Environ Monit Assess (2013) 185:2647–2669
DOI 10.1007/s10661-012-2737-3

An assessment of fecal indicator and other bacteria from an urbanized coastal lagoon in the City of Los Angeles, California, USA

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Received: 22 January 2012 / Accepted: 13 June 2012 / Published online: 6 July 2012

Abstract A study was performed in Del Rey Lagoon, City of Los Angeles, to determine if the lagoon was as a source or sink for fecal indicator bacteria (FIB: total coliforms, *Escherichia coli,* enterococci) and to screen for the presence of other potentially pathogenic bacteria. The lagoon receives tidal flows from the adjacent Ballona Estuary whose water usually is contaminated with FIB originating from the highly urbanized Ballona Creek Watershed. During 16 sampling events from February 2008 through March 2009, replicate water samples (n=3) were collected 1 h prior to the high tide and 1 h prior to the following low tide. FIB concentrations were measured by the defined substrate method (IDEXX, Westbrook, Me) followed by culturing of bacterial isolates sampled from positive IDEXX Quanti-Tray wells and were identified using the Vitek 2 Compact (bioMérieux, Durham, NC). Mean concentrations of FIB often differed by an order of magnitude from flood to ebb flow conditions. The lagoon tended to act as a sink for total coliforms based on the ratio of mean flood to ebb densities ($R_{F/E}$) >1.0 during 56 % of the sampling events and during ebb flows, as a source for *E. coli* and enterococci ($R_{F/E}$ <1.69 % of events). Approximately 54 species were identified from 277 isolates cultured from the IDEXX Quanti-Trays. Of these, 54 % were species known to include pathogenic strains that can be naturally occurring, introduced in runoff, or originated from other sources. Diversity and cluster analyses indicated a dynamic assemblage that changes in species composition with day-to-day fluctuations as well as tidal action. The concept of monitoring the lagoon and estuary as a sentinel habitat for pathogenic assemblages is discussed.

Keywords Coastal lagoon · Urban runoff · Tidal fluctuation · Fecal indicator bacteria · Pathogenic bacteria · Microbial ecology

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Introduction

California's coastline is a major resource for state fisheries, recreation, tourism, biodiversity, and esthetics, so achieving and maintaining good water

quality is of prime concern for beach and resource managers. This objective has been a challenge at many of the state's urban coastal beaches and embayments having poor water quality due to microbial pollution from fecal indicator bacteria (FIB) and associated pathogenic organisms. Although FIB only indicate the possible presence of pathogens, epidemiological studies have linked elevated FIB concentrations to swimmer illness in waters contaminated by human sewage (Cabelli et al. 1982; Dufour 1984; Haile et al. 1999; Wade et al. 2003) from exposure to a variety of waterborne pathogens (Maier et al. 2000; Hlavsa et al. 2011). However, FIB are poorly linked to human health effects in waters impacted by contaminated runoff but no obvious sewage inputs (Calderon et al. 1991; Colford et al. 2007). These microorganisms can come from a variety of sources including contaminated runoff from storm drains and freshwater inputs (Jiang et al. 2001; Reeves et al. 2004; Stein and Tiefenthaler 2004; Ackerman et al. 2005), feces from birds and other wildlife (Ricca and Cooney 1998; Alderisio and DeLuca 1999; Ferguson et al. 2003; Grant et al. 2001; Surbeck et al. 2006), outflows from coastal wetlands or other embayments (Steets and Holden 2003; Surbeck et al. 2006), and reservoirs of microorganisms residing within beach sands and other sediments (Davies et al. 1995; Ferguson et al. 2005; Lee et al. 2006; Yamahara et al. 2009). Chief among these in California would be contaminated runoff impacting the surf zone water of beaches (Noble et al. 2000), especially during wet weather (Noble et al. 2003).

Consequences of poor water quality are significant. Swimmers, surfers, and other people exposed to beach waters contaminated by pathogenic microorganisms have a greater risk of illness (Stewart et al. 2008), while frequent beach closures due to poor water quality can result in great economic losses for a region. For example, during the dry and wet seasons of 2000, Given et al. (2006) estimated total losses for Los Angeles County beaches to range from $14.2 to $35.1 million depending on the dose–response model used. To obtain water quality standards, the US Environmental Protection Agency has implemented various regulatory actions to reduce microbial pollution of marine and estuarine waters listed as impaired under Section 303(d) of the Federal Clean Water Act. Chief among these is the total maximum daily load (TMDL) strategy where the assimilative capacity of a body of water for a target pollutant is determined numerically, and then loads for that pollutant are allocated among point, nonpoint, and natural sources in order to achieve the water quality objective (Cal EPA 2010).

Santa Monica Bay in Southern California is impacted by runoff from the urbanized Ballona Creek Watershed, the largest draining into the bay. This watershed has an area of 340 km^2, is 83 % developed, and encompasses several cities including a major portion of Los Angeles (Bay et al. 1999). Its waterways have been transformed into a series of underground pipes conveying runoff to Ballona Creek, an open concrete channel in the lower portion of the watershed. An annual flow of approximately 65 million m^3 of runoff with mean daily winter/spring flows of 2.3 m^3/s and mean daily summer/fall flows of 0.6 m^3/s impacts receiving waters and habitats of the Ballona Wetlands and Estuary, Del Rey and Ballona Lagoons, Santa Monica Bay, and beaches adjacent to the mouth of Ballona Creek (Dojiri et al. 2003; PWA 2006). To date, TMDLs for metals (Cd, Cu, Pb, Ag, and Zn), toxic organic compounds (chlordane, DDTs, PCBs, and total PAHs), trash, and FIB have been established by the state to control these pollutants in the Ballona Creek and Estuary (see http://www.waterboards.ca.gov/losangeles/water_issues/programs/tmdl/ for details).

One component of the Ballona Estuary bacterial TMDL is to determine if the Del Rey Lagoon contributes significant concentrations of FIB to the estuary, thus contributing to water quality impairment not only of the estuary but to adjacent recreational beaches. According to the TMDL plan for the Ballona Estuary, the Del Rey Lagoon is considered a nonpoint source for bacteria entering waters of the estuary. However, no studies have been conducted to understand how FIB levels fluctuate within the lagoon, especially during tidal exchanges, and how this relates to the lagoon serving as a sink or source of FIB for the estuary. Studies of FIB dynamics within tidal channels of the nearby Ballona Wetlands showed that concentrations of these bacteria can fluctuate up to three orders of magnitude over a 24-h period, and that the wetlands can act as a FIB sink under daytime flood–tide conditions or a source during stronger ebb flows when sediments are suspended (Dorsey 2006; Dorsey et al. 2010). Wetland processes in general have been demonstrated to reduce FIB concentrations through exposure to UV light (Jillson et al. 2001; Karathanasis et al.

2003; Whitman et al. 2008), settling onto submerged plant surfaces (Karathanasis et al. 2003), and predation by protozoans (Surbeck et al. 2010). Conversely, ebb flows from a salt marsh wetland can result in elevated FIB densities in receiving surf zone waters of adjacent beaches (Grant et al. 2001; Steets and Holden 2003).

Given the popularity of the lagoon shoreline for recreation, it is important to have knowledge of its microbial water quality. This study therefore addresses two questions:

1. Is the lagoon a sink or source of FIB to the adjacent estuary?
2. Are potentially pathogenic bacteria present within the lagoon waters, and does their diversity differ tidally?

To address the first question, densities of FIB (total coliforms, *Escherichia coli*, and enterococci) were measured in water entering the lagoon during flood tides and leaving on the ebb flows. Here we define the lagoon as sink when FIB concentrations are reduced through various processes. Conversely, the lagoon would act as a source if FIB concentrations increase and are released to the adjacent receiving waters of the estuary. If the lagoon was a source of FIB, then the ratio of flood to ebb FIB densities would be <1.0, indicating that more FIB outwell from the lagoon on ebb tide flows. Conversely, ratios >1.0 indicate that FIB densities in the lagoon's surface water were reduced through various processes such as inactivation by UV light, sedimentation, or predation by microorganisms. This sampling scheme was conducted over a variety of tides ranging from spring to neap tides to examine how ebb and flood densities; hence, the ratio may differ with varying tidal conditions. This approach was used to help elucidate FIB dynamics in the nearby Ballona Wetland tidal channels (Dorsey 2006).

The second question was approached by attaining a partial measure of the bacterial diversity of flood and ebb flows from water samples collected for the FIB analyses. Comparing tidal diversity may provide information on the source of bacteria, particularly pathogens, and lead to measures to control their presence thus reducing risk to people exposed to the lagoon water. Although only a fraction of culturable bacteria could be isolated and identified using the same media as used for the FIB analyses, this approach was treated as a preliminary screening method, providing a measure of the relative diversity of resident bacteria and the presence of possible pathogens.

Methods

Del Rey Lagoon

The Del Rey Lagoon is in the City of Los Angeles' Playa Del Rey suburb located just south of Marina Del Rey (Fig. 1). The lagoon is the remnant of a once larger coastal lagoon system located at the mouth of Ballona Creek. It was split into a southern (Playa Del Rey Lagoon and Regional Park) and northern portion (Ballona Lagoon) after Ballona Creek was straightened and its banks armored as part of a flood-control project by the US Army Corps of Engineers in the early 1930s (PWA 2006).

