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Microbial Pollution Characterization at a TMDL Site in Michigan: Effect of Hydrological Conditions on Pollution Loading

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ABSTRACT

Communities throughout the United States are developing and implementing watershed management plans to address nonpoint sources of pollution and meet Total Maximum Daily Load (TMDL) requirements. Once a TMDL is established, a watershed management plan is developed and implemented to reduce contaminant sources and attain TMDL goals. Developing an effective TMDL and remediation plan should take into account fluctuation of pollution loadings and the timing of first-flush events. The objective of this study is to investigate the effect of hydrological conditions on microbial pollutant levels at a TMDL site during spring and summer storm events. A total of 64 water samples were collected from Sloan Creek in mid-Michigan in the spring/summer of 2015. All samples were analyzed to quantify concentrations of *E. coli*, bovine-associated *Bacteroides* (BoBac) gene markers, and human-associated *Bacteroides* (HuBac) gene markers. Discharge was the driving force of microbial contaminant loading in the studied water body. *E. coli* concentrations had significant strong correlation with precipitation and discharge, and BoBac concentrations were positively related to discharge. *E. coli,* BoBac and HuBac patterns suggested first-flush phenomena occurred during summer storms. *E. coli* permit exceedance rates increased from 31% before first-flush, to 100% during and after first-flush in the summer. The resulting information may help develop a plan for restoring impaired waters and establish the maximum amount of pollutants that the body of water can receive during different hydrological conditions.

Introduction

Communities throughout the United States are developing and implementing watershed management plans to address nonpoint sources of pollution and meet Total Maximum Daily Load (TMDL) requirements. Once a TMDL is established, a watershed management plan is developed and implemented to reduce contaminant sources and attain TMDL goals (USEPA, 2008). In addition to identifying pollution sources, effective watershed management plans should identify the timing of maximum pollutant loading and its relationship to hydrological events.

It is well known that storm water runoff, from urban and agricultural areas, significantly contributes to the microbial pollution loads of surface water. Changes in hydrological conditions have been shown to be associated with microbial contamination levels in surface waters (Almeida and Soares, 2012; Kistemann et al., 2002). Rainfall and discharge have been shown to correlate with *Escherichia coli* (*E. coli*) concentrations in urban and agricultural watersheds (Bach et al., 2010; Krometis et al., 2007; Reischer et al., 2008). Rainfall events can lead to increasing discharge and first-flush phenomena, which are associated with high contaminant concentrations. Initial runoff may significantly increase contaminant concentrations in surface waters due to compounds accumulating on the ground surface during dry periods. In addition, first flush phenomena may raise significant public health issues, such as the spread of epidemiologic diseases (Kleinheinz et al., 2010). Thus, studying first-flush phenomena can help to understand patterns of contaminant release and to improve land management decisions.

Researchers have primarily focused on chemicals in surface runoff when studying first-flush phenomena. The major chemical contaminants that have been studied were Na^+, Cl^-, K^+, TSS, and DOC (Deletic, 1998; Evans and Davies, 1998; Lee et al., 2004). Limited studies have focused on first-flush assessment of microbial pollutants. Doyle (2008) observed an increased concentration in total coliform and *E. coli* in rainwater harvesting system in Rwanda following a first-flush event. Similarly, a first-flush of *E. coli* was observed in an urban catchment in Australia (Bach et al., 2010). Hathaway and Hunt, 2011 used *E. coli*, fecal coliforms, and *Enterococci* as indicators in an urban watershed with mixed land use conditions, and first-flush phenomena of *E. coli* was observed. Several studies have been conducted in North Carolina. Stumpf et al. (2010) investigated *E. coli* and *Enterococci* in a headwater catchment, but there was no first-flush phenomena observed;

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Table 1
Primers used in this study for human and bovine associated *Bacteroides* genetic markers.

	Forward	Reverse	Probe	Reference
Human-associated *Bacteroides B. thetaiotaomicron* α-1–6 mannanase (HuBac)	TCGTTCGTCAGCAGT -AACA	AAGAAAAAGGGACAGTGG	6FAM-ACCTGCTG-NFQ	Yampara-Iquise et al. (2008)
Bovine-associated *Bacteroides* 16srRNA (BoBac)	BoBac367f (GAAG(G/A)CTGA ACCAGCCAAGTA)	BoBac467r (GCTTATTCATA CGGTACATACAAG)	BoBac402Bhqf (TGAAGGATGAAGGTTC TATGGATTGTA AACTT)	Layton et al. (2006)

Rowny and Stewart, 2012 studied fecal coliform and *E. coli* in an urban watershed, but no first-flush phenomena were observed.

