Los Coches Creek Microbial Source Tracking Study









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EXECUTIVE SUMMARY

Urban stormwater runoff in Southern California presents a unique challenge for stormwater managers as it typically contains high concentrations of fecal indicator bacteria (FIB) and may contain human fecal contamination. Los Coches Creek is a tributary of the San Diego River that is subject to the 2010 Beaches and Creeks Bacterial TMDL and was identified as a priority for source tracking studies to find sources of human fecal contamination. In this study, an adaptive stream census approach is used with traditional FIB measurements and a human fecal marker (HF183) measured by droplet digital polymerase chain reaction (ddPCR) to identify specific human fecal sources within Los Coches Creek watershed during wet weather.

A total of 28 sites across Los Coches Creek, its tributary Flinn Springs Creek, and a residential neighborhood catchment within Flinn Springs Creek were sampled during 3 subsequent storm events in winter 2017-2018 and 2018-2019. Each storm used a sampling design to isolate the largest concentrations of human fecal marker. Potential human fecal sources included leaking sanitary sewer infrastructure, cross-connections between sanitary and storm drain systems, onsite wastewater treatment systems, and homeless encampments, amongst other potential sources.

Ultimately, individual onsite wastewater treatment systems were identified as a source of human fecal contamination in the residential neighborhood catchment of Los Coches Creek. Wet weather samples collected directly below these onsite wastewater treatment systems generated amongst the highest HF183 concentrations during the study (>10,000 gene copies per 100 ml). The wet weather runoff volume from the onsite wastewater treatment system catchment comprised 1% of the cumulative total runoff volume but discharged 97% of the HF183 mass discharged from the catchment. The outfall from this catchment had amongst the highest HF183 concentrations measured within the Flinn Creek tributary of Los Coches Creek watershed. CCTV surveys of the sanitary system in the catchment showed no signs of compromise and CCTV surveys of the storm drain system showed no evidence of cross-connections. Other sources in the catchment had low to non-detectable HF183 concentrations.

Although it is clear these onsite wastewater treatment systems are a source of HF183 during wet weather, they are not the only source of HF183 to Los Coches Creek. Based on the widespread occurrence of HF183 during storm sampling, additional human fecal sources remain. Finally, there was a clear disconnect between HF183 and FIB concentrations, reinforcing the need for human specific markers for adaptive microbial source tracking since FIB alone will not identify the highest risk sources for remediation.

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INTRODUCTION

Urban stormwater runoff in southern California presents stormwater managers with a difficult water quality challenge. Wet weather discharges typically contain high concentrations of fecal indicator bacteria (FIB), such as total and fecal coliforms and *Enterococcus* ranging from 10^2-10^5 MPN per 100 ml (Schiff and Kinney 2001; Gannon and Busse 1989; Brownell et al. 2007; Tiefenthaler et al. 2011; Parker et al. 2010; Griffith et al. 2010; Steele et al. 2017; Steele et al. 2018). Most wet weather runoff is ultimately discharged to marine bathing beaches where there is an observed increase in FIB concentrations from median *Enterococcus* concentrations of 10^1-10^2 MPN per 100 ml during dry weather and 10^1-10^4 MPN per 100 ml following storm events (e.g., Ackerman and Weisberg 2003; Noble et al. 2003, Steele et al. 2018). Recently, the Surfer Health Study demonstrated illness risk for surfers entering the ocean following rainstorms in San Diego (Arnold et al. 2017).

A good example of the difficult challenges associated with FIB and wet weather is in Los Coches Creek, a tributary of the San Diego River. Previous studies performed by SCCWRP measured levels of FIB exceeding water quality objectives (> 104 *Enterococcus* CFU per 100 ml for a single sample) during wet weather at the mouth of Los Coches Creek where it connects to the San Diego River (Steele et al. 2017). As a result, Los Coches Creek may contribute to the FIB concentrations in the San Diego River, which has been placed on the State's list of impaired waterbodies and is subject to a Total Maximum Daily Load (TMDL) to reduce the FIB levels.

While FIB can come from both human and non-human sources, human fecal contamination generally represents the greatest health risk for body contact recreation. Non-human fecal sources such as birds, dogs, cats, and other wildlife typically have less concentrated and less infectious pathogens compared to human sources. The Surfer Health Study measured both human and non-human sources of fecal contamination. Human specific genetic fecal markers (HF183) and pathogens (e.g., norovirus) were detected in nearly every wet weather sample collected at the mouth of the San Diego River (Steele et al. 2018). This frequency of occurrence is not unique to the San Diego River. Similar common occurrences of HF183 were monitored in wet weather discharges from over two dozen southern California streams and rivers (Cao et al. 2017).

Los Coches Creek, while not frequently monitored, has had previous detections of HF183 during wet weather (e.g., Steele et al. 2017) making it a good candidate for source tracking to determine the specific sources of human fecal contamination. The goal of this study was to identify the specific source(s) of human fecal contamination to Los Coches Creek during wet weather. This goal will be accomplished utilizing a relatively new, census-based adaptive sampling design.

Project Setting

Los Coches Creek is an approximately 9.5-mile tributary of the San Diego River located in the upper part of the lower San Diego River watershed. It originates just east of Lakeside and northeast of El Cajon and flows from east to west before turning northwest to meet the San Diego River. Land use in the Los Coches Creek watershed is a mix of primarily residential areas with open space, light industrial, commercial, and agriculture areas as well (Figure 1).

Wastewater infrastructure is mixed and contains sanitary sewer and onsite wastewater treatment systems, commonly referred to as septic systems. Most of the residential and commercial areas have sanitary sewer infrastructure, while a small percentage of the residential areas (particularly in the upper part of the Los Coches Creek watershed) and the agricultural areas have onsite wastewater treatment systems. In addition to the contribution of surface runoff to the microbial water quality in the watershed, leaks from the sanitary sewer and onsite wastewater treatment systems may serve as sources for human fecal contamination during rainstorms and in years where above-average rainfall may contribute to groundwater table rise. Other potential sources include homeless encampments, cross-connections between the sewer and storm drain systems, poorly maintained private sewer laterals, and sewer overflow or sewage spills from broken infrastructure.

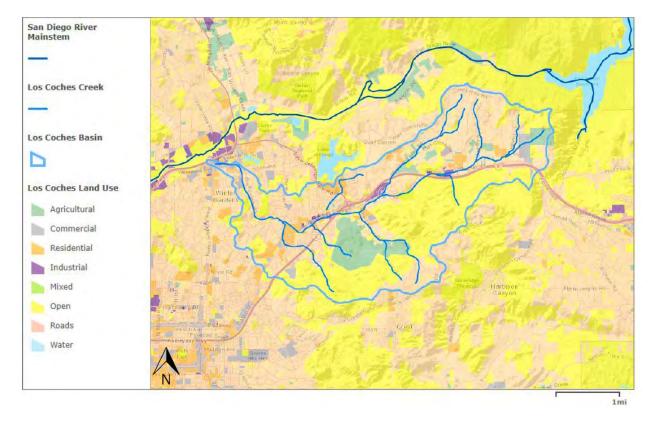


Figure 1. Map showing land use categories in Los Coches Creek Watershed.

