Evaluation of Indicator Bacteria Export from an Urban Watershed

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Abstract: Studies have shown stormwater runoff is a contributor to elevated indicator bacteria concentrations in surface waters. These elevated concentrations may indicate a heightened human health risk and lead to water quality violations as concentrations exceed regulatory standards. Total Maximum Daily Loads (TMDLs) are commonly established for indicator bacteria, requiring modeling and watershed planning to reduce loadings. However, factors correlated to indicator bacteria build-up and transport in urban watersheds have not been fully explored. Thus, efforts to manage indicator bacteria in urban watersheds are hampered. A watershed monitoring study in Raleigh, North Carolina, used flow weighted samples to provide detailed mass loadings of fecal coliform, *E. coli*, and enterococcus from a 5.1 ha (12.5-acre) residential watershed. Loads were compared to multiple antecedent and in-storm hydrologic and meteorological parameters. The objective of this study was to determine which, if any, parameters can be linked to changes in indicator bacteria export from urban watersheds and to identify patterns in intra-storm indicator bacteria transport. This study will lead to a further understanding of how indicator bacteria can be modeled and managed in urban watersheds.

Keywords: Stormwater, Indicator Bacteria, Pathogens, Runoff, Watershed

Introduction

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, and 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, high resolution indicator bacteria data sets 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.

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. Such processes likely complicate efforts to find patterns for indicator bacteria in urban stormwater runoff.

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. 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* ($p < 0.05$). McCarthy (2008) evaluated the influence of antecedent weather parameters on *E. coli* concentrations in stormwater runoff from four urban watersheds in Melbourne, Australia. 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. 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.

The purpose of this study was to add to the limited understanding of indicator bacteria export from urbanized watersheds. Statistical analysis was used to explore relationships between all three commonly used indicator bacteria species, antecedent climate variables, and in-storm hydrologic variables. Understanding such relationships is important in developing TMDLs for impacted watersheds and in determining factors that must be considered when evaluating a stormwater BMP’s potential for indicator bacteria removal.

Materials and Methods

Site Description

The experimental watershed was located in Raleigh, NC, in a medium density residential neighborhood with approximately 35% imperviousness (Figure 1). An estimated 15% of the watershed was connected impervious area, primarily roadways. The watershed was approximately 5.1 ha (12.5 acres) with a mature tree canopy (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.

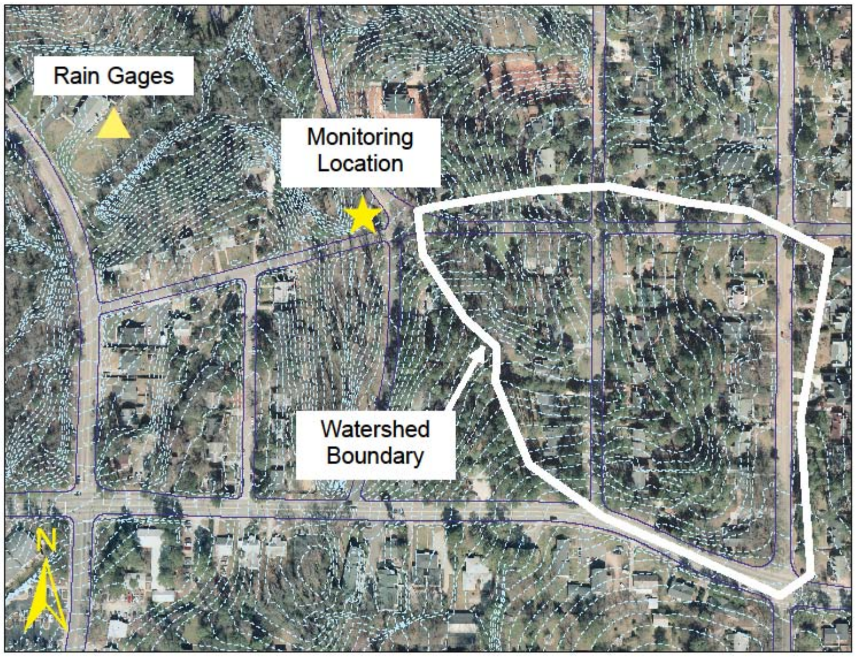

Figure 1: Aerial view of watershed

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 developed for triangular-rectangular compound weirs by Jan et al. (2006). The bubbler module was used in conjunction with an ISCO Avalanche refrigerated sampler which was equipped with a tray of 14 polypropylene bottles.

