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Fecal Indicator Bacteria Levels Do Not Correspond with Incidence of Human-Associated HF183 *Bacteroides* 16S rRNA Genetic Marker in Two Urban Southern California Watersheds

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Abstract The variability of levels of fecal indicator bacteria (FIB) and a human-associated genetic marker (HF183) during wet and dry weather conditions was investigated at two urban coastal watersheds in Southern California: Santa Monica Canyon channel (SMC) and Ventura Harbor, Keys, and Marina. Seventy-eight to 86 % of the samples collected from SMC sites exceeded standard water quality standards for FIB (n=59 to 76). At SMC, HF183 was present in 58 % of the samples (n=78) and was detected at least once at every sample site. No individual site at SMC appeared as a hotspot for the measured indicators, pointing to a likely chronic issue stemming from urban runoff in wet and dry weather. In Ventura, the Arundell Barranca, which drains into Ventura Harbor and Marina, was a source of FIB, and HF183 was most frequently detected off of a dock in the Marina. Rainfall significantly increased FIB levels at both SMC and Ventura; only at Ventura did HF183 detection increase with wet weather. Sample locations that were high in FIB were geographically distinct from the sites that were high in HF183 in Ventura, which supports the importance of measuring host-associated parameters along with FIB in chronically impaired watersheds to guide water quality managers in pollution remediation efforts.

Keywords Fecal indicator bacteria · FIB · Tiered approach · Bacteroides · Water quality · Source tracking · HF183 · Sand

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1 Introduction

Tracking pollution sources at impaired beaches is critical to ensuring that urban beaches stay healthy. Currently, fecal indicator bacteria (FIB) are used globally to measure water quality and characterize general fecal contamination and have been linked to health risks in recent epidemiological studies (Heaney et al. 2012; Wade et al. 2010). However, FIB cannot be used to identify specific sources of fecal pollution in a watershed, as they can originate from both human and non-human sources (Ram et al. 2007) and have also been

shown to persist and regrow in the environment and sand (Mika et al. 2009; Imamura et al. 2011; Lee et al. 2006; Yamahara et al. 2009). Measuring host-associated fecal indicators can help characterize hazards at chronically impaired beaches and contribute to an effective mitigation strategy (Santo Domingo et al. 2007).

Molecular methods are often used to measure, detect, and quantify low levels of host-associated markers in the environment (Santo Domingo et al. 2007; Bernhard and Field 2000a; Layton et al. 2006; Seurinck et al. 2006). To identify human fecal pollution, many PCR and qPCR assays for the *Bacteroides-Prevotella* group have been developed (see, e.g., Converse et al. 2009; Kildare et al. 2007; Bernhard and Field 2000b).

The HF183 SYBR assay was used in this study, as it has been applied in diverse settings (see, e.g., Ahmed et al. 2009; Seurinck et al. 2005; Kirs et al. 2011), as well as in our study region (Izbicki et al. 2012; Walters et al. 2011; Sercu et al. 2011) to assess human fecal pollution. Further, this method has been shown to be highly sensitive, specific, and repeatable in a multilaboratory method evaluation study (Boehm et al. 2013; Layton et al. 2013; Ebentier et al. 2013).

Understanding links between FIB and host-associated markers may improve and advance the utility of these indicators in impaired watersheds (Drozd et al. 2013; Wilkes et al. 2013; Marti et al. 2013). For example, source-tracking techniques can inform remediation strategies by identifying pollution sources such as faulty sewer infrastructure (Korajkic et al. 2010; Dickerson et al. 2007) or agriculture (Hagedorn et al. 1999). However, it is important to continue characterizing relationships between FIB and human-associated markers such as HF183, as previous studies report that they are not always well-correlated (see, e.g., Reischer et al. 2008; Boehm et al. 2003; Flood et al. 2011).

This study investigated the temporal and spatial variability of standard FIB and a marker of human-associated pollution (HF183) in two chronically impaired, densely populated locations: Santa Monica Canyon (SMC), which has a creek discharging high levels of FIB to a popular state beach, and Ventura Harbor, Marina, and Keys, which receive water from the Arundell Barranca (AB) channel, which drains a mixed-use watershed that includes agriculture. Specific aims of the study were to characterize (1) the distribution and frequency of FIB; (2) the distribution and frequency of HF183 as well as potential locations for remediation; (3) the effect of rainfall on concentrations of FIB and HF183; and (4) the relationship between FIB and HF183.

