Doheny State Beach Microbial Source Tracking Study

Conducted by
the Southern California Coastal Water Research Project Authority (SCCWRP)
as part of the Source Identification Protocol Project (SIPP)

Southern California Coastal Water Research Project

April 2014

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Southern California Coastal Water Research Project Authority, Costa Mesa, CA

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Disclosure Statement
Funding for this project has been provided in full or in part through an agreement with the State Water Resources Control Board. The contents of this document do not necessarily reflect the views and policies of the State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Contents

Background

Doheny State Beach (DSB) was chosen as a microbial source tracking study site as part of SIPP due to its history of chronically poor microbial water quality and evidence of increased health risk among swimmers. Doheny has consistently ranked among Heal the Bay's Top 10 "Beach Bummer" list, including #7 in 2013 and several #1 rankings in years past.[1] This history led to a dry weather epidemiology study at DSB carried out by SCCWRP and colleagues in 2007-08. The study clearly identified a health risk to swimmers at Doheny.[2] Adverse health outcomes in swimmers were correlated to levels of fecal indicator bacteria (FIB) when the berm separating San Juan Creek (SJC) and the ocean was open; suggesting that one route of exposure to the causative agents of illness is through contaminated water from SJC. However, there was still a positive relationship between water exposure and illness even when the berm was closed. Human enteric viruses were detected sporadically throughout the epidemiology study, but were not correlated to illness in swimmers or levels of FIB[3].

DSB is located in the city of Dana Point, in southern Orange County, CA. The beach is situated at the mouth of SJC. In late spring, groundwater from winter storms allows near continuous flows from SJC directly to the ocean, while in summer SJC becomes blocked by a seasonal sand berm that forms a brackish lagoon. This lagoon is home to large numbers of gulls, pelicans, and other waterfowl most of the year. SJC receives urban runoff from a number of storm drains, some of which flow year-round. The northern end of DSB is bound by a breakwater that protects the adjacent Dana Point Harbor. North Creek, which mainly carries nuisance flow from parking lots and irrigation, flows near the breakwater in wet weather and terminates in a pond in summertime.

Several characteristics of DSB are similar to other California beaches, and thus it is hoped that the approaches used in the present study may inform microbial source tracking efforts in other locales. These characteristics include: popularity among tourists and surfers; limited surf zone circulation near a jetty or breakwater; freshwater stream inputs containing urban runoff; seasonal lagoon and sand berm; large resident population of avian wildlife; and aging sewer infrastructure.

Potential Sources

Three suspected dry weather sources of fecal microbes were identified: urban runoff from storm drains, sanitary sewer infrastructure, and wildlife (gull population).

1) Most storm drain flow from sub-drains to SJC is diverted in the summer months, but among sub-drains that flow year-round, many have historically high levels of fecal indicator bacteria (FIB) and/or human fecal DNA markers.[4] "Reconnaissance" sampling for SIPP in summer 2011 confirmed those findings. One or more homeless persons used an especially large culvert near the beach as a latrine and evidence of open defecation was often observed.

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[1] Heal the Bay, via http://brc.healthebay.org

[2] Colford, J.M., Schiff, K.C., Griffith, J.F., Yau, V., Arnold, B.F., Wright, C.C., Gruber, J.S., Wade, T.J., Burns, S., Hayes, J., McGee, C., Gold, M., Cao, Y., Noble, R.T., Haugland, R., Weisberg, S.B., 2012. Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. Water Research 46, 2176–2186.

[3] Love, D.C., Rodriguez, R.A., Gibbons, C.D., Griffith, J.F., Yu, Q., Stewart, J.R., Sobsey, M.D., 2013. Human viruses and viral indicators in marine water at two recreational beaches in Southern California, USA. Journal of Water and Health In Press, doi:10.2166/wh.2013.078

[4] Orange County Public Works, unpublished data.

2) Within the park boundaries, the sanitary sewer infrastructure at DSB includes 1902 m of gravity collection lines and 207 m of force mains (Figure 1). If the integrity of this infrastructure were compromised, then untreated sewage could potentially reach the ocean via groundwater.
3) The outlet of SJC is blocked by a sand berm during dry weather, which forms a brackish lagoon. This area is home to large numbers of gulls, pelicans, and other waterfowl most of the year, which contribute to high FIB levels in the lagoon. The birds may also be depositing FIB on the sand and algal wrack, thus continually reseeding the surf zone with bacteria during each tidal cycle.

Figure 1. GIS map showing stormwater and sanitary sewer infrastructure surrounding Doheny State Beach. DP = Dana Point; SJC = San Juan Capistrano; SCWD = South Coast Water District.

Stakeholder Involvement

Numerous stakeholders contributed to this project. The City of Dana Point was a key partner throughout, facilitating meetings among stakeholders on a regular basis. We worked directly with Orange County Public Works and California State Parks to carry out the field portion of the study. The above agencies as well as South Coast Water District, City of San Juan Capistrano, Orange County Health Care Agency, CalTrans, San Diego Regional Water Quality Control Board, and members of the Clean Beach Task Force were asked to provide input to the study team and briefed on the results. In addition, Doheny State Beach was a focus of the Bight '08 Project microbiology group and we coordinated our efforts with theirs. After our study was completed, we continued to consult with State Parks and Orange County Public Works regarding their next steps forward to address infrastructure concerns.

Our experience was that some agencies were not immediately forthcoming with relevant information. For example, we did not learn of postponed sanitary system maintenance and repairs until after our study had been conducted; we could have adapted our study design accordingly were this information shared with us beforehand. It is crucial that MST study teams be provided with the most recent sanitary infrastructure assessments, including information on known problems or postponed maintenance, early on in the study design process. Similarly, we did not learn of a network of groundwater monitoring wells present in the area until years later; these wells would have been a great asset in our dye study.

Hypotheses

Based on the potential sources described above, we developed three main hypotheses, each with a few sub-hypotheses.

H1: Fecal microbes are transported to DSB from urban runoff in the SJC watershed

- H1.1: Effluent from upstream storm drains contribute to high FIB at creek terminus/lagoon
- H1.2: When the sand berm is in place, microbes are transported by tidal action through the berm to the ocean
- H1.3: When the sand berm is open, microbes are transported to the ocean by creek flow and tidal action in the estuarine zone of the creek terminus

H2: Fecal microbes are leaking from faulty sanitary sewer infrastructure

- H2.1: Groundwater containing microbes discharges to the surf zone from inland sources during low tides
- H2.2: Degraded sanitary sewer lines are leaking untreated sewage into the groundwater

H3: Fecal bacteria are deposited into water by waterfowl

- H3.1: Levels of FIB in water are related to the number of birds on the beach
- H3.2: The bacterial community in water contains signatures of gull droppings

Project Approach[5]

The contribution of urban runoff (H1) was evaluated by measuring weekly fluxes of FIB and human-associated qPCR markers at various inputs to lower San Juan Creek and the beach. A total of 12 sites (including ocean, surface water, and storm drain effluent) were sampled at 6 a.m. weekly for 5 weeks (Figure 2). Microbial analyses of the samples were both culture-based (EPA 1600, IDEXX Enterolert, and Colilert) and molecular (HF183 Taqman and HumM2 qPCR assays). Salinity, temperature, pH, turbidity, and flow rate (where possible) were also measured at each site. To address whether FIB are transported from the lagoon to the sea through the closed sand berm (H1.2), two transects across the berm were sampled during the 30-hour study (described in the following paragraph). Water level, salinity, and FIB in mid-berm wells were examined in context of tide height and direction.

[5] For further details on project approach and methods, see Appendix A: Technical Report.

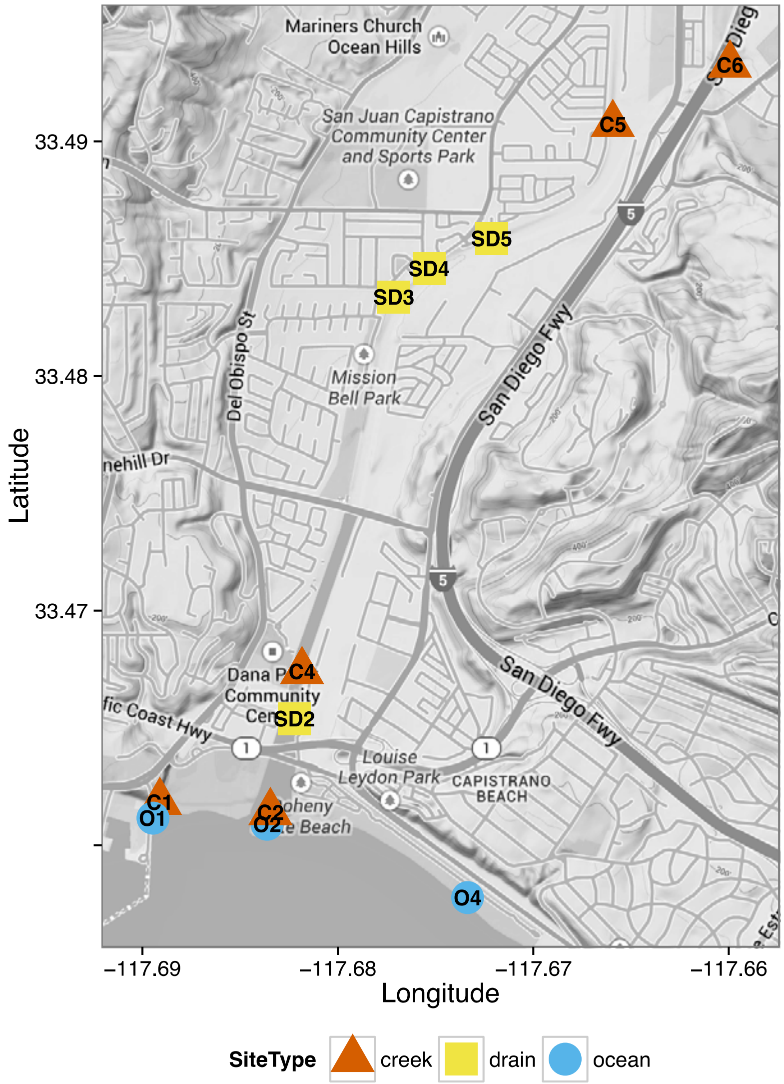

Figure 2. Urban runoff (H1) weekly monitoring locations. Sites C4, C6, and SD5 had intermittent flow during the study (Aug-Sept 2011). See Table 1 for further details on each sampling site.

