Evaluating Correlating Factors and the First Flush Phenomenon for Indicator Bacteria in Stormwater Runoff

Final Report

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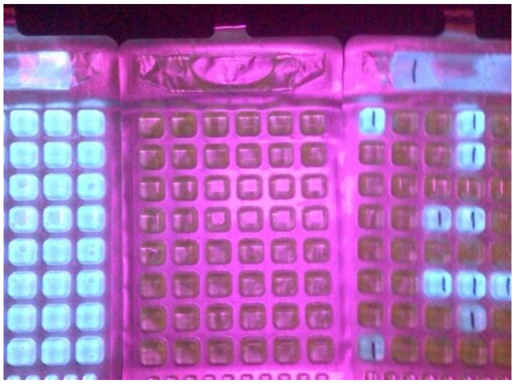

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Purpose

The purpose of this report is to document monitoring and data analysis activities undertaken by North Carolina State University to evaluate: (1) factors affecting indicator bacteria concentrations in stormwater runoff and (2) the existence of a first flush for indicator bacteria in stormwater runoff. All data were collected from an urbanized, residential watershed in Raleigh, North Carolina. This study provides information to the regulatory and scientific communities which will aid in more accurate modeling, Total Maximum Daily Load calculations, and BMP performance evaluations.

Executive Summary

Microbial quality in surface waters is a concern across North Carolina, the United States, and elsewhere due to human reliance on surface waters for food, recreation, and other life sustaining activities. Although pathogens are of utmost concern, indicator bacteria are typically used for regulatory purposes to indicate the presence of fecal matter, and thus the possible existence of pathogens. Total Maximum Daily Loads are established for surface waters impacted by excessive indicator bacteria. Analyses are required to categorize sources of indicator bacteria and a plan is developed to restore water quality in the impacted water by way of various management/control practices. Stormwater runoff has been shown to have high indicator bacteria concentrations, contributing to microbial degradation in surface waters.

Although numerous studies have been performed to establish patterns of indicator bacteria transport and export in estuarine and riverine systems, relatively little research has been performed for urban stormwater (prior to runoff entering surface water). This report provides an analysis of variables which may influence indicator bacteria export from an urban watershed. Event Mean Concentrations (EMCs) of *E. coli* and fecal coliform exhibited significant seasonal variation ($p < 0.05$). Based on multiple linear regression analyses, EMCs were also influenced by antecedent meteorological conditions, with temperature and moisture being important in explaining variability among sampling events. Further analysis provided a traditional first flush assessment of data collected from the urban watershed. Although total suspended solids (TSS) exhibited a first flush in the watershed, no first flush effect was noted for *E. coli* and enterococcus, and the first flush effect for fecal coliform was relatively weak. Seasonal

variations in first flush strength were observed, likely due to differences in pollutant sources between seasons. These studies emphasized the importance of seasonality and antecedent conditions in indicator bacteria transport and export from urban watersheds. Further, the lack of a substantial first flush effect suggests stormwater control measures ("SCMs," also known as Best Management practices or "BMPs") cannot sequester proportionally more indicator bacteria as a result of greater mass delivery during the beginning of storm events. Therefore, SCM's, designed to detain 80% of runoff (e.g., SCM's in piedmont NC that capture runoff from 1.00 inch of precipitation) have the opportunity to treat approximately 80% of indicator species.

Related Documents

This report is a compilation of two manuscripts and a doctoral dissertation which were generated using data collected during the process of this study. Portions of this report may be published in the following documents:

- Hathaway, J.M., W.F. Hunt, and O.D. Simmons III. (accepted). "Statistical evaluation of factors affecting indicator bacteria in urban stormwater runoff" *Journal of Environmental Engineering.*
- Hathaway, J.M., and W.F. Hunt. (in review). "Evaluation of First Flush for Indicator Bacteria and Total Suspended Solids in Urban Stormwater Runoff" *Water, Air, and Soil Pollution.*
- Hathaway, J.M. (in review). "An Evaluation of Indicator Bacteria Transport in Stormwater Runoff and Removal in Stormwater Control Measures." doctoral dissertation, North Carolina State University, Raleigh, NC.

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1 Introduction

Indicator bacteria are commonly used to denote the presence of fecal contamination in surface waters. Although there is some debate as to how well these bacteria relate to the presence of fecal pathogens, they offer a relatively inexpensive and expedient way to test the microbiological quality of surface waters (Arnone and Walling 2007). Various fecal indicator bacteria are currently used in the United States and elsewhere, including fecal coliform, *Escherichia coli* (*E. coli)*, and enterococcus. In 1976, the United States Environmental Protection Agency (USEPA) set guidelines for indicator bacteria concentrations in surface waters based on fecal coliform (USEPA 1976). Further study suggested *E. coli* and enterococcus as better indicators of public health risk, leading to a change in USEPA recommendations in 1986 (USEPA 1986). Despite this change, fecal coliform is still used by many states (USEPA 2003), leading to the existence of large fecal coliform water quality data sets.

Studies have shown indicator bacteria concentrations in urban stormwater and streams under storm flow are commonly above regulatory levels for surface waters (Hathaway et al. 2009, Characklis et al. 2005, Krometis et al. 2009, Line et al. 2008). This represents a potential public health risk from fecal pathogens, as stormwater runoff can be conveyed to surface waters which act as recreational areas. Studies have shown increased health impacts to swimmers near stormwater outfalls in Santa Monica Bay, CA (Haile et al. 1999). Further, shellfish waters are sometimes closed for fishing after storm events due to elevated indicator bacteria concentrations, representing a loss of revenue in coastal areas (NCDENR 2009).

Total Maximum Daily Loads (TMDLs) are established for watersheds with fecal contamination. These TMDLs require basic modeling of targeted watersheds to determine sources and potential treatment opportunities. Until recent studies by McCarthy (2008), large indicator bacteria data sets containing multiple samples per storm event were uncommon for urban stormwater, making trend analysis difficult and limiting modeling efforts. Further, limited study has been performed to determine factors which influence indicator bacteria concentrations in urban environments. Identifying such factors is necessary to understand the mechanisms which drive indicator bacteria build up and transport in urban environments.

Microorganism survival can be affected by numerous environmental factors. These factors include temperature, moisture conditions, pH, predation, exposure to sunlight (UV radiation), and nutrient availability (USEPA 2001, Arnone and Walling 2007, Stevik et al. 2004, Ferguson et al. 2003). Environmental factors must be joined with hydrologic factors such as rainfall intensity and amount to further understand build-up and-wash off relationships.

At the watershed scale, a number of studies have evaluated correlating factors for indicator bacteria export from watersheds impacted by urbanization. However, many of these studies were performed in streams or estuaries (Kelsey et al. 2004, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006, Elder 1987, Eleria and Vogel 2005, Ferguson et al. 1996, Fries et al. 2006, Ortega et al. 2009, Mallin et al. 2000). Thus, while valuable information may be gleaned from these studies, the results are likely influenced by processes specific to streams or estuaries. Processes likely differ for indicator bacteria in urban stormwater runoff which has yet to enter a stream or estuary.

Various studies have correlated indicator bacteria in streams and estuaries to physical parameters such as land use, antecedent rainfall, discharge, rainfall depth, duration of storm event, intensity of storm event, and seasonality (Kelsey et al. 2004, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006, Elder 1987, Eleria and Vogel 2005, Ferguson et al. 1996). Water quality parameters such as salinity, water temperature, turbidity, pH, total suspended solids concentration, and various nutrients have also been related to indicator bacteria concentrations (Fries et al. 2006, Line et al. 2008, Ortega et al. 2009, Kelsey et al. 2004).

Recent studies by McCarthy (2008) and Selvakumar and Borst (2006) have specifically evaluated urban stormwater runoff. Selvakumar and Borst (2006) studied nine stormwater outfalls in Monmouth County, NJ, over fourteen storm events. At least seven storms were monitored for each outfall and tested for total coliforms, fecal coliforms, fecal streptococci, enterococcus, *E. coli*, *Pseudomonas aeruginosa*, and *staphylococcus aureus*. Results of this study showed significant differences in all pathogens and indicator bacteria with season and significant differences with land use for all except *E. coli* ($p < 0.05$). Concentrations in the summer were not significantly different from fall and spring concentrations, and winter was determined to have the lowest concentrations. High density residential watersheds were found to have higher concentrations of bacteria than low density residential or landscaped commercial watersheds.

McCarthy (2008) evaluated *E. coli* concentrations in stormwater runoff from four urban watersheds in Melbourne, Australia. *E. coli* concentrations were correlated to various climate, hydrologic, and water quality variables, with a large number of variables being identified as significantly correlated. Climate variables were averaged for antecedent periods of one day, two days, seven days, fourteen days, and twenty-eight days. Multiple linear regression was used to condense these relationships into a smaller number of explanatory variables. Multiple linear regression analyses were held to two total explanatory variables, one an antecedent climate variable and one either a flow or precipitation variable. Although selected variables changed from site to site, McCarthy (2008) identified a reduced model of average rainfall intensity and vapor pressure as significant for all four watersheds, although each variable was not significant within the reduced model for each watershed. The reduced model had R^2 values between 0.62 and 0.8 for the watersheds. Other reduced models found to be significant for at least one of the watersheds included variables such as maximum rainfall intensity, relative humidity, and air temperature. Other commonly used indicator bacteria species, fecal coliform and enterococcus, were not evaluated. It should also be noted that the climate in Melbourne, Australia, varies from that of the Southeast United States, with less precipitation and smaller variations in temperature during the year.

Many pollutants in stormwater runoff are commonly thought to exhibit a “first flush” transport pattern. Essentially, that a larger proportion of pollutant mass or higher pollutant concentrations are expected during the initial stages of a storm event (Sansalone and Cristina 2004). First flush patterns have been evaluated in urban stormwater runoff for multiple pollutants including sediments, oil and grease, metals, nutrients, chemical oxygen demand, pH, temperature, and conductivity (Barrett et al. 1998, Bertrand-Krajewski et al. 1998, Characklis and Wiesner 1997, Deletic 1998, Flint and Davis 2007, Lee et al. 2002, Sansalone and Buchberger 1997, Sansalone and Cristina 2004, Stenstrom et al. 1984). However, first flush patterns have not been consistently noted in urban watersheds and may depend on such factors as storm size, rainfall intensity, watershed characteristics, and various hydrologic and transport factors (Deletic 1998, Sansalone and Cristina 2004).

