Environmental Microbiology (2007) doi:10.1111/j.1462-2920.2007.01319.x

Frequent occurrence of the human-specific *Bacteroides* fecal marker at an open coast marine beach: relationship to waves, tides and traditional indicators

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Summary

Molecular genetic markers, such as those from fecal *Bacteroides* microorganisms, can link microbial pollution with its source, and have been used successfully in studies of sheltered aquatic environments. Their applicability to wave-driven, open coast environments has not been tested. We assessed the contribution of a tidal outlet to surf zone water quality in coastal Orange County, California, USA by measuring three traditional culture-based fecal indicator bacteria (FIB) as well as the human-specific *Bacteroides* molecular marker (HF marker) at four shoreline locations. We found that total and fecal coliform levels were higher during low tides than high tides at two of the four stations, and that this effect was strongest at the mouth of the tidal lagoon and decayed with distance from the outlet. The HF marker was detected in 23% and 47% of samples from the tidal outlet and 26% and 41% of samples from an adjacent recreational beach in 2005 and 2006 respectively. Surprisingly, the station farthest from the tidal outlet had the highest occurrence of the HF marker. We found no relationship between FIB abundance and occurrence of the HF marker for individual samples, but that when the data were considered together by year, higher FIB abundance was correlated with a higher incidence of the HF marker. DNA sequences of the HF marker recovered from this site were > 99% similar to those recovered from other states and countries, suggesting low global diversity of this marker. These data provide strong support for the idea that multiple time points and physical conditions should be considered when assessing coastal water quality.

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Introduction

As recreational beach closures and advisories become more frequent across the nation (Dorfman, 2006), coastal managers are searching for tools that allow for discrimination between different sources of pollution. Of particular interest are tools that allow identification of pollution that originates from a human fecal source, as these sources are likely to be enriched in human pathogenic bacteria and viruses. One of these beaches is Huntington Beach, California, USA. Huntington State Beach (HSB) has been the subject of numerous investigations regarding the source and variability of fecal indicator bacteria (FIB) to the nearshore environment (Grant *et al*., 2001; Boehm *et al*., 2002; Noble and Xu, 2004; Rosenfeld *et al*., 2006). FIB include three groups of organisms: total coliform bacteria, fecal coliforms (FC) and enterococci (ENT). While FIB themselves are not responsible for causing illness, their presence in fresh and marine waters has been correlated with an increased risk of gastrointestinal illness among bathers (Cabelli *et al*., 1979; Kay *et al*., 1994; Haile *et al*., 1999). Wet-weather pollution is typically attributed to stormwater runoff associated with rainfall events (Dwight *et al*., 2002; Reeves *et al*., 2004; Ahn *et al*., 2005). The sources of dry-weather pollution events, the season of greatest economic and social importance to Southern California beaches (Given *et al*., 2006), remain more elusive (Noble *et al*., 2006a; Rosenfeld *et al*., 2006). Urban runoff channelled through the nearby Talbert Marsh and Santa Ana River (SAR) have been suggested as potential sources of dry weather pollution (Grant *et al*., 2001; Kim *et al*., 2004) and in response to this, in the year 2000 the Orange County Sanitation District (OCSD) began diverting and treating up to 2.6 million gallons of dry-weather urban runoff per day (CH2M Hill, 2004). Whether or not high levels of FIB are also associated with the presence of human-sourced waste in dry weather pollution events is the focus of this study.

The physical environment can play an important role in modulating the levels of FIB and other contaminants in the coastal ocean, whether from urban runoff or other sources. Water temperature (Boehm *et al*., 2004), sunlight (Sinton *et al*., 1999; Boehm *et al*., 2002) and

wave-driven currents (Noble and Xu, 2004; Grant *et al*., 2005) may all influence the abundance of FIB in the surf zone. Tides, in particular, modulate the position of the land-water interface and bring the coastal ocean in contact with potentially polluted surfaces on land. The management implications of tidal control of microbial pollution are clear; beach managers desiring to make conservative decisions about coastal water quality would have to take tide level into consideration when monitoring FIB levels or conducting site assessments. Currently in Orange County, water samples are taken in the early morning, irrespective of tide level. In other parts of California, samples of beach water quality may be taken as infrequently as every 2 weeks.

If urban runoff is a source of FIB to coastal waters, how often does it also contain waste from human sources? Recent epidemiological sources have suggested that when non-human sources contribute significantly to FIB levels, the dose–response relationship between FIB and gastrointestinal illness breaks down (Colford *et al*., 2007). One approach to answering this source-tracking question is the molecular detection of alternative indicator organisms. *Bacteroides* spp. organisms occur at levels up to three orders of magnitude larger than fecal coliform bacteria in human sewage (Gerba, 2000), and exhibit 'host-specific' patterns meaning that fecal organisms from different animals can be distinguished on the basis of genetic sequence (Bernhard and Field, 2000a; Dick *et al*., 2005). An additional advantage to this method is that *Bacteroides* spp. organisms are obligate anaerobes, limiting their potential for regrowth in the marine water column and surface sediments (Walters and Field, 2006). A test of multiple source-tracking methods in spiked seawater samples found that the host-specific *Bacteroides* assay performed the best of 12 methods examined, correctly identifying 100% of human fecal or sewage spiked samples with no false positives (Griffith *et al*., 2003). In field studies, the host-specific *Bacteroides* assay has been used to assess agriculturally impacted bays (Bernhard and Field, 2000b; Shanks *et al*., 2006), groundwater (Boehm *et al*., 2003), urban watersheds (Noble *et al*., 2006b), and freshwater lakes and rivers (Bower *et al*., 2005; Walters *et al*., 2007) to document the presence of human-sourced microbial pollution. The applicability of this assay to open coast beaches—which comprise the majority of the North American coastline and support the highest numbers of bathing public—has not been tested.

In this study, we conducted a time-intensive sampling of an approximately 9-km stretch of shoreline extending north and south of a major tidal outlet, sampled at low and high tide every night for 1 month in each of two summers (Fig. 1). The study was designed to address issues broadly applicable to coastal management and risk monitoring, specifically to determine: (i) if tidal variability affects microbial pollution levels at bathing beaches to the extent it should be considered in monitoring plans, (ii) the sources of beach advisory-causing indicator bacteria emanating from tidal outlets and whether they present a risk to human health and (iii) if culture-independent molecular source-detection methods can be used effectively in open coast environments.

