Milwaukee Metropolitan Sewerage District

P2738: Determining Sediment and Bacterial Sources and Linkages to Inform and Evaluate Total Maximum Daily Load (TMDL) Implementation

Final Report

June 30, 2020

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Executive Summary

Large rain events deliver significant levels of pollution to the Milwaukee River and Milwaukee harbor estuary. In particular, high concentrations of total suspended solids (TSS) result in turbid water plumes that are visually apparent and unappealing. In addition, fecal pollution can enter waterways during large rain events through urban runoff, agricultural runoff, or sewer system discharges. While this pollution is not visible, it carries with it significant risk to human health.

In order to appropriately mitigate these pollutants, we need to understand the sources and timing of pollutant loads as they are released into the Milwaukee harbor. **If pollutants are coupled, i.e. follow the same dynamics and are potentially from the same source, mitigation strategies can be targeted toward addressing multiple pollutants and high-frequency sources that are most likely to have the greatest benefit to overall watershed health.** This is particularly important for addressing Total Maximum Daily Load regulations in the Milwaukee River watershed in order to delist the Area of Concern designation for the Milwaukee harbor estuary. Furthermore, an understanding of differences between TSS and bacteria fate in the Milwaukee River watershed and nearshore Lake Michigan is critical for informing recreational water quality health advisories.

The goals of this project were to: (1) understand the sources of suspended solids, microbial communities, and fecal pollution in turbid water plumes; (2) determine the coupling of bacterial signals and suspended solids in upstream and downstream sources; and (3) examine how solids and bacteria in turbid water plumes impact beaches and the nearshore environment. Within this project, we have used cutting edge techniques, including microbial source tracking, microbial community sequencing, and hydrodynamic modeling, to better understand how large rain events impact the Milwaukee watershed.

During 2018-2019, we collected 1,170 water and 167 sediment samples at various locations in the Milwaukee River watershed (Figure 1). These samples were processed by various techniques to understand the composition of sediment, loading of sediment and fecal coliforms, coupling of these pollutants, and their fates in the harbor and Lake Michigan during rain events.

Key findings from our analysis are:

1. TSS loads are highly event dependent, suggesting that mitigation of high runoff events would have an important impact on overall pollutant reductions during the course of a year; in general, upstream rural and agricultural zones account for the majority of TSS load contributions.
2. Fecal indicator bacteria (FIB) loads are highly associated with human fecal marker detection in the river system during rain events, in the absence of a combined sewer overflow (CSO), demonstrating the contributions of sewage contaminated stormwater.
3. Microbial communities shift at short time scales during storm events, and characterizing communities from specific sources (river sediment, outfall discharge) can uncover additional pollution markers.
4. Weather patterns and flow regimes in the nearshore dictate the impact of rain events on nearby beaches; in general, large rain events, which sometimes result in CSOs, produce enough TSS & FIB loads to impact beaches.

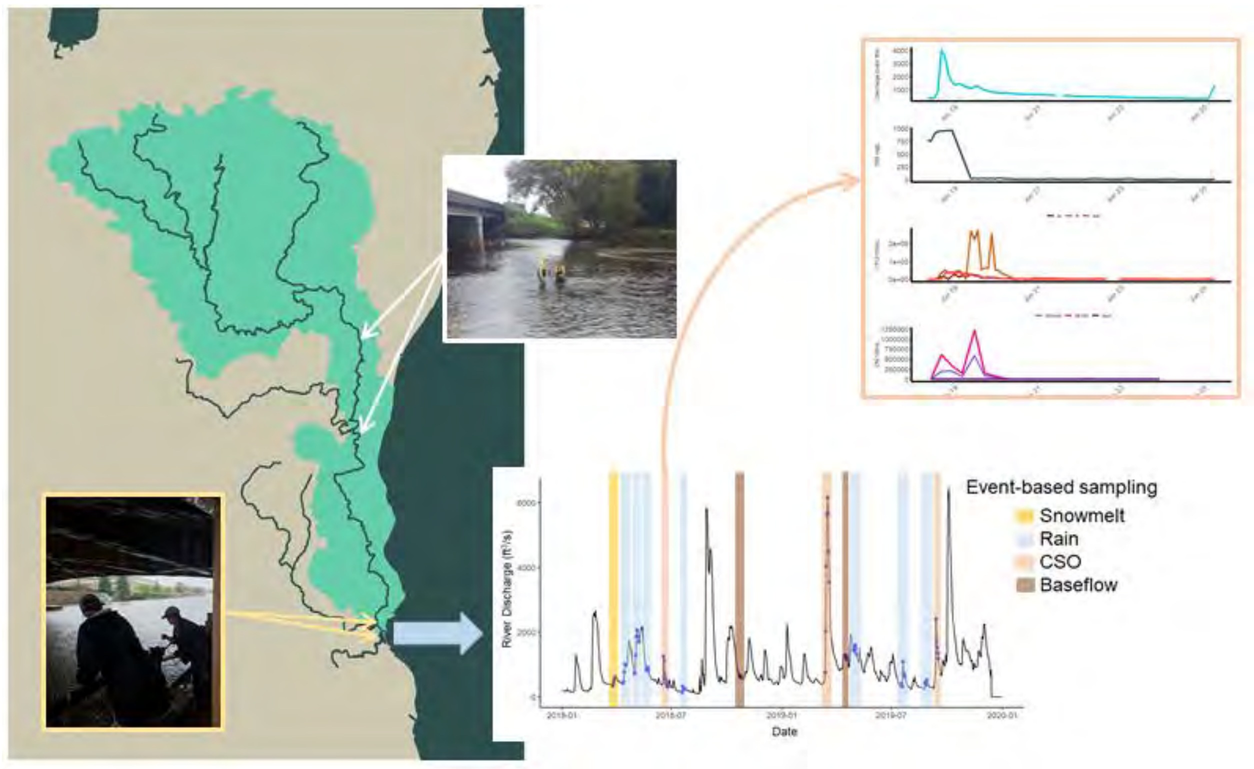


Figure 1. Graphical abstract of key sampling locations, periods, and parameters. Sampling focused on two upstream sites (white arrows) and two downstream sites (yellow arrows) primarily during specific runoff events (snowmelt, rain, CSO, baseflow), resulting in TMDL parameters sampled at high resolution over variable river hydrographs.

Overall, our findings suggest that mitigation focused on controlling runoff and subsequent pollutant delivery during the few large rain events per year would be highly beneficial, as these events account for a disproportionate loading of pollutants to the harbor. Coordination with upstream municipalities will also be essential for TSS mitigation, as large proportions of TSS load are sourced from upstream rural regions. In preliminary work, we found high total phosphorus (TP) concentrations are associated with elevated TSS concentrations and ruminant fecal marker detection, suggesting substantial nutrient inputs from upstream agricultural sources. Furthermore, our data suggest that microbial community sequencing data can help resolve TSS sources when chemical data are not discriminating. Additionally, hydrodynamic modeling suggests that heavy rain events with or without subsequent CSOs are associated with plume impacts at beaches. This work contributes to overall efforts in the Milwaukee estuary Area of Concern (AOC) to define microbial tracers for various pollutants of concern and validate predictive beach water quality models, which would be valuable for future monitoring and mitigation of pollution events and appropriate protection of public health.

Findings from this project can be useful as the Milwaukee watershed communities implement TMDL reductions. Based on our analysis, TSS and FIB pollutants are not coupled, and addressing each may require a separate management focus.

- For FIB, downstream contributions outweigh upstream contributions. Continuing to monitor for and identify sanitary sewage infiltration into stormwater systems should be a priority. Further, there are other sources of FIB such as surface runoff into the stormwater system. These sources are less of a health concern and therefore a lower priority in terms of reducing FIB as a proxy for pathogen contamination.

- We found that the majority of TSS is linked to upstream sources and is a mix of riverbank scouring/bottom resuspension and overland runoff. Overall, TSS loads were found to be proportional to flow, suggesting that TSS increases in the river system are controlled by hydrological events and therefore a focus on limiting peak flow, possibly by increasing stormwater retention in order to reduce erosive forces during extreme events in upstream regions, would be important for limiting TSS inputs.
- Ruminant fecal markers (e.g. cattle) are also detectable in the downstream urban region. This signal of agricultural runoff was not directly proportional to flow, meaning that other factors modulate the intensity of agricultural inputs to the system. For example, stochastic factors (not controllable) such as the timing and placement of rainfall may be critical in controlling these pollutant inputs; or deterministic factors, such as the seasonal spreading of manure may be a controlling factor. Deciphering what controls agricultural pollutants entering the watershed warrants further consideration, particularly since large rainfalls often coincide with field applications in the spring season.

We suggest addressing pollutant mitigation during large rain events, which will likely require a multifaceted approach. Climate models predict increased frequency of heavy rain events. While infrequent, large storms account for a major portion of TSS, and TMDL levels were most often exceeded during large storms. Mitigation strategies could consider stormwater retention systems, which may be useful in both urban and rural areas to reduce TSS and bacterial pollution.

Introduction

Background. Rain events, in particular heavy rain events, have long been known to contribute high concentrations of pollutants, including total suspended solids (TSS), fecal coliforms, and *Escherichia coli*, to urban watersheds [1, 2], and with subsequent impacts on human and ecosystem health [3, 4]. However, there are many factors that affect the concentrations and quantities of pollutants that enter waterways during wet weather events. Both spatial variables, such as surrounding land use, and temporal variables, such as antecedent dry periods or seasonal effects, have been linked to increased fecal coliform concentrations [1]. In contrast, while precipitation drives pollutants into waterways through overland runoff, pollutant concentrations during wet weather events can also decrease due to higher flow rates and concomitant dilution [1].

Previous attempts to identify fecal indicator bacteria (FIB) contamination sources based on FIB species distributions have been generally unsuccessful [5]. Furthermore, risk assessment based on FIB concentrations is limited by the differential fates of different species or sources of indicator bacteria [6]. In recent years, advancements in microbial source tracking and DNA sequencing have provided new methods for identifying pollutant sources in complex mixed-use watersheds [2, 7–9], thus improving our ability to understand both health risk and relevant mitigation strategies [10]. In particular, quantitative PCR (qPCR) assays, which target specific microbial DNA sequences, have been developed with high specificity for human or ruminant fecal sources, which strongly correlated to urban and agricultural land uses, respectively [9]. Furthermore, a frequent lack of correlation between human fecal markers and FIB concentrations suggests potentially different health risks under various flow scenarios [9]. In addition to qPCR, DNA sequencing has been explored as another means to uncover pollutant sources, as this method detects the entire community of bacteria in a sample. Microbial community composition has been observed to correlate with flow rates and *E. coli* concentrations in urban watersheds [2]. Furthermore, the distinct microbial communities in sediment and water suggest that microbes may be useful as sensitive tracers for sediment [11, 12]. This method may be particularly relevant when typical physicochemical sediment tracers do not vary significantly among relevant sources [12].

