Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Sources of Low Level Human Fecal Markers in Recreational Waters of Two Santa Barbara, CA Beaches: Roles of WWTP Outfalls and Swimmers

Dong Li^a, Laurie C. Van De Werfhorst^a, Brandon Steets^b, Jared Ervin^b, Jill L.S. Murray^c, Avery Blackwell^b, Naresh Devarajan^a, Patricia A. Holden^{a,*}

^a Bren School of Environmental Science & Management, University of California, Santa Barbara, United states

^b Geosyntec Consultants, Santa Barbara, CA 93101, United states

^c Creeks Division, Department of Parks & Recreation, City of Santa Barbara, CA, United states

ARTICLE INFO

Keywords: Fecal contamination Beach water Human marker hf183 Bather shedding

ABSTRACT

Worldwide, fecal indicator bacteria (FIB) evidence coastal water contamination for which sources are unknown. Here, for two FIB-impacted Santa Barbara recreational beaches, hypothesized fecal sources were investigated over three dry seasons (summers) using nearly 2000 field samples of water (ocean, creek, groundwater), sand, sediments, effluent and fecal sources. In years 1 and 2, gull and dog feces were identified as the probable main FIB sources to surf zone waters, yet HF183 human fecal markers were consistently detected. Determining HF183 sources was therefore prioritized, via year 3 sub-studies. In lower watersheds, human and dog wastes were mobilized by small storms into creeks, but no storm drain outfalls or creeks discharged into surf zones. Beach area bathrooms, sewers, and a septic system were not sources: dye tracing discounted hydraulic connections, and shallow groundwater was uncontaminated. Sediments from coastal creeks and downstream scour ponds, nearshore marine sediments, and sands from inter- and supratidal zones contained neither HF183 nor pathogens. Two nearby wastewater treatment plant (WWTP) outfalls discharged HF183 into plumes that were either deep or distant with uncertain onshore transport. Regardless, local sources were evidenced, as surf zone HF183 detection rates mostly exceeded those offshore and nearshore (around boat anchorages). The presence of swimmers was associated with surf zone HF183, as swimmer counts (on weekdays, holidays, weekends, and during races) significantly correlated (p<0.05, n = 196) to HF183 detections. Besides comprehensively assessing all possible fecal sources, this study provides new explanations of chronic low-level human markers in recreational beach surf zones, suggesting likely lowest achievable HF183 thresholds.

Introduction

Fecal contamination of coastal zones, particularly recreational waters, is of great concern to public health. Human pathogens in contaminated coastal waters cause severe illness worldwide, with annually more than 120 million gastrointestinal and 50 million severe respiratory illnesses estimated to be associated with swimming or bathing in polluted coastal waters (Shuval, 2003). To monitor fecal contamination in coastal waters, fecal indicator bacteria (FIB) including total coliform (TC), fecal coliform or *Escherichia coli* (EC), and enterococci (ENT) are used as indicators due to their abundances in feces (Harwood et al., 2014). California law mandates (by Assembly Bill 411, or AB411) weekly FIB testing from April to October in surf zone waters of recreational beaches that exceed 150 million user-days annually by tourists and residents who swim, wade, surf, and dive (https://www.waterb oards.ca.gov/water_issues/programs/beaches/beach_water_quality/).

Swimming advisories, beach closures, stormwater green infrastructure, and sanitary infrastructure investigations with abatement actions are management practices that are implemented where FIB concentrations exceed water quality criteria.

However, FIB may originate from animal feces that are of low risk to human health due to host specificity of pathogens, particularly enteric viruses (Sinclair et al., 2009). FIB can also persist in the environment (Field and Samadpour, 2007; Harwood et al., 2014). Epidemiological studies conclude that FIB are unreliable sole measures of public health risk (Arnold et al., 2013; Colford et al., 2007). To determine public health relevant fecal sources, microbial source tracking (MST) prioritizes quantifying genetic markers encoding 16S rRNA of host-coevolved

https://doi.org/10.1016/j.watres.2021.117378

Received 23 January 2021; Received in revised form 11 June 2021; Accepted 17 June 2021 Available online 20 June 2021 0043-1354/@ 2021 Elsevier Ltd. All rights reserved.





^{*} Corresponding author. *E-mail address:* holden@bren.ucsb.edu (P.A. Holden).

or -associated microorganisms such as the HF183 human fecal DNA marker (Boehm et al., 2013 and 2015; Field and Samadpour, 2007; Harwood et al., 2014; Mayer et al., 2018).

In urban settings, potential human fecal sources include leaking sanitary sewers and sewage overflows (Sikich et al., 2018). Coastal watersheds may have fecal contaminated creeks draining through coastal lagoons, or other transitional waters, to beach waters (Riedel et al., 2015). Runoff-generating rain events can mobilize human or animal fecal surface deposits in watersheds, with storm drainage conveying fecal materials to coastal waters (U.S. Environmental Protection Agency, 2004). Watershed sediments, urban creek outlets or scour ponds, groundwater, and beach sands (Russell et al., 2013) can be reservoirs of fecal materials that are released gradually to surf zone waters (Ishii et al., 2007). Harbors with boats, and moored boats in ocean anchorage areas, could be fecal sources to surf zone waters. WWTP treated effluent ocean outfalls can discharge fecal markers (Boehm et al., 2002). Onshore, beach campers are possible human fecal contamination sources (Noble et al., 2000). Yet, such multiple sources are rarely investigated comprehensively.

Previously, drainages, creeks, and rivers were shown to discharge fecal contamination into California recreational beaches (Cao et al., 2017; Ervin et al., 2014; Goodwin et al., 2016; Riedel et al., 2015; Sikich et al., 2018). Enhanced Watershed Management Programs which are costly to local and State water government agencies (California Water Boards Los Angeles Region 4, 2018; Ervin et al., 2014; Goodwin et al., 2016; San Diego Regional Water Quality Control Board, 2017; Sikich et al., 2018) have been implemented for abating such fecal contamination. However, sources of low but chronic human fecal markers in coastal California recreational beach surf zone waters during the summer AB411 periods remain unresolved (Cao et al., 2017; Jennings et al., 2018; McQuaig et al., 2012; Riedel et al., 2015; Russell et al., 2013; Santoro and Boehm, 2007). Systematic evaluation of all possible human fecal sources is needed (Griffith et al., 2013).

Here, two popular recreational beaches with historically-elevated surf zone FIB concentrations, Leadbetter Beach and East Beach, in Santa Barbara, CA, were investigated in a 3-year study to determine fecal sources. Besides FIB and human, dog, and gull fecal markers, pathogens including human adenovirus and Salmonella bacteria were also determined in this study to evaluate the potential risk to human health. For the first time in coastal urban California during dry weather, all hypothesized human fecal sources and transmission routes were rigorously tested, including watersheds, urban creek outlets and scour ponds, groundwater transport, supratidal and intertidal beach sands, storm drains, sanitary sewers, septic systems, harbor facilities with boats, moored boats in anchorage areas, recycled water, the treated WWTP effluent discharged from two nearby ocean outfalls, as well as beach campers and swimmers. After excluding almost all potential human fecal sources and transmission routes, the results demonstrated that consistent human marker HF183 detections in surf zones at low concentration levels correlated with swimmer counts, with the potential additional influence of WWTP effluent. Surf zone recreation has been recognized as a source of FIB to the surf zone (Elmir et al., 2007; (Elmir et al., 2009)), but this is the first time that counts of swimmers were found to be significantly correlated to human-associated fecal markers in a field study of recreational beaches. The results inform expectations of human fecal contamination and markers in recreational beaches for future water quality criteria that may regard human fecal markers, with potential benefit to regulators, managers, and researchers.

Materials and methods

Beaches, overall study structure, and sample analyses

Two urban Santa Barbara, California recreational beaches were studied: Leadbetter Beach (LB) at Honda Creek and East Beach (EB) at Sycamore Creek. Arroyo Hondo (AH) was the rural reference beach

(Fig. S1). LB and EB were selected due to historically elevated surf zone FIB concentrations, with LB and EB exceeding the U.S. Environmental Protection Agency Beach Action Advisory (BAV) in 2013 by 18% and 5% respectively (NRDC, 2014). This study was structured as an MST program (Griffith et al., 2013) during the AB411 regulatory period in California, i.e. the dry season summer months when recreational beach use is highest. For this study, dry weather was defined as <0.1'' of rainfall in the preceding 72 h. Rain events that occurred during the AB411 season were sampled if predicted to be $\geq 0.2''$. MST was planned for 2 successive years (Y1-Y2; 2015-16) to understand FIB sources to LB and EB, such that Y1 results could inform Y2 plans. However, during Y1 and Y2, low HF183 human fecal marker concentrations were consistently found in EB and LB (but not at AH) surf zone waters. Therefore, studies designed for a third summer (Y3; 2017) regarded HF183 sources to the LB and EB surf zones. To assess if secondarily-treated wastewater was an HF183 marker surf zone source, the two nearby wastewater treatment plant outfalls were studied. The El Estero WWTP outfall discharge plume depth, and marker and indicator concentrations, were simulated, with results compared to outfall diffuser field sample analyses. The Montecito Sanitary District (MSD) WWTP outfall, whose plume trajectory and potential to contaminate shorelines had been studied previously (details in the SI), was sampled from the diffuser and results interpreted with respect to the prior study report.

Overall, 761 samples including water and sediments were collected across Y1-Y3 for analyzing fecal indicators, fecal markers, and pathogens (Table S1-S8). Further, 1227 samples were collected during dye studies (details in the SI). The procedures for water and sediment sample handling, physicochemical sample analyses, FIB analyses, and DNA extraction and analysis are detailed in the SI. In brief, water samples were vacuum filtered until the point of refusal. For sediments and sands, approximately 250 g of each composite sample was collected. The regionally tested host associated fecal source markers (Boehm et al., 2013) HF183 and HumM2 (as human-associated fecal markers), Dog-Bact (as the dog marker), and Gull2TaqMan (as the gull marker) were assessed by quantitative polymerase chain reaction (qPCR), as before (Li et al., 2020). qPCR of Entero1A and ttr genes was used to quantify Enterococcus and Salmonella spp., respectively (Li et al., 2020). The HumM2 qPCR assay was only performed to confirm the presence of human fecal DNA, using samples positive for HF183. All qPCR assays were performed in triplicate. Samples with two or more replicates amplifying within the range of the standard curve were considered to be within the range of quantification (ROQ) and were quantified. Samples with two or more replicates amplifying below the lowest standard were considered detected but not quantifiable (DNQ), and samples with one or zero replicates amplifying were considered not detected (ND), as described previously (Ervin et al., 2014). Human adenovirus was quantified using a droplet digital PCR (ddPCR) method (Steele et al., 2018). The host specificity of the HF183 human fecal marker (Green et al., 2014) was evaluated using fresh gull and bird feces collected from study beaches (details in the SI).

Microbial source tracking in Y1 and Y2

Y1 and Y2 MST included the Honda Creek (Fig. S2) and Sycamore Creek (Fig. S3) watersheds, which drain to LB and EB, respectively. Each of the two watershed surface drainages (for LB: Honda Creek culvert outlet, which carried the enclosed flow of upstream Honda Creek; for EB: Sycamore Creek) terminated in scour ponds consisting of small pools disconnected from the surf zone due to intact beach sand berms, a typical summertime phenomenon in southern California (Rich and Keller, 2013). Other lower watershed features were mapped (Fig. S2, S3) and physically inspected. In Y1 of MST, hypothesized LB and EB FIB sources (Table S9) were tested by analyzing grab (ca. 4 L) samples of water from beach and lower watershed locations (Fig. S4, S5) during 6 dry weather temporally distributed events (one to five surf zone locations each beach) and 2 rain events (one location each beach). Dye

testing (Rhodamine WT; details in the SI) with groundwater sampling (Fig. S6, S7; SI) was performed to assess potential subsurface contamination from beach bathrooms.

In Y2 of MST, LB and EB surf zone and lower watershed creek sites were sampled (Fig S4, S5) to assess differences from Y1. Based on Y1 results, additional triplicated sampling was of creek sediments and intertidal sands to determine if these were fecal contamination reservoirs (Fig. S4, S5); creek water and surf zone (3 locations each beach) during the two rain events, to evaluate erosion of creek bank fecal deposits into flowing water; nearshore sediments (once), and paired surf zone and nearshore water, to determine if there was an off- to onshore gradient indicative of either moored boats or WWTP outfalls as fecal sources (Fig. S8); surf zone waters and sediments at AH to determine if EB and LB patterns were regional (Fig. S1, S9). Additional sampling events included groundwater sampling near a septic system at EB (Fig. S10), and sampling a previously leaking (then repaired) EB bathhouse sanitary sewer lateral (Fig. S11). Dye studies were performed once to test subsurface hydraulic connectivity via groundwater between creek termini scour ponds and the surf zone (Fig. S12, S13), and the integrity of nearby sanitary sewers (Fig. S14, S15). Details of the sampling methods, sample processing, and dve studies are in the SI.

MST in Y3: sub-studies to discern HF183 human marker sources

Based on results of Y1 and Y2 MST, sub-studies were performed in Y3 to determine sources of low, yet chronic, HF183 human marker concentrations in the surf zones, emphasizing EB due to its greater use. Other hypothesized HF183 sources (beach sewers and septic systems, lower watershed contamination with beach groundwater transport, and storm drain discharges) had been tested during Y1-Y2 MST. In Y3, there were six hypothesized HF183 sources to be tested: daytime swimmer shedding (Fig. S16 and Table S10); overnight water defecation (Fig. S17); supratidal sand fecal deposit erosion via tidal action (Yamahara et al., 2007); offshore to onshore fecal marker transport from moored boats (Fig. S18); the El Estero WWTP outfall plume with its potential to intersect onshore (Fig. S18), using sampling and simulations by the UM3 (Updated Merge) model from the U.S. EPA Visual Plumes model system (Frick et al., 2003); the MSD WWTP outfall plume for which findings in a prior microbiological and physical report were reassessed in light of sampling herein; sewage infrastructure in the Santa Barbara Harbor (Fig. S20) and at Stearns Wharf (Fig. S21). The details of the hypotheses tested with related study sites, number of samples, dry vs. wet weather conditions, and years in this study are summarized in Table S11.

Statistical analyses

Nonparametric statistical analyses including Wilcoxon tests (Mann-Whitney for two categories, or Kruskal-Wallis with Steel-Dwass pairwise comparisons for three or more categories), Fisher's Exact Test, and Spearman's ρ rank correlation were performed using JMP10 (SAS, Cary, NC). For statistical analyses of most qPCR assay results, DNQ values were set to 1.8 (log scale) and ND values were set to 1.3 (log scale). For *Salmonella* qPCR results, DNQ values were set to 1 (log scale) and ND values were set to 0.5 (log scale). For human adenovirus ddPCR results, ND values were set to 0. For correlations between human fecal marker results and counts of people in water, on sand, or bedding, Spearman's rho correlation analysis was performed using a substitution value (50 copies/100 mL) for DNQ results, 0 for ND, and actual concentrations for ROQ results. Additional details are described in the SI.

Results

Reference beach fecal contamination and host-associated marker specificity

Human- and dog-associated markers, and pathogens (*Salmonella* spp. and human adenovirus) were not detected in AH (reference beach) surf zone, lower watershed water, or beach sand samples (Table S1), discounting regional contamination. AH surf zone waters, and some sand samples harbored Gull2TaqMan markers (including at ROQ levels), but FIB concentrations were low.

Fresh gull and other seabird feces from the Santa Barbara area (Table S1) did not contain human markers, DogBact markers, or human adenovirus; thus birds were not sources of these analytes at LB or EB.

Fecal indicators, markers and pathogens in surf zone waters

During dry weather in Y1 and Y2 (52 and 48 surf zone samples, respectively), exceedances of single sample AB411 FIB criteria (10,000 TC, 400 EC, 104 ENT, all MPN/100 mL) were rare in surf zone waters (Table S2), with 8.3%-11.5% of EC at LB and 3.7-3.8% of ENT at EB exceeding regulatory criteria overall. Gull2TaqMan and DogBact markers were prevalent, with Gull2TaqMan detected in all surf zone waters of both beaches, and DogBact detected in 48.1% to 100% of the samples for both beaches per year. HF183 was consistently detected in both surf zones, at a frequency ranging from 44.4% to 73.1% per year. However, HF183 markers were at DNQ levels which contrasted with the ROQ levels for most Gull2TaqMan and DogBact marker results (Fig. 1). *Salmonella* spp. were not detected, and human adenovirus was detected in one sample.

No significant interannual variations were observed for FIB or any host marker in the LB and EB surf zones across Y1 and Y2 (Table S2). The surf zone concentrations of TC, EC, ENT and ENT by Entero1A were closely correlated (Spearman's ρ ranging from 0.32 to 0.74, all p<0.001, n = 100). FIB and Gull2TaqMan marker concentrations were correlated (ρ from 0.30 to 0.46, all p<0.003, n = 100); concentrations of DogBact markers were correlated with TC and ENT concentrations (both ρ >0.22, p<0.03, n = 100). These results suggested that gulls were contributing FIB to the surf zone during dry weather; dogs were also a potential surf zone FIB source. FIB and HF183 results were uncorrelated. During rain events, Gull2TaqMan and DogBact marker concentrations were more



Fig. 1. Gull2TaqMan, DogBact and HF183 fecal marker concentrations in surf zone waters at LB and EB during dry weather in 2015 and 2016. The x-axis (not to scale) represents the sampling order at EB and LB, with data plotted as increasing time (from left to right). Detected but not quantifiable (DNQ) and not detected (ND) levels were set to 1.8 and 1.3 (log scale) and shown with solid and open circles, respectively. The total number of data points plotted is 116 (Table S2).

correlated with TC, EC, and Entero1A (Table S2) (ρ from 0.52 to 0.76, all p<0.04, n = 16), suggesting that gull and dog fecal deposits on beaches were transported into surf zone waters during wet weather.

Fecal indicators, markers and pathogens in coastal creek waters and sediments

Compared to surf zone waters, LB and EB watershed coastal creek waters (34 and 56 water samples, respectively) (Fig. S4, S5) contained higher (Mann-Whitney test, all p < 0.0001) FIB concentrations in dry weather, with 83.3%-100% of TC, 33.3%-63.4% of EC, and 40%-100% of ENT results exceeding AB411 criteria in both watersheds during both Y1 and Y2 (Table S3). Lower concentrations of Gull2TaqMan (positive in less than 25% of samples), DogBact (less than 8%), and HF183 (less than 50%) occurred in watershed, compared to surf zone, waters in both watersheds during both Y1 and Y2 (all p < 0.0001), indicating that coastal creek waters were not surf zone fecal marker sources. Creek water fecal markers and FIB were uncorrelated in dry weather. However, FIB and fecal markers increased (Mann-Whitney test, all p<0.0001) during rain events, with 50%-71.4% detection frequencies for Gull2TaqMan, 40%-100% for DogBact, and 40%-85.7% for HF183 in both watersheds per year, mostly at ROQ levels (Fig. 2). The Salmonella detection frequency also increased from less than 5% to 20%-57.1% after rain events in both watersheds per year; human adenovirus was sporadic. FIB concentrations were correlated with DogBact (all ρ >0.55, p<0.0002, n = 35), HF183 (ρ ranging from 0.34 to 0.47, p < 0.05, n = 35), and Salmonella (all $\rho > 0.32$, p < 0.05, n = 35) concentrations. Furthermore, DogBact was correlated to HF183 and Gull2TaqMan, and *Salmonella* (all ρ >0.52, p<0.002, n = 35). These results suggested that fecal markers, particularly DogBact and HF183, and Salmonella, were mobilized with fecal deposits into creek waters during runoff-generating rainfall events. Although more dry weather HF183 detections appeared for LB creek waters during Y2 versus Y1 (Table S3), there were no significant interannual variations in fecal markers during either dry or wet weather.

