Flow Fingerprinting Fecal Pollution and Suspended Solids in Stormwater Runoff from an Urban Coastal Watershed

CRISTIANE Q. SURBECK,[†] SUNNY C. JIANG,[‡] JONG HO AHN,[†] AND STANLEY B. GRANT*,[†]

Department of Chemical Engineering and Materials Science, Henry Samueli School of Engineering, and Department of Environmental Health, Science, and Policy, School of Social Ecology, University of California, Irvine, California 92697

Field studies were conducted to characterize the concentration vs streamflow relationships (or "flow fingerprints") of fecal pollution and suspended solids in stormwater runoff from the Santa Ana River watershed, the largest watershed in southern California. The concentrations of fecal indicator bacteria and F^+ coliphages (viruses infecting *E. coli*) exhibit little-to-no dependence on streamflow rates, whereas the concentrations of total suspended solids (TSS) exhibit a very strong (power-law) dependence on streamflow rates. The different flow fingerprints observed for fecal pollutants, on one hand, and TSS, on the other hand, reflect different sources and transport pathways for these stormwater constituents. The flow-independent nature of fecal indicator bacteria and F^+ coliphages is consistent with the idea that these contaminants are ubiquitously present on the surface of the urban landscape and rapidly partition into the surface water as the landscape is wetted by rainfall. The flow-dependent nature of TSS, on the other hand, is usually ascribed to the shear-induced erosion of channel bed sediments and/or the expansion of drainage area contributing to runoff. The apparent ubiquity of fecal indicator bacteria and F^+ coliphages, together with the very high storm-loading rates of fecal indicator bacteria and the low detection frequency of human adenovirus and human enterovirus, suggest that fecal pollution in stormwater runoff from the Santa Ana River watershed is primarily of nonhuman waste origin.

Introduction

In the United States, water quality impairments are usually managed using total maximum daily loads (TMDL) management plans (*1*), which require information on the sources of pollution and the transport pathways by which pollutants are mobilized. In the case of fecal pollution, there are two primary approaches that are currently available to address this requirement. First, Microbial Source Tracking (MST) methods have been developed to identify sources of fecal indicator bacteria in surface waters, for example, from cows, dogs, and humans (*2*). MST methods work well in simple environmental settings where the number of possible fecal sources is relatively limited and adequately characterized—a condition unlikely to be satisfied in stormwater runoff from large urban watersheds (*3*). Second, distributed watershed models of pollutant transport in surface water can be used to define relationships among land use, water quality, and stormwater runoff (*4*−*6*). However, application of distributed models to fecal indicator bacteria and fecal indicator viruses is complicated by the fact that once microbial indicators enter the environment, their fate and transport are likely to be affected by poorly characterized ecological processes, such as the proliferation of environmentally adapted strains of fecal indicator bacteria (*7*, *8*). Consequently, fecal indicator bacteria and viruses are unlikely to accumulate and wash off at reproducible and land-use specific rates—an assumption inherent in most distributed watershed models. Indeed, at this point in time, about the only generalization that can be derived from the published literature is that fecal indicator bacteria concentrations are very high in stormwater runoff from watersheds throughout the world (*9*−*13*).

In this paper, we test the following hypothesis: concentration vs flow relationships (or flow fingerprints) harbor information on the sources and transport pathways of pollutants in stormwater runoff from urban watersheds. To test this hypothesis, we conducted a set of field studies in a coastal watershed in southern California in order to do the following: (1) characterize the flow fingerprints of fecal pollution (as measured by fecal indicator bacteria and F^+ coliphages) and suspended solids (as measured by TSS) in stormwater runoff; (2) assess the variability of the observed flow fingerprints across multiple sites in the watershed and multiple storm events; and (3) compare the results of the flow fingerprints of fecal indicator bacteria and F^+ coliphages to more direct measures of human fecal pollution, specifically molecular assays for human adenovirus and human enterovirus.

Materials and Methods

Sampling Sites. Sampling was carried out during three storms at three sites located along a coastal-to-inland transect: (1) the McFadden Avenue crossing (MCF in Figure 1) of the Santa Ana River approximately 13 km upstream of the river's ocean outlet, (2) the Imperial Highway crossing (IMP in Figure 1) of the Santa Ana River approximately 16 km downstream of Prado Dam, and (3) the Remmington Avenue crossing of Cucamonga Creek (CUC in Figure 1), a tributary of the Santa Ana River in the upper basin. These three sampling sites differ with respect to the size of their drainage area, land use, channel cross section, channel bottom, and factors affecting the storm hydrograph (Table 1). Photographs of these sampling sites, and additional information on the Santa Ana River watershed, are included in the Supporting Information (Figure S1). The antecedent dry period was longest for Storm 1 (9 days), intermediate for Storm 2 (5 days), and shortest for Storm 3 (3 days).

Streamflow Data. Streamflow rates were obtained from the U.S. Geological Survey for gauging stations within 1.5 km of the MCF and CUC. Streamflow rates at IMP were provided by the Orange County Water District.

Sample Collection and Analysis. Table 1 summarizes the storm sampling dates and sample analyses performed. During each storm, samples (n = 24−35) were collected in 2-L autoclaved Nalgene bottles (Nalge Company, Rochester, NY) at frequencies ranging from 4 samples per hour (during peak flow) to 2 samples per day (toward the end of a storm). All

* Corresponding author phone: (949) 824-8277; fax: (949) 824-2541; e-mail: sbgrant@uci.edu.
[†] Henry Samueli School of Engineering.
[‡] School of Social Ecology.