Results and discussion

Physical variables

All sites were similar in their mean temperature and salinity throughout the study period for both years (Table 1). The lack of a salinity depression in ebb flow from the SAR is a consequence of the very small contribution of fresh, urban runoff (less than 1%) to the total flow, and the importance of evaporation in the shallow lagoon. The open coast stations had higher turbidity than SAR attributable to resuspension of particles by breaking waves in the surf zone; a phenomena that occurs to a lesser extent in the river mouth. Solar heating and oxygen depletion in the upper reaches of the SAR tidal prism are reflected in the higher mean water temperature and lower dissolved oxygen content measured at the mouth during low tides as water from the lagoon is carried back to the ocean.

In both years, wave direction was predominantly from the south, with 93% and 90% of wave observations originating between 125° and 215° from true north in 2005 and 2006, respectively (*Supplementary materials*, Fig S1). Waves originating from these directions are predicted to drive an upcoast alongshore current in the vicinity of the SAR outlet. Using the highly simplified model of Longuet-Higgins (Longuet-Higgins, 1970), the mean magnitude of this current was 56 cm s^{-1} in 2005 and 52 cm s^{-1} in 2006, sufficient enough to transport water from the SAR outlet to HSB and Huntington Pier (HP) from the onset of the ebbing tide to sample collection. This estimate is on the order of previous littoral current estimates made from models and in-situ dye tracer experiments (Boehm, 2003; Grant *et al*., 2005).

FIB abundance

Time series of all the FIB data collected in this study are presented in Supplementary Fig. S2. In 2005, FIB abundance was greatest at the SAR and adjacent HSB (Table 1). The most frequently exceeded water quality standard was the FC standard; 57 and 27% of the samples, at SAR and HSB, respectively, exceeded the California state single-sample standard for contact recreation for FC. ENT levels, however, were relatively low at all stations throughout 2005 with only five samples

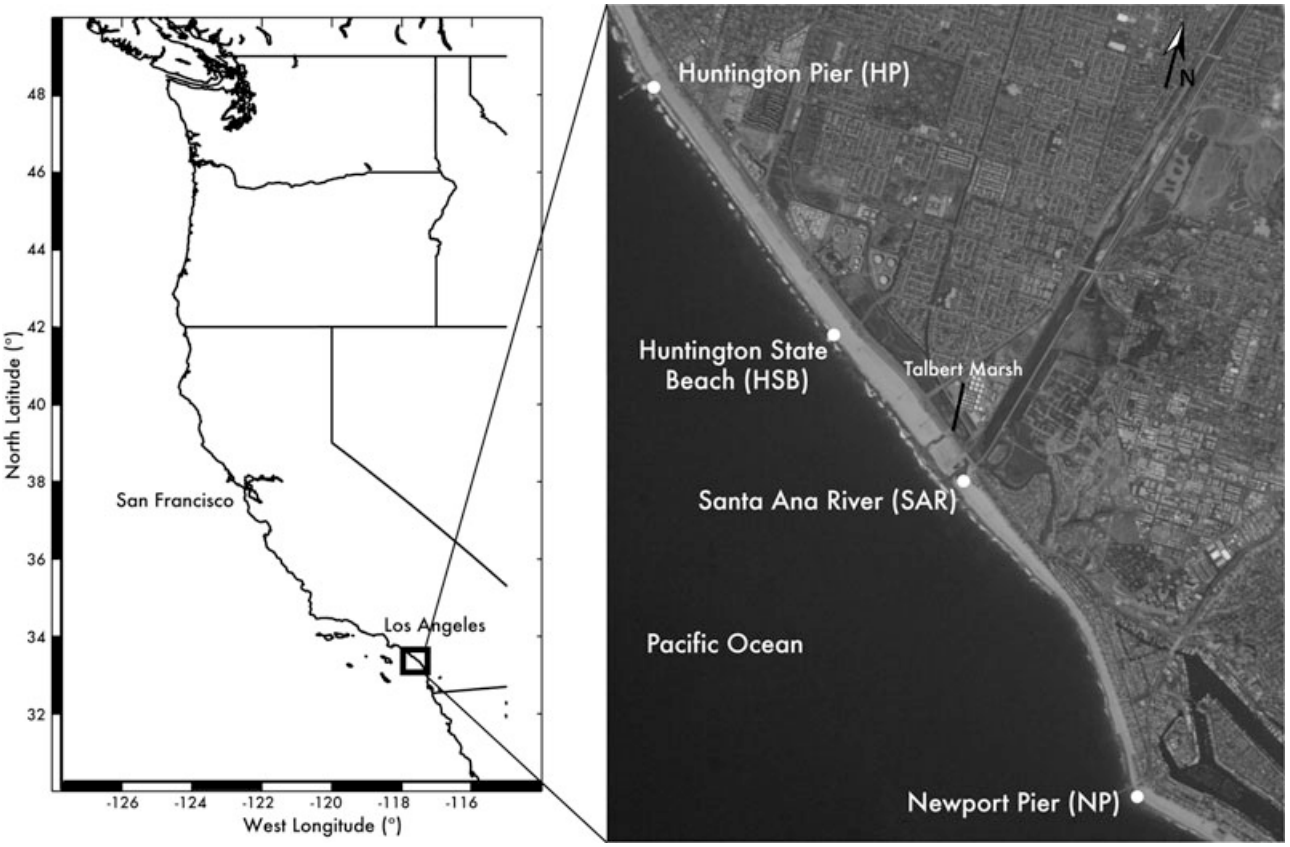

Fig. 1. Locations of coastal water and sediment sampling in Orange County, California, USA. Coastline generated using the Coastline Extractor from the National Geophysical Data Center (http://rimmer.ngdc.noaa.gov/coast/). Satellite image courtesy of the Image Science and Analysis Laboratory, National Aeronautics and Space Administration, Johnson Space Center (Image ISS005-E-18661, http://eol.jsc.nasa.gov).

exceeding the state single-sample ENT standard for all stations. This was particularly interesting given the history of high ENT levels at HSB, where these bacteria were responsible for over 70% of beach postings during the summers of 1999–2000 (Grant *et al*., 2001). Farther from the tidal outlet of the SAR, both HP and Newport Pier (NP) had low FIB abundances with just six and two samples, respectively, exceeding state single-sample FC standards. The only station to exceed the total coliform standard (TC) at any time in 2005 was SAR, which did so seven times.