2 Methodology

2.1 Site Background

This study focused on two locations in Southern California with water bodies that have been identified as impaired due to high levels of indicator bacteria. The first is Santa Monica Canyon (SMC), which is listed on the 303(d) list for impaired water bodies due to high levels of indicator bacteria (303(d) List/305(b) Report). Two channels comprise SMC in the study area (Fig. 1a). The West Channel (West) drains the Rustic and Sullivan Canyon watersheds, where it is in a natural state and becomes lined with concrete about one half mile upstream from its confluence with the eastern channel, Entrada Channel (Entrada). West and Entrada Channels merge and discharge through an open concrete channel at the southern end of Will Rogers State Beach. The combined watershed drains 10,147 acres, and dry weather flow is roughly 3.5 million gallons per day (Wilson 2001). During high-use season from April to October, dry weather flow is diverted to Hyperion sewage treatment plant to improve water quality at Will Rogers State Beach (Wilson 2001). SMC has many sources of human and nonhuman pollution to the watershed, including a golf course, horse ranch, waterfowl, pet waste, intermittently flowing storm drains, and onsite wastewater treatment systems.

The second study location is Ventura Harbor, Keys, and Marina. The Los Angeles Regional Water Quality Control Board (LARWQCB) has identified Harbor Cove beach (ENC), located near the harbor entrance, as impaired based on the elevated bacterial densities and has imposed total maximum daily loads (TMDLs) for enterococci (ENT) based on the current EPA water quality standards (WQS). Ventura Harbor is a popular tourist destination and is a mixed-use developed harbor consisting of a marina, a residential development called the Ventura Keys, and some residential parks and commercial areas. Ventura Harbor is protected by a breakwater perpendicular to the main entrance of the harbor as well as three jetties: one is north of the opening and two are south of the opening. Once inside, the harbor splits into two channels, which head south to the mixed-use Ventura Marina and north to branch out into the three individual

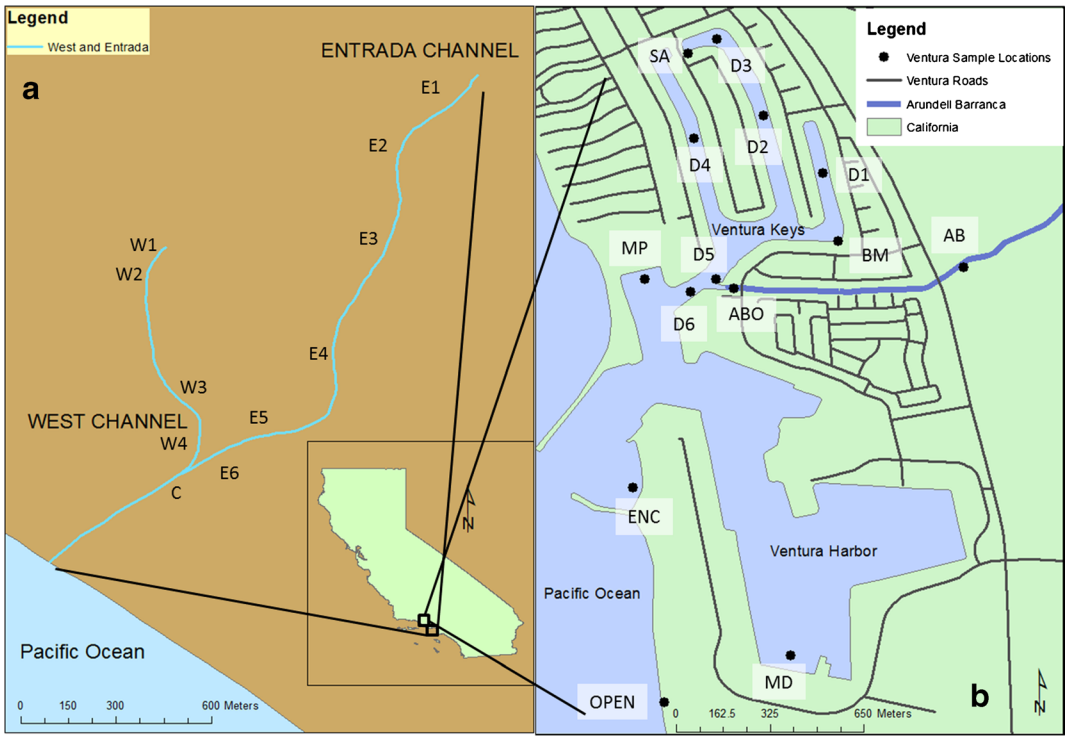

Fig. 1 **a** Map of sampling locations at Santa Monica Canyon. Sample locations in West Channel are W1-W4, in Entrada Channel are E1-E6, and at the Confluence is C. **b** Map of sampling locations at Ventura: D1-D6 = Dinghy Sample Sites in the Keys; AB = Arundell Barranca; ABO = Arundell Barranca Outlet MP = Marina Park; MD = Marina Dock; SA = Sailor, Keys Beach; BM = Beachmont, Keys Beach; ENC = Harbor Cove Beach; OPEN = Surfers' Knoll Beach

"fingers" of the Ventura Keys (Fig. 1b). The outlet for AB, which drains a watershed including agricultural, municipal, natural, and urban sources, is located near the entrance to the Ventura Keys residential development.

