

Microbiological impairment of urban rivers and streams: role of sediment and wastewater effluent

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Abstract

In densely populated areas of the arid southwest US, treated wastewater effluent is often the dominant source of water flowing in streams and rivers, particularly during dry weather. In this study, we set out to determine if treated wastewater effluent in one such river – the middle Santa Ana River in southern California – is a source of enterococci, a fecal indicator bacteria commonly used by regulatory agencies to assess river water quality. A variety of analytical and ecological approaches were employed, including culture-dependent and culture-independent assays of enterococci, molecular markers of human sewage (HF183 *Bacteroides*), chemical markers of treated wastewater (enantiomer fraction of the pharmaceutical compound propranolol and ethylenediaminetetraacetic acid), and microcosm studies designed to evaluate the survival of enterococci in wastewater effluent amended with stream sediments or macroalgae. Collectively, these studies reveal that enterococci in the Santa Ana River originate, at least in part, from growth in streambed sediments exposed to wastewater effluent. While streambed sediments appear to be a primary source of enterococci bacteria, wastewater effluent may play a secondary role by providing, for example, growth limiting nutrients or seeding the sediments with enterococci species and strains capable of growing in the environment.

KEYWORDS: enterococci, urban stream, treated wastewater effluent, southern California, sediment

Introduction

Pathogens and fecal indicator bacteria (FIB) are a leading cause of water quality impairment in urban streams and rivers [1-3]. Watershed transformations that accompany urbanization—increased impervious surface cover, hydraulic modifications of rivers and streams, and increased pathogen loading from point sources (effluent from wastewater treatment plants, WWTPs) and non-point sources (sanitary sewer overflows, illicit sewage discharges, bird and animal fecal matter)—all contribute to water quality degradation [2, 4]. In southern California, the semi-arid climate and high population densities create especially challenging conditions, in which it is frequently the case that urban streams are impaired for multiple contaminants (FIB, nutrients, metals, toxics), treated wastewater effluent is the dominant source of surface water, polluted dry and wet weather runoff discharge directly to streams without treatment, and the volume of runoff increases dramatically during storms [5-7]. For these and other reasons, the cost of implementing total maximum daily loads (TMDLs) for impaired streams in southern California is likely to be unprecedented, with estimates ranging as high as \$100 billion (2007 U.S. dollars) [6].

While dry and wet weather runoff is a well-documented source of FIB and pathogens to streams and coastal waters [2, 5, 7-9], even highly treated and disinfected WWTP effluent may contribute to water quality degradation in some cases. There are several reports, including most recently for the Los Angeles River, of situations where FIB concentrations decrease just downstream of a WWTP effluent discharge (presumably reflecting dilution of polluted stream water with FIB-free effluent), and then increase significantly with distance downstream [10, 11]. Possible explanations for this spatial pattern include [12]: (1) WWTP effluent contains live cells that escape treatment and disinfection and grow in the stream post-release; (2) WWTP effluent contains organic material or nutrients that stimulate the growth of FIB populations resident in the receiving water [13]; (3) WWTP effluent contains injured cells in a viable but nonculturable (VBNC) state that regain culturability (*i.e.*, resuscitate) in the stream

post-release [14]; and/or (4) FIB are from an unaccounted for non-point source that is unrelated to the WWTP discharge.

In this paper we describe field and laboratory studies aimed at identifying the source of enterococci (ENT) in an earthen stream where the flow consists entirely of disinfected and highly treated effluent from a large WWTP in southern California. The choice of ENT as a target water quality parameter is motivated by the fact that the U.S. EPA recommends its use for assessing water quality of both fresh and marine recreational waters [2]. We also explore the possibility that WWTP effluent and urban runoff possess unique microbiological-chemical fingerprints that can be used to apportion their relative contribution to flow in urban streams.