Presently, the lagoon is approximately 0.02 km^2 (5.2 ac) in area and surrounded by a residential community with parkland at its southern end (Fig. 1). It is a full tidal system influenced by mixed semidiurnal tides. Water enters and departs the lagoon from the Ballona Estuary via a single tide gate positioned at the north end of the lagoon. Runoff enters the lagoon from the surrounding drainage area of 0.10 km^2 (24.5 ac)(CLA 2007). Although the parkland at the southern end of the lagoon is popular for recreation, no swimming is allowed within the lagoon due to impaired water quality based on historically elevated levels FIB.

Field sampling

FIB densities were measured for 16 days from February 2008 through March 2009 (Table 1). Only dry-weather days were sampled to elucidate tidal patterns among the FIB groups. If rain occurred, then sampling was postponed to 4 days after the rain event, presumably enabling all rain-related runoff to flush from the lagoon.

During each sampling day, sets of replicate samples (n=3) were collected during the flood and ebb flows. Sampling days were selected to include a variety of tidal conditions ranging from neap to spring tides with tidal ranges ranging from 0.14 to 2.73 m, respectively (Table 1). Sampling was done approximately 1 h prior to each high and low tide peaks to ensure that water

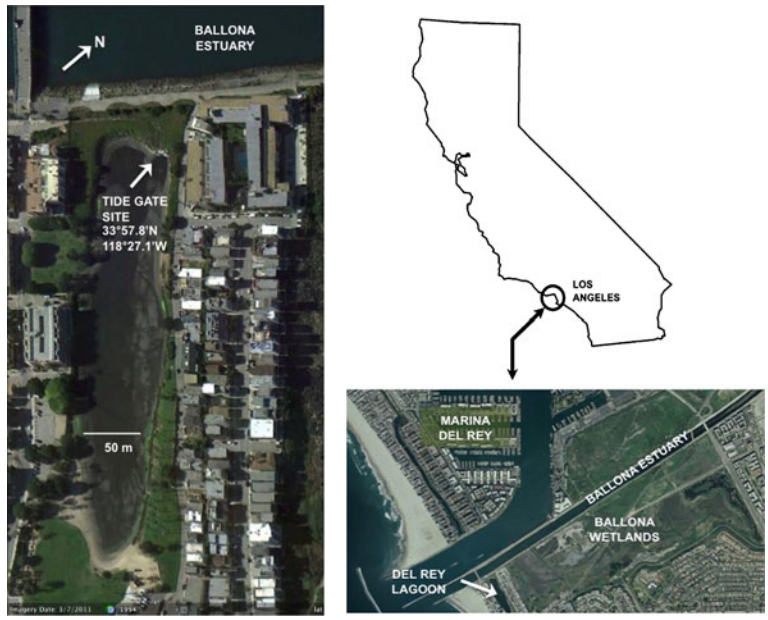

Fig. 1 Location of the sampling site at the north tide gate in Del Rey Lagoon, Playa Del Rey in the City of Los Angeles, California. (Images from Google Earth)

was moving and not at slack level. Only dry-weather days were sampled to avoid confounding effects from runoff.

Samples were collected at the north end of the lagoon by the tide gate (Fig. 1). Replicates were collected within 2 m of the tide gate over a period of about 1 min. Samples were collected in sterile 120 mL polypropylene containers, placed on ice, transported back to the laboratory, and tested within 1 h of collection.

Water quality measurements of temperature (in degree Celsius), salinity (in parts per thousand), pH, and

Table 1 Sampling dates, tidal information and collection times for the Del Rey Lagoon study

Date	Tidal condition		Tidal height (m), time (hours)[a]		Sample times (hours)	
	Neap vs. spring	Range (m)	High	Low	Flood	Ebb
7 Feb. 2008	Spring	2.19	1.81, 0836	−0.38, 1542	0830	1430
13 Feb. 2008	Neap	0.14	0.88, 1448	0.74, 1900	1328	1801
11 Mar. 2008	Neap	0.59	1.03, 1300	0.44, 1800	1120	1610
20 Mar. 2008	Spring	1.60	1.59, 0918	−0.01, 1618	0930	1427
25 Mar. 2008	Neap	0.39	0.95, 1224	0.56, 1706	1119	1614
8 Apr. 2008	Neap	0.65	1.12, 1206	0.47, 1700	1100	1530
22 Apr. 2008	Neap	0.38	1.0, 1136	0.62, 1612	1000	1500
14 May 2008	Neap	0.94	1.16, 0630	0.22, 1230	0530	1130
3–4 Jun. 2008	Spring	2.73	2.16, 2124	−0.57, 0448	2020	0330
14–15 Jul. 2008	Spring	1.79	1.71, 1936	−0.08, 0312	1815	0210
12 Sept. 2008	Neap	0.75	1.34, 0854	0.59, 1412	0835	1413
3 Oct. 2008	Neap	0.39	1.37, 1724	0.98, 1200	1044	1832
17 Oct. 2008	Spring	2.17	2.00, 1054	−0.17, 1815	1030	1754
14 Nov. 2008	Spring	2.57	2.16, 0854	−0.41, 1615	0821	1525
30 Jan. 2009	Spring	1.15	1.31, 1100	0.16, 1727	0953	1557
20 Mar. 2009	Spring	1.22	1.28, 0536	0.06, 1321	0525	1221

[a]From NOAA tidal NOS Station 9410840 Santa Monica, CA (http://tidesandcurrents.noaa.gov)

dissolved oxygen (in milligrams per liter) were made in situ using an YSI 600 sonde (YSI Yellow Springs, OH). In the laboratory, turbidity in nephelometric turbidity units (NTU) was measured for each replicate sample using a HACH model 2100 N turbidimeter (HACH, Loveland, CO).

Beginning March 2008, estimates were made of bird species and densities to determine their use of the lagoon. Estimates were made during each flood and ebb flow sample collection time on each sampling date.

Bacterial analyses

FIB concentrations were determined using chromogenic substrate tests (APHA et al. 1998, Standard Methods Section 9223 B). Idexx media Colilert®-18 was used for total coliforms and *E. coli* and Enterolert® media for enterococci (IDEXX, Westbrook, Me). Samples were quantified using Idexx Quanti-Tray® 2000 97-well trays.

A variety of other bacterial species will grow in the media used in defined substrate methods, many of which occur mainly in environmental samples (Stevens et al. 2003). In comparing traditional lactose fermentation with methods based upon β-D-galactosidase, Fricker and Eldred (2009) isolated bacteria from Colilert-18® liquid extracted from Quanti-Tray® wells testing positive for coliforms and then identified them using the Vitek microbial identification system. This approach was used to obtain a relative measure bacterial richness in the lagoon for each tidal flow during the latter part of the study for six sampling events beginning with the 16 September 2008 collection. From each set of three replicate Quanti-Trays®, 10 wells testing positive for coliforms and 10 for enterococci were randomly selected. Positive wells were sampled to ensure that we would isolate bacteria and to identify species of *Enterococci*, many of which are important from a public health perspective. A volume of 10 μL was extracted, mixed into 10 mL of sterile DI water to yield a 0.001 % suspension, immediately streaked onto tryptic soy agar (TSA), and then incubated either at 35 °C for suspensions from the *E. coli* wells or 41 °C for those from the enterococci wells. After 24–48 h of incubation, one colony of each morphological type was selected from each TSA plate, suspended in 3 mL of sterile DI water, streaked onto nutrient blood agar (5 % sheep blood in TSA) to obtain pure isolates, and then incubated as done for the initial TSA plates. One isolate from each blood agar plate then was identified using the Vitek® 2 Compact (V2C) biochemical identification system (bioMérieux Vitek Systems Inc., Hazelwood, MO) according to manufacturer's specifications. Isolates acquired from the Colilert®-18 and Enterolert® medias were identified using Vitek gram-negative and gram-positive cards, respectively.

Data analyses-FIB

For each tidal event, the mean concentration of each FIB group was tested between flood and ebb conditions using the Student's *t* test on $\log_{10}$-transformed data. Ratios of flood to ebb FIB mean concentration ($R_{F/E}$) were calculated for each sampling event to determine if the lagoon was acting as a sink ($R_{F/E}>1$) or source ($R_{F/E}<1$) of FIB.

Pearson correlations were performed between FIB densities and water quality parameters to elucidate relationships among variables. Regression analyses were done to determine the extent to which variation in bacterial density was explainable by salinity, temperature, pH, dissolved oxygen, mean NTU, and tidal height. The data set used for these analyses began with 32 records, each record representing either the flood or ebb tide collection for a date. Each record included mean densities for each FIB group, mean turbidity, tidal height, and YSI measurements (temperature, salinity, pH, and dissolved oxygen). Three incomplete records were deleted from the analyses due to missing data for the 20 March 2008, 14 May 2008, and 4 June 2008 collections, so the final data set equaled 29 records for both the Pearson correlations and regression analyses.

Data analyses-bacterial community

Bacterial isolates identified using the V2C provided a relative measure of the bacterial species richness sampled during flood or ebb tide flows, while acknowledging that these isolates represent only a fraction of the bacteria community present.