In recent years, *Bacteroides* genetic markers have been used to identify fecal contamination in water and provide a powerful tool to trace the source of fecal contamination (Field and Samadpour, 2007). Several *Bacteroides* quantitative polymerase chain reaction (qPCR) assays have been developed to detect fecal sources from multiple animal and human sources (Bernhard and Field, 2000; Dick et al., 2005; Field and Samadpour, 2007; Layton et al., 2006; Okabe et al., 2007). However, first-flush studies or studies focusing on the effect of hydrological conditions on microbial source tracking (MST) tools such as *Bacteroides* concentrations have not been reported yet. Such studies are important in understanding the loading patterns of pollution originating from different sources, and can provide valuable information for addressing TMDL sites. In this study, the concentrations of the traditional fecal contamination indicator, *E. coli*, and the concentrations of the genetic markers for Human-associated *Bacteroides* (HuBac) and Bovine-associated *Bacteroides* (BoBac) were quantified in water samples collected from Sloan Creek, a TMDL site in mid-Michigan, in the Great Lakes Basin. The purpose of this study was to assess the impact of spring and early summer rainfall events on microbial contaminant loadings. This research provides an understanding of microbial contaminant sources during different types of hydrological events (storm events and dry events) in a mixed watershed and helps with developing watershed management plans.

The sampling scheme and study design should be given special attention when conducting a microbial contamination loading study, taking into consideration all regional hydrological conditions and pollution dynamics (Kay et al., 2007; Reischer et al., 2008). This study established and evaluated a comprehensive sampling plan for the Sloan Creek with consideration given to spring and summer seasonal hydrological conditions. The sampling scheme was designed to characterize and compare spring hydrological conditions (snow-melt) with the summer hydrological events (rainfall).

Materials and methods

Site description and sampling

Sloan Creek (Ingham County, MI) is a tributary of the Red Cedar River, which flows about 50 miles through rural and agricultural land in the south-central lower peninsula of Michigan. The Red Cedar drains into the Grand River and subsequently to Lake Michigan. In the state of Michigan, the daily maximum geometric mean is 300 *E. coli*/100 mL for total body contact recreation, and 1000 *E. coli*/100 ml for partial body contact recreation (MDEQ, 2016). The Sloan Creek sub-watershed is a small sub-watershed within the Red Cedar River watershed, and was selected for this study because it previously exceeded State of Michigan *E. coli* water quality standards for total and partial body contact recreation (ICD Red Cedar Monitoring Project 2013, MDEQ 2014). Sloan Creek flows through both agricultural and residential areas. Suspected sources of bacteria in the sub-watershed include human, agricultural and wildlife fecal inputs.

South-central Michigan has hot summers with frequent precipitation. The average maximum temperature in the summer is 27.8 °C and the average annual precipitation is 962 mm of which 59% falls during the cropping season (the months of May-October), when fertilizers are applied on the land surface in agricultural areas (Ingham Conservation District, 2012). Agricultural land is 45% of the Sloan Creek sub-watershed. Precipitation data for the study period were obtained from the Michigan Automated Weather Network (MAWN) East Lansing Michigan State University (MSU) Hort, Michigan station, (42.6734, −84.4870). Discharge data in Sloan Creek was collected from a United States Geological Survey gauging station (USGS 04112000) located at Sloan Creek near the City of Williamston, Michigan (42.6758, −84.3638). The base flow in Sloan Creek is 0.0283 m^3/s.

A six-month sampling scheme was designed to collect samples ($N = 64$) at least twice per week, in addition to sampling during rain events in spring and summer 2015, from March 22nd to August 26th. The sampling site was located in Legg Park, Michigan, at the mouth of Sloan Creek where it drains into the Red Cedar River. One-liter autoclaved sampling bottles were rinsed three times with water samples prior to collection. Two liters of water samples were collected for *E. coli* and *Bacteroides* measurements. Grab water samples were collected, stored on ice, and analyzed in the laboratory within 3 (±1) hours.

