Greater Milwaukee Watersheds Stormwater Report

Report: March 19, 2008 through October 23, 2012

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TABLE OF CONTENTS

9.0 APPENDICES

List of Figures

List of Illustrations

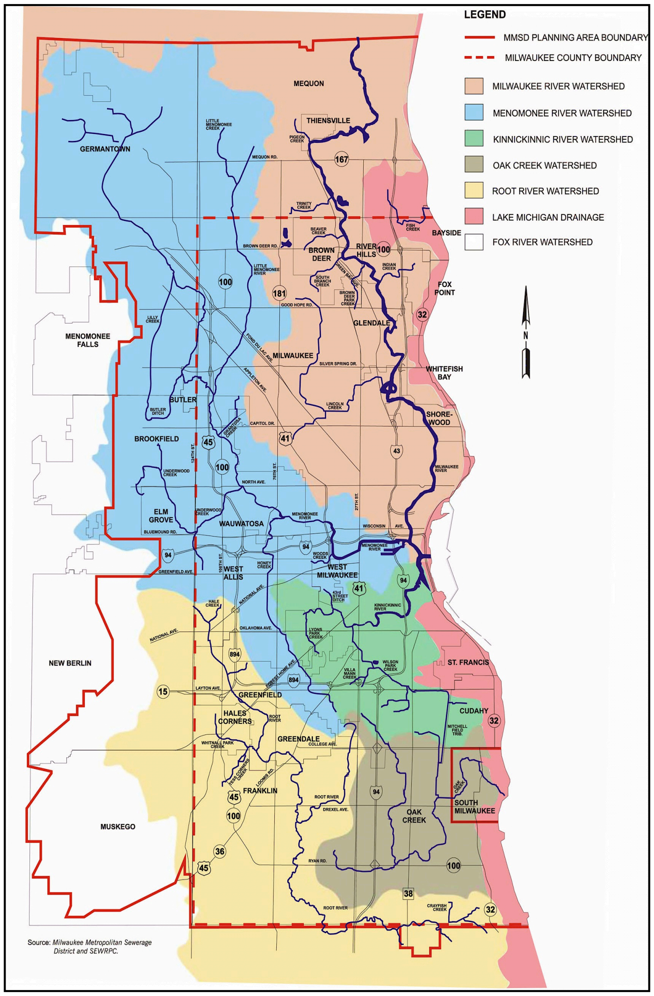
List of Tables

EXECUTIVE SUMMARY

Background and Significance

The overall goal of this project was comprehensive sampling and data analysis to determine the contribution of sanitary sewage contamination to urban stormwater discharges within the Milwaukee Metropolitan Sewerage District (MMSD) service area. This research advances a high priority commitment for MMSD, whose mission is to protect public health and the environment and improve water quality. A previous report for MMSD by the McLellan Laboratory documents results from investigations from 2006-2009 and highlights early advancements in fecal indicator bacteria source tracking. Then as well as now, high levels of fecal indicator organisms routinely contaminate the rivers of Milwaukee's watersheds **(Figure ES-1)** and Lake Michigan beaches in the absence of reported sewage overflows. Our research approach addresses our working hypothesis that sewage from failing infrastructure migrates into the stormwater system and is a major cause of water quality impairments and public health risk in urban waterways.

To improve water quality for Milwaukee's rivers and beaches, it is critical to determine what the major sources of pollution are so that remediation strategies can be formulated and implemented. Water quality standards and monitoring are based on indicator bacteria that are only a general proxy for the presence of fecal pollution and disease-causing organisms (e.g. pathogens). A goal of this research was to evaluate correlations between the presence of human specific sewage indicators and less informative general indicators of fecal pollution. As in our previous report, we continue to find very low or no level of correlation between human fecal indicators and general fecal indicators in stormwater outfall samples. This demonstrates that human sources are only one contributor (of many) to fecal indicator bacteria. From household pets to urban wildlife, a rain event brings a number of fecal sources together in urban stormwater conveyance systems. The advantage of identifying the human signal in fecal pollution sources is its association with health risk. Even low levels of human contribution may carry pathogens, regardless of the contribution of other fecal indicator bacteria.



Source: Milwaukee Metropolitan Sewerage District and SEWRPC.

Figure ES-1. Greater Milwaukee Watersheds

Results and Discussion

The McLellan Laboratory has improved accuracy of the DNA based methodology we use for detecting bacteria from human sources of fecal pollution. As reported previously, the approach is based on detecting a species of human specific *Bacteroides* (HB), first described by Field and co-workers (Bernhard and Field 2000). This bacterium is present in almost all humans, but it is rarely present in other animals – unlike traditional fecal contamination indicators. The McLellan Lab and others have found that HB is a sensitive and specific indicator of sanitary sewage contamination. A second human specific indicator, Lachnospiraceae (lachno2) is also used to improve accuracy. Using quantitative polymerase chain reaction (qPCR), close estimations of HB concentration in water samples can be made and used to compare levels of sewage pollution at different sampling sites.

Outfall Coverage

From 2008 through 2012 over 1,300 samples (including up-the-pipe stormwater and grab samples) were collected by field crews from MMSD and Milwaukee Riverkeeper. The majority of sampling efforts concentrated on the Menomonee and Kinnickinnic River watersheds. A combined 213 stormwater outfalls were sampled over this five year period **(Figure ES-2)**. Samples were analyzed for the general fecal indicator bacteria *Escherichia coli* (EC) and enterococci (ENT) by plate counts of colony forming units (CFU). When plate counts for either organism exceeded 1000 CFU/100 ml sample water, qPCR analysis for the human fecal marker (HB) followed. Samples were considered positive for HB when the copy number for the genetic marker was greater than 1000 per 100 ml of sample. Some outfalls were frequently positive for sewage contamination, as measured by HB concentration, and others were only intermittently positive. Overall, the Menomonee River watershed had a higher percentage of outfalls that were positive for sewage than the Kinnickinnic River watershed.

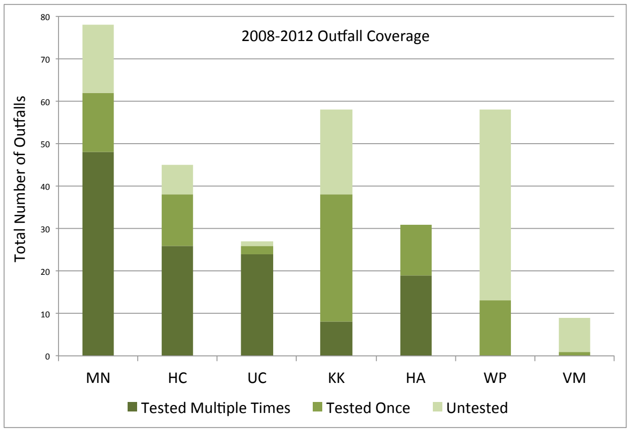


Figure ES-2. *Number of outfalls in each watershed sampled multiple times, only once or never from 2008-2012. Y-axis shows the approximate total number of outfalls present on each river. Menomonee River (MN), Honey Creek (HC), Underwood Creek (UC), Kinnickinnic River (KK), Holmes Avenue Creek (HA), Wilson Park Creek (WP), Villa Mann Creek (VM).*

- **Percent of HB positive stormwater outfalls in each watershed:**
  - ***Menomonee Watershed Outfalls (n=126)***
    - *76% on the Menomonee River*
    - *61% on Honey Creek*
    - *69% on Underwood Creek*
  - ***Kinnickinnic Watershed Outfalls (n=82)***
    - *16% on the Kinnickinnic River*
    - *10% on Holme's Ave Creek*
    - *23% on Wilson Park Creek*

Figures **ES-3A** and **ES-3B** map outfall sites that release sewage in each watershed. During this study period, the Menomonee River watershed was targeted for repeat sampling more often than the Kinnickinnic River watershed. Ongoing work is targeting the Kinnickinnic River watershed. Overall, these results represent wide coverage of the two watersheds that were recommended for further investigation based on results published in ***Greater Milwaukee Watersheds Pathogen Source Identification Report*** (2009), which indicated that sanitary sewage contamination of stormwater is a serious concern.

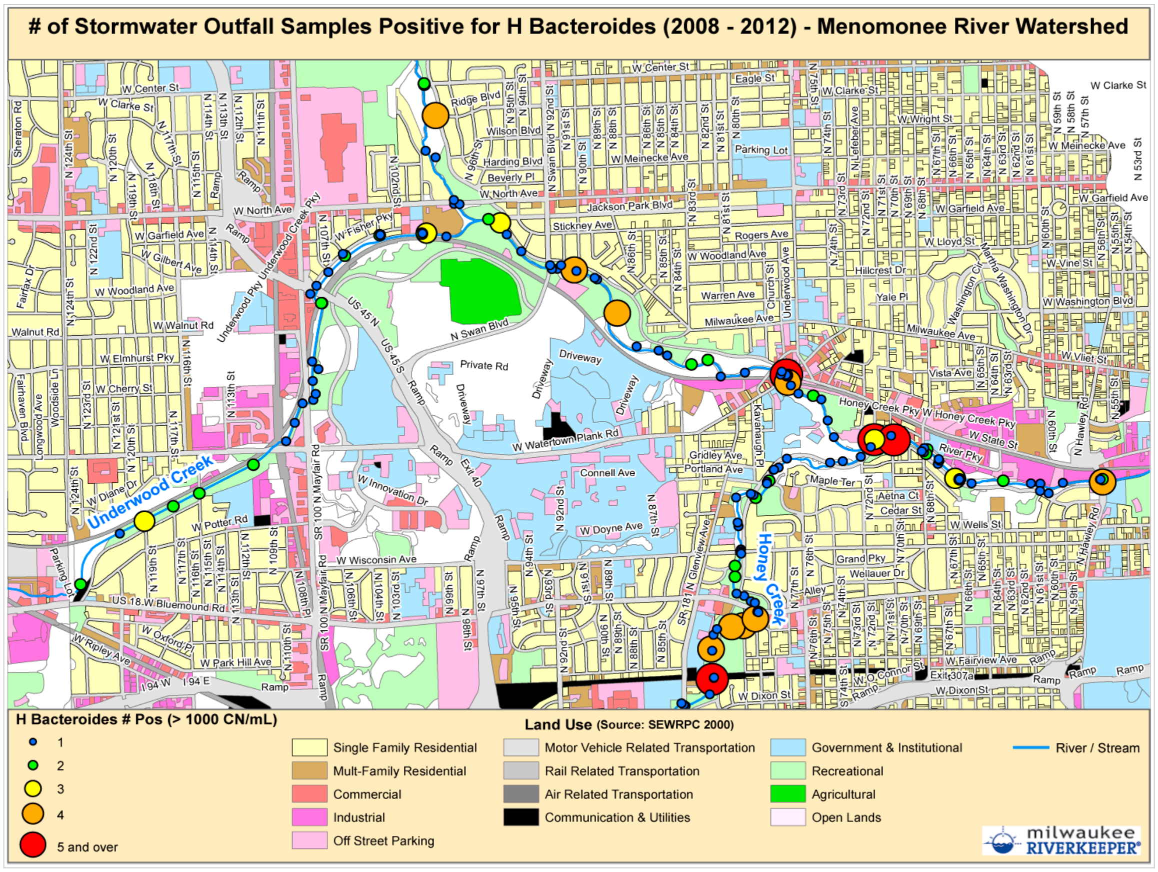


***Figure ES-3A**. Map of outfalls positive for sewage contamination, as measured by HB, in the Menomonee watershed (from 2008-2012).*

