28	3/20/2012	3,000	PC-02	No tile outlets, no direct drainage to creek
40	1	Hartford	28	4/11/2012	8,000	PC-02	No tile outlets, no direct drainage to creek
40	1	Hartford	28	4/23/2012	8,000	PC-02	No tile outlets, no direct drainage to creek
40	1	Hartford	28	5/3/2012	8,000	PC-02	No tile outlets, no direct drainage to creek
40	1	Hartford	28	5/9/2012	8,000	PC-02	No tile outlets, no direct drainage to creek
8	N1	Hartford	33	5/18/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N1	Hartford	33	8/14/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N2	Hartford	33	5/19/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N2	Hartford	33	8/15/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N3	Hartford	33	5/21/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N3	Hartford	33	8/16/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N4	Hartford	33	5/22/2012	8,000	PC-03	Tile outlet to Pine Creek
8	N4	Hartford	33	8/17/2012	8,000	PC-03	Tile outlet to Pine Creek
8	S1	Hartford	33	5/23/2012	8,000	PC-03	Tile outlet to Pine Creek
8	S1	Hartford	33	8/20/2012	8,000	PC-03	Tile outlet to Pine Creek
Dowd CB	N	Hartford	35	3/31/2012	10,000	No map	Pine Creek Tributary
Dowd CB	S	Hartford	35	4/2/2012	10,000	No map	Pine Creek Tributary
42	3	Keeler	4	6/19/2012	7,000	PC-03	no tile outlets
42	3	Keeler	4	7/12/2012	7,000	PC-03	no tile outlets
42	4	Keeler	4	6/23/2012	7,000	PC-03	no tile outlets
42	4	Keeler	4	7/13/2012	7,000	PC-03	no tile outlets
42	5	Keeler	4	7/5/2012	7,000	PC-03	no tile outlets
42	5	Keeler	4	7/16/2012	7,000	PC-03	no tile outlets
42	6	Keeler	4	7/6/2012	7,000	PC-03	no tile outlets

Field	Subfield	Township	Section	Application Date	Application Rate (gallon/acre)	Downstream Sampling Point	Tile outlet
42	6	Keeler	4	7/18/2012	7,000	PC-03	no tile outlets
42	2	Keeler	9	7/9/2012	7,000	No map	
42	2	Keeler	9	7/20/2012	7,000	No map	
42	7	Keeler	9	7/10/2012	7,000	No map	
42	7	Keeler	9	7/25/2012	7,000	No map	
42	7	Keeler	9	7/26/2012	7,000	No map	
42	7	Keeler	9	7/27/2012	7,000	No map	
42	7	Keeler	9	8/17/2012	7,000	No map	
42	7	Keeler	9	8/21/2017	7,000	No map	

Appendix 3: Microbial Source Tracking Methods, Assumptions and Results

Sample collection: Stream samples were obtained in the field by hand using sterile (autoclaved) 1-liter polypropylene storage bottles. Collected samples were placed on ice immediately and delivered to the laboratory for analysis and long-term storage.

***E. coli* analysis:** Detection of *E. coli* in water samples was performed by growth in IDEXX Colilert® medium. Colilert® contains the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide. Hydrolysis of the substrate by *E. coli*-specific enzymes is detected by illumination of the culture with long-wave ultraviolet light. For quantitation, a standard volume of water sample was diluted to 100 mL in sterile water, to which was added the contents of one packet of Colilert®. Due to the levels of *E. coli* in the samples, in general 10 mL of environmental sample was diluted to 100 mL for the assay. Each sample with medium/reagent was poured into an IDEXX Quanti-Tray®/2000 tray, sealed, and incubated at 35 C. [1] After 24 hours, most-probably number (MPN) of *E. coli* colony-forming units (cfu) was determined by counting tray wells showing fluorescence upon UV illumination based on the IDEXX MPN table. For samples that were diluted 1:10 as described, the measured MPN values were multiplied by ten to compensate for dilution. Thus, reported *E. coli* measurements are equivalent to cfu/100 mL.

Long-term storage: In order to preserve material from water samples for later molecular analysis, 600 mL of each sample collected were filtered through .45-micron filters (147 mm diameter). The filters were transferred into 50-mL plastic centrifuge tubes containing 40 mL of sterile water. Tube containing the filters were vortexed at high speed for 5 minutes, then agitated on a rocking platform in the cold room for 30 minutes to release material adhered to the filter. The filters were removed and the tubes were centrifuged for 20 minutes at 4,500 x g. Liquid was decanted from the tubes, leaving 0.5 – 1 mL of supernatant remaining. Pelleted material was resuspended in the remaining supernatant and transferred into a sterile 1.5 mL cryostorage tube and stored at -80 C.