To address Total Maximum Daily Load (TMDL) regulations, municipalities must introduce appropriate control measures and best management practices (BMPs). However, the efficacy of BMPs is dependent on the temporal and spatial variation of pollutants in a watershed; thus, a strong understanding of hydrograph dynamics and pollutant sources is important to ensure effective mitigation. Typical water quality monitoring tends to focus on low frequency (i.e. weekly or monthly), high duration (i.e. multi-year) sampling campaigns in order to understand average pollutant loadings under typical conditions. However, due to the nature of pollutant loadings during storm events and "first flush" phenomena, these monitoring campaigns may not adequately capture extreme events that can have substantial impacts on overall pollutant loads [1]. In addition, turbidity and *E. coli* concentrations are often found to be correlated [13], and during storm events bacterial indicators have been found to be more strongly associated with particles than under baseflow conditions [14]. If sediment and fecal pollutants are strongly coupled, BMPs designed to mitigate both pollutants simultaneously from critical sources may be most efficient and cost effective.

Previous work in our lab has demonstrated widespread human fecal pollution in stormwater outfalls and rivers in the Milwaukee area, which is independent of FIB concentrations and tends to peak early in storm events [15, 16]. Additionally, we observed that when combined sewer overflows (CSOs) occur, these events are the dominant contributors of human fecal loads to waterways in Milwaukee, in addition to the less intense but more persistent loads associated with non-CSO rain events [16]. Human fecal pollution markers are also frequently detected in the Milwaukee harbor, and high concentrations are indicative of human adenovirus detection [17] and are at times associated with increased turbidity [18]. Additionally, we have demonstrated significant shifts in microbial community compositions under different rainfall conditions in the Milwaukee harbor [19]. Milwaukee area beaches have had frequent issues with high FIB concentrations, leading to beach closures [20]. Beaches are highly impacted by localized sources such as nearby stormwater outfalls and gull fecal pollution [21]. However, during large rain events, urban and agricultural runoff to the Milwaukee River, CSOs, or sewage contributions from leaky sewer infrastructure can also impact beaches [5, 20]. Previous hydrodynamic modeling showed that fecal coliform “footprints” from the Milwaukee River during CSOs can extend up to eight kilometers into Lake Michigan depending on weather conditions [22].

Total Maximum Daily Load (TMDL) in the Milwaukee Harbor Estuary. In 2018, TMDLs were established for phosphorus, sediment, and bacteria in the Milwaukee River Basin [23], which includes the watersheds of the Menomonee, Kinnickinnic, and Milwaukee Rivers. The Milwaukee River watershed is significantly larger than the Menomonee and Kinnickinnic, contributing approximately 75% of the total flow that enters Lake Michigan through the Milwaukee harbor estuary. The Milwaukee River watershed covers approximately 700 square miles, and while the southern reaches of the Milwaukee River are in highly urbanized areas, approximately 79% of land use in the watershed is rural, particularly concentrated in the upstream regions. A summary of TMDL criteria set for the Milwaukee River are listed in Table 1, along with TMDL study findings on the present state of the river basin. Of note, while pathogens and sediment are the primary pollutants of concern, these pollutants are challenging to measure directly and therefore are evaluated in terms of fecal coliform and TSS, respectively. Within the data analyzed in establishing TMDL limits, a key finding was that regulated point source discharges, such as industrial discharge, sewage treatment plants, and CSOs, make up only a small percent of total pollutants that enter the river basin. Therefore, gaining greater understanding of key non-point sources will be vital for effectively meeting TMDL criteria. The TMDL study used water quality models calibrated with biweekly monitoring data. While this allowed for an accurate depiction of the watershed under different flow conditions, it cannot necessarily provide an accurate depiction of the drastic pollutant loading changes that occur sporadically and over short time intervals during intense rainfall events. By characterizing these loads at higher resolution during wet weather events, our analyses below can guide watershed managers toward understanding (1) where predominant nonpoint pollutants enter the Milwaukee River during wet weather and (2) when the loads from these pollutants are highest.

Methods & Data Summary

For the present study, sample collection was conducted during field seasons (typically March thru October) of 2018 and 2019. In order to characterize impacts of various runoff events on

Table 1. TMDL criteria and baseline study findings for the Milwaukee River [23]

	TSS	**Fecal Coliform**	**TP**
Baseline Average Concentration	25.1 mg/L	50 – 2,300 cells/100 mL	0.129 mg/L
Baseline Annual Load	29,200 tons	41,000 trillion cells	274,500 lb
Target Concentration	12 mg/L	200 (GM) or 400 (STV) units/100mL	0.1 mg/L
Percent Reduction	69.3%	NA*	63%

* Fecal coliform recommended loads vary with flow conditions, so no average percent reduction is provided

TSS and FIB dynamics in the Milwaukee River watershed, both sediment and water samples were collected at varying frequencies to target specific research questions. A summary of total samples collected is provided in Table 2, and a map of primary sampling locations is provided in Figure 2. The JI and CHR sampling sites represent the downstream watershed, both equipped with permanent sampling stations where composite samplers could be deployed. The CHR site is located on the Milwaukee River just north of the confluence with the Menomonee River and therefore represents the bulk of total flow contributed by the Milwaukee River watershed. The JI site is located at the outlet between the inner and outer harbors downstream of the confluence of the Milwaukee, Menomonee, and Kinnickinnic Rivers and therefore was selected to understand direct impacts of the rivers on the harbor estuary and Lake Michigan. The SKV and PNR sites represent the upstream watershed and were primarily selected for ease of access and proximity to agricultural land use areas. The PNR location is also shared with a USGS gauge station, providing additional reference data at this site. In total, 1,170 water samples and 167 sediment samples were collected over two years.

Water samples were collected in an event-dependent manner using high-frequency automated sample collectors at two locations in the downstream reaches of the watershed (CHR & JI, Figure 2). Events were defined based on precipitation measurements averaged across three rain gauges operated by MMSD (WS1203, WS1211, WS1221) and were categorized as either baseflow (no more than 0.1" precipitation during entire sampling and previous 48-hr period), light rain (0.5"-2"), heavy rain (>2"), CSO (CSO occurred, usually during heavy rain), or snowmelt (typically the first major runoff event of the year after river ice-off). Note that we use the term "CSO" to identify rainfall or other runoff events that resulted in a CSO, meaning that samples associated with these events include contributions from a combination of runoff, rainfall, and CSO discharges. In total, our study collected samples over thirteen events (3 CSOs (including one which occurred during snowmelt), 2 heavy rain events, 4 light rain events, 2 baseflow periods, and 1 snowmelt event). These samples cover a total of 1,226 hours during two years, thus characterizing river flows during approximately 7% of the year. In addition, samples were also collected during harbor surveys and beach surveys for select events (Table 2).

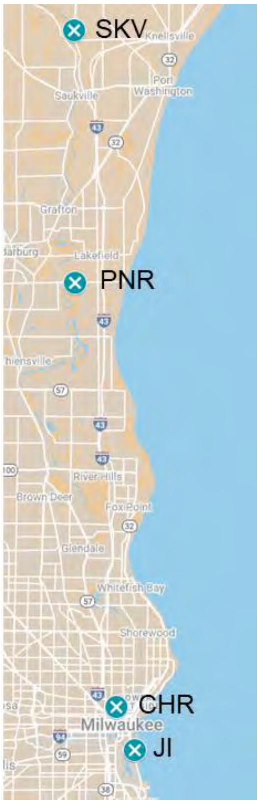


Figure 2. Map of primary sampling sites on the Milwaukee River. Detailed sample location information is provided in Appendix A.

Sediment samples in 2018 were primarily collected using deployed sediment traps at three locations: SKV, PNR, and CHR (see Figure 2). These traps were typically deployed and collected on weekly intervals. Sondes were also deployed continuously with sediment traps during 2018 to collect various environmental metadata, including turbidity. In 2019, sediment sample collection was transitioned to event-based sampling in conjunction with water sampling. These samples were collected only from the downstream CHR location, and collection frequency was increased to daily during selected events. In addition, sediment grab samples were also collected during upstream and harbor surveys for select events (Table 2).

A summary of sample processing details is provided in Table 3. Briefly, all water samples collected were processed for FIB (*E. coli*, enterococci, and fecal coliform) and TSS concentrations using standard methods [24–27]. In addition, total DNA was extracted from select samples and used to evaluate microbial source tracking markers by qPCR (specifically human [17, 28] and ruminant [29, 30] markers) and/or microbial community composition by 16S rRNA gene sequencing. Total phosphorus was also measured in select samples by MMSD. All sediment samples were processed for gamma counts, Pb-210 concentrations, and C/N ratios. Additionally, select sediment samples were further processed by ICP-MS to determine the ionic composition of collected sediment. Total DNA was also extracted from select sediment samples and used to determine qPCR marker signals and/or microbial community composition in collected sediments. A summary of all water sample data collected, plotted against each event's storm hydrograph, is provided in Appendix B.

In addition to the above sample processing, rainfall and streamflow data were obtained from MMSD rain gauges and USGS streamflow gauges, respectively. This data, along with other climatic variables collected from NOAA, were used as inputs to a previously developed hydrodynamic model of the Milwaukee harbor and nearshore environment (see Bravo et al, 2017). This model was originally developed for tracking fecal coliform fate in the nearshore and was adapted in this study to also model *E. coli*, TSS, and human *Bacteroides* fecal marker (HB).

Table 2. Sample collection summary. Additional detailed sample location information is provided in Appendix A.

