Tracking Human Fecal Sources In An Urban Watershed During Wet Weather

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SCCWRP Technical Report 1002

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EXECUTIVE SUMMARY

Identifying and remediating human fecal sources in wet weather discharges is currently amongst the largest problems vexing urban stormwater managers nationally. In this study, new technology – droplet digital polymerase chain reaction or ddPCR – is used to identify human fecal sources during wet weather in a watershed known to elicit public health concerns at the beach near its discharge following storm events. Thirteen sites located along the mainstem of the river or the end of major tributaries were sampled during two storm events between 2016 and 2017 wet seasons. Results indicated uniformly high $10^3 – 10^4$ *Enterococcus*/100 mL at every site during both events. Human sources appeared to be widely distributed in this watershed; all sites in both storm events had detectable levels of HF183 ranging from $10^1 – 10^4$ gene copies/100 mL. At the tributary with the greatest HF183 concentrations during the first storm, an illicit connection was identified and eliminated during the dry season between sampled storm events. However, HF183 concentrations of 10^2 gene copies/100 mL persisted in the second storm. This leads to the conclusion that human fecal sources are not arising from single locations, but are numerous and diffuse, potentially including exfiltration from the sanitary sewer collection system, septic leakage, and/or homeless populations (sanitary and storm drainage systems are completely separated in this watershed). HF183 concentrations were greater at all but one site during the second and larger storm event indicating that human sources may vary by storm size, which is consistent with recent epidemiologic data. Norovirus concentrations were patchily distributed reinforcing that ddPCR (or traditional QPCR)-based assays for this pathogen are not recommended as a primary source tracking tool.

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INTRODUCTION

Fecal indicator bacteria associated with wet weather discharges to receiving waters are perhaps the most difficult urban stormwater problem nationally (Noble et al. 2003, Parker et al. 2010, Sauer et al. 2011). Current water quality criteria focus on fecal indicator bacteria, like *Enterococcus,* because they are predictive of health risk from contact with recreational waters (Prüss 1998, Wade et al. 2003, Colford et al. 2007, Wade et al. 2010). *Enterococcus* concentrations in wet weather discharges and receiving waters are routinely high and exceed water quality criteria during and immediately following storm events, regardless of rainfall characteristics, land use, and season (Geldreich et al. 1968, Schiff et al. 2001, Noble et al. 2003, He and He, 2008, Sauer et al. 2011). As a result, identifying sources of *Enterococcus* in discharges for remediation has proved difficult. This challenge dramatically increases when searching for human specific sources of *Enterococcus* because this genus is not human specific. *Enterococcus* can arise from many different sources including warm-blooded animals such as pet waste or wildlife (Jiang et al. 2007, Lu et al. 2013, Ervin et al. 2014), soils (Anderson et al. 2005, Byappanahalli et al. 2012), and colonization of storm drains and beach wrack (Olapade et al. 2006, Sercu et al. 2009, Mote et al. 2012).

Recent studies have shown that surfers in San Diego, California, USA who entered the ocean following wet weather discharges, had a greater risk of gastrointestinal illness compared to surfers who did not enter the ocean or entered the ocean during dry weather (Arnold et al. 2017). The increased risk of gastrointestinal illness during wet weather was also associated with increasing *Enterococcus* concentrations and the presence of human specific pathogens in the wet weather discharge (Soller et al. 2017).

While epidemiology studies like those in San Diego compel stormwater managers to identify and remediate human sources of fecal contamination, only recently has technology been capable of accomplishing upstream source tracking during wet weather. Particularly in urban watersheds, the genetic tools previously available for source tracking, such as quantitative polymerase chain reaction (qPCR), were prone to major methodological challenges including, but not limited to, interferences from humic compounds (Cao et al. 2012).

Droplet digital polymerase chain reaction (ddPCR) is an emerging technology that improves upon qPCR, potentially enhancing human fecal source tracking during wet weather. ddPCR overcomes interferences because it does not require a calibration curve for quantification like qPCR (Cao et al. 2015). Additionally, the use of droplet generation and detection increases sensitivity thereby lowering the quantitative detection limit. Because of these advantages, ddPCR has now been adapted to some of the most sensitive and specific human source tracking genetic tools such as *Bacteroidales* HF183 (Cao et al. 2015, Boehm et al. 2013).