Various methodologies have been employed to evaluate the first flush effect. In a review by Sansalone and Cristina (2004), first flush analyses were placed into three categories based on the approach taken by the researchers: mass based, concentration based, and empirically based. For detailed first flush analyses, mass based procedures have been commonly used. Within the mass based procedure, there are multiple methodologies which have been used to evaluate the first flush; however, Sansalone and Cristina (2004) showed similar conclusions would be made when each of the methods was applied to a common experimental data set.

Despite numerous studies characterizing the first flush for pollutants in urban stormwater, relatively few detailed studies have been performed for indicator bacteria, particularly *Escherichia coli* (*E. coli*) and enterococcus. *E. coli* and enterococcus are commonly used to regulate microbial water quality in the United States, Europe, Australia, New Zealand, and elsewhere. A report by the California Department of Transportation (2000) determined a first flush was not visible for fecal coliform in highway runoff during eight storms at two locations. However, the first flush was evaluated based primarily on qualitative analysis of pollutographs. Similar conclusions resulted from an analysis of fecal coliform and fecal streptococcus data taken from the National Stormwater Quality Database (Maestre and Pitt 2004). Non-parametric statistical comparisons were made between samples taken within the first 30 minutes of a storm event and composite samples for the same event. No statistical differences in concentrations were found. Other studies by Krometis et al. (2007) showed decreased concentrations of *E. coli* and fecal coliforms in the latter portions of storm flow in streams, indicating a potential first flush effect. Conversely, enterococci was found to remain relatively consistent throughout the storm. Krometis et al. (2007) concluded that the greatest percentage of settlable microbes was exported in the first 50% of runoff volume. However, indicator bacteria transport processes in streams may differ from those in urban stormwater systems.

Recent studies by McCarthy (2009) provided detailed analysis of the first flush for *E. coli* in four urban watersheds in Melbourne, Australia. McCarthy (2009) showed a consistent first flush was not present for any of the four watersheds; however, a first flush effect was statistically found in the medium density residential watershed. Further, McCarthy (2009) tested associations between the first flush strength and antecedent climate parameters, storm characteristics, and flow characteristics. No variable was identified which could consistently explain variations in the first flush strength for all sites. It should be noted that the weather patterns in Melbourne, Australia, differ from those in the Southeastern United States, potentially leading to differences in microbial behavior. Differences in weather include higher average yearly rainfall, higher average summer temperatures, and lower average winter temperatures in Raleigh, NC (ABOM 2009, SCONC 2009).

Such evaluations are important, as Best Management Practices (BMPs) are designed to treat the runoff associated with a pre-determined water quality rainfall depth. To facilitate efficient use of land and monetary resources, this depth is often selected under the perception that a first flush exists. Subsequently, capture and treatment of the initial portion of the storm is believed to result in maximum pollutant capture relative to runoff volume capture. Determining if a first flush exists for indicator bacteria is important in determining the efficiency of stormwater BMPs for treatment of microbes. Stormwater BMPs are also known as Sustainable Urban Drainage Systems (SUDS) and Water Sensitive Urban Designs (WSUDs).

The purpose of this study was to add to the limited understanding of indicator bacteria export from urbanized watersheds. Since indicator bacteria relationships may vary based on watershed characteristics and location, relationships proposed by previous researchers were explored, including seasonal variation and correlations between indicator bacteria types. Further, statistical analyses were used to explore relationships between all three commonly used indicator bacteria species, antecedent climate variables, and in-storm hydrologic variables. These analyses helped determine if responses to explanatory variables varied based on indicator bacteria type. Such differences may be important as the USEPA has suggested the use of enterococcus as an indicator for marine waters and either *E. coli* or enterococcus as an indicator for fresh waters (USEPA 1986). Understanding such relationships is important in developing TMDLs for impacted watersheds and in determining factors that must be considered when evaluating a stormwater Best Management Practice’s (BMP) potential for indicator bacteria removal.

Further, understanding indicator bacteria transport patterns may result in better decisions regarding public health. The presence of a first flush would suggest high concentrations of indicator bacteria may reach recreational waters quickly after a storm begins. Further, variations in indicator bacteria concentrations during storm events have important ramifications for monitoring, particularly with the common use of grab samples for indicator bacteria.

The objectives of this study were to build upon the current understanding of microbial processes by: (1) performing a statistical analysis of factors influencing indicator bacteria concentrations in urban stormwater runoff, (2) determining the influence of seasonality on indicator bacteria concentrations in urban runoff, (3) evaluating the presence of a first flush in urban stormwater for multiple indicator bacteria, all of which are used in some capacity in the United States and elsewhere, (4) examining correlating factors between first flush strength and antecedent climate conditions to see if relationships exist for the humid, warm, Southeastern United States, (5) comparing results for indicator bacteria to those of Total Suspended Solids (TSS), which has been shown to exhibit a first flush effect and includes particles on which microbes are known to attach.

2 Site Description and General Methods

2.1 Site Description

The experimental watershed was located in Raleigh, NC, in a medium density residential neighborhood with approximately 35% imperviousness (Figure 2.1). An estimated 15% of the watershed was connected impervious area, primarily roadways. As is common in many residential neighborhoods in North Carolina, rooftops were typically not tied directly into the stormwater system. There were no stormwater BMPs installed in the watershed as it was developed prior to implementation of USEPA stormwater regulations. The watershed was approximately 5.1 ha (12.5 acres) with a mature tree canopy and geodetic coordinates (35.80^{o}N, 78.67^{o}W). Residents were commonly seen walking dogs during site visits. The stormwater and wastewater sewer systems were separate in the watershed, and sewer cross-connection was not expected as the stormwater outfall for the watershed was noted to be completely dry on multiple occasions during the late summer/early fall. During the rest of the year, base flow was noted, indicating possible groundwater intrusion into the stormwater system. The stormwater outfall was a 76-cm (30-inch) reinforced concrete pipe which fed a tributary to Beaverdam Branch.

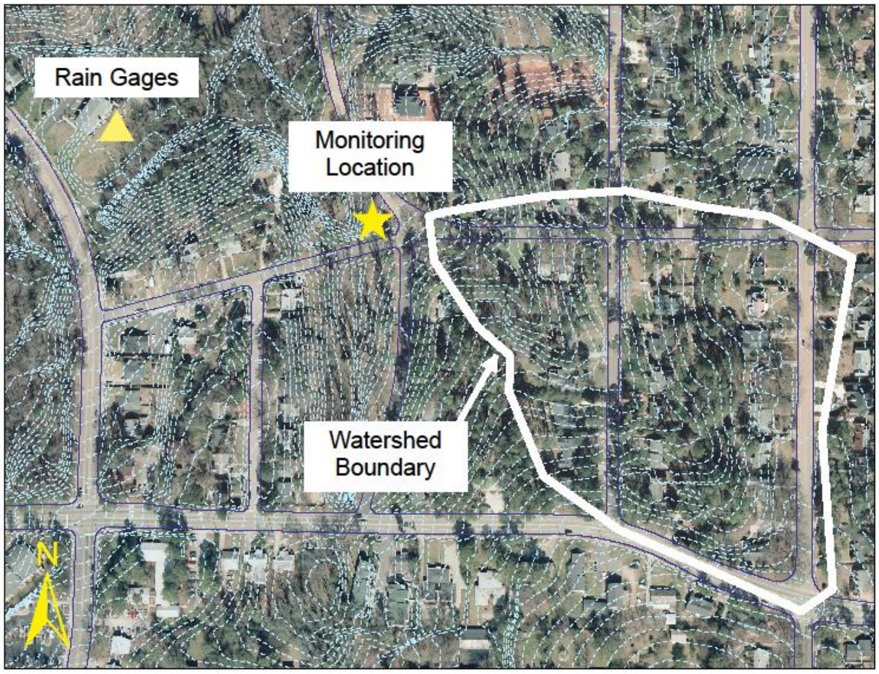


Figure 2.1: Arial Image of Experimental Watershed

2.2 General Monitoring Methods

A compound weir was installed at the end of the culvert. Sufficient vertical distance was present between the weir invert and the receiving channel to avoid submerged conditions at the weir. An ISCO 730 bubbler module was used to record depth in the pipe. The depth was converted to flow using a stage-discharge relationship (Equation 1) developed for triangular-rectangular compound weirs by Jan et al. (2006).

$$Q = \frac{8}{15} C_{td} \sqrt{2g} \tan\left(\frac{\theta}{2}\right) \; h_{2e}^{5/2} - h_{1e}^{5/2} \; + \frac{2}{3} C_{rd} \sqrt{2g} \; 2b_1 \; h_1^{3/2} \qquad (1)$$

Where: Q = flow in (m^3/s)
C_{td} = discharge coefficient for triangular, sharp-crested weir (0.58)
g = 9.81 m/s^2
θ = triangular weir angle (90^o)
h_{2e} = effective head above triangular weir invert (m)
h_{1e} = effective head above rectangular weir invert (m)
C_{rd} = discharge coefficient for rectangular weir (from Bos 1989)
b_1 = rectangular weir length on one side of triangular weir (m)
h_1 = head above rectangular weir (m)

The bubbler module was used in conjunction with an ISCO Avalanche refrigerated sampler which was equipped with a tray of 14 polypropylene bottles. Sampler intake tubing and bubbler tubing was fixed to the invert of the stormwater pipe. Evaluations by McCarthy et al. (2008b) indicated this collection point was not significantly different for indicator bacteria evaluations than those where samples were drawn from the top of the water column.

Prior to each anticipated storm, all bottles, pump tubing, and sampler tubing were washed, rinsed with deionized water, and autoclaved at 121^{o}C for 20 minutes to maintain sterility. Discrete, flow paced samples were collected and distributed sequentially into the 14 bottles during storm events. Storm events were defined as any rainfall event which produced runoff in excess of base flow during which 5 samples could be collected. All events exceeded 0.4 cm. Flow pacing was manipulated prior to and during the storm to achieve an adequate characterization of the storm. If adjustments were required, flow pacing was increased as the storm progressed to allow the greatest resolution during the initial portion of the storm when flow rates and concentrations were expected to have the highest variability. Although base flow was not present in the stormwater outfall during the entire study, base flow samples were collected from the stormwater outfall on 5 occasions. Care was taken not to disturb any sediment in the bottom of the pipe during these base flow sample events. Base flow samples were used to gain additional information about the system, but were not used in any analyses. Stormwater samples were collected from the monitoring location and transported to the Department of Biological and Agricultural Engineering at North Carolina State University where they were refrigerated until analyzed.