Alpha diversity rarefaction, and rank abundance The alpha diversity (α-diversity) of an ecological community is an evaluation of both the number of species (i.e., species richness) and the number of individuals in each species (species abundance) that are sampled at a specified site (Whittaker 1972). To assess the α-

diversity of bacterial communities, we used the Shannon diversity index (H), which is calculated as follows:

$$H = -\sum_{i=1}^{S} [p_i \times \ln(p_i)] \quad (1)$$

Where S is the total number of species sampled and p_i represents the proportional species abundance. The term p_i is calculated as follows:

$$p_i = \frac{n_i}{N} \quad (2)$$

Where n_i is the number of individuals in the ith species and N is the total number of individuals encountered across all S species. Shannon diversity index values can range from $0 \rightarrow \ln(S)$, and large values of H are indicative of sites with both high species richness and high equitability among relative species abundances (species dominance is low).

To compare the α-diversity of different bacterial communities, it is necessary to employ a rarefaction subsampling technique that will allow for the evaluation of diversity index values that are computed with equal population sizes (Sanders 1968). For each of the sites, the rarefaction procedure reduces N at random intervals and computes a respective value of H, until N=0. The resulting plot between the computed values of N and resulting values of H is known as a rarefaction curve (Gotelli and Colwell 2001), which serves to compare diversity indices based on equal sampling sizes. The rarefaction curves for this study were computed with EstimateS v8.2 using data triplets of sampling date, species identification, and species abundance for each of the flood and ebb flow tidal states.

Rank–abundance curves were used to evaluate the species richness and species abundances equitability (species evenness) in both flood and ebb bacterial assemblages. A rank–abundance curve is a plot of the proportional species abundance (p_i) and the respective rank in abundance, such that the highest value of p_i receives a rank of 1, the second highest a rank of 2, and so on.

Richness estimates beta diversity, and cluster analysis Estimating species richness is an important tool in surveying species-rich communities and is commonly used as a technique where it is impractical to sample exhaustively (Gotelli and Colwell 2001). The program EstimateS v8.2 was used to predict (i.e., estimate) the species richness of both flood and ebb collections based on the species occurrence patterns in the sampling data gathered during each sampling event. This program with description of the indices used herein is available at the EstimateS web site (http://viceroy.eeb.uconn.edu/EstimateSPages/AboutEstimateS.htm). The beta diversity (β-diversity) of an ecological community is an evaluation of the number of unique species among specified sites and measures the rate of change in species composition (Whittaker 1972). In order to assess the β-diversity of the bacterial assemblages in both the flood and ebb flows, similarity indices comparing shared species were calculated using EstimateS v8.2 and included the Bray–Curtis, Morisita-Horn, Chao-Sorenson, Chao-Jaccard, Sorensen Classic, and Jaccard Classic. A cluster analysis was then used to evaluate the temporal variability of the species composition in the bacterial assemblages for the different flood and ebb tidal conditions encountered. The proportional species abundance (p_i) was calculated for each sampling date during each flood and ebb state, which was used to generate a similarity matrix that formed the source for a Euclidean distance cluster diagram using Statistica v9.1 (StatSoft 2010).

Results

Water quality measurements

Summary statistics for the water quality parameters during the flood and ebb tide flows are presented in Table 2. Water temperatures ranged from 12.9 to 25.3 °C reflecting seasonal changes. The mean temperatures differed significantly (p=0.04) between flood and ebb tide conditions with the flood tide water being cooler by 2.3 °C on average with up to a 7.3 °C difference as recorded during the 20 March 2008 sampling event. Salinities were typical of estuarine and marine waters, ranging from 24.3 to 33.6 ppt. Water often was more saline by up to 2.5 ppt (13 February 2008 sampling event) as it entered the lagoon from the Ballona Estuary, but overall mean salinities did

Table 2 Summary of water quality parameters measured during bacterial studies in Del Rey Lagoon

Statistic	Temp (°C)		Salinity (ppt)		O_2 (mg/L)		pH		Turbidity (NTU)	
	Flood	Ebb	Flood	Ebb	Flood	Ebb	Flood	Ebb	Flood	Ebb
n	16	14	16	14	15	14	16	14	48	48
Mean	17.20	19.51	29.08	28.61	8.23	8.03	7.96	7.95	1.96	10.76
S.D.	3.34	2.25	2.34	2.46	4.80	5.68	0.26	0.36	1.06	13.15
Min	12.89	14.83	25.70	24.28	2.56	2.88	7.55	7.15	0.92	1.34
Max	25.25	22.05	33.58	31.82	20.51	25.36	8.39	8.34	5.98	46.80
p	0.0365*		0.5957 ns		0.9221 ns		0.9336 ns		<0.0001*	

Significant differences between mean flood and ebb flow values were tested using the Student's *t* test

ns not significant at $p>0.05$

*$p<0.05$ (significant)

not differ significantly ($p=0.60$) between flood and ebb flows. However, a pattern was evident that greater salinities occurred during the more extreme high and low tidal levels (Fig. 2). Dissolved oxygen (DO) displayed the greatest variation with values ranging from 2.6 to 25.4 mg/L. The mean DO values for flood vs. ebb flows did not differ significantly ($p=0.92$). When DO values regressed against tidal levels, dissolved oxygen tended to increase with higher tide level, however, this trend was not significant ($R^2=0.02$, $p=0.53$). Values of pH were consistent ($p=0.93$), averaging 7.96 and 7.95 for flood and ebb tide flows, respectively. The mean values of turbidity (NTU) differed significantly between flood and ebb flows ($p<0.01$) with flood conditions averaging 1.96 NTU compared to 10.76 during ebb flows. Correlations among the various water quality parameters generally were weak with the exception of those for turbidity vs. tidal height ($r=-0.56$, $p<0.01$) and salinity vs. pH ($r=-0.55$, $p=0.02$). In the case of turbidity, waters became more turbid as the tide receded, especially during spring tide conditions, presumably due to sediments suspended during swifter ebb flows.

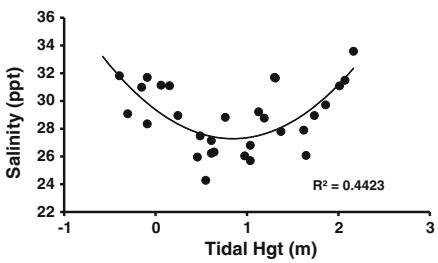

Fig. 2 The relationship between salinity and tidal height. A second-order polynomial was used to fit the line to data points

FIB concentrations

Densities of total coliforms, *E. coli*, and enterococci differed in that the lagoon tended to act nearly equally as a sink and source for total coliforms, while mainly a source for *E. coli* and enterococci (Table 3). Mean densities of total coliforms ranged from 10^2 to 10^4 most probable number (MPN)/100 mL with overall flood and ebb flow densities being similar. Over the 16 sampling events, $R_{F/E}$ for total coliforms averaged 2.9 with values >1.0 occurring 56 % of the time, suggesting that the lagoon tended to act both as a sink or source for total coliforms nearly equally. Densities of both *E. coli* and enterococci tended to be greater during the ebb flows (Table 3), ranging from 10^1 to 10^2 MPN/100 mL. Although the mean $R_{F/E}$ for *E. coli* was 3.6, due mainly to a single event in October 2008, 69 % of the events had ratios <1.0 suggesting that the lagoon was acting as a source of *E. coli* on the ebb tidal flows. The average $R_{F/E}$ for enterococci was 0.9, and like *E. coli,* the majority of the sampling events (69 %) had ratios <1.0, indicating that the lagoon was acting mainly as a source of enterococci.

Multiple regressions between the FIB groups and water quality parameters resulted in significant results for enterococci and total coliforms. Increasing enterococci concentrations was best explained by increasing levels of turbidity and salinity ($R^2=$

Table 3 Mean densities (in most probable number per 100 mL) of fecal indicator bacteria measured in Del Rey Lagoon during flood and ebb flow, February 2008–March 2009