The goal of this study is to use ddPCR for identifying upstream sources of human fecal contamination during wet weather. The study will source track the same San Diego watershed whose discharge resulted in illness to surfers following storm events. The primary goal is to assess if human fecal sources, as defined by the presence and concentration of HF183 and norovirus, are confined to a single location (i.e., tributary) or widespread and measured systemically throughout the watershed. If the human-associated fecal markers occur only downstream of a single location, this would be consistent with a point source of contamination

(e.g., a broken or overflowing sewage pipe). If the human source is systemic, the human source markers should be more diffuse (i.e., widespread sewage collection system exfiltration, multiple large homeless encampments, etc.) and should occur in most samples, irrespective of location. Regardless, the findings from this study represent the first stage in a multi-stage approach to tracking human fecal sources upstream in a large watershed during wet weather.

MATERIAL AND METHODS

Study Design

This study utilized a mass-based design to support upstream source tracking (Noble et al. 2006). Thirteen sites were sampled for this study, seven at the base of major tributaries and six mainstem locations below the confluence of the major tributaries (Figure 1). One mainstem site was sampled at the top of the watershed and a second in the estuary at the very end of the watershed just prior to reaching the Pacific Ocean. Two storms were collected, approximately 12 months apart, in January 2016 and again in February 2017. Rainfall amounts and runoff flow were measured at each site. *Enterococcus*, HF183, and human norovirus concentrations were measured in composite samples collected from every site. This enables both concentration and mass-based comparisons among sites and storms.

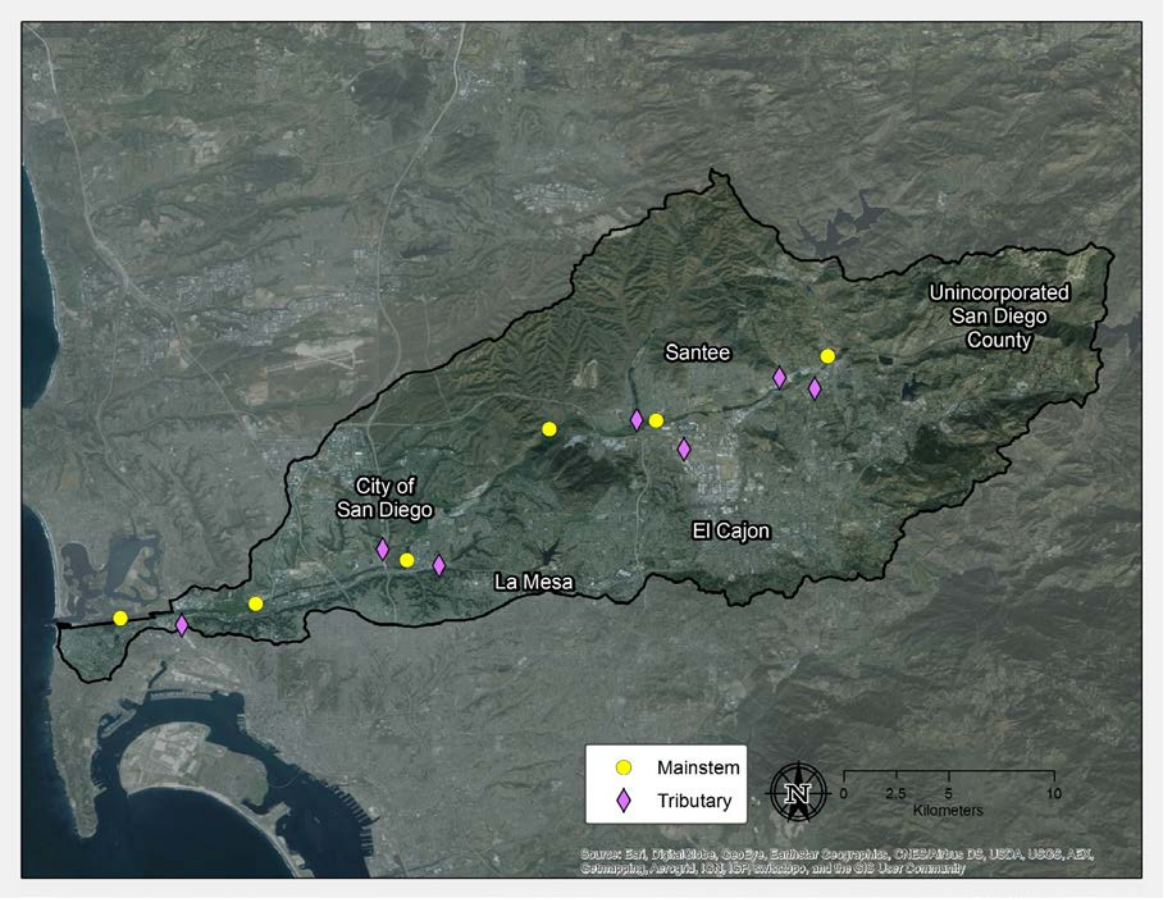

Figure 1. Map of the San Diego River Watershed including study sampling sites.