Sanitary infrastructure integrity (H2) was evaluated with an intensive 30-hour study of bacterial water quality and a simultaneous rhodamine dye test of the local collection systems owned by State Parks and Orange County. We assessed the movement of fresh groundwater to the ocean by measuring water level and salinity in a 4-inch slotted PVC well installed mid-beach. The beach face was also examined for freshwater seeps during the lowest tides. The flow rate of one seep was measured with a handmade flowmeter[6] 36 hours before the study began. At the start of the 30-hour study, approximately 2 L of rhodamine WT dye was flushed into 6 toilets adjacent to the beach within a half-hour. Subsequently, 12 sites (including ocean, surface water and groundwater) were sampled every 2 to 3 hours, 14 times each (Figure 3). These sites included two transects through the berm (lagoon -> berm well -> ocean) as illustrated in Figure 3. Samples were processed for microbial analyses (as above) within 6 hours of sample collection. Rhodamine samples were transported on ice to the laboratory, stored at 4°C in the dark for up to 2 weeks, and fluorescence measured with a Turner 10-AU fluorometer at 570 nm. Salinity, temperature, turbidity, and pH were also measured in all samples.

[6] Modeled after http://edis.ifas.ufl.edu/pdffiles/SG/SG06000.pdf

Table 1. Detailed description of sampling sites; see also Figures 2 and 3.

Site ID	Site Type	Study Phase(s)	Latitude	Longitude	Site Description	Notes
C1	Creek	Weekly, 30-hr	-117.6891067	33.46183962	North Creek, where it ponds closest to the ocean	
C2	Creek	Weekly, 30-hr	-117.6834516	33.46140243	SJC lagoon, near the middle of the berm	
C3	Creek	30-hr	-117.6825095	33.461421	SJC lagoon, at the berm near the southern bank	
C4	Creek	Weekly	-117.6818303	33.46741378	SJC upstream of the sewer main and SOCWA	
C5	Creek	Weekly	-117.6659322	33.4907318	Trabuco Creek, upstream SJC tributary	
C6	Creek	Weekly	-117.6599373	33.49326054	SJC, main fork above Trabuco creek	
SD2	Drain	Weekly	-117.6822238	33.46539578		Monitored by OCPW, HF183 found in our recon sampling
SD3	Drain	Weekly	-117.6771357	33.48337008		Monitored by OCPW, historically high FIB
SD4	Drain	Weekly	-117.6753309	33.48458204		Monitored by OCPW, HF183 found in our recon sampling
SD5	Drain	Weekly	-117.6721322	33.48586164		Monitored by OCPW, historically high FIB
GW1	Ground-water	30-hr	-117.6893835	33.4614896	Mid-beach between C1 and O1	Likely contains subsurface flows that drain to the ocean
GW2	Ground-water	30-hr	-117.6835258	33.46118955	In the berm, midpoint between C2 and O2	
GW3	Ground-water	30-hr	-117.6825106	33.46122778	In the berm, midpoint between C3 and O3	
O1	Surf zone	Weekly, 30-hr	-117.6894603	33.46115713	Near breakwater at North Creek outlet	Frequently high FIB levels
O2	Surf zone	Weekly, 30-hr	-117.6836227	33.46089239	Mid-berm	
O3	Surf zone	30-hr	-117.6825308	33.46096667	In line with south bank of SJC	
O4	Surf zone	Weekly	-117.673363	33.45776996	Reference beach site	Usually no FIB
R1	Surf zone	30-hr	-117.6882826	33.46129505	Midway between O1 & the north end of the berm	Rhodamine only
R2	Surf zone	30-hr	-117.6847792	33.46115378	At the north end of the berm by old concrete piling	Rhodamine only
R3	Surf zone	30-hr	-117.6814188	33.46109225	Across from 1st lifeguard tower south of SJC, adjacent to campground	Rhodamine only

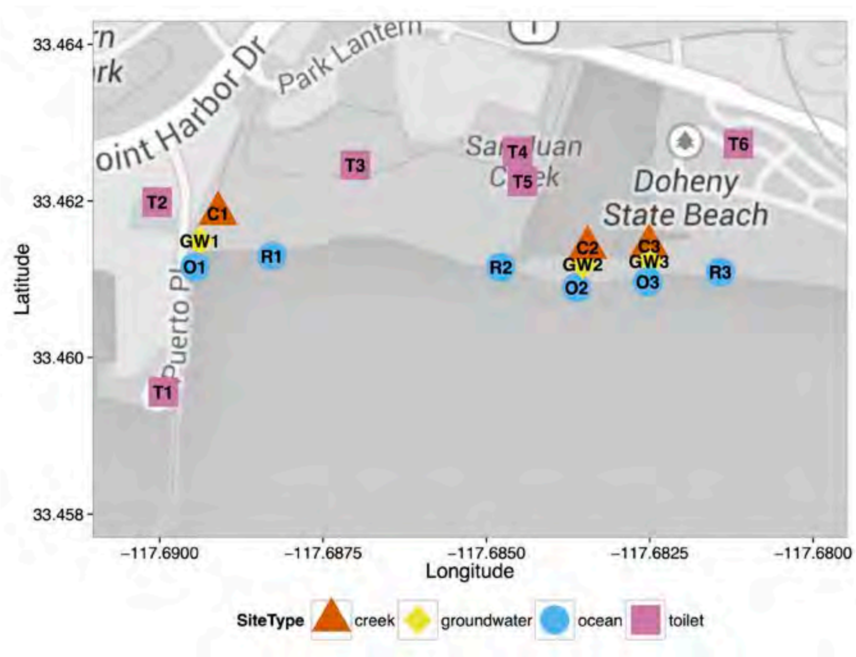

Figure 3. 30-hour study sites. Pink squares indicate the toilets that received a 2.25 L pulse of rhodamine; yellow diamonds indicate groundwater wells, which were monitored during the first 30 hours only; ocean sites labeled "R" were tested for rhodamine only (no water quality measurements). See Table 1 for further details on each sampling site.

The contribution of avian wildlife (H3) was evaluated by comparing weekly bird counts to FIB levels in the lagoon, characterizing the fecal bacteria of this population, and estimating FIB fluxes from birds to the lagoon. We performed visual counts of birds present within 150 feet of the creek termini and ocean sites in conjunction with weekly sampling. Flux estimates based on bird counts were used to approximate FIB loading from avian wildlife and compared to observed FIB counts in the lagoon.

Revised Hypotheses

Due to restrictions on heavy equipment on the beach, we were unable to install the berm wells deep enough to obtain sufficient pore water during the 30-hour study. As a result, only 1 or 2 samples were collected from each berm well and the data relevant to H1.2 are anecdotal at best. Due to the very dry winters of 2012 and 2013, H1.3 was not tested and remains an open question for future study. Strong evidence that the sanitary collection system in the park was leaking precluded the use of microbial community analysis methods, so per the recommendations developed in the Source Identification Protocol Manual, H3.2 was not evaluated. Further, while our initial hypotheses were developed with the intent of measuring human viruses, the poor performance of viral markers in the SIPP Method Evaluation Study led to a revision of the hypotheses to include bacteria (general fecal and human-associated) only.

Project Outcomes[7]

Our weekly sampling survey revealed chronic "hotspots" of microbial pollution affecting SJC (Figure 4). The high magnitude and frequency of human markers in storm drain effluent suggested the presence of sewage infiltration into storm drains; homeless encampments; and/or illegal cross-connections (Figure 5). While loading of FIB and markers from storm drain discharges was substantial, the intermittent

[7] For detailed analysis and discussion of project results, see Appendix A: Technical Report.

surface water flow in SJC indicates that FIB and human markers discharging from the upstream drains may be mitigated by natural infiltration in dry weather.

