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

Blythe A. Layton
Meredith R. Raith
John F. Griffith

Southern California Coastal Water Research Project

SCCWRP Technical Report 860

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

Blythe A. Layton, Meredith R. Raith, and John F. Griffith

Southern California Coastal Water Research Project Authority
Costa Mesa, CA

April 2015

Technical Report 860

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 microbial source tracking 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 fecal indicator bacteria and human-associated DNA 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.

ACKNOWLEDGEMENTS

Numerous stakeholders contributed to this project. We worked directly with Orange County Public Works and California State Parks to carry out the field portion of the study. The City of Dana Point, 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 consulted and asked to provide input to the study team. Finally, Doheny State Beach was a focus of the Bight '08 project's microbiology group, and we coordinated our efforts with theirs. 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.

TABLE OF CONTENTS

Executive Summary ii
Acknowledgements iii
Introduction 1
Methods 2
- Field Site 2
- Potential Sources 2
- Approach 3
- Urban Runoff Assessment 3
- Sanitary System Assessment 3
- Bird Population Assessment 4
- Fecal Indicator Bacteria Analysis 4
- Quantitative PCR 4
- Data Analysis 5

Results 6
- Urban Runoff 6
- Sanitary Infrastructure 6
- Avian Wildlife 6

Discussion 8
- Urban Runoff 8
- Sanitary Infrastructure 8
- Avian Wildlife 9

Tables 10
Figures 12
Literature Cited 20

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, soils, or decaying plant material (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 FIB host sources, especially those that present the greatest human health risk (Boehm et al. 2013). 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. This document 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 (McQuaig et al. 2012; Love et al. 2014). 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: (1) the exact agents of disease, (2) how those agents came to be in the creek (via wildlife, storm drains, etc.), and (3) whether they originated from humans or animals. These questions motivated the source tracking work described in this study.

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, which originates in the Santa Ana Mountains, has a rocky bottom even in developed stretches 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). A second surface water source to the beach, North Creek, drains approximately 150 acres of primary residential and light commercial complexes in the City of Dana Point. The majority of runoff from this subwatershed is treated by an ozone system. North Creek flows to the ocean in wet weather and terminates in a pond in summertime. 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).

Potential Sources

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 small portion of runoff entering North Creek does not go through the ozone treatment system and may carry nuisance flow from parking lots and irrigation. In addition, homeless persons inhabit the area and practice open defecation near surface water, including North Creek and within 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, at an annual average rate of 17.3 million gallons per day. 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; 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; these sources were not investigated here.

Approach

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, and (3) waterfowl are the predominant source of FIB to the lagoon. 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 via 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 06:00 weekly for 5 weeks (August 17-September 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 (sterile deionized 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 a Marsh McBirney Flowmate Model 2000 Portable Flowmeter) for flowing drains and at the upstream creek sites (C4-C6).

Sanitary System Assessment

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 on August 27, 2011, and ended at 15:00 on August 28, 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 ($1x10^9$ ppb) 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 2-3 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 and stored at 4ºC in the dark for up to 2 weeks; fluorescence was measured with a Turner 10-AU fluorometer at 570 nm. Relative fluorescence unit (RFU) measurements were converted to ppb

using a linear 4-point standard curve ranging from 5 to 100 ppb. Reference standard dilutions were based on the dye manufacturer's starting concentration of $1x10^9$ ppb. Samples with an RFU value below the y-intercept of the standard curve were considered non-detect. qPCR was performed on a subset of the initial samples collected during the 30-hour, intensive sampling period as described below.

To assess the movement of fresh groundwater to the beach, we measured the water level and salinity in a 4" 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 06:00. 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 hours of sample collection. Culturable *E. coli* were measured with IDEXX Colilert-18. 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 were filtered onto Millipore HTTP 0.45 µm polycarbonate filters and flash-frozen in liquid nitrogen. Filters were stored at -80ºC for up to 4-5 months. qPCR assays were performed on samples with >104 MPN *Enterococcus* (ENT) 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 -20ºC for up 6 weeks before ENT 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).

Data Analysis

Weekly data were analyzed spatially after taking the mean (arithmetic or log-mean, as appropriate) for each variable. The 30-hour 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 $\tau = 0.56$, $p = 0.017$). HF183 was related to *E. coli* ($\tau = 0.38$, $p = 0.04$), but ENT 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).

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, 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, $p < 0.001$).

Sanitary Infrastructure

Rhodamine dye tracer results indicated 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 dye 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. When tide height data were binned into rising and falling tides, Rhodamine detection was significantly associated with rising tides (Fisher's exact test, $p = 0.001$).

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 35 $L/m^2/min$.

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; 100% of those detections were BLOQ. Detection of HF183 and rhodamine were not related (Fisher's exact test, $p = 0.2$).

Avian Wildlife

Bird counts correlated significantly to ENT qPCR measurements, as well as culturable ENT and *E. coli* in creek samples ($\tau = 0.71$, 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 mg to 36.6 mg feces/bird (Table 3).

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 $p < 0.05$). When ocean ENT values were lagged one time point after C2, the correlation with O2 became even stronger: $\tau = 0.72$; $p = 0.006$.

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 Doheny State Beach (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 4-5). 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 lack of correlation between ENT and human markers suggests ENT may have a non-human origin in both drain and creek samples. The storm drain pipes and culverts where the effluents were sampled contained biofilms that could harbor ENT, as in Ferguson et al. (2011). The high magnitude and frequency of human markers in storm drain effluent suggest 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. (2009) found high levels of the HF183 human marker in storm drain discharges in Santa Barbara. 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 SD3, SD4, and SD5.