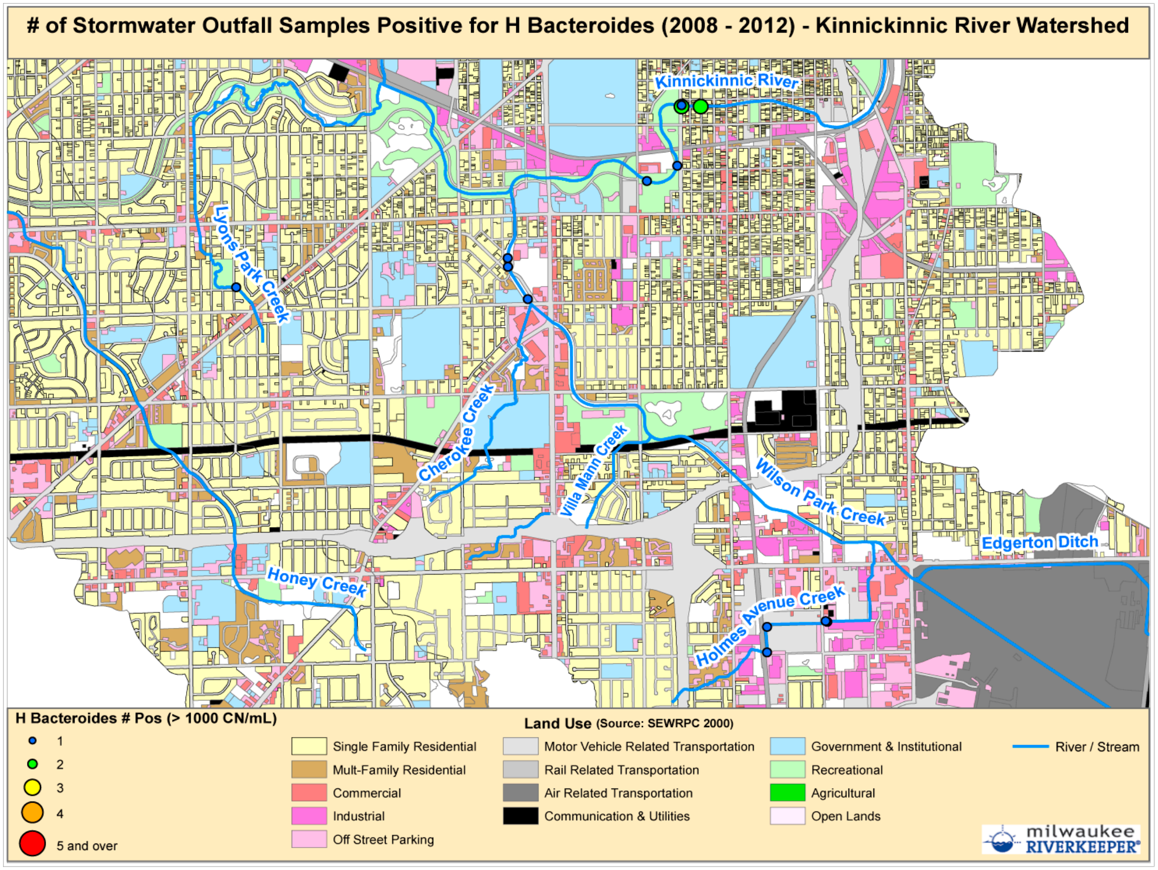


Figure ES-3B*. Map of outfalls positive for sewage contamination, as measured by HB, in the Kinnickinnic watershed (from 2008-2012). Additional sampling is planned for 2013-2015.*

Outfall Categories

Two major mitigation actions that would reduce sewage pollution to receiving waters are removal of illicit lateral connections to stormwater pipes and maintenance/replacement of the degrading sewer line responsible for leaking sewage. To help distinguish between the two issues we grouped sites into Category A and Category B outfalls. Category A outfalls were classified as those that flow in dry weather and show evidence of sanitary sewage contamination in dry weather, which may be an indication of illicit connections to stormwater pipes (there was no prior rainfall). This is based on the assumption that cross connections would result in contaminated discharges during wet and dry weather. In contrast, Category B outfalls were classified as those with evidence of sewage contamination only after rainfall, which may be more likely contaminated due to sewage leakage from failing infrastructure. However, cross-connections cannot necessarily be ruled out in this case.

We found that in wet weather Category A outfalls had a 21-fold higher average (4.3-fold higher geometric mean) for the HB sewage indicator than Category B outfalls did **(Table ES-1)**. This demonstrates that Category A outfalls (i.e. those that have evidence of sewage contamination in dry weather) are major contributors to fecal bacteria in rivers. To evaluate the overall contribution to sewage pollution by Category A outfalls versus Category B outfalls, we compared a subset of outfalls with known drainage areas. Based on average HB concentrations and outfall drainage area, we estimated sewage loads for 7 Category A outfalls and 33 Category B outfalls. We found that although Category A outfalls only represented 18% of the subset outfalls, they were responsible for approximately 68% of the wet weather sewage pollution load. Additionally, Category A outfalls released sewage during dry weather, meaning their sewage contribution could be much higher when this constant input is factored in. This calculation was a very course estimate of load, but we think it is an important and useful distinction between the two outfall types. It means that reducing the number of illicit lateral connections to stormwater pipes (evidenced by Category A outfalls) could have a major impact on the amount of sewage pollution reaching receiving waters.

Table ES-1. *Wet weather average and geometric mean for HB, EC and ENT found in samples from Category A and Category B outfalls. HB (CN/100 ml); EC and ENT (CFU/100 ml).*

	Number of Outfalls	HB Average (Geometric Mean)	EC Average (Geometric Mean)	ENT Average (Geometric Mean)
Category A outfalls	21	460,135 (10,803)	126,175 (11,128)	97,883 (16,529)
Category B outfalls	73	21,624 (2,515)	33,360 (4,504)	126,660 (10,161)

Influence of Drainage Area on Sewage Pollution Load

We found only a slight upward trend in HB concentration dependent on increasing outfall drainage area. This lack of association can be attributed to other factors at play in the level of sewage contamination that reaches a terminal outfall, such as age of infrastructure, land use, etc. In this study we found many small drainage areas that contain highly contaminated outfalls and contribute relatively large concentrations to receiving waters. Thus we utilized small drainage area combined with large estimated sewage load as a level of outfall remediation prioritization.

Up-the-Pipe Investigations

We examined the correlation of standard water quality parameters to HB to determine if there were simple measures that could be used for up-the-pipe investigations of sewage contamination. We found up-the-pipe samples showed fairly good correlations between HB and EC and less correlation with ENT, ammonia (NH_3) and total phosphorus (TP) in dry weather samples.

- Dry Weather Sample Pearson's Correlations (r):
    - HB and EC r = .77
    - HB and ENT r = .56
    - HB and NH_3 r = .47
    - HB and TP r = .40

The correlations were not surprising, as during dry weather contaminated flow through storm pipes likely represents illicit cross-connections, adding HB and the other fecal bacteria at relatively consistent ratios in the stormwater system. Additionally, both NH_3 and TP are present in domestic wastewater and would be expected to correlate to some degree with HB.

During wet weather there were no significant correlations between HB and EC, NH3 or TP in pipe samples, due to incoming stormwater diluting domestic wastewater to varying degrees. Although not recommended, up-the-pipe investigation during dry weather could utilize EC or ENT as a proxy for sewage contamination, but it should be noted that there are instances where there are low EC and ENT counts but strong evidence of human fecal markers (sewage).

Conclusions and Recommendations

In Greater Metropolitan Milwaukee, stormwater collection and conveyance systems are designed to capture storm runoff and discharge it, untreated, directly into the city's major rivers and tributaries. Sanitary sewage should not be present in outfall discharge, but the McLellan Laboratory has found widespread contamination of the outfalls tested here.

Recommendations for follow-up investigation include**:**

- Magnitude and frequency of outfall contamination
- Outfalls with contaminated flow during dry weather
- Outfalls with small drainage areas, but large sewage load estimates

- A heat map of outfalls was created based on the magnitude and frequency of HB contamination. Outfalls were divided into three priority groups:

  Tier 1 outfall sites were positive over 80% of the time (HB > 1,000 copy number/100 ml).
  Tier 2 outfall sites were positive in 50-80% of samples and had very high HB (> 10,000 copy number/100 ml).
  Tier 3 priority outfalls were positive for HB at least 50% of the time.

- Though Category A outfalls are less frequent than Category B outfalls, we estimate that they are responsible for a major proportion of the HB pollution escaping into receiving waters (perhaps higher than 70% in some reaches). The outfalls make follow-up investigation easier as they flow during dry weather.

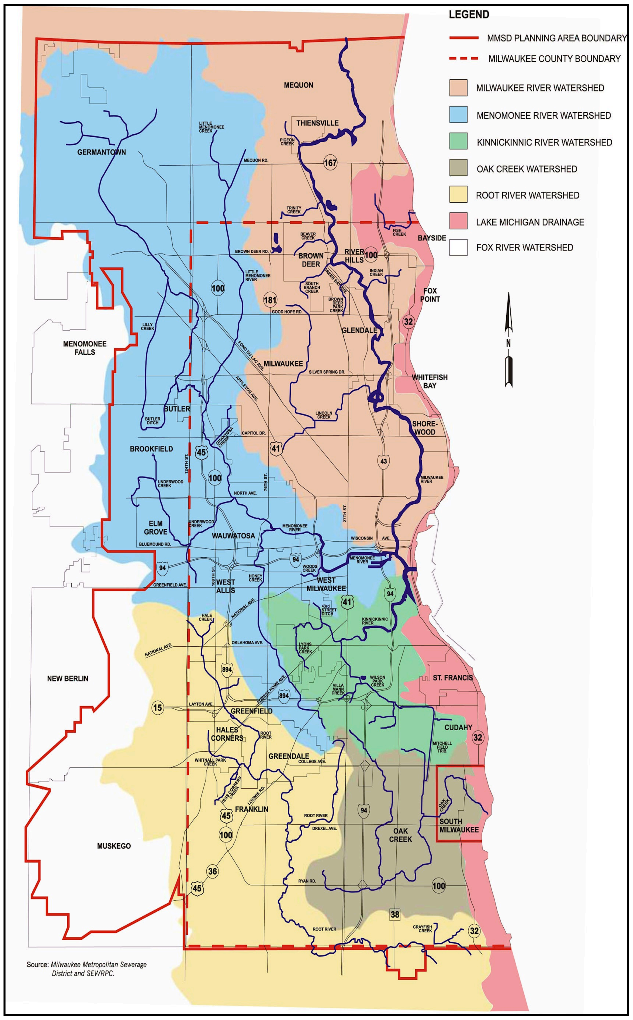
- Some small drainage areas contained highly contaminated outfalls and are an important source of pollution to receiving waters. Small drainage areas with large concentrations of HB are good targets for remediation since small areas make follow-up more straightforward with a greater chance of success.

Targeted repairs to infrastructure help direct resources to the sites that will maximize improved water quality and minimize monetary output. A challenge of this work was to prioritize up-the-pipe investigation with the hope of pinpointing remediation efforts to the worst sites. The recommended outfall prioritization scheme provides an efficient approach to district remediation effort.