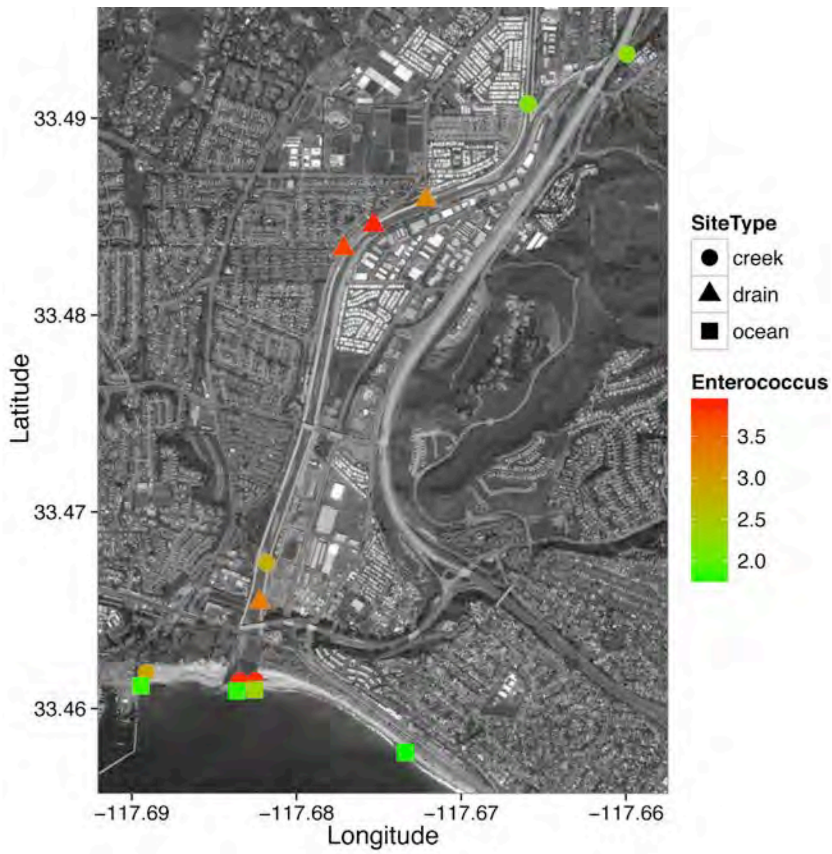

Figure 4. Map of *Enterococcus* log MPN per 100 ml, mean of all samples per site (including weekly monitoring and 30-hour study).

The dye test indicated that the integrity of the sanitary collection system within DSB was compromised. rhodamine was first detected in the ocean within 9 hours, and persisted for 7 days (Figure 6). The site with the highest signal during the 30-hour study (Site O3, Figure 7) is adjacent to the outfall noted in Figure 1; this subsurface infrastructure may be acting as a preferential flow path for groundwater discharging to the ocean.

Several lines of evidence indicate groundwater is discharging to the surf zone: salinity and water level in the mid-beach well changed dramatically with the tides; brackish seeps were observed in the beach face during low tides; and rhodamine was detected in the surf zone (Figure 7). This suggests that groundwater is a pathway for human sewage leaking from faulty infrastructure to reach the ocean. We hypothesize that this pathway was the likely source of human enteric viruses observed at Doheny in prior studies.[8]

[8] McQuaig, S., Griffith, J.F., 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.

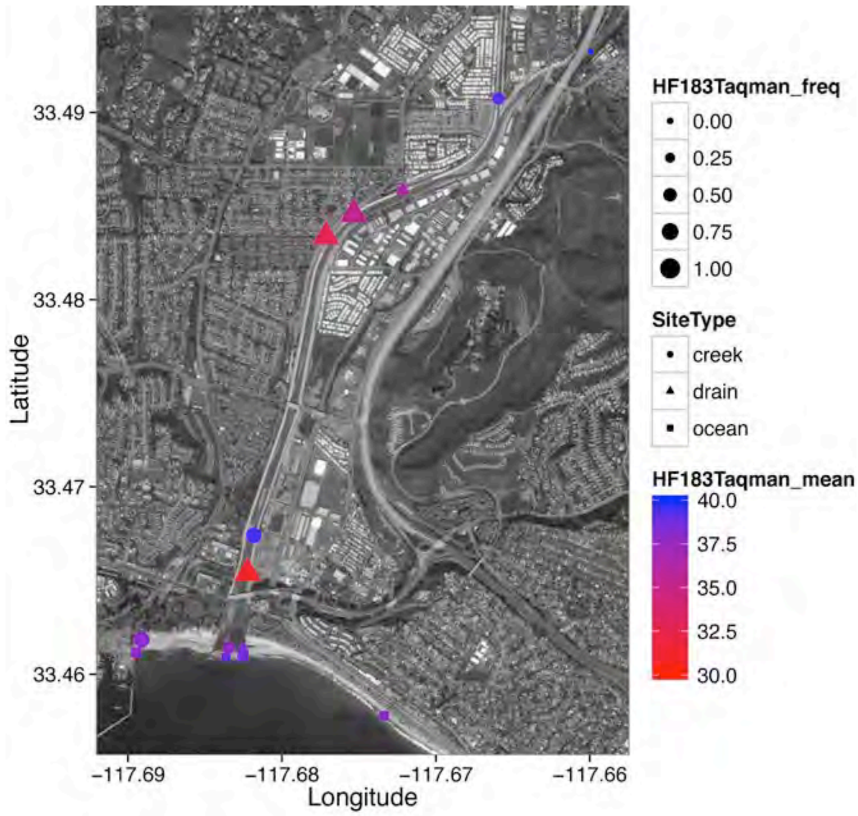

Figure 5. Map of HF183 Cq values, mean of all samples per site (including weekly monitoring and 30-hour study). A lower Cq value indicates a higher level of HF183 in the sample, and the size of the marker indicates the frequency of detection.

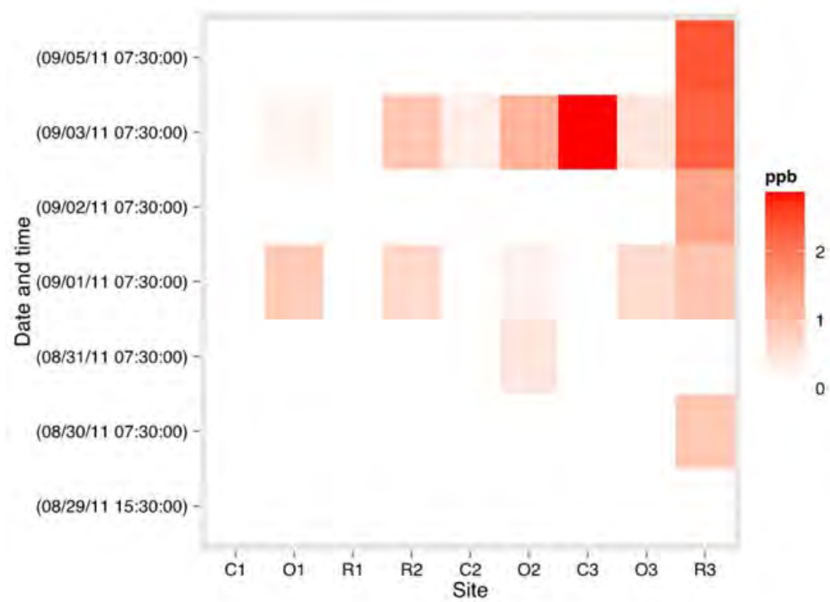

Figure 6. Rhodamine detection during the week following the 30-hr study; the units are parts per billion. Time increases moving up the y-axis. The sites on the x-axis are ordered from west to east.