Water quality, as measured by FIB abundance, was worse in the second year of the study with nearly half of the samples taken at SAR and HSB exceeding the California state single-sample standard for water quality recreation for FC in 2006 (25 of 54 at each station). In contrast to SAR and HSB, NP had only four exceedences for FC during the same period and HP had only 7. Nearly 30% (16 of 54) of the samples taken at HSB in 2006 exceeded the state standards for ENT abundance, an anomaly among the four stations. The other three stations together had less than half the ENT exceedences of HSB (six samples total between HP, SAR and NP). Once again in 2006, the TC standard was violated the fewest number of times, with only one sample at HSB and three samples at SAR exceeding this metric. Our data represent a conservative (i.e. greater) report of FIB abundance because all samples were taken at night when FIB levels are at their maximum (Boehm *et al*., 2002).

There was a significant effect of tide level (high vs. low) on FIB abundance. Mean total TC abundance was greater during low tide at two stations: the tidal outlet (SAR) and the station directly upcoast of the outlet (HSB) for both years (Fig. 2, Student's *t*-test, $P < 0.001$). Fecal coliform concentrations were also significantly greater at low tide at SAR in both years, and at HSB in 2005. This tidal effect was also observed at the next station upcoast (HP), although the effect was not significant. Downcoast of the SAR outlet at NP, there were no significant differences in abundance between high and low tide for any of the three FIB.

ENT abundance was significantly greater in low tide samples, at HSB in 2005 and SAR in 2006. However, in contrast to TC and FC, ENT abundance was greatest at HSB and not the tidal outlet at SAR. This suggests a different source of ENT to the surfzone at HSB than the other fecal indicators that is located upcoast of SAR.

Table 1. Arithmetic mean and standard deviation (parentheses) of physical water quality parameters and log mean and standard deviation (parentheses) of biological water quality parameters measured during the study period at four stations in Orange County, CA.

	HP		HSB		SAR		NP	
	High tide	Low tide	High tide	Low tide	High tide	Low tide	High tide	Low tide
August 2005								
Temperature (°C)	19.49	18.23	19.32	18.27	18.92	20.93	19.07	18.27
	(0.97)	(1.50)	(1.03)	(1.47)	(1.19)	(1.44)	(1.17)	(1.52)
Salinity (psu)	32.89	33.09	33.13	32.98	33.26	33.34	33.12	33.08
	(0.79)	(0.26)	(0.37)	(0.73)	(0.24)	(0.29)	(0.35)	(0.31)
Turbidity (NTU)	46	61	59	25	8	9	27	35
	(32)	(127)	(108)	(29)	(2)	(5)	(20)	(34)
Dissolved oxygen	97	93	92	94	94	76	94	93
(% saturation)	(20)	(17)	(20)	(16)	(27)	(23)	(21)	(24)
Total coliforms	2.73	2.82	2.75	3.20	2.92	3.80	2.54	2.49
(log MPN/100 ml)	(0.28)	(0.30)	(0.41)	(0.34)	(0.38)	(0.31)	(0.30)	(0.38)
E. coli	1.77	2.01	1.95	2.51	2.32	2.85	1.46	1.22
(log MPN/100 ml)	(0.71)	(0.54)	(0.47)	(0.37)	(0.47)	(0.29)	(0.89)	(0.60)
Enterococci	0.720	0.89	1.22	1.35	0.57	0.892	0.500	0.331
(log MPN/100 ml)	(0.74)	(0.65)	(0.55)	(0.68)	(0.67)	(0.55)	(0.64)	(0.51)
July 2006								
Temperature (°C)	20.99	20.40	20.62	20.65	20.47	24.00	20.81	20.82
	(2.68)	(2.51)	(2.72)	(2.44)	(2.84)	(1.62)	(2.58)	(2.37)
Salinity (psu)	33.51	33.86	33.34	33.87	33.92	33.73	33.71	33.74
	(0.65)	(0.15)	(0.63)	(0.14)	(0.15)	(0.32)	(0.42)	(0.43)
Turbidity (NTU)	121	46	365	60	12	17	131	100
	(178)	(56)	(434)	(120)	(4)	(8)	(324)	(231)
Dissolved oxygen	92	89	93	90	84	67	85	87
(% saturation)	(12)	(20)	(12)	(12)	(22)	(27)	(31)	(32)
Total coliforms	2.64	2.75	2.79	3.24	2.30	3.79	2.21	2.16
(log MPN/100 ml)	(0.56)	(0.58)	(0.35)	(0.54)	(0.34)	(0.27)	(0.57)	(0.30)
E. coli	2.00	2.01	2.37	2.66	1.75	3.04	1.62	1.35
(log MPN/100 ml)	(0.67)	(0.67)	(0.42)	(0.71)	(0.62)	(0.47)	(0.90)	(0.61)
Enterococci	0.795	0.700	1.49	1.50	0.566	1.11	0.641	0.473
(log MPN/100 ml)	(0.68)	(0.72)	(0.73)	(0.91)	(0.60)	(0.75)	(0.83)	(0.68)

Stations are listed north to south.
HP, Huntington Pier; HSB, Huntington State Beach; NP, Newport Pier; SAR, Santa Ana River mouth.

Spearman's rank correlation (a non-parametric test of covariation) was used to explore the FIB abundance relationships between sites. TC and FC levels between the SAR and HSB stations are well correlated ($P < 0.001$), but are not correlated between other sites. ENT levels are also correlated between SAR and NP in 2006, although this is likely a statistical artefact of the large number of samples below detection limits for ENT at these sites.

Taken together, the results of the tide level and covariation analyses suggest that tidal flushing of the SAR is a major source of both TC and FC to the recreational beaches north of the river mouth as has been suggested by previous researchers (Grant *et al*., 2005). This conclusion is further supported by the predicted upcoast current in the surfzone created by incoming waves incident to the shoreline that could transport FIB discharged from the river mouth along the coast. The SAR does not, however, appear to be responsible for the large ENT levels observed at HSB. Kim and coworkers (Kim *et al*., 2004) analysed hourly data from four 48-h periods at points north and south of the river outlet and reached similar conclusions regarding the sources of TC and ENT to the surfzone at HSB, i.e. that the SAR was a source of TC but not ENT to the shoreline and that ENT were likely originating from the outlet of the Talbert Marsh. As low points for urban runoff, and as areas frequented by sea birds, wetlands are frequently implicated as important sources of FIB to coastal waters (Grant *et al*., 2001; 2005; Sanders *et al*., 2005; Evanson and Ambrose, 2006).