While loading of FIB and human markers from storm drain discharges was substantial, these discharges were not the primary source of beach contamination. Surface water flow in San Juan Creek was intermittent and did not reach the surf zone; the creek bed fully dried out between sites SD3 and C4 during the latter part of the study. More work is needed to establish the magnitude of microbial loading from stormwater runoff that reaches Doheny State Beach in 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). This site corresponds to the location of SOCWA's outfall pipe; thus, this subsurface infrastructure could be acting as a preferential pathway for groundwater flow to the surf zone. While the dye was visible at the local wastewater treatment plant within the same day it was introduced to the system (SOCWA, pers. comm.), previous work with an autonomous glider indicated the outfall plume is unlikely to reach the shore (Jones 2009). The detection of rhodamine for 7 days 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 Doheny State Beach, and plans to test the system again once repairs are complete.

Several lines of evidence indicate groundwater is discharging to the surf zone: (1) Salinity and water level data from GW1 indicate subsurface tidal exchange near North Creek, (2) brackish seeps were observed in the beach face during low tides, and (3) 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 (McQuaig et al. 2012; Love et al. 2014). In the present study, the lack of correlation between rhodamine and bacterial indicators is likely due to differing sources and transport mechanisms. FIB has many possible sources other than groundwater, and bacteria (e.g., *Bacteroides* containing the HF183 marker) could be effectively filtered out during travel through the subsurface, as observed previously at Stinson Beach, CA (de Sieyes et al. 2008). In contrast, dye (and potentially human viruses) would travel relatively easily; thus, we do not necessarily expect a correlative relationship between FIB and rhodamine.

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 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. Previous studies indicate that FIB can readily mobilize through surficial beach sands subjected to periodic (tidal) wetting (Yamahara et al. 2009; Russell et al. 2012), which supports the hypothesis that FIB could be transported through the berm during tidal changes in pore water level. Note that bacterial transport through the berm, which is relatively porous, is much more likely to occur than bacterial transport via groundwater, which travels through relatively dense soils and sediments. Future work at Doheny should include quantification of through-berm transport. The paucity of berm well samples in the present study precluded any mixing or transport calculations with this dataset. Regardless, the magnitude of FIB added to the surf zone via through-berm transport is potentially far overshadowed by beach-based sources.

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 (Imamura et al. 2011; Halliday and Gast 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.

TABLES

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)

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

Table 3. Observed *Enterococcus* (ENT) and bird abundance in the lagoon (site C2); estimated total *Enterococcus* 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