2.2 Sample Collection and Analysis

2.2.1 Collection

SMC Water samples (n=130) were collected on 20 days from January 2008 to February 2009 (17 dry days and three wet days). There were a total of 11 sampling sites through the channel system (Fig. 1a). A total of eight storm drain samples were collected throughout the study. Six storm drains were sampled on 1 day in a storm drain survey and two were collected concurrent with the monthly sampling events. Water was collected at each site (1–2 L per sample) and transported on ice for laboratory analysis. Water samples collected between January 2008 and August 2008 were analyzed for FIB only (n=44); samples collected between August 2008 and February 2009 were analyzed for both FIB and HF183 (n=86).

Ventura Samples were collected monthly between May 2008 and April 2009. Water (n=176) and sediment (n=60) samples were collected from 14 locations throughout the Ventura Harbor, Keys, and Marina. Water samples were collected at six locations throughout the Keys (D1-D6), with D5 and D6 being sampling sites located just east and west of the mouth of AB; at two enclosed beaches within the Ventura Keys (BM and SA); at two locations within the marina, at Marina Park (MP) and at the dock near boats, shops, and restaurants (MD); at a jetty-enclosed beach, Harbor Cove (ENC); and at an open beach, Surfers' Knoll (OPEN). Sand samples were collected from all four beaches (BM, SA, ENC, and OPEN) (Fig. 1b).

In addition to the monthly surveys, rainy day intensive surveys were performed on April 2–4, 2008 and February 4–6, 2009. Rainfall in April 2008 was minimal, with only 0.1 in. falling on April 2 and 0.07 in. on April 3. Only total coliform (TC) concentrations were

detected during this storm; accordingly, only TC data was analyzed. A larger storm was sampled in February 2009; 1.6 in. of rain fell over February 5–6 and an additional 1.36 in. of rain fell through February 9. One set of samples was collected on February 4, the day before the storm began. Samples were again collected at 10 am and 4 pm on February 5, the first day of the storm, and at 10 am on February 6 on the second day of the storm. After an interceding dry day, samples were taken on February 11 to determine whether the FIB levels were still elevated 2 days after a large storm. All water samples were analyzed for both FIB and HF183, except those from the April 2008 rainstorm in which only FIB was measured. Water was collected at each site (1–2 L per sample) and transported on ice for laboratory analysis.

2.2.2 Physicochemical Parameters

Selected samples were measured for total dissolved solids (TDS), water temperature, electrical conductivity, and pH (HANNA instruments #H198130, Smithfield, RI); total suspended solids (TSS) (Environmental Sciences Section Method 340.2); and dissolved oxygen (DO) (YSI model 55/12 FT, YSI Inc, Yellow Springs, OH).

2.2.3 FIB

All water samples were analyzed for TC, *Escherichia coli* (EC), and ENT using defined substrate technology (IDEXX Laboratories, Inc, Westbrook, ME). Colilert®-18 (IDEXX) was used to quantify TC and EC, and Enterolert® (IDEXX) was used to quantify ENT. A subset (n=14) of samples was also analyzed for EC using an in-field method, covalently linked immunomagnetic separation/ATP quantification (Lee et al. 2010). Single-sample marine recreational WQS were used to measure water quality [TC (10,000 MPN 100 mL^{-1}), fecal coliforms (FC) (400 MPN 100 mL^{-1}), and ENT (104 MPN 100 mL^{-1})]. EC were used as a conservative proxy for FC.

Sediment was collected using sterile 50 mL Falcon Tubes (BD BioSciences, USA) by sterile gloved hands. Sediment was removed from the upper 1.5-cm surface of a given site, weighed (10 g total), and placed into sterile 120 mL Nalgene bottles. Each sediment sample was washed vigorously by hand for 2 min in 50 mL PBS (pH 7.0±0.2) and allowed to settle for 1 min. The supernatant was then transferred into a separate sterile 120 mL Nalgene bottle. This "wash" step was repeated for a combined supernatant total of 100 mL, which was then analyzed for FIB.

2.2.4 DNA Extraction and Quantification

Water samples were vacuum-filtered through 47 mm diameter, 0.45 μm pore size Fisherbrand nitrocellulose filters (Fisher Scientific, Pittsburgh, PA) until the total volume was filtered or the sample stopped passing through the filter. DNA was extracted from filters using the Mobio UltraClean Fecal DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA), and extracts were stored at −80 °C until processed for HF183. DNA was extracted according to the manufacturer's protocol, with the exception of 90 s of bead-beating in a BioSpec Mini-Beadbeater-8 (BioSpec Products, Bartlesville, OK) instead of vortexing for 10 min at maximum speed. Total DNA concentration in the sample extract was analyzed using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Molecular Probes, Inc, Eugene, OR) with a Stratagene Mx3000P Real-Time PCR System and Mx3000P Software (Agilent Technologies, Santa Clara, CA). Sample extract aliquots (1 μL) were run concurrently with a standard curve of lambda DNA standard provided in the Quant-iT™ kit ranging from 0.1 to 20 ng of DNA μL^{-1} in a total reaction volume of 30 μL.

