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Evaluation of Chemical, Molecular, and Traditional Markers of Fecal Contamination in an Effluent Dominated Urban Stream

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In this paper we present a quantitative sanitary survey of the Middle Santa Ana River, in southern California, utilizing a variety of source tracking tools, including traditional culture-dependent fecal markers (*Enterococcus* and *Escherichia coli* by IDEXX), speciation of enterococci isolates, culture-independent fecal markers (human-specific HF183 *Bacteroides* and *Enterococcus* by quantitative polymerase chain reaction, qPCR), and chemical markers of sewage and wastewater (nutrients, enantiomeric fraction (EF) of propranolol and ethylenediaminetetraacetic acid). To facilitate comparison of these different methods, data are interpreted in a loading (i.e., mass per time) framework that enables a quantitative apportionment of fecal markers and nutrients to specific source waters in the Middle Santa Ana River. Multiple lines of evidence support the hypothesis that *Enterococcus* in the Middle Santa Ana River originates primarily from in situ growth in streambed sediments, not from significant and persistent sources of untreated human waste. The EF of propranolol of tertiary treated wastewater effluent is in the range (0.42 to 0.71) previously reported for raw sewage, making EF of propranolol an unsuitable marker for fecal pollution, at least at this site. The human fecal marker HF183 *Bacteroides* was detected at a few sites, although not in a source of disinfected and tertiary treated wastewater effluent. Based on the results presented here and prior experience at other sites in southern California, HF183 *Bacteroides* would appear to be a candidate marker of fecal contamination for inland waters, although more qPCR measurements in disinfected wastewater effluent are needed to account for variations due to treatment plant performance and other factors. More generally, our results support the notion that regrowth of fecal indicator bacteria (FIB) in river sediments may lead to a decoupling between FIB and pathogen concentrations in the water column and thus limit the utility of FIB as an indicator of recreational waterborne illness in inland waters.

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Introduction

Fecal indicator bacteria (FIB) are the number one cause of river and stream impairments in the United States (*1*), and developing mitigation strategies to address these impairments has been described as the "challenge of the 21st century" (*2*). The use of FIB to assess surface water quality is motivated by epidemiological studies, carried out primarily at recreational beaches impacted by point sources of partially or untreated sewage, that correlate recreational waterborne illness to *Escherichia coli* (EC) and enterococci bacteria (ENT) concentrations in freshwaters and ENT concentrations in marine waters (*3*). Mounting evidence suggests, however, that FIB can naturalize and grow in sediment and aquatic environments, casting doubt on their universal utility as a marker for fecal pollution, in general, and as a sentinel for recreational waterborne illness, in particular (*4, 5*). FIB growth has been documented in water and/or sediment collected from freshwater streams (*6*), freshwater ponds (*7*), freshwater beaches (*8*), estuaries and tidal creeks (*9*−*11*), and marine beaches (*12, 13*). The growth of naturalized FIB appears to be regulated by ambient concentrations of phosphorus and/or organic carbon (*14, 15*), including assimilable carbohydrates in sediments (*16*). Nutrient-limited growth of naturalized FIB may explain frequently observed correlations between FIB and nutrient concentrations in freshwater (*17, 18*), marine water (*19*), and groundwater (*20*) systems. An additional challenge is translating the substantial health risk research data available for coastal sites to inland waters, such as flowing streams (*21*). In light of these concerns, the United States Environmental Protection Agency (EPA) is currently evaluating a number of alternative indicators that might better signal fecal pollution, and recreational waterborne illness risk, in different regions of the country, and in different recreational settings (*5*).

In this study we set out to address some of the critical and unresolved issues surrounding monitoring for fecal pollution and recreational waterborne illness risk in rivers where flow is dominated by disinfected wastewater effluent. In particular, this study had the following objectives: (1) Compare several different methods (traditional, molecular, and chemical) for measuring fecal pollution in a wastewater effluent dominated river; (2) Interpret the data in a loading context that allows for the quantitative apportionment of markers between different source waters in the river; (3) Evaluate the importance of river sediments as a contributor of FIB, and ENT in particular, in the water column of a nutrient rich river; and (4) Compare culture based and qPCR measurements of enterococci bacteria.