High concentrations of FIB, such as TC over 1000 MPN/g dry sediment detected in 65.2% of samples (n = 23 by combining LB and EB watersheds together), were quantified in creek sediments (Table S4) —a characteristic of freshwater environments (Pachepsky and Shelton, 2011)—but HF183 was not detected, and only one sediment sample at



Fig. 2. Heat-map of the log concentrations of host fecal markers in waters of lower Sycamore Creek and the bird refuge (7 sampling sites upstream of EB, Fig. S5) and Honda Creek and a drain on the beach (4 sampling sites upstream of LB, Fig. S4) during dry and wet weather of 2015 and 2016. The vertical axis represents individual sampling sites relevant to EB and LB MST (left) displayed for each of the three host fecal markers (right: gull (Gull2TaqMan), dog (Dog-Bact), human (HF183)); the horizontal axis represents individual sampling events. The highest value (1.0E+06 copies per 100 mL) is indicated in red, and not detected (ND) is indicated in blue. Detected but not quantifiable (DNQ) is in pink and all DNQ samples were also emphasized with green rectangle dashed lines. Gray represents no data. Samples are ordered according to the time of the event, from left to right (not to scale). A total of 125 samples are represented (Table S3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

site E11 was positive for DogBact. Gull2TaqMan was detected in sediments near the Honda Creek culvert outlet (LB), where shorebirds congregated. Human adenovirus was detected at one site (E10, Fig. S5; Table S4).

Beach sands, scour ponds, beach infrastructure, and recycled water

Neither supratidal sands (collected from the average high tide elevation) nor intertidal sands showed evidence of fecal contamination (Table S4): FIB concentrations were very low to nondetectable in beach sands, and no fecal markers or pathogens were detected. Groundwater sampling and dye studies, which were performed to determine whether creek termini scour ponds were delivering contamination to surf zones (Fig. S12 and S13), indicated neither (HF183 or DogBact) fecal markers nor pathogens in groundwater (Table S5), and very low LB (Fig. S22, S23) and EB (Fig. S24, S25) groundwater velocities towards the ocean. Dye tests of sewer infrastructure (Fig. S6, S7, S14, S15, S21) indicated no leakage (Fig. S10, S11, S20) were uncontaminated (details in the SI Results). Recycled water was not a source of fecal contamination (SI Results).

Nearshore and offshore water, sediments, and moored boats

By synchronously sampling surf zone, nearshore, and offshore LB and EB waters in Y2 (Fig. S8) and surf zone, nearshore, and boat anchorage waters in Y3 (Fig. S18), FIB and fecal markers, particularly HF183, decreased from the surf zone to nearshore to offshore (Fig. 3). HF183 markers were detected in 40.9% of surf zone (n = 88), 30.4% of nearshore (n = 56), and 6.7% of offshore (n = 15) water samples during two years; Gull2TaqMan markers in 100%, 81%, and 73%; and DogBact markers in 61%, 19%, and 0% of samples, respectively (Table S6). FIB and HF183 detections in boat anchorage areas (Fig. S18)-located between nearshore and offshore-fell within these gradients, with an HF183 detection frequency of 20% (n = 20). This discounted human waste discharge from moored boats as an HF183 surf zone source. Yet, DNQ level detections of HF183 were occasionally observed in the nearshore and offshore, along with the surf zone water samples, and on one date (10/5/2017) higher concentrations were measured in the nearshore (Fig. S31, SI Results), indicating periodic offshore or nearshore sources. Markers were mostly uncorrelated with FIB, except that Gull2TaqMan concentrations correlated with EC and Entero1A (p of 0.41 and 0.39, both p < 0.02, n = 179). Pathogens were not detected in nearshore or offshore waters. Nearshore marine sediments were devoid of fecal markers including HF183, and contained low to nondetectable FIB (Table S4).

Treated wastewater effluent

The El Estero WWTP raw sewage contained 2.0E+08, 9.3E+06, and 3.1E+04 copies/100 mL of HF183, DogBact, and human adenovirus, respectively (Table S7). Concentrations averaged 3.6E+05, 6.9E+03, and 1.6E+01 copies/100 mL for HF183, DogBact and human adenovirus, respectively, in samples acquired at one of the outfall diffuser ports (Table S7 and Fig. S18) which were a mixture of surrounding seawater and effluent. Lower HF183 concentrations (on average, 4.4E+02 copies/100 mL), and no DogBact or human adenovirus, were found in the discharge of an MSD WWTP outfall diffuser port (Table S7 and Fig. S18).

The fate of the diffused El Estero WWTP outfall discharge was simulated using the UM3 plume model (details in the SI). Under the three modeling scenarios, the plume was not predicted to surface on the Y3 offshore sampling dates and, while the Y2 plume was predicted to be more buoyant, it was also unlikely to surface. At marine sampling locations directly above the outfall diffuser (Fig. S19), fecal markers and pathogens were detected at 18 m depth from the surface, but not at 1 m



Fig. 3. FIB (a), HF183 (b), and Gull2TaqMan and DogBact (c) concentrations in surf zone, nearshore, anchorage, and offshore waters during dry weather in 2016 and 2017. The x-axis represents the order of the sampling events (first to last, from left to right, not to scale) in each area. (a) FIB concentrations <10 MPN/100 mL were set to 0.5 (log scale). (b, c) detected but not quantifiable (DNQ) and not detected (ND) for DNA markers were set to 1.8 and 1.3 (log scale) and shown with solid and open circles, respectively. A total of 179 data points are displayed (Table S6).

depth and rarely at 9 m depth (Table S7). Predicted HF183, Entero1A, and human adenovirus concentrations in the plume after trapping were within range or similar to those measured in the collected 18 m depth water samples. Although it is possible that the effluent plume could reach the surf zone via surface currents that can move onshore in the region (Ohlmann et al., 2012), fecal indicator, marker and pathogen concentrations in the plume would attenuate during transport due to diffusion and decay and thus the frequency or magnitude of plume constituents in EB or LB surf zones were highly uncertain. Similarly, the results from a prior study of the MSD WWTP outfall would support that the surfaced plume could migrate towards EB sampling sites. However, the comparatively low HF183 concentrations measured at the MSD outfall in this study, coupled with the previously observed dilution of microbiological constituents along the surfaced plume trajectory, and that there was no significant increase in HF183 marker detection at the surf zone and nearshore locations closest to the outfall, indicated that the MSD WWTP outfall plume was also an unlikely source of HF183 to EB (SI Results).

Surf zone water defecation, recreation and spatial analysis studies

Possible water defecation overnight in the surf zone by beach campers was investigated in a targeted study at EB (Fig. S17) by comparing HF183 concentrations in the early morning (ca. 7 am) with those in the previous late afternoon (ca. 6 pm), and further with the following mid-afternoon (ca. 3 pm); this timing also allowed for inferring possible daytime sunlight-mediated marker decay across the study sites. The HF183 detection frequency was higher in the late afternoon (48%, n = 25) as compared to the early morning (8%, n = 25) and mid-afternoon (4%, n = 25; Table S8) (Wilcoxon test, both p < 0.03), thus relegating overnight water defecation as an unlikely source of HF183 in the surf zone.

On holidays and busy (high visitation) weekends, HF183 detection frequencies in the surf zone were also higher in the mid-afternoon (ca. 3 pm) (26.7% positive, n = 15) as compared to the morning (6.7%, n = 15) or at the same time (ca. 3 pm) on weekdays (4%, n = 25) (Table S8), possibly due to more people in the surf zone (7.4 versus 0.2 and 2.4 per site on average; Table S10). The highest HF183 detection frequency was observed before (ca. 5 pm) and after swimming races (ca. 7 pm; Fig. S16), with HF183 present in 73% of surf zone samples (both n = 15), followed by 67% in the next early morning (n = 15) (Table S8). The concentrations of HF183 pre- and post- race events were higher than those at nearly the same time (ca. 6 pm) on weekdays during the water defecation study (p = 0.006, n = 55), corresponding to the higher number of swimmers during races (p < 0.0001; Table S10).

However, across all morning Y1-Y3 EB surf zone samples (from sites E01 to E05; Fig. S5; n = 113 total, 20 to 27 per site) there was no significant difference in HF183 based on sampling location (p = 0.8878, Wilcoxon), suggesting an in-common HF183 source. Even when including the dry weather afternoon samples taken at the same sites (n = 179 total, 32 to 45 per site), there was still no significant difference in HF183 detection and/or concentration between the sites (p = 0.7603, Wilcoxon). Similarly, there was no significant difference in HF183 detection between the three EB nearshore sampling locations (Fig. S8 and S18: sites E-1NS, E-2NS, and E-3NS; n = 24 total, 8 per site) sampled across Y2 and Y3 (2016 and 2017; p = 1.0000, Wilcoxon). These results could support a chronic, albeit low level and varying, effect of the WWTP outfalls on surf zone HF183.

Regardless, there was a significant association between the presence of HF183 in LB and EB surf zones versus surf zone swimmer counts at the time of sampling (Table S10) (Fisher's Exact Test p = 0.03; Spearman's ρ = 0.17, p = 0.02; logistic regression p = 0.01, n = 196) that could imply a role of swimmers on surf zone HF183 detections. This included samples taken during weekdays, holidays and high visitation weekends, swimming races, and also the morning sampling campaigns when people were counted. In contrast, negative correlations were found between HF183 concentrations and the numbers of people on sand or observed bedding (data not shown). The association between HF183 concentrations and the counts of swimmers (Fig. 4) was more significant when only considering the samples collected at EB (Fisher's Exact Test p =0.02; Spearman's $\rho = 0.21$, p = 0.007; Logistic regression p = 0.01, n =165), or in the afternoon (Spearman's $\rho = 0.23$, p = 0.03, n = 85) but not in the morning ($\rho = 0.09$, p = 0.32, n = 111). These results point to influences of swimmers on surf zone HF183 detections, perhaps separable and against a backdrop of outfall discharge plumes potentially reaching the shore.

Discussion

Considering the frequencies of dogs and gulls or other seabirds at beaches, it is not surprising that such hosts are often identified as major FIB sources in surf zones (Converse et al., 2012; Goodwin et al., 2016;



Fig. 4. Boxplot of HF183 concentrations in EB surf zone samples with number of swimmers counted. Center lines show the medians; box limits indicate the 25th and 75th percentiles: whiskers extend 1.5 times the interguartile range from the 25th and 75th percentiles, and outliers are represented by open dots. DNQ results are plotted as 50 copies/100 mL. EB.M: EB morning (sample size n = 25), O.A.6: water defecation late afternoon (ca. 6 pm) (n = 20), O.M: water defecation next morning (n = 25), O.A.3: water defecation next early afternoon (ca. 3 pm) (n = 20), H.M: holiday morning (n = 15), H.A.3: holiday afternoon (ca. 3 pm) (n = 15), S.A.5: swimming afternoon pre-race (ca. 5 pm) (n = 15), S. A.7: swimming afternoon post-race (ca. 7 pm) (n = 15), S.M: swimming next morning (n = 15). "Water defecation" refers to the Y3 substudy in which surf zone sampling was timed across 24 hour periods to assess potential direct contamination overnight by beach campers. "Holiday" refers to the sub-studies that emphasized sampling on typically high visitation holidays and weekends for these tourist beaches of LB and EB. "Swimming" refers to dates of race events wherein swimmers were densely congregating at the beach pre-race with some swimming prior to the race event, then entering the water at once and swimming as a large group until the event end. The total number of samples represented in this plot is 165 (Table S10).

Wright et al., 2009), as shown in this study. However, animal feces typically pose fewer risks to public health due to the host specificity of many pathogens. Here, similarly to prior studies of recreational beaches (Cao et al., 2017; Jennings et al., 2018; McQuaig et al., 2012; Riedel et al., 2015; Russell et al., 2013; Santoro and Boehm, 2007), low levels of human fecal markers were consistently detected in surf zone waters. HF183 did not nonspecifically amplify from gull or seabird feces in this study, or from marine mammal feces as shown previously (Shanks et al., 2010). A low-level amplification of HF183 from dog fecal DNA was reported previously (Boehm et al., 2013). However, to generate a low DNQ level of HF183 (10 copies/100 mL in this study), 8.8E+07 times more dog feces than human feces are needed (Boehm et al., 2013; Ervin et al., 2013 and 2014). This would result in a corresponding DogBact concentration of 9.4E+08 copies/100 mL which is much higher than the DogBact concentrations quantified herein (less than 1.0E+05 copies/100 mL). Thus the presence of HF183 in this study's surf zone water samples could not be attributed to dog fecal contamination, and instead signaled human fecal sources.

Freshwater flows from storm drains and urban creeks can convey watershed sources of fecal contamination into surf zones, owing partly to at-risk intersections of storm drains and sewage pipes, as discussed before (Izbicki et al., 2009). The California AB411 monitoring period coincides with little rainfall for the Mediterranean-type climate of southern California. Yet, the few small rain events during this study were able to mobilize dog and human fecal wastes into creek waters which resulted in elevated fecal indicators, markers and pathogens, as observed previously (Steele et al., 2018). Dog fecal contamination in urban creeks can be controlled through targeted pet waste disposal education and management for creek-side residences (Ervin et al., 2014). However, creek sediments—previously found to harbor human fecal bacteria and consistently contaminate surface water, particularly during storm flows (Frey et al., 2015; Kim and Wuertz, 2015)—were not reservoirs for fecal markers and pathogens in this study. Furthermore, similarly to a previous study elsewhere (Russell et al., 2013), and similarly to a prior study in this region (Izbicki et al., 2009), beach groundwater was not contaminated with fecal markers, indicating that the bermed creek outlets here were hydrologically disconnected from surf zones. Further, as per the dye studies, sewers in the surf zone vicinities were not leaking.

Compared to other potential fecal origins to surf zones, those from nearshore and offshore have been less investigated. Moored boats could illegally discharge human waste into the ocean, although this was not evidenced herein. HF183 concentrations in the effluent plume from the El Estero WWTP ocean outfall matched the plume modeling output for deep waters at a distance from shore, but salinity, light intensity, dilution, and food webs can contribute to marker attenuation (Carneiro et al., 2018). Still, on one sampling date, higher HF183 concentrations were observed in the nearshore as compared to the surf zone, indicating a source of HF183 related to the nearshore environment (Fig. S31). Owing to complex circulation processes in the Santa Barbara Channel including upwelling (Harms and Winant, 1998), far field modeling approaches (Brooks, 1960) were not utilized to simulate the plume movement beyond its initial dilution. One or more WWTP outfalls, such as El Estero or MSD, discharging HF183 into a plume that consistently migrates to shore could account for chronically observed HF183 with inter-site spatial homogeneity at EB, as the sources would be continuous. Further, given typical diurnal patterns of WWTP flows (Tchobanoglous et al., 2003), lower morning relative to late afternoon HF183 detections, as observed here, might relate to temporal patterns of HF183 discharge at the outfall diffusers and associated plume migration to shore, neither of which was studied here. Thus, the WWTP effluent plumes were not ruled out as sources of either HF183 or other fecal indicators to LB or EB. Yet, given the majority on- to offshore spatial gradients of HF183 detections, it is unlikely that effluent plumes were the main sources of HF183.

Remarkably, there were higher levels of surf zone HF183 during swimming race events, holidays and high visitation weekends, in contrast to low visitation weekdays. Further, surf zone HF183 correlated to counts of swimmers. Beach campers have been previously hypothesized as human fecal contamination sources in recreational beach waters due to water defecation (Noble et al., 2000). However, the HF183 night-to-day patterns when assessing potential water defecation did not support this hypothesis. Rather, swimmers appeared to be sources of HF183 in surf zone waters. An estimate of either 152 swimmers at an average published fecal shedding amount (0.14 g per person), or only 2 bathers at a worst case fecal shedding amount (10 g per person) (Gerba, 2000), or 0.2 fecal discharge events (Rose et al., 2015), could generate a low ROQ level (100 copies/100 mL) of HF183 (Ervin et al., 2013) in a narrow swath of surf zone spanning over the sampling sites at LB or EB (1.9E+08 L of water; estimated by measuring the distance spanning the three nearshore sampling locations and the distance halfway from the nearshore locations to the surf zone using GIS, assuming an average depth of 1.5 m as extrapolated from scientific diver depth measurements at the nearshore locations, then averaging the volume from each beach). In addition, applied to a smaller section of the narrow surf zone swath (i. e. encompassing the swim race section of 2 of the EB 5 surf zone sampling sites totaling 7.5E+07 L, calculated by multiplying the original surf zone swath estimate above by 0.4), an HF183 signal of 100 copies/100 mL could be generated by 61 swimmers at the average shedding amount, or by 1 bather at the worst case fecal shedding amount, or by 0.08 fecal events. Similar or even higher levels of HF183 were recorded before races, and people counted on beach sands-as these counts included people preparing for the swim race events-showed a significant correlation with the concentration of HF183 in preand after-race surf zone samples (Spearman's $\rho = 0.40$, p = 0.03, n = 30) (Table S10). Further, the correlation between swimmer count and HF183 was stronger in the afternoon, when there were more swimmers, relative to the morning. Most research emphasizes surf zone water sampling in the morning to avoid effects of sunlight mediated

accelerated human marker decay. Late afternoon sampling of surf zone water—or at least sampling to coincide with the presence of swimmers—should be considered in future research.

The low levels of HF183 recorded in the surf zones of this study were well below estimated thresholds for public health risk (4100 copies/100 mL) (Boehm et al., 2015; Brown 2017), with only one exception (Site L04) (Fig. 3b, Table S6, S8). Consistently, pathogens including human adenovirus and Salmonella bacteria were rarely detected in the surf zones. Beaches in California deemed as priorities and listed for potential study as well as implementation of capital improvements towards remediation are based on FIB concentrations (Sikich et al., 2018). Given the disconnect between FIB and human fecal contamination (Mika et al., 2014) as in this study, human fecal markers such as HF183 could be used more routinely to indicate human fecal contamination. In cases such as this study where HF183 was found to correlate with swimmer counts, human fecal marker detection frequency relatedness to variations in beach visitation, swimmers, or particularly racers, could be monitored for understanding potential public health ramifications and the need for management of human behavior through education. Regardless, this study points to a low threshold of HF183 that is attainable at urban recreational beaches, when almost all other potential human fecal sources besides bathing humans and remote effluent plumes are ruled out. These results may also be relevant for enclosed beaches with intractable sources of HF183 that may be a result of high swimmer loads and lack of dilution. In addition, these results suggest that natural exclusion studies or quantitative microbial risk assessment (QMRA)-based site specific criteria may need to allow for consistent, low-levels of HF183. However, as the potential for chronic contamination of coastal zones by WWTP effluent plumes has many chemical and microbiological pollution implications, further studies of plume migration using modeling and multi-tracer field studies is also warranted.