2.2.5 qPCR Analyses

Samples were analyzed for the human-associated *Bacteroides* 16S rRNA gene marker (HF183) using qPCR. DNA was amplified using the primers and reaction conditions described in Seurinck et al. (2005). Briefly, amplification occurred in 25 μL reaction volumes containing at least 2 ng of sample DNA, 1× SYBR GreenER PCR Master Mix (Life Technologies, Grand Island, NY), 0.25 mM of each primer, and DNase- and RNAse-free water (Fisher BioReagents, Pittsburgh, PA). Samples were run in duplicate and the reactions were performed in 96-well reaction plates using a Stratagene Mx3000P system (Agilent Technologies, Santa Clara, CA). The qPCR temperature program was 2 min at 50 °C, 10 min at 95 °C, then 45 cycles of 30 s at 95 °C, 1 min at 53 °C, and 1 min at 60 °C. Samples containing one or more HF183 copies (cps) μL^{-1} and a melting temperature at 77.5±0.5 °C indicated positive, correct amplification of HF183. Any PCR

product with a melting temperature deviating more than 0.5 °C from the expected temperature was classified as nondetect. A known concentration of target-containing plasmid DNA was used to generate standard curves and validate the melting temperature. For HF183 quantification, a six-point standard curve using plasmid DNA was run in duplicate alongside the environmental samples on every plate. The presence of environmental interference was assessed by running samples spiked with 2×10^5 cps μL^{-1} of plasmid DNA alongside the sample duplicates. Values were calculated using Mx3000P Software. If less than 8×10^4 cps μL^{-1} of HF183 were detected in the spiked samples, then the samples were diluted twofold and reanalyzed. This process was repeated up to three times as needed. If interference was still present after the third dilution, samples were omitted from further analyses. Six samples (out of 86) were eliminated from HF183 analysis at SMC due to interference. Twenty-four water samples (out of 176) were eliminated from HF183 analysis at Ventura due to interference, as were all sand samples.

2.3 Statistics and Data Analyses

Statistical analyses were run using SPSS statistical software (SPSS Inc., Chicago, IL). As FIB results were similar over the entire time period from January 2008 to February 2009, only a subset of FIB samples from August 2008 to February 2009, which were also measured for HF183, were used in the statistical analyses. Concentrations of indicators and physicochemical parameters did not follow a normal distribution and were log-transformed prior to analysis. However, log-transformed data were not found to be normal using Shapiro-Wilk's test; thus, all data analyses were performed using nonparametric tests. Spearman's rank correlation tests were used to determine the relationships between all parameters. Kruskal-Wallis and Mann–Whitney *U* tests were used to discern differences between sample locations and weather types for the parameters analyzed. Data were further analyzed for patterns and relationships using Fisher's exact test for frequency of water quality standard exceedance for FIB and the frequency of detection for HF183.

The limit of detection (LOD) for the qPCR assay was defined as 1 copy μL^{-1} and the limit of quantification (LOQ) was defined as 100 cps μL^{-1}. Samples in the range of 1 to 100 cps μL^{-1} were classified as detectable. All samples that were positive for HF183 were validated using a melt curve analysis.

3 Results

3.1 Dynamics of FIB at SMC and Ventura

FIB contamination was ubiquitous at SMC throughout the sampling period. WQS for TC, EC, and ENT were exceeded in 80 % (55/69), 78 % (59/76), and 86 % (51/59) of samples, respectively. Maximum values exceeded 62,700 MPN 100 mL^{-1} and 24,100 MPN 100 mL^{-1} for ENT and EC, respectively. Samples were collected from SMC sites at Entrada (E1-E6), West (W1-W4), Confluence (C), and from storm drains. When averaged across all time points, EC was present at significantly different concentrations among these locations (Kruskal-Wallis, $\chi^2(3)=12.733$, $p=0.005$). Over the course of the study, EC was highest at the confluence (5,600 MPN 100 mL^{-1}), followed by Entrada Channel (3,500 MPN 100 mL^{-1}). In contrast, ENT concentrations were not found to be significantly different among these locations (Kruskal-Wallis, $\chi^2(3)=3.998$, $p=0.262$).