Materials and methods

Study area. The Santa Ana River (SAR) drains a 4,406 km² watershed in southern California that is home to approximately 6 million residents (as of 2000) and a large flood control structure, Prado dam, used to limit flow in the river during storms and provide base flow for groundwater recharge in the lower reaches of the river during dry weather periods [15]. The river drains three of the twelve most populous counties in the United States (San Bernardino, Riverside, and Orange) before discharging to the Pacific Ocean at Newport Beach and Huntington Beach [16]. As with many urban rivers in southern California, much of the SAR is listed by the U.S. Environmental Protection Agency (EPA) as impaired for recreational body contact, due to elevated concentrations of fecal coliform. Enteroviruses have also been detected in the ocean near the mouth of the river during storms [8]. Approximately 80 to 100 percent of the river's dry weather flow is from wastewater effluent [17] discharged from 12 WWTPs, each of which contribute a minimum of 3,785 m³day⁻¹ of effluent. The four largest WWTPs on the river account for 73% of the flow at 600,000 m³day⁻¹ [18].

The Riverside Regional Water Quality Treatment Plant is one of the largest WWTPs discharging to the SAR. This WWTP employs secondary biological and tertiary wastewater treatment, including activated sludge with filtration and chlorination/dechlorination. Treated wastewater effluent

(approximately $120,000 \text{ m}^3\text{day}^{-1}$) from this WWTP is discharged through a culvert into a soft bottom stream, referred to here as the “WWTP stream”, which contains no other source of surface water. Effluent flows down the WWTP stream for approximately 0.6 km, at which point the WWTP stream merges with a flood control channel referred to as the Anza Channel. The Anza Channel contains only runoff from the surrounding urban landscape (flow of approximately $6,100 \text{ m}^3\text{day}^{-1}$). The combined flow from the WWTP stream and Anza Channel flows downstream of the confluence for another 0.4 km before discharging to the SAR in the City of Riverside (Fig. 1 and Table S1).

Synoptic sampling. During dry weather in the summer of 2007, two 24-hour synoptic sampling studies were conducted. The first study (Study 1) was carried out July 31st to August 1st, and the second study (Study 2) was carried out August 29th to August 30th. Each study included four sampling events timed approximately six hours apart, during which water (both Studies 1 and 2) and sediment (only Study 2) samples were collected from, respectively, 13 and 10 sampling locations (see sampling sites, Fig. 1). Analyses performed on these samples are summarized in Table 1.

Water samples were grabbed close to the center of the stream into (1) two autoclaved 500 mL polypropylene (PP) bottles for culture-dependent FIB, pH, and nutrient assays; (2) one 4 L sterile glass amber bottle for chemical marker assays; and (3) one 2 L sterile PP bottle for culture-independent (qPCR) assays of ENT and the human fecal marker HF183 *Bacteroides*. Immediately following collection in the field, water samples for culture-dependent FIB assays were amended with one mL of 0.1 N sodium thiosulfate to remove any residual chlorine (the WWTP dechlorinates its effluent prior to discharge), and water samples slated for culture-independent assays were passed through 22-25 micron pore size Miracloth (Calbiochem/EMD Biosciences, San Diego, CA) to remove large particles. Sediment samples were collected into sterile 50 mL plastic centrifuge tubes (Fisherbrand, Pittsburgh, PA) from the upper 3 cm of the streambed at the stream’s thalweg. Water temperature (Infrared gun, Fluke, Everett, WA) and pH (Model 720A+ Thermo Orion, Waltham, MA) were measured on site. After collection, all water and sediment samples were immediately capped and stored on ice in the dark. Culture-dependent FIB assays were conducted within 6 h at an onsite field laboratory. Water samples

for culture-independent and chemical marker assays were shipped by same-day or overnight courier to laboratories at UC Santa Barbara and UC Berkeley, respectively. Splits of water samples were sent to TestAmerica (Irvine, CA) for dissolved organic carbon (DOC) and ANR Analytical Lab (Davis, CA) for Total Kjeldahl Nitrogen (TKN), PO_4 , NH_4 , and NO_3 . All nutrient analyses were performed using standard methods [19].