The wet season extends from October to April, but San Diego typically receives the majority of rainfall from January to March (Ackerman and Weisberg 2003). Rainfall in the Los Coches Creek watershed is variable from year to year and generally receives greater rainfall than the coastal areas. The recorded 24-hour rainfall in nearby Lakeside had ranged from < 0.01 inches to 3.77 inches in 24 hours since 1988 (Figure 2). During the two seasons for the current study, the total rainfall was below the annual average of 10 inches in the 2018 water year (7.37 in), but well above the annual average in the 2019 water year (19.5 as of this report). The discharge from the creek itself ranges from dry years where the peak flow remains below 100 cu. ft per second during any storm to wet years where the peak flow tops 1100 cu. ft per second (Figure 2).

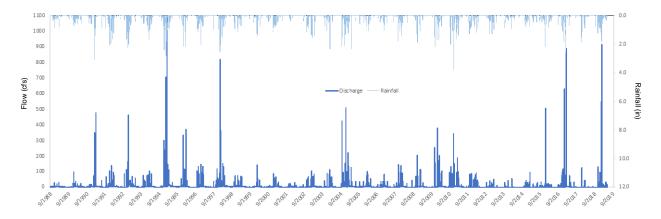


Figure 2. 20-year historical discharge (i.e., flow) at the Los Coches Creek station (USGS 11022200) and rainfall from the NWS stations in lakeside (NOAA GHCND: USC00044710, US1CASD0103).

Study Design

There are many dozens of stormwater outfalls to the mainstem of Los Coches Creek, making it infeasible to measure each outfall individually during the same storm event. Therefore, we applied an adaptive stream census study design to this study. This study design samples the watershed over a series of three storms, starting by dividing the mainstem of the creek into reaches and tributaries and identifying those reaches/tributaries that have the highest levels of HF183. In the second storm, we focus on the reach/tributary with the greatest HF183 in storm 1, attempting to find the contributing catchment with the greatest HF183 concentrations. In the third storm, spatial scales are now small enough to sub-divide the catchment into specific sources of potential fecal contamination. Here, HF183 concentrations are supplemented with flow data to ensure mass estimates of HF183 can be quantified for confirming source tracking conclusions. Once the greatest HF183 sources are identified in this catchment, additional follow up verifications can occur during dry weather such as visual observations, review of maintenance records, additional sampling, and/or closed-circuit television (CCTV) surveys. The watershed-tributary-catchment sites ultimately chosen for Los Coches Creek are shown in Figure 3.

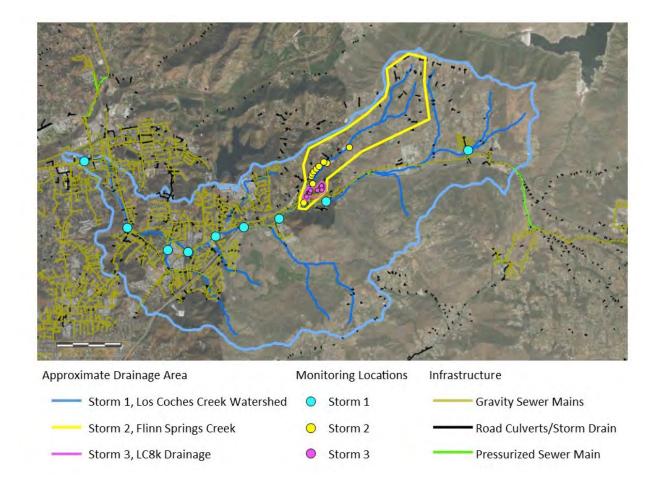


Figure 3. Sites from adaptive sampling across 3 storms in the Los Coches Creek watersheds. Each storm area outline shows the approximated boundaries of the watershed being targeted for Storm 1 (blue outline), Storm 2 (yellow outline), and Storm 3 (pink outline). Blue circles indicate location of Storm 1 sites, yellow circles are Storm 2 sites, and pink circles are Storm 3 sites.

Detailed Methods

Sampling methods

Precipitation measurements, flow estimation, and water sampling was performed following Steele et al. (2017) with the exception of water sampling being comprised of time-weighted, rather than flow-weighted composites. Briefly, precipitation was measured using SigmaTM 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 at a subset of 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). Time-paced composite samples were collected for water quality analyses. A minimum of 24 aliquots were targeted 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. Composite samples were collected for six hours before transport to the laboratory to maintain holding times for microbiological analyses. For storm 1 and 2 up to two composite samples were collected per site for a total sample time of 12 hours, or until flow decreased below sampling intakes. For storm 3 one 6-hour composite was collected.

Laboratory Analysis Methods

FIB Cultivation

Cultivable *Enterococcus* and Total Coliforms and *E. Coli* were measured using Enterolert and Colilert-18 respectively. The concentration was determined using 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 FIB 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

Filtration was performed following the California Source Tracking Manual (Griffith et al. 2013) and the Surfer Health Study (Steele et al. 2017, 2018). Briefly, 100 ml of stormwater was filtered in triplicate on a vacuum manifold through 47 mm diameter, 0.4 μ m polycarbonate filters (Millipore Type HTTP, Millipore, Bedford, MA) to capture bacterial DNA. 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). DNA from a halophilic, alkaliphilic archaeon (*Natronomonas pharaonis*) which does not naturally occur in surface waters or sewage 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 halophile DNA were processed for every extraction in the same manner as the samples.

Human-associated Bacteroidales (HF183) and Enterococcus gene copies were measured using a duplex digital PCR assay following a previously published protocol (Cao et al. 2015, Steele et al. 2018).

RESULTS AND DISCUSSION

Storm 1

Site Selection

Site selection for storm 1 was based on regular spacing of sites along the mainstem. In this way, large increases in HF183 can be detected from one segment to the next. The criteria for site selection in storm 1 included:

- Approximately equal numbers of inputs (either tributaries or major outfalls) from one segment to the next
- Access for sampling (not private property)
- Ability to deploy automated samplers
- Safety of field crews

Additional criteria considered

- Ability to rate flow
- Near existing flow or rain gauge
- Bracketing the ends of the watershed

In total, 10 sites were selected (LC1-LC10, Figure 4) in storm 1, which covered most of the Los Coches Creek mainstem (Figures 3, 4). For the most part, tributaries and outfalls were not sampled during storm 1. Instead, the placement of storm 1 sites between group of major tributaries and outfalls allowed for upstream prioritization of these inputs for storm 2.

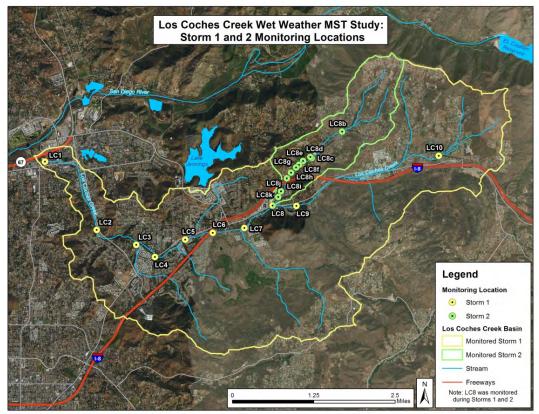


Figure 4. Sampling sites in the Los Coches Watershed for Storm 1 and Storm 2. Yellow symbols are Storm 1 sites and green symbols are Storm 2 sites.

Rainfall & Flow

On February 25, 2018, SCCWRP and its partners began mobilization for the first storm event (Storm 1), which arrived on February 27. Although 0.25 inches of rainfall was forecasted in the watershed, 0.93 inches was recorded at the San Diego County Flood Control rain gauge #27025, located in Flinn Springs County Park (Table 2, Figure 4). Composite samples were successfully collected in 15-minute intervals throughout the first 12 hours of the storm at all 10 stations, comprising 20 composite samples (two consecutive six-hour composite samples per site) made up of 471 aliquots out of a possible 480 aliquots (Figure 5). This effort represented > 99% sampling success for this storm.