Conclusions

Low levels of chronic HF183 in recreational beach surf zone waters have long been observed, pointing to ongoing public health risks. In a comprehensive three-year summertime assessment of two urban California beaches, surf zone FIB contamination was mainly attributed to gull and dog wastes. After excluding almost all other human fecal sources, HF183 in surf zones was attributable to swimmers, particularly in the afternoon, but two WWTP outfalls in the vicinity could not be ruled out as sources. This study demonstrates how all hypothesized fecal sources can be systematically tested to reveal—for two urban recreational beaches—a prevalence of non-human FIB sources, an absence of tested pathogens in most samples, and a lower achievable limit of HF183.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the State of California Clean Beach Initiative, using funds from Proposition 84, and from funds provided by Mr. Henry (Sam) Wheeler. We acknowledge the assistance of Tania Gomez Ayala, Marina Feraud, Mitchell Maier, Patrick Roehrdanz, Ying Wang, Dr. Monika Mortimer, Christoph Pierre, Christian Orsini, and Eric Hessell at UCSB as well as Geosyntec Consultants' researchers including Stacy Isaac, Adam Questad, and Maia Colyar. We also thank Hillary Hauser of Heal the Ocean, Cathleen Garnand of Santa Barbara County, Project Clean Water, Todd Heldoorn of the City of Santa Barbara El Estero Wastewater Treatment Plant (WWTP), Mick Kronman of the City of Santa Barbara, Waterfront Division, and Willie Brummett of Santa Barbara County, Environmental Health Services. We acknowledge the generous consultation of several researchers including Dr. Yiping Cao, Professor Libe Washburn, and Dr. Carter Ohlmann.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117378.

References

- Arnold, B.F., Schiff, K.C., Griffith, J.F., Gruber, J.S., Yau, V., et al., 2013. Swimmer illness associated with marine water exposure and water quality indicators: impact of widely used assumptions. Epidemiology 24, 845–853.
- Boehm, A.B., Sanders, B.F., Winant, C.D., 2002. Cross-shelf transport at Huntington Beach. Implications for the fate of sewage discharged through an offshore ocean outfall. Environ. Sci. Technol. 36, 1899–1906.
- Boehm, A.B., Soller, J.A., Shanks, O.C., 2015. Human-associated fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environ. Sci. Technol. Lett.* 2, 270–275.
- Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., Weisberg, S.B., 2013. Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. Water Res 47, 6812–6828.
- Brooks, N.H., 1960. Diffusion of sewage effluent in an ocean current. In Proceedings of the First International Conference on Waste Disposal in the Marine Environment, Pearson EA. Ed. Pergamon: pp 246–267.
- Brown, K.I., Graham, K.E., Soller, J.A., Boehm, A.B., 2017. Estimating the probability of illness due to swimming in recreational water with a mixture of human- and gullassociated microbial source tracking markers. Environ. Sci. Process Impacts 19, 1528–1541.
- California Water Boards Los Angeles Region 4. Watershed Management Programs, 2018. https://www.waterboards.ca.gov/losangeles/water_issues/programs/stormwater/ municipal/watershed_management/. (Accessed 2 July 2021).
- Cao, Y., Raith, M.R., Smith, P.D., Griffith, J.F., Weisberg, S.B., et al., 2017. Regional assessment of human fecal contamination in southern California coastal drainages. Int. J. Environ. Res. Public Health 14, E874.
- Carneiro, M.T., Cortes, M.B.V., Wasserman, J.C., 2018. Critical evaluation of the factors affecting Escherichia coli environmental decay for outfall plume models. *Rev. Ambient. Água.* 13, 1–18.
- Colford, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., et al., 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology 18, 27–35.
- Converse, R.R., Kinzelman, J.L., Sams, E.A., Hudgens, E., Dufour, A.P., Ryu, H., Santo-Domingo, J.W., Kelty, C.A., Shanks, O.C., Siefring, S.D., Haugland, R.A., Wade, T.J., 2012. Dramatic improvements in beach water quality following gull removal. Environ. Sci. Technol. 46, 10206–10213.
- Elmir, S.M., Shibata, T., Solo-Gabriele, H.M., Sinigalliano, C.D., Gidley, M.L., Miller, G., Plano, L.R.W., Kish, J., Withum, K., Fleming, L.E., 2009. Quantitative evaluation of enterococci and Bacteroidales released by adults and toddlers in marine water. Water Res 43, 4610–4616.
- Elmir, S.M., Wright, M.E., Abdelzaher, A., Solo-Gabriele, H.M., Fleming, L.E., Miller, G., Rybolowik, M., Shih, M.T.P., Pillai, S.P., Cooper, J.A., Quaye, E.A., 2007. Quantitative evaluation of bacteria released by bathers in a marine water. Water Res 41, 3–10.
- Ervin, J.S., Russell, T.L., Layton, B.A., Yamahara, K.M., Wang, D., Sassoubre, L.M., Cao, Y.P., Kelty, C.A., Sivaganesan, M., Boehm, A.B., Holden, P.A., Weisberg, S.B., Shanks, O.C., 2013. Characterization of fecal concentrations in human and other animal sources by physical, culture-based, and quantitative real-time PCR methods. Water Res 47, 6873–6882.
- Ervin, J.S., Van De Werfhorst, L.C., Murray, J.L., Holden, P.A., 2014. Microbial source tracking in a coastal California watershed reveals canines as controllable sources of fecal contamination. Environ. Sci. Technol. 48, 9043–9052.
- Field, K.G., Samadpour, M., 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Res 41, 3517–3538.
- Frey, S.K., Gottschall, N., Wilkes, G., Grégoire, D.S., Topp, E., Pintar, K.D., Sunohara, M., Marti, R., Lapen, D.R., 2015. Rainfall-induced runoff from exposed streambed sediments: an important source of water pollution. J. Environ. Qual. 44, 236–247.
- Frick, W.E., Roberts, P.J.W., Davis, L.R., Keyes, J., Baumgartner, D.J., George, K.P., 2003. Dilution models for effluent discharges. (Visual Plumes, 4th ed. United States Environmental Protection Agency, Athens, Georgia.
- Gerba, C.P., 2000. Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality. Quantitative Microbiology 2, 55–68.
- Goodwin, K.D., Gruber, S., Vondrak, M., Crumpacker, A., 2016. Watershed assessment with beach microbial source tracking and outcomes of resulting gull management. Environ. Sci. Technol. 50, 9900–9906.
- Green, H.C., Haugland, R.A., Varma, M., Millen, H.T., Borchardt, M.A., Field, K.G., Walters, W.A., Knight, R., Sivaganesan, M., Kelty, C.A., Shanks, O.C., 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Appl. Environ. Microb.* 80, 3086–3094.

D. Li et al.

Griffith, J.F., Layton, B.A., Boehm, A.B., Holden, P.A., Jay, J.A., et al., 2013. The California Microbial Source Identification Manual: a Tiered Approach to Identifying Fecal Pollution Sources to Beaches. Southern California Coastal Water Research Project. Technical Report 804.

- Harms, S., Winant, C.D., 1998. Characteristic patterns of the circulation in the Santa Barbara Channel. J. Geophys. Res. Oceans 103, 3041–3065.
- Harwood, V.J., Staley, C., Badgley, B.D., Borges, K., Korajkic, A., 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol. Rev. 38, 1–40.
- Ishii, S., Hansen, D.L., Hicks, R.E., Sadowsky, M.J., 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. Environ Sci Technol 41, 2203–2209.
- Izbicki, J.A., Swarzenski, P.W., Reich, C.D., Rollins, C., Holden, P.A., 2009. Sources of fecal indicator bacteria in urban streams and ocean beaches, Santa Barbara, California. Ann. Environ. Sci. 3, 139–178.
- Jennings, W.C., Chern, E.C., O'Donohue, D., Kellogg, M.G., Boehm, A.B., 2018. Frequent detection of a human fecal indicator in the urban ocean: environmental drivers and covariation with enterococci. Environ. Sci. Process Impacts 20, 480–492.
- Kim, M., Wuertz, S., 2015. Survival and persistence of host-associated Bacteroidales cells and DNA in comparison with *Escherichia coli* and Enterococcus in freshwater sediments as quantified by PMA-qPCR and qPCR. Water Res 87, 182–192.
- Mayer, R.E., Reischer, G.H., Ixenmaier, S.K., Derx, J., Blaschke, A.P., et al., 2018. Global distribution of human-associated fecal genetic markers in reference samples from six continents. Environ. Sci. Technol. 52, 5076–5084.
- McQuaig, S., Griffith, J., Harwood, V.J., 2012. Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Appl. Environ. Microbiol.* 78, 6423–6432.
- Mika, K.B., Ginsburg, D.W., Lee, C.M., Thulsiraj, V., Jay, J.A., 2014. Fecal indicator bacteria levels do not correspond with incidence of human-associated HF183 Bacteroides 16S rRNA genetic marker in two urban southern California watersheds. Water Air Soil Pollut 225, 1–11.
- Noble, R.T., Dorsey, J.H., Leecaster, M., Orozco-Borbón, V., Reid, D., Schiff, K., Weisberg, S.B., 2000. A regional survey of the microbiological water quality along the shoreline of the southern California bight. In: Sandhu, S.S., Melzian, B.D., Long, E.R., Whitford, W.G., Walton, B.T. (Eds.), Monitoring Ecological Condition in the Western United States. Soringer. Dordrecht.
- Ohlmann, J.C., LaCasce, J.H., Washburn, L., Mariano, A.J., Emery, B., 2012. Relative dispersion observations and trajectory modeling in the Santa Barbara Channel: lagrangian statistics and modeling. J. Geophys. Res. Oceans 117, C05040.
- Pachepsky, Y.A., Shelton, D.R., 2011. Escherichia coli and fecal coliforms in freshwater and estuarine sediments. Crit. Rev. Environ. Sci. Technol. 41, 1067–1110.
- Rich, A., Keller, E.A., 2013. A hydrologic and geomorphic model of estuary breaching and closure. Geomorphology 191, 64–74.

- Riedel, T.E., Thulsiraj, V., Zimmer-Faust, A.G., Dagit, R., Krug, J., Hanley, K.T., Adamek, K., Ebentier, D.L., Torres, R., Cobian, U., Peterson, S., Jay, J.A., 2015. Longterm monitoring of molecular markers can distinguish different seasonal patterns of fecal indicating bacteria sources. Water Res 71, 227–243.
- Rose, C., Parker, A., Jefferson, B., Cartmell, E., 2015. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Crit. Rev. Env. Sci. Technol.* 45, 1827–1879.

Russell, T.L., Sassoubre, L.M., Wang, D., Masuda, S., Chen, H., Soetjipto, C., Hassaballah, A., Boehm, A.B., 2013. A coupled modeling and molecular biology approach to microbial source tracking at Cowell Beach, Santa Cruz, CA, United States. Environ. Sci. Technol. 47, 10231–10239.

- San Diego Regional Water Quality Control Board, 2017. Cost-benefit analysis: San Diego Region Bacteria Total Maximum Daily loads. (San Diego Regional Water Quality Control Board, p. 416. San Diego, CA).
- Santoro, A.E., Boehm, A.B., 2007. Frequent occurrence of the human-specific Bacteroides fecal marker at an open coast marine beach: relationship to waves, tides and traditional indicators. *Environ. Microbiol* 9, 2038–2049.
- Shanks, O.C., White, K., Kelty, C.A., Sivaganesan, M., Blannon, J., Meckes, M., Varma, M., Haugland, R.A., 2010. Performance of PCR-based assays targeting Bacteroidales genetic markers of human fecal pollution in sewage and fecal samples. Environ. Sci. Technol. 44, 6281–6288.
- Shuval, H., 2003. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. J. Water Health 1, 53–64.
- Sikich, S.A., Luce, S., Pease, K., King, M., Vu, K., 2018. Heal the Bay's 2011-2012 Annual Beach Report Card. Available at. https://healthebay.org/wp-content/uploads/2018/ 07/BRC_2017-2018_07-12-18.pdf.
- Sinclair, R.G., Jones, E.L., Gerba, C.P., 2009. Viruses in recreational water-borne disease outbreaks: a review. J. Appl. Microbiol. 107, 1769–1780.
- Steele, J.A., Blackwood, A.D., Griffith, J.F., Noble, R.T., Schiff, K.C., 2018. Quantification of pathogens and markers of fecal contamination during storm events along popular surfing beaches in San Diego, California. Water Res 136, 137–149.
- Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater engineering: Treatment and Reuse. Metcalf and Eddy, Inc. Boston: McGraw-Hill.
- U.S. Environmental Protection Agency, 2004. In: Report to Congress: impacts and control of CSOs and SSOs. U.S. Environmental Protection Agency, Office of Water, Washington.
- Wright, M.E., Solo-Gabriele, H.M., Elmir, S., Fleming, L.E., 2009. Microbial load from animal feces at a recreational beach. *Mar. Pollut. Bull.* 58, 1649–1656.
- Yamahara, K.M., Layton, B.A., Santoro, A.E., Boehm, A.B., 2007. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. Environ Sci Technol 41, 4515–4521.

SUPPLEMENTAL INFORMATION

Sources of Low Level Human Fecal Markers in Recreational Waters of Two Santa Barbara, CA Beaches: Roles of WWTP Outfalls and Swimmers

Dong Li¹, Laurie C. Van De Werfhorst¹, Brandon Steets², Jared Ervin²,

Jill L. S. Murray³, Avery Blackwell², Naresh Devarajan¹, Patricia A. Holden¹*

¹Bren School of Environmental Science & Management, University of California, Santa Barbara ²Geosyntec Consultants, Santa Barbara, CA 93101

³Creeks Division, Department of Parks & Recreation, City of Santa Barbara, CA 93102

*Corresponding author:

Patricia A. Holden

E-mail: holden@bren.ucsb.edu

Supplemental Materials and methods

Selected study beaches

Two urban Santa Barbara, California recreational beaches were studied: Leadbetter Beach (LB; 34.40222 latitude, -119.696944 longitude) and East Beach at Sycamore Creek (EB; 34.416389 latitude, -119.66667 longitude). Arroyo Hondo (AH; 34.473333 latitude, -120.141111 longitude) was the rural reference beach to the west of Santa Barbara (Fig. S1). The regional climate is Mediterranean, with an annual average rainfall of approximately 47.5 cm (Santa Barbara, 1900-2020; Santa Barbara County Public Works Department, http://countyofsb.org/pwd/yearlyrain.sbc) occurring mostly in the winter (November through March). The AB411 period summer months (April-October) are mostly dry with few runoffgenerating storms. LB and EB were selected due to historically elevated surf zone FIB concentrations, with LB and EB exceeding the U.S. Environmental Protection Agency Beach Action Advisory (BAV) in 2013 by 18 % and 5% respectively (NRDC, 2014). Although not a focus of this study, nearby East Beach at Mission Creek, located between LB and EB (34.41222 latitude, -119.687222 longitude), exceeded the U.S. EPA BAV in 2013 by 18% (NRDC, 2014). Because fecal contamination sources to LB and EB had not been studied, researching these popular bathing beaches was prioritized. For this study, dry weather was defined as <0.1" of rainfall in the preceding 72 hours. Rain events that occurred during the AB411 season were sampled if predicted to be ≥ 0.2 ".

MST overview in Y1-Y2

MST in Y1 (and Y2) followed the recommended steps in California (Griffith et al., 2013) of mapping watersheds; conducting field reconnaissance to confirm and gather additional

information regarding potential fecal sources; formulating fecal source hypotheses; performing dye studies to directly test for leaking infrastructure, conducting field sampling; and laboratory plus data analyses to test the hypotheses. For both the Honda Creek (Fig. S2) and Sycamore Creek (Fig. S3) watersheds, which drain to LB and EB, respectively, MST was geographically confined to the lower watershed and beach areas located downstream of the most upstream observed pooled surface water (Fig. S2, S3). Each of the two watershed surface drainages (for LB: Honda Creek culvert outlet, which carried the enclosed flow of upstream Honda Creek; for EB: Sycamore Creek) terminated in scour ponds that consisted of small pools not flowing overland into the surf zone due to their respective sand berms which remained intact during the study periods, a phenomenon typical in summertime southern California (Rich and Keller, 2013). Other relevant lower watershed features were sanitary sewers and storm drains including pipe crossings, locations of reclaimed wastewater being used for irrigation, and septic systems (Fig. S2, S3), as these had the potential to contribute human waste markers to creeks or drains with further conveyance to the beaches. Three potential fecal sources-gull, dog and human-were identified through evaluating mapped and observed lower watershed plus beach area features (Fig. S2, S3) including locations of sanitary sewers relative to nearby storm drains that discharged into the Honda Creek culvert outlet or into Sycamore Creek or to the beaches, the Santa Barbara Harbor near LB, the bird refuge (with adjacent zoo) upstream of EB, sewers and septic systems in the beach areas, the City of Santa Barbara El Estero WWTP whose outfall extended into the offshore submarine environment between LB and EB, the Montecito Sanitary District (MSD) WWTP outfall to the far east of EB, boat moorings mainly offshore of EB, shorebird congregations, wrack and dogs on the beaches, animal fecal deposits, beach area camps, and flowing drains. Further, the following were hypothesized as reservoirs potentially

harboring fecal contamination that could be mobilized into the surf zones via direct flow, subsurface flow, or periodic overland runoff processes: fecal deposits on creek banks, creek and culvert sediments, water and sediments in scour ponds at watershed termini, flowing storm drains, beach bathroom laterals, beach area sewer mains or septic systems, boats in the Santa Barbara Harbor, secondarily-treated WWTP effluent discharging offshore through the associated outfalls, recycled water (tertiary wastewater effluent) used for inland irrigation of public areas, and coastal marine sediments.

Y3 Substudy descriptions

To test if daytime swimmers were shedding HF183 markers to the surf zone, sampling was performed when the most swimmers were expected: during race events, and on holidays and high visitation weekends. Because EB race events are more attended relative those at LB, EB was the focus for sampling race events and, for consistency, during holidays and weekends. Surf zone samples were collected (Fig. S16) for 3 of the EB race events, including before the race, just after, and the following morning; intertidal beach sand samples were also collected for assessing potential direct contamination during the events. On high visitation recreational weekends, surf zone waters were sampled during mornings and afternoons, and intertidal sands were sampled in the morning. Locations (Fig. S17) were the same as in Y1 and Y2 MST (Fig. S5). To test the hypothesis that water defecation was occurring by overnight beach campers, surf zone waters were sampled during the evening of one day, and the subsequent morning and afternoon, repeating 5 times at 5 surf zone locations (Fig. S17). Inferred direct human influences, including potential water defecation directly into the surf zone and shedding during surf zone recreation on holidays and high visitation weekends or during swimming races, were related to

the amount of bedding on the beach, the numbers of swimmers or players in the surf zones, and people on beach sands, all recorded during sampling.