In Ventura, samples were collected at sites in the Keys (D1-D6, BM, and SA), AB (AB and ABO), and the Harbor (MD, MP, ENC, OPEN). Marine WQS for FIB were exceeded in only 2 to 7 % of all dry weather samples collected (Table 1). On average, AB was significantly higher than all other sample locations for TC, EC, and ENT (Mann–Whitney, $p<0.001$). AB appeared to be a dry weather source of TC and ENT in the Ventura Keys, as the highest levels detected in the Keys were at D5 and D6 (sample locations nearest the AB outlet). Although average EC and ENT levels were consistently higher in the Keys (D1-D6, BM, and SA) compared to the Harbor (MD, MP, ENC, OPEN), this difference was not statistically significant (Mann–Whitney, $p>0.2$). TC

Table 1 Exceedance rates of FIB and frequency of detection of HF183 in wet and dry weather at Ventura

Bacteria	Dry rate (%)	N	Wet rate (%)	N
TC	5	6/121	56	31/55
EC	2.5	3/121	60	33/55
ENT	7.4	9/121	60	33/55
HF183	12	12/100	19	10/52

was almost significantly higher in the Keys samples (Mann–Whitney, $p=0.055$).

FIB (TC, EC, ENT) concentrations in sand were significantly higher in samples taken from Keys beaches (BM, SA) compared to those from the Harbor (ENC, OPEN) (Mann–Whitney, $p<0.001$). Sand collected from BM, which was located nearer the AB outlet, had higher levels of TC and ENT than SA. ENT was detected in 60 % (6/10) of sand samples at Keys beaches.

3.2 Physicochemical Parameters

Among all of the physicochemical parameters measured at SMC, significant differences among locations were observed only in nitrate (Kruskal-Wallis, $\chi^2(2)=12.862$, $p=0.002$) and ammonia (Kruskal-Wallis, $\chi^2(2)=13.191$, $p=0.001$). Entrada Channel had higher levels than West Channel of both nitrate (Mann–Whitney, $p=0.001$) and ammonia (Mann–Whitney, $p<0.001$).

In Ventura, AB was significantly higher in nitrate (Mann–Whitney, $p<0.001$) and temperature (Mann–Whitney, $p=0.029$) than all other sample locations. The AB sites also were significantly less saline than the remainder of the sites, which were in marine waters (Mann–Whitney, $p<0.001$). However, there was no statistical difference in DO, pH, ammonia, DNA, or TSS between the AB sites and other sample locations.

3.3 Levels of and Patterns in HF183

HF183 was detected in 58 % of the water samples collected during the SMC study (45/78) but was never present at quantifiable levels. HF183 was detected most frequently in storm drain samples (75 %, 6/8), followed by West Channel (65 %, 17/26), then the confluence (56 %, 5/9), and finally, in Entrada (41 %, 16/39). HF183 was detected in ocean water near the SMC outlet on a single day (out of three sample days) that HF183 also was detected in the confluence sample.

At SMC, HF183 was detected more frequently in the three most downstream sample locations in Entrada (50 to 60 %, $n=4$ to 10) than in the three most upstream (22 to 28 %, $n=4$ to 9). In contrast, HF183 presence was detected most frequently in the two most upstream samples taken in West Channel, W1 (85 %, $n=7$) and W2 (100 %, $n=4$). The frequency of detection decreased to ≤60 % for the remainder of downstream and confluence samples.

At Ventura, HF183 was detected in 14 % of samples (22/152) and quantifiable in 4.6 % (7/22). HF183 was never detected at the open beach location or at D6, near the mouth of AB. HF183 was detected only once at eight sites (MP, SF, D1, D3, D4, D5, AB, and ABO); all detections at these sites occurred during wet weather with the exception of D3. HF183 was detected twice at BM and three times at ENC.

The Ventura Marina Dock sample location had both the highest frequency of detection and concentration of HF183. HF183 was detected in 54 % (7/13) of the samples and quantifiable in 31 % (4/13) of the samples. ENC had the second highest HF183 detection rate, 21 % (3/14). Interestingly, on one of the dry days in November 2008, HF183 was quantifiable at both D2 (1,500 cps 100 mL^{-1}) and D3 (1,200 cps 100 mL^{-1}), located within the same channel within the Keys.

3.4 Rainfall Effects on FIB, HF183, and Physicochemical Parameters

Rainfall led to significant increases in FIB concentrations at both study areas. At SMC, wet weather samples exceeded WQS 100 % of the time (12/12) for both ENT and EC. FIB concentrations ranged from 3 to 10 times higher in wet weather compared to dry weather at SMC; concentrations of ENT and EC also were found to be significantly higher during wet weather (Mann–Whitney, $p<0.001$).