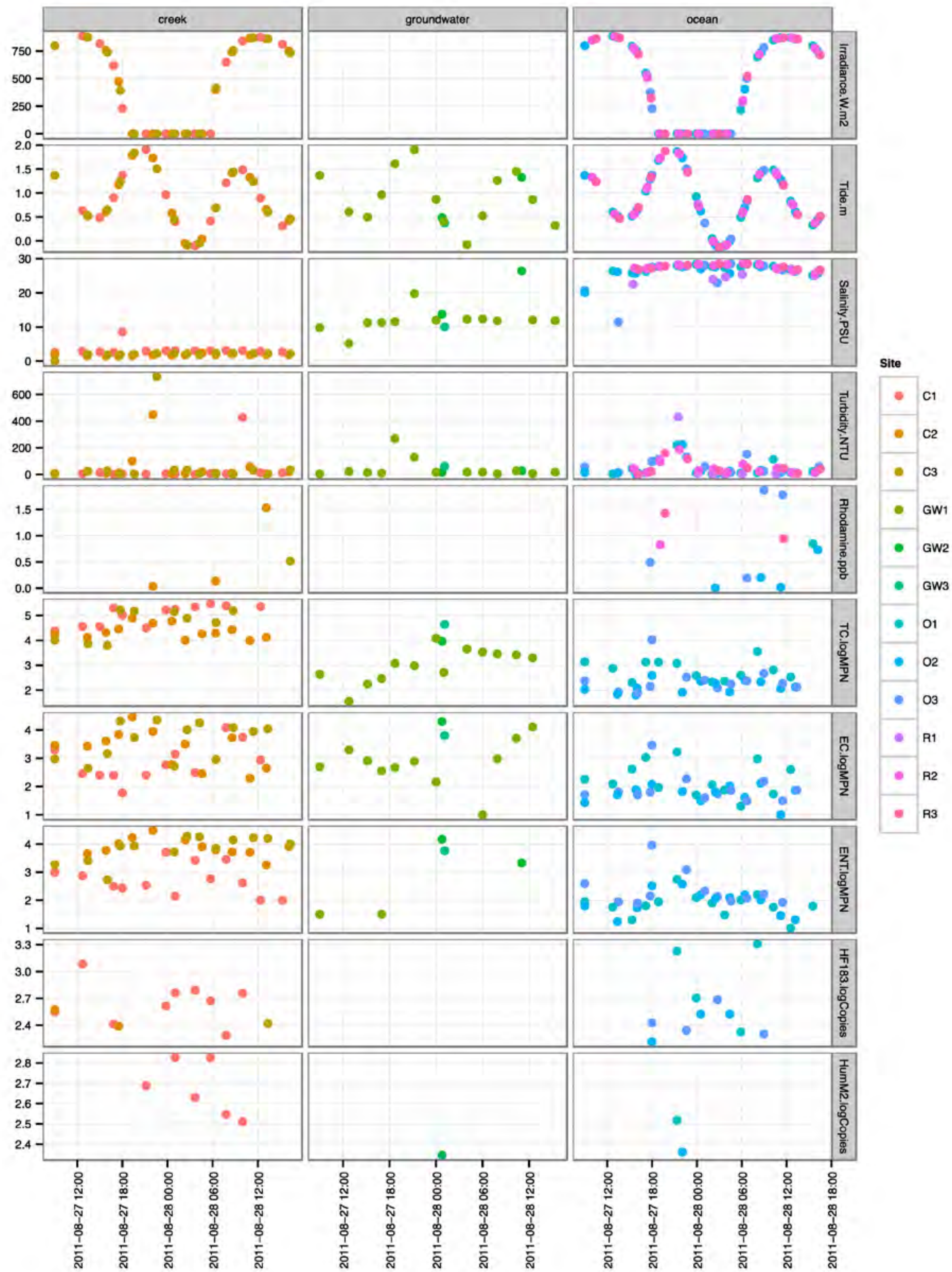

Figure 7. Time series of physical, chemical, and biological measurements during the 30-hour study. The creek sites are shown in the left column; groundwater sites in the center column; and ocean sites in the right column. Each row shows data for a different analyte, from top to bottom: irradiance (W/m^2), tide height (m), salinity (PSU), rhodamine (ppb), culturable *Enterococcus* (log MPN/100 ml), and HF183 human markers (log copies per 100 ml).

According to our flux estimates (see Appendix A), birds are likely the primary source of FIB to the lagoon. Bird fecal bacteria may also impact the surf zone via thru-berm transport, beach deposits washed into the ocean by waves, and direct deposit to water. Several observations suggest that through-berm transport of FIB may occur: 1) our few berm pore water samples were high in FIB; 2) strong correlations of FIB levels among the lagoon and adjacent ocean sites suggest the berm is "leaky"; and 3) the substrate uncovered during berm well installation was cobble and very coarse sands, which provide poor entrainment of bacteria. Last, strong correlations among FIB, tide height, and turbidity in ocean samples suggest a beach-based source of FIB (see Appendix A). Our field observations indicate that the beach source may be bird droppings deposited on sand and wrack.

Lessons Learned

- An obvious FIB source (gull population) can mask an underlying sanitary infrastructure problem
- rhodamine dye testing coupled with fluorometric detection is an inexpensive and effective method of detecting infrastructure leaks
- Stakeholder engagement and communication before, during and following completion of the study is key to gaining valuable information that informs study results and in spurring subsequent stakeholder action to address problems related to degraded sanitary sewer infrastructure.

Next Steps

Orange County Public Works and the City of Dana Point are presently investigating and remediating the sources of human fecal material to drain LO1SO2 (SD2 in our study). Similarly, the City of San Juan Capistrano is investigating the infrastructure relevant to the upstream storm drains that had high levels of human fecal material (Sites SD3, SD4, SD5).

Orange County Public Works conducted a separate dye test of their sanitary system adjacent to DSB, and found no evidence of leaks.

Under contract to California State Parks (State Parks), South Coast Water District (SCWD) performed CCTV inspections of the sanitary sewer infrastructure in DSB and found numerous defects. Accordingly, State Parks is currently repairing the gravity collection system and plans to test the system again once repairs are complete. In addition, State Parks is moving forward with overdue repairs to a major force main that runs along the northern boundary of DSB Park.

Appendix A: Technical Report

Use of dye tracers and qPCR to identify human fecal contamination at Doheny State Beach, Dana Point, CA

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Southern California Coastal Water Research Project Authority

Executive Summary

Doheny State Beach (Dana Point, CA) has a history of chronically poor microbial water quality, increased swimmer illness and the presence of human-associated bacterial and viral markers. Here we conduct a phased, tiered MST approach to investigate three potential fecal contamination sources during dry weather: urban runoff discharges to adjacent San Juan Creek, potential leaks in sanitary infrastructure, and avian wildlife. The contribution of urban runoff was evaluated by measuring weekly fluxes of FIB and human-associated qPCR markers at various inputs to lower San Juan Creek and the beach. Sanitary infrastructure was evaluated with an intensive 30-hour study of bacterial water quality and a simultaneous rhodamine dye test of the local collection system. The contribution of avian wildlife was evaluated by comparing weekly bird counts to FIB levels in the lagoon, characterizing the fecal bacteria of this population and estimating fluxes of FIB from birds to the lagoon. While upstream storm drain outlets consistently contained high levels of FIB and human markers (4.42 ±2.20 log HF183 copies/second/drain), this source was unlikely to make significant contributions to the problems at the beach because creek flow was intermittent and did not reach the beach during most of the study period. In contrast, leaking sanitary lines were clearly a contributor as fluorometric measurement of beach and lagoon water samples after rhodamine introduction to the nearby sanitary collection system revealed pervasive diffuse leaks. Birds in the lagoon were found to be a primary source of FIB to the lagoon, and possibly to the surf zone via through-berm transport and beach deposits washed into the ocean by waves. Several observations suggest that through-berm transport of FIB is occurring: 1) berm pore water samples were high in FIB; 2) the berm substrate is cobble and coarse sands, which provide for good transport of bacteria, and 3) there was a correlation between *Enterococcus* concentrations in the lagoon and the nearby ocean sampling site.

Introduction

When California beaches exceed water quality standards for fecal indicator bacteria (FIB), the bacterial sources are often unknown. FIB can originate from numerous sources, including human sewage; urban runoff; manure from livestock operations or wildlife; or even regrowth in biofilms and soils (e.g. Piggot et al., 2012). Effective management of bacterial water quality and mitigation requires knowledge of the (potentially different) source(s) of fecal contamination and of FIB.

Microbial source tracking (MST) methods can identify any FIB host sources, especially those that present the greatest human health risk. However, the performance of these methods can vary widely. To identify optimal MST methods for use in California, the State Water Resources Control Board funded a comprehensive study, known as the Source Identification Protocol Project (SIPP). The first phase of the SIPP demonstrated the ability of MST technologies to identify various types of feces and wastewaters from California (Boehm et al., 2013). Researchers concluded that MST technologies are ready for routine, standardized deployment in California coastal watersheds. The next step in this deployment was to conduct field case studies using the top-performing MST methods, in an effort to provide examples and guidance to future investigators. The present work describes one such case study.

Recently, a major epidemiological study identified a relationship between health risk and swimming at Doheny State Beach in Dana Point, CA (Colford et al., 2012). Concurrent water quality analyses revealed the presence of human bacterial and viral DNA markers (Love et al., 2014; McQuaig et al., 2012). Further, researchers observed a correlative relationship between FIB and adverse health effects in swimmers when the adjacent San Juan Creek was connected to the ocean, which suggested the etiologic agents of illness are transported via San Juan Creek. The remaining unknowns were the exact agents of disease, how those agents came to be in the creek (via wildlife, storm drains, etc.), and whether they originated from humans or animals. These questions motivated the present source tracking work.

The most basic question driving this study is: What are the sources of FIB and human fecal contamination at Doheny State Beach? We sought to answer this question by evaluating three potential contributors of fecal microbes: urban runoff, sanitary sewer infrastructure, and avian wildlife. These potential sources were evaluated by measuring FIB and human-associated qPCR markers in surface water, seawater, and groundwater over various temporal and spatial scales. Further, a dye tracer (rhodamine WT) was used to test the integrity of local sewer and sanitary infrastructure. Flux calculations were used to compare contributions from multiple sources.

Methods

Field Site

Doheny State Beach is located in the city of Dana Point, in southern Orange County, CA. The beach is adjacent to Dana Point Harbor and spans the outlet of San Juan Creek. The creek originates in the Santa Ana Mountains and remains relatively natural in developed areas, with a rocky bottom and continuous, connected flows from upstream tributaries (Arroyo Trabuco and Oso Creek) in all but the driest months of the year. Of the 396 km^2 in the San Juan Creek watershed, 41% are undeveloped public lands; 20% are residential; 15% are agricultural and 13% are commercial (Orange County Public Works). Doheny State Beach Park comprises 0.25 km^2 (62 acres), including several day use areas and a small campground. Its sanitary infrastructure includes 1902 m of gravity collection lines and 207 m of pressurized lines (Figure 1).

Figure 1. GIS map of Doheny State Beach showing sewer and stormwater infrastructure. DP = Dana Point; SJC = San Juan Capistrano; SCWD = South Coast Water District.