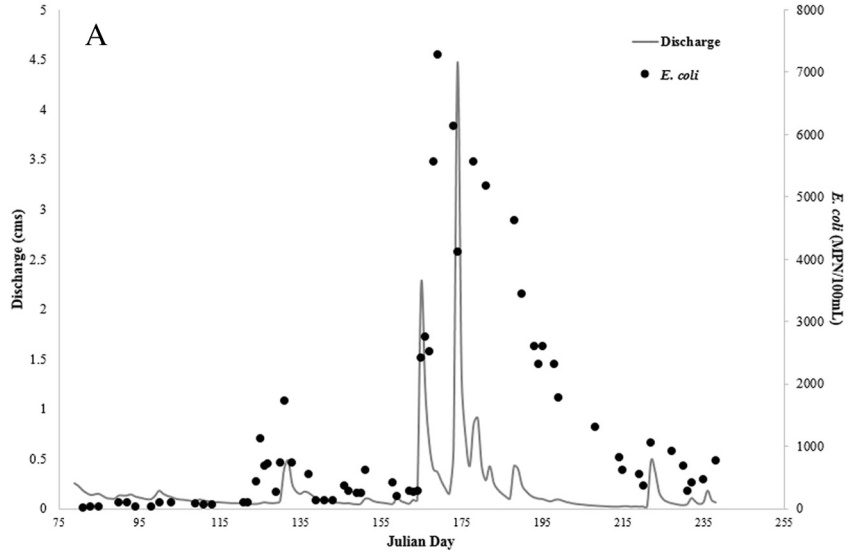
E. coli quantification

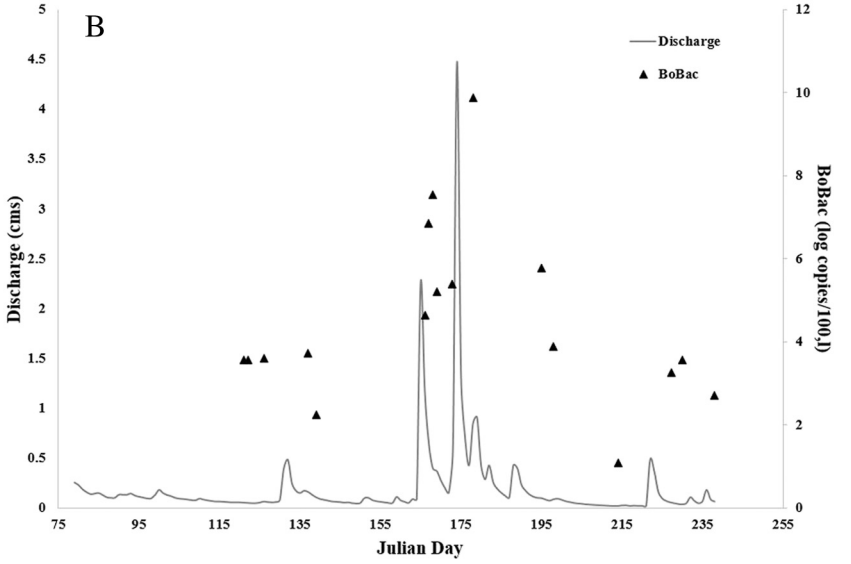
E. coli enumeration was performed using Colilert-18®, which has a detection limit of 1 Most Probable Number per 100 mL (MPN/100 mL). Samples were diluted (1:10 and 1:100) with deionized water to make a 100 mL solution. Colilert-18® was added to each sample, dissolved by shaking, poured into a Quanti–Tray/2000 tray, and the trays were incubated overnight at 35 °C for 18 h (Colilert-18 procedure). The wells of the trays were counted and the most probable number (MPN) per 100 mL of sample was calculated according to the manufacturer's instructions. The measurement was performed twice on each sample and their corresponding dilutions and average MPN values were calculated.