In Ventura, levels of all three types of FIB were significantly higher in wet weather compared to dry weather (Mann–Whitney, $p<0.001$), as was nitrate (Mann–Whitney, $p<0.001$), with exceedance rates ranging from 56 to 60 % (Table 1). The FIB storm signal decayed rapidly, with both ENT and EC concentrations returning to pre-storm levels within 2 days after the storm. Salinity was significantly lower during wet weather than dry during the February 2009 storm (Mann–Whitney, $p<0.001$) but not during the April 2008 storm (Mann–Whitney, $p=0.155$). TSS and ammonia were not significantly different between wet and dry weather (Mann–Whitney, $p\geq0.276$). Concentrations for all FIB were 8 to 24 times higher in wet weather compared to dry weather.

Although HF183 at SMC was observed slightly more frequently during wet weather (67 %, $n=12$) compared to dry weather (56 %, $n=66$), no significant difference was observed (Fisher's exact test, $p=0.544$). In Ventura, HF183 was detected significantly more frequently

during wet weather (22 %, 12/55) than dry weather (8 %, 10/121) (Fisher's exact test, p=0.024). The afternoon of February 5 during the 2009 storm event had the highest number of sites positive for HF183 of any sampling period (5 sites out of 13). In addition, salinity was significantly lower in samples with detectable HF183 (Mann–Whitney, p=0.048).

3.5 Relationships Between HF183, FIB, and Physicochemical Parameters

Relationships between the concentrations of any FIB or physicochemical parameters were examined to find significant differences when grouped by the presence of the HF183 marker. At SMC, neither FIB nor physicochemical parameters (Mann–Whitney, p>0.05) were significantly different when HF183 was present or absent (Fig. 2). Samples were also grouped based on FIB WQS exceedance in order to determine whether HF183 was more frequently detected in samples in which WQS were exceeded. At SMC, HF183 was not detected more frequently in samples that exceeded WQS than those that did not for TC, EC, or ENT (Fisher's exact test, p=0.552, p=0.274, p=1.00, respectively).

At Ventura, both EC and ENT were markedly higher in samples with HF183 (Mann–Whitney, p=0.004 and p=0.029, respectively), whereas TC was not (Fig. 3). HF183 was detected more frequently in samples that exceeded WQS for EC and ENT (Fisher's exact test, p=0.023 and p=0.030, respectively) but not for TC (Fisher's exact test, p=0.265). Salinity also was significantly lower in samples with HF183 compared to those without (Mann–Whitney, p=0.048) (Fig. 3). Additionally, the ratios of HF183 to ENT and EC were examined for samples collected from Ventura that had quantifiable HF183. Notably, five samples (out of seven), including MD, D2, and D3, had ratios of HF183 to both EC and ENT that either met or exceeded the range observed in sewage (Jay lab, unpublished data).

4 Discussion

Understanding and identifying pollution sources are important elements of a strategy to mitigate chronic water quality issues. At two geographically distinct but chronically impaired sample locations, we identified potential sources of fecal contamination that could be responsible for water quality exceedances at the respective locations. FIB were detected frequently at both SMC and Ventura. While high levels of FIB were present irrespective of wet and dry weather at SMC, high levels of FIB at Ventura were driven by both location (highest at AB, elevated in the Keys) and weather (e.g., rain events). WQS were exceeded at SMC at all locations in 25 to 89 % of all samples collected; high exceedance rates are hypothesized to show a chronic pollution source that is present regardless of weather or sample time (Peed et al. 2011). FIB results observed at SMC are consistent with other works studying urban watersheds (Walters et al. 2011; Sassoubre et al. 2011).

Poor water quality issues in Ventura, in contrast to SMC, appeared to be linked to weather and location. While we could not isolate a particular hotspot of contamination in SMC, we were able to identify the AB,

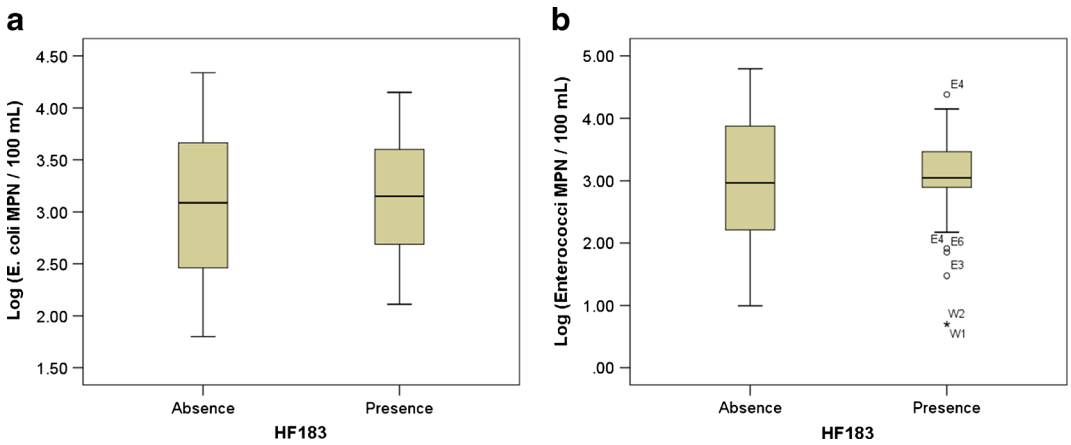

Fig. 2 Box plots binned by HF183 absence or presence at SMC. **a** EC concentrations; **b** ENT concentrations