Approach
The study was initiated by walking the watershed and meeting with stakeholders to identify the most likely contamination sources. The first possible source identified was contaminated surface water runoff. San Juan Creek receives urban runoff from a number of storm drains, many of which are diverted to wastewater treatment in the summer months. The drains that do flow year-round have historically high levels of FIB and/or human fecal DNA markers (unpublished data, Orange County Public Works). A second surface water source to the beach, North Creek, mainly carries nuisance flow from parking lots and irrigation. North Creek flows to the ocean in wet weather and terminates in a pond in summertime. In addition, homeless persons inhabit the area and practice open defecation near surface water, including North Creek and storm drains flowing to San Juan Creek.

The second possible source identified was nearby sanitary sewer infrastructure. The South Orange County Wastewater Authority (SOCWA) treatment plant is situated on San Juan Creek a few hundred yards inland, and its outfall pipe runs underneath the creek and out to sea. The secondary-treated effluent is discharged approximately two miles offshore. A large city sewer main runs north-to-south under San Juan Creek, near where the Pacific Coast Highway crosses the creek above ground. The adjacent Dana Point Harbor has several restrooms whose sewer lines run along the breakwater near the beach. Finally, the public day-use restrooms, lifeguard restrooms and campground restrooms at Doheny State Beach all share sewer lines that run near the beach and underneath San Juan Creek.

The third possible source was the local avian wildlife population. The drainage system forms a lagoon immediately upstream of its connection to the ocean and this lagoon is home to large numbers of gulls, pelicans, and other waterfowl. Feces from wildlife inhabiting the lagoon, beach, and nearshore water contribute large amounts of bacteria to the environment.

Several other possible sources were identified, such as boats or pump-out stations in Dana Point Harbor as well as sand and algal wrack. Sand and wrack may act as a reservoir of FIB and continually reseed the surf zone with each tidal cycle (Yamahara et al., 2009). However, consensus among stakeholders was that these sources were minor and were not investigated here.

After all of the potential sources were identified, three major hypotheses were developed: 1) urban runoff in the lower San Juan Creek watershed contributes fecal pollution to the creek terminus/lagoon; 2) sewage leaking from faulty infrastructure is transported to the surf zone via groundwater discharge; 3) waterfowl are the predominant source of FIB to the lagoon. The contribution of urban runoff was evaluated by measuring weekly fluxes of fluxes of FIB and human-associated qPCR markers at various inputs to lower San Juan Creek and the beach. Sanitary infrastructure was evaluated with an intensive 30-hour study of bacterial water quality and a simultaneous rhodamine dye test of the local collection system. The contribution of avian wildlife was evaluated by comparing weekly bird counts to FIB levels in the lagoon, characterizing the fecal bacteria of this population and estimating FIB fluxes from birds to the lagoon. Details about each of these elements are provided below.

Urban Runoff Assessment
We determined the contribution of FIB from various points in the watershed during the dry season by collecting water samples weekly at 12 targeted locations: 5 creek sites, 4 drain sites and 3 ocean (knee depth) sites (Figure 2). All sites were sampled at 6:00 a.m. weekly for 5 weeks (August 17-Sept 14, 2011). Each drain was flowing throughout the study except SD5, which was dry on two sampling dates. Salinity and temperature were measured in the field with a YSI sonde; pH and turbidity were measured in the laboratory. Field blank samples (dI water) were carried during each sampling event and processed

identically to environmental samples. Microbial analyses of the samples were both culture-based (EPA 1600, IDEXX Enterolert and Colilert) and molecular (HF183 Taqman and HumM2 qPCR assays); see analysis-specific sections below. FIB and marker fluxes were calculated based on flow rates (measured with an electronic flow meter) for flowing drains and at the upstream creek sites (C4 - C6).

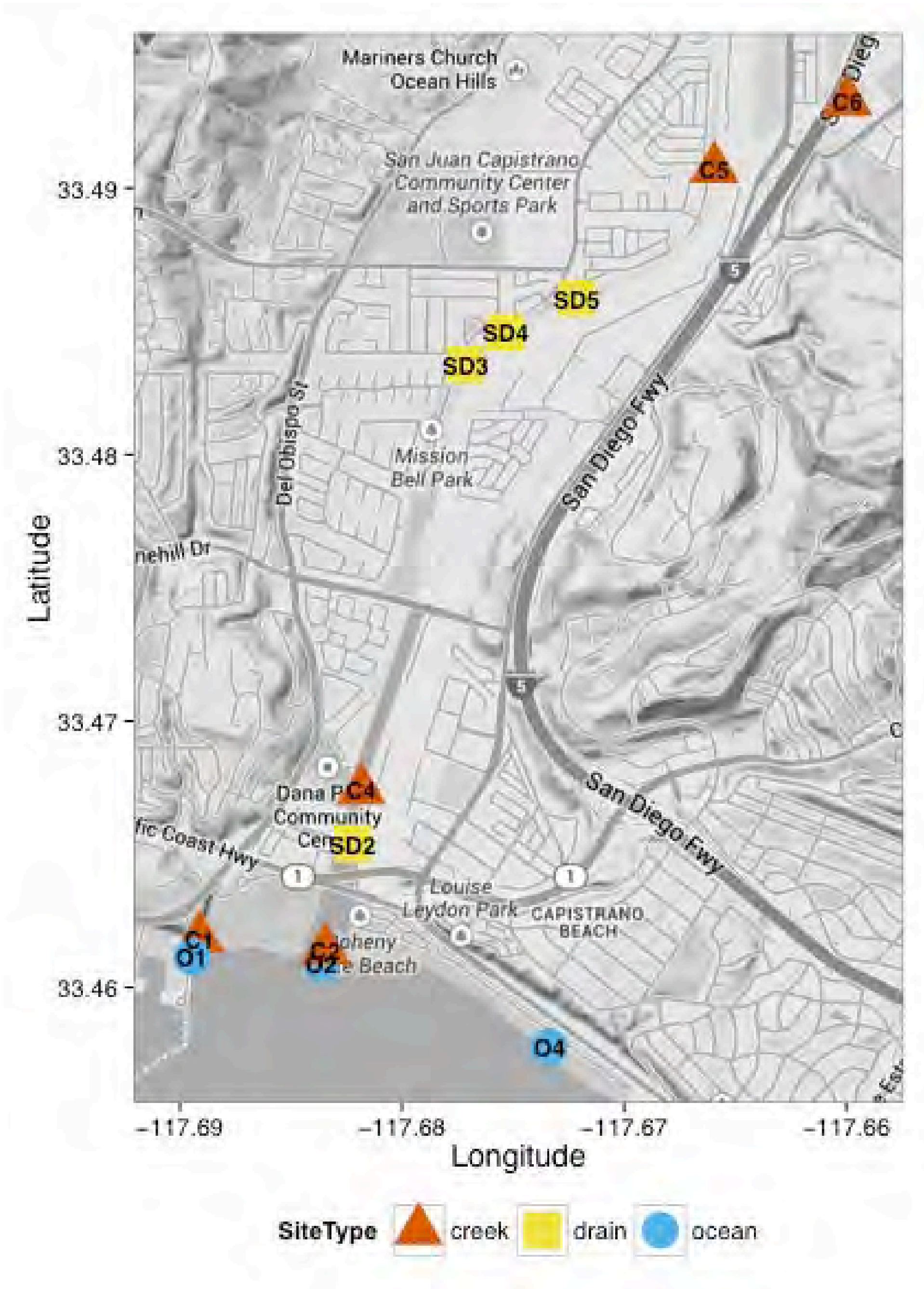

Figure 2. Urban runoff weekly monitoring Sites. C4, C6, and SD5 had intermittent flow during the study (Aug-Sept 2011).

Sanitary System Source Evaluation
We tested the integrity of the sewer infrastructure adjacent to the beach during a short-term intensive sampling period over a spring tide event. This 30-hour study began at 09:00 a.m. on 27 August 2011 and ended at 1:00 p.m. on 28 August 2011, encompassing 3 high and 3 low tides, including the peak tide range of the tidal cycle. Approximately 2.25 L of full-strength rhodamine WT was flushed down toilets in each of 6 restrooms adjacent to the beach ("T" sites in Figure 3). This rhodamine "pulse" to the sewer system occurred within a half-hour prior to start of sampling. Water was collected from 12 sites (Figure 3) every 2 to 3 hours for 30 hours (14 sampling events total). The creek and ocean sites were monitored daily for rhodamine for 7 days after the 30-hour study. Samples were processed as described below for FIB and qPCR within 6 hours of sample collection. Rhodamine samples were transported on ice to the laboratory, stored at 4°C in the dark for up to 2 weeks, and fluorescence measured with a Turner 10-AU fluorometer at 570 nm. qPCR was performed on a subset of the initial samples collected during the 30 hours of intensive sampling period as described below.

Figure 3. 30-hour study sites. Pink squares indicate the toilets that received a 2.25 L pulse of rhodamine; yellow diamonds indicate are groundwater wells, which were monitored during the first 30 hours only; ocean sites labeled "R" were tested for rhodamine only (no water quality measurements).