Los Coches Creek responded quickly to the rainfall and there was an initial rise, peak and slight fall in flow during the first 7 hours of the storm. The remaining five hours saw an increase in rainfall and a second peak in the hydrograph (Figure 5). Most of the hydrograph was covered by the 12 hours of sampling and the samples represented all but the last tail of the hydrograph. Peak flow at the discharge into the San Diego River (LC1/SDR11) was measured to be 156 cubic feet per second and the total volume was calculated to be 2,625,778 cubic feet. These values are in the middle of the range that was observed in the previous San Diego River study for 2017 (Table 2). These samples were determined to provide a good characterization of a reasonably large storm for Los Coches Creek.

 Table 2. San Diego River and Los Coches Wet Weather MST Precipitation, measured flow, and total storm volume.

Study	Date	Precipitation (inches)	Peak Flow (cu. ft. per sec.)	Total Volume (cu. ft.)
San Diego River	1/31/16	0.56	52.7	1,871,979
Wet Weather MST	2/18-2/19/17	1.00	135	3,224,517
Los Coches Creek Wet Weather MST	2/27/18	0.93	156	2,625,778

Human Fecal Marker (HF183) and Fecal Indicator Bacteria Concentrations

HF183 concentrations ranged from 1,000-14,080 copies per 100 ml in during Storm 1 (Table 3, Figure 5). At nearly every site, the HF183 concentration increased during the second 6-hour composite. Los Coches Creek at Silva Rd (LC10) and Tributary 5 or Flinn Springs Creek (LC8) were the exceptions. Flinn Springs Creek had the highest overall concentration (14,080 copies per 100ml) in addition to a drop from the first 6-hour composite sample to the second 6-hour composite. The increase in HF183 concentration in the mainstem sites downstream from LC10 and LC8 during the second 6-hour composite were likely at least partially due to the higher upstream input during the first 6-hour composite. Los Coches at Flinn Springs site increased to 4640 copies per 100 ml similar to the 4600 copies per 100ml at the Los Coches at Silva Road first 6-hour composite. The site immediately downstream from Flinn Springs Creek increased from 4,280 HF183 copies per 100 ml in the first composite to 10,800 copies per 100 ml in the second composite, and the next three mainstem sites downstream (sites LC6-LC4) also had concentrations that increased from 1000-3000 copies per 100 ml in the first 6-hour composite to 8440-9800 copies per 100 ml in the second six hour deposit. Sites further downstream also increased in concentration, but to a lesser extent, potentially reflecting dilution by storm water with lower HF183 concentrations.

FIB concentrations at every site exceeded regulatory limits for single samples (Table 4). *Enterococcus* concentrations ranged from 9340 to more than 2.4 million MPN per 100 ml across both composites, *E. coli* concentrations ranged from 1200 to 86,000 MPN per 100 ml, and Total Coliforms concentrations ranged from 51,200 to 2,909,000 MPN per 100 ml. The FIB concentrations, while universally high, did not follow the same pattern as the HF183. The highest concentrations of *Enterococcus, E. coli*, and Total Coliforms was at Los Coches Creek at Footbridge (LC5). This is another example of the decoupling between FIB and HF183 concentrations that can take place in stormwater and underscores the importance of using human fecal markers to detect the major human fecal inputs.

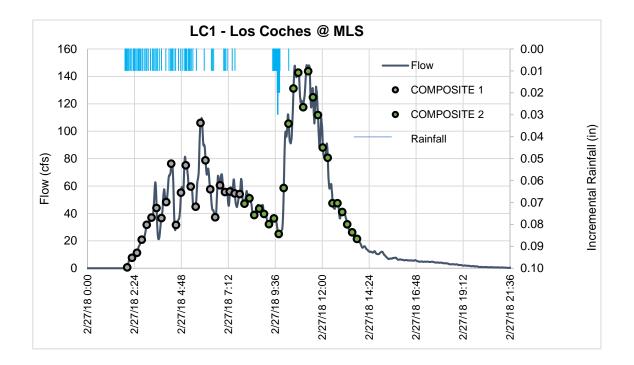


Figure 5. Hydrograph at LC1 (SDR11) Los Coches Creek discharge to the San Diego River and LC8 (Flinn Springs Creek) during storm 1. Rainfall is shown in blue and sample collection times are represented by the circles in the plot.

Table 3. Human specific genetic marker HF183 concentrations (gene copies/100 ml) in Los Coches Creek during storm 1.

Site Name	Site Number	First 6 Hour Composite	Second 6 Hour Composite
Los Coches @ Silva	LC10	4,600	2,480
Los Coches @ Flinn Springs County Park	LC9	1,680	4,640
Trib 5 MLS Flinn Springs Ck	LC8	14,080	5,880
Los Coches @ Rios Cyn Rd	LC7	4,280	10,800
Los Coches @ Gaucho Ln US	LC6	3,000	9,600
Los Coches @ Footbridge	LC5	1,000	8,440
Los Coches @ USGS	LC4	2,360	9,800
Trib 1 MLS	LC3	1,000	1,240
Los Coches @ Los Coches Ct	LC2	2,760	3,880
Los Coches @ MLS	LC1 (SDR 11 ¹)	2,800	3,120

1 – Site Number from the San Diego River Upstream Source Tracking Study.

Site Number	Enterococcus		E. coli		Total Coliforms	
	First 6 hour composite	Second 6 hour composite	First 6 hour composite	Second 6 hour composite	First 6 hour composite	Second 6 hour composite
LC10	20,460	19,350	3,790	4,040	122,300	104,620
LC9	14,830	26,130	14,140	19,890	185,000	57,800
LC8	19,890	24,890	19,890	15,650	166,400	51,200
LC7	38,730	29,090	17,220	14,210	162,400	248,100
LC6	30,760	29,090	13,960	14,010	307,600	201,400
LC5	>2,419,600	127,400	86,000	21,430	2,909,000	387,300
LC4	36,540	34,480	17,250	14,700	156,500	155,310
LC3	14,390	9,340	4,200	3,320	75,400	137,600
LC2	34,480	32,550	10,670	1,200	115,300	146,700
LC1 (SDR11 ¹)	21,870	36,540	17,820	14,140	241,960	165,800

Table 4. FIB concentrations (MPN per 100ml) for Los Coches Creek during storm 1.

1 – Site Number from the San Diego River Upstream Source Tracking Study.

Storm 1 Summary

Results from Storm 1 (Tables 3, 4, Figure 5) indicated several basic conclusions and identified the next steps in the adaptive management framework of this study design:

- Indicator bacteria concentrations exceeded water quality objectives at every sample location.
- Human specific genetic marker (HF183) was detected at every sample location, including the most upstream site.
- Site LC8 had amongst the highest HF183 concentrations of any sample location during storm 1. Sites upstream of Site LC8 detected HF183, but at an order of magnitude lower concentrations.
- Sites downstream of LC8 increased in HF183 concentration in the second half (second composite) of storm 1, likely in response to the inputs from LC8.
- LC8 was at the terminus of Flinn Springs Creek, which became the targeted tributary for the second storm in the adaptive sampling design.



Figure 6. Map showing HF183 concentrations as bubbles at sites along Los Coches Creek during the storm 2/27/2018. The map on the left (Composite 1) shows the HF183 concentrations in the first 6-hour composite and the map on the right (Composite 2) shows the HF183 concentrations at the sites where a second 6-hour composite was collected. Bubble diameter and color are scaled to concentration of HF183 gene copies per 100ml. Site names were taken in the main channel of the tributary and white site names were taken at outfalls along the tributary.