A fortnightly variability in FIB concentrations associated with the spring-neap tide cycle has been observed at this site and across Southern California (Boehm *et al*., 2002; Boehm and Weisberg, 2005; Rosenfeld *et al*., 2006). Some researchers have suggested this fortnightly cycle is actually an artefact of the once-daily morning sampling regime (Noble *et al*., 2006a). Early morning summer time 'spring-tide' sampling at the field site are always 'low-tide' samples, thus it is difficult to distinguish between the effect of tide level (high vs. low) and tide range (spring vs. neap) on summer time FIB levels using regional monitoring data. The data collected in this study can be used to separate these effects because samples were taken throughout the spring-neap tidal cycle at high and low

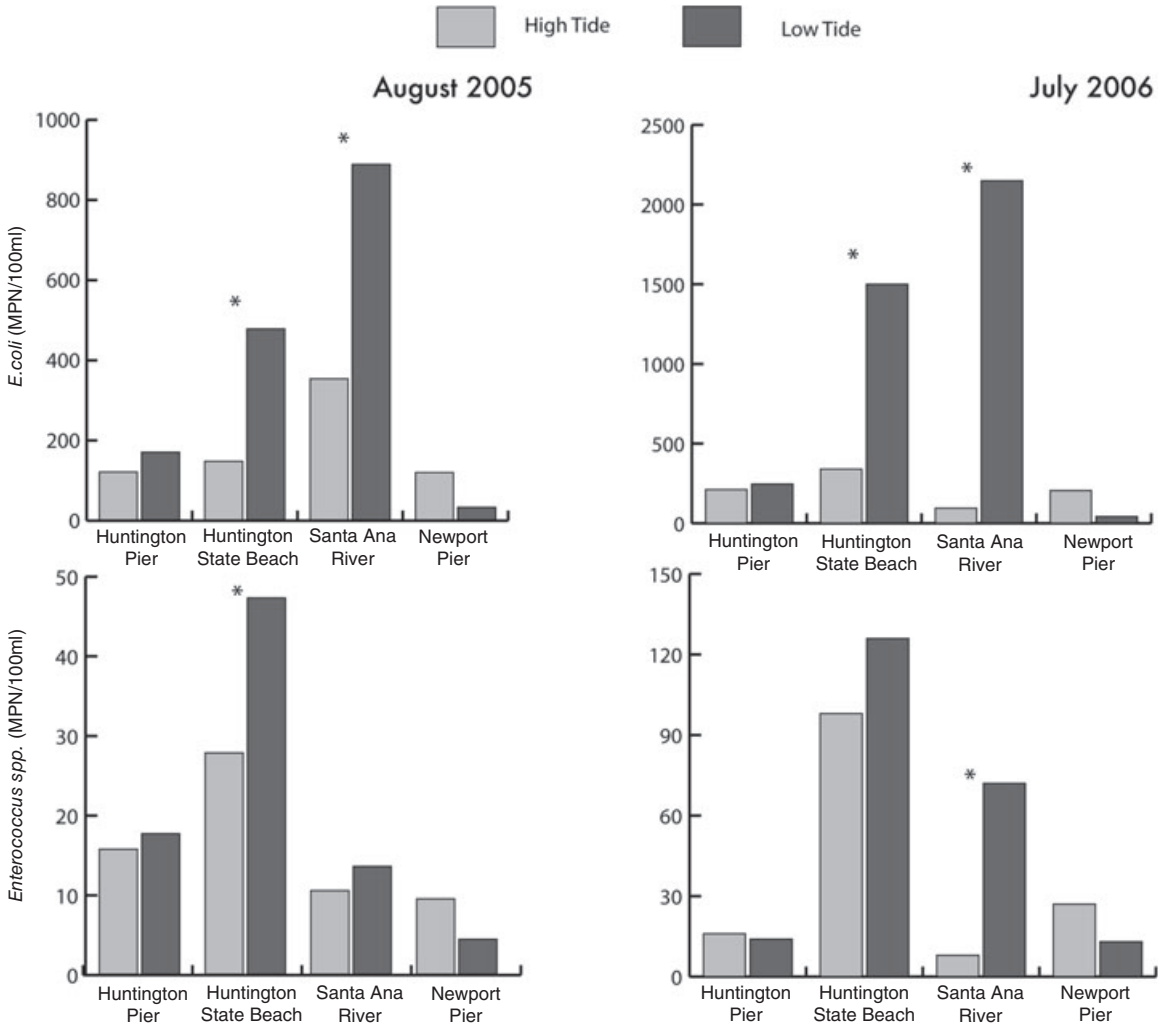

Fig. 2. Mean fecal indicator bacteria (FIB) abundance at high and low tides at four stations in southern California (listed from north to south). Note that different scales are used on the vertical axis for each plot. Samples were taken at night during August 2005 and July 2006. Significant differences between high tide and low tide (Student's *t*-test on log-transformed data, $P < 0.05$) are indicated with an asterisk (*).

tide. We used an N-way ANOVA including tide level, tide range and the interaction between the two to determine significant effects on FIB levels for each site in each year (Table 2). In 2005, the only site with a significant effect of tide range was HSB for TC ($F = 6.86$, $P = 0.01$). Newport Pier showed a significant interaction of tide level and tide stage for FC ($F = 4.88$, $P < 0.05$). Spring-neap effects were more apparent in the 2006 data set. Huntington State Beach, SAR and NP all had significant spring-neap variability in TC levels; HP, HSB and NP had significant effects for FC; and SAR and NP for ENT. In all cases, the effect was that FIB levels were greater during the spring tide. Our data together with the aforementioned reports of spring-neap variability indicate that multiple tide conditions (range and level) need to be considered when assessing water quality in open coast environments, especially adjacent to tidal outlets.