FIGURES

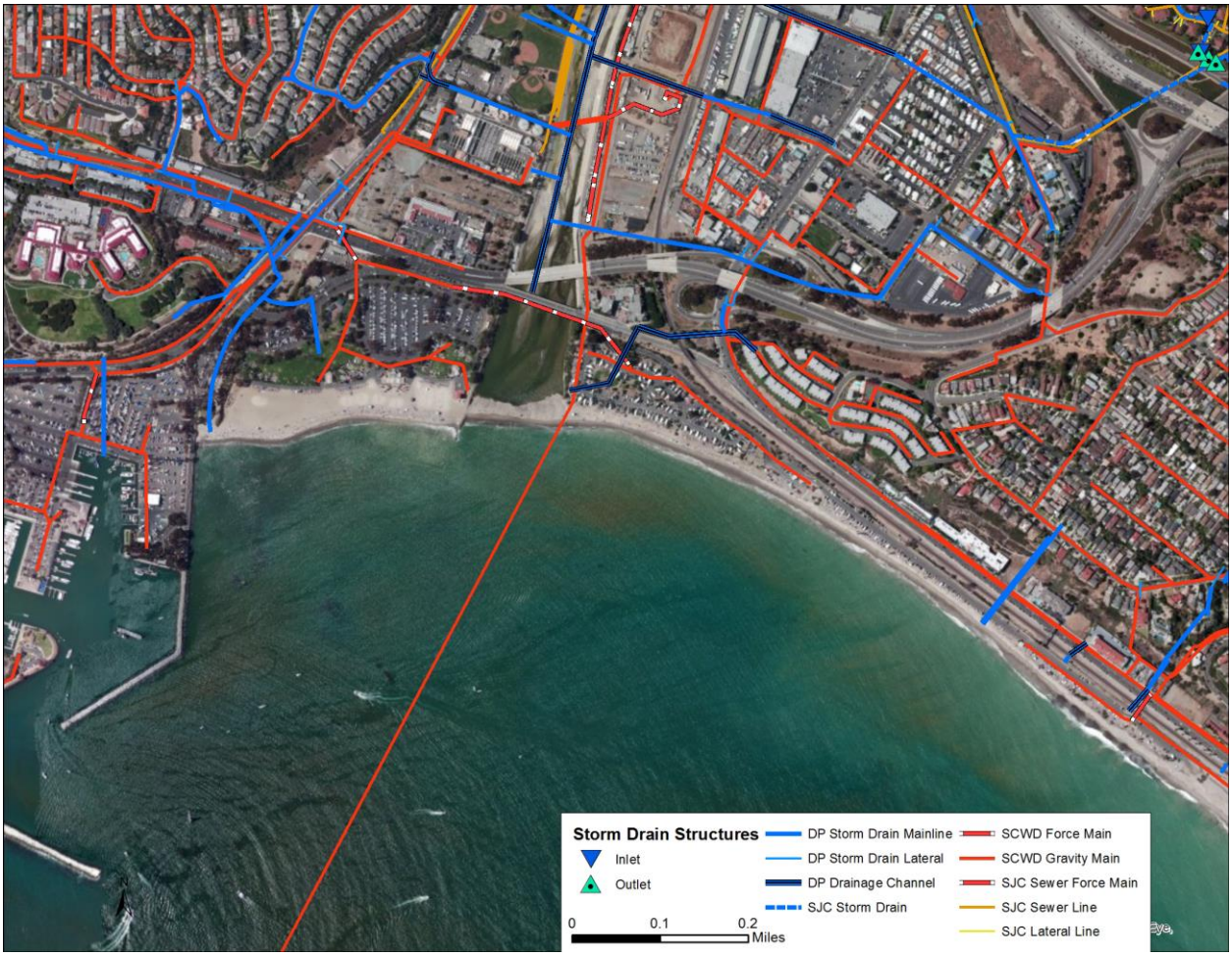

Figure 1. GIS map of Doheny State Beach showing sewer and stormwater infrastructure. DP = Dana Point; SJC = San Juan Capistrano; SCWD = South Coast Water District.

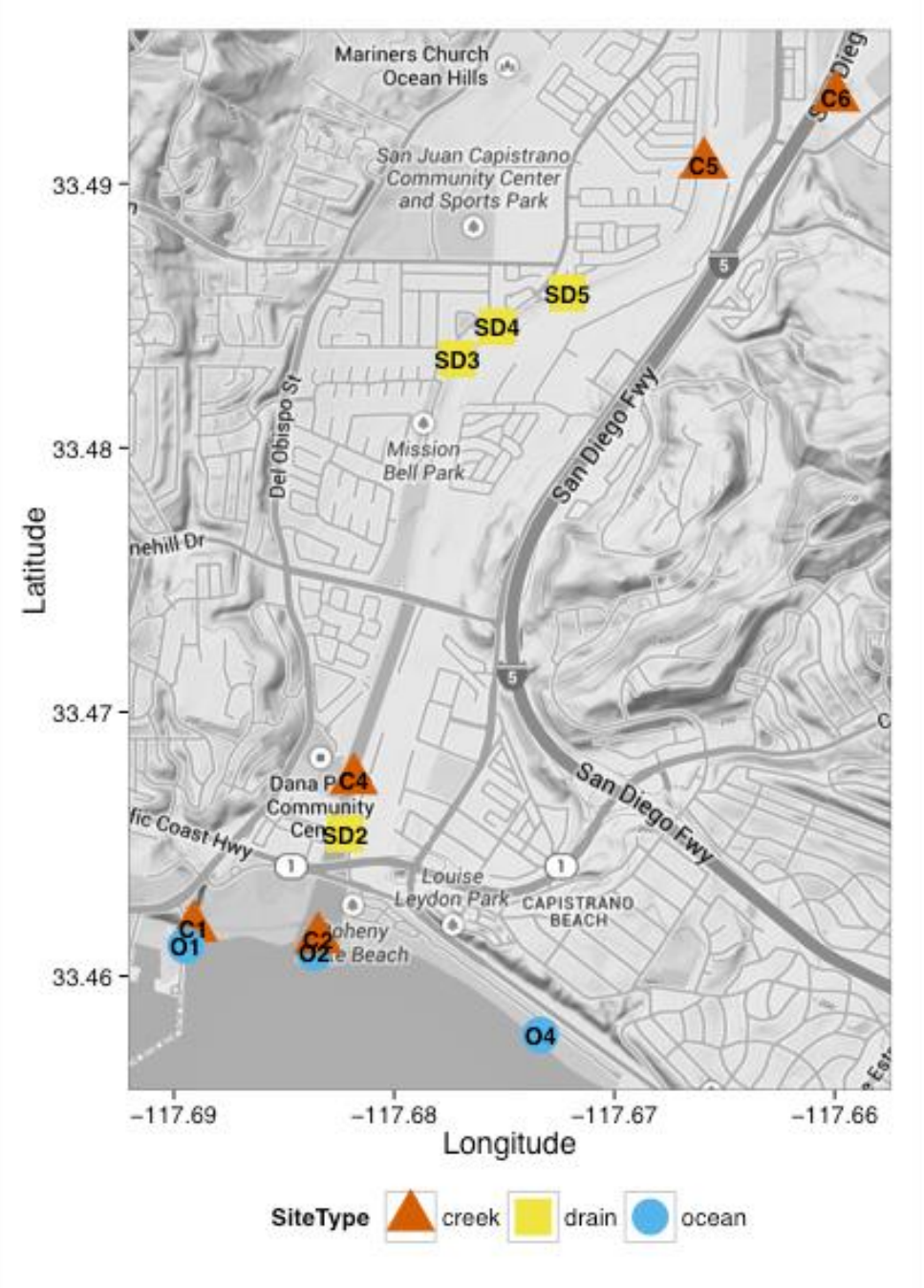

Figure 2. Urban runoff weekly monitoring sites. C4, C6, and SD5 had intermittent flow during the study (Aug.-Sept. 2011).