Materials and Methods

Site Description. The Santa Ana River drains a 4406 km^2 watershed that is home to approximately 6 million residents (as of 2000) in San Bernardino, Riverside, and Orange Counties (*22*) (upper map, Figure 1). The Santa Ana River is divided geographically into Upper and Lower basins by a large flood control structure, Prado Dam, used to limit flow

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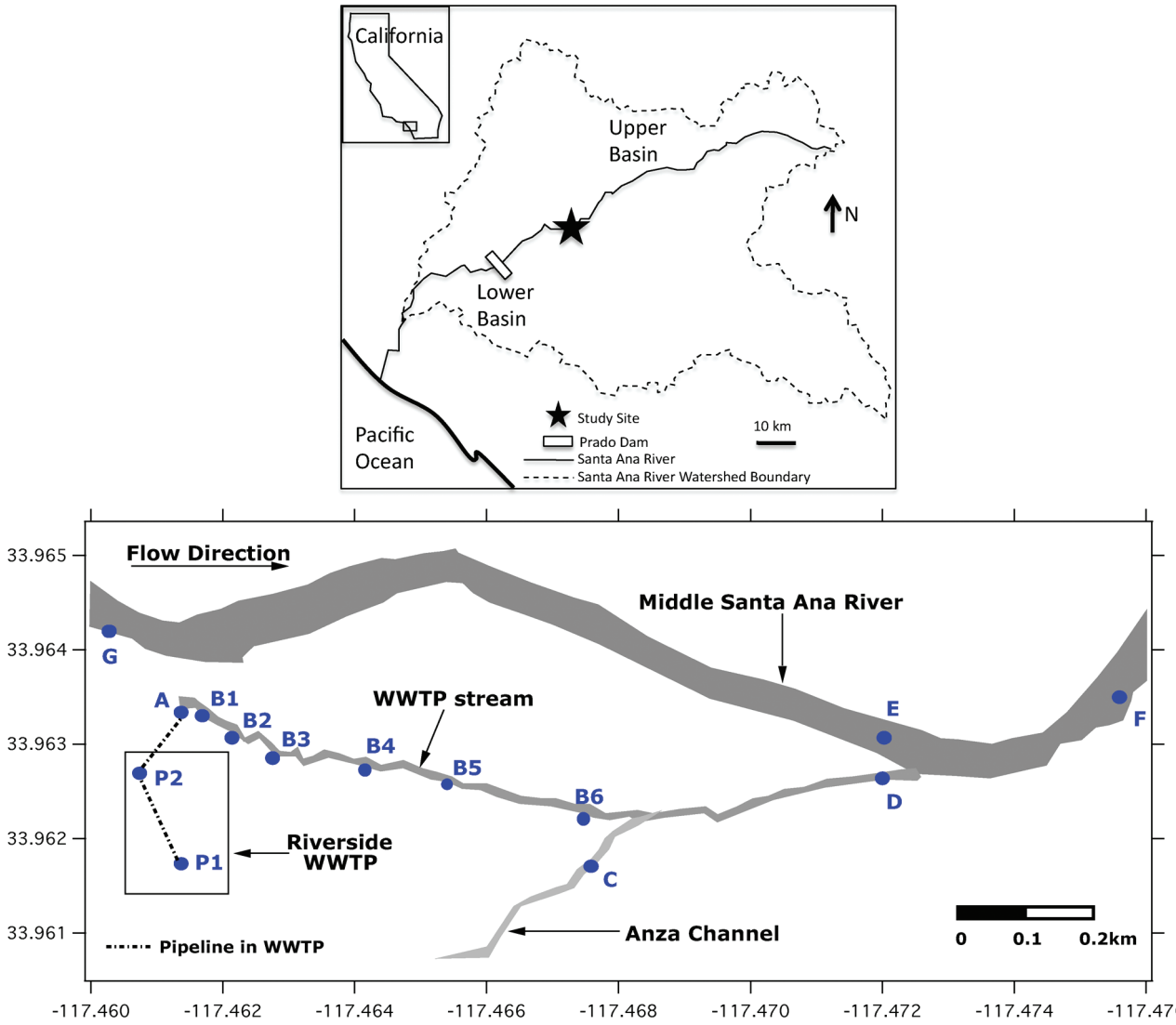

FIGURE 1. Regional (top panel) and local (bottom panel) maps of the field site located in the City of Riverside, County of Riverside, southern California. Note that the map in the bottom panel has been rotated 180° around the north-south axis, so that the order of sampling sites corresponds to the horizontal axes of Figures 2 and 4.

in the river during storms and provide base flow for groundwater recharge in the lower reaches of the river during dry weather periods (*23*). During dry weather, approximately 85% of flow in the Santa Ana River is disinfected tertiary treated wastewater effluent (*24*) discharged from 12 wastewater treatment plants (WWTPs), each of which contribute a minimum of 3700 m^3 day^{-1} (*25*). A previous study suggested that the Middle Santa Ana River, located upstream of Prado Dam, may be impacted by untreated human sewage, based on the enantiomeric fraction (EF) of the pharmaceutical compound propranolol measured there (*26*).

