
Origins and mechanisms of watershed and land use based sources of fecal indicator bacteria in urban stormwater

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ABSTRACT

Stormwater runoff is a well documented source of fecal indicator bacteria (FIB). Routine stormwater monitoring programs focus on quantification of average FIB concentration, or fluxes at the terminal watershed discharge point. While important for overall status and trends assessment, such monitoring provides little insight in assessing land use impacts on aquatic ecosystems, or into the mechanisms and processes that influence FIB concentrations in stormwater. The goal of this study was to quantify the sources, patterns of concentrations and fluxes of three FIB (i.e. *Escherichia coli*, enterococci, and total coliforms) from representative land use (LU) types in both urban and non-urban watersheds within the greater Los Angeles, California region. Bacteria concentrations were measured over the entire storm duration from 8 different LU types over 13 storm events in 5 southern California watersheds during the 2000-2001 through 2004-2005 storm seasons. In addition, runoff samples were collected from 8 bottom of the watershed mass emission (ME) sites during 13 storm events. Intra-storm and intra-season patterns were investigated in order to identify mechanisms that influence patterns of FIB concentrations. Finally, this study compared the estimates of stormwater FIB concentrations to existing water quality standards to provide context for the contribution of stormwater to overall FIB concentrations in the region. Mean event mean concentrations (EMCs) at LU sites ranged from 10^3 to 10^5 MPN/100 ml for *E. coli*, and enterococci, and 10^4 to 10^6 MPN/100 ml for total coliforms. Recreational LU sites contributed significantly higher storm EMCs than other LU types. Early season storms (October-December) repeatedly produced higher EMCs than comparably sized late season (April-May) storms. For most storms sampled, the highest bacterial concentrations occurred during the early phases of stormwater runoff with peak concentrations usually preceding peak flow.

INTRODUCTION

Fecal indicator bacteria in urban stormwater is known to occur from many sources, including runoff from fertilized cropland, animal wastes associated with residential development, failing septic or sewer systems, and urban storm drains (Cook and Baker 2001, Moog and Whiting 2002, Jamieson *et al.* 2003). Water quality modeling is an increasingly common tool for investigating bacteria loading processes and for evaluating management options (Ackerman and Stein 2008). Models are useful because once validated they can be used to explore patterns over a wide range of conditions in a relatively efficient manner. However, calibration and validation of models requires mechanistic data on bacteria runoff not typically collected as part of routine monitoring programs. Most existing stormwater monitoring programs focus on quantification of average concentration or load at the terminal watershed discharge point. While important for overall status and trends assessment and for demonstrating permit compliance, such monitoring provides little insight into the mechanisms and processes that influence FIB concentrations in stormwater.

Model calibration and validation requires quantification of differences in bacteria runoff between LU categories. In addition, within-storm and within-season patterns are needed in order to identify mechanisms that influence patterns of FIB concentrations. In particular, managers need to better understand the sources, processes, and mechanisms that affect runoff and associated FIB concentrations. Specifically, data is needed to better understand how patterns of FIB concentrations vary over the course of a single storm, how FIB flux varies over the course of a storm season, and how applicable national or regional estimates of LU based concentrations are to southern California.

The goal of this study is to provide detailed data necessary for model calibration and for development

of management strategies. To accomplish this, sources, patterns of concentrations, and fluxes of FIB from representative LU types in both urban and non-urban watersheds within the greater Los Angeles, California region were quantified.

METHODS

Study Areas

Stormwater bacteria data were collected from 10 urban and 2 non-urban watersheds in the greater Los Angeles metropolitan area in southern California. Urban watersheds were densely populated (approximately 90 residents/km²; US Census Bureau 2000) ranging from 49 to 94% developed. In contrast, the two non-urban watersheds each had less than 5% urban area.

Within the urban and non-urban watersheds, stormwater runoff was sampled from a range of homogenous LU sites as well as ME sites that integrate runoff from all the LU types in contributing watersheds (Table 1; Figure 1). The ME sites consist of natural streams in the two undeveloped watersheds and engineered flood control channels (highly modified rivers) in the developed watersheds, all of which ultimately discharge to recreational beaches and harbors along the Pacific Ocean. The 8 ME sites ranged in size from 31 to 2,161 km² and were sampled during the 2000-2001 through 2004-2005 storm seasons (Table 1). The 19 homogeneous LU sites were sampled over the same time period and represent 8 LU types. LU categories included high density residential, low density residential, commercial, industrial, agricultural, recreational (horse), transportation, and open space.