Watershed Description

The San Diego River watershed extends across 1,124 km^2 and is comprised largely of urban land uses and extensive open lands, with limited agricultural uses (SANDAG 2015). The upper watershed is separated from the lower watershed by two major dams, which capture runoff from predominately open space including national forest. Below these dams, the watershed area is 450 km^2, and the top three land uses are open lands (44%), single family residential (20%), and roads (8%). During the two storms measured, no water was discharged from the dams.

The wet season in San Diego extends from October to April, but the greatest historical average precipitation falls in the months of January through March (Ackerman et al. 2003). Precipitation in the 2016 wet season (18.9 cm) was below the long-term average of 25.5 cm/year, while the precipitation in the 2017 wet season (29.8 cm) exceeded the long-term average (NOAA NCDC).

Because of the few, but intense rainfall events in San Diego, storm drain systems are physically separate from sanitary sewer collection systems in the San Diego River watershed effectively enhancing flood protection and nullifying combined sewer overflows.

Field and Laboratory Methods

Precipitation and Flow Measurement

Precipitation was measured using Sigma™ tipping bucket rain gages (Hach, Loveland, CO), which measure precipitation in 0.025 cm increments. Flow was measured using stage-discharge relationships, stage-velocity measurements, or both. Over 6,300 data points were collected to establish stage-discharge relationships across multiple storm events across all sites. Stage measurements were made using either a Sigma 950 Submerged AV sensor bubbler (Hach, Loveland, CO) or Onset HOBO level logger pressure transducer (Onset Computer Co., Bourne, MA). Velocity measurements were made using a Hach acoustic Doppler sensor (Hach, Loveland, CO). Accuracy for stage-discharge relationships was estimated at 2-20% and stage-velocity measurements at 0.3-55%. Flow was not measured at the Ingraham Bridge site, located within the estuary, since this waterbody exhibits two-way tidal flows twice daily.

Stormwater Sampling

Flow-paced composite samples were collected for water quality analyses. A minimum of 12 samples were collected per composite to minimize bias and maximize precision (Leecaster et al. 2002). Samples were collected autonomously using peristaltic pumps and sterilized Teflon tubing with a stainless-steel intake screen mounted to the channel bottom. Most sites had no flow during dry weather. Sampling was initiated when stage increased > 5 cm, sufficient to cover flow sensors and pump intakes. At the tidal site and at the Upper Eucalyptus Hills site where flow could not be measured accurately, time weighted composite samples were collected. To ensure stormwater flows were sampled at the tidal site, sampling was conducted when salinity dropped below 22 parts-per-thousand (approximately one-third freshwater).

Composite samples were collected for six hours before transport to the laboratory to maintain holding times for microbiological analyses. Up to three composite samples were collected per site for a total sample time of 18 hours, or until flow decreased below 5 cm stage.

Enterococcus Cultivation

Cultivable *Enterococcus* were measured using Enterolert and the Quantitray 2000™ system (IDEXX, Westbrook, ME), as per the manufacturer's instructions, with three dilutions covering a 100,000-fold range of concentration. Field and equipment blanks were collected and tested for *Enterococcus* contamination in the same manner as regular samples. Laboratory blanks were performed using sterile phosphate buffered saline solution.

Filtration and Extraction of Bacteria and Viruses

Briefly, 100 ml of stormwater was filtered in triplicate on a vacuum manifold through 47 mm diameter, 0.4 μm polycarbonate filters (Millipore Type HTTP, Millipore, Bedford, MA) to capture bacterial DNA. To capture viral RNA and DNA, 250-500 ml of stormwater was adjusted to pH 3.5 using 20% HCl, and $MgCl_2$ was added at a final concentration of 25 mM. The adjusted stormwater was then filtered in triplicate on a vacuum manifold through 47 mm diameter, 0.45 mixed cellulose ester filters (Millipore Type HA, Millipore, Bedford, MA). The filters were folded and placed into microcentrifuge tubes. Tubes were flash frozen in liquid nitrogen, and stored at -80°C until extraction. A filter blank was also collected for every sampling event as follows: autoclaved PBS solution was filtered, flash frozen in liquid nitrogen and stored at -80°C until extraction.