To test if supratidal sand held human fecal contamination, e.g. from direct deposits of observed beach campers, sand was sampled at 5 EB locations and 5 LB locations on one day, with the possibility of-if the sand was positive for FIB, markers, or pathogens-assessing potential marker transport via tidal action (Yamahara et al, 2007). To test if offshore HF183 sources, including from boats in anchorage areas and the El Estero WWTP outfall, were influencing the surf zones, the Y2 sampling in the surf zone to offshore transect (Fig. S8) was repeated in Y3, but with added sites in the anchorage area instead of the offshore locations in Y1 (Fig. S18). The El Estero WWTP effluent or commingled discharge (effluent + seawater) was sampled at the WWTP to determine source strength of HF183 markers. In addition, scientific divers sampled directly from the El Estero outfall (Fig. S18, S19), the nearer of the two WWTP outfalls to the EB and LB beach areas, on 4 occasions, and from the Montecito Sanitary District WWTP outfall (Fig. S18) on 2 dates. To simulate whether the effluent plume from the El Estero WWTP ocean outfall would surface, the UM3 (Updated Merge) model from the U.S. EPA Visual Plumes model system (Frick et al., 2003), a mixing zone modeling application consisting of five models, was utilized for single and multi-port submerged discharges in the near-field mixing zone (details follow herein). Also, although more distant and to the east of EB, the MSD WWTP outfall (Fig. S8, S18) was considered for its potential effects on EB surf zone HF183 detections, based on a reexamination of a prior study and its associated report (Ohlmann et al. 2010).

Sampling of surf zone water and groundwater was performed on 3 occasions in and nearby the Santa Barbara Harbor, directly to the east of LB (Fig. S20), to further evaluate

possible Harbor infrastructure or boats as HF183 sources. A dye study of the Stearns Wharf bathrooms and associated sewers, located to the east of the Harbor (Fig. S21), was performed on one occasion, to determine infrastructure integrity as related to HF183 contamination that could conceivably transport either east to EB or westward to LB.

Sample collection, physicochemical analyses and FIB measurement

Overall, 761 samples including water from the watersheds, groundwater, surf zones, nearshore, offshore, raw sewage, treated wastewater effluent, wastewater ocean outfall discharge, and recycled water, supratidal and intertidal beach sand, as well as sediments from the watersheds and nearshore were collected in the AB411 season from 2015 through 2017 for the analyses of fecal indicators, fecal markers, and pathogens (Tables S1-S8). Further, 1227 surface, surf zone, and groundwater samples were collected during dye studies to assess hydraulic connections between watershed termini scour ponds and surf zones; septic, sewer or bathroom lateral contamination of beach groundwater; and potential sewer leakage into the Santa Barbara Harbor and at Stearns Wharf.

Surf zone water samples were collected in ankle to knee deep water into sterile polypropylene bottles (4 L). Waters from creeks and scour ponds were collected from the surface by dipping a sterile beaker into the waterbody and then dispensing into the sample bottle. Water from flowing drains was collected similarly using sterile beakers and bottles. Submarine groundwater on the beach was collected by coring into the sand with a 3.2 inch soil auger (AMS, American Falls, ID) and then pumping to the surface with a portable peristaltic pump (Geotech, Denver, CO). Additionally, raw sewage, treated wastewater effluent, and recycled water from the El Estero WWTP were collected using sterile beakers and bottles. Subsurface marine water was collected by boat using a 2 or 5 L Van Dorn bottle, which was rinsed at least 3 times with sterile Nanopure water prior to sample collection. The Van Dorn bottle was lowered at each site with both ends open. When reaching the sampling depth, the bottle was moved side to side to flush the interior before the ends were snapped closed. Final effluent samples from WWTP outfall diffuser ports were also collected similarly using the Van Dorn bottles by scientific divers. The divers descended with both ends of the Van Dorn bottle open, and flushed the interior of the bottle before positioning adjacent to a diffuser port. The bottle was positioned against the port for at least 30 s before snapping the ends closed to capture the effluent sample. Dissolved oxygen, electrical conductivity, and temperature of water samples were recorded in the field using an HQ40d multiparameter meter (Hach, Loveland, OH). Water samples, except raw sewage, were passed through sterile 25 µm pore size Miracloth (Calbiochem, San Diego, CA) to remove large debris, and stored on wet ice until processing (within 6 h).

For sediments from creeks and scour ponds, supratidal sands, intertidal sands, and nearshore sediments, approximately 250 g of each composite wet sediment or sand sample was collected by sampling and combining sediments or sands from five individual locations at each sampling site. Each sand or sediment sample was collected by using a sterile 50 mL polypropylene tube (BD Biosciences, Bedford, MA) and scraping along the surface (1-7 cm depth) at each location. Samples were then combined and homogenized in clean Ziplock plastic bags and stored on wet ice before delivery to the lab. Nearshore sediments were collected by scientific divers using modified sterile 60 mL syringes with rubber stoppers. Five cores were collected at each sampling site including a center location followed by locations 3-4 m to the north, east, south, and west directions. At each location, the top 2 cm was brushed away before the barrel of the syringe was uncapped and pushed into the sediment until approximately three-quarters full, at which point the

stopper was replaced on the syringe and the plunger gently pushed to expel any water. Sample cores were then stored in clean Ziplock bags and stored on wet ice until processing in the lab. Particle grain sizes of sediment or sand samples were measured with a CILAS 1190 Laser-particle size analyzer (CILAS, Madison, WI) after dispersing 8-10 g of sediment or sand into 30 mL of deionized water for 5 min. Moisture and total organic matter content were estimated by measuring the mass loss of approximately 20 g of sample at 60 °C and (on ignition) 500 °C, respectively in a muffle furnace (IsotempTM, Fisher Scientific, USA). Fresh gull feces (observed as originating from a gull) and bird feces (wet feces on sand from unknown bird species) were collected from the study beaches by bird baiting onto a clean (new) tarp and scraping the fresh feces into a sterile sampling container (Sterileware Samplit Scoop and Container System, Bel-Art SP Scienceware, Wayne, NJ). Multiple feces were combined into a single composite sample for gull feces, and a composite for bird feces.

Total coliform (TC), *E. coli* (EC) and enterococci (ENT) were quantified using the IDEXX Quanti-Tray/2000 method, following the manufacturer's protocols (IDEXX Laboratories, Westbrook, MA). Aliquots from each water sample were diluted tenfold using sterile Nanopure water, prior to FIB analysis. Sample duplicates were analyzed for at least every 10 samples processed. A method blank using sterile Nanopure water was included for each batch of reagents. For sand or sediment, 5 g was suspended in 25 mL of sterile Nanopure water or 0.2 % hexametaphosphate (Sigma Aldrich, USA), respectively, shaken for 5 min to dislodge microorganisms and settled for 1 min. The supernatant was collected and analyzed using the same protocol for water samples. FIB exceedances were based on California's AB411 single sample surf zone criteria for TC (10000 MPN per 100 mL), EC (400 MPN per 100 mL), and ENT (104 MPN per 100 mL) (Sikich et al., 2018).

DNA extraction, qPCR and ddPCR

Water samples (up to 2 L) were vacuum filtered in the laboratory through sterile 0.22 µm filters (MicroFunnel Filter Funnels, PALL Co.) until the point of refusal. The volume of water filtered was recorded. For each sampling event, a blank was included by filtering approximately 1.5 L of sterile Nanopure water. Filters were stored at -20 °C until DNA extraction using the DNeasy PowerWater Kit (Qiagen, Carol Stream, IL) following the manufacturer's protocol. For pathogen analysis including Salmonella spp. and human adenovirus, water samples (up to 2 L) were vacuum filtered through sterile 0.45 µm HAWP filters (EMD Millipore, Billerica, MA) until the point of refusal. Filters were stored at -80 °C until combined DNA and RNA extraction using the RNeasy PowerWater Kit (Qiagen) and following the manufacturer's protocol except for omitting DNase to allow the elution of DNA plus RNA. The DNeasy PowerSoil Kit (Qiagen, Carol Stream, IL) was used to extract DNA from sand and sediments. Duplicate extractions (0.25-0.5 g wet) were performed for each sand or sediment sample and combined onto a single spin filter prior to the washing and elution steps in the kit. For all (water, sand, and sediment samples), an extraction blank without any filter was included in each batch of extractions. The DNA concentration was quantified using the Quant-iT dsDNA Broad-Range Assay Kit (Invitrogen, Carlsbad, CA) on a Cytation3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT).

The presence of human, dog, and gull fecal materials was determined using quantitative polymerase chain reaction (qPCR) of extracted DNA with HF183 (Bernhard and Field, 2000; Green et al., 2014) and HumM2 (Shanks et al., 2009) as human-associated fecal markers, DogBact (Dick et al., 2005; Sinigallianio et al., 2010) as the dog marker, and Gull2TaqMan as

the gull marker (Lu et al., 2008; Sinigallaiano et al., 2010). These host associated markers were selected based on their regional performances as thoroughly evaluated previously (Boehm et al., 2013). The Entero1A marker was used to quantify ENT in units of cell equivalents (c.eq.) per 100 mL, to compare with culture-based measurements (Haugland et al., 2012; Oana et al., 2002). The *ttr* gene was analyzed to quantify *Salmonella* spp. (Malorny et al., 2004). A previous droplet digital PCR (ddPCR) method was used for human adenovirus quantification with a Bio-Rad QX200 ddPCR system (Hercules, CA) (Steele et al., 2018). Details of qPCR and ddPCR performance follow (herein). The host specificity of the HF183 human fecal marker (Green et al., 2014) was evaluated using fresh gull and bird feces collected from the study beaches.

Sample inhibition was assessed using an internal amplification control (IAC) incorporated into the HF183 TaqMan assay (Green et al., 2014). The inhibition threshold and competition threshold were calculated for each plate, and a sample was considered inhibited when its IAC quantification cycle (Cq) was greater than the inhibition threshold and less than the competition threshold. If this occurred, the sample was diluted and re-analyzed. The HumM2 qPCR assay is considered more specific but less sensitive to human feces compared to the HF183 assay (Boehm et al., 2013) and was performed only on HF183 positive samples to confirm the presence of human fecal DNA detected by HF183. All qPCR assays were performed using TaqMan environmental master mix (Life Technologies, Grand Island, NY) on a Bio-Rad CFX96 real-time PCR detection system (Hercules, CA). Synthesized plasmid DNA containing qPCR targeted sequences or genomic DNA from bacterial cultures were serially diluted to generate standard curves for all qPCR assays. All samples and standards were analyzed in triplicate, with triplicate no-template controls included for each microtiter plate. Any plate with amplification of a notemplate control replicate was discarded and the samples were re-analyzed. Filter and extraction blanks were incorporated to assess contamination during sample filtration and DNA extraction, respectively. If any filter or extraction blanks amplified, the corresponding samples were flagged and not used in further analysis. Regression analysis was performed on the pooled standard curve and outliers were removed based on standardized residual values of >+3 or <-3. The Lower Limit of Quantification (LLOQ) for each assay was calculated by taking the average Cq value of the non-outlier standard replicates at the lowest concentration included in the standard curve. Samples with at least two replicates amplifying within the range of the standard curve were considered to be within the range of quantification (ROQ) and were quantified. Samples with replicates amplifying below the LLOQ were considered detected but not quantifiable (DNQ), and samples with one or zero replicates amplifying were considered not detected (ND) (Sinigalliano et al., 2013). A summary of the pooled standard curve parameters for each assay and sampling year of this project is provided in Table S24.

The reaction mixture of ddPCR was made for droplet generation using a Bio-Rad Droplet Generator (Hercules, CA) with droplet generation oil. Generated droplets were PCR amplified, including three positive control replicates (VR-930D, Human Adenovirus 41; ATCC, Manassas, VA) and five negative (no template) control replicates per plate. Fluorescence was measured using a Bio-Rad QX200 Droplet Reader (Hercules, CA) and analyzed using associated QuantaSoft software. The fluorescence threshold was manually set at approximately one standard deviation (500-700 fluorescence units) above the negative control signal. A total of \geq 20,000 droplets for two reactions were generated per sample. The average upper limit of quantification was 10⁵ gene copies per 100 mL. For the plate to be included in the analysis, all negative (no template) control reactions were required to have no positive droplets on the plate. To consider a sample positive and included in further analysis, each sample was required to have

a minimum of three positive droplets. All samples were analyzed in duplicate. If a sample had one positive replicate and one negative replicate, a third replicate was analyzed. Samples with two or more positive replicates were considered positive and averaged; samples with one or no replicates amplifying were considered ND.

Dye studies

Dye tests were performed to assess sanitary infrastructure as a potential source of fecal contamination including sanitary sewers associated with bathrooms at the beaches and on Stearns Wharf, as well as sanitary sewer infrastructure under roads and the parking lots proximate to LB and EB, respectively. Since there was no observed surface flow from creek outlets into the surf zones during the study periods, dye studies were conducted for watershed outlets and scour ponds to evaluate potential routes of fecal contamination through the subsurface. A non-toxic fluorescent dye Rhodamine WT was introduced into sanitary sewer infrastructure (manholes or toilets) by flushing (bathrooms) or adding directly (sanitary sewers). For creek outlets and scour ponds, fluorescent dye was added to the subsurface through a temporary well placed near each creek outlet. Surface and groundwater sampling locations were selected near suspected sources to determine whether fecal contamination was entering the surf zone and to monitor areas where dye would most likely travel. Background water samples from each location were collected before adding dye. The fluorescence of background samples was highly variable depending on the sampling locations and water matrix types. Any storm drains in the beach area that had flow or internally-pooled water were also sampled.

During the studies, dye concentrations in collected water samples were analyzed in the lab using a Cytation3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT). The excitation wavelength was 546 nm and the emission wavelength was 590 nm, as suggested (Wilson et al., 1986). Standards of 1, 10, and 100 ppb dye in Nanopure water (Barnstead, Thermo Scientific) were used to create standard curves. Matrix effects were evaluated for all water samples by spiking standards into background samples. Recoveries of 54%-78% were obtained for surf zone water, 82%-147% for groundwater, and 90%-210% for surface water. The limit of detection in each type of water was defined as the mean plus three times the standard deviation of the background measurements. The maximum background concentration for each water matrix was then defined as twice the limit of detection, and any sample below that was not considered to be a dye detection.

Furthermore, dye addition and groundwater elevations were used in the scour pond wells to estimate the groundwater velocity. The gradient was calculated by linearly fitting the groundwater elevations to the well coordinates, and then used in combination with the time and distance at which dye was detected to approximate the groundwater velocity over the study period.

Dye tests were first performed to determine whether sanitary sewer infrastructure associated with the bathrooms at EB and LB were sources of fecal contamination to the surf zone. Restrooms at the Cabrillo Bathhouse at EB and both the public and restaurant restrooms at LB were included in this study. Surface and groundwater sampling locations were selected near the restrooms at both beaches to determine whether sewage exfiltration was entering the surf zone and to monitor areas where dye would most likely travel if leaking was present, as shown in Fig. S6 and S7. At EB, surf zone sampling locations were positioned 100 m to the east (SZ1) and west (SZ2) of the Cabrillo Bathhouse. This was to account for movement due to ocean currents, wind, or wave action after potential entry into the surf zone. A third surf zone location (SZ3) was

positioned 300 m west of the bathhouse to account for the sewer line which runs the length of the parking west of the bathhouse. Other surface water sampling locations at this beach included the Sycamore Creek outlet (SW1) and the Milpas drain (SD). Trickling flow was observed from the Milpas drain over the first 6 days of the study; no flow at the drain outfall was observed after this and there was no surface connection to the surf zone. Three groundwater sampling locations were selected at this beach. One well (GW1) was positioned in the sand in front of the west side of the bathhouse. A second well (GW2) was positioned in the sand near the entrance to the parking lot on the west side of the bathhouse. This well was near a sewer line manhole in the parking lot. A third well (GW5) was installed toward the end of the study in order to collect additional samples from the west end of this sewer line near sampling location SD. At LB, surf zone sampling locations were positioned between the bathrooms near the Honda Creek culvert outlet (SZ5), as well as 100 m to the east (SZ4) and west (SZ6) of this location. Surface water was also collected from the Honda creek culvert outlet (SW2). Groundwater sampling locations were positioned in the sand directly in front of the public restroom (GW3) and the restaurant restroom (GW4).

Prior to well installation, Dig Alert was contacted to ensure that no underground infrastructure was co-located with proposed well locations at either beach. Four sampling wells were installed at EB (GW1 and GW2) and LB (GW3 and GW4) on Jun 12, 2015, and GW5 was installed on Jun 24, 2015. To install each well, dry surface sand was first removed from the area using a shovel. A 3.25 in hand auger (AMS, Inc., American Falls, ID) was used to core down to groundwater. An additional four to five cores (augers full of sand) were removed to loosen the sand one foot below the groundwater surface. A PVC sampling pipe (Environmental Service Products, Irvine, CA), consisting of a 5 ft screened section of 1in PVC with a threaded cap on

one end and a 5 ft section of 1 in PVC casing on the other end, was then placed in the auger hole and pushed so that the screened section of PVC was approximately one foot below the groundwater surface. Sand was packed in around the sampling pipe, and the pipe was then cut such that 18 in extended above the sand surface. Autoclaved 0.25 in polypropylene tubing (Cole-Parmer, Vernon Hills, IL) was then pushed into the bottom of the sampling pipe and cut at the top. A 1 in PVC slip cap was used to close the top of the sampling pipe between sampling events. Wells were tested by pumping a water sample to the surface after installation to ensure that sufficient volume could be collected at each location.

Before dye was added, background samples were collected on Jun 15, 2015 from all sampling locations except GW5. At EB, one gallon of 2.5% Rhodamine WT dye (Cole-Parmer, Vernon Hills, IL) was individually flushed in the men's and women's restrooms at the Cabrillo Bathhouse between 9:01 am to 9:09 am on Jun 15, 2015. Dye was first observed visually in the sewer manhole just west of the Cabrillo Bathhouse and then confirmed by using a 600OMS V2 Sonde equipped with a rhodamine WT optical probe (YSI, Inc., Yellow Springs, OH). At LB, one gallon of dye was individually flushed in the men's public restroom east of the Shoreline Beach Cafe at 9:55 am and in the men's restroom at the Shoreline Beach Cafe at 10:03 am on Jun 15, 2015. One gallon of dye was then individually flushed in the women's restrooms at the public restroom at 10:15 am and at the Shoreline Beach Cafe at 10:18 am. Dye was first observed visually in the sewer manhole in the parking lot across Shoreline Drive and further confirmed using the YSI probe. The details of each sample collected are shown in Table S12. A total of 208 water samples were collected over 12 days (112 from East Beach and 96 from LB). Samples were collected in 50 mL tubes (Cole-Parmer, Vernon Hills, IL) and immediately placed on ice in the dark. Groundwater was pumped for at least one minute to clear water from the

tubing and well before sampling was performed. Two samples were selected for analysis of FIB, host fecal markers, and pathogens. These results are presented in Table S5.

Dye tests were then performed for sanitary sewers beneath Shoreline Drive near LB (Fig. S14) as well as the sanitary sewer beneath the parking lot west of the Cabrillo Bathhouse near EB (Fig. S15). Dye tests were also performed to assess dry weather flow transmission from the Honda Creek culvert outlet and scour pond at LB (Fig. S12), and dry weather flow from the Sycamore Creek outlet and scour pond at EB (Fig. S13). Fluorescent dye was added directly to the sanitary sewer lines and groundwater sampled in the vicinity between the surf zone and the sewers. Dye transmission via the subsurface to the surf zone was evaluated by sampling through a temporary well placed near each creek outlet to determine whether scour ponds at the watershed or creek termini were hydraulically connected to the surf zone via the subsurface.