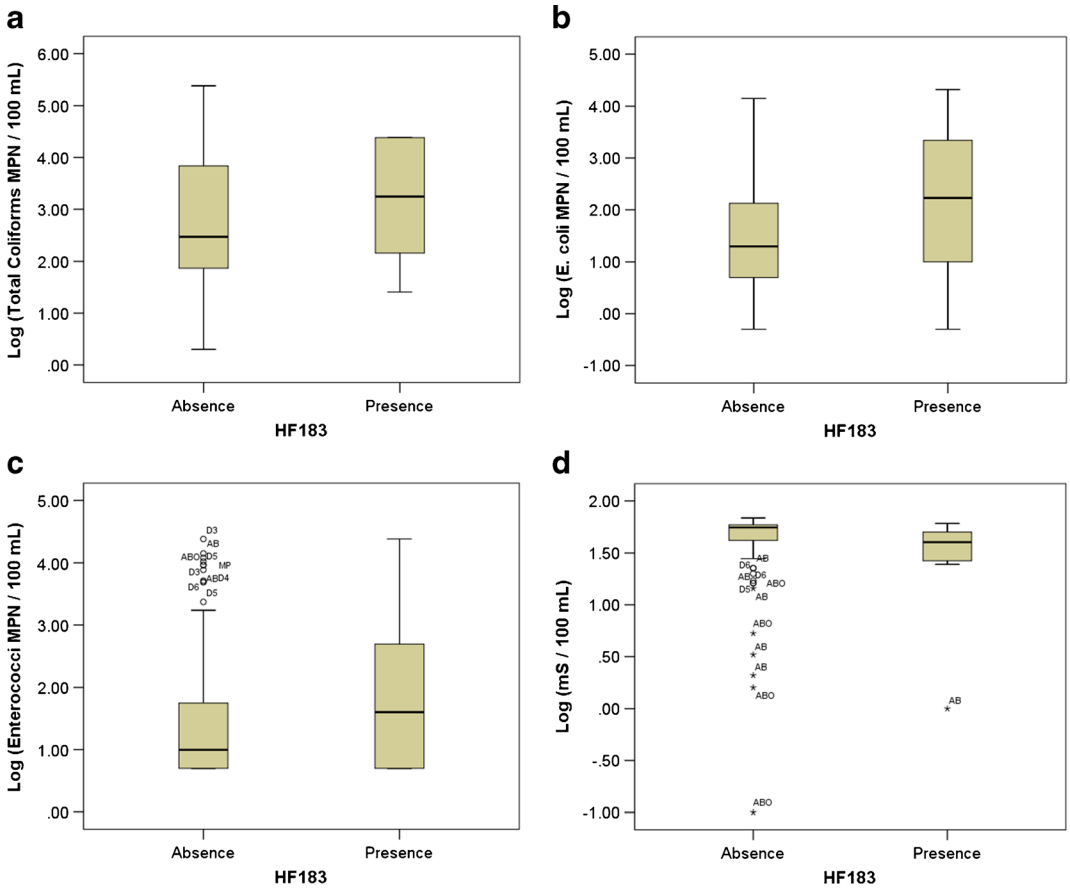

Fig. 3 Box plots of concentrations grouped by the presence/absence of HF183 marker at Ventura for **a** TC, **b** EC, **c** ENT, and **d** mS

which drains agricultural land uses among others, as a dry weather source of FIB in Ventura. The presence of nitrate observed in AB is consistent with agriculture as well. Other factors that may contribute to poor water quality in Ventura include areas of low flow and circulation (e.g., the Ventura Keys and Harbor), which tend to harbor more bacteria in water (Clarke et al. 2007) and sand (Lee et al. 2006), and can then lead to an increase in FIB levels in overlying water (Lee et al. 2006; Phillips et al. 2011). Furthermore, Ventura Harbor is utilized by a large multipurpose boating community, and frequent boat use and boat waste discharges have been generally shown to be a potential source of and to affect circulation of bacteria (Ho et al. 2011; Sobsey et al. 2003).