Presence of the HF marker at coastal beaches and relationships to biological and physical water quality conditions

Our detection limit for the HF marker was < 1 µl of primary sewage per litre of seawater, including losses to filtration and DNA extraction. All negative reactions spiked with positive control showed amplification of the HF marker, indicating minimal inhibition of the polymerase chain reaction (PCR).

Evidence of human-sourced pollution was apparent at all stations examined (SAR, HSB and NP). We detected the presence of the HF marker in 27 and 53 samples or approximately 25 and 50% of the samples analysed in 2005 and 2006, respectively (Table 3). Surprisingly, at the NP station, a station with relatively low FIB abundances, 29 and 67% of samples tested positive for the HF marker

Table 2. Stations and fecal indicator bacteria (FIB) groups showing a significant effect ($P < 0.05$) of spring-neap tidal range (RANGE) or an interaction between tide range and tide level (high vs. low, RANGE × LEVEL) as tested by an N-way ANOVA with FIB abundance as the dependent variable and tide RANGE and the interaction between tide range and tide level included as random effects.

Year	Station	FIB	F	*P*	Model terms
2005					
	HSB	TC	6.86	0.012	RANGE
	NP	FC	4.88	0.032	RANGE × LEVEL
2006					
	HSB	TC	7.64	< 0.001	RANGE
	HSB	TC	4.18	0.046	RANGE × LEVEL
	NP	TC	7.58	0.008	RANGE
	NP	TC	4.69	0.035	RANGE × LEVEL
	SAR	TC	7.45	0.009	RANGE
	HP	FC	6.30	0.015	RANGE
	NP	FC	9.12	0.004	RANGE
	NP	FC	4.12	0.048	RANGE × LEVEL
	NP	ENT	8.85	0.004	RANGE
	NP	ENT	7.85	0.007	RANGE × LEVEL
	SAR	ENT	9.45	0.003	RANGE

In all cases, spring or spring-low tides had higher FIB abundance.
ENT, enterococci; FC, fecal coliforms; TC, total coliforms.

in 2005 and 2006 respectively. Sites did not differ significantly in the frequency of HF marker detection. However, the HF marker occurrence rate was higher in 2006 than 2005 for all sites, ranging from 26 to 29% in 2005 and 41–67% in 2006 ($\chi^2 = 14.2$, $P < 0.001$). The tidal variability observed in FIB abundances was not observed for the HF marker; there was no significant difference in its occurrence between high and low tide or spring and neap tide at any site. The HF marker was not detected in any of the four Talbert Marsh sediment samples analysed.

There was no direct relationship between any FIB abundance or interaction of FIB abundances and the presence of the HF marker as tested with an N-way ANOVA. In fact, of the 80 samples testing positive for the HF marker, only two were above the California state single-sample water quality standards for all three FIB. Considering all California state single-sample standards, the best FIB predictor of HF marker occurrence was samples that simultaneously exceeded both the TC and FC standard, 28 of which also had the HF marker. The ratio of TC bacteria to FC (TC : FC) is also used by the state of California as an indicator of potentially health-harming water quality (Haile *et al.*, 1999). When samples are grouped by presence/absence of the HF marker, there is no significant difference in the TC : FC ratio. One possibility for this discrepancy between traditional indicators and the HF marker could be that prefiltering disrupted relationships between FIB and the HF marker, as FIB samples were not prefiltered before analysis. Specifically, our results may underestimate the presence of the HF marker as any particle-associated *Bacteroides* were not detected. While no statistically significant relationship between FIB and the HF marker in individual samples could be determined, it is interesting to note that when each study year is taken as a 'survey' of water quality, 2006 emerged as the poorer water quality year both in terms of overall FIB abundance and occurrence of the HF marker.

As presence of the HF marker could not be predicted using culture-based microbial water quality (i.e. FIB), we also explored the relationship between the occurrence of the HF marker and the physical water quality parameters examined during the study. Neither temperature, turbidity, salinity, nor per cent saturation dissolved oxygen differed between HF positive and HF negative samples either

Table 3. Occurrence of the human-specific fecal *Bacteroides* molecular marker at three sites in Orange County, California detected by conventional PCR.

	August 2005				July 2006			
	Samples screened	Low tide	High tide	Site occurrence rate	Samples screened	Low tide	High tide	Site occurrence rate
Huntington State Beach	47	7/24	5/23	0.26	49	12/26	8/23	0.41
Santa Ana River	48	3/24	8/24	0.23	49	10/24	13/25	0.47
Newport Pier	14	3/9	1/5	0.29	15	5/9	5/6	0.67
Total	109	13/57	14/52		107	27/58	26/49	

Fractions denote number of samples positive out of the total number of samples screened.