HF183 cps/100 mL



Storm 2

Site Selection

Based on the HF183 results from Storm 1, the project team focused on Flinn Springs Creek (LC8) for Storm 2. Within two weeks of Storm 1, ten new sampling locations were identified, verified, and installed (where permitted) along Flinn Springs Creek (LC8B-LC8J; Figures 3,4). The original LC8 site was also included. Seven of the 11 sites focused on major inputs (e.g., outfalls) to Flinn Springs Creek. Four of the 11 sites (LC8, LC8J, LC8C, and LC8B) were located on the mainstem of Flinn Springs Creek, in locations where particular land uses (e.g., residential and commercial) or source types (e.g., sanitary sewers, onsite wastewater treatment systems) could be isolated. There were only two sites, both on the mainstem, where flow could be accurately measured (LC8 and LC8C).

Rainfall & Flow

Although all equipment for storm 2 was in place by mid-March, water year 2018 was below average rainfall (Figure 2) and the storms anticipated for March and April 2018 did not materialize. There were two storms which had forecasts of > 0.25 inches, but both resulted in false start due to much lower than anticipated rainfall (Table 5). In both cases Flinn Springs Creek was not hydrologically connected and could not be sampled. The remainder of the season had no further qualifying storm events with rainfall forecasted > 0.25 inches, therefore the decision was made to sample again in the beginning of the next water year.

Table 5. False starts for Storm 2 in Spring 2018

Date	Forecasted Precipitation ¹ (inches)	Actual Precipitation ² (inches)	Outcome	Notes
3/17/18	0.32 ³	0.204	Storm 2 False Start	Observed flows were intermittent due to lack of rainfall and quickly infiltrated. No hydrologic connectivity between sites along Flinn Springs Creek.
3/22-23/18	0.765	0.044	Storm 2 False Start	Rainfall dissipated before reaching the Lakeside area.

1 - Forecasted amount calculated based on forecast amounts with probability of precipitation (POP) greater than 60%.

2 – Precipitation Received at Time of Sample Completion

3 - Source: National Weather Service Forecast for Lakeside, CA - Hourly Weather Graph Accessed 3/16/18 22:00

4 – Source: San Diego County Flood Control Gauge #27025

5 - Source: National Weather Service Forecast for Lakeside, CA - Hourly Weather Graph Accessed 3/21/18 22:00

In the fall of 2018, the sites were re-occupied and Storm 2 was captured on 11/29/2018. Precipitation was measured at 1.20 inches at LC8C using a rain gauge installed at the site and 1.09 inches nearby at Flinn Springs County Park using San Diego County Flood Control Gauge #27025. Time-weighted composite samples were successfully collected at each of 11 sites during Storm 2. Of the composite samples collected, 425 out of 432 attempted aliquots (> 98%) were successfully collected¹.

¹ Aliquots where water was not collected due to lack of flow at the site were not considered unsuccessful because they were representative of site conditions.

The rainfall was light for the first 9 hours and heavier for the last 3 hours and the mainstem sites along Flinn Springs Creek (LC8, LC8J, LC8C, LC8B) only produced sufficient flow for a single 6-hour composite to be collected (Figure 7). The peak flow at the upstream site LC8C was 1.53 cu. ft. per second and the total volume was 2,294 cu. ft. At the downstream site at the confluence of Flinn Springs Creek and Los Coches Creek (LC8) the peak flow was 6.43 cu. ft. per second and the total volume was 28,882 cu. ft. The outfall sites (LC8D, LC8E, LC8F, LC8G, LC8H, LC8I, LC8K) responded to both the light rain and the heavier rain and the flow was sufficient to collect 2 6-hour composites, however flow was not measured at these sites.

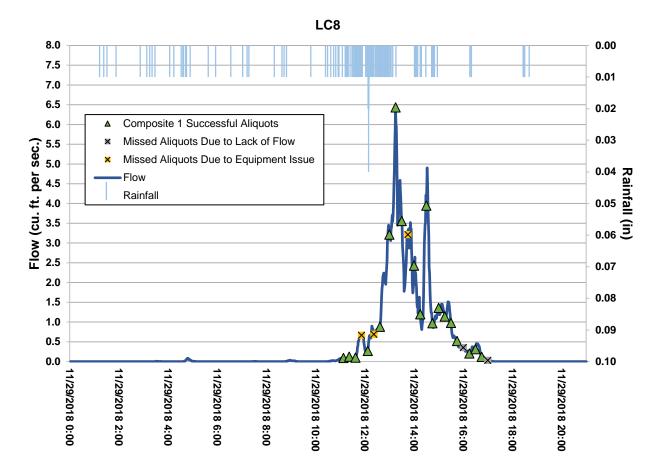


Figure 7. Hydrograph for LC8 during storm 2. Rainfall is shown in light blue, flow in dark blue, and sample collection times are represented by triangles in the plot.

Human Fecal Marker (HF183) and Fecal Indicator Bacteria Concentrations

Human fecal marker concentrations ranged from 348-4280 copies per 100 ml during Storm 2 (Table 6, Figure 8) with the highest concentrations at LC8F, LC8G, LC8I, and LC8K. A decrease in concentration from the first 6 hours to the second 6 hours was measured at most sites where 2 composite samples were collected. Only LC8F and LC8K increased slightly and LC8G stayed the same from the first to the second composite. About 55% (6 of 11 sites) had HF183 values in the 10³ copies per 100 ml range for the first composite, while the remainder had HF183

values in the 10² copies per 100 ml range. For the second composite, only 43% (3 of 7 sites) had HF183 values in the 10³ copies per 100 ml range (Figure 8). LC8I had the highest concentration at 4280 copies per 100 ml in the first composite. LC8K and LC8F, in addition to LC8I remained in the 10³ copies per 100 ml range in both samples. Each of these sites represented good potential sites for next round of adaptive stream census sampling.

LC8 had a much lower concentration in storm 2 (760 copies per 100 ml) compared to storm 1 (10,480 copies per 100 ml). This could be due to the different rate at which the rain fell in storm 2, or it could be due to storm 2 being earlier in the wet season and, therefore, before groundwater levels had risen. The hydrograph showed a much more sluggish response of Flinn Springs Creek during Storm 2 compared to Storm 1, suggesting that the rain was likely infiltrating rather than running off during the initial, less intense rainfall portion of the storm.

Table 6. Human fecal marker (HF183) concentrations at sites along Flinn Springs Creek during the Storm 2 (11/29/2018). Sites are in order from the confluence with Los Coches Creek (LC8) upstream along Flinn Springs Creek (LC8b). Concentrations are reported for the first 6 hour composite at all sites and for the second 6 hour composite where flow continued beyond 6 hours. Concentrations in copies per 100 ml.