10.1021/es060701h CCC: $33.50
Published on Web 06/13/2006

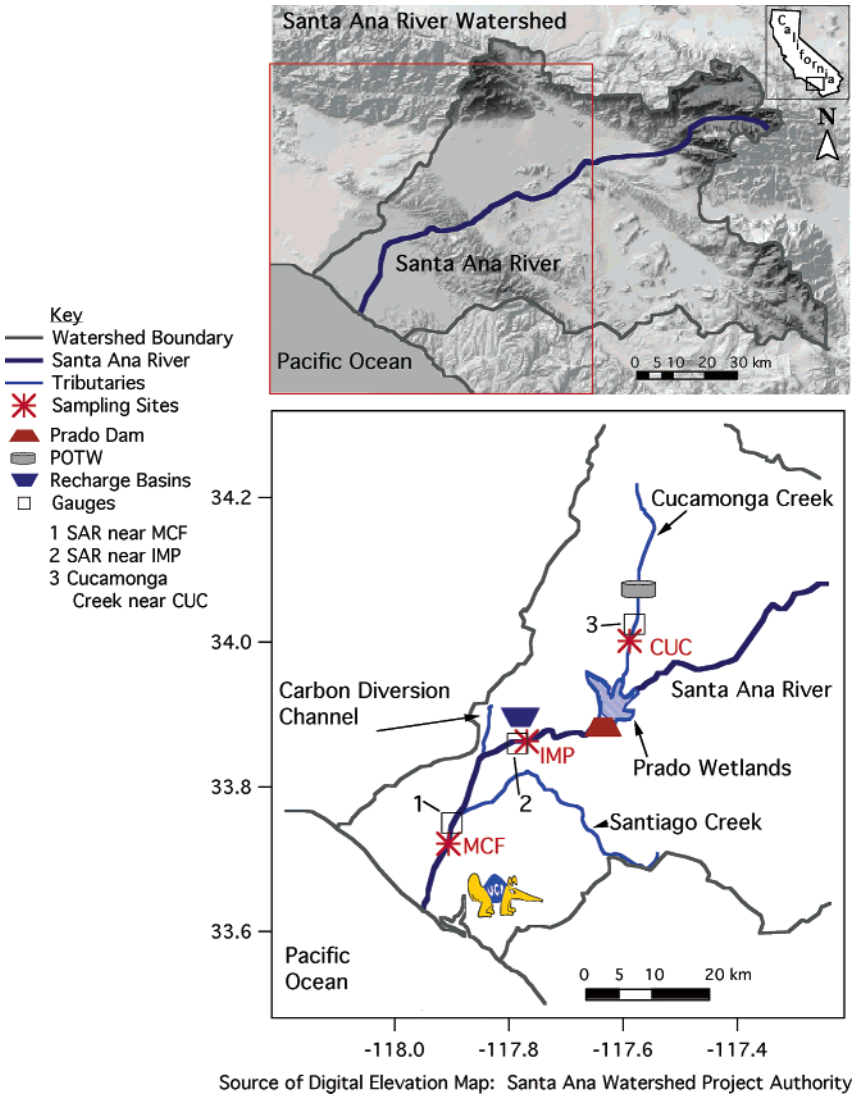

FIGURE 1. A map showing the location of the three sampling sites in the Santa Ana River (SAR) watershed (McFadden Avenue, MCF; Imperial Highway, IMP; and Cucamonga Creek, CUC). Also shown are the locations of groundwater recharge basins, Prado Dam, gauge stations, publicly owned treatment works (POTW), and the University of California Irvine (denoted by anteater) where sample analyses took place.

TABLE 1. Features of the Sampling Sites, Storms Sampled, and Analyses Performed

	MCF[a] site	IMP[b] site	CUC[c] site
storms sampled[d]	Storm 1, 2, 3	Storm 2	Storm 3
parameters analyzed	FIB,[e] TSS,[f] viruses[g]	FIB, TSS, viruses	FIB, TSS, viruses
drainage area (km^2)	4400	4000	200
cross section description	concrete-lined, trapezoidal	soft bottom, rip rap sides, rectangular	concrete-lined, trapezoidal
factors affecting storm hydrograph	upstream dams (Prado, Imperial, Five Coves), water reclamation facilities, storm drains	upstream dam (Prado), downstream dam (Imperial), water reclamation facilities, storm drains	POTW discharge, storm drains
surrounding land use	residential, commercial	residential, commercial	agricultural, residential

[a] MCF = McFadden Avenue crossing of Santa Ana River. [b] IMP = Imperial Highway crossing of the Santa Ana River. [c] CUC = Remmington Avenue crossing of Cucamonga Creek. [d] Storm 1: 11–13 Nov, 2003; Storm 2: 2–4 Feb, 2004; Storm 3: 21–23 Feb, 2004. [e] FIB = fecal indicator bacteria. [f] TSS = total suspended solids. [g] At MCF, viruses were assayed only during Storms 2 and 3.

samples were analyzed for fecal indicator bacteria and TSS. Selected samples (15 total) collected during Storms 2 and 3 were analyzed for the presence of human pathogenic viruses (human adenovirus and human enterovirus by nested polymerase chain reaction (PCR) and reverse transcription polymerase chain reaction (RT-PCR), respectively) and fecal indicator viruses (F^+ coliphage by culture enrichment or plaque assay). Further information on the logic used to select

