



City of Malibu

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August 6, 2010

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CALIFORNIA
COASTAL COMMISSION
SOUTH CENTRAL COAST DISTRICT

Chair Bonnie Neely and the Members of the
California Coastal Commission
Attn: A. Tysor
89 South California Street, Suite 200
Ventura, CA 93001

Re: 8/12/10 Agenda Item Malibu Lagoon State Park, City of Malibu, Los Angeles County
CCC CDP Application No.: 4-07-098

Dear Chair Neely and Members of the Commission:

I write in support of the long-awaited Malibu Lagoon Restoration Project ("project"); and, although the project provides an opportunity for improvements to an impaired water system, I also write to convey significant potential environmental consequences that must be addressed in the permit. The City of Malibu is committed to improving and protecting water quality and, to that end, the City appreciates your consideration of the following concerns.

The City requests that before the permit is approved, the Coastal Commission do the following:

1. Satisfy its obligations under CEQA by studying and identifying the potential environmental consequences of the project. The hydrology in the Lagoon is complex and recent studies (attached and discussed in detail below) have shed new light on the existence of bacteria and nutrients in the water. These findings suggest that the proposed work has the potential to increase bacteria and nutrients in the water. The Commission must fully understand the potential impacts expected from disrupting this complex hydrologic system before approving the permit.
2. Impose adequate mitigation measures for the project to mitigate and prevent degradation of water quality in the area.
3. Require monitoring before, during and after the project. The proposed bi-annual monitoring plan is not adequate to understand the baseline water condition before the project begins. Without a baseline from which to measure, the scientists cannot determine the impact that this project will have on water quality. The City also requests the Commission require more frequent monitoring to identify promptly activities that are degrading the water quality. The project will likely increase Total Coliform, Fecal Coliform and Enterococcus at Surfrider Beach during the height of recreational activities. The City and other watershed agencies can potentially be held responsible for



bacteria exceedances at Surfrider Beach and it is imperative that the monitoring be frequent enough to enable the permittee to identify and promptly cease activities that degrade the water quality in the area. The permit only contemplates potential impacts on Malibu Creek fresh water and needs to properly account for potential impacts at Surfrider Beach as well.

Additionally, the City has the following comments with respect to the water quality in the Lagoon.

- 1) The Santa Monica Bay Beaches Bacterial Total Maximum Daily (TMDL) Load limits are set for three constituents (Total Coliform, Fecal Coliform and Enterococcus)(FIB); however, the permit only references Total Coliform in one chart, and Fecal Coliform in another reference. The monitoring plan should address and require monitoring for all constituents subject to TMDLs in the area. Periodic sampling for human markers may also help participating agencies understand the source of bacteria.
- 2) Lagoons and estuaries, like Malibu Lagoon, are known to cause a net increase in bacteria loads especially when the physical conditions constrain naturally functioning systems. Research shows that high fecal indicator bacteria at Surfrider Beach and other coastal sites is most likely from bird feces in the sand and kelp, decaying vegetation and naturally occurring bacteria released from the lagoon sediments. (See exhibits 12, 14, 15, 21, 22, 24, 26 and 31). Further, monitoring results are particularly affected if the sample is taken at high tide and early in the day.

Recent studies by the United States Geological Survey (USGS) demonstrate that even when the Malibu Lagoon sand berm is closed, that fecal indicator bacteria can pass through the berm and affect sampling results at Surfrider Beach, if certain conditions are present. Additionally, multiple studies show that even when no human markers were present, fecal indicator bacteria limits were exceeded at Surfrider Beach. (See 13, 28, 32).

The project proposes to increase tidal exchange. This exchange is not clearly defined but presumably, the number of events and length of time the berm is open may increase during construction and as a result of the project. The historical monitoring records show that fecal indicator bacteria loads increase, and often exceed regulatory limits more often, when the berm is open.

- 3) The City supports the following restoration elements; however, as adequate environmental analysis demonstrates, the project has the potential to increase FIB and nutrient levels in Malibu Lagoon and Surfrider Beach. To mitigate such adverse impacts consider the following:
 - a. Re-contouring the slopes for the 12-acre western arms of the lagoon to create broad shallow slopes will increase the surface area and lagoon sediment area. This may increase the release of naturally occurring bacteria that will increase the potential for FIB exceedances at the three sample sites at Surfrider Beach, SMB – MC 1, 2 and 3 – commonly known as Malibu Lagoon, Malibu Pier and Malibu

Colony. Malibu Colony sample station is actually seaward of the lagoon at the most western extent. These exceedances can occur when the berm is open or closed. Re-contouring could also affect nutrients released from the lagoon sediment. The project is expected to more effectively reduce stagnation and increase oxygen availability in the lower depths of the lagoon through improved horizontal mixing. The recent Lagoon studies indicate that the lower depths host the naturally occurring bacteria, which will now be more readily released to the surface waters where samples are collected.

- b. Revegetation may increase bacteria produced from the natural decaying process – more bank surface area and more vegetation will result in higher levels of bacteria. There may be some time periods when slow transit time can help remove bacteria by the vegetation; but in most scenarios, there will be a net increase of bacteria. It is noted that improved circulation and increased tidal flow, a goal of the project, will decrease contact time with lagoon plants capable of removing some bacteria. The project anticipates that there will be an increase in the discharge volumes and events at Surfrider Beach and affect water quality at sampling sites.
- c. The project proposes to increase the mudflats on the Eastern bank near the Adamson Boat House. This activity will increase foraging and bird habitat, surface area sediment contact, and bird feces, which is likely to increase the bacteria in Lagoon waters and can impact water quality at Surfrider Beach. Exhibit 20, water sampling location map, shows no sampling proposal for this element of the project. FIB exceedances were experienced in this specific area of Malibu Lagoon, as discussed in the recent UCLA and USGS studies. The City requests the permit be revised to include a 9th sampling location between the newly created mudflats and boathouse channel and the Lagoon sand berm.
- d. The staff report, environmental analysis and permit fail to consider the potential of spreading the invasive New Zealand mudsnails (known to inhabit Malibu Lagoon). The permit should include conditions to ensure that equipment and tools used at the project site are subject to the current protocol to prevent the spread of the snails to other reaches or other creeks where the equipment may do future work. Soil disposal activities must be similarly conditioned. Vegetation removal and dispersal could also transfer this highly invasive species. Many of the background studies referenced in the staff report were conducted prior to the invasion of the New Zealand mudsnail; hence, the staff report and permit does not adequately address this concern.
- e. Include a provision to indemnify the City for any water quality violations that arise from exceedances caused by or primarily contributed to as a result of the Lagoon Restoration Project.

- f. The background studies referenced in the staff report have been superseded with more recent studies that can provide relevant information about the complex hydrology in the Lagoon and surrounding areas. The staff report only refers to a 1999 URS Greiner Woodward Clyde study and the 2004 Stone Environmental study related to impacts from onsite wastewater treatment systems. These references have been enhanced by extensive relevant studies conducted by UCLA (28, 32) and USGS (21, 22, 24, 26, and 31), and SCCWRP (13, 14, 20).
- g. Historical drainage from the Malibu Colony residential neighborhood may be significantly impacted by the project. The City has agreed to allow two, 4 inch drain diversions into its Civic Center stormwater treatment facility (SWTF) if there is a bypass to account for overflow. This will allow for diversion of dry-weather urban runoff and limited stormwater flows so that it can be treated and disinfected; however, the surface flows have not been accounted for in the permit. Malibu Colony residents have reported concerns that the project's proposed solid block wall could obstruct historically occurring surface flows from the rear yards with potential flood impacts, if a mitigation measure is not required. The City requests a revision to the project plan to account for these surface flows and prevent significant flooding during rain events.

Again, the City supports a more efficient, better functioning lagoon system, but requests that the project be properly conditioned to prevent any unintended water quality degradation. Adequate environmental analysis is essential to determine the appropriate mitigation measures.

The following questions and comments from the staff report should also be addressed before the permit is issued:

Page 11 3.A.e. If construction equipment cannot be cleaned on the temporary berm, parking lot or trails, where does the permit specify that cleaning will take place?

Page 12 4. The dewatering Plan focuses on protection of aquatic species; however, human health could be affected depending on when and where the water is discharged and the results of increasing flow rates within the Lagoon.

Page 15 iv., c. and d. Sediment samples should also include FIB analysis and appropriate human markers analysis. Vertical profiles should be conducted quarterly, note all physical conditions, and be performed throughout the day to account for heat, sunlight, and tidal influences.

Page 16 & 17 Success Measures and Supplemental Measures

The report fails to discuss the increase in bacteria and resulting potential impact on human health for swimmers and surfers at Surfrider Beach. Since research shows that lagoons and estuaries contribute bacteria to the near shore sampling locations, what measures will be required if there is an increase in bacteria?

Page 24 16. Any excavated material should be monitored for the presence of New Zealand mudsnail.

Page 28 Dewatering

It is not clear from the project description where water will be discharged. If it is discharged into the main channel of the Lagoon, the discharge will cause an unnatural breach of the Lagoon berm and increase FIB loads at Surfrider Beach. The proposed filtration methods using carbon and resin vessels will not disinfect, and using only chlorine for disinfection at these flow rates is not recommended. The proposed list of constituents for testing only includes fecal coliform. All three FIB must be monitored during the dewatering process.

The City is also concerned about the dewatering process and the disinfection required prior to dispersal near Surfrider Beach. The staff report does not provide enough information about the Los Angeles Regional Water Quality Control Board's dewatering permit, the discharge location(s) and the actual constituents to be monitored. The City requests more information on the permit and has requested also requested a copy from the RWQCB.

Page 33 Riparian Forest Picnic Area

It is not clear whether or not this area will include a public toilet facility. The project should include a public facility, as the nearest facility is quite far from this area. City staff has observed toilet paper remains in the area, indicating that visitors may not go find the nearest public facility.

Page 33 "Adamson House Wall" is actually the Malibu Colony Wall

As noted earlier, an unintended consequence of the solid concrete masonry wall may increase flooding in the neighborhood directly to the south of the project if historical surface flows are subsequently impeded. There was no analysis of potential impacts or mitigation measures in any engineering study provided in the CCC staff report or environmental review documents for the project.

Page 42 and 43 Water Quality Conditions

The reference studies were conducted prior to 2005. The baseline for nutrients and bacteria should be determined from more contemporary studies and from studies that utilize state-of-the-art analytical methodologies. There has been extensive research conducted in the groundwater and surface waters that migrate to Malibu Creek and Lagoon and significant capital improvement projects have been completed since 2005. Aging onsite wastewater treatment systems along Malibu Creek have been replaced with the most advanced treatment systems. Since 2007, almost all surface flows from the Malibu Lagoon sub-watershed have been intercepted, filtered and disinfected resulting in the elimination of bacteria and significant removal of nutrients from the developed areas in the Malibu Civic Center.

In 2009, USGS conducted extensive nutrient and bacteria monitoring throughout the Civic Center area, near shore, Lower Malibu Creek, Malibu Lagoon and upcoast in the Malibu Colony and just off shore in both dry- and wet-weather conditions when the berm was closed and open. The primary source of bacteria is from natural sources such as avian feces deposited into the Creek and Lagoon, decaying vegetation, avian feces in the kelp and sand. Using the most up to date analysis, no human bacteria was found at Surfrider Beach by the University of California at Los Angeles (28, 32) nor in Malibu Creek and Lagoon or Surfrider Beach by researchers from SCCWRP in 2005 (13, 14 and 20) and in the extensive investigations by USGS in 2009 (21, 22, 24, 26, 31).

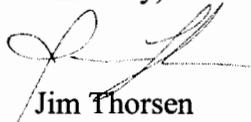
The Commission's staff report comments on Page 43 that the source of fecal indicator bacteria is from wastewater treatment facilities upstream and leaching from septic systems in the immediate vicinity of the Lagoon. This statement is not accurate; recent scientific data indicate that bacteria comes from avian sources and kelp, among other things. These attached studies should be considered, as they provide the best data on Lagoon Hydrology.

The City also requests the opportunity to review and comment on the final water monitoring plan before the Executive Director approves the plan. All water quality sampling during and post-construction must reflect the regulatory requirements for Santa Monica Bay Beaches Bacteria TMDL, since the project description anticipates a high likelihood that the berm will breach and/or there will be direct discharge to Santa Monica Bay. This would include Total coliform, Fecal coliform and Enterococcus. The staff report states that the permit allows for the discharge of 1.3 million gallons per day into Santa Monica Bay. Chlorination alone, without ozone or ultraviolet disinfection, is an uncertain process, especially for the high volume and flow rate anticipated.

Lastly, the City would request that the Project Manager provide contact information where he or she can be reach 24 hours a day, seven days a week. The public will generally contact the City of Malibu with emergency concerns and having this information will reduce response time.

Thank you for your consideration of these comments

Sincerely,



Jim Thorsen
City Manager

Enclosures: City of Malibu Comment Letter Reference Documents

Cc: Mayor Wagner and Honorable Members of the Malibu City Council
Christi Hogin, City Attorney
Los Angeles Regional Water Quality Control Board

City of Malibu Comment Letter Reference Documents

	Title	Author	Date	Notes
12	Enumeration and Spaciation of Enterococci Found in Marine and Intertidal Sediments and Coastal Water in Southern California	Ferguson, Moore, et al.	January 2005	Journal of Applied Microbiology
13	Multi-Tiered Approach Using Quantitative Polymerase Chain Reaction For Tracking Sources of Fecal Pollution to Santa Monica Bay	Noble, Griffith, Blackwood, et al.	February 2005	Southern California Coastal Water Research Project Also published in American Society for Microbiology
14	Modeling the Dry-Weather Tidal Cycling of Fecal Indicator Bacteria in Surface Waters of an Intertidal Wetland	Sanders, Arega, and Sutula	July 2005	Department of Civil and Environmental Engineering, UC Irvine and Southern California Coastal Water Research Project
15	Final Report: Identification and Control of Non-Point Sources of Microbial Pollution in a Coastal Watershed	Sanders, Grant, Horne, et al.	February 2006	United States Environmental Protection Agency-National Center for Environmental Research
20	Fecal Indicator Bacteria (FIB) Levels During Dry Weather from Southern California Reference Streams	Tiefenthaler, Stein and Lyon	January 2008	Southern California Coastal Water Research Project
21	Coastal groundwater dynamics off Santa Barbara, California: Combining geochemical tracers, electronic seepmeters, and electrical resistivity	USGS Swarzenski, Izbicki	April 2009	Estuarine, Coastal and Shelf Science published by Elsevier
22	Sources of Fecal Indicator Bacteria in Urban Streams and Ocean Beaches, Santa Barbara	Izbicki, Swarzenski, et al	September 2009	United States Geologic Survey, Annals of Environmental Science
24	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	Interim Reports: 10/29/09, 1/11/10, 2/18/10	City of Malibu - United States Geologic Survey – Dry Weather
26	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	May 2010	City of Malibu - United States Geologic Survey – Wet Weather Power Point Presentation to RWQCB staff
28	Malibu Lagoon Bacterial Study PowerPoint Presentation	Ambrose, Jay et al	05/25/10	UCLA Bacterial study comparing FIB with Human-Specific bacteriodes in Lower Malibu Creek, Malibu Lagoon and Surfrider Beach
31	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	Report 6/25/10	City of Malibu - United States Geologic Survey – Wet Weather
32	Malibu Lagoon Bacterial Study	Ambrose, Jay et al	Report July 2010	UCLA Bacterial study comparing FIB with Human-Specific bacteriodes in Lower Malibu Creek, Malibu Lagoon and Surfrider Beach

Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California

D.M. Ferguson, D.F. Moore, M.A. Getrich and M.H. Zhouandai

Orange County Public Health Laboratory, Newport Beach, CA, USA

2004/1187: received 14 October 2004, revised 24 January 2005 and accepted 25 January 2005

ABSTRACT

D.M. FERGUSON, D.F. MOORE, M.A. GETRICH AND M.H. ZHOWANDAI. 2005.

Aims: To determine the levels and species distribution of enterococci in intertidal and marine sediments and coastal waters at two beaches frequently in violation of bacterial water standards.

Methods and Results: Faecal indicator bacteria were extracted from sediment and enumerated using membrane filtration. High levels of enterococci were detected in intertidal sediments in a seasonal river and near a storm drain outlet. Low levels were found in marine sediments at 10 m depths and in surf zone sand. Bacterial isolates presumptively identified as *Enterococcus* on mEI media were speciated. The predominant species found in both water and sediment included *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus casseliflavus* and *Enterococcus mundtii*. A number of isolates (11–26%) from regulatory water samples presumptively identified as enterococci on mEI media were subsequently identified as species other than *Enterococcus*. At both study sites, the distribution of species present in water was comparable with those in sediments and the distribution of species was similar in water samples passing and exceeding bacterial indicator standards.

Conclusions: High levels of *Enterococcus* in intertidal sediments indicate retention and possible regrowth in this environment.

Significance and Impact of the Study: Resuspension of enterococci that are persistent in sediments may cause beach water quality failures and calls into question the specificity of this indicator for determining recent faecal contamination.

Keywords: beach pollution, enterococci, faecal indicator bacteria, marine sediments, water quality.

INTRODUCTION

In 1999, California adopted new, more extensive ocean recreational water quality standards (AB411 1999). The United States Environmental Protection Agency (USEPA) numerical standards for enterococci, total coliform and faecal coliform bacteria (USEPA 1986), which are used to indicate faecal contamination in marine waters, were implemented along with regulations for increased testing of recreational water. In southern California, the implementa-

tion of all three faecal indicator bacteria standards along with intensified testing led to an increased number of beach sites that exceeded standards (Noble *et al.* 2003). Beaches that fail any of these standards must be posted with warning signs or closed for swimming. The *Enterococcus* standard has proven to be the most sensitive of the three indicator bacteria. In the summer dry weather season, 60% of water quality failures are the result of exceedances of the *Enterococcus* standard alone (Noble *et al.* 2003). Summer beach postings and closings have resulted in public pressure on governmental agencies to take action to improve recreational water quality.

The two beaches studied here are representative of southern California open ocean and harbour pocket beaches

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with ongoing bacterial indicator failures during summer dry weather. Numerous studies conducted at both sites ruled out obvious large point sources of faecal contamination such as leaking sewer lines and outfalls. Nonpoint sources, including urban runoff were suggested, but no definitive source(s) were identified (Grant *et al.* 2001; Boehm *et al.* 2002; Kim *et al.* 2004; Noble and Xu 2004). Subsequently, water quality improvement projects, including storm drain diversions were implemented. Yet, indicator failures at these beaches continue. In this study, we investigate a less obvious nonpoint source of indicator bacteria: intertidal or marine sediments. Laboratory and field studies have demonstrated long-term survival of indicator bacteria such as *Escherichia coli* and other faecal coliforms in sediments (Gerba and McLeod 1976; LaLiberte and Grimes 1982). High densities of faecal coliforms (Valiela *et al.* 1991), faecal streptococci (Sayler *et al.* 1975; Obiri-Danso and Jones 2000) and enterococci (Anderson *et al.* 1997) found in marine sediments are suggestive of natural or environmental sources of contamination to overlying water. Regrowth of *E. coli* and enterococci was shown to occur in river sediments (Desmarais *et al.* 2002) and in soil, water and plants (Byappanahalli *et al.* 2003). Recently, indicator bacteria in sediments was directly linked to beach water quality failures. In England, resuspension of sewage impacted intertidal sediments was suggested as the cause of exceedances of regulatory standards (Obiri-Danso and Jones 2000). In New Zealand, resuspension of enterococci in sediments impacted by stream and storm water contributed to elevated levels in beach water (Le Fevre and Lewis 2003).

The objective of this study was to determine if intertidal or marine sediments harbour faecal indicator bacteria that could contribute to recreational water pollution at Huntington State Beach and Dana Point Harbor Baby Beach. The levels of indicator bacteria in marine and intertidal sediments from areas most likely to impact these beaches were determined. Enterococci isolated from sediments and recreational water were further characterized by identification to species level. The distribution of *Enterococcus* and enterococci-related species were compared in sediments *vs* beach water and in water samples passing or failing regulatory bacterial standards to determine possible relationships.

MATERIALS AND METHODS

Study sites

Dana Point Baby Beach is a small pocket beach *c.* 118 m wide and located inside an artificial harbour. A breakwater allows minimal current flow and protects the beach from ocean swell and currents (Fig. 1). Two storm drains discharge runoff from local residences, businesses, streets and parking lots to the west or east end of the beach. Beach

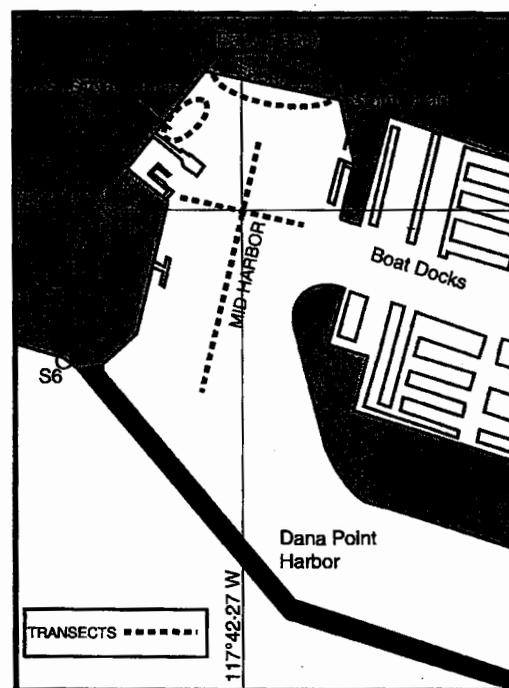


Fig. 1 Sampling locations at Dana Point Baby Beach

usage includes swimming and kayak launching. Boat docks and a pier are located adjacent to the beach. Remediation actions that have been implemented include plugging and diverting storm drains during the summer to prevent urban runoff flow into the beach, installing bird netting below the pier and restricting bird feeding to reduce direct faecal contamination. The beach water is sampled once a week at four sampling sites and tested for total and faecal coliforms and enterococci. During the study period, there were failures because of at least one bacterial indicator group on 32 of 90 (35.6%) sampling days; 66% of all indicator failures were caused by *Enterococcus*.

Huntington State Beach spans *c.* 7.2 km and is bordered by the Santa Ana River (SAR) and Talbert Marsh (TM) outlet on the south-east and Huntington City Beach on the north-west (Fig. 2). The SAR is a seasonal river/flood control channel where tidal flows in the channel can reach as far as 7.7 km inland during spring tides (Grant *et al.* 2001). Approximately 535 200 m³ of sediment comprised of gravel, sand and mud lies in the channel from the mouth to *c.* 5.8 km upriver. The channel is lined with cement walls or rock boulders. All major contributing storm drains are diverted during the summer, so the water in the SAR is almost exclusively tidally induced flow with minimal urban runoff. The Talbert Marsh outlet channel is located 290 m north-west of the SAR. Storm drains leading into the marsh

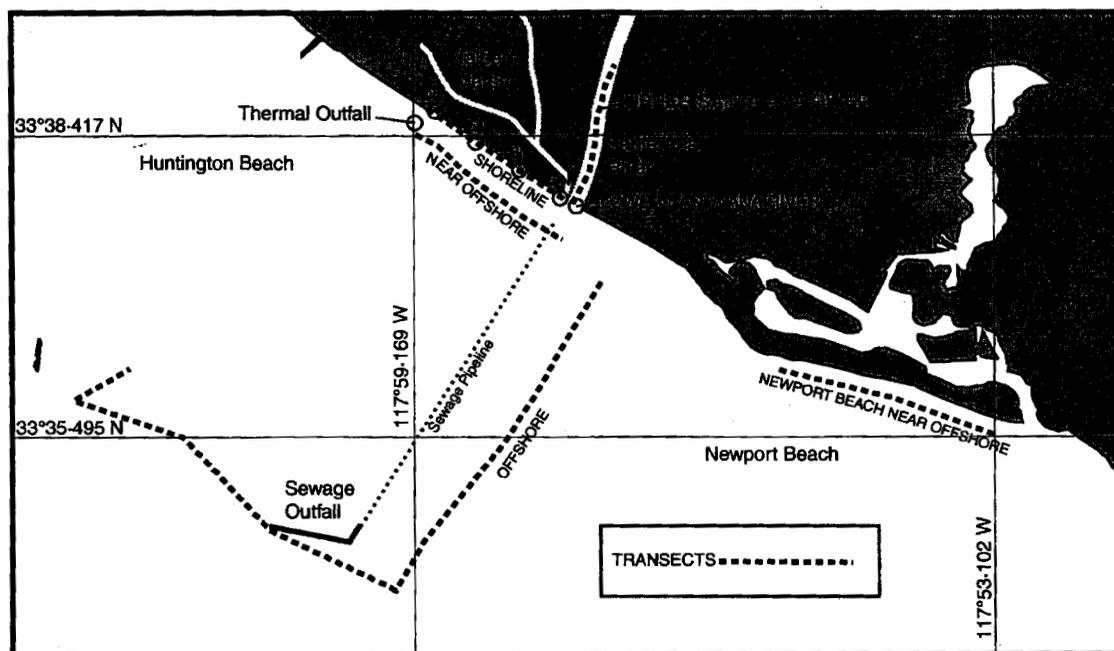


Fig. 2 Sampling locations at Huntington State Beach

are also diverted during summer. A sewage outfall lies 7.6 km offshore from the SAR mouth and releases $c. 10^6 \text{ m}^3$ per day of chlorine treated sewage in 60 m of water. A thermal outfall of a power plant, located $c. 700 \text{ m}$ offshore discharges a maximum of $1.9 \times 10^6 \text{ m}^3$ per day of water at 10 m depth. Previous studies did not find direct evidence implicating the sewage (Noble and Xu 2004) or thermal outfalls (Kim *et al.* 2004) as sources of pollution to the beach. Surf zone water from regulatory sampling sites: 0N, 3N, 6N and 9N corresponding to 0, 914, 1829 and 2743 m north-west of the SAR are monitored five times weekly for indicator levels (Fig. 2). During the study period, beach failures for at least one bacterial indicator occurred on 8 of 31 (25.8%) sampling days; 57% of all indicator failures were because of *Enterococcus*.

Sampling strategy

To determine sediment indicator bacteria densities, samples were taken along transects at onshore beach and river sites (intertidal) and offshore (marine) areas suspected of impacting the beach water and from two control sites adjacent to beaches with generally low to nondetectable levels of indicators. The transect locations are shown in Figs 1 and 2 and described in Table 1. At Baby Beach, water and sediment samples were collected between 7 August 2002 and 20 November 2003. At Huntington State Beach, the water samples were collected

between 5 August and 15 September 2003 for regulatory monitoring purposes. Sediments from the SAR were collected between 23 December 2003 and 24 January 2004 along an upper and lower transect that was delineated by the Pacific Coast Highway Bridge. Near offshore sediment samples were collected about 330 m offshore Huntington State Beach at 10 m depths. The north-west end of this transect was in the thermal outfall area. Offshore sediment samples were collected around the sewage outfall. The Newport Beach near offshore control transect starts at $c. 4.0 \text{ km}$ south-east of the mouth of the SAR (Fig. 2).

Sample collection methods

Offshore. Sediment samples from the ocean bottom were collected by boat using a Van Veen grab sampler (Kahl Scientific Instrument, El Cajon, CA, USA) that was rinsed between sampling stations by submerging it in seawater. A portion (100 ml) of the water overlaying the sediment was collected to compare the levels of indicator bacteria in water to sediment. The water was then decanted and $c. 75 \text{ g}$ of the top 2 cm of sediment was aseptically scraped into a 100 ml sterile bottle.

Intertidal. Sediment samples were collected from the intertidal river or shoreline sites at negative tide levels to avoid collecting overlying water. Approximately 75 g of the

Table 1 Description of sediment transects

	Sediment type	Number transects	Number samples	Transect length (m)	Transect spacing (m)	Water depth (m)
Dana Point Baby Beach						
Shoreline	Intertidal	21	168	120	10	NA
West Storm Drain	Intertidal	27	269	60	3	NA
Mid-Harbor	Marine	2	14	380	20	0.5-6
S-6 (Control)	Intertidal	NA*	13	NA	NA	NA
Huntington State Beach						
Upper Santa Ana River	Intertidal	2	35	2520	90	NA
Lower Santa Ana River	Intertidal	1	15	400	30	NA
Shoreline	Intertidal	1	10	3600	300	NA
Near offshore	Marine	2	31	3200	160	10
Offshore (sewage outfall)	Marine	1	10	15 240	670-2597	10-51
Newport Beach near offshore (control)	Marine	1	15	4950	330	10

*NA, not applicable.

top 2 cm of sediment was collected into a sterile bottle, taking care to avoid bird droppings. Water samples from the beach shoreline sites were collected at ankle depth using a sterile bottle (100 ml) that was clamped to a sampling pole. The pole was extended to obtain samples at ankle depth at a short distance away from the sample collector.

Sample processing

Water and sediments were held at 5–10°C and analysed for faecal indicator levels within 6 h of collection. To extract bacteria from sediments, 10 g of sediment was suspended in 100 ml of 1% (w/v) sodium metaphosphate (Valiela *et al.* 1991) and sonicated at the rate of 30% output using a Branson Sonifier® Cell Disruptor 450 (13 mm tip; Branson Ultrasonics, Danbury, CT, USA) for 30 s. Sonication time and intensity were previously optimized in our laboratory (D. M. Ferguson, D. F. Moore and M. A. Getrich, unpublished data). Suspended sediment and water samples were analysed using the membrane filtration method as per Standard Methods (APHA 1998). Total coliforms were enumerated using mENDO agar incubated for 24 h at 35°C. Faecal coliforms were enumerated using mFC agar incubated for 24 h at 44.5°C. Enterococci were enumerated using mEI agar incubated for 22–24 h at 41°C (USEPA 2000). Faecal indicator levels were reported as colony forming units (CFU) per 100 ml of water or CFU per 10 g of wet weight sediment.

As marine sediments are mixed with water trapped within sediment macropores, the concentration of indicators present in the overlying water was determined to account for bacteria present in the water fraction of sediment. The water content of each sediment sample was determined as the difference in weight before and after drying sediments overnight in an oven at 105°C.

Enterococci speciation

Colonies on mEI media that had blue halos were considered presumptive for *Enterococcus* species as per USEPA Method 1600 (USEPA 2000). Up to five colonies per sample were subcultured onto Trypticase™ soy agar with 5% sheep blood (BBL, Bethesda, MD, USA) and incubated at 35°C for 24 h. In some cases, there were fewer than five colonies present per sample. Isolates were identified to species level using the API™ 20 Strep identification system (API; bioMérieux, St Louis, MO, USA) and additional biochemical testing. The biochemical test results were interpreted using published standard biochemical identification charts (Facklam and Collins 1989; Facklam and Elliot 1995; Facklam 2002; American Society for Microbiology 2003). Biochemical tests included: carbohydrate fermentation with 1% mannitol, sorbitol, arabinose, raffinose, sucrose, lactose and inulin; Motility Test Medium w/TTC, pyrrolidonyl arylamidase (PYR) and leucine arylamidase (LAP) using disc tests (Remel, Inc., Lenexa, KS, USA); bile esculin, growth in 6.5% NaCl and at 45°C in brain–heart infusion broth, deamination of arginine in Moeller's decarboxylase broth (BBL, Franklin Lakes, NJ, USA); and catalase. Isolates that were not identified to species level that had positive reactions to PYR and LAP using API, esculin hydrolysis, growth at 45°C and tolerance to 6.5% NaCl were identified as *Enterococcus* species (American Society for Microbiology 2003).

Data analysis

The Pearson chi-square test in SPSS, version 12.0 for Windows, 2003 (Chicago, IL, USA) was used to test the statistical differences between enterococci species distribution in regulatory water samples passing and exceeding single sample standards.

RESULTS

Faecal indicator bacteria levels in sediments

The levels and percentage of samples positive for total coliforms, faecal coliforms and enterococci found in sediments from the two study sites are summarized in Table 2. Sediments from the Upper SAR transect adjacent to Huntington State Beach and West Storm Drain area at Dana Point Baby Beach had the highest percentage of positive samples as well as the highest geometric mean and maximum concentrations for all three indicator bacteria. At the Upper SAR, total coliforms and enterococci were found in 91.4% and 100% of 35 samples, respectively, with corresponding geometric mean concentrations of 1876 and 5922 CFU 10 g⁻¹. At the West Storm Drain area, total coliforms and enterococci were found in 61.8% and 66.5% of 269 samples, respectively, with corresponding geometric mean concentrations of 85 and 79 CFU 10 g⁻¹. Maximum concentrations were at the 10⁵ CFU 10 g⁻¹ level, or about 4 log higher than the geometric mean levels. Faecal coliforms were detected less frequently and at geometric mean concentrations that were about 1 log lower than total coliforms and enterococci.

At both study sites, indicator bacteria were also detected in shoreline and near offshore sediments but less frequently and at lower concentrations. Of the three indicators, *Enterococcus* was most abundant, followed by total coliforms and faecal coliforms with maximum geometric mean concentrations of 17, 9 and 3 CFU 10 g⁻¹ respectively. Most samples collected from a section of the Huntington Beach transect in a thermal outfall area of a power plant were below detection limits for indicators. As for the sewage outfall area, enterococci and faecal coliforms were not detected, however three sediment samples collected closest to the outfall pipe had low levels of total coliforms. Only a few sediment samples from near offshore Newport Beach (control area) were positive for indicators as compared with Huntington Beach near offshore, with similar bacterial concentrations found at both sites. At Dana Point Baby Beach, sediments collected from sites distant to the West Storm Drain, including the Mid-Harbor and a shoreline control site located outside the harbour, were generally below detection limit for all indicator bacteria.

Overall, *Enterococcus* was present more often and at higher concentrations in sediment samples when compared with total and faecal coliforms. Of a total of 580 samples from both study sites, 57.5% were positive for *Enterococcus*, 42.7% for total coliforms and 22.9% for faecal coliforms. Of all three indicators, the geometric mean levels of *Enterococcus* was highest in all transects except for the Baby Beach West Storm Drain and Huntington Beach offshore transects.

Water overlying marine sediment samples may contain bacteria that could affect the measurement of the bacterial

Table 2 Faecal indicator bacteria levels in sediment samples

	Total coliforms				Faecal coliforms				Enterococci				
	Number samples	% Positive samples	Concentration*		% Positive samples	Concentration		% Positive samples	Concentration		% Positive samples	Concentration	
			Geomean	Maximum		Geomean	Maximum		Geomean	Maximum		Geomean	Maximum
Dana Point Baby Beach													
Shoreline	168	35.3	9	51 000	17.3	3	15 500	48.8	17	200 000			
West Storm Drain	269	61.8	85	191 000	30.1	6	20 200	66.5	79	268 000			
Mid Harbor	14	7.1	1	200	0.0	NA	NA	7.1	1	200			
S-6 (control)	13	0.0	NA	NA	0.0	NA	NA	15.4	1	10			
Huntington State Beach													
Upper Santa Ana River	35	91.4	1876	200 000	77.1	137	3500	100.0	5922	77 400			
Lower Santa Ana River	15	13.3	2	100	0.0	NA	NA	73.3	21	940			
Shoreline	10	20.0	3	140	10.0	2	200	40.0	6	500			
Near offshore	31	9.7	1	20	3.2	1	20	48.4	6	200			
Offshore (sewage outfall)	10	30.0	5	500	0.0	NA	NA	0.0	NA	NA			
Newport Beach near offshore (control)	15	6.7	1	20	0.0	NA	NA	33.3	3	50			

*Colony forming units 10 g⁻¹ (wet weight).
% Positive samples, samples with values greater than detection limit; NA, not applicable.

concentration in sediment. In this study, the bacterial concentrations in overlying water were at least 2 logs lower than concentrations in corresponding sediment samples. Thus, the calculated bacterial concentrations in sediment were not because of overlying water.

Spatial and temporal variation of faecal indicator concentrations in sediment

The spatial and temporal variability of the concentration of all three indicator bacteria in sediments was determined for a single transect at Dana Point Baby Beach. Sampling sites included two intertidal and two marine sites along a 6.1 m transect running eastward from the mouth of the West Storm Drain. The intertidal sites were located within 3.0 m of the drain mouth. Sediments at the marine sites, located further away, were below the waterline. Sediments were collected at six different times (at 1 to 2-week intervals) over a 14-week period during the summer dry season (Fig. 3). On most of these sampling days, bacterial levels and frequency in species observed were highly variable between sites.

Indicators were more consistently detected and present in higher concentrations in samples from the intertidal sites when compared with the marine sites. The geometric mean concentrations of all three indicators were approximately 2 logs higher here than at the marine sites. There was also higher variability in bacterial concentrations in sediments from the marine sites.

Distribution of *Enterococcus* and enterococci-related species in sediment samples and shoreline water

The species distribution of isolates presumptively identified as *Enterococcus* using mEI agar was determined for sediment

and adjacent shoreline water samples. Shoreline water samples were obtained from regulatory agencies responsible for monitoring indicator bacteria on a routine basis; samples from all other sites were collected for the purposes of this study. A total of 1361 isolates from sediment and shoreline water samples from both beaches were speciated (Table 3). In general, *Enterococcus faecalis* and *Enterococcus faecium* were the most common species found in both sediment and water samples. *Enterococcus hirae*, *Enterococcus casseliflavus* and *Enterococcus mundtii* were also frequently seen when compared with *Enterococcus gallinarum*, *Enterococcus durans* and *Enterococcus avium*. Surprisingly, a high percentage of isolates from sediment (8.2–15.0%) and shoreline water (11.4–25.5%) were non-*Enterococcus* species (Table 3). These isolates, which appeared identical to enterococci on mEI media, included *Streptococcaceae* and related organisms (Bascomb and Manafi 1998) such as *Streptococcus bovis*, other *Streptococcus* spp., *Aerococcus* spp., as well as species that could not be identified with the methods used.

Enterococcus faecalis was the predominant species isolated from shoreline water at both Huntington Beach (39.8%) and Baby Beach (33.2%), West Storm Drain water (35.6%) and Huntington State Beach near offshore sediments (68.8%). *Enterococcus faecium* was the predominant species isolated from sediments at the West Storm Drain (35.2%) and the SAR (51.4%) (Table 3).

Enterococcus species distribution during single sample failure periods

The overall species distribution of samples from shoreline water at both study sites was similar, with the exception of a higher incidence of *Streptococcus* spp., particularly *S. bovis*, at Huntington State Beach (Table 3). The source(s) of these organisms to the beach are uncertain. To better understand

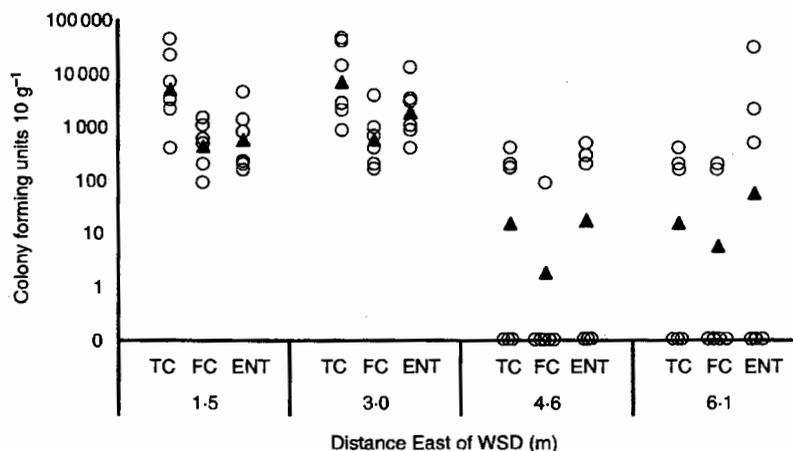


Fig. 3 Temporal and spatial variability of faecal indicator concentrations in sediments from four West Storm Drain sites sampled six times over 14 weeks, O, concentration; ▲, geometric mean; TC, total coliforms; FC, faecal coliforms; ENT, *Enterococcus*

Table 3 Enterococcus species distribution in water and sediment samples

No. samples	No. isolates	Number (%) of isolates												
		<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. hirae</i>	<i>E. casseliflavus</i>	<i>E. mundtii</i>	<i>E. gallinarum</i>	<i>E. durans</i>	<i>E. avium</i>	ENT*	S. bovis	STR	AER	Other, not ENT†
Dana Point Baby Beach														
169	349	116 (33.2)	74 (21.2)	40 (11.5)	42 (12.0)	29 (8.3)	4 (1.1)	1 (0.3)	1 (0.3)	3 (0.8)	11 (3.2)	0 (0.0)	0 (0.0)	28 (8.0)
26	45	16 (35.6)	6 (13.3)	0 (0.0)	15 (33.3)	1 (2.2)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (13.3)
73	105	11 (10.5)	37 (35.2)	15 (14.3)	14 (13.3)	9 (8.6)	2 (1.9)	1 (1.0)	3 (2.8)	1 (1.0)	3 (2.8)	0 (0.0)	0 (0.0)	9 (8.6)
Huntington State Beach														
144	576	229 (39.8)	75 (13.0)	73 (12.7)	36 (6.2)	9 (1.6)	5 (0.9)	1 (0.2)	0 (0.0)	1 (0.2)	102 (17.7)	27 (4.7)	5 (0.9)	13 (2.2)
47	206	41 (19.9)	106 (51.4)	19 (9.2)	14 (6.8)	7 (3.4)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	3 (1.4)	1 (0.5)	8 (3.9)	5 (2.4)
20	80	55 (68.8)	9 (11.2)	2 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	11 (13.8)	0 (0.0)	0 (0.0)	1 (1.2)
479	1361	468 (34.4)	307 (22.6)	149 (10.9)	121 (8.9)	55 (4.0)	12 (0.9)	5 (0.4)	5 (0.4)	6 (0.4)	130 (9.6)	28 (2.0)	13 (1.0)	62 (4.6)

*Four isolates unidentified *Enterococcus* spp., one *Enterococcus raffinosus* isolate and one *Enterococcus malodrans* isolate.

†Sixty unidentified non-*Enterococcus* spp., one *Lactococcus* spp. and one *Helicobacter* spp. ENT, *Enterococcus* spp.; STR, *Streptococcus* spp.; AER, *Aerococcus* spp.

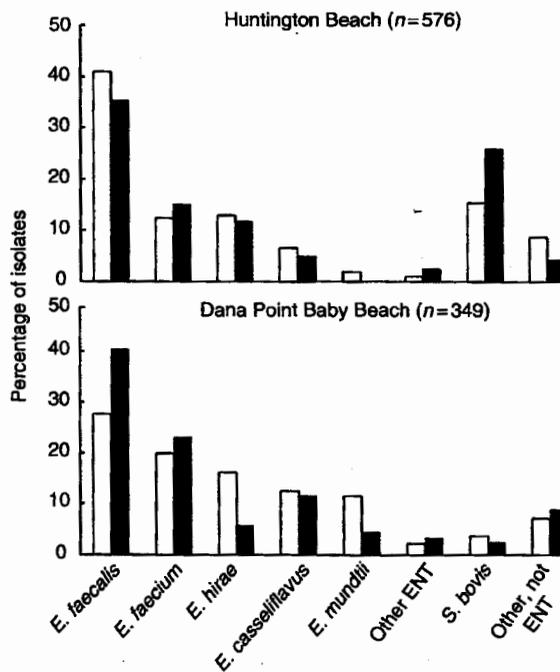


Fig. 4 Distribution of *Enterococcus* and related species in recreational marine water samples collected during ambient (□) and exceedance (■) conditions

possible relationships between contamination events and changes in species distribution, enterococci species composition in water samples with levels in water above and below the single sample standard (≥ 104 CFU 100 ml^{-1}) was compared (Fig. 4). There was no significant difference in the species distribution in samples at both Baby Beach and Huntington State Beach in samples collected during beach failures when compared with ambient conditions ($P = 0.13$ and $P = 0.10$, respectively; Pearson chi-square test).

DISCUSSION

In southern California, it is well recognized that a major cause of bacterial pollution of coastal waters is urban runoff in rivers/channels and storm drains that discharge into the ocean (Dwight *et al.* 2002; Reeves *et al.* 2004). The data presented here points to another source associated with urban runoff. Intertidal sediments harbouring high levels of indicator bacteria can be resuspended in water and transported to beaches by waves and wind, leading to water quality failures. We found a concentration gradient of faecal indicator bacteria in sediments: extremely high densities in the Santa Ana River near Huntington Beach and West Storm Drain area at Dana Point Baby Beach; significantly lower concentrations in shoreline and near offshore sedi-

ments at both beaches and even lower or nondetectable levels in offshore and control site sediments. These results indicate that shoreline waters at Huntington State Beach and Baby Beach may be recipients of faecal indicator bacteria originating from intertidal sediments in the SAR that contain high levels of bacteria. Field studies conducted at the Huntington Beach area suggest that indicator bacteria from the SAR and TM sediments are resuspended and flushed to the ocean during ebb tides and transported to the beach by surf zone and tidal currents (Grant *et al.* 2001; Kim *et al.* 2004). This resuspension and transport process is more pronounced during spring tide conditions, which occurs during full and new moon periods. At these times the greatest volume of tidal water flows inland into coastal outlets such as the TM and SAR and back out to the ocean, which is also when most of the beach failures at Huntington State Beach occur during the dry weather season (Boehm *et al.* 2004; Noble and Xu 2004). Other possible reasons for the indicator concentration gradient observed may be related to differences in sediment type, organic content and amount of UV exposure at the intertidal, onshore and offshore locations, parameters which were not measured at all sites in this study.

The high densities of total coliforms, faecal coliforms and enterococci found in intertidal sediments in the SAR and Baby Beach are similar to sediment indicator levels found at several different geographical locations: a tidally influenced river in Florida (Solo-Gabriele *et al.* 2000), an embayment in New Zealand (Le Fevre and Lewis 2003), an estuary in Massachusetts (Valiela *et al.* 1991) and freshwater creeks and lakes in Michigan (Byappanahalli *et al.* 2003) and in Wisconsin (LaLiberte and Grimes 1982).

The low levels of indicator bacteria found in sediments around the sewage outfall area offshore Huntington State Beach indicate that the discharge pipe may not be a constant source of contamination to these sediments. This finding is in contrast to a similar study conducted at Morcambe Bay, a bathing beach in England. Here, high levels were found in bay sediments receiving sewage effluent from an outfall pipe (ranging from untreated through to secondary treatment) and agricultural runoff from streams and rivers (Obiri-Danso and Jones 2000). The low levels found at Huntington State Beach may be the result of chlorination of the wastewater by the sewage treatment plant and the dilution or dispersion of bacteria by ocean currents. Wastewater entering the plant contains approximately 10^7 to 10^8 total coliforms per 100 ml and is reduced to 10^5 per 100 ml for total coliforms and 10^4 for faecal coliforms and enterococci after disinfection. The effluent is discharged from the outfall pipe that is engineered to achieve a 180 : 1 dilution in ocean water. In this study, finding higher levels of indicator levels at storm drain impacted sediments as opposed to the outfall area was surprising. In fact, the geometric mean levels in

storm drain impacted intertidal sediments were about one order of magnitude higher concentration when compared with the sewage impacted sediments at Morcambe Bay.

At Dana Point Baby Beach, contamination of beach water during summer dry weather appears to be related to the proximity of the storm drain to the beach, retention and/or regrowth of indicator bacteria in sediments and resuspension of indicator bacteria because of wave action in the harbour. In a previous study at this location, we determined that exceptional surf heights of 2–3 m that topped the breakwater and greater wave action correlated with a considerable increase in indicator levels at the beach (BBSSR 2003). A similar study conducted at a protected beach in New Zealand also showed that storm and stream water contributed high numbers of enterococci to sediments around these discharge points and that resuspension of sediments because of wave action led to elevated levels in water (Le Fevre and Lewis 2003). Increased bacterial levels because of resuspended sediments can occur as a result of increased turbulence due to runoff, animal traffic, sustained winds, storms, boats and dredging activities (Gerba and McLeod 1976; Sherer *et al.* 1992; Obiri-Danso and Jones 2000).

Repeated sampling of Baby Beach intertidal sediments around the West Storm Drain indicated high temporal and spatial variability in indicator concentrations. Although total coliforms and enterococci were consistently detected within 3.0 m of the storm drain, higher concentrations of enterococci were also found in two samples collected furthest from the drain where the levels were generally low. Determining the causes of temporal and spatial variability of indicator concentrations in sediment was not included in this study. Further studies on sediment characteristics that can affect bacterial growth and decay rates, such as temperature, moisture content, nutrient content, particle size, surface area and biofilm formation are needed to understand the potential flux of indicator bacteria from sediments to water.

Indicator levels ranging from 10^3 to 10^5 CFU 10 g^{-1} of sediment suggest the occurrence of long-term survival and regrowth of indicator bacteria in this environment. It has generally been accepted that faecal indicator bacteria do not survive for very long in seawater. In seawater, 90% of total coliforms, *E. coli* and enterococci die off in about 2.2, 19.2 and 60 h respectively (Bartram and Rees 2000). However, prolonged survival may be possible in marine and freshwater sediments. Indicator bacteria have been shown to persist in storm drain impacted sediments for up to 6 days following storm events without further supplementation of bacteria from runoff (Marino and Gannon 1991). Davies *et al.* (1995) showed that *E. coli* remains culturable in marine sediment for up to 68 days. In addition, laboratory studies have shown that faecal indicators survive longer in water supplemented with sediment (Gerba and McLeod 1976; Sherer *et al.*

1992). Survival in sediment may be enhanced because of protection from UV inactivation and predation, moisture, buffered temperatures and availability of nutrients originating from algae, debris and plankton (Whitman and Nevers 2003). Phytoplankton are most active in late spring to early summer and late summer to early fall, which are also the periods when bacterial levels in coastal waters increase (Dowd *et al.* 2000). Seaweed (Anderson *et al.* 1997), seawrack (Valiela *et al.* 1991) and zooplankton (Maugeri *et al.* 2004) provide both nutrients and surfaces for indicator bacteria to survive in the marine environment. Recently, groundwater discharge at Huntington Beach was found to be a source of nitrogen and orthophosphate to the surf zone that may enrich intertidal sediments and allow bacteria to persist (Boehm *et al.* 2004).

At both study sites, enterococci were found more frequently and in higher concentrations in intertidal sediment samples than total and faecal coliforms. *Enterococcus* spp. may be more abundant in intertidal sediments because these organisms are more resilient in seawater and are not as easily inactivated by sunlight when compared with *E. coli* (Bartram and Rees 2000). Enterococci are also capable of growing at a wider range of temperature (between 10 and 45°C) and pH (4.8–9.6) as well as in the presence of 28% sodium chloride (Huycke 2002).

Presumptive enterococci isolates were speciated to better understand the sources and ecology of these organisms in the marine environment. Of 1361 isolates tested, the predominant species identified in water and sediment, in order of occurrence were *E. faecalis*, *E. faecium* and *E. hirae*. These results are similar to the distribution reported for environmental strains elsewhere (Stern *et al.* 1994; Pinto *et al.* 1999; Dicuonzo *et al.* 2001; Ott *et al.* 2001; Harwood *et al.* 2004). *Enterococcus faecalis* and *E. faecium* are also the predominant *Enterococcus* spp. in the intestinal microflora of humans and animals and are considered opportunistic pathogens (Willey *et al.* 1999). *Enterococcus hirae* is a member of animal microflora, but has been found to occasionally cause infections in humans (Tannock and Cook 2002). *Enterococcus gallinarum* and the yellow pigmented species, *E. casseliflavus* and *E. mundtii*, are associated with plants and soil and are rarely associated with human infection (Pinto *et al.* 1999). In this study, these three 'environmental' associated species comprised 13.8% of all isolates tested. Thus, the species distribution of enterococci in insects, plants and sediments as well as in pristine and faecal-contaminated waters is important when assessing this group as faecal indicators (Leclerc *et al.* 1996).

During beach failures, the species distribution of enterococci and related species in shoreline waters was similar to the distribution found during ambient conditions. This distribution in water was also comparable with intertidal

sediment samples with high concentrations of enterococci. These findings suggest that there may be constant loading of a stable enterococcal population from intertidal sediments and other sources to water that increases because of changes in environmental conditions, resulting in frequent failures. The enterococci species distribution found in sediments and water were similar to that of humans, animals and birds. Thus, species distribution was not useful in pinpointing major source(s) of beach contamination in this study. However, this determination could be useful to finding sources of contamination in other sites where 'environmental' species may be predominant.

Comparison of the enterococci species composition in water *vs* sediments in highly contaminated areas could provide additional information in assessing sediments as a source. Knowledge of the predominant species present in specific sites could also be useful to investigators using or developing microbial source tracking methods targeting enterococci.

The API Strep system and traditional biochemical tests and identification charts used to speciate enterococci and related organisms in this study are culture-based methods designed to identify clinical isolates. Further studies are needed using PCR or 16S rRNA sequencing to identify environment isolates, particularly the noncultivable strains.

There was a high incidence of non-*Enterococcus* species (17.1%) using mEI media. The majority of these isolates (9.6%) were identified as *Streptococcus bovis*, a member of the faecal streptococcus group. This finding was unexpected as *Streptococcus* spp. are not known to persist in marine water (Geldreich and Kenner 1969). The mEI media used to isolate enterococci in this study was formulated to differentiate enterococci from other genera of the faecal streptococcal group (Messer and Dufour 1998). Like enterococci, *S. bovis* is also β -D-glucosidase-positive, which is indicated on mEI media by the formation of a blue halo around the colony. Marine water samples from Baby Beach and Huntington Beach had false-positive rates (occurrence nonenterococci species) of 11.2% and 25.5% respectively. These rates are much higher than 6%, as reported by the EPA (USEPA 2000).

