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Storm Drains are Sources of Human Fecal Pollution during Dry Weather in Three Urban Southern California Watersheds

BRAM SERCU,[†,‡] LAURIE C. VAN DE WERFHORST,[†,‡] JILL MURRAY,[§] AND PATRICIA A. HOLDEN*[,†]

Donald Bren School of Environmental Science and Management, University of California, Santa Barbara, California 93106-5131, Marine Science Institute, University of California, Santa Barbara, California 93106-5131, and City of Santa Barbara, Santa Barbara, California 93102-1990

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Coastal urbanized areas in Southern California experience frequent beach water quality warnings in summer due to high concentrations of fecal indicator bacteria (FIB). Remediation can be difficult, as sources are often unknown. During two summers, we sampled three urbanized watersheds in Santa Barbara, CA at sites with historically high FIB concentrations to determine if human fecal matter was influencing water quality. By quantification of a human-specific *Bacteroides* marker (HBM), human waste was evidenced throughout both transects, and concentrations were highest in the discharges of several flowing storm drains. The HBM concentrations in storm drain discharges varied by up to 5 orders of magnitude on the same day. While the exact points of entry into the storm drain systems were not definitively determined, further inspection of the drain infrastructure suggested exfiltrating sanitary sewers as possible sources. The HBM and FIB concentrations were not consistently correlated, although the exclusive occurrence of high HBM concentrations with high FIB concentrations warrants the use of FIB analyses for a first tier of sampling. The association of human fecal pollution with dry weather drainage could be a window into a larger problem for other urbanized coastal areas with Mediterranean-type climates.

Introduction

Microbiological coastal water quality is indicated by quantifying total coliform, either fecal coliform or *Escherichia coli*, and enterococci (*1*). These indicators are not necessarily pathogens, but epidemiology studies link them to swimmer illnesses, particularly when there are known pollution point sources (*2, 3*). Unfortunately, most pollution sources leading to beach closures are unknown (*4*). Finding human-associated sources by quantifying fecal indicator bacteria (FIB) is difficult because FIB are not specific to humans, survive in the environment (*5, 6*) to different degrees compared to pathogens (*7*), and can become nonculturable (*8, 9*). The first two issues lead to costly false-positive-based beach closures, and the third issue can lead to contaminated waters being posted as clean, thus not protecting public health.

In urban areas, FIB and pathogens may arise near shore from leaking sewer lines or septic systems (*10, 11*), natural features such as decaying wrack (*12*), algae (*13*), and coastal marshes with enterococci-generating waterfowl (*14*). Away from shore, urban infrastructure may discharge high loads of FIB and pathogens even in dry weather (*15*−*18*). However, high concentrations of FIB also arise from eroded sediments in channels and storm drains (*16, 18*) and possibly from soil banks and beyond (*19*). Thus, the simple association of FIB with urban infrastructure like storm drains reveals little about either the presence or source of specific wastes. To determine if human waste is present in fecal-indicator-contaminated waters, the human-specific *Bacteroides* genetic marker (HBM) can be analyzed (*20, 21*).

Although high FIB concentrations in Mediterranean climates often occur with precipitation (*20, 22*), and most of the annual FIB load is transported to the ocean during storms (*18*), human exposure is greatest during the dry summer months because of intensive beach use. Here, we studied dry-weather creek and drain waters from three urban reaches in Santa Barbara, CA for FIB and evidence of human-associated waste. We hypothesized human waste as a source of water contamination because the watershed reaches were within urbanized areas with low wildlife abundances. Longitudinal transects spanned fresh, to brackish lagoon, to coastal ocean waters, with sites historically high in dry-weather FIB concentrations. The study goal was to determine if human waste coincided with high FIB in this setting.

Materials and Methods

Study Sites and Sampling. We studied lower Mission and Laguna watersheds (ML) and Arroyo Burro watershed (AB), in the City of Santa Barbara, CA (Figure 1 and Supporting Information). Santa Barbara has a Mediterranean climate with an average rainfall of 359 mm, mostly (>85%) occurring in the period November through March. June through August are dry with an average total rainfall of 3 mm (*23*). In phase I, sampling occurred along the lower portions of each watershed (M1−M9 and A1−A10, Figure 1). Sites were selected based on historical FIB data from the City of Santa Barbara, and included flowing creek reaches, urban storm drains discharging into the creek, creek outlets into lagoons, and the coastal ocean. Working downstream to upstream, each site was sampled daily for three consecutive days (June 28−30, 2005 for ML and August 23−25, 2005 for AB) at approximately the same time. No rainfall had occurred for at least 48 days prior. Creek flows were constant at 0.016 $m^3 s^{-1}$ and 0.013 $m^3 s^{-1}$ at sites M5 and A5, respectively (SBC LTER: http://www.lternet.edu/sites/sbc/). Flow rates from discharging storm drains (sites M6 and A9) were measured by determining the time to fill a 1 L beaker (5−10 replicates). A follow-up study was performed on August 4, 2005, when bacteria concentrations and flow rates were measured again at locations M5 and M6 to calculate drain and creek contaminant fluxes.

In phase II, the storm drain networks upstream of M6 and A9 (Figure 1) were sampled. On August 2, 2005, M6 and three upstream drain locations were sampled (DM1, DM2, DM4, Figure 1). From August 15 to 17, 2006, M6 and upstream drain locations DM1−DM9 were sampled for three consecutive days. Location DM1 was at a continuous deflection

* Corresponding author e-mail: holden@bren.ucsb.edu; tel: 805-893-3195; fax: 805-893-7612.
[†] Donald Bren School of Environmental Science and Management, University of California, Santa Barbara.
[‡] Marine Science Institute, University of California, Santa Barbara.
[§] City of Santa Barbara.