Site Number	Site Type	HF183		
		First 6 hour composite	Second 6 hour composite	
LC8B	Main Tributary	1,520		
LC8C	Main Tributary	432		
LC8D	Outfall	840	680	
LC8E	Outfall	2,640	200	
LC8F	Outfall	2,160	2360	
LC8G	Outfall	348	348	
LC8H	Outfall	760	324	
LC8I	Outfall	4,280	2,720	
LC8J	Main Tributary	1,120		
LC8K	Outfall	2,280	2,480	
LC8	Main Tributary Just Prior to Discharge to Los Coches Creek	760		

FIB concentrations at every site exceeded regulatory limits for single samples (Table 7). *Enterococcus* concentrations ranged from 5,172 to 137,200 MPN per 100 ml, *E. coli* concentrations ranged from 630 to 125,900 MPN per 100 ml, and Total Coliforms ranged from 15,850 to 7,701,000 MPN per 100 ml. The FIB and HF183 did not follow the same pattern as far as increasing or decreasing in concentration from the first composite sample to the second. The site with the highest FIB concentration was not the site with the highest HF183 concentration providing further evidence that FIB is not the most reliable indicator of human fecal pollution.

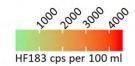
Table 7. Fecal Indicator bacteria (FIB) concentrations at sites along Flinn Springs Creek during Storm 2 (11/29/2018). Sites are in order from the confluence with Los Coches Creek (LC8) upstream along Flinn Springs Creek (LC8b). Concentrations are reported for the first 6 hour composite at all sites and for the second 6 hour composite where flow continued beyond 6 hours. Concentrations in MPN/100 ml.

Site Number	Enterococcus		E. coli		Total Coliforms	
	First 6 hour composite	Second 6 hour composite	First 6 hour composite	Second 6 hour composite	First 6 hour composite	Second 6 hour composite
LC8B	137,200		125,900		1,076,000	
LC8C	17,230		2,110		1,076,000	
LC8D	20,350	9,090	2,720	1,870	1,986,300	1,259,000
LC8E	15,000	14,140	1,850	1,350	52,700	866,400
LC8F	57,940	32,550	7,170	7,630	2,014,000	3,448,000
LC8G	6,131	5,172	630	850	148,300	98,800
LC8H	118,700	20,980	57,940	17,890	7,701,000	959,000
LC8I	34,480	38,730	630	7,120	15,850	1,616,000
LC8J	48840		14,210		2,909,000	
LC8K	34,480	95,800	51,720	122,300	727,000	1,274,000
LC8	38,730		18,720		2,098,000	

Storm 2 Summary

The second storm of the adaptive stream census sampled Flinn Springs Creek where the highest concentrations of HF183 were measured at LC8 in storm 1. While the HF183 concentrations at LC8 were not as high as Storm 1, HF183 was detected and FIB concentrations were above regulatory thresholds at all Flinn Springs Creek sites for storm 2. Some conclusions drawn from storm 2 include:

- Three sites had consistently higher concentrations: LC8F, LC8I, and LC8K across both composite samples.
- The LC8F site drained a small area which did not have extensive sewer infrastructure. This sub-tributary is a good candidate for future investigation (see summary in Appendix 1).
- The LC8I outfall drained an area of primarily onsite wastewater treatment systems on private property and did not have sewer infrastructure. This sub-tributary was flagged for further investigation using CCTV or other methods (see summary in Appendix 1).
- The LC8K outfall, while not discharging the highest HF183 concentrations of all the inputs, had the most persistently elevated HF183 concentrations. This indicates a relatively consistent human fecal source and likely the most consistent health risk. The LC8K catchment contained more infrastructure than the other catchments including both sewered and onsite wastewater treatment residential areas.
- Because of the persistently elevated HF183 concentrations and the potential for various infrastructure contributions, the LC8K catchment was selected as the best candidate for the final phase of sampling in storm 3.



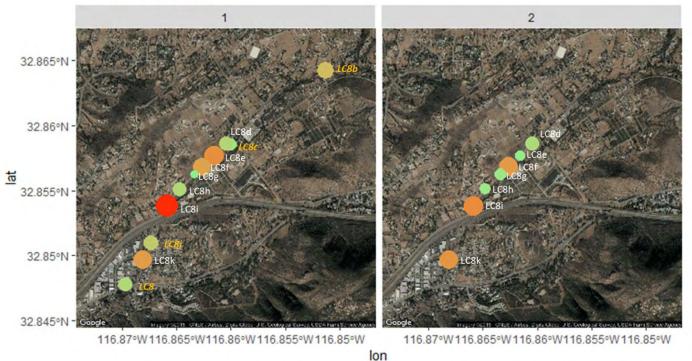


Figure 8. Map showing HF183 concentrations as bubbles at sites along Tributary 5 of Los Coches Creek during the storm 11/29/2018. The map on the left (1) shows the HF183 concentrations in the first 6-hour composite and the map on the right (2) shows the HF183 concentrations at the sites where a second 6-hour composite was collected. Bubble diameter and color are scaled to concentration of HF183 gene copies per 100 ml. Yellow site names were taken in the main channel of the tributary and white site names were taken at outfalls along the tributary.

Storm 3

Site Selection

LC8K was chosen for sampling in the third storm based on the persistent concentration of HF183, and the capability of isolating different sections of the catchment for source tracking (Figures 1, 3). Seven sites were selected in total (Figure 9). Two sites were located at the head of the catchment, both of which drain residential land use with onsite wastewater treatment systems (LC8K-6, LC8K-5). Two sites were located on the northern and southern edges of the catchment, both which drain residential land use with sanitary sewer collection systems (LC8K-2, LC8K-1). Two sites were selected in the middle of the catchment, along the mainstem of LC8K, all of which drain residential land use with sanitary sewer collection systems (LC8K4, LC8K3).

The last site was the outfall from the catchment LC8K, which conveys flow from the LC8K catchment to the larger Flinn Springs catchment. In addition, two street curb and gutter samples were also taken, upstream from LC8K-3 and upstream from LC8K-4 to measure the contribution of HF183 from curb-and-gutter runoff from the residential area shown below (Figure 9). This attribution of sampling sites allowed for isolation of different HF183 sources (onsite wastewater treatment vs sanitary) and differentiation of contributions from various portions of the catchment (eastern, northern, southern, and mid-catchment) as flow moves from east to west.



Figure 9. Map of sampling locations for Storm 3 at LC8K. Monitoring locations are described by red circles, sanitary sewer manhole covers are pink dots, flow direction is shown as a green arrow, and the drainage area is outlined in orange.

Rainfall & Flow

A qualifying storm was forecast to produce 0.56 inches on January 31, 2019. Rainfall presented as a moderate drizzle (approximately 0.1 inches/hour) through 16:00. This was followed by a period of more intense rainfall (> 0.2 inches/hour) until 18:00, and another period of light to moderate drizzle until 20:00. Rainfall was recorded at multiple locations throughout the LC watershed, either by Wood Environment & Infrastructure (Wood E&I) at LC8K-1 or San Diego County Flood Control at Flinn Springs County Park (#27025). The rain gauge at LC8K-1 reported 0.88 inches precipitation and the gauge at Flinn Springs County Park reported 0.89 inches.

Time-weighted composite samples were successfully collected at 6 of the 7 targeted sites during Storm 3. The 7th site (onsite wastewater treatment-influenced location LC8K-6) did not produce flow at any time during the storm event. Of the 6 composite samples targeted at flowing sites

(197 aliquots attempted across all site-composites), 195 aliquots (99%) were successfully collected. Reliable level and velocity data were collected at all sites when flow was present, with the exception of site LC8K-5. At LC8K-5, logged level data was deemed poor quality due to a malfunction in the level sensor. However, the velocity data collected during Storm 3 was good quality. Level and velocity data were collected on a subsequent storm and the relationship was used to recalculate flow for Storm 3 with ground-truthing based on level observations taken during Storm 3.