Sample Type	Event #	Dates	Event type	Locations	# of samples
water	1	3/26-3/30/2018	snowmelt	CHR, JI	57
water	2	4/11-4/15/2018	rain	CHR, JI	76
water	3	5/1-5/9/2018	rain	CHR, JI	116
water	4	5/21-5/24/2018	rain	CHR, JI, beaches, harbor	49
water	5	6/18-6/25/2018	CSO	CHR, JI, beaches, harbor	252
water	6	7/19-7/24/2018	rain	CHR, JI	90
water	NA	Oct 2018	(weekly)	SKV, PNR	6
water	7	10/22-10/24/2018	baseflow	CHR, JI	24
water	8	3/13-3/20/2019	snowmelt + CSO	JI	72
water	9	4/16-4/18/2019	baseflow	CHR, JI	24
water	10	4/29-5/4/2019	rain	CHR, JI	110
water	11	7/18-7/23/2019	rain	CHR, JI, beaches	152
water	12	8/26-8/29/2019	rain	CHR, JI, harbor	70
water	13	9/13/-9/17/2019	CSO	CHR, JI	72
sediment trap	NA	Mar - Oct 2018	(weekly)	SKV, PNR, CHR	107
sediment trap	9	4/16-4/18/2019	baseflow	CHR	2
sediment trap	10	4/29-5/4/2019	rain	CHR	7
sediment trap	11	7/18-7/23/2019	rain	CHR	10
sediment trap	12	8/26-8/29/2019	rain	CHR, harbor	15
sediment trap	13	9/13-9/17/2019	CSO	CHR	8
sediment grab	NA	9/26/2019	NA*	various upstream	11
soil core	NA	12/19-12/27/2019	NA*	various	78
				Total Samples:	1408

*Event types are not listed for sediment grab and soil core samples because these samples are expected to primarily characterize the locations from which they were sampled as opposed to the conditions during which they were sampled

Table 3. Summary of sample processing methods.

Metric	# of Samples
FIB (fecal coliform, enterococci, *E. coli*)	1,139
TSS	942
TP	103
qPCR (HB, BacR)	217
16S rRNA gene sequencing	198
Gamma counts	160
Pb-210	157
C/N	100
Ionic composition	120

Results Summary and Example Event

In total, our sampling characterized eleven runoff/rainfall events and two baseflow periods in 2018-2019 (Figure 3). Rainfall during rain and CSO events ranged between 0.5-3 inches. Three captured events resulted in combined sewer overflows (CSOs); these events were in June 2018, March 2019, and September 2019. The March 2019 CSO was unique in that very minimal precipitation was recorded; instead it resulted from a rapid snowmelt event. No samples were able to be collected from the Cherry Street sampling location during this event due to ice cover.

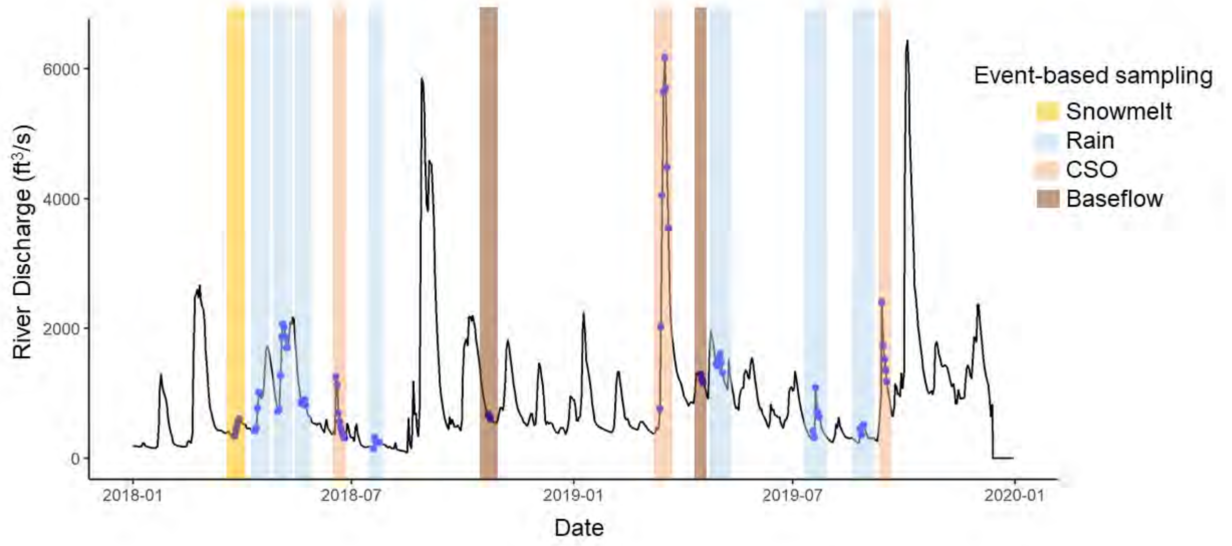


Figure 3. Milwaukee River hydrograph during 2018-2019 based on USGS streamflow gauge (station #04087000). Event-based sample collection periods are highlighted, and points along the hydrograph represent specific days of sample collection.

Time-series data from event sampling were originally visualized by comparison to their respective storm hydrographs (Figure 4). In doing so, a few key trends were observed. First, we typically observed high pulses of FIB concentrations early in storm hydrographs. These initial FIB pulses often occurred in conjunction with increases in human fecal marker concentrations, suggesting sewer infrastructure failures outside of reported sewer overflows. Peak fecal coliform concentrations for each event ranged from 700 CFU/100 mL during a light rain (event 4) to 272,000 CFU/100 mL during a CSO (event 6). Peak human fecal marker concentrations ranged from 1,898 CN/100 mL to 1,218,994 CN/100 mL. Of the six events with qPCR markers evaluated at the CHR site, three events exhibited elevated human fecal marker concentrations above a previously defined health threshold (7,800 CN/100 mL [31]) for between 2-38 hours.

Some events were also characterized by increases in TSS concentrations prior to FIB peaks. These high TSS periods were not associated with human or ruminant fecal markers, suggesting other inputs not associated with our microbial source tracking markers, such as riverbed resuspension or streambank erosion. Finally, we also observed increased detection of ruminant

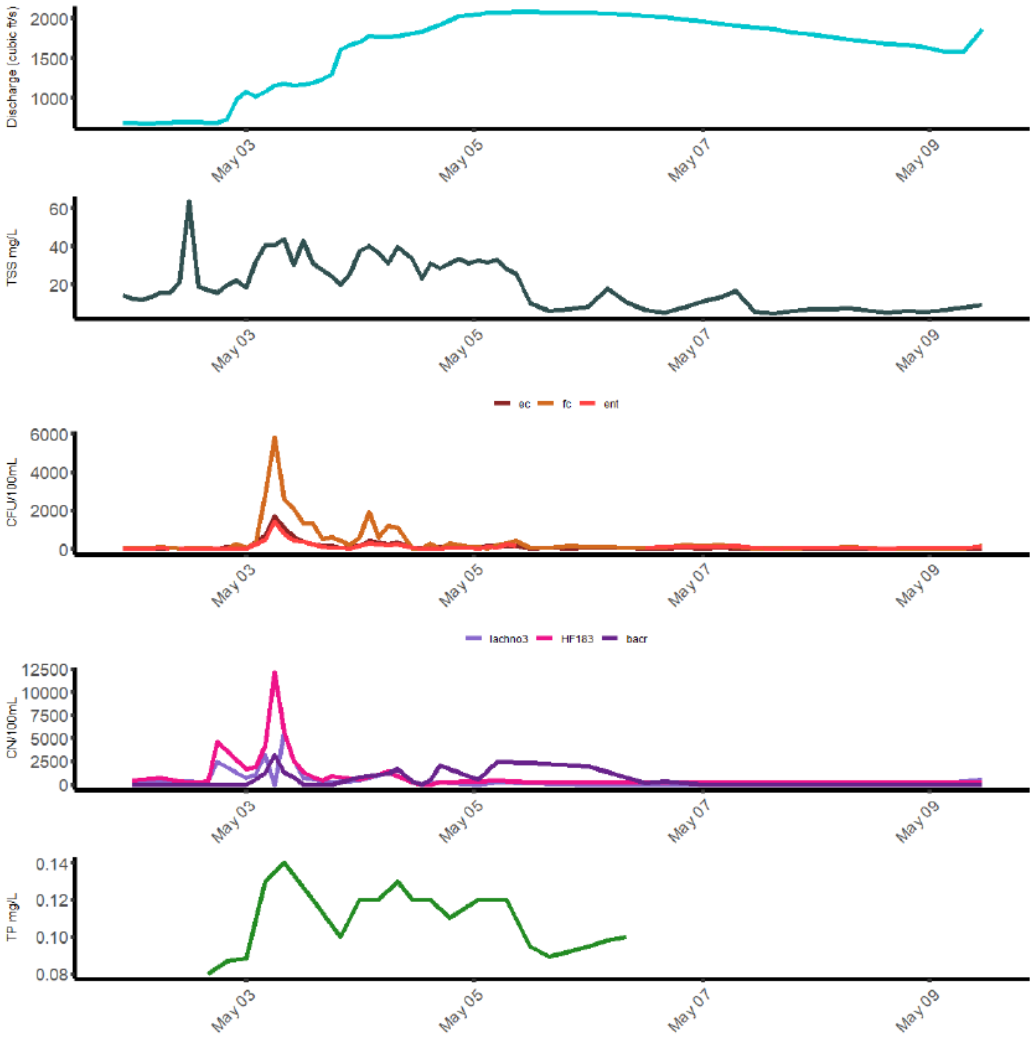


Figure 4. Hydrographs representing Milwaukee river discharge, TSS, FIB, qPCR markers, and total phosphorus (TP) for a rain event in May 2018.

fecal markers at late stages of storm hydrographs, demonstrating inputs from upstream agricultural sources. However, observed concentrations were varied, and at times the marker was at low concentration or non-detectable. Ruminant marker detection is likely dependent on a variety of factors, such as timing of manure application, specific locations of rainfall, assay sensitivity in the case of excessive dilution, or duration of event-based sampling.

After reviewing storm hydrographs individually, we aimed to further understand the general patterns of TSS and FIB loading. Our analysis presented below therefore focuses on: (1) overall sediment composition, loading estimates, and sources, (3) FIB loading and coupling with TSS, (4) microbial community dynamics and sediment tracer identification, and (5) nearshore fate and beach impacts of FIB and TSS.

Results & Analysis

1. TSS Loads, Sources, and Composition

Mass balance considerations:

Our first analysis goal was to calculate and evaluate the total sediment loads observed in the Milwaukee River using data from sediment traps, event-based TSS measurements, and continuous turbidity monitoring. Using a previously calibrated direct conversion between turbidity and TSS, total daily sediment loads in 2018 were estimated from continuous turbidity monitoring and flow from discharge data taken at the USGS monitoring stations near Pioneer Road and Cherry Street. Based on this data, total sediment loads in the Milwaukee River were estimated at approximately 6,000,000 kg at Pioneer Road and 8,160,000 kg at the Cherry Street Bridge for the period of June 4 through October 18. The implication being that, on average, approximately 75% of sediment load observed downstream was expressed at the upstream Pioneer Road location, i.e. there was only a 25% net increase in sediment loads between these two sites (Figure 5).