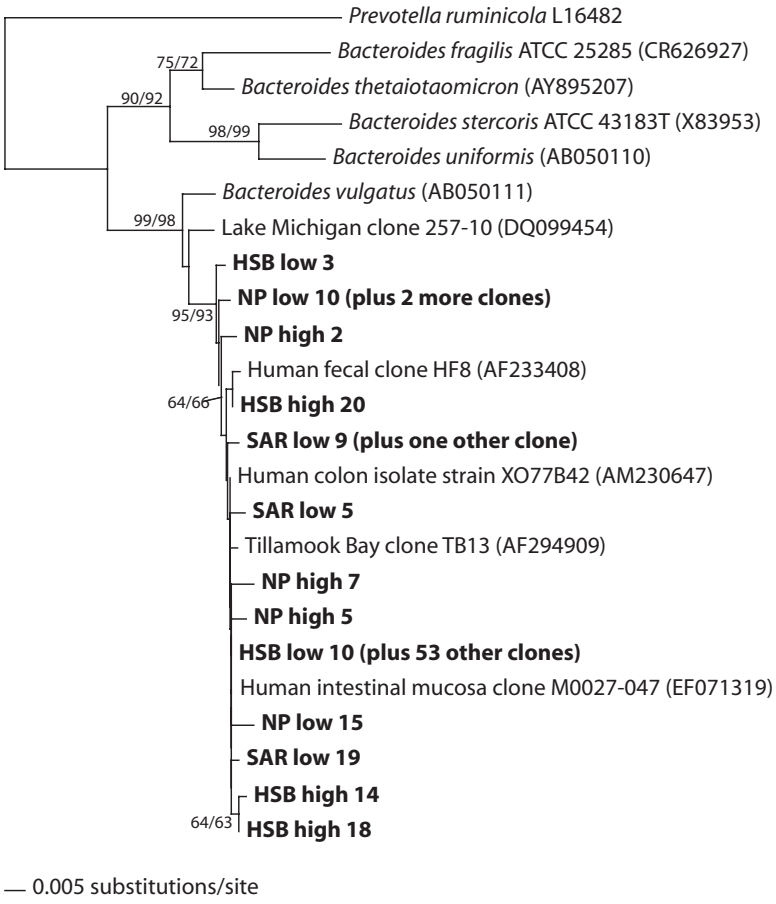

Fig. 3. Neighbour-joining Jukes-Cantor corrected phylogenetic tree of an approximately 540-bp segment of the 16S rRNA gene for fecal Bacteroidetes from cultured isolates and uncultured clones. *Prevotella rumincola* was used as the outgroup. Bootstrap support values (Jukes-Cantor/Maximum Likelihood) for 1000-replicates are given above branches with > 50% support. Sequences in bold are from this study; clone names indicate the site and tide conditions the sample was taken from. Numbers in parentheses indicate GenBank nucleotide accession numbers. Sequences from this study have been deposited in GenBank under Accession numbers EF215457–EF215527.

when grouped by site or analysed collectively, with one exception. The average turbidity (NTU) was significantly higher in samples where the HF marker occurred at SAR in 2005 (average of 8 vs. 11 NTU in samples negative and positive for the HF marker, respectively, $F = 5.45$, $P < 0.05$). Increased turbidity may be associated with greater inputs of urban runoff. Stepwise regression models had slightly better predictive power of the HF marker. The best-fit model of HF marker occurrence included only salinity as an independent variable ($R^2 = 0.56$, $P = 0.005$). Increased salinity could indicate that seawater has spent a long amount of time in tidal wetlands and lagoons, becoming hypersaline and potentially acquiring increased levels of pollutants. However, this correlative result should be interpreted with caution as there were no significant relationships between salinity and FIB or the HF marker for any individual site.

Although the occurrence of the HF marker could not be predicted by standard measures of biological and physical water quality, its occurrence was much more prevalent in the second year of the study (2005 vs. 2006). Several factors may account for this. The most obvious difference between the two sampling years was the widespread dinoflagellate bloom throughout the 2005 sampling period. If organisms that carry the HF marker have a high affinity for particle attachment, this may have influenced the occurrence of the HF marker if these organisms were associated with the large number of algal cells in the water column that were then prefiltered out. A second possibility is that there was less mixing of offshore and surfzone waters during 2006 due to reduced episodic upwelling of cool waters; mean water temperatures were 1–2°C higher at each site during the 2006 sampling period (Table 1). The result of reduced upwelling could be that land-based pollutants had a longer residence time in the surfzone during the 2006-study period. The persistence of the HF marker in seawater has not been examined, although it has been shown to persist at least 25 days in 12°C freshwater (Seurinck *et al*., 2005).

DNA sequencing of the HF marker

All HF marker sequences recovered in this study were greater than 99% identical to one another (Fig. 3). The most common sequence type, accounting for 53 of the 69 clones, was 100% similar to a strain isolated from the

human intestine (GenBank AM230647) as well as several uncultured sequence types obtained from the human digestive tract (Eckburg *et al*., 2005; Gophna *et al*., 2006). This group was also 99% identical to the HF8 human intestinal clone used in the design the HF183F PCR primer [(Bernhard and Field, 2000a), GenBank AF233408]. Of the small amount of variability that did exist in the HF sequence data, there was no clear clustering of specific sequence types with particular sites or tide stages. There appears to be little genetic variability in the sequence type targeted by the HF183F/708R primer set as there is $>99\%$ sequence similarity between sequences obtained from multiple states within the USA [e.g. Oregon (Bernhard and Field, 2000a), Michigan (Bower *et al*., 2005) and California (this study)] and multiple countries [e.g. Belgium (Seurinck *et al*., 2005), Canada (Eckburg *et al*., 2005; Gophna *et al*., 2006) and Australia (GenBank EF071319, unpublished)].

Implications for coastal water quality management

On one hand, the presence of the HF marker in nearly one-third of the samples assayed is surprising given that nearly all studies of shoreline pollution in this area have concluded that FIB in the surfzone have a non-sewage source. FIB abundances, particularly ENT, are generally attributed to shorebirds or sediments residing in the nearby Talbert Marsh (Choi *et al*., 2003; Grant *et al*., 2005; Sanders *et al*., 2005). Discrepancies between FIB levels and human markers have previously been reported at this site. A study of the lower SAR found that human fecal sterols were not correlated with FIB abundance and did not find any evidence of sewage or human-sourced feces (Noblet *et al*., 2004).

On the other hand, the presence of human-sourced markers in urban runoff appears to be widespread, thus their presence in receiving waters should not be a surprise. The health effects of swimming in runoff-contaminated water were documented by an epidemiology study in nearby Santa Monica Bay, indicating that urban runoff likely contains human pathogens (Haile *et al*., 1999). A study by Noble and coworkers (Noble *et al*., 2006b) found the HF marker in 86% of samples in Ballona Creek, an urban watershed in Los Angeles County. Jiang and coworkers (Jiang *et al*., 2001) also found evidence of human fecal contamination as measured by human adenovirus in about one-third of samples taken in urban-runoff impacted creeks across Southern California. Likewise, the HF marker was detected in one-third (3/9) of freshwater beaches examined in a study of Milwaukee-area beaches on Lake Michigan (Bower *et al*., 2005) and between 25 and 76% of samples taken in a Pacific north-west watershed and its receiving coastal bay (Shanks *et al*., 2006). A study using antibiotic resistance profiles of *Enterococcus* spp. bacteria at our study site in Orange County also found that human sources were present in the coastal environment (Choi *et al*., 2003). It is important to note that the HF marker does not discriminate between different sources of human waste; it may originate from direct sources, urban runoff, or untreated sewage.