The field site is located where the Riverside WWTP discharges to the Middle Santa Ana River (lower map in Figure 1). At this WWTP, approximately 120,000 m^3 day^{-1} of domestic and industrial wastewater undergoes secondary biological and tertiary wastewater treatment, including activated sludge, alum and/or polymer injection, filtration through 16 dual media filters, chlorination through three chlorine contact basins, and dechlorination by sulfur dioxide. Effluent from the WWTP is discharged to an earthen bottom channel, referred to here as the "WWTP stream" that receives no other source of surface water during dry weather. Effluent flows down the WWTP stream for 550 m, where it merges with an earthen bottom flood control channel called Anza Channel. The Anza Channel receives only runoff from the surrounding urban landscape. These two streams mix and, during this study, flow downstream another 400 m before merging with the Middle Santa Ana River in the City of Riverside.

Water Sampling. Water samples ($N = 104$) were collected from within the WWTP downstream of the chlorination and dechlorination contact basins (sites P1 and P2, respectively), at the WWTP discharge pipe (site A), along the WWTP stream (sites B1 through B6), on the Anza Channel (site C), downstream of the confluence between the WWTP stream and the Anza Channel (site D), and in the Middle Santa Ana River upstream (site E) and downstream (site F) of the confluence. Eight sampling campaigns occurred during July and August 2007, twice (12:30 and 18:30) on 7/31/07, twice (4:45 and 7:40) on 8/1/07, once (19:00) on 8/29/07, and three times (07:00, 09:50, and 13:05) on 8/30/07. All water samples intended for FIB analysis were immediately amended with sodium thiosulfate after collection, and held on ice in the dark until processed, within a holding time of six hours. Water temperature was recorded (Infrared gun, Fluke, Everett, WA) in the field, and all water samples were analyzed in the field laboratory (located at the Riverside WWTP) for pH (Model 720A+ Thermo Orion, Waltham, MA) and culturable FIB, including total coliform (cTC), *Escherichia coli* (cEC), and enterococci bacteria (cENT) using chromogenic substrate tests known commercially as Colilert-18 and Enterolert

implemented in a 97 well quantitray format (IDEXX, Westbrook, ME). Separate water samples collected from a subset of sites (A, B3, B5 or B6, C, D, E, and F) were analyzed for dissolved organic carbon (DOC), ammonium, nitrate, total Kjeldahl nitrogen (TKN), soluble inorganic phosphorus (orthophosphate), enantiomeric fraction (EF) of the pharmaceutical compound propranolol, ethylenediaminetetraacetic acid (EDTA), total DNA, and the human-specific marker HF183 *Bacteroides* and enterococci bacteria (qENT) both measured by quantitative PCR (qPCR). Detailed methods are presented in the Supporting Information (SI). The reference above to sites "B5 or B6" reflects the fact that, for the latter suite of analytes, water samples were collected from site B6 during the first four sampling campaigns (12:30 and 18:30 on 7/31/07; 4:45 and 7:40 on 8/1/07) and from site B5 during the last four sampling campaigns (19:00 on 8/29/07; 07:00, 9:50, and 13:05 on 8/30/07). In the results presented later, summary statistics (averages and standard deviations) were computed for samples collected at sites B5 and B6 separately and for samples collected from both sites B5 and B6 (denoted "B5+B6").

Sediment Sampling. On the third and fourth sampling dates (8/29/07 and 8/30/07), sediment samples were collected coincident with water sampling (19:00 on 8/29/07 and 07:00, 09:50, and 13:05 on 8/30/07) at sites B1, B2, B3, B4, B5, B6, C, D, E, and F. Sediment samples ($N = 52$) were collected into two sterile 50 mL plastic centrifuge tubes (Fisherbrand, Pittsburgh, PA) from the upper 3 cm of the streambed at the stream's thalweg. The centrifuge tubes were emptied of excess water, capped, amended with sodium thiosulfate, capped, and then stored on ice in the dark until processed. Sediment samples were analyzed for organic carbon content based on loss on ignition and FIB (cTC, cEC, and cENT) using an extraction protocol modified from Craig et al. (*27*); sediment extract was analyzed for FIB using the IDEXX Colilert-18 and Enterolert tests described above. Four sediment samples from each site were pooled, thoroughly mixed, and analyzed for bulk density, grain size distribution (silt, clay, and sand fractions), and porosity. Detailed sediment analysis methods are presented in the SI.

Enterococcus Speciation. Subsequent to the field sampling described above, on 9/14/09 presumptive ENT isolates were cultured from water and sediment samples collected at sites B5, B6, C, D, E, F and G using EPA Method 1600 and speciated using automated substrate utilization tests (Vitek 2 Compact, bioMérieux, Inc., Durham, NC). Detailed procedures are presented in the SI.