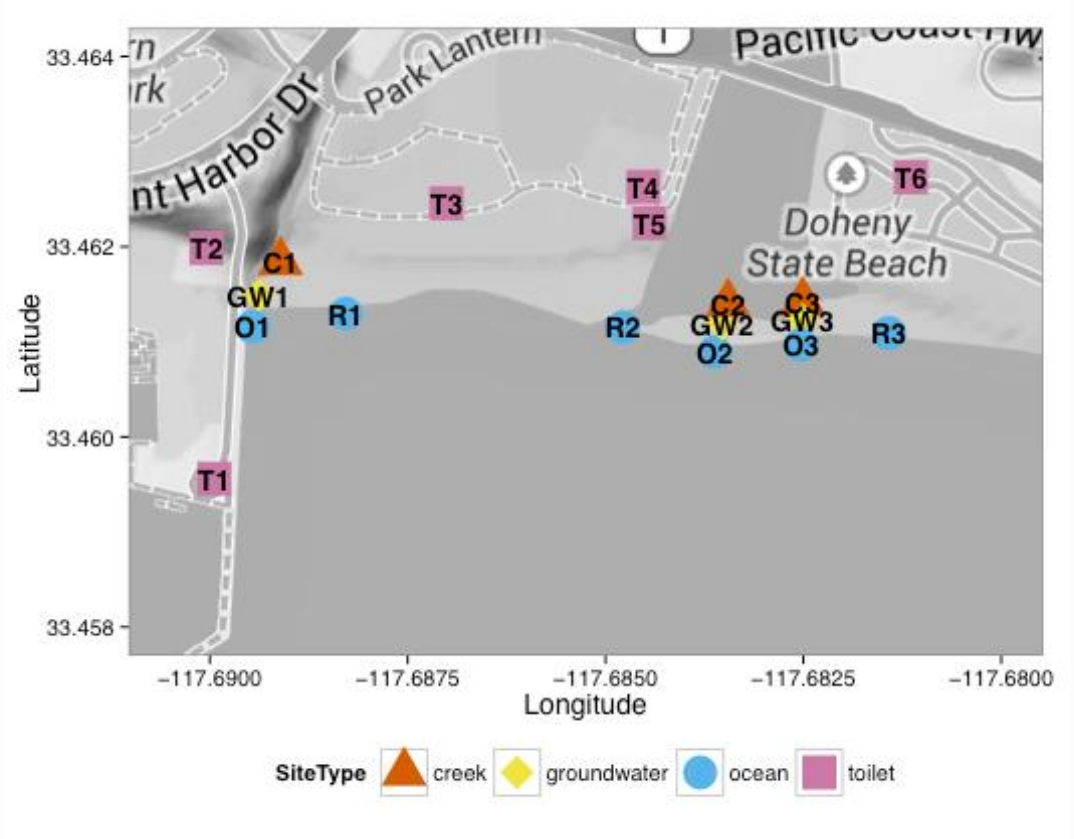

Figure 3. 30-hour study sites. Pink squares indicate the toilets that received a 2.25 L pulse of rhodamine; yellow diamonds indicate are groundwater wells, which were monitored during the first 30 h only; ocean sites labeled "R" were tested for rhodamine only (no water quality measurements).