Date	Flood		Ebb		*p* value from *t* test	$R_{F/E}$
	Mean	S.D.	Mean	S.D.		
			Total coliforms			
07 Feb. 2008	2,848.0	699.7	963.0	82.0	0.0017*	3.0
13 Feb. 2008	1,868.0	554.6	2,058.0	669.8	0.7674 ns	0.9
11 Mar. 2008	565.0	72.1	433.3	93.6	0.1270 ns	1.3
20 Mar. 2008	1,366.0	445.6	1,452.0	354.3	0.7518 ns	0.9
25 Mar. 2008	2,194.0	405.0	818.0	54.8	0.0010*	2.7
08 Apr. 2008	699.3	40.6	388.7	9.0	<0.0001*	1.8
22 Apr. 2008	732.7	133.0	510.0	40.0	0.0340*	1.4
14 May 2008	234.3	51.9	108.0	34.0	0.0250*	2.2
03 Jun. 2008	782.0	134.3	1,728.0	368.4	0.0090*	0.5
14–15 Jul. 2008	819.0	615.7	7,332.0	1,066.0	0.0045*	0.1
12 Sept. 2008	18,390.0	10,060.0	24,200.0	0.6	0.4000 ns	0.8
03 Oct. 2008	11,130.0	1,895.0	1,485.0	76.8	<0.0001*	7.5
17 Oct. 2008	5,087.0	1,283.0	2,261.0	1,064.0	0.0501 ns	2.2
14 Nov. 2008	2,969.0	1,008.0	922.7	366.0	0.0150*	3.2
30 Jan. 2009	683.3	371.4	1,044.0	362.7	0.2658 ns	0.7
20 Mar. 2009	465.7	93.0	3,217.0	400.1	0.0001*	0.1
			E. coli			
07 Feb. 2008	45.0	16.4	44.7	6.4	0.9011 ns	1.0
13 Feb. 2008	70.3	6.4	378.3	102.3	0.0005*	0.2
11 Mar. 2008	45.0	12.1	213.3	47.9	0.0022*	0.2
20 Mar. 2008	226.3	149.6	338.3	43.8	0.2149 ns	0.7
25 Mar. 2008	70.3	16.8	200.7	51.3	0.0082*	0.4
08 Apr. 2008	24.0	24.3	125.7	32.5	0.0262*	0.2
22 Apr. 2008	48.3	16.8	13.3	5.8	0.0155*	3.6
14 May 2008	51.3	17.9	16.7	5.8	0.0188*	3.1
03 Jun. 2008	16.7	5.8	66.7	17.8	0.0069*	0.3
14–15 Jul. 2008	235.3	210.0	277.0	47.6	0.4684 ns	0.8
12 Sept. 2008	448.3	380.3	586.0	90.4	1.000 ns	0.8
03 Oct. 2008	173.0	45.9	181.3	33.7	0.7699*	1.0
17 Oct. 2008	241.0	83.5	146.0	2.0	0.1000 ns	1.7
14 Nov. 2008	163.0	29.4	173.0	103.3	0.9150 ns	0.9
30 Jan. 2009	42.0	32.5	608.3	113.1	0.0083*	0.1
20 Mar. 2009	137.7	24.8	1,349.0	270.0	0.0001*	0.1
			Enterococci			
07 Feb. 2008	38.0	24.3	45.3	30.6	0.9111 ns	0.8
13 Feb. 2008	20.3	10.5	45.7	38.2	0.4614 ns	0.4
11 Mar. 2008	13.3	5.8	10.3	0.6	0.4410 ns	1.3
20 Mar. 2008	48.7	33.9	116.7	37.5	0.1594 ns	0.4
25 Mar. 2008	13.3	5.8	34.3	5.8	0.0163*	0.4
08 Apr. 2008	10.0	0.0	10.0	0.0	no test	1.0
22 Apr. 2008	48.3	16.8	13.3	5.8	0.0155*	3.6

Table 3 (continued)

Date	Flood		Ebb		*p* value from *t* test	$R_{F/E}$
	Mean	S.D.	Mean	S.D.		
14 May 2008	51.3	17.9	16.7	5.8	0.0180*	3.1
03 Jun. 2008	13.3	5.8	66.7	17.8	0.0040*	0.2
14–15 Jul. 2008	52.7	29.3	93.0	14.7	0.1067 ns	0.6
12 Sept. 2008	39.0	50.2	34.3	21.8	0.7886 ns	1.1
03 Oct. 2008	23.7	6.4	90.0	29.2	0.0052*	0.3
17 Oct. 2008	56.0	26.0	196.3	61.0	0.0144*	0.4
14 Nov. 2008	86.3	41.1	112.7	35.6	0.4454 ns	0.8
30 Jan. 2009	10.0	0.0	134.0	56.0	0.0005*	0.1
20 Mar. 2009	30.7	10.5	88.7	31.8	0.0300*	0.3

Also presented for each sampling event are results of *t* tests between flood and ebb FIB densities and corresponding ratio of flood to ebb mean density ($R_{F/E}$)

ns not significant at $p>0.05$

*$p<0.05$ (significant)

0.69, $p<0.01$). Total coliform variation was best explained by diminished levels of salinity and dissolved oxygen ($R^2=0.54$, $p<0.01$), possibly associated with cooler water entering the lagoon during flood flows. Regression models for total coliforms and *E. coli* were similar in that both FIB groups were negatively associated with diminishing levels of salinity and pH, although the regression model for *E. coli* was not significant ($R^2=0.05$, $p=0.31$).

Birds

The most common birds observed on or around the lagoon were ring-billed gulls *Larus delawarensis*, American coots *Fulica americana*, and mallards *Anas platyrhynchos* (Table 4), particularly during spring and fall. During low tides, various species of shorebirds were observed feeding on the exposed mudflat, the most common being whimbrels *Numenius phaeopus*, Dowitchers *Limnodromus* sp., and various species of plovers. The most conspicuous large birds were a resident group of seven graylag geese *Anser anser*.

For each FIB group, Spearman's rank correlations were done between the number of birds per acre (Table 4) and mean concentrations of FIB during ebb flows (Table 3). However, there was no significant relationship between bird densities and the FIB groups (total coliforms: $r=-0.34$, $p>0.05$; *E. coli*: $r=0.14$, $p>0.05$; enterococci: $r=0.07$, $p =>0.05$).

Bacterial diversity

Of the 277 isolates cultured from the IDEXX Quanti-Tray wells, approximately 54 species representing 24 genera were identified using the V2C system (Table 5). Nine isolates, 3.2 % of the total, were unidentifiable using the V2C, while 19 taxa (6.9 %) were reported as species complexes requiring additional tests to acquire final species designations. Genera with the greatest number of isolates included species of *Enterococcus* with 87 and *Vibrio* with 54. Species of *Vibrio* tended to be more prevalent during the months of September and October when water temperatures were warmer relative to the cooler winter months (Pearson $r=0.61$, $p=0.03$). About half (55 %) of the species represented by the isolates have been implicated as opportunistic or direct human pathogens (Table 6), but detailed molecular tests would be required to determine if these strains were actually pathogenic.

The rarefaction curves illustrate that the Shannon diversity index of the isolates collected during ebb flows was higher than for the flood flows (Fig. 3a). The rank–abundance curves also demonstrate that ebb flows had higher species richness and higher species evenness than the flood flows (Fig. 3b). However, estimates of species richness based on sampling patterns show that flood flows are projected to have a greater number of species (Fig. 4). Samples from both

Table 4 Birds observed at Del Rey Lagoon during each sampling event, March 30, 2008 to March 20, 2009

Common name	Species	20 Mar. 2008	25 Mar. 2008	8 Apr. 2008	22 Apr. 2008	14 May 2008	14 Jul. 2008*	12 Sept. 2008	3 Oct. 2008	17 Oct. 2008	14 Nov. 2008	30 Jan. 2009	20 Mar. 2009[a]
Brown pelican	*Pelecanus occidentalis*	0	0	0	2	0	0	0	0	0	0	0	0
Double-crested cormorant	*Phalacrocorax auritus*	0	0	0	0	0	0	0	0	0	1	0	0
Brandt's cormorant	*Phalacrocorax penicillatus*	1	0	0	0	0	0	0	0	1	0	0	0
Great egret	*Ardea alba*	1	0	1	0	0	0	4	6	2	2	1	0
Snowy egret	*Egretta thula*	1	0	5	3	8	0	1	3	2	1	1	3
Greylag goose	*Anser anser*	7	7	7	7	7	7	7	7	7	7	7	7
Canadian goose	*Branta canadensis*	0	3	0	0	0	0	0	0	0	0	0	0
Mallard duck	*Anas platyrhynchos*	8	15	8	3	9	5	113	86	92	52	44	7
Lesser scaup	*Aythya affinis*	0	0	0	0	0	0	0	0	0	9	50	0
Bufflehead	*Bucephala albeola*	0	0	0	0	0	0	0	0	0	18	13	0
Red-breasted merganser	*Mergus serrator*	0	0	0	0	0	0	0	0	0	2	0	0
American coot	*Fulica americana*	150	200	105	3	0	0	0	20	70	180	174	97
Black-bellied plover	*Pluvialis squatarola*	0	1	5	0	0	0	4	2	7	1	0	93
Semi-palmated plover	*Charadrius semipalmatus*	0	0	0	0	0	0	0	8	0	0	0	0
Killdeer	*Charadrius vociferus*	0	0	50	0	0	0	0	0	0	0	0	0
Plover, unidentified	Charadriinae, unid.	0	0	0	40	0	0	0	0	0	0	0	0
Sand piper, unidentified	*Calidris* sp.	0	12	200	35	0	0	0	0	0	0	0	0
Dowitcher	*Limnodromus* sp.	0	0	5	11	2	0	3	2	0	2	0	11
Whimbrel	*Numenius phaeopus*	0	24	122	1	1	0	0	1	0	0	0	0
Willet	*Tringa semipalmata*	0	0	0	0	0	0	2	4	1	0	9	4
Marbled godwit	*Limosa fedoa*	0	0	20	51	0	0	0	4	0	0	2	5
California gull	*L. californicus*	1	0	1	0	0	0	0	0	0	0	0	0
Ring-billed gull	*Larus delawarensis*	66	14	55	15	6	0	0	0	0	4	50	8
Herrmann's gull	*L. heermanni*	0	0	0	0	0	0	21	3	2	6	24	7
Western gull	*L. occidentalis*	9	2	5	0	0	0	8	11	0	7	3	3
Total individuals observed		244	278	589	171	33	12	163	157	184	292	378	245
Total individuals/acre		46.9	53.5	113.3	32.9	6.3	2.3	31.3	30.2	35.4	56.2	72.7	47.1

Counts were summed for the flood and ebb tide collections for each sampling event. Species that are year-around residents are presented in bold (Cooper 2006)

[a] Ebb tide observations only

ebb and flood flows had 21 bacterial species in common. Similarity analyses show that indices of shared species vary between 38 and 92 % (Fig. 5). Additionally, the cluster analysis indicates that there was high variation in terms of both species abundance and composition of bacterial species with respect to both sampling date as well as ebb- and flood flow events (Fig. 6).