Five groundwater (GW1-GW5) and three surf zone sample locations (SZ1-SZ3) were chosen in front of the sewer line between the west end of LB and the parking lot (Fig. S14). Additionally, pooled water inside storm drains (SD1 & SD3) and pooled water above a buried storm drain (SD2) (Fig. S14) that was previously sampled in 2015 as site L07 (Fig. S4) was collected. There was no surface connection from any of the storm drains to the surf zone during the study. Groundwater monitoring wells were installed at nine locations (SP1-SP9; Fig. S12) near the Honda Creek culvert outlet. A tenth well was installed to introduce dye to the subsurface (SP0). Wells were located 10, 30, and 60 ft from the dye addition well SP0, respectively. Three surf zone sampling locations (SZ4-SZ6) were chosen from the area in front of the creek outlet. Meanwhile, three groundwater monitoring wells were installed along the sewer line west of the Cabrillo Bathhouse at EB (GW1-GW3; Fig. S15). Three surf zone locations (SZ1-SZ3) were also chosen in front of the same area. Groundwater monitoring wells were installed at nine

locations (SP1-SP9) near the Sycamore Creek outlet (Fig. S13). A tenth well (SP0) was installed to introduce dye to the subsurface. Wells were located 5, 10, and 60 ft from the dye addition well SP0, respectively. Three surf zone locations (SZ4-SZ6) were chosen in front of the creek outlet (Fig. S13). Groundwater monitoring wells were installed at LB on Aug 8, 2016. The depth to groundwater was between 2 and 4 ft below ground surface for all wells installed at LB. Groundwater wells were installed at EB on Aug 22, 2016. The depth to groundwater was around 5.5 ft below ground surface for the EB wells installed west of the Cabrillo Bathhouse and between 1.5 and 2 ft below ground surface for the scour pond wells. Six samples from EB and eleven samples from LB were selected for analysis of FIB, host fecal markers, and pathogens. These results are presented in Table S5.

The date for the addition of dye was selected to coincide with a neap tide, when groundwater discharge to the ocean is largest, thus maximizing groundwater movement through the subsurface. Before dye was added, five rounds of background sampling were conducted for all the groundwater and surf zone locations associated with sanitary sewer tests. One round of background sampling was conducted for groundwater and surf zone locations associated with score pond subsurface flow evaluations. Consistent with previous dye testing, 2.5% Rhodamine WT dye was used for all dye tests.

For LB, dye was introduced to the sewer lines and scour pond on Aug 10, 2016. At 7:05 am one gallon of dye was added to the sewer trunk line on Shoreline Drive, which had significant flow. At 7:16 am one gallon was added to the smaller sewer line running north of the trunk line on Shoreline Drive, which had no flow at the time. Dye was observed at a manhole nearby at 7:35 am. At 8:00 am 200 mL of dye was added to the scour pond at well and flushed with 500 mL of water. For EB, dye was introduced to the sewer line and scour pond on Aug 24, 2016. At

7:32 am two gallons of dye were added to the sewer line at the manhole east of the Cabrillo Bathhouse and flushed with 1 L of water. At 7:38 am 200 ml of dye was added to the scour pond and was flushed with 500 mL of water. Dye addition and water sample collection times are shown in Table S13.

Finally, a dye test was performed to determine whether sanitary sewer infrastructure on Stearns Wharf was a source of fecal contamination to the nearby surf zone and nearshore waters (Fig. S21). Sewage infrastructure conveys sewage from six bathrooms on the wharf to the sewer trunk line by both gravity and pressurized lines via 4 small lift (pump) stations. Pump stations were triggered manually prior to dye addition at 8 am to ensure that holding tanks were empty prior to testing. Dye was added in the morning between 8 and 9 am when activities, and therefore flows into the system, were minimal. A total of four gallons of fluorescent dye was added to six bathrooms on the wharf. Toilets were flushed multiple times to ensure that dye was transported to each pump station. Pumps stations were then manually triggered again at 10 am to ensure that dye was transported into the sewer lines running from the wharf to the sewer trunk line. This process allowed the concentration of dye being pumped from the holding tank of each lift station to be similar. The normal operations of the pump stations throughout the day then flushed all remaining dye out of the system, which was verified through visual observation at a downstream manhole. Five rounds of background sampling of water around the Wharf were conducted prior to dye addition to determine the background fluorescence signal in nearby waters. After dye addition, grab samples were collected at 16 locations around the perimeter of the wharf approximately every 30 minutes for a period of 4 hours (Table S14). A downstream manhole near the road was opened to confirm that dye travelled through the wharf infrastructure visually. No samples were selected for analysis of FIB, host fecal markers, or pathogens.

Groundwater testing

To determine if an onsite septic system at the Clark Estate on the east side of EB was a source of fecal contamination to groundwater or to the nearby surf zone at EB, four temporary groundwater monitoring wells (GW4-GW7) were installed near the property boundary between the beach cottage and the surf zone on Aug 25, 2016 (Fig. S10). Groundwater sampling was conducted during a neap tide when groundwater discharge was highest on Aug 25, 29, and 31, 2016 for analysis of FIB, host fecal markers, and pathogens. Results are shown in Table S5.

In early November, 2016, a sewage leak was identified by the City of Santa Barbara who repaired the responsible sewer lateral serving the beach bathroom at Chase Palm Park of EB. To determine if the leaking sewage was a source of fecal contamination to groundwater or to the nearby surf zone, four temporary groundwater monitoring wells (GW1-GW4) were installed between the bathroom and surf zone on Dec 5, 2016 (Fig. S11). Three wells were installed as near to the bathroom as possible, to sample groundwater nearest the sewage leak. One well was installed just above the high tide line to sample groundwater before discharging to the surf zone. Groundwater samples and a surf zone sample (SZ1) were collected on Dec 5 and 8, 2016 for the analysis of FIB, human marker HF183, and human adenovirus. Results are shown in Table S5. The sampling date Dec 8 was immediately following a neap tide when submarine groundwater discharge was highest.

The Santa Barbara Harbor was addressed as a possible fecal contamination source to LB by sampling 2 surf zone sites L01 and L02 at the east end of LB (Fig. S20), 3 temporary groundwater wells LB-GW6 to LB-GW8, one site inside the harbor (southwest corner) (L-IH) and one site outside of the harbor breakwater (east end) (L-OH) on Jul 18, Aug 15, and Sep 20,

2017. Samples were analyzed for FIB, human marker HF183, and human adenovirus. The results are shown in Table S5.

El Estero WWTP Outfall Effluent Plume Modeling

To determine whether the effluent plume from the El Estero WWTP outfall (Fig. S8, S18) would surface on the 2017 offshore study sampling dates, the UM3 (Updated Merge) model from the U.S. Environmental Protection Agency's (U.S. EPA) Visual Plumes model system was utilized. Visual Plumes is a mixing zone modeling application consisting of five models and certified by the California State Water Resources Board for use in ocean outfall design. The UM3 model is used for simulating single and multi-port submerged discharges in the near-field mixing zone. Model inputs include physical characteristics of the diffuser design (number of ports, port spacing, angle of ports, port elevation on pipe, port depth in water column), effluent parameters (salinity, temperature, concentration of pollutant(s), discharge flow rate), and ambient conditions (salinity, temperature, current direction and speed, background concentration). Model parameters used were selected in consultation with a physical oceanographer (Dr. Libe Washburn, UCSB).

Whenever possible, actual measured values were used in the model. The model input values that are in English (inches, feet) units are reported here as such, for accuracy in describing the simulation. The El Estero outfall pipe is 48 inch internal diameter (ID) and has a 720 ft diffuser section with 60 ports, staggered on opposite sides of the pipe. The ports are located 6 inches above the horizontal centerline (Jenkins, 2014). The pipe rests on top of a rubble mound bed, with the diffuser section at a water depth of 70 to 75 feet. As half of the port diameters are 3.5 inches and half are 4 inches, 3.75 inches was used in the UM3 model. The model assumes that all ports are on one side of the pipe; thus, as recommended in the software manual, all ports

were treated as if they were on the same side with half the spacing (Frick et al., 2003). Based on the specifics above, a port elevation of 35 inches and a port depth of 73 feet were used in the model. As the ports are cylindrical holes in the pipe, a discharge coefficient of 0.61 was used, as recommended in the software manual (Frick et al., 2003). The vertical discharge angle used was 0 degrees (i.e. horizontal). The horizontal discharge angle used was 0 degrees (i.e. East) on $\frac{8}{29}$ and 180 degrees (i.e. West) on the other dates, based on the direction of the average surface currents during the sampling period as described below. Effluent (and commingled discharge when applicable) salinity and temperature were as measured in the samples collected on each sampling date by UCSB for this study or from the El Estero WWTP results (probe or grab sample). An average flow rate of the effluent or commingled discharge during the sampling timespan (7-11 am) on each sampling date was used as input to the model. Actual concentrations of HF183 markers (copies/100mL), Entero1A markers (cell equivalents/100mL), and human adenovirus (copies/100mL) results from the effluent and commingled samples collected on each offshore sampling date by UCSB were used. A summary of the model input parameters for the Diffuser Tab is shown in Table S15.

For ambient conditions, current speed and direction on the day and timespan (7-11 am) of the offshore sampling events were collected and averaged from one location inshore and one location offshore of the outfall location from the High Frequency Radar surface current data available on the Southern California Coastal Ocean Observing System website (SCOOS, 2018). The HF radar surface current measurements are valid from the water surface to 1 m depth. The speed of the currents at depth near the outfall on the offshore study sampling dates is unknown. Therefore the model was run under three different scenarios: applying the surface current speed uniformly at depth (all markers), using 9 cm/sec at depth (HF183 only), and 55 cm/sec at depth

(HF183 only). The latter two values were obtained from the El Estero WWTP discharge permit (NPDES) monitoring period 11/13/11-11/24/12 results from an Acoustic Doppler Current Profiler (ADCP; located near the outfall at 6.4 m above the seabed as previously summarized; Jenkins, 2014; Tierra Data, 2013), with 9 cm/sec being the average current speed at this depth (15.85 m) and 55 cm/sec the maximum. The current direction at depth was assumed to be the same as measured at the surface. No ambient temperature or salinity measurements were made at depth near the outfall outside of the plume influence during this study. For use in the model, temperature (from 3 m, 4 m, 7 m, and 10 m depths) and salinity measurements (from 4 m depth) were obtained from instruments moored at Mohawk Reef in Santa Barbara (N34.39323, W119.73012) as part of the Santa Barbara Coastal Long Term Ecological Research project (Santa Barbara Coastal LTER, 2020), and the averaged results from the sampling timespan (7-11 am) on each of the offshore sampling dates were used. The UM3 model was utilized both with and without decay rates for the HF183 marker (1.6 d^{-1} at surface, 1.4 d^{-1} from 1 m to depth) (Mattioli, et al., 2017). The background concentrations for HF183, Entero1A, and human adenovirus in the ambient water were considered to be zero. A summary of the model input parameters for the Ambient Tab is shown in Table S16.

Y3 studies of potential water defecation and human waste contamination during surf zone recreation

During the studies of human behavior including water defecation and surf zone recreation on holidays and high visitation weekends or during swimming races, as well as morning sampling campaigns when the number of people were recorded, the counts of people were grouped for the correlation analysis into people "in water" including swimmers or anyone recreating in the surf zone, people "on sand" who were recreating but not in the water (walking, sitting on sand, performing fitness exercises, etc.), and the "bedding" category including people sleeping or camping on the beach (Table S10). When exact numbers of people with tents or bedding were counted, those numbers were used; otherwise, an occupancy of 2 people was assumed for each tent or camp. On offshore sampling dates, swimmers were also counted from the sampling boat and assigned to the closest surf zone location.

Although human HF183 markers were consistently present in surface waters inside of the Harbor (Table S5), and surf zone site L01 at the east most sampling site of LB (Fig. S4 and S20) might have been impacted via defects in the breakwater structure, all site L01 samples including those from the Harbor sampling dates were included in the correlation analysis. This is due to the fact that the concentrations of FIB, fecal markers and pathogens at site L01 were not significantly different from the other LB surf zone sites (Table S2, S5, S6). Site M01 sample results (Fig. S8 and S18, Table S6) were not included in the correlation analysis, since these were from the terminus of a different (Mission Creek) watershed.

Statistical analyses

Over or under range values were adjusted prior to graphing and statistical analysis to be above the highest, and below the lowest, quantified value, respectively. FIB values were treated as follows: <10 = 0 (log scale), >24196 = 25000. Fisher's Exact Test was also performed using detection or non-detection of HF183, and presence or absence of people in the water, on the sand, and bedding.

Supplemental Results

Studies of infrastructure at the beaches

Above background dye detections were not measured in any groundwater or surf zone waters collected during dye studies (Fig. S26-S29), except for a single positive detection recorded for groundwater seven days after the initial dye addition at a distance of 120 ft from the sewer main associated with the bathroom at EB (Fig. S30). A groundwater velocity of 17.1 ft/day was thus deduced, which was much higher than velocities estimated near scour ponds, possibly due to preferential subsurface water flow paths through construction backfill or along underground infrastructure. Although this dye detection suggested possible sewage exfiltration from the sewer, no dye was detected in any other groundwater or surf zone water sample, and subsequent dye monitoring of the same sewer did not result in any further detections. Furthermore, human HF183 and dog markers, and *Salmonella* and human adenovirus, were not detected in the groundwater samples (Table S5). Therefore, sewage exfiltration from compromised infrastructure was unlikely to cause fecal contamination in the EB surf zone.

Although human adenovirus was detected in the one EB storm drain sampled in 2015 (Fig. S7, Table S5), no human HF183 markers were detected and the drain did not flow to the surf zone. Human HF183 marker and adenovirus were not detected in groundwater near the Santa Barbara Harbor with its public bathrooms and sewage pump stations (Fig. S20). However, HF183 markers were consistently detected in surface waters inside of the Harbor (Table S5), suggesting that leaking sanitary sewer infrastructure or boat activities in the Harbor might contribute to human fecal contamination at the most eastern surf zone sampling site of LB via defects in the breakwater structure. Still, the concentrations of FIB, markers, and pathogens at this site were not significantly different from other LB sites, and the average concentration of human marker HF183 was actually lower than for other LB sites (Tables S2 and S5-S6).

Dye study results

Groundwater sampling and dye studies were used to determine whether creek termini scour ponds were hydraulically connected, via the subsurface, to surf zones (Fig. S12 and S13). The dye added to the Honda Creek culvert outlet (LB) took over 17 days to travel 15 ft from the addition point (Fig. S22), during which groundwater gradients were towards the ocean. Groundwater transport velocity was thus estimated to be 0.47 to 1.3 ft/day (Fig. S23), suggesting that groundwater travel from the creek outlet to the surf zone (high tideline) would take more than 70 days at an average velocity. The groundwater velocity from the Sycamore Creek outlet was lower, with a maximum of 0.13 ft/day estimated, given that no dye was detected within 2 ft of the addition point over 17 days (Fig. S24 and S25). No human HF183 or dog fecal markers, or pathogens, were detected in groundwater (Table S5), confirming that fecal markers and pathogens were not transported via groundwater.

Dye tests were performed to determine if sewer infrastructure (Fig. S6, S7, S14, S15, S21) was leaking and impacting groundwater or surf zone waters. Dye was not detected above background in groundwater or surf zone waters (Fig. S26-S29), except for the single detection described above (Fig. S30). No fecal markers or pathogens were detected in storm drain waters or the groundwater at monitoring wells (Fig. S10, S11, S20).

Recycled water

Starting in 2016, public spaces were irrigated with El Estero WWTP's recycled water, a potential source of FIB and of HF183 to the lower EB and LB watersheds and beach areas. Recycled water samples contained low FIB concentrations. HF183 markers ranged from DNQ levels to

1692 copies/100 mL, and human adenovirus was detected in one sample at a low level (14 copies/100 mL; Table S7). For the LB watershed samples, recycled water might have explained the significant increase in HF183 concentrations from 2015 to 2016 (when pooled together by year but not by site, Wilcoxon, p = 0.0001, n=34; Table S3). However, EB watershed HF183 concentrations showed no significant differences across 2015 and 2016 (Table S3), either when pooled by year (p = 1.0, n=56) or when compared at the site level (p > 0.6, n=56). Regardless, because (by dye study results, as before) the scour ponds were hydraulically disconnected from the surf zones, recycled water in the watersheds could not have been a source of HF183 to the surf zones. While recycled water could have been used on landscaping just downstream of the scour ponds, there was no apparent effect, since HF183 surf zone concentrations did not vary from 2015 (when there was no use of recycled water) to 2016, when samples were either pooled by year (both p values = 0.3, n=50; Table S2) or by site (for EB p > 0.2; for LB p > 0.06, both n=50; Table S2).

HF183 marker concentration patterns in on- to offshore sampling transects, with comparison to the El Estero WWTP effluent

In 2016, synchronized water sampling was performed in the surf zone, nearshore, and offshore sites of EB and LB, to test the hypothesis that the treated El Estero WWTP effluent plume was responsible for chronic low level HF183 detections in the surf zone. A similar study was performed in 2017, except that, instead of offshore sampling, boat anchorage area sampling was added (Table S6); also, in 2017, sampling occurred of treated effluent discharging directly from the outfall diffusers and in the water column in the ocean above the diffusers (Table S7). Overall, a gradient (more to less) in HF183 detections appeared in the direction of the surf zone to the

offshore in both 2016 and 2017. In 2016, across EB and LB samples, approximately 24% of surf zone samples (n=33) had detectable or quantifiable (DNQ or ROQ) HF183, while approximately 14% of nearshore samples (n=21) had only detectable (DNQ) HF183, and only approximately 7% of offshore samples (n=15) had detectable (DNQ) HF183. Similarly, in 2017, HF183 was detected in 51% of the surf zone samples (n=55), mostly at DNQ level and three at ROQ level. In the nearshore and boat anchorage samples, 40% (n=35) and 20% (n=20) of samples contained HF183, respectively, mostly at DNQ level and three at ROQ level. Thus, the overall percentage of HF183 detections was relatively greater in the surf zone in both 2016 and 2017. However, when examining each individual sampling date, as shown in Fig. S31, Oct 5, 2017 HF183 concentrations in the nearshore source(s) of HF183 on that date. One possible source is the El Estero WWTP effluent plume.

El Estero WWTP Effluent Outfall Visual Plumes Modeling Results

The UM3 model text output contains depth, ambient current, plume diameter, concentration of pollutant(s), dilution, distance from the diffuser (x, y coordinates), and time for each step. Steps are flagged when the plume is trapped, reaches maximum rise, or surfaces. The plume will rise until the density of the plume equals the density of the ambient water, which results in the plume becoming trapped at that depth. At that point, the plume will cease to rise and will continue to diffuse and dilute horizontally into the surrounding water. When the plume is trapped, the model also flags the depth of the shallowest part of the average plume boundary (where plume dilution equals the average plume-element dilution), which is called the maximum rise. If the plume density does not equal that of the ambient water until shallow depths, the plume may reach the

surface. The model also provides a graphic representation of the predicted plume elevation, looking horizontally through the water column.

Under the first scenario (uniform currents at surface and depth), the UM3 model results from all five offshore sampling dates in 2017 indicated that the plume did not surface (Fig. S32 – S36) and was trapped at a depth of 50-66' in 54 to 192 seconds after being discharged (Table S17). The maximum rise of the average plume boundary was 41-63' in 105 to 381 seconds (Table S18). Due to the short period of time from discharge to becoming trapped, the predicted concentration of HF183 markers in the plume did not greatly differ between modeling scenarios with and without the decay rate (Tables S17 – S18). Therefore the other modeling scenarios do not include decay. Dilution factors ranged from 151-486 at the plume trap level, and from 219-714 at the maximum rise (Tables S17- S18).