Sampling and Analysis

A total of 20 discrete storms were sampled, with each site being sampled between 1 and 7 individual storms. Rainfall ranged from 0.12 to 9.68 cm per storm and antecedent conditions varied from 0 to 142 days without measurable rain. Rainfall was measured using a standard tipping bucket at each site that recorded at 0.025-cm increments. Water quality sampling was initiated when flows were greater than base flows by 20%, continued through peak flows, and ended when flows subsided to less than 20% of base flow. Flow at ME sites was estimated at 15-minute intervals using existing, county maintained flow gauges or stage recorders in conjunction with historically derived and calibrated stage-discharge relationships. At ungauged ME sites and pre-

Table 1. Sampling sites with corresponding watershed size and number of storms sampled. NA = size not available.

	Watershed Size (km ²)	No. of Storms Sampled
Mass Emission Sites		
LA River above Arroyo Seco	1460	3
LA River at Wardlow	2161	4
Verdugo Wash	65	4
Arroyo Seco	130	2
Ballona Creek	338	7
Dominguez Channel	187	2
Santa Monica Canyon	41	2
Open Space Arroyo Sequit	31	4
Land Use Sites		
High Density Residential	0.017	3
High Density Residential	0.518	2
High Density Residential	1	2
Low Density Residential	0.977	3
Low Density Residential	0.177	1
Commercial	NA	1
Commercial	2.453	1
Commercial	0.059	3
Industrial	0.004	3
Industrial	0.001	1
Industrial	2.771	1
Industrial	0.008	1
Agricultural	0.985	4
Agricultural	0.8	1
Recreational	0.026	2
Transportation	0.01	1
Transportation	0.002	1
Open Space	9.49	1
Open Space	2.89	1

viously unmonitored LU sites, stream discharge was measured as the product of the channel cross-sectional area and the flow velocity. Velocity was measured using an acoustic Doppler velocity (AV) meter. The AV meter was mounted to the invert of the stream channel, and velocity, stage, and instantaneous flow data were transmitted to a data logger/controller on query commands found in the data logger software.

Between 10 and 15 discrete grab samples were collected per storm at approximately 30- to 60-minute intervals for each site-event based on optimal sampling frequencies in southern California described by Leecaster *et al.* (2001). Samples were collected more frequently when flow rates were high or rapidly changing and less frequently during lower flow periods.

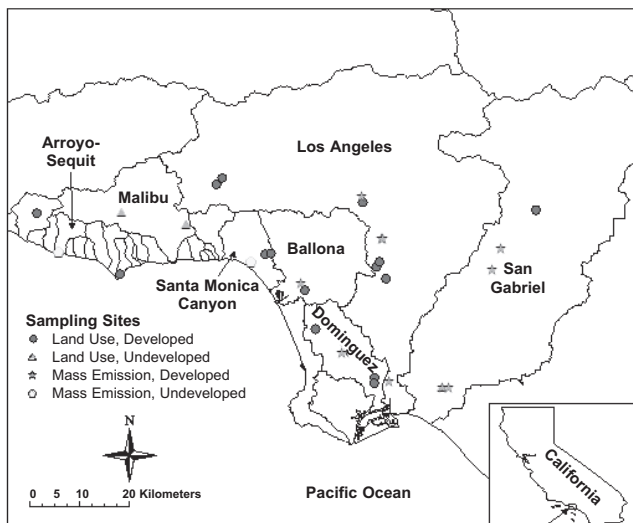


Figure 1. In-river mass emission sampling sites and watersheds within the greater Los Angeles region, California. Watersheds indicated with dots contained LU sites that drain catchments that are >90% undeveloped.

All water samples were collected by one of three methods: 1) peristaltic pumps with Teflon tubing and stainless-steel intakes that were fixed at the bottom of the channel or pipe pointed in the upstream direction in an area of undisturbed flow, 2) direct filling of the sample bottle either by hand or affixed to a pole, or 3) indirect filling using an intermediate bottle for securing large volumes. After collection, the samples were stored in pre-cleaned glass bottles on ice with Teflon-lined caps until they were shipped to the laboratory for analysis.