Measuring HF183 in parallel with FIB provided further information to identify SMC as a region with chronic contamination. Frequent HF183 detection at SMC (58 %) as well as high frequency of HF183 detection in intermittent storm drain flows (75 %) points to chronic contamination caused by urban runoff during dry weather. Storm drains impacted by leaking sewers have been identified as a source of HF183 elsewhere in the Southern California bight (Sercu et al. 2011), and further mapping and sampling of storm drains in SMC could provide additional insight into specific HF183 sources such as the aging infrastructure in the watershed (Kirs et al. 2011; Sercu et al. 2011). The presence of HF183 upstream did not correspond with HF183 at the closest downstream site, which points to a complex environment with many inputs, dilution, UV inactivation, etc. affecting the presence and persistence of the marker in the stream (Flood et al. 2011). Further, no seasonal variation of HF183 was observed, which also is consistent with other studies (Tambalo et al. 2012; Schriewer et al. 2010).

In addition to chronic contamination, frequency of HF183 detection pointed to several local sources of HF183, including a transient group that was observed on several occasions camping in the channel next to the water and near the confluence (50 to 60 % detection) at

SMC and, in Ventura, a commercial area with boats, shops, restaurants, and a public restroom located near MD (54 % detection). Results from this study show that host-associated markers can provide valuable information to guide water quality managers even if present at detectable rather than quantifiable levels and lead to the identification of sources of microbial contamination (Korajkic et al. 2010; Hagedorn et al. 1999; Kinzelman and McLellan 2009; Parker et al. 2010). Illicit discharge of boat waste also was identified as a further potential source of HF183 in the Ventura Keys. In one Keys Channel sampled in November, HF183 concentrations (1200–1500 cps 100 mL^{-1}) and the ratio of HF183 to FIB measured at both locations were consistent with calculations based on the discharge of 10 gal of boat sewage into the volume of this Keys channel (1,600 cps 100 mL^{-1}). Although standard FIB levels also were consistent with these calculations, this event would not have been identified without measuring HF183 since FIB levels were ≤10 MPN 100 mL^{-1} and would not have drawn attention to the possibility of an illicit boat discharge there. Thus, measuring human-associated markers could greatly accelerate the source identification process and, thus, mitigation of a chronically impaired watershed.

Rainfall also impacted standard FIB concentrations and HF183 differently at each study site. In SMC, FIB levels increased with rainfall, whereas the frequency of detection of HF183 was unimpacted. These findings are consistent with previous studies in the same region (Surbeck et al. 2006). In Ventura, rainfall resulted in significant increases in both FIB levels and HF183 detection rates. HF183 was detected with the most frequency during the second day of the 2009 rain event. FIB levels, however, increased on the first day of the storm and stayed high throughout; such pattern differences may arise from different sources and/or modes of transport into the watershed for these indicators (Surbeck et al. 2006; Converse et al. 2011). Reasons for the increased HF183 presence observed in Ventura during rain could include a rising water table or flushing of leaking septic and sewage systems into storm drains and out into the environment (Peed et al. 2011; Converse et al. 2011). We also observed that storm conditions and rain volumes can affect FIB and other indicators differently, in accord with other work (Converse et al. 2011).

Using agricultural source markers (e.g., ruminant- or horse-associated markers) in areas like Ventura could be very useful for local water quality managers, as has been shown in previous studies (Wilkes et al. 2013; Marti et al. 2013). Ratios of HF183 to FIB could also be a valuable management tool for identifying human sources of pollution. In this study, this ratio was compared to that of human sewage and was found to be comparable in some samples taken from Ventura MD, as well as D2 and D3 (Jay lab, unpublished data) where boats could represent a source of HF183. In Ventura, sample sites with high concentrations of FIB and HF183 did not coincide, which indicates that FIB may not be an effective indicator of sewage pollution at this site. Thus, it is important to understand multiple contributing sources of contamination in a study area to help both identify and address areas for remediation (Schriewer et al. 2010; Reischer et al. 2011).

5 Conclusions

Markedly different patterns in FIB levels and HF183 detection were observed at both of these impaired sites. Chronic pollution from urban runoff during dry weather rather than any single identifiable source of pollution was identified in SMC as FIB exceeded water quality standards 78 % of the time, and low levels of HF183 were detected frequently at all sample locations. In Ventura, AB was determined to be a dry weather source of FIB to the Ventura Keys, MD was identified as an area with high frequencies of HF183 detection, and a potential illicit discharge of boat waste was identified in a Keys channel. Further, Ventura Keys sand and water were higher in FIB than the Harbor, which may result from quiescent waters and frequent boat activity in the Keys.

Examining nitrate concentrations and ratios of HF183 to FIB lent further support to identifying potential sources of fecal pollution to Ventura. The presented work provides further support that measuring multiple parameters (e.g., FIB, HF183, nitrate) associated with the pollution sources specific to the chronically impaired watershed of interest can strengthen a water quality study and increase the ability of water quality managers to identify and remediate sources of fecal pollution.

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