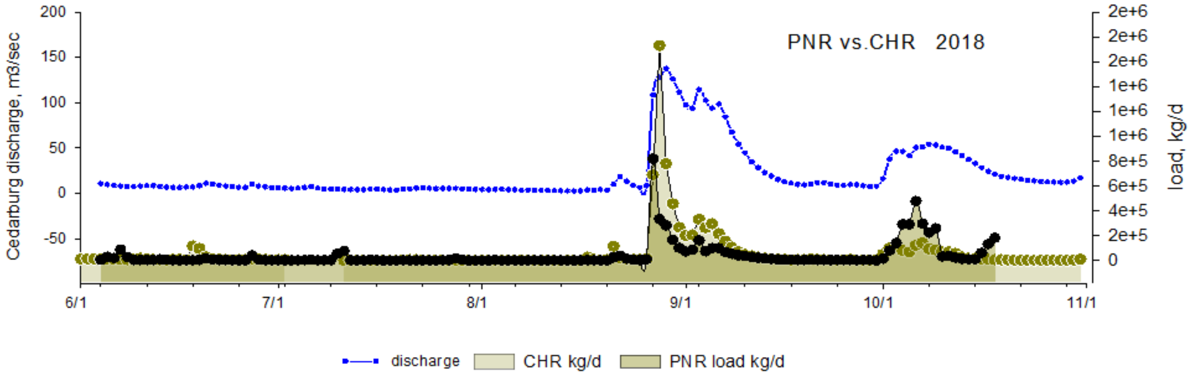


Figure 5. Sediment loads in 2018 based on turbidity and flow at CHR and PNR locations, plotted with upstream river discharge. Loads at both stations track the hydrograph very closely and the load at the Pioneer Road station (PNR) is ~ 75% of the load estimated at the downstream Cherry Street location (CHR).

Similar complimentary sediment load analysis was carried out using direct TSS concentrations measured at Cherry Street during event-based sampling (Figure 6). In total, TSS loads of 7,230,000 kg were measured at Cherry Street during our sampled events, with 5,470,000 kg of this load attributed to the three events sampled during June thru October 2018. This estimate suggests that 67% of the TSS load during this three-month period occurred during only 17 days, which was captured by our event-based sampling. Additionally, within the total event-based sampling conducted, more than 75% of TSS loads measured occurred during only two events: a CSO in May 2018 and a snowmelt CSO in March 2019 (Figure 7). Based on the previous TMDL analysis of the Milwaukee River watershed, our estimates suggest that we captured approximately 27% of annual TSS loads during sampling for only 7% of the year. This further demonstrates that mitigating high runoff events would have an important impact on overall pollutant reductions during the course of a year.

As there is a relatively strong correlation between total river discharge and TSS load during events not associated with CSOs (Pearson $p<0.05$, $R^2=0.96$), we used this relationship to

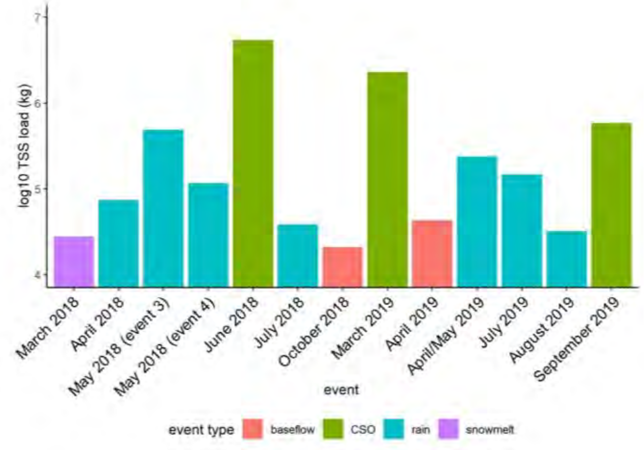


Figure 6. TSS loads in the Milwaukee River for each event sampled. Note that for the March 2019 event, no data was available at CHR due to ice cover, so JI data is shown instead.

estimate on average the percent of excess TSS load that may be attributed to CSO discharge. On average, 59% of TSS load during CSO-associated events is in excess of expected loads during typical non-CSO runoff conditions. However, we also note that this excess cannot be determined to be entirely attributed to CSO discharge, as there may be additional characteristics of these events (e.g. high rainfall intensity, antecedent weather conditions, etc) that could also lead to increased TSS loads.

Estimates of source functions based upon tracers:

Urban runoff frequently exhibits elevated concentrations of a number of trace elements. Notably in Milwaukee, heavy metals like Pb, Cr, Ni and Co, may be observed at concentrations in suspended sediment at more than 50% higher than in suspended and bottom sediments in upstream reaches of the river. Particulate lead concentrations in urban sourced sediments, for example, are often double that for riparian soils, upstream river bottom sediments, and upstream rural suspended sediments (Figure 8). Under high flow conditions, however, suspended sediments in the urban reach are more characteristic of upstream sediment sources, consistent with the implication that the major source of plume sediments during these events are derived from upstream, exurban areas of the watershed. In fact, both upstream river bottom and suspended sediments tend to have lower Pb concentrations than riparian surface soils – potentially pointing to riverbank and gully erosion as being significant sources of sediment transported downstream in addition to surface runoff.

In a complementary approach, we also estimated the proportion of TSS loads at the downstream Cherry Street site that may have been sourced from upstream inputs using varying relationships between human and ruminant microbial source tracking markers during event-based sampling. Ruminant microbial source tracking (MST) markers were previously detected in the downstream Milwaukee River a few days after large rain events (see example hydrograph Figure 4),

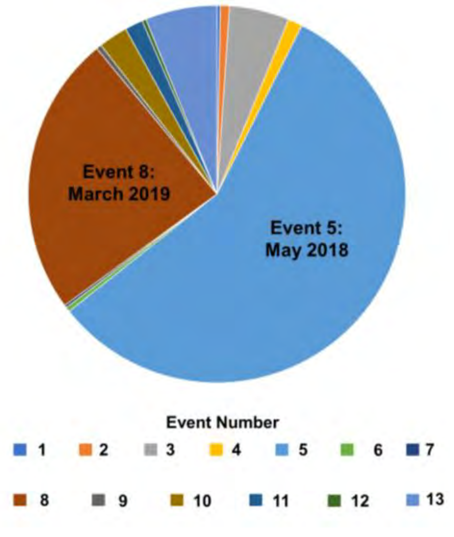


Figure 7. Proportions of TSS loads allocated to each sampled event.

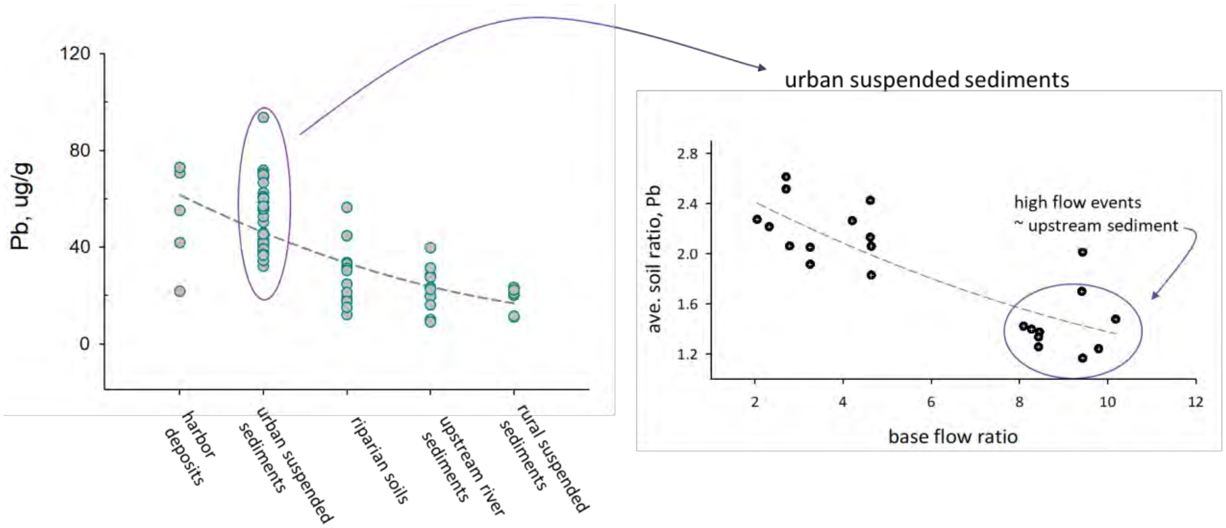


Figure 8. a). Concentration of lead (Pb) in micrograms Pb per gram (ug/g) of sediment for various sediment sources in the Milwaukee River watershed. Both harbor bottom sediments (deposits) and urban suspended sediments (Cherry Street sediment traps) show significantly higher concentrations of Pb than soils collected from the riparian zone along the river (14 locations), sediments collected from the river bottom upstream (11 locations), and suspended sediments collected via traps at upstream locations (Pioneer Road and Saukville sampling stations).

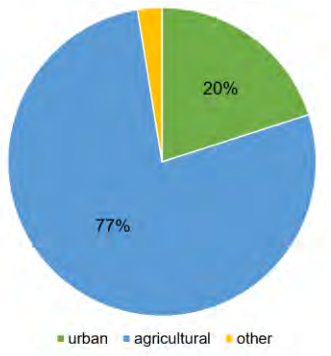


Figure 9. Proportions of TSS loads sourced based on MST markers.

suggesting their entry into the water column with upstream agricultural and rural runoff and potential utility as a tracer for upstream inputs. Similarly, human MST markers are frequently detected in the Milwaukee River, particularly during wet weather events, and are assumed to indicate significant inputs from leaking urban sewer infrastructure and/or CSOs. Of note, samples with detectable ruminant MST markers were always characterized by detectable human MST markers, suggesting that some human MST marker could be entering the water column in upstream areas with the ruminant marker; however, with the frequent human MST marker detection in downstream samples, it is not possible to definitively determine if human MST marker in ruminant-positive samples is the result of upstream or downstream inputs. By assuming that all samples with detectable ruminant MST marker primarily contain water and pollutants carried from upstream agricultural sources, we calculated the proportion of TSS loading at the downstream Cherry Street location associated with upstream agricultural inputs. Using a subset of samples that were analyzed using qPCR (n = 126 samples from 5 total events), we determined that approximately 77% of TSS loads measured at Cherry Street during event-based sampling were due to upstream agricultural inputs (Figure 9). While TSS concentrations were not significantly different between urban or rural contributions, we observed significantly higher TSS loads when ruminant MST markers were detected, compared with samples when human markers alone were detected (Figure 10). This conclusion is in close agreement to data presented in Figure 7 based on turbidity measurements and heavy metal tracers. TSS inputs from agricultural sources appear to make up a significant fraction of total TSS loads in the downstream Milwaukee River estuary.