Laboratory Microcosms. Fourteen microcosms were prepared by mixing, in 4 L sterilized Erlenmeyer flasks, effluent from the WWTP stream (with or without filter sterilization) and varying wet weights of sediment or macroalgae. The sediment and macroalgae laboratory microcosms were incubated in a constant temperature room (temperatures reported in Table 2), and water samples (10 mL) were aspirated from the microcosms every three hours for the first 12 hours, again at 24 hours, and then every 24 hours for an additional 3 days. All water samples were analyzed for culture-dependent FIB using defined substrate tests (Colilert-18 and Enterolert, described later). Effluent, sediment, and macroalgae were collected from the WWTP stream as follows. Effluent was collected from the surface of the WWTP stream (at sites B1, B5, and B6 depending on the microcosm) into autoclaved 2 L PP bottles. A split of the sample was immediately filter-sterilized by passage through a tangential flow filter (Pall Filtron Centramate, Pall Corporation, East Hills, NY) using a 0.2 μm pore size cartridge. Macroalgae was collected aseptically from the sides of the WWTP stream at site B1 and placed into 500 mL autoclaved PP bottles. Sediment was collected into sterile 500 mL PP bottles from the upper 3 cm of the streambed at the channel center of site B5. Water, sediment, and macroalgae samples were transported on ice in the dark to UC Irvine, where microcosms were prepared within 6 hours of sample collection. Microcosms were stirred in the morning, during sampling, and in the evening of each day to reduce the formation of spatial gradients.

Field Microcosms. Twelve microcosms were prepared by combining filtered or unfiltered effluent from the WWTP stream with varying wet weights of sediment or macroalgae in 4 L dialysis membrane bags (MWCO 12000-14000 Da, Spectra/Por Dialysis Membrane, Rancho Dominguez, CA).

The dialysis bags were sealed, affixed to a crate, and immersed just below the surface of the water column in the WWTP stream at site B5. Following deployment, water samples (10 mL) were aspirated from the dialysis bag every three to four hours for the first 24 hours; a final water sample was collected 48 hours after the start of the experiment. All water samples were analyzed for culture-dependent FIB using defined substrate tests (Colilert-18 and Enterolert). Protocols for collecting effluent, sediment, and macroalgae from the WWTP stream are the same as that described above for laboratory microcosms.

Culture-dependent FIB assays (Colilert-18 and Enterolert). Culture-dependent concentrations of *Escherichia coli* (EC) and ENT were determined using the defined substrate tests Colilert-18 and Enterolert, implemented in a quantitative (96 well-quantitray) format (IDEXX, Westbrook, ME). Analysis of water samples followed manufacturer's protocol, with 10 mL of water sample diluted into 90 mL of deionized water. Analysis of sediment samples involved an FIB extraction step, followed by analysis of the extract using Colilert-18 and Enterolert. The FIB extraction step was similar to the protocol in Craig et al. [20], but optimized for southern California sediments by the Orange County Public Health Laboratory (Joe Guzman, personal communication). Briefly, 10 g of wet sediment was suspended in a 150 mL sterile bottle with 100 mL of 1% sodium metaphosphate, followed by sonication for 30 seconds (Branson Sonifier 450, Danbury, CT). The mixture was then allowed to settle for 20 minutes, and 10 mL of the supernatant was analyzed using Colilert-18 and Enterolert.

Culture-independent (qPCR) assays for HF183 Bacteroides and ENT. The UltraClean Water DNA Kit (MoBio Laboratories, Carlsbad, CA) was used to collect bacteria from the water and extract the DNA. DNA was extracted following manufacturer's recommendations followed by ethanol precipitation. Total DNA was quantified using the Quant-iT PicoGreen® dsDNA kit (Molecular Probes/Invitrogen, Carlsbad, CA). All quantitative PCR was performed in 25 µl volumes, using iQ 96-well Real-Time PCR plates and optical sealing tape (BioRad, Hercules, CA), in an iQ5 thermocycler (Bio-Rad, Hercules, CA). The human-specific HF183 *Bacteroides* 16S rRNA genetic marker was quantified using SYBR® Green I detection, using the qPCR Core kit for SYBR® Green I (Eurogentec,