A tipping bucket rain gage was installed approximately 190 m (630 ft) from the watershed outfall and 560 m (1850 ft) from the outer boundary of the watershed (Figure 2.1). A HOBO data logger stored data from the tipping bucket rain gage and a manual rain gage was placed on site to verify precipitation depths. Data were used to generate depth and intensity values for rainfall events. Additional climate data were obtained from a weather station at the Lake Wheeler Road Field Laboratory located approximately 8.3 km (5.2 mi) from the experimental watershed. The weather station is operated by the North Carolina Agricultural Research Service. Climate data preceding the storm were averaged at various time intervals to establish antecedent conditions. Data were averaged for the 1, 2, 7, 14, and 28 days prior to a given storm event, similar to the methodology employed by McCarthy (2008). Climate data which were used for correlation analysis were air temperature, relative humidity, vapor pressure, solar radiation, potential evapotranspiration (PET), and precipitation total. Vapor pressure was not collected at the Lake Wheeler Road Field Laboratory. Thus, it was calculated using standard equations (NOAA 2009). Historical average temperature and precipitation data for Raleigh, NC, are presented in Table 2.1.

Table 2.1: Historical Climate data for Raleigh, NC, from 1973-2000 (SCONC 2009)

Parameter	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Average Daily Maximum Temperature (^{o}C)	9.33	11.7	16.2	21.4	25.3	29.1	31.1	29.9	26.7	21	16.3	11.2
Average Daily Minimum Temperature (^{o}C)	-1.1	0.17	4.33	8.78	13.7	18.3	20.8	20.1	16.6	9.67	5.33	0.83
Average Total Rainfall (cm)	11.3	8.97	11.3	7.57	10.2	10.3	11	10.9	10.8	9.6	7.77	8.15

2.3 Sample Analysis

Bacteria analyses were performed within 24 hours of sample collection. Fecal coliform and *E. coli* were enumerated using Colilert (defined substrate technologies; IDEXX, Westbrook, Maine). The Colilert method was modified to detect fecal coliform and *E. coli* by incubating the samples at 37^{o}C for 1 to 3 hours followed by incubation at 44.5^{o}C for 21 to 23 hours (Yakub et al. 2002). Enterococcus was enumerated using Enterolert (defined substrate technologies; IDEXX, Westbrook, Maine). The Enterolert method was performed per manufacturer guidelines by incubating at 41^{o}C for 24 hours. Positive (stock culture) and negative (dilution blank) controls were used during laboratory analyses, although enterococcus standards were not available until the latter two thirds of the study. Samples typically required either a 100:1 or 1000:1 dilution due to high bacteria counts. Base flow samples were only tested for *E. coli* and fecal coliform to examine the potential presence of sewer cross connections.

Further sample analysis was performed at the North Carolina Center for Applied Aquatic Ecology (NCCAAE). An aliquot was taken from each discrete sample, composited in an acidified bottle, sent to NCCAAE, and tested for TKN using the EPA 351.2 method (USEPA 1983). The remainder of each discrete sample was tested for total suspended solids (TSS) analysis using SM 2540D (APHA 1998).

3 Statistical Evaluation of Factors Affecting Indicator Bacteria in Urban Stormwater Runoff

3.1 Abstract

An urban watershed in Raleigh, North Carolina, was monitored for indicator bacteria during 20 rain events. Results showed elevated levels of *E. coli*, enterococcus, and fecal coliform. Samples were compared based on seasonality and were found to be statistically different ($p < 0.05$), with pairwise comparisons indicating significantly lower concentrations of *E. coli* and fecal coliform during the winter ($p < 0.05$). Enterococcus concentrations were substantially lower in the winter and fall, but no significant differences were found between seasons during pairwise comparisons ($p < 0.05$). Correlation analyses showed multiple significant relationships between antecedent climate parameters, flow characteristics, and indicator bacteria concentrations. More detailed multiple linear regression yielded explanatory variables related to antecedent climate conditions. Variables were generally related to temperature and moisture conditions in the atmosphere and soil. The results of this study show indicator bacteria concentrations significantly vary based on season; however, this variability can partially be explained by antecedent climate data.

3.2 Materials and Methods Specific to Section 3

3.2.1 *Data Analysis*

To estimate loading for a given storm, discrete sample concentrations for indicator bacteria and TSS were multiplied by the volume corresponding to the sample (equation 2). Discrete samples which exceeded the maximum detectable concentration for the analysis were not included in loads analysis.

$$Load = \sum_{i=1}^{n} c_i V_i \qquad (2)$$

Where:
c_i = concentration at time i
V_i = volume of runoff during time i

Loads were then divided by the total volume of stormwater produced by a given storm to generate an Event Mean Concentration (EMC) for each storm (equation 3 – USEPA 2002).

$$EMC = \frac{\sum_{i=1}^{n} c_i V_i}{\sum_{i=1}^{n} V_i} \qquad (3)$$

3.2.2 Statistical Analysis

All statistical analyses were performed using SAS 9.1 (SAS 2001). Indicator bacteria were used as dependent variables; thus, they were checked for normality using histograms and a Kolmogorov-Smirnov test (Hollander and Wolfe, 1999). Indicator bacteria EMCs were found to be log-normally distributed and were used in this format throughout the statistical analyses. All statistical analyses were performed at an alpha = 0.05 significance level unless otherwise noted.

Seasonal variations in indicator bacteria concentration were first explored with a distribution free Kruskal-Wallis test to determine if there were any significant differences among all seasons (Hollander and Wolfe, 1999). Pairwise analyses were then performed between seasons using a distribution free Wilcoxon Rank Sum test (Hollander and Wolfe, 1999). This additional analysis allowed comparisons between all combinations of seasonal indicator bacteria concentrations.

A distribution free Spearman rank correlation test was used to explore correlations between indicator bacteria EMCs and climate, flow, and precipitation variables (Hollander and Wolfe, 1999). Using the PROC CORR procedure in SAS 9.1, both a Spearman's rank correlation coefficient (ρ) and a p-value were generated. Thus, any correlation could be verified for statistical significance.

To determine which flow, rainfall, or antecedent climate variables best explained the variability of indicator bacteria concentrations in urban watersheds, multiple linear regression analyses were used. Multiple linear regression analyses utilized the STEPWISE selection procedure in the

PROC REG function of SAS 9.1. This procedure generally involves adding variables to the model piecemeal in order of largest F statistic. Due to the large number of predictor variables being considered in the analysis, only the first three variables selected by the procedure were used as explanatory variables as to not overparameterize the model. After the selection procedure, the three selected variables were placed in a model and evaluated using the PROC REG function. Variance Inflation Factors (VIF) were generated to ensure multicollinearity was not a problem among the predictor variables (Ott and Longnecker, 2001). Further, residuals were plotted and checked for normality to assure that the assumptions of the model were not violated.

3.3 Results and Discussion

3.3.1 Summary Statistics

Between October, 2008, and September, 2009, twenty storm events were monitored. Storms ranged in size from 0.41 to 5.6 cm (0.16 to 2.2 inches). Five events were monitored for each of the four seasons during the period. At least 5 discrete samples were collected during each event. On average, ten discrete samples were collected per event (Figure 2.2). TSS was evaluated for each discrete sample for thirteen of the sample events. TSS was not evaluated until later in the study, so the fall season was not represented by these samples. TKN concentrations were measured for 16 rain events. Again, the fall season was not well represented for this parameter.

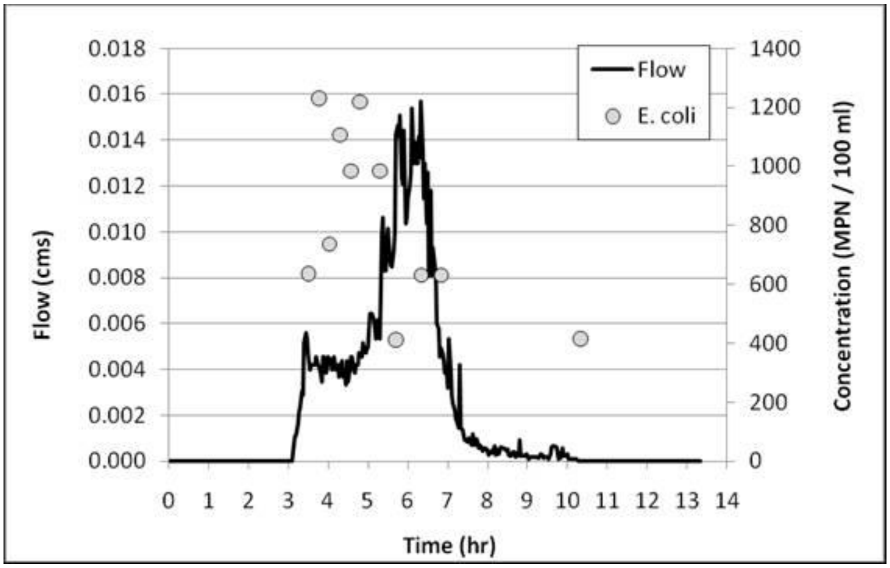


Figure 3.1: Example of sample collection spacing during rain event on 2/18/2009

Concentrations for each storm and summary statistics are provided in Table 2.2. EMCs for *E. coli* ranged from 700 to 84,700 MPN / 100 ml (MPN = Most Probable Number); EMCs for fecal coliform ranged from 1500 to 342,400 MPN / 100 ml; and EMCs for enterococcus ranged from 1300 to 181,800 MPN / 100 ml. Although the maximum EMC for each indicator bacteria was high, McCarthy (2008) showed similar maximum *E. coli* concentrations for two of four watersheds in Melbourne, Australia.