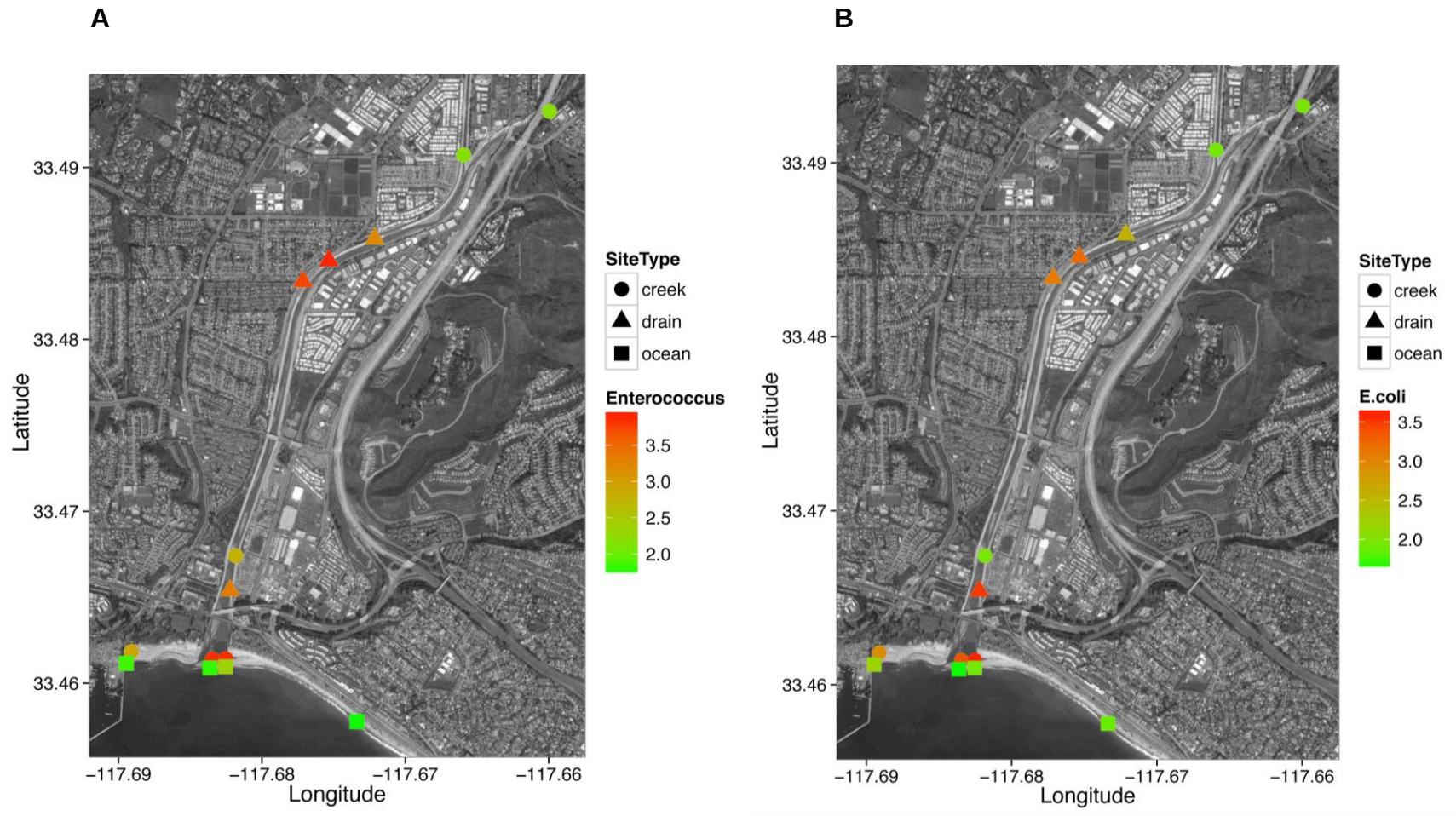

Figure 4. Maps of (A) *Enterococcus* and (B) *E. coli* MPN per 100 ml, log mean of all samples per site, including weekly and 30-hour monitoring; *n* values vary by site (range 5-26).

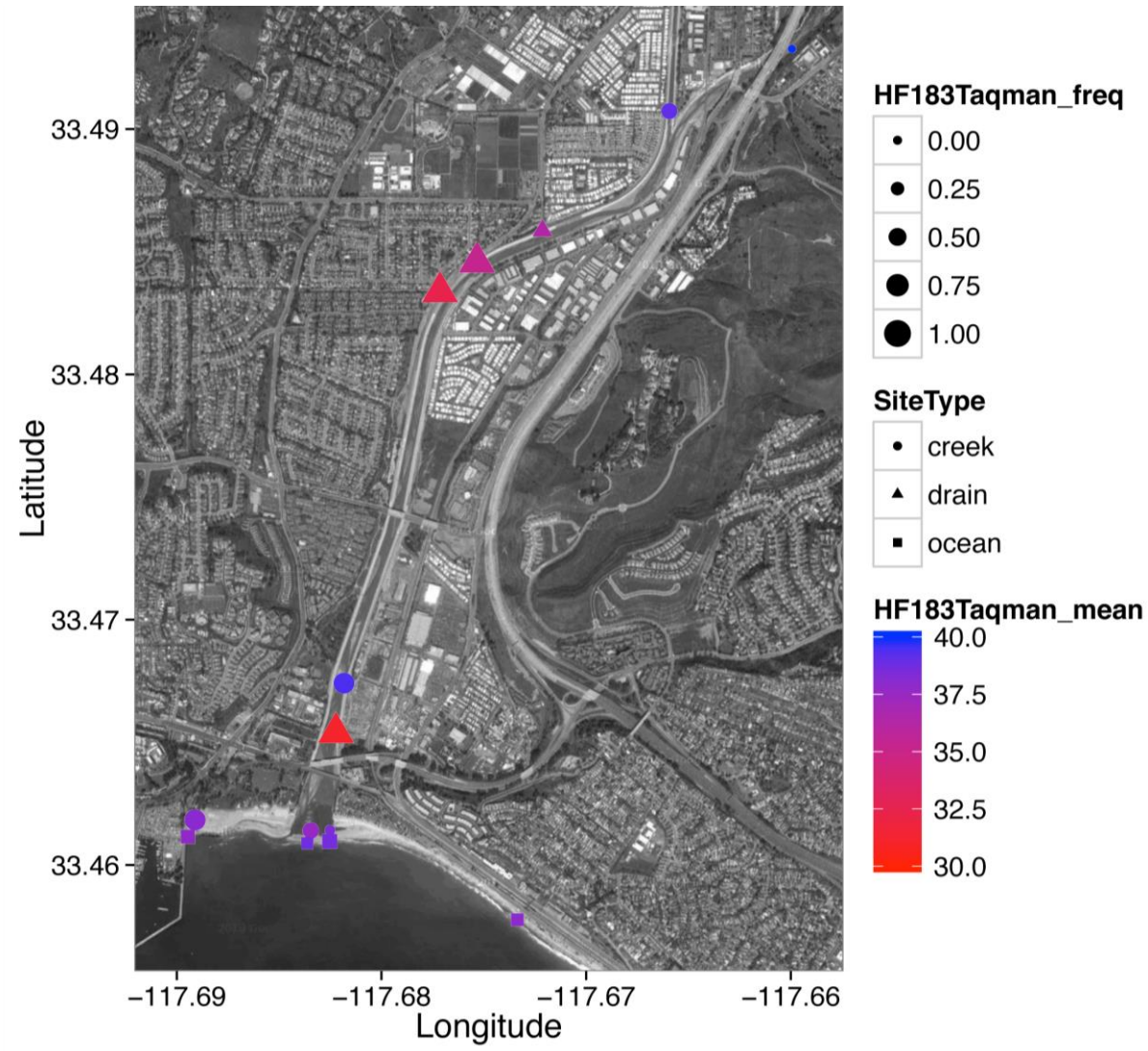

Figure 5. Map of HF183 Cq values, mean of all samples per site (includes weekly and 30-hour monitoring). The size of the marker indicates the frequency of detection. A lower Cq value indicates a higher level of HF183 in the sample.