1.0 Introduction

Potential for Pathogens in Stormwater Runoff

It is well known that urban stormwater runoff is a major contributor of non-point source of fecal pollution entering the rivers of Greater Metropolitan Milwaukee. In fact, stormwater runoff is the largest contributor of FC bacteria to the Kinnickinnic, Milwaukee and Menomonee Rivers (68.7%, 59%, and 83.7% of total FC loads, respectively) (SEWRPC 2008). While fecal coliforms (FC), including *Escherichia coli* (EC), are not generally pathogenic, their concentration in recreational waters is used to evaluate the risk of sewage associated human pathogens. High concentrations of fecal bacteria frequently cause river samples to exceed State of Wisconsin water quality standards and the Milwaukee Metropolitan Sewerage District (MMSD) has recognized FC and EC as a priority pollutant in the Greater Milwaukee Watersheds (**Figure 1**). However, although it is easy to detect these bacteria using standardized methods, they are not the best indicator of potential human pathogens in stormwater runoff or receiving waters.



Source: Milwaukee Metropolitan Sewerage District and SEWRPC.

Figure 1. *Watersheds in the Greater Milwaukee area.*

During storm events, high levels of pollutants are washed off the land, then transported and discharged directly into local waterways. Pollution from FC (including EC) and enterococci (ENT) is abundant in terrestrial runoff that harbors feces from domesticated animals and wildlife. Unrecognized sanitary sewage input from failing infrastructure and septic systems, or from overflows and illicit connections can also contribute to the bacterial pollutant load. While bacterial loads from animal waste carried in strormwater may not harbor many human pathogens, sewage infiltrating into the stormwater conveyance system has a substantially higher possibility of contamination with human pathogens. Human sources of fecal contamination are known to carry human pathogens, therefore, any contamination from sanitary sewage can be considered a potentially serious public health risk.

Characterizing sources of fecal pollution is essential to understanding the health risk that is associated with fecal indicators in urban stormwater. Little is known about the type of pathogens present, however, there is increasing evidence that urban stormwater may pose a serious human health risk (O'Shea and Field 1992, Haile et al. 1999, Gaffield et al. 2003, Rajal et al. 2007). One way to differentiate human from non-human fecal indicator bacteria is to utilize host specific genetic markers. Certain types of bacteria are only found in the human gut and not found in other hosts. Because most gut bacteria are sensitive to the presence of oxygen, they are difficult to grow in the laboratory and must be quantified using other methods. Quantitative polymerase chain reaction (qPCR) offers a relatively straightforward way to assay for host specific microorganisms in environmental samples. Based on the amplification of targeted DNA sequence (e.g., DNA sequence found exclusively in a bacterium specific to humans), the measured qPCR product corresponds to the amount of the targeted sequence that was present in the original sample.

The McLellan Laboratory uses qPCR to amplify and quantitate the human *Bacteroides* (HB) genetic marker (Bernhard and Field 2000). Because *Bacteroides* are generally in much higher abundance in fecal pollution than are FC, they can be considered a very sensitive indicator of fecal or sanitary sewage contamination. Moreover, certain species of *Bacteroides* have been found to be common in humans, but not other sources of fecal pollution such as cows or gulls (Bernhard and Field 2000, Dick et al. 2005, Lamendella et al. 2007). Sauer et al. (2011) found that sanitary sewage infiltration of stormwater outfalls was a major contributor to poor water quality in Milwaukee rivers. In this study initial sampling in a highly contaminated area showed close to half of the investigated outfalls had samples with a composition of at least 25% sanitary sewage. River water within all of the subwatersheds studied had very high levels of HB as did the outfalls discharging to these rivers. The author's findings support the concept that outfall discharge directly influences quality of receiving waters (**Figure 2**) and demonstrate the importance of using an alternative fecal indicator to identify sources in runoff pollution.

***Figure 2**. Stormwater outfall on Honey Creek.*

2.0 Goals and Objectives

The overall goal of our stormwater project was to perform comprehensive sampling and data analysis to determine the contribution of sanitary sewage contamination to urban stormwater within a targeted area of the Milwaukee Metropolitan Sewerage District (MMSD) service area. This research advances a high priority commitment for MMSD, whose mission is to protect public health and the environment and improve water quality.

Our research approach addresses our working hypothesis that sewage from failing infrastructure migrates into the stormwater system and is a major cause of water quality impairments and public health risk in urban waterways. An earlier report for MMSD by the McLellan Laboratory documents stormwater investigations from 2006-2009 and set the goal of intensive outfall sampling focused on two Milwaukee watersheds. Targeted outfalls included in the Menomonee Watershed were located along the Menomonee River, Honey Creek and Underwood Creek. The Kinnickinnic Watershed was sampled at outfalls on the Kinnickinnic River, Holme's Avenue Creek and Wilson Park Creek.

In this project we:

- Identified stormwater outfalls with sewage contamination as measured by HB levels.
- Prioritized outfalls by relevant findings for further investigation by MMSD
    - Percentage of time outfall is contaminated
    - Level of outfall contamination
    - Outfalls with sewage contaminated discharge during dry weather
    - Influence of drainage area on outfall contamination levels
- Identify failing infrastructure and illicit connections to storm pipes for remediation.

3.0 Methods

3.1 Sample Collection

Over 1,300 stormwater samples, both inline and grab samples, were collected from 2008-2012 and included 213 outfalls in Greater Milwaukee watersheds (**Appendix A – Maps**). In total, on the Menomonee River 62 outfalls were sampled. Additionally, two Menomonee tributaries, Honey Creek and Underwood Creek, were sampled at 38 and 26 outfalls respectively. Kinnickinnic watershed sampling totaled 37 outfalls on the Kinnickinnic River, 31 outfalls on Holme's Avenue Creek, 13 outfalls on Wilson Park Creek and 1 outfall on Villa Mann Creek. **Figure 3** shows total outfall coverage in the two major rivers and tributaries targeted in this study. Up-the-pipe grab samples and automated inline samples were collected by MMSD as part of their stormwater monitoring program. Samples were collected during rain events and during dry weather where necessary. Milwaukee Riverkeeper collected grab samples at 197 of the 213 outfalls in this study and the McLellan Lab collected the remaining outfalls. All Riverkeeper outfalls were assessed for residual flow in dry weather. In the field, all crews kept samples on ice and delivered them to the McLellan lab for analysis immediately after collection.

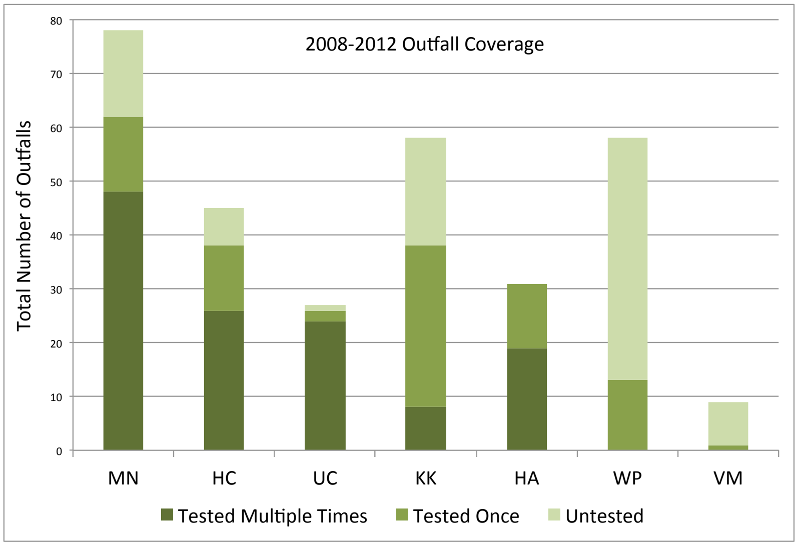


__Figure 3.__ Number of outfalls in each watershed sampled multiple times, only once or never from 2008-2012. Y-axis shows the approximate total number of outfalls present on each river. Menomonee River (MN), Honey Creek (HC), Underwood Creek (UC), Kinnickinnic River (KK), Holmes Avenue Creek (HA), Wilson Park Creek (WP), Villa Mann Creek (VM).

Outfall sites were chosen based obtaining wide coverage of the Menomonee and Kinnickinnic watersheds in the Greater Milwaukee Metropolitan area, which was a recommendation of the 2009 ***Greater Milwaukee Watersheds Pathogen Source Identification Report***. A concentrated sampling effort in these two areas of concern was expected to deliver the most useful information for remediation efforts.

3.2 *E. coli* and Enterococcus Enumeration

All water samples were analyzed within 12 hours using the USEPA method for EC enumeration (USEPA 2002). Samples were filtered through a 0.45 µm pore size 47 mm nitrocellulose filter and placed on modified m-TEC and MEI agar. The volume of sample filtered was varied according to the estimated contamination level. Plates were incubated for 18 hours and colony forming units (CFUs) were counted and recorded. When plate counts for either organism exceeded 1000 CFU/100 ml sample, qPCR analysis for HB followed.

3.3 Quantitative Polymerase Chain Reaction (qPCR)

All water samples were filtered within 12 hours for DNA extraction. A volume of 100 to 200 ml of sample was filtered onto a 0.22 µm pore size 47 mm nitrocellulose filter and stored at -80°C. The frozen filters were broken into small fragments using a metal spatula. DNA was extracted using the MPBIO FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Anna, CA) and DNA was eluted using 150ul of DES.

Quantitative PCR was carried out using an Applied Biosystems StepOne Plus™ Real-Time PCR System Thermal Cycling Block (Applied Biosystems; Foster City, CA) with Taqman hydrolysis probe chemistry. We used previously published primers and probe for human *Bacteroidales* (Kildare et al. 2007) with the exception that the HF183F was used as the forward primer (Bernard and Fields 2000). Standard curves were generated during each run and consisted of a linearized plasmid containing the *Bacteroides* human target sequence (**Figure 4**). Standard curves were run with DNA serially diluted from $1.5X10^6$ to $1.5X10^1$ copies/reaction. Standards were run in triplicate, and each sample was run in duplicate in a final volume of 25ul with a final concentration of 1uM for each primer, 80nM for the probe, 5ul of sample DNA, and 12.5ul of 2X Taqman® Gene Expression Master Mix Kit (Applied Biosystems; Foster City, CA). Amplification conditions consisted of the following cycles: 1 cycle at 50° C for 5 minutes to activate the uracil-N-glycosylase (UNG); 1 cycle at 95°C for 10 minutes to inactivate the UNG and activate the Taq polymerase; and 40 cycles of 94°C for 15 seconds and 1 minute at 60°C.