Filters for bacterial DNA analyses were extracted using commercial kits (DNA EZ RWO4, GeneRite, Mammoth Junction, NJ, USA) following previously published methods (Cao et al. 2015, Boehm et al. 2013, Layton et al. 2013). Salmon testes DNA was added to the lysis buffer prior to extraction as an external extraction and inhibition control following previously published methods using USEPA method 1611 (Haugland et al. 2005). Negative Extraction Controls (NEC) containing only lysis buffer and salmon testes DNA were processed for every extraction in the same manner as the samples.

Filters for viral RNA were extracted by two methods over the course of the study. Method A: PowerViral Environmental RNA/DNA Extraction Kit (formerly MoBio Laboratories, Carlsbad, CA, presently AllPrep PowerViral DNA/RNA kit, QIAGEN, Germantown, MD) with addition of bead-beating, 1:1 phenol:chloroform, and β-mercaptoethanol according to the manufacturer's instructions. Method B: NucliSens MiniMag RNA Extraction Kit (BioMerieux, Durham, NC) according to the manufacturer's instructions. NECs were prepared regardless of method by the addition of 10 ng mouse lung RNA (Zyagen, San Diego, CA) to the lysis buffer and processed in the same manner as the sample in order to serve as carrier nucleic acids and as a combined extraction and inhibition control. Extracted samples were processed on the same day whenever possible to avoid nucleic acid degradation by freeze-thaw cycles.

Genetic-based *Enterococcus*, human-associated molecular source marker (HF183), and human norovirus genotype I and II were measured using ddPCR (Schiff et al. 2016, Steele et al. in review). Human-associated *Bacteroidales* and *Enterococcus* were measured using a duplex digital PCR assay following a previously published protocol (Cao et al. 2015). Human norovirus gene copies were quantified using a digital RT-PCR assay following a previously published protocol (Schiff et al. 2016, Steele et al. in review).

Quality Assurance

Bubblers, pressure transducers, AV sensors, and pump volumes were tested and calibrated prior to storm sampling. Field and equipment blanks detected no microbial targets. All laboratory blanks did not detect microbial targets and all duplicate samples met the data quality objective of < 25% reproducible percent difference.

RESULTS

The 2017 storm was roughly three times larger than the 2016 storm on the San Diego River (Table 1). Between January 31 and February 1, 2016, precipitation ranged from 1.1 to 1.7 cm, monotonically increasing from the lower to upper watershed. In contrast, between February 17 and 18, 2017, precipitation ranged from 2.69 to 3.48 cm, monotonically decreasing from the lower to upper watershed.

Sampling success was near 100% (Table 1). The most upstream main channel site (Channel Road) observed no flow; this site is located directly below two large dams and both flood control structures reported no discharge during both wet seasons.

Watershed discharge at the most downstream, Fashion Valley, was 3.7 times larger during the 2017 storm event compared to 2016 storm event (Table 1). The Fashion Valley site recorded 18.9 million ft^3 discharge volume during the January 31-February 1, 2016 storm, and 69.2 million ft^3 during the February 17-18, 2017 storm. The difference in runoff volumes was a function of precipitation quantities and cumulative precipitation for the wet season (beginning October 1). Antecedent precipitation was 1.4 times greater 2017 (20.5 cm) compared to 2016 (14.9 cm), likely increasing soil moisture content and decreasing depth to water table.

All sites had detectable levels of *Enterococcus*, but no site consistently had anomalously high or low concentrations, and no spatial pattern was evident (Table 2). *Enterococcus* concentrations ranged from 10^2 to 10^4 MPN/100 ml during both storm events. The highest *Enterococcus* concentrations were at Los Coches in both the 2016 and 2017 storms, but comparable concentrations exceeding 10^4 MPN/100 ml occurred at multiple sites each year. There was no consistent relationship with rainfall; the higher precipitation and stormwater volume in 2017 did not produce a consistent increase or decrease in concentration at each site. *Enterococcus* concentrations at Los Coches, Forrester Creek, and Morena Blvd all decreased in in 2017 compared to 2016.

Table 1. Storm characteristics and sampling success.