To our knowledge, this is the first publication showing high concentrations of faecal indicator bacteria in intertidal sediments impacted by storm drains. The levels of enterococci found in shoreline and near offshore sediments could be a result of continuous loading of faecal indicator from highly contaminated sediments in areas associated with urban runoff. Exceedances in enterococci standards may also occur because of resuspension of bacteria-laden sediment in water. This occurrence supports the suggestion made by others that an evaluation of faecal indicators in sediments may be a more stable index of overall or long-term water quality than the overlying water (LaLiberte and Grimes

1982; Sherer *et al.* 1992; Obiri-Danso and Jones 2000). The long-term persistence/regrowth of indicators in sediments, particularly enterococci, calls into question the reliability of this indicator for determining recent faecal contamination of water.

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Southern California Coastal Water Research Project

Multi-Tiered Approach Using Quantitative Polymerase Chain Reaction For Tracking Sources of Fecal Pollution to Santa Monica Bay, California

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ABSTRACT

The ubiquity of fecal indicator bacteria such as *Escherichia coli* and enterococcus make tracking sources in urban watersheds extremely challenging. In this study, a multi-tiered approach was used to assess sources of fecal pollution in Ballona Creek, an urban watershed that drains to Santa Monica Bay (SMB), CA. A mass-based design at six mainstem sites and four major tributaries was used to quantify the flux of enterococcus and *E. coli* using traditional culture-based methods, and three additional indicators including enterococcus, *Bacteroides* sp. and enterovirus, using quantitative polymerase chain reaction (QPCR). Sources and concentrations of fecal indicator bacteria were ubiquitously high throughout Ballona Creek and no single tributary appeared to dominate the fecal inputs. The flux of enterococcus and *E. coli* averaged 10^9 to 10^{10} cells/hr and were as high at the head of watershed as they were at the mouth prior to its discharge into SMB. In contrast, the site furthest upstream had the most frequent occurrence and generally the greatest concentrations of enterovirus. Ninety-two percent of the samples that tested positive for enterovirus also tested positive for *Bacteroides* sp. A similar survey in Malibu Creek, a nearby non-urban watershed, found low levels of traditional fecal indicator bacteria and no detectable enterovirus or *Bacteroides* sp. The influent and effluent from three structural best management practices (BMPs) were evaluated for removal efficiency. Results indicated that those with ultraviolet (UV) treatment worked better than a constructed treatment wetland for reducing enterococcus concentrations using culture-based methods, but also degrading its DNA based on QPCR measurements.

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INTRODUCTION

Santa Monica Bay (SMB), California, is home to some of the most popular beaches in the world. It is located adjacent to metropolitan Los Angeles where more than 50 million beachgoers visit SMB shorelines every year, which is more than all other beaches in California combined (SMBRC 2005). However, there are serious concerns about beach water quality because of continued exceedences of water quality thresholds based on fecal indicator bacteria such as total coliforms, fecal coliform or *E. coli*, and enterococcus, particularly in areas impacted by urban runoff. Thirteen percent of the shoreline mile-days in SMB exceeded water quality thresholds between 1995-2000 with over 50% of these exceedences located near storm drains (Schiff et al 2003). In contrast, sewage spills were relatively rare accounting for less than 0.1% of the water quality exceedences and subsequent warnings to swimmers. Moreover, swimming near storm drains in SMB can lead to an increased risk of swimming-related illnesses. Haile et al (1999) demonstrated that swimmers near storm drain discharges in SMB had a higher likelihood of respiratory and/or gastrointestinal symptoms compared to swimmers more than 400 m from a storm drain.

Despite the impairments to water quality and risk to human health, identifying and eliminating the sources of bacteria responsible for the beach warnings remains elusive. The difficulty in identifying and eliminating the sources of bacteria results from two important factors. First, the traditional indicators of fecal pollution on which the water quality thresholds were derived are not specific to humans. These fecal indicator bacteria can be shed from any warm-blooded organism including wild and domesticated animals (Geldreich 1978). Therefore, source tracking turns into a challenging scenario when these diffuse and frequently intermittent or episodic fecal releases occur. The second difficulty when identifying and eliminating sources of fecal indicator bacteria is their ubiquity in the environment. Unlike many of the pathogens of interest, fecal indicator bacteria may survive and even grow in the environment. For example, fecal indicator bacteria were able to persist in beach wrack impacting beaches in Cape Cod, MA (Weiskel et al. 1996).

Viruses are one tool that could prove useful in source tracking studies because they are the pathogen of interest. Viruses are known to cause a significant portion of waterborne disease from water contact, mostly from ingestion of sewage contaminated water and seafood (Fogarty et al. 1995). Until recently, however, methods for virus detection and quantification have relied on growth-based endpoints that are much too slow to be effective source tracking tools. Recently developed molecular techniques, such as Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRT-PCR) can detect and quantify viral genetic material directly from water samples. Results of tests conducted previously in Southern California (Noble and Fuhrman, 2000; Tsai et al., 1993; Tsai et al., 1994), in Florida (Griffin et al., 1999; Rose et al., 1997), and Europe (Pina et al., 1998) using conventional RT-PCR or PCR have detected a host of genetic material from human specific viruses including enterovirus, hepatitis A virus, rotavirus, and adenovirus in urban runoff discharges or seawater samples. The major drawback to

using viruses as source tracking tools, however, is their dependence on a large human population in order to have sufficient numbers for detection (Noble et al. 2003).

A different approach would be to use alternative bacterial indicators for source tracking that might be much more abundant in fecal waste discharges. This alternative approach could prove useful if host specific bacterial indicators could be found. Of the facultative anaerobic organisms common in human fecal flora, enterococci have been found in almost all subjects with a mean level of \log_{10} 8.9 per gram feces (Klessen et al, 2000). Another option would be *Bacteroides* sp., which make up approximately one-third of the human fecal microflora, considerably outnumbering the fecal coliforms. *Bacteroides* sp. belongs to a group of nonspore forming obligate anaerobes, so there is little concern over persistence or regrowth in the environment. More importantly, human specific *Bacteroides* sp. markers have been developed increasing the value of this potential indicator (Bernhard and Field 2000a, Bernhard and Field 2000b).

Both virus and alternative bacterial indicators such as *Bacteroides* sp. have been shown to be potentially useful source tracking tools. Griffith et al (2003) concluded that genetic based methods, such as PCR consistently provided the best information when attempting to conduct source tracking on mixed source samples. *Bacteroides* sp. correctly identified human sources of fecal pollution when present in mixed water samples delivered blind to the laboratory. Likewise, human enterovirus measurements had virtually no false positives, a problem that plagued many other methods in that study. However, the human marker identified in *Bacteroides* sp. may be present in additional hosts or the primers used to detect the human marker may cross react with species from nonhuman hosts (Kreader 1995). Similarly, enterovirus consistently and correctly detected human sewage when present, but had difficulty determining human sources when only one or a few likely uninfected individuals contributed fecal material. Since no method has all of the traits to be the consummate bacterial source tracking tool, a multi-tiered multi-indicator approach has been recommended by some investigators (Stewart et al 2003). By using multiple tools, investigators can utilize the strengths of each to ascertain inputs and track fates that will ultimately lead to successful management solutions.

This objective of this study was to identify the contributions and quantify the loading of fecal contamination to the SMB using a multi-tiered approach. The first tier included traditional fecal indicator bacteria measurements. The second tier included newly developed methods for enterococcus, *Bacteroides* sp., and enterovirus. All of these newly developed methods rely on QPCR or QRT-PCR, which has not been applied previously for source tracking studies in urban watersheds until now. The multi-tiered approach was applied using a mass-based design to quantify inputs and flux through an urban watershed to the beach. A subsidiary objective included using the multi-tiered approach through a relatively undeveloped watershed. Finally, the multi-tiered approach was used to determine the effectiveness of a variety of structural best management practices (BMPs) that were aimed at reducing bacterial inputs from urban watersheds.

MATERIALS AND METHODS

This study was conducted in three phases. The first phase quantified inputs of flow, bacteria concentrations and virus particles, then tracked them through an urban watershed over time. This mass-based design was applied in the Ballona Creek watershed, the largest tributary to SMB. Ballona Creek is over 85% developed and currently has the largest inputs of fecal indicator bacteria to SMB (Figure 1). The second phase quantified bacteria concentrations and virus particles in the Malibu Creek watershed, the second largest tributary to SMB. Malibu Creek is only 12% developed and has a large lagoon system at its terminus prior to discharging across the beach to the world famous Surfrider Beach. Although no flow was measured in Malibu Creek to provide flux estimates, this system provided the opportunity to measure concentrations at several points through the lagoon and as it enters the ocean to assess shoreline mixing and dilution. The third phase examined the effectiveness of three BMPs to reduce bacteria and virus concentrations. The three BMPs, only two of which were located in the Santa Monica Bay watershed, included a multimedia filtration system with inline ultraviolet (UV) treatment, a filtration-aeration system with an inline UV treatment system, and a constructed wetland. For each of the BMPs, an influent-effluent approach was used to estimate treatment effectiveness.

Sample Collection and Filtration

Ballona Creek

Samples were collected at six mainstem and four of the major tributaries to the Ballona Creek system. The six mainstem sites extended from where the system daylight at Cochran Avenue to Inglewood Avenue, which is located at the head of tide just prior to discharge into SMB (Table 1). The four tributaries represented the four largest hydrodynamic inputs to the system and were located in reaches between each of the mainstem sampling sites.

Flow was calculated as the product of flow rate and wetted cross-sectional area. Doppler area-velocity sensors were used to measure flow rate. Pressure transducers that measure stage, along with verified as-built cross sections, were used to estimate wetted cross-sectional area. One minute instantaneous flow was logged electronically during the entire six hour sampling period. Both the area-velocity sensors and pressure transducers were calibrated prior to sampling.

One hour composite water samples were collected at each site between 8:00 AM and 2:00 PM on August 26, 2004. The six hour sampling period corresponds to the approximate hydrodynamic travel time from Cochran Avenue to Inglewood Avenue (Ackerman et al, 2004). Four liter composite samples at each site were created after combining ten individual 400 ml grab samples collected every 6 minutes into a single container. In total, 60 composite samples were collected at Ballona Creek as a result of sampling 6 hours at 10 different sites.

Table 1. Ballona Creek sampling sites.

Site	Description	GPS Coordinates (NAD 83 datum)
Cochran Ave.	mainstem	34 02.662N 118 21.237W
Fairfax Drain	tributary	34 02.298N 118 22.136W
Adams Ave.	mainstem	34 02.009N 118 22.494W
Adams Drain	tributary	34 02.009N 118 22.494W
Rodeo/Higuera	mainstem	34 01.305N 118 22.693W
Benedict Box Channel	tributary	34 00.925N 118 23.432W
Overland Ave.	mainstem	33 00.429N 118 23.771W
Sawtelle Ave.	mainstem	33 59.816N 118 24.164W
Sepulveda Channel	tributary	33 59.512N 118 24.693W
Inglewood Ave.	mainstem	33 59.394 N 118 24.696W

Malibu Creek

One hour composite samples were collected at five sites along the mainstem of Malibu Creek (Table 2). The sites stretched from the Cold Creek tributary to the head of the lagoon, near the mouth of the lagoon, in the discharge across the beach, and in the wave wash immediately in front of the discharge across the beach. Composite samples were collected in a similar fashion as Ballona Creek with the following exceptions. A single composite sample was collected at each site daily on three consecutive days (November 10, 11 and 12, 2004) coinciding with low tide to ensure that the flow direction was from the lagoon, across the beach, and into the wave wash. No flow information was collected since most of the sites were hydrologically unrateable.

Table 2. Malibu Creek sampling sites.

Site	Description	GPS Coordinates (NAD 83 datum)
Bridge at Cold Creek	Mainstem	34 04.865N 118 42.262W
Bridge at Cross Creek	Mainstem	34 02.578N 118 41.052W
Head of Malibu Lagoon	Lagoon	34 02.154N 118 41.036W
Mouth of Malibu Lagoon	Lagoon	34 01.920N 118 40.810W
Malibu Creek Wavewash	Mixing Zone	34 01.920N 118 40.810W

BMPs

Three BMPs were selected for sampling. The three sites included: the Santa Monica Urban Runoff (SMURRF) treatment facility located in Santa Monica; the Clear Creek, Inc. MURF™ pilot treatment facility located in Paradise Cove; and a constructed wetland (WET CAT) located in Laguna Niguel. The SMURRF consists of a grit screen to remove debris and trash, a dissolved aeration system to separate the oil and grease, and a microfiltration system to remove solids inline with a UV treatment device. The MURF system uses a combination of proprietary multi-media filtration and UV treatment. The WET CAT is a 2.1 acre constructed wetland with no other in line treatment. Grab samples were collected from the influent and effluent at each BMP.

Filtration

After collection, samples were placed on ice in a cooler and transported immediately to the University of Southern California for processing. For each composite sample, 200-600 ml of sample volume was vacuum filtered through replicate 47mm 0.45µm polycarbonate filters (Poretics, Inc.) using a filter funnel and receiver (Millipore, Inc.) for bacterial marker analysis by QPCR. In addition, replicate filtrations were conducted using 47 mm diameter 0.7 µm nominal pore size, type GF/F microporous filters (Whatman, Inc.), and replicate Type HA Millipore mixed ester cellulose acetate/nitrate, 0.45 µm pore size filters (for subsequent enterovirus analysis). The polycarbonate filters were immediately placed into a 1.5 ml screw-cap tube and placed on dry ice until storage at -80°C. Type HA filters were either placed into a Whirl-pak bag for analysis by the Fuhrman laboratory (EnteroA), or into a 1.5 ml screw-cap tube for subsequent analysis by the Noble laboratory (EnteroB). Type GF/F filters were cut into quarters, each quarter placed in a 1.5 ml screw cap tube and placed on dry ice until storage at -80°C.

Indicator Bacteria Analyses Using Chromogenic Substrate

Concentrations of *E. coli* and enterococcus were measured by chromogenic substrate methods using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME). *E. coli* was measured using the Colilert-18® reagents, while enterococci were measured using Enterolert™ reagents. Both tests used the Quanti-Tray/2000 for enumeration of cells. Samples were incubated overnight per the manufacturer's instructions and inspected for positive wells. Conversion of positive wells from these tests to a most probable number (MPN) was done following Hurley and Roscoe (1983).

Enterovirus Analyses Using QRT-PCR

Samples were analyzed for enteroviruses using two separate, but similar methods conducted in two separate laboratories, EnteroA (Fuhrman laboratory) and EnteroB (Noble laboratory). For EnteroA, filters were extracted using the RNeasy mini kit (Qiagen Cat. No.74106) and QIAvac 24 vacuum manifold (Qiagen Cat. No.19403). The extraction protocol was modified from the manufacturer's instructions as follows: 1ml lysis buffer RLT (with 10µl β-mercaptoethanol) was added directly into each Whirl-Pak bag, allowed to soak the filter for ten minutes, and the resulting extracts (lysates) were carefully removed by pipet into 2 ml microcentrifuge tubes (droplets hanging in the bag and water clinging to the filter were first squeezed to the bottom corner of the bag by manually applying pressure to the outside of the bag). If there was visible filter or sample debris, the particulate matter was removed by brief centrifugation. Then one volume of 70% ethanol (usually 1 ml) was added to the extract and mixed by pipetting. Samples were transferred to the RNeasy spin columns, filtered through with the QIAvac at approximately 500 mm Hg vacuum, and were washed on the manifold once with 700 µl RW1 solution, and twice with 500 µl RPE solution to remove contaminants. The columns were cleared of remaining droplets of buffer by centrifugation into a 2 ml collection tube (14,000 rpm, Eppendorf 5415 microfuge, 2 minutes), and the buffer discarded. The RNA

was eluted from the columns into a 1.5 ml collection tube with 50 μ l volumes of RNase free water by centrifugation (Eppendorf 10,000 rpm, 2 min.), after allowing the water to stay in the column 1 min. This filter extraction step typically took up to two hours for 15 samples.

For each PCR reaction, 5 μ l of the 50 μ l RNA was analyzed by QRT-PCR on a Mx3000P Thermal Cycler (Stratagene, Inc.). The PCR protocol was modified from the single-tube RT-PCR method previously developed for sludge samples by Monpoeho et al. (2001). Primers and probe, not changed from that original published method (except for the BHQ quencher), were reverse primer Ev1 [5'-GATTGTCACCATAAGCAGC-3'], forward primer Ev2 [5'-CCCCTGAATGCGGCTAATC-3'], synthesized by Qiagen and Ev-probe [5'-FAM-CGGAACCGACTACTTTGGGTGTCCGT-BHQ-Phosphor-3'], synthesized by Sigma Genosys. A GenBank BLAST search done on 3 June 2004 revealed that only human (not other animal) enteroviruses matched all three primer and probe sequences. Each PCR reaction contained 5 μ l RNA extract and 20 μ l master mix, each 20 μ l master mix contained: 1X Taq gold buffer (ABI), 5.5mM MgCl₂ (ABI), 500uM dNTPs (ABI), 6% glycerol (Sigma Chemical Co.), 2% PVP 40 (polyvinylpyrrolidone, av. MW 40,000, Sigma Chemical Co.), 500nM Ev1, 400nM Ev2, 120nM Ev-probe, 1.5 μ g T4 gene 32 protein (Ambion), 10 units of RNAsin (ABI), 2.5 units of AmpliTaq gold (ABI) and 5 units MULV reverse transcriptase (ABI). Each RNA extract was analyzed in duplicate. Enterovirus RNA was transcribed into cDNA at 50°C for 45 minutes, the cDNA was amplified by PCR, after a 95°C 10 minute hot start, for 50 cycles at 94°C for 15 sec and 60°C for 1min. Fluorescence measurements were made during the extension step, every cycle at 60°C. Calculations for quantification were done by the Stratagene QPCR software in real time, with raw data saved for possible reanalysis. Parameters (e.g. fluorescence threshold) were set manually after PCR was done to generate a standard curve with optimal statistics (usually $r^2 > 0.95$, slope around 3.3) and unknowns were calculated based on that standard curve. Standards were prepared using the poliovirus stock described above. Standards used in the high concentration set were 10-fold dilutions ranging from high to low concentration. For Enterob, a similar approach was used. Samples were extracted using the RNeasy mini kit (Qiagen Cat. No.74106), with additional of 2.0% polyvinylpyrrolidone (PVP)-40 (final concentration) and the filter fully homogenized in the screw cap tube. After homogenization, approximately 700 μ l of the RLT/filter slurry was applied to a QiaShredder column (until the QiaShredder was full) and spun at max speed, $\geq 8000 \times g$, for 2 minutes. It was often necessary to perform two spins to ensure the entire volume of RLT/filter slurry was shredded. The supernatant fluid was then carefully removed and placed into a new 1.5 ml tube. The volume of solution in each tube was estimated by pipetting and 0.4 volumes of potassium acetate were added. Tubes were mixed by inversion and incubated on ice for 15 minutes. The mixture was then spun at 4°C for 15-30 minutes and the supernatant transferred to a new 1.5 ml microfuge tube. Following this, the protocol for the Qiagen RNeasy Plant and Fungi RNA isolation was followed starting at step 5. Five μ l of extracted RNA from the previous procedure was added to 5X RT Buffer, 6 mM MgCl₂, 500 nM dNTPs (final concentration), 700 nM EV1 Reverse primer, 700 nM EV1 Forward primer, and 300 nM EV-BHQ TaqMan probe, 10 units of RNAsin, 2.5 units of Taq polymerase, and 5 units MULV reverse transcriptase. The Cepheid Smart Cycler® was programmed to: 1 hour

RT at 37°C followed by a 15 minute hold at 95°C for *Taq* activation, then 45 cycles of 94°C 15 seconds (denature), 60°C 1 minute (anneal/extension-optics on).

QRTPCR results were available three hours after the start of analysis, making the total PCR preparation and analysis time less than 5 hours for 15 samples. Results are reported as equivalent virus particles per unit sample volume, meaning that this is where the QRTPCR calculation indicated the sample appeared relative to the standard curve prepared from poliovirus standards.

Bacterial Analyses Using QPCR

The polycarbonate filters were processed for DNA extraction using the UltraClean™ Fecal DNA Isolation Kit (MoBio Laboratories, Inc., 12811-50) as per manufacturer's alternative protocol. Eluted DNA extracts were stored at -20°C until use.

Table 3. Primer and probe sequences for PCR detection of enterococci

Sequence Name	Nucleotide Sequence 5' to 3'	Length	GC (%)	T _m (°C)	Detection System*
ECST748F ¹	5'aga aat tcc aaa cga act tg-3'	20mer	35	51.2	
ENC854R ¹	5'-cag tgc tct acc tcc atc att-3'	21mer	47.6	57.9	
GPL813TQ ¹	5'Cy3-tgg ttc tct ccg aaa tag ctt tag ggc ta-BHQ-2-3'	29mer	44.8	65.3	Taqman

¹Ludwig and Schleifer, 2000.

Total enterococci primers and probe were constructed using the rDNA regions around the target site of a well established enterococci group specific primer (ENC854R) (Table 3). The primer ECST748F targets enterococci, lactococci, and several clostridia. The target site of the probe GPL813TQ is present in rDNA from a variety of representatives of gram-positive bacteria with a low G+C DNA content (Ludwig and Schleifer, 2000).

Table 4. Master mix using individual reagents

Reagents	Final conc. (μM)	Initial vol (μl)
Water		3.9
10X Taq buffer (Mg ⁺⁺ free)	1	2.5
250mM MgCl ₂	5	0.5
10mM DNTPs	0.5	1.25
10μM ENC854R	1	2.5
10μM ECT748F	1	2.5
10μM GPL813 TQ Cy3 Probe	0.08	0.2
5U/u Taq polymerase	0.05	0.5

The Master Mix of reagents (Table 4) yields a final volume of 20 μ l, to which 5 μ l of sample (either DNA extract from an environmental sample, or 5 μ l of lysed cell suspension or genomic equivalents) was added for a final volume of 25 μ l. The samples were run under the following optimized assay conditions for PCR: 1 cycle initial hold at 95°C for 2 min, and 45 cycles of denaturation (94°C) for 15 seconds, and annealing (60°C) for 30 seconds, the optics were turned on during the annealing step. The Cepheid Smart Cycler was set with the following specific parameters for this assay. The Dye Set was set for FCTC25. The Ct analysis mode was set for growth curve (linear) analyses, with a manual threshold typically set at between 5 and 15 fluorescence units. The background subtract level was set at a minimum of 12 and a maximum of 40. The BoxCar averaging feature was set at 0. For quality control, combined *E. faecalis* and *E. faecium* were used as our calibration strain for the total enterococci primer and probe set. Control bacteria preparations were prepared by boiling bacteria for 5 minutes, centrifuging 1 min at 12,000 rpm in a Beckman Microcentrifuge, and immediate storage on ice. *E. faecalis* and *E. faecium* cells were enumerated using either SYBR Green I epifluorescence microscopy (Noble and Fuhrman, 1998) and/or using Enterolert® or the EPA 1600 methods (APHA 1992). This yielded information on both the cell numbers in the sample, and the number of metabolically active cells present in the sample. Serial dilutions of the standards were made in duplicate in DEPC-treated sterile water, and four point standard curves are run in concert with the unknown samples on the Smart Cycler II instrument. Total enterococci primers were tested with all 19 validly described species of the genus enterococci, and demonstrated amplification of rDNA of all strains, with varying efficiencies.

Bacteroides sp. Using Conventional PCR

Amplification of the human-specific Bacteroides/Prevotella marker generally followed the procedure of Bernhard and Field (2000), with PCR primers that amplify partial 16S rRNA from the human fecal (HF) specific group. DNA was extracted a MoBio Ultra Clean fecal extraction kit. A range of extracted DNA quantities (2 – 5 μ l, representing 1-70 ng per assay, with most samples in the range of 5-20 ng) was tested to avoid problems with inhibition. DNA was amplified with Bacteroides-Prevotella specific primers Bac708r CAATCGGAGTTCTTCGTG and HF183f ATCATGAGTTCACATGTCCG. Each 50- μ l PCR mixture contained the following reagents: 1 X Taq polymerase buffer (Promega), each primer at a concentration of 1 μ M, each deoxynucleoside triphosphate at a concentration of 200 μ M, 1.25U of Taq polymerase (Promega), 0.64 μ g of bovine serum albumin (Sigma) per μ l and 1.5mM MgCl₂. The thermal cycler was run under the following conditions, 2 min 95°C, then 25 cycles of 95°C for 30 sec, 60°C for 30 sec and 72 °C for 30 sec followed by a 5-min extension at 72°C. Then 1 μ of each PCR product was re amplified using the same conditions as above for another 25 cycles. PCR products were visualized in a 2% agarose gel stained with 1X SYBR Gold (Molecular Probes) and compared to a 100bp DNA ladder (Promega). Positive results had 525 bp products. The positive control was human fecal sample extracted with a QIAamp stool kit. Negative controls use water instead of sample. All negative samples are spiked (in a second PCR

Multi-tiered approach to source tracking using QPCR

run) with 0.1 ng of positive control to determine possible inhibition. Inhibited samples are re-run with less DNA.

RESULTS

Ballona Creek

Total volume discharged from Ballona Creek during the six-hour sampling period was 13,390 m³ (Figure 2). Of this volume, 97% was attributed to monitored inputs from Cochran, Fairfax, Adams and Benedict, and Sepulveda tributaries. The largest volume was contributed at Cochran Avenue where the creek daylights from beneath downtown Los Angeles. Flow remained relatively stable over the study period at all sites with little variation or pattern in discharge. For example, the coefficient of variation for flow at the most downstream site, Inglewood Avenue, was less than 8% approaching the resolution of our flow monitoring devices.

The flux of fecal indicator bacteria remained relatively constant moving downstream in Ballona Creek (Figure 3). The average flux of *E. coli* ranged from 1.1 X 10¹⁰ to 5.3 X 10¹⁰ cells/hr at the six mainstem sites. The average flux of enterococcus ranged from 6.6 X 10⁸ to 1.4 X 10⁹ cells/hr at the six mainstem sites. In both cases, there was no discernable increase in bacterial flux; no two mainstem sites were significantly different from one another for either *E. coli* or enterococcus.

The flux of fecal indicator bacteria decreased over time (Figure 4). The average flux of enterococcus was highest at 9:00 AM (2.9 X 10⁹ cells/hr) and monotonically decreased throughout the study period. The lowest flux was measured at 2:00 PM (3.0 X 10⁹ cells/hr). Similar patterns were observed for *E. coli* (data not shown). In contrast to the culture-based methods, the QPCR method for measuring enterococcus did not decrease over time. The flux of enterococcus ranged from 2.7 X 10¹⁰ to 4.7 X 10¹⁰ cells/hr with the 9:00 AM and 2:00 PM samples being nearly equivalent.

The relative pattern of enterococcus contributions between tributaries was similar at all time periods (Figure 5). Benedict tributary always had the greatest flux of fecal indicator bacteria followed by Sepulveda, Fairfax and Adams tributaries. A similar pattern was also observed for *E. coli*. The flux of enterococcus from Benedict tributary ranged from 4.1 X 10⁹ to 1.4 X 10¹⁰ cells/hr throughout the sampling period while the flux of enterococcus from Adams tributary ranged from 3.7 X 10⁵ to 4.4 X 10⁶ cells/hr. On average, Benedict tributary contributed 81% of the enterococcus loading from all four tributaries.

The hourly flux of enterococcus (using culture-based methods) from each of the four main tributaries approximated the load being passed down Ballona Creek (Figure 5). Regardless of hour, the flux from each of the tributaries was within a factor of 10¹ compared to its nearest downstream site on the mainstem of Ballona Creek. The only exception was the Adams tributary, which was as much as four orders of magnitude less than its nearest downstream site. The mainstem showed virtually no response to any of these tributary inputs, including Adams. Enterococcus flux remained virtually unchanged from upstream to downstream of each of the tributary inputs (Figure 5, Figure 3).

Measurements of *Bacteroides* sp. and enterovirus indicated the presence of human fecal contamination throughout the system (Table 5). *Bacteroides* sp. was present in 12 of 36 mainstem samples (33%). Enterovirus was present in 14 of 36 mainstem samples (44%). The concordance among these measurements was nearly complete; almost every location that detected *Bacteroides* sp. was also positive for enterovirus. Only two samples were positive for enterovirus and not *Bacteroides* sp. These two samples were furthest downstream or latest in the day.

Table 5. Number of enterovirus genomes (per 100 ml) detected.

Distance Upstream (km)	Time of Day					
	9:00	10:00	11:00	12:00	1:00	2:00
6.3	106*	71*	93*	70*	67*	
5.4		41*	19**	25*		
4.7		17*		113*	51	
2.6				79*		
1.5				13*		39
0						

* Human *Bacteroides* marker also detected.

** PCR reaction for human *Bacteroides* marker inconclusive due to inhibition.

Spatial and temporal patterns in enterovirus concentration were evident in the Ballona Creek system (Table 3). Main channel locations in the upper reaches of the study area were more likely to be positive for enteroviruses than downstream sites. The most consistently positive site was Cochran Ave., where 89% of the samples contained measurable levels of enterovirus. In addition, the highest concentrations of enterovirus were measured at Cochran Ave. during four of the six time periods. A general pattern in enterovirus detection was observed during the course of the day. Enterovirus was detected earliest in the day at upstream sites. Enterovirus was detected most frequently late in the day at the downstream sites. The 12:00 sampling interval had the most frequent detection of enterovirus with the highest concentrations observed at the middle sites in the watershed. Enterovirus was not detected in high concentrations in any of the tributaries; only Adams tributary had any detectable enterovirus.

Malibu Creek

Malibu Creek had a similar pattern of fecal indicator bacteria concentrations at each sampling event throughout the study period (Figure 6). Concentrations decreased along the mainstem as it flowed from Cold Creek to Cross Creek, then increased as it flowed through the estuary until it discharged into the ocean at Malibu Beach. The increase in fecal indicator bacteria through the lagoon averaged 10^2 MPN/100 ml for both

enterococcus and *E. coli*. The dilution factor from the discharge to the shoreline as a result of wave induced mixing averaged 0.86 for *E. coli* and 0.34 for enterococcus. Despite the increase in fecal indicator bacteria concentrations, none of the Malibu samples were positive for enteroviruses or *Bacteroides* sp.

BMP's

Both of the BMPs that incorporated UV treatment systems were more effective than the constructed wetland at removing fecal indicator bacteria (Table 6). Albeit concentrations were low in the influent, the constructed wetland did not reduce concentrations of *E. coli* or enterococcus using either culture-based or QPCR methods in the effluent. Both UV treatment systems, however, reduced influent concentrations of *E. coli* and enterococcus in the effluent to near or below method reporting levels. Enterococcus concentrations by QPCR were reduced by an order of magnitude, which was not as great as the culture-based method. No enterovirus or *Bacteroides* sp. were detected in any of the BMP influent or effluent samples analyzed.

Table 6. BMP effectiveness for indicator bacteria removal measured by culture-based (chromogenic substrate, CS) or quantitative polymerase chain reaction (QPCR) methods.

Indicator	Constructed Wetland		Filtration+UV		Filter+DAF+UV	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
<i>E. coli</i> (CS) (MPN/100 ml)	<10	36	58	20	147	<10
Enterococcus (CS) (MPN/100 ml)	<10	47	184	15	42	<10
Enterococcus (QPCR) (Cells/100 ml)	163	110	5004	729	No Data	No Data
Enterovirus (genomes/100 ml)	-	-	-	-	No Data	No Data

* no *Bacteroides* sp. detected

- no enterovirus detected

DISCUSSION

The Ballona Creek watershed is a system severely impacted by fecal pollution. The flux of fecal indicator bacteria was as high at the head of the watershed as it was at the mouth of the creek where it discharges into SMB. Although we focused on flux of these fecal indicator bacteria, it is important to note that 92% of all samples collected from Ballona Creek in this study, including 100% of the samples just upstream of SMB, exceeded the water quality thresholds established by the State of California. The presence of human enterovirus and human specific markers of *Bacteroides* sp. further documents the fecal inputs and should increase an environmental manager's awareness of the possible human health risks associated with these discharges.

Our study is not the first to examine the presence of viruses in urban runoff entering shorelines in SMB and other southern California urban watersheds. For example, Gold *et al.* (1990) and Gold *et al.* (1992) found viruses in repeated samples from multiple storm drains to SMB using both cell culture and RTPCR techniques. Haile *et al.* (1999) detected human specific viruses in all three storm drains tested in their epidemiology study of SMB. Noble and Fuhrman (2001) found human enteric virus genomes in the nearshore marine waters of SMB. Jiang *et al.* (2001) found human adenovirus in samples collected at 12 sites between Malibu and the Mexican border.

The multi-tiered approach used in this study can assist watershed managers in determining sources and efficiently abating the most significant inputs of fecal indicator bacteria. If managers relied solely on the patterns in fecal indicator bacteria from Ballona Creek, then the only option would be to treat the entire 37 m³/s discharge furthest downstream at Inglewood Ave. because the flux of fecal indicator bacteria was similar from all sources. The use of multiple tools, however, allows managers to prioritize the most important sources. In this case, the presence of human enterovirus was greatest from the Cochran Ave. site, where the system daylights from the underground storm drain system beneath Los Angeles and the discharge volume is one-third the volume at Inglewood Ave. Since Cochran Ave. had the most frequent occurrence and highest concentrations of enterovirus, this source would appear to be the most likely candidate for future management actions. The co-occurrence of the human *Bacteroides* sp. marker at most of the locations and time periods where enterovirus was quantified, most notably in all of the Cochran Ave. samples, provides the reassurance most managers would need before planning future management steps.

The lack of correlation between bacterial indicator levels and levels of human pathogenic viruses has been observed in previous studies (Dufour, 1984; Elliott and Colwell, 1985) and demonstrates the value of a multi-tiered approach used herein for source identification. For example, analysis of wild shellfish from the Atlantic coast of France indicated no significant correlation between fecal coliforms and enteroviruses or hepatitis A virus (LeGuyader *et al.*, 1993; Leguyader *et al.*, 1994), and viruses have sometimes been found in oysters without coliform contamination (Goyal *et al.*, 1984; Yamashita *et al.*, 1992). Noble and Fuhrman (2001) detected enterovirus in 35% of the 50 shoreline

samples they examined over a five year period and no significant statistical relationship to any of the standard bacterial indicators was found. Noble *et al* (2000) measured virus and fecal indicator bacteria in dry weather urban runoff in drains along 300 km of shoreline from Santa Barbara to San Diego. Despite 46% of the storm drains containing detectable enterovirus, there was no correlation with fecal indicator bacteria concentrations.

The results of this study indicated that Ballona Creek presents a greater risk to human health than Malibu Creek. There were no enterovirus or *Bacteroides* sp. detected in any sample from the Malibu Creek watershed. The bacterial concentrations were lower at Malibu Creek than at Ballona Creek; none of the Malibu Creek samples from the bottom of the watershed (at Cross Creek) exceeded water quality thresholds established by the State of California. Interestingly, fecal indicator bacteria concentrations increased as water flowed through the lagoon at the base of the Malibu watershed. No enterovirus or *Bacteroides* sp. was detected in these samples either, although other studies have detected human specific sources of viruses in this discharge (John Griffith *personal communication*).

The use of QPCR to measure fecal indicator bacteria presents unique opportunities and challenges. The advantage of QPCR for measuring fecal indicator bacteria is speed potentially providing measurements in less than four hours (Griffith *et al* 2004). However, culture-based methods only quantify viable bacteria, while QPCR measures the DNA from both cultivable and noncultivable microbes. This was most apparent in the temporal trends from Ballona Creek. Samples of enterococcus using culture-based methods generally decreased as the day progressed, most likely as the result of degradation from sunlight (Noble *et al.* 2004). Ballona Creek is a 40m wide concrete-lined channel concentrating solar energy into the shallow creek in the channel invert. The QPCR results, however, remained steady indicating that the bacterial DNA was still intact even though the enterococci were not viable.

The data from this study suggested the UV treatment systems were more effective than the constructed wetland at reducing fecal indicator bacterial concentrations. We suspected the UV treatment system would be effective since this method is a well-known mechanism for degrading bacteria (Fujioka *et al.* 1981, Davies and Evison, 1991, Davies-Colley *et al.* 1994, Noble *et al* 2004). Not only did the UV system reduce concentrations of enterococci using culture-based techniques, but it also degraded its DNA as shown by large reductions in enterococcus by QPCR. On the other hand, the effectiveness of the treatment wetland remains incompletely quantified. Although levels of fecal indicator bacteria were similar before and after flowing through the wetland, concentrations were very low to begin with. Monitoring by others at this treatment wetland suggest that it has been effective at reducing fecal indicator bacteria concentrations using culture-based methods (Nancy Palmer, City of Laguna Niguel *personal communication*). More study, particularly with the QPCR, will be needed before the wetland effectiveness can be fully quantified.

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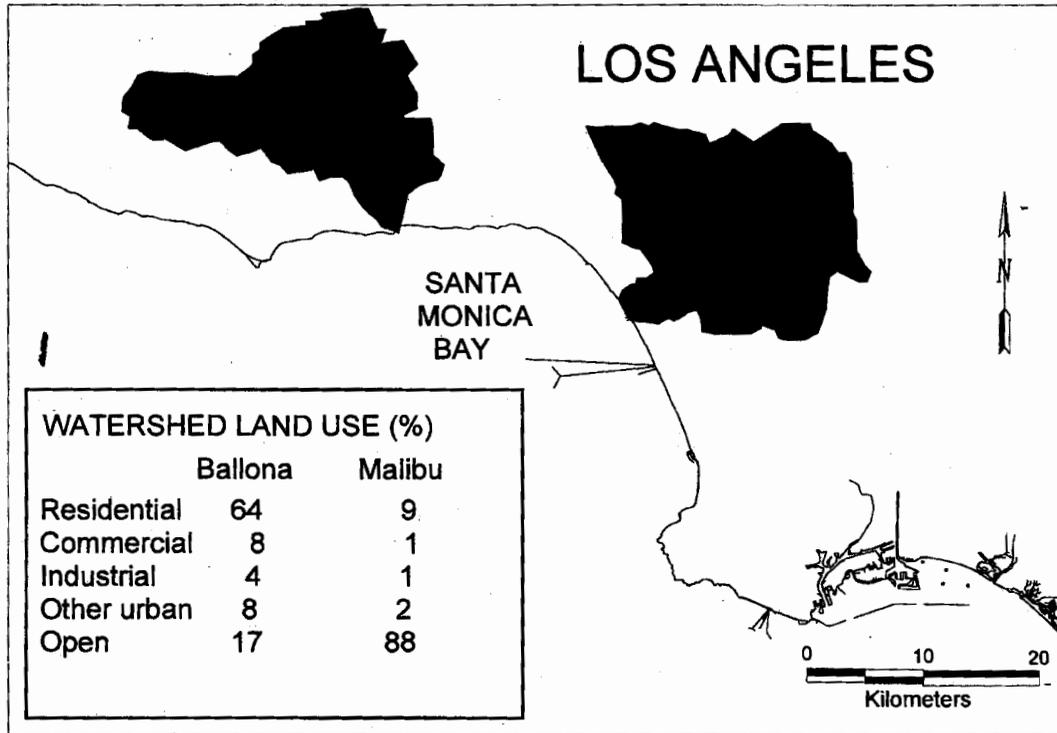


Figure 1: Map of Santa Monica Bay, CA indicating the locations and land use distribution for Ballona Creek and Malibu Creek watersheds.

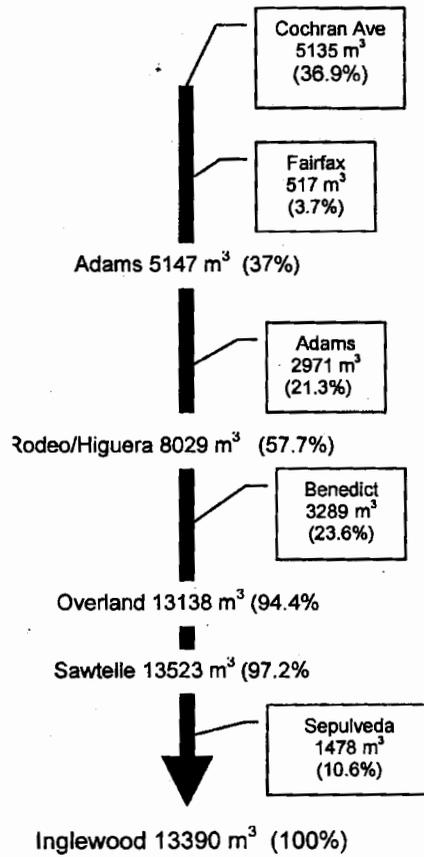


Figure 2. Schematic diagram depicting additive flow in main channel and percent contribution from each tributary.

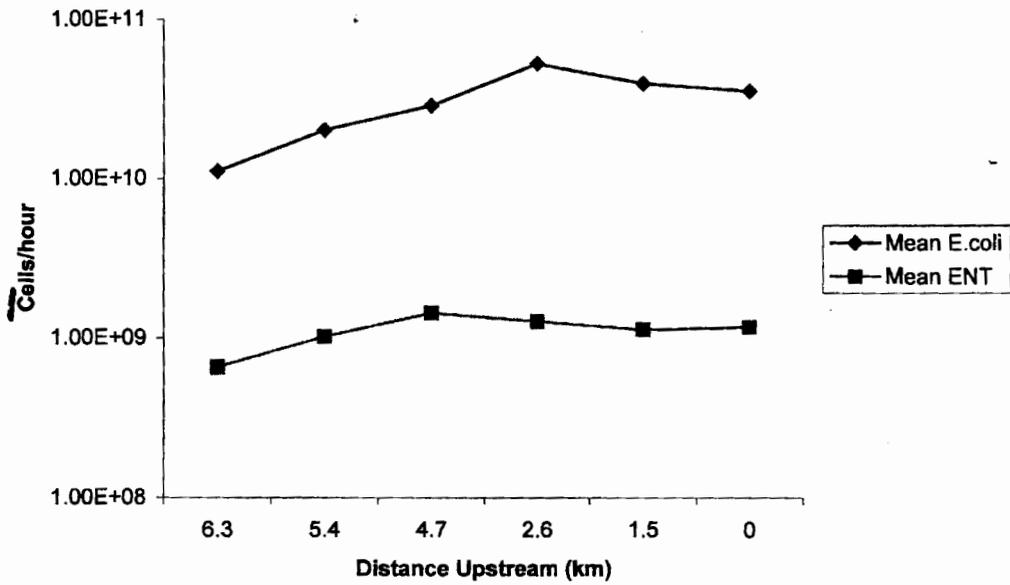


Figure 3. Mean hourly flux of *E. coli* and enterococcus at each station in main channel of Ballona Creek measured using the IDEXX™ method.

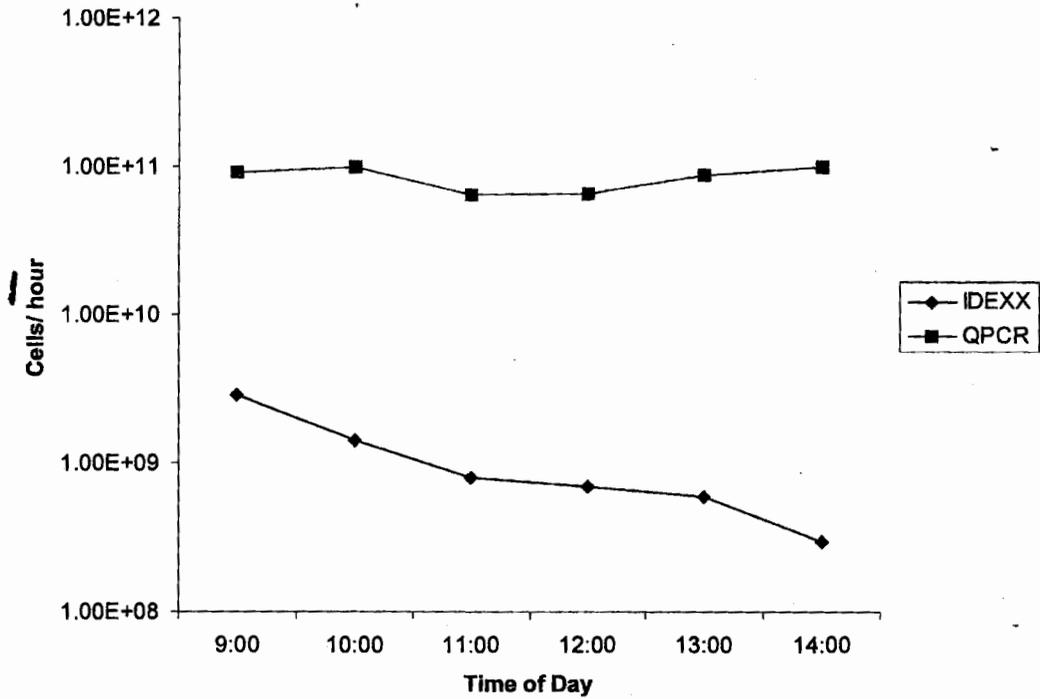
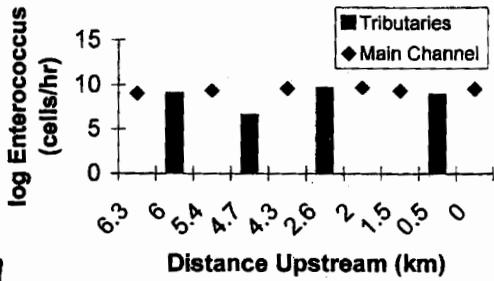
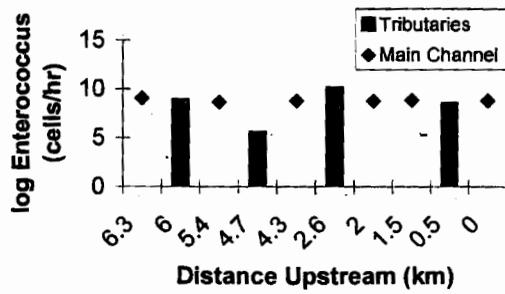


Figure 4. Mean hourly flux of enterococcus along the main channel of Ballona Creek as measured using both IDEXX the QPCR methods.

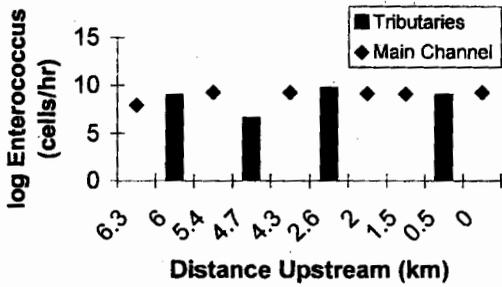
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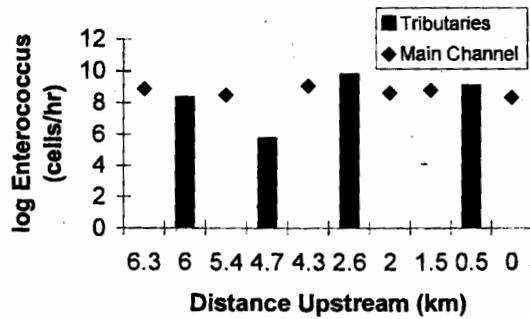
d)



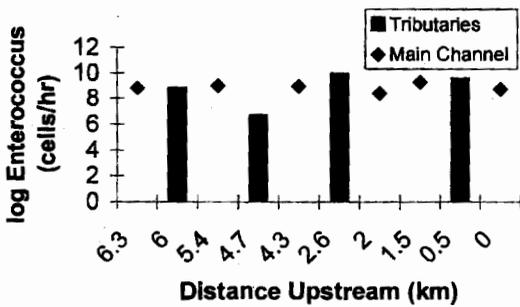
b)



e)



c)



f)

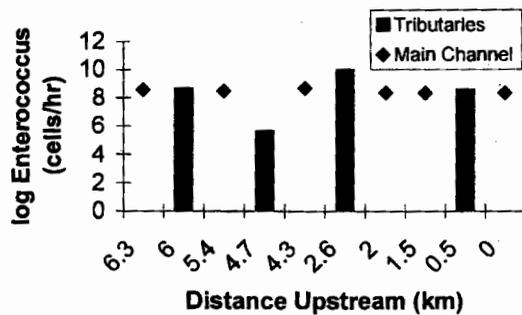
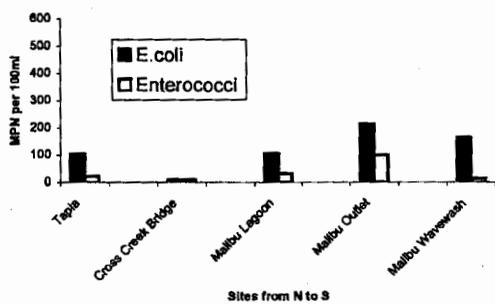


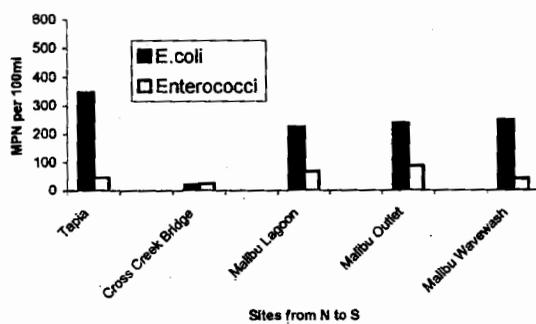
Figure 5. Enterococcus loading in main channel and tributaries of Ballona Creek at a) 9:00, b) 10:00, c) 11:00, d) 12:00, e) 13:00, f) 14:00.

Multi-tiered approach to source tracking using QPCR

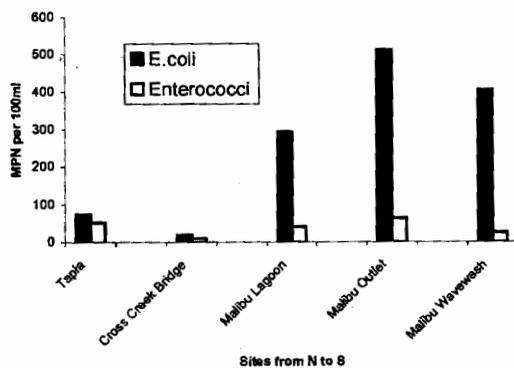
a)



b)



c)



Figures 6. Fecal indicator bacteria concentrations in Malibu Creek on a) 11/10/04 b) 11/11/04 and c) 11/12/04



Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland

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Abstract

Recreational water quality at beaches in California and elsewhere is often poor near the outlets of rivers, estuaries, and lagoons. This condition has prompted interest in the role of wetlands in modulating surface water concentrations of fecal indicator bacteria (FIB), the basis of water quality standards internationally. A model was developed and applied to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California, in response to loads from urban runoff, bird feces, and resuspended sediments. The model predicts the advection, dispersion and die-off of total coliform, *Escherichia coli*, and enterococci using a depth-integrated formulation. We find that urban runoff and resuspension of contaminated wetland sediments are responsible for surface water concentrations of FIB in the wetland. Model predictions show that urban runoff controls surface water concentrations at inland sites and sediment resuspension controls surface water concentrations near the mouth. Direct wash-off of bird feces into the surface water is not a significant contributor, although bird feces can contribute to the sediment bacteria load. The key parameters needed to accurately predict FIB concentrations, using a validated hydrodynamic model, are: the load due to urban runoff, sediment erodibility parameters, and sediment concentrations and surface water die-off rates of enteric bacteria. In the present study, literature values for sediment erodibility and water column die-off rates are used and average concentrations of FIB are predicted within 1/2 log unit of measurements. Total coliform are predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. Since wetland-dependent animals are natural sources of FIB, and FIB survive for long periods of time and may multiply in wetland sediments, these results highlight limitations of FIB as indicators of human fecal pollution in and near wetlands.

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Keywords: Enteric bacteria; Intertidal wetland; Coastal; Water quality; Sediment

1. Introduction

Fecal indicator bacteria (FIB) groups such as total coliform (TC), fecal coliform (FC), *Escherichia coli* (EC), and enterococci (ENT) are utilized world wide to measure health hazards in bathing and shellfish harvesting waters (Thomann and Mueller, 1987). Water samples at popular beaches and harvesting waters are

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routinely tested for FIB, which are thought to signal the presence of pathogens but are not necessarily pathogenic (US EPA, 1986). Chronic exceedances of California criteria have placed coastal water bodies such as Tomales Bay, Moss Landing Harbor, Morro Bay, Ventura Harbor, Marina Del Rey Harbor, Newport Bay, and Mission Bay on lists of pathogen impaired water bodies (CalEPA, 2002). Exceedances are also common at open ocean beaches, particularly near the outlets of storm drains, rivers, estuaries, and lagoons. Numerous coastal water bodies are impaired worldwide according to such standards.

Pathways by which FIB enter coastal waters include urban and agricultural runoff, waste water discharges, sewage leaks and spills, and fecal deposits by wildlife, notably birds. A complex web of processes influence the distribution of FIB in surface waters including flushing by ocean water, die-off, predation, sedimentation and resuspension, and regrowth on sediments, vegetation, and debris (Savage, 1905; Goyal et al., 1977; Roper and Marshall, 1979; Jensen et al., 1979; LaBelle et al., 1980; Grimes et al., 1986; Thomann and Mueller, 1987; Davies et al., 1995; Oshiro and Fujioka, 1995; Anderson et al., 1997; Byappanahalli and Fujioka, 1998; Solo-Gabriele et al., 2000; Grant et al., 2001). Use of FIB as indicators of human pathogens is complicated by these processes, particularly in wetlands where wildlife is abundant and nutrient rich sediments support growth of bacteria. So long as FIB remain the basis of regulations governing coastal water quality, a need will exist to identify the forcing factors (river inputs, storm drains, etc.) supporting FIB populations so that appropriate and cost-effective management measures can be implemented.

