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Densities of Fecal Indicator Bacteria in Tidal Waters of the Ballona Wetlands, Los Angeles County, California

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Abstract.—Densities of fecal indicator bacteria (FIB) represented by total coliforms, *E. coli* and enterococci were measured within tidal channels of the Ballona Wetlands (Los Angeles County) to see if the wetlands act as a sink or source for these bacteria and to measure increases in FIB densities during wet weather. Samples were collected on 10 days over a 1-yr period beginning February 2003 at four sites within the wetlands and one site in Ballona Creek opposite the west tide gate. Incoming flood and outgoing ebb tides were sampled during each sampling event at each station. Water from Ballona Creek may be a significant source of indicator bacteria in the wetlands. Within the tidal channels, densities for total coliforms typically ranged from 10^3-10^4 MPN/100 ml, but ranged up to 10^6 during runoff events. Densities of *E. coli* and enterococci were orders of magnitude less than those measured for total coliforms, generally ranging from 10^{1} – 10^{4} for *E*. $\text{coll},$ and $10^{1}-10^{5}$ for enterococci; greater densities were associated with runoff events. Densities of FIB tended to be up to three times greater during flood than ebb tide conditions depending on the tidal range. This result suggests that more FIB may be entering the wetlands on flood tides than leaving during ebbs, so that these bacteria either are being destroyed, sinking into the tidal channel sediments and plant surfaces, or both. This hypothesis needs to be tested by further identifying other possible FIB sources within the wetlands, and increasing the study design's statistical power to better characterize the flux of these bacteria entering and leaving the wetlands.

Wetlands, both freshwater and salt marsh, are noted for their water purification abilities. For example, suspended sediments will settle into the marsh systems including any associated metal and organic pollutant loads while nutrients will be utilized by plants (Keddy 2000; Mitsch and Gosselink 2000) and feces-derived bacteria are eliminated from the water through destruction by sunlight and other mechanisms (Mayo 1995; Sinton *et al.* 2002). Given these qualities, wetland systems are being used to purify sewage effluents and runoff (Kadlec and Knight 1996; Schueler and Holland 2000).

Conversely, wetlands such as coastal salt marshes can be sources of fecal bacteria depending on degrees of biological activity and input of contaminated runoff from surrounding urban areas. Water contaminated with feces-derived coliform and enterococci bacteria outwelling from coastal marshes has been shown to cause water quality problems at adjacent ocean beaches where swimmers and surfers recreate (Grant *et al.* 2001). If bacterial bathing water standards are not met, then beaches can be posted with advisories or closed resulting in millions of dollars of lost revenues to local businesses. Understanding the sources of fecal bacteria,

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and their circulation dynamics within coastal marshes, and outwelling to adjacent beaches will help refine regulatory strategies to protect the public.

The goal of this study was to characterize levels of fecal indicator bacteria during different tidal flows within the Ballona Wetlands, a salt marsh located in Los Angeles County along the southern border of Marina Del Rey boat harbor. The central question for this study was: Are concentrations of fecal indicator bacteria greater entering the wetlands from Ballona Creek than leaving the wetland system? To begin addressing this question, densities of fecal indicator bacteria (FIB: total coliforms, *E. coli,* enterococci) were measured in the creek and throughout the wetland during different tidal flows (flood and ebb conditions), and amplitudes (neap vs. spring). To date, FIB measurements have been made in Ballona Creek and Estuary by various storm water monitoring agencies (e.g. LADPW Watershed Management Division: http://ladwp.org/wmd) or runoff research studies (e.g. Dorsey and Lindaman 2004, Stein and Tiefenthaler 2004). Contrastingly, only a single water sample characterizing coliform densities has been collected in the Ballona Wetlands. This work was done in 1998 by a consulting group performing an environmental survey for the Playa Vista development project (Camp Dresser & McKee 1998 as reported in U.S. Army Corps of Engineers 1999). Results reported herein are the first major effort to characterize FIB densities in this wetland system.