Base flow samples had substantially lower concentrations of indicator bacteria than those collected during rain events. Fecal coliform concentrations ranged from 204 to 2890 MPN / 100 ml with a median concentration of 730 MPN / 100 ml. *E. coli* concentrations ranged from < 10 to 626 MPN / 100 ml with a median concentration of 136 MPN / 100 ml. These numbers compare well to studies of indicator bacteria concentrations in drainage entering stormwater BMPs during dry conditions (Krometis et al. 2009) and to average concentrations of indicator bacteria in stream base flow (Characklis et al. 2005). Relatively low base flow concentrations compared to those during storm events, combined with completely dry conditions in the stormwater outfall during some periods of the study, suggests a lack of sewer cross-connections in the stormwater system. However, base flow is likely due to groundwater intrusion into the pipe system, which could potentially be influenced by groundwater contaminated with fecal indicator bacteria from leaking sewer lines.

Table 3.1: Indicator bacteria, TSS, and TKN concentrations for each storm

Date	Season	Rain (cm)	Number of Samples Collected	*E. coli* EMC (MPN / 100 ml)	Fecal Coliform EMC (MPN / 100 ml)	Enterococcus EMC (MPN / 100 ml)	TSS EMC (mg/ L)	TKN (mg/L)
10/17/2008	Fall	2.03	14	32,483	134,175	2,682	.	.
11/4/2008	Fall	2.69	16	16,539	46,186	3,225	.	.
11/14/2008	Fall	2.82	13	12,491	72,199	13,504	.	.
11/25/2008	Fall	0.95	5	3,475	14,623	5,111	.	.
12/20/2008	Fall	2.97	9	10,943	25,695	11,179	.	2.38
1/6/2009	Winter	2.55	7	4,653	7,564	14,373	.	0.82
1/28/2009	Winter	1.24	11	8,913	14,115	6,568	.	5.10
2/11/2009	Winter	0.41	8	13,480	18,009	2,728	309	6.94
2/18/2009	Winter	1.70	11	710	1,469	1,306	87	1.84
3/13/2009	Winter	2.11	10	8,806	13,145	7,687	106	3.71
3/26/2009	Spring	1.80	8	12,868	17,024	4,261	309	4.22
4/2/2009	Spring	0.94	5	46,157	98,350	50,503	196	2.66
5/8/2009	Spring	1.85	10[a]	84,688	165,032	44,229	160	2.12
5/14/2009	Spring	0.56	7[b]	43,965	96,248	181,846	125	2.66
6/4/2009	Spring	3.94	12	59,302	113,567	30,371	122	2.71
7/17/2009	Summer	1.40	10	26,882	185,230	29,181	181	2.99
7/25/2009	Summer	0.41	6	4,487	63,327	3,175	44	2.28
8/5/2009	Summer	1.65	12	74,658	342,405	53,633	97	1.77
8/28/2009	Summer	1.50	12	29,081	118,925	20,962	49	2.44
9/7/2009	Summer	5.59	18	18,280	55,558	16,566	33	1.21
Arithmetic Mean =		1.96	10.2	25,643	80,142	25,155	140	2.87
Median =		1.75	10.0	15,010	59,442	12,342	122	2.55
Standard Deviation =		1.25	3.5	24,323	82,931	40,380	90	1.52

a. Note: only 9 enterococcus samples
b. Note: only 5 enterococcus samples

3.3.2 *Seasonal Variation*

Seasonal variations in indicator bacteria are shown in Figure 2.3. All indicator bacteria had lower concentrations during the winter; however, this was less pronounced for enterococcus. The highest average *E. coli* and enterococcus concentrations were observed during the spring, while the highest fecal coliform concentrations were observed during the summer.

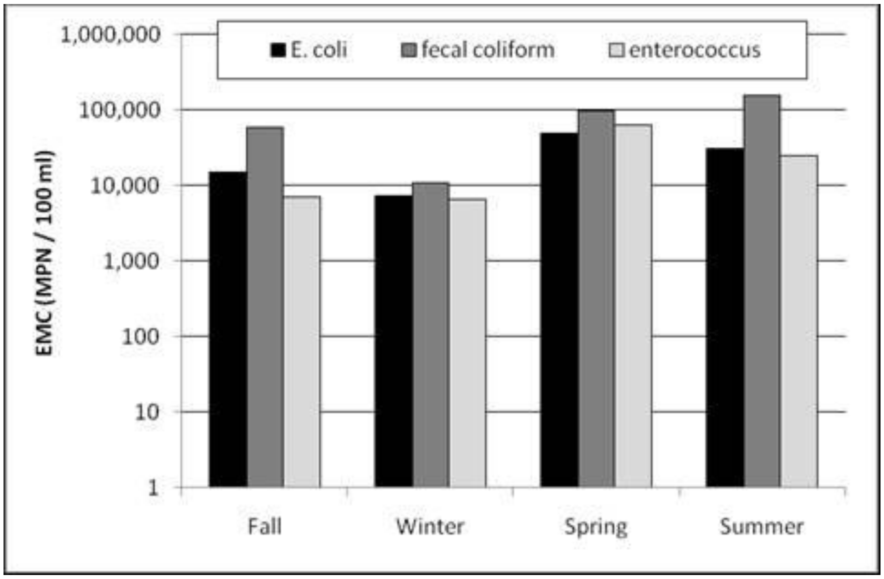


Figure 3.2: Average seasonal EMC for each indicator bacteria

The results of the Kruskal-Wallis test indicated a seasonal difference in EMC for *E. coli*, fecal coliform, and enterococcus. The Wilcoxon Rank Sum tests allowed detailed investigation of pairwise differences between all seasons for each indicator bacteria. For *E. coli*, significant differences were found between winter - spring and fall - spring. For fecal coliform, fall - winter, winter - spring, and winter - summer were all significantly different. Although the Kruskal-Wallis test indicated seasonal differences for enterococcus, none of the pairwise comparisons was statistically significant.

Winter concentrations were consistently associated with significant differences among indicator bacteria. These data are similar to observations made by Selvakumar and Borst (2006), Line et al. (2008), Young and Thackston (1999), and Schoonover and Lockaby (2006), who noted lower concentrations of indicator bacteria in surface waters during the winter. The results of the seasonal analysis are presented in Table 2.3.

Table 3.2: Analysis of seasonal differences in indicator bacteria concentrations

Indicator Bacteria	Kruskal-Wallis (p-values)	Wilcoxon Rank Sum (p-values)					
		Seasonal Pair Evaluated					
		fall - winter	fall - spring	fall - summer	winter - spring	winter - summer	spring - summer
E. coli	***0.026***	0.1745	***0.0283***	0.2506	***0.0163***	0.0758	0.2506
fecal coliform	***0.0097***	***0.0163***	0.2506	0.1172	***0.0163***	***0.0090***	0.4647
enterococcus	***0.0412***	1.0000	0.0556	0.0952	0.0556	0.0556	0.3095

Note: Significant relationships are bold and italicized

3.3.3 Correlation Analysis

Correlations among indicator bacteria are shown in Table 2.4. Fecal coliform and *E. coli* were enumerated concurrently using the Colilert method and thus were not analyzed for correlation due to concerns over independence of the data. Correlations between enterococcus and the other indicator species were statistically significant. Enterococcus correlations to *E. coli* and fecal coliform had Spearman coefficients of 0.68 and 0.58, respectively.

Studies by Ortega et al. (2009) and Fries et al. (2006) showed poor correlations between various indicators in estuaries. However, a study of indicator bacteria in urban stormwater runoff by Selvakumar and Borst (2006) showed high correlations between fecal coliform and *E. coli* (Pearson correlation coefficient of 0.771), moderate correlations between *E. coli* and enterococcus (Pearson correlation coefficient of 0.425), and moderate correlations between fecal coliform and enterococcus (Pearson correlation coefficient of 0.534). Although these relationships are expected to be variable between watersheds, it may be possible to monitor both *E. coli* and fecal coliform for a period of time to correlate these two indicators for a given watershed. This would aid in using fecal coliform datasets when developing TMDLs based on *E. coli* (provided bacteria sources have not significantly changed since the data were collected). Such analysis was performed in a case study for Lower Geddes Pond in Michigan as presented by USEPA (2001).The same correlations may not be possible; however, when fecal coliform data are desired for use in enterococcus based TMDLs due to relatively low correlations noted in this study and in Selvakumar and Borst (2006).

Table 3.3: Correlations between indicator bacteria

Indicator Bacteria	enterococcus	
	P-value	p-value
E. coli	0.68	0.001
fecal coliform	0.58	0.008

TSS and TKN were also analyzed for correlations with indicator bacteria. However, very poor correlations were found, none of which were statistically significant. Spearman coefficients for correlation between TSS and *E. coli*, fecal coliform, and enterococcus were 0.203, 0.022, and 0.132, respectively. Spearman coefficients for correlation between TKN and *E. coli*, fecal coliform, and enterococcus were 0, -0.097, and -0.229, respectively.

Results for studies which correlated indicator bacteria and TSS have been variable. McCarthy et al. (2007) showed significant correlations for TSS and *E. coli* for two of four watersheds studied in Melbourne, Australia ($p < 0.05$). However, studies of surface waters by Fries et al. (2006) and Line et al. (2008) showed poor correlation between indicator bacteria and TSS concentrations. Although it is understood that indicator bacteria attach to and are transported with particles (Characklis et al. 2005, Krometis et al. 2007, Fries et al. 2006), TSS concentrations should not be used to infer microbiological quality in surface waters.

Although TKN would intuitively be linked to indicator bacteria, due to the potential of similar sources for the two parameters, studies have shown varied correlations between nitrogen species and indicator bacteria. A study by Line et al. (2008) showed no correlation between fecal coliform concentrations and NO_3-N or NH_3-N in three watersheds in North Carolina. Conversely, McCarthy (2008) showed positive correlations between NH_3-N and *E. coli* for 3 out or 4 watersheds monitored in Melbourne, Australia. Due to varied conclusions regarding correlations between nitrogen species and indicator bacteria, there does not seem to be a clear interaction. Numerous significant correlations were found between each indicator bacteria and various hydrologic and antecedent climate variables (Table 2.5). Despite the inclusion of multiple flow and precipitation metrics, only peak flow was found to be significantly correlated to indicator bacteria EMCs. However, peak flow is directly related to rainfall intensity, thus signifying the importance of rainfall and flow characteristics in bacteria transport in urban watersheds.

Antecedent rainfall conditions likely impact both the amount of indicator bacteria build-up and the amount of moisture present in a watershed, but this was only statistically true for enterococcus in this correlation analysis. It should be noted that enterococcus consistently showed poorer correlation to explanatory variables.