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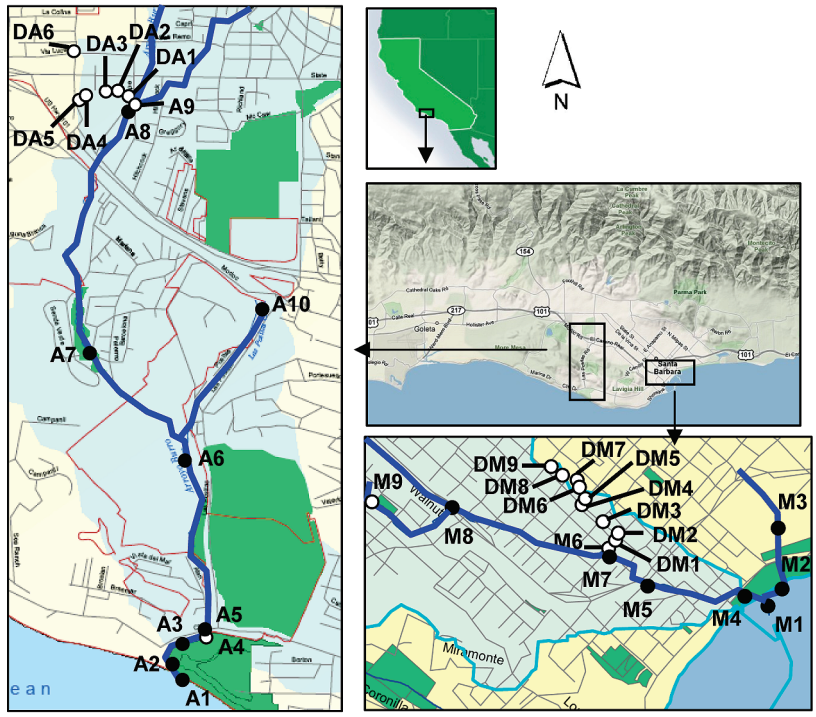

FIGURE 1. Maps (not to scale) of the sampling locations in the studied watersheds in Santa Barbara, CA. The largest magnifications show the numbered sampling locations in the creek (black symbols) and in the storm drains (white symbols).

separation (CDS) unit (for removing debris) just upstream of M6; DM2−DM5 were drains, and DM6−DM9 were sumps in a shopping mall. The A9 drain system in AB was also sampled for three consecutive days from September 5 to 7, 2006 (A9 and DA1−DA6, Figure 1). Samples DA1−DA6 were from drains located in the paved parking lot and roadways of a shopping center.

Water samples (approximately 2 L) were grabbed using a sterile beaker, passed through 25 μm pore size Miracloth (Calbiochem, San Diego, CA), and stored on ice until processing (within 6 h). When access was difficult (e.g., in deep manholes), water was pumped into the beaker using an ISCO 6712 sampler (Teledyne Isco, Inc., Lincoln, NE), with flushing between samples using sterile Nanopure water. Dissolved oxygen, temperature, and salinity were measured in the field with a YSI Model 85 meter (YSI Inc., Yellow Springs, OH), and pH was measured in the laboratory with a Corning pH meter 430 (Corning, NY). FIB (total coliform, *E. coli*, and enterococci) most probable numbers (MPNs) were quantified using the Quanti-Tray/2000 method, according to manufacturer's instructions (IDEXX Laboratories, Westbrook, MA). Water samples were diluted in sterile Nanopure water prior to analysis (between 1:10 and 1:1000, depending on the sample).

Fecal Source Samples. While this study was oriented toward determining if human sewage was present in creeks and storm drains, human feces and other host wastes (based on their relevance) were analyzed for potential analytical cross-reactivity. Waste samples included sewage, septage, human, gull, raccoon, dog, and cat feces. Raw sewage samples were collected from the influent at the El Estero Wastewater Treatment Plant (Santa Barbara, CA) on three separate dates (12/14/04, 10/24/05, 4/10/06). Septage was obtained from MarBorg Industries (Santa Barbara, CA) during septic tank pumping (9/8/2005) at a public restroom in a park that is visited by >100 persons per day. Human feces were from 3 individuals at a local hospital laboratory (12/14/2004). Gull feces were collected on two separate occasions (12/14/2004 and 3/28/2006) by baiting onto clean, plastic tarps. On each date, feces from a minimum of 3 individual gulls were scraped with Samplit Sterile Scoop & Container System disposable sampling scoops (Sterileware, Bel-Art Products, Pequannock, NJ) and composited into the attached vessel. Raccoon feces from 3 healthy individuals from the Santa Barbara Wildlife Care Network were similarly scooped from individual cages and composited. Dog and cat feces were composited from 3 healthy individuals.

DNA Extraction. The UltraClean Water DNA Kit (MoBio Laboratories, Carlsbad, CA) was used to extract the DNA from water samples. Water samples, sewage, and septage samples were vacuum filtered through 0.22 μm filters until either the collected volume was filtered or the point of refusal. Filters were stored at −20 °C until extraction. DNA was extracted according to the manufacturer's protocols, followed by ethanol precipitation. DNA was extracted from fecal samples using approximately 0.25 g wet weight feces in the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's protocols. Total DNA was quantified using the Quant-iT PicoGreen dsDNA kit (Molecular Probes/Invitrogen).