Discussion

Differences were apparent in the bacterial composition of water flooding in from the estuary compared to water draining from the lagoon during ebb flows. These differences reflected the varying FIB concentrations and diversity of other bacterial species identified in this study.

Table 5 Bacterial species identified from Del Rey Lagoon during six sampling events from September 2008 through March 2009

Species	12 Sept. 2008		3 Oct. 2008		17 Oct. 2008		14 Nov. 2008		30 Jan. 2009		20 Mar. 2009	
	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB
Acinetobacter haemolyticus									1	1		
Aerococcus urinae							1					
Aeromonas sobria	1					1						
Aeromonas sobria/Vibrio cholerae				1								
Dermacoccus nishinomiyaensis/ Kytococcus sedentarius	1							1				
Escherichia coli	4	2	2	4		7	5	10	8	7	10	6
Escherichia coli/Shigella sonnei									1			
Enterobacter asburiae/cloacae	1											
Enterobacter cloacae							1					
Enterococcus casseliflavus	2	6	2	2	6	5	1	2	4		1	
Enterococcus casseliflavus/gallinarum	2	3	4	2	2		2	2		2		
Enteroccocus durans/hirae										1		
Enterococcus faecium						1			6	1		3
Enteroccocus faecium/durans												2
Enteroccocus faecium/durans/ Lactococcus garvieae												1
Enterococcus faecalis				3				2		2	3	
Enterococcus gallinarum	1			1	2	2	1					3
Enterococcus hirae		1					1					
Gemella begeri/Leuconoctoc mesenteroides/Staphylococcus capitus											1	
Gemella bergeri											1	
Kocuria varians				1								
Lactococcus garvieae	1											
Leuconostoc mesenteroides ssp. *cremoris*		1										
Leuconostoc mesenteroides ssp. *dextranicum*												1
Morganella morganii ssp. *morganii*									1			
Oligella ureolytica/Aeromonas salmonicida										1		
Pantoea spp.							1		1			
Pasteurella pneumotropica										1		
Proteus mirabilis							1					
Proteus vulgaris/penneri							3					
Rothia mucilaginosa											1	
Serratia plymuthica				1			1					
Shewanella putrefaciens	1	1		1		1	2	2	1			
Sphingomonas paucimobilis							2			1		3
Staphylococcus equorum	1											
Staphylococcus hominis ssp. *hominis/ novobiosepticus*												1
Staphylococcus lentus			1		1	1						
Streptococcus gallolyticus spp. *pasteurianus*								1			1	
Streptococcus gallolyticus ssp. *gallolyticus*										1		
Streptococcus gallolyticus ssp.[b]										1		

Table 5 (continued)

Species	12 Sept. 2008		3 Oct. 2008		17 Oct. 2008		14 Nov. 2008		30 Jan. 2009		20 Mar. 2009	
	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB	FLD	EBB
Stretococcus infantarius complex[a]										1		
Streptococcus mutans										2		
Streptococcus oralis/mitis											1	
Streptococcus pneumoniae						1						
Streptococcus pluranimalium			1									
Vagococcus fluvialis	1			1		1						
Vibrio alginolyticus			1									
Vibrio cholerae	5	6	11	2	7	2						
Vibrio fluvialis		1			1	1						
Vibrio fluvialis/cholerae		1		2	1							1
Vibrio fluvialis/metschnikovii/vulnificus					1							
Vibrio metschnikovii										1		1
Virbio parahaemolyticus		1		2		2	2					
Vibrio vulnificus				1	1							
Unidentified isolate	1				1		1	1		2	3	
Total isolates	22	23	22	24	23	25	25	21	23	25	22	22
Total no. spp	13	10	7	14	10	12	15	8	8	15	9	10

Identifications were based on isolates obtained from Idexx Quanti-Tray® 2000 wells using Colilert® -18 and Enterolert® media, and identified with the Vitek 2 Compact

[a] *Streptococcus coli, Streptococcus bovis, Streptococcus gallolyticus* ssp. *gallolyticus, Streptococcus gallolyticus* ssp. *pasteurianus*

[b] *Streptococcus gallolyticus, Streptococcus gallolyticus* ssp. *pasteurianus, Streptococcus mutans*

FIB variation

Concentrations of all FIB groups often differed by up to an order of magnitude over a 6-h period from flood through ebb tide. Concentrations of total coliforms tended to diminish after flooding into the lagoon while *E. coli* and enterococci concentrations could increase during strong ebb flows during spring tides. Dorsey (2006) and Dorsey et al. (2010) measured similar tidal variation in FIB concentrations upstream in the Ballona Wetlands.

The observed tidal variation could have implications for FIB compliance testing at recreational beaches near the mouths of tidally influenced drainage areas. In California, most agencies monitoring beach water quality collect daily or weekly shoreline water samples (one grab sample per station) to determine if FIB concentrations meet state standards. Samples typically are collected during morning hours and then processed by mid-afternoon with results available within 18–24 h. FIB concentrations could be in compliance with standards during the morning collection but exceed standards in the afternoon as tidal conditions change. This situation was demonstrated in the lagoon. When looking at individual replicate data, *E. coli* were within compliance of California recreational water standards (CDPH 2010) for single samples (400 MPN/100 mL) during the morning flood flows but failed these standards on eight occasions during the afternoon ebb flows. Likewise, enterococci failed the standard of 103 MPN/100 mL 12 times during the afternoon ebb flows even though standards were met for this FIB group earlier in the day during the flood flow. As discussed below, elevated densities in the afternoon ebb flows probably were associated with suspended sediments occurring during periods of low spring tides.

These results are inconsequential for the lagoon since swimming is banned but could be important for Dockweiler and Venice Beaches, popular recreational beaches in Santa Monica Bay lying adjacent to the mouth of the Ballona Estuary (Fig. 1). Ballona Creek

Table 6 Notes on the pathogenicity of bacterial species identified in water samples from Del Rey Lagoon

Species	Comment
Acinetobacter haemolyticus	Opportunistic pathogens implicated in infections of the respiratory and urinary tracts, wounds and can cause septicemia; resistant to antibiotics (Murray et al. 1998)
Aerococcus urinae	Opportunistic pathogen implicated in urinary tract infections, bacteremia, endocarditis (Zhang et al. 2000; Murray et al. 1998)
Aeromonas sobria	Ubiquitous in fresh and brackish water, causing gastroenteritis and wound infections in healthy people, systemic disease in immunocompromised patients; resistant to many antibiotics (Daily et al. 1981; Champsaur et al. 1982; Murray et al. 1998)
Kytococcus sedentarius	Common skin bacteria that can cause pitted keratolysis; has been associated with prosthetic valve endocarditis (Singh and Naik 2005; Bannerman and Peacock 2007; English 2010)
Escherichia coli[a]	Toxigenic strain O157:H7 associated with illness from swimming in contaminated fresh waters (Craun et al. 2005)
Enterobacter cloacae[a]	Naturally found in terrestrial and aquatic habitats; implicated in variety of nosocomial infections; major human pathogen (Sanders and Sanders 1997; Halda-Alija et al. 2001; Abbott 2007)
Enterococcus spp.[a]	Many species (e.g., *E. faecalis, E. faecium, E. durans, E. avium, E. gallinarum, E. casselifalvis)* considered prominent nosocomial pathogens; resistant to multiple antibiotics (Gordon et al. 1992; Moellering 1992; Huycke et al. 1998; Murray et al. 1998; Teixeira et al. 2007)
Gemella spp.	Strains have been implicated in endocarditis, meningitis, and other infections (Facklam and Elliott 1995)
Kocuria varians	Inhabits human skin, can become opportunistic pathogen in immunologically suppressed patients (Shashikala et al. 2008)
Lactococcus garvieae	Mainly a fish pathogen but has been isolated from endocarditis infections (Fefera et al. 1998; Deguchi et al. 1991)
Proteus mirabilis[a]	Prominent in nosocomial urinary tract infections; resistant to several antibiotics (de Champs 2000; Abbott 2007; Lockhart et al. 2007).
Proteus vulgaris or *penneri*[a]	Implicated in nosocomial abdominal and urinary tract and subcutaneous infections (Krajden et al. 1987; Engler et al. 1990; Abbott 2007)
Rothia mucilaginosa	Opportunistic pathogen implicated in cases of endocarditis, meningitis, peritonitis, and other infections (Ruoff 2002)
Serratia plymuthica	Uncommon human pathogen implicated in both nosocomial and community acquired infections, mainly in immunocompromised patients; resistant to many antibiotics (Carrero et al. 1995; Murray et al. 1998)
Sphingomonas paucimobilis	Nosocomial infections caused by contaminated water supplies in hospitals (Perola et al. 2002).
Staphylococcus equorum	Associated with nosocomial infections (Vandenesch et al. 1995)
Staphylococcus hominis ssp hominis or *novobiosepticus*	*S. hominis* indigenous to human skin, occasionally causative agent in infections associated with catheters, urinary tract infections, septicemia (Kloos and Bannerman 1994; Bannerman and Peacock 2007)
Staphylococcus lentus	Member of the *S. sciuri* group implicated in nosocomial urinary tract infections, resistant to chloramphenicol, ciprofloxacin, and norfloxacin (Stepanovic et al. 2003)
Streptococcus mutans	Member of the Viridans Group of streptococci, common in human mouth, most frequently associated with dental caries (Murray et al. 1998; Facklam 2002; Spellerberg and Brandt 2007)
Streptococcus oralis or *mitis*	*S. mitis* group can become aggressively pathogenic in immunologically compromised individuals, causing septicemia, respiratory infections (Hardle and Whlley 1997; Spellerberg and Brandt 2007)
Streptococcus pneumoniae[a]	Leading cause of community acquired pneumoniae, other serious acute infections; resistant to many antibiotics (Facklam 2002; Spellerberg and Brandt 2007)
Vibrio spp.[a]	Haliophilic vibrios naturally occurring in coastal marine and estuarine waters; *V. alginolyticus, V. parahaemolyticus, V. cholerae, V. fluvialis* and *V. vulnificus* can cause mild to severe cytotoxic effects depending on the strain and associated virulence factors; implicated in gastrointestinal, septicemia, cellulitis, and ear, skin and eye infections from consumption of under cooked seafood or wound infections after exposure in contaminated water (Schmidt et al. 1979; Hardy and Klontz 1996; Baffone et al. 2000, Murray et al. 1998, Harwood et al. 2004; Abbott et al. 2007)