DNA isolation: Following storage, samples chosen for source-tracking analysis were thawed. DNA was isolated from the contents by isolation and purification using Qiagen's QIAamp® DNA mini kit reagents and spin columns. Isolation of DNA was performed as described in the manufacturer's instructions; in brief, material collected by filtration was subjected to increasing temperatures in the presence of lysozyme to induce lysis of bacteria. Following lysis, material was applied to the binding column by centrifugation. Columns were washed and purified DNA was eluted using buffers supplied in the kit. In most cases, concentration of DNA was measured using a Qubit® (Invitrogen) fluorometer using Broad Range DNA measurement reagents. DNA yields were 1 – 5 micrograms DNA per mL in a volume of 200 μL of water.

Detection of general and host-specific *Bacteroides*: Quantitative polymerase chain reaction (qPCR) based on amplification of ribosomal DNA (rDNA) sequences was used to assess the presence and amount of genetic material originating from *Bacteroides* in purified DNA. Unlike E coli and other coliforms, organisms of the *Bacteroides* genus and related bacteria are obligate anaerobes. As such they cannot replicate in the environment and may be a more direct marker of the presence of fecal material. For qPCR, a single non-host specific reverse primer (262R) was used matched to one of a presumed host-specific forward (32F, HF183, CF128, PF163). The numbering system for the primers is based on their analogous position in the *E. coli* rDNA gene; F designates a primer oriented in the forward direction relative to the transcription of the

[1] "Quanti-tray/2000 Procedure."

rDNA gene and R designates a reverse primer. H denotes a human specific host, C denotes cow specific, and P denotes pig specific.

General (non-host specific) Bacteroides: 32F = AACGCTAGCTACAGGCTT [2]
Human specific forward: HF 183 = ATCATGAGTTCACATGTCCG [3]
Cow specific forward: CF128 = CCAACYTTCCCGWTACTC (Y = C or T; W = A or T) [4]
Pig specific forward: PF163 = GCGGATTAATACCGTATGA [5]
All-purpose reverse primer: 262R = TACCCCGCCTACTA TCTAATG [6]

Primers were obtained from Eurofins/MWG-Operon and dissolved in water at a working stock concentration of 10 μM.

qPCR was performed using Bio-Rad SsoAdvanced™ SYBR Green Supermix. For each reaction, 1 μL of Qiagen-purified DNA was combined with 1 μL of forward and reverse primer in a volume of 10 μL, to which were added 10 μL of SYBR Green mastermix. Reactions were carried out in either 96-well plates or 8-tube strips. Once mixed, reactions were placed in a Bio-Rad CFX96™ real-time PCR instrument. Reaction conditions were: 10 min at 95 C pre-heat; 30 sec at 95 C, 30 sec at 57 C, and 1 min at 72 C for 40 cycles, and melt curve detection from 65 C to 95 C.

Threshold cycle values (C_t) were recorded for those reactions that generated an optically detectable amplification product in 36 cycles or fewer. qPCR results were taken as valid if the melt curve for the product was at the correct temperature, indicative of amplification of the desired target.

If an amplification reaction gave an ambiguous result (that is, a melt curve displaying an off-target denaturation temperature), the product was analyzed by gel electrophoresis. Electrophoresis was performed on 2% agarose or 12% cross-linked polyacrylamide,[7] stained using ethidium bromide, and imaged on a UV transilluminator.
In samples for which valid qPCR results were obtained, the C_t for the environmental sample was compared to a standard C_t value using the ΔC_t method[8]. Positive control standard for the general (32F) and human specific (HF183) was purified from sewage obtained from the local sewage treatment plant. Control for cow (CF128) amplification was purified from cow manure

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[2] Bernhard and Field, "Identification of Nonpoint Sources of Fecal Pollution in Coastal Waters by Using Host-Specific 16S Ribosomal DNA Genetic Markers from Fecal Anaerobes."
[3] Bernhard and Field, "A PCR Assay To Discriminate Human and Ruminant Feces on the Basis of Host Differences in Bacteroides-Prevotella Genes Encoding 16S rRNA."
[4] Ibid.
[5] Dick et al., "Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification."
[6] Seurinck et al., "Detection and Quantification of the Human-specific HF183 Bacteroides 16S rRNA Genetic Marker with Real-time PCR for Assessment of Human Faecal Pollution in Freshwater."
[7] Ausubel, *Short Protocols in Molecular Biology*.
[8] Livak and Schmittgen, "Analysis of Relative Gene Expression Data Using Real-time Quantitative PCR and the 2(-Delta Delta C(T)) Method."

from a local dairy. Amounts of each (general, human, and cow) was expressed as parts per million relative to the appropriate standard, calculated as ppm = $2^{\{-(Ct,sample\ \ -C_{t,standard})\}} \times 10^6$. For example, if an environmental sample upon amplification with human-specific primers yielded a C_t of 35.0 compared to a C_t from the sewage standard of 25.0, the amount of human-specific fecal material was reported as $2^{\{-(35.0-25.0)\}} \times 10^6$ = 976 ppm. Because the sewage and cow manure standards were diluted (1:1000) prior to qPCR analysis, the corresponding ppm concentrations in the environmental samples were further multiplied by 10^{-3}.