Human-Specific *Bacteroides* qPCR. The human-specific HF 183 *Bacteroides* 16S rRNA marker was quantified using SYBR Green I detection, as in Seurinck et al. (*21*), with the addition of fluorescein (Eurogentec, Belgium) in an iQ5 thermocycler (Bio-Rad, Hercules, CA). Reactions were run in 25 μL volumes, with 10 ng of sample DNA. Samples were diluted with molecular biology grade water (Sigma-Aldrich, St. Louis, MO). Samples were run in triplicate, including standards (5.6×10^1 to 5.6×10^7 targets) and nontemplate control. Standards were created by diluting purified PCR amplicons from raw sewage DNA extracts, using the human-specific HF 183 *Bacteroides* primers (*20*). Standard amplicon concentrations were quantified using the Quant-iT PicoGreen dsDNA kit (Molecular Probes/Invitrogen). Any qPCR well replicate that did not amplify, or that produced a Ct value below that of the lowest standard, was treated as a zero value. To ensure correct target amplification, a melt curve was verified for each sample. No severe PCR inhibition was found for the phase I samples, by checking DNA yield after

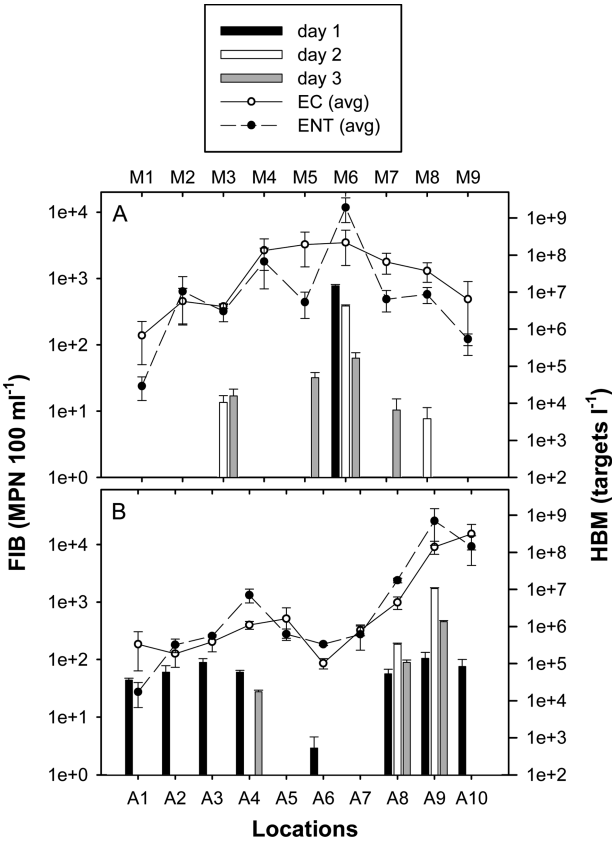

FIGURE 2. Concentrations of HBM and fecal indicators in (A) ML and (B) AB watersheds. HBM concentrations are shown as bars, for each day separately, while averaged 3-day concentrations are shown for EC and ENT as line graphs. Samples were from June 28 to 30, 2005 for ML and August 23 to 25, 2005 for AB. Error bars for EC and ENT represent SE of the mean across three days. Error bars for HBM represent the SE of replicate qPCR reactions for a single sample. Sites M6 and A9 are storm drains discharging during dry weather. ML, Mission and Laguna; AB, Arroyo Burro; HBM, human-specific *Bacteroides* marker; EC, *E. coli*; ENT, *Enterococcus* spp.; SE, standard error.

amplifying 16S rDNA using bacterial primers, even though less diluted template (50 ng) was used in those reactions. Potential inhibition in the phase II samples was also examined by running 2-fold template dilutions. No consistent increase in final HBM copy numbers per liter was observed after dilution, indicating no significant inhibition.

Statistical Analysis. FIB (*E. coli* and enterococci) and HBM concentrations were analyzed via one-way ANOVA and Dunnett's T3 multiple comparison, in SPSS version 12 (SPSS Inc., Chicago, IL). Only FIB results within the proper dilution range were statistically analyzed.

Results

Physicochemical Parameters, Indicator Bacteria and Human-Specific *Bacteroides* Across Watersheds. Dissolved oxygen was highest in two lagoons (M4 and A2) and low at a few locations (M3, M5, M6, A10). Water temperature was constant except for a low at M1 (ocean) and a high at M6 (drain); pH varied little, and salinity varied expectedly along the ocean to freshwater gradient (Table S1 in the Supporting Information). Total coliform values were not reported because at least one-third of the data were out of range. For ML, *E. coli* (EC) concentrations plateaued at sites M4, M5, or M6 (the drain), while enterococci (ENT) concentrations peaked at M6 (Figure 2A); for AB, the highest EC and ENT concentrations appeared at sites A9 and A10 (Figure 2B). However,

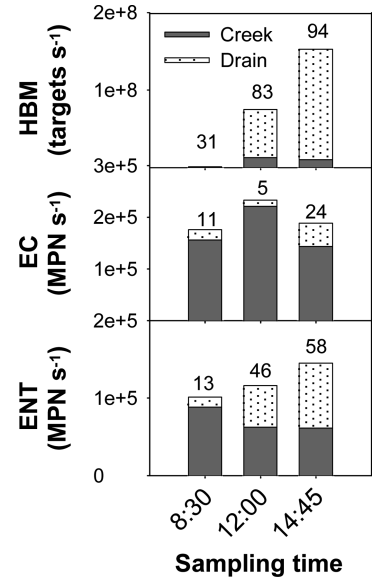

FIGURE 3. Relative contributions of upstream Mission Creek (M7) and an urban drain (M6) to bacterial loads (HBM, EC, ENT) in Mission Creek downstream of the mixing point of creek and drain, for three sampling times on 08/04/05. The percentage contribution of the drain (M6) is indicated above the bars.

posthoc testing for ML and AB revealed no significant differences between the sites for either EC or ENT.