Statistical Analysis. Spearman's rank correlation (ρ) for nonparametric data was used to characterize the covariation between measured variables, and the nonparametric Kruskal–Wallis test was used to evaluate if median concentrations were significantly different by site (SPSS Statistics v. 17, Chicago, IL). At some sites, a subset of analyte measurements were above or below the detection limits of the analytical method (i.e., there were censored values), and in these cases mean and standard deviations were estimated using Regression on Order Statistics (*28*) implemented as a macro in the computer program Excel (personal communication, M. Kayhanian). In several cases the Regression on Order Statistics method did not yield a maximum likelihood estimate because there were too many censored values or, more rarely, a few censored values together with a large number of samples with the same value. In these cases we reported either the limit of detection (all censored values) or the mode (too many of the same value).

Results

Tables S1 and S2 report summary statistics for measurements on water and sediment samples collected during the eight sampling campaigns in July and August 2007, respectively;

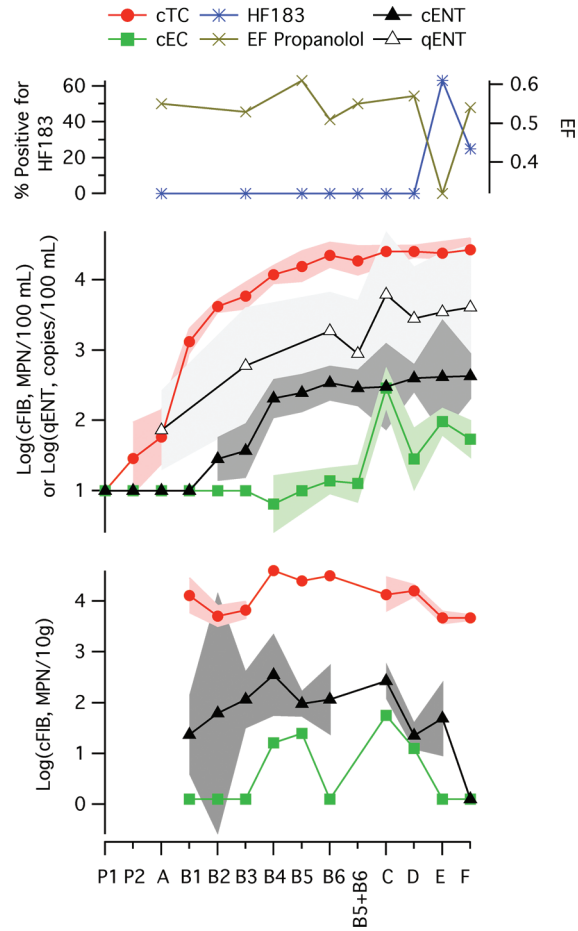

FIGURE 2. Averages and standard deviations of the fecal pollution markers measured during the field study. Top panel: Water column measurements of HF183 *Bacteroides* and EF of propranolol. Middle panel: Water column measurements of cTC, cEC, cENT, and qENT. Bottom panel: Sediment measurements of cTC, cEC, and cENT.

the raw data from each sampling campaign are reported in Tables S3–S14 (see SI). These data are described below.

Culture-Based and Culture-Independent Measurements of FIB and HF183 Bacteroides. *Water Column Measurements.* Measurements of FIB in the water column are plotted against sample location in the middle panel of Figure 2. Culture-dependent FIB concentrations (reported here in units of most probable number (MPN) of bacteria per 100 mL of water sample, or MPN/100 mL) are near or below the lower-limit of detection (<10) at sites within the WWTP (sites P1 and P2) and at the effluent discharge pipe (site A), increase substantially with distance downstream in the WWTP stream (sites B1-B6) and are elevated at all other sites downstream (sites C, D, E, and F). In general, the concentration of qENT in the river is ~10 times higher and exhibits significantly more variability than the concentration of cENT, consistent with other studies comparing these two assays (*29*). Within the WWTP stream the concentration of cENT increases significantly ($p = 0.013$) from site A (<10 MPN/100 mL) to B6 (~300 MPN/100 mL). The concentration of qENT also increases significantly ($p = 0.02$) from site A (~10^2 copies/100 mL) to B5+B6 (~10^3 copies/100 mL), excluding sampling events for which qENT was below the detection limit at both sites (sampling events 07:00 and 09:50 on 8/30/07, see Tables S8 and S9). The human fecal marker HF183 *Bacteroides* was not detected at sites A, B3, B5, B6, C, and D (0% detection in top panel Figure 2), which indicates concentrations <900 targets/L (equivalent to 56 copies/PCR reaction). Approxi-