Results – Hope college MST study in Black River and Pine Creek

Microbiology:
Measurement of viable *E. coli* by IDEXX Colilert® reagent revealed high bacterial counts (exceeding 300 cfu/100 mL standard) throughout the watershed and over most of the sampling events. At most sites sampled, *E. coli* amounts peaked in early summer and dropped into late summer/fall. Slightly higher *E. coli* levels were found following rain events, though with a less dramatic difference between rain and dry samplings than have been seen in a different west Michigan watershed (Lake Macatawa and tributaries in Ottawa County, MI) as tested in this laboratory and others. No particular "hot spots" were observed, suggesting that the *E. coli* is of non-point source origin.

Source tracking:
Organisms within the *Bacteroides* and related genera are obligate anaerobes found in large abundance in the mammalian gut. Unlike *E. coli* and other coliforms, *Bacteroides* cannot grow in the environment. Thus, tests for *Bacteroides* in surface water rely not on culture of live organisms but on molecular detection of the bacteria's genomic DNA. This testing is done by isolation of DNA from an environmental sample and testing that DNA by quantitative PCR (qPCR). Because qPCR relies on detection of particular DNA sequence, selection of appropriate target sequence can distinguish the subtle differences in DNA not only between one bacterial species and another but, in the case of *Bacteroides*, between different sub-strains based on the host mammal species. The DNA sequence targeted in this analysis is within the 16S rDNA gene, the gene for the main RNA component of the ribosome known as the 16S rRNA (ribosomal RNA). 16S rDNA sequences have been compiled for thousands of eubacteria and are the basis for many metagenomic studies. Also unlike *E. coli*, the *Bacteroides* 16S rDNA fingerprint is very short-lived in environmental waters, with a half-life of 1-3 days (decreasing with increasing temperature). Thus the presence of *Bacteroides* 16s rDNA in a water sample is thought to be more indicative of a recent and direct fecal incursion into the water than the presence of *E. coli*.

For this study, qPCR primer sequences were chosen to identify four categories of *Bacteroides*:
1) A "general" or non-host specific primer set was used to indicate fecal contamination by any mammal.
2) A human-specific set which has been shown to have a high degree of selectivity and specificity as an indicator of human host, both in the form of direct fecal isolates and collective municipal sewage.
3) A cow-specific set, which is largely unique to bovine host *Bacteroides* (either from cow feces taken directly or from likely environmental sources such a manure lagoons), with cross-reactivity to other ruminants in many cases.
4) A pig-specific set, which has been confirmed on swine feces and on manure sweepings from a hog farm. It has been less well-validated than the human and cow specific probes.

These primer sets are referred to as general, human, cow, and pig, respectively.

Following work-up and analysis, direct fecal contamination in the Black River and Pine Creek samples, as indicated by *Bacteroides* presence, is overall quite low. Although regulatory standards that correlate directly to *Bacteroides* levels by qPCR do not exist, it seems reasonable to assume a cut-off relative to common suspect fecal contaminants such as municipal sewage or animal facility manure storage lagoons at a dilution of one part to 100,000 (5 log10). This is consistent USEPA guidelines on groundwater i. This comparison can be made with qPCR data by comparing Ct values between representative sources and environmental samples.

It must be kept in mind that Ct represents number of “doublings” of target DNA until fluorescence reaches the threshold cycle. Thus, if the Ct for a given environmental sample is four cycles greater than that for the appropriate standard, the relative amount of target DNA sequence is 2 raised to the power of the difference in Ct between sample and standard (2ΔCt), so 24 or 16-fold lower in concentration. For those more accustomed to comparing log10 values, one “log” (a ten-fold dilution) is equal to a ΔCt of 3.32 cycles (because 23.32=10). The 5 x log10 standard mentioned above would correspond to a ΔCt of 16.6 between standard and sample. Because reliable qPCR results occur in the range of Ct’s between 20 and 35, in practice we have pre-diluted our standards by 1:1000 (3 log10). With this dilution, the cutoff becomes 1:100 (2 log10), with the result that a ΔCt of 6.64 between diluted standard and sample indicates a risk factor of 1:100,000 relative to undiluted standard. Standards used for this work were input from a local municipal wastewater treatment plant for general and human *Bacteroides*, and manure from the storage lagoon of a local dairy farm (located in Jenison, Michigan). For ease of comparison, ΔCt values were converted to parts per million (ppm) relative to undiluted standard; therefore reported values of 10 ppm or greater correspond to the 1:100,000 cutoff.