The HBM concentrations in ML varied over several orders of magnitude (Figure 2A). On all sampling days, the maximum HBM concentrations ($P < 0.033$) were at M6 (1.7×10^5 to 1.5×10^7 targets L^{-1}), i.e., the discharging storm drain. HBM were observed at M3 for 2 out of 3 days, and only once at each M5, M7, and M8. Loads of HBM and FIB (targets or MPN per second) transported by the drain at M6 into ML were calculated as the product of drain discharge and concentrations. The drain delivered 31−94% of the load of HBM to ML, while its contribution to ML FIB was lower (5−24% for EC and 13−58% for ENT; Figure 3). In AB, sites A9 (drain) and A8 (downstream of the drain) consistently had the highest HBM concentrations (Figure 2B). Site A4 had HBMs on 2 out of 3 days and sites A1−A3, A6, and A10 had detectable HBM markers on the first day only.

All nonhuman fecal sources that were analyzed for HBM (gull, raccoon, cat, and dog) were negative. The HBM concentrations in human feces, septage, and sewage averaged $2.9 \pm 0.1 \times 10^7$ g^{-1} wet feces, $3.9 \pm 0.1 \times 10^9$ L^{-1}, and $7.8 \pm 0.2 \times 10^9$ L^{-1}, respectively (average ± standard error). The limit of quantification (LOQ), based on averaged sewage results, was approximately 0.0001% sewage (i.e., on the order of 0.5×10^3 to 10^4 L^{-1}).

Storm Drain Tracking. During phase I, storm drains M6 and A9 flowing during dry weather contained the highest HBM and FIB concentrations. Therefore, in follow-up studies, several locations were sampled within the drain networks to find the sources. In ML, high HBM concentrations were consistently observed at M6, but also in the storm drain system at DM1 and DM2 (Figure 4A). Significant temporal variability of the HBM signals occurred, with orders of magnitude concentration differences during August 15−17, 2006. Upstream from DM2, HBMs were only detected once (at DM4). The EC and ENT concentrations (Figure S1B, C) showed similar trends in that they were high between M6 and DM2. The more upstream drain samples from sites DM3−DM5 contained generally lower FIB concentrations, whereas the sump samples (DM6−DM9) contained the lowest FIB concentrations and only one positive result for DM9.

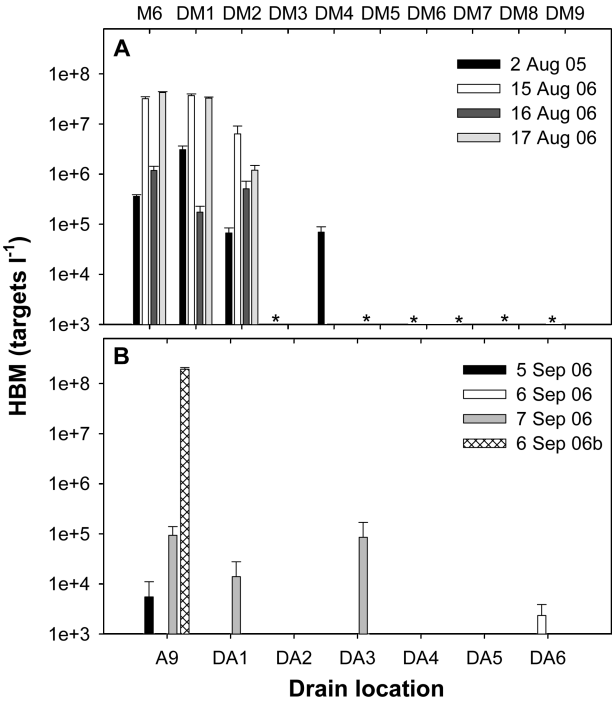

FIGURE 4. Concentrations of HBM in (A) M6 drain network in ML, and (B) A9 drain network in AB. * indicates where no samples were taken in 2005. Error bars represent SE of the mean ($n \geq 3$) of qPCR replicates for a single sample. ML, Mission and Laguna; AB, Arroyo Burro; HBM, human-specific *Bacteroides* marker.

During the 2006 storm drain sampling in AB (Figure 4B), lower HBM concentrations were measured at drain A9 ($\leq 9.4 \times 10^4$ targets L^{-1}) compared with 2005 ($\geq 1.4 \times 10^5$ targets L^{-1}, Figure 2B). Upstream, HBM were found once at each DA1, DA3, and DA6 (Figure 4B). The EC concentrations in the A9 drain network (Figure S2B) were higher than anticipated, and therefore some data were out of range (>24,196 MPN 100 mL^{-1}). Still, the EC concentrations appeared higher than in the M6 drain system, and increased from upstream to downstream (DA6 to DA4) on all 3 days (Figure S2B). The ENT concentrations in the A9 drain system (Figure S2B) were also higher than in the M6 drain system, and, not considering the out of range data, constant except for September 6, 2006. On that day, a sudden flow increase was observed in the A9 drain network. Whereas no HBMs were detected at A9 two hours earlier, $1.9 \pm 0.2 \times 10^8$ targets L^{-1} were associated with this pulse flow (Figure 4B), and EC and ENT increased at least 3 orders of magnitude (Figure S2B, C).