Laboratory Analysis

FIB analysis

Concentrations of *E. coli* and enterococci were measured by chromogenic substrate technology using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME). *E. coli* was measured using Colilert-18 media, while enterococci were measured using Enterolert media. Ten-fold and 100-fold dilutions of water samples were made with deionized water containing the appropriate media and sodium thiosulfate, mixed to dissolve, dispensed into trays (Quanti-Tray/2000), and heat-sealed. Samples were incubated overnight according to the manufacturer's instructions and inspected for positive wells. Conversion of positive wells from these tests to a most probable number (MPN) was done following (Hurley and Roscoe 1983).

Total Suspended Solids (TSS)

TSS were analyzed by filtering a 10- to 100-ml aliquot of stormwater through a tared 1.2- μm Whatman GF/C glass fiber filter (GFF). The filters plus solids were dried at 60°C for 24 hours, cooled, and weighed.

Data Analysis

Four basic analyses were used to characterize temporal patterns and determine sources of FIB in stormwater. Prior to analysis bacterial concentrations were log-transformed to improve normality. Non-detectable results were assigned a value of one-half the minimum detection limit, based on the inability to log transform a value of zero.

For the first analysis, event flow-weighted mean (FWM) concentrations and flux rates among undeveloped and developed ME sites were compared to determine if significant differences existed among watershed types. The FWM concentrations from the homogeneous LU sites were also compared. Using only those samples for a single storm, the event FWM was calculated according to Equation 1:

$$FWM = \frac{\sum_{i=1}^n C_i * F_i}{\sum_{i=1}^n F_i} \quad (1)$$

where FWM = flow-weighted mean for a particular storm, C_i = individual runoff sample concentration of i th sample, F_i = instantaneous flow at the time of i th sample, and n = number of samples per event.

Mass loading was calculated as the product of the FWM concentration and the storm volume during the sampling period. Flux estimates facilitated loading comparisons among watersheds of varying sizes. Flux was calculated as the ratio of the mass loading per storm and watershed area. Differences in concentration or flux between ME or LU sites were investigated using a one-way ANOVA, with a $p < 0.05$ significance level followed by Tukey-Kramer *post-hoc* test for multiple comparisons (Sokal and Rohlf 1995).

The second data analysis used Spearman Rank correlation coefficients (ρ ; a nonparametric measure

of correlation) to explore the relationships between FIB concentrations, stormwater runoff volume, and TSS (Townsend 2002). Significant relationships were determined based on a $p < 0.05$.

The third and fourth analyses investigated temporal patterns in FIB concentrations at two scales. Variability in flow and FIB concentration within storm events were investigated for the presence of a concentration-based first flush by examining the time-concentration series relative to the hydrograph using a plot we term a pollutograph. Seasonal patterns in FIB concentration were investigated by plotting EMCs as a function of cumulative annual rainfall prior to the date of the storm being sampled. For this analysis, all ME sites were analyzed as a group to look for differences between early- (October-December) and late season (April-May) storms across the sampling region.

RESULTS

Rainfall

On an annual basis, there are two distinct climatic periods in southern California: a dry (semi-arid) period from late May to mid-October, and a wet period from mid-October through late May. Winter storms typically provide 85 to 90% of the annual average rainfall (38.4 cm), with about 30 cm of total precipitation being distributed over 3 to 5 large and 8 to 10 small storms (Ackerman and Weisberg 2003). Annual rainfall quantities in Los Angeles can be highly variable. The 2004-2005 rainfall season brought 94.6 cm of rain to downtown Los Angeles, making it the second wettest season in Los Angeles since records began in 1877 (National Weather Service 2005). In contrast, during the 2001-2002 season rainfall totaled a mere 11.2 cm, 27 cm below the seasonal average. The study period encompassed a representative range of rainfall conditions.

FIB Concentration and Flux from Specific Land Uses

Geometric mean *E. coli*, enterococci, and total coliform EMCs and fluxes were significantly greater at ME sites from developed compared to undeveloped watersheds (ANOVA, $p = 0.006$). For example the geometric mean EMC at the developed Ballona Creek watershed was two orders of magnitude higher than at the undeveloped open space Arroyo Sequit watershed (10^4 MPN/100 ml vs. 10^2 MPN/100 ml). Furthermore, the higher concentrations and fluxes from developed watersheds were generated by sub-

stantially less rainfall than necessary to generate flux from open space watersheds (2.07 ± 1.22 cm for storms in developed watersheds vs 6.49 ± 3.79 cm for storms in undeveloped watersheds).