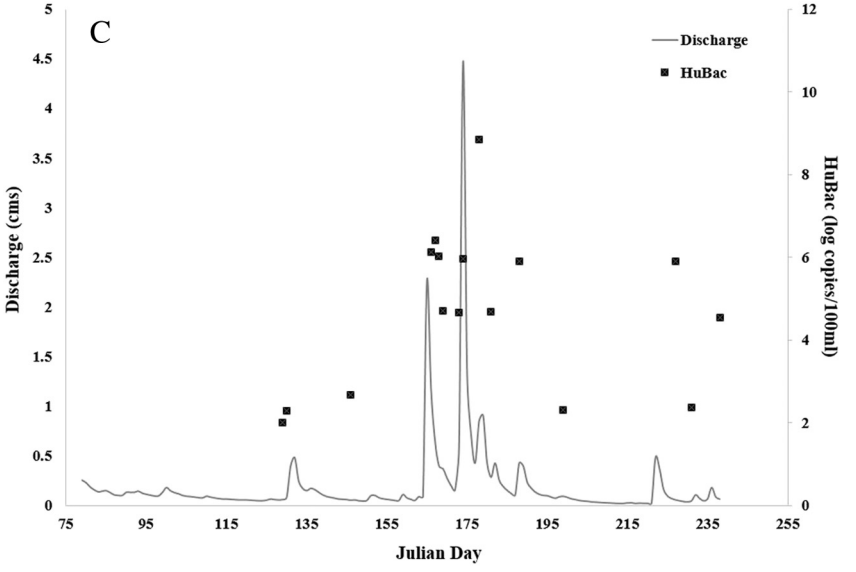
Bacteroides quantification

All water samples were tested for Hubac and BoBac molecular markers quantitatively using qPCR. 500 mL of water sample were filtered through 0.45 μm hydrophilic mixed cellulose esters filter (Pall Corporation 66278) under partial vacuum. The filter was placed into a 50 mL sterile disposable centrifuge tube containing 45 mL of sterile phosphate buffered saline PBS, vortexed on high for 10 min, and then centrifuged (30 min; 4500 ×g; 20 °C) to pellet the cells. Samples were concentrated down to 2 mL by decanting 43 mL from the tube and the remaining pellets were stored at −80 °C until DNA could be extracted. After thawing the samples, 100 μL of DNA was extracted from 400 μL of pellet using MagNa Pure Compact System automatic machine (Roche Applied Sciences, Indianapolis, IN) with the corresponding kit (MagNA Pure Compact Nucleic Acid Isolation Kit I), according to the

Fig. 1. River discharge and concentrations of a) *E. coli*, b) bovine-associated *Bacteroides (BoBac)*, c) human-associated *Bacteroides (HuBac)*. Julian days 79–238 correspond to March 20th 2015–August 26th 2015


A
Discharge
E. coli
Discharge (cms)
E. coli (MPN/100mL)
Julian Day


B
Discharge
BoBac
Discharge (cms)
BoBac (log copies/100,l)
Julian Day


C
Discharge
HuBac
Discharge (cms)
HuBac (log copies/100ml)
Julian Day

manufacturer instructions. Two host-specific qPCR methods were utilized to identify and quantify potential sources of fecal pollution within the sub-watershed. Primers used are listed in Table 1.

All qPCR quantification analysis was carried out with LightCycler® 1.5 Instrument (Roche Applied Sciences, Indianapolis, IN) and LightCycler 480 Probes Master kit with a total reaction volume of 20 μL. DNA extracted from samples was analyzed in triplicate with 5 μL of extract used for template. The crossing point (Cp) value for each qPCR reaction was automatically determined by the LightCycler® Software 4.0. One copy of the targeted gene is assumed present per cell; thus, one gene copy number corresponded to one cell equivalent. Gene copies were then converted and reported using the unit "copies/100 mL".

In order to prepare the standard curves to quantify the gene numbers, the DNA was extracted from American Type Culture Collection (ATCC), number 29148D-5, genomic DNA for *B. thetaiotaomicron*, because of its high host-specificity (Xu et al., 2003). Bovine feces obtained from the Michigan State University dairy farm was used for DNA extraction for BoBac. The amplified PCR products for the target genes were cloned into one shot chemically competent *E. coli* using TOPO TA Cloning kit for Sequencing (Invitrogen Inc., Carlsbad, CA, USA) according to the protocol provided by the manufacturer. Plasmids were extracted with QIAprep Spin MiniPrep kit (Valencia, CA, USA) and were sequenced at the Research Technology Support Facility (RTSF) at Michigan State University to confirm the insertion of the target inside the vector. The DNA concentration in plasmids was quantified using Qubit Fluorometric Quantitation (Thermo Fisher Scientific) and then serially diluted ten-fold to construct qPCR standard curves. Triplicates of dilutions ranging from 10^8 to 10^0 were used for the standard curves. One plasmid standard was included during each qPCR run as a positive control and molecular-grade water was used in place of DNA template for negative controls.

Data analyses

The association of all measured microbial contaminants with river discharge and rainfall was investigated. Statistical analyses were performed using SPSS Statistics software (Version 22) with a significance $\alpha = 0.05$. *Bacteroides* concentrations were log-transformed to achieve normality and meet the assumptions of a parametric test. Simple t-tests were used to determine the differences in mean concentrations of target organisms among each other and with the precipitation and the discharge. The t-test was two-tailed, with alpha levels, or the probability of rejecting the null hypothesis when it is true, set at $p < 0.05$. Pearson's correlation coefficient was used to test the relationship between *E. coli*, *Bacteroides* markers, precipitation, and discharge.