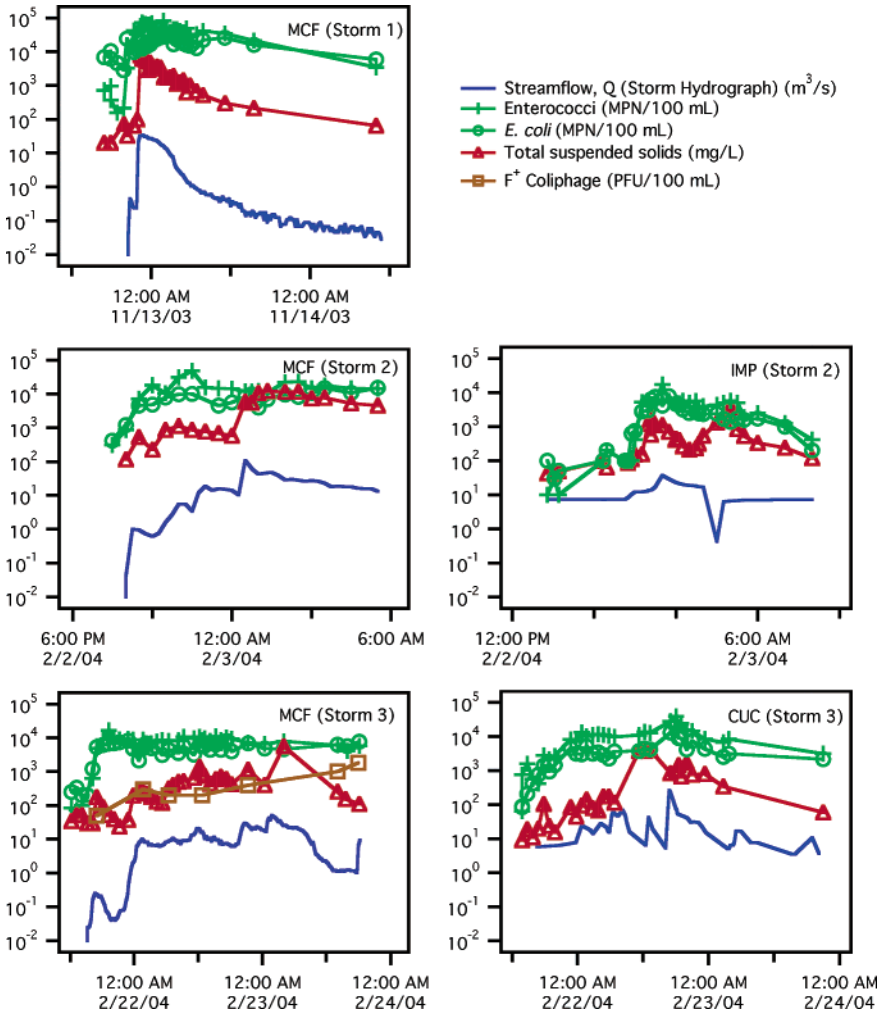

FIGURE 2. Hydrographs and pollutographs by site and storm. Pollutographs for TSS follow the hydrographs, while pollutographs for fecal indicator bacteria and F^+ coliphages do not follow the hydrographs.

this particular suite of pollutants is provided in the Supporting Information.

Fecal Indicator Bacteria and Total Suspended Solids. Water samples were kept on ice in the dark and transported to UCI (denoted by the anteater in Figure 1) within 6 h of collection. At the laboratory, samples were diluted and analyzed for total coliform and *Escherichia coli* (*E. coli*) using the Colilert test and enterococci bacteria using the Enterolert test (IDEXX, Westbrook, ME). These defined substrate tests yield the concentration of fecal indicator bacteria in units of most probable number of bacteria per 100 mL of sample (MPN/100 mL). TSS measurements were carried out using Standard Method 2540D (*14*).

Fecal Indicator Viruses (F^+ Coliphage). Selected samples were analyzed for the presence/absence of F^+ coliphage by a two-step enrichment method (USEPA Method 1601). At MCF during Storm 3, F^+ coliphage was quantified using the plaque-forming unit assay as originally described by Adams (*15*). See Supporting Information for details.

Human Pathogenic Viruses (Human Adenovirus and Human Enterovirus). Five hundred milliliters of water sample was concentrated to a final volume of ~500 μL using a Centricon Plus-80 ultrafiltration system with 100 kDa molecular mass cutoff membrane (Millipore Inc., Billerica, MA). Viral nucleic acid was purified/extracted from concentrates using QIAmp Viral RNA Mini Kit (Qiagen Inc., Valencia, CA) following manufacturer's protocols. Detection of enteroviruses used a RT-PCR procedure developed by Tsai et al. (*16*). An internal probe hybridization was used to confirm PCR amplicon following the protocol developed by Jiang and Chu (*17*). For adenovirus detection, a nested PCR protocol was used as previously described by Pina et al. (*18*). A real-time PCR protocol developed by He and Jiang (*19*) was also used for adenovirus quantification. See Supporting Information for details.

Total Pollutant Loads and Event Mean Concentrations (EMC). Loadings of fecal indicator bacteria (in units of MPN/s) and TSS (in units of mg/s) were calculated by multiplying the pollutant concentration into the streamflow recorded (or linearly interpolated) at the time of sample collection. These instantaneous loading rates were integrated over the storm hydrograph (using the trapezoidal rule) to obtain the total mass and most probable number of TSS and fecal indicator bacteria discharged per storm. Event mean concentrations (EMC) of fecal indicator bacteria and TSS were calculated as the ratio of the total pollutant mass and most probable number divided by the total volume of water discharged during the storm (*20*).