For any given variable, it was common to have multiple antecedent periods of time correlated to bacteria concentrations. For example, air temperature was significantly correlated to all three indicator bacteria when averaged for 1, 2, 7, 14, and 28 days before the storm event. This is likely due to a given climate variable not changing substantially over a 28 day period.

Temperature and vapor pressure consistently provided significant correlations with all indicator bacteria, similar to relationships found by McCarthy (2008). Various studies have shown indicator bacteria concentrations in surface waters are higher during warmer parts of the year (Selvakumar and Borst 2006, McCarthy 2008, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006). The strong positive correlation between temperature and indicator bacteria concentration is somewhat unexpected as many studies have shown that die off rates for indicator bacteria are higher as temperature increases (Kibbey et al. 1978, Van Donsel et al. 1967, Ferguson et al. 2003, Crane and Moore 1986). However, interactive effects between such factors as temperature and moisture may result in more complicated relationships (Wang et al. 2004, Kibbey et al. 1978).

This paradox has been examined by McCarthy et al (2008), Crane and Moore (1986), and Tiefenthaler et al. (2009). Based on the conclusions of these and other studies, the possible explanations for the increase in indicator bacteria concentration with increased temperatures include: (1) Increased sources of indicator bacteria due to domestic and wild animal activity and (2) increased persistence due to seasonal variations in environmental conditions such as temperature, humidity, and rainfall patterns. Essentially, indicator bacteria die-off is likely based on a combination of factors which vary from season to season (Crane and Moore, 1986). Temperature probably acts as a surrogate for such seasonal variations and interactions in this analysis.

Table 3.4: Results of correlation analysis (only significant relationships shown)

Variable	*E. coli*		fecal coliform		enterococcus	
	ρ	p-value	ρ	p-value	ρ	p-value
peak flow	0.4842	0.0305	0.5699	0.0087	0.5444	0.0131
antecedent dry period	-	-	-	-	-0.4737	0.0349
Rain Total 7 days	-	-	-	-	0.6102	0.0043
Rain Total 14 days	-	-	-	-	0.5790	0.0075
Rain Total 28 days	-	-	-	-	0.5323	0.0157
Air Temperature 1 day	0.6511	0.0019	0.8316	<.0001	0.5053	0.0231
Air Temperature 2 days	0.6767	0.0011	0.8346	<.0001	0.5158	0.0199
Air Temperature 7 days	0.6346	0.0027	0.8241	<.0001	0.5173	0.0195
Air Temperature 14 days	0.6000	0.0052	0.8226	<.0001	0.5368	0.0147
Air Temperature 28 days	0.5624	0.0098	0.8000	<.0001	0.5098	0.0217
Vapor Pressure 1 day	0.6571	0.0016	0.8812	<.0001	0.5774	0.0077
Vapor Pressure 2 days	0.6421	0.0023	0.8346	<.0001	0.5489	0.0122
Vapor Pressure 7 days	0.6541	0.0018	0.8481	<.0001	0.5805	0.0073
Vapor Pressure 14 days	0.5624	0.0098	0.8030	<.0001	0.5639	0.0096
Vapor Pressure 28 days	0.5609	0.0101	0.7774	<.0001	0.5143	0.0203
PET 1 day	0.5323	0.0157	0.6105	0.0042	-	-
PET 2 days	0.4993	0.0250	0.6165	0.0038	-	-
PET 7 days	0.4556	0.0435	0.4947	0.0266	-	-
PET 14 days	0.6311	0.0028	0.7183	0.0004	0.5386	0.0143
PET 28 days	0.6030	0.0049	0.7729	<.0001	0.5203	0.0187
Relative Humidity 7 days	0.6135	0.0040	0.7744	<.0001	0.5203	0.0187
Relative Humidity 14 days	0.5218	0.0183	0.6977	0.0006	0.4602	0.0412
Relative Humidity 28 days	-	-	0.4626	0.0400	-	-
Solar Radiation 2 days	-	-	0.5038	0.0235	-	-
Solar Radiation 14 days	0.6000	0.0052	0.6301	0.0029	0.4872	0.0293
Solar Radiation 28 days	0.5895	0.0062	0.6797	0.0010	0.5068	0.0226

Other contradictory relationships were observed in the correlation analysis. PET consistently had a positive correlation to indicator bacteria concentrations. This was unexpected, as greater desiccation was expected as evaporation within the watershed increased. However, correlation analysis between air temperature and PET showed a strong relationship, indicating that as air temperature increases, so too does PET. Thus, the true affect of PET may not be illustrated in the data, as it is overwhelmed by that of other factors. Air temperature is also related to vapor pressure, relative humidity, and solar radiation. All would be expected to increase during the warmer months in the Southeastern United States.

Multiple linear Regression

The multiple linear regression analysis condensed the results of the correlation analysis into a smaller number of explanatory variables that best described the indicator bacteria concentrations. Final reduced models showed VIFs for all variables were less than 10, indicating little autocorrelation among the selected variables (Ott and Longnecker 2001). This was of particular importance given the relationship between temperature and many other climate variables. The reduced models for each indicator bacteria are presented in Table 2.6.

Table 3.5: Results of multiple linear regression analysis

Indicator Bacteria	**Variable 1**			**Variable 2**			**Variable 3**			**Overall (R^2)**
	name	VIF	p	name	VIF	p	name	VIF	p	
E. coli	2 day average air temperature	1.01	<.0001	28 day total rain	1.01	0.0059	2 day total rain	1.02	0.0078	0.7462
fecal coliform	2 day average air temperature	2.00	0.0058	14 day average relative humidity	1.95	0.0335	7 day total rain	1.09	0.0555	0.802
enterococcus	7 day total rain	1.06	0.0094	14 day average relative humidity	1.06	0.0275	-	-	-	0.526

The reduced model for *E. coli* included the average air temperature and total rain amount for 2 days preceding the rain event, and the total rain amount for the 28 days preceding the event. The coefficient of determination (R^2) for this model was 0.75, with each variable being significant in the model. The parameter estimate for total rain for 2 days preceding an event was negative, indicating that if rain is occurring frequently in the days preceding a given storm, the indicator bacteria source in the watershed is reduced by wash-off dynamics. Conversely, the total rain amount for 28 days preceding the event is likely an indication of how wet the watershed is, but not necessarily whether indicator species have been subject to wash-off. Moist soils would be expected to facilitate slower die-off of indicator bacteria (Kibbey et al. 1978).

The reduced model for fecal coliform included the antecedent 2 day average air temperature, the antecedent 7 day total rainfall, and antecedent 14 day relative humidity. The coefficient of determination (R^2) for this model was 0.80, and only the antecedent 7 day total rainfall had a p-value above 0.05 ($p = 0.055$). Parameter estimates for each variable were positive, indicating that each variable leads to an increase in fecal coliform concentrations. Thus, these variables all contribute to the build-up/persistence of fecal coliform in the watershed. Specifically, antecedent 7 day total rainfall and 14 day average relative humidity seem to relate to the amount of moisture in the watershed and atmosphere leading up to the event. Atmospheric moisture was also considered important in evaluations of *E. coli* export from urban watersheds in Melbourne, Australia (McCarthy 2008).

The reduced model for enterococcus included the total 7-day antecedent rainfall total and the antecedent 14-day average relative humidity. Both of these variables were also selected for the fecal coliform reduced model; however, no other variables were selected by the model based on the default SAS significance threshold of $p < 0.15$. The coefficient of determination (R^2) for this model was 0.53. As with fecal coliform, all variables in the reduced model seem related to build-up/persistence of the enterococcus in the watershed, and atmospheric and/or soil moisture seem to be important factors. Statistical modeling of enterococcus yielded lower R^2 values than *E. coli* or fecal coliform. Generally, enterococcus is considered to have a slower die off rate than *E. coli* and fecal coliform in the environment (USEPA 2001). Thus, slight climate variations may have less of an impact on enterococcus or may be harder to detect.

From these data, it seems antecedent conditions do have an impact on indicator bacteria and may help explain the variability seen in concentrations of indicator bacteria in urban watersheds. Similar conclusions were made by McCarthy (2008). However, selected variables indicate high complexity for indicator bacteria in urban watersheds. Simple linear regression of one antecedent climate variable is not sufficient for indicator bacteria modeling (McCarthy et al. 2007). In general, variables selected for the models were related to antecedent climate instead of storm-specific hydrologic or rainfall characteristics. Commonly included were variables that could be related to differences in atmospheric and soil moisture, such as total rain preceding the event and relative humidity. Antecedent rainfall totals were also found to influence indicator bacteria concentrations in Murrells Inlet in South Carolina by Kelsey et al. (2004). Temperature was also important for *E. coli* and fecal coliform; however, temperature possibly acted as a surrogate for changes in the watershed associated with seasonal differences.

3.3.4 Conclusions

Flow weighted stormwater samples were collected for 20 events in a medium density residential neighborhood in Raleigh, North Carolina. *E. coli* and fecal coliform concentrations were significantly lower during winter storm events ($p < 0.05$). Enterococcus concentrations during the winter and fall were also lower, but the differences were not statistically significant ($p < 0.05$).

Correlation analysis showed numerous significant relationships between indicator bacteria concentrations, antecedent climate variables, and flow variables. Simple correlation analysis appeared to misconstrue the effect of climate variables on indicator bacteria concentrations. Many relationships that appeared during the correlation analysis were not logical and were likely the result of multicollinearity between variables.

A multiple linear regression analysis allowed a more detailed examination of these relationships. Temperature and variables related to soil and atmospheric moisture appeared to be important in explaining the variability of indicator bacteria concentrations. All three indicator bacteria seemed to show similar behavior in regard to antecedent climate based on the variables selected by the multiple linear regression. However, statistical models for enterococcus were not as predictive. Enterococcus is generally regarded as more persistent in the environment (USEPA 2001). Thus, temporal climate variations may be harder to associate to enteroccocus concentrations. Therefore, caution should be taken when applying modeling techniques from one indicator bacteria to another.