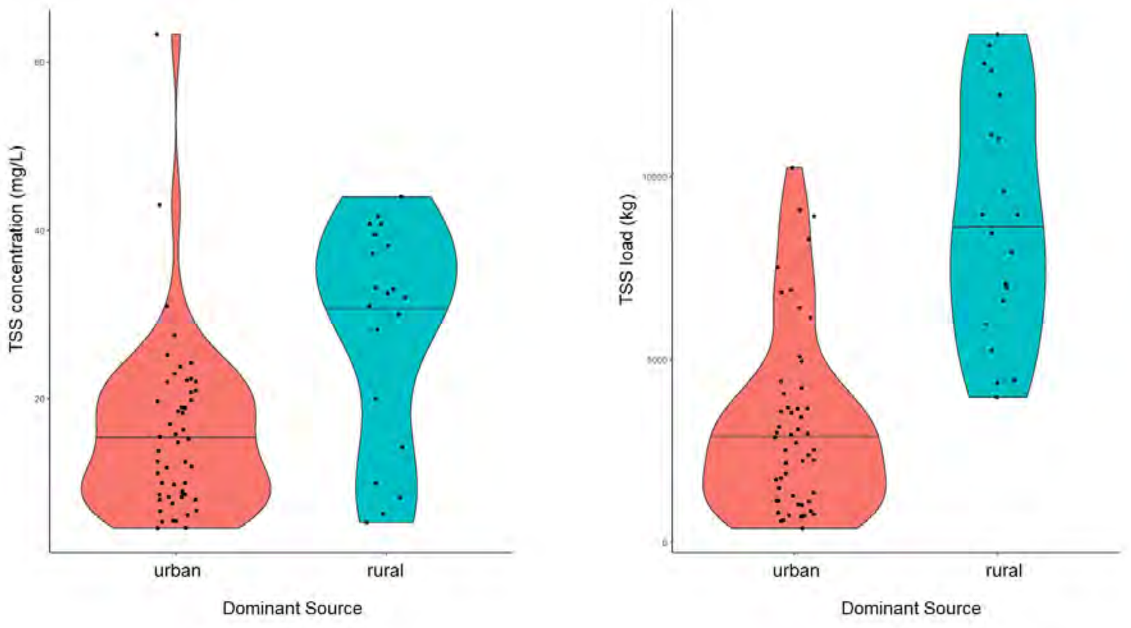


Figure 10. Distributions of TSS concentrations and loads associated with urban or rural MST markers.

^{131}I, a unique human source tracer:

The radioisotope of iodine, I-131, is a commonly used radioactive pharmaceutical excreted in human waste. I-131 is an 'artificial' radioisotope, i.e. does not have a natural source, and has a half-life of only 8 days. Consequently, once introduced into the environment from human wastewater discharges, I-131 disappears via radioactive decay relatively quickly, which means its presence in the environment is an unequivocal indicator of recent and contemporary human derived inputs. During 2018, we detected I-131 activity pulses in suspended sediments on only a few occasions (Figure 11), and all of these sediment samples were from upstream sampling locations (SKV & PNR). In all cases these inputs were not associated with a concomitant increase in flow, i.e. these inputs were not linked to the hydrograph, indicating an independent point source of I-131 to the river. No I-131 signal was detected at the Cherry Street station in 2018, even though I-131 is present in detectable amounts at the Jones Island and South Shore WWTPs effluents.

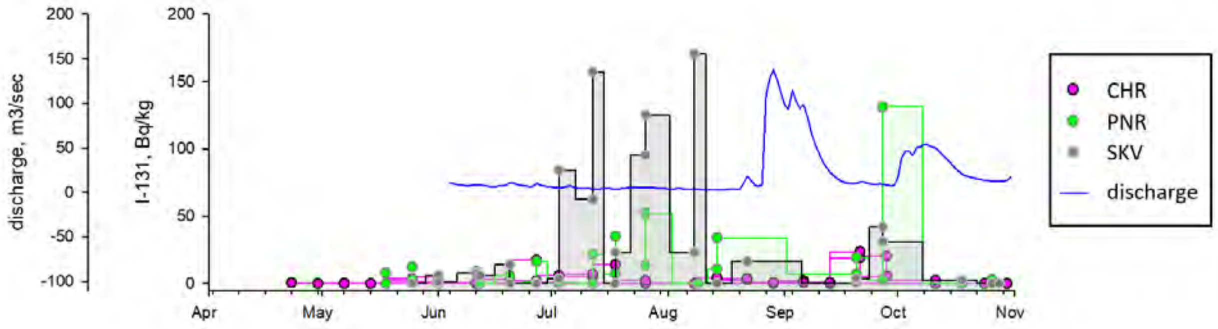


Figure 11. Occasional pulses of detectable I-131 (in Bq/kg sediment, 1 Bq = 1 disintegration per sec) were observed only in the upstream portions of the river during 2018. Four or five spikes in activity were seen (green at Pioneer, gray at Saukville), none of which was associated with the hydrograph, i.e. were unrelated to river flow and discharge (blue line, plotted against the second y-axis).

Mass transport to the river:

Apart from upstream versus downstream contributions, another important consideration for understanding sediment loading is the contribution of riverbank erosion. To investigate this, estimates of the speed with which sediments are transported to and within the river system were determined from measurements of two relatively short-lived, naturally occurring radionuclides, Be-7 (half-life 53 days) and Pb-210 (half-life 22.3 years). Both of these nuclides are derived from the atmosphere and therefore are essentially uniformly distributed across the landscape. The river itself represents an insignificant surface area within the watershed, so that any activity showing up in suspended sediments in the river results from material washed off the land surface. Because the two nuclides have very different half-lives, the time they persist within the system varies directly as a function of their respective decay rates. Over the time scales active in a river system (days-weeks), the activity of the longer-lived radionuclide, Pb-210, essentially remains constant. On the other hand, Be-7, with its much shorter half-life will exhibit within-river decay. The ratio of the two nuclides (Be-7/Pb-210) will therefore decrease proportionally to the time the sediment spends in the river as the shorter-lived nuclide decays. This change in the relative activities is then used to estimate the “apparent age” of suspended sediments collected over time in the river. In this study, we found the suspended sediments generally ranged in “age” between 100-200 days. Trapped downstream sediments appear to be slightly younger (~ 150 days) than upstream sediment (~ 165 days), implying a slightly faster transport of sediment in the urban reaches of the system. Since the input of the radionuclides to the surface of the watershed is known, uniform, and must support the mass transport within the river itself, we also used this radionuclide pair to estimate the area of the watershed ‘swept’ or contributing to the sediment flux into the river In general, the surface area required to supply these radionuclides is on the order of 100 meters of the riparian zone on both sides of the river, implying also that transport rates off the land surface are at a minimum ~ a meter per day. The extent to which river bank erosion, or other sources of sediments essentially “unlabeled” by these radionuclides, contribute to the mass of material moving downstream would affect this apparent age by diluting the activities observed. A smaller fraction of faster moving material could result in the activities measured, a result which seems likely and would warrant more detailed analysis within a small catchment.

Major Conclusions:

- Based on evaluation of TSS loads using both MST markers and overall mass balance calculations, this study shows that TSS loads in the Milwaukee River basin are strongly influenced by upstream sources.
- Dominant TSS sources in individual events are highly variable, and a focus on controlling rural inputs during early season events (i.e. following manure application) would mitigate the largest impacts.

2. *FIB Loading and Coupling with TSS*

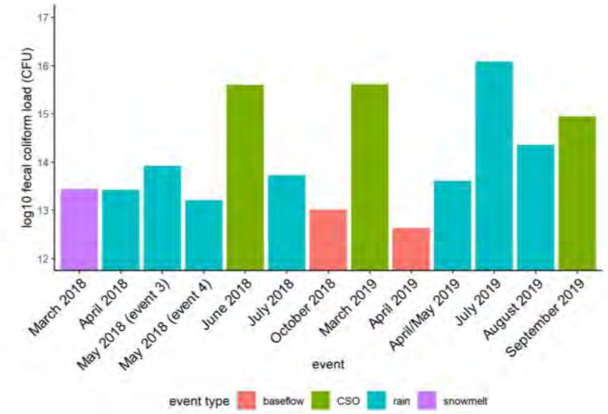


Figure 12. FC loads in the Milwaukee River for each event sampled. Note that for event 2019_e1, no data was available at CHR due to ice cover, so JI data is used instead.

Fecal bacteria are another important pollutant identified in the Milwaukee River watershed TMDL. Mirroring our analysis for TSS, we similarly calculated loads and estimated sources of fecal coliform in the downstream Milwaukee River. Overall, we measured loads of 2.9×10^{15} CFU fecal coliform across all sampled events (Figure 12). Interestingly, based on previous TMDL analyses, our load estimates suggest that we detected only approximately 4% of total fecal coliform loads in the watershed during sampling for approximately 7% of a year. This is in contrast to the TSS loads where 27% of the yearly load was captured. It is important to note that our calculations were based on measurements at the downstream CHR site, whereas TMDL modeling relies on data taken upstream, and decay during transit likely occurs at appreciable levels.

In order to efficiently mitigate high TSS and FIB loads that occur during storm events, it is important to understand whether or not these pollutants are coupled; that is, whether they come from the same sources and/or concurrently exhibit high concentration pulses. To investigate whether or not TSS and FIB are coupled, we first assessed FIB sources using qPCR MST markers. Overall, urban contributions (i.e. those associated with human fecal marker detection) make up the vast majority of fecal coliform loads detected in the downstream Milwaukee River (Figure 13), accounting for 91% of total loads. This high percentage of fecal coliform load associated with human fecal markers holds true even when a CSO event is removed from the dataset, which is consistent with event hydrographs showing

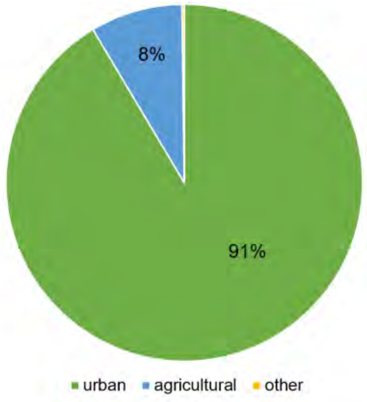


Figure 13. Proportions of fecal coliform loads sourced based on MST markers.

peak FIB concentrations at the same time as peak human fecal marker concentrations even during events without a CSO. This is in contrast to the identical analysis for TSS, which concluded only 20% of TSS loads were attributed to urban inputs. This difference is likely due to the decay of most upstream associated fecal coliform during transport from upstream to downstream and/or the comparatively high concentration sources associated with urban environments, such as leaky sewer lines. In addition, while the two events contributing dominant TSS loads also made up a large proportion of fecal coliform loads, the largest fecal coliform loads measured during event sampling were attributed to a rain event in July 2019, accounting for 56% of total loads (Figure 14). However, a similar high fecal coliform load rain event in the absence of a CSO did not occur during the first season of sample collection (2018), making it difficult to determine if such loads are typical or if this particular event was an anomaly for unknown reasons. On average, fecal coliform loads during CSO-associated events are 22x higher than during non-CSO-associated rain events. Thus while it appears rural inputs and early season events account for dominant TSS loads, fecal coliform loads from rain events can be highly variable and are not fully accounted for by CSO contributions.