SITE	TRIB/ MS	Latitude	Longitude	Precipitation (cm)		Discharge Volume (Million cu. Ft.)		Sample Percent Capture	
				2016	2017	2016	2017	2016	2017
SDR @ Channel	MS	32.86519	-116.927016	1.42	2.84	-[a]	-[a]	-[a]	-[a]
Eucalyptus Hills	TRIB	32.86747	-116.944943	1.42	2.84	-[b]	0.2	100	100
Los Coches	TRIB	32.85709	-116.929587	1.42	2.54	1.9	3.1	81	97.5
SDR @ Carlton Hills	MS	32.84333	-116.997522	1.27	2.92	4.1	8.5	100	100
Forrester Ck	TRIB	32.83119	-116.98562	1.60	2.69	3.7	13.5	100	100
Sycamore Cyn Ck	TRIB	32.84398	-117.006431	1.27	2.92	1.8	5.4	100	96.9
SDR @ Mission Trails	MS	32.838879	-117.045112	1.65	3.38	9.7	33.2	100	100
SDR @ Mission Rd	MS	32.78386	-117.10413	1.22	3.45	14.5	42.4	100	100
Alvarado Ck	TRIB	32.78145	-117.0904	1.24	3.45	4.9	11.9	100	100
Murphy Cyn Ck	TRIB	32.79448	-117.112834	1.04	3.45	2.1	5.2	100	99.5
SDR @ Fashion Valley	MS	32.76508	-117.168689	1.04	3.48	18.9	69.2	100	100
Morena Blvd Drain	TRIB	32.76036	-117.201957	1.04	3.48	0.06	0.6	100	95.2
SDR @ Ingraham	MS	32.75901	-117.226552	1.04	3.48	-[c]	-[c]	100	100

[a] No flow occurred at this site. No sample collected due to lack of flow.
[b] No flow collected at this site in 2016
[c] No flow collected at this site due to tidal surcharge

Table 2. Wet weather concentrations of fecal indicator bacteria (*Enterococcus*), human genetic marker (HF183) and human pathogen (Norovirus I & II) in the San Diego River Watershed.

SITE	Tributary or Main Stem	Enterococcus (Culture)		HF183 (ddPCR)		Norovirus (ddPCR)	
		2016	2017	2016	2017	2016	2017
SDR @ Channel	MS	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]
Eucalyptus Hills	T	10,250	20,278	1,480	2,984	<[b]	<[b]
Los Coches	T	30,342	21,492	199	5,971	26	<[b]
SDR @ Carlton Hills	MS	2,644	5,020	113	317	<[b]	112
Forrester Ck	T	18,444	9,324	3,084	3,100	<[b]	<[b]
Sycamore Cyn Ck	T	3,619	4,540	378	889	<[b]	<[b]
SDR @ Mission Trails	MS	8,176	8,078	1,334	1,697	<[b]	<[b]
SDR @ Mission Rd	MS	270	1,824	17	3,212	12	<[b]
Alvarado Ck	T	1,203	7,031	144	5,173	<[b]	<[b]
Murphy Cyn Ck	T	4,396	10,142	2,148	3,466	<[b]	<[b]
SDR @ Fashion Valley	MS	866	6,402	554	2,270	49	<[b]
Morena Blvd Drain	T	14,400	7,043	16,240	2,449	280	168
SDR @ Ingraham	MS	491	4,099	238	1,533	<[b]	148

[a] No flow so no sample collected
[b] Below detection limit

All sites had detectable levels of HF183, but no site consistently had anomalously high or low concentrations, and no spatial pattern was evident (Table 2). Concentrations of HF183 ranged from 10^1 to 10^4 during the 2016 event, and 10^2 to 10^3 during the 2017 event. Morena Blvd. was the only site to decrease in HF183 concentration from 2016-2017. There was no consistent relationship between *Enterococcus* and HF183, suggesting that these indicators came from different watershed sources. Likely, there were more than just human sources of *Enterococcus*.

While the 2017 storm had approximately double the rainfall of the 2016 storm, the HF183 mass discharged at the last gaged site on the mainstem (Fashion Valley) had roughly 15 times the mass observed in 2016 (Figure 2, Table 2). The mass accumulation along the mainstem increased by almost 90% between Carlton Hills and Mission Road (between river kilometer 28.6 and 14.7). Although Forester Creek, which had the greatest tributary input of HF183, connects with the mainstem within this span, the increase between Mission Trails and Mission Road, where the bulk of the increase takes place, had no measured tributary inputs (Figure 3). Systemwide, the cumulative tributary HF183 mass inputs accounted for approximately 85% of the mass discharge from the watershed, suggesting that 15% of the HF183 mass inputs arise from in-channel sources or small unsampled drains that discharge directly to the mainstem.