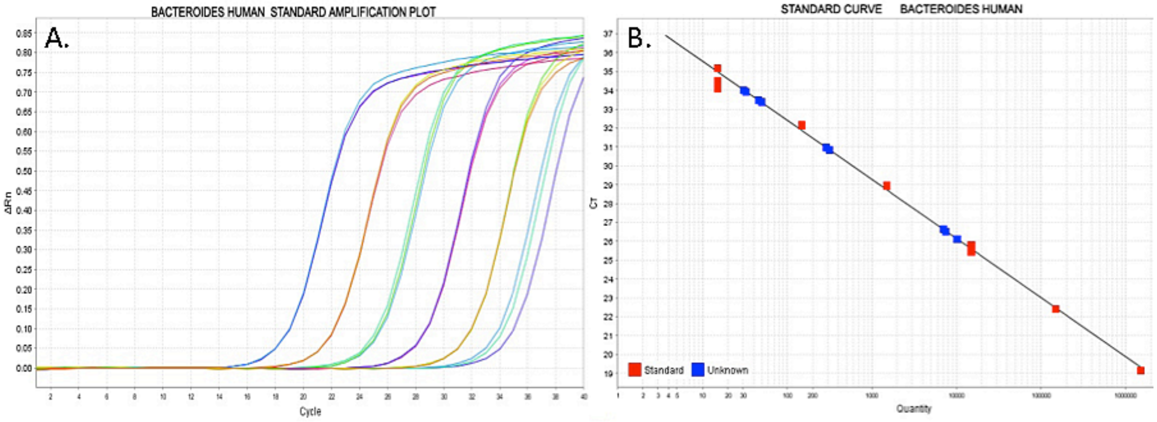


Figure 4. *Plot A illustrates a typical amplification of known quantities of HB DNA (a standard). The line in Plot B is a generated with information from Plot A and is a best fit to the concentrations of the HB standard (red squares) measured in Plot A. The unknown quantity of PCR amplified HB present in an environmental sample (blue squares) is then plotted along the line and its concentration is ascertained.*

3.4 Data Management

All samples from MMSD were tracked by their Laboratory Information Management System (LIMS) number and associated with a McLellan Lab Fate and Transport (FT) number. Samples collected by Milwaukee Riverkeeper or the McLellan Lab were assigned an FT number for tracking. Sites also have location informative site codes assigned to them by the group sampling them (i.e., MMSD, Riverkeeper or McLellan Lab). When outfall sites had more than one site code name (same site collected by different groups) they were binned with the Riverkeeper code for data analysis. Data was logged into a laboratory notebook and then transferred to an MS Access® database and Excel® spreadsheets (2008-2012 HB data contained in an Excel® spreadsheet in **Appendix B** of this report). Database entries were reviewed for accuracy by the McLellan Lab project manager and the Riverkeeper field manager. MMSD staff, Riverkeeper staff and McLellan Laboratory staff meet on a regular basis to discuss field and laboratory experimental progress and data management and reporting. Final reports are submitted to MMSD and Dr. McLellan gives presentations to disseminate the information.

3.5 Data Analysis

Data analysis was completed using Excel®, R version 3.0.1 (R Development Core Team 2012) open source programming language and the R Commander version 1.9-6 (Fox 2005) graphical user interface. Data was log10 transformed to a normal distribution for statistical analysis. Pearson's correlation analysis was used to test for associations between fecal bacteria indicator levels as well as associations between HB levels and MMSD-measured water quality variables. Holm's method of adjusted p-values was used to evaluate significance ($\alpha=0.05$) of Pearson's correlation coefficient.

4.0 Results and Discussion

The primary goal of this project was to evaluate levels of sewage contamination in stormwater conveyance systems by comprehensive sampling and analysis of outfall discharge in targeted Greater Milwaukee Watersheds. To that end, 213 outfalls were sampled and over 1,300 samples were analyzed from 2008-2012. The data presented here includes the historic data previously reported (McLellan and Sauer 2009) and newly analyzed data from 2009-2012. The research approach addresses our working hypothesis that sewage from failing infrastructure migrates into the stormwater system and is a major cause of water quality impairments and public health risk in urban waterways.

4.1 Overview of Human Specific *Bacteroides* and Bacterial Indicator Organisms

The relationship between levels of traditional bacterial indicators of fecal pollution and levels of human specific *Bacteroides* was examined. In outfall samples, bacterial indicators EC and ENT showed a good correlation (Pearson's $r = 0.71$, $p \leq 0.01$). However, outfall samples tested for HB showed low or no level of correlation with EC and ENT (Pearson's $r = 0.28$, $p \leq 0.01$ and $r = 0.1$, $p > 0.05$ respectively; ($n=376$)). This demonstrates that human sources are only one contributor (of many) to fecal indicator bacteria and targeting human specific bacterial signals is important to specifically address pathogen contamination. From domestic pets to urban wildlife, a rain event introduces diverse and varied fecal sources into urban stormwater conveyance systems. The advantage of identifying the human signal lies in the fact that even low human contribution may carry pathogens and therefore could pose a significant health risk, regardless of the contribution of other fecal indicator bacteria. Thus, in this report we rely on presence and levels of human specific indicators to evaluate levels of sewage contamination in stormwater outfall samples.

In **Table 1** overall average and geometric mean concentrations of traditional fecal indicator bacteria (FIB) are shown. Also shown are the average and geometric mean of HB concentrations from outfalls that tested positive for this alternative indicator in at least 50% of samples tested. In this study we considered outfalls that were positive more than 50% of the time as persistently positive for HB, and therefore a major concern. We also set thresholds of > 1000 CN of HB as "positive" and > 10,000 as highly contaminated. In the Menomonee watershed, outfalls discharging to the main stem of the Menomonee River and Underwood Creek had the highest average and geometric mean HB counts, but EC and ENT tended to be higher in Honey Creek outfalls. This demonstrates the problem with using EC or ENT to evaluate levels of sewage pollution – although levels of these FIB were higher in Honey Creek outfalls, levels of human specific marker was higher in Menomonee River and Underwood Creek outfalls. The limited sampling effort in the Kinnickinnic watershed showed the Kinnickinnic River had the highest average and geometric mean levels of both traditional FIB and HB. Further sampling is underway in this watershed.

***Table 1**. Number of samples tested for each watershed from 2008-2012, average and geometric mean for EC and ENT levels. Also, number of outfalls tested more than once for HB, the number of outfalls positive more than 50% of the time (persistently positive) and the HB average and geometric mean for these persistently positive outfalls.*

	All Outfalls Tested				**Outfalls tested more than once for HB**		
Receiving Waters	# of Samples	# of Outfalls	**EC** Average (Geomean)	**ENT** Average (Geomean)	# of Outfalls[1]	# That Tested HB Positive ≥50% of the Time	**HB** Average[2] (Geomean)
Menomonee River	212	62	30,840 (2,085)	43,523 (3,919)	39	30	233,445 (9,701)
Honey Creek	137	38	23,111 (4,439)	246,727 (10,742)	21	10	10,446 (5,330)
Underwood Creek	90	26	16,299 (947)	30,480 (2,092)	22	14	47,101 (13,370)
Kinnickinnic River	57	38	180,433 (772)	18,825 (939)	3	3	6,827 (5,293)
Holmes Ave Creek	55	31	1,928 (146)	2,512 (532)	5	1	949 (614)
Wilson Park Creek	13	13	10,340 (307)	2,822 (138)	0	NA	NA

[1] number of outfalls tested more than once

[2] average and geomean is of outfalls that are positive more than 50% of the time

4.2 Identification of Sewage Contaminated Stormwater Outfalls

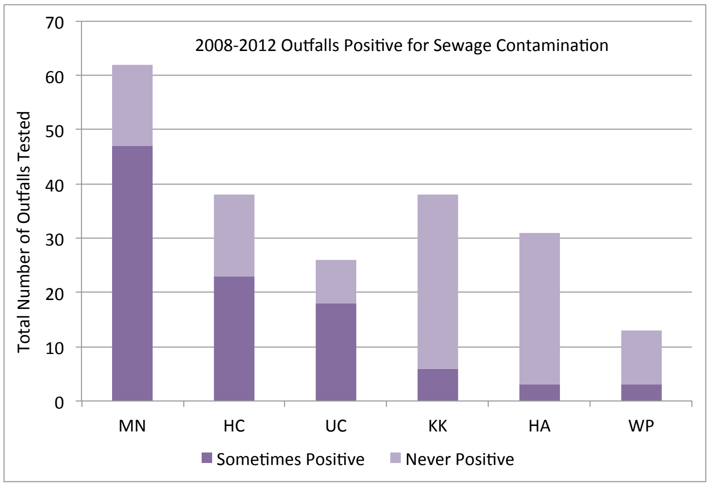


***Figure 5**. Number of outfalls that leaked sewage into receiving waters compared to the number that did not leak. For leaky outfalls – some leaked intermittently and others leaked regularly; some leaked in dry weather and others did not. Menomonee River (MN), Honey Creek (HC), Underwood Creek (UC), Kinnickinnic River (KK), Holmes Avenue Creek (HA), Wilson Park Creek (WP).*

Evidence of sanitary sewage contamination was assessed by two human specific markers, human *Bacteroides and* human *Lachnospiraceae.* These human markers were widely distributed throughout the watersheds targeted in this study. **Figure 5** shows the number of outfalls that showed evidence of sewage in each watershed and subwatershed tested. A "positive" designation for HB was given to samples when greater than 1,000 copy numbers of the genetic marker for the bacterium were detected (>1,000 CN/100 ml). Low levels of contamination are found almost ubiquitously; therefore we determined 1,000 CN/100 ml is an appropriate benchmark to designate an outfall as positive for untreated sewage contamination. Untreated sewage contains 3.4 x 10^7 HB CN/100 ml on average, with very steady levels of both HB and *Lachnospiraceae* (Newton et al. 2011), therefore what we call positive corresponds to 0.003% untreated sewage.

Table 2 provides annual and summary results for outfalls in two major rivers and their tributaries. In the Menomonee watershed, 76% of stormwater outfalls along the Menomonee River tested positive for HB either occasionally or consistently. Two Menomonee tributaries, Honey Creek and Underwood Creek, tested positive in 61% and 69% respectively of outfalls tested. Overall, the Kinnickinnic watershed had fewer positive outfalls with the Kinnickinnic River and its tributaries, Holme's Avenue Creek and Wilson Park Creek, testing positive at 16%, 10% and 23% respectively. Ongoing sampling efforts will provide additional coverage of the Kinnickinnic River, therefore the overall assessment of the % of outfalls affected is incomplete. Results from individual stormwater samples (outfalls and up-the pipe) are shown in **Appendix B**. These results represent wide coverage of two Greater Milwaukee Watersheds and indicate that stormwater inputs continue to create a public health risk at these rivers and their tributaries, and by extension, to Lake Michigan and local beaches.

***Table 2**. Number of outfalls surveyed, number of those that ever showed contamination with sewage (as measured by HB levels) and number samples tested from 2008 through 2012 along the Menomonee and Kinnickinnic watersheds.*

		Number of outfalls tested	Contaminated outfalls	Number of samples tested
2008	Menomonee River	36	16 (44%)	62
	Honey Creek	27	8 (30%)	72
	Underwood Creek	18	2 (11%)	19
	Kinnickinnic River	6	0 (0%)	18
	Holmes Ave. Creek	NT	NT	NT
	Wilson Park Creek	NT	NT	NT
2009	Menomonee River	13	10 (77%)	14
	Honey Creek	7	2 (29%)	7
	Underwood Creek	4	2 (50%)	4
	Kinnickinnic River	7	2 (29%)	7
	Holmes Ave. Creek	17	1 (6%)	18
	Wilson Park Creek	NT	NT	NT
2010	Menomonee River	50	35 (70%)	96
	Honey Creek	10	4 (40%)	10
	Underwood Creek	9	2 (22%)	9
	Kinnickinnic River	18	0 (0%)	18
	Holmes Ave. Creek	12	0 (0%)	12
	Wilson Park Creek	NT	NT	NT
2011	Menomonee River	21	15 (71%)	38
	Honey Creek	12	9 (75%)	22
	Underwood Creek	7	3 (43%)	9
	Kinnickinnic River	8	3 (38%)	11
	Holmes Ave. Creek	20	2 (10%)	25
	Wilson Park Creek	NT	NT	NT
2012	Menomonee River	2	2 (100%)	2
	Honey Creek	24	9 (38%)	26
	Underwood Creek	25	13 (52%)	49
	Kinnickinnic River	3	1 (33%)	3
	Holmes Ave. Creek	NT	NT	NT
	Wilson Park Creek	13	3 (23%)	13
Total 2008-2012	**Menomonee River**	**62**	**47 (76%)**	**212**
	Honey Creek	**38**	**23 (61%)**	**137**
	Underwood Creek	**26**	**18 (69%)**	**90**
	Kinnickinnic River	**38**	**6 (16%)**	**57**
	Holmes Ave. Creek	**31**	**3 (10%)**	**55**
	Wilson Park Creek	**13**	**3 (23%)**	**13**

Although many outfalls were positive (>1,000 CN/100 ml) for human sewage indicators, some outfalls were intermittently positive and others gave a consistent sewage pollution signal. Out of 92 outfalls that were consistently tested, 60 outfalls (including 2 that discharge directly into Lake

Michigan) tested positive at least 50% of the time. In this group we found a high proportion of outfalls that average > 10,000 CN/100 ml HB. Out of these 60 frequently contaminated outfalls, 23 (38%) had HB averages >10,000 CN/100 ml, whereas this high average was only seen in 1 of 32 (3%) of the outfalls showing less frequent human sewage pollution. Thus, we designate outfalls that are positive at least 50% of the time (i.e. persistently positive) as a priority for investigation and remediation.