San Diego, CA), with primers and identical reaction conditions as in Seurinck et al. [21] except for the addition of fluorescein (Eurogentec, San Diego, CA) to enable collection of well factors. Quantitative PCR for *Enterococcus spp.* was performed using the TaqMan® Universal PCR Master Mix, no Amperase® UNG (Applied Biosystems, Foster City, CA), with primers ECST748F/ENC854R and the FAM/TAMRA labeled probe GPL813TQ (Operon Biotechnologies, Huntsville, AL) [22]. For more detailed information regarding the methods for DNA extraction, detection of HF183 markers, and the qPCR for *Enterococcus spp.* refer to Supplemental Information (SI).

Chemical marker assays. Water samples were analyzed for ethylenediaminetetraacetic acid (EDTA) using a modified version of the method described by Ridge and Sedlak (2004) based on the measurement of EDTA as an Fe (III) complex [23]. Water samples were analyzed for enantiomer fraction (EF) of the pharmaceutical compound propranolol using a modified version of the method described by Fono and Sedlak (2005) [24] (see SI for details).

Principal Component Analysis. During Studies 1 and 2, water samples were collected from not only the WWTP stream, but also from the following sites: Anza Channel (site C), downstream of the confluence of the WWTP stream and Anza Urban Runoff channel (mixed effluent/runoff stream, Site D), and upstream and downstream of the confluence between the mixed effluent/runoff stream and the SAR (sites E and F, respectively) (map in Fig. 1). Altogether, samples from 8 sites (A, B3, B5, B6, C, D, E, and F) were analyzed for 13 different chemical and microbiological constituents (Table 1, Table S1 and Table S2). Principal Component Analysis (PCA) was carried out (SPSS 16.0, Chicago, IL) on this large data to determine if source waters at our field site possess unique biological-chemical fingerprints. Only samples for which all analytes were measured were included in the PCA (n= 44), and measurements were first converted to standardized z-scores to allow comparison of analytes with different units. The five most significant principal components (PCs) were further analyzed using Varimax rotation to elucidate the most influential analytes in those five PCs [25].

Results

ENT in the WWTP stream. The concentration of culture-dependent ENT was near or below the detection limit (<10 most probable number (MPN)/100 mL) in water samples collected from within the WWTP (sites P1 and P2) and at the discharge culvert (site A) (blue and black circles, bottom left panel, Fig. 1). However, once effluent entered the earthen channel, the concentration of culture-dependent ENT rapidly increased with distance downstream, from $<10^1$ at site B1 to $10^{2.5}$ at site B6 (units of MPN/100 mL). The downstream rebound in culture-dependent ENT was observed during all eight daytime and nighttime sampling events conducted during Studies 1 and 2 (Table S2). Measurements of culture-independent ENT by qPCR also rebound rapidly with distance downstream, from 10^2 DNA copies/L at site A to $10^{4.5}$ DNA copies/L at site B6 (blue and grey bars, bottom left panel, Fig. 1).

ENT in the WWTP streambed sediment. Culture-dependent ENT were detected at high concentrations (*ca.* 10^3 MPN/10 g dry weight) in all sediment samples collected from the WWTP stream (bottom right panel, Fig. 1).

Human fecal marker in the WWTP stream. HF183 *Bacteroides* was not detected in water samples collected from the WWTP stream during Studies 1 and 2, implying its concentration in the water column was < 900 targets/L (equivalent to 56 copies/rxn).

Chemical markers in the WWTP stream. EDTA and EF measured in the WWTP stream exhibit no consistent downstream trends. When averaged over the two studies, EDTA concentrations varied from $0.71 \pm 0.22 \mu\text{M}$ at site A to $0.85 \pm 0.23 \mu\text{M}$ at sites B5 (Study 2) and B6 (Study 1), which are within the range reported for other wastewater effluents [26, 27]. Because the propranolol concentrations were relatively low during Study 2, EF ratios were estimated only for water samples collected from the WWTP stream during Study 1. During Study 1, EF ratios were relatively constant with distance downstream in the WWTP stream, ranging from 0.49 ± 0.06 at site A to 0.51 ± 0.05 at site B6 (Table 1). EF ratios measured in samples of WWTP effluent at site A (before the effluent enters the earthen

channel) are higher than typically seen for WWTP effluent, and in the range previously reported for raw sewage [24, 28].