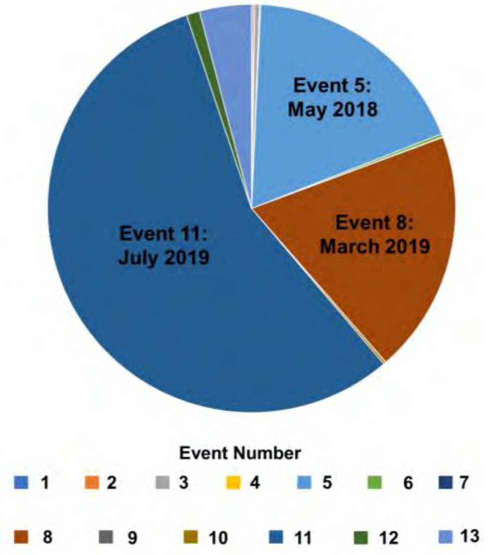


Figure 14. Fecal coliform load proportions by event

The differences in dominant sources (i.e. upstream or downstream) of TSS and FIB loads suggest that these pollutants are typically not strongly coupled during rain events. To further understand the timing and dynamics of TSS and FIB loading at short time scales during rain events, we evaluated whether or not these pollutants exhibited a "first flush" phenomenon. A first flush can be defined as occurring when a large proportion of the total pollutant load is expressed during the initial portion of a rain event. For example, if 60% of TSS load was expressed during the first 30% of storm event flow volume, this could suggest a first flush dynamic. To explore this phenomenon, we calculated normalized cumulative storm volumes and normalized cumulative pollutant loads for each event. This analysis revealed different dynamics between TSS and fecal coliform for the majority of events sampled (Figure 15). TSS loads for the majority of events sampled appear to follow the 45° line, suggesting loading rates proportional to the volume of river flow during each event. However, some events, including a heavy rain event and two of the measured CSOs, show a first flush phenomenon for TSS. In particular, for the CSO event sampled in June 2018, nearly 75% of total TSS load was expressed before the first 25% of storm volume. Fecal coliform loading curves show much stronger pulses, and the majority of events expressed strong first flush phenomena. This data suggests that, while higher loadings of both TSS and fecal coliform are expected during storms, the dominant loads are typically un-coupled, suggesting different primary sources in the watershed.

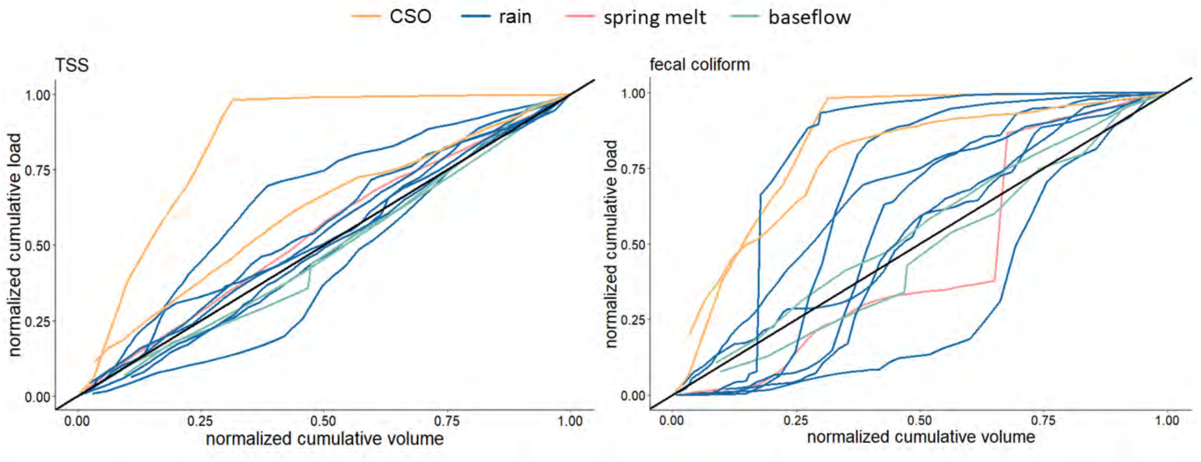


Figure 15. Normalized cumulative loading of TSS and fecal coliform at Cherry Street across all sampled events.

Patterns of total phosphorus concentrations

While our study's focus was primarily on connections between TSS and fecal bacteria, we also evaluated total phosphorus concentrations during certain storm events. Total phosphorus (TP) is also included in the Milwaukee watershed's TMDL requirements, with a target concentration of 0.1 mg/L in river reaches. In total, we measured TP at the CHR site only in 103 samples during five rain events. Among the samples collected, TP concentrations above the TMDL target threshold were associated with higher TSS concentrations (Figure 16). Additionally, of the 103 samples evaluated for TP, a smaller subset (n=22) were evaluated for qPCR MST markers. Within this subset, 67% of samples with high TP concentrations (> 0.1 mg/L) had detectable ruminant marker, while only 30% of samples with low TP concentrations (≤ 0.1 mg/L) had detectable ruminant marker. This data suggests that TP in the Milwaukee River may be driven by agricultural inputs and coupled with TSS. However, as this analysis was based on a small subset of samples, further work is needed to determine these relationships more conclusively.

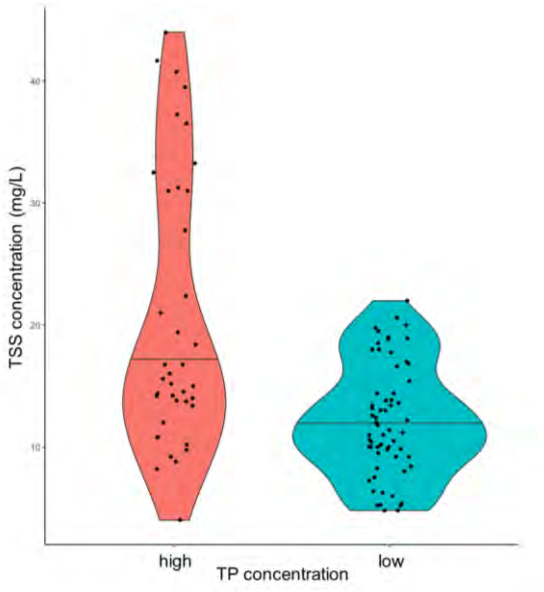


Figure 16. Distribution of TSS concentrations between samples with high (>0.1 mg/L) or low (≤0.1 mg/L) TP concentrations.

Major Conclusions:

- Fecal coliform loads vary strongly by event and are primarily associated with urban inputs
- The largest fecal coliform load measured during event-based sampling was during a large rain event in July 2019, in the absence of a documented CSO.
- Dynamics of TSS and fecal coliform loading during storm events are typically uncoupled, with fecal coliform exhibiting more frequent first flush phenomena than TSS.

3. *Bacterial Community Co-occurrence with TSS and Source Markers*

As noted above, qPCR MST markers designed to detect human and ruminant fecal pollution were used to estimate TSS loads that may have been sourced from upstream agricultural versus downstream urban sources. However, TSS can also be sourced from riverbed resuspension or bank erosion, which may not be associated with the fecal pollution that MST markers were designed to detect. To address this limitation, we explored the use of microbial community sequencing to provide more information on TSS pollution sources. Microbial communities adapt to the environments in which they thrive, and there are significant differences between bacteria that thrive in freshwater, sediment, or fecal environments. Therefore, we similarly evaluated total microbial communities using 16S rRNA gene amplicon sequencing to determine if other microbial markers may be indicative of high TSS concentrations or particular sources of sediment in the water column.

We targeted our sequencing efforts toward understanding the differences between high TSS concentrations during observed "first flush" conditions and subsequent FIB peak concentrations. Water samples were sequenced from a total of nine events, and four example events with early high TSS phases followed by FIB peaks are shown in Figure 17. In addition to