Several researchers have recently reported on models to predict FIB concentrations in coastal waters. Use of such models in coordination with field monitoring programs can help to identify the relative impact of various sources (e.g., a river versus a storm drain), characterize the mechanisms governing the fate of these organisms (e.g., flushing versus die-off) and predict the efficacy of a range of potential management measures. Kashefipour et al. (2002) used a model consisting of depth-integrated continuity, momentum, and transport equations to predict FIB concentrations in the Ribble Estuary, England. Fiandrino et al. (2003) used a model consisting of three dimensional continuity, momentum, and transport equations to predict FIB concentrations in Thau lagoon, France. Steets and Holden (2003) used a one-dimensional model to predict FIB concentrations in Arroyo Burro lagoon, California.

In this study we use a model to simulate dry-weather tidal cycling of TC, EC, and ENT concentrations in surface waters of Talbert Marsh, an intertidal wetland in Huntington Beach, California. Runoff from an urbanized watershed drains to the marsh, the marsh accommodates a high concentration of shore birds,

and high sediment concentrations of FIB have been measured. Grant et al. (2001) reported that Talbert Marsh was a net source of ENT to coastal waters and hypothesized that it was due to a combination of bird feces and interactions with sediments and vegetation. Sediments act as a reservoir of FIB (Goyal et al., 1977, e.g.), and suspension and deposition cycles are germane to the estuarine environment (Mehta and Dyer, 1990). In Talbert Marsh, it is not clear whether FIB concentrations are predominantly controlled by urban runoff, erosion of contaminated sediments, bird feces, or some combination of these factors. Therefore, the model is applied to examine and rank the influence of these "forcing factors." The modeling effort described in this paper is unique relative to previously published studies in that non-point loads of FIB (bird feces, erosion of contaminated sediments) are incorporated into a multi-dimensional, time-dependent formulation for the first time.

The model in this study consists of depth-integrated continuity and momentum equations to simulate circulation, and depth-integrated transport equations to simulate surface water concentrations of FIB resulting from urban runoff, bird droppings and resuspended sediments. The model is parameterized using either in situ data or previously published values of model parameters. The model is applied to predict FIB over a 15 day period beginning May 2, 2000, coincident with an extensive field monitoring effort previously reported (Grant et al., 2001, 2002). This work demonstrates the power of first-principle models to elucidate the mechanisms and pathways by which near-shore coastal waters are polluted by FIB.

2. Methods and materials

2.1. Site description

Talbert Watershed, shown in Fig. 1, is a 3300 ha catchment along the southern California coastline in the cities of Huntington Beach and Fountain Valley. On average, the watershed receives 29 cm of rainfall, over 90% of which falls between November and April. Daily high/low temperatures average 23/17°C in September and 17/8°C in January. The watershed slopes mildly (10^{-4}) towards the ocean and is drained by a network of channels that, due to the low elevation and mild slope of the watershed, are flooded by tides. Talbert Channel is the main stem of the network. Inland 2 km from the mouth, Huntington Beach Channel branches west and extends 5 km inland; and 8 km from the mouth, Fountain Valley Channel branches east. High tide floods Talbert Channel to the Fountain Valley Channel junction and the length of the Huntington Beach channel. Depths in the channels are comparable to the



Fig. 1. View of Talbert Marsh, channel network, and surrounding watershed as low tide. Channels and watershed extends several kilometers further inland than indicated in the figure. At high tide, the southwestern and southern portions of Talbert Marsh are flooded. PCH, BRK, and AES indicate monitoring stations.

tidal amplitude, roughly 1 m. Near the outlet, Talbert Marsh occupies roughly 10 ha of what used to be an extensive (1200 ha) tidal marsh environment that was filled for development over the past century. Talbert Marsh was created in 1990 when remnant marsh was flooded following the removal of a Talbert Channel levee. The channel bed consists of beach sand and silts near the outlet and within a flood delta that penetrates a short distance into the marsh. Further inland, the marsh and channel bed consists of organic rich silts and muds, except the upper reaches of Talbert Channel and Fountain Valley Channel where the bed is lined with concrete. In this study, the Talbert Marsh and tidal channels are collectively referred to as the wetland. From a perspective of flushing the wetland may be divided into two zones: a poorly mixed zone inland where residence times are at least a week, and a well-mixed zone near the mouth that is flushed each tide cycle

with ocean water. The interface between these zones oscillates with the ebb and flow of the tides.

The watershed is heavily developed as is common to the greater Los Angeles basin, and it contains separate networks of storm and sanitary sewers. Storm sewers direct runoff into street drains that funnel to the wetland. In the lower half of the watershed where the topography is lowest, runoff collects in one of several roughly 500 m³ forebays that are intermittently drained by pump stations. A program is now in place to divert dry-weather runoff from the storm sewer to the sanitary sewer for treatment. This program began on a limited basis in Fall 1999 and encompassed the entire watershed by Summer 2001. During the 15 day period that is the focus of this study, pump stations were operated in two different modes. During the first 8 days, pumpstations were not activated so runoff either collected in the forebay or was diverted to the sanitary sewer system.

During the remaining 7 days, pumpstations intermittently discharged untreated runoff to the channel network. Over the entire 15 day period, there was dry-weather baseflow in Talbert Channel that entered the wetland.

Monitoring stations referenced in this paper include Pacific Coast Highway (PCH), Brookhurst Street (BRK), and AES Corp. (AES). These are shown in Fig. 1.

2.2. FIB modeling

A hydrodynamic model was developed to simulate surface water concentrations of FIB in the wetland, from the outlet of Talbert Marsh to the head of the Huntington Beach Channel and, along Talbert Channel, to the Fountain Valley Channel junction. The model consists of depth-integrated continuity, momentum, and transport equations (Arega and Sanders, 2004), similar to the approach adopted by Kashefipour et al. (2002). The flow equations (continuity and momentum) were forced by the ocean tide just offshore of the marsh, and by runoff flowing into the upper reaches of the wetland. Ocean tide forcing was based on tide levels recorded at NOAA station 9410660, Los Angeles and archived online at <http://tidesonline.nos.noaa.gov/>. The discharge of runoff at the Talbert Channel inflow boundary, Q_R , was assumed steady over the study period. To model pumpstation operations, the discharge of runoff from each pumpstation, Q_P , was assumed uniform and steady over the final seven days of the study period, but zero over the first eight days. Seven

pumpstations that discharge directly into the wetland were incorporated into the model. Runoff data (Q_R and Q_P) were obtained from other reports (Grant et al., 2001, 2002; Chu, 2001) and appear in Table 1. Topographic data necessary for flow predictions were obtained from as-built plans of the concrete-line portions of the channels and a field survey of the Talbert Marsh (Chu, 2001). A uniform Manning coefficient was used to account for bed resistance (Arega and Sanders, 2004).

Simultaneous with the flow prediction described above, surface water FIB were predicted by solving the following transport equations:

$$\begin{aligned} \frac{\partial}{\partial t}(hc_i) + \frac{\partial}{\partial x}(\bar{u}hc_i) + \frac{\partial}{\partial y}(\bar{v}hc_i) \\ = \frac{\partial}{\partial x} \left(hE_{xx} \frac{\partial c_i}{\partial x} + hE_{xy} \frac{\partial c_i}{\partial y} \right) \\ + \frac{\partial}{\partial y} \left(hE_{yx} \frac{\partial c_i}{\partial x} + hE_{yy} \frac{\partial c_i}{\partial y} \right) \\ - hl_i + a_i + \sum_{k=1}^{N_{PS}} \mathcal{L}_k \delta(x - x_k^i, y - y_k^i), \end{aligned} \quad (1)$$

where h = depth [m] and \bar{u} , \bar{v} = components of the depth-averaged fluid velocity [m/s], E_{xx} , E_{xy} , E_{yx} , and E_{yy} = elements of the dispersion tensor [m²/s], c_i , ($i = 1, \dots, N_b$) = water column concentration of FIB [MPN/m³], N_b = number of FIB groups tracked by the model, l_i = water column loss rate [MPN/m³/s], a_i = flux of FIB to water column at sediment/water interface [MPN/m²/s], and \mathcal{L}_k = FIB loading rate of the i th FIB group at the k th inflow point [MPN/s], N_{PS} is the

Table 1
Measured, cited, and computed parameters used to estimate loading and die-off models

Parameter	Units	Total coliform		<i>E. coli</i>		Enterococci	
		Value	Uncertainty	Value	Uncertainty	Value	Uncertainty
k^a	m ² /Watts/h	0.0018	±10%	0.0017	±10%	0.00097	±10%
c_R^b	MPN/100 mL	1.5×10^4	$2.2 \times 10^4/1.0 \times 10^4$	9.8×10^2	$1.4 \times 10^3/7.1 \times 10^2$	1.8×10^3	$2.4 \times 10^3/1.3 \times 10^3$
r^b	MPN/bird/day	2.8×10^7	$8.4 \times 10^7/2.1 \times 10^6$	1.5×10^7	$1.0 \times 10^8/1.2 \times 10^7$	7.2×10^6	$2.6 \times 10^7/5.2 \times 10^6$
s^b	MPN/g	5.2×10^3	$1.3 \times 10^4/2.0 \times 10^3$	2.1×10^2	$8.5 \times 10^2/5.1 \times 10^1$	6.8×10^2	$1.6 \times 10^3/2.9 \times 10^2$
Parameter	Units	Value	Uncertainty (%)	Parameter	Units	Value	Uncertainty (%)
Q_R	m ³ /d	1000	±50	\bar{n}_b	—	174	±50
Q_P	m ³ /d	300	±50	$(\bar{\tau})$	Pa	0.08	±50
\bar{A}_S	ha	32	±50	τ_0	Pa	0.75	±50
E_0^c	kg/m ² /s	1×10^{-4}	±50	τ_c^c	Pa	0.25	±50

Except where noted, a conservative estimate of 50% uncertainty was adopted. Note that the mathematical model is presented using SI units, so conversion factors need not appear in model equations. Commonly used units are presented here to facilitate comparison with previous works and other studies.

^aDie-off rates based on Sinton et al. (1999).

^bMeasured in situ, uncertainty based on standard error.

^cErodibility rates based on Uncles and Stephens (1989).

number of inflow points where runoff is added to the wetland (pump stations and tributary inflow), x_s^k and y_s^k = coordinates of each inflow point [m], and δ = Dirac delta function [$1/m^2$]. The dispersion tensor accounts for longitudinal dispersion (Elder, 1959) and transverse mixing (Ward, 1974), and it is computed locally depending on the orientation of the currents (Arega and Sanders, 2004). Note that SI units are adopted for the purpose of presenting the mathematical model, so conversion factors need not appear in model equations. However, many model parameters are reported in Table 1 with commonly used units to facilitate comparison with previous works and other studies.

Eq. (1) was solved using $N_b = 9$ to predict the distribution of TC, EC, and ENT resulting from urban runoff, bird feces, and sediment resuspension. Groups 1–3 correspond to TC, EC, and ENT concentrations resulting from runoff sources, 4–6 from bird sources, and 7–9 from sediment sources. All model predictions account for surface water die-off using first order kinetics as follows:

$$I_i(x, y, t) = k_i I(t) c_i(x, y, t), \quad (2)$$

where k_i = die-off rate constant [$m^2/Watts/s$] based on Sinton et al. (1999), and $I(t)$ = solar intensity [$Watts/m^2$]. Die-off rates used in the model were taken from Sinton et al. (1999), and solar intensity data for the study period were obtained from Grant et al. (2001).

The model does not account for settling. Suspended sediments in the $1-10^3 \mu m$ size range are typical of intertidal wetlands adjacent to sandy ocean beaches, but FIB in southern California coastal waters are either free-living (planktonic, roughly $1 \mu m$ in size) or associated with very fine sediments, probably in the $10 \mu m$ range or less (Ahn et al., 2005). The relative influence of settling and die-off is defined by the ratio $w_s/k_i h$, where w_s is the settling velocity. Using Stokes Law to model the settling velocity in terms of particle size (e.g. Nazaroff and Alvarez-Cohen, 2001, Chapter 4), the average solar radiation rate for the study period ($288 \text{ Watts}/m^2$), die-off rates reported by Sinton et al. (1999), and a depth of 1 m which is typical for the wetland, this ratio is unity for ENT when particle diameter $d = 10 \mu m$ and for TC and EC when $d = 13 \mu m$. When $d = 5 \mu m$, this ratio is 0.3 for ENT and 0.2 for TC and EC. The nonlinear dependence is due to the quadratic relationship between settling velocity and particle size. Without a clear understanding of the partitioning of FIB between free-living and particle-associated states, and knowledge of the median diameter of particles with attached FIB, selecting an appropriate settling velocity is difficult. Certainly, without settling terms the model will underestimate water column FIB losses if these organisms are associated with particles in the $10-20 \mu m$ range or larger.

Therefore, this assumption should be reconsidered if the model significantly overpredicts FIB concentrations.

For all predictions, the concentration of FIB in water entering the wetland from the ocean was set to zero. For the urban runoff predictions ($i = 1-3$), point loads of FIB were specified at runoff inflow points and the non-point loading term, a_i , was set to zero. The loading rate was set equal to the volumetric flow rate multiplied by the concentration of FIB in runoff, c_R , which was specified based on average Talbert Watershed urban runoff concentrations reported by Reeves et al. (2004).

FIB loading to surface waters by bird feces ($i = 4-6$) was modeled as a spatially distributed (around the water line) and temporally variable non-point source. It was assumed that all bird feces fell exclusively on the shoals of the marsh, were subject to sunlight induced die-off, and upon flooding by the tide were instantaneously and completely transferred to the water column. Hence, loading in the model occurs at water's edge during the rising tide. This approach was motivated by bird surveillance data, which showed birds congregated on shoals during low tides (Grant et al., 2001). The following mass balance equation was solved to track the build-up and die-off of FIB on the shoals of the marsh,

$$\frac{dm_i(x, y, t)}{dt} = d_i(t) - k_i I(t) m_i(x, y, t), \quad (3)$$

where m_i = the surficial FIB density [MPN/m^2] and d_i = FIB loading rate [$MPN/m^2/s$]. Note that the die-off rate constant for the marsh banks is identical to that used for surface water. The FIB loading rate was computed as,

$$d_i(t) = n_b(t) r_i / A_{IT}(t), \quad (4)$$

where $A_{IT}(t)$ = the exposed (or dry) inter-tidal surface area [m^2], $n_b(t)$ = bird population measured hourly in the marsh and r_i = rate of FIB loading per bird [$MPN/bird/s$]. The exposed inter-tidal surface area (or area of the exposed shoals) was determined from the marsh topography as the difference between the exposed surface area of the marsh and the exposed surface area under high spring tide conditions. This varied from 0 to 4.5 ha depending upon the tide stage. Table 1 presents bird loading rates used in the model, which were based upon samples collected Talbert Marsh. The sampling methodology is described in (Grant et al., 2001), but only ENT concentrations are reported. TC and EC were quantified from the same samples using defined substrate tests (IDEXX, Westbrook, Maine), but the data have not previously been reported.

FIB loading rates of birds vary widely depending upon species, habitat, diet, and feeding habits. Hussong et al. (1979) reported fecal coliform loading rates for wild swan and Canadian geese of 10^6-10^9 and 10^4-10^7 $MPN/bird/day$, respectively, Gould and Fletcher (1978)

reported fecal coliform loading rates for several gull species in the range of 10^6 – 10^7 MPN/bird/day. Alderisio and DeLuca (1999) reported fecal coliform loading rates of roughly 10^8 and 10^5 MPN/bird/day for ring-billed gulls and Canadian geese, respectively. Rates reported in Table 1 for shore birds in Talbert Marsh are similar, roughly 10^7 MPN/bird/day for all three indicator groups. During the study period bird populations ranged from 0 to 1180 (Grant et al., 2001).

After being flooded by the rising tide, the wash-off of surficial bacteria from the marsh banks contributes to the sediment/water interface loading rate, a_i appearing in Eq. (1), as follows:

$$a_i(x, y, t) = m_i(x, y, t)\delta(t - t_f), \quad (5)$$

where t_f = the instant land is flooded by the rising tide [s] and δ = Dirac delta function [1/s]. Hence, the transfer of surficial FIB from the banks of the marsh to surface waters is modeled as an instantaneous exchange that is triggered by moment the bank is flooded by the rise of the tide. After transfer to surface waters, $m_i = 0$ until the banks are again dry at which point the build-up process resumes.

FIB loading to surface waters by sediments ($i = 7$ – 9) was modeled as a spatially distributed and temporally variable non-point. The non-point loading term a_i in Eq. (1) was formulated to account for the transfer of FIB to the water column that occurs when FIB laden particulate matter and pore water on the bed is mobilized by turbulent shear. The mobilization of estuarine sediments occurs after a threshold in turbulent shear has been exceeded, and in proportion to the excess of turbulent shear above the threshold (Partheniades, 1965; Mehta and Dyer, 1990). Whether or not the same is true for FIB is not clear, for FIB may be free living in sediment pore water, attached to sediment grains, or incorporated into microbial biofilms; and how these phases of FIB respond to shear is not known. Therefore, a novel approach was taken. The FIB loading term was developed by dimensional analysis with the following conditions in mind: (a) that the transfer rate of FIB from sediments to surface waters be proportional to the shear rate; and (b) that FIB liberated from the sediments over a tide cycle be equal to FIB present (either attached to particles or free-living in pore water) in the erodible layer of surficial sediments. Therefore, the following rate expression was used,

$$a_i(x, y, t) = s_i E \frac{\tau(x, y, t)}{\tau_0} \left(\frac{\tau_0}{\tau_c} - 1 \right), \quad (6)$$

where s_i = geometric mean concentration of FIB per mass of sediment [MPN/kg], E = entrainment rate parameter [$\text{kg}/\text{m}^2/\text{s}$], τ = spatially and temporally varying shear stress at the bed [Pa] computed by the hydrodynamic model, τ_c = critical shear stress for

erosion [Pa], and τ_0 = reference stress [Pa] representative of erosive conditions in the wetland.

The reference stress was computed based on water level and velocity data collected at BRK (Arega and Sanders, 2004). BRK serves as a good reference point due to its central location. Using a drag coefficient of 0.003 which is typical of estuaries, a fluid density of $1 \text{ g}/\text{cm}^3$, and a velocity of $0.5 \text{ m}/\text{s}$, the reference stress was estimated to be $\tau_0 = 0.75 \text{ Pa}$. A velocity of $0.5 \text{ m}/\text{s}$ was used for this calculation since the peak flood velocity varies from 0.4 to $0.6 \text{ m}/\text{s}$ over the spring-neap cycle, while the peak ebb velocity varies from 0.1 to $0.4 \text{ m}/\text{s}$. Site specific entrainment rate and critical shear parameter estimates were not available, so values reported in the literature by Uncles and Stephens (1989) and Tattersall et al. (2003) were used. All model parameters are reported in Table 1.

Note that measured concentrations of FIB in Talbert Marsh sediments were utilized to estimate s_i . No attempt was made to model the cycling of FIB in submerged sediments. To estimate the concentration of FIB in sediments, cores were collected at low tide within the inter-tidal zone and immediately transported to the laboratory. Overlying water was siphoned off the top and the cores were sectioned in 1 cm intervals with an extruder. For the few cores with a high sand content, sediment was scraped from the core tube in specified intervals to avoid slumping. Each sediment section was homogenized. A 5 g sample was suspended with 45 ml of a 0.5 M mono potassium phosphate buffer solution in a sterilized glass centrifuge tube for enteric bacteria analysis (APHA, 1992, Methods 9221 and 9050C). The sample was agitated for 1 min with a vortex mixer, then centrifuged at 2000 rpm for 5 min . The supernatant was then analyzed for TC, EC, and ENT using defined substrate tests with dilutions to the supernatant made with DI water (IDEXX, Westbrook, Maine). The remaining sediment from each 1 cm section was oven dried at 50°C , and stored for analysis of grain size. The concentration s_i was taken as the geometric mean of FIB concentrations in the top 1 cm of each sample, and is reported in Table 1.

An important assumption of this formulation is that sediment concentrations are constant over the two-week study period. Unpublished sediment data collected on a daily to weekly basis in nearby Santa Ana River wetlands show sediment concentrations of FIB increase at least one log unit immediately following storms, and subsequently decrease over a period of several days to weeks; but during dry-weather periods sediment concentrations are relatively uniform (Ambrose, 2004). This assumption would no longer be appropriate were the model used for wet-weather conditions or to predict variability on seasonal time scales.

The hydrodynamic equations, FIB transport equations, and mass balance equation for FIB build-up/

die-off on inter-tidal mudflats were integrating using a common time step of 0.2 s on an unstructured grid of 11732 quadrilateral cells encompassing all the wetted and inter-tidal portions of the channel network shown in Fig. 1. The flow and transport equations were solved by a finite volume numerical method described and validated for this study site by Arega and Sanders (2004). The build-up/die-off model for the load due to bird feces was solved using a backwards Euler discretization, for stability purposes and without concern for time-stepping errors due to the very small time step. The time of flooding, t_f appearing in Eq. (5) is determined in the model as the moment that all four nodes of a cell first become submerged by the rising tide. The solution of this model gives a spatially and temporally varying prediction of FIB concentrations in the wetland resulting from loading by urban runoff, bird feces, and sediment resuspension.

To summarize, nine different FIB concentration fields were predicted for the 15 day period beginning May 2, 2000 based on three different sources of three different FIB groups. Urban runoff loads were modeled by several point sources located at inland sites. Bird feces loads were modeled by a build-up, wash-off model: bacteria concentrations build up on inter-tidal mudflats and wash off (to surface waters) with the rising tide. Sediment loads were modeled by a non-point source that is scaled by the shear stress on the bed. For all nine predictions, the model accounts for FIB advection, dispersion, and die-off. Initial conditions for the model were obtained by a spin-up procedure. Starting with an FIB concentration of zero, predictions were made for two sequential 15 day periods, and results of the second 15 day period were saved and used for analysis purposes. Forcing data such as the ocean tide record, solar radiation data, and bird census data were simply duplicated into 30 day records. Finally, predictions were compared to FIB measurements at PCH and BRK monitoring stations (Fig. 1) reported by Grant et al. (2001, 2002). Water level, velocity, and turbidity data for PCH and BRK reported by Grant et al. (2001) were also utilized for model validation purposes.

2.3. Uncertainty in Model Predictions

Uncertainty in FIB predictions is due to several factors including: (a) approximations inherent to the mathematical representation of FIB transport processes; (b) errors incurred during the numerical solution of the mathematical model; and (c) uncertainty in model parameters and in particular, parameters that characterize point and non-point loads of FIB. Uncertainties in parameter values were estimated based on standard errors or literature reported values, where possible. Otherwise, a conservative estimate of 50% was used. Table 1 presents uncertainty estimates. In cases invol-

ving FIB concentrations, uncertainties may be 200–500%. By comparison, uncertainty associated with the mathematical model and numerical method are relatively small, roughly 20% and 1%, respectively, based on previous modeling efforts (Arega and Sanders, 2004). Therefore, the propagation of uncertainty in the model was ignored for the purpose of determining uncertainties in predicted FIB concentrations, and emphasis was placed on the uncertainty in loading terms (Holman, 1978). Hence, the relatively uncertainty in FIB predictions was assumed to be equal to the relative uncertainty in the corresponding FIB load. Based on the preceding model formulation for urban runoff, bird, and sediment loads of FIB, spatially and temporally averaged loading rates follow as:

$$L_R = (Q_R + \tau Q_P)C_R, \quad (7)$$

$$L_B = \overline{n_b}r, \quad (8)$$

$$L_S = sE \frac{(\overline{\tau})}{\tau_0} \left(\frac{\tau_0}{\tau_c} - 1 \right) \overline{A}_S, \quad (9)$$

where the overbar notation indicates a time-average value, the angled brackets indicate a spatial average, A_S represents the submerged surface area of the wetland, and the subscripts R, B, and S denote loads from runoff, bird droppings, and sediments, respectively. The upper limit of uncertainty was estimated by a conventional variational method (Taylor and Kuyatt, 1994), but this method predicted negative loads at the lower limit. Hence, the lower limit of the loads were estimated by computing the load based on lower limit parameter values. After upper and lower uncertainties for each of the nine FIB loads were estimated, these were normalized by the corresponding load to obtain relative uncertainties.

3. Results

Model predictions of water level and velocity during the study period compare well to measurements, as shown in Fig. 2. This indicates that the dominant circulation pattern in the wetland, which drives the mixing and flushing of FIB, is resolved. The spatial distribution of TC predictions at mid-flood tide are shown in Fig. 3 for the case of loading by bird feces (left panel), urban runoff (center panel) and sediment resuspension (right panel). For the case of loading by urban runoff, where FIB enter the wetland far inland along the channels and transport to the marsh during the ebb, the mid-flood condition highlights the transport of (assumed to be) FIB-free ocean water into the main channel of the marsh while remnant wetland water is displaced either into the fringes of the marsh or inland along the channels (note the gradient in FIB between the

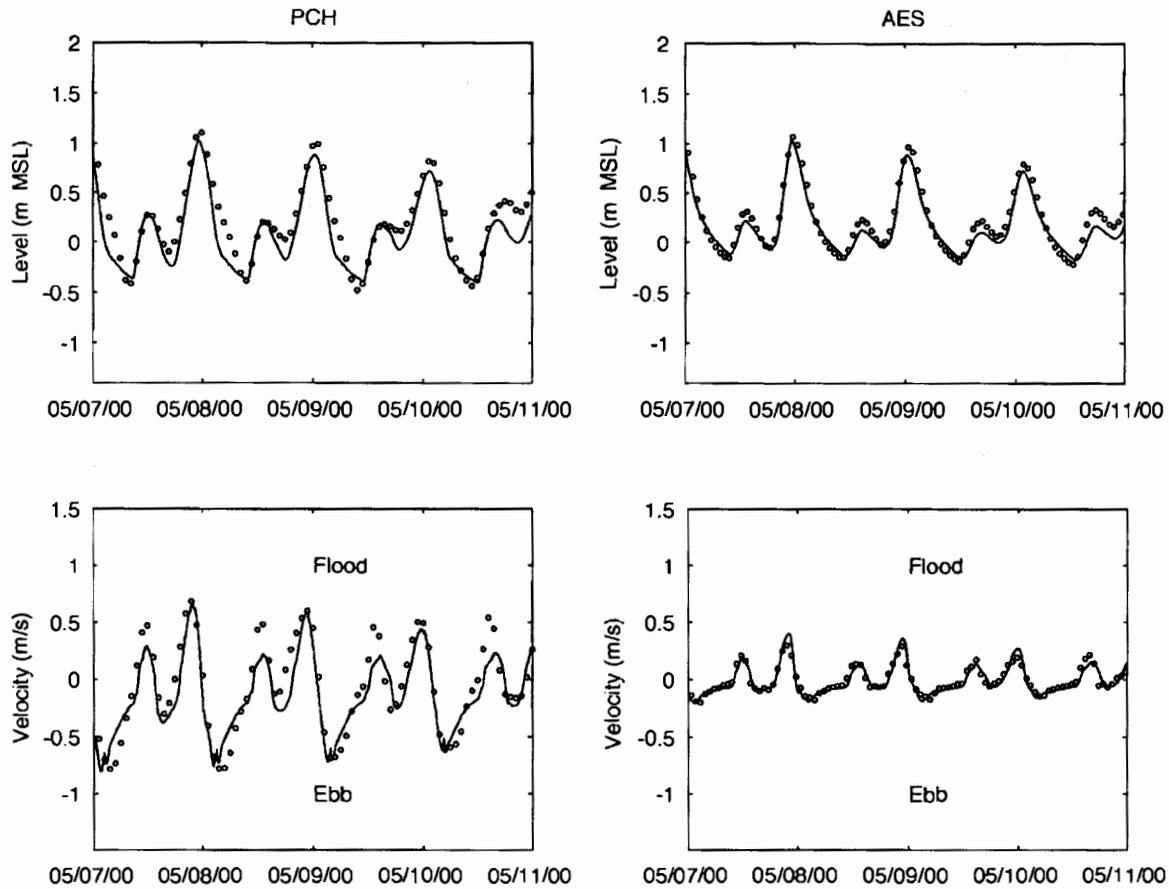


Fig. 2. Comparison of model predicted water level (top) and velocity (bottom) to data reported by Grant et al. (2001). Solid line corresponds to model prediction, symbols correspond to data.

main channel and the fringes of the marsh). For the case of loading by bird feces, model predictions illustrate the concentration of TC near the banks and over the shoals of the marsh. This is an expected response since FIB loading is modeled at the interface between wet and dry land. For the case of loading by sediment resuspension, FIB concentrations are relatively uniform across the marsh, compared to forcing by urban runoff or bird droppings. A similar distribution is predicted for EC and ENT.

Model predictions and measurements of FIB for the two-week study period are shown for BRK and PCH in Figs. 4 and 5, respectively, along with water level and turbidity. The tide record shows the spring-neap-spring transition. Note that water levels in the marsh do not drop far below -0.5 m-MSL due to hydraulic choking which occurs during the ebb at the outlet, where the minimum bed elevation is close to -0.7 m-MSL . FIB predictions vary considerably depending upon the type of loading, both in terms of magnitude and variability,

particularly at 1 and 2 cycles per day. In addition, the variability of each prediction appears unique. Therefore, the phasing and magnitude of FIB predictions for each load type (i.e., urban runoff, bird feces, or sediment) can be utilized to help determine the contribution towards observed FIB concentrations. Pearson correlation coefficients were computed to quantify how well each prediction captured the variability, or phasing, of measured FIB concentrations and are shown in Table 2. Mean values of each prediction, and uncertainty based on loading rate uncertainty, are listed in Table 3. Mean values of measured FIB, along with standard errors based on $N = 360$ are also shown for comparison purposes. The "combined" FIB time series referenced in Tables 2 and 3 represent the sum of the three FIB predictions (i.e., urban runoff, bird feces, and sediment), a valid operation for linear transport equations. That is, the combined FIB time series is precisely what the model would have predicted had each of the forcing factors been incorporated into a single

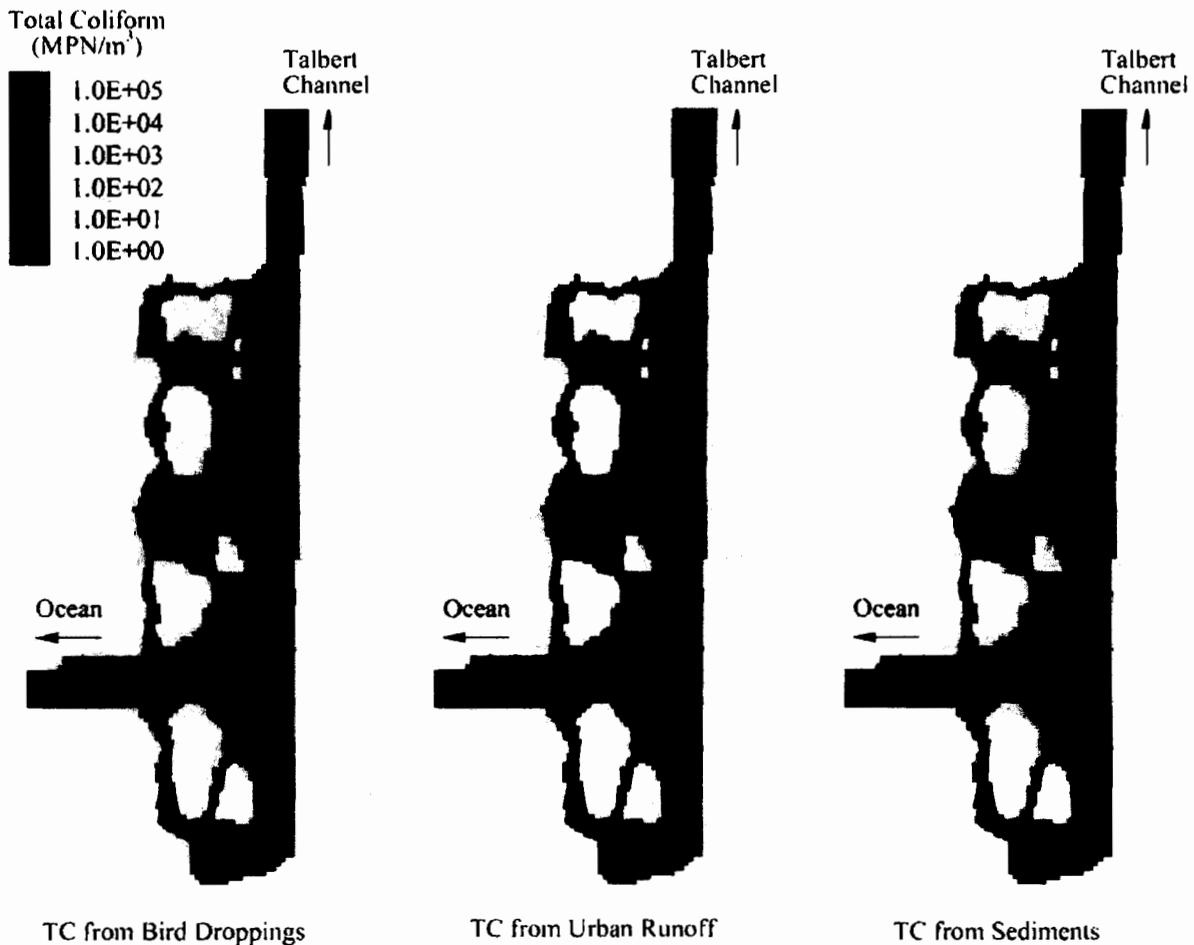


Fig. 3. Contours of total coliform in Talbert Marsh predicted by the model for mid-flood tide. Black lines indicate velocity direction and relative magnitude.

simulation. To obtain the mean value, the combined time series was first log-transformed. The combined series is not shown in Figs. 4 and 5, but at any given instant it basically tracks the largest of the three curves representing different forcing factors.

TC at PCH are predicted remarkably well based on loading by sediment resuspension, as shown in Fig. 5. The mean of log transformed measurements, $\log_{10}(\text{TC}) = 2.17(\pm 0.04)$, or “log mean”, compares well with the log mean of predictions $\log_{10}(\text{TC}) = 2.25(+0.45/-1.02)$; and there is a moderate correlation ($R^2 = 0.58$, $p_{N=360} < 0.01$) between log transformed predictions and measurements on an hourly basis. Predictions based on loading by urban runoff compare best to measurements at the end of the ebb tide, particularly during the second week of the study when pump stations contributed runoff to the channels, but not at other phases of the tide and this is reflected by a weaker but significant correlation ($R^2 = 0.37$, $p_{N=360} < 0.01$). Predictions based

on bird feces loading appear at least three orders of magnitude too small to account for observed TC.

Similar trends can be observed at BRK. Predictions based on both urban runoff and sediment loading are large enough to account for measured FIB, though in this case measurements correlate better to the prediction based on runoff ($R^2 = 0.56$, $p_{N=360} < 0.01$) than sediment resuspension ($R^2 = 0.26$, $p_{N=360} < 0.01$). The prediction based on bird feces loading is too small to account for observed TC. When predictions based on all three forcing factors are added together (valid for linear transport equations), the prediction at BRK correlates slightly better ($R^2 = 0.58$, $p_{N=360} < 0.01$) and the magnitude of the signals compare well (Table 3).

For ENT and EC, trends in model predictions are similar to TC. However, trends in measured FIB differ. Both ENT and EC measurements compare best to predictions based on sediment resuspension loading, both in terms of geometric mean concentrations

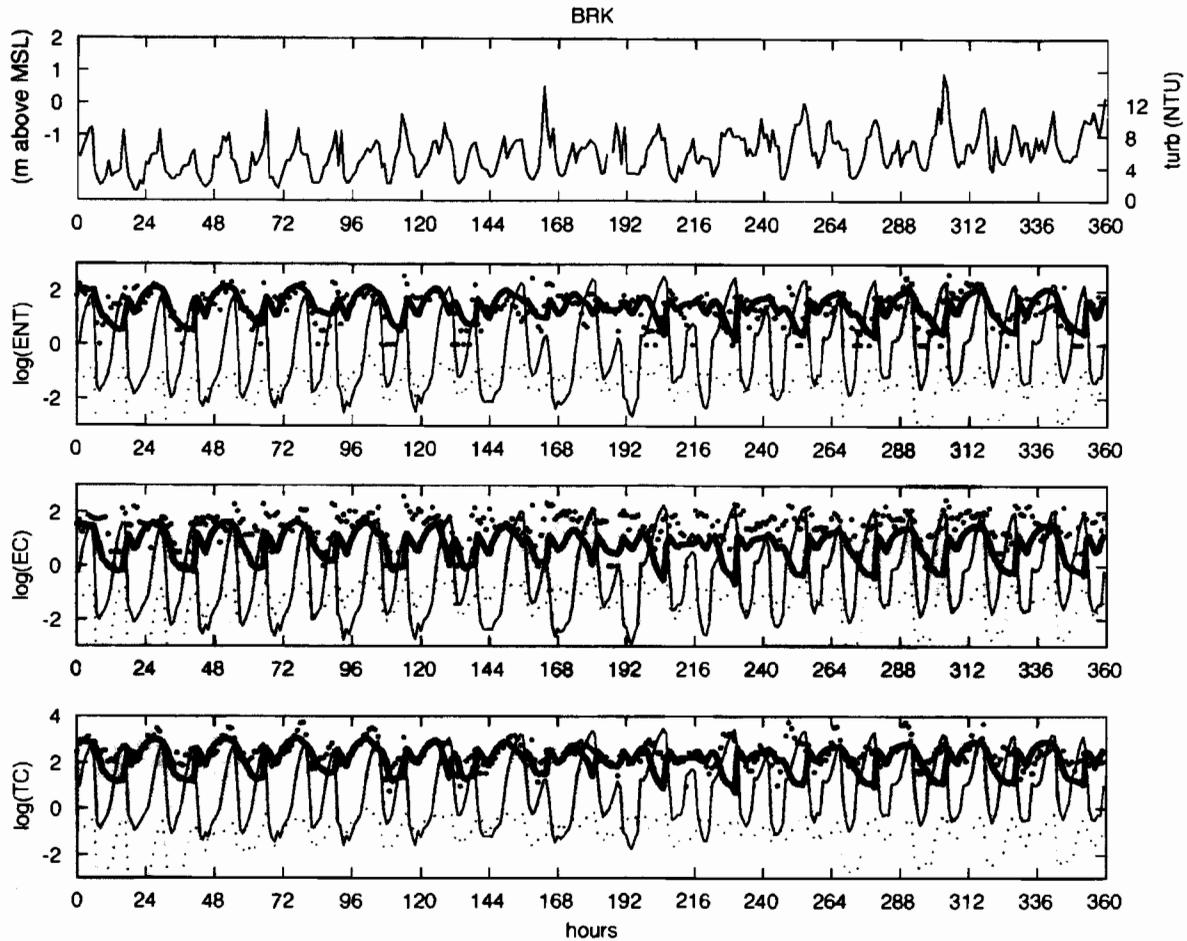


Fig. 4. BRK results. Water level and turbidity reported by Grant et al. (2001) shown in top panel. Bottom three panels show FIB concentrations: data from Grant et al. (2001, 2002) (dots), prediction based on sediment loading (heavy line), prediction based on runoff loading (light line), and prediction based on bird loading (broken line). FIB concentrations reported as log₁₀ (MPN/100 ml).

(Table 3) and the correlation coefficient (Table 2). Predictions based on bird feces loading are several log units too small to account for measured concentrations. Predictions based on urban runoff loading are comparable in magnitude only at the end of the ebb tide, and do not correlate to measurements.

Correlations between turbidity measurements and FIB measurements over the first six days were also computed and these appear in Table 4 (Due to drift in the turbidity data, the second week of data was excluded.) Turbidity correlates best to TC, compared to ENT and EC, and the correlation is stronger at BRK than PCH. Correlations between turbidity measurements and FIB predictions based on loading by urban runoff and sediment resuspension were also computed. Predictions based on urban runoff loads serve as an index of particulate material transported from upstream (fine mineral particles, detritus, and plankton) where

flow is quiescent, while predictions based on sediment loads serve as an index of material eroded locally in the lower reaches of the wetland where the shear is greatest. At BRK the turbidity signal correlates better with FIB predictions based on runoff forcing ($R^2 = 0.70$, $p_{N=144} < 0.01$) than FIB predictions based on sediment resuspension forcing ($R^2 = 0.19$, $p_{N=144} < 0.01$). At PCH the turbidity signal correlates slightly better with the prediction based on sediment resuspension ($R^2 = 0.57$, $p_{N=144} < 0.01$) than the prediction based on runoff ($R^2 = 0.47$, $p_{N=144} < 0.01$).

4. Discussion

Hydrodynamic model predictions show that tidal cycling of TC, EC, and ENT in Talbert marsh surface waters is driven primarily by two processes: advection of

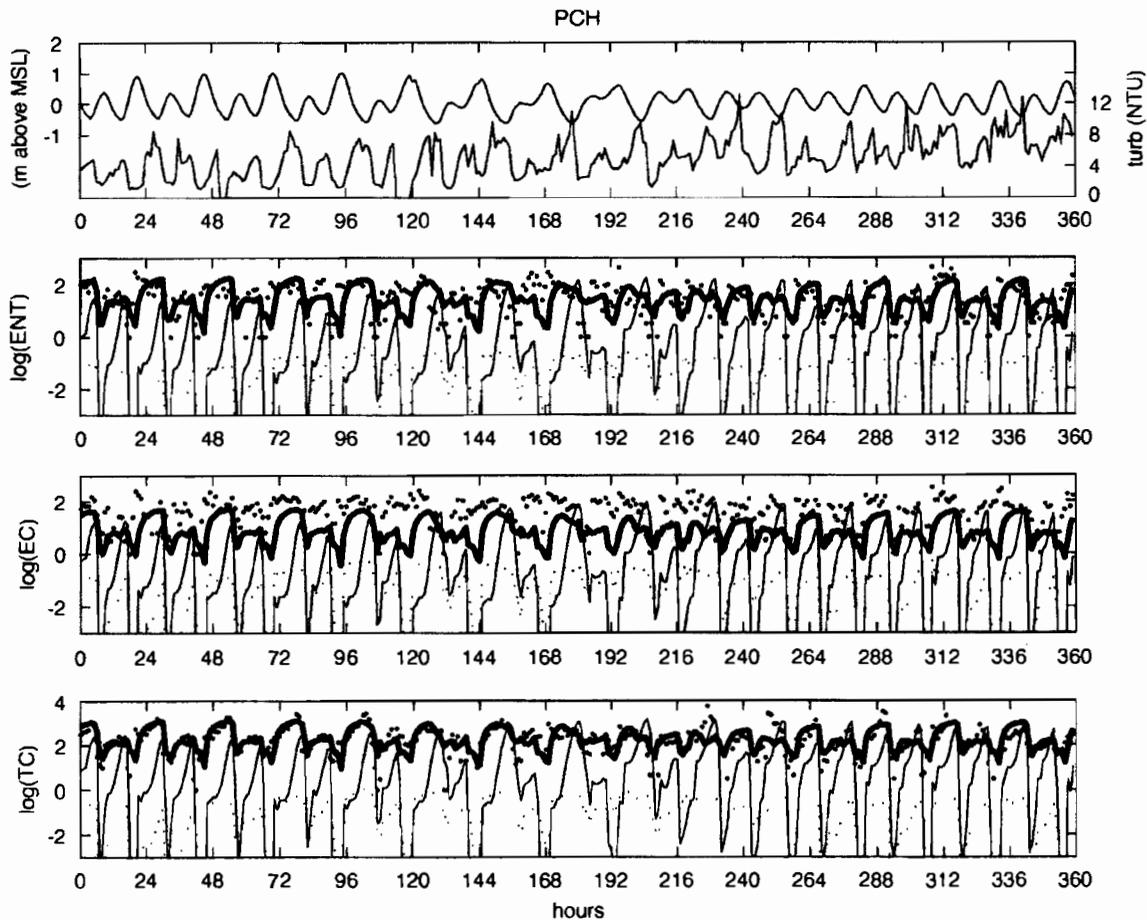


Fig. 5. PCH results. Water level and turbidity reported by Grant et al. (2001) shown in top panel. Bottom three panels show FIB concentrations: data from Grant et al. (2001, 2002) (dots), prediction based on sediment loading (heavy line), prediction based on runoff loading (light line), and prediction based on the bird loading (broken line). FIB concentrations reported as \log_{10} (MPN/100 ml).

FIB from inland sources (urban runoff) and entrainment of FIB from sediments. Loads of FIB from urban runoff control surface water concentrations inland within the poorly flushed zone while tidal resuspension controls surface water concentrations in the well-flushed zone near the mouth. Therefore, water quality models for FIB in hydrodynamically active wetland surface waters should at minimum account for loads from point sources (storm drains, channels, etc.), loads from resuspended sediments, transport by advection and turbulent dispersion/diffusion, and die-off. The present model captures tidal variability of TC better than EC or ENT suggesting either that processes important to EC and ENT transport are not included in the model, or perhaps the model oversimplifies one or more of the processes that are included in the model. For example, the spatial distribution of EC and ENT in sediments

may differ substantially from the TC distribution due to differences in survival and/or regrowth rates. Many studies have shown that FIB can survive for long periods or regrow attached to sediments and vegetation (Savage, 1905; Roper and Marshall, 1979; LaBelle et al., 1980; Davies et al., 1995; Desmarais et al., 2002). In tropical watersheds, regrowth has been cited as the dominant factor affecting bacteria loading in streams (Hardina and Fujioka, 1991; Fujioka et al., 1999). The ability of bacteria to secrete extracellular polymers (collectively termed microbial biofilms) may be one reason why survival and regrowth of FIB is enhanced in sediments (Decho, 2000). A model capable of simulating sediment concentrations of FIB, accounting for these factors, might lead to better EC and ENT predictions. In cases where the size of particles with attached FIB is known, settling can also be included in the model if size

Table 2
Pearson correlation between log transformed enteric bacteria measurements and model predictions ($N = 360$)

	Station	Bird source	Sed. source	Runoff source	Combined
Total	BRK	0.36*	0.26*	0.56*	0.58*
coliform	PCH	0.40*	0.58*	0.37*	0.55*
<i>E. coli</i>	BRK	0.33*	0.35*	0.04	0.39*
	PCH	0.28*	0.33*	0.10	0.22*
Enterococci	BRK	0.27*	0.47*	-0.02	0.36*
	PCH	0.23*	0.34*	0.04	0.24*

*Significant at the 0.01 level (2-tailed).

Table 3
Comparison between predicted and measured geometric mean bacteria concentrations [$\log_{10}(\text{MPN}/100 \text{ ml})$]

	Station	Bird source	Sed. source	Runoff source	Combined	Measured
Total	BRK	-1.12 (+0.49/ - 0.43)	2.14 (+0.45/ - 1.02)	0.81 (+0.20/ - 0.47)	2.42 (+0.45/ - 1.02)	2.38 (± 0.03)
coliform	PCH	-1.39 (+0.49/ - 0.43)	2.25 (+0.45/ - 1.02)	-0.18 (+0.20/ - 0.47)	2.33 (+0.45/ - 1.02)	2.17 (± 0.04)
<i>E. coli</i>	BRK	-1.38 (+0.83/ - 0.40)	0.76 (+0.63/ - 1.21)	-0.36 (+0.20/ - 0.44)	1.10 (+0.63/ - 1.21)	1.49 (± 0.03)
	PCH	-1.65 (+0.83/ - 0.40)	0.86 (+0.63/ - 1.21)	-1.35 (+0.20/ - 0.44)	0.98 (+0.63/ - 1.21)	1.53 (± 0.03)
Enterococci	BRK	-1.56 (+0.56/ - 0.44)	1.39 (+0.43/ - 0.98)	-0.11 (+0.18/ - 0.44)	1.61 (+0.43/ - 0.98)	1.34 (± 0.03)
	PCH	-1.85 (+0.56/ - 0.44)	1.42 (+0.43/ - 0.98)	-1.10 (+0.18/ - 0.44)	1.49 (+0.43/ - 0.98)	1.38 (± 0.04)

Uncertainty of predictions is shown along with standard error of measurements ($N = 360$).

Table 4
Pearson correlation between turbidity measurements and bacteria predictions and measurements for first six days of study ($N = 144$)

	Station	Sed. source	Runoff source	Combined	Measured
Total	BRK	0.19	0.70*	0.51*	0.56*
coliform	PCH	0.57*	0.47*	0.59*	0.41*
<i>E. coli</i>	BRK	0.20	0.70*	0.56*	0.20
	PCH	0.57*	0.48*	0.60*	0.14
Enterococci	BRK	0.26*	0.70*	0.53*	0.31*
	PCH	0.58*	0.47*	0.60*	0.14

*Significant at the 0.01 level (2-tailed).

dependent settling rates are also known. This would be particularly important if FIB were associated with particles larger than 10–15 μm , in which case accurate settling data would be crucial for reliable predictions.

Both turbidity and FIB are generally associated with fine particles, but in this as well as previous studies (Goyal et al., 1977; Jensen et al., 1979) a strong association between the two has not been observed. In Talbert Marsh, peaks in turbidity and TC are observed at low tide, when brackish water from the upper reaches of the wetland is translated furthest seaward (Figs. 4 and 5). Hence, urban runoff is clearly contributing to the TC

signal. On the other hand, there are not clearly defined peaks in the EC and ENT measurements and in many cases EC and ENT are elevated when turbidity values are relatively small. If sediments are the source of these FIB, a possibility strongly supported by model predictions shown here, shear stresses on the bed must be large enough to disturb and saltate surficial sediments, large enough to mix small particles, colloidal matter, and FIB through the water column, but not large enough to suspend the sandy sediments more than a short distance above the bed. Recall that sediments consist of beach sands and silts near the outlet. Hence, water quality

models designed to account for the effects of sediment resuspension should be sensitive to differences between the rate of sediment entrainment, and the rate of FIB entrainment. Sediment entrainment formulations adopt the notion that mass transfer occurs when the shear stress on the bed exceeds a certain threshold (Mehta and Dyer, 1990). The entrainment of FIB in surficial pore water or incorporated into microbial biofilms may occur at a much smaller threshold.

The significance of loading due to sediment resuspension explains why tidal wetlands serve to “generate” FIB, as was reported by (Grant et al., 2001). That is, FIB associated with sediment particles, colloidal organic matter, or free living in porewater are supplied to the water column when bottom sediments are disturbed and/or scoured by tidal currents. FIB input to wetlands from wet or dry weather surface water runoff may be temporarily stored in sediments and later resuspended during storm events or during tidal scouring. The relative magnitude of resuspension effects versus die-off and settling effects is likely to control whether or not coastal wetlands are net generators or net accumulators. The results of this study are important to temper expectations that hydrodynamically-active wetlands such as estuaries or streams can provide passive treatment of urban runoff with high concentrations of FIB.

Reeves et al. (2004) reported that over 99% of the annual load of FIB from Talbert Watershed runoff is shed during storm events, while less than 1% is shed during dry-weather periods. It is therefore likely that sediments serve to couple FIB loads from storm water runoff to dry-weather water quality. Additional studies are warranted to characterize the variability of FIB in sediments over seasonal to tidal time scales and in response to storm events, to characterize the spatial variability of FIB, and to understand the mechanisms driving this variability. Do these organisms die-off, deposit, stimulate regrowth, and/or pass through the wetland? Microbiological source tracking methods (DNA fingerprinting, etc.) could also be applied to assess whether FIB in sediments are linked to human sources of fecal pollution (Simpson et al., 2002; Scott et al., 2002).

5. Conclusions

This study successfully employed a first-principle model to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California. Model predictions show that surface water concentrations of TC, EC, and ENT in the wetland are driven by loads from urban runoff and resuspended wetland sediments. The model more accurately predicts TC than EC or ENT.

The crucial role that sediments play in the cycling of FIB is highlighted by this study. Sediments function as a reservoir of FIB that may accumulate FIB due to regrowth or settling, or shed FIB when tidal currents or storm flows scour away or even just disturb surficial particles. This finding is important to temper expectations that hydrodynamically-active wetlands serve to “treat” FIB from runoff and other sources, and it also explains why wetlands can function as net generators of surface water FIB. That is, generation occurs when the entrainment rate exceeds the rate of die-off and settling.

Additional studies should be conducted to characterized the “memory” of sediments relative to FIB. Knowing the extent to which dry-weather sediment concentrations of FIB are linked to wet-weather runoff loads, dry-weather runoff loads, regrowth or other factors such as bird droppings would help determine which factors predominately control dry-weather water quality. Additional studies should also be conducted to evaluate the size and settling velocities of particles associated with FIB, and the partitioning of FIB between free-living and particle-associated states. Improved predictions of FIB might result from separately modeling free-living and particle-associated FIB.

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Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland

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Abstract

Recreational water quality at beaches in California and elsewhere is often poor near the outlets of rivers, estuaries, and lagoons. This condition has prompted interest in the role of wetlands in modulating surface water concentrations of fecal indicator bacteria (FIB), the basis of water quality standards internationally. A model was developed and applied to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California, in response to loads from urban runoff, bird feces, and resuspended sediments. The model predicts the advection, dispersion and die-off of total coliform, *Escherichia coli*, and enterococci using a depth-integrated formulation. We find that urban runoff and resuspension of contaminated wetland sediments are responsible for surface water concentrations of FIB in the wetland. Model predictions show that urban runoff controls surface water concentrations at inland sites and sediment resuspension controls surface water concentrations near the mouth. Direct wash-off of bird feces into the surface water is not a significant contributor, although bird feces can contribute to the sediment bacteria load. The key parameters needed to accurately predict FIB concentrations, using a validated hydrodynamic model, are: the load due to urban runoff, sediment erodibility parameters, and sediment concentrations and surface water die-off rates of enteric bacteria. In the present study, literature values for sediment erodibility and water column die-off rates are used and average concentrations of FIB are predicted within 1/2 log unit of measurements. Total coliform are predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. Since wetland-dependent animals are natural sources of FIB, and FIB survive for long periods of time and may multiply in wetland sediments, these results highlight limitations of FIB as indicators of human fecal pollution in and near wetlands.