Microcosm studies. Two statistics are reported for each microcosm in Table 2: the ratio of final to initial ENT concentration (ENT_f/ENT_i) and a first-order die-off rate k (or time to 90% die-off, T_{90}). With one exception (B1 water amended with macroalgae in the laboratory), the ratio ENT_f/ENT_i was less than unity, implying that the concentration of ENT in the microcosms declined over the 2 to 4 day period of measurement. First-order decay rates estimated from the laboratory ($T_{90}= 20$ to 28 hours) and field microcosms ($T_{90}= 14$ to 23 hours) are similar. While ENT concentrations declined over time in any given microcosm, the initial ENT concentration was generally higher in microcosms amended with more sediment (i.e., lower water:sediment ratios, Fig.2B, C, E, and F). Microcosms amended with macroalgae also had higher ENT concentrations, compared to the no-macroalgae control (Fig. 2A and D).

Principal Component Analysis. PCA was performed to determine if data collected during the synoptic studies provide a “chemical-biological fingerprint” of source waters (Fig. 3 and Table S4 and S5). The top five PCs account for 77 percent of the data variance, and the top two PCs (PC1 and PC2) account for 35 and 14 percent of the total variance, respectively. After Varimax rotation, the top two PCs account for 21 and 18 percent of the total variance. Cross-plots of Varimax rotated PC1 and PC2 loadings clearly discriminate WWTP stream samples (group I in top panel, Fig. 3) from runoff samples (group II in top panel, Fig. 3). Varimax rotated PC1 is characterized by significant positive coefficients for PO_4 , TKN, and EDTA, while Varimax rotated PC2 is characterized by significant positive coefficients for N:P and NO_3 . PC1 and PC2 loadings for water samples collected from site F (group IV, top panel in Fig. 3) plot closer to WWTP effluent (group I), consistent with estimates from EDTA measurements that 70% of the water at site F is a mixture of WWTP effluent and urban runoff from site D. PC1 and PC2 loadings for water samples collected from site E (group III, top panel in Fig. 3) plot

closer to urban runoff (group II), implying that water collected from the SAR upstream of the location E is dominated by urban runoff.

Discussion

Source of ENT in the WWTP stream

The most important finding reported in this study is that ENT concentrations in highly treated and disinfected effluent increase rapidly with distance downstream of a WWTP discharge (>100 fold increase over 0.5 km). This rebound in ENT could be attributable to several different processes, as outlined next.

Untreated sewage inputs. HF183 is a sensitive indicator of fresh human fecal contamination in surface waters [21, 29, 30]. The fact that this marker was not detected in the WWTP stream suggests that the rebound of ENT is not attributable to unaccounted for sources of human waste, such as raw sewage or bather shedding (on occasion, people were observed bathing in the WWTP stream). The relative constancy of the EF ratio and EDTA concentration between sites A and B6 is consistent with this conclusion.

Resuscitation of VBNC cells. Both culture-dependent (Enterolert) and culture-independent (qPCR) measurements of ENT increased dramatically from site A to B6. If the rebound in culture-dependent ENT was caused by resuscitation of VBNC cells, the concentration of culture-independent ENT should remain constant with distance downstream, contrary to observations. Instead, the downstream rebound of culture-independent ENT implies that new ENT cells are being added to the effluent as it flows downstream.