Results

Flow Fingerprinting Fecal Indicator Bacteria, Fecal Indicator Viruses, and Total Suspended Solids. At all three sites (MCF, IMP, and CUC) and during all three storms (Storms 1, 2, and 3), the concentration of fecal indicator bacteria behaved in a similar manner (Figure 2). At the onset of a storm the concentration of fecal indicator bacteria abruptly increased by 1 or more orders of magnitude and then

TABLE 2. Spearman's Rank Correlations between Parameters Monitored during the Storms

		Spearman's rank correlations				
		MCF (Storm 1)	MCF (Storm 2)	MCF (Storm 3)	IMP (Storm 2)	CUC (Storm 3)
TSS[a] vs Q[b]		0.94[d]	0.76[d]	0.87[d]	0.054	0.79[d]
bacteria[c] vs Q	total coliform	NC[e]	0.22	−0.071	0.41	0.69[d]
	E. coli	0.10	0.30	−0.14	0.72[d]	0.86[d]
	enterococci	0.86[d]	0.093	0.18	0.62[d]	0.91[d]
bacteria vs TSS	total coliform	NC[e]	0.46	0.012	0.86[d]	0.80[d]
	E. coli	0.21	0.37	−0.080	0.70[d]	0.80[d]
	enterococci	0.88[d]	0.17	0.28	0.72[d]	0.82[d]

[a] TSS = total suspended solids, mg/L. [b] Q = streamflow, m³/s. [c] Units of MPN/100 mL. [d] $p < 0.01$. [e] NC = not calculated because some concentration measurements were above the upper limit of detection 241 960 MPN/100 mL.

remained more-or-less constant over the remainder of the storm hydrograph. The concentration of TSS, on the other hand, increased and decreased in parallel with measured streamflow at most sites (Spearman's rank correlation (Sp) between TSS and streamflow is 0.76−0.94, two-tailed significance (p) < 0.01, Table 2). During the single storm and site where the concentrations of F+ coliphages were measured (Storm 3 at MCF), the concentrations of these viruses increased with time from 50 plaque-forming units (PFU)/100 mL at the onset of the storm to 1800 PFU/100 mL at the end of the monitoring period (Figure 2). The concentration of F+ coliphage was not determined at the other sites and storms, although presence/absence tests for F+ coliphage were conducted (results described below). The F+ coliphage measurements at MCF were not significantly correlated with either streamflow (Sp = 0.072, $p = 0.88$) or TSS (Sp = −0.058, $p = 0.91$).

For the single site that exhibited multiple log-cycle change in streamflow (MCF), log−log plots of concentration versus streamflow rate reveal distinct flow fingerprints for TSS, on one hand, and fecal indicator bacteria, on the other hand (Figure 3). TSS concentrations increase as a power law of streamflow, Q. In particular, the data fall along a linear trend when plotted on a log−log basis (top panel of Figure 3): $TSS \sim Q^x$, where $x = 0.46-0.64$. The concentration of fecal indicator bacteria, on the other hand, remains high and relatively constant over a 4-log-cycle increase in Q above base flow conditions (bottom three panels, Figure 3). Coefficients of variation calculated from the fecal indicator bacteria data are lower than those calculated for TSS and streamflow (Table 3).

At MCF, where three storms were monitored, event mean concentrations (EMCs) and peak loading rates were highest during Storm 1, intermediate during Storm 2, and lowest during Storm 3 (Table 4). However, the total pollutant discharged during each storm did not follow this declining trend; i.e., during Storm 3 at MCF, the total pollutant discharged was higher compared to the other storms (Table 4). Across the three sampling sites, the lowest EMCs and peak loading rates were measured at IMP (Table 4).

Presence/Absence Testing for F+ Coliphage and Human Pathogenic Viruses (Human Adenovirus and Human Enterovirus). Fifteen samples collected during Storms 2 and 3 at MCF, IMP, and CUC were analyzed for F+ coliphage. All samples, except two collected from CUC, tested positive for F+ coliphage, both before and after the onset of the storms (Table 5).

PCR assays of adenovirus and enterovirus yielded negative results for all samples, except one sample collected at CUC early in Storm 3 that tested positive for human adenovirus by nested-PCR. High levels of PCR inhibitors were found in all samples, significantly reducing the sensitivity of the PCR assay. Figure 4 shows that 0.25 μL of stormwater nucleic acid