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Final Report: Identification and Control of Non-Point Sources of Microbial Pollution in a Coastal Watershed

NCER Research Project Search

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Investigators: [Sanders, Brett](#), [Grant, Stanley B.](#), [Horne, Alex](#), [Keller, Robin](#), [Sobsey, Mark D.](#)

Institution: [University of California - Irvine](#), [University of California - Berkeley](#), [University of North Carolina at Chapel Hill](#)

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Description:

Objective:

The objectives of this study were to: (1) characterize the magnitude and variability of fecal indicator bacteria (FIB) loads in the watershed along an inland to coastal gradient that includes street gutters, storm channels, tidal channels, and the surf-zone at Huntington Beach; (2) examine linkages between FIB and other indicators of human pathogens; (3) develop strategies to control FIB loads during nonstorm periods; and (4) aid decisionmaking by examining the perspectives of stakeholders, including beachgoers, environmentalists, local businesses, public health officials, and wastewater utility managers on various aspects of beach pollution problems, such as the causes, health risks, and responsibility to pay.

California beaches are a critical component of the culture and economy of California and are threatened by coastal pollution. Beach recreation in California accounts for \$5.5 billion of the Gross State Product (King and Symes, 2003). Nowhere has there been greater attention on beach pollution than at Huntington Beach in southern California.

Huntington Beach, consisting of Huntington State Beach and Huntington City Beach, is located along a northwest to southeast striking section of the Pacific coastline between Los Angeles and San Diego, in Orange County, California. Several areas of Huntington State Beach have suffered chronic beach postings and closures over the past several years as a result of elevated concentrations of FIB in the surf zone (Kim and Grant, 2004). This beach is very popular (more than 5 million visitors per year), and the combination of surf zone pollution and significant beach usage implies that a large number of people (perhaps as many as 50,000) may acquire highly credible gastroenteritis from swimming and surfing in this area each year (Turbow, et al., 2003). FIB pollution at Huntington State Beach is thought to be caused by a combination of sources, including dry and wet weather runoff from the

surrounding community, bird droppings deposited in the Talbert Marsh, and regrowth of bacteria on vegetation and marsh sediments (Grant, et al., 2001; Reeves, et al., 2004). Additional potential sources of FIB include the offshore discharge of partially treated sewage effluent (Boehm, et al., 2002a), the offshore discharge of power plant cooling water that contains FIB from plant wash-down and other activities (Boehm, et al., 2002b), resuspension of contaminated sediments (Sanders, et al., 2004), bather shedding, the accumulation of bird droppings along the shoreline and offshore, the exfiltration of sewage-contaminated groundwater, and contributions from watershed outlets located north and south of the study area, including the Los Angeles River, the San Gabriel River, and outlets for Huntington Harbor and Newport Bay (Kim, et al., 2004).

This project focuses on the Talbert Watershed in Huntington Beach and Fountain Valley, California, which drains to Huntington Beach and is a significant stressor of Huntington Beach water quality. The Talbert Watershed encompasses 3,400 hectares in the cities of Huntington Beach and Fountain Valley. The watershed is urbanized and consists of residential developments, commercial districts, plant nurseries, and light industry. This area of southern California has separate storm water and sanitary sewer systems, therefore, dry and wet weather runoff flows to the ocean without treatment. Runoff from the Talbert Watershed is conveyed along street gutters to inlets that connect to underground storm water pipelines. These pipelines connect to a network of three flood control channels (Fountain Valley, Talbert, and Huntington Beach) that converge near the ocean at a constructed wetland known as the Talbert Marsh. Ocean water floods both the Talbert Marsh and the lower reaches of the open channels during rising tides (flood tides), and a brackish mixture of ocean water and runoff drains from the system during falling tides (ebb tides). The Talbert Watershed is nearly flat and only a few feet above sea level. This geographical setting hinders drainage by gravity alone, so a system of transfer stations is used in the lower reaches of the Talbert Watershed to pump runoff into the open channels from storm water pipelines. Each transfer station, or pump station, consists of a forebay, where runoff can be stored, and several pumps. Pumping of runoff to the channels occurs intermittently during dry weather periods and continuously during storms. Talbert Marsh is a 10-hectare remnant of what used to be an extensive (1,200 hectare) saltwater wetland and dune system in coastal Orange County. The majority of this wetland system was drained and filled over the past century for agricultural reclamation and urban development. Most of what remained of the historical wetland, including Talbert Marsh, was cut off from tidal flushing by the construction of the Pacific Coast Highway and channelization of the surrounding area for flood control. As part of a habitat restoration effort, tidal flushing in the Talbert Marsh was restored in 1990 when a new tidal inlet was constructed. Since its restoration, Talbert Marsh has become a typical southern California tidal saltwater marsh with open water, wetland, and upland habitats (Grant, et al., 2001). Pickle weed (*Salicornia virginica*) is the dominant macrophytic vegetation, and the marsh is utilized by several special-status bird species, including the California least tern, brown pelican, and Belding's savannah sparrow.

Summary/Accomplishments (Outputs/Outcomes):

To achieve the objectives, extensive monitoring of Talbert Watershed surface waters was conducted to measure the spatio-temporal variability of FIB loads (total coliform, *Escherichia coli*, and *Enterococcus*) and analysis was performed to examine the factors that control fate and transport. Monitoring also was performed to examine the association between FIB and other indicators of fecal pollution. Both one-dimensional and two-dimensional hydrodynamic models were developed to analyze the FIB loads in tidal channels and into the surf-zone and to develop a predictive tool that can be used to examine how bacteria loads would be altered by operational changes to the infrastructure. Surveys were performed to measure stakeholder preferences in the context of multi-

stakeholder, multi-objective beach pollution problems and to support decisionmaking analysis.

Closure and posting of Huntington Beach, California, during the study period was the source of widespread media attention. In response, members of the research team redirected efforts and/or engaged in a number of additional studies to better understand the factors controlling surface water quality in the Huntington Beach surf zone, as well as the response of stakeholders to the unfolding pollution problem. For example, co-principal investigator (PI) Keller focused attention on the decisionmaking of beachgoers (to swim or not to swim) in response to warning signs posted on the beach. Co-PI Keller also focused attention on the decisionmaking of public agencies, who were under great public pressure to remedy the pollution problem but had little understanding of its cause. To better understand the pollution problem, co-PI Grant analyzed short- and long-term FIB monitoring data to identify trends in Huntington Beach bathing water quality. The observed variability was examined in the context of historical management measures, such as passage of the Clean Water Act, construction of a new ocean outfall, and efforts to prevent urban runoff from draining directly to the beach. Co-PI Grant also developed a method to identify and rank the sources of pollution to the surf-zone using high-frequency monitoring data collected along the beach. PI Sanders teamed with University of California (UC) Irvine and UC San Diego researchers to examine the potential for Orange County Sanitation District effluent, discharged roughly 7 km offshore of Huntington Beach, to be transported onshore by internal tides. After the Talbert Marsh was identified as a contributor of FIB to the Huntington Beach surf zone, co-PI Sobsey focused attention on potential health risks associated with water contaminated with bird feces. In particular, marsh bird feces and surface water was examined for *Campylobacter*, *Salmonella*, and male-specific coliphages.

During dry weather, concentrations of FIB were highest in inland urban runoff, intermediate in tidal channels harboring variable mixtures of urban runoff and ocean water, and lowest in ocean water at the base of the watershed. This inland-to-coastal gradient is consistent with the hypothesis that urban runoff from the watershed contributes to coastal pollution. On a year-round basis, the vast majority (> 99%) of FIB loading occurs during storm events when runoff diversions, the management approach of choice, are not operating. During storms, the load of FIB in runoff follows a power law of the form $L \sim Q^n$, where L is the loading rate (in units of FIB per time), Q is the volumetric flow rate (in units of volume per time), and the exponent n ranges from 1 to 1.5. This power law and the observed range of exponent values are consistent with the predictions of a mathematical model that assumes FIB in storm runoff originate from the erosion of contaminated sediments in drainage channels or storm sewers. (Reeves, et al., 2004)

During dry weather periods, urban runoff controls surface water concentrations of FIB in channels where flushing is weak, and resuspension of FIB from the sediment/water interface controls surface water concentrations near the mouth where flushing by ocean water occurs once per day. The reservoir of FIB at the sediment/water interface is probably linked to settling of bacteria from both dry and wet weather urban runoff, deposition of animal feces, decaying vegetation, and bacterial regrowth. It is not clear whether the FIB are primarily attached to sediments, suspended in pore water, or incorporated into microbial biofilms. Nevertheless, surface water concentrations of FIB are rapidly amplified as turbulence in water column increases. A result is that dry weather urban runoff has little direct impact on surf zone water quality, but significant indirect impact given FIB loads from runoff accumulate at the sediment/water interface and are subsequently resuspended and exported to the surf-zone by tidal currents (Grant, et al., 2001; Arega and Sanders, 2004; Sanders, et al., 2004).

During the project period, dry-weather diversions of urban runoff to the sanitary sewer system were implemented to mitigate impacts to the surf-zone, at a cost of at least \$6 million to the County of

Orange and City of Huntington Beach. The efficacy of this approach is unclear, because the vast majority of watershed loads are shed during wet weather, whereas during dry weather, the tidal channels and marsh serve to dissipate loads by promoting die-off and settling. On the other hand, diversions presumably serve to reduce loads of other contaminants, including oil, grease, heavy metals, and so forth and, therefore, may be justified on these grounds. To evaluate whether the diversions are justified on the basis of FIB control, a better understanding of the cycling of FIB in sediments is needed. The alternative is to focus management efforts on wet weather controls. For example, if erosion of sediments is driving the loading of FIB, then regular removal of contaminated sediments accumulating in the storm sewer system might be an appropriate management strategy. The creation of distributed wetland treatment systems, in which contaminants in urban runoff are removed near their source, might also prove useful for reducing downstream impacts (Reeves, et al., 2004).

Research lead by PI Sanders shows that numerical modeling can be performed to predict FIB loads in tidal wetlands, analytes that are notoriously difficult to model because of poorly characterized non-conservative processes. The key parameters needed for accurate predictions of FIB loads, using a validated hydrodynamic model, are: (1) the load as a result of urban runoff; (2) sediment erodibility parameters; and (3) sediment concentrations and surface water die-off rates of enteric bacteria. For channels in the Talbert Watershed, literature values for sediment erodibility and water column die-off rates were used and average concentrations of indicator bacteria were predicted within one-half log unit of measurements. Total coliform were predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. This work is important because it represents the first case where first-principle models were successfully applied to predict FIB in an estuarine setting with significant nonpoint sources. The approach adopted here is highly transferable and could benefit both wetland restoration and water quality compliance efforts on a widespread basis (Sanders, et al., 2004).

Plume tracking studies conducted by UC Irvine and UC San Diego researchers, including PI Sanders, show that Orange County Sanitation Department (OCSD) effluent occasionally moves shoreward toward Huntington Beach into water less than 20 m deep. Analyses of current and temperature observations indicate cold water is regularly advected crossshelf, into and out of the nearshore, at both semi-diurnal and diurnal frequencies. Isotherms typically associated with the wastefield near the outfall are observed just outside the Huntington Beach surf zone, where the total depth is less than 6 m, highlighting the extent of the cross-shelf transport. This advection is attributed to a mode 1 internal motion, or internal tide. Based on this analysis, it is not possible to rule out the possibility that the OCSD plume contributes to poor bathing-water quality at Huntington Beach (Boehm, et al., 2002a). Concerned over potential shoreline impacts, OCSD began a disinfection program in 2002 and initiated a roughly \$300 million program to build the necessary infrastructure for full secondary treatment.

Analysis of Huntington Beach monitoring data lead by co-PI Grant shows that the concentration of FIB varies over time scales that span at least seven orders of magnitude, from minutes to decades. Sources of this variability include historical changes in the treatment and disposal of wastewater and dry weather runoff, El Niño events, seasonal variations in rainfall, spring-neap tidal cycles, sunlight-induced mortality of bacteria, and nearshore mixing. On average, total coliform concentrations have decreased over the past 43 years, although point sources of shoreline contamination (storm drains, river outlets, and submarine outfalls) continue to cause transiently poor water quality. These transient point sources typically persist for 5 to 8 years and are modulated by the phase of the moon, reflecting the influence of tides on the sourcing and transport of pollutants in the coastal ocean. Indicator bacteria are very sensitive to sunlight; therefore, the time of day when samples are

collected can influence the outcome of water quality testing. These results demonstrate that coastal water quality is forced by a complex combination of local and external processes and raise questions about the efficacy of existing marine bathing water monitoring and reporting programs (Boehm, et al., 2002b). Further analysis led by co-PI Grant reveals that protocols used to decide whether to post a sign are prone to error. Errors in public notification (referred to here as posting errors) originate from the variable character of pollutant concentrations in the ocean, the relatively infrequent sampling schedule adopted by most monitoring programs (daily to weekly), and the intrinsic error associated with binary advisories in which the public is either warned or not. We derived a probabilistic framework for estimating posting error rates, which at Huntington Beach range from 0 to 41 percent, and show that relatively high sample-to-sample correlations (> 0.4) are required to significantly reduce binary advisory posting errors. Public misnotification of coastal water quality can be reduced by utilizing probabilistic approaches for predicting current coastal water quality, and adopting analog, instead of binary, warning systems (Kim and Grant, 2004).

Research lead by co-PI Sobsey on the potential health risks of bathing water contaminated by bird feces has led to only preliminary findings. Specifically, *Campylobacter* and male specific coliphages were identified in Talbert Marsh bird feces and in marsh surface waters near the marsh. *Salmonella* was found only in bird feces samples and not water samples. Analysis continues to understand the relationship between microbes in bird feces and surrounding surface waters, and potential health impacts.

Research lead by co-PI Keller indicates that stakeholders share diverse opinions about the causes of beach pollution, the risks to beachgoers, and the responsibility to pay. In the context of a multi-objective decision model, stakeholders disagree on the appropriate weights of objectives. For example, local businesses heavily weigh economics whereas beachgoers heavily weigh health risks. Stakeholders also disagree on the severity of pollution problems. For example, environmentalists believe the probability of an environmental health problem is high when beaches are posted, but beachgoers do not. Relative to beachgoers' perceptions of potential health risks, surveys showed a peer effect: decisions to enter the water at posted beaches were strongly affected by whether or not others were in the water (Biswas and Keller, 2004; Biswas, et al., 2004).

Conclusions:

The vast majority of FIB loads in runoff from the Talbert Watershed are shed during storms and are associated with particles that appear to be scoured from the water collection system, including street gutters, storm pipes, and storm channels. Loads in runoff during dry weather periods account for roughly 1 percent of the annual runoff load and dissipate within the tidal channels by a combination of die-off and settling.

Loads exported from the watershed to the surf zone during dry weather period are deflected along the shoreline by wave driven currents and can cause exceedances of water contact recreation standards. Model predictions show the origin of such loads is the scouring by tidal currents of FIB at the sediment/water interface of tidal channels and Talbert Marsh. FIB at the sediment/water interface are linked to urban runoff FIB loads during both dry and wet weather periods, bird droppings, decaying vegetation, and bacterial regrowth. Because intertidal wetlands are to some extent natural generators of FIB, these results call into question the exclusive use of FIB as the basis of water contact recreation standards at beaches near the outlet of these water bodies.

On the basis of FIB control, the efficacy of dry weather diversions in Talbert Watershed is unclear,

although diversions presumably serve to mitigate other types of pollution as well. A better understanding of the cycling of FIB between the water column and sediments is needed to evaluate the linkages between wet weather and dry weather loads in relation to sediment interactions.

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Journal Article	Boehm AB, Sanders BF, Winant CD. Cross-shelf transport at Huntington Beach. Implications for the fate of sewage discharged through an offshore ocean outfall. <i>Environmental Science & Technology</i> 2002;36(9):1899-1906	R828011 (2001) R828011 (Final)	<ul style="list-style-type: none"> • Full-text: ACS Publications Full Text <small>EXIT Disclaimer</small> • Other: ACS Publications PDF <small>EXIT Disclaimer</small>
Journal Article	Grant SB, Sanders BF, Boehm AB, Redman JA, et al. Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surf zone water quality. <i>Environmental Science and Technology</i> 2001;35(12):2407-2416.	R828011 (2000) R828011 (2001) R828011 (Final)	<i>not available</i>
Journal Article	Reeves RL, Grant SB, Mrse RD, Copil-Oancea CM. Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California. <i>Environmental Science & Technology</i> 2004;38(9):2637-2648.	R828011 (Final)	<ul style="list-style-type: none"> • Full-text: ACS Full Text <small>EXIT Disclaimer</small> • Other: ACS PDF <small>EXIT Disclaimer</small>
Journal Article	Sanders BF, Arega F, Sutula M. Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. <i>Water Research</i> . 2005;39(14):3394-3408.	R828011 (Final)	<i>not available</i>

Supplemental Keywords:

urban runoff, non-point sources, coastal wetlands, flood control channels, active control, passive control, decision model, coastal watershed, contaminant transport, decision making, ecosystem modeling, indicator organisms, man-made wetlands, microbial pollution, non-point sources, pathogens, pollution identification and control, pump stations, recreational area, runoff, stakeholders, storm water, stormwater drainage, suburban watersheds, tidal influence, urban runoff, , Water, Geographic Area, Scientific Discipline, RFA, Water & Watershed, Ground Water, Wet Weather Flows, Watersheds, Environmental Chemistry, Environmental Monitoring, Engineering, State, runoff, water quality, California (CA), stakeholders, fecal coliform, coastal watershed, fate and transport, escherichia coli (e. coli), decision model, indicator organisms, ecosystem modeling, stormwater drainage, decision making, active control, pump stations, enterocci, man-made wetlands, storm water, pollution identification and control, community values, contaminant transport, suburban watersheds, recreational area, pathogens, flood control, urban runoff, microbial pollution, non-point sources, bacteriophage, clostridium, forebay water

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Enviro Science & Technology

Bird Droppings Are Blamed for Bacteria

By Stanley Allison

June 02, 2001 in print edition B-9

A team of UC Irvine researchers has concluded that waterfowl and other animal droppings from a saltwater marsh and the Santa Ana River are a significant source of bacteria contaminating the ocean waters off Huntington Beach.

In a report that will be published in the June 15 issue of Environmental Science and Technology, the researchers point to inherent flaws in the design of the man-made saltwater Talbert Marsh.

Stanley Grant, the UCI professor who led the 18-month study of the ocean contamination problem at Huntington Beach, said water containing fecal bacteria, pesticides, nutrients and other materials filters through the marsh and then flows into the ocean in about 40 minutes—which is too fast.

For the marsh to act as a natural cleanser and remove contaminants, the water must spend at least a week filtering through the wildlife preserve, Grant said.

Even though other sources such as urban runoff from the Santa Ana River may have contributed to the contamination that resulted in four miles of beach closures for most of the summer of 1999, the levels of bacteria from the marsh were hundreds of times more than the state limits, the researchers said.

The team's conclusions contradict the accepted environmental theory that wetlands purify contaminated water flowing into the ocean.

The findings suggest that approximately 4.6 million saltwater marshes in the U.S. could be similarly affected, Grant said.

Mark Gold, a spokesman for the conservation group Heal the Bay, said that finding animal droppings in a nature preserve is nothing new, and insists that marshes still serve as a cleanser for other, more hazardous, contaminants.

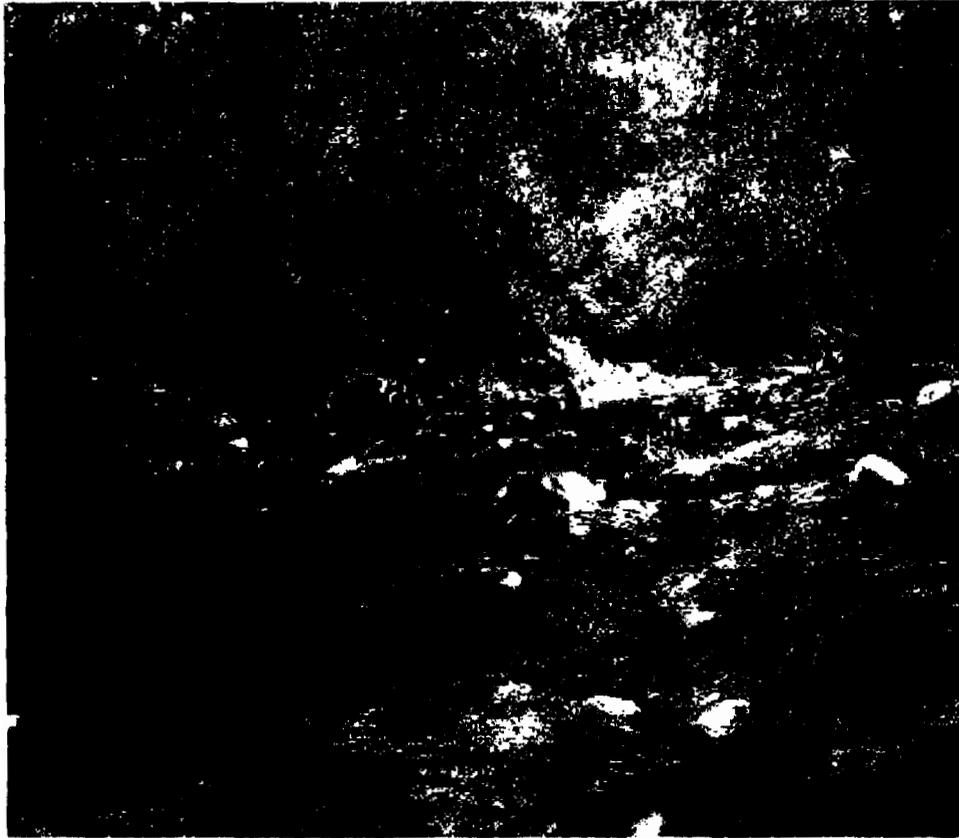
"It's not surprising that wetlands are sources of fecal bacteria," Gold said. "What wetlands are great at doing is removing nutrients and metals."

The 25-acre wetlands preserve is on the inland side of Pacific Coast Highway at Brookhurst Street. Part of the Talbert watershed that encompasses 12 square miles in Huntington Beach and Fountain Valley, it attracts thousands of migratory birds and other wildlife each year.

The UCI researchers also say that the nearby AES power plant contributes to the shore's contamination. The study suggests that partly treated sewage released four miles offshore from the Orange County Sanitation District treatment plant is being pulled back to the shore by tides and the plant as it draws water to cool its towers.

Fecal Indicator Bacteria (FIB) Levels During Dry Weather from Southern California Reference Streams

*Liesl L. Tiefenthaler
Eric D. Stein
Gregory S. Lyon*



Southern California Coastal Water Research Project

Technical Report 542 - January 2008

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ABSTRACT

High levels of fecal indicator bacteria (FIB) in surface waters is a common problem in urban areas that often leads to impairment of beneficial uses such as swimming or other contact recreation. Once impaired, common management and regulatory solutions include development of Total Maximum Daily Loads (TMDLs) and other water quality management plans. A critical element of these plans is establishment of a "reference" level of exceedances against which to assess management goals and TMDL compliance. Unfortunately, existing "background" or reference data on contributions of FIB from undeveloped catchments during dry weather is limited to a small number of locations measured at few time points. The goal of this study was to provide information on indicator bacteria contributions from natural streams in undeveloped catchments throughout southern California during dry weather, non-storm conditions. Specific questions addressed were: a) What are the "background" ranges of concentrations of FIB associated with dry weather flow from reference areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas? To help establish a regional reference data set, bacteria levels (i.e. *Escherichia coli* (*E. coli*), enterococci and total coliforms)) were measured from 15 unimpaired streams in 11 southern California watersheds weekly for one full year. A total of 590 water samples were collected from spring 2006 through spring 2007. Results were compared with data from the developed Ballona Creek watershed and to established State of California bacteria standards. Concentrations measured from reference areas were typically between one to two orders of magnitude lower than levels found in developed watersheds. The absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin. Nearly 82% of the time, samples did not exceed daily and monthly bacterial indicator thresholds, demonstrating good bacteriological water quality in natural streams throughout southern California. *E. coli* had the lowest daily percent exceedance (1.5%). A total of 13.7% of enterococci exceeded daily thresholds. The average measured enterococci levels of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml and a minimum of 160 MPN/100 ml. Indicator bacteria levels fluctuated seasonally with an average of 79% of both enterococci and total coliforms exceedances occurring during summer months (June-August). Temperature, at all sites, explained about one-half the variation in total coliforms density suggesting that stream temperatures regulated bacterial populations. Studies of human health risk associated with natural bacteria levels have not been conducted, but the levels observed in this study are below those reported to cause risk in freshwater systems with known human sources of FIB. Accounting for natural background levels will allow for management targets that are more reflective of the contributions from natural sources. Additional monitoring during wet weather is warranted to further characterize background bacterial contamination in southern California reference waterbodies.

Keywords: Dry Weather Water Quality, Indicator Bacteria, Reference Condition, Background Water Quality, TMDL

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INTRODUCTION

The presence of fecal indicator bacteria (FIB) in surface waters is a prevalent concern for many municipalities, health departments, and regulatory agencies. Persistent or excessive bacteria levels often result in reduced opportunities for beneficial uses such as swimming, and may lead to waterbodies being listed as impaired under Section 303(d) of the Clean Water Act. Approximately 280 waterbodies are listed as impaired in the Los Angeles, Santa Ana, and San Diego regions (http://www.swrcb.ca.gov/tmdl/303d_lists.html). Management of impaired water bodies may involve development of Total Maximum Daily Loads (TMDLs), issuance of National Pollutant Discharge Elimination System (NPDES) permits, or development of water quality plans that are intended to reduce bacteria levels to a point where water quality standards are met and beneficial uses are protected. An important step in the development of TMDLs and other water quality management plans is to identify all sources of the constituent(s) of concern in order to accurately quantify loads and set appropriate management or regulatory targets. One of the challenges in developing appropriate targets is accounting for biogenic inputs, or the natural contribution from undeveloped catchments.

Most watersheds consist of both developed and undeveloped areas, both of which can contribute bacteria to streams via surface runoff. Bacteria associated with runoff from urban surfaces are well documented (Gore & Storrie Ltd. and Proctor & Redfern Ltd. 1981, USEPA 1993). For example, (Stein *et al.* 2007) observed that recreational (horse) and agricultural land uses in Los Angeles, CA contributed substantially higher storm fluxes for *Escherichia coli* (*E. coli*). Additional investigations by Bay and Schiff (1998), Noble *et al.* (2000) and Stein and Tiefenthaler (2005) found freshwater outlets such as storm drains to be especially high contributors of dry-weather FIB contamination.

Natural areas can also be a source of bacteria originating from wildlife, including birds and mammals, pets, and livestock (Griffith *et al.* 2006). Grant *et al.* (2001) found that enterococci bacteria generated in a restored wetland had greater effect on coastal water quality than dry season urban runoff. The presumed sources of these bacteria were birds that used the tidal salt marsh as habitat. (Ahn *et al.* 2005) also recognized that natural sources could be significant contributors to total bacteria levels in urban storm water in southern California. However, most previous studies have focused on either short measurements during or immediately following storm water runoff or on bacteria in coastal waters (beaches). Few studies have attempted to quantify naturally occurring background levels of bacteria in streams during baseflow (i.e. non-storm) conditions over an extended period of time. This data gap is critical because the non-storm period is when streams and the coastal waters they drain to receive the most human use and thus the potential risk is highest.

The goal of this study is to establish a "reference" level of bacteria that can be used to set appropriate water quality management targets. More specifically, we address the following questions: a) What are the "background" ranges of concentrations of FIB associated with dry-weather runoff from natural areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas?

METHODS

The overall approach to the study was to characterize dry weather bacteria levels at a set of sites that is representative of existing natural conditions in southern California. The specific study design consisted of an intensive sampling regime with collection of weekly dry weather bacteria data for an entire year.

Sampling Sites

Fifteen sites were selected for inclusion in the study based on criteria developed by Stein and Yoon (2007, Stein and Yoon In press). Criteria were designed to ensure that sampling would capture natural conditions without influence from any land-based anthropogenic input. The criteria included: 1) contributing drainage area should be at least 95% undeveloped. 2) sites should be in a relatively homogenous setting in terms of underlying geology and landcover, 3) sites should have either year-round or prolonged dry-weather flow to allow sampling during at least a portion of the dry season, and 4) sites should not be within watersheds that have burned during the previous three years. Although fire can be a natural occurrence, inclusion of sites in burned catchments would have added a confounding factor and, therefore, were excluded. Catchment land use was determined by plotting watershed boundaries over (year 2003) land cover maps from the (National Oceanographic Administration (NOAA) 2003) Coastal Change Analysis Program (CCAP) - <http://www.csc.noaa.gov/crs/lca/ccap.html>. The 15 selected sites are located across five counties (Los Angeles, Orange, Riverside, San Bernardino and San Diego) and ten different watersheds: Los Angeles River, Los Alisos Canyon, Malibu Creek, Soltice Canyon, San Juan Creek, Santa Ana River, San Jacinto, Cucamonga, Santa Margarita, and San Dieguito (Figure 1, Table 1, and Appendix A).

Sampling

Weekly dry-season sampling was conducted at all 15 sites from May 15, 2006 through May 31, 2007. A site was eligible for sampling if it had not received measurable rainfall for at least 24 h and flow was no more than 20% above baseflow. Weekly sampling continued as long as there was measurable stream flow. For intermittent streams, sampling was suspended once the stream was too low to sample. Based on these criteria, the duration of sampling ranged from 9 to 55 weeks (Table 1). Water samples were collected as composite grab samples, with equivalent volumes collected from three different points across the stream (approximately 10, 50, and 90% distance across). These samples were taken from the flowing portion of the streams at a depth sufficient to exclude surface scum without introducing bottom sediment. A replicate water sample was collected in the same way after completion of the initial water sample for approximately 25% of the samples. A field blank sample was also collected at each site once a month. All water samples were collected in presterilized 125 ml high-density polyethylene (HDPE) sample bottles. Collected water samples were immediately placed on ice and transported to the laboratories within 6 h of sample collection for subsequent analyses.

At each sampling location and during each round of sample collection, water quality readings (i.e. temperature (°C), dissolved oxygen (DO) mg/L, pH, turbidity, and conductivity ($\mu\text{S}/\text{cm}$)) were measured using hand held field probes (i.e. Orion 125, YSI 63 and Horiba U-10). Measurements were taken in triplicate at each transect. In addition, physical and biological

parameters of the site and general climatic conditions were recorded and documented (using both data forms and photo documentation). Stream discharge was measured as the product of the channel cross-sectional area and flow velocity. Channel cross sectional area was measured in the field. At each sampling event, velocity was measured using a Marsh-McBirney Model 2000 flow meter (Frederick, MD). The velocity, width, and depth were measured at three points along each transect. Flow for each transect subsection was computed and summed for a total flow for the transect. Values from three transects were averaged to estimate overall flow at each site (Rantz 1982).

Laboratory Analysis

Water quality samples were analyzed for four bacteria indicators; *E. coli*, enterococci, total coliforms and *Bacteroides thetaiotaomicron*. Enterococci, total coliforms and *E. coli* were measured by the chromogenic substrate method using Enterolert® for enterococci and Colilert® for *E. Coli* and total coliforms (Idexx 24 h, Inc.). This commercially available product uses a Multiple Tube Fermentation (MTF) type format with defined substrate technology to detect the presence or absence of bacteria indicator density in a water sample. In this medium, the detection of coliform densities is based upon a color change caused by the reaction of a fluorogen with a bacterial enzyme. This assay is read within 24 hours and coliform densities are reported as most probable number (MPN)/100 milliliters (ml). Given the large geography covered by the study and the short holding time required for bacterial analysis, eight laboratories cooperated on sample analysis. Laboratory intercalibration studies were completed to ensure consistent methodology, data quality, and repeatability between laboratories. All laboratories had had good repeatability for all three bacterial indicators and all results fell within the median log comparability criteria. The low variability between labs indicated that interlab differences should not be a confounding factor in interpreting the results of the study. Details of the laboratory intercalibration study are provided in Appendix C.

Bacteroides thetaiotaomicron are anaerobic bacteria that comprise the majority of microorganisms that inhabit the human digestive tract. As such, they may be a more reliable measure of human fecal matter or pathogens than *E. coli* (Bernhard and Field 2000a,b). Samples were analyzed for either presence or absence of *B. thetaiotaomicron* as a negative control for human bacteria sources. This analysis was initiated at a sampling site when the State of CA single-sample water quality thresholds for both *E. coli* and enterococci were exceeded for two consecutive weeks. The presence of *B. thetaiotaomicron* would suggest that bacteria observed in the surface waters were predominantly of human origin. *B. thetaiotaomicron* was measured by DNA extraction followed by polymerase chain reaction (PCR) as described by (Brinkman *et al.* 2003).

Data Analysis

Three analyses were used to characterize FIB levels from natural streams. First the 30-d geomeans, variances, and ranges of concentrations, and fluxes were calculated to provide an estimate of expected baseline bacterial levels. Flux estimates facilitated region wide comparisons among watersheds of varying sizes. Flux was calculated as the ratio of the 30-d geomean or mean yearly bacterial concentration (MPN/100 ml) and contributing watershed area (km²) at a specific site. Second, dry weather FIB concentrations were compared with the state of CA standards for single-sample and 30-d geomean maximum allowable densities (Table 2).

Cumulative density frequency plots (CDFs) were produced to compare observed bacterial concentrations to the CA quantitative standards and to calculate accumulated relative exceedance percentages. Third, water quality statistics from natural sites were compared with previous data collected from watercourses draining developed areas of the greater Los Angeles basin to determine if significant differences existed between natural and developed areas (Stein *et al.* 2007, Stein and Yoon 2007).

Bacteria data were analyzed for differences between perennial vs. intermittent streams, between developed and undeveloped watersheds, and to assess temporal patterns. Differences in concentration or flux were tested using a one-way analysis of variance (ANOVA), with a significance level $p < 0.05$ (Sokal and Rohlf 1995). Differences based on flow regime were assessed using a Tukey-Kramer post-hoc test for multiple comparisons; differences between developed and undeveloped sites were investigated by comparing median values using a Kruskal-Wallis one-way ANOVA on ranks.

Spatial and temporal patterns were also investigated using Pearson's r correlation coefficient to determine if there were strong associations between FIB concentrations and continuous variables (i.e. temperature and flow; Helsel and Hirsch 2002); the null hypothesis, in this case, is that the correlation coefficient is zero.

RESULTS

Background Bacteria Concentrations and Fluxes

Annual median bacteria fluxes from the natural sites were 2 ± 1.4 MPN/100 ml/km², 3 ± 1.7 MPN/100 ml/km², and 106 ± 61.4 MPN/100 ml/km² for *E. coli*, enterococci, and total coliforms, respectively. *E. coli* and enterococci, median density values at the natural sites (based on single-sample measurements) were 10 MPN/100 ml and 20 MPN/100 ml respectively, while median density values in Ballona Creek are typically in the 10³ range. Densities and fluxes were significantly lower for all indicator bacteria at the natural sites relative to data from developed areas ($p < 0.001$, Figure 2).

Only two sites exceeded State water quality standards for both *E. coli* and enterococci for two or more weeks during the yearlong study. During the period of exceedance, *E. coli* levels ranged from 327 to 9804 MPN/100 ml while enterococci ranged from 388 to 7270 MPN/100 ml. Repeat exceedances were seen most commonly for enterococci. In both cases, the *thetaiotaomicron* samples were negative, suggesting that the bacterial populations represented by the FIB were probably derived from non-human sources.

Frequency of Exceedance of Bacteria Standards at Natural Sites

A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 14% of enterococci exceeded the daily threshold of 104 MPN/100 ml (Figure 3). The average enterococci level of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml (Orange County) and a minimum of 160 MPN/100 ml (San Bernardino County). For *E. coli*, 1.5% of the measurements exceeded the single sample standard of 235 MPN/100 ml with a maximum and a minimum of 5500 MPN/100 ml and 241 MPN/100 ml, respectively (Orange County). For total coliforms, 3% exceeded the single sample standard of 10,000 MPN/100 ml.

A total of 39% of enterococci samples from the natural sites exceeded the 30-d geomean water quality standard of 33 MPN/100 ml. The average enterococci level of these exceedances was 47 MPN/100 ml, with a maximum of 744 MPN/100 ml and a minimum of 3 MPN/100 ml. For *E. coli*, approximately 1% exceeded the 30-d geomean threshold of 126 MPN/100 ml with a maximum and a minimum of 146 MPN/100 ml and 1 MPN/100 ml, respectively (Orange County). For total coliforms, 45% exceeded the 30-d geomean of 1000 MPN/100 ml with a maximum and a minimum of 5040 MPN/100 ml and 23 MPN/100 ml, respectively.

Seventy-five percent of enterococci and 83% of total coliforms exceedances occurred during the summer months (June-August, Table 4). In August all indicator thresholds were exceeded with 12.5%, 62.5% and 75% of *E. coli*, enterococci and total coliforms samples exceeding monthly thresholds, respectively (Table 4).

Temporal and Spatial Patterns in FIB Levels

Bacteria levels for all three indicators were significantly higher during the summer than during all other seasons (Table 4, $p < 0.01$). For example, 30-d geomeans for total coliforms were near the water quality standard in May 2006 with levels approximately 878 MPN/100 ml \pm 3.2 SD, increased substantially during the summer, exceeding the criterion, peaking in July at 2586 MPN/100 ml \pm 3.1 SD (Figure 4b). Total coliform geomeans decreased gradually throughout the winter nearing zero in February, 2007 (289 MPN/100 ml \pm 4.2 SD), as stream temperatures fell below 10°C, before gradually returning to baseline geomeans throughout spring, 2007 (Figure 4a and b). Similar seasonal patterns were observed for *E. coli* and enterococci (Figure 5a and b).

Orange County had the highest daily and monthly water quality exceedances for both *E. coli* and total coliforms (12.9%; 25% and 3.2%; 100%, respectively, Table 3). For enterococci, approximately 47% of the San Diego County samples exceeded the daily threshold and 100% exceeded the monthly standard (Table 3). However, the Orange County and San Diego County streams had no flow in winter due to an unusually low 2006-2007 rainfall season, so the results are from only the spring and early summer months and do not represent annual averages that may occur in perennially flowing streams.

Perennial vs. Non-perennial Streams

Background bacteria levels differed based on the duration of stream flow (Table 1, Appendix A). *E. coli* and enterococci densities were significantly different in perennial vs. intermittent streams ($p < 0.05$, Figure 6). Mean \log_{10} concentrations for *E. coli* and enterococci at perennial streams were 1.0 ± 0.4 and 1.3 ± 0.5 , respectively. Intermittent streams had higher mean \log_{10} concentrations for *E. coli* and enterococci (1.6 ± 0.5 and 1.8 ± 0.6 , respectively). There were no statistical differences between stream types for total coliform densities (mean 2.7 ± 0.6 vs. 3.3 ± 0.4).

Relationship of Bacteria Levels to Environmental Variables

Of the five environmental variables measured (temperature, conductivity, dissolved oxygen, pH, turbidity), only stream temperature exhibited a significant correlation with seasonal FIB levels. Water temperature varied by about 5-10°C at each of the sites, reaching a maximum of 28°C on warm sunny afternoons. Streams located in the foothills (Mill Creek, San Bernardino Co.) or where the creek was significantly shaded had the lowest average temperatures (Table 1, Appendix B). For example streams in San Bernardino County ranged from 650 m to 1200 m in elevation and averaged 12.7°C. The highest monthly average water temperatures (20.4 °C) were recorded in Orange County where streams were approximately 200 m in altitude. Stream temperature and total coliforms were significantly positively correlated (Table 5, $p < 0.001$, $r^2 = 0.48$). A weaker, but still significant, positive correlation existed between stream temperature and *E. coli* or enterococci ($p < 0.04$, $r^2 = 0.20$ and $p < 0.04$, $r^2 = 0.26$, respectively). The Pearson's r for these two correlations was between 0.2 and 0.3 suggesting that similar processes may have controlled the relationship between stream temperature and FIB. A strong negative correlation existed between dissolved oxygen and both conductivity or stream temperature (Table 5, $p < 0.05$, $r^2 = -0.5$; $p < 0.001$, $r^2 = -0.84$, respectively). However, few statistically significant relationships existed among the other physical variables.

Total coliform densities increased exponentially at temperatures above 10°C (Figure 7, $r^2 = 0.48$). Dissolved oxygen concentrations varied inversely with stream temperatures throughout the study (Figure 4a). Monthly mean DO concentrations decreased sharply to approximately 8 mg/L at stream temperatures above 15°C, and concentrations increased to approximately 11 mg/L at stream temperatures below 10°C.

DISCUSSION

Enterococci, *E. coli* and total coliforms (FIB) are commonly used indicators of the possible presence of pathogenic (disease-causing) microorganisms in streams and the ocean. As shown in this study, these FIB can be found in natural streams, with populations increasing during warm summer months and persisting through winter. However, the densities observed in natural streams were usually below State water quality objectives, which are set below levels typically thought to impair beneficial uses (Geldreich 1978, Toranzos 2007). Furthermore, the absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin (Carson *et al.* 2005). There are three possible sources of FIB observed in natural streams: External inputs from sources such as waterfowl, animals, or soil erosion; internal sources of bacterial growth and colonization within the stream associated with decomposition of organic matter; or a combination of the two (Byappanahalli *et al.* 2003, Toranzos 2007).

Higher bacteria levels observed during the summer suggest that factors existed which promote bacteria growth and regrowth in streams. The positive relationship between temperature and bacteria levels suggests that heat induced growth may be a contributing factor to seasonally high bacteria levels. In addition, warmer temperatures influence the dissolved oxygen content of the water. Decreased oxygen solubility associated with higher temperature may combine with lower dissolved oxygen levels producing algal blooms, which have been shown in previous studies to support growth of *E. coli* and enterococci in freshwater (Byappanahalli *et al.* 2003, Byappanahalli *et al.* 2007). These conditions may in turn accelerate death and decomposition of organic matter in the stream, further enhancing in situ bacterial growth. Increases in organic decomposition have been shown to increase survival and regrowth of enteric bacteria and viruses (Novotny and Olem 1994). This hypothesis is further supported by the negative correlation observed between conductivity and dissolved oxygen. Conductivity is closely correlated with total dissolved solids, which are typically comprised of inorganic and organic substances, a potential source of biological oxygen demand (BOD).

Higher FIB densities and incidence of water quality standard exceedences during the summer is consistent with the observations of others such as Noble *et al.* (2000) and Sieracki (1980). Nuzzi and Burhans (1998) compared the responses among indicator bacteria at 143 New York beach sites and found that survival was longer in the summer, but that the duration could be mediated by exposure to UV radiation from sunlight. More recently, growth or regrowth of fecal indicator bacteria in tropical and temperate soils during the summer months has also been reported (USEPA 2000, Ishii *et al.* 2006). Whitman *et al.* (1999) attributed a gradual increase of *E. coli* bacteria in water and sand at beaches during summer to higher survival and growth at warmer temperatures.

Another explanation for higher FIB levels during the summer could be higher external sources due to different patterns of use by wildlife and birds. A number of studies have shown that wildlife and other animals can be sources of bacteria in run-off (Baxter-Potter and Gilliland 1988, Bagshaw 2002, Stein *et al.* 2007). Previous studies have quantified that wildlife and bird feces contain high levels of FIB. Cox *et al.* (2005) measured fecal coliform levels of $10^3 - 10^5$ CFU/g from native wildlife in Australian watersheds. Ricca and Cooney (1998) reported that droppings from feral populations of pigeons, geese and herring gulls from the environment

around Boston Harbor, MA, USA contained up to 10^8 CFU/100 ml of enterococci. Bacteria from wildlife and birds can be associated with FIB levels in streams used by these animals. Noblet *et al.* (2004) found that birds were a likely source of intermittently high levels of FIB observed in the lower Santa Ana River watershed and the nearby surf zone in southern California. Similarly, Harwood *et al.* (2000) reported that animals were the dominant sources of indicator bacteria at Florida sample sites with relatively low anthropogenic impact. Bacterial source tracking studies conducted in Michigan suggested that feces from pets and raccoons were important contributors to FIB levels in streams and storm sewers (Ram *et al.* 2007). Moreover, levels increased in the late summer and fall coincident with increased raccoon den mobility following breeding.

Decreased stream flow may have also contributed to higher bacteria levels during the summer months. Although there was no statistically significant relationship between flow and bacterial densities, in all cases densities increased exponentially when stream flow decreased below approximately $0.5 \text{ m}^3/\text{s}$ (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially. Therefore, the combined range of bacteria levels for perennial and intermittent streams observed in this study should reflect expected levels in natural streams throughout southern CA.

Relatively minor perturbations in the contributing watershed can cause sites to quickly deviate from background conditions. Four sites originally considered, but later rejected from the study had bacteria levels 2-3 log units greater than the natural sites retained, but significantly lower than levels observed in the developed Ballona Creek watershed (Figure 8). The watersheds of these four sites were almost entirely natural open space, but had small portions subject to agricultural or transportation related runoff. In one instance, a portion of the contributing watershed was affected by a recent fire. These small perturbations in the watershed led to dramatic changes in bacteria levels that moved sites away from reference conditions. Although these sites were not included in the analysis of background conditions, they provide valuable insight into the sensitivity of natural watersheds to small increases in anthropogenic sources.

Although this study focused on background FIB levels during dry weather (non-storm) conditions, comparison of these results to background levels in storm water is important because FIB are major constituents of concern in storm water runoff that can result in impairment of receiving waters (Noble *et al.* 2003, Schiff *et al.* 2003, Stein and Tiefenthaler 2005). Stein and Yoon (2007) reported geometric mean FIB levels from natural streams during storms of 125, 140, and 4,460 MPN/100 ml for *E. coli*, enterococci and total coliforms, respectively. These levels are generally 1.5 - 2 log units higher than geomean levels observed in this study during dry weather conditions (Figure 9). As is the case in urban areas, bacteria levels in natural systems are significantly lower during dry weather conditions than during storms, although the higher levels observed during storms are much more transient in nature. Griffith *et al.* (2006) reported that one-fifth of all samples collected within three days of rainfall from beaches at the bottom of natural catchments exceeded water quality thresholds for at least one bacterial indicator.

Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize “background” bacterial contamination in southern California reference waters following storms.

The results of this study indicated that streams in undeveloped watersheds contain low levels of FIB of non-human origin. An important management question is whether the levels observed pose a potential health risk. Wade *et al.* (2003) reviewed 27 studies and concluded that *E. coli* levels between 45 and 170 CFU/100 ml in freshwater pose a relative human health risk level of 1.22 (i.e. low level risk). We observed 30-day geometric mean *E. coli* levels ranging from 2 – 138 MPN/100 ml, with an overall 30-day geometric mean of 41 ± 20 MPN/100 ml. Because the mean levels observed in this study were below the “low risk” range reported by Wade *et al.* (2003), it could be concluded that background levels in natural streams have a low likelihood of posing a human health risk. However, this conclusion should be made with caution because previous exposure and risk studies were conducted in areas known to receive wastewater or storm water discharges containing human fecal sources. In contrast, the FIB levels observed in this study were of non-human origin, so the actual risk is unknown.

Conclusion and Future Research

This study yielded the following conclusions about FIB levels in natural streams during dry weather conditions:

1. ***Fecal indicator bacteria typically occur in natural streams during dry weather conditions at levels below State water quality standards.*** Annual mean concentrations (both single sample and 30-day geometric mean) were below established water quality criteria for all three indicators. A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 1.5%, 14%, and 3% of *E. coli*, enterococci, and total coliforms, respectively, exceeded single sample water quality criteria.
2. ***Fecal indicator bacteria in natural streams are most likely of non-human origin.*** All samples tested for the presence of *B. thetaiotaomicron* were negative, indicating non-human sources in natural streams. FIB levels in natural streams likely result from a combination of natural inputs, such as wildlife, birds, and soil erosion and instream bacterial growth facilitated by high summer temperatures and presence of decaying organic matter.
3. ***Dry weather fecal indicator bacteria in natural streams are typically two orders of magnitude lower than those observed in streams draining developed watersheds.*** Data from the developed Ballona Creek watershed were typically in the 10^3 MPN/100 ml range for *E. coli* and enterococci. Even slight watershed modifications appear to result in a relatively rapid departure from background FIB levels.
4. ***Fecal indicator bacteria levels exhibit seasonal patterns.*** Mean bacteria levels and frequency of exceedance of water quality standards were higher during the warmer summer months for all three bacteria indicators. This suggests that summer is a critical period for assessing background bacteria levels. Past studies indicate that fecal indicator

bacteria levels in natural streams during storms are one to two orders of magnitude higher than those observed during dry weather conditions; however, the duration of these elevated levels is unknown. Studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of “background” bacterial contamination in southern California reference waters following storms.

5. ***Bacteria levels in natural streams were generally higher during lower flow conditions.*** For all three indicators, densities increased exponentially when stream flow decreased below approximately 0.5 m³/s (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially.
6. ***Dry weather fecal indicator bacteria levels were one to two orders of magnitude lower than those observed in natural streams during storm conditions.*** Past studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of “background” bacterial contamination in southern California reference waters following storms.
7. ***Fecal indicator bacteria in natural streams occurred at levels below those reported to pose health risks due to freshwater contact recreation.*** However, past risk assessments have all occurred in waters that are known to receive bacteria inputs of human origin. No epidemiology studies have been conducted on FIB of non-human origin, so the precise risk is unknown.

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Table 1. List of natural stream sampling sites, characteristics and their median monthly fecal indicator bacteria densities (MPN/100 ml).

Site Name	Watershed	County	Catchment Size (km ²)	Number Sampling Weeks/Yr	Mean Flow (m ³ /sec)	<i>E. coli</i>		Geomean (30-d) Enterococci		Total coliforms	
						(MPN/100 ml)	SD	(MPN/100 ml)	SD	(MPN/100 ml)	SD
Arroyo Seco	LA River		41.50	47	0.04	15.24	2.22	20.48	2.45	1291.90	2.85
Cold Creek	Malibu Creek		1.43	49	0.00	13.59	1.89	15.33	2.42	443.30	4.33
Lachusa Canyon	Los Alisos Canyon	Los Angeles	3.86	49	0.01	16.08	2.24	20.55	2.26	1486.50	2.14
Solstice Canyon	Solstice Canyon		8.74	49	0.01	16.97	2.28	20.64	2.43	1109.21	2.68
Chesebro Creek	Malibu Creek		7.55	49	0.00	90.30	5.49	68.25	4.24	2940.41	2.88
Bell Creek	San Juan		17.97	12 ^a	0.02	80.45	4.30	164.60	5.48	2008.67	3.16
San Juan Creek	San Juan	Orange	99.94	9 ^a	0.03	74.66	2.46	25.25	3.29	2848.15	1.66
Santiago Creek	Santa Ana		17.02	10 ^a	0.02	22.99	2.84	34.75	3.06	1869.15	1.98
Hurkey Creek	San Jacinto	Riverside	29.73	29	0.01	18.89	4.38	36.92	4.75	688.57	3.33
Mill Creek	Santa Ana		15.21	55	0.08	2.06	2.68	12.74	3.32	75.00	2.98
Cucamonga Creek	Cucamonga	San Bernardino	24.10	52	0.14	11.14	1.66	26.35	3.33	399.64	2.39
Day Creek	Santa Ana		11.70	55	0.32	11.02	1.58	25.18	2.87	545.71	2.41
Cajon Creek	Santa Ana		82.82	52	0.08	54.98	3.18	159.21	2.49	4794.47	2.04
Stone Creek	Santa Margarita	San Diego	7.00	50	0.00	138.18	3.86	52.72	3.58	1728.44	3.21
Boden Creek	San Dieguito		19.81	18 ^a	0.01	45.33	6.14	98.26	2.86	1658.46	2.54
		Mean	25.89	39	0.05	40.79	3.15	52.08	3.26	1592.51	2.70
		SD	14.54	9	0.04	19.84	0.71	25.32	0.47	622.94	0.34

^aIntermittent stream

Table 2. State of California marine water quality standards for fecal indicator bacteria as established in Assembly Bill 411. Currently a freshwater quality standard for total coliforms does not exist.

Fecal Indicator Bacteria	CA Maximum Allowable Density (MPN/100 ml)	
	single-sample	30-day geometric mean
Enterococci	104	33
<i>E. coli</i>	235	126
Total coliforms	10,000	1000

Additional Indicator

Bacteroides thetaiotaomicron Presence / absence of a human source

Table 3. Assessment of percent exceedances between counties in southern California during the present study. A ¹ represents those counties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes.

	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total Coliforms
Daily			
Los Angeles County	0.0	6.3	0.0
Orange County ¹	12.9	38.7	3.2
San Bernardino	0.0	13.1	0.0
San Diego ¹	5.3	47.4	0.0
Monthly			
Los Angeles County	0.0	7.7	46.2
Orange County ¹	25.0	75.0	100.0
San Bernardino	0.0	23.1	0.0
San Diego ¹	0.0	100.0	80.0

Table 4. Percent single-sample exceedance of fecal indicator bacteria (FIB) levels in natural streams during dry weather from May 2006-May 2007. Numbers in bold are significantly different ($p < 0.01$).