Our results indicate that, in addition to the growing body of research illustrating the negative impacts of wet-weather runoff to coastal environments, dry-weather runoff can also have significant implications for coastal water quality. Furthermore, we demonstrate that although single FIB measurements cannot accurately predict the presence of human pollution sources, when integrated over a longer period of time there is better agreement between traditional, culture-based indicators of pollution and molecular detection methods, underscoring the importance of a 'site-assessment' approach to understanding microbial pollution rather than single-point sampling. Linking the presence of the HF marker in seawater to human-health outcomes through epidemiology studies will be the next step in understanding its utility as a coastal water quality indicator. Future work with the HF marker in coastal environments should focus on understanding its persistence in seawater, verifying its specificity in a range of geographic locations and matrix types, and quantifying its abundance.

Experimental procedures

Study site and sample collection

Water samples were collected during August 2005 and July 2006 at four stations in coastal Orange County, California, USA (33°38′N, 117°58′W, Fig. 1). The four stations are as follows: the mouth of the SAR, a tidal lagoon that receives no freshwater input other than urban runoff during the summer months; HSB, a wave-driven, steeply sloping beach approximately 2 km north of the SAR outlet; HP, a wave-driven beach approximately 5 km north of the SAR outlet; and NP, a site approximately 4 km to the south with historically lower fecal indicator counts (Noble *et al*., 2006a). Because we wanted to isolate the effect of tide stage on microbial pollution to the greatest extent possible, samples were collected only at night at high and low tides between sunset and sunrise to minimize the effects of sunlight on fecal indicator abundance. Previous work has shown that by mid-day, up to 80% of FIB measurements in marine waters are below detection limits by culture-based methods (Boehm *et al*., 2002). Samples were collected within 30 min of the high/low tide as predicted by XTide (http://tbone.biol.sc.edu/tide/) for the Newport Harbor Entrance, California. In total, 460 samples were collected for this study: 57 low-tide samples and 58 high-tide samples at each of four stations.

Water samples were collected in waist-depth water using 1-l acid-washed HDPE bottles triple-rinsed with sample water before sample collection. Samples were transported back to

the laboratory within 1 h of collection for further processing as described below. An in-situ water quality probe (YSI-6600, Yellow Springs, Yellow Springs, OH) was used to measure temperature, salinity, dissolved oxygen and turbidity at the time of sample collection.

Wave data and littoral current calculation

Wave height, direction and beach slope at the study site were obtained from the Coastal Data Information Project (http://cdip.ucsd.edu) for the Huntington Beach Nearshore Buoy (Buoy 172). Wave heights were converted to breaker heights by assuming a linear deep-water dispersion relation between wave height and wavelength and using the relationship (Gaughan and Komar, 1975):

$$H_b = \frac{0.4(H_o n L_o)^{-0.33}}{H_o}$$

Where H_b is the height of the breaker, H_o is the deep-water wave height, n is a scaling parameter equal to one, and L_o is the wavelength. This breaker height was then used in a calculation of alongshore-current speed (q_l) within the surfzone using the Longuett-Higgins relationship (Longuet-Higgins, 1970):

$$q_l = 20.7 S \sqrt{g H_b} \sin\alpha \cos\alpha$$

where S is the beach slope, H_b is the breaker height, and α is the angle waves impinge on the shoreline.

Transport in the nearshore environment results from a complex interplay of tidal currents, wave-driven currents and barotropic flows (Feddersen *et al*., 1998). While the model presented above is a gross simplification of the nearshore environment, it provides a rough approximation of a potential transport mechanism during the study period. A more in-depth treatment of nearshore circulation at this site using numerical modelling is addressed by Grant and coworkers (Grant *et al*., 2005) and is beyond the scope of this study.

Enumeration of fecal indicator organisms

Total coliform (TC), EC and ENT bacteria were enumerated in 10 ml of each water sample diluted to 100 ml in Butterfield's buffer and analysed using colorimetric and fluorometric defined-substrate assays implemented in a 97-well format (Colilert-24 and Enterolert-24, IDEXX, Westbrook, ME). Positive wells were scored and converted to most-probable number abundance (MPN/100 ml) according to the manufacturer's instructions. California recreational water quality standards are written in terms of TC, FC and ENT. For analysis purposes, we used EC abundance as a proxy for FC. *Escherichia coli* are a subset of FC, thus the actual number of FC is higher. The California state single-sample water quality standards established by Assembly Bill 411 (AB411) for contact recreation in units of MPN/100 ml are: 10 000 for TC, 400 for FC and 104 for ENT.

Molecular detection of human-specific fecal markers

Presence of the human-specific *Bacteroides* fecal marker (hereafter HF marker) was assayed in water samples from SAR ($n = 92$), HSB ($n = 95$) and NP ($n = 29$) using the conventional PCR method of (Bernhard and Field, 2000a), optimized for this study. This assay targets a segment of the 16S rRNA gene using the HF183F/Bac708R primer set. Four sediment samples from the Talbert Marsh, proposed to be an important source of FIB to the study area (Grant *et al*., 2001) were also analysed for the presence of the HF marker.

Sample collection. One litre of seawater was prefiltered using glass-fibre filters (934-AH, ~1.2 μm pore size; Whatman, Clifton, NJ) and vacuum filtered onto 45 mm diameter, 0.22 μm pore size Durapore membrane filters (Millipore Corporation, Billerica, MA). A large red-tide dinoflagellate bloom throughout the 2005 sampling period precluded collecting samples without the use of a prefilter. Therefore, prefilters were also used during the 2006 sampling period for consistency. Filters were flash frozen in cryotubes using liquid nitrogen and stored at −80°C until further analysis. Two sediment samples from the approximate high tide line and two samples from a subtidal channel were taken within the Talbert Marsh. Cores were collected in cut-off syringes and frozen at −80°C until processing.

Extraction of DNAs. Filters were shredded in the cryotube on dry ice using a sterile scalpel before DNA extraction. DNAs were extracted using a hot sodium dodecyl sulfate (SDS)/proteinase K lysis (Fuhrman *et al*., 1988) and purified using a commercial DNA purification kit (DNeasy, Qiagen). Briefly, 800 μl of lysis buffer (0.75 M sucrose, 20 mM EDTA, 400 mM NaCl, 50 mM Tris) and SDS (1% final concentration) were added to the shredded filters and incubated for 1 min at 99°C. Proteinase K (Invitrogen) was then added to a final concentration of 0.5 mg ml^{-1} and filters were incubated at 55°C for approximately 4 h. The supernatant was then transferred to a clean 2 ml centrifuge tube and the filter was washed with an additional 200 μl of lysis buffer that was then combined with the supernatant and purified using DNEasy columns following the manufacturer's protocol. The purified DNAs were eluted in 100 μl of DNase/RNase-free water (Gibco) and quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) with an accuracy of approximately 10% for extracts prepared with this method. DNAs were extracted from 0.5 g of the surface layer of each sediment core using the Qbiogene FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) with a final elution in 100 μl of DNase-/RNase-free water. Extraction blanks were run at a rate of approximately one per 20 extractions. These extractions were then screened using conventional PCR as described below.