Although watershed studies can provide useful observations of microbial transport and fate, variable relationships likely exist based on watershed characteristics. Further, transport mechanisms within urban stormwater conveyances are presumably different than those in lotic systems; thus, care should be taken when extrapolating between data collected from stormwater outfalls and data collected within streams or estuaries. The intent of this analysis was to determine important climatic variables for this watershed and to compare those variables to those determined for other watersheds within scientific literature. The reduced models provided in this analysis are unlikely to be applicable to other watersheds with adequate confidence. However, as discussed herein, common relationships were identified which will aid the scientific community in the continued development of microbial transport and fate models. In particular, climate does appear to influence indicator bacteria concentrations in stormwater runoff from urban watersheds. Process-based approaches will ultimately be required to develop models which are robust with respect to watershed location and characteristics.

The results of this study have multiple implications for watershed management:

(1) Indicator bacteria exported via urban stormwater can be a substantial source of non-point pollution in watersheds. Based on the magnitude of indicator bacteria concentrations, stormwater runoff should be carefully considered in TMDLs.
(2) Per USEPA guidance, TMDLs must account for seasonal variations in indicator bacteria. As noted in this and other studies, these variations can be significant and should be carefully considered.
(3) Stormwater best management practices should be evaluated for differences in performance based on season. Poor performance during warmer months, combined with high influent concentrations, could make watershed restoration efforts which employ these practices of reduced benefit. This is of particular concern during warm months when recreational use of surface waters is high.
(4) Antecedent climate conditions can explain some of the variability noted for indicator bacteria concentrations in urban stormwater. Such relationships seem complex and likely will require incorporation of many variables. Atmospheric and soil moisture conditions appear important at the watershed scale, which is intuitive based on the impact of moisture on indicator bacteria in laboratory studies. However, further understanding of these relationships would result in more efficient management of recreational waters.

4 Evaluation of First Flush for Indicator Bacteria and Total Suspended Solids in Urban Stormwater Runoff

4.1 Abstract

An urban watershed in Raleigh, NC, was evaluated for *Escherichia coli (E. coli)*, fecal coliform, enterococcus, and Total Suspended Solids (TSS) over 20 storm events. Sampling procedures allowed collection of multiple discrete samples per event, resulting in a relatively detailed description of mass export for each storm. Data were evaluated to determine if a first flush effect was present for indicator bacteria and TSS in stormwater runoff. Analyses suggested there was a significant first flush effect for fecal coliform and TSS, although the first flush effect for fecal coliform was relatively weak. For *E. coli* and enterococcus, no significant first flush effect was noted. Generally, the first flush effect was not always present for indicator bacteria and, if present, tended to be weak. The first flush effect for TSS was substantially stronger than that of any indicator bacteria. Further analysis showed poor correlation between first flush strength and antecedent climate variables, storm characteristics, and flow characteristics. However, seasonal differences for first flush strength were noted. Specifically, winter storms showed a stronger first flush effect for all indicator bacteria. The results of this study indicate that stormwater runoff presents a public health hazard due to elevated indicator bacteria levels for all portions of the storm event. Further, stormwater management practices cannot be expected to treat proportionally more indicator bacteria when sized for the water quality event. Instead, removal will simply be a function of a management practice's volume capture and microbe sequestration efficiency.

4.2 Materials and Methods Specific to Section 4

4.2.1 Data Analysis

Discrete, flow paced samples were collected during the course of each storm event. The concentrations of TSS and indicator bacteria in each sample were compiled with flow data collected at two minute intervals to calculate pollutant mass export for each sampling interval. These data were then used to generate dimensionless relationships for volume and mass for each storm event. Cumulative volume and mass were calculated for each storm at each sampling time (t_k). These cumulative values were then normalized by the total mass or volume of the storm (Equations 1 and 2).

$$v(t_k) = \frac{\sum_{i=0}^{i=k} Q_i \times t_i}{\sum_{i=0}^{i=t} Q_i \times t_i} \qquad (1)$$

$$m(t_k) = \frac{\sum_{i=0}^{i=k} Q_i \times t_i \times C_i}{\sum_{i=0}^{i=t} Q_i \times t_i \times C_i} \qquad (2)$$

In equations 1 and 2, v(t_k) and m(t_k) are the cumulative volume or mass at any time t_k normalized by the total volume or mass for a given event. Each pair of normalized values (v(t_k) and m(t_k)) were plotted for a given storm event to evaluate the presence of a first flush. The first flush was indicated by the plot of normalized values lying above a 45° line, thus signifying the largest proportion of mass left the watershed in the initial portion of the rain event (Figure 3.2). However, the first flush is typically evaluated at some percentage of the total runoff volume, whereby the total mass exported at this percent volume is compared to a chosen threshold value. The percent of total volume used for evaluation varies. Sansalone and Cristina (2004) and Deletic (1998) evaluated the first flush at 20% of the total storm volume, Flint and Davis (2007) at 25%, and Bertrand-Krajewski et al. (1998) suggested a value of 30%. To provide comparison to similar studies, 30% will be used, similar to that of McCarthy (2009). Essentially, the proportion of total mass transported during the first 30% of the total storm volume was determined and labeled FF_{30} (as in McCarthy 2009), with a first flush effect being noted if FF_{30} is greater than 30% (Figure 3.2). The strength of the first flush effect was also evaluated based on the magnitude of the FF_{30}.

Other criterion have been used which rely on exact thresholds to verify a first flush effect. Thus, data will also be evaluated via a threshold used by Wanielista and Yousef (1993) and Flint and Davis (2007). Both authors selected a criterion whereby 50% of the total mass must be transported in the first 25% of runoff for a storm to be defined as truly exhibiting a first flush.

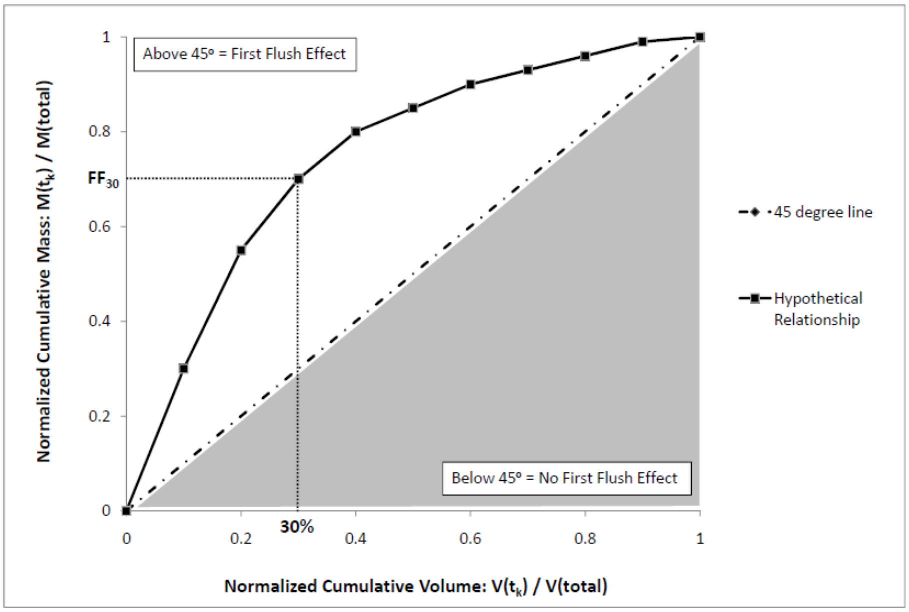


Figure 4.1: Illustration of data analysis method

4.3 Results and Discussion

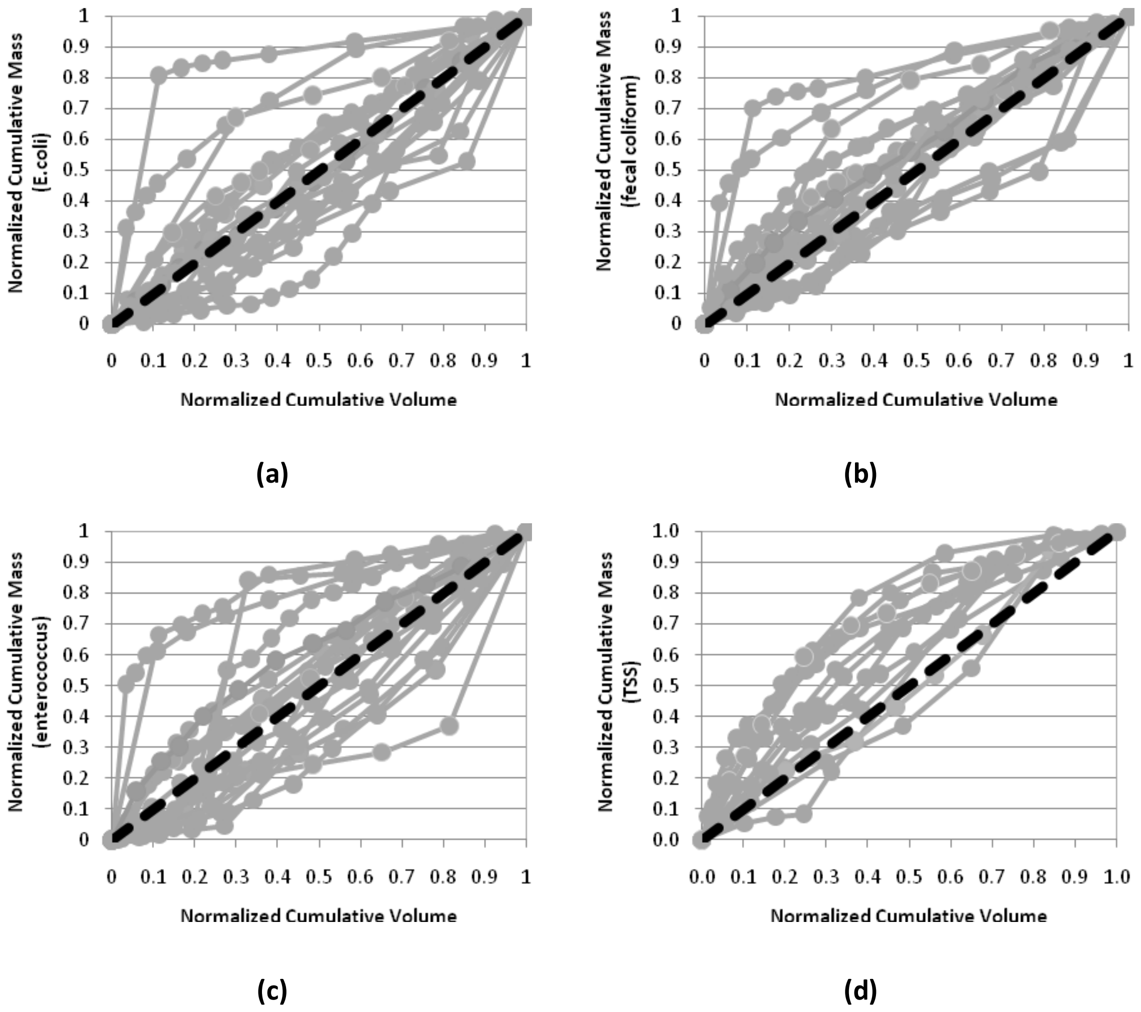
Overall, 20 storms were evaluated for indicator bacteria between October 2008 and September 2009. Analysis for TSS began in February 2009 and continued for 13 events. An average of 10 discrete samples were collected for each storm, with no storm having fewer than 5 discrete samples. Event mean concentrations were developed by Hathaway and Hunt (in review) and are summarized in Table 3.3.