To assess the movement of fresh groundwater to the beach, we measured the water level and salinity in a 4-inch slotted PVC well installed mid-beach near the breakwater during the 30-hour study (Site GW1, Figure 3). The beach face was also examined for freshwater seeps during the lowest tides. The flow rate of one seep was measured with a handmade flow meter (modeled after http://edis.ifas.ufl.edu/pdffiles/SG/SG06000.pdf) approximately 36 hours before the study began. The study was conducted during the peak of a spring tide to take advantage of the maximum head difference between the beach aquifer and the sea.

Bird Population Assessment
Visual counts of birds present at the creek termini and nearby ocean sites were performed in conjunction with weekly sampling. The birds within 150 feet of each site were counted at the time of sampling, which began at 6:00 a.m. Correlation analysis was used to provide evidence of an association between bird counts and FIB. Flux calculations based on prior fecal characterizations were used to evaluate FIB loading to the lagoon from avian wildlife.

Through-berm transport could be an important pathway for bird fecal bacteria deposited in the lagoon to contaminate the surf zone. To address whether FIB are transported from the lagoon to the sea through the closed sand berm, two transects across the berm were sampled during the 30-hour study. Each transect consisted of three sites: creek, berm well, and surf zone (Figure 3). Water flow through the berm was observed by comparing water level, salinity and FIB across transects in context of tide height and direction.

Fecal Indicator Bacteria Analysis
Culture-based analyses occurred within 6 h of sample collection. Culturable *E. coli* were measured with IDEXX Colilert. Culturable enterococci were measured with EPA Method 1600 and IDEXX Enterolert. Only Enterolert data are presented here because the EPA Method 1600 data were frequently above the upper limit of detection (too numerous to count/TNTC). Two sample volumes (1 ml, 10 ml) were assayed in duplicate for all culture-based measurements.

Quantitative PCR
Up to 100 ml of sample was filtered onto Millipore HTTP 0.45 µm polycarbonate filters and flash-frozen in liquid nitrogen. Filters were stored at -80°C for up to 4 to 5 months. qPCR assays were performed on samples with >104 MPN *Enterococcus* per 100 ml. DNA was extracted from filters using Gene-Rite EZ kits and a BioSpec bead-beater. qPCR was performed the same day as DNA extraction for the human fecal markers HF183 Taqman (Haugland et al., 2010) and HumM2 (Shanks et al., 2010). DNA extracts were then frozen at -20°C for up 6 weeks before *Enterococcus* qPCR (EPA, 2010) was performed. The qPCR protocols were identical to the SIPP method evaluation study (Layton et al., 2013) except Environmental MasterMix (Applied Biosystems, Life Technologies, Carlsbad, CA) was used. The lower limit of quantification (LLOQ) was 10 cell (or genome) equivalents per Entero1A reaction, and 10 copies of linearized plasmid standard for both HF183 and HumM2 (Table 1). The qPCR data were handled with similar QA/QC procedures used by the core labs during the SIPP Method Evaluation study (Ebentier et al., 2013).

Table 1. Quantitative PCR assay parameters ("master" standard curves generated by combining standard curve data from each plate). %E = amplification efficiency; LLOQ = Lower Limit of Quantification; Cq = quantification cycle; cp/rxn = copies per reaction.

Assay	Slope	Y-intercept	R^2	%E	LLOQ (Cq)	LLOQ (cp/rxn)	% detect at LLOQ (n)
Entero1A (EPA, 2010)	-3.54	40.23	0.987	91.7%	36.37	10	100% (16)
HF183 (Haugland et al., 2010)	-3.40	41.12	0.992	96.7%	37.59	10	81% (16)
HumM2 (Shanks et al., 2009)	-3.34	41.38	0.990	99.4%	37.99	10	88% (16)

Data Analysis

Weekly data was analyzed spatially after taking the mean for each variable. The 30-h time series of FIB, salinity, well depth, rhodamine and qPCR data was analyzed for trends related to tide height and direction as well as sunlight. Six-minute tide data were obtained from NOAA, using Station ID: 9410230 (Scripps Pier in La Jolla, CA, roughly 89 km south of Doheny; http://tidesandcurrents.noaa.gov/waterlevels.html?id=9410230). Weather data, including sunlight irradiance, were obtained from a station at Loyola Marymount University, approximately 105 km north of Doheny (http://www.nrel.gov/midc/lmu/). Correlation among variables was assessed using Kendall's τ (chosen based on the nonparametric nature of the data and the presence of rank ties). Frequencies were compared among groups with Fisher's exact test. The flux of FIB and DNA markers from the upstream inputs was calculated from measured flow rates and microbial concentrations. The theoretical input of bird feces to the lagoon was estimated by assuming the volume of the lagoon as a triangular prism 175 m wide, 100 m long, and 1 m maximum depth. These values were estimated from satellite imagery of the site and field observations.

Results

Urban runoff

Storm drain discharges contained high levels of FIB and human fecal contamination. Overall, HF183 Taqman and HumM2 markers were detected in 89% and 61% of storm drain samples, respectively. Of those, 13% and 27% were below the limit of quantification (BLOQ). Marker and FIB fluxes were consistently high, while flow rates were small for both drain and creek sites (Table 2). HF183 Taqman and HumM2 magnitudes were highly correlated in drain samples (Kendall's τ = 0.56, p = 0.017). HF183 was related to *E. coli* (τ = 0.38, p = 0.04), but *Enterococcus* did not correlate with human markers. Several sites had intermittent flow during the study, resulting in small sample sizes: C4 (n = 3), C6 (n = 2), and SD5 (n = 3).

Table 2. Flow rates and microbial fluxes from storm drains and upstream San Juan Creek sites. Values are mean ± standard deviation. ND = not detected. When sites had inconsistent detection of the human markers, a value of 0.1 log copies/s was substituted for ND to calculate the mean.

Site	cfs	m^3/s	*E. coli* log MPN/s	*Enterococcus* log MPN/s	Total coliform log MPN/s	Entero1A log copies/s	HF183 Taqman log copies/s	HumM2 log copies/s
C4	0.45 ± 0.41	0.0126 ± 0.0116	3.95 ± 0.41	4.76 ± 0.57	6.1 ± 0.18	6.05 ± 0.39	2.74 ± 2.28	ND
C5	2.39 ± 0.54	0.0677 ± 0.0153	4.75 ± 0.15	5.01 ± 0.24	7.1 ± 0.11	6.68 ± 0.24	2.13 ± 2.78	ND
C6	0.52 ± 0.36	0.0149 ± 0.0102	4.09 ± 0.36	4.33 ± 0.14	6.08 ± 0.23	5.58 ± 0.68	ND	ND
SD2	0.2 ± 0.06	0.0057 ± 0.0016	5.24 ± 0.36	5.07 ± 0.39	6.78 ± 0.52	6.63 ± 0.35	6.52 ± 0.61	5.35 ± 0.51
SD3	0.05 ± 0.03	0.0014 ± 0.0009	4.07 ± 0.93	4.7 ± 0.77	5.38 ± 0	6.23 ± 0.63	5.49 ± 1.46	3.9 ± 2.42
SD4	0.03 ± 0.03	0.0008 ± 0.0007	3.88 ± 0.7	4.58 ± 1.17	5.75 ± 0.68	6.02 ± 0.66	4.22 ± 0.92	1.47 ± 1.89
SD5	0.03 ± 0.02	0.0009 ± 0.0006	3.49 ± 0.84	4.1 ± 0.46	5.44 ± 0.44	5.31 ± 0.51	1.44 ± 2.32	ND

Spatial analysis revealed that the drains and the lagoon were 'hotspots' of FIB over the study area (Figure 4). Similarly, the drain sites also had the highest magnitude and frequency of HF183 detection (Figure 5). North Creek (Site C1) also had relatively frequent, though low-level, detections of the human marker. Site C6 was the only site with a 0% frequency of HF183; however, Site C6 was sampled only twice due to dry stream flow conditions. Detection frequencies of HF183 were significantly different among sites (having at least 5 qPCR measurements; Fisher's exact test, p <0.001).