To define first-flush phenomena quantitatively the total river discharge during the sampling period was accumulated and divided into three events of equal amounts. Our goal was to evaluate the impact of a set amount of cumulative discharge on pollutant concentrations. The cumulative discharge of the sampling period was 30.28 cm (m^3/s) and the event size of 10 cm cumulative discharge was chosen. The purpose of dividing into equal amounts of cumulative discharge was to capture the impact of event size on the first flush. The distribution of mean concentration of microbial contaminants *E. coli*, BoBac, and HuBac in each event was characterized by using the non-parametric Wilcoxon rank sum test. According to Bach et al., 2010, a first-flush phenomenon is confirmed if there is a statistically significant difference of pollutant concentrations between the events. In case the differences are not statistically significant, an alternative amount of equal cumulative discharge should be selected. The 5% significance level was used for event grouping (Bach et al., 2010). First-flush events were confirmed with Mass/Volume (M/V) method (plots not shown). The M/V method assesses and quantifies first-flush phenomena by using dimensionless cumulative pollutant mass load vs. cumulative runoff volume curves (Bertrand-Krajewski et al., 1998). This theory sets the criteria for first flush phenomenon when over 80% of the total pollutant mass load is transported within the first 30% of total discharge volume (Bertrand-Krajewski et al., 1998).

Results and discussion

E. coli temporal variation

Sloan Creek was monitored for the presence of the microbial fecal indicator *E. coli*. The detection rate of *E. coli* was 100%. Monitoring results for *E. coli* concentrations are shown in Fig. 1. High concentrations of *E. coli* were found within 24 to 72 h following each rain event. 59% (38 of 64) of the measured water samples exceeded the water quality standards given the single sample limit of *E. coli* is 300 MPN/100 mL for total body contact recreation (MDEQ, 2016).

In our study, the highest concentration of *E. coli* was measured on day 169 (June 18th, 2015), four days after the first large rain event of the season on day 165 (June 14th, 2015, 42.42 mm). A sharp increase was observed on June 18th, 2015 (7270 MPN/100 mL) compared with day 163 (June 12th, 2015, 272.3 MPN/100 mL), then *E. coli* continued to decrease gradually until the end of the season and fell back within the base flow conditions. On day 222 (August 10th, 2015) the largest rain event of the season was recorded (57.66 mm), but *E. coli* levels did not increase significantly (Fig. 1a). Apparently *E. coli* was depleted from the land surface and soil after the series of earlier rainfall events.

Host-specific Bacteroides temporal variation

Human and bovine *Bacteroides* gene markers shared similar patterns as *E. coli* (Fig. 1b and c). The detection rate for bovine *Bacteroides* was similar to human *Bacteroides*, and they were 26.6% and 25% respectively. The highest concentration of bovine and human *Bacteroides* occurred on day 178 (June 27th, 2015) after the highest discharge peak with the concentration of 7.6×10^9 copies/100 mL and 6.99×109 copies/100 mL respectively.

Bacteroides markers underwent some delay on the time series. The highest hits for the *Bacteroides* markers were not contemporaneous with *E. coli*. Both *Bacteroides* markers had a sharp increase on day 166 (June 15th, 2015) within 24 h of the first large rain event. HuBac was raised from undetected on day 165 (June 14th, 2015) to 1.35×10^6 copies/100 mL on day 166 (June 15th), and reached its highest concentration on June 27th, 2015 (6.99×10^8 copies/100 mL). BoBac behaved similarly to HuBac; it rose from undetected on June 14th to 4.3×10^4 copies/100 mL on day 166 (June 15th) with its highest concentration on day 178 (June 27th, 2015). Later in the season on day 222 (August 10th) the largest rain event was recorded, and *E. coli* had a sharp increase from 357.5 MPN/100 mL on day 220 (August 8th, 2015) to 1046.2 MPN/100 mL on day 222 (August 10th, 2015). Similarly, the BoBac concentration was raised from undetected to 6.97×10^4 copies/100 mL. No human marker was detected during this event. This pattern indicates that there was a strong loading of fecal contamination originating from bovine sources to the stream during the largest rainfall event.