[a] Species have strains considered to be significant human pathogens

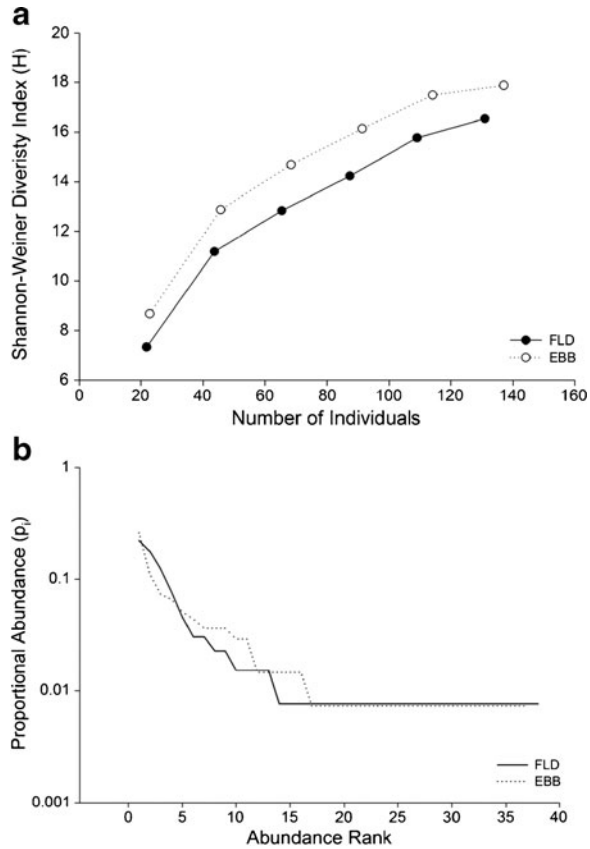

Fig. 3 Rarefaction curve for the Shannon diversity index (**a**) and rank–abundance curve (**b**) of bacterial assemblages detected in the flood (*FLD*) and ebb (*EBB*) flows

is a significant source of FIB (Dorsey and Lindaman 2004; Stein and Tiefenthaler 2005; Tiefenthaler et al. 2009) as is the estuary (Dorsey 2006), particularly during ebb flows (Dorsey et al. 2010). Morning compliance sampling during a flood tide could indicate that standards are met, but 6 h later during the ebb flow, standards could be exceeded, thus placing swimmers at greater risk of exposure to possible pathogens. Boehm et al. (2002) measured FIB variability along the open ocean beach at Huntington Beach where the surf zone was impacted with flow from the Santa Ana River and Talbert Marsh. FIB varied over time scales ranging from minutes to hours depending on tidal and daylight conditions. Given this variability, especially around the mouths of major drainages, Boehm et al. (2002) recommend that compliance monitoring focus on obtaining a geometric mean from a series of sample collected over a longer period, such as a week. This approach would enable beach managers to judge water quality based on 30-day standards while still being able to respond to spikes in FIB concentrations measured from instantaneous samples.

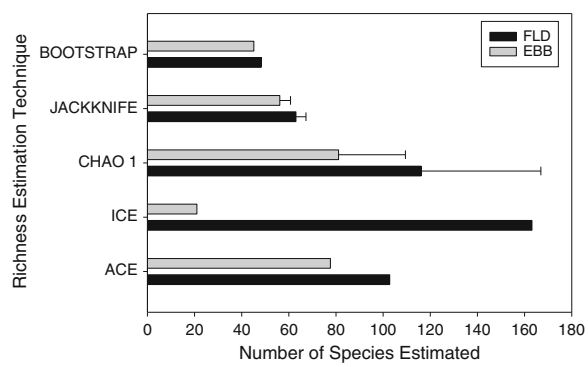

Fig. 4 Comparison of the number of bacterial species estimated in flood (*FLD*) and ebb (*EBB*) flows by means of different richness-estimation techniques. *Error bars* indicate one standard deviation unit

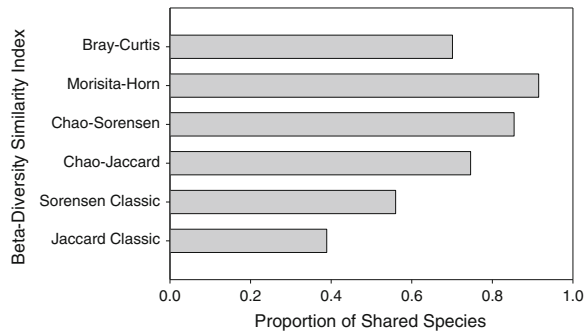

Fig. 5 The proportion of shared species between bacterial assemblages detected in flood (*FLD*) and ebb (*EBB*) flows by means of different similarity-analysis techniques

The lagoon as a sink for FIB

The Ballona Estuary tended to be a major source of FIB for the lagoon, especially for total coliforms whose mean $R_{F/E}$ value over the study period equaled 1.83 (Table 3). In turn, the estuary is contaminated mainly by runoff from Ballona Creek. Within the creek itself, Stein and Tiefenthaler (2004) and Tiefenthaler et al. (2009) measured dry weather geometric mean concentrations of *E. coli* and enterococci ranging from 10^2 to 10^3 MPN/100 mL and 10^4 for total coliforms. In the lower reaches of the creek, Dorsey and Lindaman (2004) measured similar densities for *E. coli* and enterococci during dry weather with total coliforms averaging 10^4–10^5 MPN/100 mL. During FIB loading studies within the Ballona Wetlands, concentrations in the estuary generally were an order of magnitude greater than those found in the wetlands, following similar trends with mean concentrations ranging from 10^2 to 10^4 MPN/100 mL for total coliforms and 10^1–10^3 MPN/100 mL for both *E. coli* and enterococci (Dorsey et al. 2010).

The lagoon tended to be a sink for total coliforms based on values of $R_{F/E}$ >1.0 for 56 % of the sampling events and a mean ratio of 2.9. Exposure to sunlight could explain this trend. During this study, sampling usually was done during daylight hours with flood tides occurring during morning hours, ebb flows later in afternoons or early evenings (Table 1). During the day, solar UV light likely was a factor in reducing densities of FIB within the surface waters of the lagoon. Sunlight has been demonstrated to reduce FIB densities within wetland and other water systems (Jillson et al. 2001; Boehm et al. 2002; Stinton et al. 2002; Karathanasis et al, 2003; Noble et al. 2004; Mayo 2004; Vymazal 2005; Whitman et al. 2008).