The second modelling scenario utilized actual surface current speeds and assumed a slower current speed of 9 cm/sec at 15.85 m depth, which was the average reported current speed near the outfall in 2011-12, as described above (Jenkins, 2014). This resulted in the plume trapping shallower as compared to the first scenario (average 54' vs. 59'), along with a shallower maximum rise (48' vs. 55') (Tables S19 – S20). Dilution factors were also reduced to 119-347 at the plume trap level, and 179 to 565 at the maximum rise level. HF183 marker concentrations were slightly higher as compared to the first scenario (Tables S17 – S19). Two dates (9/25/17 and 10/5/17) had noticeably higher plume trapping and maximum rise depths than the other three dates (Tables S19 – S20). This was true under all three modeling scenarios, but most apparent in the second scenario. The predicted average plume boundary would rise to 29' on 9/25/17 and 39' on 10/5/17 (Figures S35 – S36). This was likely caused by the decreased ambient temperature gradients on these dates versus the other dates (Tables S16). On 9/25/17, the difference between
the 3 m and 10 m temperature measurements was only 0.3° C, and for 10/5/17 this was 0.5° C. In contrast, the temperature measurements between these depths on the other three dates ranged from 1.7 to 2.7° C.

The third scenario also used the actual surface current speeds but assumed that the currents were 55 cm/sec at 15.85 m depth, which was the maximum reported current speed near the outfall from a previous study in 2011-12 (Jenkins, 2014). This scenario resulted in the plume trapping deeper than in the first two scenarios (average 65') and a deeper maximum rise (average 63') (Tables S21 – S22). Dilution factors were increased to 301 to 880 at the plume trap level, and 430 to 1283 at the maximum rise level (Tables S17 – S22). Predicted HF183 marker concentrations were therefore reduced in comparison to the previous two scenarios (Tables S17 – S22).

The overall average plume trap level for all three modeling scenarios is 60' and the maximum rise 55'. The deepest boat sampling location (LE-3DF) over the diffuser was 18 m (59'), which falls within the predicted depths of the plume. At this location, based on the quantities of HF183 markers measured, it appears that part of the plume was sampled on four of the sampling dates (7/6/17, 7/25/17, 8/29/17, 10/5/17). Concentrations of HF183 on those dates at 18 m ranged from 340 to 36640 copies/100 mL, which is similar to the predicted concentrations with no decay (2129 to 14030 copies/100mL) of the three modeling scenarios (Tables S17 – S22). Entero1A markers were detected on all five dates, with three of them within the range of quantification (7/6/17, 8/29/17, 10/5/17) at 293 to 705 cell equivalents/100 mL, all within the predicted range of 117 to 821 cell equivalents/100 mL from the first model scenario (Tables S17 – S18). Human adenovirus was detected in one sample at 18 m (7/6/17) at 18

copies/100 mL, within the predicted concentrations (0.2 to 21 copies/100 mL) of the first model scenario (Tables S17 - S18).

The model was also run using an average ambient temperature gradient for each date (19.1°C at 1 m, 18.3°C at 9 m, 17.0°C at 18 m), to examine the plume elevation differences between a freshwater effluent only discharge (as in 2016 and early 2017 field season) and a more saline commingled discharge of seawater plus effluent (later 2017 field season). Using the inputs described in the first scenario and substituting the ambient temperature values, the average freshwater effluent discharge (7/6/17, 7/25/17) was trapped 1.3' shallower (59.8' vs. 61.1') and had a maximum rise of 1.1' shallower (55.6' vs. 56.7') as compared to the commingled discharge (8/29/17, 9/25/17, 10/5/17). More saline discharge, such as when the desalination plant adds brine discharge to the freshwater effluent, would be expected to result in deeper plume trap and maximum rise levels than those observed in 2017.

In Summary:

- Under the three modeling scenarios performed, the plume was not predicted to surface on any of the 2017 offshore sampling dates.
- Predicted HF183, Entero1A, and human adenovirus marker concentrations in the plume after trapping were within predicted range or similar to those measured in the 18 m depth water samples collected.
- When using an ambient temperature gradient in under the first modeling scenario on all 5 dates, freshwater effluent discharge resulted in ~1' shallower plume trap and maximum rise levels than when commingled (seawater plus effluent) was discharged.
- Although the 2016 offshore study dates were not analyzed with the UM3 model, the discharge during the study period was freshwater effluent. Since the 2017 freshwater

effluent discharge dates resulted in plume trap levels ranging from 58 to 68', and a maximum rise of 54 to 67', it is unlikely that the plume surfaced in 2016 either. Running the model for these 3 dates would be needed to confirm this.

Montecito Sanitary District (MSD) WWTP Effluent Plume Report Reassessment and 2017 Sample Analyses

The Montecito Sanitary District (MSD) WWTP outfall (Figure S8, S18) discharges ca. 457 m offshore, with a 30 m diffuser section that has 10 duckbill ports, staggered on opposite sides at an approximately 45° angle near the top of the pipe, at a water depth of approximately 11 m (Ohlmann et al., 2010). The effluent plume was studied in 2007-08 (Ohlmann et al., 2010). During that 1-year study, global positioning system (GPS)-tracked drifters (that moved with the upper 1-m surface ocean currents away from the deployment location) were used to ascertain the directionality and speed of the surfaced effluent plume as it moved away from the outfall diffusers. Water samples were collected at multiple locations including from within the drifter trajectories; the surf zone water was also sampled from onshore at locations observed to be nearest to each of the projected drifter destinations, although drifters were retrieved prior to surf zone entry to avoid wave associated damage. Water samples (surf zone, and taken from a boat within the drifter trajectories) were analyzed for the HF183 marker using a SYBR Green assay (Seurinck et al., 2005), a method that produces results comparable to the TaqMan assay (Green et al., 2014) used herein. The original report results (Ohlmann et al., 2010) indicated that on 6 of the 50 dates sampled (12%), drifters released at the MSD outfall traveled in the direction of EB and were retrieved in the nearshore between surf zone sites E01 and E02 of the study herein. Based on plume dilution calculations for the ocean overlying the diffuser, and dilution

calculations along the drifter trajectories, ranges of HF183 concentrations from ND to ROQ were predicted. While most actual measurements from ocean samples acquired within the drifter trajectories were ND, some measurements were ROQ and thus measurable. However, no HF183 detections were found in the EB surf zone during the study.

The MSD outfall was sampled in this study in 2017 as a potential source of chronic HF183 markers to the surf zone at EB (Fig. S18). Historically, although the MSD outfall is relatively shallow and located close to shore (compared to the El Estero outfall), HF183 markers were detected less frequently (33%) in the sampled effluent prior to discharge from MSD (during 2007-08) versus El Estero (100%; during 2016-17) and, when detected, the geomean of HF183 concentrations were approximately 3 orders of magnitude less from MSD than El Estero ($1.0x10^3$ vs. $2.7x10^6$ copies/100 mL) (Table S23). During this current study, this was confirmed by sampling directly from the diffuser ports located closest to shore at both outfalls in 2017. The geomean of HF183 markers from a diffuser port at the MSD outfall was $3.4x10^2$ copies/100 mL (n=2), and from El Estero $2.8x10^5$ copies/100 mL (n=4) (Table S7).

On one date during this study (Oct 5, 2017), the on- to offshore spatial gradient sampling indicated the potential for an offshore HF183 source. However, on this date, the MSD outfall HF183 concentration was 164 copies/100 mL (Table S7), and both the surf zone (E01) and nearshore (E-1NS) locations closest to the MSD outfall had no detectable HF183, nor did any of the other surf zone (E02-E05) and nearshore (E-2NS and E-3NS) locations from East Beach near Sycamore Creek on that date (Table S6). Further, when examining all dry weather surf zone samples from sites E01-E05 in 2015 through 2017, there was no significant difference in HF183 concentration between the sites (Wilcoxon, p=0.8, n=179), indicating the lack of a concentration gradient towards the MSD outfall. Similarly, there was no significant difference in HF183

concentrations between nearshore sites E-1NS to E-3NS (Wilcoxon, p=1.0, n=24). Thus, while the influence of the MSD WWTP outfall cannot be ruled out, it is highly unlikely that human markers discharging from the MSD outfall were responsible for the HF183 concentrations observed during this study in the EB surf zone.

In summary, this assessment of a prior study of the MSD WWTP outfall discharge surface plume (Ohlmann et al., 2010) did not rule out that this outfall, located further east relative to the El Estero WWTP outfall, could have been impinging on surf zone HF183 detections at EB during this study. Regardless, the HF183 concentrations from the MSD outfall were comparatively low; further, there was no spatial gradient alongshore in the EB monitoring sites that would suggest an influence of an MSD outfall plume arriving from the east.

References

- Bernhard, A.E., Field, K.G., 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 66, 4571-4574.
- Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C.,Wang, D., Weisberg, S.B., 2013. Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Res.* 47, 6812-6828.
- Dick, L.K., Bernhard, A.E., Brodeur, T.J., Santo Domingo, J.W., Simpson, J.M., Walters, S.P., Field, K.G., 2005. Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification. *Appl. Environ. Microbiol.* 71, 3184-3191.

- Frick, W.E., Roberts, P.J.W., Davis, L.R., Keyes, J., Baumgartner, D.J., George, K.P., 2003. Dilution Models for Effluent Discharges. 4th ed. (Visual Plumes) ed.; United States Environmental Protection Agency: Athens, Georgia.
- Green, H.C., Haugland, R.A., Varma, M., Millen, H.T., Borchardt, M.A., Field, K.G., Walters,
 W.A., Knight, R., Sivaganesan, M., Kelty, C.A., Shanks, O.C., 2014. Improved HF183
 quantitative real-time PCR assay for characterization of human fecal pollution in ambient
 surface water samples. *Appl. Environ. Microb.* 80, 3086-3094.
- Jenkins, S.A., 2014. Hydrodynamic Modeling of Brine Dilution and Dispersion from the El Estero Wastewater Treatment Plant Outfall, Santa Barbara, CA.
- Lu, J.R., Santo Domingo, J.W., Lamendella, R., Edge, T., Hill, S., 2008. Phylogenetic diversity and molecular detection of bacteria in gull feces. *Appl. Environ. Microbiol.* 74, 3969-3976.
- Mattioli, M.C., Sassoubre, L.M., Russell, T.L., Boehm, A.B., 2017. Decay of sewage-sourced microbial source tracking markers and fecal indicator bacteria in marine waters. *Water Res.* 108, 106-114.
- Oana, K., Okimura, Y., Kawakami, Y., Hayashida, N., Shimosaka, M., Okazaki, M., Hayashi, T., Ohnishi, M., 2002. Physical and genetic map of *Enterococcus faecium* ATCC19434 and demonstration of intra- and interspecific genomic diversity in enterococci. *FEMS Microbiol. Lett.* 207, 133-139.
- Santa Barbara Coastal LTER, L. Washburn, C. Gotschalk, and D. Salazar. 2020. SBC LTER: Ocean: Currents and biogeochemistry: moored CTD and ADCP data from Mohawk Outside Spar (MKO), ongoing since 2005 ver 12. Environmental Data Initiative.

https://doi.org/10.6073/pasta/8fef527ca2bf889da28114b09455295c. Accessed 2020-05-27.

- SCOOS, Southern California Coastal Ocean Observing System, 2018. In Surface Current Mapping, Santa Barbara. Available at: <u>http://www.sccoos.org/data/hfrnet/</u>
- Shanks, O.C., Kelty, C.A., Sivaganesan, M., Varma, M., Haugland, R.A., 2009. Quantitative PCR for genetic markers of human fecal pollution. *Appl. Environ. Microbiol.* 75, 5507–5513.
- Sikich, S.A., Luce, S., Pease, K., King, M., Vu, K., 2018. Heal the Bay's 2011-2012 Annual Beach Report Card. Available at: <u>https://healthebay.org/wp-</u> content/uploads/2018/07/BRC_2017-2018_07-12-18.pdf
- Sinigalliano, C.D., Fleisher, J.M., Gidley, M.L., Solo-Gabriele, H.M., Shibata, T., Plano, L.R.W., Elmir, S.M., Wanless, D., Bartkowiak, J., Boiteau, R., Withum, K., Abdelzaher, A.M., He, G.Q., Ortega, C., Zhu, X.F., Wright, M.E., Kish, J., Hollenbeck, J., Scott, T., Backer, L.C., Fleming, L.E., 2010. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Res.* 44, 3763-3772.
- Sinigalliano, C.D., Ervin, J.S., Van De Werfhorst, L.C., Badgley, B.D., Balleste, E., Bartkowiak,
 J., Boehm, A.B., Byappanahalli, M., Goodwin, K.D., Gourmelon, M., Griffith, J.,
 Holden, P.A., Jay, J., Layton, B., Lee, C., Lee, J., Meijer, W.G., Noble, R., Raith, M.,
 Ryu, H., Sadowsky, M.J., Schriewer, A., Wang, D., Wanless, D., Whitman, R., Wuertz,
 S., Domingo, J.W.S., 2013. Multi-laboratory evaluations of the performance of *Catellicoccus marimammalium* PCR assays developed to target gull fecal sources. *Water Res.* 47, 6883-6896.

Tierra Data, 2013, City of Santa Barbara El Estero Wastewater Treatment Plan, 2013 Ocean Outfall Monitoring, NPDES #CA0048143, December 2013, 202 pp.

List of Supplemental Tables

Table S1. Sampling details and results of surf zone, lower watershed water, and beach sand at the Arroyo Hondo reference beach as well as fresh gull and unidentified seabird feces collected from the Santa Barbara area.

Table S2. Sampling details and results of surf zone water at LB and EB in 2015 and 2016

Table S3. Sampling details and results of coastal creek waters at LB and EB in 2015 and 2016

Table S4. Sampling details and results of coastal creek and nearshore sediments as well as supratidal and intertidal beach sands at LB and EB

Table S5. Sampling details and results of surface and groundwater for infrastructures and scour ponds at LB, EB, and the Santa Barbara Harbor.

Table S6. Synchronous sampling details and results of surf zone, nearshore, and offshore including boat anchorage in 2016 and 2017.

Table S7. Sampling details and results of raw sewage, treated effluent, recycled water, ocean outfall diffuser effluent, and marine water over diffuser in 2016 and 2017.

Table S8. Sampling details and results of surf zone water samples from LB and EB in 2017.

Table S9. Microbial source tracking (MST) hypotheses tested in Y1 (2015) at Leadbetter Beach (LB) and East Beach (EB), Santa Barbara, CA.

Table S10. Counts of people in water, on sand, and bedding as well as human marker concentrations for LB and EB surf zone samples in 2017.

Table S11. Study sites, hypotheses tested, number of samples by substrate type, weather condition, and year summarized in this study.

Table S12. Dates and times for which dye study samples were collected at locations (represented by x in the table) for studying sanitary sewer infrastructure associated with the bathrooms at EB and LB in 2015.

Table S13. Dye addition and sampling times for sanitary sewers beneath Shoreline Drive near LB, and dry weather flow from the Honda Creek culvert outlet and scour pond at LB, as well as the sanitary sewer beneath the parking lot and dry weather flow from the Sycamore Creek outlet and scour pond at EB in 2016.

Table S14. Dye addition and sampling times for sanitary sewer infrastructure on Stearns Wharf in 2017.

Table S15. Summary of Visual Plumes UM3 model input parameters for the Diffuser Tab.

Table S16. Summary of Visual Plumes UM3 model input parameters for the Ambient Tab.

Table S17. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming uniform current speed at surface and depth</u>.

Table S18. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, assuming uniform current speed at surface and depth.

Table S19. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming a current speed of 9 cm/sec at 15.85 m depth</u>.

Table S20. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, <u>assuming a current speed of 9 cm/sec at 15.85 m depth</u>.

Table S21. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming a current speed of 55 cm/sec at 15.85 m depth</u>.

Table S22. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, <u>assuming a current speed of 55 cm/sec at 15.85 m depth</u>.

Table S23. Summary of HF183 detection and concentrations in effluent discharged by El Estero and MSD.

Table S24. qPCR standard curve summary.

List of Supplemental Figures

Fig. S1 Location of reference beach Arroyo Hondo (AH, far west yellow pin) in relation to Leadbetter Beach (LB, yellow pin most immediately to the east of AH) and East Beach at Sycamore Creek (EB. far east yellow pin).

Fig. S2 a. Location of the Honda Creek watershed and Leadbetter Beach (LB) in Santa Barbara, CA. b. Map of LB showing the (green) watershed boundary, sewer mains, storm drains, and atrisk storm drains (green triangles)—where sanitary sewers cross in close spatial proximity.

Fig. S3 a. Location of the Sycamore Creek watershed and East Beach (EB) at Sycamore in Santa Barbara, CA. b. Map of EB at Sycamore showing the (green) watershed boundary, sewer mains, storm drains, and at-risk storm drains (yellow triangles)—where sanitary sewers cross in close spatial proximity.

Fig. S4 LB surf zone and watershed sampling locations in 2015 and 2016 including 5 surf zone sites (L01-L05), Honda Creek culvert outlet (L06), flowing storm drain (L07), Honda Creek culvert (L08), and creek water before entering the culvert (L09).

Fig. S5 EB surf zone and watershed sampling locations in 2015 and 2016 including 5 surf zone sites (E01-E05), bird refuge outlet (E06), lower bird refuge (E07), upper bird refuge (E08), flowing water discharging from pipe (E09), the Sycamore creek outlet (E10), and 2 creek sites (E11 and E12).

Fig. S6 Dye addition (stars, at the bathrooms) and sampling locations (temporary sampling wells GW3-GW4, surface water site SW2, and surf zone sites SZ4-SZ6) at LB to determine the potential leaking from sanitary sewer infrastructure associated with the bathrooms at LB in 2015. Fig. S7 Dye addition and sampling locations (temporary sampling wells GW1-GW2 and GW5, surface water site SW1, storm drain site SD, and surf zone sites SZ1-SZ3) at EB to determine the potential leaking from sanitary sewer infrastructure associated with the bathrooms (star locations) at EB in 2015.

Fig. S8 Sampling locations at surf zone, nearshore and offshore of LB, EB, and EB at Mission Creek in 2016.

Fig. S9 Surf zone (sites A01-A05) and creek (site A06) sampling locations at reference beach Arroyo Hondo.

Fig. S10 Groundwater well locations (GW4-GW7) near an onsite septic system at the Clark Estate on the east side of EB in 2016.

Fig. S11 Groundwater well locations (GW1-GW4) and surf zone sampling location (SZ1) near the leaking sewer lateral serving the beach bathroom at Chase Palm Park of EB in 2016.

Fig. S12 Groundwater (SP1-SP9) and surface water (SZ4-SZ6) sampling locations at the Honda Creek culvert outlet, LB.

Fig. S13 Groundwater (SP1-SP9) and surface water (SZ4-SZ6) sampling locations at the Sycamore Creek outlet, EB.

Fig. S14 Groundwater (GW1-GW5), surface water (SZ1-SZ3), and storm drain (SD1-SD3) sampling locations at LB for sanitary sewers beneath Shoreline Drive near LB in 2016.