Consistent with the high concentration fluctuation described above, a high short-term temporal variability of the HBM signal (up to 3 orders of magnitude) is observed when coevaluating all concentrations from the storm drain outlets (Figure 2, Figure 4, and summarized in Figure S3). The variability time scale is on the order of hours to days. By one-way ANOVA, mean HBM concentrations were not significantly different on a time scale of months to years (Figure S3), suggesting chronic contamination ($P = 0.46$ for AB and $P = 0.47$ for ML). Qualitatively, the origin of HBM in the M6 drain discharge did not appear to be a sanitary sewer cross connection because there was neither visual evidence of large sewage-solids in the drain system, nor were there any televised cross connections or aberrant lateral flows between M6 and DM2 (personal communication from the City of Santa Barbara). Similarly, for A9, there was no visual evidence of sewage solids indicative of a sanitary sewer cross connection.

Correlations between HBM and FIB. Spearman rank correlations (ρ) between the log-transformed HBM, EC, and ENT concentrations were determined for all water samples and all drain samples, separately and pooled (Figure S4), while omitting out of range data (FIB) and data < LOQ (HBM). The log-transformed EC and ENT concentrations were significantly correlated ($P \leq 0.001$), with correlations of 0.74 (creeks), 0.69 (drains), and 0.80 (all samples). Log-transformed HBM and EC concentrations were only correlated in the drains ($\rho = 0.64$, $P = 0.001$) and when all samples were combined ($\rho = 0.67$, $P < 0.001$), while log-transformed HBM and ENT concentrations were significantly correlated in the combined data set only ($\rho = 0.58$, $P < 0.001$). The HBM concentrations were below the LOQ (500 targets L^{-1} in Figure S4) for most samples, therefore correlations between HBMs and FIB were based on within-range data only (i.e., 40 out of 110).

Discussion

This study sought to determine if human waste was a source of dry weather contamination in three Santa Barbara, California creeks where FIB were historically high, and to determine human waste environmental origins. Human waste markers were present throughout each system, and were entering the creeks from storm drains discharging flow continuously during dry weather.

Quantitative PCR for HBM indicated relatively concentrated human waste in the storm drains, and to a lesser extent in the creeks. Given that we measured average HBM concentrations of 7.8×10^9 markers L^{-1} in sewage (i.e., within the ranges found before (*21*)), M6 was discharging an equivalent of ~0.001−1% sewage into ML, and A9 and A4 were discharging ~0.0001−0.1% and 0−0.001% sewage, respectively, into AB. While elevated FIB concentrations in dry weather urban drain flow has been observed before (*17, 24*), this is the first report showing extensive human fecal pollution in separated storm drain systems. The temporal and spatial variations of HBM concentrations in the drains support that the HBM signal is not due to a false-positive background signal. A few other studies have shown the presence of HBM (*25, 26*) in urban creeks receiving storm drainage during dry weather. Here, storm drains were clearly implicated, particularly by the high HBM loads of drain M6 to Mission Creek.

In general, PCR assays using the HF183 marker are not cross-reactive for nonhuman fecal sources, including dogs (*21, 27−29*). Using the HF183 marker with qPCR and SYBR Green detection, we also did not observe any cross-reactivity with nonhuman fecal sources, similarly as before (*21*). However, one recent study, testing the same HBM assay using more fecal replicates, found 14% and 25% cross-reactivity for cat and dog feces, respectively (*30*). Therefore, it is theoretically possible that part of the HBM signal could be attributed to such pets. We expect this fraction to be minimal within the storm drains, however, as this infrastructure is shielded from the environment during the summer, and there is no reasonable point of entry via either subdrains or catch basins. Also, amplicons with a different melting temperature than the sewage-derived human marker were not detected. Although melting curve analysis only works if amplicons from different animal sources are not identical, it was useful to distinguish HBM amplicons in human versus chicken fecal waste (*21*).

On 08/04/05, the U.S. Geological Survey sampled several locations relevant for this study (M1, just upstream of M5, M6, M9, A1, and near A5) for real-time quantitative reverse transcriptase PCR detection of human enteroviruses (*31*) (John Izbicki, U.S. Geological Survey, written communication, 2008). Human enteroviruses were detected in duplicate

samples only at storm drain outlet M6, where we found the highest HBM concentrations. Enterovirus concentrations between 1882 and 7556 genomes/L were present, slightly higher than those found before in an urban drain just downstream of where the storm drain daylights from underground (*26*). Although the data set is limited for human enteroviruses, their distribution reinforces the HBM results here that revealed human fecal pollution.

Three potential sources could explain the elevated HBM concentrations in the drains: (i) in situ growth, (ii) direct contamination, e.g. through illicit cross-connections, and (iii) indirect contamination from nearby sanitary sewer lines. Theoretically, growth of *Bacteroides* spp. in aerated water may occur (*32, 33*), although PCR-based assays indicated only decay of spiked human-specific or other *Bacteroides* spp. in freshwater above 20 °C (*21, 34, 35*). While potential growth of *Bacteroides* spp. in biofilms or nonflowing drain sections (e.g., CDS unit) deserves further research, the temporal variability of the HBM signal here indicates a repeated external source rather than a continuous input from regrowth. Also, the detection of human enteroviruses (see above) in one drain indicates that human fecal pollution rather than bacterial regrowth causes high HBM concentrations. For M6, direct contamination from cross connections is unlikely because there were no visible sewage solids during sampling; this observation is supported by the City's television footage. In the A9 drain system, sewage solids were absent as well, but no televising was performed. Still unclear is the source of the observed pulse flow with very high HBM concentrations but no visual evidence for sewage contamination. However, for the M6 drain system, sewage may exfiltrate from buried sewer pipes and flow through unsaturated soil into storm drains. Maps of the storm drains and sewer lines (source, City of Santa Barbara), show that both the M6 and A9 drain systems are at depths similar to those of nearby sanitary sewer lines. Sanitary and storm sewers are known to exfiltrate and receive infiltration, and groundwater can be contaminated by exfiltration from sanitary sewers (*36*−*38*). Still, sanitary-to-storm sewer exfiltration is only one hypothesis: the exact origins of HBM within the storm drain system remained undefined, partly because of the confounding effects of variable flow during sampling.