Geometric mean *E. coli* concentrations and EMCs were significantly higher from the recreational LU site compared to the commercial, high density residential, industrial, and transportation LU sites (i.e., 5.3×10^5 MPN/100ml $\pm 1.7 \times 10^5$, $p = 0.004$; Figure 2). Agricultural LU sites contributed the second greatest mean indicator bacteria EMCs, but were not statistically different from all other LU sites (i.e., 4.0×10^4 MPN/100 ml $\pm 1.4 \times 10^4$ *E. coli*). Flux patterns between LU sites were similar, with agricultural and recreational sites producing the greatest flux, typically in the range of 10^{14} colonies/km².

Correlation between FIB and TSS Concentration

A Spearman's correlation matrix (Table 2) of TSS, stream flow and FIB indicates that *E. coli* was significantly positively correlated ($p < 0.0001$) with TSS from agricultural, recreational and open LU sites. *E. coli* concentrations from low-density residential and industrial LU sites were weakly correlated with TSS. Enterococci was significantly correlated

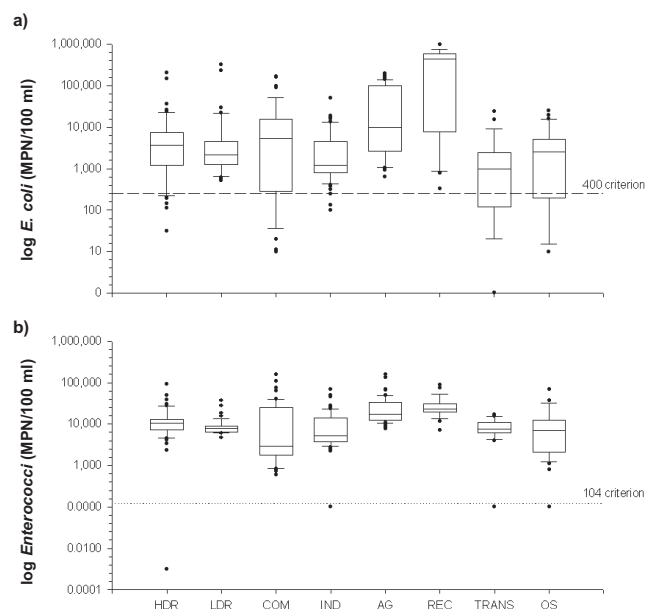


Figure 2. Distribution of *E. coli* (a) and enterococci (b) concentrations during the 2000-2001 through 2004-2005 wet seasons from land use (LU) sites. HDR = high density residential, LDR = low density residential, COM = commercial, IND = industrial, AG = agricultural, REC = recreation with horses, TRANS = transportation and OS = undeveloped open space.

Table 2. Correlations between total suspended solids (TSS), stream flow, and fecal indicator bacteria (FIB) during storm condition; Spearman's correlation coefficient (ρ) and the number of samples (n). Bold numbers indicate significant correlations ($p < 0.05$); Bold numbers with asterisk (*) indicate significant correlations ($p < 0.01$).

Land Use Site	Total Suspended Solids			Stream Flow		
	<i>E. coli</i>	Enterococci	Total Coliforms	<i>E. coli</i>	Enterococci	Total Coliforms
High Density Residential	-0.082 (42)	0.023 (42)	-0.020 (42)	*0.611 (42)	-0.056 (42)	0.066 (42)
Low Density Residential	-0.364 (37)	*-0.603 (37)	-0.180 (37)	0.239 (37)	0.04 (37)	-0.269 (37)
Commercial	0.246 (47)	0.354 (47)	*0.416 (47)	*0.772 (47)	*0.819 (47)	*0.796 (47)
Industrial	*-0.389 (55)	-0.304 (55)	-0.130 (55)	-0.251 (55)	-0.248 (55)	-0.133 (55)
Agricultural	*0.553 (44)	*0.616 (44)	0.356 (44)	0.281 (44)	*0.436 (44)	*0.688 (44)
Recreational	*0.694 (20)	*0.767 (20)	*0.732 (20)	-0.0162 (20)	*0.587 (20)	-0.092 (20)
Transportation	0.519 (20)	*0.741 (20)	*0.672 (20)	*-0.712 (20)	0.392 (20)	-0.347 (20)
Open	*0.670 (30)	*0.461 (30)	0.174 (30)	0.255 (30)	0.223 (30)	-0.199 (30)

($p < 0.0001$) with total suspended solids from low density residential, agricultural, recreational and transportation LU sites and all correlations with the exception of the low density residential site were positive. Enterococci counts from commercial and open LU sites were weakly and positively correlated with TSS. FIB concentrations were significantly correlated ($p < 0.0001$) with stream flow at the commercial, high density residential and agricultural LU sites, but not at other sites. Stream flow and TSS were not significantly correlated.