Most samples subjected to *Bacteroides* qPCR analysis fell below this cutoff, and where tested but below this limit are recorded as BDL (below detection limit) on the accompanying spreadsheet. Even where detected, *Bacteroides*, whether general or host-specific, showed levels in the tens of ppm relative to standards. Only at three sites did measured *Bacteroides* DNA approach or exceed 100 ppm relative to standard, all three during the July rain event sampling.

Two of the sites that proved positive were on Pine Creek, PC01 and PC02. The general assay revealed *Bacteroides* DNA at concentrations comparable to sewage at a 500 or 700 ppm dilution. In the host-specific assays, this material matched sequence specific to cow host. In that assay, cow specific DNA is compared to that present in a dairy manure lagoon and revealed an equivalence to lagoon material at a 70 or 300 ppm dilution.

The third positive site was on the Black River, BR06 (also July rain event). The general assay level corresponded to 93 ppm sewage. This sample proved positive for human specific *Bacteroides* and by that assay measured 220 ppm sewage equivalence.

All other sites tested (15 samples tested for general and all three species-specific *Bacteroides*, and a number of further individual tests) were below the 1:100,000 cutoff and are considered of minimal risk by direct fecal contamination. Sites tested with valid below-threshold results are indicated as “BDL” for below detection limit.

In sum, *Bacteroides* genomic DNA was not found in a widespread manner across the sampling sites and sampling events. Where found, at one site it matched human-specific material, and at the other two sites, which are neighboring each other, the *Bacteroides* DNA matched cow (or other ruminant) material. At no sites was DNA matching to *Bacteroides* from pig host. Even where found, the concentration of *Bacteroides* DNA remained at quite low at hundreds of ppm, i.e. less than one part per thousand, relative to likely source contaminants.

i "Ground Water Rule Source Assessment Guidance Manual - Guide_gwr_sourcewaterassessments.pdf."

Microbial Source Tracking Results

Date/Sampling Location	E. coli cfu/ 100mL	human		general		pig	cow	
		Ct	ppm std	Ct	ppm std	Ct	Ct	ppm std
July 17 - wet 1								
Pine Creek								
PC-01	>2420	33.94	39	24.50	557	BDL	33.60	69
PC-02	>2420	34.07	36	24.09	740	BDL	31.47	300
PC-03	>2420	35.69	12	28.66	31			
PC-04	>2420						BDL	
Black River								
BR-01	>2420	BDL		BDL		BDL	BDL	
BR-02	1898	35.75	11	33.09	1	BDL	BDL	
BR-03	>2420	BDL		BDL		BDL	BDL	
BR-04	>2420	BDL		BDL		BDL	BDL	
BR-05	>2420	BDL		BDL		BDL	BDL	
BR-06	>2420	31.44	220	27.08	93	BDL	34.87	29
BR-07	>2420	BDL		36.55	0		BDL	
Mill Creek								
MC-01	>2420	36.09	9	30.07	12	BDL	BDL	
August 3 - dry 2								
PC-01	5184	BDL		BDL				
August 18 - wet 2								
Pine Creek								
PC-01	1007	BDL		BDL		BDL	39.07	2
PC-02	1296	BDL		BDL		BDL	BDL	
PC-03	1587	BDL						
Black River								
BR-01	322	BDL		BDL		BDL	BDL	
BR-02	1654	BDL		BDL		BDL	33.91	55
BR-03	1364	BDL						
BR-06	524	BDL						
Mill Creek								
MC-01	5577	BDL		BDL		BDL	BDL	
August 30 - dry 3								
PC-01	331	BDL						
October 18 - wet 3								
Pine Creek								
PC-01	621	BDL		BDL				
PC-03	5796					BDL		
Black River								
BR-02	709	BDL						
BR-03	985	BDL						
BR-06	458	BDL						
Fecal standards:								
Municipal sewage (pretreatment) at 1:1000 dilution (Ct = 29.26 with human specific primers; 23.66 with general prime								
Cow manure (dairy lagoon) at 1:1000 dilution (Ct 29.74)								

Appendix 4: Beach Related Techniques

Stormwater Control Measures – North Beach, Racine, WI


Altered Beach Grooming Strategies
OLD
NEW