The good correlations between EC and ENT concentrations in drains and creeks suggest similar sources and fates (decay, etc.) for these indicators. HBM concentrations correlated less with either EC or ENT concentrations, and importantly, any observed correlations were only valid in the quantifiable HBM concentration range. Samples having HBM concentrations < LOQ contained FIB concentrations spanning the entire range observed throughout this study, and thus did not support correlations between HBM and FIB. Our correlation analyses agree with previous observations of similar decay rates for EC and ENT (*5, 39*), but faster decay of DNA-based *Bacteroides* markers (*35*). Also, the limited number of samples harboring quantifiable HBM cautions for carefully interpreting any correlations to FIB: high HBM concentrations co-occurred with high FIB concentrations, but low HBM concentrations did not necessarily translate to low FIB concentrations. Thus, while sites were selected based on historically high FIB, the correlation analyses suggest it would not be possible to predict where HBM concentrations would be measurable based on FIB. Rather, a two-step approach as was performed here is still necessary, i.e., establish where FIB appear to be high, then resample to determine the presence and possible origins of human fecal pollution.

For protecting human health, assessing the presence of human-specific markers is likely valuable. However, if source tracking FIB sources is the goal, then other host-specific markers are also needed. For urban areas, markers for pets and raccoons are relevant. Recently, dog-specific qPCR assays (*30*) were published, but their sensitivity (63%) and specificity (33% detection of raw sewage) may not be optimal for samples with a high human component. As far as we know, neither cat- nor raccoon-specific markers are available. Further development and testing of host-specific markers will increase the understanding of FIB sources in the environment.

This study showed that urban drains can discharge human fecal waste into creeks during dry weather, but our findings do not necessarily extend to other urbanized areas. Some studies have reported either no or low levels of human-specific markers in storm sewers during dry weather (*40*−*42*). How many drains in urban environments are discharging human-associated waste? Based on our study and those prior (i.e., (*17*) and (*39*)), we also wonder to what degree storm drains discharge human-specific waste that migrates downstream into coastal zones during dry weather. Ultimately, a full quantification of the phenomenon, better models and appropriate decay parameters for relating upstream to downstream concentrations, as well as learning the ultimate origins of infrastructure-associated contamination will be crucial for informing coastal water quality management in urban settings.

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Supporting Information Available

More detailed descriptions of the study sites and data that are not provided in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

Manuscript title:

Storm drains are sources of human fecal pollution during dry weather in three urban Southern California watersheds.

Bram Sercu, Laurie C. Van De Werfhorst, Jill Murray, Patricia A. Holden

Contains:

8 Pages

1 Table

4 Figures

Materials and Methods

Study sites. The three adjacent watersheds all belong to the Santa Barbara coastal hydrologic unit, and consist of creeks flowing south from their headwaters in the mountains to the Santa Barbara Channel (1,2). The Mission Creek watershed area is approximately 7,203 acres. The upper watershed is dominated by open space (mixed forest and chaparral), while the lower watershed is urbanized with mostly residential and commercial land uses, and some open space. Sampling locations M4-M9 and DM1-DM9 belonged to the Mission watershed. The Laguna Creek watershed is the most urbanized of the three watersheds studied, and comprised of approximately 2020 acres of almost entirely urban land. Only 22% (mainly upper watershed) consists of open space, while the remaining area consists of residential and some commercial land uses. Sampling locations M2 and M3 belonged to this watershed. Mission Creek and Laguna Creek converge in a lagoon on the beach that periodically flows directly to the ocean (location M1). The Arroyo Burro watershed encompasses approximately 6,311 acres, with mostly open space in the upper watershed; commercial and residential in the middle watershed and suburban and rural residential with some open space in the lower watershed. Sampling locations A2-A10 and DA1-DA6 were all in this watershed. All watersheds have few or no agricultural lands. Both Arroyo Burro and Mission Creek are Water Quality Limited Segments in the Clean Water Act Section 303-(d) List and both terminate at beaches frequently posted with warnings against recreational use based on fecal indicator bacteria levels that Santa Barbara County measures weekly.

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Tables

Table S1. Sampling locations and physical-chemical parameters, indicated as mean (SE, n=3) for phase I samples.

Site ID	Description	DO (mg/l)	Temp (°C)	Salinity (ppt)	pH
Mission/Laguna					
M1	Surf zone	8.7 (0.3)	16.1 (0.4)	33.2 (0.3)	8.2 (0.1)
M2	Lagoon	7.4 (0.6)	19.2 (0.4)	2.5 (1.4)	7.7 (0.2)
M3	Creek	5.5 (0.2)	19.7 (0.0)	0.5 (0.0)	7.4 (0.0)
M4	Lagoon	10.0 (0.4)	18.9 (0.8)	6.4 (2.1)	8.3 (0.1)
M5	Creek	5.2 (0.3)	18.4 (0.1)	0.5 (0.2)	7.8 (0.0)
M6	Drain	5.8*	21.8*	0.0*	7.8 (0.0)
M7	Creek	6.9 (0.5)	18.7 (0.2)	0.7 (0.0)	7.8 (0.0)
M8	Creek	nd	nd	nd	7.9 (0.1)
M9	Drain	7.7 (0.2)	19.5 (0.5)	0.0 (0.0)	7.9 (0.0)
Arroyo Burro					
A1	Surf zone	8.4 (0.3)	17.7 (0.5)	30.6 (1.8)	8.2 (0.0)
A2	Lagoon	13.4 (0.6)	18.7 (0.5)	0.1 (0.0)	8.1 (0.0)
A3	Lagoon	8.5 (0.7)	18.4 (0.6)	1.1 (0.7)	7.9 (0.0)
A4	Drain	8.8 (0.5)	17.6 (0.5)	0.0 (0.0)	8.2 (0.0)
A5	Creek	7.3 (0.5)	17.9 (0.6)	0.2 (0.2)	7.8 (0.0)
A6	Creek	7.8 (0.5)	17.9 (0.6)	0.0 (0.0)	7.9 (0.0)
A7	Creek	8.2 (0.4)	18.0 (0.3)	0.0 (0.0)	7.9 (0.0)
A8	Creek	nd	nd	nd	8.1 (0.0)
A9	Drain	nd	nd	nd	8.1 (0.0)
A10	Creek	2.7 (1.3)	18.4 (0.8)	0.0 (0.0)	7.7 (0.2)