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. 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 1). 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. 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).

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°C for 1 to 3 hours followed by incubation at 44.5°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°C for 24 hours. Further total suspended solids (TSS) analysis was performed at the North Carolina Center for Applied Aquatic Ecology using SM 2540D (APHA 1998).

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. Discrete samples which exceeded the maximum detectable concentration for the analysis were not included in loads analysis. 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 1 – USEPA 2002).

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

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).

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

$$m(t_k) = \frac{\sum_{i=0}^{i=k} Q_i \times t_i \times C_i}{\sum_{i=0}^{i=t} Q_i \times t_i \times C_i} \qquad (3)$$

In equations 2 and 3, $v(t_k)$ 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 $m(t_k)$) was plotted for a given storm event to evaluate the presence of a first flush. The presence of a first flush is generally indicated by the plot of normalized values lying above a 45° line, thus meaning the largest proportion of mass leaves the watershed in the initial portion of the rain event. All statistical analyses were performed using SAS 9.1 (SAS 2001).

Results and Discussion

Summary Statistics
Between October, 2008, and September, 2009, twenty storm events were monitored. 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. 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.

Concentrations for each storm and summary statistics are provided in Table 1. EMCs for *E. coli* ranged from 700 to 85,200 MPN / 100 ml; 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.

Table 1: Summary statistics for monitored events

Statistic	Rain (cm)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococcus (MPN / 100 ml)	TSS (mg/L)
Mean	1.96	25,671	83,581	25,155	140
Median	1.75	15,010	59,442	12,342	122
Standard Deviation	1.25	24,393	87,098	40,380	90
Max	5.59	85,233	342,405	181,846	309
Min	0.41	710	1,469	1,306	33

Seasonal Variation

The results of a Kruskal-Wallis test indicated a seasonal difference in EMC for *E. coli*, fecal coliform, and enterococcus. A Wilcoxon Rank Sum tests allowed detailed investigation of 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 were 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.

Table 2: Analysis of seasonal variations of indicator bacteria

Indicator Bacteria	**Kruskal-Wallis**	**Wilcoxon Rank Sum**					
		fall - winter	fall - spring	fall - summer	winter - spring	winter - summer	spring - summer
E. coli	***0.026***	0.2222	***0.0317***	0.3095	***0.0159***	0.0952	0.3095
fecal coliform	***0.0102***	**0.0159**	0.3095	0.1508	**0.0159**	**0.0159**	0.6905
enterococcus	***0.0412***	1.0000	0.0556	0.0952	0.0556	0.0556	0.3095

Note: Significant relationships are bold and italicized

Multiple linear Regression

A multiple linear regression analysis was performed using PROC REG. This analysis provided a small 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 3.

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 may not be 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.

Table 3: 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	2 day total rain	1.01	0.0059	28 day total rain	1.02	0.0079	0.75
fecal coliform	2 day average air temperature	1.99	0.0059	14 day average relative humidity	1.95	0.0364	7 day total rain	1.09	0.0410	0.80
enterococcus	7 day total rain	1.06	0.0094	14 day average relative humidity	1.06	0.0275	-	-	-	0.53

Analysis of First Flush Effect – General Observations

Plots of normalized flow vs. normalized mass for TSS and each indicator bacteria are presented in figures 2a – 2d for each event. Plots for indicator bacteria showed fairly consistent distribution of storms on either side of the 45^o line for *E. coli* and enterococcus. Fecal coliform appeared slightly more distributed above the 1:1 line. TSS appeared to have a stronger first flush effect than the indicator bacteria with few storms lying below the 45° line. Most storms for indicator bacteria fairly closely followed the 45° line, indicating relatively consistent mass loading throughout the storm event.

There were some storm events during which 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 may also possible that the land use contributing to stormwater runoff near the end of the storm event, likely pervious areas, have high concentrations of indicator bacteria. In this experimental watershed, such pervious areas may be residential lawns and landscaping where domestic animals are common.