Correlation between microbial indicators, discharge, and rainfall

Our results showed that *E. coli* concentrations were significantly elevated and strongly correlated with precipitation ($p = 0.001$, $r = 0.422$) and discharge ($p = 0.001$, $r = 0.414$). Similar to these findings, bacterial loading rates in rivers and beaches have been shown to increase during hydrologic events (Daly et al., 2013; Kistemann et al., 2002; Kleinheinz et al., 2010; Krometis et al., 2007; Rowny and Stewart, 2012; Stumpf et al., 2010). A strong relationship between fecal indicator bacteria and wet weather has been documented previously. For example, Reeves et al., 2004 carried out a series of field studies to identify the spatial distribution of fecal indicator bacteria in dry and wet weather runoff, and indicated that stormwater runoff was an important factor

Table 2
Correlation analysis (2-tailed) for microbial contaminants and hydrological conditions. The number of samples that were involved in the statistical analysis for *E. coli*, BoBac, and Hubac were 64, 17, 16 respectively.

Parameters		*E. coli*	BoBac	HuBac
Precipitation (mm)	Pearson Correlation	.422**	.407	.216
	Sig.	.001	.105	.422
Discharge (cms)	Pearson Correlation	.414**	.642**	.383
	Sig.	.001	.005	.144

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

Table 3
E. coli exceedance based on hydrological events. The total river discharge during the sampling period was accumulated and divided into three hydrological events of equal volume, as explained in the methods section. The overall *E. coli* exceedance rate was 59% (based on 300 MPM/100 mL limit)

Event	Day range	Date	Exceedance rate
Event 1:	Day 79–164	March 20th to June 13th	31%
Event 2:	Day 165–175	June 14th to June 24th	100%
Event 3:	Day 176–238	June 25th to August 26th	95%

correlating with nonpoint source pollution. A long-term water quality study (1994–2010) estimated the loads from streams and drains supplying an estuary in Australia and it suggested that stormwater was a significant source of *E. coli* during wet-weather flow (Daly et al., 2013). In the Hoosic River watershed in Massachusetts, bacterial levels were found to be higher in summer than in winter and higher during storms than during base flow conditions (Traister and Anisfeld, 2006). Similarly, during winter the lowest microorganism concentrations in Navesink River watershed in New Jersey were observed (Selvakumar and Borst, 2006). Additionally, the importance of precipitation and streamflow in the transport of protozoan and bacterial pathogens and fecal indicator bacteria has been frequently reported (Dorner et al., 2006; Ferguson et al., 2003; Wu et al., 2011). Our study also illustrated that late season storms had a greater frequency of water quality exceedances compared to early season storm events, possibly because in early spring the soil temperature is still low, which makes the deposited pollution hard to release into the stream. Another reason could be the effect of cold temperature on *E. coli*.

In addition to *E. coli*, our study demonstrated that bovine-associated *Bacteroides* concentrations were significantly correlated with discharge ($p = 0.005$, $r = 0.642$). There was no significant relationship with discharge and precipitation for HuBac (Table 2).

Our study investigated the effect of selected hydrometeorology factors (precipitation and discharge) on the increased concentration of *E. coli* and host-specific *Bacteroides*. However, *E. coli* concentrations were also reported to be positively correlated with antecedent climate, rainfall intensity, stream water temperature, and sediment (Crabill et al., 1999; Liao et al., 2014; McCarthy et al., 2012; Oun et al., 2017). These factors might affect water quality along with precipitation and river discharge, leading to the elevated *E. coli* and *Bacteroides* concentrations. Furthermore, the persistence and fate of microbes after they are released to the natural environment should be considered when studying the variation of the concentrations.

Effect of hydrological events on pollution loadings

Both discharge and rainfall fluctuated during the sampling period (Fig. 2); two main storms were observed during the sampling period which caused drastic increases to the discharge in the creek. The first storm was on day 165 (June 14th, 2015) with precipitation of 42.42 mm and the second on day 173 (June 22nd, 2015) with precipitation of 43.94 mm. The cumulative discharge of the sampling period was 30.28 m^3/s (cms), and the event size 10 cm was chosen for the merits of calculation. Therefore, there were three events in this study (Fig. 2). As explained in the methods section, the purpose of dividing into equal volume events was to capture the impact of first flush. The base flow condition for Sloan Creek is 0.0283 cm according to the USGS gauging station 04112000. When the increment of surface runoff (event size) 10 cm was used, day 79–164 were grouped into Event 1; day 165–175 were grouped into Event 2; and day 176–238 were grouped into Event 3. The *E. coli* exceedance rates for each event are shown in Table 3. Our results showed that *E. coli* exceedance rates raised from 59% before first flush to 100% during first flush in the summer.