	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total coliforms
Season			
Spring 06	0.0	41.7	75.0
Summer	12.5	75.0	83.3
Fall	0.0	0.0	28.6
Winter	0.0	0.0	11.1
Spring 07	0.0	22.2	44.4
Month			
May 2006	0.0	27.3	45.5
June 2006	0.0	66.7	75.0
July 2006	0.0	72.7	90.9
August 2006	12.5	62.5	75.0
September 2006	0.0	42.9	57.1
October 2006	0.0	0.0	14.3
November 2006	0.0	0.0	28.6
December 2006	0.0	0.0	14.3
January 2007	0.0	0.0	0.0
February 2007	0.0	12.5	25.0
March 2007	0.0	22.2	11.1
April 2007	0.0	11.1	44.4
May 2007	0.0	25.0	62.5
Annual	1.0	26.4	41.8

Table 5. Correlation table (r^2 values) between water quality variables and fecal indicator bacteria (FIB) during dry weather in natural streams in southern California between May 2006-May 2007. Significant correlations ($p<0.04$) are shown in bold, while significant correlations ($p<0.001$) are both bolded and in italics.

Parameter	Pearson r^2 -values				
	DO (mg/L)	Flow (m ³ /s)	<i>E. coli</i>	Enterococci (MPN/100 ml)	Total Coliform
Conductivity	-0.50	0.48	0.22	0.01	0.19
Dissolved Oxygen	-	0.12	0.18	0.21	0.16
pH	0.32	0.09	0.11	0.02	0.04
Flow	0.12	-	-0.06	-0.02	-0.08
Temperature (°C)	-0.84	0.02	0.20	0.26	0.48
Turbidity	0.19	0.00	0.02	1.44	0.07

Bolded values = $p<0.05$

Bolded italic values = $p<0.001$

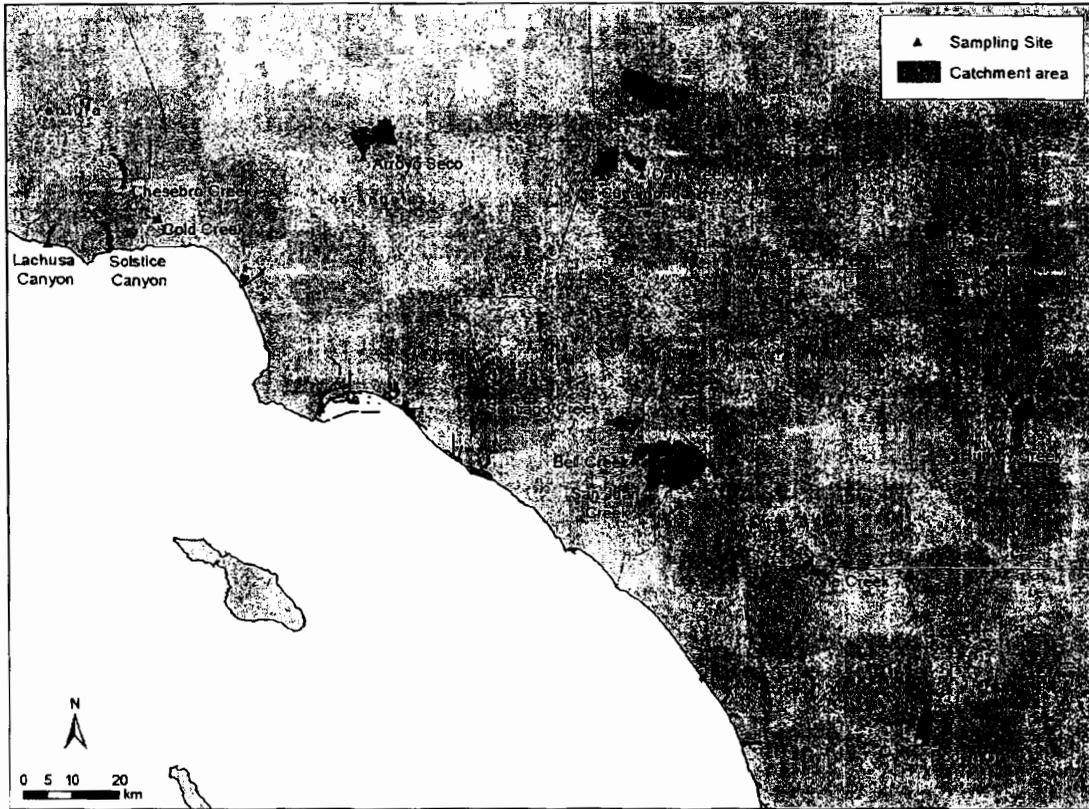


Figure 1. Map of natural stream sampling sites and their respective catchments within southern California.

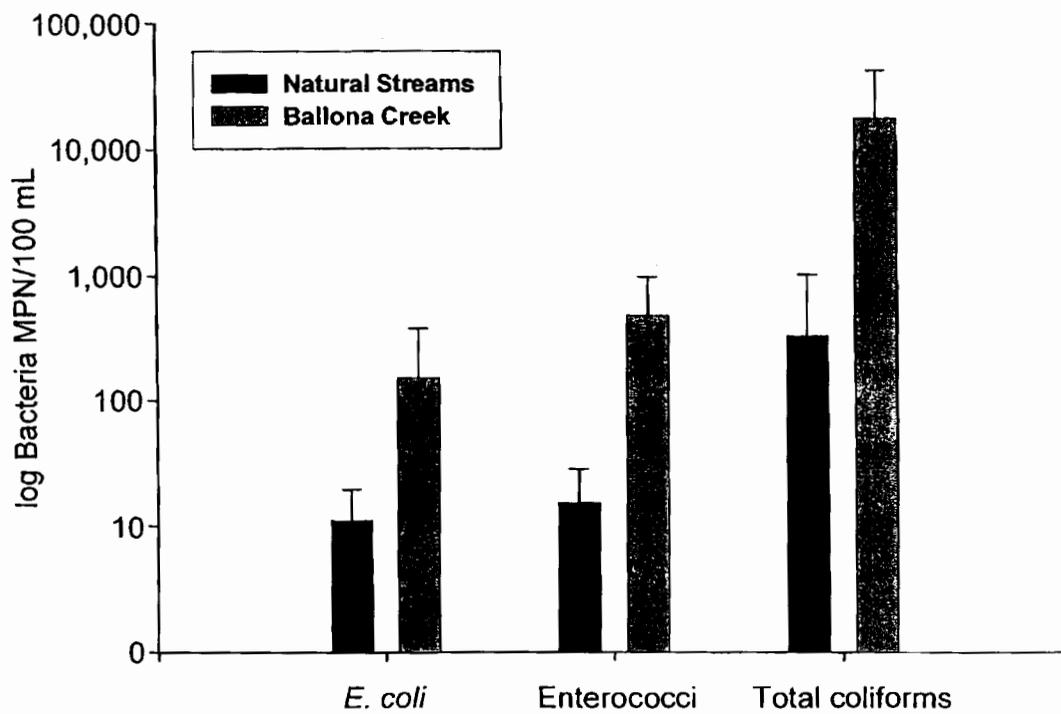


Figure 2. Comparison of dry weather log₁₀ fecal indicator bacteria (FIB) densities (\pm standard deviations) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006-May 2007 in southern California, USA.

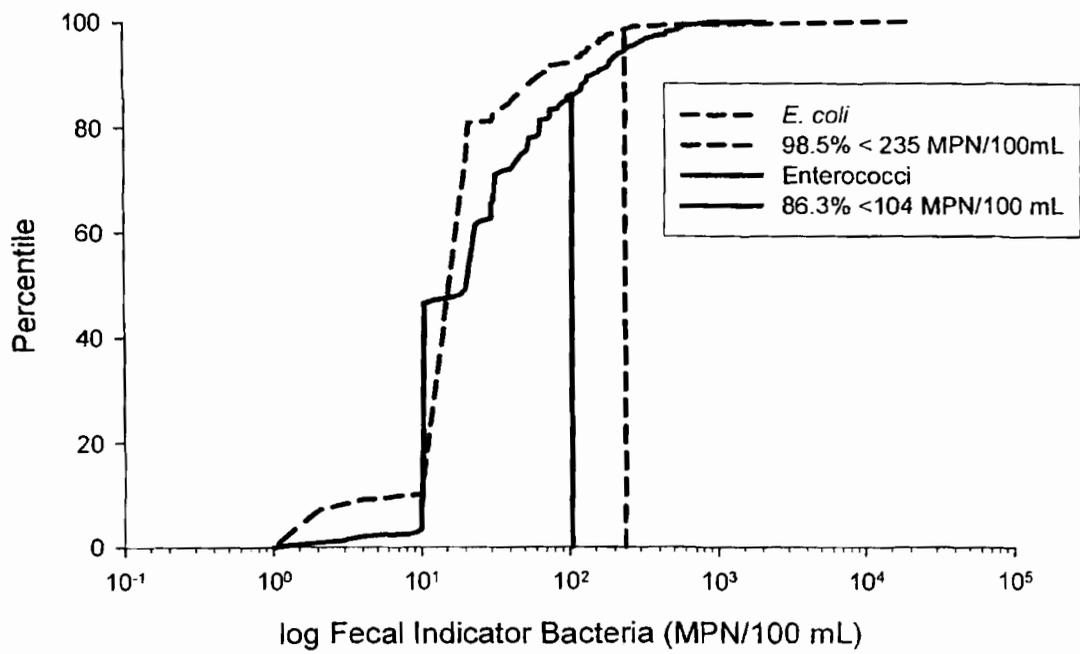


Figure 3. Dry season fecal indicator bacteria cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California, USA.

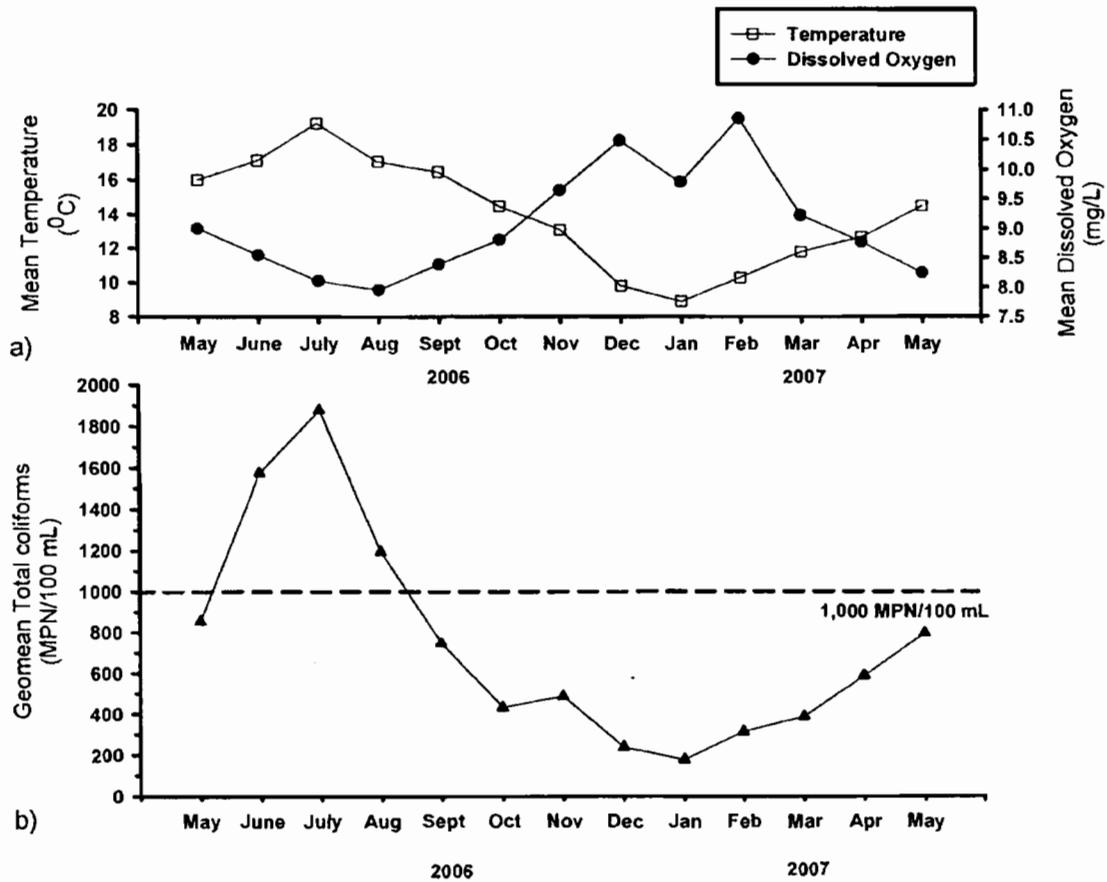


Figure 4. Mean monthly temperature (°C) and dissolved oxygen (mg/L) comparison (a) and geomean total coliform densities in natural streams in southern California (b) between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons ($p < 0.01$). *E. coli* and enterococci exhibited similar results. The dotted line indicates the 30-d geomean for total coliforms equal to 1,000 MPN/100 ml. All points above the line represent bacteria water quality exceedances.

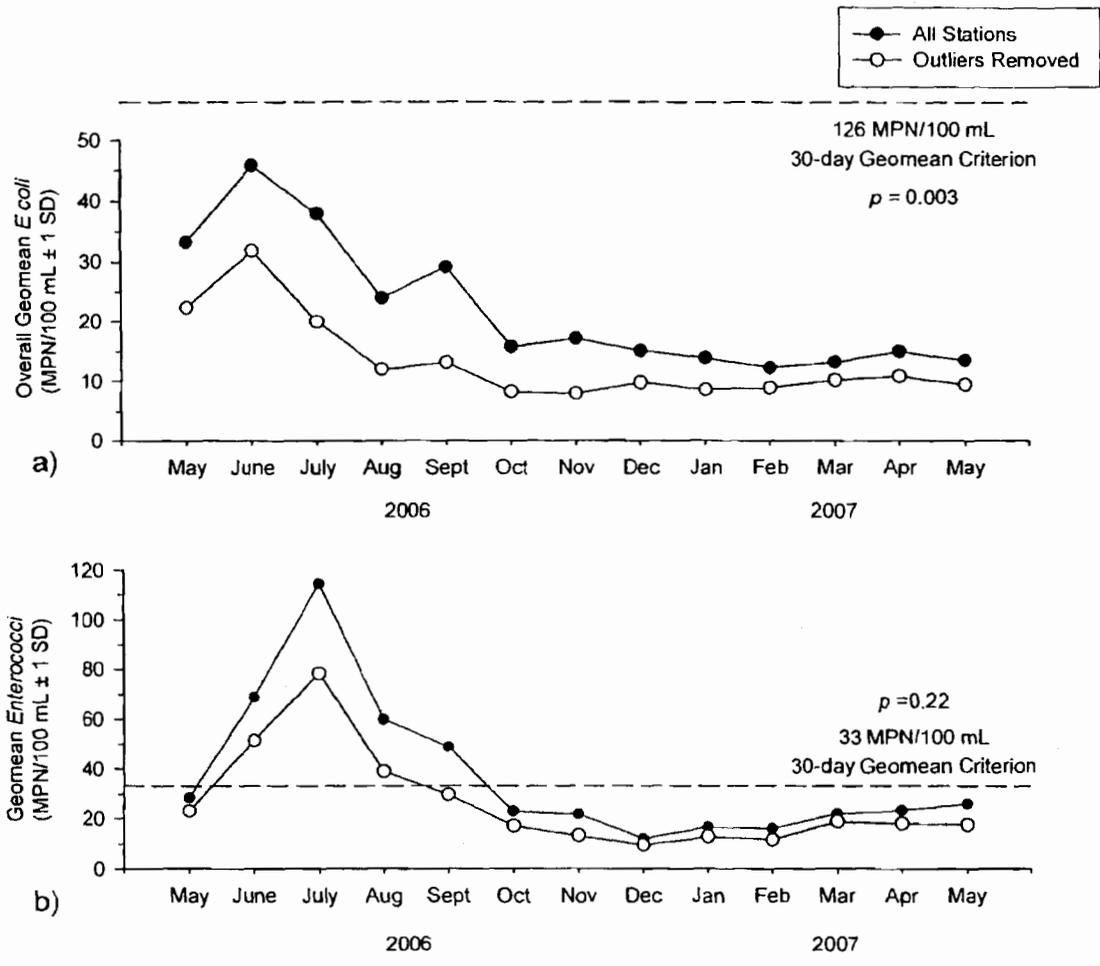


Figure 5. *E. coli* a) and enterococci b) geomean densities in natural streams in southern California between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons. The dashed line indicates the monthly water quality standard equal to 235 MPN/100 ml and 104 MPN/100 ml for *E. coli* and enterococci respectively. All points above the line represent bacteria water quality exceedances.

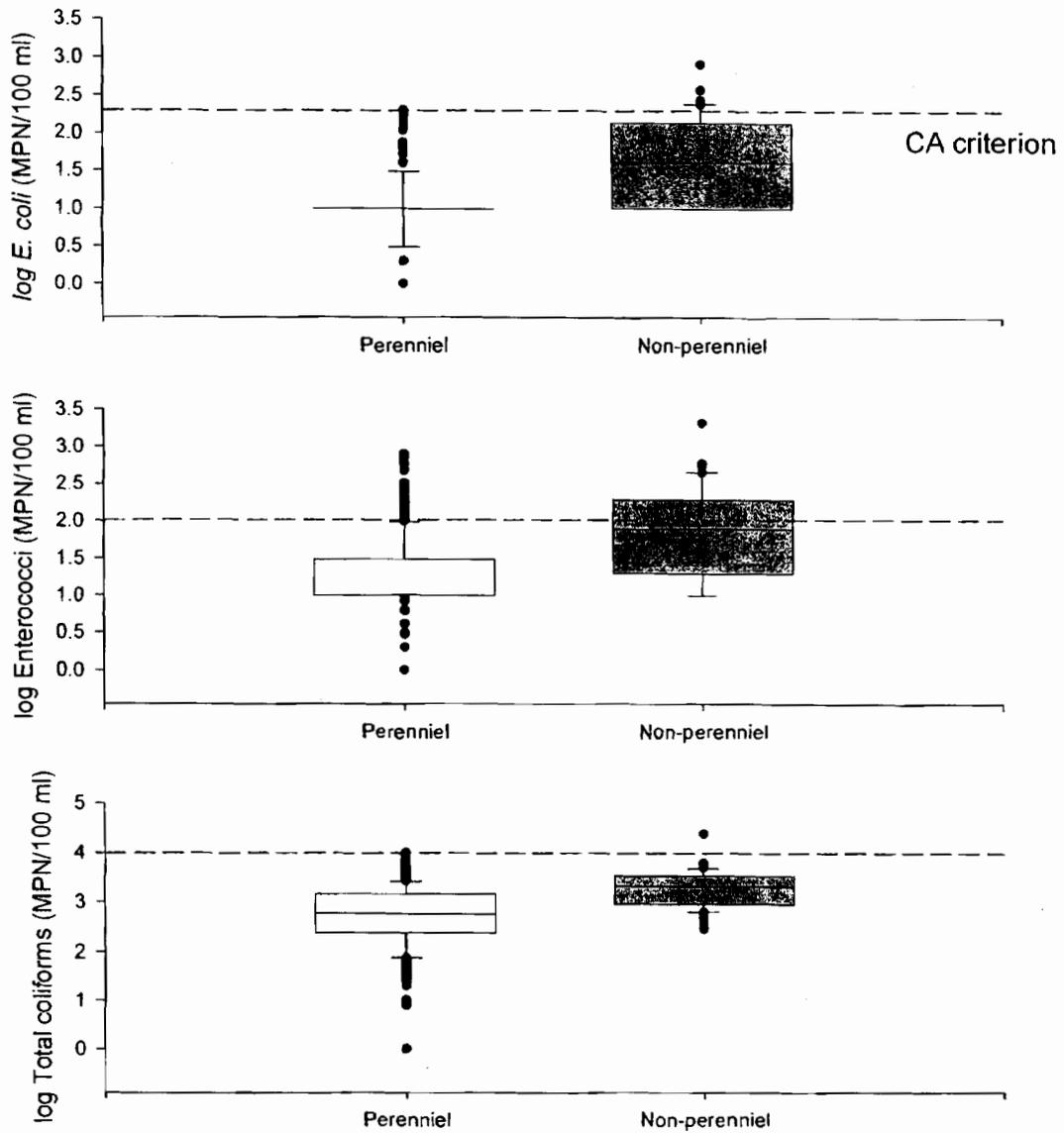


Figure 6. Perennial and non-perennial stream comparison of log₁₀ fecal indicator bacteria densities (MPN/100 ml) in southern California during the present study. The dotted line indicates the State single-sample bacterial water quality criterion. Significant differences in indicator densities existed between streams but ranges generally overlapped ($p < 0.05$). Boxplots show mean, median, 25th and 75th percentiles.

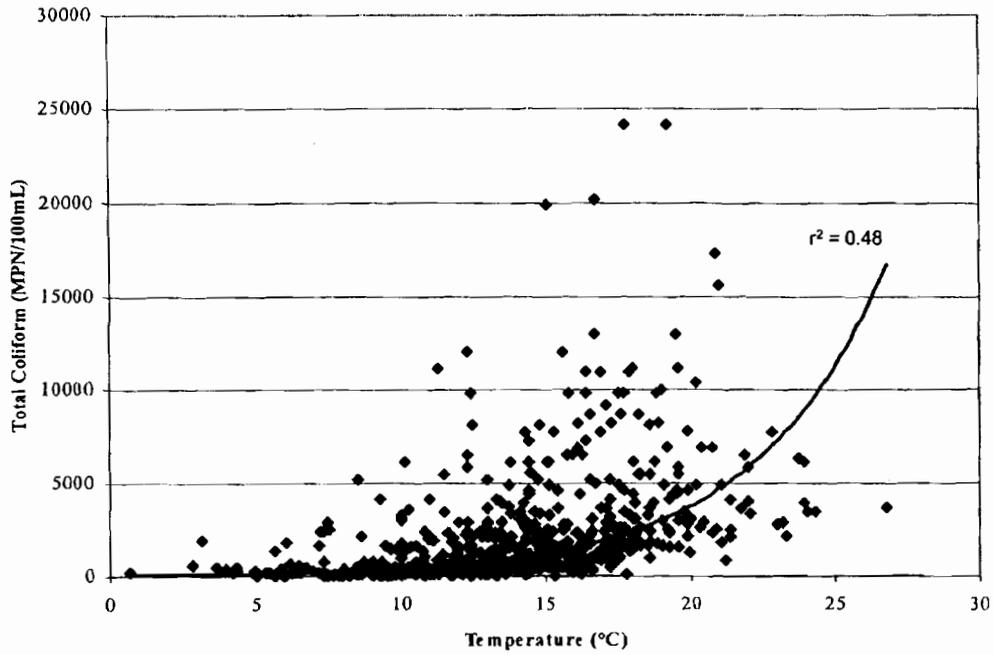


Figure 7. Natural stream temperatures in southern California versus total coliform densities (MPN/100 ml) during dry weather for an entire year. Solid line indicates the exponential trend line ($r^2 = 0.48$).

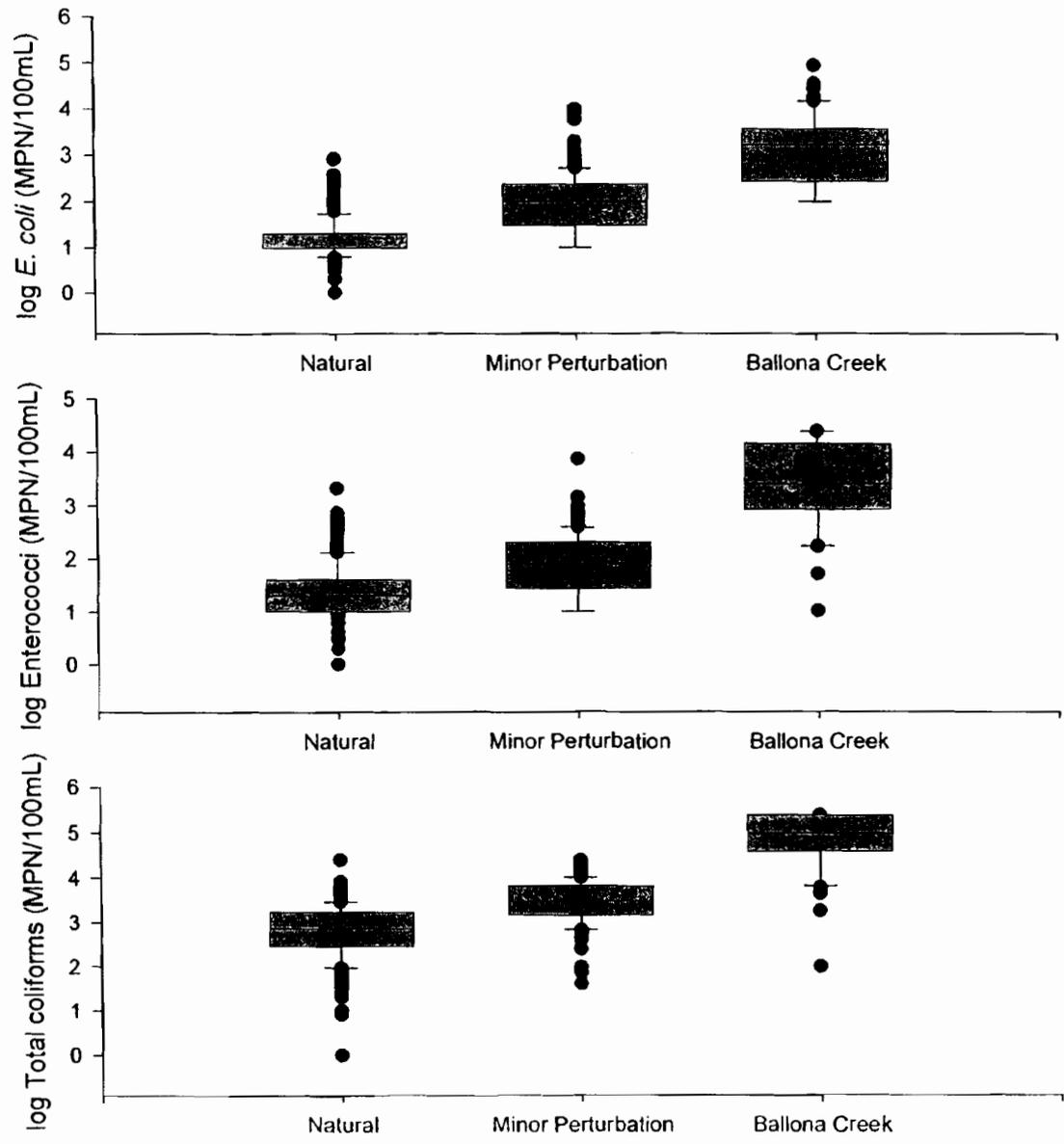


Figure 8. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams, streams with minor perturbations, and in developed Ballona Creek watershed in southern California, USA. Natural streams were significantly lower than all other streams ($p < 0.001$). Minor perturbation streams were significantly lower than developed Ballona Creek ($p < 0.001$).

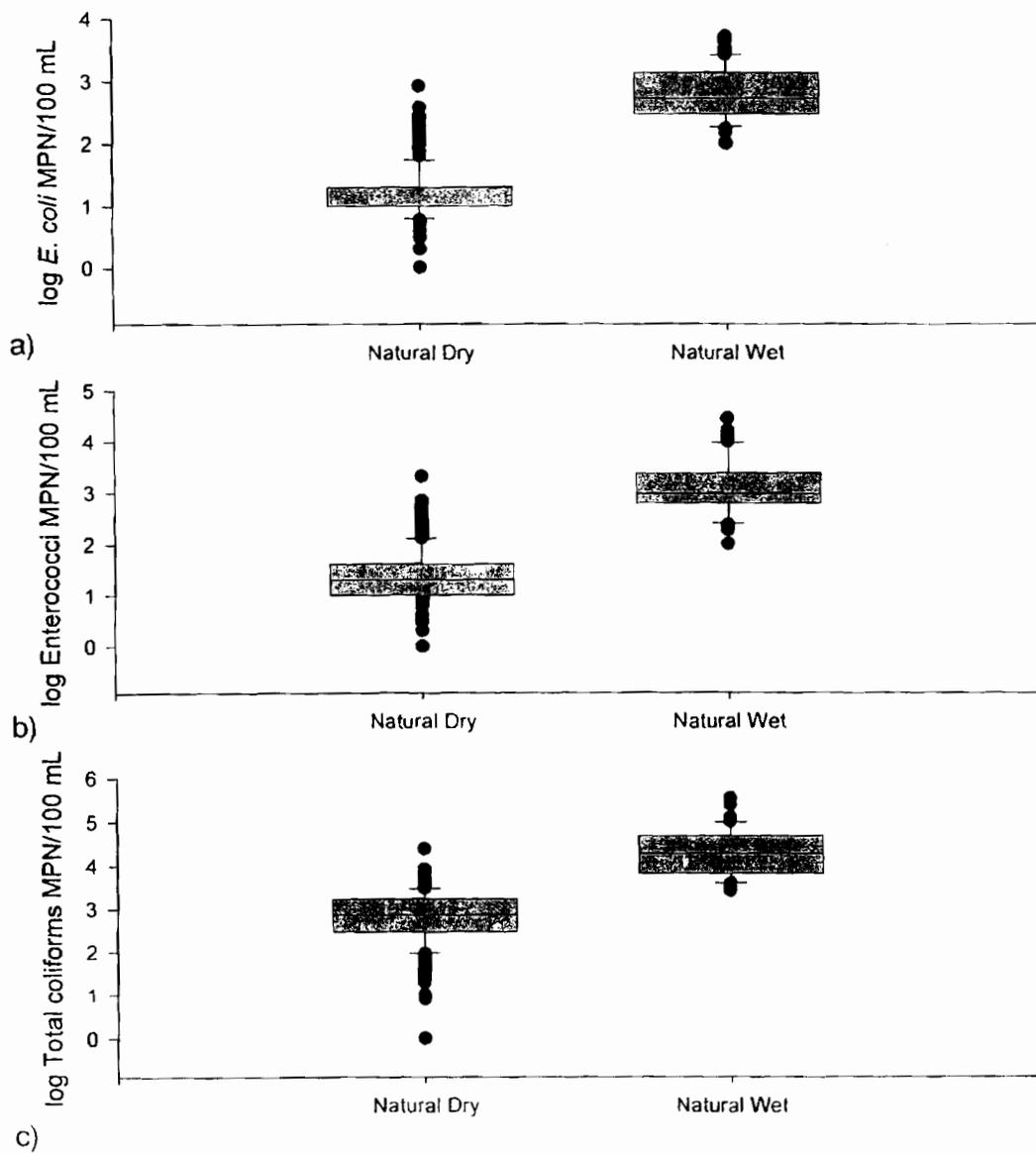


Figure 9. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams during dry weather (present study) compared to wet weather (Natural Loadings; 2003-2005 and Los Angeles River watershed; 2001-2005) studies in southern California, USA. Dry weather bacteria concentrations were significantly lower than wet weather concentrations ($p < 0.001$).

**APPENDIX A - SUMMARY BACTERIA DATA FOR ALL NATURAL
STREAM SITES**

Table A1. List of natural stream sampling sites, characteristics and their daily fecal indicator bacteria densities (MPN/100 ml).

Sampling site	Watershed	Concentration (MPN/100 ml)											
		E. coli			Enterococci			Total coliforms					
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max			
Arroyo Seco	LA River	10	15.2	148	10	20.5	250	10	1291.9	6867			
Cold Creek	Malibu Creek	10	13.6	108	10	15.3	480	10	443.3	6131			
Lachusa Canyon	Los Alisos Canyon	10	16.1	161	10	20.6	197	146	1486.5	8164			
Solstice Canyon	Solstice Canyon	10	17.0	200	10	20.6	262	10	1109.2	5475			
Chesebro Creek	Malibu Creek	10	90.3	9804	10	68.2	7270	96	2940.4	24192			
Bell Creek	San Juan	10	80.5	820	10	164.6	2098	292	2008.7	24196			
San Juan Creek	San Juan	20	74.7	259	10	25.2	299	1664	2848.2	6294			
Santiago Creek	Santa Ana	10	23.0	134	10	34.7	228	469	1869.1	3873			
Hurkey Creek	San Jacinto	10	18.9	5500	10	36.9	780	210	688.6	7700			
Mill Creek	Santa Ana	1	2.1	20.9	1	12.7	190	1	75.0	435			
Cucamonga Creek	Cucamonga	6	11.1	180	10	26.3	580	10	399.6	2000			
Day Creek	Santa Ana	4	11.0	160	10	25.2	240	31	545.7	9800			
Cajon Creek	Santa Ana	10	55.0	520	20	159.2	960	730	4794.5	13000			
Stone Creek	Santa Margarita	10	138.2	5830	10	52.7	1408	40	1728.4	15530			
Boden Creek	San Dieguito	10	45.3	18600	10	98.3	563	388	1658.5	20140			
Mean		9.40	40.79	2829.66	10.07	52.08	1053.67	273.80	1592.51	10253.13			
StDev		2.04	19.84	2662.11	1.82	25.32	911.35	222.68	622.94	3837.71			

Table A2. Monthly *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	<i>E. coli</i> Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	10.0	37.5	56.1	11.5	26.0	12.5	10.0	10.0	10.0	10.0	10.0	16.5	10.0
Lachusa Canyon	82.8	30.7	20.0	12.5	28.5	10.0	10.0	14.1	10.0	10.0	16.0	10.0	25.1
Cold Creek	14.4	42.1	10.0	27.6	10.0	14.2	10.0	10.0	10.0	10.0	10.0	10.0	20.0
Solstice Canyon	32.2	59.6	11.9	15.2	29.9	10.0	10.0	40.0	12.6	10.0	15.2	10.0	20.0
Chesebro Creek	150.3	276.0	444.2	233.5	1336.8	111.3	27.1	58.7	11.9	25.3	65.8	28.9	10.0
Bell Creek	25.9	125.6	104.0	146.0									
San Juan Creek	36.0	121.6	84.2										
Santiago Creek	10.0	22.8	53.6										
Hurkey Creek	5500.0	18.9	14.1						22.6	10.0	10.0	10.0	
Cucamonga Creek	10.0	10.0	10.0	12.4	10.0	10.0	10.0	20.6	10.0	13.2	10.0	10.0	10.0
Mill Creek	10.0	10.0	5.0	2.6	2.8	1.4	1.0	1.0	1.1	2.0	1.0	1.0	1.0
Day Creek	10.0	20.0	10.0	11.0	10.0	13.2	10.0	10.0	10.0	11.9	10.0	10.0	10.0
Capon Creek	38.3	180.1	146.9	104.1	225.3	96.6	76.1	42.4	35.3	12.6	12.6	10.0	10.0
Stone Creek	65.7	129.2	269.5	134.6		156.1	441.1	57.8	240.1	82.8	20.2	99.4	112.1
Boden Creek	1082.5	26.1									21.5	63.5	30.7

Table A3. Monthly enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Enterococci Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	41.0	63.0	105.7	23.9	54.6	18.1	11.9	10.0	10.0	10.0	10.0	10.0	14.1
Lachusa Canyon	20.2	13.2	15.1	17.4	21.6	20.1	11.5	14.1	14.6	34.0	82.3	24.7	17.6
Cold Creek	12.6	18.8	115.6	16.6	16.5	10.0	10.0	10.0	10.0	10.0	11.5	17.6	10.0
Solstice Canyon	25.1	23.8	39.8	61.0	47.5	16.9	13.2	10.0	12.6	10.0	12.5	10.0	35.1
Chesebro Creek	59.0	200.3	563.1	146.5	252.2	31.1	41.1	24.9	11.9	29.0	51.5	26.8	62.0
Bell Creek	12.6	402.1	467.8	158.0									
San Juan Creek	20.2	47.5	10.0										
Santiago Creek	14.6	59.0	40.8										
Hurkey Creek	380.0	121.6	744.2						18.9	10.0	10.0	19.5	
Cucamonga Creek	33.9	90.2	241.1	85.8	31.2	14.3	10.0	14.1	10.0	11.9	12.6	18.4	10.0
Mill Creek	10.0	10.0	20.2	35.8	16.5	23.2	14.8	4.0	16.1	3.2	22.6	25.8	3.9
Day Creek	21.5	43.3	125.8	92.1	42.4	18.8	24.6	11.9	11.5	21.4	10.0	11.9	14.1
Cajon Creek	87.1	307.1	486.6	367.7	253.0	157.0	217.8	56.6	66.9	100.3	74.1	95.2	200.0
Stone Creek	53.6	163.0	192.3	133.8	79.0	31.8	53.4	12.6	46.1	18.5	11.9	45.9	74.2
Boden Creek	143.4	208.4									69.4	44.9	98.4

Table A4. Monthly total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Total coliforms Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	708.0	1854.8	4200.8	1859.1	2506.6	1480.0	1155.9	534.0	134.9	588.4	1547.1	1843.3	2926.5
Lachusa Canyon	1611.2	1825.6	2724.7	3350.6	2074.7	998.6	1139.4	1206.9	725.0	1655.0	807.2	1009.0	2176.8
Cold Creek	997.6	1743.3	3567.4	1312.3	1347.5	488.0	250.7	109.2	70.7	78.3	123.9	218.3	277.4
Solstice Canyon	1064.2	1404.8	2278.4	2998.4	1048.4	499.8	550.8	654.2	761.3	1218.5	529.5	1783.9	2549.3
Chesebro Creek	2546.1	4655.0	9044.6	8141.9	8332.1	4770.4	2142.9	1017.2	789.6	1085.4	1515.9	1722.6	2540.4
Bell Creek	518.6	4780.6	2513.8	1483.0									
San Juan Creek	1748.1	3406.8	4139.9										
Santiago Creek	1189.6	1846.4	2985.1										
Hurkey Creek	6500.0	2102.0	5040.8										
Cucamonga Creek	419.1	688.2	1334.1	650.0	740.5	362.5	364.9	122.4	348.1	224.5	326.7	347.1	
Mill Creek	170.6	224.0	126.4	139.1	35.3	91.9	151.7	27.5	155.4	253.2	318.7	434.9	720.0
Day Creek	311.1	746.5	1146.1	1320.5	668.1	267.3	417.4	374.0	30.8	24.0	48.3	52.0	115.7
Cajon Creek	5915.7	8730.8	7512.4	3300.6	7335.3	9693.4	2667.5	3993.7	2747.3	2242.1	2946.6	5451.8	8200.0
Stone Creek	347.3	3493.6	4887.8	5727.9	7310.9	2482.6	1959.5	321.2	734.3	617.5	673.7	1610.4	1151.6
Boden Creek	7229.3	3207.2									603.5	1295.2	1302.1

Table A5. Dry season *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	<i>E. coli</i> Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	25.0	22.4	13.7	10.0	13.3
Lachusa Canyon	Los Alisos Canyon	45.9	15.9	13.4	12.0	13.7
Cold Creek	Malibu Creek	24.8	16.5	11.2	10.0	11.9
Solstice Canyon	Solstice Canyon	49.7	19.7	12.6	12.6	13.0
Chesebro Creek	Malibu Creek	213.3	531.7	56.0	21.5	32.8
Bell Creek	San Juan	48.3	115.8			
San Juan Creek	San Juan	74.2	75.2			
Santiago Creek	Santa Ana	16.8	31.4			
Hurkey Creek	San Jacinto	119.5	16.9		15.3	10.0
Cucamonga Creek	Cucamonga	10.0	10.8	12.7	11.1	10.0
Mill Creek	Santa Ana	10.0	4.2	1.2	1.3	1.0
Day Creek	Santa Ana	17.4	10.3	10.9	10.6	10.0
Cajon Creek	Santa Ana	84.7	126.2	102.7	20.1	11.2
Stone Creek	Santa Margarita	95.1	181.5	292.4	80.7	82.9
Boden Creek	San Dieguito	148.1	14.1		10.0	43.1

Table A6. Dry season enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Enterococci Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	54.6	49.4	15.6	10.0	11.0
Lachusa Canyon	Los Alisos Canyon	15.9	17.1	14.2	30.6	35.0
Cold Creek	Malibu Creek	15.2	35.0	10.0	10.0	14.5
Solstice Canyon	Solstice Canyon	22.1	52.9	14.7	12.2	13.7
Chesebro Creek	Malibu Creek	118.8	365.1	33.7	21.1	46.1
Bell Creek	San Juan	60.1	338.0			
San Juan Creek	San Juan	26.8	23.4			
Santiago Creek	Santa Ana	24.2	49.9			
Hurkey Creek	San Jacinto	127.2	386.5		14.2	14.9
Cucamonga Creek	Cucamonga	47.0	138.9	14.1	11.3	16.5
Mill Creek	Santa Ana	10.0	20.6	11.4	12.4	8.0
Day Creek	Santa Ana	28.7	77.4	20.5	13.7	11.9
Cajon Creek	Santa Ana	140.7	383.2	145.4	89.6	96.7
Stone Creek	Santa Margarita	83.1	151.8	40.0	22.0	51.2
Boden Creek	San Dieguito	154.0	305.7		28.6	81.6

Table A7. Dry season total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Total coliforms Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	1066.8	2610.9	1230.6	422.2	2163.9
Lachusa Canyon	Los Alisos Canyon	1663.9	2899.9	1092.8	1034.7	1099.7
Cold Creek	Mailbu Creek	1069.4	2133.9	295.8	97.2	180.3
Solstice Canyon	Solstice Canyon	1278.2	2165.1	543.3	616.5	1900.3
Chesebro Creek	Mailbu Creek	3776.6	8814.0	2535.0	889.0	2281.4
Bell Creek	San Juan	1169.5	2955.9			
San Juan Creek	San Juan	2001.5	4426.6			
Santiago Creek	Santa Ana	1417.1	2465.3			
Hurkey Creek	San Jacinto	2952.6	3345.5		326.8	310.5
Cucamonga Creek	Cucamonga	508.0	958.8	254.1	216.2	500.4
Mill Creek	Santa Ana	185.3	104.5	82.8	31.6	79.5
Day Creek	Santa Ana	425.2	1001.3	374.8	348.5	795.6
Cajon Creek	Santa Ana	6926.3	5634.3	5220.2	2595.1	5267.6
Stone Creek	Santa Margarita	1343.4	5682.0	2193.0	516.7	1361.0
Boden Creek	San Dieguito	5146.1	2216.8		514.8	1163.2

Table A8. Annual dry season fecal indicator bacteria geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Annual Dry Season Geomeans		
		<i>E. coli</i>	Enterococci	Total Coliforms
Arroyo Seco	LA River	15.2	20.5	1291.9
Lachusa Canyon	Los Alisos Canyon	16.1	20.6	1486.5
Cold Creek	Malibu Creek	13.6	15.3	443.3
Solstice Canyon	Solstice Canyon	17.0	20.6	1109.2
Chesebro Creek	Malibu Creek	90.3	68.2	2940.4
Bell Creek	San Juan	80.5	164.6	2008.7
San Juan Creek	San Juan	74.7	25.2	2848.2
Santiago Creek	Santa Ana	23.0	34.7	1869.1
Hurkey Creek	San Jacinto	18.9	36.9	688.6
Cucamonga Creek	Cucamonga	11.1	26.3	399.6
Mill Creek	Santa Ana	2.1	12.7	75.0
Day Creek	Santa Ana	11.0	25.2	545.7
Cajon Creek	Santa Ana	55.0	159.2	4794.5
Stone Creek	Santa Margarita	138.2	52.7	1728.4
Boden Creek	San Dieguito	45.3	98.3	1658.5

**APPENDIX B - SUMMARY OF PHYSICAL PARAMETERS AT ALL
NATURAL STREAM SITES**

Table B1. Annual dry season averages of measured physical parameters in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Physical Parameter Averages					
	Conductivity µs	DO mg/L	Flow Rate m ³ /s	pH	Temperature °C	Turbidity
Arroyo Seco	411.9	na	0.038	na	13.8	na
Lachusa Canyon	1431.1	na	0.006	na	16.2	na
Cold Creek	604.0	na	0.005	na	13.8	na
Solstice Canyon	1051.6	na	0.011	na	15.4	na
Chesebro Creek	3089.0	na	0.005	na	11.9	na
Bell Creek	738.8	8.7	0.018	8.0	18.7	1.1
San Juan Creek	518.8	10.4	0.028	8.2	21.1	0.7
Santiago Creek	636.9	9.6	0.017	8.1	22.2	0.5
Hurkey Creek	129.9	na	0.006	7.8	11.6	na
Cucamonga Creek	9.8	9.8	0.138	8.0	12.3	na
Mill Creek	0.7	9.4	0.080	8.0	10.6	12.3
Day Creek	13.7	9.9	0.317	8.0	12.6	1.8
Cajon Creek	37.7	8.7	0.082	7.9	15.7	8.0
Stone Creek	1171.6	7.2	0.002	7.5	16.4	16.1
Boden Creek	1012.0	7.5	0.005	7.8	15.3	6.1

APPENDIX C - INTERLABORATORY CALIBRATION RESULTS

RESULTS

SCCWRP is currently coordinating an investigation of bacteria levels in reference drainages throughout southern California. This is a cooperative study involving multiple jurisdictions that are each contributing to the project through combinations of in-kind services and direct funding. Because numerous analytical labs will be participating in analysis of fecal indicator bacteria, it was necessary to conduct a laboratory intercalibration study to ensure that comparable results could be achieved from all participating laboratories. This memo summarizes the results of this intercalibration study.

Eight laboratories from five counties participated in the calibration exercise, a performance-based approach used to evaluate analytical accuracy, reproducibility of results and to ensure that data from participating laboratories were comparable (Table C1). The calibration exercise occurred on March 22th, 2006 and consisted of each lab receiving six common samples for analysis (Table C2). Necessary dilutions or aliquot volumes were processed to insure that reportable values could be determined. Bacterial results were reported for total coliform, *Escherichia coli* (*E. coli*), and enterococcus as organism type per 100 ml of sample. Precision was examined by assessing repeatability variance (based on intralaboratory data) and reproducibility variance (based on interlaboratory data). All participating labs were required to fall within a +/- 0.5 median log count comparability goal (Noble *et al.* 2000).

All laboratories had low repeatability variability for all three constituents and all results fell within the median log comparability criteria. Based on all results there were not large variations between the laboratories (i.e. neither of the laboratories were consistently higher or lower for any parameters) in a given sample or for all samples. However, one lab (CSD) reported higher values than the rest, but this can be explained by their inadvertent double diluting of the sample. Also, both Truesdail and Weck laboratories tended to report lower values than the rest. These laboratories should be extra cautious and invest extra efforts in data interpretation in order to not bias the results of bacterial concentrations on the low side.

Figures C1-3 are an example of how the laboratories compared with the different analyses and how well the laboratories were able to reproduce results. These plots are representative of all the data and illustrated good comparability between the analytical labs. As a result of this study we conclude that there should be no bias introduced into the dataset due to sample analysis by different laboratories. All the data and plots are available from SCCWRP upon request.

Table C1. List of participating laboratories and counties involved in the reference bacteria/watershed interlaboratory calibration.

Laboratory Name	County
E. S. Babcock, & Sons, Inc.	Riverside
City of San Diego	San Diego
CRG Marine Laboratories, Inc.	Los Angeles
HCA Public Health Laboratory	Orange
Truesdale Laboratories, Inc.	Orange
Weck Laboratories	Los Angeles
Aquatic Bioassay & Consulting Laboratory (ABC)	Ventura
Weston Solutions, Inc.	San Diego

Table C2. List of the six common samples and their representative sewage dilutions in (ml) which each laboratory received for the interlaboratory calibration.

Media	Dilution (ml)
DI Water	-
Santiago Creek	-
Sewage Dilution 1	3
Sewage Dilution 2	1.5
Sewage Dilution 3	1
Sewage Dilution 4	0.5

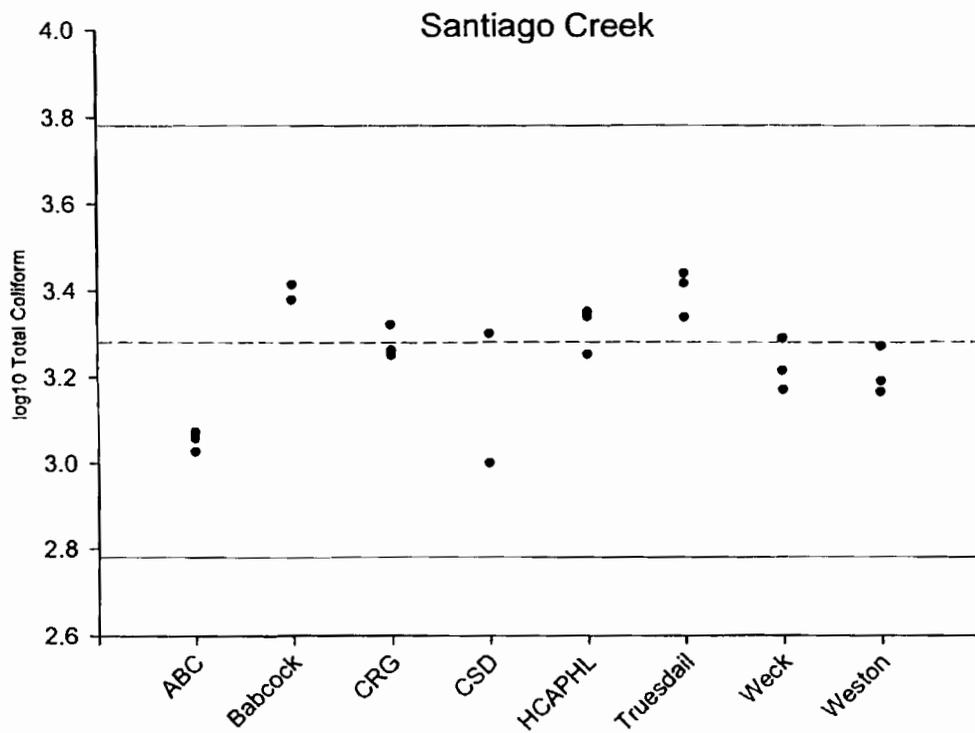


Figure C1. Laboratory comparison results for log transformed total coliform data at Santiago Creek, Orange County. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

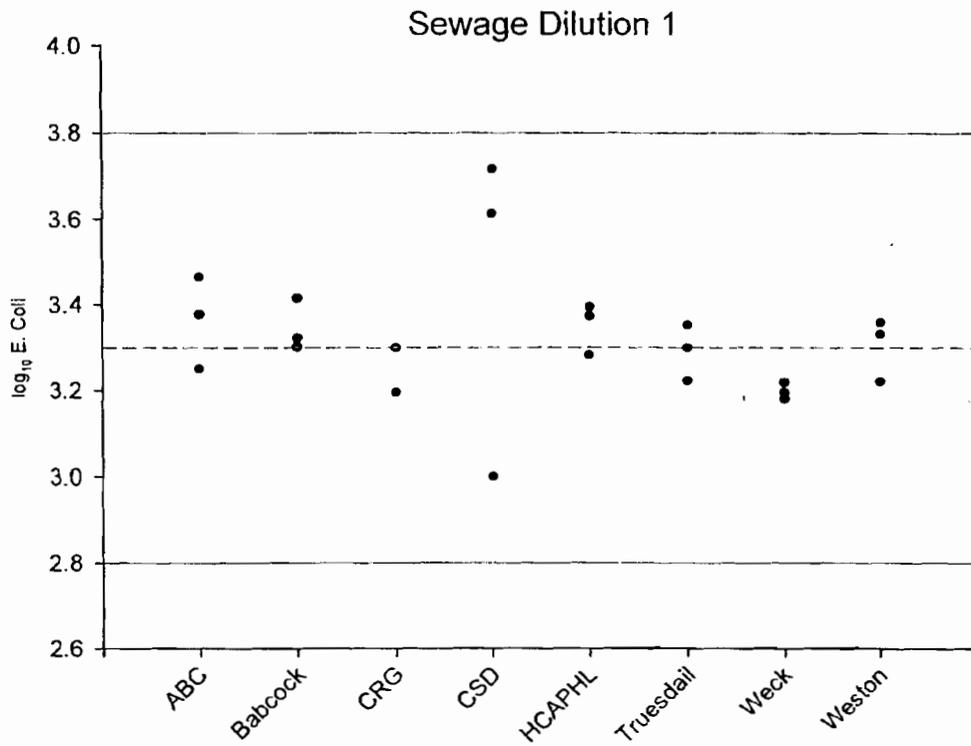


Figure C2. Laboratory comparison results for *E. coli* using a 3 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

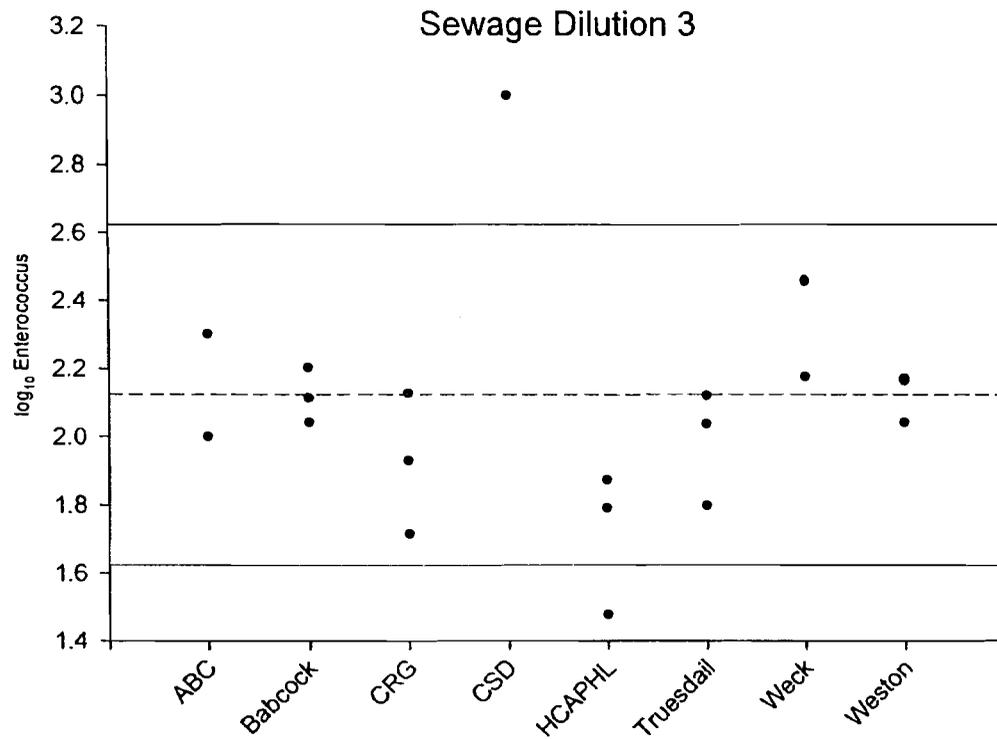


Figure C3. Laboratory comparison results for Enterococcus using a 1 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.