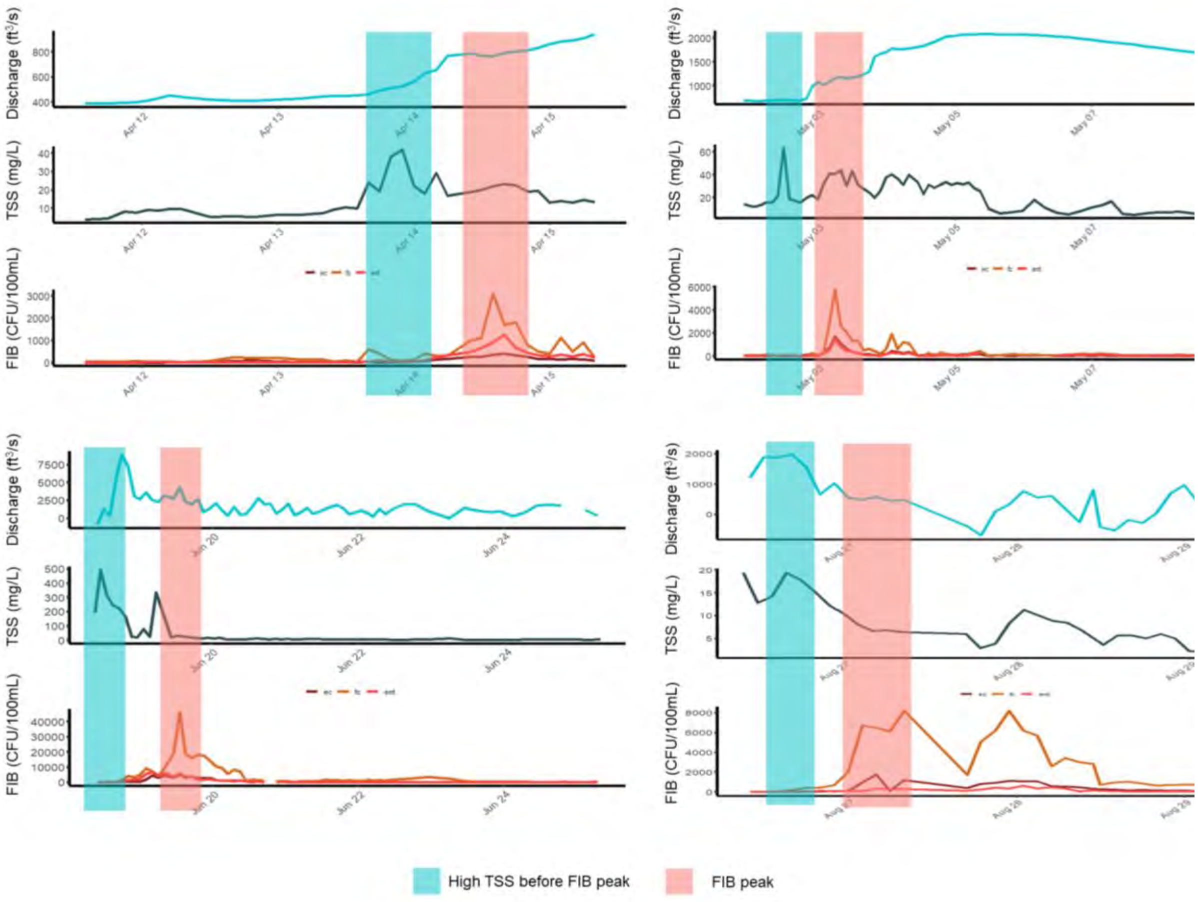


Figure 17. Hydrographs of 4 events selected for sequencing. Highlighted areas represent initial TSS flush and FIB peak areas that were selected for sequencing.

water samples, we also sequenced an additional 31 sediment samples, including upstream sediment traps, downstream sediment traps, and upstream sediment grab samples.

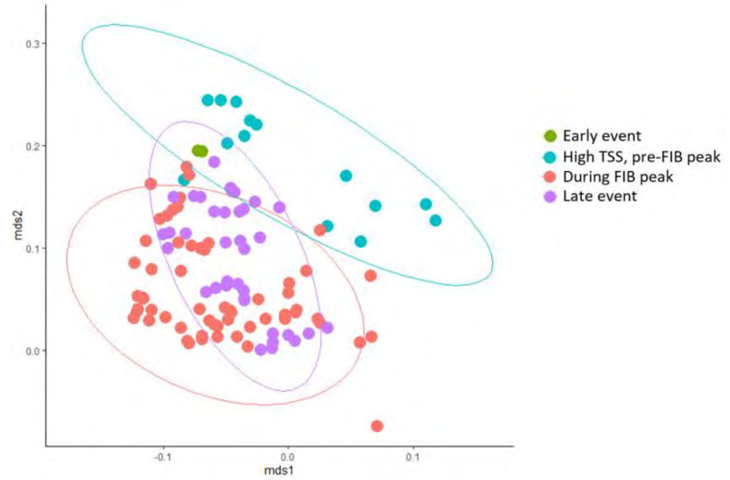


Figure 18. NMDS ordination of microbial communities during summer events. Each point represents an individual sample's bacterial community composition. Axes are arbitrary nondimensional values representing variance of the bacterial communities. Points that are close to each other have more similar bacterial communities, and points far from each other have more dissimilar communities.

Each sample contained on average 50,000 bacterial sequences, and the total dataset comprised 20,110 unique sequences representing different bacterial species. In order to better understand the composition and potential source of high TSS concentrations that peaked without similarly high FIB concentrations, we compared the total bacterial community compositions of each sample. Our analysis shows that microbial communities in the Milwaukee River exhibit compositional shifts at short time scales during rain events. In particular, during high TSS concentrations, significant shifts are observed between periods prior to and during peak FIB concentrations (Figure 18) during summer events. Shifts at Jones Island are typically larger than at Cherry Street, suggesting that some additional microbial community variability at the Jones Island location is due to the influx of pulses from disparate sources, such as the Kinnickinnic River or Lake Michigan. By analyzing specific sequences that differed between TSS and FIB peak phases across both CHR and JI sites and four different events, we identified 73 sequences associated with high TSS in the absence of high FIB, including *Crenothrix*, Sandaracinaceae, and *Sulfuritalea*, which are commonly isolated from freshwater sediments. These sequences are also not associated with ruminant MST marker detection, suggesting that they may serve as markers of more generalized sediment inputs.

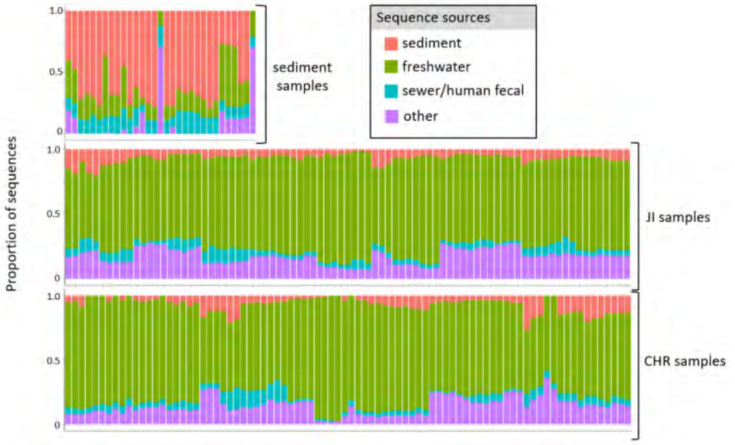


Figure 19. Relative proportions of freshwater, sewer, and sediment sequences in water and sediment samples.

Future work can attempt to identify these sequences within additional sediment samples in order to better understand the source of early TSS peaks during the storm hydrograph. With our previous knowledge of bacteria that associate with freshwater, human fecal/sewage, and sediment environments, we will be able to uncover the imprint of each of these communities within Milwaukee River and harbor water samples at different stages in the storm hydrograph. For example we can clearly identify sediment communities within water samples (Figure 19), and the proportion of sequences associated with sediment varies based on location and event. Among the full set of sediment sequences, we were also able to identify 9 sequences that are highly specific to riverbed samples as opposed to sediment trap samples, suggesting that the presence of these sequences in the water column may indicate significant riverbed resuspension. These sequences were detected in 29% of sequenced water samples overall, with more frequent detection in samples from CSO-associated events (36%) than non-CSO-associated rain events (29%) or baseflow samples (0%), and are not associated with significantly higher TSS concentrations, suggesting that riverbed resuspension is an important, though not dominant, source of TSS during runoff events.

Major Conclusions:

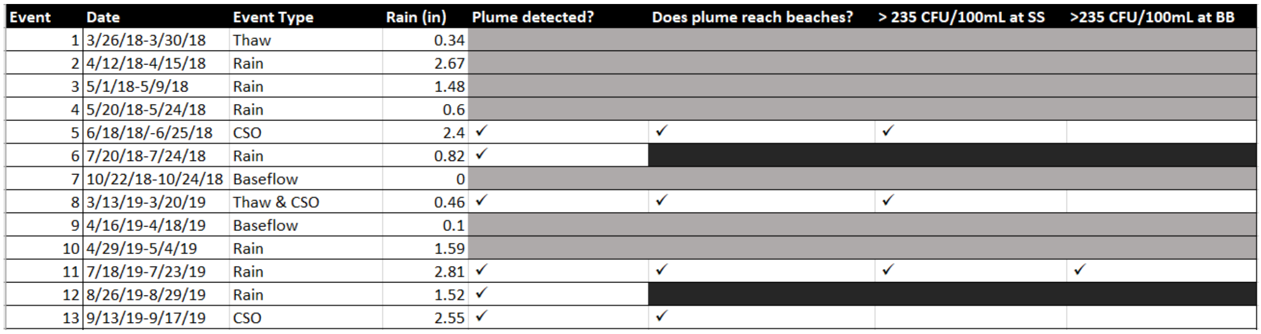
- Microbial community compositions (i.e. the types of sequences detected) shift at short time scales during storm events. These community shifts demonstrate that source markers related to transient high TSS conditions or riverbed resuspension can be developed.
- 73 unique bacterial sequences appear associated with high TSS concentrations in the absence of high FIB or MST marker concentrations, suggesting their utility for understanding and identifying general sediment transport.

4. *Hydrodynamic Modeling in the Nearshore*

Our analysis of samples collected in the upstream and downstream reaches of the Milwaukee River allowed us to conclude that TSS loads are mostly associated with rural inputs and FIB loads are mostly associated with urban inputs. However, the loading magnitude and timing of these pollutants in the storm hydrograph was highly event dependent. Therefore, in order to understand how these pollutants may influence the nearshore during different events, we used a hydrodynamic model to illustrate the fate of both FIB and TSS in Lake Michigan. This model was previously constructed and validated with our collaborator Dr. Hector Bravo and was adapted by Dr. Bahram Khazaei for the data in this study. For each event sampled, the fate of FIB plumes in nearshore Lake Michigan were modeled and visualized. Additionally, for a select set of events, TSS plumes were also evaluated. In evaluating model results, plumes were considered “detected” if the model predicted fecal coliform concentrations exceeding 50 CFU/100 mL at the Milwaukee River mouth. Additionally, plumes were considered to reach beaches if the predicted concentration of fecal coliform exceeded the beach advisory threshold (235 CFU/100 mL) at either South Shore Beach or Bradford Beach.

In general, FIB plumes were observed for approximately half (6/13) of events sampled (Table 4). When plumes were visualized in modeling results (Figure 20) their persistence varied substantially, from only 4 days for a moderate 1.5-inch rain event in August 2019 to 19 days for a large rain event associated with a CSO in March 2019. In general, 4 out of 6 observed plumes impacted beaches, and all of these four events were associated with a CSO or a heavy rain event. However, while our model demonstrated that events with reported CSOs frequently resulted in plumes reaching nearshore beaches, the model itself does not differentiate between CSO discharge and general event runoff. As both CSOs and plumes are more likely to occur during heavy rain events with high discharge, it is not possible to differentiate the specific impacts of CSO discharge from our model. Interestingly, one heavy rain event in April 2018 did not result in a detectable pollutant plume. In contrast to the heavy rain event in July 2019 which did result in a pollutant plume, April 2018 was characterized by less widespread rainfall and substantially lower peak discharge from the Milwaukee River and at Jones Island. These differences demonstrate the importance of various climatic factors in determining the impact of rain events in the nearshore environment. Additionally, we want to note that while nine out of 13 events did not appear to impact beaches, our model only accounted for inputs from the Milwaukee harbor estuary. There are other sources of FIB in the watershed near beaches, such as stormwater outfalls or overland runoff, that could have impacted beaches during these events, but these sources were not included in our model.