Peak flow at the sites in the LC8K sub-tributary ranged from no flow to 2.98 cu. ft per second, and total storm volume at the sites ranged from no flow to 19,290 cu. ft. The percent of the total volume captured in representative composite samples ranged from 91.9% to 99.7% across all sites (Table 8). LC8K-3 had the bulk of the flow, far greater than the upstream sites of LC8K-6, LC8K-5, and LC8K-4 showing the inputs from the sanitary sewer neighborhood produced most of the flow in this sub-tributary. In spite of the difference in flow and volume, the storm drain sites responded to the precipitation over a similar period with nearly all of the flow captured during the 6-hour composite sampling (Figure 10).

Site	Drain Type	Peak Flow (cu. ft sec ⁻¹)	Captured Volume (cu. ft)	Total Storm Volume (cu. ft)	Percent Captured
LC8K-6	Outfall	No Flow	NA	NA	NA
LC8K-5	Outfall	0.05	188	199	94.5
LC8K-4	Main	0.06	311	321	96.8
LC8K-3	Main	1.67	13953	13985	99.8
LC8K-2	Outfall	0.07	485	516	94.1
LC8K-1	Outfall	0.24	1553	1691	91.9
LC8K	Main Just Prior to Discharge to Flinn Springs Creek	2.98	18854	19290	97.7

Table 8. Flow and volume of stormwater in the LC8K sub-tributary during Storm 3. LC8K-6 did not flow.

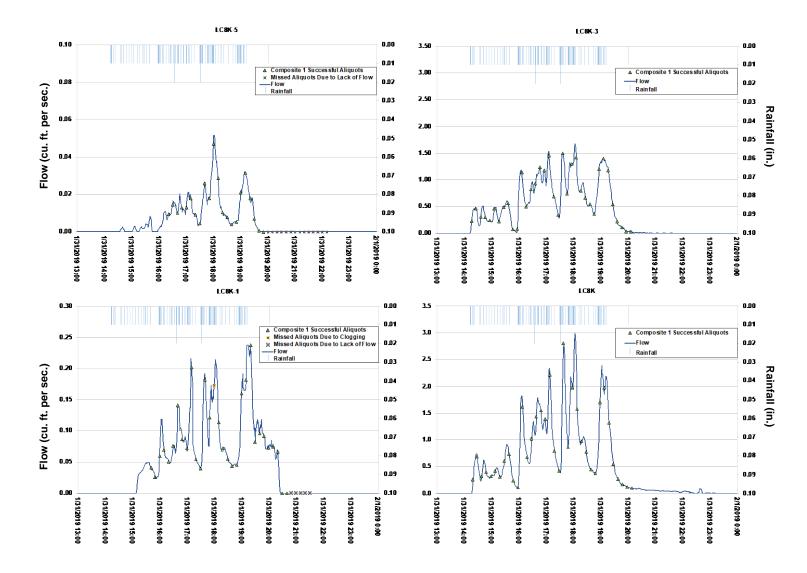


Figure 10. Stream flow and precipitation at LC8K-5 (A), LC8K-3 (B), LC8K-1 (C), LC8K (D). Precipitation values are taken from the gauge at LC8K-1.

Human Fecal Marker (HF183) and Fecal Indicator Bacteria Concentrations

Human fecal marker concentrations ranged from 52-10,880 HF183 gene copies per 100 ml in the composite samples from the LC8K sub-tributary. The curb-and-gutter drain grab samples upstream from LC8K-3 and LC8K-4 were below detection. The upstream portions of the LC8K sub-tributary (LC8K-5, LC8K-4) showed the highest concentrations of human fecal marker in the 6-hour composite samples (Table 9, Figure 9). HF183 concentrations at LC8K-5 were about 10-fold greater concentration compared to the next highest concentration measured in the LC8K sub-tributary. The lower values seen in the downstream composites, is likely due to dilution from inputs downstream of LC8K-5 some of which had higher flow and volume and all of which had much lower HF183 concentrations (Table 8). This suggests that the residential onsite wastewater treatment systems represent a large source of human fecal contamination in the LC8K catchment and could be an important source to Flinn Springs Creek.

FIB concentrations were above regulatory thresholds in nearly every sample. In the composite samples, *Enterococcus* concentrations ranged from 38,730-111,900 MPN per 100 ml, *E. coli* concentrations ranged from 8,130-30,760 MPN per 100 ml, Total Coliforms ranged from 69,700-644,000 MPN per 100 ml. FIB concentrations were higher in the grab samples upstream from LC8K-3 over those upstream from LC8K-4: *Enterococcus* concentrations were 1,935 and 1,187 MPN per 100 ml, *E. coli* concentrations were 3,730 and 100 MPN per 100 ml, Total Coliform concentrations were 77,010 and 15,290 MPN per 100 ml. In contrast to the human marker concentrations, FIB concentrations were highest in the two drainages in the downstream portion of the LC8K sub-tributary (LC8K-2, LC8K-1; Table 9, Figure 9). This provides further evidence that FIB do not correlate with specific markers of human fecal pollution and that FIB concentrations alone are not always sufficient to distinguish the sources with the highest potential public health risk. We do note, however, that all samples where HF183 was detected also had FIB concentrations well above regulatory thresholds, suggesting that FIB, although non-specific, can still serve as a first step in identifying problems in a tiered investigatory approach.

HF183 concentrations at LC8K during Storm 3 were lower and the FIB concentrations were higher when compared to Storm 2 concentrations (Tables 6, 8). Storm 2 precipitation was greater (1.09 inches vs 0.89 inches recorded at Flinn Springs County Park) and occurred earlier in the 2019 water year compared to Storm 3. The precipitation in the 2019 water year was above average and it is possible factors such as washoff and/or dilution could have contributed to the differences in LC8K between the two storm events. It is also possible that these factors could have altered groundwater conditions changing HF813 contributions if a groundwater transport mechanism was involved. Ultimately, the adaptive stream census design is not appropriate to determine the cause of increases or decreases in HF183 concentrations between storm events. However, concentrations of FIB and HF183 at sites within the LC8K catchment during Storm 3 met or exceeded concentrations observed at LC8K during Storm 2, suggesting a level of comparability in human sources between Storms 2 and 3.

Station	Drainage Sewage Infrastructure	HF183 (copies/ 100 ml)	Entero- coccus (MPN/ 100 ml)	<i>E. coli</i> (MPN/ 100 ml)	Total Coliforms (MPN/ 100 ml)
LC8K-6	Onsite Wastewater Treatment	NA	NA	NA	NA
LC8K-5	Onsite Wasewater Treatment	10880	72,700	8,130	155,300
LC8K-4	Sewer	1680	38,730	8,820	172,200
LC8K-3	Sewer	52	41,060	15,150	69,700
LC8K-2	Sewer	420	111,900	15,760	>241,960e
LC8K-1	Sewer	84	72,700	30,760	644,000
LC8K	Sewer	112	81,640	21,870	146,700
LC8K-4-Curb Grab	Sewer	BD	1,187	100	15,290
LC8K-3- Curb Grab	Sewer	BD	1,935	3,730	77,010

 Table 9. Concentrations of Human Marker (HF183) and FIB from samples collected during Storm 3.

HF183 Mass

The total mass of HF183 discharged from the LC8K catchment during Storm 3 was 5.98 x 10⁸ gene copies (Figure 12). Despite comprising only 1% of the total discharge volume, site LC8K-5 discharged 97% of the total HF183 mass. This portion of the catchment drained exclusively onsite wastewater treatment system residential land use, and only three single family homes. The remaining portions of the catchment were comprised of sanitary sewer residential land use, cumulatively discharging 99% of the storm volume and 3% of the HF183 mass.