City of Malibu

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August 6, 2010

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CALIFORNIA
COASTAL COMMISSION
SOUTH CENTRAL COAST DISTRICT

Chair Bonnie Neely and the Members of the
California Coastal Commission
Attn: A. Tysor
89 South California Street, Suite 200
Ventura, CA 93001

Re: 8/12/10 Agenda Item Malibu Lagoon State Park, City of Malibu, Los Angeles County
CCC CDP Application No.: 4-07-098

Dear Chair Neely and Members of the Commission:

I write in support of the long-awaited Malibu Lagoon Restoration Project ("project"); and, although the project provides an opportunity for improvements to an impaired water system, I also write to convey significant potential environmental consequences that must be addressed in the permit. The City of Malibu is committed to improving and protecting water quality and, to that end, the City appreciates your consideration of the following concerns.

The City requests that before the permit is approved, the Coastal Commission do the following:

1. Satisfy its obligations under CEQA by studying and identifying the potential environmental consequences of the project. The hydrology in the Lagoon is complex and recent studies (attached and discussed in detail below) have shed new light on the existence of bacteria and nutrients in the water. These findings suggest that the proposed work has the potential to increase bacteria and nutrients in the water. The Commission must fully understand the potential impacts expected from disrupting this complex hydrologic system before approving the permit.
2. Impose adequate mitigation measures for the project to mitigate and prevent degradation of water quality in the area.
3. Require monitoring before, during and after the project. The proposed bi-annual monitoring plan is not adequate to understand the baseline water condition before the project begins. Without a baseline from which to measure, the scientists cannot determine the impact that this project will have on water quality. The City also requests the Commission require more frequent monitoring to identify promptly activities that are degrading the water quality. The project will likely increase Total Coliform, Fecal Coliform and Enterococcus at Surfrider Beach during the height of recreational activities. The City and other watershed agencies can potentially be held responsible for



bacteria exceedances at Surfrider Beach and it is imperative that the monitoring be frequent enough to enable the permittee to identify and promptly cease activities that degrade the water quality in the area. The permit only contemplates potential impacts on Malibu Creek fresh water and needs to properly account for potential impacts at Surfrider Beach as well.

Additionally, the City has the following comments with respect to the water quality in the Lagoon.

- 1) The Santa Monica Bay Beaches Bacterial Total Maximum Daily (TMDL) Load limits are set for three constituents (Total Coliform, Fecal Coliform and Enterococcus)(FIB); however, the permit only references Total Coliform in one chart, and Fecal Coliform in another reference. The monitoring plan should address and require monitoring for all constituents subject to TMDLs in the area. Periodic sampling for human markers may also help participating agencies understand the source of bacteria.
- 2) Lagoons and estuaries, like Malibu Lagoon, are known to cause a net increase in bacteria loads especially when the physical conditions constrain naturally functioning systems. Research shows that high fecal indicator bacteria at Surfrider Beach and other coastal sites is most likely from bird feces in the sand and kelp, decaying vegetation and naturally occurring bacteria released from the lagoon sediments. (See exhibits 12, 14, 15, 21, 22, 24, 26 and 31). Further, monitoring results are particularly affected if the sample is taken at high tide and early in the day.

Recent studies by the United States Geological Survey (USGS) demonstrate that even when the Malibu Lagoon sand berm is closed, that fecal indicator bacteria can pass through the berm and affect sampling results at Surfrider Beach, if certain conditions are present. Additionally, multiple studies show that even when no human markers were present, fecal indicator bacteria limits were exceeded at Surfrider Beach. (See 13, 28, 32).

The project proposes to increase tidal exchange. This exchange is not clearly defined but presumably, the number of events and length of time the berm is open may increase during construction and as a result of the project. The historical monitoring records show that fecal indicator bacteria loads increase, and often exceed regulatory limits more often, when the berm is open.

- 3) The City supports the following restoration elements; however, as adequate environmental analysis demonstrates, the project has the potential to increase FIB and nutrient levels in Malibu Lagoon and Surfrider Beach. To mitigate such adverse impacts consider the following:
 - a. Re-contouring the slopes for the 12-acre western arms of the lagoon to create broad shallow slopes will increase the surface area and lagoon sediment area. This may increase the release of naturally occurring bacteria that will increase the potential for FIB exceedances at the three sample sites at Surfrider Beach, SMB – MC 1, 2 and 3 – commonly known as Malibu Lagoon, Malibu Pier and Malibu

Colony. Malibu Colony sample station is actually seaward of the lagoon at the most western extent. These exceedances can occur when the berm is open or closed. Re-contouring could also affect nutrients released from the lagoon sediment. The project is expected to more effectively reduce stagnation and increase oxygen availability in the lower depths of the lagoon through improved horizontal mixing. The recent Lagoon studies indicate that the lower depths host the naturally occurring bacteria, which will now be more readily released to the surface waters where samples are collected.

- b. Revegetation may increase bacteria produced from the natural decaying process – more bank surface area and more vegetation will result in higher levels of bacteria. There may be some time periods when slow transit time can help remove bacteria by the vegetation; but in most scenarios, there will be a net increase of bacteria. It is noted that improved circulation and increased tidal flow, a goal of the project, will decrease contact time with lagoon plants capable of removing some bacteria. The project anticipates that there will be an increase in the discharge volumes and events at Surfrider Beach and affect water quality at sampling sites.
- c. The project proposes to increase the mudflats on the Eastern bank near the Adamson Boat House. This activity will increase foraging and bird habitat, surface area sediment contact, and bird feces, which is likely to increase the bacteria in Lagoon waters and can impact water quality at Surfrider Beach. Exhibit 20, water sampling location map, shows no sampling proposal for this element of the project. FIB exceedances were experienced in this specific area of Malibu Lagoon, as discussed in the recent UCLA and USGS studies. The City requests the permit be revised to include a 9th sampling location between the newly created mudflats and boathouse channel and the Lagoon sand berm.
- d. The staff report, environmental analysis and permit fail to consider the potential of spreading the invasive New Zealand mudsnails (known to inhabit Malibu Lagoon). The permit should include conditions to ensure that equipment and tools used at the project site are subject to the current protocol to prevent the spread of the snails to other reaches or other creeks where the equipment may do future work. Soil disposal activities must be similarly conditioned. Vegetation removal and dispersal could also transfer this highly invasive species. Many of the background studies referenced in the staff report were conducted prior to the invasion of the New Zealand mudsnail; hence, the staff report and permit does not adequately address this concern.
- e. Include a provision to indemnify the City for any water quality violations that arise from exceedances caused by or primarily contributed to as a result of the Lagoon Restoration Project.

- f. The background studies referenced in the staff report have been superseded with more recent studies that can provide relevant information about the complex hydrology in the Lagoon and surrounding areas. The staff report only refers to a 1999 URS Greiner Woodward Clyde study and the 2004 Stone Environmental study related to impacts from onsite wastewater treatment systems. These references have been enhanced by extensive relevant studies conducted by UCLA (28, 32) and USGS (21, 22, 24, 26, and 31), and SCCWRP (13, 14, 20).
- g. Historical drainage from the Malibu Colony residential neighborhood may be significantly impacted by the project. The City has agreed to allow two, 4 inch drain diversions into its Civic Center stormwater treatment facility (SWTF) if there is a bypass to account for overflow. This will allow for diversion of dry-weather urban runoff and limited stormwater flows so that it can be treated and disinfected; however, the surface flows have not been accounted for in the permit. Malibu Colony residents have reported concerns that the project's proposed solid block wall could obstruct historically occurring surface flows from the rear yards with potential flood impacts, if a mitigation measure is not required. The City requests a revision to the project plan to account for these surface flows and prevent significant flooding during rain events.

Again, the City supports a more efficient, better functioning lagoon system, but requests that the project be properly conditioned to prevent any unintended water quality degradation. Adequate environmental analysis is essential to determine the appropriate mitigation measures.

The following questions and comments from the staff report should also be addressed before the permit is issued:

Page 11 3.A.e. If construction equipment cannot be cleaned on the temporary berm, parking lot or trails, where does the permit specify that cleaning will take place?

Page 12 4. The dewatering Plan focuses on protection of aquatic species; however, human health could be affected depending on when and where the water is discharged and the results of increasing flow rates within the Lagoon.

Page 15 iv., c. and d. Sediment samples should also include FIB analysis and appropriate human markers analysis. Vertical profiles should be conducted quarterly, note all physical conditions, and be performed throughout the day to account for heat, sunlight, and tidal influences.

Page 16 & 17 Success Measures and Supplemental Measures

The report fails to discuss the increase in bacteria and resulting potential impact on human health for swimmers and surfers at Surfrider Beach. Since research shows that lagoons and estuaries contribute bacteria to the near shore sampling locations, what measures will be required if there is an increase in bacteria?

Page 24 16. Any excavated material should be monitored for the presence of New Zealand mudsnail.

Page 28 Dewatering

It is not clear from the project description where water will be discharged. If it is discharged into the main channel of the Lagoon, the discharge will cause an unnatural breach of the Lagoon berm and increase FIB loads at Surfrider Beach. The proposed filtration methods using carbon and resin vessels will not disinfect, and using only chlorine for disinfection at these flow rates is not recommended. The proposed list of constituents for testing only includes fecal coliform. All three FIB must be monitored during the dewatering process.

The City is also concerned about the dewatering process and the disinfection required prior to dispersal near Surfrider Beach. The staff report does not provide enough information about the Los Angeles Regional Water Quality Control Board's dewatering permit, the discharge location(s) and the actual constituents to be monitored. The City requests more information on the permit and has requested also requested a copy from the RWQCB.

Page 33 Riparian Forest Picnic Area

It is not clear whether or not this area will include a public toilet facility. The project should include a public facility, as the nearest facility is quite far from this area. City staff has observed toilet paper remains in the area, indicating that visitors may not go find the nearest public facility.

Page 33 "Adamson House Wall" is actually the Malibu Colony Wall

As noted earlier, an unintended consequence of the solid concrete masonry wall may increase flooding in the neighborhood directly to the south of the project if historical surface flows are subsequently impeded. There was no analysis of potential impacts or mitigation measures in any engineering study provided in the CCC staff report or environmental review documents for the project.

Page 42 and 43 Water Quality Conditions

The reference studies were conducted prior to 2005. The baseline for nutrients and bacteria should be determined from more contemporary studies and from studies that utilize state-of-the-art analytical methodologies. There has been extensive research conducted in the groundwater and surface waters that migrate to Malibu Creek and Lagoon and significant capital improvement projects have been completed since 2005. Aging onsite wastewater treatment systems along Malibu Creek have been replaced with the most advanced treatment systems. Since 2007, almost all surface flows from the Malibu Lagoon sub-watershed have been intercepted, filtered and disinfected resulting in the elimination of bacteria and significant removal of nutrients from the developed areas in the Malibu Civic Center.

In 2009, USGS conducted extensive nutrient and bacteria monitoring throughout the Civic Center area, near shore, Lower Malibu Creek, Malibu Lagoon and upcoast in the Malibu Colony and just off shore in both dry- and wet-weather conditions when the berm was closed and open. The primary source of bacteria is from natural sources such as avian feces deposited into the Creek and Lagoon, decaying vegetation, avian feces in the kelp and sand. Using the most up to date analysis, no human bacteria was found at Surfrider Beach by the University of California at Los Angeles (28, 32) nor in Malibu Creek and Lagoon or Surfrider Beach by researchers from SCCWRP in 2005 (13, 14 and 20) and in the extensive investigations by USGS in 2009 (21, 22, 24, 26, 31).

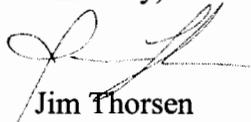
The Commission's staff report comments on Page 43 that the source of fecal indicator bacteria is from wastewater treatment facilities upstream and leaching from septic systems in the immediate vicinity of the Lagoon. This statement is not accurate; recent scientific data indicate that bacteria comes from avian sources and kelp, among other things. These attached studies should be considered, as they provide the best data on Lagoon Hydrology.

The City also requests the opportunity to review and comment on the final water monitoring plan before the Executive Director approves the plan. All water quality sampling during and post-construction must reflect the regulatory requirements for Santa Monica Bay Beaches Bacteria TMDL, since the project description anticipates a high likelihood that the berm will breach and/or there will be direct discharge to Santa Monica Bay. This would include Total coliform, Fecal coliform and Enterococcus. The staff report states that the permit allows for the discharge of 1.3 million gallons per day into Santa Monica Bay. Chlorination alone, without ozone or ultraviolet disinfection, is an uncertain process, especially for the high volume and flow rate anticipated.

Lastly, the City would request that the Project Manager provide contact information where he or she can be reach 24 hours a day, seven days a week. The public will generally contact the City of Malibu with emergency concerns and having this information will reduce response time.

Thank you for your consideration of these comments

Sincerely,



Jim Thorsen
City Manager

Enclosures: City of Malibu Comment Letter Reference Documents

Cc: Mayor Wagner and Honorable Members of the Malibu City Council
Christi Hogin, City Attorney
Los Angeles Regional Water Quality Control Board

City of Malibu Comment Letter Reference Documents

	Title	Author	Date	Notes
12	Enumeration and Spaciation of Enterococci Found in Marine and Intertidal Sediments and Coastal Water in Southern California	Ferguson, Moore, et al.	January 2005	Journal of Applied Microbiology
13	Multi-Tiered Approach Using Quantitative Polymerase Chain Reaction For Tracking Sources of Fecal Pollution to Santa Monica Bay	Noble, Griffith, Blackwood, et al.	February 2005	Southern California Coastal Water Research Project Also published in American Society for Microbiology
14	Modeling the Dry-Weather Tidal Cycling of Fecal Indicator Bacteria in Surface Waters of an Intertidal Wetland	Sanders, Arega, and Sutula	July 2005	Department of Civil and Environmental Engineering, UC Irvine and Southern California Coastal Water Research Project
15	Final Report: Identification and Control of Non-Point Sources of Microbial Pollution in a Coastal Watershed	Sanders, Grant, Horne, et al.	February 2006	United States Environmental Protection Agency-National Center for Environmental Research
20	Fecal Indicator Bacteria (FIB) Levels During Dry Weather from Southern California Reference Streams	Tiefenthaler, Stein and Lyon	January 2008	Southern California Coastal Water Research Project
21	Coastal groundwater dynamics off Santa Barbara, California: Combining geochemical tracers, electronic seepmeters, and electrical resistivity	USGS Swarzenski, Izbicki	April 2009	Estuarine, Coastal and Shelf Science published by Elsevier
22	Sources of Fecal Indicator Bacteria in Urban Streams and Ocean Beaches, Santa Barbara	Izbicki, Swarzenski, et al	September 2009	United States Geologic Survey, Annals of Environmental Science
24	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	Interim Reports: 10/29/09, 1/11/10, 2/18/10	City of Malibu - United States Geologic Survey – Dry Weather
26	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	May 2010	City of Malibu - United States Geologic Survey – Wet Weather Power Point Presentation to RWQCB staff
28	Malibu Lagoon Bacterial Study PowerPoint Presentation	Ambrose, Jay et al	05/25/10	UCLA Bacterial study comparing FIB with Human-Specific bacteriodes in Lower Malibu Creek, Malibu Lagoon and Surfrider Beach
31	Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu	Izbicki, Swarzenski, et al	Report 6/25/10	City of Malibu - United States Geologic Survey – Wet Weather
32	Malibu Lagoon Bacterial Study	Ambrose, Jay et al	Report July 2010	UCLA Bacterial study comparing FIB with Human-Specific bacteriodes in Lower Malibu Creek, Malibu Lagoon and Surfrider Beach

Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California

D.M. Ferguson, D.F. Moore, M.A. Getrich and M.H. Zhouandai

Orange County Public Health Laboratory, Newport Beach, CA, USA

2004/1187: received 14 October 2004, revised 24 January 2005 and accepted 25 January 2005

ABSTRACT

D.M. FERGUSON, D.F. MOORE, M.A. GETRICH AND M.H. ZHOWANDAI. 2005.

Aims: To determine the levels and species distribution of enterococci in intertidal and marine sediments and coastal waters at two beaches frequently in violation of bacterial water standards.

Methods and Results: Faecal indicator bacteria were extracted from sediment and enumerated using membrane filtration. High levels of enterococci were detected in intertidal sediments in a seasonal river and near a storm drain outlet. Low levels were found in marine sediments at 10 m depths and in surf zone sand. Bacterial isolates presumptively identified as *Enterococcus* on mEI media were speciated. The predominant species found in both water and sediment included *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus casseliflavus* and *Enterococcus mundtii*. A number of isolates (11–26%) from regulatory water samples presumptively identified as enterococci on mEI media were subsequently identified as species other than *Enterococcus*. At both study sites, the distribution of species present in water was comparable with those in sediments and the distribution of species was similar in water samples passing and exceeding bacterial indicator standards.

Conclusions: High levels of *Enterococcus* in intertidal sediments indicate retention and possible regrowth in this environment.

Significance and Impact of the Study: Resuspension of enterococci that are persistent in sediments may cause beach water quality failures and calls into question the specificity of this indicator for determining recent faecal contamination.

Keywords: beach pollution, enterococci, faecal indicator bacteria, marine sediments, water quality.

INTRODUCTION

In 1999, California adopted new, more extensive ocean recreational water quality standards (AB411 1999). The United States Environmental Protection Agency (USEPA) numerical standards for enterococci, total coliform and faecal coliform bacteria (USEPA 1986), which are used to indicate faecal contamination in marine waters, were implemented along with regulations for increased testing of recreational water. In southern California, the implementa-

tion of all three faecal indicator bacteria standards along with intensified testing led to an increased number of beach sites that exceeded standards (Noble *et al.* 2003). Beaches that fail any of these standards must be posted with warning signs or closed for swimming. The *Enterococcus* standard has proven to be the most sensitive of the three indicator bacteria. In the summer dry weather season, 60% of water quality failures are the result of exceedances of the *Enterococcus* standard alone (Noble *et al.* 2003). Summer beach postings and closings have resulted in public pressure on governmental agencies to take action to improve recreational water quality.

The two beaches studied here are representative of southern California open ocean and harbour pocket beaches

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with ongoing bacterial indicator failures during summer dry weather. Numerous studies conducted at both sites ruled out obvious large point sources of faecal contamination such as leaking sewer lines and outfalls. Nonpoint sources, including urban runoff were suggested, but no definitive source(s) were identified (Grant *et al.* 2001; Boehm *et al.* 2002; Kim *et al.* 2004; Noble and Xu 2004). Subsequently, water quality improvement projects, including storm drain diversions were implemented. Yet, indicator failures at these beaches continue. In this study, we investigate a less obvious nonpoint source of indicator bacteria: intertidal or marine sediments. Laboratory and field studies have demonstrated long-term survival of indicator bacteria such as *Escherichia coli* and other faecal coliforms in sediments (Gerba and McLeod 1976; LaLiberte and Grimes 1982). High densities of faecal coliforms (Valiela *et al.* 1991), faecal streptococci (Sayler *et al.* 1975; Obiri-Danso and Jones 2000) and enterococci (Anderson *et al.* 1997) found in marine sediments are suggestive of natural or environmental sources of contamination to overlying water. Regrowth of *E. coli* and enterococci was shown to occur in river sediments (Desmarais *et al.* 2002) and in soil, water and plants (Byappanahalli *et al.* 2003). Recently, indicator bacteria in sediments was directly linked to beach water quality failures. In England, resuspension of sewage impacted intertidal sediments was suggested as the cause of exceedances of regulatory standards (Obiri-Danso and Jones 2000). In New Zealand, resuspension of enterococci in sediments impacted by stream and storm water contributed to elevated levels in beach water (Le Fevre and Lewis 2003).

The objective of this study was to determine if intertidal or marine sediments harbour faecal indicator bacteria that could contribute to recreational water pollution at Huntington State Beach and Dana Point Harbor Baby Beach. The levels of indicator bacteria in marine and intertidal sediments from areas most likely to impact these beaches were determined. Enterococci isolated from sediments and recreational water were further characterized by identification to species level. The distribution of *Enterococcus* and enterococci-related species were compared in sediments *vs* beach water and in water samples passing or failing regulatory bacterial standards to determine possible relationships.

MATERIALS AND METHODS

Study sites

Dana Point Baby Beach is a small pocket beach *c.* 118 m wide and located inside an artificial harbour. A breakwater allows minimal current flow and protects the beach from ocean swell and currents (Fig. 1). Two storm drains discharge runoff from local residences, businesses, streets and parking lots to the west or east end of the beach. Beach

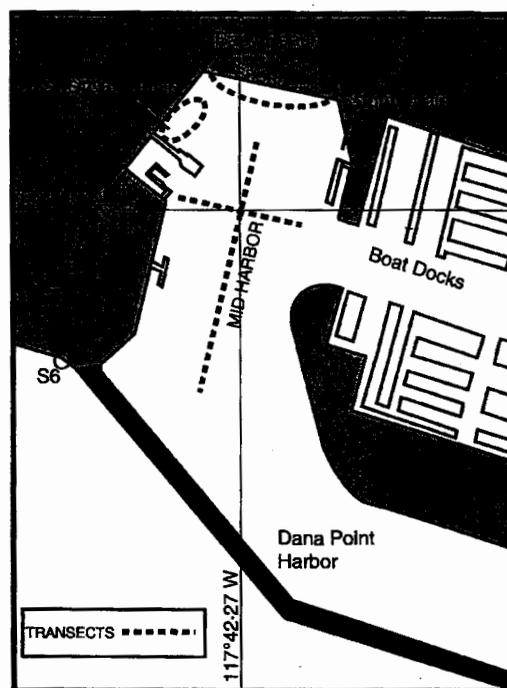


Fig. 1 Sampling locations at Dana Point Baby Beach

usage includes swimming and kayak launching. Boat docks and a pier are located adjacent to the beach. Remediation actions that have been implemented include plugging and diverting storm drains during the summer to prevent urban runoff flow into the beach, installing bird netting below the pier and restricting bird feeding to reduce direct faecal contamination. The beach water is sampled once a week at four sampling sites and tested for total and faecal coliforms and enterococci. During the study period, there were failures because of at least one bacterial indicator group on 32 of 90 (35.6%) sampling days; 66% of all indicator failures were caused by *Enterococcus*.

Huntington State Beach spans *c.* 7.2 km and is bordered by the Santa Ana River (SAR) and Talbert Marsh (TM) outlet on the south-east and Huntington City Beach on the north-west (Fig. 2). The SAR is a seasonal river/flood control channel where tidal flows in the channel can reach as far as 7.7 km inland during spring tides (Grant *et al.* 2001). Approximately 535 200 m³ of sediment comprised of gravel, sand and mud lies in the channel from the mouth to *c.* 5.8 km upriver. The channel is lined with cement walls or rock boulders. All major contributing storm drains are diverted during the summer, so the water in the SAR is almost exclusively tidally induced flow with minimal urban runoff. The Talbert Marsh outlet channel is located 290 m north-west of the SAR. Storm drains leading into the marsh

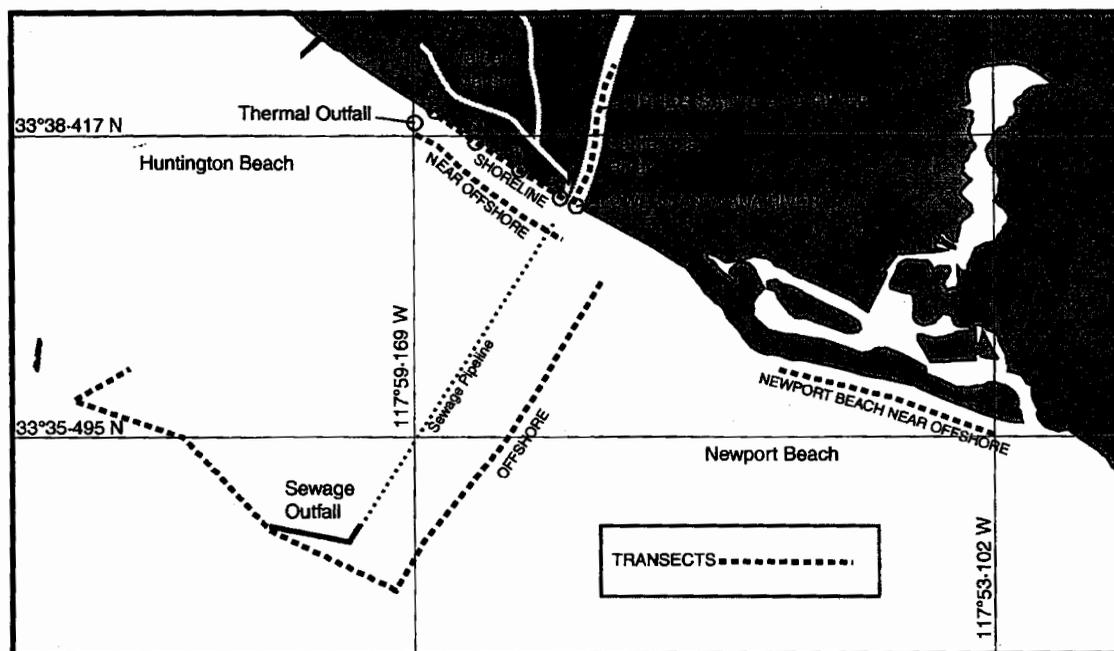


Fig. 2 Sampling locations at Huntington State Beach

are also diverted during summer. A sewage outfall lies 7.6 km offshore from the SAR mouth and releases $c. 10^6 \text{ m}^3$ per day of chlorine treated sewage in 60 m of water. A thermal outfall of a power plant, located $c. 700 \text{ m}$ offshore discharges a maximum of $1.9 \times 10^6 \text{ m}^3$ per day of water at 10 m depth. Previous studies did not find direct evidence implicating the sewage (Noble and Xu 2004) or thermal outfalls (Kim *et al.* 2004) as sources of pollution to the beach. Surf zone water from regulatory sampling sites: 0N, 3N, 6N and 9N corresponding to 0, 914, 1829 and 2743 m north-west of the SAR are monitored five times weekly for indicator levels (Fig. 2). During the study period, beach failures for at least one bacterial indicator occurred on 8 of 31 (25.8%) sampling days; 57% of all indicator failures were because of *Enterococcus*.

Sampling strategy

To determine sediment indicator bacteria densities, samples were taken along transects at onshore beach and river sites (intertidal) and offshore (marine) areas suspected of impacting the beach water and from two control sites adjacent to beaches with generally low to nondetectable levels of indicators. The transect locations are shown in Figs 1 and 2 and described in Table 1. At Baby Beach, water and sediment samples were collected between 7 August 2002 and 20 November 2003. At Huntington State Beach, the water samples were collected

between 5 August and 15 September 2003 for regulatory monitoring purposes. Sediments from the SAR were collected between 23 December 2003 and 24 January 2004 along an upper and lower transect that was delineated by the Pacific Coast Highway Bridge. Near offshore sediment samples were collected about 330 m offshore Huntington State Beach at 10 m depths. The north-west end of this transect was in the thermal outfall area. Offshore sediment samples were collected around the sewage outfall. The Newport Beach near offshore control transect starts at $c. 4.0 \text{ km}$ south-east of the mouth of the SAR (Fig. 2).

Sample collection methods

Offshore. Sediment samples from the ocean bottom were collected by boat using a Van Veen grab sampler (Kahl Scientific Instrument, El Cajon, CA, USA) that was rinsed between sampling stations by submerging it in seawater. A portion (100 ml) of the water overlaying the sediment was collected to compare the levels of indicator bacteria in water to sediment. The water was then decanted and $c. 75 \text{ g}$ of the top 2 cm of sediment was aseptically scraped into a 100 ml sterile bottle.

Intertidal. Sediment samples were collected from the intertidal river or shoreline sites at negative tide levels to avoid collecting overlying water. Approximately 75 g of the

Table 1 Description of sediment transects

	Sediment type	Number transects	Number samples	Transect length (m)	Transect spacing (m)	Water depth (m)
Dana Point Baby Beach						
Shoreline	Intertidal	21	168	120	10	NA
West Storm Drain	Intertidal	27	269	60	3	NA
Mid-Harbor	Marine	2	14	380	20	0.5-6
S-6 (Control)	Intertidal	NA*	13	NA	NA	NA
Huntington State Beach						
Upper Santa Ana River	Intertidal	2	35	2520	90	NA
Lower Santa Ana River	Intertidal	1	15	400	30	NA
Shoreline	Intertidal	1	10	3600	300	NA
Near offshore	Marine	2	31	3200	160	10
Offshore (sewage outfall)	Marine	1	10	15 240	670-2597	10-51
Newport Beach near offshore (control)	Marine	1	15	4950	330	10

*NA, not applicable.

top 2 cm of sediment was collected into a sterile bottle, taking care to avoid bird droppings. Water samples from the beach shoreline sites were collected at ankle depth using a sterile bottle (100 ml) that was clamped to a sampling pole. The pole was extended to obtain samples at ankle depth at a short distance away from the sample collector.

Sample processing

Water and sediments were held at 5–10°C and analysed for faecal indicator levels within 6 h of collection. To extract bacteria from sediments, 10 g of sediment was suspended in 100 ml of 1% (w/v) sodium metaphosphate (Valiela *et al.* 1991) and sonicated at the rate of 30% output using a Branson Sonifier® Cell Disruptor 450 (13 mm tip; Branson Ultrasonics, Danbury, CT, USA) for 30 s. Sonication time and intensity were previously optimized in our laboratory (D. M. Ferguson, D. F. Moore and M. A. Getrich, unpublished data). Suspended sediment and water samples were analysed using the membrane filtration method as per Standard Methods (APHA 1998). Total coliforms were enumerated using mENDO agar incubated for 24 h at 35°C. Faecal coliforms were enumerated using mFC agar incubated for 24 h at 44.5°C. Enterococci were enumerated using mEI agar incubated for 22–24 h at 41°C (USEPA 2000). Faecal indicator levels were reported as colony forming units (CFU) per 100 ml of water or CFU per 10 g of wet weight sediment.

As marine sediments are mixed with water trapped within sediment macropores, the concentration of indicators present in the overlying water was determined to account for bacteria present in the water fraction of sediment. The water content of each sediment sample was determined as the difference in weight before and after drying sediments overnight in an oven at 105°C.

Enterococci speciation

Colonies on mEI media that had blue halos were considered presumptive for *Enterococcus* species as per USEPA Method 1600 (USEPA 2000). Up to five colonies per sample were subcultured onto Trypticase™ soy agar with 5% sheep blood (BBL, Bethesda, MD, USA) and incubated at 35°C for 24 h. In some cases, there were fewer than five colonies present per sample. Isolates were identified to species level using the API™ 20 Strep identification system (API; bioMérieux, St Louis, MO, USA) and additional biochemical testing. The biochemical test results were interpreted using published standard biochemical identification charts (Facklam and Collins 1989; Facklam and Elliot 1995; Facklam 2002; American Society for Microbiology 2003). Biochemical tests included: carbohydrate fermentation with 1% mannitol, sorbitol, arabinose, raffinose, sucrose, lactose and inulin; Motility Test Medium w/TTC, pyrrolidonyl arylamidase (PYR) and leucine arylamidase (LAP) using disc tests (Remel, Inc., Lenexa, KS, USA); bile esculin, growth in 6.5% NaCl and at 45°C in brain–heart infusion broth, deamination of arginine in Moeller's decarboxylase broth (BBL, Franklin Lakes, NJ, USA); and catalase. Isolates that were not identified to species level that had positive reactions to PYR and LAP using API, esculin hydrolysis, growth at 45°C and tolerance to 6.5% NaCl were identified as *Enterococcus* species (American Society for Microbiology 2003).

Data analysis

The Pearson chi-square test in SPSS, version 12.0 for Windows, 2003 (Chicago, IL, USA) was used to test the statistical differences between enterococci species distribution in regulatory water samples passing and exceeding single sample standards.

RESULTS

Faecal indicator bacteria levels in sediments

The levels and percentage of samples positive for total coliforms, faecal coliforms and enterococci found in sediments from the two study sites are summarized in Table 2. Sediments from the Upper SAR transect adjacent to Huntington State Beach and West Storm Drain area at Dana Point Baby Beach had the highest percentage of positive samples as well as the highest geometric mean and maximum concentrations for all three indicator bacteria. At the Upper SAR, total coliforms and enterococci were found in 91.4% and 100% of 35 samples, respectively, with corresponding geometric mean concentrations of 1876 and 5922 CFU 10 g⁻¹. At the West Storm Drain area, total coliforms and enterococci were found in 61.8% and 66.5% of 269 samples, respectively, with corresponding geometric mean concentrations of 85 and 79 CFU 10 g⁻¹. Maximum concentrations were at the 10⁵ CFU 10 g⁻¹ level, or about 4 log higher than the geometric mean levels. Faecal coliforms were detected less frequently and at geometric mean concentrations that were about 1 log lower than total coliforms and enterococci.

At both study sites, indicator bacteria were also detected in shoreline and near offshore sediments but less frequently and at lower concentrations. Of the three indicators, *Enterococcus* was most abundant, followed by total coliforms and faecal coliforms with maximum geometric mean concentrations of 17, 9 and 3 CFU 10 g⁻¹ respectively. Most samples collected from a section of the Huntington Beach transect in a thermal outfall area of a power plant were below detection limits for indicators. As for the sewage outfall area, enterococci and faecal coliforms were not detected, however three sediment samples collected closest to the outfall pipe had low levels of total coliforms. Only a few sediment samples from near offshore Newport Beach (control area) were positive for indicators as compared with Huntington Beach near offshore, with similar bacterial concentrations found at both sites. At Dana Point Baby Beach, sediments collected from sites distant to the West Storm Drain, including the Mid-Harbor and a shoreline control site located outside the harbour, were generally below detection limit for all indicator bacteria.

Overall, *Enterococcus* was present more often and at higher concentrations in sediment samples when compared with total and faecal coliforms. Of a total of 580 samples from both study sites, 57.5% were positive for *Enterococcus*, 42.7% for total coliforms and 22.9% for faecal coliforms. Of all three indicators, the geometric mean levels of *Enterococcus* was highest in all transects except for the Baby Beach West Storm Drain and Huntington Beach offshore transects.

Water overlying marine sediment samples may contain bacteria that could affect the measurement of the bacterial

Table 2 Faecal indicator bacteria levels in sediment samples

	Total coliforms			Faecal coliforms			Enterococci		
	Number samples	% Positive samples	Concentration*	% Positive samples	Concentration	% Positive samples	Concentration	% Positive samples	Concentration
			Geomean						
Dana Point Baby Beach									
Shoreline	168	35.3	9	51 000	17.3	3	15 500	48.8	17
West Storm Drain	269	61.8	85	191 000	30.1	6	20 200	66.5	79
Mid Harbor	14	7.1	1	200	0.0	NA	NA	7.1	1
S-6 (control)	13	0.0	NA	NA	0.0	NA	NA	15.4	1
Huntington State Beach									
Upper Santa Ana River	35	91.4	1876	200 000	77.1	137	3500	100.0	5922
Lower Santa Ana River	15	13.3	2	100	0.0	NA	NA	73.3	21
Shoreline	10	20.0	3	140	10.0	2	200	40.0	6
Near offshore	31	9.7	1	20	3.2	1	20	48.4	6
Offshore (sewage outfall)	10	30.0	5	500	0.0	NA	NA	0.0	NA
Newport Beach near offshore (control)	15	6.7	1	20	0.0	NA	NA	33.3	3

*Colony forming units 10 g⁻¹ (wet weight).
% Positive samples, samples with values greater than detection limit; NA, not applicable.

concentration in sediment. In this study, the bacterial concentrations in overlying water were at least 2 logs lower than concentrations in corresponding sediment samples. Thus, the calculated bacterial concentrations in sediment were not because of overlying water.

Spatial and temporal variation of faecal indicator concentrations in sediment

The spatial and temporal variability of the concentration of all three indicator bacteria in sediments was determined for a single transect at Dana Point Baby Beach. Sampling sites included two intertidal and two marine sites along a 6.1 m transect running eastward from the mouth of the West Storm Drain. The intertidal sites were located within 3.0 m of the drain mouth. Sediments at the marine sites, located further away, were below the waterline. Sediments were collected at six different times (at 1 to 2-week intervals) over a 14-week period during the summer dry season (Fig. 3). On most of these sampling days, bacterial levels and frequency in species observed were highly variable between sites.

Indicators were more consistently detected and present in higher concentrations in samples from the intertidal sites when compared with the marine sites. The geometric mean concentrations of all three indicators were approximately 2 logs higher here than at the marine sites. There was also higher variability in bacterial concentrations in sediments from the marine sites.

Distribution of *Enterococcus* and enterococci-related species in sediment samples and shoreline water

The species distribution of isolates presumptively identified as *Enterococcus* using mEI agar was determined for sediment

and adjacent shoreline water samples. Shoreline water samples were obtained from regulatory agencies responsible for monitoring indicator bacteria on a routine basis; samples from all other sites were collected for the purposes of this study. A total of 1361 isolates from sediment and shoreline water samples from both beaches were speciated (Table 3). In general, *Enterococcus faecalis* and *Enterococcus faecium* were the most common species found in both sediment and water samples. *Enterococcus hirae*, *Enterococcus casseliflavus* and *Enterococcus mundtii* were also frequently seen when compared with *Enterococcus gallinarum*, *Enterococcus durans* and *Enterococcus avium*. Surprisingly, a high percentage of isolates from sediment (8.2–15.0%) and shoreline water (11.4–25.5%) were non-*Enterococcus* species (Table 3). These isolates, which appeared identical to enterococci on mEI media, included *Streptococcaceae* and related organisms (Bascomb and Manafi 1998) such as *Streptococcus bovis*, other *Streptococcus* spp., *Aerococcus* spp., as well as species that could not be identified with the methods used.

Enterococcus faecalis was the predominant species isolated from shoreline water at both Huntington Beach (39.8%) and Baby Beach (33.2%), West Storm Drain water (35.6%) and Huntington State Beach near offshore sediments (68.8%). *Enterococcus faecium* was the predominant species isolated from sediments at the West Storm Drain (35.2%) and the SAR (51.4%) (Table 3).

Enterococcus species distribution during single sample failure periods

The overall species distribution of samples from shoreline water at both study sites was similar, with the exception of a higher incidence of *Streptococcus* spp., particularly *S. bovis*, at Huntington State Beach (Table 3). The source(s) of these organisms to the beach are uncertain. To better understand

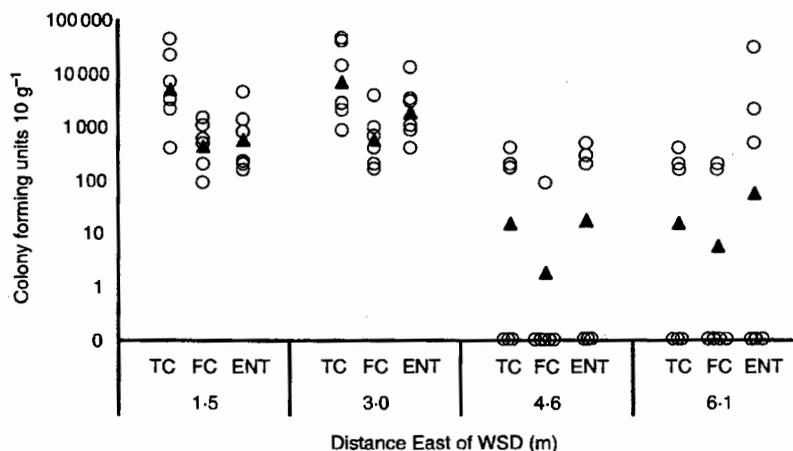


Fig. 3 Temporal and spatial variability of faecal indicator concentrations in sediments from four West Storm Drain sites sampled six times over 14 weeks, O, concentration; ▲, geometric mean; TC, total coliforms; FC, faecal coliforms; ENT, *Enterococcus*

Table 3 Enterococcus species distribution in water and sediment samples

No. samples	No. isolates	Number (%) of isolates												
		<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. hirae</i>	<i>E. casseliflavus</i>	<i>E. mundtii</i>	<i>E. gallinarum</i>	<i>E. durans</i>	<i>E. avium</i>	ENT*	S. bovis	STR	AER	Other, not ENT†
Dana Point Baby Beach														
169	349	116 (33.2)	74 (21.2)	40 (11.5)	42 (12.0)	29 (8.3)	4 (1.1)	1 (0.3)	1 (0.3)	3 (0.8)	11 (3.2)	0 (0.0)	0 (0.0)	28 (8.0)
26	45	16 (35.6)	6 (13.3)	0 (0.0)	15 (33.3)	1 (2.2)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (13.3)
73	105	11 (10.5)	37 (35.2)	15 (14.3)	14 (13.3)	9 (8.6)	2 (1.9)	1 (1.0)	3 (2.8)	1 (1.0)	3 (2.8)	0 (0.0)	0 (0.0)	9 (8.6)
Huntington State Beach														
144	576	229 (39.8)	75 (13.0)	73 (12.7)	36 (6.2)	9 (1.6)	5 (0.9)	1 (0.2)	0 (0.0)	1 (0.2)	102 (17.7)	27 (4.7)	5 (0.9)	13 (2.2)
47	206	41 (19.9)	106 (51.4)	19 (9.2)	14 (6.8)	7 (3.4)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	3 (1.4)	1 (0.5)	8 (3.9)	5 (2.4)
20	80	55 (68.8)	9 (11.2)	2 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	11 (13.8)	0 (0.0)	0 (0.0)	1 (1.2)
479	1361	468 (34.4)	307 (22.6)	149 (10.9)	121 (8.9)	55 (4.0)	12 (0.9)	5 (0.4)	5 (0.4)	6 (0.4)	130 (9.6)	28 (2.0)	13 (1.0)	62 (4.6)

*Four isolates unidentified *Enterococcus* spp., one *Enterococcus raffinosus* isolate and one *Enterococcus malodrans* isolate.

†Sixty unidentified non-*Enterococcus* spp., one *Lactococcus* spp. and one *Helicobacter* spp. ENT, *Enterococcus* spp.; STR, *Streptococcus* spp.; AER, *Aerococcus* spp.

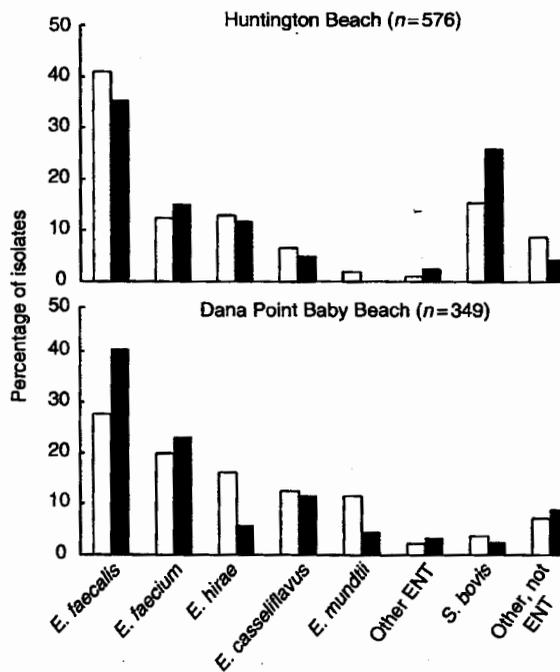


Fig. 4 Distribution of *Enterococcus* and related species in recreational marine water samples collected during ambient (□) and exceedance (■) conditions

possible relationships between contamination events and changes in species distribution, enterococci species composition in water samples with levels in water above and below the single sample standard (≥ 104 CFU 100 ml^{-1}) was compared (Fig. 4). There was no significant difference in the species distribution in samples at both Baby Beach and Huntington State Beach in samples collected during beach failures when compared with ambient conditions ($P = 0.13$ and $P = 0.10$, respectively; Pearson chi-square test).

DISCUSSION

In southern California, it is well recognized that a major cause of bacterial pollution of coastal waters is urban runoff in rivers/channels and storm drains that discharge into the ocean (Dwight *et al.* 2002; Reeves *et al.* 2004). The data presented here points to another source associated with urban runoff. Intertidal sediments harbouring high levels of indicator bacteria can be resuspended in water and transported to beaches by waves and wind, leading to water quality failures. We found a concentration gradient of faecal indicator bacteria in sediments: extremely high densities in the Santa Ana River near Huntington Beach and West Storm Drain area at Dana Point Baby Beach; significantly lower concentrations in shoreline and near offshore sedi-

ments at both beaches and even lower or nondetectable levels in offshore and control site sediments. These results indicate that shoreline waters at Huntington State Beach and Baby Beach may be recipients of faecal indicator bacteria originating from intertidal sediments in the SAR that contain high levels of bacteria. Field studies conducted at the Huntington Beach area suggest that indicator bacteria from the SAR and TM sediments are resuspended and flushed to the ocean during ebb tides and transported to the beach by surf zone and tidal currents (Grant *et al.* 2001; Kim *et al.* 2004). This resuspension and transport process is more pronounced during spring tide conditions, which occurs during full and new moon periods. At these times the greatest volume of tidal water flows inland into coastal outlets such as the TM and SAR and back out to the ocean, which is also when most of the beach failures at Huntington State Beach occur during the dry weather season (Boehm *et al.* 2004; Noble and Xu 2004). Other possible reasons for the indicator concentration gradient observed may be related to differences in sediment type, organic content and amount of UV exposure at the intertidal, onshore and offshore locations, parameters which were not measured at all sites in this study.

The high densities of total coliforms, faecal coliforms and enterococci found in intertidal sediments in the SAR and Baby Beach are similar to sediment indicator levels found at several different geographical locations: a tidally influenced river in Florida (Solo-Gabriele *et al.* 2000), an embayment in New Zealand (Le Fevre and Lewis 2003), an estuary in Massachusetts (Valiela *et al.* 1991) and freshwater creeks and lakes in Michigan (Byappanahalli *et al.* 2003) and in Wisconsin (LaLiberte and Grimes 1982).

The low levels of indicator bacteria found in sediments around the sewage outfall area offshore Huntington State Beach indicate that the discharge pipe may not be a constant source of contamination to these sediments. This finding is in contrast to a similar study conducted at Morcambe Bay, a bathing beach in England. Here, high levels were found in bay sediments receiving sewage effluent from an outfall pipe (ranging from untreated through to secondary treatment) and agricultural runoff from streams and rivers (Obiri-Danso and Jones 2000). The low levels found at Huntington State Beach may be the result of chlorination of the wastewater by the sewage treatment plant and the dilution or dispersion of bacteria by ocean currents. Wastewater entering the plant contains approximately 10^7 to 10^8 total coliforms per 100 ml and is reduced to 10^5 per 100 ml for total coliforms and 10^4 for faecal coliforms and enterococci after disinfection. The effluent is discharged from the outfall pipe that is engineered to achieve a 180 : 1 dilution in ocean water. In this study, finding higher levels of indicator levels at storm drain impacted sediments as opposed to the outfall area was surprising. In fact, the geometric mean levels in

storm drain impacted intertidal sediments were about one order of magnitude higher concentration when compared with the sewage impacted sediments at Morcambe Bay.

At Dana Point Baby Beach, contamination of beach water during summer dry weather appears to be related to the proximity of the storm drain to the beach, retention and/or regrowth of indicator bacteria in sediments and resuspension of indicator bacteria because of wave action in the harbour. In a previous study at this location, we determined that exceptional surf heights of 2–3 m that topped the breakwater and greater wave action correlated with a considerable increase in indicator levels at the beach (BBSSR 2003). A similar study conducted at a protected beach in New Zealand also showed that storm and stream water contributed high numbers of enterococci to sediments around these discharge points and that resuspension of sediments because of wave action led to elevated levels in water (Le Fevre and Lewis 2003). Increased bacterial levels because of resuspended sediments can occur as a result of increased turbulence due to runoff, animal traffic, sustained winds, storms, boats and dredging activities (Gerba and McLeod 1976; Sherer *et al.* 1992; Obiri-Danso and Jones 2000).

Repeated sampling of Baby Beach intertidal sediments around the West Storm Drain indicated high temporal and spatial variability in indicator concentrations. Although total coliforms and enterococci were consistently detected within 3.0 m of the storm drain, higher concentrations of enterococci were also found in two samples collected furthest from the drain where the levels were generally low. Determining the causes of temporal and spatial variability of indicator concentrations in sediment was not included in this study. Further studies on sediment characteristics that can affect bacterial growth and decay rates, such as temperature, moisture content, nutrient content, particle size, surface area and biofilm formation are needed to understand the potential flux of indicator bacteria from sediments to water.

Indicator levels ranging from 10^3 to 10^5 CFU 10 g^{-1} of sediment suggest the occurrence of long-term survival and regrowth of indicator bacteria in this environment. It has generally been accepted that faecal indicator bacteria do not survive for very long in seawater. In seawater, 90% of total coliforms, *E. coli* and enterococci die off in about 2.2, 19.2 and 60 h respectively (Bartram and Rees 2000). However, prolonged survival may be possible in marine and freshwater sediments. Indicator bacteria have been shown to persist in storm drain impacted sediments for up to 6 days following storm events without further supplementation of bacteria from runoff (Marino and Gannon 1991). Davies *et al.* (1995) showed that *E. coli* remains culturable in marine sediment for up to 68 days. In addition, laboratory studies have shown that faecal indicators survive longer in water supplemented with sediment (Gerba and McLeod 1976; Sherer *et al.*

1992). Survival in sediment may be enhanced because of protection from UV inactivation and predation, moisture, buffered temperatures and availability of nutrients originating from algae, debris and plankton (Whitman and Nevers 2003). Phytoplankton are most active in late spring to early summer and late summer to early fall, which are also the periods when bacterial levels in coastal waters increase (Dowd *et al.* 2000). Seaweed (Anderson *et al.* 1997), seawrack (Valiela *et al.* 1991) and zooplankton (Maugeri *et al.* 2004) provide both nutrients and surfaces for indicator bacteria to survive in the marine environment. Recently, groundwater discharge at Huntington Beach was found to be a source of nitrogen and orthophosphate to the surf zone that may enrich intertidal sediments and allow bacteria to persist (Boehm *et al.* 2004).

At both study sites, enterococci were found more frequently and in higher concentrations in intertidal sediment samples than total and faecal coliforms. *Enterococcus* spp. may be more abundant in intertidal sediments because these organisms are more resilient in seawater and are not as easily inactivated by sunlight when compared with *E. coli* (Bartram and Rees 2000). Enterococci are also capable of growing at a wider range of temperature (between 10 and 45°C) and pH (4.8–9.6) as well as in the presence of 28% sodium chloride (Huycke 2002).

Presumptive enterococci isolates were speciated to better understand the sources and ecology of these organisms in the marine environment. Of 1361 isolates tested, the predominant species identified in water and sediment, in order of occurrence were *E. faecalis*, *E. faecium* and *E. hirae*. These results are similar to the distribution reported for environmental strains elsewhere (Stern *et al.* 1994; Pinto *et al.* 1999; Dicuonzo *et al.* 2001; Ott *et al.* 2001; Harwood *et al.* 2004). *Enterococcus faecalis* and *E. faecium* are also the predominant *Enterococcus* spp. in the intestinal microflora of humans and animals and are considered opportunistic pathogens (Willey *et al.* 1999). *Enterococcus hirae* is a member of animal microflora, but has been found to occasionally cause infections in humans (Tannock and Cook 2002). *Enterococcus gallinarum* and the yellow pigmented species, *E. casseliflavus* and *E. mundtii*, are associated with plants and soil and are rarely associated with human infection (Pinto *et al.* 1999). In this study, these three 'environmental' associated species comprised 13.8% of all isolates tested. Thus, the species distribution of enterococci in insects, plants and sediments as well as in pristine and faecal-contaminated waters is important when assessing this group as faecal indicators (Leclerc *et al.* 1996).

During beach failures, the species distribution of enterococci and related species in shoreline waters was similar to the distribution found during ambient conditions. This distribution in water was also comparable with intertidal

sediment samples with high concentrations of enterococci. These findings suggest that there may be constant loading of a stable enterococcal population from intertidal sediments and other sources to water that increases because of changes in environmental conditions, resulting in frequent failures. The enterococci species distribution found in sediments and water were similar to that of humans, animals and birds. Thus, species distribution was not useful in pinpointing major source(s) of beach contamination in this study. However, this determination could be useful to finding sources of contamination in other sites where 'environmental' species may be predominant.

Comparison of the enterococci species composition in water *vs* sediments in highly contaminated areas could provide additional information in assessing sediments as a source. Knowledge of the predominant species present in specific sites could also be useful to investigators using or developing microbial source tracking methods targeting enterococci.

The API Strep system and traditional biochemical tests and identification charts used to speciate enterococci and related organisms in this study are culture-based methods designed to identify clinical isolates. Further studies are needed using PCR or 16S rRNA sequencing to identify environment isolates, particularly the noncultivable strains.