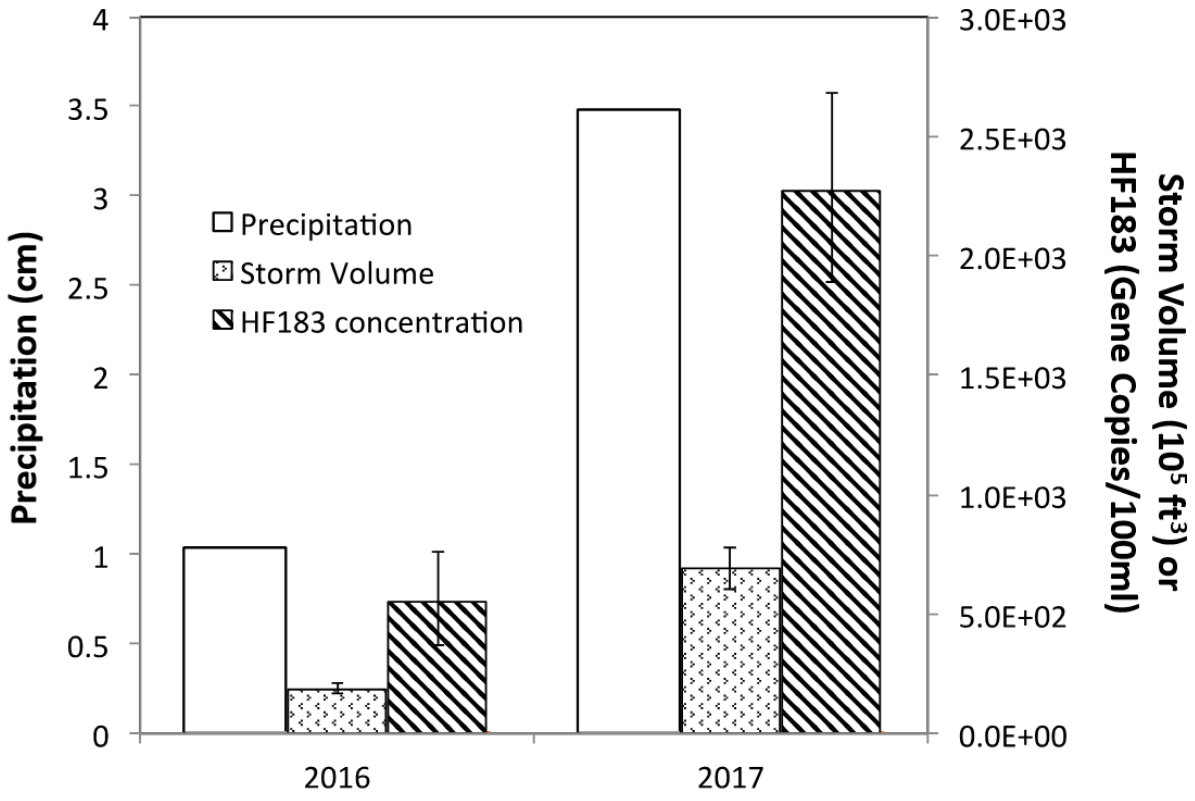

Figure 2. Comparison of precipitation, storm volume (per 10^5 $ft.^3$) and HF183 concentration from the San Diego River watershed between the 2016 and 2017 storm events.

In contrast to *Enterococcus* and HF183, pathogens were patchily detected (Table 2). Norovirus was detected in less than 31% of the 13 sites across both storms. Only one site detected norovirus in both storms (Morena Blvd Drain). Infrequent and inconsistent detection of norovirus precluded any correlation to HF183 or *Enterococcus*. However, detection of any human enteric virus in stormwater samples confirms the presence of human fecal material.