Temporal Patterns in FIB Concentrations

Within-storm variability

E. coli and enterococci concentrations varied with time as a function of flow over the course of storm events (Figures 3 and 4). In all cases, bacterial concentrations increased markedly preceding peak flow. Enterococci concentrations stayed high for a relatively short period at the developed Ballona Creek site (2.4×10^5 MPN/100 ml) and then decreased back to base levels within two hours (Figure 3a). In contrast, *E. coli* concentrations were more variable exhibiting two separate peaks around 2.6×10^4 MPN/100 ml and an order of magnitude

lower than enterococci concentrations (Figure 3b). Although the pattern of an early peak in concentration was comparable in both urban and non-urban watersheds, in the undeveloped watersheds the peak concentration tended to occur later in the storm and persist for a longer duration (i.e., three to four hours; Figure 4). Furthermore, flow continued above base flow conditions for a longer duration in the non-urban watersheds. However, FIB concentrations steadily decreased following the early peak in storm.

Within-season variability

Antecedent dry period (expressed as cumulative annual rainfall) was strongly correlated with FIB concentrations from ME sites in an exponential manner ($r^2 = 0.67 - 0.92$; Figure 5). Early season storms generally had higher enterococci and total coliforms EMCs than late season storms both within and between watersheds, even when rainfall quantities were similar. For all indicators, there was an inflection point in the concentration vs. rainfall curve at around 5 cm, which represents the first one to three storms of the season. For example, the early season storm from Ballona Creek in water year 2004 had an enterococci EMC two times larger (3.0×10^4 MPN/100 ml) than the storm that occurred at the end

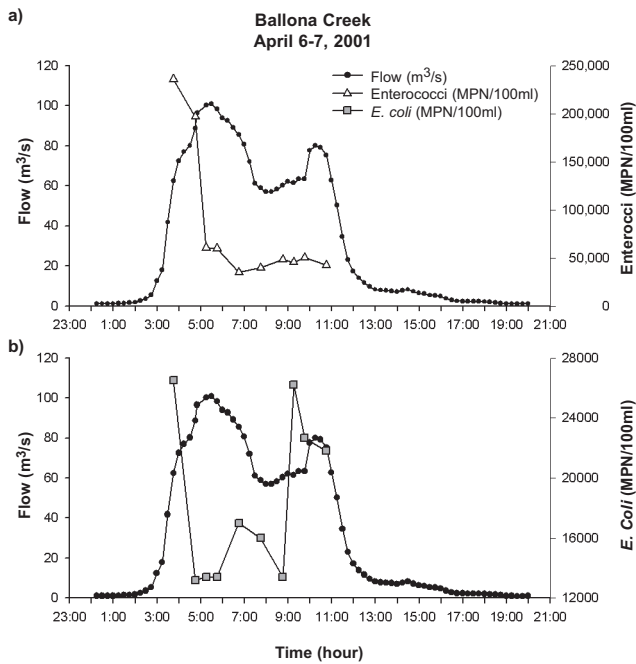


Figure 3. Enterococci (a) and *E. coli* (b) concentrations with time for a storm event from the developed Ballona Creek watershed. Total coliforms concentrations showed a similar pattern.

of the rainy season in water year 2003 (1.6×10^4 MPN/100 ml), despite the early and late season storms resulting from comparable rainfall (approx. 3.0 cm). The results for *E. coli* EMCs from early and late season storms were comparable. When all watersheds were analyzed together, *E. coli*, enterococci, and total coliforms concentrations decreased with increasing cumulative annual rainfall until approximately 5 cm (average annual rainfall is 33 cm), beyond which the effect is markedly less dramatic (Figure 5).