Table 4. Summary of results from nearshore modeling for *E. coli* (EC) and fecal coliform (FC) plumes.



Event	Date	Event Type	Rain (in)	Plume detected?	Does plume reach beaches?	> 235 CFU/100mL at SS	>235 CFU/100mL at BB
1	3/26/18-3/30/18	Thaw	0.34				
2	4/12/18-4/15/18	Rain	2.67				
3	5/1/18-5/9/18	Rain	1.48				
4	5/20/18-5/24/18	Rain	0.6				
5	6/18/18/-6/25/18	CSO	2.4	✓	✓	✓	
6	7/20/18-7/24/18	Rain	0.82	✓			
7	10/22/18-10/24/18	Baseflow	0				
8	3/13/19-3/20/19	Thaw & CSO	0.46	✓	✓	✓	
9	4/16/19-4/18/19	Baseflow	0.1				
10	4/29/19-5/4/19	Rain	1.59				
11	7/18/19-7/23/19	Rain	2.81	✓	✓	✓	✓
12	8/26/19-8/29/19	Rain	1.52	✓			
13	9/13/19-9/17/19	CSO	2.55	✓	✓		

Another important observation from our modeling results was the differences in modeled plume impacts from TSS and FIB depending on the event. For example, early in our sampled July 2019 rain event (Figure 20), a highly concentrated fecal coliform plume was modeled in the inner harbor in the absence of significant TSS concentrations. In contrast, during a rain event resulting in a CSO in June 2018 (Figure 21), a concentrated TSS plume is visible in our model prior to corresponding FIB impacts. This discrepancy is important as the TSS portion of the plume is what is likely "observed" by the public and demonstrates that the observed plume does not always co-occur with fecal bacteria, especially during early periods of runoff events.

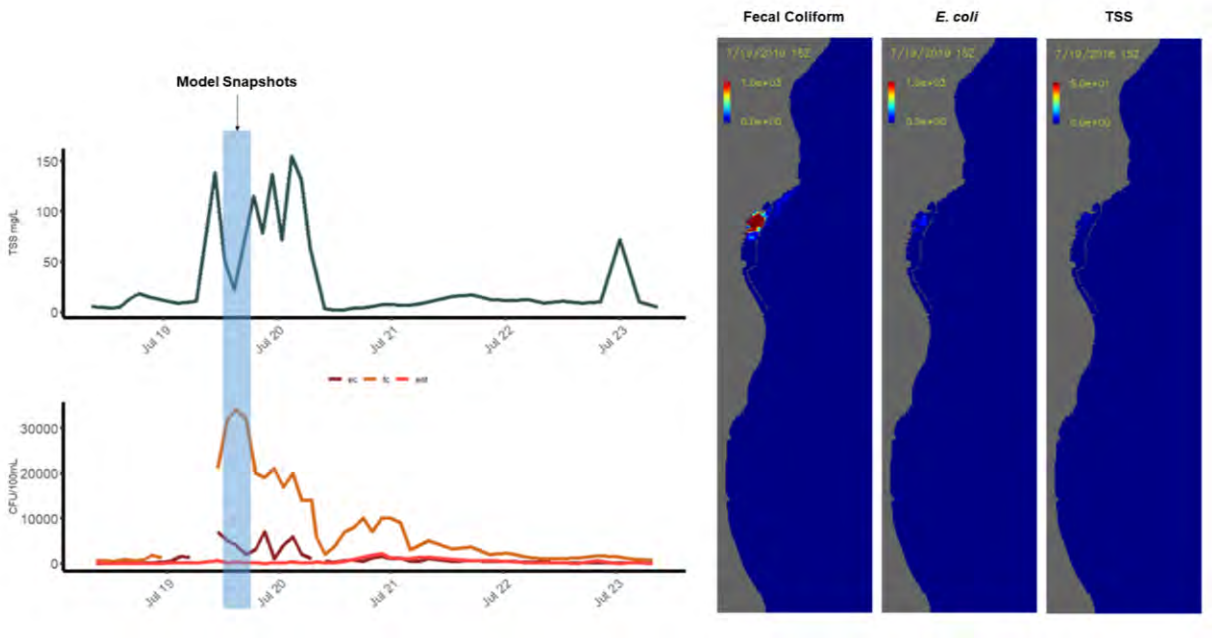


Figure 20. Hydrodynamic model snapshots at CHR of fecal coliform, *E. coli*, and TSS impacts in the nearshore from July 2019 rain event.

Major Conclusions:

- Based on a hydrodynamic model, fecal bacteria plumes from the Milwaukee harbor reached beaches and persisted for more than a week when associated with CSOs
- When not associated with a documented CSO, modeled fecal bacteria plume occurrence and persistence varied for each event and was not necessarily associated with total rainfall.
- Timing of modeled TSS and fecal bacteria plumes varied, with TSS plumes preceding or lagging behind fecal bacteria depending on the event.

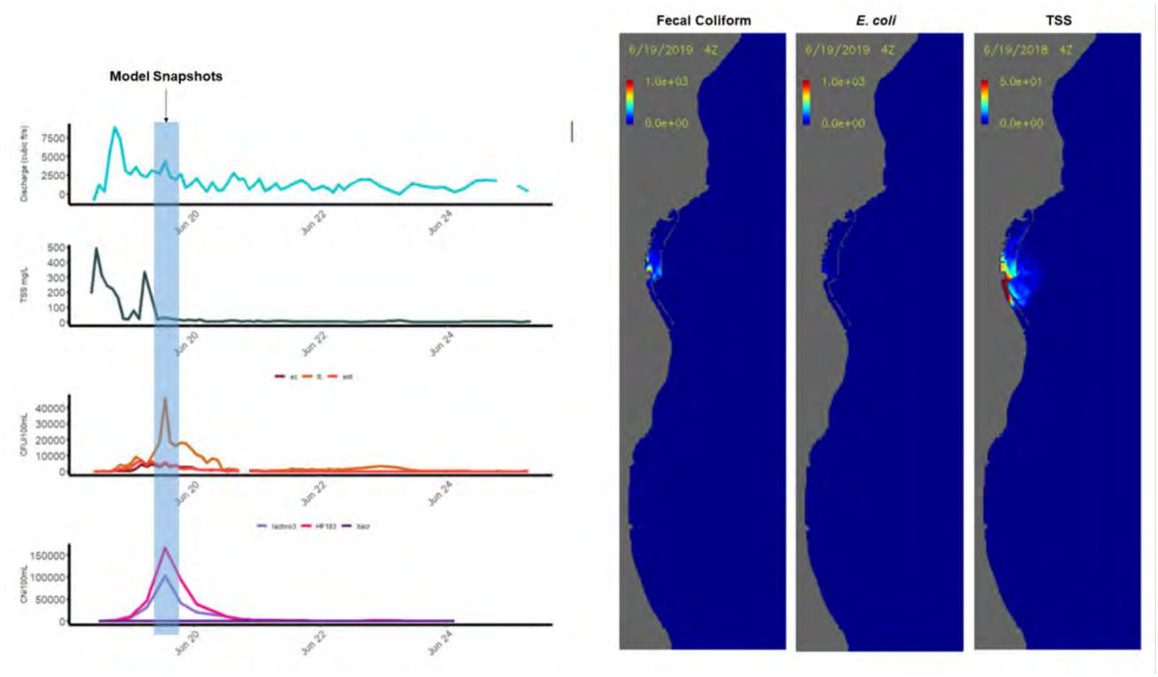


Figure 21. Hydrodynamic model snapshots at CHR of fecal coliform, *E. coli*, and TSS impacts in the nearshore from June 2018 rain + CSO event. Videos of model results from this event are available at: https://people.sfs.uwm.edu/mclellanlab/climate-2/

Conclusions and Recommendations

Overall, our study provides important empirical data that corroborates previous TMDL analyses in the Milwaukee River watershed and contributes additional information to help focus pollutant mitigation efforts. Our key conclusions are:

1. TSS loads are highly event dependent, but in general, upstream rural zones, including significant agricultural inputs, account for the majority of TSS load contributions.
2. FIB loads are highly associated with human fecal marker detection in the river system during rain events, regardless of whether or not a combined sewer overflow (CSO) also occurred.
3. Microbial communities shift at short time scales during storm events, and partitioning communities based on defined databases can uncover additional pollution markers.
4. Weather patterns and flow regimes in the nearshore dictate the impact of rain events on nearby beaches; in general, large rain events & CSOs produce enough TSS & FIB loads to impact beaches.

Based on these results, we found that the largest benefit to reducing loads of TSS or FIB would involve mitigation strategies that address the high flow periods during large rain events, which account for a disproportionate amount of pollutant loading to the harbor. When considering loads from the large rain events, including those with CSO occurrence, captured in our data set, reducing pollutant loads during these events to levels comparable to smaller rain events would reduce total TSS loads measured by 59% and total fecal coliform loads by 77%. As extreme weather events are expected to increase in frequency in the Great Lakes region, stormwater retention to mitigate peak flow conditions will likely become more valuable in the future. Furthermore, 50% of events evaluated had human fecal marker concentrations exceed a health risk threshold in the Milwaukee River, typically reaching peak concentrations during the rising limb of the hydrograph. This suggests that early “first flush” periods pose the greatest risk to river recreation. Additionally, coordination with upstream municipalities will be essential for TSS mitigation, as large proportions of total load during certain rain events are sourced from upstream regions. While large rain events contribute the dominant loads over the course of a year, mitigation strategies should likely focus both on reducing overall runoff volumes to minimize erosion, as well as pollutant concentrations by reducing pollutant sources before runoff occurs. High total phosphorus (TP) concentrations are highly associated with ruminant fecal marker detection, suggesting substantial nutrient inputs from upstream agricultural sources, which warrants future study. Finally, our work evaluated microbial community signals to identify new tracers for sediment inputs uncoupled from fecal inputs, and our modeling results will feed directly into additional work being done to remove the Area of Concern designation for Milwaukee area beaches.

Overall, this study demonstrates that TSS and fecal bacteria tend to be uncoupled in the Milwaukee River watershed. Mitigating TSS will require actions in the upstream rural reaches of the watershed, while mitigating fecal bacteria will require actions in the downstream urban areas. However, despite their distinct sources, both pollutants demonstrated loads that were strongly event-dependent and focused around large rain events. While focusing on the impact of peak flow and extreme event mitigation will be important, these events are much more difficult to predict, and therefore mitigation strategies that can increase stormwater retention and reduce pollutant sources during both minor and major event flows would likely be the most valuable.

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