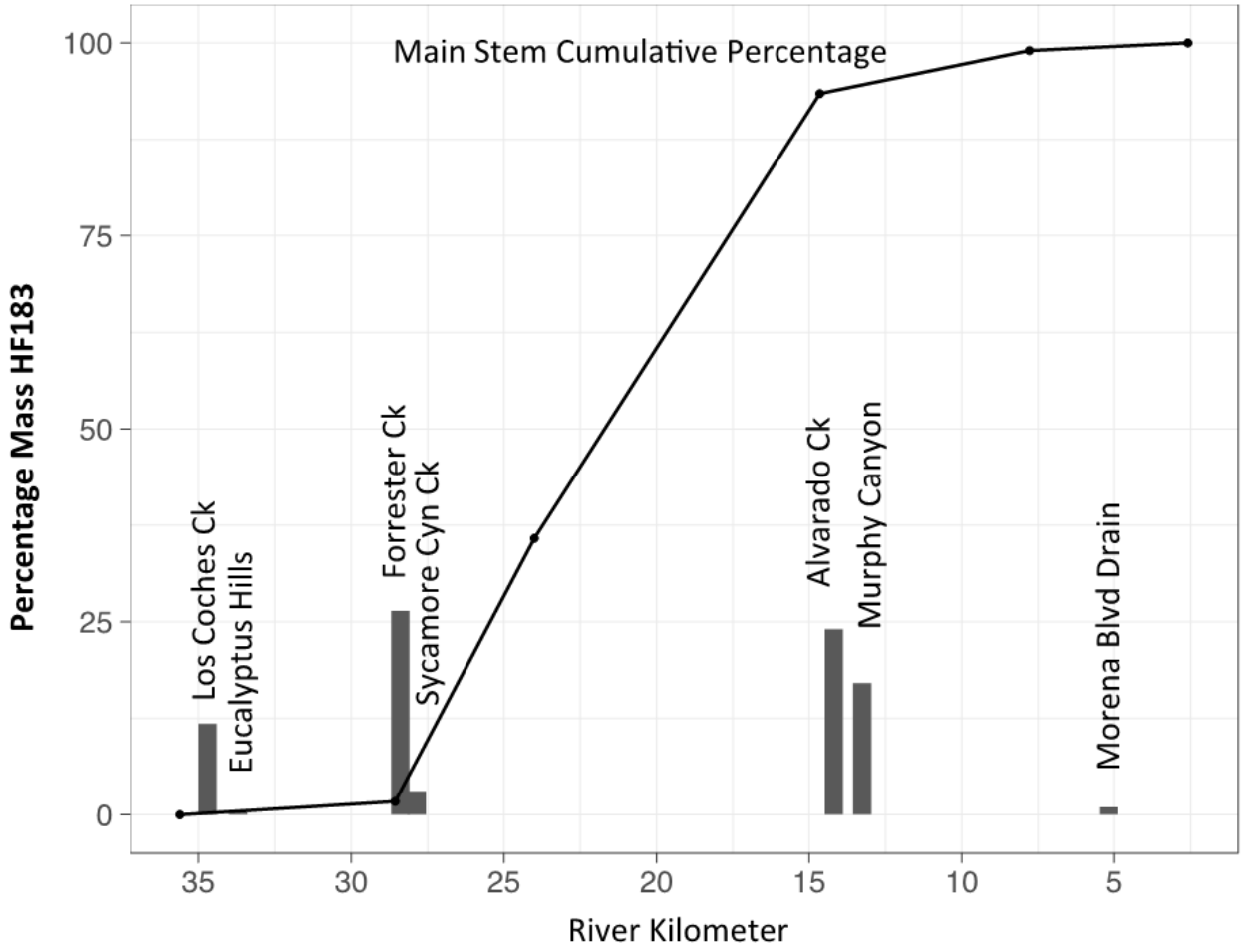

Figure 3. Accumulation of HF183 mass (%) along the mainstem of the San Diego River (line), and the relative mass inputs (as a percentage of the most downstream mainstem site) from major tributaries during the February 2017 storm.

DISCUSSION

It appears that human fecal inputs occur ubiquitously throughout the San Diego River watershed during wet weather. HF183 was detected at every site in both sampled storm events. This ubiquitous human signal occurred in both large and small tributaries, and along the mainstem. At the Morena Blvd site following the first storm, upstream dry weather investigations identified an illicit connection and illegal dumping. Yet, even when these human sources were removed, HF183 was still detected during the second storm albeit at lower concentrations. No wet weather sanitary sewer overflows were reported during the sampled events.

Human sources in dry weather discharges are not unique. Sercu et al. (2011) found HF183 in a small coastal catchment, ultimately linked to a leaking sewage collection system. Noble et al. (2006) measured pathogens in dry weather discharges from Ballona Creek, a large urban watershed draining the City of Los Angeles. Jiang et al. (2001) measured human pathogens in

coastal dry weather discharges from Orange County, California. However, studies measuring HF183 and pathogens in wet weather discharges have rarely been conducted (Soller et al. 2017).