About 24% of samples in this study (n=281) were assayed concurrently for human *Bacteroides* and human *Lachnospiraceae* genetic markers to confirm human contamination results and assess if there were false positives. The two human sewage indicators matched as both being present or absent in 81% of samples tested. In instances where the two markers don't match, we believe age of the pollution signal and differential survival rates in some environmental conditions may be playing a role. Future comparisons of larger datasets with concurrent human *Bacteroides* and human *Lachnospiraceae* assays should resolve these differences.

4.3 Outfalls Categories

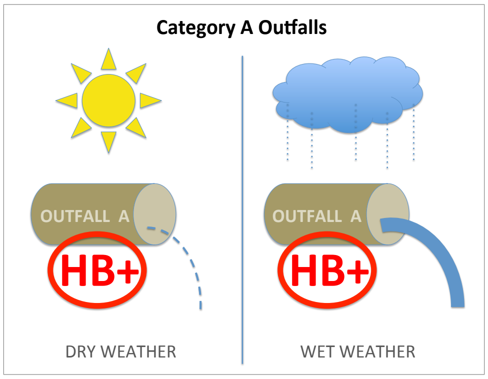
Most outfall sites were checked for discharge during dry weather (i.e., stormwater outfalls that flow when there has not been a recent rain event). Outfalls that flow in dry weather and show evidence of sanitary sewage contamination in those samples may be an indication of illicit connections to stormwater pipes (there was no prior rainfall). In contrast, outfalls that have evidence of sewage contamination only after rainfall, are more likely contaminated due to sewage leakage from failing infrastructure. We surveyed for these two outfall types and compared their contributions to sewage pollution.

Out of the 213 outfalls in this study, 136 were found to flow during dry weather and 77 outfalls either did not flow or were not checked for flow. Dry weather flow samples with high FIB counts were assayed for HB. Outfalls were subsequently categorized based on a combination of flow pattern and sewage contamination. **Table 3** summarizes the outfall categories and is followed by a description of each category. There were 48 outfalls that could not be categorized. These outfalls were either never checked for dry weather flow, or were never sampled in wet weather and therefore could not be assigned a category. The undefined outfalls will be categorized during future sampling efforts.

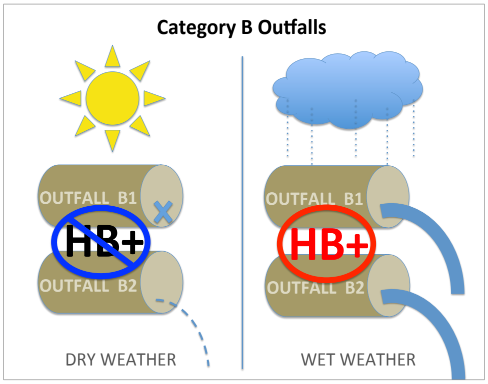
	Outfalls				
Category	**A**	**B**	C	Undefined	ALL
Number	**21**	**73**	71	48	213

***Table 3**. Outfall categories summary. Category A and Category B outfalls (Bolded) show evidence of sewage contamination.*

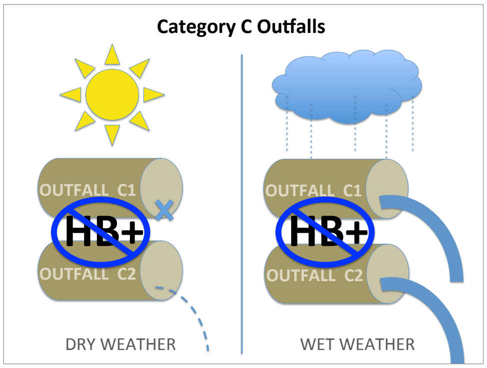
Illustration 1. *Stormwater outfall categories*



Category A Outfalls – Of the 136 outfalls found to flow during dry weather, 21 were HB-positive *during dry weather* (HB ≥ 1,000 CN/100 ml) and were classified as Category A outfalls. Of the 21 Category A outfalls, 10 outfalls were tested for HB in wet weather and all were strongly HB-positive, with individual outfall averages ranging from HB ≥ 4,000 CN/100 ml to HB ≥ 2,000,000 CN/100 ml. Although the remaining 11 outfalls were not checked in wet weather, we predict they will also be HB-positive based on the strong positive results of the 10 outfalls that were checked, therefore these 11 outfalls are Category A* (i.e., presumptive Category A).



Category B Outfalls – Outfalls that are not HB-positive in dry weather but are HB-positive in wet weather are designated Category B. For this category dry weather results include outfalls that do not flow in dry weather and outfalls that do flow in dry weather, but samples are not HB contaminated. The uncontaminated dry weather flow may be due to water from outdoor sources (washing a car, watering the lawn, etc.) in the stormwater conveyance system. In either case, these outfalls are HB-positive in wet weather samples. A total of 73 outfalls fell into this category.



Category C Outfalls – Our final category contained 71 outfalls. Outfalls that were not HB-positive in dry or wet weather samples were designated Category C outfalls. As was seen in Category B outfalls, there may or may not be flow in dry weather, but when flow is present it is not HB-positive. This category represents outfalls that never showed any evidence of sanitary sewage contamination. They were clean.

4.3.1 FIB and HB Results of Outfalls that Flow in Dry Weather

A summary of data results for the 136 outfalls that flow in dry weather is provided in **Table 4**. The 21 Category A/A* outfalls are in red. Most outfalls that flowed in dry weather did not show evidence of sanitary sewage contamination and fell into other categories. Generally, only outfalls with EC or ENT counts >1,000 CFU/100 ml were assayed for HB. When FIB counts are lower than 1,000 CFU/100 ml it is unlikely that HB is present. Some dry weather flow outfalls remain to be tested for HB, but during dry weather most outfalls listed in **Table 4** do not appear to be discharging sewage pollution.

Table 4. *Dry weather averages for HB, EC and ENT for 136 outfalls that flow in dry weather. Category A outfalls are in red. HB (CN/100 ml); EC and ENT (CFU/100 ml). Outfalls with EC or ENT counts > 1,000 CFU/100 ml are being currently being assayed for HB.*

SITE	Ave HB	Ave EC	Ave ENT
FMRHAC08		0	0
FMRHAC12		0	0
FMRHAC26		0	0
FMRHAC28		14	37
FMRHAC29		TNTC	TNTC
FMRHC01		24	93
FMRHC04	225	32,000	22,000
FMRHC08		13,350	17,800
FMRHC09		110	160
FMRHC19		2,200	1,970
FMRHC20		100	90
FMRHC22	803	15,200	2,390
FMRHC23		3,440	130
FMRHC29		60	50
FMRHC30	590	650	280
FMRHC31		22,800	3,800
FMRHC32		200	4,700
FMRHC33	15,330	1,450	1,215
FMRHC35	225	100	60
FMRHC36		540	30
FMRHC38		130	270
FMRHC40		170	840
FMRHC42		50	510
FMRHC45		570	3,000
FMRKK02		33	100
FMRKK03		2	180
FMRKK04	6,806	200	670
FMRKK05	49,229	TNTC	169,000
FMRKK06		13,300	490
FMRKK07		6	0
FMRKK08	225	8	0
FMRKK10		0	580
FMRKK11		10	10
FMRKK12	225	100	1,090
FMRKK14		100	190
FMRKK19		61,000	2,080
FMRMN40		150	280
FMRMN41		210	230
FMRMN44	164,082	1,001	1,468
FMRMN46		220	250
FMRMN48		220	340
FMRMN49		270	470
FMRMN51		460	490
FMRMN52		0	0
FMRMN54		1,000	1,200
FMRMN55		400	310
FMRMN58	4,202	730	505
FMRMN59		250	340
FMRMN60		390	340
FMRMN61		350	430
FMRMN62	602	24	0
FMRMN63		190	410
FMRMN65		4,600	128,000
FMRMN66		0	0
FMRMN70		172	200
FMRMN71		0	0
FMRMN72		3,800	900
FMRMN73	2,532,847	TNTC	11,400
FMRMN74	225	3,400	87
FMRMNMP03	6,905	140	100
FMRMNPH	530,407	0	20
FMRUC01	1	30	1,340
FMRUC03		630	410
FMRUC04		4,600	1,900
FMRUC06		70	80
FMRUC08	272,381	770	5,300
FMRUC11		3,000	850
FMRUC12		50	440
FMRUC13		100	10
FMRUC14		0	0
FMRUC15		0	20
FMRUC16	630	7,700	3,400

SITE	Ave HB	Ave EC	Ave ENT
FMRKK28		2,310	1,840
FMRKK31		390	1,080
FMRKK32		40	10
FMRKK34		90	140
FMRKK35		420	460
FMRKK39		270	60
FMRKK40	225	1,100	1,100
FMRKK42	225	670	1,400
FMRKK50		940	370
FMRKK51		10	50
FMRKK52	225	1,690	7,100
FMRKK54	244,511	4,650,740	2,525
FMRKK56	346	14,400	124,000
FMRKK60	225	0	400
FMRMN01		3,000	209
FMRMN04		0	0
FMRMN06		16	137
FMRMN07		720	760
FMRMN08	347	1,620	7,300
FMRMN10	15,286	3,100	3,700
FMRMN12	4,814	5,300	4,100
FMRMN13	225	20,200	6,400
FMRMN15	10,547	3,100	2,900
FMRMN18	680	1,270	820
FMRMN24		160	120
FMRMN27		0	0
FMRMN29	138,241	1,610	1,325
FMRMN33		52,039	26,537
FMRMN34		20	40
FMRMN35		150	250
FMRMN38		230	160
FMRMN39	34,551	160	126
FMRUC17		10	20
FMRUC18	21,886	2,180	1,970
FMRUC20		380	260
FMRUC21		470	440
FMRUC28		50	50
FMRUC29		0	0
FMRVM09	225	39,000	32,000
FMRWPC05	10,698	2,200	600
FMRWPC08	200,217	8,100	3,400
FMRWPC11	776	106,000	3,700
FMRWPC15	1,168	1,000	0
FMRWPC20		100	300
FMRWPC22		0	0
FMRWPC25	566	2,500	2,800
FMRWPC26	315	3,550	300
FMRWPC31	225	10,700	20,700
FMRWPC36		0	0
FMRWPC44		0	0
FMRWPC48	945	210	4,850
FMRWPC49		58	36
RUSSAVE		92	171
SHC110		13,800	5,000
SKK010		3,570	5
SKK10	1	70	0
SKK11	1	0	20
SKK12	408	0	10
SKK13	99	560	6,300
SLC020		70	0
SLP010		730	0
SMN020	46,388	1,990	0
SOC010		90	8
SWWB09	17,054	2,905	0

4.3.2 Category A Outfalls: Are these the Majority of Problem Outfalls?

We wanted to know if dry weather surveys could replace wet weather surveys in identifying the outfalls that are major contributors to sewage discharge during rain events. We found that only 19% of the outfalls that were positive for human marker in wet weather were Category A outfalls (i.e., had HB-positive flows during dry weather). So, dry weather surveys missed 81% of outfalls found to be positive by rain surveys and thus did not identify the problem in terms of gross number of outfalls that have sewage contamination. (See **Appendix B.1** for a full table of assay results for outfall samples.) Importantly, rain surveys remain necessary for identification and monitoring of many problem outfalls.