There was a high incidence of non-*Enterococcus* species (17.1%) using mEI media. The majority of these isolates (9.6%) were identified as *Streptococcus bovis*, a member of the faecal streptococcus group. This finding was unexpected as *Streptococcus* spp. are not known to persist in marine water (Geldreich and Kenner 1969). The mEI media used to isolate enterococci in this study was formulated to differentiate enterococci from other genera of the faecal streptococcal group (Messer and Dufour 1998). Like enterococci, *S. bovis* is also β -D-glucosidase-positive, which is indicated on mEI media by the formation of a blue halo around the colony. Marine water samples from Baby Beach and Huntington Beach had false-positive rates (occurrence nonenterococci species) of 11.2% and 25.5% respectively. These rates are much higher than 6%, as reported by the EPA (USEPA 2000).

To our knowledge, this is the first publication showing high concentrations of faecal indicator bacteria in intertidal sediments impacted by storm drains. The levels of enterococci found in shoreline and near offshore sediments could be a result of continuous loading of faecal indicator from highly contaminated sediments in areas associated with urban runoff. Exceedances in enterococci standards may also occur because of resuspension of bacteria-laden sediment in water. This occurrence supports the suggestion made by others that an evaluation of faecal indicators in sediments may be a more stable index of overall or long-term water quality than the overlying water (LaLiberte and Grimes

1982; Sherer *et al.* 1992; Obiri-Danso and Jones 2000). The long-term persistence/regrowth of indicators in sediments, particularly enterococci, calls into question the reliability of this indicator for determining recent faecal contamination of water.

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Multi-Tiered Approach Using Quantitative Polymerase Chain Reaction For Tracking Sources of Fecal Pollution to Santa Monica Bay, California

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Multi-Tiered Approach Using Quantitative Polymerase Chain Reaction For Tracking Sources of Fecal Pollution to Santa Monica Bay, California

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ABSTRACT

The ubiquity of fecal indicator bacteria such as *Escherichia coli* and enterococcus make tracking sources in urban watersheds extremely challenging. In this study, a multi-tiered approach was used to assess sources of fecal pollution in Ballona Creek, an urban watershed that drains to Santa Monica Bay (SMB), CA. A mass-based design at six mainstem sites and four major tributaries was used to quantify the flux of enterococcus and *E. coli* using traditional culture-based methods, and three additional indicators including enterococcus, *Bacteroides* sp. and enterovirus, using quantitative polymerase chain reaction (QPCR). Sources and concentrations of fecal indicator bacteria were ubiquitously high throughout Ballona Creek and no single tributary appeared to dominate the fecal inputs. The flux of enterococcus and *E. coli* averaged 10^9 to 10^{10} cells/hr and were as high at the head of watershed as they were at the mouth prior to its discharge into SMB. In contrast, the site furthest upstream had the most frequent occurrence and generally the greatest concentrations of enterovirus. Ninety-two percent of the samples that tested positive for enterovirus also tested positive for *Bacteroides* sp. A similar survey in Malibu Creek, a nearby non-urban watershed, found low levels of traditional fecal indicator bacteria and no detectable enterovirus or *Bacteroides* sp. The influent and effluent from three structural best management practices (BMPs) were evaluated for removal efficiency. Results indicated that those with ultraviolet (UV) treatment worked better than a constructed treatment wetland for reducing enterococcus concentrations using culture-based methods, but also degrading its DNA based on QPCR measurements.

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INTRODUCTION

Santa Monica Bay (SMB), California, is home to some of the most popular beaches in the world. It is located adjacent to metropolitan Los Angeles where more than 50 million beachgoers visit SMB shorelines every year, which is more than all other beaches in California combined (SMBRC 2005). However, there are serious concerns about beach water quality because of continued exceedences of water quality thresholds based on fecal indicator bacteria such as total coliforms, fecal coliform or *E. coli*, and enterococcus, particularly in areas impacted by urban runoff. Thirteen percent of the shoreline mile-days in SMB exceeded water quality thresholds between 1995-2000 with over 50% of these exceedences located near storm drains (Schiff et al 2003). In contrast, sewage spills were relatively rare accounting for less than 0.1% of the water quality exceedences and subsequent warnings to swimmers. Moreover, swimming near storm drains in SMB can lead to an increased risk of swimming-related illnesses. Haile et al (1999) demonstrated that swimmers near storm drain discharges in SMB had a higher likelihood of respiratory and/or gastrointestinal symptoms compared to swimmers more than 400 m from a storm drain.

Despite the impairments to water quality and risk to human health, identifying and eliminating the sources of bacteria responsible for the beach warnings remains elusive. The difficulty in identifying and eliminating the sources of bacteria results from two important factors. First, the traditional indicators of fecal pollution on which the water quality thresholds were derived are not specific to humans. These fecal indicator bacteria can be shed from any warm-blooded organism including wild and domesticated animals (Geldreich 1978). Therefore, source tracking turns into a challenging scenario when these diffuse and frequently intermittent or episodic fecal releases occur. The second difficulty when identifying and eliminating sources of fecal indicator bacteria is their ubiquity in the environment. Unlike many of the pathogens of interest, fecal indicator bacteria may survive and even grow in the environment. For example, fecal indicator bacteria were able to persist in beach wrack impacting beaches in Cape Cod, MA (Weiskel et al. 1996).

Viruses are one tool that could prove useful in source tracking studies because they are the pathogen of interest. Viruses are known to cause a significant portion of waterborne disease from water contact, mostly from ingestion of sewage contaminated water and seafood (Fogarty et al. 1995). Until recently, however, methods for virus detection and quantification have relied on growth-based endpoints that are much too slow to be effective source tracking tools. Recently developed molecular techniques, such as Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRT-PCR) can detect and quantify viral genetic material directly from water samples. Results of tests conducted previously in Southern California (Noble and Fuhrman, 2000; Tsai et al., 1993; Tsai et al., 1994), in Florida (Griffin et al., 1999; Rose et al., 1997), and Europe (Pina et al., 1998) using conventional RT-PCR or PCR have detected a host of genetic material from human specific viruses including enterovirus, hepatitis A virus, rotavirus, and adenovirus in urban runoff discharges or seawater samples. The major drawback to

using viruses as source tracking tools, however, is their dependence on a large human population in order to have sufficient numbers for detection (Noble et al. 2003).

A different approach would be to use alternative bacterial indicators for source tracking that might be much more abundant in fecal waste discharges. This alternative approach could prove useful if host specific bacterial indicators could be found. Of the facultative anaerobic organisms common in human fecal flora, enterococci have been found in almost all subjects with a mean level of \log_{10} 8.9 per gram feces (Klessen et al, 2000). Another option would be *Bacteroides* sp., which make up approximately one-third of the human fecal microflora, considerably outnumbering the fecal coliforms. *Bacteroides* sp. belongs to a group of nonspore forming obligate anaerobes, so there is little concern over persistence or regrowth in the environment. More importantly, human specific *Bacteroides* sp. markers have been developed increasing the value of this potential indicator (Bernhard and Field 2000a, Bernhard and Field 2000b).

Both virus and alternative bacterial indicators such as *Bacteroides* sp. have been shown to be potentially useful source tracking tools. Griffith et al (2003) concluded that genetic based methods, such as PCR consistently provided the best information when attempting to conduct source tracking on mixed source samples. *Bacteroides* sp. correctly identified human sources of fecal pollution when present in mixed water samples delivered blind to the laboratory. Likewise, human enterovirus measurements had virtually no false positives, a problem that plagued many other methods in that study. However, the human marker identified in *Bacteroides* sp. may be present in additional hosts or the primers used to detect the human marker may cross react with species from nonhuman hosts (Kreader 1995). Similarly, enterovirus consistently and correctly detected human sewage when present, but had difficulty determining human sources when only one or a few likely uninfected individuals contributed fecal material. Since no method has all of the traits to be the consummate bacterial source tracking tool, a multi-tiered multi-indicator approach has been recommended by some investigators (Stewart et al 2003). By using multiple tools, investigators can utilize the strengths of each to ascertain inputs and track fates that will ultimately lead to successful management solutions.

This objective of this study was to identify the contributions and quantify the loading of fecal contamination to the SMB using a multi-tiered approach. The first tier included traditional fecal indicator bacteria measurements. The second tier included newly developed methods for enterococcus, *Bacteroides* sp., and enterovirus. All of these newly developed methods rely on QPCR or QRT-PCR, which has not been applied previously for source tracking studies in urban watersheds until now. The multi-tiered approach was applied using a mass-based design to quantify inputs and flux through an urban watershed to the beach. A subsidiary objective included using the multi-tiered approach through a relatively undeveloped watershed. Finally, the multi-tiered approach was used to determine the effectiveness of a variety of structural best management practices (BMPs) that were aimed at reducing bacterial inputs from urban watersheds.

MATERIALS AND METHODS

This study was conducted in three phases. The first phase quantified inputs of flow, bacteria concentrations and virus particles, then tracked them through an urban watershed over time. This mass-based design was applied in the Ballona Creek watershed, the largest tributary to SMB. Ballona Creek is over 85% developed and currently has the largest inputs of fecal indicator bacteria to SMB (Figure 1). The second phase quantified bacteria concentrations and virus particles in the Malibu Creek watershed, the second largest tributary to SMB. Malibu Creek is only 12% developed and has a large lagoon system at its terminus prior to discharging across the beach to the world famous Surfrider Beach. Although no flow was measured in Malibu Creek to provide flux estimates, this system provided the opportunity to measure concentrations at several points through the lagoon and as it enters the ocean to assess shoreline mixing and dilution. The third phase examined the effectiveness of three BMPs to reduce bacteria and virus concentrations. The three BMPs, only two of which were located in the Santa Monica Bay watershed, included a multimedia filtration system with inline ultraviolet (UV) treatment, a filtration-aeration system with an inline UV treatment system, and a constructed wetland. For each of the BMPs, an influent-effluent approach was used to estimate treatment effectiveness.

Sample Collection and Filtration

Ballona Creek

Samples were collected at six mainstem and four of the major tributaries to the Ballona Creek system. The six mainstem sites extended from where the system daylight at Cochran Avenue to Inglewood Avenue, which is located at the head of tide just prior to discharge into SMB (Table 1). The four tributaries represented the four largest hydrodynamic inputs to the system and were located in reaches between each of the mainstem sampling sites.

Flow was calculated as the product of flow rate and wetted cross-sectional area. Doppler area-velocity sensors were used to measure flow rate. Pressure transducers that measure stage, along with verified as-built cross sections, were used to estimate wetted cross-sectional area. One minute instantaneous flow was logged electronically during the entire six hour sampling period. Both the area-velocity sensors and pressure transducers were calibrated prior to sampling.

One hour composite water samples were collected at each site between 8:00 AM and 2:00 PM on August 26, 2004. The six hour sampling period corresponds to the approximate hydrodynamic travel time from Cochran Avenue to Inglewood Avenue (Ackerman et al, 2004). Four liter composite samples at each site were created after combining ten individual 400 ml grab samples collected every 6 minutes into a single container. In total, 60 composite samples were collected at Ballona Creek as a result of sampling 6 hours at 10 different sites.

Table 1. Ballona Creek sampling sites.

Site	Description	GPS Coordinates (NAD 83 datum)
Cochran Ave.	mainstem	34 02.662N 118 21.237W
Fairfax Drain	tributary	34 02.298N 118 22.136W
Adams Ave.	mainstem	34 02.009N 118 22.494W
Adams Drain	tributary	34 02.009N 118 22.494W
Rodeo/Higuera	mainstem	34 01.305N 118 22.693W
Benedict Box Channel	tributary	34 00.925N 118 23.432W
Overland Ave.	mainstem	33 00.429N 118 23.771W
Sawtelle Ave.	mainstem	33 59.816N 118 24.164W
Sepulveda Channel	tributary	33 59.512N 118 24.693W
Inglewood Ave.	mainstem	33 59.394 N 118 24.696W

Malibu Creek

One hour composite samples were collected at five sites along the mainstem of Malibu Creek (Table 2). The sites stretched from the Cold Creek tributary to the head of the lagoon, near the mouth of the lagoon, in the discharge across the beach, and in the wave wash immediately in front of the discharge across the beach. Composite samples were collected in a similar fashion as Ballona Creek with the following exceptions. A single composite sample was collected at each site daily on three consecutive days (November 10, 11 and 12, 2004) coinciding with low tide to ensure that the flow direction was from the lagoon, across the beach, and into the wave wash. No flow information was collected since most of the sites were hydrologically unrateable.

Table 2. Malibu Creek sampling sites.

Site	Description	GPS Coordinates (NAD 83 datum)
Bridge at Cold Creek	Mainstem	34 04.865N 118 42.262W
Bridge at Cross Creek	Mainstem	34 02.578N 118 41.052W
Head of Malibu Lagoon	Lagoon	34 02.154N 118 41.036W
Mouth of Malibu Lagoon	Lagoon	34 01.920N 118 40.810W
Malibu Creek Wavewash	Mixing Zone	34 01.920N 118 40.810W

BMPs

Three BMPs were selected for sampling. The three sites included: the Santa Monica Urban Runoff (SMURRF) treatment facility located in Santa Monica; the Clear Creek, Inc. MURF™ pilot treatment facility located in Paradise Cove; and a constructed wetland (WET CAT) located in Laguna Niguel. The SMURRF consists of a grit screen to remove debris and trash, a dissolved aeration system to separate the oil and grease, and a microfiltration system to remove solids inline with a UV treatment device. The MURF system uses a combination of proprietary multi-media filtration and UV treatment. The WET CAT is a 2.1 acre constructed wetland with no other in line treatment. Grab samples were collected from the influent and effluent at each BMP.

Filtration

After collection, samples were placed on ice in a cooler and transported immediately to the University of Southern California for processing. For each composite sample, 200-600 ml of sample volume was vacuum filtered through replicate 47mm 0.45µm polycarbonate filters (Poretics, Inc.) using a filter funnel and receiver (Millipore, Inc.) for bacterial marker analysis by QPCR. In addition, replicate filtrations were conducted using 47 mm diameter 0.7 µm nominal pore size, type GF/F microporous filters (Whatman, Inc.), and replicate Type HA Millipore mixed ester cellulose acetate/nitrate, 0.45 µm pore size filters (for subsequent enterovirus analysis). The polycarbonate filters were immediately placed into a 1.5 ml screw-cap tube and placed on dry ice until storage at -80°C. Type HA filters were either placed into a Whirl-pak bag for analysis by the Fuhrman laboratory (EnteroA), or into a 1.5 ml screw-cap tube for subsequent analysis by the Noble laboratory (EnteroB). Type GF/F filters were cut into quarters, each quarter placed in a 1.5 ml screw cap tube and placed on dry ice until storage at -80°C.

Indicator Bacteria Analyses Using Chromogenic Substrate

Concentrations of *E. coli* and enterococcus were measured by chromogenic substrate methods using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME). *E. coli* was measured using the Colilert-18® reagents, while enterococci were measured using Enterolert™ reagents. Both tests used the Quanti-Tray/2000 for enumeration of cells. Samples were incubated overnight per the manufacturer's instructions and inspected for positive wells. Conversion of positive wells from these tests to a most probable number (MPN) was done following Hurley and Roscoe (1983).

Enterovirus Analyses Using QRT-PCR

Samples were analyzed for enteroviruses using two separate, but similar methods conducted in two separate laboratories, EnteroA (Fuhrman laboratory) and EnteroB (Noble laboratory). For EnteroA, filters were extracted using the RNeasy mini kit (Qiagen Cat. No.74106) and QIAvac 24 vacuum manifold (Qiagen Cat. No.19403). The extraction protocol was modified from the manufacturer's instructions as follows: 1ml lysis buffer RLT (with 10µl β-mercaptoethanol) was added directly into each Whirl-Pak bag, allowed to soak the filter for ten minutes, and the resulting extracts (lysates) were carefully removed by pipet into 2 ml microcentrifuge tubes (droplets hanging in the bag and water clinging to the filter were first squeezed to the bottom corner of the bag by manually applying pressure to the outside of the bag). If there was visible filter or sample debris, the particulate matter was removed by brief centrifugation. Then one volume of 70% ethanol (usually 1 ml) was added to the extract and mixed by pipetting. Samples were transferred to the RNeasy spin columns, filtered through with the QIAvac at approximately 500 mm Hg vacuum, and were washed on the manifold once with 700 µl RW1 solution, and twice with 500 µl RPE solution to remove contaminants. The columns were cleared of remaining droplets of buffer by centrifugation into a 2 ml collection tube (14,000 rpm, Eppendorf 5415 microfuge, 2 minutes), and the buffer discarded. The RNA

was eluted from the columns into a 1.5 ml collection tube with 50 μ l volumes of RNase free water by centrifugation (Eppendorf 10,000 rpm, 2 min.), after allowing the water to stay in the column 1 min. This filter extraction step typically took up to two hours for 15 samples.

For each PCR reaction, 5 μ l of the 50 μ l RNA was analyzed by QRT-PCR on a Mx3000P Thermal Cycler (Stratagene, Inc.). The PCR protocol was modified from the single-tube RT-PCR method previously developed for sludge samples by Monpoeho et al. (2001). Primers and probe, not changed from that original published method (except for the BHQ quencher), were reverse primer Ev1 [5'-GATTGTCACCATAAGCAGC-3'], forward primer Ev2 [5'-CCCCTGAATGCGGCTAATC-3'], synthesized by Qiagen and Ev-probe [5'-FAM-CGGAACCGACTACTTTGGGTGTCCGT-BHQ-Phosphor-3'], synthesized by Sigma Genosys. A GenBank BLAST search done on 3 June 2004 revealed that only human (not other animal) enteroviruses matched all three primer and probe sequences. Each PCR reaction contained 5 μ l RNA extract and 20 μ l master mix, each 20 μ l master mix contained: 1X Taq gold buffer (ABI), 5.5mM MgCl₂ (ABI), 500uM dNTPs (ABI), 6% glycerol (Sigma Chemical Co.), 2% PVP 40 (polyvinylpyrrolidone, av. MW 40,000, Sigma Chemical Co.), 500nM Ev1, 400nM Ev2, 120nM Ev-probe, 1.5 μ g T4 gene 32 protein (Ambion), 10 units of RNAsin (ABI), 2.5 units of AmpliTaq gold (ABI) and 5 units MULV reverse transcriptase (ABI). Each RNA extract was analyzed in duplicate. Enterovirus RNA was transcribed into cDNA at 50°C for 45 minutes, the cDNA was amplified by PCR, after a 95°C 10 minute hot start, for 50 cycles at 94°C for 15 sec and 60°C for 1min. Fluorescence measurements were made during the extension step, every cycle at 60°C. Calculations for quantification were done by the Stratagene QPCR software in real time, with raw data saved for possible reanalysis. Parameters (e.g. fluorescence threshold) were set manually after PCR was done to generate a standard curve with optimal statistics (usually $r^2 > 0.95$, slope around 3.3) and unknowns were calculated based on that standard curve. Standards were prepared using the poliovirus stock described above. Standards used in the high concentration set were 10-fold dilutions ranging from high to low concentration. For Enterob, a similar approach was used. Samples were extracted using the RNeasy mini kit (Qiagen Cat. No.74106), with additional of 2.0% polyvinylpyrrolidone (PVP)-40 (final concentration) and the filter fully homogenized in the screw cap tube. After homogenization, approximately 700 μ l of the RLT/filter slurry was applied to a QiaShredder column (until the QiaShredder was full) and spun at max speed, $\geq 8000 \times g$, for 2 minutes. It was often necessary to perform two spins to ensure the entire volume of RLT/filter slurry was shredded. The supernatant fluid was then carefully removed and placed into a new 1.5 ml tube. The volume of solution in each tube was estimated by pipetting and 0.4 volumes of potassium acetate were added. Tubes were mixed by inversion and incubated on ice for 15 minutes. The mixture was then spun at 4°C for 15-30 minutes and the supernatant transferred to a new 1.5 ml microfuge tube. Following this, the protocol for the Qiagen RNeasy Plant and Fungi RNA isolation was followed starting at step 5. Five μ l of extracted RNA from the previous procedure was added to 5X RT Buffer, 6 mM MgCl₂, 500 nM dNTPs (final concentration), 700 nM EV1 Reverse primer, 700 nM EV1 Forward primer, and 300 nM EV-BHQ TaqMan probe, 10 units of RNAsin, 2.5 units of Taq polymerase, and 5 units MULV reverse transcriptase. The Cepheid Smart Cycler® was programmed to: 1 hour

RT at 37°C followed by a 15 minute hold at 95°C for *Taq* activation, then 45 cycles of 94°C 15 seconds (denature), 60°C 1 minute (anneal/extension-optics on).

QRT-PCR results were available three hours after the start of analysis, making the total PCR preparation and analysis time less than 5 hours for 15 samples. Results are reported as equivalent virus particles per unit sample volume, meaning that this is where the QRT-PCR calculation indicated the sample appeared relative to the standard curve prepared from poliovirus standards.

Bacterial Analyses Using QPCR

The polycarbonate filters were processed for DNA extraction using the UltraClean™ Fecal DNA Isolation Kit (MoBio Laboratories, Inc., 12811-50) as per manufacturer's alternative protocol. Eluted DNA extracts were stored at -20°C until use.

Table 3. Primer and probe sequences for PCR detection of enterococci

Sequence Name	Nucleotide Sequence 5' to 3'	Length	GC (%)	T _m (°C)	Detection System*
ECST748F ¹	5'aga aat tcc aaa cga act tg-3'	20mer	35	51.2	
ENC854R ¹	5'-cag tgc tct acc tcc atc att-3'	21mer	47.6	57.9	
GPL813TQ ¹	5'Cy3-tgg ttc tct ccg aaa tag ctt tag ggc ta-BHQ-2-3'	29mer	44.8	65.3	Taqman

¹Ludwig and Schleifer, 2000.

Total enterococci primers and probe were constructed using the rDNA regions around the target site of a well established enterococci group specific primer (ENC854R) (Table 3). The primer ECST748F targets enterococci, lactococci, and several clostridia. The target site of the probe GPL813TQ is present in rDNA from a variety of representatives of gram-positive bacteria with a low G+C DNA content (Ludwig and Schleifer, 2000).

Table 4. Master mix using individual reagents

Reagents	Final conc. (μM)	Initial vol (μl)
Water		3.9
10X Taq buffer (Mg ⁺⁺ free)	1	2.5
250mM MgCl ₂	5	0.5
10mM DNTPs	0.5	1.25
10μM ENC854R	1	2.5
10μM ECT748F	1	2.5
10μM GPL813 TQ Cy3 Probe	0.08	0.2
5U/u Taq polymerase	0.05	0.5

The Master Mix of reagents (Table 4) yields a final volume of 20 μ l, to which 5 μ l of sample (either DNA extract from an environmental sample, or 5 μ l of lysed cell suspension or genomic equivalents) was added for a final volume of 25 μ l. The samples were run under the following optimized assay conditions for PCR: 1 cycle initial hold at 95°C for 2 min, and 45 cycles of denaturation (94°C) for 15 seconds, and annealing (60°C) for 30 seconds, the optics were turned on during the annealing step. The Cepheid Smart Cycler was set with the following specific parameters for this assay. The Dye Set was set for FCTC25. The Ct analysis mode was set for growth curve (linear) analyses, with a manual threshold typically set at between 5 and 15 fluorescence units. The background subtract level was set at a minimum of 12 and a maximum of 40. The BoxCar averaging feature was set at 0. For quality control, combined *E. faecalis* and *E. faecium* were used as our calibration strain for the total enterococci primer and probe set. Control bacteria preparations were prepared by boiling bacteria for 5 minutes, centrifuging 1 min at 12,000 rpm in a Beckman Microcentrifuge, and immediate storage on ice. *E. faecalis* and *E. faecium* cells were enumerated using either SYBR Green I epifluorescence microscopy (Noble and Fuhrman, 1998) and/or using Enterolert® or the EPA 1600 methods (APHA 1992). This yielded information on both the cell numbers in the sample, and the number of metabolically active cells present in the sample. Serial dilutions of the standards were made in duplicate in DEPC-treated sterile water, and four point standard curves are run in concert with the unknown samples on the Smart Cycler II instrument. Total enterococci primers were tested with all 19 validly described species of the genus enterococci, and demonstrated amplification of rDNA of all strains, with varying efficiencies.

Bacteroides sp. Using Conventional PCR

Amplification of the human-specific Bacteroides/Prevotella marker generally followed the procedure of Bernhard and Field (2000), with PCR primers that amplify partial 16S rRNA from the human fecal (HF) specific group. DNA was extracted a MoBio Ultra Clean fecal extraction kit. A range of extracted DNA quantities (2 – 5 μ l, representing 1-70 ng per assay, with most samples in the range of 5-20 ng) was tested to avoid problems with inhibition. DNA was amplified with Bacteroides-Prevotella specific primers Bac708r CAATCGGAGTTCTTCGTG and HF183f ATCATGAGTTCACATGTCCG. Each 50- μ l PCR mixture contained the following reagents: 1 X Taq polymerase buffer (Promega), each primer at a concentration of 1 μ M, each deoxynucleoside triphosphate at a concentration of 200 μ M, 1.25U of Taq polymerase (Promega), 0.64 μ g of bovine serum albumin (Sigma) per μ l and 1.5mM MgCl₂. The thermal cycler was run under the following conditions, 2 min 95°C, then 25 cycles of 95°C for 30 sec, 60°C for 30 sec and 72 °C for 30 sec followed by a 5-min extension at 72°C. Then 1 μ of each PCR product was re amplified using the same conditions as above for another 25 cycles. PCR products were visualized in a 2% agarose gel stained with 1X SYBR Gold (Molecular Probes) and compared to a 100bp DNA ladder (Promega). Positive results had 525 bp products. The positive control was human fecal sample extracted with a QIAamp stool kit. Negative controls use water instead of sample. All negative samples are spiked (in a second PCR

Multi-tiered approach to source tracking using QPCR

run) with 0.1 ng of positive control to determine possible inhibition. Inhibited samples are re-run with less DNA.

RESULTS

Ballona Creek

Total volume discharged from Ballona Creek during the six-hour sampling period was 13,390 m³ (Figure 2). Of this volume, 97% was attributed to monitored inputs from Cochran, Fairfax, Adams and Benedict, and Sepulveda tributaries. The largest volume was contributed at Cochran Avenue where the creek daylighted from beneath downtown Los Angeles. Flow remained relatively stable over the study period at all sites with little variation or pattern in discharge. For example, the coefficient of variation for flow at the most downstream site, Inglewood Avenue, was less than 8% approaching the resolution of our flow monitoring devices.

The flux of fecal indicator bacteria remained relatively constant moving downstream in Ballona Creek (Figure 3). The average flux of *E. coli* ranged from 1.1 X 10¹⁰ to 5.3 X 10¹⁰ cells/hr at the six mainstem sites. The average flux of enterococcus ranged from 6.6 X 10⁸ to 1.4 X 10⁹ cells/hr at the six mainstem sites. In both cases, there was no discernable increase in bacterial flux; no two mainstem sites were significantly different from one another for either *E. coli* or enterococcus.

The flux of fecal indicator bacteria decreased over time (Figure 4). The average flux of enterococcus was highest at 9:00 AM (2.9 X 10⁹ cells/hr) and monotonically decreased throughout the study period. The lowest flux was measured at 2:00 PM (3.0 X 10⁹ cells/hr). Similar patterns were observed for *E. coli* (data not shown). In contrast to the culture-based methods, the QPCR method for measuring enterococcus did not decrease over time. The flux of enterococcus ranged from 2.7 X 10¹⁰ to 4.7 X 10¹⁰ cells/hr with the 9:00 AM and 2:00 PM samples being nearly equivalent.

The relative pattern of enterococcus contributions between tributaries was similar at all time periods (Figure 5). Benedict tributary always had the greatest flux of fecal indicator bacteria followed by Sepulveda, Fairfax and Adams tributaries. A similar pattern was also observed for *E. coli*. The flux of enterococcus from Benedict tributary ranged from 4.1 X 10⁹ to 1.4 X 10¹⁰ cells/hr throughout the sampling period while the flux of enterococcus from Adams tributary ranged from 3.7 X 10⁵ to 4.4 X 10⁶ cells/hr. On average, Benedict tributary contributed 81% of the enterococcus loading from all four tributaries.

The hourly flux of enterococcus (using culture-based methods) from each of the four main tributaries approximated the load being passed down Ballona Creek (Figure 5). Regardless of hour, the flux from each of the tributaries was within a factor of 10¹ compared to its nearest downstream site on the mainstem of Ballona Creek. The only exception was the Adams tributary, which was as much as four orders of magnitude less than its nearest downstream site. The mainstem showed virtually no response to any of these tributary inputs, including Adams. Enterococcus flux remained virtually unchanged from upstream to downstream of each of the tributary inputs (Figure 5, Figure 3).

Measurements of *Bacteroides* sp. and enterovirus indicated the presence of human fecal contamination throughout the system (Table 5). *Bacteroides* sp. was present in 12 of 36 mainstem samples (33%). Enterovirus was present in 14 of 36 mainstem samples (44%). The concordance among these measurements was nearly complete; almost every location that detected *Bacteroides* sp. was also positive for enterovirus. Only two samples were positive for enterovirus and not *Bacteroides* sp. These two samples were furthest downstream or latest in the day.

Table 5. Number of enterovirus genomes (per 100 ml) detected.

Distance Upstream (km)	Time of Day					
	9:00	10:00	11:00	12:00	1:00	2:00
6.3	106*	71*	93*	70*	67*	
5.4		41*	19**	25*		
4.7		17*		113*	51	
2.6				79*		
1.5				13*		39
0						

* Human *Bacteroides* marker also detected.

** PCR reaction for human *Bacteroides* marker inconclusive due to inhibition.

Spatial and temporal patterns in enterovirus concentration were evident in the Ballona Creek system (Table 3). Main channel locations in the upper reaches of the study area were more likely to be positive for enteroviruses than downstream sites. The most consistently positive site was Cochran Ave., where 89% of the samples contained measurable levels of enterovirus. In addition, the highest concentrations of enterovirus were measured at Cochran Ave. during four of the six time periods. A general pattern in enterovirus detection was observed during the course of the day. Enterovirus was detected earliest in the day at upstream sites. Enterovirus was detected most frequently late in the day at the downstream sites. The 12:00 sampling interval had the most frequent detection of enterovirus with the highest concentrations observed at the middle sites in the watershed. Enterovirus was not detected in high concentrations in any of the tributaries; only Adams tributary had any detectable enterovirus.

Malibu Creek

Malibu Creek had a similar pattern of fecal indicator bacteria concentrations at each sampling event throughout the study period (Figure 6). Concentrations decreased along the mainstem as it flowed from Cold Creek to Cross Creek, then increased as it flowed through the estuary until it discharged into the ocean at Malibu Beach. The increase in fecal indicator bacteria through the lagoon averaged 10^2 MPN/100 ml for both

enterococcus and *E. coli*. The dilution factor from the discharge to the shoreline as a result of wave induced mixing averaged 0.86 for *E. coli* and 0.34 for enterococcus. Despite the increase in fecal indicator bacteria concentrations, none of the Malibu samples were positive for enteroviruses or *Bacteroides* sp.

BMP's

Both of the BMPs that incorporated UV treatment systems were more effective than the constructed wetland at removing fecal indicator bacteria (Table 6). Albeit concentrations were low in the influent, the constructed wetland did not reduce concentrations of *E. coli* or enterococcus using either culture-based or QPCR methods in the effluent. Both UV treatment systems, however, reduced influent concentrations of *E. coli* and enterococcus in the effluent to near or below method reporting levels. Enterococcus concentrations by QPCR were reduced by an order of magnitude, which was not as great as the culture-based method. No enterovirus or *Bacteroides* sp. were detected in any of the BMP influent or effluent samples analyzed.

Table 6. BMP effectiveness for indicator bacteria removal measured by culture-based (chromogenic substrate, CS) or quantitative polymerase chain reaction (QPCR) methods.

Indicator	Constructed Wetland		Filtration+UV		Filter+DAF+UV	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
<i>E. coli</i> (CS) (MPN/100 ml)	<10	36	58	20	147	<10
Enterococcus (CS) (MPN/100 ml)	<10	47	184	15	42	<10
Enterococcus (QPCR) (Cells/100 ml)	163	110	5004	729	No Data	No Data
Enterovirus (genomes/100 ml)	-	-	-	-	No Data	No Data

* no *Bacteroides* sp. detected

- no enterovirus detected

DISCUSSION

The Ballona Creek watershed is a system severely impacted by fecal pollution. The flux of fecal indicator bacteria was as high at the head of the watershed as it was at the mouth of the creek where it discharges into SMB. Although we focused on flux of these fecal indicator bacteria, it is important to note that 92% of all samples collected from Ballona Creek in this study, including 100% of the samples just upstream of SMB, exceeded the water quality thresholds established by the State of California. The presence of human enterovirus and human specific markers of *Bacteroides* sp. further documents the fecal inputs and should increase an environmental manager's awareness of the possible human health risks associated with these discharges.

Our study is not the first to examine the presence of viruses in urban runoff entering shorelines in SMB and other southern California urban watersheds. For example, Gold *et al.* (1990) and Gold *et al.* (1992) found viruses in repeated samples from multiple storm drains to SMB using both cell culture and RTPCR techniques. Haile *et al.* (1999) detected human specific viruses in all three storm drains tested in their epidemiology study of SMB. Noble and Fuhrman (2001) found human enteric virus genomes in the nearshore marine waters of SMB. Jiang *et al.* (2001) found human adenovirus in samples collected at 12 sites between Malibu and the Mexican border.

The multi-tiered approach used in this study can assist watershed managers in determining sources and efficiently abating the most significant inputs of fecal indicator bacteria. If managers relied solely on the patterns in fecal indicator bacteria from Ballona Creek, then the only option would be to treat the entire 37 m³/s discharge furthest downstream at Inglewood Ave. because the flux of fecal indicator bacteria was similar from all sources. The use of multiple tools, however, allows managers to prioritize the most important sources. In this case, the presence of human enterovirus was greatest from the Cochran Ave. site, where the system daylights from the underground storm drain system beneath Los Angeles and the discharge volume is one-third the volume at Inglewood Ave. Since Cochran Ave. had the most frequent occurrence and highest concentrations of enterovirus, this source would appear to be the most likely candidate for future management actions. The co-occurrence of the human *Bacteroides* sp. marker at most of the locations and time periods where enterovirus was quantified, most notably in all of the Cochran Ave. samples, provides the reassurance most managers would need before planning future management steps.

The lack of correlation between bacterial indicator levels and levels of human pathogenic viruses has been observed in previous studies (Dufour, 1984; Elliott and Colwell, 1985) and demonstrates the value of a multi-tiered approach used herein for source identification. For example, analysis of wild shellfish from the Atlantic coast of France indicated no significant correlation between fecal coliforms and enteroviruses or hepatitis A virus (LeGuyader *et al.*, 1993; Leguyader *et al.*, 1994), and viruses have sometimes been found in oysters without coliform contamination (Goyal *et al.*, 1984; Yamashita *et al.*, 1992). Noble and Fuhrman (2001) detected enterovirus in 35% of the 50 shoreline

samples they examined over a five year period and no significant statistical relationship to any of the standard bacterial indicators was found. Noble *et al* (2000) measured virus and fecal indicator bacteria in dry weather urban runoff in drains along 300 km of shoreline from Santa Barbara to San Diego. Despite 46% of the storm drains containing detectable enterovirus, there was no correlation with fecal indicator bacteria concentrations.

The results of this study indicated that Ballona Creek presents a greater risk to human health than Malibu Creek. There were no enterovirus or *Bacteroides* sp. detected in any sample from the Malibu Creek watershed. The bacterial concentrations were lower at Malibu Creek than at Ballona Creek; none of the Malibu Creek samples from the bottom of the watershed (at Cross Creek) exceeded water quality thresholds established by the State of California. Interestingly, fecal indicator bacteria concentrations increased as water flowed through the lagoon at the base of the Malibu watershed. No enterovirus or *Bacteroides* sp. was detected in these samples either, although other studies have detected human specific sources of viruses in this discharge (John Griffith *personal communication*).

The use of QPCR to measure fecal indicator bacteria presents unique opportunities and challenges. The advantage of QPCR for measuring fecal indicator bacteria is speed potentially providing measurements in less than four hours (Griffith *et al* 2004). However, culture-based methods only quantify viable bacteria, while QPCR measures the DNA from both cultivable and noncultivable microbes. This was most apparent in the temporal trends from Ballona Creek. Samples of enterococcus using culture-based methods generally decreased as the day progressed, most likely as the result of degradation from sunlight (Noble *et al.* 2004). Ballona Creek is a 40m wide concrete-lined channel concentrating solar energy into the shallow creek in the channel invert. The QPCR results, however, remained steady indicating that the bacterial DNA was still intact even though the enterococci were not viable.

The data from this study suggested the UV treatment systems were more effective than the constructed wetland at reducing fecal indicator bacterial concentrations. We suspected the UV treatment system would be effective since this method is a well-known mechanism for degrading bacteria (Fujioka *et al.* 1981, Davies and Evison, 1991, Davies-Colley *et al.* 1994, Noble *et al* 2004). Not only did the UV system reduce concentrations of enterococci using culture-based techniques, but it also degraded its DNA as shown by large reductions in enterococcus by QPCR. On the other hand, the effectiveness of the treatment wetland remains incompletely quantified. Although levels of fecal indicator bacteria were similar before and after flowing through the wetland, concentrations were very low to begin with. Monitoring by others at this treatment wetland suggest that it has been effective at reducing fecal indicator bacteria concentrations using culture-based methods (Nancy Palmer, City of Laguna Niguel *personal communication*). More study, particularly with the QPCR, will be needed before the wetland effectiveness can be fully quantified.

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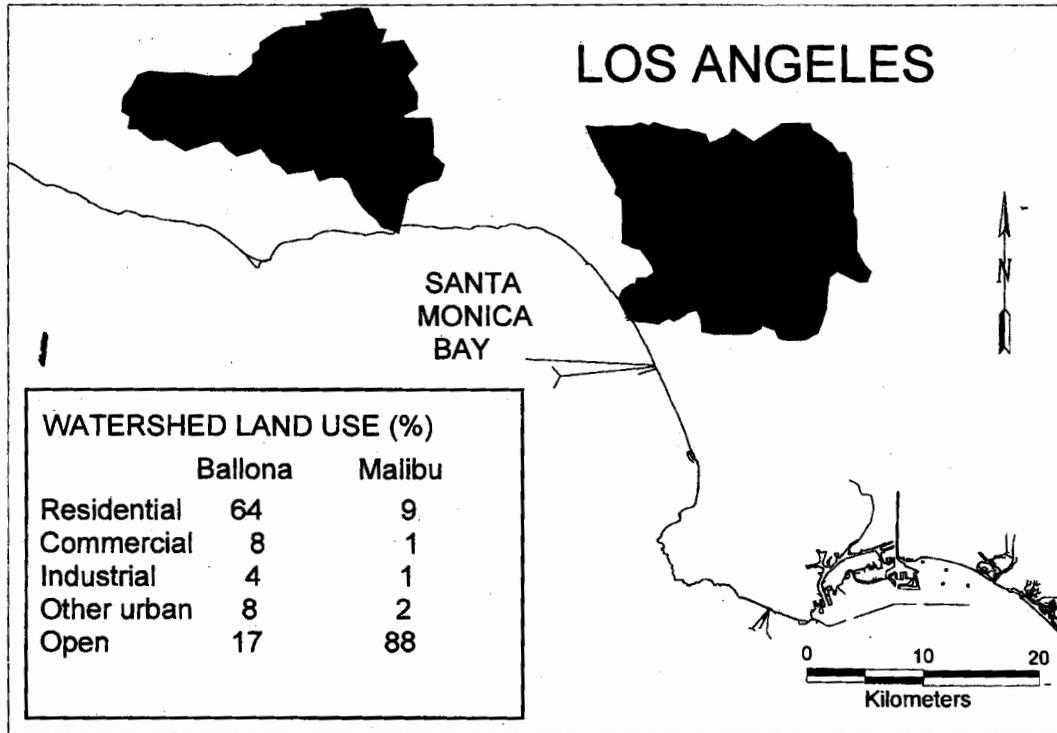


Figure 1: Map of Santa Monica Bay, CA indicating the locations and land use distribution for Ballona Creek and Malibu Creek watersheds.

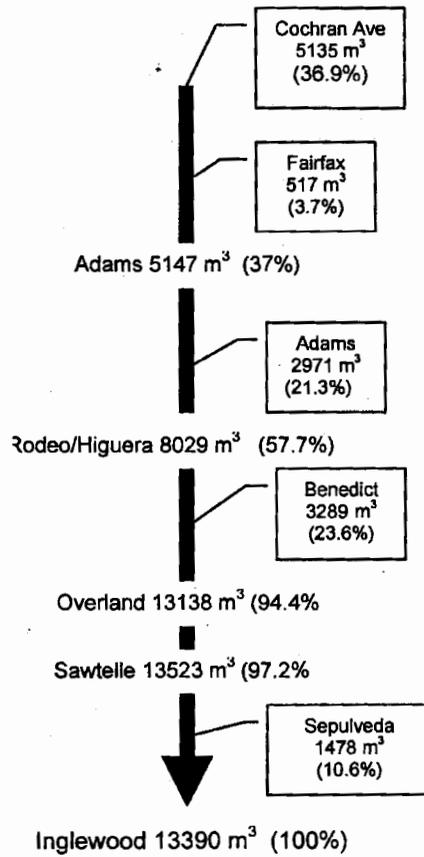


Figure 2. Schematic diagram depicting additive flow in main channel and percent contribution from each tributary.

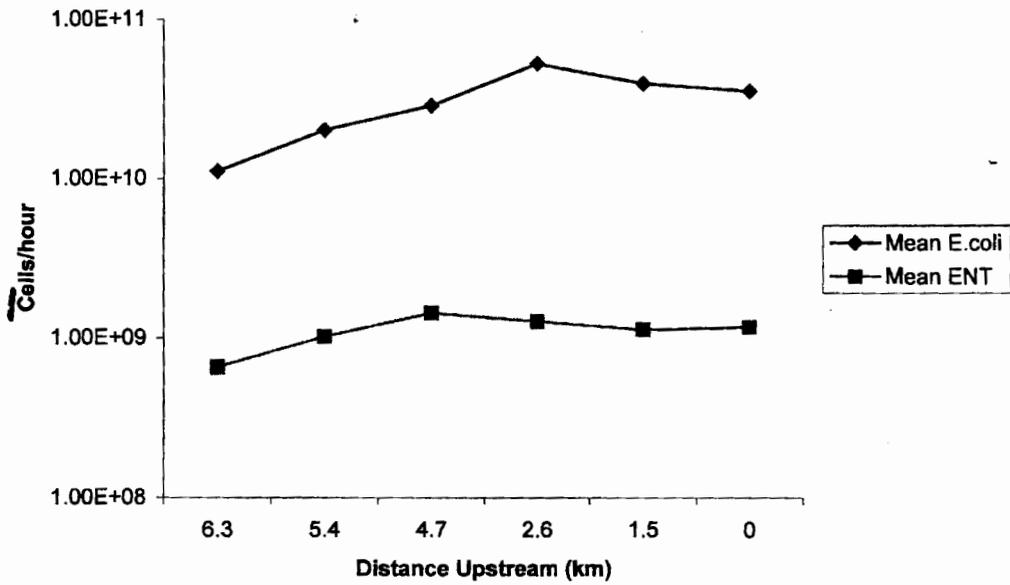


Figure 3. Mean hourly flux of *E. coli* and enterococcus at each station in main channel of Ballona Creek measured using the IDEXX™ method.

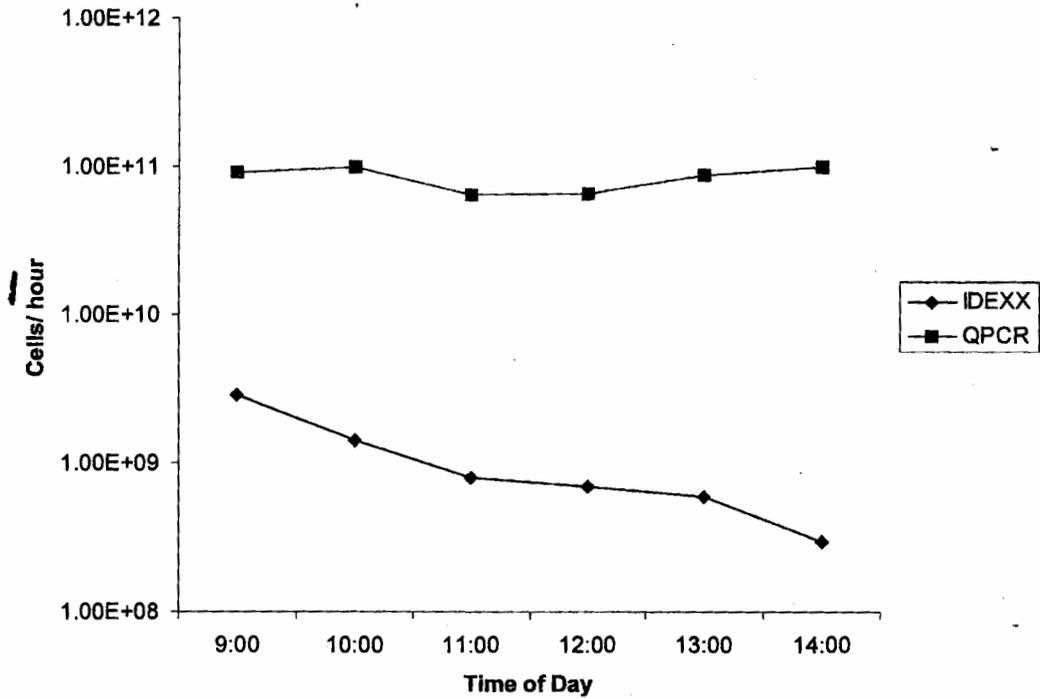
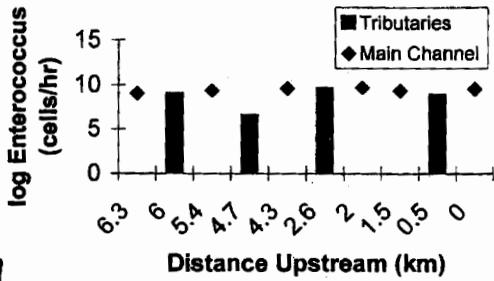
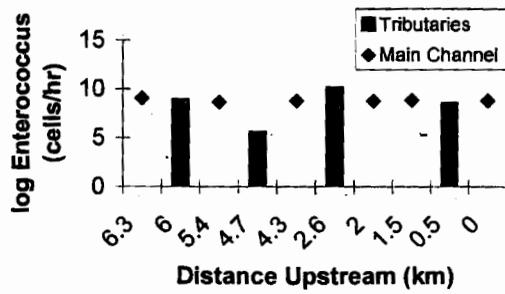


Figure 4. Mean hourly flux of enterococcus along the main channel of Ballona Creek as measured using both IDEXX the QPCR methods.

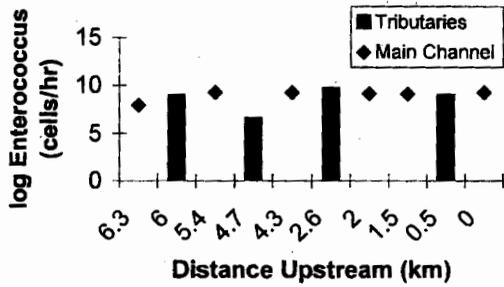
a)



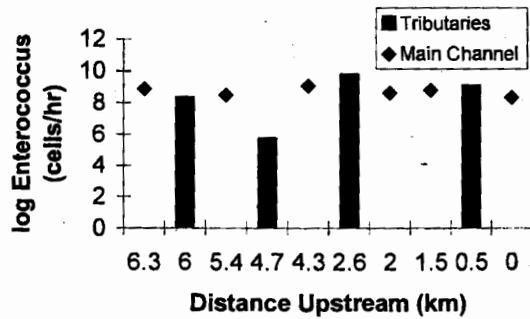
d)



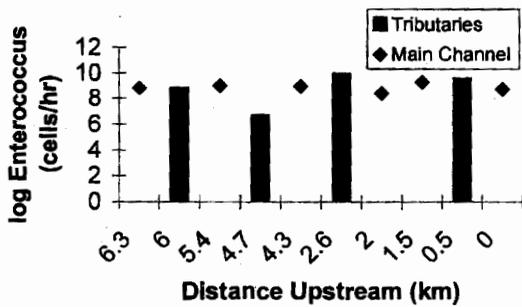
b)



e)



c)



f)

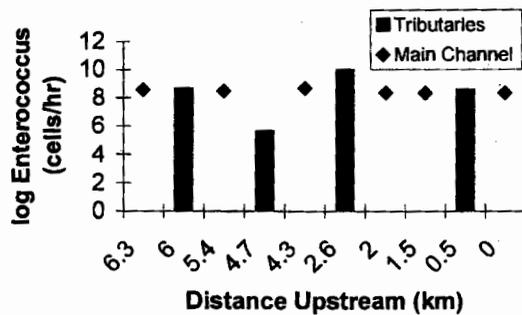
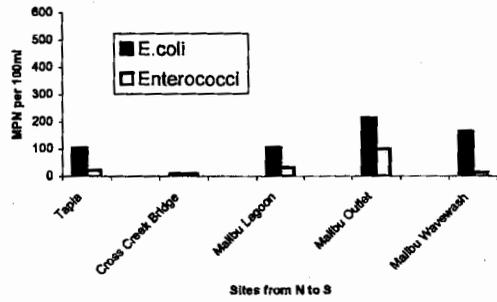


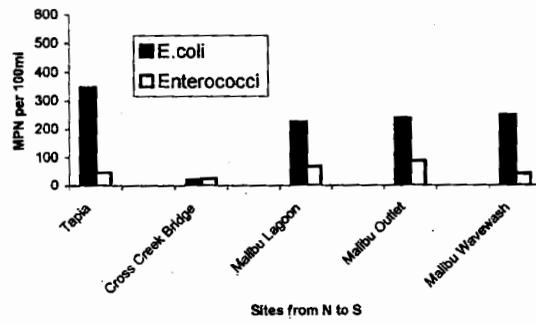
Figure 5. Enterococcus loading in main channel and tributaries of Ballona Creek at a) 9:00, b) 10:00, c) 11:00, d) 12:00, e) 13:00, f) 14:00.

Multi-tiered approach to source tracking using QPCR

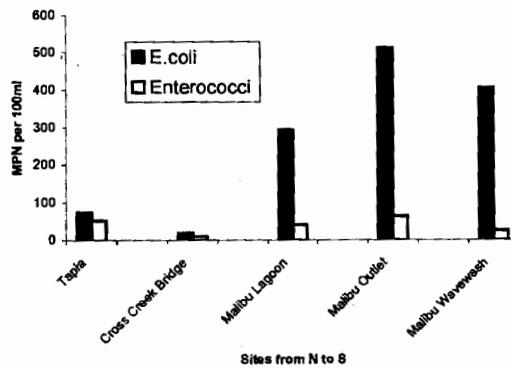
a)



b)



c)



Figures 6. Fecal indicator bacteria concentrations in Malibu Creek on a) 11/10/04 b) 11/11/04 and c) 11/12/04



Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland

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Abstract

Recreational water quality at beaches in California and elsewhere is often poor near the outlets of rivers, estuaries, and lagoons. This condition has prompted interest in the role of wetlands in modulating surface water concentrations of fecal indicator bacteria (FIB), the basis of water quality standards internationally. A model was developed and applied to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California, in response to loads from urban runoff, bird feces, and resuspended sediments. The model predicts the advection, dispersion and die-off of total coliform, *Escherichia coli*, and enterococci using a depth-integrated formulation. We find that urban runoff and resuspension of contaminated wetland sediments are responsible for surface water concentrations of FIB in the wetland. Model predictions show that urban runoff controls surface water concentrations at inland sites and sediment resuspension controls surface water concentrations near the mouth. Direct wash-off of bird feces into the surface water is not a significant contributor, although bird feces can contribute to the sediment bacteria load. The key parameters needed to accurately predict FIB concentrations, using a validated hydrodynamic model, are: the load due to urban runoff, sediment erodibility parameters, and sediment concentrations and surface water die-off rates of enteric bacteria. In the present study, literature values for sediment erodibility and water column die-off rates are used and average concentrations of FIB are predicted within 1/2 log unit of measurements. Total coliform are predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. Since wetland-dependent animals are natural sources of FIB, and FIB survive for long periods of time and may multiply in wetland sediments, these results highlight limitations of FIB as indicators of human fecal pollution in and near wetlands.

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Keywords: Enteric bacteria; Intertidal wetland; Coastal; Water quality; Sediment

1. Introduction

Fecal indicator bacteria (FIB) groups such as total coliform (TC), fecal coliform (FC), *Escherichia coli* (EC), and enterococci (ENT) are utilized world wide to measure health hazards in bathing and shellfish harvesting waters (Thomann and Mueller, 1987). Water samples at popular beaches and harvesting waters are

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routinely tested for FIB, which are thought to signal the presence of pathogens but are not necessarily pathogenic (US EPA, 1986). Chronic exceedances of California criteria have placed coastal water bodies such as Tomales Bay, Moss Landing Harbor, Morro Bay, Ventura Harbor, Marina Del Rey Harbor, Newport Bay, and Mission Bay on lists of pathogen impaired water bodies (CalEPA, 2002). Exceedances are also common at open ocean beaches, particularly near the outlets of storm drains, rivers, estuaries, and lagoons. Numerous coastal water bodies are impaired worldwide according to such standards.