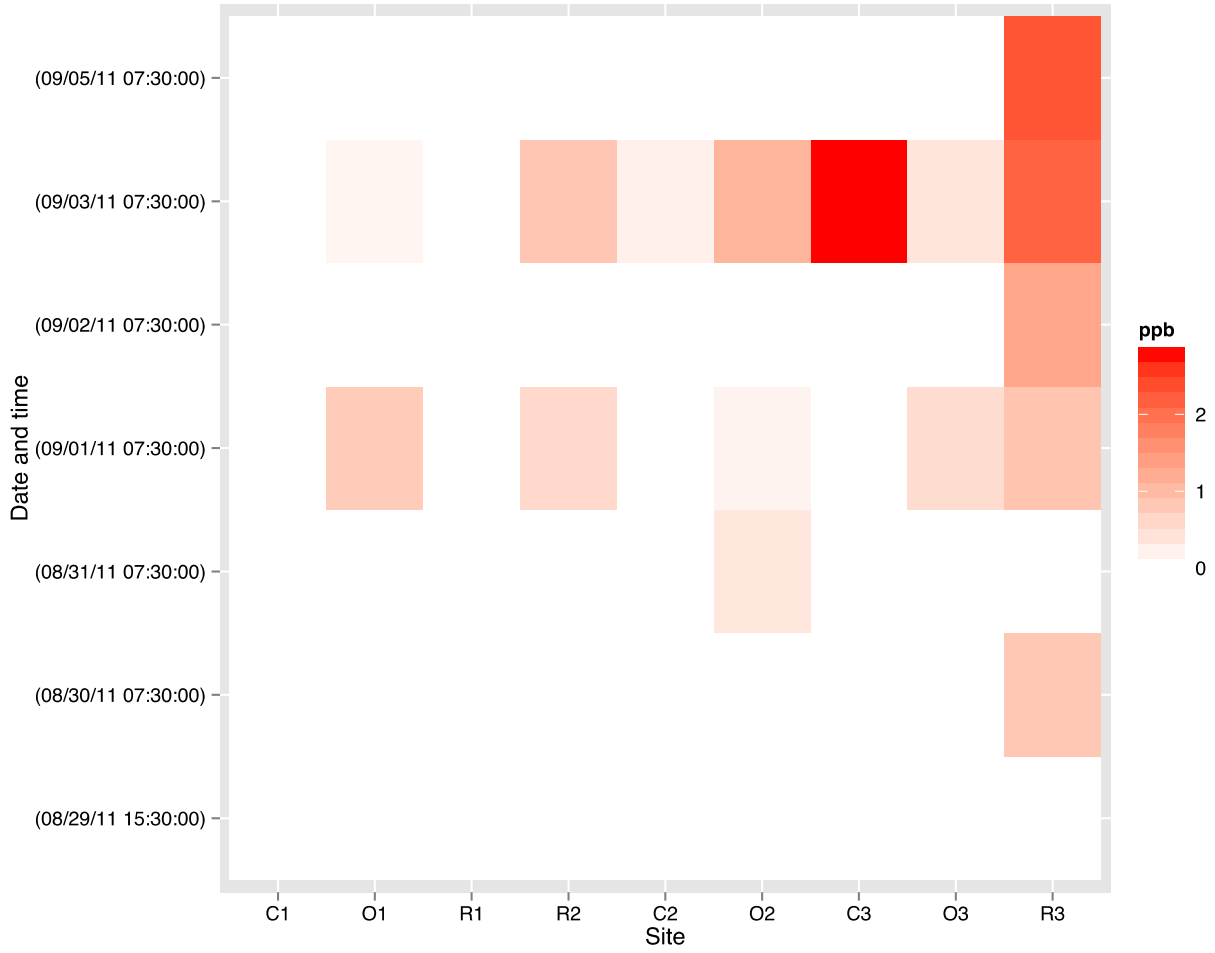

Figure 6. Rhodamine detection during the week following the 30-hour study. The order of the sites along the x-axis follows their location along the beach, west to east. Moving up the y-axis moves forward in time.