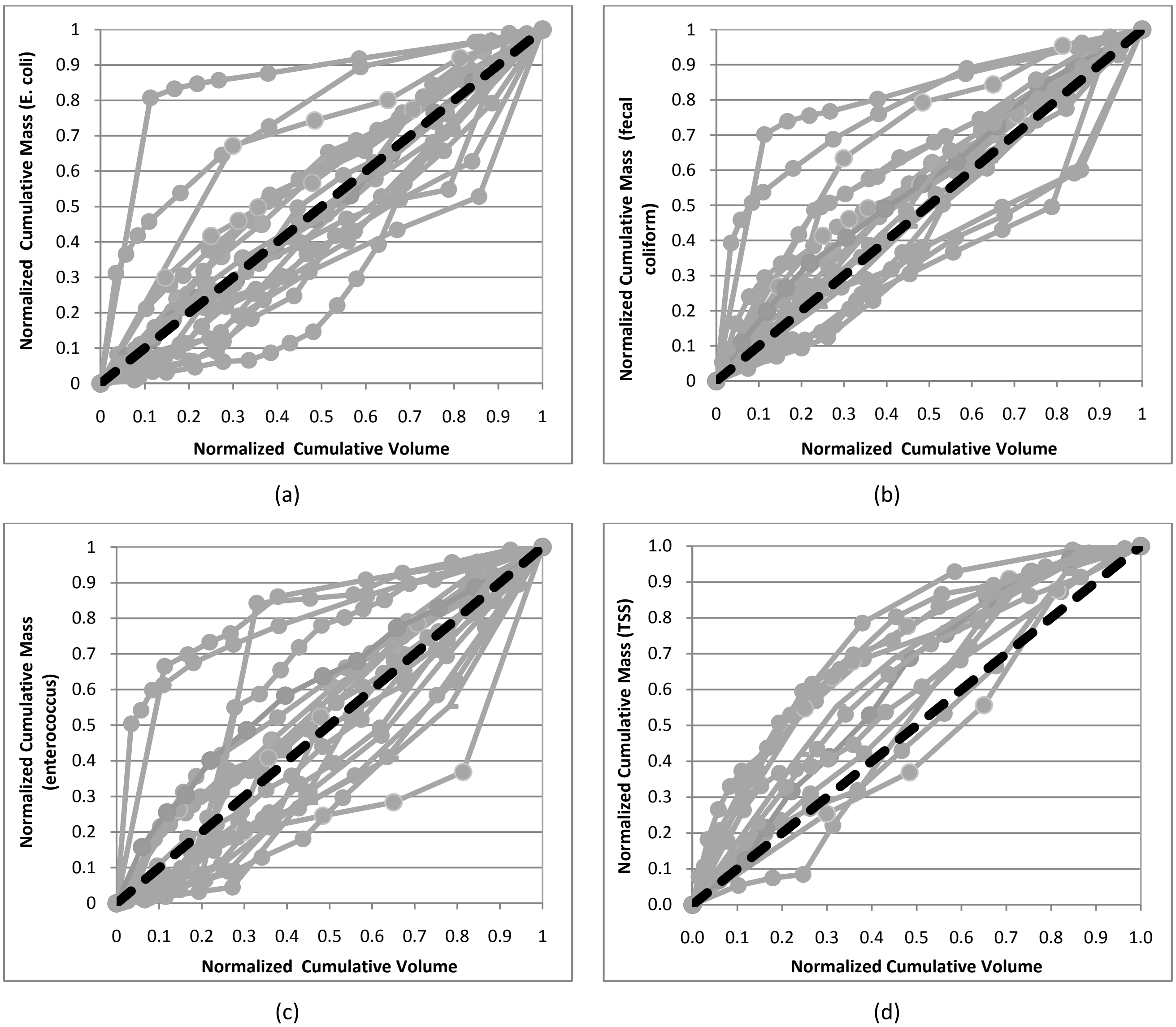

Figure 2: Normalized cumulative mass vs. normalized cumulative volume for (a) *E. coli*, (b) fecal coliform, (c) enterococcus, and (d) TSS.

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 ($p < 0.05$).

A multiple linear regression analysis allowed a more detailed examination of antecedent climate and indicator bacteria 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. Thus, caution should be taken when applying modeling techniques from one indicator bacteria to another.

Examination of the first flush effect indicated that no consistent first flush could be noted for *E. coli* and enterococcus. Conversely, the first flush effect was more apparent for fecal coliform and even stronger for TSS. Further analysis is needed to determine factors which may affect the first flush for indicator bacteria and to characterize the first flush strength for all monitored parameters.

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

(1) TMDLs are required to account for variations in indicator bacteria concentrations based on seasonal differences. As noted in this and other studies, these variations can be significant and should be carefully considered.
(2) 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.
(3) Indicator bacteria may not consistently exhibit a first flush effect, even in watersheds were the first flush effect is apparent for other water quality parameters.

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