4.3.3 Category A Outfalls: Are these Major Contributors to Untreated Sewage Contamination?

We also wanted to assess if surveying Category A outfalls was a good way to identify outfalls that have the highest concentrations of sewage contamination. We compared rain event data for the average and geometric mean concentrations of HB coming out of Category A versus Category B outfalls. We analyzed the data this way for two reasons; first, to distinguish contributions from probable illicit connections (Category A outfalls) vs. failing infrastructure (Category B outfalls), and second, to determine which type of outfall provides the biggest overall sewage contribution to receiving waters. **Table 5** summarizes the data for HB, EC and ENT concentrations found in rain event samples.

Category A outfalls had a 21-fold higher average (4.3-fold higher geometric mean) for HB counts than Category B outfalls (**Table 5**). Thus, outfalls with probable illicit connections (Category A) were not only a constant source of sewage pollution, contaminating receiving waters in both dry and wet weather, but wet weather samples had higher average concentrations of HB and EC than Category B outfalls. Category A outfalls also had higher geometric mean concentrations of all FIB measured than Category B outfalls (**Table 5**). So although dry flow surveys may not be useful in determining the gross number of contaminated outfalls as illustrated in Section 4.3.2, they are useful in distinguishing outfalls that are major contributors of high sewage concentrations.

Table 5. *Wet weather average and geometric mean for HB, EC and ENT found in samples from Category A and Category B outfalls. HB (CN/100 ml); EC and ENT (CFU/100 ml).*

	Number of Outfalls	HB Average (Geometric Mean)	EC Average (Geometric Mean)	ENT Average (Geometric Mean)
Category A outfalls	21	460,135 (10,803)	126,175 (11,128)	97,883 (16,529)
Category B outfalls	73	21,624 (2,515)	33,360 (4,504)	126,660 (10,161)

If illicit connections are the primary cause of Category A outfalls, our results suggest that tracking and eliminating these connections could have a large impact on the level of sewage contamination reaching receiving waters. However, Category A outfalls are not as common as Category B outfalls so a comparison of outfall sewage contributions should include load estimates derived from associated drainage areas.

4.4 Influence of Drainage Area and Loads

Assuming other parameters held equal, the size of drainage areas associated with outfalls might be a predictor of the contribution to the level of sewage contamination coming from the terminal outfall. An outfall draining an older conveyance system in a large area would have more potential to come into contact with sewage input from failing infrastructure, including failing laterals, or illicit connections than an outfall in a smaller drainage area of the same age. We found only a slight upward trend in HB concentration dependent on increasing drainage area (**Figure 6**) and attribute this lack of association to the many other factors at play in the level of sewage contamination that reaches an outfall. In this study, we found many small drainage areas contain highly contaminated outfalls.

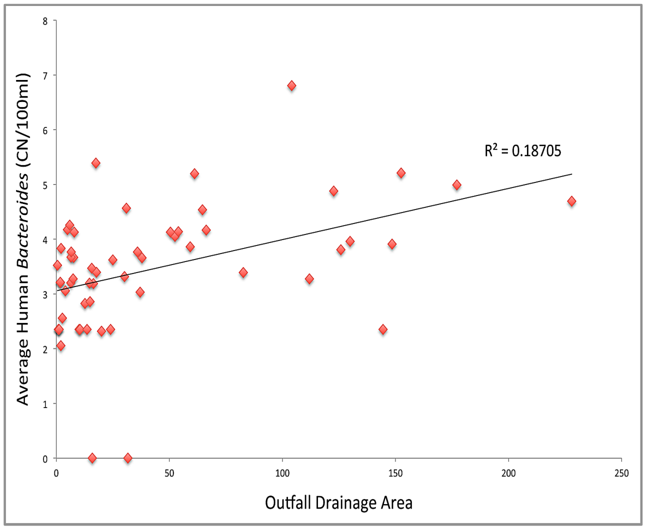


Figure 6. *Outfall drainage area versus levels of HB for samples where both variables were known. The largest drainage area (976 acres) was removed from the scatterplot and y-axis is log10 scale to help visualization.*

To utilize outfall drainage area as a level of remediation prioritization, we estimated the load of HB at outfalls with known drainage (**Table 6**). A rough estimated load was calculated by multiplying average HB concentration for an outfall by the number of acres in the drainage area for that outfall. Comparing these estimates of load gives good outfall candidates for further investigation. Simply based on ease of investigation, small drainage areas with large loads are prime targets for remediation. In **Table 6,** there are 15 outfalls (rows that are bold and red) that have comparatively small drainage areas (< 60 acres), high average load estimates (> 30,000 CN/100 ml) and associated outfalls that are regularly (> 50% of the time) positive for HB, making them especially good candidate areas for investigation. An additional 7 outfalls have small drainage areas and high load estimates, but the associated outfall is only positive intermittently (≤ 50% of the time) or was only checked once, making them a lower priority. However, small drainage areas may make even intermittent problems easier to track and repair.

Examining the influence of drainage area on level of sewage contamination from the outfalls in **Table 6**, it is clear that large drainage areas tend to have higher levels of HB contamination than smaller drainage areas. Thus large drainage areas appear to be more susceptible to sewage infiltration. However it is also evident that particularly small drainage areas (i.e., FMRKK59, FMRMN70, FMRMN41) can be highly contaminated and are important sources of pollution to receiving waters.

Table 6. *Outfall with drainage area (DA), average HB count and estimated average load of HB. Bold Red: Small drainage areas with large load estimates and frequent high HB concentrations at associated outfall. Pink have small DA but only intermittently high HB concentrations at outfalls. (Outfalls with a shared drainage are footnoted as such.)*

Outfall Site Code	DA in Acres	Average HB	Estimated Load (Ave. HB x DA)	Outfall Site Code	DA in Acres	Average HB	Estimated Load (Ave. HB x DA)
FMRHAC01	1.3	225	293	FMRMN06	7.4	1,882	13,924
FMRHAC12	14.7	0	0	FMRMN12	82.7	2,469	204,186
FMRHAC29	15.9	0	0	**FMRMN13**	**54.0**	**13,670**	**738,162**
FMRHAC30	31.7	0	0	FMRMN19	129.9	9,089	1,180,645
FMRHC12[1]	111.9	3,556	397,916	FMRMN23	0.5	3,337	1,669
FMRHC29	12.7	668	8,484	**FMRMN24**	**17.8**	**2,476**	**44,064**
FMRHC30	**35.9**	**5,817**	**208,837**	**FMRMN26**	**15.7**	**2,933**	**46,042**
FMRHC31	59.2	7,262	429,881	FMRMN27	16.4	1,546	25,354
FMRHC32	**7.7**	**4,661**	**35,886**	FMRMN29	122.7	76,021	9,327,818
FMRHC33[2]	125.8	9,376	1,179,454	FMRMN30	1.0	225	225
FMRHC34[3]	20.0	184	3,680	FMRMN33	14.9	724	10,793
FMRHC35	**31.0**	**36,666**	**1,136,643**	**FMRMN35**	**50.5**	**13,467**	**680,058**
FMRHC38[1]	111.9	225	25,178	FMRMN38	4.0	1,142	4,568
FMRHC40[2]	125.8	1,598	201,079	FMRMN39	152.5	161,654	24,652,286
FMRHC42[3]	20.0	225	4,500	FMRMN41	4.9	14,868	72,853
FMRHC43	2.0	113	226	FMRMN44	177.1	97,707	17,303,821
FMRHC45	1.8	1,600	2,880	FMRMN48	25.0	4,194	104,850
FMRKK01	**14.7**	**2,351**	**34,560**	FMRMN49	6.4	1,585	10,142
FMRKK04	2.1	6,806	14,293	**FMRMN51**	**6.6**	**5,856**	**38,650**
FMRKK05	227.9	49,229	11,219,289	**FMRMN52**	**37.9**	**4,553**	**172,559**
FMRKK08	13.6	225	3,060	FMRMN53	0.9	225	203
FMRKK12	10.1	225	2,273	FMRMN54	37.1	1,078	39,994
FMRKK40	66.3	225	14,918	FMRMN58	148.5	8,100	1,202,801
FMRKK42	24.0	225	5,400	FMRMN59[4]	976.5	7,494	7,317,891
FMRKK52	144.5	225	32,513	FMRMN60[4]	976.5	13,866	13,540,149
FMRKK54	**17.5**	**244,511**	**4,278,943**	FMRMN61[4]	976.5	13,986	13,657,329
FMRKK57	10.5	225	2,363	**FMRMN64**	**52.5**	**11,187**	**587,318**
FMRKK58	6.5	4,696	30,521	FMRMN65	2.7	360	972
FMRKK59	**7.9**	**13,434**	**106,129**	FMRMN70	5.9	18,032	106,387
FMRMN01	1.8	1,639	2,867	FMRMN73	104.1	6,370,235	663,141,411
FMRMN04	**30.2**	**2,077**	**62,715**	**FMRMN74**	**61.1**	**157,321**	**9,612,313**

[1] FMRHC12 and FMRHC38 shared 111.9 acre drainage area

[2] FMRHC33 and FMRHC40 shared 125.8 acre drainage area

[3] FMRHC34 and FMRHC42 shared 20 acre drainage area

[4] FMRMN59, FMRMN60 and FMRMN61 shared 976.5 acre drainage area

4.4.1 Influence of Drainage Area on Category A Outfall Loads

Although results indicate that Category A outfalls are good candidates for high priority up-the-pipe investigation, they are relatively rare in comparison to the number of Category B outfalls. To determine which type of outfall makes the biggest overall sewage contribution to receiving waters, we approximated loads from a subset of Category A and B outfalls with known drainage areas (n=7 and n=33, respectively). For rain event data, averaged HB counts for each outfall were multiplied by drainage area to give load estimates. Load estimates were summed for each outfall category to give total contribution of HB from Category A or B outfalls over the study period (2008-2012).

- *Summed loads from Category A outfalls w DA (n=7): 112,362,856 HB CN/100 ml*

- *Summed loads from Category B outfalls w DA (n=33): 52,795,723 HB CN/100 ml*

- *Percent of Category A outfalls: 7/(7+33) = .18 (18%)*

[The proportion of Category A to Category B outfalls used in the subset for this analysis approximates the proportion of total Category A outfalls in relation to Category B outfalls, therefore we estimate that the subset is a good approximation of the whole.]

- *Percent contribution by Category A: 112,362,856 / (112,362,856 + 52,795,723) = 0.68 (68%)*

- *Cat A Load / Cat B Load: 112,362,856 HB CN/100 ml / 52,795,723 HB CN/100 ml ≈ 2 times more HB from Category A outfalls*

In the outfall subset used for these load estimates, only 18% were Category A outfalls, but they delivered 68% of the measured sewage pollution. Thus, Category A outfalls not only have a higher average and geometric mean for the HB indicator of sewage contamination than Category B outfalls, but even though they are relatively infrequent, they appear to contribute the largest HB load to receiving waters. Additionally, they contaminate receiving waters during dry weather and although the load on those days may not be large, the number of those days is generally greater than the number of rain days in the Greater Milwaukee area. As a source of frequent small sewage pollution loads, Category A outfalls could have a very large impact on water quality, especially when considering concentrations in nearby river sites.