Fig. S15 Groundwater (GW1-GW3) and surface water (SZ1-SZ3) sampling locations at EB for assessing sanitary sewer leakage beneath the parking lot west of the Cabrillo Bathhouse in 2016.

Fig. S16 Reef & Run swimming race event map (offshore inset) superimposed on EB to show the proximity of sampling sites to the event locations.

Fig. S17 Sampling locations for the water defecation study and surf zone recreation on holidays and busy weekends at EB.

Fig. S18 Sampling locations at surf zone, nearshore and in boat anchorage areas (outlined with white lines) of LB, EB, and EB at Mission Creek in 2017.

Fig. S19 Schematic diagram of sampling sites for treated effluent or commingled discharge (effluent mixed with seawater from the desalination plant) at the WWTP before discharging to the ocean outfall, the effluent leaving one of the diffuser ports closest to shore, and marine water collected vertically over the diffusers at 1, 9, and 18 m depths.

Fig. S20 Sampling locations for the Santa Barbara Harbor.

Fig. S21 The Stearns Wharf bathrooms, sewage lift stations and dye sampling locations in 2017.

Fig. S22 Scour pond and groundwater sampling dye concentration results after dye addition at the Honda Creek culvert outlet (SP0), at LB.

Fig. S23 LB groundwater velocity calculated for the Honda Creek culvert outlet (site SP0 in Fig. S14).

Fig. S24 Scour pond and groundwater sampling dye concentration results after dye addition at the Sycamore Creek outlet (CO), at EB.

Fig. S25 EB groundwater velocity calculated for the Sycamore Creek outlet.

Fig. S26 Groundwater results after dye addition at LB for sanitary sewers beneath Shoreline Drive near LB.

Fig. S27 Surf zone results after dye addition at LB for sanitary sewers beneath Shoreline Drive near LB and during the scour pond dye study.

Fig. S28 Groundwater results after dye addition at EB for the sanitary sewer beneath the parking lot west of the Cabrillo Bathhouse.

Fig. S29 Surf zone results after dye addition at EB for the sanitary sewer beneath the parking lot west of the Cabrillo Bathhouse.

Fig. S30 Fluorescence results from sampling location GW2 at EB in 2015.

Fig. S31 HF183 concentrations in surf zone, nearshore, anchorage, and offshore samples during 8 sampling events in 2016-2017 shown in Fig. Fig. S10 and S20. A: surf zone; B: nearshore; C: anchorage; D: offshore. a-h: 8 sampling events in 2016-2017, individually on Jul 28, Aug 23, and Sep 27, 2016, and Jul 6, Jul 25, Aug 29, Sep 25, and Oct 5, 2017.

Figure S32. Visual Plumes UM3 model graphic output of the predicted plume elevation from 7/6/17, looking horizontally through the water column.

Figure S33. Visual Plumes UM3 model graphic output of the predicted plume elevation from 7/25/17, looking horizontally through the water column.

Figure S34. Visual PlumesUM3 model graphic output of the predicted plume elevation from 8/29/17, looking horizontally through the water column.

Figure S35. Visual Plumes UM3 model graphic output of the predicted plume elevation from 9/25/17, looking horizontally through the water column.

Figure S36. Visual Plumes UM3 model graphic output of the predicted plume elevation from 10/5/17, looking horizontally through the water column.

Table S9. Microbial source tracking (MST) hypotheses tested in Y1 (2015) at Leadbetter Beach (LB) and East Beach (EB), Santa Barbara, CA.

Beach	Hypothesis
LB	Bathrooms at the beach are contributing FIB to the beach.
LB	Honda creek culvert is contributing FIB and human markers to the beach
	(surface or subsurface)
LB	Gulls and/or dogs are contributing FIB and markers directly to the beach/surf
	zone
LB	Flowing drainage pipe on west end of beach is contributing FIB (and markers)
	directly to the beach.
LB	Boats in the harbor are contributing FIB and human markers that are carried by
	surface currents.
LB	Honda creek and/or culvert contains FIB and human markers that then flow
	directly to the beach.
LB	Gulls and dogs are contributing FIB and markers directly to the creek.
EB	Bathroom at the beach is contributing FIB to the beach
EB	Sycamore Creek is contributing FIB and (human) markers directly to the beach
EB	Gulls and/or dogs are contributing FIB and markers directly to the beach/surf
	zone
EB	Parcels on septic to the east and/or boats moored offshore are contributing FIB
	and human markers to the surf zone
EB	Sycamore creek contains FIB and (human) markers that then flow directly to the

beach		

Table S11. Study sites, hypotheses tested, number of samples by substrate type, weather condition, and year summarized in this study.

Study site	Hypothesis	Number of samples	Weather	Year
Arroyo Hondo	Regional	18 surf zone and	Dry	2016
beach	background	watershed water samples,		
	contamination	9 intertidal sand samples		
		(Table S1)		
EB	Nonspecific	2 fresh gull and other	Dry	2016
	amplification from	seabird composite fecal		
	gull or bird feces	samples (Table S1)		
LB	Gulls and/or dogs	50 surf zone water	Dry and Wet	2015 and 2016
	contribute FIB and	samples under dry		
	markers directly to	weather, and 8 surf zone		
	the beach/surf zone	water samples under wet		
		weather (Table S2)		
EB	Gulls and/or dogs	50 surf zone water	Dry and Wet	2015 and 2016
	contribute FIB and	samples under dry		
	markers directly to	weather, and 8 surf zone		
	the beach/surf zone	water samples under wet		
		weather (Table S2)		
LB watershed	Honda creek culvert	34 watershed water	Dry and Wet	2015 and 2016
	contributes FIB and	samples under dry		
	fecal markers to the	weather, 13 watershed		
	beach	water samples under wet		
		weather, and 6 sediment		
		samples under dry weather		
		(Tables S3 & S4)		2015 12016
EB watershed	Sycamore Creek	56 watershed water	Dry and Wet	2015 and 2016
	focal markers to the	samples under dry		
	head	weather, 22 watershed		
	Deach	water samples under wet		
		samples under dry weather		
		(Tables S3 & S4)		
LB beach sand	Supratidal sands or	5 supratidal and 15	Drv	2016 and 2017
ED beden sand	intertidal sands act	intertidal sands under dry	Dry	2010 and 2017
	as source of FIB	weather (Table S4)		
	and fecal markers			
EB beach sand	Supratidal sands or	5 supratidal and 15	Drv	2016 and 2017
	intertidal sands act	intertidal sands under drv	5	
	as source of FIB	weather (Table S4)		
	and fecal markers			
LB creek termini	Creek termini scour	3 groundwater samples	Dry	2016
scour pond	pond contribute FIB	under dry weather (Table	5	
-	and fecal markers to	S5)		
	surf zone through			
	groundwater			
EB creek termini	Creek termini scour	3 groundwater samples	Dry	2016
scour pond	pond contribute FIB	under dry weather (Table		
	and fecal markers to	S5)		
	surf zone through			
	groundwater			

LB infrastructure and storm drain	Infrastructure and storm drain contribute to FIB and human fecal markers in surf zone	3 surface water and 5 groundwater (Table S5)	Dry	2016
EB infrastructure, septic system, and sewage pipeline	Infrastructure, septic system, and sewage pipeline contribute to FIB and human fecal markers in surf zone	1 surface water, 2 surf zone water and 24 groundwater samples (Table S5)	Dry	2015 and 2016
Recycled water	Recycled water contributes to HF183 human fecal markers in surf zone	6 recycled water samples (Table S7)	Dry	2016
The Santa Barbara Harbor	Sanitary sewer infrastructure or boat activities in the harbor contribute to HF183 human fecal markers in surf zone	6 surface water, 6 surf zone water and 9 groundwater samples (Table S5)	Dry	2017
LB and EB nearshore, offshore, and moored boats	Nearshore, offshore, and moored boats contribute to FIB and HF183 human fecal markers in surf zone	88 surf zone water, 56 nearshore water, 15 offshore water, 20 anchorage water, 7 nearshore sediments samples (Table S6)	Dry	2016 and 2017
Raw sewage, treated effluent, ocean outfall diffuser effluent, and marine water over diffuser	Treated wastewater effluent contribute to HF183 human fecal markers in surf zone	1 raw sewage, 11 treated effluent, 3 commingled effluent, 6 ocean outfall diffuser effluent, and 15 marine water over diffuser samples (Table S7)	Dry	2016 and 2017
EB surf zone water defecation	Water defecation overnight in the surf zone by beach campers contribute to FIB and HF183 human fecal markers in surf zone	75 surf zone water samples under dry weather (Table S8)	Dry	2017
EB and LB surf zone	Swimmers in surf zone contribute to HF183 human fecal markers in surf zone	30 surf zone water samples during holidays and high visitation weekends, 45 surf zone water samples during swimming races, and 56 surf zone water samples during the morning sampling campaigns when people were counted (Table S8)	Dry and wet (microcell storm on 9/3/2017, 10 samples from holidays and high visitation weekends taken on 9/4/2017)	2017

Table S12. Dates and times for which dye study samples were collected at locations (represented by x in the table) for studying sanitary sewer infrastructure associated with the bathrooms at EB and LB in 2015. Sampling locations are shown in Fig. S6 (LB) and Fig. S7 (EB).

	T.					EB						Ι	LB		
Date	Time	SW1	SZ1	SZ2	SZ3	GW1	GW2	GW5	SD	SW2	SZ4	SZ5	SZ6	GW3	GW4
	7 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/15	12 pm	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
0/15	4 pm	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
	8 pm	Х	Х	Х	Х	Х	Х			Х	Х	Х	Х	Х	Х
	2 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/16	8 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
	8 pm	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/17	8 am	Х	Х	Х	Х	Х	Х		Х	X	Х	Х	Х	Х	Х
6/18	8 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/19	8 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/20	8 am	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х	Х	Х
6/21	8 am	Х	Х	Х	Х	Х	Х			Х	Х	Х	Х	Х	Х
6/22	8 am	Х	х	Х	Х	Х	Х			Х	Х	Х	Х	Х	Х
6/23	12 pm		Х	Х	Х		Х	Х		Х	Х	Х	Х		
6/24	8 am		Х	Х	Х		Х	Х		Х	Х	Х	Х		
6/25	8 am		Х	Х	Х		Х	Х		Х	Х	Х	Х		
6/26	8 am		Х	Х	Х		Х	Х		Х	Х	Х	Х		

Table S13. Dye addition and sampling times for sanitary sewers beneath Shoreline Drive near LB, and dry weather flow from the Honda Creek culvert outlet and scour pond at LB, as well as the sanitary sewer beneath the parking lot and dry weather flow from the Sycamore Creek outlet and scour pond at EB in 2016. The locations of the sampling sites are depicted in Fig. S12 and S14 (LB) and Fig. S13 and S15 (EB).

Description	LB Date	EB Date	Time
-			
	8/8	8/22	2pm, 8pm
Background sample collection			
	8/9	8/23	8am, 2pm, 8pm
Dve addition	8/10	8/24	7am
	0/10	0/24	7 ann
Groundwater and surface water	8/10	8/24	8am, 2pm, 8pm
Stould which and suffice which			
sample collection	8/11	8/25	8am, 8pm
sumple concerton			
	8/12-8/19	8/26-9/2	8am daily

Table S14. Dye addition and sampling times for sanitary sewer infrastructure on Stearns Wharf in 2017. The locations of the sampling sites are depicted in Fig. S21.

Description	Date	Time
	10/30	8 am, 10 am, 12 pm
Background compling		
Background sampling		
	11/1	7 am, 7:30 am
D	11/1	0.0
Dye addition	11/1	8-9 am
		8.15 am 8.45 am 9.00 am 9.30 am 10.00 am 10.30 am
C	11/1	0.15 uni, 0.15 uni, 9.00 uni, 9.50 uni, 10.00 uni, 10.50 uni,
Surface water sampling	11/1	
		11:00 am, 11:30 am, 12:00 pm, 12:45 pm

Table S15. Summary of Visual Plumes UM3 model input parameters for the Diffuser Tab. As described in the text, a discharge coefficient of 0.61 was used in the model. 2017 is the simulation year.

												HF183	Entero1A	Human adenovirus
		Port	Port	Vert.	Horiz.	No.	Port	Port	Effluent	Effluent	Effluent	Effluent	Effluent	Effluent
		Dia.	elevation	angle	angle	of ports	spacing	depth	flow	salinity	temp	concentration	concentration	concentration
	Date	(in)	(in)	(deg)	(deg)		(ft)	(ft)	(MGD)	(psu)	(°C)	(copies/100mL)	(c.eq./100mL)	(copies/100mL)
•	7/6/17	3.75	35	0	180	60	6	73	6.10	1.2	27.5	1793402	50910	1393
	7/25/17	3.75	35	0	180	60	6	73	5.56	4.2	27.9	2649183	182964	511
	8/29/17	3.75	35	0	0	60	6	73	8.66	16.2	24.3	1693120	125662	3250
	9/25/17	3.75	35	0	180	60	6	73	12.29	19.3	21.8	1896638	95913	195
	10/5/17	3.75	35	0	180	60	6	73	12.71	16.2	22.6	1810157	133369	80

Table S16. Summary of Visual Plumes UM3 model input parameters for the Ambient Tab. The model was run under three different scenarios: applying the surface current speed uniformly at depth (all markers), using 9 cm/sec at 15.85 m depth (HF183 only), and 55 cm/sec at 15.85 m depth (HF183 only). All three scenarios for each date used the same current direction. Surface current speed and direction was obtained from the High Frequency Radar surface current data available on the Southern California Coastal Ocean Observing System website, and the ambient temperature and salinity from instruments moored at Mohawk Reef in Santa Barbara as part of the Santa Barbara Coastal Term Ecological Research project in Santa Barbara, as described in the text.

	current	Current	Ambient	Ambient
	speed	direction	Salinity	temperature
Date	(cm/s)	(N-deg)	(psu)	(°C)
7/6/2017	23.80	271.73	33.5 (at 4 m)	16.7 (at 3 m), 16.5 (at 4 m), 15.9 (at 7 m), 14.9 (at 10 m)
7/25/2017	11.00	254.17	33.4 (at 4 m)	19.0 (at 3 m), 18.9 (at 4 m), 18.6 (at 7 m), 17.3 (at 10 m)
8/29/2017	13.65	45.98	33.4 (at 4 m)	18.1 (at 3 m), 17.9 (at 4 m), 17.2 (at 7 m), 15.4 (at 10 m)
9/25/2017	17.03	275.41	33.4 (at 4 m)	16.3 (at 3 m), 16.2 (at 4 m), 16.0 (at 10 m)
10/5/2017	15.34	271.42	33.4 (at 4 m)	18.6 (at 3 m), 18.5 (at 4 m), 18.1 (at 10 m)

Surface

Table S17. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming uniform</u> current speed at surface and depth. 2017 is the simulation year.

Human w/decay Depth Amb-cur P-dia HF183 HF183 Entero1A adenovirus Dilutn Time x-posn y-posn Date 0 (ft) (**ft**) **(s)** (ft) (cm/s) (in) (cp/100mL) (cp/100mL) c.eq./100mL cp/100mL 7/6/17 64.2 23.8 171.1 4101.6 4106.1 116.6 3.2 424.8 -56.2 1.6 67.7 7/25/17 63.0 11.0 179.8 10976.5 10986.6 758.8 2.1 235.1 -24.0 -5.5 57.1 8/29/17 54.3 66.0 13.7 184.5 11062.7 11072.5 821.1 21.2 150.5 23.5 16.5 9/25/17 50.0 17.0 524.7 3843.9 3855.9 195.0 0.4 486.0 -119.4 10.0 192.3 10/5/17 15.3 410.5 387.3 0.2 339.6 -79.7 133.6 54.1 5244.8 5256.1 1.6

Table S18. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, <u>assuming uniform current speed at surface and depth</u>. 2017 is the simulation year.

				w/decay			Human					
									Х-			
	Depth	Amb-cur	P-dia	HF183	HF183	Entero1A	adenovirus	Dilutn	posn	y-posn	Time	
Date	(ft)	(cm/s)	(in)	(cp/100mL)	(cp/100mL)	c.eq./100mL	cp/100mL	0	(ft)	(ft)	(s)	
7/6/17	61.5	23.8	248.6	2812.7	2818.6	80.0	2.2	618.8	-105.9	3.1	131.1	
7/25/17	59.9	11.0	285.4	7356.9	7370.0	509.0	1.4	350.5	-42.7	-10.6	109.6	
8/29/17	63.2	13.7	286.0	7607.7	7620.7	564.6	14.6	218.6	40.9	32.1	104.8	
9/25/17	41.5	17.0	796.7	2609.5	2625.6	132.8	0.3	713.8	-226.0	19.9	380.7	
10/5/17	47.5	15.3	626.0	3597.2	3612.3	266.1	0.2	494.1	-144.3	3.2	258.3	

				no decay					
	Depth	Amb-cur	P-dia	HF183	Dilutn	x-posn	y-posn	Time	
Date	(ft)	(cm/s)	(in)	(cp/100mL)	0	(ft)	(ft)	(s)	
7/6/2017	58.0	9.0	223.5	7148.7	244.0	-25.3	0.6	67.0	
7/25/2017	61.7	9.0	180.6	12620.2	204.7	-20.6	-4.4	56.2	
8/29/2017	64.3	9.0	191.2	14030.4	118.6	18.7	10.7	53.6	
9/25/2017	40.3	10.8	617.6	5399.3	347.1	-73.1	5.3	191.3	
10/5/2017	48.1	9.5	435.0	7359.8	242.5	-53.9	0.9	130.0	

Table S19. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming a current speed of 9 cm/sec at 15.85 m depth</u>.

Table S20. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, <u>assuming a current speed of 9 cm/sec at 15.85 m depth</u>.

	Depth	Amb-cur	P-dia	HF183	Dilutn	x-posn	y-posn	Time
Date	(ft)	(cm/s)	(in)	(cp/100mL)	0	(ft)	(ft)	(s)
7/6/2017	53.7	9.0	389.7	4664.8	373.9	-43.7	1.1	127.4
7/25/2017	58.2	9.0	311.1	8191.0	315.3	-36.2	-8.6	109.2
8/29/2017	60.8	9.0	336.9	9307.2	178.7	30.5	20.6	102.4
9/25/2017	29.0	12.8	1025.2	3315.5	565.3	-135.3	10.9	374.8
10/5/2017	39.0	10.7	776.1	4612.6	387.0	-94.3	1.9	254.4

Table S21. Visual Plumes UM3 model output for the **Plume Trap Level** on the five offshore sampling event dates, <u>assuming a current speed of 55 cm/sec at 15.85 m depth</u>.

		Amb-						
	Depth	cur	P-dia	HF183	Dilutn	x-posn	y-posn	Time
Date	(ft)	(cm/s)	(in)	(cp/100mL)	0	(ft)	(ft)	(s)
7/6/2017	66.6	55.0	116.3	2763.3	631.2	-123.6	3.6	67.6
7/25/2017	68.1	55.0	95.46	4878.2	529.4	-102.0	-28.2	58.1
8/29/2017	69.3	55.0	105.4	5531.5	300.9	74.9	68.9	55.7
9/25/2017	59.7	55.0	306.5	2128.8	880.4	-354.6	32.7	193.6
10/5/2017	62.6	55.0	230.9	2789.1	640.0	-244.7	5.8	131.9

Table S22. Visual Plumes UM3 model output for the **Maximum Rise Plume Level** on the five offshore sampling event dates, <u>assuming a current speed of 55 cm/sec at 15.85 m depth</u>.