During this study, there were three occasions when total coliform densities did not diminish between flood and subsequent ebb flows. These events occurred when all samples were collected at night (3 June

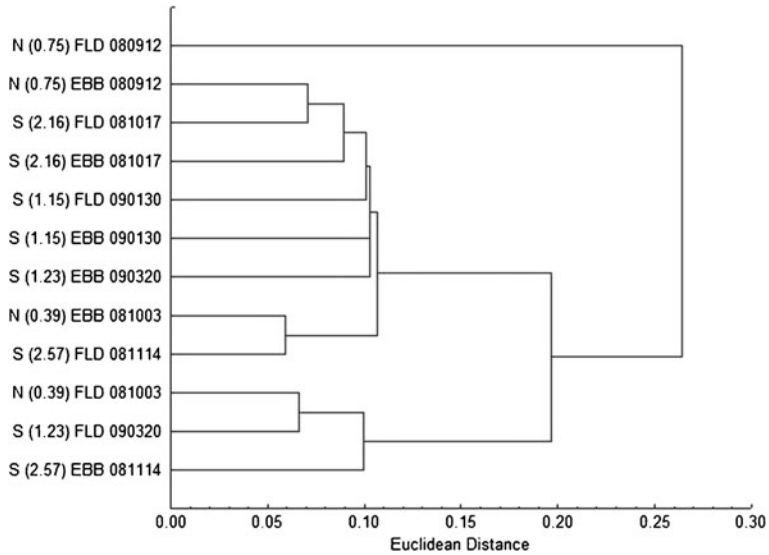

Fig. 6 The variation among bacterial assemblages (in terms of both species composition and species abundance) with respect to both tidal flow (FLD or EBB) and sampling date (expressed as year–month–day or YYMMDD). For each date, the neap (*N*) or spring (*S*) tidal conditions are given along with the tidal range between the high and low tides sampled that day

Table 7 Predicted vs. observed percent reduction (or increase) of FIB in the Del Rey Lagoon based on light inactivation coefficients (k_d) determined by Noble et al. (2004). Sampling events conducted during night hours in June and July 2008 were not included in this analysis

Date	Δt (h)	Total coliforms			*E. coli*			Enterococci		
		k_d	Predicted % reduction (–)	Observed % reduction (–) or addition (+)	kd	Predicted % reduction (–)	Observed % reduction (–) or addition (+)	kd	Predicted % reduction (–)[a]	Observed % reduction (–) or addition (+)
7 Feb. 2008	6	0.025	−29.2	-66.2	0.048	−48.5	−0.7	0.243	−24.5	+19.2
13 Feb. 2008	4.5	0.025	−22.8	+10.2	0.048	−39.2	+438.1	0.243	−38.9	+125.1
11 Mar. 2008	5	0.025	−25.0	-23.3	0.048	−42.5	+374.0	0.243	+23.4	−22.6
20 Mar. 2008	5	0.025	−25.0	+6.3	0.048	−42.5	+49.5	0.243	−66.3	+139.6
25 Mar. 2008	5	0.025	−25.0	-62.7	0.048	−42.5	+185.5	0.243	+23.4	+157.9
8 Apr. 2008	4.5	0.025	−22.8	-44.4	0.048	−39.2	+423.8	0.243	+24.0	0
22 Apr. 2008	5	0.025	−25.0	-30.4	0.048	−42.5	−72.5	0.243	−66.0	−72.5
14 May. 2008	6	0.037	−40.0	-53.9	0.137	−84.9	−67.4	0.257	−32.1	−67.4
12 Sept. 2008	6	0.037	−40.0	+31.6	0.137	−84.9	+30.7	0.257	−10.7	−12.1
3 Oct. 2008	8	0.037	−49.4	-86.7	0.137	−92.0	+4.8	0.257	+380.0	+279.7
17 Oct. 2008	7	0.037	−44.9	-55.6	0.137	−89.0	−39.4	0.257	+12.4	+250.5
14 Nov. 2008	7	0.025	−33.2	-68.9	0.048	−53.9	+6.1	0.243	−41.8	+30.6
30 Jan. 2009	6	0.025	−29.2	+52.8	0.048	−48.5	+1348.3	0.243	+187.1	+1240.0
20 Mar. 2009	7	0.025	−33.2	+590.9	0.048	−53.9	+879.7	0.243	+63.6	+188.9

Δt time between flood and ebb flow sample collection, k_d lower light conditions for winter days, larger coefficients for summer light conditions (from Noble et al. 2004)

[a] Positive values occurred when enterococci densities were close or at method detection levels

2008 and 14–15 July 2008), or the flood flow samples were collected before sunrise (20 March 2009). For these events, $R_{F/E}$ values for total coliforms were <1.0 with significantly greater mean densities during the ebb tides (Table 3). More than likely, there was insufficient exposure of total coliforms to UV light over this period to effectively reduce their concentrations.

Noble et al. (2004) determined light inactivation coefficients (k_D) for FIB in a series of experiments where sea- and freshwater matrices were inoculated with sewage and runoff, then exposed to full and reduced (simulating winter insolation) sunlight for a period of about 1 week. Results indicated that inactivation rates were similar in both fresh and seawater matrices, and that enterococci degraded more rapidly in sunlight than *E. coli*. Because the study of Noble et al. was conducted at a facility about 4.5 km from the Del Rey Lagoon, we used the k_D coefficients generated from this study to see if FIB in the lagoon were inactivated at similar rates during the daylight hours (Table 7). Densities of total coliforms fell on 9 out of 14 occasions. These reductions averaged 54.7 %, slightly more than the average of 30.4 % predicted by the k_D coefficients. In contrast, *E. coli* and enterococci had reduced densities on only 4 of the 14 dates examined, indicating that the lagoon may act as a source for these bacteria as discussed below. On the four occasions that densities did fall over the day, the observed percent reductions were similar to the predicted values. We conclude from this analysis that light does reduce densities of FIB, particularly total coliforms in this case, but that other factors, discussed below, tend to reintroduce FIB back into the lagoon's water.

The lagoon as a source for FIB

Over the 16 sampling events, $R_{F/E}$ values for *E. coli* and enterococci were <1 on 11 occasions or 68 % of the time (Table 3), suggesting that the lagoon was a source for these FIB groups. It was not apparent why *E. coli* displayed this pattern since it did not significantly correlate with other FIB groups, water quality

parameters, or tidal levels. It is possible that a source for this FIB would be the many species of birds using the lagoon (Table 4) as discussed below. In contrast, densities of enterococci were associated with greater salinities, turbidity, and lower tidal levels. Greater salinities were measured during both higher and lower tidal levels associated with spring tides (Fig. 2). Flood flows would bring more saline water in from the estuary with corresponding loads of enterococci. During spring ebb flows, salinities also were relatively greater, possibly from evaporation in the tidal channels as water levels and volume fell throughout the day. During these spring tide ebb flows, densities of enterococci were elevated as tidal heights diminished, presumably due to suspension of enterococci with bottom sediments. During spring low tides when water levels are less than 1.0 m mean lower low water, water within the lagoon drains from a narrow central channel approximately 3–5 m wide. At the tide gate, swifter ebb flows within the channel tend to suspend sediments, increasing turbidity and associated FIB. Based on a regression between enterococci and low tide heights (R^2= 0.36, p<0.01, y=−0.61X+1.76), a tidal height of ≤0.35 m would result in enterococci densities ≥103 MPN/100 mL, exceeding bathing water standards for the State of California (CDPH 2010). A similar situation was documented in the nearby Ballona Wetlands where Dorsey et al. (2010) measured greater current speeds, turbidity, and FIB spikes associated with spring tide ebb flows.

Sediments can have viable populations of enterococci (Ferguson et al. 2005; Lee et al. 2006; Yamahara et al. 2009) and *E. coli* (Lee et al. 2006; Ishii et al. 2007), resulting in increased concentrations within the water column if sediment FIB reservoirs are suspended through storm or tidal actions (Sanders et al. 2005). Evanson and Ambrose (2006) found that within a wetland system impacted by urban runoff, the sediment-associated FIB populations may be distinct from those in the overlying water column based on the ratio of total coliforms/*E. coli* (TC/EC). In their study, sediment populations generally had ratios >10 compared to overlying water where TC/EC ratios were <10, suggesting possible human fecal contamination (Haile et al. 1999). Within the Del Rey Lagoon, additional studies like that of Evanson and Ambrose (2006) would be required to conclusively determine if increased FIB concentrations during ebb flows are caused by tidally suspended sediments.

Birds using the lagoon as a resource are another potentially significant source of FIB impacting lagoon water. Bird feces have been shown to be a prime source of fecal material, driving up FIB densities within the water and shoreline sediment (Alderisio and Deluca 1999; Ricca and Cooney 1998). During this study, we observed flocks of American coots and mallards reaching densities of 100 individuals or more, while various species of gull collectively approached similar densities (Table 4). A variety of shorebird species were common, especially during low tides when they were observed foraging along the lagoon's exposed mudflat. American coots, mallards, Western gulls, and killdeers are year-round residents of the area while the other species of waterfowl, gulls, and shorebirds observed are mainly winter residents (Cooper 2006). Based on correlation analyses, there was no significant relationship between the densities of FIB groups and the densities of birds observed at the lagoon.

Regarding enterococci specifically, Ferguson et al. (2011) and von Bitner et al. (2011) found *Enterococcus faecalis and Enterococcus faecium* to predominate in sediments where flocks of shorebirds fed and roosted, suggesting that the source of this species were the birds. In Del Rey Lagoon, these two species represented 24 % of the 87 *Enterococcus* isolates (Table 5). Further studies at the lagoon should document the seasonal use of the lagoon by birds, more closely linking their densities per unit area with changing FIB concentrations.