To examine the change of concentrations over the absolute cumulative surface runoff and to characterize the distribution of the events,

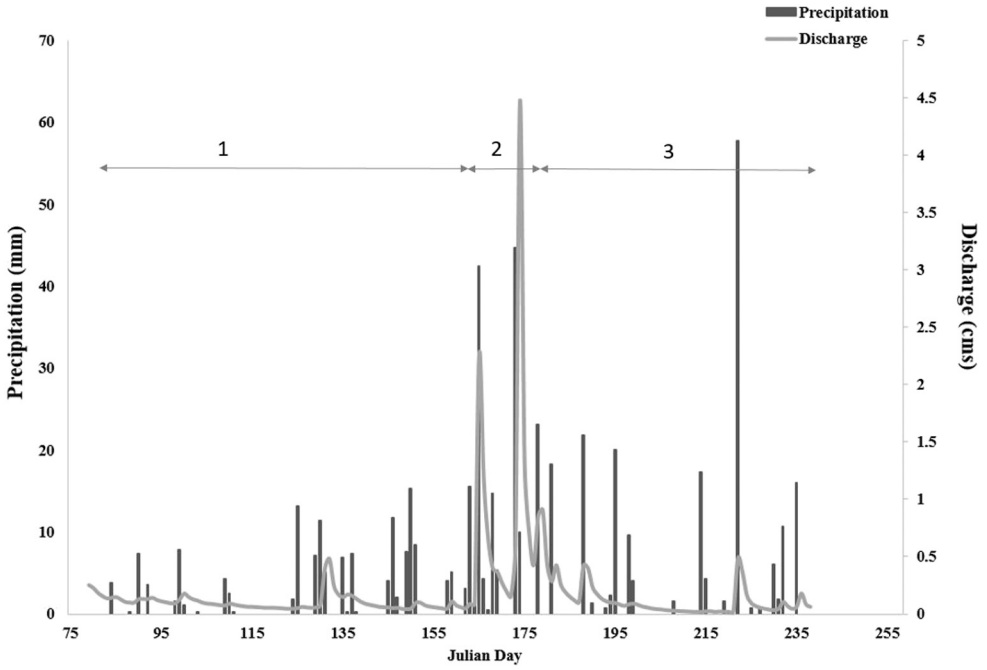


Fig. 2. Rainfall and hydrograph of Sloan Creek discharge during the study period. River discharge levels are expressed as daily mean values. Event 1 ranges from Julian day 79–164; Event 2 ranges from day 165–175; Event 3 ranges from day 176–238. Each hydrological event was of equal total volume of water.

box-and-whisker plots were constructed for each event (Fig. 3) and the non-parametric Wilcoxon Rank Sum test was used to group events of statistically similar concentrations. In addition, the non-parametric Wilcoxon rank sum test was conducted for the three microbial contamination indicators at the three different hydrological events (Table 4).