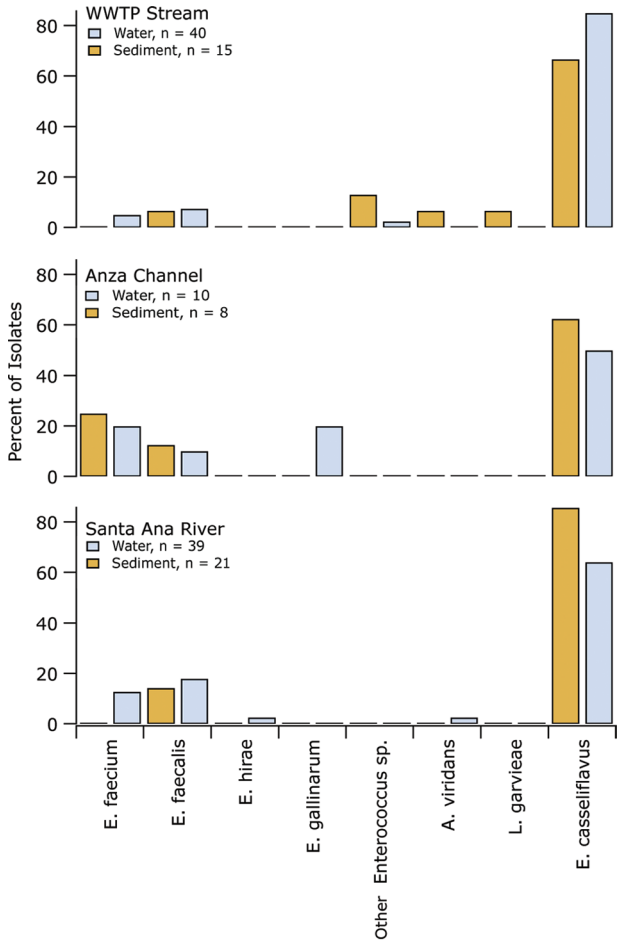

FIGURE 3. Species distribution of *Enterococcus* isolates cultured from water (blue bars) and sediment (brown bars) on September 14 (2009) from the WWTP effluent stream (top panel), urban runoff channel (middle panel), and Santa Ana River (bottom panel).

mately 60% and 25% of samples collected in the Middle Santa Ana River at sites E and F, respectively, were positive for HF183 *Bacteroides* (top panel, Figure 2).

Sediment Measurements. FIB concentrations in sediments were relatively constant over the WWTP stream (sites B1 through B6) and Anza Channel (site C) and lower in sediment collected from the Middle Santa Ana River (sites E and F) and downstream of the confluence of Anza Channel and the WWTP stream (site D) (bottom panel, Figure 2). Organic carbon measured by loss on ignition is significantly correlated with sediment concentrations of cTC ($p = 0.001$, $\rho = 0.50$), cEC ($p = 0.002$, $\rho = 0.47$), and cENT ($p = 0$, $\rho = 0.62$). Sediments exhibit relatively little variability in bulk density (1.3 to 1.8 g/mL), although sites in the WWTP stream (sites B1−B6) have higher silt and clay fractions, compared to the Middle Santa Ana River (sites E and F).

Enterococcus Speciation. A total of 133 presumptive ENT were isolated from water ($N = 90$) and sediment ($N = 43$) samples from the WWTP stream (sites B5 and B6), Anza Channel (site C), and Middle Santa Ana River (sites E, F, and G) during a single sampling event on 9/14/09 between 10:00 and 12:00. Of these 133 isolates, 130 were confirmed *Enterococcus* species, two were identified as *Aerococcus viridans* (one from a water sample collected at site E and another from a sediment sample collected at site B5), and one was identified as *Lactococcus garvieae* (from a sediment sample collected at site B6) (Figure 3). Of the 130 confirmed *Enterococcus* isolates, *E. casseliflavus* was the dominant species isolated in water and sediment samples collected from the WWTP stream (85 and 77%), Anza Channel (50 and 63%), and Santa Ana River (66 and 86%) (Figure 3). Fewer *E. faecium* and *E. faecalis* were cultured from the WWTP stream (0 to 8%), Anza Channel (10 to 25%), and Santa Ana River (0 to 18%). Other species identified include *E. gallinarum* (20% of isolates from Anza Channel), *E. hirae* (<1% of all samples), and several species isolated only from sediment samples collected from the WWTP stream (*E. cecorum*, *E. saccharolyticus*, and *E. columbae)*.