Table 4.1: Summary statistics for collected data

Statistic	Rain (cm)	Event Mean Concentrations (EMCs)			
		E. coli (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococcus (MPN / 100 ml)	TSS (mg/L)
Geometric Mean	1.59	15,396	43,148	11,475	114
Mean	1.96	25,643	80,142	25,155	140
Median	1.75	15,010	59,442	12,342	122
Standard Deviation	1.25	24,323	82,931	40,380	90
Max	5.59	84,688	342,405	181,846	309
Min	0.41	710	1,469	1,306	33

4.3.1 Analysis of First Flush Effect – General Observations

Plots of normalized flow vs. normalized mass for TSS and each indicator bacteria are presented in Figures 3.3a – 3.3d. Plots showed fairly even distribution of storms on either side of the 45° line for *E. coli* and enterococcus. On average, fecal coliform appeared to be slightly distributed above the 45° line. TSS appeared to have a substantially stronger first flush effect than the indicator bacteria with few storms lying below the 45° line. Most storms for indicator bacteria were nearly as likely to be below the 45° line as above.



(a) (b)

(c) (d)

Figure 4.2: Normalized volume vs. normalized mass for (a) *E. coli*, (b) fecal coliform, (c) enterococcus, and (d) TSS

During some storm events, the final portion of runoff had the largest indicator bacteria concentrations, described by McCarthy (2009) as an "end flush." McCarthy (2009) attributed this end flush to possible wastewater intrusion into the stormwater pipe. However, it is also possible that the land use contributing to stormwater runoff near the end of the storm event, likely pervious areas, had high concentrations of indicator bacteria. In this experimental watershed, such pervious areas were frequently residential yards where domestic animals were common.

4.3.2 Analysis of First Flush Effect – FF30

The FF_{30} was determined for each pollutant and compiled in Table 3.4. The FF_{30} can describe the strength of the first flush effect for a given storm event. As FF_{30} increases, the percentage of total mass delivered in the first 30% of runoff increases. The first flush effect was not evident for any pollutant for all storm events. However, some degree of first flush effect was determined for *E. coli* during 45% of events, for enterococcus during 50% of events, for fecal coliform during 70% of events , and for TSS during 77% of events.

On average, the FF_{30} for *E. coli* was 35% with a maximum of 86%. Enterococcus also had an average FF_{30} of 35%, with a max of 79%. Fecal coliform was slightly higher with an average FF_{30} of 39% and high of 78%. TSS had the highest average FF_{30} at 46%, with a maximum of 67%. Wilcoxon Signed Rank analyses showed that *E. coli* and enterococcus did not exhibit a median FF_{30} significantly different than 30% ($p < 0.05$). The median FF_{30} for fecal coliform was significantly different than 30% ($p = 0.047$), as was the median FF_{30} for TSS ($p = 0.009$). Further statistical analysis indicated that although the first flush effect was stronger for some pollutants, no statistical differences in FF_{30} could be found for any of the pollutants (Table 3.5). This is likely due to the high amount of variability in FF_{30} that was noted for each pollutant, as evidenced by the standard deviations in Table 3.4.

Table 4.2: FF_{30} for collected data

Date	FF_{30} (as %)			
	E. coli	enterococcus	fecal coliform	TSS
10/17/2008	6	56	36	-
11/4/2008	41	27	31	-
11/14/2008	25	26	27	-
11/25/2008	18	9	35	-
12/20/2008	23	20	45	-
1/6/2009	39	38	40	-
1/28/2009	43	46	53	-
2/11/2009	86	79	78	67
2/18/2009	42	39	37	34
3/13/2009	66	74	71	60
3/26/2009	18	36	19	19
4/2/2009	67	20	63	25
5/8/2009	14	14	16	29
5/14/2009	45	37	45	62
6/4/2009	29	48	40	41
7/17/2009	27	67	20	62
7/25/2009	33	19	27	52
8/5/2009	14	10	19	47
8/28/2009	29	16	32	64
9/7/2009	28	21	53	40
mean =	35	35	39	46
median =	29	31	37	47
st dev =	20	21	17	16
minimum =	6	9	16	19
maximum =	86	79	78	67

Table 4.3: Wilcoxon signed-rank analysis of differences in FF_{30} (p-values)

Pollutant	fecal coliform	enterococcus	TSS
E. coli	0.165	0.5217	0.2163
fecal coliform	-	0.2162	0.3396
enterococcus	-	-	0.1909

These results are similar to first flush evaluations on *E. coli* for four watersheds in Melbourne, Australia, by McCarthy (2009). McCarthy (2009) observed that none of the watersheds consistently exhibited a first flush for *E. coli*. However, a significant first flush effect was identified for a medium density residential watershed ($p < 0.05$). Average FF_{30} for *E. coli* for the four watersheds studied by McCarthy (2009) was between 30 and 40%, similar to the results of this analysis.

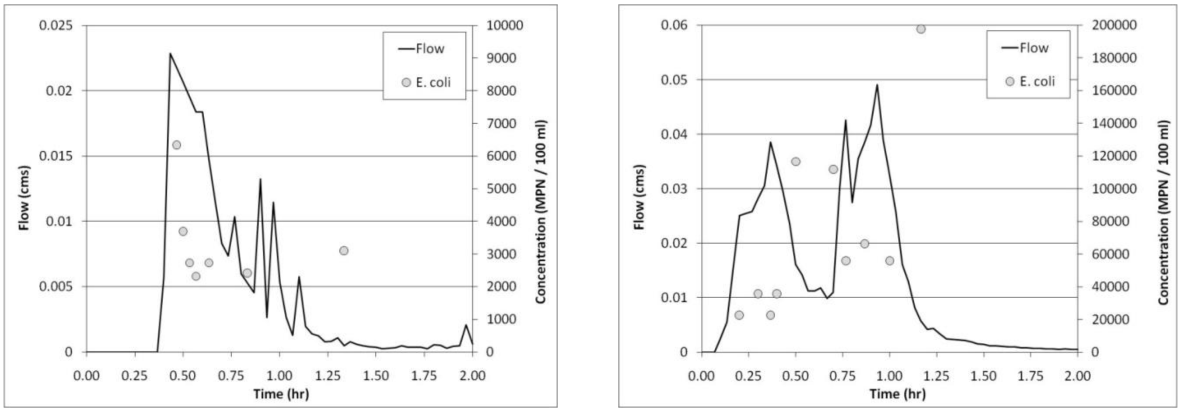
Conversely, sediments have been shown to exhibit some degree of first flush effect in such studies as Flint and Davis (2007) who showed 70% of storms exhibited flushing for TSS, Bertrand-Krajewski et al. (1998) where 80% of storms from watersheds with separate sewer systems had normalized mass vs. normalized volume curves above the 45^{o} line for TSS, Sansalone and Cristina (2004) who showed the percent of total mass of both dissolved solids and suspended sediment concentrations was higher than percent of total volume at the threshold of 20%, and Deletic (1998) where the first 20% of runoff carried 25.5% and 30.8% of suspended solids for two watersheds studied. It should be noted that the methodologies employed by these studies varied and that conclusions as to whether a first flush effect was exhibited were based on varying thresholds. Thus, conclusions for each study may differ from this analysis, where a first flush is identified simply by the percent total mass being larger than the percent total volume during the beginning of the runoff event.

Despite variations in methodologies, the results of this study and others in scientific literature imply that sediments can exhibit a first flush effect. Thus, TSS can be considered a type of control which shows that the first flush effect *is possible* given the hydrologic regime in this watershed. However, *E. coli* and enterococcus did not follow this pattern. This indicates the potential for different sources and/or transport mechanisms for TSS and indicator bacteria in urban watersheds. It should be noted that fecal coliform exhibited a stronger first flush effect that other indicator bacteria.

Differences in TSS and indicator bacteria transport were further explored by performing correlation analyses on TSS and indicator bacteria for each discrete sample taken during a given storm event. Spearman correlation coefficients generated for each storm event were averaged. This allowed further description of intra-event relationships between TSS and indicator bacteria. TSS was poorly correlated to indicator bacteria with average spearman coefficients of 0.08, 0.10, and 0.01 for TSS – E. coli, TSS – fecal coliform, and TSS – enterococcus, respectively. This further supports the assertion that TSS and indicator bacteria may have different sources and/or transport patterns in urban stormwater runoff, and that high concentrations of TSS do not necessarily correspond to high concentrations of indicator bacteria. Although indicator bacteria can attach to and travel with particles (Characklis et al. 2005, Krometis et al. 2007), complicating factors likely make relationships between TSS and indicator bacteria hard to identify, particularly considering the large range of particle sizes represented by TSS measurements. Such complicating factors include differences in attachment based on particle size (Davies and Bavor 2000), differences in sorption based on soil type (Mankin et al. 2007), and natural variability /analytical uncertainty for both indicator bacteria and TSS (Characklis et al. 2005).