While we have used a subset of outfalls for these calculations, it is our goal to gain comprehensive coverage of the watershed and be able to provide more empirical data on loads from Category A outfalls (presumed cross connections) *vs.* Category B outfalls (failing infrastructure, other mechanisms)

4.5 Up-the-pipe Outfall Investigation

MMSD collected neighborhood stormwater pipe samples (up-the-pipe samples) that were analyzed for human specific *Bacteroides*. Collaboratively, 21 investigations were conducted. Samples taken up the pipe from the terminal outfall were numbered consecutively with A, B, C, etc.

We examined the correlation of standard water quality parameters to HB to determine if there were simple measures that could be used for up the pipe investigations. We found up-the-pipe samples showed fairly good correlations between HB and traditional fecal indicators, especially EC (**Table 7**) and especially during dry weather. The correlations were not surprising, as during dry weather contaminated flow through storm pipes likely represents illicit cross-connections, adding HB and the other fecal bacteria at relatively consistent ratios in the stormwater system. However during wet weather, samples are "diluted" with storm runoff carrying high levels of fecal bacteria from other animals into the stormwater conveyance system – a situation that lowers the concentration of HB while maintaining or increasing the other fecal indicators found in the sample. Therefore, wet weather correlations between HB and fecal indicators are not only weaker (less predictable) but are also negative due to the diluting effect of runoff carrying terrestrial sources of EC and ENT. Up the pipe investigation during dry weather could utilize EC or ENT as a proxy, but it should be noted that there are instances where there are low EC and ENT but strong evidence of human genetic markers (SHC31A, SHC31B, SMN06D, SMN29A, SMN44A and SMN73A are examples highlighted in red Appendix B.2).

Like fecal indicator bacteria, water quality measurements for ammonia (NH_3) and total phosphorus (TP) showed higher correlations with HB levels in dry weather samples than in wet weather samples. Both NH_3 and TP are present in domestic wastewater and when illicit connections are responsible for dry flow found in stormwater pipes, both pollutants would be expected to increase as HB levels increase. Unlike HB, NH_3 and TP are also present to varying degrees in stormwater runoff, so in wet conditions we do not expect a correlation with HB as storm pipes fill with runoff.

As expected total suspended solids (TSS) had no significant correlation with HB in dry weather collections. However during rain there is a slight negative correlation (as runoff scours the landscape and enters stormwater pipes, a rise in TSS concentration accompanies a dilution in HB concentration). ENT, on the other hand, showed a significant positive correlation with TSS, which suggests that the scouring effect is increasing the concentration of ENT as well as TSS in runoff.

Table 7. *Pearson's correlation coefficients (r) for HB and water quality measurements in up-the-pipe samples. Data was log transformed and significant correlations are in bold (*$\alpha = 0.05$*). Panel* ***A*** *shows correlations in dry weather samples (n=71) and panel* ***B*** *correlations in wet weather samples (n=105). HB was measured by qPCR assay (CN/100 ml), while EC and ENT were measured by plate count (CFU/100 ml). Ammonia (*NH_3*), Total Phosphorus (TP), Total Suspended Solids (TSS).*

A. Pearson's Correlations - Up-the-pipe in DRY conditions

	HB	EC	ENT	NH_3	TP	TSS
HB	1	**0.77**	**0.56**	**0.47**	**0.40**	0.23
EC	**0.77**	1	**0.87**	**0.49**	**0.52**	**0.39**
ENT	**0.56**	**0.87**	1	**0.46**	**0.52**	**0.47**
NH_3	**0.47**	**0.49**	**0.46**	1	**0.46**	**0.34**
TP	**0.40**	**0.52**	**0.52**	**0.46**	1	**0.61**
TSS	0.23	**0.39**	**0.47**	**0.34**	**0.61**	1

B. Pearson's Correlations - Up-the-pipe in WET conditions						
	HB	EC	ENT	NH_3	TP	TSS
HB	1	-0.24	**-0.30**	-0.11	0.00	**-0.34**
EC	-0.24	1	**0.83**	-0.03	-0.02	0.21
ENT	**-0.30**	**0.83**	1	-0.05	0.03	**0.31**
NH_3	-0.11	-0.03	-0.05	1	0.12	0.17
TP	0.00	-0.02	0.03	0.12	1	**0.49**
TSS	**-0.34**	0.21	**0.31**	0.17	**0.49**	1

Sewage Sniffing Dogs

A possible option for covering more ground and moving up-the-pipe to potential sewage sources faster, is the use of sewage sniffing dogs. In a preliminary study we compared our HB sewage indicator to the sewage indications of trained dogs. We co-sampled from eight manholes on both dry weather and wet weather field days with two dogs and handlers from Environmental Canine Services, LLC. Dogs indicated whether or not they detected sewage by sitting or barking after standing over an open manhole and sniffing pipe contents from street level. We found that our HB qPCR results agreed with results from each dog 88% of the time on the dry day and 88% or 75% of the time (depending on the dog) on the rainy day (**Table 8**). The only disagreement with dog results on the dry day was attributed to a heavy smell of wet asphalt nearby the manhole in question that overwhelmed dog noses (by visual inspection, the manhole clearly had an illicit sewage connection). On the rain day, HB in samples was diluted 10-100 fold by runoff, but Logan was as accurate as he had been on the more concentrated dry day samples and Sable was only slightly less accurate. To search out illicit connections quickly and cover a large area, the sewage sniffing dogs are an excellent option.

***Table 8**. Sewage sniffing dog agreement with HB and EC results.*

	Accuracy		HB		EC	
Weather	qPCR Agreement with Logan	qPCR Agreement with Sable	Average (CN/100 ml)	Range	Average (CFU/100 ml)	Range
No Rain	7 of 8 (88%)	7 of 8 (88%)	7,524,952	(1 M - 29 M)	2,891,857	(34 K - 20 M)
Rain	7 of 8 (88%)	6 of 8 (75%)	99,323	(14 K - 130 K)	6,650	(1 K - 14 K)

Sewage Sensors

A near-future possibility for quick detection of sewage pollution, both in-line and in receiving waters, is an optical sensor currently being developed by USGS. The sensor will analyze water samples for specific optical properties that correlate well to organic waste compounds (OWCs), human pathogens and alternative bacterial indicators of sewage contamination. To develop the sensor, samples are being split and analyzed broadly for absorbance and fluorescence characteristics, as well as for OWCs, human viruses and alternative indicators. Wavelengths that show the best association with other sewage contamination measures will be used to develop a set of portable sensors for rapid field assessment of sewage pollution.

4.6 Prioritization

We suggest a list of priority outfalls for remediation. This outfall prioritization scheme provides an efficient approach to district remediation efforts based on the following:

Magnitude and frequency of outfall contamination:

- Tier 1 Outfalls – Positive over 80% of the time (HB > 1,000 copy number/100 ml).
- Tier 2 Outfalls – Positive 50-80% of the time and very high HB (> 10,000 copy number/100 ml).
- Tier 3 Outfalls – Positive at least 50% of the time (HB > 1,000 copy number/100 ml).

From 2008-2012 92 outfalls were tested multiple times for HB contamination. These outfalls were prioritized according to the percentage of time they were positive for HB (>1,000 CN/100 ml) or had very high (>10,000 CN/100 ml) average counts of the bacterium (**Figure 7**). Of the 92 outfalls, 23 were always positive when tested for HB and 24 had very high (red and dark red bars) average HB levels. Outfalls with very high HB levels, included 7 of the 10 Category A outfalls previously described. The high load of contamination associated with Category A outfalls makes these outfalls especially good targets for remediation and the fact that they run in dry weather should help make source tracking easier. Three of the four outfalls with the highest contamination are Category A (dark red bars in **Figure 7** with average HB >100,000 CN/100 ml and positive 100% of the time).

We separated outfalls into three tiers of prioritization based how often they were contaminated and how high average HB concentrations were. The 27 highest priority Tier 1 sites were positive over 80% of the time – seven of the Tier 1 sites were also Category A outfalls. Tier 2 priority outfalls were chosen based on having very high HB in 50-80% of samples tested – eight outfalls fell into this category and two of the eight were Category A outfalls. Tier 3 priority outfalls were simply positive for HB at least 50% of the time. Outfalls that are Category A (in green boxes) as well as falling into one of the three priority tiers in **Figure 7**, are considered especially good targets for remediation and lead to our next category of prioritization.

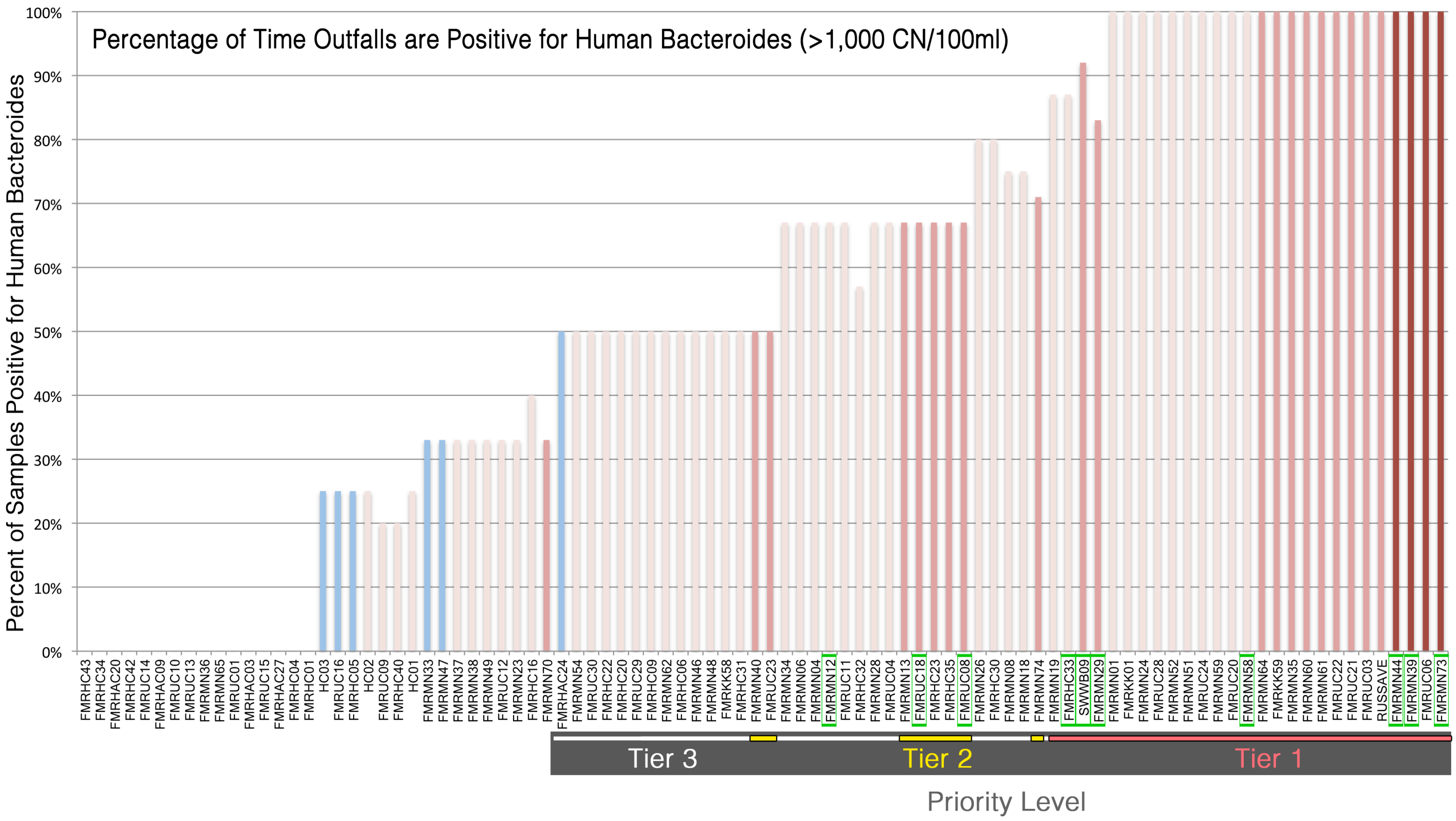


***Figure** 7. Outfalls sampled multiple times for HB (n=92) plotted for percentage of HB tested samples that were positive for the human marker and heat mapped for mean HB level found at each outfall. Heat map colors: Blue <1,000 CN/100 ml; pink >1,000 CN/100 ml; Red >10,000 CN/100 ml; Dark Red >100,000 CN/100 ml. Outfalls outlined in green are Category A. Outfalls of concern are prioritized as Tier 1, 2 or 3.*