Chemical Markers of Fecal Pollution. EDTA concentrations at sites A (0.71 ± 0.23 μM) and B5+B6 (0.85 ± 0.23 μM) are within the range reported for other wastewater effluents (*30*) (Table S1). The pharmaceutical compound propranolol was detected in the WWTP stream at sites A and B5+B6 (~10 ng/L, ranging from 1 to 41 ng/L) but not in the Anza Channel at site C (<0.5 ng/L) (Table S1). The EF of propranolol, which has been proposed as an indicator of untreated sewage (*26*), was lower (0.32) in the Santa Ana River at site E and relatively higher (0.55) at sites A and B5+B6 (top panel, Figure 2). EF ratios measured at sites A and B5+B6 are in the range previously reported for raw sewage (*26*).

Mass Loading of Fecal Pollution Markers and Nutrients. Mass loading rates were calculated from the concentration data in Table S1 and flow rates in the SI. Averaging over all analytes, the mass recovery across the confluence—i.e., the percent of mass flowing into the confluence (at sites B5+B6, C, and E) that exits the confluence (at site F)—is 96 ± 30%, ranging from a low of 69% (for orthophosphate) to a high of 163% (for cEC) (Table S15). The dominant source of pollutant loading at site F varies by analyte (Figure 4): (1) FIB (cTC, cEC, cENT, qENT) and nitrate originate primarily from site E; (2) EDTA originates primarily from the WWTP stream (site B5+B6); and (3) propranolol, TKN, orthophosphate, and DOC are roughly split between the WWTP stream (site B5+B6) and site E. Anza Channel (site C) is a minor contributor to pollutant loading at site F, with the exception of cEC for which site C contributes approximately 30% of the loading at site F. Loading rates of cTC, cENT, and qENT exhibit a significant increase (>10-fold) along the length of the WWTP stream, from the discharge pipe at site A to the end of the channel at site B5+B6. Mass loading rates for the remaining analytes exhibit no measurable change (DOC, nitrate, TKN, orthophosphate, cEC, EDTA) or a modest decrease (propranolol) from site A to B5+B6.

Discussion

U.S. EPA recommends the use of EC or ENT to assess recreational water quality at freshwater beaches and rivers (*3*). Most sites sampled during our dry weather study of the Middle Santa Ana River exceeded the EPA recommended geometric mean criteria for ENT of 33 MPN/100 mL; note that a geometric mean of 33 MPN/100 mL is equivalent to a log-mean value of 1.5 (see log-mean values for cENT illustrated in Figure 2 and listed in Table S1). A loading analysis of cENT and qENT in the Santa Ana River and two of its tributaries-Anza Channel and the WWTP stream-indicates that 70 to 80% of ENT measured downstream of the confluence (site F) are from the Santa Ana River upstream of the confluence (site E), while the remaining is from the WWTP stream (site B5+B6) and Anza Channel (site C) (Figure 4). The fact that the WWTP stream contributes any FIB to the Santa Ana River is surprising, given that water in this stream is tertiary treated and disinfected wastewater effluent. Along the length of the WWTP stream, the geometric means and estimated loading rates of cENT and qENT increase >10-fold from the point where disinfected effluent is discharged (site A) to the downstream end of the WWTP stream (site B5+B6), a distance of 550 m. The downstream increase of cENT in the WWTP stream was observed during all eight sampling campaigns, including day and night sampling carried out