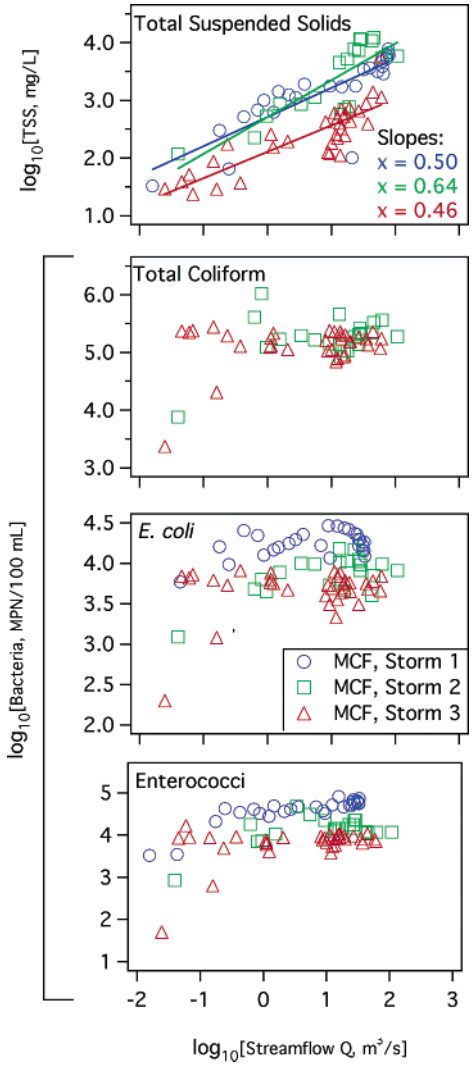

FIGURE 3. Log−log plots of pollutant concentration vs streamflow Q. TSS and streamflow are related by a power law of the form $TSS \sim Q^x$, where $x = 0.46-0.64$. Bacteria and streamflow are not related by a power law; bacteria concentrations stay constant and high over multiple log cycles of Q.

extract contains enough PCR inhibitors to cause complete inhibition of seeded adenovirus genome, while the nested-PCR assay is capable of detecting 2 orders of magnitude lower concentrations (2−10 copies of genome) in the absence of PCR inhibitors. We estimate that when a sample tested negative for human adenovirus, the concentration of viral genomes in the sample is less than 80 genome equivalence/mL (see Supporting Information for computation of detection

TABLE 3. Mean, Standard Deviation, and Coefficient of Variation of Streamflows and Pollutants across the Storm Hydrographs

	parameter[a]	arithmetic mean[b] ± standard deviation	coefficient of variation
MCF (Storm 1)	streamflow	$(3.00 \pm 3.16) \times 10^1$	1.0
	E. coli	$(1.87 \pm 0.59) \times 10^4$	0.32
	enterococci	$(4.70 \pm 1.66) \times 10^4$	0.35
	TSS	$(2.51 \pm 2.28) \times 10^3$	0.91
MCF (Storm 2)	streamflow	$(2.18 \pm 2.48) \times 10^1$	1.1
	total coliform	$(2.29 \pm 2.12) \times 10^5$	0.92
	E. coli	$(7.81 \pm 3.76) \times 10^3$	0.48
	enterococci	$(1.55 \pm 0.99) \times 10^4$	0.64
	TSS	$(4.60 \pm 4.40) \times 10^3$	0.96
MCF (Storm 3)	streamflow	$(1.38 \pm 1.61) \times 10^1$	1.2
	Total coliform	$(1.56 \pm 0.66) \times 10^5$	0.42
	E. coli	$(5.26 \pm 1.84) \times 10^3$	0.35
	enterococci	$(7.73 \pm 2.92) \times 10^3$	0.38
	TSS	$(5.28 \pm 9.37) \times 10^2$	1.8
	F^+ coliphage	$(5.64 \pm 6.25) \times 10^2$	1.1
IMP (Storm 2)	streamflow	$(1.44 \pm 0.79) \times 10^1$	0.55
	total coliform	$(3.59 \pm 2.02) \times 10^4$	0.56
	E. coli	$(2.86 \pm 1.96) \times 10^3$	0.69
	enterococci	$(4.21 \pm 3.19) \times 10^3$	0.76
	TSS	$(6.91 \pm 7.91) \times 10^2$	1.2
CUC (Storm 3)	streamflow	$(3.34 \pm 4.90) \times 10^1$	1.5
	total coliform	$(8.06 \pm 4.14) \times 10^4$	0.51
	E. coli	$(5.37 \pm 4.27) \times 10^3$	0.80
	enterococci	$(1.35 \pm 0.79) \times 10^4$	0.58
	TSS	$(9.88 \pm 12.9) \times 10^2$	1.3

[a] Units are as follows: for streamflow, m^3/s; for total coliform, *E. coli*, and enterococci, MPN/100 mL; for TSS, mg/L; and for F^+ coliphage, PFU/100 mL. [b] Arithmetic mean used for purposes of comparison.

limit). This converts to less than 10^{-2} PFU of infectious adenovirus per mL according to plaque efficiency studies (*21*). The detection limit for enterovirus is estimated to be 0.016 PFU per mL in these samples according the single reaction detection limit of 0.002 PFU reported previously (*16*).

Discussion

In general, we expect that the concentration vs streamflow pattern, or flow fingerprint, of stormwater constituents will depend on many factors, including the timing, location, and intensity of rainfall in the watershed, land use, pollution sources, human manipulation of runoff by civil infrastructure (e.g., dams and diversion structures), and a myriad of transport and transformation processes that occur as stormwater runoff flows downstream. Surprisingly, despite these complexities, the results presented in this paper indicate the following: (1) there are unique and reproducible flow fingerprints associated with fecal pollution, as measured by fecal indicator bacteria and F^+ coliphages; and (2) the flow fingerprints for fecal pollution differ from those for TSS.

Flow fingerprints for fecal pollution in stormwater runoff from the Santa Ana River were similar for all four analytes tested (total coliform, *E. coli*, enterococci, and F^+ coliphage), across all sampling sites (MCF, IMP, and CUC), and across all three storms. In all cases, the concentration of fecal pollution increases abruptly at the onset of stormwater runoff and remains elevated (or increase steadily) over the storm hydrograph. The relative insensitivity of fecal pollutant concentrations to streamflow is evident from the storm pollutographs (Figure 2), from concentration vs streamflow plots (Figure 3), from the generally poor correlation observed between fecal indicator bacteria and streamflow (Table 2), and from the relatively low coefficient of variation values observed for fecal indicator bacteria (Table 3). Flow fingerprints for TSS, on the other hand, are characterized by a highly flow-dependent process, as evidenced by the pollutgraphs (Figure 2), the power-law relationship between TSS and streamflow rate (Figure 3), the generally high correlation between TSS and streamflow (Table 2), and the relatively large coefficient of variation values calculated for TSS and streamflow (Table 3).