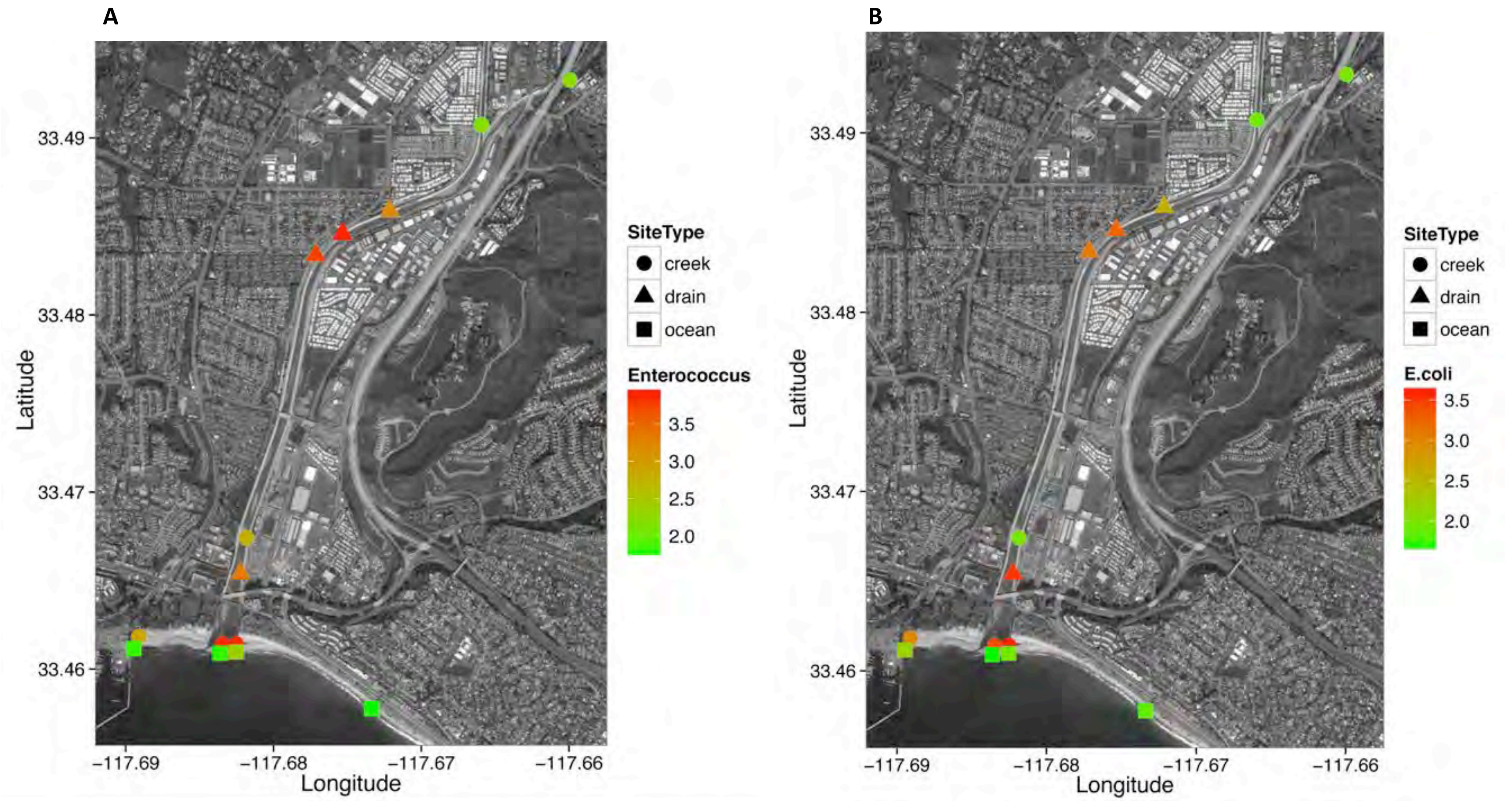

Figure 4. Maps of A) *Enterococcus* and B) *E. coli* MPN per 100 ml, log mean of all samples per site, including weekly and 30-hour monitoring; *n* values vary by site (range 5 - 26).

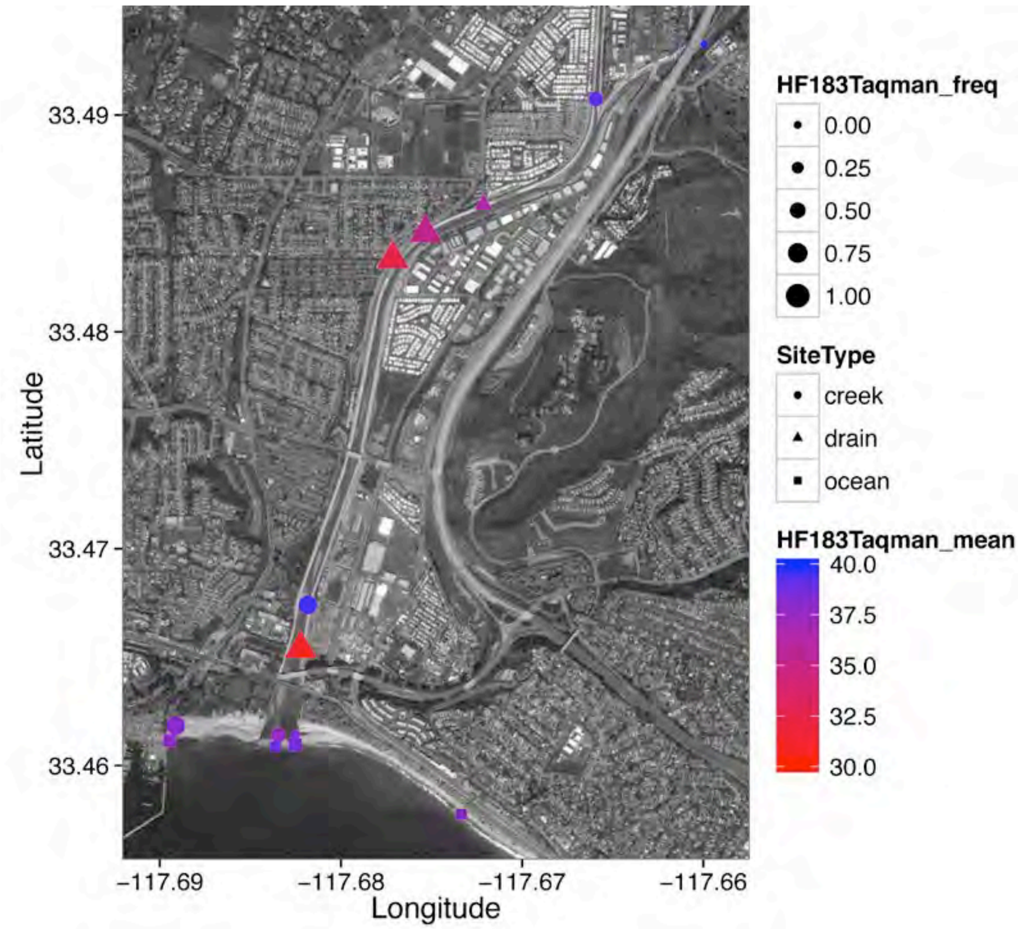

Figure 5. Map of HF183 Cq values, mean of all samples per site (includes weekly and 30-hour monitoring). The size of the marker indicates the frequency of detection. A lower Cq value indicates a higher level of HF183 in the sample.

Sanitary Infrastructure

Dye tracer results indicate the wastewater collection system near the beach was leaking. Rhodamine was first detected at Site O3 within 9 hours, and remained detectable for 7 days after the pulse was introduced (Figure 6). The dye was detected at low levels, with a maximum concentration of 2.8 ppb (Site C3). The highest frequency of rhodamine detection was observed at Sites O2 and R3 (63% of measurements, n = 20). Rhodamine concentrations did not correlate with microbial indicators, and rhodamine detection had no association with tide height.

Groundwater inputs to the surf zone were observed indirectly. Water level and salinity changes were observed in the mid-beach well (Site GW1, Figure 7; water level data not shown). In addition, a slight freshening at some ocean sites was observed at low tides. Brackish seeps in the beach face were observed at very low tides, with an estimated flow rate of 34.8 L/m^2/min.

Both the frequency and magnitude of positive human marker results in seawater are consistent with a diffuse source of human fecal material. The HF183 marker was detected in 54.2% of ocean samples measured with qPCR (n = 24); 85% of those detections were BLOQ. HumM2 was detected in 13% of ocean samples, and 100% of those detections were BLOQ. Detection of HF183 and rhodamine were not related (Fisher's exact test, p = 0.2).

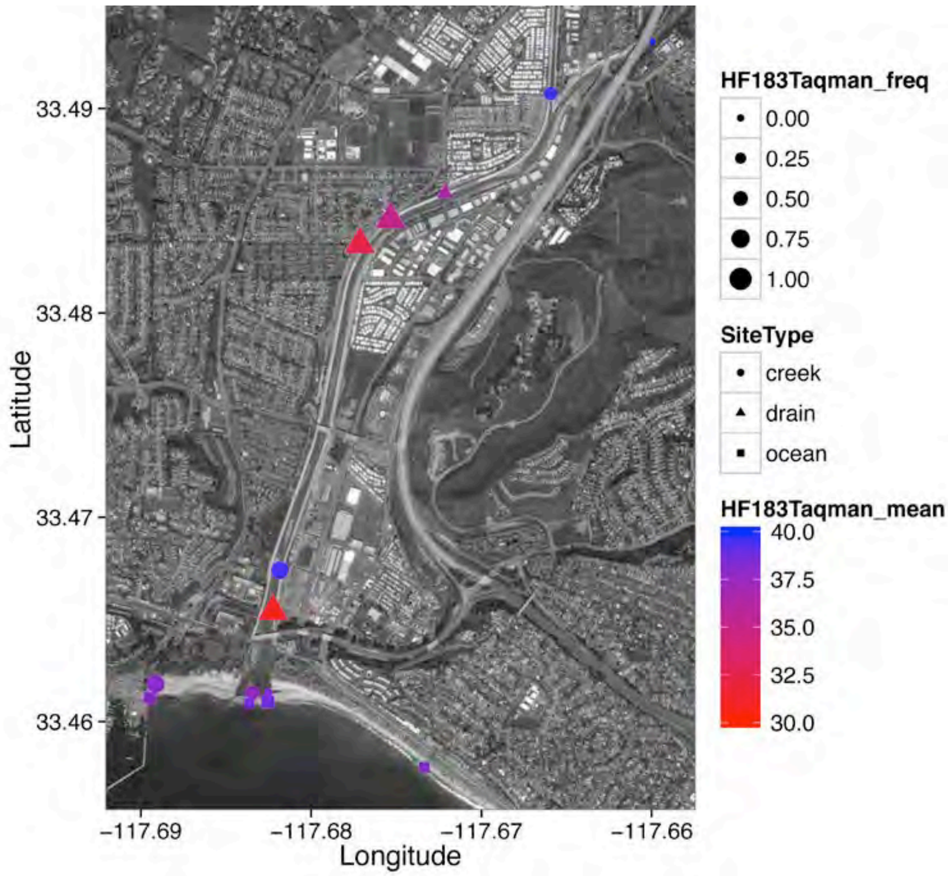

Figure 5. Map of HF183 Cq values, mean of all samples per site (includes weekly and 30-hour monitoring). The size of the marker indicates the frequency of detection. A lower Cq value indicates a higher level of HF183 in the sample.