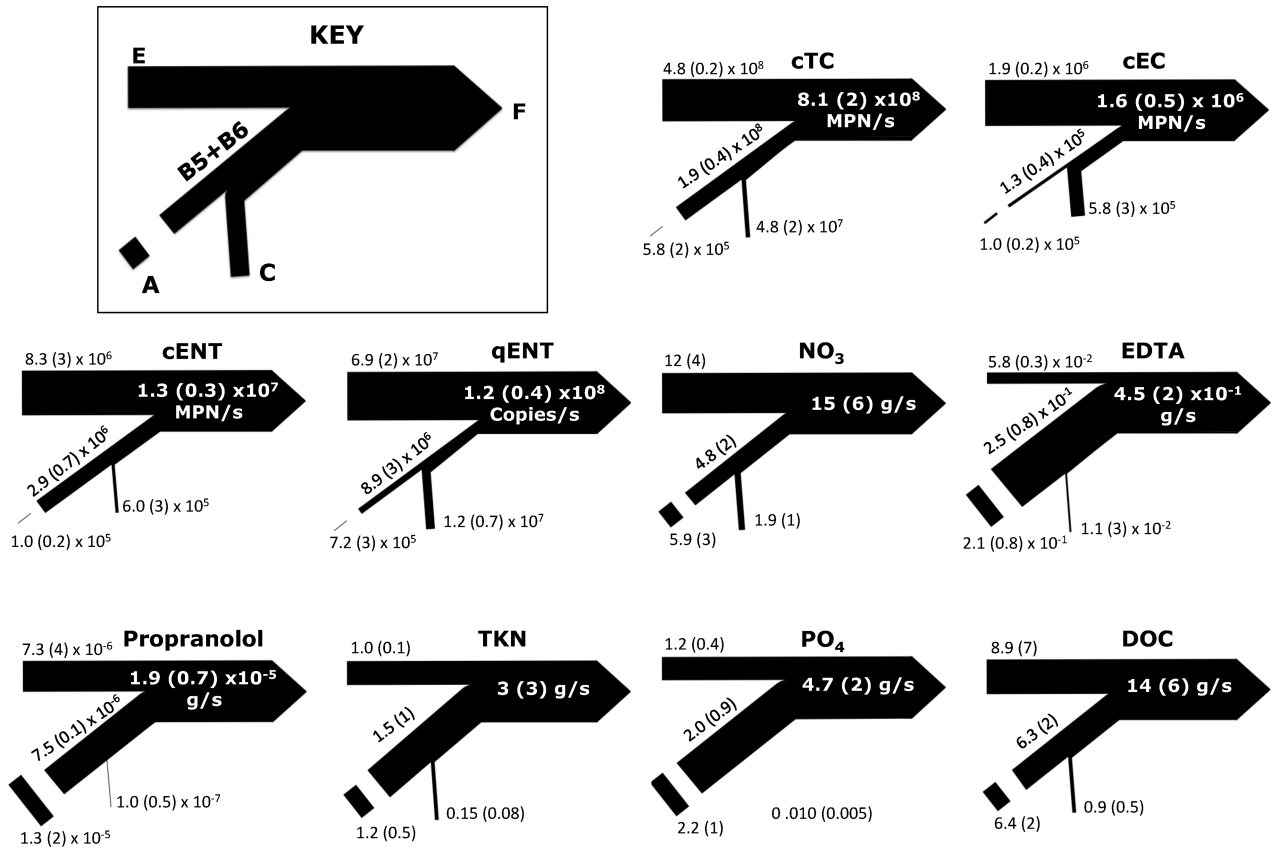

FIGURE 4. Mass loading rates of pollutants measured in source waters (sites B, C, and E) and blended water downstream of the confluence (site F). Numbers indicate mean loading rate (units MPN/s, g/s, or copies/s) for source and blended waters; also shown are the standard deviations of loading estimates (in parentheses). Because of uncertainties in flow and concentration estimates, loading rates are reported here to only two significant digits.

over two consecutive months in the summer of 2007 (Figure 2 and Tables S3−S10).

What is the cause of the rapid and persistent downstream increase of ENT in the WWTP stream? It does not appear to be, at least predominantly, growth of ENT in the water column. Based on dye tracer studies (data not shown), the average flow velocity in the WWTP stream is 0.6 m/s, and therefore water parcels take, on average, 15 min to travel from sites A to B6. Assuming first-order growth kinetics, a >10-fold increase in cENT concentration from sites A to B6 implies an unrealistically short cell doubling time of <15 min. By comparison, the doubling time of ENT growing under optimal conditions in the laboratory is approximately 40 min (*31*), and microcosm studies conducted at the same site indicate that cENT die, rather than grow, when they are suspended in WWTP effluent (data not shown). Resuscitation of chlorine injured ENT cells also appears unlikely, because the geometric means of both cENT and qENT increase significantly, and by approximately the same multiplicative factor (ca. 30-fold, see Figure 2 and Table S1), as effluent flows from sites A to B6. Thus, it appears that new ENT cells are being added to the effluent as it flows downstream, or, put another way, the downstream increase of cENT does not reflect the conversion of ENT cells from a nonculturable to a culturable state. In theory disaggregation of ENT flocs, possibly discharged from the WWTP plant, might lead to an increase in the cENT concentration with distance downstream in the effluent channel, although this hypothesis is inconsistent with the observation that qENT also increased with distance downstream (i.e., the cell equivalent concentration obtained by qENT should not be affected by the aggregation state of cells in a given sample). While homeless populations and bather shedding might contribute ENT cells to the stream, the intermittent nature of these sources implies that they are unlikely to yield the highly reproducible and persistent downstream increase in cENT and qENT signals measured in the water column and the relatively stable cENT signal measured in the sediment. Taken as a whole, the data are most consistent with the hypothesis that environmental growth of ENT in riverbed sediment is a primary source of ENT in the water column of the WWTP stream: (1) cENT concentration is elevated in river bed sediments; (2) the water column concentration of both cENT and qENT increase with distance downstream; and (3) during the one day that cENT isolates were cultured from the stream and subsequently speciated, a single species of cENT, *E. casseliflavus*, predominated in both water and sediment samples. *E. faecalis* and *E. faecium* are the dominant *Enterococcus* species in human and animal intestinal flora, human fecal matter, and urban wastewater and sludge (*32*), while *E. casseliflavus* is more often associated with decaying vegetation and has been described as an epiphyte (*11*). Previous studies in southern California report that *E. casseliflavus* is frequently isolated from dry-weather urban runoff (*11*) although to our knowledge this is the first study to report that *E. casseliflavus* also dominates the enterococci population in water and sediment from a wastewater effluent stream. To the extent that the primary source of *E. casseliflavus* in the SAR is environmental growth, ENT concentrations in the water column are likely to be decoupled from both the concentration of human pathogens and recreational waterborne illness risk (*21*). On the other hand, *E. faecium* and *E. faecalis* were isolated at low frequencies from all three source waters (WWTP stream, Anza Channel, and Santa Ana River), and strains of both