A final source of FIB entering the lagoon could be runoff from the local community and adjacent park. The City of Los Angeles has noted four small storm drains that empty directly into the lagoon (CLA 2007). Although flows from these drains are unknown, we have observed them to convey only a small volume of runoff, so their contribution of FIB to the lagoon may be irrelevant. Nonetheless, their flows should be measured to estimate FIB loading. Irrigation of the parkland adjacent to the lagoon also could result in contaminated runoff entering the water. For example, when American coots are present, they often congregate in large groups on the parkland located at the southern end of the lagoon. During irrigation, water not soaking into the ground could carry their feces into the lagoon.

Despite the FIB spikes during spring tide ebb flows, water flowing from the lagoon still might be diluting water flowing from the more contaminated estuary into Santa Monica Bay. Dorsey et al. (2010) reported

this situation for ebb flows departing the Ballona Wetlands' west and east tide gates located at 206 and 388 m, respectively, upstream from the lagoon's tide gate. Further measurements would need to be conducted comparing ebb flows from the lagoon with those of the estuary to determine if dilution occurs and under what tidal circumstances. If additional investigations indicate that lagoon ebb flows dilute those of the estuary, then we would not consider the lagoon to be a significant source of FIB to the adjacent Ballona Estuary.

Bacterial isolates and public health considerations

Bacterial isolates identified from the Idexx Quanti-Trays® using the V2C system provided a measure of the bacterial community composition even though the following factors could affect the diversity measurements:

1. Only a fraction of potentially culturable species was sampled from the Quanti-Tray wells.
2. A selectivity bias would be present due to components in the Colilert®-18 and Enterolert® media. Both these broth-type media are subject to species selection bias as opposed to solid media, allowing for faster growing or hardier species and strains to predominate. However, this limitation did not appear to be a major problem in this study given the high diversity of species measured (Table 5).
3. By sampling only positive Quanti-Tray® wells, *E. coli* and species of enterococci would be enriched, thus biasing the diversity results.

Despite these drawbacks, a variety of species were isolated, providing a measure of diversity for the various tidal flows. Also, identifying various *Enterococcus* species was of public health interest since many have been implicated as human pathogens (Teixeira et al. 2007).

This method indicated that during ebb flow conditions, the assemblage of bacterial isolates were more α-diverse than when water flooded into the lagoon from the estuary (Fig. 3a), and ebb flows had a greater evenness (relative species abundance) in the community composition. Rarefaction curves for the ebb and flood flows (Fig. 3a) never reached their respective asymptotes, indicating that there are more species yet to be encountered at the two tidal conditions using this identification system. Based on sampling patterns, all species richness estimates predict that flood flow will overtake ebb flow in terms of the greatest number of bacterial species (Fig. 4). However, it is unclear how a greater richness estimate would impact species evenness, a measure of the equitability of individuals among flood flow species. This complexity is important because changes in species evenness during flood flow conditions will also serve to impact (by either increasing or decreasing) the bacterial species index of diversity. Species unique to flood and ebb flows reflect a medium-to-low β-diversity of shared species between the two bacterial assemblages. However, the cluster analysis (Fig. 6) points to a dynamic assemblage that changes species composition with respect to both day-to-day fluctuations and tidal action, as represented by mix of tidal flows (ebb vs. flood) and ranges (neap vs. spring) within clusters. The variability and dynamic nature of the bacterial assemblages measured in the flood and ebb flows is not surprising given the potentially great number of bacterial species present in the lagoon and estuary systems. Bacterial assemblages would represent a mix of marine indigenous species and those externally introduced from contaminated urban runoff.

Of the total number of species identified, approximately 56 % represent species that have strains reported to be associated with human illnesses (Table 6). These included natural marine pathogens, mainly *Vibrio* spp., and many opportunistic pathogens implicated in nosocomial infections. The presence of these species could be of public health concern under the following defining conditions for the disease triangle (Scholthof 2007): (1) the pathogenic strain of the species must be present and in sufficient abundance to cause infections, (2) the person must be exposed to the pathogen and susceptible to infection, and (3) environmental conditions must conducive for maintaining the pathogen.

With regard to the first condition above, Stewart et al. (2008) point out that not all strains of an infectious bacterial species are equally pathogenic. For example, results presented herein showed that species of *Vibrio*, particularly *Vibrio cholerae*, were more common in the lagoon during warmer water months (Table 5). Further molecular testing is required to determine whether the *Vibrio* strains found in this study are toxigenic. Of the 103 recognized strains of *V. cholerae*, only *V. cholerae* O1 and *V. cholerae* O130 cause

the severe gastrointestinal illness associated with many epidemics (Abbott et al. 2007). A third strain, *V. cholerae* Non-O1, causes not only a milder form of watery diarrhea but also has been implicated in cases of severe septicemia (Abbott et al. 2007). Therefore, microbial risk analyses in bodies of water where people can be exposed to natural and introduced pathogens need to include genomic analyses or other techniques such as agglutination for pathogenic species of *Vibrio*, to identify those strains of microorganisms expressing virulence genes required for human illness (Stewart et al. 2008).

The second condition above, human exposure and degree of susceptibility to pathogens, is limited in the lagoon since it is posted for no swimming, and shellfish consumption is banned during the warm water months of May through October when this source of seafood can be contaminated by blooms of harmful dinoflagellates and other toxic alga. However, children playing in the sand along the lagoon's shoreline could be at greater risk for illness and infections. A child would have less of an immune response compared to an adult, conceivably acquiring wound infections from digging in the sand or gastrointestinal illness if ingesting contaminated sand. Bacteria capable of producing such illnesses were present in the lagoon (Tables 5 and 6), but the question remains—were environmental conditions favorable for sustaining pathogenic strains in sufficient concentrations to cause increased health risks? This question would also apply to the bathing beaches surrounding the mouth of the Ballona Estuary, especially during periods of ebb flows when swimmers would be exposed to potentially greater levels of pathogens. With regard to shellfish consumption, we recommend that this source of seafood be banned year around given the potentially pathogenic species of bacteria recorded in this study.

Knowledge of pathogen loading into coastal waters via nonpoint source runoff is limited (Stewart et al. 2008), and the association with human illness may not be predicted using traditional FIB indicators (Colford et al. 2007). Stewart et al. (2008) discuss how coastal habitats and their resident populations of marine organisms (e.g., shellfish) can harbor pathogens introduced from terrestrial sources like contaminated runoff or sewage effluents. These habitats and organisms act as sentinels to be monitored, providing information on emerging pathogens, potential health impacts to human users, and general health of the ecosystem. The Del Rey Lagoon and adjacent Ballona Estuary should be considered as such a habitat sentinel. Periodic monitoring of the non-FIB microbial assemblage utilizing molecular methods such as PCR or microarrays (Girones et al. 2010) should be performed on key ecosystem components of this system such as flood and ebb flow water, sediments, and edible seafood (clams and mussels). Also included in such monitoring would be the contribution of microorganisms by birds into this habitat. Such sentinel monitoring would provide valuable information to resource managers and regulators on the overall health of the system and aid in assessing progress towards improved water quality as regulatory programs like TMDLs are implemented and conducted.

Conclusions

1. Concentrations of all FIB groups often differed by up to an order of magnitude over a tidal period.
2. The lagoon tended to act as a sink for densities of total coliforms. In contrast, *E. coli* and enterococci concentrations were greater during ebb flow conditions for the majority of the sampling events. Enterococci were associated with the stronger spring tide ebb flows, suggesting that the lagoon was a source for this FIB group during these tidal conditions. Also, another potentially significant source of these two FIB groups could be from the many species of birds using the lagoon as a resource.
3. Ebb flow conditions showed the highest α-diversity of bacterial isolates. We also detected a medium-to-low β-diversity of shared species between ebb and flood flows. Rarefaction curves indicated that bacterial diversity for both ebb and flood flow conditions are still increasing. Estimates of species richness (based on patterns in the sampling data) projected that flood flow will eventually overtake ebb flow in terms of species richness. The cluster analysis of bacterial isolates also reflected a complex and dynamic bacterial assemblage of both marine indigenous and externally introduced species that varied in terms of species

abundance and composition between sampling dates and ebb vs. flood flow conditions.

4. Approximately 56 % of the identified isolates represented potential opportunistic human pathogens; many of which have been implicated in nosocomial infections. Pathogenic strains within a few of these species groups, such as *Vibrio alginolyticus* could be harmful to people recreating along the lagoon's shoreline (mainly children) due to infections of open wounds, or consuming shellfish during winter months when harvesting is permitted.

Acknowledgments We wish to acknowledge the hard work and dedication of Marcus Raymond and Bridget Hellige, our students who spent many hours in the field and lab making this study possible. Donna Ferguson, Dr. Yiping Cao (Southern California Coastal Water Research Project), and Dr. David Turbow (TUI University, College of Health Sciences) reviewed the manuscript, providing valuable comments and suggestions. We wish to thank Dr. Gerald McGowan (City of Los Angeles, Bureau of Sanitation, Environmental Monitoring Division) for providing initial suggestions for the study along with Idexx and Vitek testing supplies. A Loyola Marymount University Continuing Faculty Grant (to JHD), Rains Research Assistant Grant, and a grant from the Southern California Academy of Sciences (Research Training Program) helped support this research.

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