Growth in the water column. From the measured rebound in ENT concentrations with distance downstream, and assuming all of the rebound is attributable to growth of ENT in the water column, we calculate apparent doubling times of 1.7 +/- 0.5 and 1.8 +/- 0.13 minutes for Studies 1 and 2, respectively (based on a 13 minute travel time between sites A and B4, data not shown). These apparent doubling times are at least 20 times less than the fastest doubling time reported in the literature for ENT,

approximately 40 minutes under optimal growth conditions in the laboratory [31]. Further, the laboratory and field microcosm data indicate that ENT does not survive well in WWTP effluent, even after amendment with sediment or macroalgae (T_{90} values range from 14 to 28 hours). Thus, it is unlikely that the rebound of ENT in the WWTP stream is caused by growth of ENT in the water column.

Growth on macroalgae. In laboratory and field microcosms, the addition of macroalgae resulted in higher initial concentrations of ENT, implying that macroalgae can release ENT to the surrounding water. This result is consistent with other studies reporting that plant material is a source of ENT [32-34]. However, macroalgae was found exclusively at site B1 (where effluent from the WWTP pools after leaving the discharge culvert, personal observation) while most of the rebound of ENT occurred downstream of site B1.

Growth in sediment. Several lines of evidence support the hypothesis that streambed sediments are the primary source of ENT in the WWTP stream: (1) ENT are present in streambed sediments at relatively high concentrations (ca., 10^3 MPN/10 g dry weight); (2) when field and laboratory microcosms were amended with sediment collected from the WWTP stream, the concentrations of ENT in the water phase increased in proportion to the weight of sediment added; and (3) sediment samples collected from all sites in the WWTP stream had high ENT concentrations and therefore, unlike macroalgae, sediments are not a geographically localized source of ENT.

Practical implications

Ferguson et. al. [35] measured elevated ENT concentrations in tidally washed sediment collected from various locations along the coastline in Orange County, California, and hypothesized that resuspension of sediment was a source of ENT in the overlying water column. Studies in marine and freshwater environments, and across a variety of climates, support the idea that sediments can harbor high concentrations of fecal bacteria, and contribute to bacterial standard violations in the overlying water column [36-39]. Our study adds to these previous findings by demonstrating that sediment can be a source of ENT in wastewater effluent dominated urban streams. In southern California, TMDL

implementation efforts for urban streams are likely to focus on capturing and treating dry weather runoff [6]. However, our results suggest that such efforts may not eliminate ENT from urban streams if, as appears to be the case at our field site, these bacteria grow in effluent-exposed streambed sediments.

Our results also raise questions about the utility of ENT as an indicator of human health risk. On the one hand, ENT cells growing in sediment obviously have little utility as direct “indicators” of human waste. Because recreational water quality standards are derived from epidemiological studies conducted primarily at sewage-impacted freshwater or marine beaches [40], their validity in settings where ENT are from non-sewage sources is questionable [41]. On the other hand, the growth of ENT in sediments exposed to human wastewater effluent might constitute a health concern, given that antibiotic resistant strains of *Enterococcus faecium* are increasingly responsible for human infections, particularly in hospital settings [42].

Research at this site is ongoing, with the goal of quantifying the flux of ENT cells across the sediment-water interface and identifying environmental variables that trigger ENT proliferation in streambed sediments.

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Table 1. Sample analyses performed during each sampling synoptic study sampling location by site*.

Synoptic Study	Date and Time	EC	ENT	EDTA	EF	HF183	Total DNA	ENT DNA	Nutrients
1-1	7/31/07 12:31	All	All	Sub1	Sub1	Sub1	Sub1	Sub1	Sub1
1-2	7/31/07 18:30	All	All	Sub1	Sub1	Sub1	Sub1	Sub1	Sub1
1-3	8/1/07 4:45	All	All	Sub1	Sub1	Sub1	Sub1	Sub1	Sub1
1-4	8/1/07 7:37	All	All	Sub1	Sub1	Sub1	Sub1	Sub1	Sub1
2-1	8/29/07 18:59	All, Sed	All	Sub2	Sub2	Sub2	Sub2	Sub2	Sub2
2-2	8/30/07 7:00	All, Sed	All	Sub2	Sub2	Sub2	Sub2	Sub2	Sub2
2-3	8/30/07 9:50	All, Sed	All	Sub2	Sub2	Sub2	Sub2	Sub2	Sub2
2-4	8/30/07 13:05	All, Sed	All	Sub2	Sub2	Sub2	Sub2	Sub2	Sub2