The power-law streamflow dependence of TSS concentrations has been described in previous studies *(22, 23)* and could reflect a number of different hydrologic processes, including the shear-induced erosion of sediments off the urban landscape and/or the expansion of the watershed area contributing to streamflow during periods of intense rainfall (*24*). The relative weak streamflow dependence observed for fecal indicator bacteria and F^+ coliphages, on the other hand, suggests that these pollutants are mobilized into surface water runoff by a largely flow-independent process. The flow fingerprints observed here for fecal pollution are not obviously consistent with the buildup/wash-off paradigm employed in most distributed watershed models. Specifically, in a scenario typically ascribed to the buildup/wash-off paradigm, pollutant concentrations peak during the initial phases of the storm and decline thereafter (a so-called "first-

TABLE 4. Summary of Event Mean Concentrations (EMC), Pollutant Loading, and Streamflow Rates during the Storms

	MCF (Storm 1)	MCF (Storm 2)	MCF (Storm 3)	IMP (Storm 2)	CUC (Storm 3)
Event Mean Concentration					
total coliform, MPN/100 mL	NC[a]	2.16×10^5	1.54×10^5	3.02×10^4	1.06×10^5
E. coli, MPN/100 mL	1.98×10^4	8.95×10^3	5.44×10^3	2.42×10^3	8.44×10^3
enterococci, MPN/100 mL	5.66×10^4	1.50×10^4	7.58×10^3	3.93×10^3	1.90×10^4
total suspended solids, mg/L	3.67×10^3	6.61×10^3	1.96×10^3	4.84×10^2	1.11×10^3
Total Pollutant Discharge					
total coliform, MPN/storm	NC[a]	1.62×10^{15}	6.56×10^{15}	2.36×10^{14}	5.07×10^{15}
E. coli, MPN/storm	2.02×10^{14}	6.71×10^{13}	2.31×10^{14}	1.89×10^{13}	4.03×10^{14}
enterococci, MPN/storm	5.77×10^{14}	1.13×10^{14}	3.22×10^{14}	3.07×10^{13}	9.09×10^{14}
total suspended solids, mg/storm	3.76×10^{12}	5.00×10^{12}	1.04×10^{13}	3.78×10^{11}	4.87×10^{12}
Peak Loading					
total coliform, MPN/s	NC[a]	2.29×10^{11}	1.07×10^{11}	2.42×10^{10}	3.13×10^{11}
E. coli, MPN/s	1.43×10^{10}	8.76×10^9	4.16×10^9	2.28×10^9	2.94×10^{10}
enterococci, MPN/s	5.81×10^{10}	1.27×10^{10}	4.75×10^9	6.41×10^9	6.33×10^{10}
TSS, mg/s	6.08×10^8	6.27×10^8	6.91×10^7	4.15×10^7	1.95×10^8
total streamflow, m^3/storm	1.02×10^6	7.50×10^5	4.25×10^6	7.81×10^5	4.78×10^6
peak streamflow, m^3/s	7.99×10^1	1.08×10^2	6.08×10^1	2.90×10^1	2.28×10^2

[a] NC = not calculated because some concentration measurements were above the upper limit of detection 241 960 MPN/100 mL.

TABLE 5. Summary of Virus Results

	F^+ coliphage	human adenovirus	human enterovirus
MCF (Storm 1)	not sampled	not sampled	not sampled
MCF (Storm 2)	+ (3 of 3)[a]	− (3 of 3)	− (3 of 3)
MCF (Storm 3)	+ (7 of 7)	− (7 of 7)	− (7 of 7)
IMP (Storm 2)	+ (3 of 3)	− (3 of 3)	− (3 of 3)
CUC (Storm 3)	− (2 of 2)	+ (1 of 2); − (1 of 2)	− (2 of 2)

[a] Inside the parentheses are the numbers of positive or negative outcomes out of the total number of samples tested.

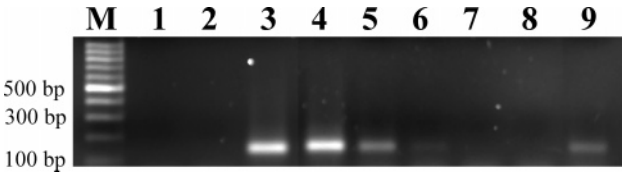

FIGURE 4. Electrophoresis gel showing the inhibition of stormwater extracts on PCR reaction. Lanes 1 and 2: PCR negative control; Lane 3: PCR positive control; Lanes 4, 5, and 6 each contains 10^0, 10^{-1}, and 10^{-2} dilution of plasmid DNA containing adenovirus hexon gene insertion in water, respectively; Lanes 7, 8, and 9 each contains 10^0 dilution of plasmid DNA and 2.5, 0.25, and 0.025 μL of stormwater nucleic acid extract from CUC, respectively. The nested-PCR reaction was inhibited by greater than 0.25 μL of stormwater nucleic acid extract in a total of 25 μL reaction (lanes 7 and 8). The PCR is capable of detecting 10^{-2} dilution of plasmid DNA (2−10 copies of hexon gene) in the absence of PCR inhibitors (lane 6). The actual plasmid DNA concentration was not quantified.