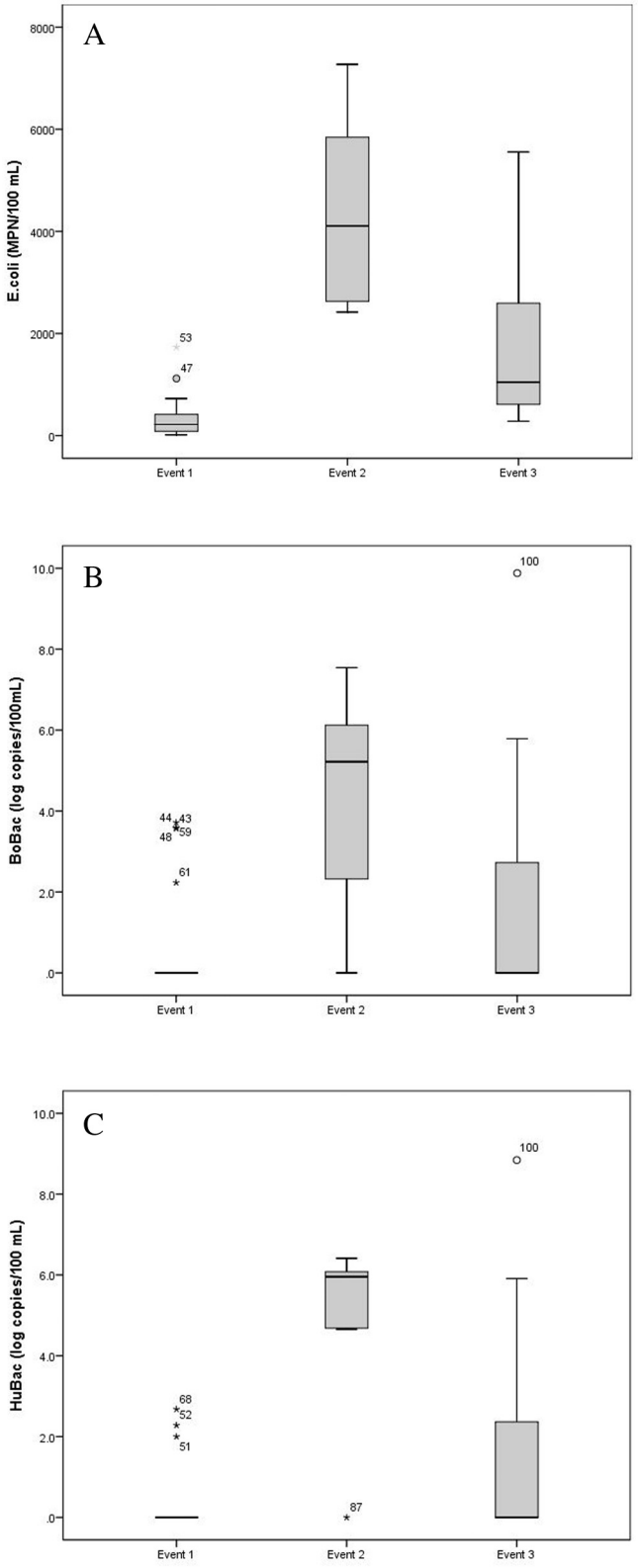


Fig. 3. Box and whisker plots for the three different hydrological events a) *E. coli*, b) bovine-associated *Bacteroides (BoBac)*, c) human-associated *Bacteroides (HuBac)*.

Table 4
Results of non-parametric Wilcoxon rank sum test for *E. coli*, bovine-associated *Bacteroides* (BoBac), and human-associated *Bacteroides* (HuBac).

Microbial contamination indicator	Comparison of average concentration between events	N (number of sample)	p-Value
E. coli	Event 1 and Event 2	43	0.000*
E. coli	Event 2 and Event 3	28	0.002*
BoBac	Event 1 and Event 2	43	0.004*
BoBac	Event 2 and Event 3	28	0.048*
HuBac	Event 1 and Event 2	43	0.000*
HuBac	Event 2 and Event 3	28	0.008*

Significance indicated by * at 0.05 level

The microbial contamination indicators *E. coli*, BoBac, HuBac all showed significant difference in Event 2 when compared to Event 1 and Event 3 ($p \leq 0.05$). First-flush phenomena is believed to have occurred during Event 2 as the mean contaminant concentration in this period was significantly greater than Event 1 and Event 3 (Bach et al., 2010).

The state of Michigan receives an average 700 to 1000 mm precipitation throughout the year, and it has a typical moist continental mid-latitude climate. In 2015, the annual precipitation of the sampling site was 741.3 mm. It snowed from November of 2014 to March of 2015, and 69% of yearly precipitation fell during March to August as rainfall events. This climate can create a long period of pollutant build-up deposited on surfaces during dry, low discharge weather (November–March) which is washed away in the spring when the snow starts to melt into surface waters. The initial storms of the spring season are usually expected to be associated with higher pollutant concentrations, which cause a first-flush phenomena. This is not the case in mid-Michigan's climate because the first-flush runoff may begin in early March when the snow starts to melt, but the soil is still frozen. In this case pollutants will not be flushed until the soil temperature starts to increase during the summer. Indeed, our results show that the first-flush phenomena for *E. coli* and *Bacteroides* occurred in early summer, rather than spring. To the best of our knowledge, this study is the first work to adopt *Bacteroides* genetic markers in the first-flush analysis.

Conclusions

This study examined the influence of hydrological conditions on *E. coli* and *Bacteroides* concentrations in Sloan Creek, located mid-Michigan in the Great Lakes Basin. *E. coli* and bovine-associated *Bacteroides* concentrations were strongly influenced by precipitation and stream discharge. The study identified the timing of first-flush phenomena. High levels of microbial contamination were observed and first-flush phenomena of fecal contaminants occurred during summer rainfall events rather than during snow melt. The study revealed that the majority of pollution loading was contributed to the river over a short period of time in early summer. Developing an effective TMDL and remediation plan should take into account fluctuation of pollutant loadings and the timing of first-flush events.

Acknowledgments

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