*All = water samples at sites P1, P2, A, B1, B2, B3, B4, B5, B6, C, D, E, F; Sub1 = water samples at sites A, B1, B3, B6, C, D, E, F; Sub2 = water samples at sites A, B1, B3, B5, C, D, E, F; Sed = sediments at sites B1, B2, B3, B4, B5, B6, C, D, E, F.

Table 2. Enterococci decay rates with standard deviations, T_{90} values, average final to initial ENT concentration ratios, and experiment duration for microcosms.

Microcosm Type	Start Date and Time	Average decay rate and standard deviation (T_{90})	Average ENT_f/ENT_i	Experiment Duration
WWTP effluent + sediment (lab study, 29 °C)	8/20/07 15:00	0.08 +/- 0.02 hr ⁻¹ (28 hrs), n=5	0.39, n=5	96 hrs
WWTP effluent + sediment (field study)	9/24/07 17:00	0.1 +/- 0.05 hr ⁻¹ (23 hrs), n= 4	0.49, n=4	48 hrs
Filter-sterilized WWTP effluent + sediment (lab study)	8/20/07 15:00	0.38 +/- 0.27 hr ⁻¹ (6 hrs), n=4	0.52, n=4	96 hrs
Filter-sterilized WWTP effluent + sediment (field study)	9/24/07 17:00	NA	0.77, n=3	48 hrs
WWTP effluent + Macroalgae (lab study, 20 °C)	8/8/07 10:00	0.12 +/- 0.07 hr ⁻¹ (20 hrs), n=4	0.88, n=4	96 hrs
WWTP effluent + Macroalgae (field study)	8/28/07 10:50	0.16 +/- 0.11 hr ⁻¹ (14 hrs), n=3	0.58, n=4	45 hrs

Figure captions

Figure 1. Top panel is a map of the field area indicating sampling sites, as identified by the alphabetical assignment. ENT bacteria concentrations measured in the WWTP effluent stream (lower left panel) and sediments (lower right panel), respectively. Acronyms include Wastewater Treatment Plant (WWTP), Santa Ana River (SAR), and enterococci (ENT). This field site is located in the City of Riverside, County of Riverside, southern California. SAR flow rates observed upstream of Site E (at USGS Site 11066460) for Studies 1 and 2 were 173,000 m³/d and 155,000 m³/d, respectively. By comparison, flow rates measured or estimated in the WWTP effluent stream and Anza Channel are 120,000 m³day⁻¹ and 6,100 m³day⁻¹, respectively.

Figure 2. Microcosm study results. Panels A, B, and C are laboratory microcosms; panels D, E, and F are field microcosms. A and D microcosms contained 25 g (wet weight) of macroalgae with 3 L of effluent from sites B1, B5, or B6 (circles, asterisks, and asterisks respectively); panels B and E are microcosms with unfiltered effluent with sediment amendments from site B5 (low ratios indicate more sediment); and panels C and F are microcosms with B5 filter-sterilized effluent with sediment amendments. Laboratory microcosms with effluent:sediment ratios of 9:1, 4.5:1, and 2.25:1 were prepared from 1800 mL of B5 effluent and 200, 400, and 800 grams of wet sediment, respectively; the 1.8:1 ratio was prepared from 1440 mL effluent and 800 g wet sediment. Field microcosm effluent:sediment ratios of 9:1, 3.6:1, and 1.8:1 were prepared from 1800 mL B5 effluent and 200, 500, and 1000 g wet sediment, respectively.

Figure 3. Principal Component Analysis results. Top panel is a cross-plot of PC2 versus PC1, with letters designating the sampling locations (see map in Figure 1), and major grouping classified by boxes

and Roman numerals. Bottom panel are the principal component loading coefficients for PC1 (black) and PC2 (red).