flush effect"). While peak loading rates and EMCs of fecal pollution appear to decline over the sequence of three storms at MCF (Table 4), there is no evidence of a "first flush" effect within a given storm at any of the sites or storms sampled here; i.e., the fecal pollutant concentrations remain relatively constant over the storm hydrograph. Indeed, the simplest conceptual model that appears to explain our data is one in which fecal pollution is present at more-or-less the same concentration in every "mud-puddle" in the rain-impacted drainage area. The concentration of fecal pollution measured downstream therefore would be expected to remain constant as these mud puddles connect and cascade into large streamflows. This "mud-puddle" hypothesis (as opposed to the "buildup/wash-off" hypothesis described above) obviously requires a near limitless supply of fecal pollution in the watershed, which then begs the question of where these pollutants might be coming from. One possibility is that fecal indicator bacteria and F^+ coliphages are ubiquitously present on the urban landscape in southern California and rapidly partition into surface water as soon as the ground is wet by rainfall. This hypothesis is supported both by loading calculations (see below) and recent studies that suggest that bacteria adapted to grow in the soil environment may contribute to the fecal indicator concentrations in surface water (*8*, *25*−*27*). The fact that F^+ coliphage is not diluted over the storm hydrograph is especially surprising, given the long-held dogma that F^+ coliphages do not replicate in soil or water (*28*). Further investigation of the ecology of F^+ coliphage in the environment may shed light on the sources of F^+ coliphage in stormwater runoff from urban watersheds.

The fact that nearly all stormwater samples tested negative for human viruses is consistent with the idea that stormwater runoff is not contaminated with high concentrations of human sewage. However, PCR methods employed here are sensitive to PCR inhibitors carried by the stormwater, and these inhibitors are co-purified during the nucleic acid extraction, which can cause false negative assay results. Therefore, we cannot rule out the presence of human viruses in the stormwater runoff at concentrations less than 1 infectious human virus per mL. However, from the observed loading rates in Table 4, it is very unlikely that fecal indicator bacteria are from human sewage sources, particularly given the fact that the storm and sewer collection systems are separate in this watershed. At MCF during Storm 1, for example, the peak enterococci bacteria loading measured during our field studies is equivalent to 11 million people defecating directly into the river at once—more than twice the people that actually inhabit the entire watershed. This last calculation assumes a human production rate of fecal streptococcus (equivalent to enterococcus) of 4.5×10^8 MPN/human/day (*29*).

The results presented in this study underscore the conclusion that mitigation of fecal indicator bacteria pollution in stormwater runoff will be extremely challenging, given the nearly unlimited potential sources of these organisms and the extremely high volume of stormwater runoff that would need to be treated. The highest streamflow rate seen in these studies was over 200 m^3/s, and to our knowledge no stormwater treatment systems are currently available that can accommodate such high flow rates. In addition, the results presented here question whether such treatment would be appropriate (at least relative to the removal of fecal indicator bacteria), given the apparent ubiquity of nonhuman sources of fecal indicator bacteria in the watershed.

Acknowledgments

This study was funded by a joint grant from the National Water Research Institute (03-WQ-001) and the U.S. Geological Survey National Institute for Water Research (UCOP-33808), with matching funds from the Counties of Orange and San Bernardino in California. C.Q.S. is funded by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Program. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA. Partial support for the human virus and fecal indicator virus study was provided by Water Environment Research Foundation award 01-HHE-2a to S.C.J. We gratefully acknowledge the many people, especially Ryan Reeves, involved in the collection of data described in this paper, and the contribution of Weiping Chu of UCI for the PCR analysis of human viruses. The authors acknowledge the feedback from numerous colleagues, most notably P. Holden, R. Linsky, R. H. McCuen, B. F. Sanders, and colleagues from the Orange County Water District. We thank three anonymous reviewers who provided comments on the manuscript. The authors dedicate this paper to the memory of Ronald Linsky, former Executive Director of the National Water Research Institute, who provided the initial impetus for carrying out this study.

Supporting Information Available

Background information on the pollutants studied, watershed description, additional description of the field site, photographs (Figure S1) of each sampling site, and additional methods and materials on fecal indicator viruses and human pathogenic viruses. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) *USEPA Protocol for Developing Pathogen TMDLs*; EPA 841-R-00-002; National Service Center for Environmental Publications: Washington, D.C., 2001.

(2) Simpson, J. M.; Santo Domingo, J. W.; Reasoner, D. J. Microbial source tracking: state of the science. *Environ. Sci. Technol.* **2002**, *36*, 5279−5288.

(3) Moore, D. F.; Harwood, V. J.; Ferguson, D. M.; Lukasik, J.; Hannah, P.; Getrich, M. S.; Brownell, M. Evaluation of antibiotic resistance analysis and ribotyping for identification of faecal pollution sources in an urban watershed. *J. Appl. Microbiol.* **2005**, *99*, 618−628.

(4) Im, S.; Brannan, K. M.; Mostaghimi, S.; Cho, J. Simulating fecal coliform bacteria loading from an urbanizing watershed. *J. Environ. Sci. Health* **2004**, *A39*, 663−679.

(5) Tong, S. T. Y.; Chen, W. Modeling the relationship between land use and surface water quality. *J. Environ. Manage.* **2002**, *66*, 377−393.