*not replicated
nd: no data available

Figures

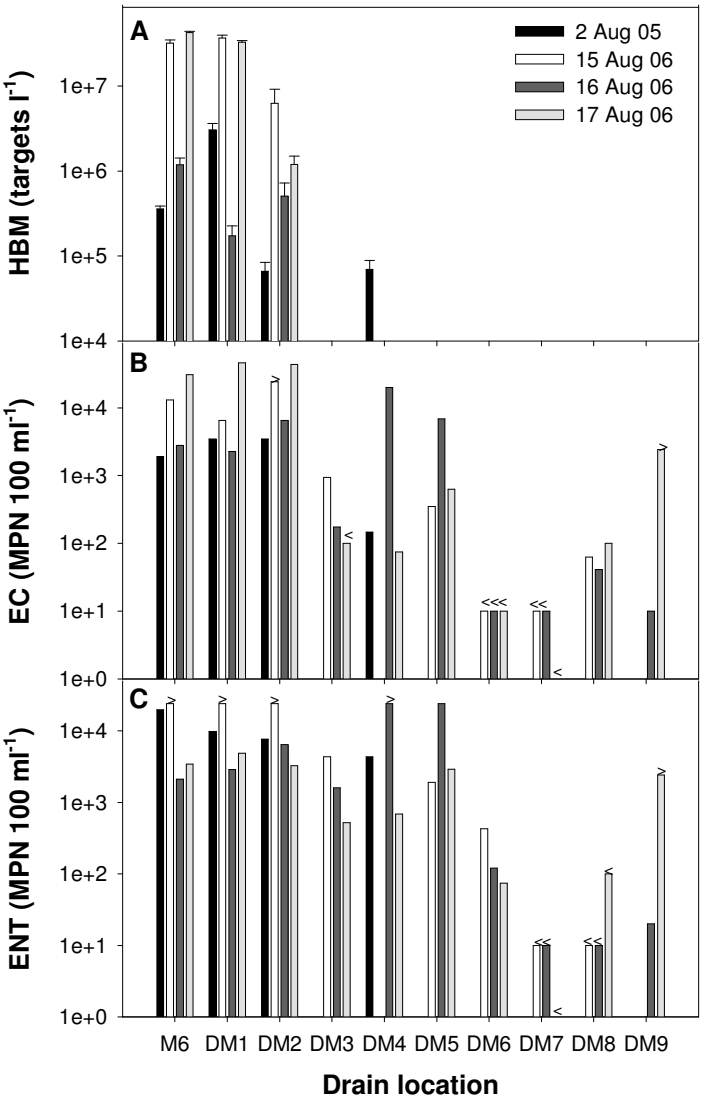

Figure S1. Concentrations of A) HBM, B) *E. coli*, and C) enterococci in the M6 drain network in the Mission Creek watershed. The absence of bars indicates that no data were available. The symbols "<" or ">" above a bar indicate out of range concentrations, with the actual concentrations being lower or higher than indicated by the bar, respectively. Error bars in part A) are the SE of the mean ($n \geq 3$) of qPCR replicates for a single sample.