4.3.3 Analysis of First Flush Effect – Threshold Methodology

Numerous studies have applied a threshold methodology to determine if a given plot of cumulative volume to cumulative mass constitutes a first flush (Deletic 1998, Sansalone and Cristina 2004, Bertrand-krajewski et al. 1998, Wanielista and Yousef 1993, Flint and Davis 2007). For this analysis, the threshold was taken to be 50% of the total mass being transported in the first 25% of runoff (similar to Flint and Davis 2007). Total mass was rounded to the nearest one percent. TSS had the greatest number of events exhibiting a first flush effect with 5 of 13 events, or 38%, having greater than 50% of the total mass transported in the first 25% of runoff. This was higher than that reported by Flint and Davis (2007), where only 17% of storms exhibited a first flush. A Wilcoxon signed rank analysis showed that TSS cumulative mass was not significantly different than 50% for the storms monitored (p = 0.057). Fecal coliform reached the first flush threshold on 5 of 20 events (25%). Cumulative mass was significantly lower than 50% (p = 0.002). *E. coli* and enterococcus had the least number of storms meeting the first flush threshold with 3 of 20 storms and 2 of 20 storms, respectively. Cumulative mass for both *E. coli* and enterococcus was significantly less than 50% (p =0.006 and p = 0.004, respectively). Figure 3.4 shows example data from storm events which exhibited (Figure 3.4a) and did not exhibit (Figure 3.4b) a first flush effect for *E. coli*.



(a) (b)

Figure 4.3: Flow vs. *E. coli* concentration for (a) 2/11/2008 – first flush effect evident and (b) 5/18/2009 – no first flush effect evident

4.3.4 Correlation analysis

Antecedent climate, storm, and runoff variables were correlated to FF_{30} for each indicator bacteria and TSS (Table 3.6). Resultant Spearman ranks are presented in Table 3.7 for relationships which were significant at $\alpha = 0.05$. Few parameters were correlated to FF_{30} for *E. coli* and fecal coliform. Further, no parameters were correlated to enterococcus and TSS. Similarly, few antecedent climate, storm, and flow parameters were found to be correlated to *E. coli* for 3 of 4 watersheds studied by McCarthy (2009). Relationships impacting the build-up and wash-off of indicator bacteria are complex, making correlation analyses difficult. Indicator bacteria transport and fate in urban watersheds is likely influenced by such factors as climate, soil properties, interactions between microbes, land use, and dynamics within stormwater conveyances (Crane and Moore 1985, Haydon and Deletic 2006, McCarthy 2008)

Parameters were generally negatively correlated to *E. coli* and fecal coliform and were either temperature itself or associated with temperature (in the Southeast United States), such as relative humidity and vapor pressure. Analysis by Hathaway and Hunt (in review) found that as temperature increased in the watershed, indicator bacteria EMCs increased. Thus, it is possible that widespread abundance of indicator bacteria during warmer temperatures results in a lack of first flush, as the supply of bacteria is not limited and not concentrated in a particular part of the watershed or stormwater conveyance system.

Table 4.4: Variables used in correlation analysis

Variable	Type
Flow duration	runoff
Average flow rate	runoff
Peak flow rate	runoff
Total runoff volume	runoff
Total rainfall	rainfall
Storm duration	rainfall
Antecedent dry period	rainfall
Antecedent period since 0.5 cm of rainfall	rainfall
Max 5 minute intensity	rainfall
Average intensity	rainfall
Air temperature	climate*
Relative humidity	climate
Vapor pressure	climate
Solar radiation	climate
Total rainfall	climate
Potential evapotranspiration	climate

*climate variables averaged over antecedent 1, 2, 7, 14, and 28 days

Table 4.5: FF$_{30}$ correlation analysis

Variable	*E. coli*		fecal coliform		Enterococcus		TSS	
	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
enterococcus	-	-	0.48	0.0323	-	-	-	-
Air Temperature 14 days	-	-	-0.46	0.039	-	-	-	-
Air Temperature 28 days			-0.50	0.0245	-	-	-	-
Relative Humidity 14 days	-0.54	0.0137			-	-	-	-
Vapor Pressure 28 days			-0.46	0.039	-	-	-	-

Significant correlations were also noted between fecal coliform and enterococcus FF$_{30}$ (correlation coefficient = 0.48, p = 0.032). *E. coli* and enterococcus had a spearman correlation coefficient of 0.44, but the relationship was not significant (p = 0.054). These data indicate that first flush strength is somewhat linear among indicator bacteria. Thus, although average FF$_{30}$ may vary, factors influencing the FF$_{30}$ may be reasonably similar among indicator bacteria types.

E. coli and fecal coliform were not tested for correlation due to concerns over independence of the data given the analytical methodology.

4.3.5 *Investigation of Seasonal Differences*

Based on the results of the correlation analysis, further examination was performed to determine the affect of season on FF_{30}. Mean seasonal FF_{30} for each indicator bacteria is presented in Figure 3.5. Kruskal-Wallis analyses were performed to determine if seasonality significantly influenced FF_{30}. This was followed by Wilcoxon Rank Sum analyses for pairwise comparisons. The results of these analyses are presented in Table 3.8.

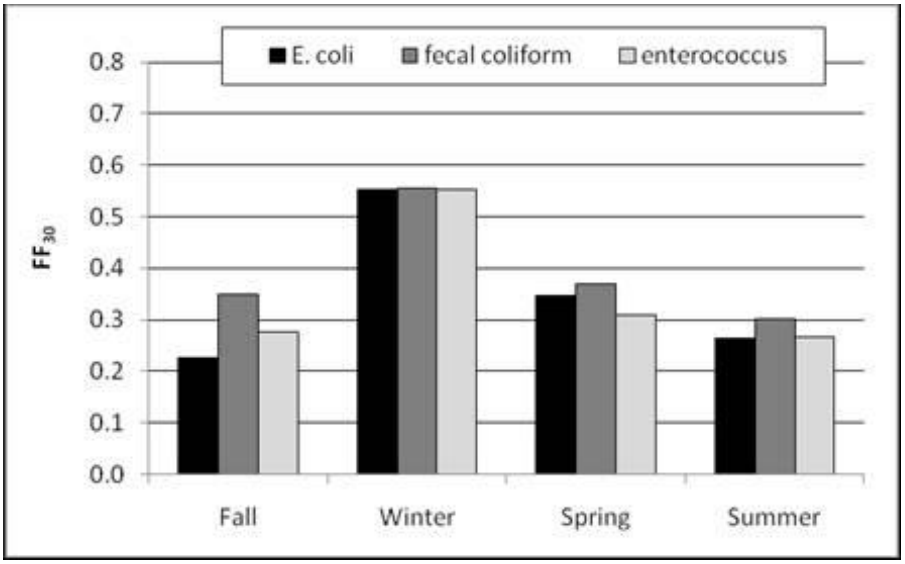


Figure 4.4: Mean seasonal FF_{30} for indicator bacteria

Table 4.6: Statistical analysis of seasonal differences in FF_{30} (p-values)

Indicator Bacteria	**Kruskal-Wallis**	**Wilcoxon Rank Sum**					
		fall - winter	fall - spring	fall - summer	winter - spring	winter - summer	spring - summer
E. coli	***0.0479***	***0.0216***	0.4633	0.4034	***0.0122***	0.2963	0.6742
fecal coliform	0.1202	***0.0367***	0.9166	0.3457	0.2963	***0.0122***	0.6742
enterococcus	0.0868	0.0601	0.7533	0.6761	0.0601	0.0601	0.5309

Significant Kruskal-Wallis values were only noted for *E. coli*; however, pairwise comparisons showed significant differences among seasons for both *E. coli* and fecal coliform ($p < 0.05$).

Significant differences were generally observed in comparisons involving the winter season. Seasonal relationships for enterococcus were not significant, but low p-values ($p = 0.06$) were noted in all analyses comparing winter. Mean seasonal FF_{30} for the winter is the highest among all seasons. No winter storm event for any of the three indicator bacteria had an FF_{30} less than 30%.

Winter indicator bacteria concentrations were shown to be lowest by Hathaway and Hunt (in review) and Selvakumar and Borst (2006). Thus, indicator bacteria during the winter may be source limited. McCarthy (2009) surmised that indicator bacteria persisting within stormwater pipes may be washed out during some rain events, giving the appearance of a first flush. It is possible this phenomenon combined with reduced sources during the winter, from such factors as diminished wild and domestic animal activity, may produce a first flush effect as concentrations lessen through the storm event. These processes are not well understood and further study is needed to verify these postulations.

4.3.6 Conclusions

An urban watershed was monitored for 20 storm events for *E. coli*, fecal coliform, enterococcus, and for 13 events for TSS. Multiple discrete samples were taken during the course of each storm event, allowing detailed analysis of mass transport from the watershed. Results show the FF_{30} for *E. coli* and enterococcus is not significantly different than 30%, demonstrating no greater proportion of mass loading at the beginning of storms ($p < 0.05$). A significant first flush effect was noted for fecal coliform and TSS ($p<0.05$), TSS having the most pronounced first flush effect. Further analysis suggested FF_{30} was not significantly different among any of the pollutants, emphasizing the variability in FF_{30} that was observed in this study. First flush strength was fairly well correlated among indicator bacteria, suggesting similar transport and fate mechanisms influence the first flush effect for microbes.

Data were also analyzed based on a threshold methodology, which contends that a true first flush effect does not exist unless a prescribed threshold is reached. For this study, a threshold of 50% of the total mass being transported in the first 25% of runoff volume was used (similar to Wanielista and Yousef 1993, Flint and Davis 2007). Based on this criterion, no pollutant showed a first flush effect more than 35% of the time. TSS met the criterion most frequently, followed by fecal coliform, *E. coli*, and enterococcus.

Statistical analyses generally showed poor correlation between explanatory variables and pollutant FF_{30}. However, seasonal differences among FF_{30} were noted for indicator bacteria. Winter FF_{30} was highest for each indicator bacteria, and pairwise statistical analyses commonly identified winter as statistically different than other seasons for *E. coli* and fecal coliform.

There are numerous implications of this research related to public health and environmental management. The results of this study suggest indicator bacteria concentrations can remain high even as stormwater flow decreases during the falling limb of the hydrograph. Thus, public health risks will continue throughout the entire runoff event, even if rainfall has ceased.

These data also suggest that treating a water quality volume (e.g. that associated with a 2.5 cm storm event) may not, on average, result in treating proportionally more indicator bacteria. No additional effectiveness should be assumed due to treatment of a first flush. This is important in determining stormwater BMP functionality for watershed restoration. BMP effectiveness will be a function of volume capture and treatment efficiency. In other words, if a designer wishes to treat 90% of the microbial load on an annual basis, the BMP needs to be sized to capture 90% of annual runoff.

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