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

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ABSTRACT

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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 implementation 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.

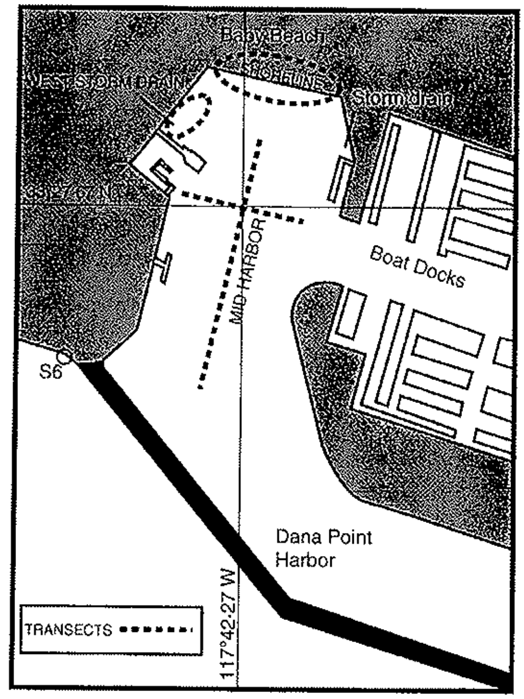

Fig. 1 Sampling locations at Dana Point Baby Beach

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 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 m^3 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

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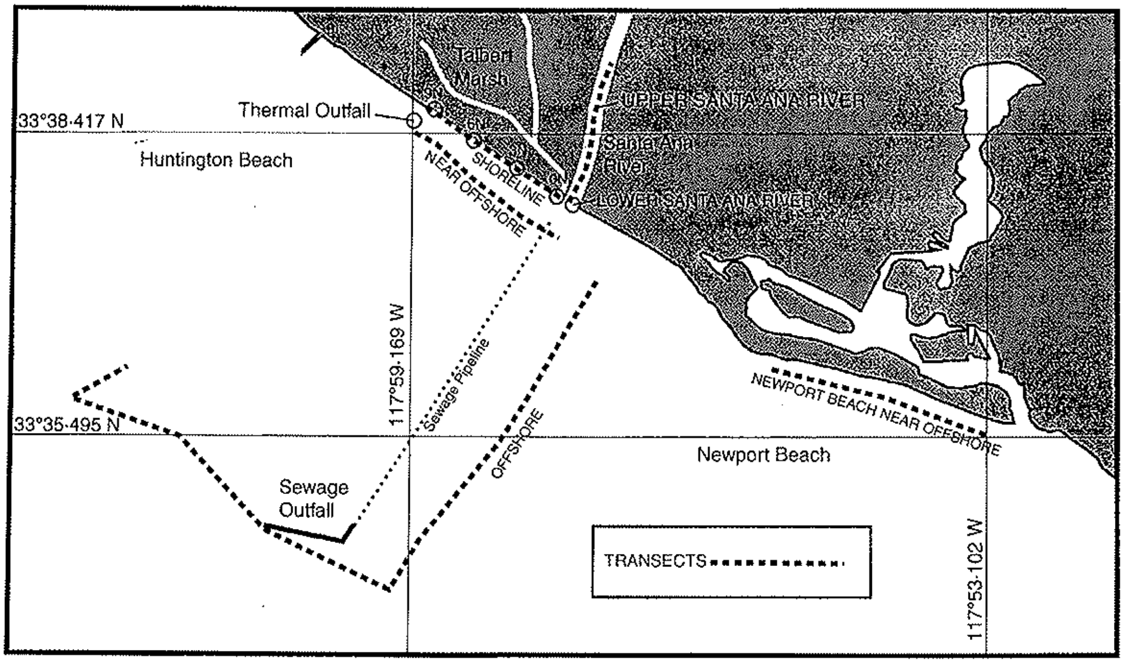

Fig. 2 Sampling locations at Huntington State Beach

are also diverted during summer. A sewage outfall lies 7·6 km offshore from the SAR mouth and releases *c.* 10^6 m^3 per day of chlorine treated sewage in 60 m of water. A thermal outfall of a power plant, located *c.* 700 m offshore discharges a maximum of $1{\cdot}9 \times 10^6$ 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 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 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