With the context of onsite wastewater treatment systems dominating human sources in the upper catchment, but storm volume dominated by the impervious cover in the lower catchment, spatial patterns of HF183 concentrations make intuitive sense. LC8K-5 located immediately downstream from the onsite wastewater treatment systems had the greatest HF183 concentrations. Surface runoff in the sanitary sewer portions of the catchment generated generous volume, but virtually no HF183 based on the non-detectable HF183 concentrations in the curb and gutter runoff samples. Therefore, as a result of dilution, HF183 concentrations decreased as onsite wastewater treatment runoff progressed downstream through the sanitary sewer portions of the catchment.



Figure 11. Map and bubble plot showing the concentration of HF183 in 6-hour composite samples in the Flinn Springs Creek LC8K sub-tributary during Storm 3. Bubble diameter and color are scaled to concentration of HF183 gene copies per 100 ml.

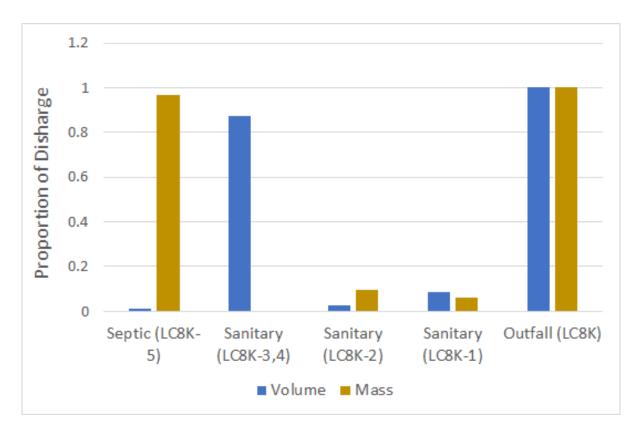


Figure 12. Measured volume and HF183 mass for Storm 3 along the mainstem of the Flinn Springs Creek LC8K sub-tributary.

Confirmation of sources

Supplementary source identification activities helped confirm the onsite wastewater treatment system(s) upstream of LC8K-5 were the primary source of HF183, by ruling out other sources. Closed circuit television (CCTV) investigation of the sewer pipes within the LC8K catchment by the San Diego County Sanitation District revealed no evidence of damage to the sewer infrastructure (see summary in Appendix 1). Similarly, CCTV investigation of the storm drains in the LC8K catchment by the San Diego County Department of Public Works, Transportation Division did not reveal any cross-connections or seepage into the LC8K storm drain system.

To confirm that there was no potential cross-reactivity of HF183 assays between human and canine feces, samples of canine feces near LC8K-5 were collected and analyzed. All HF183 results from these feces samples were non-detectable. Finally, visual inspections observed no homeless encampments or illicit dumping in the LC8K catchment.

The presence of residential onsite wastewater treatment systems in the catchment directly upstream of LC8K-5 were confirmed by reviewing onsite wastewater treatment records, parcel, and infrastructure maps (see summary in Appendix 1).

Storm 3 Summary

After narrowing outfall LC8K as a persistent location of human fecal pollution in Storm 1 and Storm 2, Storm 3 was designed to isolate different sources and locations within the LC8K catchment to pinpoint the identity and location of the HF183 inputs. Conclusions from Storm 3 included:

- Despite comprising only 1% of the total discharge volume, site LC8K-5 discharged 97% of the total HF183 mass. This portion of the catchment drained exclusively onsite wastewater treatment system residential land use, and only three single family homes.
- The remaining portions of the catchment were comprised of sanitary sewer residential land use, cumulatively discharging 99% of the storm volume and only 3% of the HF183 mass
- Futher evidence showing the onsite wastewater treatment system(s) upstream of the LC8K-5 were the primary source was found through CCTV surveys of the sanitary sewer and storm drain systems within the LC8K catchment. No cross-connections and no failing sanitary sewer collection system pipes were observed.

Synthesis

This study identified two specific locations that contributed elevated levels of human fecal marker during wet weather. The first was immediately upstream of site LC8K-5, a location comprised exclusively of residences with onsite wastewater treatment systems. A second location was immediately upstream of LC8I, a location also comprised of residences with onsite wastewater treatment systems.

Although we identified two of the largest human fecal contributions in the Flinn Springs Creek tributary, we did not identify all sources of HF183 in the Los Coches catchment. HF183 was measured at virtually every site across the three storms sampled in this study, including Los Coches sites upstream of the Flinn Springs Creek tributary. Widespread HF183 concentrations during wet weather are not uncommon in southern California and the exact sources are unknown. It is highly unlikely that all locations that detect human fecal markers will be attributable to onsite wastewater treatment systems, because detectable HF183 concentrations occur in areas without onsite wastewater treatment systems.

In this study we compared the concentration of HF183 across sites and built the study around the sites with the highest HF183 concentrations. This study did not attempt to determine the health risk associated with the human fecal contamination indicated by the HF183 concentrations. We note that we do not know what concentration of HF183 is associated with regulatory guidance of 32/1000 illnesses (USEPA 2012). We did find that FIB such as *Enterococcus* did not correlate with HF183 in this study, so extrapolating FIB to public health risk is likely tenuous. Identifying the level of HF183 in stormwater that represents a potential threat to public health will be important for setting guidance for management actions and assessing metrics of success. If identifying health risk is a goal, then additional sampling and analysis for human-specific pathogens will be required.

The adaptive stream census approach was successfully applied across 3 storms for rapid source tracking investigation. The ability to determine the concentrations of HF183 and FIB, and then use this information to re-deploy sampling instrumentation at a new set of sites within 2 weeks, allowed for effective upstream tracking in the same storm season. The advent of new technology makes this approach new and innovative, enabling a new tool in the toolbox for wet weather source tracking. Depending on the size of the watershed being investigated, this could allow for identification of specific sources within a watershed within a single season. Although more expensive than traditional FIB measurements alone, this framework can be applied in future studies to quickly identify sources for management action.

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APPENDIX 1: FLINN SPRINGS CREEK RECONNAISSANCE SUMMARY AND OUTCOMES

This appendix contains a summary of findings produced during the reconnaissance of the LC8K, LC8I, and LC8F catchments of Flinn Springs Creek on 1/18/2019. Pictures of the sites and surrounding areas of interest are included where available. Also included in this appendix are Storm Drain Schematics near LC8F and LC8I.

LC8K Survey Summary

We walked this catchment from the outfall to Flinn Springs Creek to the top of the catchment and identified four potential sources: 1) onsite wastewater treatment system contributions from houses at the top of the catchment; 2) segments of the sanitary sewer system serving the neighborhood above LC8K are above the MS4 system, so there is a possibility of sanitary to storm contributions if there are leaks in the sewer systems in close proximity; 3) the sanitary sewer system serving residences to the south of LC8K is a bit older and crosses the uppermost portions of the catchment, which could contribute wastewater if underground leakage surfaces or if sanitary sewer overflows occur; 4) illicit connections to the MS4.

Recommendation:

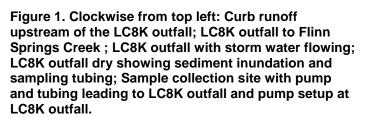
Equip this catchment for one more storm to isolate the different potential sources including; upstream inlets to isolate onsite wastewater treatment systems, mid-catchment to isolate the inverted storm-sanitary sewers, and downstream to check for contributions from illicit connections or leaking laterals.