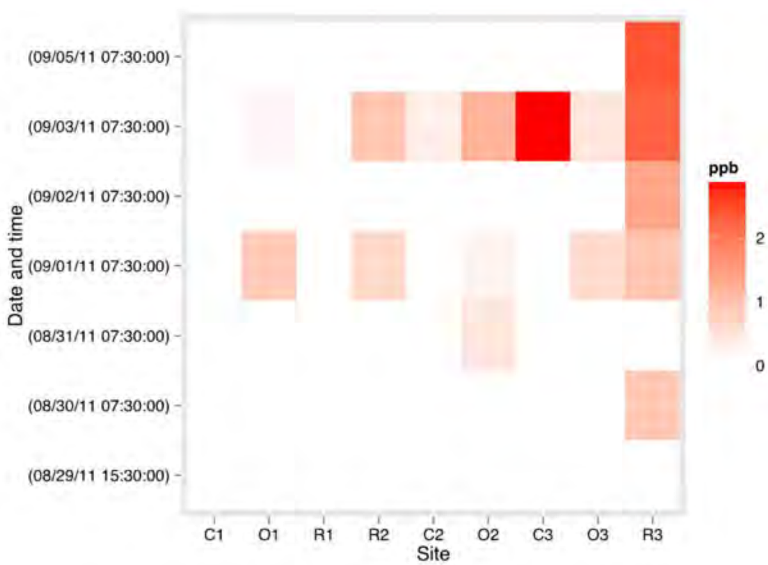

Figure 6. Rhodamine detection during the week following the 30-hr study. The order of the sites along the x-axis follows their location along the beach, west to east. Moving up the y-axis moves forward in time.

Avian Wildlife
Bird counts correlated significantly to *Enterococcus* (ENT) qPCR measurements, as well as culturable ENT and *E. coli* in creek samples (τ = 0.71, 0.70, and 0.62, respectively; p <0.001, n = 20). We performed a "back of the envelope" calculation to estimate whether the birds alone could be responsible for ENT levels in the lagoon. Using satellite measurements and field observations, we estimated the lagoon volume at 8750 m^3. The measured ENT concentrations were extrapolated (assuming the lagoon was well-mixed) to estimate the total number of ENT in the lagoon. Finally, we used published values of ENT/g of shorebird feces (Wright et al., 2009) to estimate the fecal input required per bird to provide the total ENT in the lagoon. These values ranged from 4.6 - 36.6 mg feces/bird (Table 3).

Table 3. Observed *Enterococcus* (ENT) and bird abundance in the lagoon (Site C2); estimated total ENT and mg of feces required per bird.

Site	Date and time	ENT log MPN/100 ml	Total ENT in lagoon	No. of birds	mg feces per bird
C2	08/17/11 06:00 AM	3.54	302000	200	4.6
C2	08/24/11 06:00 AM	3.72	454000	100	13.8
C2	08/31/11 06:00 AM	4.14	1210000	100	36.6
C2	09/08/11 06:00 AM	3.93	738000	120	18.6
C2	09/14/11 06:00 AM	3.36	203000	40	15.4

Through-berm transport would allow FIB from lagoon-dwelling birds to affect water quality in the surf zone. While only a few berm pore water samples were obtained due to low water levels in the wells, the brackish salinity of those samples indicates hydrologic connectivity between the lagoon and the ocean (Figure 7, Sites GW2 and GW3). The FIB levels in berm pore water samples were high, on the same order of magnitude as lagoon samples (~10^4 *E. coli* and ENT/100 ml; Figure 7). In addition, there was a strong correlation in ENT levels between Site C2 and ocean sites (τ range 0.5 - 0.69; all p <0.05).

Turbidity, tide height, and ENT (by culture and qPCR) were all significantly, positively correlated with one another in ocean samples (τ range 0.20 - 0.55, all p <0.05). While we did not specifically investigate sand and algal wrack as potential reservoirs of FIB, avian fecal deposits were observed on the sand at multiple sampling events, and juvenile herons were observed feeding in fresh wrack at low tide.

Discussion

Urban Runoff
Previous studies have documented the high levels of fecal microbes at the terminus of SJC and its relationship with surf zone water quality at DSB (Colford et al., 2012; McQuaig et al., 2012). We sought to characterize the bacterial sources affecting SJC in dry weather, and our weekly sampling survey revealed chronic "hotspots" of microbial pollution at storm drain discharges (Figures 4 and 5). The SJC sampling sites upstream of the storm drains had background FIB levels consistent with historical dry weather measurements in the upper SJC watershed (Tiefenthaler et al., 2009). The high magnitude and frequency of human markers in storm drain effluent suggests sewage infiltration into storm drains, homeless encampments, and/or illegal cross-connections. These findings are consistent with previous work in Southern California: Sercu et al. found high levels of the HF183 human marker in storm drain discharges in Santa Barbara (Sercu et al., 2009). Storm drain contamination is not limited to this region, as researchers in Wisconsin and Australia have also found the HF183 marker widespread in stormwater outfalls (Sauer et al., 2011; Sidhu et al., 2013). With respect to San Juan Creek, Orange County Public Works and the City of Dana Point are presently investigating and remediating the sources of human

fecal material to Site SD2. Similarly, the City of San Juan Capistrano is investigating the infrastructure relevant to Sites SD3, SD4, and SD5.

While loading of FIB and human markers from storm drain discharges was substantial (Table 2), these discharges were not the primary source of beach contamination. Surface water flow in San Juan Creek was intermittent during the study and did not reach the surf zone. More work is needed to establish whether microbial loading from storm drains could reach the beach during wet weather.

Sanitary Infrastructure

The rhodamine dye test indicated that the local sewage collection system integrity was compromised. The ocean site with the strongest rhodamine signal during the 30-hour study was also the first site where rhodamine was detected (Site O3, Figure 7). This site corresponds to the location of a major stormwater discharge outfall pipe (Figure 1); this subsurface infrastructure could be acting as a preferential pathway for groundwater flow to the surf zone. The detection of rhodamine for 7 days (Figure 6) could be due to the longevity of rhodamine in the environment (Smart and Laidlaw, 1977), entrainment in the surf zone, a continuous slow leak of dye from the restrooms' wet wells, or a combination of those factors. Other researchers have also used rhodamine testing of infrastructure integrity to confirm the source of human markers (Sercu et al., 2011). As a result of the present work, California State Parks has begun repairing the gravity collection system at DSB, and plans to test the system again once repairs are complete.

Several lines of evidence indicate groundwater is discharging to the surf zone. Salinity and water level data from GW1 indicate subsurface tidal exchange near North Creek; brackish seeps were observed in the beach face during low tides; and rhodamine was detected in the surf zone. This suggests that groundwater could be a pathway for leaking sewage to reach the ocean, a phenomenon that has been observed elsewhere in Southern California (Boehm et al., 2003). We hypothesize that this pathway was the likely source of human enteric viruses observed at Doheny in prior studies (Love et al., 2014; McQuaig et al., 2012).

Avian Wildlife

Birds are likely a primary source of FIB to the lagoon, and possibly also the surf zone via through-berm transport and beach deposits washed into the ocean by waves. Several observations suggest that through-berm transport of FIB is occurring: 1) berm pore water samples were high in FIB; 2) strong correlations in ENT between Site C2 and ocean sites suggest C2 may be near a "leaky" section of the berm; and 3) the substrate uncovered during berm well installation was cobble and coarse sands, which provide poor entrainment of bacteria (Fontes et al., 1991; Gargiulo et al., 2007). Taken together, these observations suggest bacteria could be transported through the sand berm from the lagoon to the surf zone in dry weather. Indeed, previous studies indicate that FIB can readily mobilize through beach sands subjected to periodic (tidal) wetting (Russell et al., 2012; Yamahara et al., 2009).

The strong positive correlations among ENT, tide height, and turbidity observed in ocean samples suggest a beach-based source of FIB (such as sand or algal wrack). Our field observations indicate that the beach source may be bird droppings deposited on sand and wrack. However, we did not specifically investigate FIB loading from sand and wrack, which can act as FIB reservoirs in the environment (Halliday and Gast, 2011; Imamura et al., 2011; Russell et al., 2012). All of these lines of evidence suggest that the bird population is a major source of FIB to all compartments of the beach environment at Doheny; however, more advanced techniques such as microbial community analysis (Cao et al., 2013) would be required to confirm that the FIB are avian in origin.

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