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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 10^5 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	% Positive	Concentration*		% Positive	Concentration		% Positive	Concentration	
	samples	samples	Geomean	Maximum	samples	Geomean	Maximum	samples	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

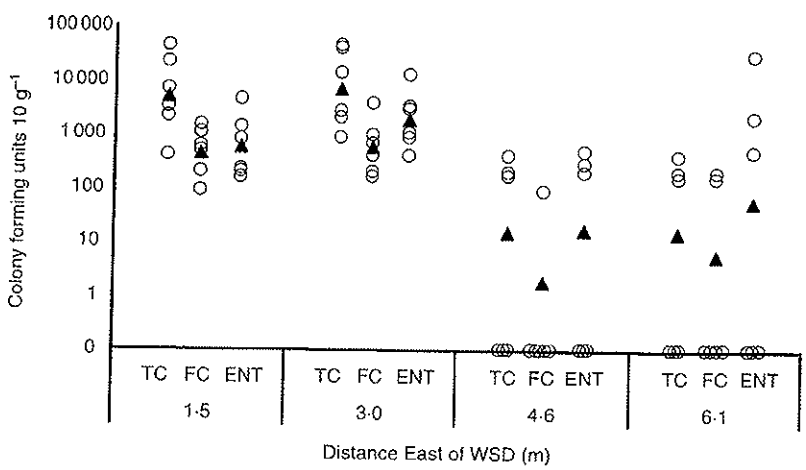

Fig. 3 Temporal and spacial variability of faecal indicator concentrations in sediments from four West Storm Drain sites sampled six times over 14 weeks, ○, concentration; ▲, geometric mean; TC; total coliforms; FC, faecal coliforms; ENT, *Enterococcus*

Table 3 Enterococcus species distribution in water and sediment samples

	No. samples	No. isolates	Number (%) of isolates: *E. faecalis*	*E. faecium*	*E. hirae*	*E. casseliflavus*	*E. mundtii*	*E. gallinarum*	*E. durans*	*E. avium*	Other ENT*	*S. bovis*	STR	AER	Other, not ENT†
Dana Point Baby Beach															
Shoreline water	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)
West Storm Drain water	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)
West Storm Drain sediments	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															
Shoreline water	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)
Upper and lower SAR sediments	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)
Near offshore sediments	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)
Total	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 malodratus* isolate.
†Sixty unidentified non-*Enterococcus* spp., one *Lactococcus* spp. and one *Helcococcus* spp.
ENT, *Enterococcus* spp.; STR, *Streptococcus* spp.; AER, *Aerococcus* spp.

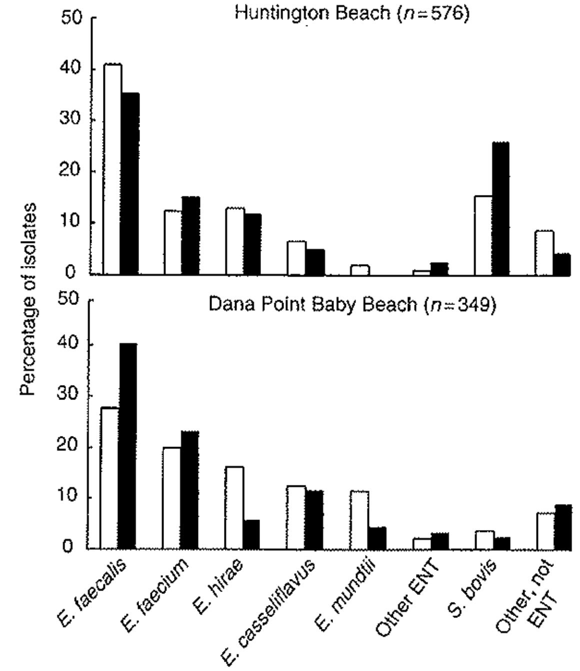

Fig. 4 Distribution of *Enterococcus* and related species in recreational marine water samples collected during ambient (□) and exceedance (■) conditions

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 ($P = 0{\cdot}13$ and $P = 0{\cdot}10$, 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 sediments 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 Moracambe 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 Moracambe 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 (BBBSSR 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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