Recommended Action Items:

- CCTV investigation to video the MS4 from LC8K outfall to check for structural integrity and the presence of illicit connections.
- Examine onsite wastewater treatment system records for the two properties at the top of the catchment
- Examine the most recent records for the sanitary system to verify structural integrity
- CCTV investigation of the sanitary sewer to check for structural integrity

Outcomes:

- CCTV inspections of the two sanitary sewer systems were performed by the County of San Diego Sanitation District and did not locate any defective pipe or joint displacement. High confidence that the sanitary sewer in the area does not leak.
- CCTV inspections of the storm drain network draining to LC8K were performed by the Department of Public Works Storm Water. Storm drains were inspected following the introduction of dye into the adjacent sewer system. The only flow observed in the storm drain system was from irrigation runoff. No dye was observed in the storm drain system.
- Properties above the LC8K catchment were confirmed to be onsite wastewater treatment systems served by onsite wastewater treatment systems, however properties within the MS4 network were confirmed to be on sanitary sewer.
- Other properties of interest along adjacent to the sampling area also had onsite wastewater treatment systems records.





LC8I Survey Summary

We walked the lower portion of catchment, nearest the beginning of the MS4 system, which originates as a catch basin inlet and runs under the street to an outfall into Flinn Springs Creek. Two sources were readily apparent: 1) the property adjacent to the inlet had several piles of manure adjacent to the road, which easily contributed fecal indicator bacteria to Flinn Springs Creek thru the MS4 catch basin; and 2) a second property had poorly graded its horse corral which is the location of its onsite wastewater treatment systems leach field, as indicated by the Department of Environmental Health records. The property owner had then dug a shallow ditch to drain the horse corral, which discharged into street and directly into the catch basin.

Recommendation

Additional sampling can be done to confirm sources, with follow up actions by the County <u>Action Items:</u>

• Additional samples could be collected from the properties to confirm indicator bacteria and/or HF183 concentrations.

Outcomes:

• Further sampling at LC8I pending



Figure 2. Clockwise from top left: Horse corral with disturbed leach field and drainage ditch; Discharge from drainage ditch to street; piles of manure adjacent to street; catch basin in street between the drainage ditch discharge and the manure piles.

LC8F Survey Summary

We walked the entire watershed from the outfall to the top of the catchment. This is a relatively new development and many properties are still under construction. The MS4 only covers a small portion of the catchment. Five potential sources were observed, but none of these sources were obvious: 1) contaminated groundwater; 2) an unknown storm drain inlet upstream of LC8F 3) mulch from the many new landscaped areas; 4) portable toilets associated with construction crews; and 5) leach field resurfacing.

Recommendation:

This catchment is likely the lowest priority of the three surveyed today. Potential strategies here would likely focus on dry weather sampling to start.

Potential Action Item:

• CCTV investigation to video the storm drain network for evidence of onsite wastewater treatment systems intrusion

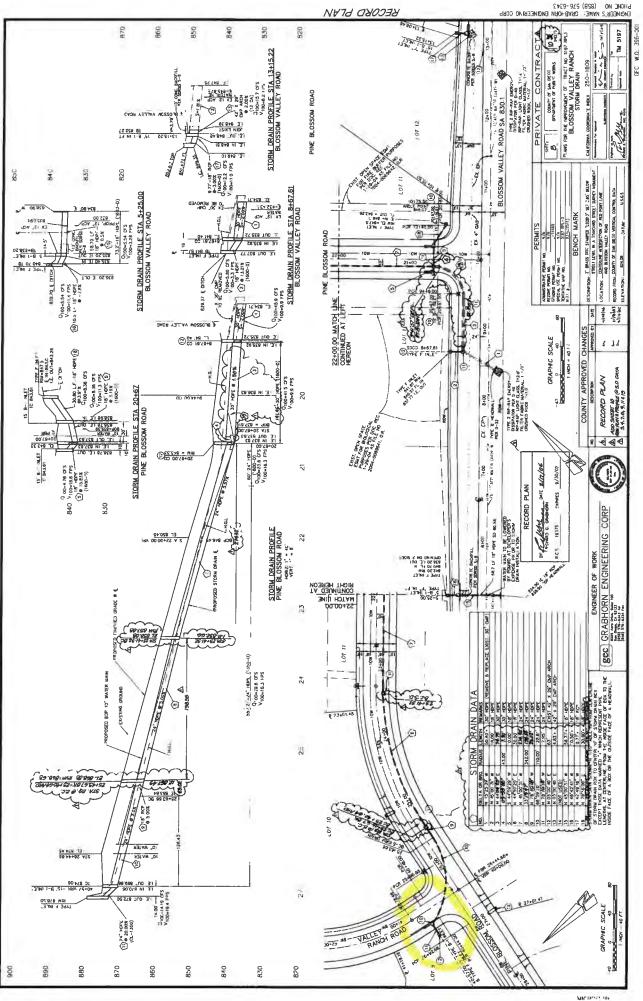
Outcomes:

- CCTV inspection pending
- Further sampling pending
- Subdivision maps for the new residential development upstream of LC8f showed the unknown storm drain found led to an inlet just inside the property line of the adjacent residence. Historic photos and maps show that the brow ditch drainage used to run directly to this inlet which was graded over and filled in at some point.

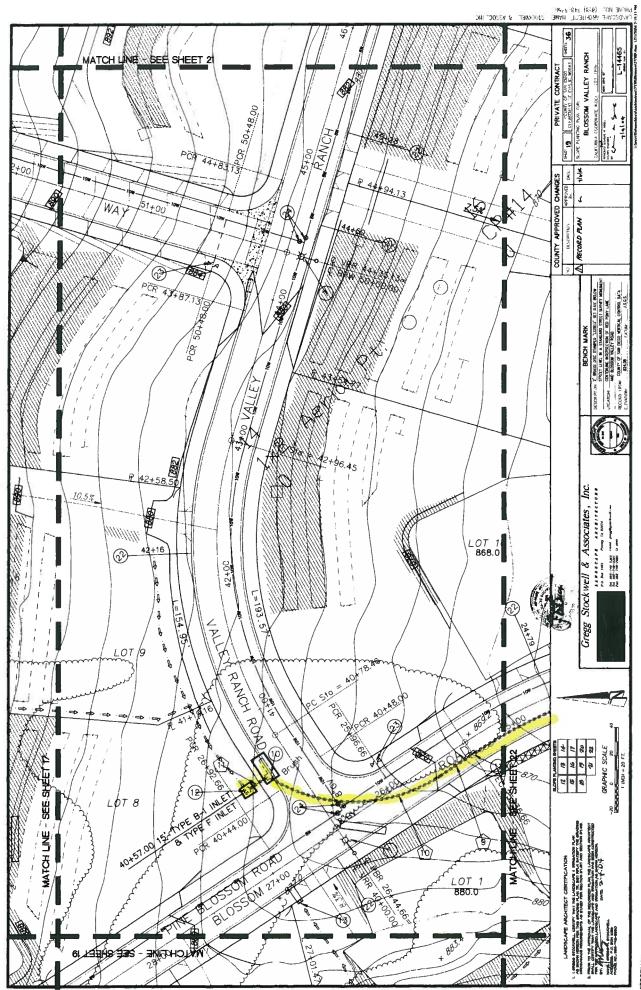


Figure 3. Pictures clockwise from top left; LC8F discharge during dry weather, LC8F discharge flowing; LC8F discharge with sampling tubing and flow meter instrumentation installed.





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