The next stage of wet weather source tracking on the San Diego River is to further refine the human source signal. There are potentially four sources of HF183 in the San Diego River watershed; exfiltration from the sewage collection system, septic system contributions, direct deposition from homeless populations, and illegal discharges of human sewage to the storm drains (e.g., discharges from recreational vehicles or connection of sewage laterals to the storm drain system). There are no combined sanitary/stormwater collection systems in this watershed - sanitary and storm drainage systems are completely separated - so combined sewer overflows are not a source. At over 3,000 linear miles, the sewage collection system in the San Diego River is large and complex. Much of the watershed, particularly the upper watershed, was developed in the last five decades. However, there are portions of the watershed that were developed over 100 years ago. Regardless, HF183 was detected from both newer and older portions of the watershed. The municipal agencies responsible for the collection system conduct routine and ongoing inspections and maintenance (including cured-in-place lining) and large volume sanitary system overflows are rarely reported (http://www.waterboards.ca.gov/water_issues/programs/sso/sso_map/sso_pub.shtml). Septic systems are concentrated in the upper watershed, however, pockets of septic systems occur throughout the watershed where an estimated 11,400 septic systems exist (pers. communication San Diego Regional Water Quality Control Board). HF183 was consistently detected at the Upper Eucalyptus Hills site, comprised almost all of septic systems with limited sewage collection infrastructure. The estimated homeless population is near 300 people (personal communication Rob Hutsel San Diego River Park Foundation) located in encampments along reaches of the San Diego River between the coast and the City of Santee located 29 km (18 miles) upstream. The largest encampments exist along the mainstem between Fashion Valley and Mission Trails. The exact number of homeless that defecate into the river directly, or latrine on the banks of the river only to get flooded as storm flows rise, is unquantified. However, HF183 was detected upstream of these sites and in-stream mass inputs of HF183 along the mainstem were estimated near 15%, in 2017. Thus, human fecal inputs are likely not exclusive to the homeless population. While it is impossible to quantify the contribution of illegal discharges, it could contribute to both the tributary and mainstem HF183 loads.

While the study was not designed to estimate the relative human fecal contamination in wet weather discharges, pilot data can provide preliminary estimates (Table 3). Eight samples of wastewater influent to the Point Loma Sewage Treatment Plant were collected over the two wet seasons. Concentrations of HF183 in untreated wastewaters averaged 10^6 gene copies/100 mL, consistent with the range of concentrations reported by others (Boehm et al. 2015, Ahmed et al. 2017). Wet weather discharge concentrations of HF183 averaged 10^3 gene copies/100 mL at the most downstream sampling location in the San Diego River. If the sewage collection system were the only source of HF183, then assuming simple dilution, an average of 0.1% human fecal inputs from sewage could account for the concentrations observed. This simple assumption is independent of mixing and decay. Ultimately, regardless of source, the widespread occurrence of HF183 points to a system-wide issue with multiple sources.

Table 3. Concentrations of *Enterococcus*, HF183, and norovirus in influent from the San Diego Point Loma wastewater treatment plant.

Sample Date	*Enterococcus* (10^5 gene copies/100 ml)	HF183 (10^6 gene copies/100 ml)	Norovirus (10^3 gene copies 100/ml)
12/22/15	1.42	2.0	17.9
1/6/16	60.0	4.2	2.9
1/20/16	5.11	4.6	25.1
2/3/16	5.22	7.5	0.8
Average	12.0	4.6	10.8

The HF183 concentrations in wet weather appeared to be related to storm size. While the second storm delivered double the rainfall and five times the runoff volume, the mass inputs of HF183 increased 15-fold at Fashion Valley, the last gaged station on the mainstem. The increased mass inputs relative to rainfall or runoff were consistent with epidemiological data suggesting that larger storms presented greater risk of gastrointestinal illness and seven other symptoms (Arnold et al. 2017). Hypotheses for the relative increased mass vary by source. For the sewage collection system, increased infiltration could result in increased exfiltration (Selvakumar et al. 2004). For the septic system, increased storm size, combined with increased antecedent rainfall, would make for greater soil moisture content and the saturation could result in greater mobilization of subsurface flows (Conn et al. 2012). For homeless populations, higher flood stages might capture more fresh fecal material deposited on river banks. It is more unlikely that higher flow would affect illicit connections or discharges.

One uncertainty from this project is the extrapolation of the sampled watershed, San Diego River, to other watersheds during wet weather. This study reports one of the first comprehensive upstream source tracking studies targeting wet weather (Sauer et al. 2011, Parker et al. 2010, Sidhu et al. 2013), limiting comparisons to judge the uniqueness of the San Diego River. Templar et al. (2016) reported increased human marker concentrations during wet weather at most sites in three watersheds discharging into the Milwaukee estuary; HF183 concentrations further increased during larger storms similar to the increased human marker concentrations observed in this study. Converse et al. (2011) found increased human marker concentrations over the entire duration of a storm hydrograph. Our study utilized two to three composite samples across the hydrograph, all of which had detectable HF183 concentrations. Results from regional wet weather studies in California indicate that human fecal sources in wet weather are not unique. Detectable HF183 concentrations were measured during wet weather in 23 of 27 end-of-watershed sites from catchments throughout southern California (Cao et al. 2017).

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