		Amb-						
	Depth	cur	P-dia	HF183	Dilutn	x-posn	y-posn	Time
Date	(ft)	(cm/s)	(in)	(cp/100mL)	0	(ft)	(f t)	(s)
7/6/2017	64.9	55.0	166.0	1859.6	937.9	-247.5	7.4	136.2
7/25/2017	66.8	55.0	134	3282.9	786.7	-209.4	-58.5	119.9
8/29/2017	67.9	55.0	147.5	3869.5	429.8	146.2	137.2	110.3
9/25/2017	55.1	55.0	444.8	1461.2	1282.6	-704.8	65.8	388.1
10/5/2017	59.1	55.0	333.9	1914.5	932.3	-474.8	11.5	259.0

Table S23. Summary of HF183 detection and concentrations in effluent discharged by El Estero and MSD. ND = not detected, ROQ = range of quantification. Effluent samples were taken from a sampling location just prior to discharge at each of the WWTPs.

Effluent	El Estero	MSD
	2016-2017	2007-2008
Ν	12	52
Median	1.9E+06	ND
Geomean*	2.7E+06	1.0E+03
Min	4.7E+05	ND
Max	2.0E+08	5.7E+05
%ND	0	67.3
%ROQ	100	32.7

*Geomean is calculated from ROQ values only

Table S24. qPCR standard curve summary.

Summary of the pooled standard curves for each sample year and assay. ROQ = range of quantification. LLOQ = lower limit of quantification. Cq = quantification cycle.

					Amplification	ROQ	LLOQ
Assay	Year	Slope	y- intercept	\mathbf{R}^2	Efficiency ^a	copies/rxn	Ca
	2015	-3.50	38.0	0.995	0.931	10^{1} - 10^{5}	34.66
HF183IAC	2016	-3.45	38.1	0.997	0.948	10^{1} - 10^{6}	34.72
	2017	-3.44	38.4	0.998	0.951	10^{1} - 10^{5}	34.79
	2015	-3.40	39.4	0.996	0.969	10^{1} - 10^{5}	36.09
HumM2	2016	-3.44	39.3	0.996	0.954	10^{1} - 10^{5}	35.91
	2017	-3.49	39.0	0.996	0.935	10^{1} - 10^{5}	35.47
	2015	-3.53	39.4	0.992	0.919	10^{1} - 10^{5}	36.17
DogBact	2016	-3.41	39.4	0.990	0.964	10^{1} - 10^{5}	35.89
_	2017	-3.54	39.8	0.995	0.915	10^{1} - 10^{5}	36.21
	2015	-3.40	39.8	0.996	0.970	10^{1} - 10^{5}	36.26
Gull2TaqMan	2016	-3.46	40.3	0.994	0.945	10^{1} - 10^{6}	36.83
	2017	-3.40	40.4	0.995	0.969	10^{1} - 10^{5}	36.98
Salmonella	2015/2016	-3.33	35.2	0.994	0.996	10^{0} - 10^{4}	35.20
Samonena	2017	-3.31	35.0	0.993	1.003	10^{0} - 10^{4}	35.01
Entero1A	2015	-3.34	37.7	0.996	0.992	10^{1} - 10^{5}	34.35
	2016	-3.38	38.4	0.997	0.975	10^{1} - 10^{6}	34.97
	2017	-3.35	37.8	0.994	0.987	10^{1} - 10^{5}	34.43

^aEfficiency = $10^{(-1/\text{slope})} - 1$



Fig. S1 Location of reference beach Arroyo Hondo (AH, far west yellow pin) in relation to Leadbetter Beach (LB, yellow pin most immediately to the east of AH) and East Beach at Sycamore Creek (EB, far east yellow pin). Not shown is East Beach at Mission Creek, another frequently FIB-contaminated beach located midway between LB and EB but not one that is part of this study such that new research of LB and EB were prioritized.



Fig. S2 Location of the Honda Creek watershed and Leadbetter Beach (LB) in Santa Barbara, CA. a. The dark blue line within the (green) watershed boundary marks the flow path of Honda Creek (upper watershed) and culvert (lower watershed). The orange circle indicates the most upstream sampling location during AB411, which was based on field observations of flowing or pooled water at that location but not upstream. The site at LB where Santa Barbara County monitors fecal indicator bacteria (FIB) weekly is marked with a red "star". b. Map of LB showing the (green) watershed boundary, sewer mains, storm drains, and at-risk storm drains (green triangles)—where sanitary sewers cross in close spatial proximity.



Fig. S3 Location of the Sycamore Creek watershed and East Beach (EB) at Sycamore in Santa Barbara, CA. a. The dark blue lines within the (green) watershed boundary mark the flow paths of tributaries (upper watershed) and the main stem (lower watershed) of Sycamore Creek. The lower watershed (south of Hwy 101, down to EB) was the focus of this study because there the main stem integrates and conveys all upstream creek waters (with their associated contaminants) further downstream and, depending on flow and rain conditions, entrains local pollution sources as the creek flows towards the watershed terminus upstream of the beach area. The orange circle indicates the most upstream sampling location during AB411, which was based on field observations of flowing or pooled water at that location but not upstream. The beach site where SB County monitors FIB weekly is marked with a red "star". b. Map of EB at Sycamore showing

the (green) watershed boundary, sewer mains, storm drains, and at-risk storm drains (yellow triangles)—where sanitary sewers cross in close spatial proximity.



Fig. S4 LB surf zone and watershed sampling locations in 2015 and 2016 including 5 surf zone sites (L01-L05), Honda Creek culvert outlet (L06), flowing storm drain (L07), Honda Creek culvert (L08), and creek water before entering the culvert (L09). Numbers on the map indicate sampling locations (e.g. site L01 is indicated by the circle containing number 1). Grey circle indicates a flowing drain that was sampled in 2015 and was covered by sand in 2016 (L07). Flow from the drain had no surface connection to the surf zone. Site L06, the Honda Creek culvert outlet, was sampled using different methods in 2015: water on top of sediment in pipes after dewatering scour pond (L06), surface sample of scour pond (L06A), and water directly from inside the pipes using a peristaltic pump (L06B). In 2016 this culvert outlet was buried by sand and was not sampled. During wet weather events, the Honda Creek was sampled as it spilled over a small wall before entering the culvert (L09). For dry weather sampling in Honda Creek, water pooled behind the wall was sampled (L09) until it dried up, at which point the closest upstream location
that still contained water was sampled (L09A or L09B). For most sampling dates, there was no flowing water between site L09/A/B and downstream site L08, except during rain events and two dry weather dates (May 20 and Jun 24, 2015). Site L09D, a drain pipe near site L09 had a trickle flow and was sampled on Sep 9, 2015 and Sep 22 2015. Sediment samples were collected from sites L06, L08 and L09 on three dates (May 26, Jul 14, and Sep 8, 2016). Intertidal sand was collected from the five surf zone locations (sites L01-L05) on three dates (May 24, Jul 12, and Sep 6, 2016). Supratidal sand was collected from locations that were aligned perpendicularly to surf zone sampling sites L01-L05 on one date (Jun 28, 2017).



Fig. S5 EB surf zone and watershed sampling locations in 2015 and 2016 including 5 surf zone sites (E01-E05), bird refuge outlet (E06), lower bird refuge (E07), upper bird refuge (E08), flowing water discharging from pipe (E09), the Sycamore creek outlet (E10), and 2 creek sites (E11 and E12). Numbers on the map indicate sampling locations (e.g. site E01 is indicated by the circle containing number 1). Grey circle indicates a flowing drain (E11D) which was observed to have flow and sampled on Sep 9, 2015. Site E09, a storm drain pipe, could only be sampled during wet weather events. During dry weather, the puddle of water in front of the pipe (E09A) was sampled. Similarly, Sycamore Creek site E12 could only be sampled during rain events. During dry weather sampling, there was no flowing water at or upstream of site E12, and the closest downstream location with pooled water was sampled (E12A or E12B). For most sampling dates, there was no flowing water or connected pools between site E12/A/B and downstream site E11. Sediment samples were collected from sites E06, E09A, E10, E11, and

E12B on three dates (May 26, Jul 14, and Sep 8, 2016). Intertidal sand was collected from the five surf zone locations (sites E01-E05) on three dates (May 24, Jul 12, and Sep 6, 2016). Supratidal sand was collected from locations that were aligned perpendicularly to surf zone sampling sites E01-E05 on one date (Jun 28, 2017).



Fig. S6 Dye addition (stars, at the bathrooms) and sampling locations (temporary sampling wells GW3-GW4, surface water site SW2, and surf zone sites SZ4-SZ6) at LB to determine the potential leaking from sanitary sewer infrastructure associated with the bathrooms at LB in 2015.



Fig. S7 Dye addition and sampling locations (temporary sampling wells GW1-GW2 and GW5, surface water site SW1, storm drain site SD, and surf zone sites SZ1-SZ3) at EB to determine the potential leaking from sanitary sewer infrastructure associated with the bathrooms (star locations) at EB in 2015.



Fig. S8 Sampling locations at surf zone, nearshore and offshore of LB, EB, and EB at Mission Creek in 2016. Numbers indicate sampling locations (e.g. site L01 is indicated by the circle containing number 1 at LB, E01 by the circle containing number 1 at EB Mission Creek site). Water from the surf zone (sites 1-5 at each beach), nearshore (NS), and offshore (OS) locations were sampled on Jul 28, Aug 23, and Sep 27, 2016. Nearshore sampling sites were near boat mooring area, which is illustrated using white lines. Red dots indicate the location of wastewater outfalls from El Estero WWTP (center) and Montecito Sanitary District (right). Sediment was collected at all seven nearshore locations by scientific divers on Jul 19, 2016 for EB and Jul 21, 2016 for LB.



Fig. S9 Surf zone (sites A01-A05) and creek (site A06) sampling locations at reference beach Arroyo Hondo. Numbers on the map indicate sampling locations (e.g. site A01 is indicated by the circle containing number 1). Dry weather sampling occurred on Jun 21, Aug 19, and Sep 16, 2016. On each date, water was collected from all six sites, and intertidal sands were collected from three surf zone locations (sites A01, A03, A05).



Fig. S10 Groundwater well locations (GW4-GW7) near an onsite septic system at the Clark Estate on the east side of EB in 2016.



Fig. S11 Groundwater well locations (GW1-GW4) and surf zone sampling location (SZ1) near the leaking sewer lateral serving the beach bathroom at Chase Palm Park of EB in 2016.



Fig. S12 Groundwater (SP1-SP9) and surface water (SZ4-SZ6) sampling locations at the Honda Creek culvert outlet, LB. SP0 was installed to introduce dye to the subsurface.



Fig. S13 Groundwater (SP1-SP9) and surface water (SZ4-SZ6) sampling locations at the Sycamore Creek outlet, EB. SP0 was installed to introduce dye to the subsurface.



Fig. S14 Groundwater (GW1-GW5) and surface water (SZ1-SZ3) sampling locations at LB for sanitary sewers beneath Shoreline Drive near LB in 2016. Also shown with red circles are the sampling locations of pooled water inside storm drains (SD1 & SD3), and pooled water above a buried storm drain (SD2) that was previously sampled in 2015 as site L07 (Fig. S4).



Fig. S15 Groundwater (GW1-GW3) and surface water (SZ1-SZ3) sampling locations at EB for assessing sanitary sewer leakage beneath the parking lot west of the Cabrillo Bathhouse in 2016.



Fig. S16 Reef & Run swimming race event map (offshore inset) superimposed on EB to show the proximity of sampling sites to the event locations. Water was collected from five surf zone locations (E03 and E2R-E5R) at three time points including the warmup (pre-race) phase, post-race, and the following morning on Jun 22 & 23, Jul 13 & 14, and Aug 17 & 18, 2017. Intertidal sand samples were collected from three sites (E-2R, E-3R, and E-4R) during the warmup (pre-race) phase.



Fig. S17 Sampling locations for the water defecation study and surf zone recreation on holidays and busy weekends at EB. Numbers indicate sampling locations (e.g. site E01 is indicated by the circle containing number 1). Shaded locations indicate areas where people had previously been observed camping. Water was collected from five surf zone locations (sites E01-E05) at three time points (1st day late afternoon, 2nd day early morning and mid-afternoon) on Jun 20 & 21, Aug 1 & 2, Aug 23 & 24, Sep 26 & 27, and Oct 17 & 18, 2017 for the water defecation study. Water samples were collected from all five surf zone locations at two time points (early morning, mid-afternoon) on Jul 3, Aug 12, and Sep 4, 2017, and intertidal sand was collected at sites E01, E03, and E05 during the early morning sampling on all three dates for the holidays and busy weekends study.



Fig. S18 Sampling locations at surf zone, nearshore and in boat anchorage areas (outlined with white lines) of LB, EB, and EB at Mission Creek in 2017. Water was collected from 11 surf zone locations (sites L01-L05 at LB, E01-E05 at EB, M01 at EB at Mission Creek), 7 nearshore locations (L1NS-L3NS, E1NS-E3NS, M1NS), 4 locations inside the boat anchorage areas off EB (E1A-E4A), from 3 depths over the diffuser section of the El Estero WWTP outfall (1, 9, and 18 m) on five dates Jul 6, Jul 25, Aug 29, Sep 25, and Oct 5, 2017. Samples were also collected of the final effluent, and commingled effluent if applicable, from the El Estero WWTP prior to discharge. On the last four dates, scientific divers also collected a sample of the effluent leaving one of the diffuser ports closest to shore. Effluent was also collected from the Montecito Sanitary District outfall diffuser on two dates Oct 5 and Oct 10, 2017.



Fig. S19 Schematic diagram of sampling sites for treated effluent or commingled discharge (effluent mixed with seawater from the desalination plant) at the WWTP before discharging to the ocean outfall, the effluent leaving one of the diffuser ports closest to shore, and marine water collected vertically over the diffusers at 1, 9, and 18 m depths. Sample data (Table S7) corresponding to this diagram were labeled as: ED (effluent sample at a diffuser port); 1DF (1 m below the surface); 2DF (9 m below the surface; 3DF (18 m below the surface).



Fig. S20 Sampling locations for the Santa Barbara Harbor. Water was collected from two surf zone locations (L01 and L02), three groundwater locations (L-GW6 to L-GW8), one location inside the harbor (L-IH), and one location outside of the harbor breakwater (L-OH) on three dates Jul 18, Aug 15, and Sep 20, 2017. Red lines (storm drains) and orange lines (sanitary sewers) indicate the location of relevant public infrastructure.



Fig. S21 The Stearns Wharf bathrooms, sewage lift stations and dye sampling locations in 2017.



Fig. S22 Scour pond and groundwater sampling dye concentration results after dye addition at the Honda Creek culvert outlet (SP0), at LB. Dye was added at 8 am on Aug 10, 2016 at location SP0. Culvert and temporary groundwater well locations (SP1 – SP9) are shown in Fig. S12. Corresponding surf zone dye concentration results from locations SZ4-SZ6 are shown in Fig. S27.



Fig. S23 LB groundwater velocity calculated for the Honda Creek culvert outlet (site SP0 in Fig. S12).



Fig. S24 Scour pond and groundwater sampling dye concentration results after dye addition at the Sycamore Creek outlet (CO), at EB. Dye was added at 7:30 am on Aug 24, 2016 at location SP0. Scour pond and temporary groundwater well locations (SP1 – SP9) are shown in Fig. S13. Surf zone sample dye concentration results from locations SZ4-SZ6 are shown in Fig. S29.



Fig. S25 East Beach groundwater velocity calculated for the Sycamore Creek outlet.



Fig. S26 Groundwater results after dye addition at LB for sanitary sewers beneath Shoreline Drive near LB. Dye was added between 7 and 7:30 am on Aug 10, 2016. Sampling locations are depicted in Fig. S14.



Fig. S27 Surf zone results after dye addition at LB for sanitary sewers beneath Shoreline Drive near LB. Dye was added between 7 and 7:30am on Aug 10, 2016. Sampling locations are depicted in Fig. S14 (SZ1 – SZ3). Also shown are the surf zone results from locations SZ4-SZ6 (Fig. S12) from the scour pond dye study. Scour pond and groundwater results are shown in Fig. S22.



Fig. S28 Groundwater results after dye addition at EB for the sanitary sewer beneath the parking lot west of the Cabrillo Bathhouse. Dye was added at 7:30 am on Aug 24, 2016. Sampling locations are depicted in Fig. S15.



Fig. S29 Surf zone results after dye addition at EB for the sanitary sewer beneath the parking lot west of the Cabrillo Bathhouse. Dye was added at 7:30 am on Aug 24, 2016. Sampling locations are depicted in Fig. S15 (SZ1 – SZ3). Also shown are the surf zone results from locations SZ4-SZ6 (Fig. S13) from the scour pond dye study. Scour pond and groundwater results are shown in Fig. S24.



Fig. S30 Fluorescence results from sampling location GW2 at EB in 2015. The sampling location is depicted in Fig. S7.



Fig. S31 HF183 concentrations in surf zone, nearshore, anchorage, and offshore samples during 8 sampling events in 2016-2017 shown in Fig. S8 and S18. a-h: 8 sampling events in 2016-2017, individually on Jul 28, Aug 23, and Sep 27, 2016, and Jul 6, Jul 25, Aug 29, Sep 25, and Oct 5, 2017.



Figure S32. Visual Plumes UM3 model graphic output of the predicted plume elevation from 7/6/17, looking horizontally through the water column. Input parameters are described in Tables S15 and S16, using uniform current speed. The plume was predicted to trap at 64.2 feet (Table S17) and have a maximum rise of 61.5 feet (Table S18). The solid line indicates the centerline of the plume, and the dots indicate the average plume boundary.



Figure S33. Visual Plumes UM3 model graphic output of the predicted plume elevation from 7/25/17, looking horizontally through the water column. Input parameters are described in Tables S15 and S16, using uniform current speed. The plume was predicted to trap at 63.0 feet (Table S17) and have a maximum rise of 59.9 feet (Table S18). The solid line indicates the centerline of the plume, and the dots indicate the average plume boundary.



Figure S34. Visual PlumesUM3 model graphic output of the predicted plume elevation from 8/29/17, looking horizontally through the water column. Input parameters are described in Tables S15 and S16, using uniform current speed. The plume was predicted to trap at 66.0 feet (Table S17) and have a maximum rise of 63.2 feet (Table S18). The solid line indicates the centerline of the plume, and the dots indicate the average plume boundary.



Figure S35. Visual Plumes UM3 model graphic output of the predicted plume elevation from 9/25/17, looking horizontally through the water column. Input parameters are described in Tables S15 and S16, using uniform current speed. The plume was predicted to trap at 50.0 feet (Table S17) and have a maximum rise of 41.5 feet (Table S18). The solid line indicates the centerline of the plume, and the dots indicate the average plume boundary.



Figure S36. Visual Plumes UM3 model graphic output of the predicted plume elevation from 10/5/17, looking horizontally through the water column. Input parameters are described in Tables S15 and S16, using uniform current speed. The plume was predicted to trap at 54.1 feet (Table S17) and have a maximum rise of 47.5 feet (Table S18). The solid line indicates the centerline of the plume, and the dots indicate the average plume boundary.