Pathways by which FIB enter coastal waters include urban and agricultural runoff, waste water discharges, sewage leaks and spills, and fecal deposits by wildlife, notably birds. A complex web of processes influence the distribution of FIB in surface waters including flushing by ocean water, die-off, predation, sedimentation and resuspension, and regrowth on sediments, vegetation, and debris (Savage, 1905; Goyal et al., 1977; Roper and Marshall, 1979; Jensen et al., 1979; LaBelle et al., 1980; Grimes et al., 1986; Thomann and Mueller, 1987; Davies et al., 1995; Oshiro and Fujioka, 1995; Anderson et al., 1997; Byappanahalli and Fujioka, 1998; Solo-Gabriele et al., 2000; Grant et al., 2001). Use of FIB as indicators of human pathogens is complicated by these processes, particularly in wetlands where wildlife is abundant and nutrient rich sediments support growth of bacteria. So long as FIB remain the basis of regulations governing coastal water quality, a need will exist to identify the forcing factors (river inputs, storm drains, etc.) supporting FIB populations so that appropriate and cost-effective management measures can be implemented.

Several researchers have recently reported on models to predict FIB concentrations in coastal waters. Use of such models in coordination with field monitoring programs can help to identify the relative impact of various sources (e.g., a river versus a storm drain), characterize the mechanisms governing the fate of these organisms (e.g., flushing versus die-off) and predict the efficacy of a range of potential management measures. Kashefipour et al. (2002) used a model consisting of depth-integrated continuity, momentum, and transport equations to predict FIB concentrations in the Ribble Estuary, England. Fiandrino et al. (2003) used a model consisting of three dimensional continuity, momentum, and transport equations to predict FIB concentrations in Thau lagoon, France. Steets and Holden (2003) used a one-dimensional model to predict FIB concentrations in Arroyo Burro lagoon, California.

In this study we use a model to simulate dry-weather tidal cycling of TC, EC, and ENT concentrations in surface waters of Talbert Marsh, an intertidal wetland in Huntington Beach, California. Runoff from an urbanized watershed drains to the marsh, the marsh accommodates a high concentration of shore birds,

and high sediment concentrations of FIB have been measured. Grant et al. (2001) reported that Talbert Marsh was a net source of ENT to coastal waters and hypothesized that it was due to a combination of bird feces and interactions with sediments and vegetation. Sediments act as a reservoir of FIB (Goyal et al., 1977, e.g.), and suspension and deposition cycles are germane to the estuarine environment (Mehta and Dyer, 1990). In Talbert Marsh, it is not clear whether FIB concentrations are predominantly controlled by urban runoff, erosion of contaminated sediments, bird feces, or some combination of these factors. Therefore, the model is applied to examine and rank the influence of these "forcing factors." The modeling effort described in this paper is unique relative to previously published studies in that non-point loads of FIB (bird feces, erosion of contaminated sediments) are incorporated into a multi-dimensional, time-dependent formulation for the first time.

The model in this study consists of depth-integrated continuity and momentum equations to simulate circulation, and depth-integrated transport equations to simulate surface water concentrations of FIB resulting from urban runoff, bird droppings and resuspended sediments. The model is parameterized using either in situ data or previously published values of model parameters. The model is applied to predict FIB over a 15 day period beginning May 2, 2000, coincident with an extensive field monitoring effort previously reported (Grant et al., 2001, 2002). This work demonstrates the power of first-principle models to elucidate the mechanisms and pathways by which near-shore coastal waters are polluted by FIB.

2. Methods and materials

2.1. Site description

Talbert Watershed, shown in Fig. 1, is a 3300 ha catchment along the southern California coastline in the cities of Huntington Beach and Fountain Valley. On average, the watershed receives 29 cm of rainfall, over 90% of which falls between November and April. Daily high/low temperatures average 23/17°C in September and 17/8°C in January. The watershed slopes mildly (10^{-4}) towards the ocean and is drained by a network of channels that, due to the low elevation and mild slope of the watershed, are flooded by tides. Talbert Channel is the main stem of the network. Inland 2 km from the mouth, Huntington Beach Channel branches west and extends 5 km inland; and 8 km from the mouth, Fountain Valley Channel branches east. High tide floods Talbert Channel to the Fountain Valley Channel junction and the length of the Huntington Beach channel. Depths in the channels are comparable to the



Fig. 1. View of Talbert Marsh, channel network, and surrounding watershed as low tide. Channels and watershed extends several kilometers further inland than indicated in the figure. At high tide, the southwestern and southern portions of Talbert Marsh are flooded. PCH, BRK, and AES indicate monitoring stations.

tidal amplitude, roughly 1 m. Near the outlet, Talbert Marsh occupies roughly 10 ha of what used to be an extensive (1200 ha) tidal marsh environment that was filled for development over the past century. Talbert Marsh was created in 1990 when remnant marsh was flooded following the removal of a Talbert Channel levee. The channel bed consists of beach sand and silts near the outlet and within a flood delta that penetrates a short distance into the marsh. Further inland, the marsh and channel bed consists of organic rich silts and muds, except the upper reaches of Talbert Channel and Fountain Valley Channel where the bed is lined with concrete. In this study, the Talbert Marsh and tidal channels are collectively referred to as the wetland. From a perspective of flushing the wetland may be divided into two zones: a poorly mixed zone inland where residence times are at least a week, and a well-mixed zone near the mouth that is flushed each tide cycle

with ocean water. The interface between these zones oscillates with the ebb and flow of the tides.

The watershed is heavily developed as is common to the greater Los Angeles basin, and it contains separate networks of storm and sanitary sewers. Storm sewers direct runoff into street drains that funnel to the wetland. In the lower half of the watershed where the topography is lowest, runoff collects in one of several roughly 500 m³ forebays that are intermittently drained by pump stations. A program is now in place to divert dry-weather runoff from the storm sewer to the sanitary sewer for treatment. This program began on a limited basis in Fall 1999 and encompassed the entire watershed by Summer 2001. During the 15 day period that is the focus of this study, pump stations were operated in two different modes. During the first 8 days, pumpstations were not activated so runoff either collected in the forebay or was diverted to the sanitary sewer system.

During the remaining 7 days, pumpstations intermittently discharged untreated runoff to the channel network. Over the entire 15 day period, there was dry-weather baseflow in Talbert Channel that entered the wetland.

Monitoring stations referenced in this paper include Pacific Coast Highway (PCH), Brookhurst Street (BRK), and AES Corp. (AES). These are shown in Fig. 1.

2.2. FIB modeling

A hydrodynamic model was developed to simulate surface water concentrations of FIB in the wetland, from the outlet of Talbert Marsh to the head of the Huntington Beach Channel and, along Talbert Channel, to the Fountain Valley Channel junction. The model consists of depth-integrated continuity, momentum, and transport equations (Arega and Sanders, 2004), similar to the approach adopted by Kashefipour et al. (2002). The flow equations (continuity and momentum) were forced by the ocean tide just offshore of the marsh, and by runoff flowing into the upper reaches of the wetland. Ocean tide forcing was based on tide levels recorded at NOAA station 9410660, Los Angeles and archived online at <http://tidesonline.nos.noaa.gov/>. The discharge of runoff at the Talbert Channel inflow boundary, Q_R , was assumed steady over the study period. To model pumpstation operations, the discharge of runoff from each pumpstation, Q_P , was assumed uniform and steady over the final seven days of the study period, but zero over the first eight days. Seven

pumpstations that discharge directly into the wetland were incorporated into the model. Runoff data (Q_R and Q_P) were obtained from other reports (Grant et al., 2001, 2002; Chu, 2001) and appear in Table 1. Topographic data necessary for flow predictions were obtained from as-built plans of the concrete-line portions of the channels and a field survey of the Talbert Marsh (Chu, 2001). A uniform Manning coefficient was used to account for bed resistance (Arega and Sanders, 2004).

Simultaneous with the flow prediction described above, surface water FIB were predicted by solving the following transport equations:

$$\begin{aligned} \frac{\partial}{\partial t}(hc_i) + \frac{\partial}{\partial x}(\bar{u}hc_i) + \frac{\partial}{\partial y}(\bar{v}hc_i) \\ = \frac{\partial}{\partial x} \left(hE_{xx} \frac{\partial c_i}{\partial x} + hE_{xy} \frac{\partial c_i}{\partial y} \right) \\ + \frac{\partial}{\partial y} \left(hE_{yx} \frac{\partial c_i}{\partial x} + hE_{yy} \frac{\partial c_i}{\partial y} \right) \\ - hl_i + a_i + \sum_{k=1}^{N_{PS}} \mathcal{L}_k \delta(x - x_k^i, y - y_k^i), \end{aligned} \quad (1)$$

where h = depth [m] and \bar{u} , \bar{v} = components of the depth-averaged fluid velocity [m/s], E_{xx} , E_{xy} , E_{yx} , and E_{yy} = elements of the dispersion tensor [m²/s], c_i , ($i = 1, \dots, N_b$) = water column concentration of FIB [MPN/m³], N_b = number of FIB groups tracked by the model, l_i = water column loss rate [MPN/m³/s], a_i = flux of FIB to water column at sediment/water interface [MPN/m²/s], and \mathcal{L}_k = FIB loading rate of the i th FIB group at the k th inflow point [MPN/s], N_{PS} is the

Table 1
Measured, cited, and computed parameters used to estimate loading and die-off models

Parameter	Units	Total coliform		<i>E. coli</i>		Enterococci	
		Value	Uncertainty	Value	Uncertainty	Value	Uncertainty
k^a	m ² /Watts/h	0.0018	±10%	0.0017	±10%	0.00097	±10%
c_R^b	MPN/100 mL	1.5×10^4	$2.2 \times 10^4/1.0 \times 10^4$	9.8×10^2	$1.4 \times 10^3/7.1 \times 10^2$	1.8×10^3	$2.4 \times 10^3/1.3 \times 10^3$
r^b	MPN/bird/day	2.8×10^7	$8.4 \times 10^7/2.1 \times 10^6$	1.5×10^7	$1.0 \times 10^8/1.2 \times 10^7$	7.2×10^6	$2.6 \times 10^7/5.2 \times 10^6$
s^b	MPN/g	5.2×10^3	$1.3 \times 10^4/2.0 \times 10^3$	2.1×10^2	$8.5 \times 10^2/5.1 \times 10^1$	6.8×10^2	$1.6 \times 10^3/2.9 \times 10^2$
Parameter	Units	Value	Uncertainty (%)	Parameter	Units	Value	Uncertainty (%)
Q_R	m ³ /d	1000	±50	\bar{n}_b	—	174	±50
Q_P	m ³ /d	300	±50	$(\bar{\tau})$	Pa	0.08	±50
\bar{A}_S	ha	32	±50	τ_0	Pa	0.75	±50
E_0^c	kg/m ² /s	1×10^{-4}	±50	τ_c^c	Pa	0.25	±50

Except where noted, a conservative estimate of 50% uncertainty was adopted. Note that the mathematical model is presented using SI units, so conversion factors need not appear in model equations. Commonly used units are presented here to facilitate comparison with previous works and other studies.

^aDie-off rates based on Sinton et al. (1999).

^bMeasured in situ, uncertainty based on standard error.

^cErodibility rates based on Uncles and Stephens (1989).

number of inflow points where runoff is added to the wetland (pump stations and tributary inflow), x_s^k and y_s^k = coordinates of each inflow point [m], and δ = Dirac delta function [$1/m^2$]. The dispersion tensor accounts for longitudinal dispersion (Elder, 1959) and transverse mixing (Ward, 1974), and it is computed locally depending on the orientation of the currents (Arega and Sanders, 2004). Note that SI units are adopted for the purpose of presenting the mathematical model, so conversion factors need not appear in model equations. However, many model parameters are reported in Table 1 with commonly used units to facilitate comparison with previous works and other studies.

Eq. (1) was solved using $N_b = 9$ to predict the distribution of TC, EC, and ENT resulting from urban runoff, bird feces, and sediment resuspension. Groups 1–3 correspond to TC, EC, and ENT concentrations resulting from runoff sources, 4–6 from bird sources, and 7–9 from sediment sources. All model predictions account for surface water die-off using first order kinetics as follows:

$$I_i(x, y, t) = k_i I(t) c_i(x, y, t), \quad (2)$$

where k_i = die-off rate constant [$m^2/\text{Watts/s}$] based on Sinton et al. (1999), and $I(t)$ = solar intensity [Watts/m^2]. Die-off rates used in the model were taken from Sinton et al. (1999), and solar intensity data for the study period were obtained from Grant et al. (2001).

The model does not account for settling. Suspended sediments in the $1\text{--}10^3 \mu\text{m}$ size range are typical of intertidal wetlands adjacent to sandy ocean beaches, but FIB in southern California coastal waters are either free-living (planktonic, roughly $1 \mu\text{m}$ in size) or associated with very fine sediments, probably in the $10 \mu\text{m}$ range or less (Ahn et al., 2005). The relative influence of settling and die-off is defined by the ratio $w_s/k_i h$, where w_s is the settling velocity. Using Stokes Law to model the settling velocity in terms of particle size (e.g. Nazaroff and Alvarez-Cohen, 2001, Chapter 4), the average solar radiation rate for the study period (288 Watts/m^2), die-off rates reported by Sinton et al. (1999), and a depth of 1 m which is typical for the wetland, this ratio is unity for ENT when particle diameter $d = 10 \mu\text{m}$ and for TC and EC when $d = 13 \mu\text{m}$. When $d = 5 \mu\text{m}$, this ratio is 0.3 for ENT and 0.2 for TC and EC. The nonlinear dependence is due to the quadratic relationship between settling velocity and particle size. Without a clear understanding of the partitioning of FIB between free-living and particle-associated states, and knowledge of the median diameter of particles with attached FIB, selecting an appropriate settling velocity is difficult. Certainly, without settling terms the model will underestimate water column FIB losses if these organisms are associated with particles in the $10\text{--}20 \mu\text{m}$ range or larger.

Therefore, this assumption should be reconsidered if the model significantly overpredicts FIB concentrations.

For all predictions, the concentration of FIB in water entering the wetland from the ocean was set to zero. For the urban runoff predictions ($i = 1\text{--}3$), point loads of FIB were specified at runoff inflow points and the non-point loading term, a_i , was set to zero. The loading rate was set equal to the volumetric flow rate multiplied by the concentration of FIB in runoff, c_R , which was specified based on average Talbert Watershed urban runoff concentrations reported by Reeves et al. (2004).

FIB loading to surface waters by bird feces ($i = 4\text{--}6$) was modeled as a spatially distributed (around the water line) and temporally variable non-point source. It was assumed that all bird feces fell exclusively on the shoals of the marsh, were subject to sunlight induced die-off, and upon flooding by the tide were instantaneously and completely transferred to the water column. Hence, loading in the model occurs at water's edge during the rising tide. This approach was motivated by bird surveillance data, which showed birds congregated on shoals during low tides (Grant et al., 2001). The following mass balance equation was solved to track the build-up and die-off of FIB on the shoals of the marsh,

$$\frac{dm_i(x, y, t)}{dt} = d_i(t) - k_i I(t) m_i(x, y, t), \quad (3)$$

where m_i = the surficial FIB density [MPN/m^2] and d_i = FIB loading rate [$\text{MPN/m}^2/\text{s}$]. Note that the die-off rate constant for the marsh banks is identical to that used for surface water. The FIB loading rate was computed as,

$$d_i(t) = n_b(t) r_i / A_{IT}(t), \quad (4)$$

where $A_{IT}(t)$ = the exposed (or dry) inter-tidal surface area [m^2], $n_b(t)$ = bird population measured hourly in the marsh and r_i = rate of FIB loading per bird [$\text{MPN}/\text{bird/s}$]. The exposed inter-tidal surface area (or area of the exposed shoals) was determined from the marsh topography as the difference between the exposed surface area of the marsh and the exposed surface area under high spring tide conditions. This varied from 0 to 4.5 ha depending upon the tide stage. Table 1 presents bird loading rates used in the model, which were based upon samples collected Talbert Marsh. The sampling methodology is described in (Grant et al., 2001), but only ENT concentrations are reported. TC and EC were quantified from the same samples using defined substrate tests (IDEXX, Westbrook, Maine), but the data have not previously been reported.

FIB loading rates of birds vary widely depending upon species, habitat, diet, and feeding habits. Hussong et al. (1979) reported fecal coliform loading rates for wild swan and Canadian geese of $10^6\text{--}10^9$ and $10^4\text{--}10^7$ $\text{MPN}/\text{bird}/\text{day}$, respectively, Gould and Fletcher (1978)

reported fecal coliform loading rates for several gull species in the range of 10^6 – 10^7 MPN/bird/day. Alderisio and DeLuca (1999) reported fecal coliform loading rates of roughly 10^8 and 10^5 MPN/bird/day for ring-billed gulls and Canadian geese, respectively. Rates reported in Table 1 for shore birds in Talbert Marsh are similar, roughly 10^7 MPN/bird/day for all three indicator groups. During the study period bird populations ranged from 0 to 1180 (Grant et al., 2001).

After being flooded by the rising tide, the wash-off of surficial bacteria from the marsh banks contributes to the sediment/water interface loading rate, a_i appearing in Eq. (1), as follows:

$$a_i(x, y, t) = m_i(x, y, t)\delta(t - t_f), \quad (5)$$

where t_f = the instant land is flooded by the rising tide [s] and δ = Dirac delta function [1/s]. Hence, the transfer of surficial FIB from the banks of the marsh to surface waters is modeled as an instantaneous exchange that is triggered by moment the bank is flooded by the rise of the tide. After transfer to surface waters, $m_i = 0$ until the banks are again dry at which point the build-up process resumes.

FIB loading to surface waters by sediments ($i = 7$ – 9) was modeled as a spatially distributed and temporally variable non-point. The non-point loading term a_i in Eq. (1) was formulated to account for the transfer of FIB to the water column that occurs when FIB laden particulate matter and pore water on the bed is mobilized by turbulent shear. The mobilization of estuarine sediments occurs after a threshold in turbulent shear has been exceeded, and in proportion to the excess of turbulent shear above the threshold (Partheniades, 1965; Mehta and Dyer, 1990). Whether or not the same is true for FIB is not clear, for FIB may be free living in sediment pore water, attached to sediment grains, or incorporated into microbial biofilms; and how these phases of FIB respond to shear is not known. Therefore, a novel approach was taken. The FIB loading term was developed by dimensional analysis with the following conditions in mind: (a) that the transfer rate of FIB from sediments to surface waters be proportional to the shear rate; and (b) that FIB liberated from the sediments over a tide cycle be equal to FIB present (either attached to particles or free-living in pore water) in the erodible layer of surficial sediments. Therefore, the following rate expression was used,

$$a_i(x, y, t) = s_i E \frac{\tau(x, y, t)}{\tau_0} \left(\frac{\tau_0}{\tau_c} - 1 \right), \quad (6)$$

where s_i = geometric mean concentration of FIB per mass of sediment [MPN/kg], E = entrainment rate parameter [$\text{kg}/\text{m}^2/\text{s}$], τ = spatially and temporally varying shear stress at the bed [Pa] computed by the hydrodynamic model, τ_c = critical shear stress for

erosion [Pa], and τ_0 = reference stress [Pa] representative of erosive conditions in the wetland.

The reference stress was computed based on water level and velocity data collected at BRK (Arega and Sanders, 2004). BRK serves as a good reference point due to its central location. Using a drag coefficient of 0.003 which is typical of estuaries, a fluid density of $1 \text{ g}/\text{cm}^3$, and a velocity of $0.5 \text{ m}/\text{s}$, the reference stress was estimated to be $\tau_0 = 0.75 \text{ Pa}$. A velocity of $0.5 \text{ m}/\text{s}$ was used for this calculation since the peak flood velocity varies from 0.4 to $0.6 \text{ m}/\text{s}$ over the spring-neap cycle, while the peak ebb velocity varies from 0.1 to $0.4 \text{ m}/\text{s}$. Site specific entrainment rate and critical shear parameter estimates were not available, so values reported in the literature by Uncles and Stephens (1989) and Tattersall et al. (2003) were used. All model parameters are reported in Table 1.

Note that measured concentrations of FIB in Talbert Marsh sediments were utilized to estimate s_i . No attempt was made to model the cycling of FIB in submerged sediments. To estimate the concentration of FIB in sediments, cores were collected at low tide within the inter-tidal zone and immediately transported to the laboratory. Overlying water was siphoned off the top and the cores were sectioned in 1 cm intervals with an extruder. For the few cores with a high sand content, sediment was scraped from the core tube in specified intervals to avoid slumping. Each sediment section was homogenized. A 5 g sample was suspended with 45 ml of a 0.5 M mono potassium phosphate buffer solution in a sterilized glass centrifuge tube for enteric bacteria analysis (APHA, 1992, Methods 9221 and 9050C). The sample was agitated for 1 min with a vortex mixer, then centrifuged at 2000 rpm for 5 min . The supernatant was then analyzed for TC, EC, and ENT using defined substrate tests with dilutions to the supernatant made with DI water (IDEXX, Westbrook, Maine). The remaining sediment from each 1 cm section was oven dried at 50°C , and stored for analysis of grain size. The concentration s_i was taken as the geometric mean of FIB concentrations in the top 1 cm of each sample, and is reported in Table 1.

An important assumption of this formulation is that sediment concentrations are constant over the two-week study period. Unpublished sediment data collected on a daily to weekly basis in nearby Santa Ana River wetlands show sediment concentrations of FIB increase at least one log unit immediately following storms, and subsequently decrease over a period of several days to weeks; but during dry-weather periods sediment concentrations are relatively uniform (Ambrose, 2004). This assumption would no longer be appropriate were the model used for wet-weather conditions or to predict variability on seasonal time scales.

The hydrodynamic equations, FIB transport equations, and mass balance equation for FIB build-up/

die-off on inter-tidal mudflats were integrating using a common time step of 0.2 s on an unstructured grid of 11732 quadrilateral cells encompassing all the wetted and inter-tidal portions of the channel network shown in Fig. 1. The flow and transport equations were solved by a finite volume numerical method described and validated for this study site by Arega and Sanders (2004). The build-up/die-off model for the load due to bird feces was solved using a backwards Euler discretization, for stability purposes and without concern for time-stepping errors due to the very small time step. The time of flooding, t_f appearing in Eq. (5) is determined in the model as the moment that all four nodes of a cell first become submerged by the rising tide. The solution of this model gives a spatially and temporally varying prediction of FIB concentrations in the wetland resulting from loading by urban runoff, bird feces, and sediment resuspension.

To summarize, nine different FIB concentration fields were predicted for the 15 day period beginning May 2, 2000 based on three different sources of three different FIB groups. Urban runoff loads were modeled by several point sources located at inland sites. Bird feces loads were modeled by a build-up, wash-off model: bacteria concentrations build up on inter-tidal mudflats and wash off (to surface waters) with the rising tide. Sediment loads were modeled by a non-point source that is scaled by the shear stress on the bed. For all nine predictions, the model accounts for FIB advection, dispersion, and die-off. Initial conditions for the model were obtained by a spin-up procedure. Starting with an FIB concentration of zero, predictions were made for two sequential 15 day periods, and results of the second 15 day period were saved and used for analysis purposes. Forcing data such as the ocean tide record, solar radiation data, and bird census data were simply duplicated into 30 day records. Finally, predictions were compared to FIB measurements at PCH and BRK monitoring stations (Fig. 1) reported by Grant et al. (2001, 2002). Water level, velocity, and turbidity data for PCH and BRK reported by Grant et al. (2001) were also utilized for model validation purposes.

2.3. Uncertainty in Model Predictions

Uncertainty in FIB predictions is due to several factors including: (a) approximations inherent to the mathematical representation of FIB transport processes; (b) errors incurred during the numerical solution of the mathematical model; and (c) uncertainty in model parameters and in particular, parameters that characterize point and non-point loads of FIB. Uncertainties in parameter values were estimated based on standard errors or literature reported values, where possible. Otherwise, a conservative estimate of 50% was used. Table 1 presents uncertainty estimates. In cases invol-

ving FIB concentrations, uncertainties may be 200–500%. By comparison, uncertainty associated with the mathematical model and numerical method are relatively small, roughly 20% and 1%, respectively, based on previous modeling efforts (Arega and Sanders, 2004). Therefore, the propagation of uncertainty in the model was ignored for the purpose of determining uncertainties in predicted FIB concentrations, and emphasis was placed on the uncertainty in loading terms (Holman, 1978). Hence, the relatively uncertainty in FIB predictions was assumed to be equal to the relative uncertainty in the corresponding FIB load. Based on the preceding model formulation for urban runoff, bird, and sediment loads of FIB, spatially and temporally averaged loading rates follow as:

$$L_R = (Q_R + \tau Q_P)C_R, \quad (7)$$

$$L_B = \overline{n_b}r, \quad (8)$$

$$L_S = sE \frac{(\overline{\tau})}{\tau_0} \left(\frac{\tau_0}{\tau_c} - 1 \right) \overline{A}_S, \quad (9)$$

where the overbar notation indicates a time-average value, the angled brackets indicate a spatial average, A_S represents the submerged surface area of the wetland, and the subscripts R, B, and S denote loads from runoff, bird droppings, and sediments, respectively. The upper limit of uncertainty was estimated by a conventional variational method (Taylor and Kuyatt, 1994), but this method predicted negative loads at the lower limit. Hence, the lower limit of the loads were estimated by computing the load based on lower limit parameter values. After upper and lower uncertainties for each of the nine FIB loads were estimated, these were normalized by the corresponding load to obtain relative uncertainties.

3. Results

Model predictions of water level and velocity during the study period compare well to measurements, as shown in Fig. 2. This indicates that the dominant circulation pattern in the wetland, which drives the mixing and flushing of FIB, is resolved. The spatial distribution of TC predictions at mid-flood tide are shown in Fig. 3 for the case of loading by bird feces (left panel), urban runoff (center panel) and sediment resuspension (right panel). For the case of loading by urban runoff, where FIB enter the wetland far inland along the channels and transport to the marsh during the ebb, the mid-flood condition highlights the transport of (assumed to be) FIB-free ocean water into the main channel of the marsh while remnant wetland water is displaced either into the fringes of the marsh or inland along the channels (note the gradient in FIB between the

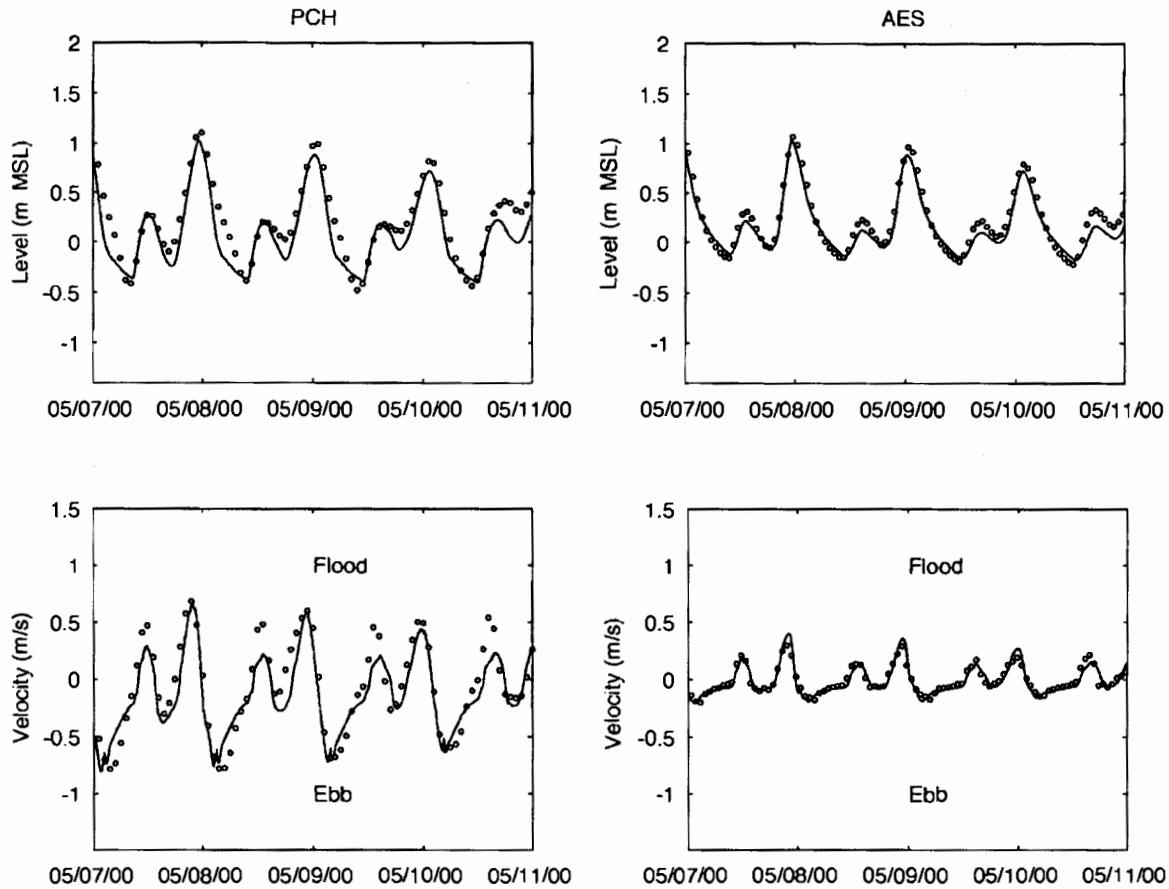


Fig. 2. Comparison of model predicted water level (top) and velocity (bottom) to data reported by Grant et al. (2001). Solid line corresponds to model prediction, symbols correspond to data.

main channel and the fringes of the marsh). For the case of loading by bird feces, model predictions illustrate the concentration of TC near the banks and over the shoals of the marsh. This is an expected response since FIB loading is modeled at the interface between wet and dry land. For the case of loading by sediment resuspension, FIB concentrations are relatively uniform across the marsh, compared to forcing by urban runoff or bird droppings. A similar distribution is predicted for EC and ENT.

Model predictions and measurements of FIB for the two-week study period are shown for BRK and PCH in Figs. 4 and 5, respectively, along with water level and turbidity. The tide record shows the spring-neap-spring transition. Note that water levels in the marsh do not drop far below -0.5 m-MSL due to hydraulic choking which occurs during the ebb at the outlet, where the minimum bed elevation is close to -0.7 m-MSL . FIB predictions vary considerably depending upon the type of loading, both in terms of magnitude and variability,

particularly at 1 and 2 cycles per day. In addition, the variability of each prediction appears unique. Therefore, the phasing and magnitude of FIB predictions for each load type (i.e., urban runoff, bird feces, or sediment) can be utilized to help determine the contribution towards observed FIB concentrations. Pearson correlation coefficients were computed to quantify how well each prediction captured the variability, or phasing, of measured FIB concentrations and are shown in Table 2. Mean values of each prediction, and uncertainty based on loading rate uncertainty, are listed in Table 3. Mean values of measured FIB, along with standard errors based on $N = 360$ are also shown for comparison purposes. The "combined" FIB time series referenced in Tables 2 and 3 represent the sum of the three FIB predictions (i.e., urban runoff, bird feces, and sediment), a valid operation for linear transport equations. That is, the combined FIB time series is precisely what the model would have predicted had each of the forcing factors been incorporated into a single

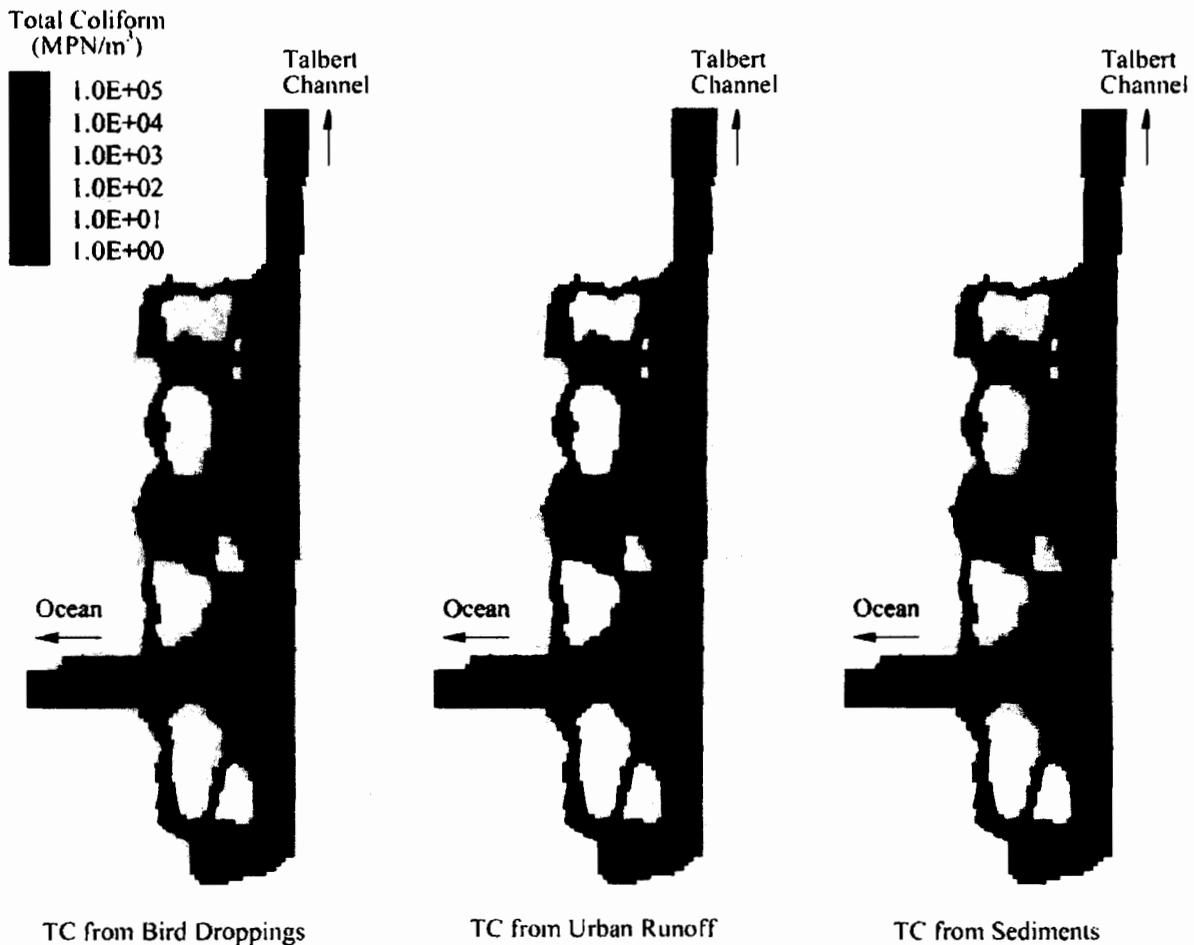


Fig. 3. Contours of total coliform in Talbert Marsh predicted by the model for mid-flood tide. Black lines indicate velocity direction and relative magnitude.

simulation. To obtain the mean value, the combined time series was first log-transformed. The combined series is not shown in Figs. 4 and 5, but at any given instant it basically tracks the largest of the three curves representing different forcing factors.

TC at PCH are predicted remarkably well based on loading by sediment resuspension, as shown in Fig. 5. The mean of log transformed measurements, $\log_{10}(\text{TC}) = 2.17(\pm 0.04)$, or “log mean”, compares well with the log mean of predictions $\log_{10}(\text{TC}) = 2.25(+0.45/-1.02)$; and there is a moderate correlation ($R^2 = 0.58$, $p_{N=360} < 0.01$) between log transformed predictions and measurements on an hourly basis. Predictions based on loading by urban runoff compare best to measurements at the end of the ebb tide, particularly during the second week of the study when pump stations contributed runoff to the channels, but not at other phases of the tide and this is reflected by a weaker but significant correlation ($R^2 = 0.37$, $p_{N=360} < 0.01$). Predictions based

on bird feces loading appear at least three orders of magnitude too small to account for observed TC.

Similar trends can be observed at BRK. Predictions based on both urban runoff and sediment loading are large enough to account for measured FIB, though in this case measurements correlate better to the prediction based on runoff ($R^2 = 0.56$, $p_{N=360} < 0.01$) than sediment resuspension ($R^2 = 0.26$, $p_{N=360} < 0.01$). The prediction based on bird feces loading is too small to account for observed TC. When predictions based on all three forcing factors are added together (valid for linear transport equations), the prediction at BRK correlates slightly better ($R^2 = 0.58$, $p_{N=360} < 0.01$) and the magnitude of the signals compare well (Table 3).

For ENT and EC, trends in model predictions are similar to TC. However, trends in measured FIB differ. Both ENT and EC measurements compare best to predictions based on sediment resuspension loading, both in terms of geometric mean concentrations

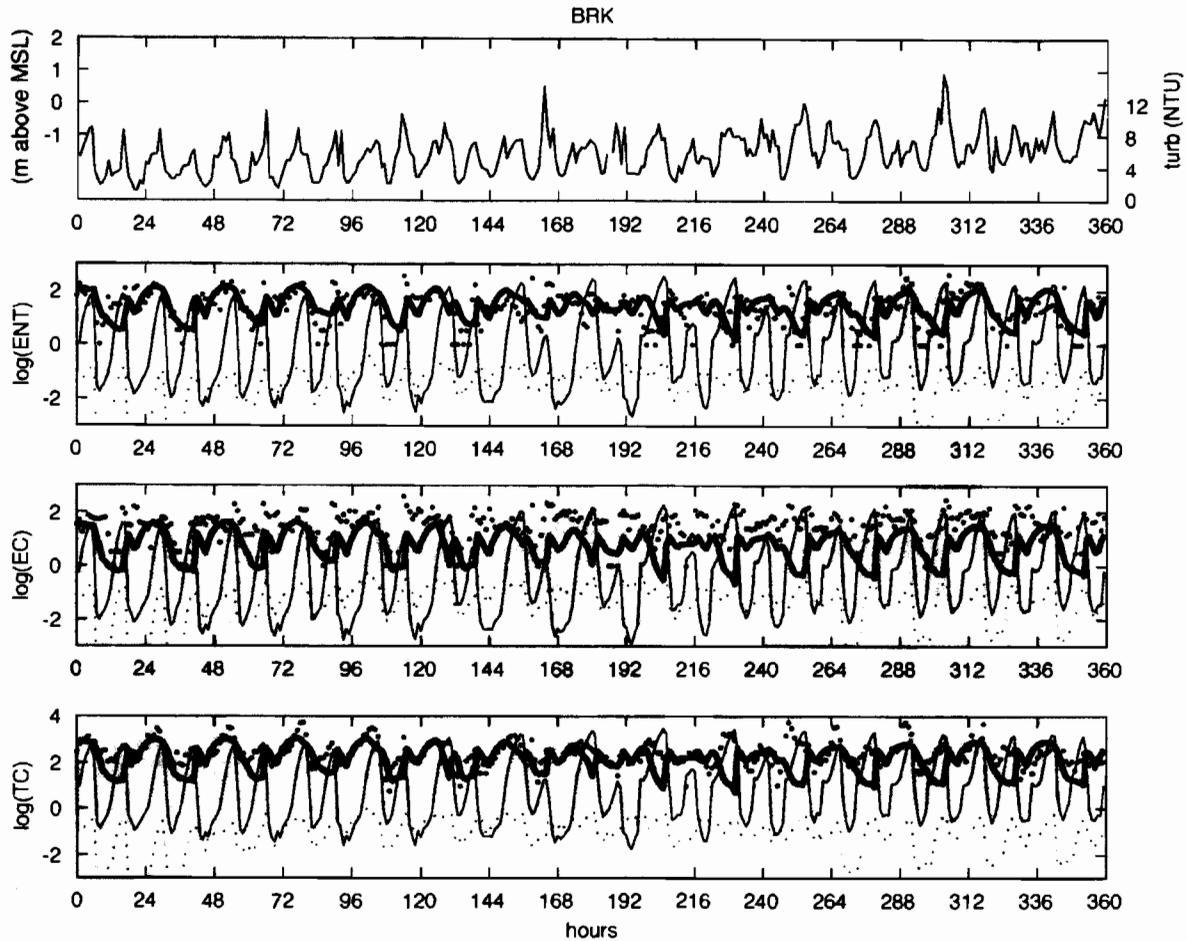


Fig. 4. BRK results. Water level and turbidity reported by Grant et al. (2001) shown in top panel. Bottom three panels show FIB concentrations: data from Grant et al. (2001, 2002) (dots), prediction based on sediment loading (heavy line), prediction based on runoff loading (light line), and prediction based on bird loading (broken line). FIB concentrations reported as log₁₀ (MPN/100 ml).

(Table 3) and the correlation coefficient (Table 2). Predictions based on bird feces loading are several log units too small to account for measured concentrations. Predictions based on urban runoff loading are comparable in magnitude only at the end of the ebb tide, and do not correlate to measurements.

Correlations between turbidity measurements and FIB measurements over the first six days were also computed and these appear in Table 4 (Due to drift in the turbidity data, the second week of data was excluded.) Turbidity correlates best to TC, compared to ENT and EC, and the correlation is stronger at BRK than PCH. Correlations between turbidity measurements and FIB predictions based on loading by urban runoff and sediment resuspension were also computed. Predictions based on urban runoff loads serve as an index of particulate material transported from upstream (fine mineral particles, detritus, and plankton) where

flow is quiescent, while predictions based on sediment loads serve as an index of material eroded locally in the lower reaches of the wetland where the shear is greatest. At BRK the turbidity signal correlates better with FIB predictions based on runoff forcing ($R^2 = 0.70$, $p_{N=144} < 0.01$) than FIB predictions based on sediment resuspension forcing ($R^2 = 0.19$, $p_{N=144} < 0.01$). At PCH the turbidity signal correlates slightly better with the prediction based on sediment resuspension ($R^2 = 0.57$, $p_{N=144} < 0.01$) than the prediction based on runoff ($R^2 = 0.47$, $p_{N=144} < 0.01$).

4. Discussion

Hydrodynamic model predictions show that tidal cycling of TC, EC, and ENT in Talbert marsh surface waters is driven primarily by two processes: advection of

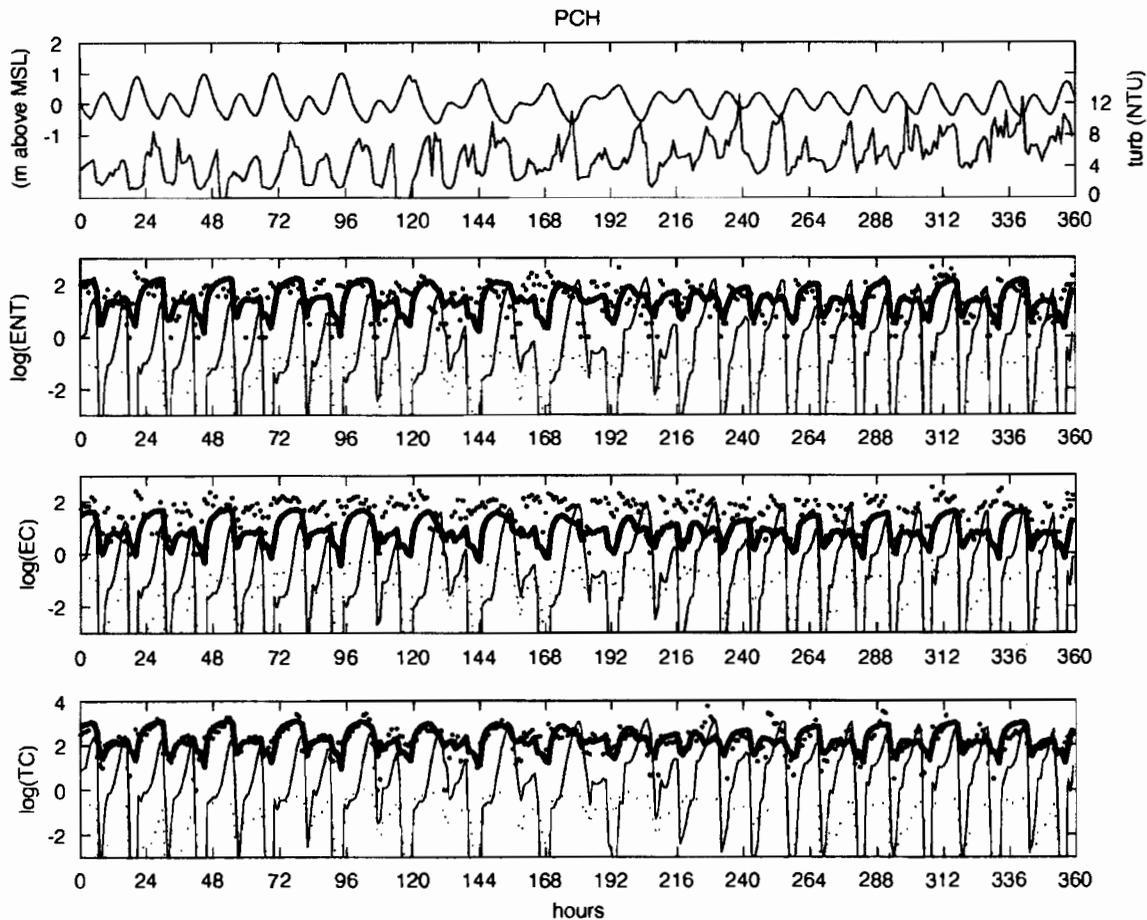


Fig. 5. PCH results. Water level and turbidity reported by Grant et al. (2001) shown in top panel. Bottom three panels show FIB concentrations: data from Grant et al. (2001, 2002) (dots), prediction based on sediment loading (heavy line), prediction based on runoff loading (light line), and prediction based on the bird loading (broken line). FIB concentrations reported as \log_{10} (MPN/100 ml).

FIB from inland sources (urban runoff) and entrainment of FIB from sediments. Loads of FIB from urban runoff control surface water concentrations inland within the poorly flushed zone while tidal resuspension controls surface water concentrations in the well-flushed zone near the mouth. Therefore, water quality models for FIB in hydrodynamically active wetland surface waters should at minimum account for loads from point sources (storm drains, channels, etc.), loads from resuspended sediments, transport by advection and turbulent dispersion/diffusion, and die-off. The present model captures tidal variability of TC better than EC or ENT suggesting either that processes important to EC and ENT transport are not included in the model, or perhaps the model oversimplifies one or more of the processes that are included in the model. For example, the spatial distribution of EC and ENT in sediments

may differ substantially from the TC distribution due to differences in survival and/or regrowth rates. Many studies have shown that FIB can survive for long periods or regrow attached to sediments and vegetation (Savage, 1905; Roper and Marshall, 1979; LaBelle et al., 1980; Davies et al., 1995; Desmarais et al., 2002). In tropical watersheds, regrowth has been cited as the dominant factor affecting bacteria loading in streams (Hardina and Fujioka, 1991; Fujioka et al., 1999). The ability of bacteria to secrete extracellular polymers (collectively termed microbial biofilms) may be one reason why survival and regrowth of FIB is enhanced in sediments (Decho, 2000). A model capable of simulating sediment concentrations of FIB, accounting for these factors, might lead to better EC and ENT predictions. In cases where the size of particles with attached FIB is known, settling can also be included in the model if size

Table 2
Pearson correlation between log transformed enteric bacteria measurements and model predictions ($N = 360$)

	Station	Bird source	Sed. source	Runoff source	Combined
Total	BRK	0.36*	0.26*	0.56*	0.58*
coliform	PCH	0.40*	0.58*	0.37*	0.55*
<i>E. coli</i>	BRK	0.33*	0.35*	0.04	0.39*
	PCH	0.28*	0.33*	0.10	0.22*
Enterococci	BRK	0.27*	0.47*	-0.02	0.36*
	PCH	0.23*	0.34*	0.04	0.24*

*Significant at the 0.01 level (2-tailed).

Table 3
Comparison between predicted and measured geometric mean bacteria concentrations [$\log_{10}(\text{MPN}/100 \text{ ml})$]

	Station	Bird source	Sed. source	Runoff source	Combined	Measured
Total	BRK	-1.12 (+0.49/ - 0.43)	2.14 (+0.45/ - 1.02)	0.81 (+0.20/ - 0.47)	2.42 (+0.45/ - 1.02)	2.38 (± 0.03)
coliform	PCH	-1.39 (+0.49/ - 0.43)	2.25 (+0.45/ - 1.02)	-0.18 (+0.20/ - 0.47)	2.33 (+0.45/ - 1.02)	2.17 (± 0.04)
<i>E. coli</i>	BRK	-1.38 (+0.83/ - 0.40)	0.76 (+0.63/ - 1.21)	-0.36 (+0.20/ - 0.44)	1.10 (+0.63/ - 1.21)	1.49 (± 0.03)
	PCH	-1.65 (+0.83/ - 0.40)	0.86 (+0.63/ - 1.21)	-1.35 (+0.20/ - 0.44)	0.98 (+0.63/ - 1.21)	1.53 (± 0.03)
Enterococci	BRK	-1.56 (+0.56/ - 0.44)	1.39 (+0.43/ - 0.98)	-0.11 (+0.18/ - 0.44)	1.61 (+0.43/ - 0.98)	1.34 (± 0.03)
	PCH	-1.85 (+0.56/ - 0.44)	1.42 (+0.43/ - 0.98)	-1.10 (+0.18/ - 0.44)	1.49 (+0.43/ - 0.98)	1.38 (± 0.04)

Uncertainty of predictions is shown along with standard error of measurements ($N = 360$).

Table 4
Pearson correlation between turbidity measurements and bacteria predictions and measurements for first six days of study ($N = 144$)

	Station	Sed. source	Runoff source	Combined	Measured
Total	BRK	0.19	0.70*	0.51*	0.56*
coliform	PCH	0.57*	0.47*	0.59*	0.41*
<i>E. coli</i>	BRK	0.20	0.70*	0.56*	0.20
	PCH	0.57*	0.48*	0.60*	0.14
Enterococci	BRK	0.26*	0.70*	0.53*	0.31*
	PCH	0.58*	0.47*	0.60*	0.14

*Significant at the 0.01 level (2-tailed).

dependent settling rates are also known. This would be particularly important if FIB were associated with particles larger than 10–15 μm , in which case accurate settling data would be crucial for reliable predictions.

Both turbidity and FIB are generally associated with fine particles, but in this as well as previous studies (Goyal et al., 1977; Jensen et al., 1979) a strong association between the two has not been observed. In Talbert Marsh, peaks in turbidity and TC are observed at low tide, when brackish water from the upper reaches of the wetland is translated furthest seaward (Figs. 4 and 5). Hence, urban runoff is clearly contributing to the TC

signal. On the other hand, there are not clearly defined peaks in the EC and ENT measurements and in many cases EC and ENT are elevated when turbidity values are relatively small. If sediments are the source of these FIB, a possibility strongly supported by model predictions shown here, shear stresses on the bed must be large enough to disturb and saltate surficial sediments, large enough to mix small particles, colloidal matter, and FIB through the water column, but not large enough to suspend the sandy sediments more than a short distance above the bed. Recall that sediments consist of beach sands and silts near the outlet. Hence, water quality

models designed to account for the effects of sediment resuspension should be sensitive to differences between the rate of sediment entrainment, and the rate of FIB entrainment. Sediment entrainment formulations adopt the notion that mass transfer occurs when the shear stress on the bed exceeds a certain threshold (Mehta and Dyer, 1990). The entrainment of FIB in surficial pore water or incorporated into microbial biofilms may occur at a much smaller threshold.

The significance of loading due to sediment resuspension explains why tidal wetlands serve to “generate” FIB, as was reported by (Grant et al., 2001). That is, FIB associated with sediment particles, colloidal organic matter, or free living in porewater are supplied to the water column when bottom sediments are disturbed and/or scoured by tidal currents. FIB input to wetlands from wet or dry weather surface water runoff may be temporarily stored in sediments and later resuspended during storm events or during tidal scouring. The relative magnitude of resuspension effects versus die-off and settling effects is likely to control whether or not coastal wetlands are net generators or net accumulators. The results of this study are important to temper expectations that hydrodynamically-active wetlands such as estuaries or streams can provide passive treatment of urban runoff with high concentrations of FIB.

Reeves et al. (2004) reported that over 99% of the annual load of FIB from Talbert Watershed runoff is shed during storm events, while less than 1% is shed during dry-weather periods. It is therefore likely that sediments serve to couple FIB loads from storm water runoff to dry-weather water quality. Additional studies are warranted to characterize the variability of FIB in sediments over seasonal to tidal time scales and in response to storm events, to characterize the spatial variability of FIB, and to understand the mechanisms driving this variability. Do these organisms die-off, deposit, stimulate regrowth, and/or pass through the wetland? Microbiological source tracking methods (DNA fingerprinting, etc.) could also be applied to assess whether FIB in sediments are linked to human sources of fecal pollution (Simpson et al., 2002; Scott et al., 2002).

5. Conclusions

This study successfully employed a first-principle model to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California. Model predictions show that surface water concentrations of TC, EC, and ENT in the wetland are driven by loads from urban runoff and resuspended wetland sediments. The model more accurately predicts TC than EC or ENT.

The crucial role that sediments play in the cycling of FIB is highlighted by this study. Sediments function as a reservoir of FIB that may accumulate FIB due to regrowth or settling, or shed FIB when tidal currents or storm flows scour away or even just disturb surficial particles. This finding is important to temper expectations that hydrodynamically-active wetlands serve to “treat” FIB from runoff and other sources, and it also explains why wetlands can function as net generators of surface water FIB. That is, generation occurs when the entrainment rate exceeds the rate of die-off and settling.

Additional studies should be conducted to characterized the “memory” of sediments relative to FIB. Knowing the extent to which dry-weather sediment concentrations of FIB are linked to wet-weather runoff loads, dry-weather runoff loads, regrowth or other factors such as bird droppings would help determine which factors predominately control dry-weather water quality. Additional studies should also be conducted to evaluate the size and settling velocities of particles associated with FIB, and the partitioning of FIB between free-living and particle-associated states. Improved predictions of FIB might result from separately modeling free-living and particle-associated FIB.

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