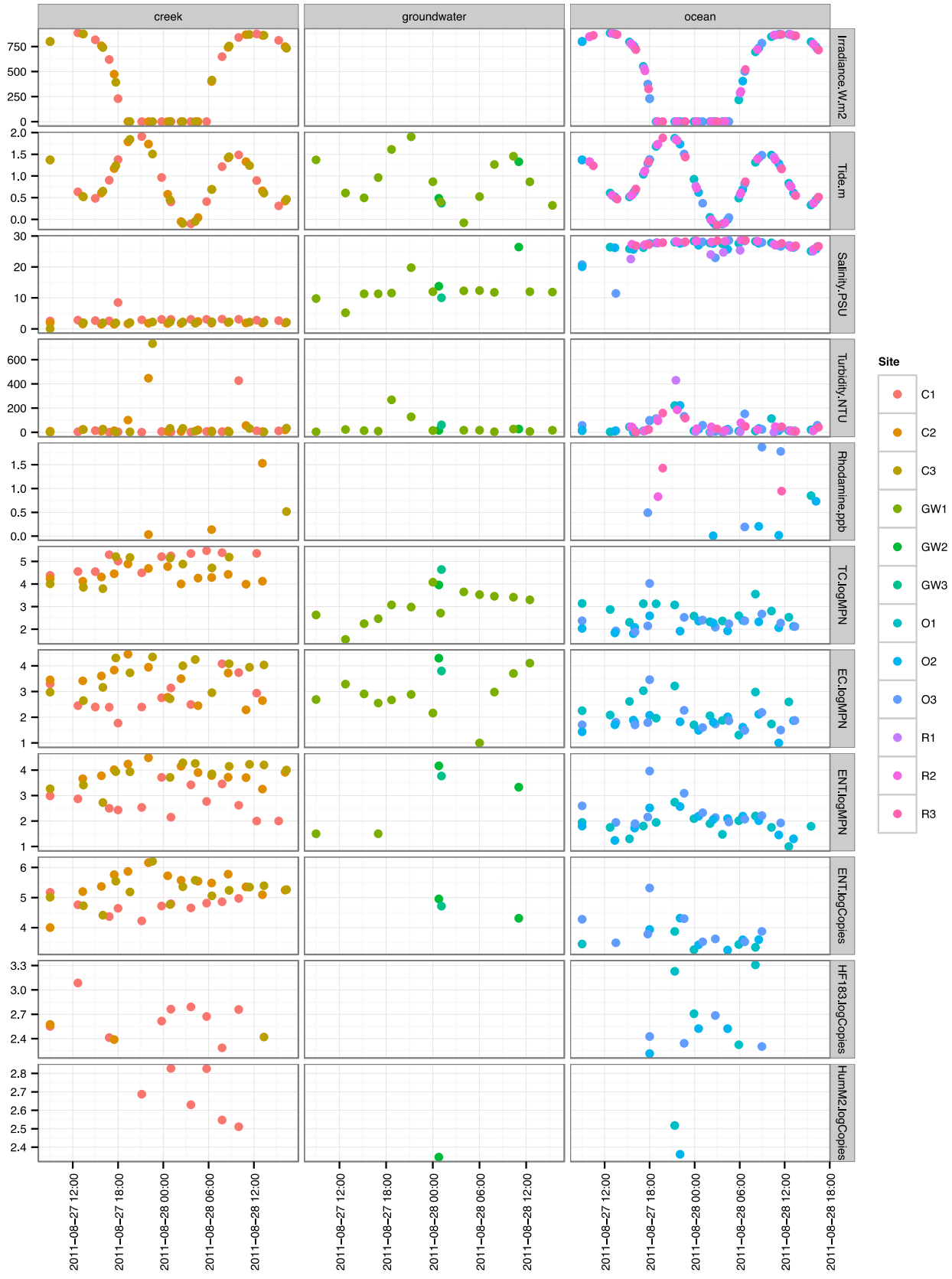
creek
groundwater
ocean
Irradiance.W.m2
Tide.m
Salinity.PSU
Turbidity.NTU
Rhodamine.ppb
TC.logMPN
EC.logMPN
ENT.logMPN
ENT.logCopies
HF183.logCopies
HumM2.logCopies
2011−08−27 12:00
2011−08−27 18:00
2011−08−28 00:00
2011−08−28 06:00
2011−08−28 12:00
2011−08−28 18:00
Site
C1
C2
C3
GW1
GW2
GW3
O1
O2
O3
R1
R2
R3

Figure 7 (previous page). 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), turbidity (NTU), rhodamine (ppb), culturable total coliform (log MPN/100 ml), culturable *E. coli* (log MPN/100 ml), culturable *Enterococcus* (log MPN/100 ml), *Enterococcus* by qPCR (log copies per 100 ml), HF183 human markers (log copies per 100 ml), and HumM2 human markers (log copies per 100 ml). (Note that the salinity meter was apparently not calibrated properly during the 30-hour study; hence, the rather low marine salinity measurements shown here. These values were inconsistent with measurements taken during weekly sampling when the meter was performing properly. However, the observed salinity trends are so informative with respect to groundwater movement that we chose to report the data as is, without any mathematical correction for the calibration bias.)

LITERATURE CITED

Boehm, A. B., J. A. Fuhrman, R. D. Mrše, and S. B. Grant. 2003. Tiered Approach for Identification of a Human Fecal Pollution Source at a Recreational Beach: Case Study at Avalon Bay, Catalina Island, California. *Environmental Science and Technology* 37:673–680.

Boehm, A. B., L. C. Van De Werfhorst, J. F. Griffith, P. A. Holden, J. A. Jay, O. C. Shanks, D. Wang, and S. B. Weisberg. 2013. Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. *Water Res* 47:6812–6828.

Cao, Y., L. C. Van De Werfhorst, E. A. Dubinsky, B. D. Badgley, M. J. Sadowsky, G. L. Andersen, J. F. Griffith, and P. A. Holden. 2013. Evaluation of molecular community analysis methods for discerning fecal sources and human waste. *Water Res* 47:6862–6872.

Colford, J. M., K. C. Schiff, J. F. Griffith, V. Yau, B. F. Arnold, C. C. Wright, J. S. Gruber, T. J. Wade, S. Burns, J. Hayes, C. McGee, M. Gold, Y. Cao, R. T. Noble, R. Haugland, and S. B. Weisberg. 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.

de Sieyes, N. R., K. M. Yamahara, B. A. Layton, E. Joyce, and A. B. Boehm. 2008. Submarine discharge of nutrient enriched fresh groundwater at Stinson Beach, California is enhanced during neap tides. *Limnology and Oceanography* 53:1434–1445.

Ebentier, D. L., K. T. Hanley, Y. Cao, B. D. Badgley, A. B. Boehm, J. S. Ervin, K. D. Goodwin, M. Gourmelon, J. F. Griffith, P. A. Holden, C. A. Kelty, S. Lozach, C. McGee, L. A. Peed, M. Raith, H. Ryu, M. J. Sadowsky, E. A. Scott, J. S. Domingo, A. Schriewer, C. D. Sinigalliano, O. C. Shanks, L. C. Van De Werfhorst, D. Wang, S. Wuertz, and J. A. Jay. 2013. Evaluation of the repeatability and reproducibility of a suite of qPCR-based microbial source tracking methods. *Water Res* 47:6839–6848.

EPA. 2010. Method A: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. U.S. Environmental Protection Agency Office of Water.