Conventional PCR screening. The PCR thermo-cycling conditions and reaction chemistry used in this study were modified slightly from the original assay and optimized for our samples. Specifically, we found that increasing the annealing temperature from 59°C to 60°C reduced non-specific amplification while maintaining the same detection limit. One μl of extracted DNAs (10–322 ng) were used as template in 50 μl reactions with the following reaction chemistry: 25 μl Failsafe Premix E (Epicentre Technologies, Madison, WI), 10 μmol forward primer HF183F (5′-ATCATGAGTTCACATGTCCG-3′), 10 μmol reverse primer Bac708R (5′-CAATCGGAG TTCTTCGTG-3′) (Bernhard and Field, 2000a), 640 ng

per microlitre of non-acetylated bovine serum albumin (Sigma) and 0.25 U LD *Taq* polymerase (Roche). Thermocycling conditions were as follows: an initial denaturing for 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 6 min. One μl of DNAs extracted from 1 l of seawater spiked with 10 μl of primary-treated sewage were used as template in the positive control reaction. Polymerase chain reaction products were visualized in 2% w/v agarose gels stained with 1× SYBR Gold (Invitrogen-Molecular Probes, Eugene, OR). Samples were scored as positive by the presence of an approximately 540-bp amplicon. A subset of negative reactions ($n = 24$) were spiked with 1 μl of positive control to test for PCR inhibition. Two negative controls were run per PCR using 1 μl of either ultra-pure water or an extraction blank.

Detection-limit determination for HF marker assay

Detection limit (in terms of volume of primary-treated sewage detectable in 1 l of seawater) was determined for the entire HF marker assay, including filtration and DNA extraction. Ten millilitres of primary sewage was spiked into 10 L of seawater. Serial dilutions of this mixture were made in 1 l volumes of seawater down to a dilution corresponding to 0.1 μl of sewage per L seawater. These samples were then filtered and processed as described above. This was performed with two separate sewage samples. The filters were extracted and subjected to PCR screening for the HF marker as described for water samples.

Cloning, sequencing and phylogenetic analysis of HF marker

Five samples with strong PCR amplification of the HF marker were chosen for cloning and DNA sequence analysis: one from SAR, two from HSB and two from NP. Triplicate 50 μl PCR reactions were pooled and gel purified using the MinElute Gel Extraction kit (Qiagen, Carlsbad, CA). Products were cloned using the TOPO-TA (Invitrogen) cloning kit with the pCR2.1 vector and TOP10 competent cells. Insert-bearing white clones were transferred to Luria–Bertani broth (w/50 μg ml^{-1} kanamycin) and PCR-screened directly for inserts using T7 and M13R vector primers. Sixteen clones were randomly selected for sequencing from each sample and sequenced using an ABI 3730XL capillary sequencer (PE Applied Biosystems, Foster City, CA). The total number of sequences obtained from each clone library varied due to differences in the length and quality of the sequencing reads. Sequences were trimmed, edited, and aligned using Sequencher v4.7 (Gene Codes) Neighbour-joining trees were produced for the full 541 bp amplicon using PAUP 4.0b10 (Sinauer Associates). PAUP was also used to generate a Jukes-Cantor corrected nucleic acid distance matrix for each site, used in subsequent DOTUR analyses. DOTUR (Schloss and Handelsman, 2005) was used to determine the total number of sequences types recoverd in the study. Bootstrapping (1000 replicates) was used to estimate the reproducibility of the trees using both Jukes-Cantor and Maximum Likelihood distance corrections. Nearest database matches to sequences from this study were determined using GenBank (http://www.ncbi.nlm.nih.gov) BLAST queries of the nucleotide database. Sequences recovered in this study have been deposited in GenBank under Accession numbers (EF215457–EF215527).

Data analysis

Student's *t*-tests, Spearman's rank correlation, and step-wise regression analyses were performed in Matlab v7.0.4 (Mathworks) using the Statistics Toolbox. All ANOVA models were tested using SPSS v.11 (SPSS). FIB data were log-transformed to meet assumptions of normality for the Student's *t*-test, where applicable and for all ANOVA models. An N-way ANOVA using Type III sums of squares was performed to determine differences in environmental parameters and FIB concentrations between samples where the HF marker was present versus absent, where a given water quality parameter was used as the dependent variable and the presence/absence of the HF marker was included as an independent variable with random effects. To further understand which physical variables influence the presence or absence of the HF marker, the proportion of HF-positive samples for each site-tide-year combination (3 sites × 2 tides × 2 years = 12 total) were used as the dependent variable in a stepwise-regression model with the mean water temperature, salinity, per cent dissolved oxygen, turbidity, TC, EC, and ENT as dependent variables. To compare the occurrence of the HF marker detection between sites, years, and tidal conditions the observed frequency of the HF marker was tested against the null hypothesis of equal probability using Fisher's exact test for individual site and tide comparisons, and the Chi-square test for the entire data set (Snedecor and Cochran, 1989).

Acknowledgements

The authors acknowledge M. Choy, N. Handler, D. Keymer, J. Marincola, N. Nidzieko, J. Nidzieko, K. Roberts, L. Silverthorn and K. Willis for tireless help with late-night sample collection and processing. P. Leahy and the staff of the Kerckhoff Marine Laboratory provided invaluable assistance and support throughout the project. S.P. Walters and three anonymous reviewers provided helpful comments on the manuscript. A.E.S. was supported by an NSF Graduate Student Research Fellowship and a National Oceanic and Atmospheric Administration Oceans and Human Health Initiative Grant (NA04OAR4600195) to A.B.B.

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Supplementary material

The following supplementary material is available for this article online:

Fig. S1. Histogram of incoming wave directions during the study period.

Fig. S2. Complete time series of FIB abundance and HF marker detection.

This material is available as part of the online article from http://www.blackwell-synergy.com