species are opportunistic pathogens (*33*), as are *E. casseliflavus* (*34*). These uncertainties underscore the need for epidemiological studies of the potential health risk associated with recreating in wastewater effluent dominated streams, like the Middle Santa Ana River (*21*).

Given that naturalized strains of enterococci bacteria appear to grow in the streambed sediments of the Middle Santa Ana River and its tributaries, it is reasonable to ask if any of the alternative analytical methods tested here-EF of propranolol, qENT, and HF183 *Bacteroides*-might be more suitable markers for fecal pollution. EF of propranolol ratios measured on both WWTP effluent and on water samples collected from the WWTP stream are in the range (0.42 to 0.71) reported previously for raw sewage (*26*), which is inconsistent with effluent samples collected from seven other WWTPs. The inability of EF to differentiate between raw sewage and disinfected and tertiary treated wastewater effluent, at least at this site, would presumably make it unsuitable as a fecal marker. Quantitative PCR measurements of ENT have been suggested as an alternative to culture-based measurements, in part because of the relatively rapid turn around time of the former compared to the latter (*35*). At our field site, measurements of cENT and qENT were significantly ($p = 0.036$) and highly (ρ=0.79) correlated, and their comparison provided useful information, such as our conclusion that resuscitation of chlorine injured ENT was unlikely to be a dominant cause of the increasing cENT signal in the WWTP stream. Further, PCR primers could be chosen to target only specific ENT species such as *E. faecalis* or *E. hirae,* that may be better indicators of human waste (*36*). HF183 *Bacteroides* was successfully used in the Middle Santa Ana River to identify a source of human sewage (*37*), and it has been used to track sources of human waste in dry weather runoff at other locales in southern California (*38*). In our study, only water collected from the Santa Ana River tested positive for HF183 *Bacteroides* (63% and 25% of samples tested positive at sites E and F, respectively); none of the disinfected effluent samples were positive, in contrast with previous studies that detected 10^5-10^6 copies/L (*39, 40*). Differences in HF183 *Bacteroides* concentrations in the disinfected effluents of this versus other studies are likely related to (1) plant performance (FIB concentrations in this study were orders of magnitude lower compared to those in ref *40*) and/or (2) filtration (this study, but not ref *39*) which would preclude sampling "free" DNA. One potential complication associated with the adoption of HF183 *Bacteroides* as an alternative fecal marker is that, at the present time, an association between HF183 *Bacteroides* and recreational waterborne illness has not been demonstrated.

From the results presented in this paper, three policy alternatives can be envisioned: (1) perform a use-attainability analysis of the field site; (2) control environmental factors (e.g., organic carbon loading) that favor the environmental growth of ENT; and/or (3) implement site-specific criteria that supersede the U.S. EPA 1986 recreational water quality criteria. It is estimated that approximately 1000 person-days of recreational activity occur in the Santa Ana River every year (T. Moore, personal communication), and thus option 1 is probably not appropriate for this site. Based on the observed correlation between cENT and organic carbon in the sediment, it is possible, although unproven, that reductions in DOC loading (e.g., by increasing treatment requirements for WWTPs on the river) might lead to reductions in ENT in the river and sediment (option 2). To the extent that ENT grow naturally in the Middle Santa Ana River and its tributaries, adoption of site-specific criteria (option 3) may be warranted, particularly if the new criteria utilize a fecal marker (or markers) known to detect human fecal waste. Based on data presented in this study and previous microbial source tracking experience in the Middle Santa Ana River watershed (*37*), HF183 *Bacteroides* might meet these criteria (subject to the caveats noted above), while the EF of propranolol would not. Although not tested here, human specific viruses might also be suitable, particularly given that they are relatively resistant to disinfection (*41*).

Acknowledgments

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Supporting Information Available

Detailed materials and methods and raw data for measurements performed on water and sediment samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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