Ferguson, D. M., Y. Cao, and J. F. Griffith. 2011. Use of concrete coupons to assess attachment, growth and diversity of *Enterococcus* in storm drains. Poster presented at the General Meeting of the American Society for Microbiology.

Fontes, D. E., A. L. Mills, G. M. Hornberger, and J. S. Herman. 1991. Physical and chemical factors influencing transport of microorganisms through porous media. *Appl. Environ. Microbiol.* 57:2473–2481.

Gargiulo, G., S. Bradford, J. Simunek, P. Ustohal, H. Vereecken, and E. Klumpp. 2007. Bacteria transport and deposition under unsaturated conditions: The role of the matrix grain size and the bacteria surface protein. *Journal of Contaminant Hydrology* 92:255–273.

Halliday, E., and R. J. Gast. 2011. Bacteria in Beach Sands: An Emerging Challenge in Protecting Coastal Water Quality and Bather Health. *Environmental Science and Technology* 45:370–379.

Haugland, R. A., M. Varma, M. Sivaganesan, C. Kelty, L. Peed, and O. C. Shanks. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. *Systematic and Applied Microbiology* 33:348–357.

Imamura, G. J., R. S. Thompson, A. B. Boehm, and J. A. Jay. 2011. Wrack promotes the persistence of fecal indicator bacteria in marine sands and seawater. *FEMS Microbiology Ecology* 77:40–49.

Jones, B. 2009. Report to SOCWA on results of Glider Deployment from July-September 2008 to map and monitor the effluent plume from the San Juan Creek Ocean Outfall.

Layton, B. A., Y. Cao, D. L. Ebentier, K. Hanley, E. Ballesté, J. Brandão, M. Byappanahalli, R. Converse, A. H. Farnleitner, J. Gentry-Shields, M. L. Gidley, M. Gourmelon, C. S. Lee, J. Lee, S. Lozach, T. Madi, W. G. Meijer, R. Noble, L. Peed, G. H. Reischer, R. Rodrigues, J. B. Rose, A. Schriewer, C. Sinigalliano, S. Srinivasan, J. Stewart, L. C. Van De Werfhorst, D. Wang, R. L. Whitman, S. Wuertz, J. Jay, P. A. Holden, A. B. Boehm, O. C. Shanks, and J. F. Griffith. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Res* 47:6897–6908.

Love, D. C., R. A. Rodríguez, C. D. Gibbons, J. F. Griffith, Q. Yu, J. R. Stewart, and M. D. Sobsey. 2014. Human viruses and viral indicators in marine water at two recreational beaches in Southern California, USA. *Journal of Water and Health* 12:136–150.

McQuaig, S., J. F. Griffith, and V. J. Harwood. 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.

Piggot, A. M., J. S. Klaus, S. Johnson, M. C. Phillips, and H. M. Solo-Gabriele. 2012. Relationship between Enterococcal Levels and Sediment Biofilms at Recreational Beaches in South Florida. Appl. *Environ. Microbiol.* 78:5973–5982.

Russell, T. L., K. M. Yamahara, and A. B. Boehm. 2012. Mobilization and transport of naturally occurring enterococci in beach sands subject to transient infiltration of seawater. *Environ. Sci. Technol.* 46:5988–5996.

Sauer, E. P., J. L. VandeWalle, M. J. Bootsma, and S. L. McLellan. 2011. Detection of the human specific *Bacteroides* genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research* 45:4081–4091. Elsevier Ltd.

Sercu, B., L. C. V. D. Werfhorst, J. Murray, and P. A. Holden. 2009. Storm Drains are Sources of Human Fecal Pollution during Dry Weather in Three Urban Southern California Watersheds. *Environmental Science and Technology* 43:293–298.

Sercu, B., L. C. Van De Werfhorst, J. L. S. Murray, and P. A. Holden. 2011. Sewage Exfiltration As a Source of Storm Drain Contamination during Dry Weather in Urban Watersheds. *Environmental Science and Technology* 45:7151–7157.

Shanks, O. C., C. A. Kelty, M. Sivaganesan, M. Varma, and R. A. Haugland. 2009. Quantitative PCR for Genetic Markers of Human Fecal Pollution. *Appl. Environ. Microbiol.* 75:5507–5513.

Shanks, O. C., K. White, C. A. Kelty, M. Sivaganesan, J. Blannon, M. Meckes, M. Varma, and R. A. Haugland. 2010. Performance of PCR-Based Assays Targeting Bacteroidales Genetic Markers of Human Fecal Pollution in Sewage and Fecal Samples. *Environmental Science and Technology* 44:6281–6288.

Sidhu, J. P. S., W. Ahmed, W. Gernjak, R. Aryal, D. McCarthy, A. Palmer, P. Kolotelo, and S. Toze. 2013. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. *Sci. Total Environ.* 463-464:488–496.

Smart, P. L., and I. M. S. Laidlaw. 1977. An evaluation of some fluorescent dyes for water tracing. *Water Resources Research* 13:15–33.

Tiefenthaler, L. L., E. D. Stein, and G. S. Lyon. 2009. Fecal indicator bacteria (FIB) levels during dry weather from Southern California reference streams. *Environ Monit Assess* 155:477–492.

Wright, M. E., H. M. Solo-Gabriele, S. Elmir, and L. E. Fleming. 2009. Microbial load from animal feces at a recreational beach. *Marine Pollution Bulletin* 58:1649–1656.

Yamahara, K. M., S. P. Walters, and A. B. Boehm. 2009. Growth of Enterococci in Unaltered, Unseeded Beach Sands Subjected to Tidal Wetting. *Appl. Environ. Microbiol.* 75:1517–1524.