Outfalls with contaminated flow during dry weather (Category A):

Even though Category A outfalls are less frequent than Category B outfalls, we estimate that they are responsible for a major proportion of the HB pollution escaping into receiving waters (perhaps higher than 70% in some reaches). The outfalls make follow-up investigation easier as they flow during dry weather. **Table 9** lists the 11 Category A* outfalls (presumptive A* outfalls) found in this study.

Table 9. *Category A outfalls considered medium priority sites for further investigation. Sites have only been checked one time and have not been checked in rain.*

SITES	WEATHER	# SAMPLES	HB (CN/100 ml)	EC (CFU/100 ml)	ENT (CFU/100 ml)
FMRKK04	no rain	1	6,806	200	670
FMRKK05	no rain	1	49,229	NA	169,000
FMRKK54	no rain	1	244,511	9,300,000	4,100
FMRMN10	no rain	1	15,286	3,100	3,700
FMRMN15	no rain	1	10,547	3,100	2,900
FMRMNMP03	no rain	1	6,905	140	100
FMRMNPH	no rain	1	530,407	0	20
FMRWPC05	no rain	1	10,698	2,200	600
FMRWPC08	no rain	1	200,217	8,100	3,400
FMRWPC15	no rain	1	1,168	1,000	0
SMN020	no rain	1	46,388	1,990	0

Outfalls with small drainage areas, but large sewage load estimates:

Outfalls with consistently low contamination tended to have smaller drainage areas than outfalls with very high levels of contamination as seen in section 4.5 of this report. However, some small drainage areas contained highly contaminated outfalls and are an important source of pollution to receiving waters. **Table 10** summarizes outfalls that are good targets for remediation based on ease of coverage. Small drainage areas with large average load estimates of HB are good targets for remediation (red rows); however, even intermittent problem sites may be priorities when the drainage area is relatively small (black rows).

Table 10. *Red Rows: Small drainage areas (< 60 acres) with large average load estimates (> 30,000 CN/100 ml) and frequent high HB concentrations (> 50% of the time) at associated outfall. Black Rows: Small drainage areas with large average load estimates but HB concentrations are only intermittently high at the associated outfall.*

Outfall Site Code	DA in Acres	Average HB	Estimated Load (HB x DA)
FMRHC30	35.9	5,817	208,837
FMRHC31	59.2	7,262	429,881
FMRHC32	7.7	4,661	35,886
FMRHC35	31.0	36,666	1,136,643
FMRKK01	14.7	2,351	34,560
FMRKK04	2.1	6,806	14,293
FMRKK54	17.5	244,511	4,278,943
FMRKK58	6.5	4,696	30,521
FMRKK59	7.9	13,434	106,129
FMRMN04	30.2	2,077	62,715
FMRMN13	54.0	13,670	738,162
FMRMN24	17.8	2,476	44,064
FMRMN26	15.7	2,933	46,042
FMRMN35	50.5	13,467	680,058
FMRMN41	4.9	14,868	72,853
FMRMN48	25.0	4,194	104,850
FMRMN51	6.6	5,856	38,650
FMRMN52	37.9	4,553	172,559
FMRMN54	37.1	1,078	39,994
FMRMN64	52.5	11,187	587,318
FMRMN70	5.9	18,032	106,387
FMRMN74	61.1	157,321	9,612,313

5.0 Case Studies

The research presented here, and in prior collaborations between MMSD and the McLellan Laboratory, has had direct effect on remediation of sewage intrusion into local stormwater conveyance systems. Problem sites that were identified and had repair work done are summarized in **Table 11**.

Table 11. *An inventory of problem sites that have been identified and fixed, or repaired to some degree.*

Outfall or Pipe:	**Miller Park**
Mean HB: (Prior to fix)	**93% of samples tested were positive for HB by gel assay.**
ID of Problem:	**Dye testing showed connection to luxury boxes**
Date of Fix:	**3/14/07-4/2/07**
Follow-up Testing:	**7% of samples tested were positive for HB by gel assay.**
Additional Investigation:	**Mean values for BEFORE samples: EC 238,000 CFU/100 ml; ENT 176,000 CFU/100 ml; Mean values for AFTER samples: EC 14,700 CFU/100 ml; ENT 17,240 CFU/100 ml; Dry weather sampling 4/8/07 all non detects for FC <20 CFU/100 ml and EC <100 MPN/100 ml. Wet weather not that different than wet weather results prior to fix.**
Outfall or Pipe:	**Hawley & 5844 W Bluemound (Barbiere Pizza) SMN73F**
Mean HB: (Prior to fix)	**This site was found by visual inspection and "repaired" before testing for HB levels.**
ID of Problem:	**Looking for SW sites to sample on 3/24/10 during dry weather for the 2012 season crews noticed significant amount of grease and sanitary waste. City was notified. Lateral apparently was improperly connected during road construction.**
Date of Fix:	**4/23/2012**
Follow-up Testing:	**Mean HB 27,111,520 CN/100 ml**
Additional Investigation:	**This is an area of concern and locations will continue to be sampled during dry weather. Mean values for BEFORE samples (MMSD): FC>600,000 CFU/ 100 ml; EC>2,400,000 MPN/100 ml. AFTER Samples for SMN73F sent to GLWI: dry summer 2012: mean ENT 127,000 CFU/100 ml; mean EC 583,666 CFU/100 ml.**
Outfall or Pipe:	**Cold Spring**
Mean HB: (Prior to fix)	**9/7/11 HC Survey; FC=5400 CFU/ml; EC= 4300 MPN/100 ml**
ID of Problem:	**8/15/11 Honey Creek Survey Data, DNR notified. City of Greenfield identified the source as a gas station that had been reconstructed with improper connection to sewer.**
Date of Fix:	**9/1/2011**
Follow-up Testing:	**FC>600,000 CFU/100 ml, EC= 190,000 MPN/100 ml**
Additional Investigation:	

Outfall or Pipe:	**29th St and KK River**
Mean HB: (Prior to fix)	**River Survey #860 on 6/6/11:** **RI-12S downstream of location FC=38,000 CFU/100 ml; EC= 77,000 MPN/100 ml** **RI-35S upstream of location FC= 10,000 MPN/100 ml; EC 8,500 MPN/100 ml**
ID of Problem:	**6/8/11 Water Quality Research staff observed Sanitary Waste on KK River Bank while sampling and City and DNR was notified. During construction at St. Lukes Hospital, an additional bathroom was improperly connected to a stormsewer.**
Date of Fix:	**Unclear, sometime between 6/28/11-8/18/11**
Follow-up Testing:	**River Survey # 863 on 8/8/11:** **RI-12S downstream of location FC 8,800 CFU/100 ml, EC=11,00MPN/100 ml** **RI-35S upstream of site FC=490 CFU/100 ml, EC= 440 MPN/100 ml**
Additional Investigation:	

Outfall or Pipe:	**MN44**
Mean HB: (Prior to fix)	**97,706 CN/100 ml**
ID of Problem:	**High counts of Fecal Indicator Bacteria and Human Indicator Bacteria at terminal outfall.**
Date of Fix:	**2012-2013 New sewer and stormwater lines were installed in much of the drainage area**
Follow-up Testing:	**5,700 CN/100 ml**
Additional Investigation:	**McLellan Lab sampling stormwater pipes in area 2013-2014**

6.0 Conclusions and Recommendations

In Greater Metropolitan Milwaukee, stormwater collection and conveyance systems are designed to capture storm runoff and discharge it, untreated, directly into the city's major rivers and tributaries. Sanitary sewage should not be present in outfall discharge, but the McLellan Laboratory has found widespread contamination of the outfalls tested here. Recommendations for follow-up investigation have been grouped by three factors: 1. Percentage of time that an outfall is contaminated and the level of contamination; 2. Contaminated flow during dry weather and; and 3. Small drainage areas with large load estimates. Some outfalls fall into more than one group, which should increase target rank (**Table 12**).

Table 12. *Summary of outfall prioritization*

OUTFALL	Tier 1	Tier 2	Tier 3	Category A/A*	DA
FMRHAC24			•		
FMRHC06			•		
FMRHC09			•		
FMRHC20			•		
FMRHC22			•		
FMRHC23		•			
FMRHC30			•		•
FMRHC31			•		
FMRHC32			•		•
FMRHC33	•			•	
FMRHC35		•			•
FMRKK01	•				•
FMRKK04				•	
FMRKK05				•	
FMRKK54				•	•
FMRKK58			•		
FMRKK59	•				•
FMRMN01	•				
FMRMN04			•		•
FMRMN06			•		
FMRMN08			•		
FMRMN10				•	
FMRMN12			•	•	
FMRMN13		•			•

OUTFALL	Tier 1	Tier 2	Tier 3	Category A/A*	DA
FMRMN15				•	
FMRMN18			•		
FMRMN19	•				
FMRMN24	•				•
FMRMN26			•		•
FMRMN28			•		
FMRMN29	•			•	
FMRMN34			•		
FMRMN35	•				•
FMRMN39	•			•	
FMRMN40		•			
FMRMN44	•			•	
FMRMN46			•		
FMRMN48			•		
FMRMN51	•				•
FMRMN52	•				•
FMRMN54			•		
FMRMN58	•			•	
FMRMN59	•				
FMRMN60	•				
FMRMN61	•				
FMRMN62			•		
FMRMN64	•				•
FMRMN73	•			•	
FMRMN74		•			•
FMRMNMP03				•	
FMRMNPH				•	
FMRUC03	•				
FMRUC04			•		
FMRUC06	•				
FMRUC08		•		•	
FMRUC11			•		
FMRUC18		•		•	
FMRUC20	•				

OUTFALL	Tier 1	Tier 2	Tier 3	Category A/A*	DA
FMRUC21	●				
FMRUC22	●				
FMRUC23		●			
FMRUC24	●				
FMRUC28	●				
FMRUC29			●		
FMRUC30			●		
FMRWPC05				●	
FMRWPC08				●	
FMRWPC15				●	
RUSSAVE	●				
SMN020				●	
SWWB09	●			●	

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