FIB Water Quality Exceedances

Finally, the study compared the estimates of stormwater FIB concentrations to existing water quality standards to provide context for the magnitude of importance of stormwater to overall FIB concentrations for the region. Fecal indicator bacteria concentrations consistently and uniformly exceeded the State of California (CA) microbiological water quality thresholds at both ME and LU sites during the present study. Bacteria counts were compared to the CA single sample criterion for ocean beaches of 104 MPN enterococci/100 ml, 400 MPN *E. coli*/100 ml, and 10,000 total coliforms MPN/100 ml) to estimate percent exceedances. Approximately 80% of all samples exceeded water quality thresholds at LU sites for

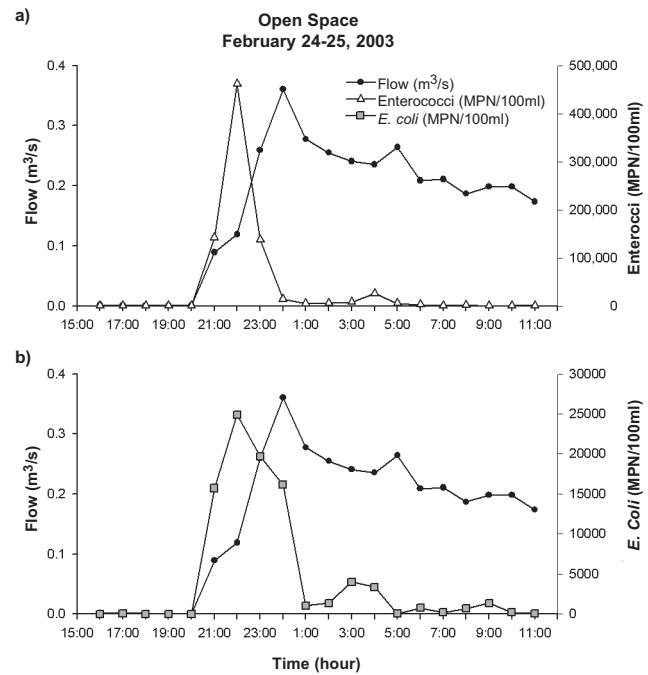


Figure 4. Enterococci (a) and *E. coli* (b) concentrations with time for a storm event from the open space land use site. Total coliforms concentrations showed a similar pattern.

at least one indicator (i.e., *E. coli* exceedance = 83%). Similarly, 98, 94, and 92%, respectively, of the ME stormwater samples for enterococci, *E. coli*, and total coliforms bacteria exceeded CA ambient water quality standards. The above comparisons were based on receiving water quality standards. If compared to the proposed freshwater standards, which are approximately 60% lower than the receiving water standards, the exceedances would be slightly higher.

DISCUSSION

Bacteria concentrations in stormwater runoff varied based on the contributing land use in the following manner: Recreational > agricultural > urban > open space. The relatively higher bacteria concentrations from recreational and agricultural LU sites may result from several mechanisms. Bacteria from fertilizers, domestic pet and wildlife wastes that are deposited, stored, or applied to the land may account for the high *E. coli* and enterococci EMCs observed at the agricultural sites during this study. Previous studies have identified high FIB concentrations at agricultural sites associated with regular application of fertilizers (Niemi and Niemi 1991, Cook and Baker 2001). In contrast, land use sites such as industrial and residential areas have proportionately less direct sources of fecal material and have lower

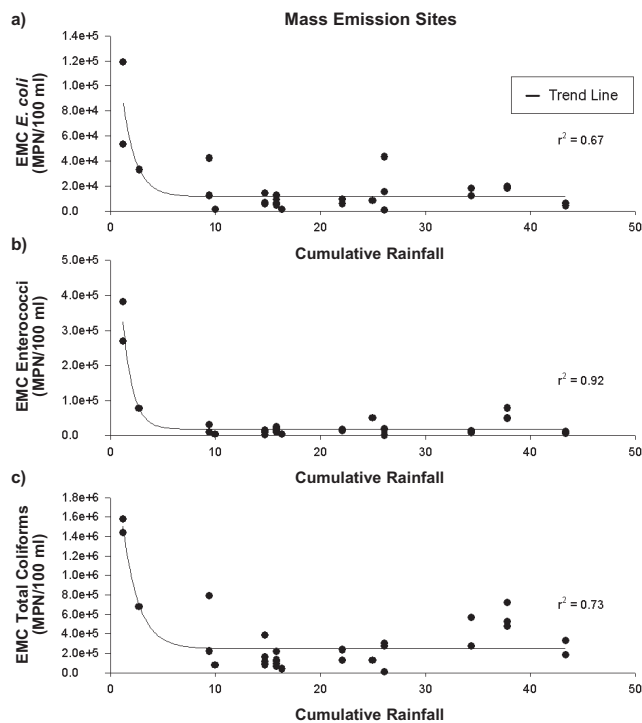


Figure 5. Cumulative annual rainfall versus event mean concentration (EMC) for *E. coli* (a) enterococci (b), and total coliforms (c) during 2000-2001 through 2004-2005 storm seasons. Data shown for mass emission (ME) sites only.

bacteria concentrations in stormwater than do mixed use LU and developing areas (i.e., recreational; Mallin and Wheeler 2000, Burnhart 1991).