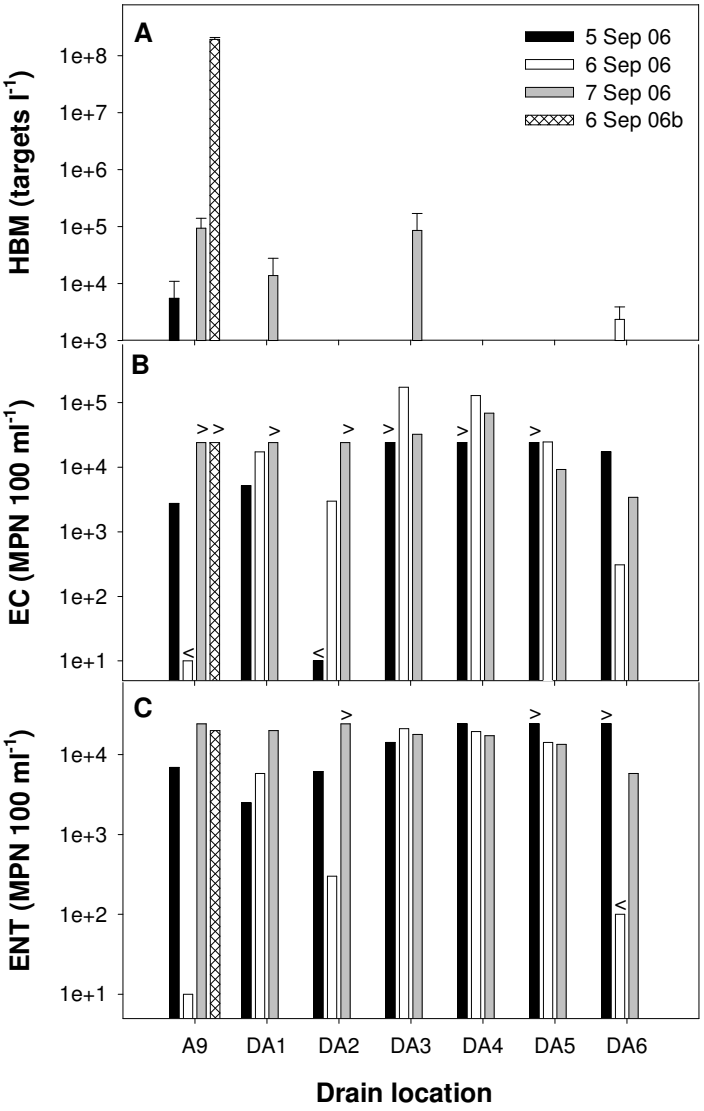

Figure S2. Concentrations of HBM and fecal indicator bacteria in the A9 drain network in the AB watershed. A) HBM, B) *E. coli*, C) enterococci. The symbols "<" or ">" above a bar indicate out of range concentrations, with the actual concentrations being lower or higher than indicated by the bar, respectively. Error bars in A) are the SE of the mean ($n \geq 3$) of qPCR replicates for a single sample.

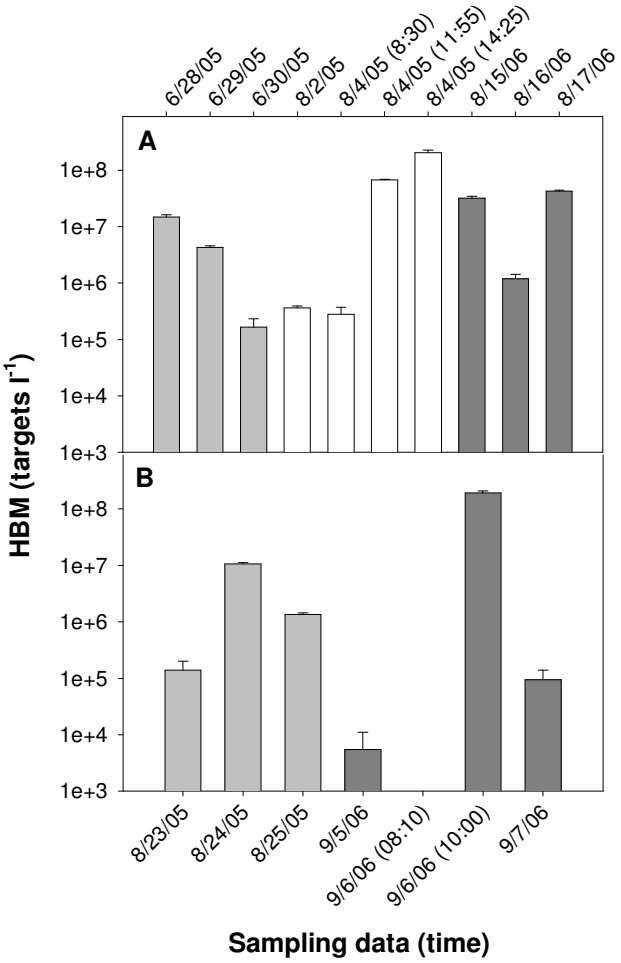

Figure S3. Summary of HBM concentrations associated with storm drain discharges into ML and AB for all sampling dates. A) Location M6. B) Location A9. Bars of the same shading were sampled within 3 days of each other. Error bars are SE of the mean ($n \geq 3$) of qPCR replicates for a single sample.

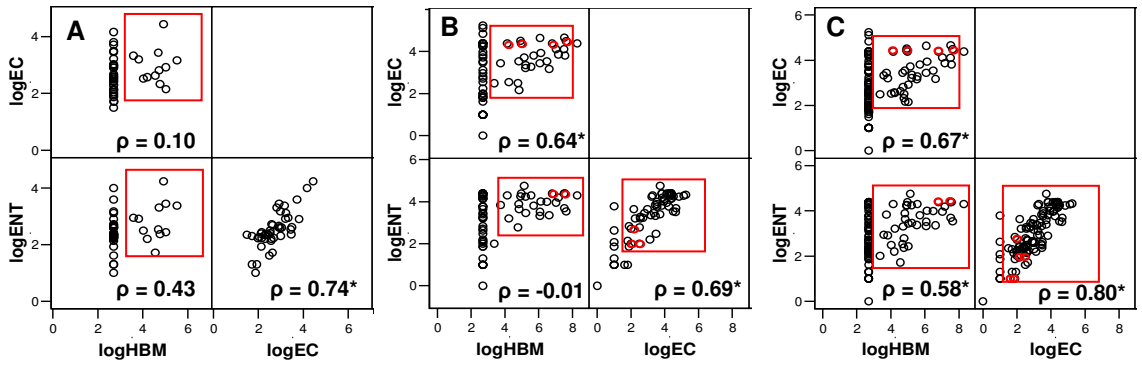

Figure S4. Scatter plots showing log-log correlation between HBM, EC and ENT for the two watersheds combined. A) creek samples only, B) drain samples only, C) creek and drain samples combined. Spearman's rank correlation coefficients (ρ) are indicated in each plot, with * if significant (all at $P \leq 0.001$). Non-significant correlations had $P \geq 0.05$. The subset of non-censored data, used for correlation analysis, was framed in the box, except red data points that were also out of range.