(6) Paul, S.; Haan, P. K.; Matlock, M. D.; Mukhtar, S.; Pillai, S. D. Analysis of the HSPF water quality parameter uncertainty in predicting peak in-stream fecal coliform concentrations. *Trans. ASAE* **2004**, *47*, 69−78.

(7) Power, M. L.; Littlefield-Wyer, J.; Gordon, D. M.; Veal, D. A.; Slade, M. B. Phenotypic and genotypic characterization of encapsulated Escherichia coli isolated from blooms in two Australian lakes. *Environ. Microbiol.* **2005**, *7*, 631−640.

(8) Ferguson, D. M.; Moore, D. F.; Getrich, M. S.; Zhowandai, M. H. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *J. Appl. Bacteriol.* **2005**, *99*, 598−608.

(9) Ahn, J. H.; Grant, S. B.; Surbeck, C. Q.; DiGiacomo, P.; Nezlin, N.; Jiang, S. Coastal water quality impact of stormwater runoff from an urban watershed in southern California. *Environ. Sci. Technol.* **2005**, *35*, 5940−5953.

(10) Chigbu, P.; Gordon, S.; Strange, T. R. Fecal coliform bacteria disappearance rates in a north-central Gulf of Mexico estuary. *Estuarine, Coastal Shelf Sci.* **2005**, *65*, 309−318.

(11) Wilkinson, J.; Kay, D.; Wyer, M.; Jenkins, A. Processes driving the episodic flux of fecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. *Wat. Res.* **2006**, *40*, 153−161.

(12) Reeves, R. L.; Grant, S. B.; Mrse, R. D.; Copil Oancea, C. M.; Sanders, B. F.; Boehm, A. B. Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California. *Environ. Sci. Technol.* **2004**, *38*, 2637−2648.

(13) Pardue, J. H.; Moe, W. M.; McInnis, D.; Thibodeaux, L. J.; Valsaraj, K. T.; Maciasz, E.; Van Heerden, I.; Korevec, N.; Yuan, Q. Z. Chemical and microbiological parameters in New Orleans floodwater following Hurricane Katrina. *Environ. Sci. Technol.* **2005**, *39*, 8591−8599.

(14) Clesceri, L. S., Greenbert, A. E., Eaton, A. D., Eds.; *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, DC, 1999.

(15) Adams, M. H. *Bacteriophages*; Interscience Publishers: New York, 1959.

(16) Tsai, Y. L.; Sobsey, M. D.; Sangermano, L. R.; Palmer, C. J. Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. *Appl. Environ. Microbiol.* **1993**, *59*, 3488−3491.

(17) Jiang, S. C.; Chu, W. PCR detection of pathogenic viruses in southern California urban rivers. *J. Appl. Microbiol.* **2005**, *97*, 17−28.

(18) Pina, S.; Puig, M.; Lucena, F.; Jofre, J.; Girones, R. Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Appl. Environ. Microbiol.* **1998**, *64*, 3376−3382.

(19) He, J.; Jiang, S. C. Quantification of enterococci and human adenoviruses in environmental samples by real-time PCR. *Appl. Environ. Microbiol.* **2005**, *71*, 2250−2255.

(20) Sansalone, J. J.; Buchberger, S. G. Partitioning and first flush of metals in urban roadway storm water. *J. Environ. Eng. − ASCE* **1997**, *123*, 134−143.

(21) Jiang, S.; Dezfulian, H.; Chu, W. Real-time quantitative PCR for enteric adenovirus serotype 40 in environmental waters. *Can. J. Microbiol.* **2005**, *51*, 393−398.

(22) Reid, I.; Bathurst, J. C.; Carling, P. A.; Walling, D. E.; Webb, B. W. Sediment Erosion, Transport and Deposition. In *Applied Fluvial Geomorphology for River Engineering and Management*; Thorne, C. R., Hey, R. D., Newson, M. D., Eds.; John Wiley & Sons: Chichester, 1997; pp 106−113.

(23) Walling, D. E.; Moorehead, P. W. The particle size characteristics of fluvial suspended sediment: an overview. *Hydrobiologia* **1989**, *176/177*, 125−149.

(24) Horowitz, A. J. *A Primer on Sediment-Trace Element Chemistry*; Lewis Publishers: Chelsea, MI, 1991.

(25) Fujioka, R.; Sian-Denton, C.; Borja, M.; Castro, J.; Morphew, K. Soil: the environmental sources of E. coli and enterococci in Guam's streams. *J. Appl. Microbiol. Symp. Supplement* **1999**, *85*, 83S−89S.

(26) Solo-Gabriele, H. M.; Wolfert, M. A.; Desmarais, T. R.; Palmer, C. J. Sources of Escherichia coli in a coastal subtropical environment. *Appl. Environ. Microbiol.* **2000**, *66*, 230−237.

(27) Byappanahalli, M.; Fowler, M.; Shively, D.; Whitman, R. Ubiquity and persistence of E. coli in a mid-western coastal stream. *Appl. Environ. Microbiol.* **2003**, *69*, 4549−4555.

(28) Woody, M. A.; Cliver, D. O. Effects of temperature and host cell growth phase on replication of F-specific RNA coliphage Qbeta. *Appl. Environ. Microbiol.* **1995**, *61*, 1520−1526.

(29) Mara, D. D. *Sewage Treatment in Hot Climates*; John Wiley and Sons: Chichester, England, 1976.

Received for review March 23, 2006. Accepted April 5, 2006.

ES060701H