The association of bacteria with stormwater particles may also explain differences in *E. coli* and enterococci concentrations from different LU sites, and can be a useful relationship for simulating bacteria washoff processes when calibrating models. Correlations of FIB with TSS from recreational and agricultural LU sites suggest associations with particulate material, but it is unclear if the particulate material resulted from soils transported to the stream from these LU sources or from erosion and resuspension of sediment already in the streambed from upland areas. Other studies have implicated streambed sediment and its resuspension (Matson *et al.* 1993, Francy *et al.* 2000, Embrey 2001) as sources and principal transport vectors for bacteria. However, the high bacterial concentrations we observed in direct runoff from recreational and agricultural land use sites suggests that bacteria associated with these areas may be directly linked with site erosion. Assessing particle size distribution over the entire storm duration at these LU sites may help clarify the dynamics of bac-

teria-particle source associations.

Interestingly, indicator bacteria concentrations were only significantly correlated ($p < 0.0001$) with flow at the commercial and high density residential LU sites even though bacteria in streams are commonly associated with suspended particles. One reason for this apparent difference may be that highly impervious sites (such as commercial and high density residential) have inherently less particle load than sites that have more impervious cover. The lower particle load may shift the FIB distribution to the dissolved phase, decreasing the relationship with TSS and increasing the relationship with flow.

Consistently higher bacteria levels during early season storms likely reflect bacteria buildup during dry periods that flushes to rivers during early season storms. The strong relationship between FIB and antecedent dry period suggests that bacterial flux associated with stormwater runoff depends on the amount of time available for build up on land surfaces, and that storm size may be a less reliable predictor of the magnitude of bacterial loading. A seasonal pattern was observed for polycyclic aromatic hydrocarbons (PAHs) and trace metals in the Los Angeles region, (Sabin *et al.* 2004, Stein *et al.* 2006, Tiefenthaler *et al.* 2008), although the inflection point occurred late in the season (~10 cm). Han *et al.* (2006) also reported that antecedent dry period was the best predictor of the magnitude of pollutant runoff from highways. Several researchers (Anderson and Rounds 2003, Ngoye and Machiwa 2004) have reported corresponding temporal trends for other contaminants and linked these patterns to the timing of particle washoff. This seasonal pattern suggests that focusing management actions on early season storms may provide relatively greater efficiency than distributing lower intensity management actions throughout the season.

FIB concentrations in stormwater were highly variable, with concentrations often ranging by factors of 10 to 100 during a single storm. The greatest bacteria concentrations occurred at or just before the peak in flow of the storm hydrograph for nearly every storm sampled. This early peak in concentration was also observed for PAHs (Stein *et al.* 2006) and trace metals (Tiefenthaler *et al.* 2008) in the greater Los Angeles area. Tiefenthaler *et al.* (2001) observed similar pollutographs that showed peak suspended-sediment concentrations preceding the peak in discharge for the Santa Ana River. Similar time vs. concentration relationships were also

observed by Characklis and Wiesner (1997), who reported that the maximum concentrations of zinc, organic carbon and solids coincided with early peak stormwater flows. Bacterial counts typically vary by up to five orders of magnitude on daily, seasonal, and inter-annual scales.

These patterns provide valuable information for model calibration. However, further research is needed to directly assess the relationship between indicator bacteria concentrations and particle size distributions in stormwater runoff from mass emission and LU sites to better understand the fate, transport and treatment of indicator bacteria in urban runoff. Stormwater borne bacteria are typically associated with particulates to varying degrees depending on the indicator bacteria and the size distribution of suspended solids in the stormwater runoff. Furthermore, the particle size distribution, and bacteria partitioning can change over the course of a storm event (Furumai *et al.* 2002). Understanding the dynamic partitioning of indicator bacteria to various size particles is important to facilitate estimation of temporal and spatial patterns of bacterial deposition in estuaries and harbors, and should be an area of future investigation.

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