Ballona Wetland Study Area

The Ballona Wetlands consist of approximately 180 acres of tidal salt marsh receiving muted tidal flows. It is the last remaining major coastal wetland in Los Angeles County (West 2001). As such, it is surrounded by extensively developed urban areas and impacted by many other human activities. Historically, rail and roadways have fragmented the wetlands since the 1800's. In 1937 The Army Corps of Engineers (COE) and the Los Angeles County Flood Control District constructed the Ballona Creek flood control channel that diverted water from the creek straight to the Santa Monica Bay, thus severely limiting tidal flow to the salt marsh. Presently, water entering the wetlands mainly is from Ballona Creek via a single tidal gate (described below). Other sources of freshwater would include intermittent flows of runoff from residential areas on the southern bluffs, and storm runoff from Culver Blvd. running through the wetland area.

In 2004, several major events occurred that began reversing the history of negative impacts to this wetland. The State of California purchased the remaining 180 acres of wetlands from a developer for eventual restoration, and the COE constructed a new set of tide gates.

Tidal flows within the wetlands are muted. Prior to April 2003 water entered the system through two tidal gates positioned along the southern bank of the Ballona Creek estuary. Both gates were poorly maintained, allowing only a limited amount of tidal flow in and out of the wetlands. The eastern gate was the main gate for the wetlands, allowing in flood and ebb flows. The western gate was a one-way flap-gate allowing only ebb flows to leave the wetlands. Due to corroded hinges, the western gate was frozen in an open position, allowing some flood flows into the wetlands.

In April 2003 the COE replaced the old eastern tide gate with a pair of selfregulating gates allowing a maximum tidal level of 1.1 m to occur (U.S. Army

Corps of Engineers 1999). In November 2003, the COE removed marine growth and corrosion from the hinges of the west tide gate so that it now functions to only allow ebb flows to leave the wetlands. The wetlands now receive flood flows only through the east tide. When the tide turns, ebb flows drain the tidal channels via both the east and west gates.

Wetland and tidal channel monitoring associated with the new tide gate system is being conducted by the City of Los Angeles (Bureau of Sanitation, Environmental Monitoring Division) to document any changes in the biological community associated with this project. If populations of native species are stable, then tidal levels may be increased by 0.1 m. This decision will be made by the California Department of Fish and Game, who, in conjunction with the California Coastal Conservancy, are now developing a restoration plan and implementation strategy to restore the Ballona Wetlands to a more naturally functioning wetland.

Methods

Station Locations and Sampling Times

Water quality measurements and FIB densities were collected along the intertidal channels at five stations during this study (Figure 1). These channels always had some water flow, even during the lowest spring tides. Stations 1 through 4 were located within the wetlands while Station 5 was in Ballona Creek. Both Station 1 and 5 were positioned at the west tide gate. This tidal gate was selected over the eastern gate for its safer access to the tidal channel, and lack of disrupting construction activity. Stations were sampled on a variety of tides ranging from neap to spring tides. Collection days, tidal conditions and ranges are given in Table 1.

Field Procedures

Water measurements and samples were collected within the tidal channels by standing on the bank and reaching out into the channel with a 10-ft rod with either a sensor or a container for a water sample. Only water from the surface was collected for testing. Water quality measurements were collected *in situ* using an YSI 600 QS sonde. At each station the sonde was positioned about 1-ft beneath the water surface, and allowed to equilibrate over about a 5-min period before the suite of measurements were taken. Measurements included water temperature (degrees C), salinity (ppt) and dissolved oxygen (mg/L). A single water sample for bacterial densities was collected in sterile 125 ml polypropylene bottles, placed on ice, and returned to the laboratory for determination of FIB densities within the 6-hr holding time. On three occasions (March 6, 16 and 28, 2003) three instantaneous replicate water samples were collected at each station to measure FIB variability.

Bacterial Determinations

Densities of FIB (total coliforms, *E. coli,* enterococci) were measured using Idexx test kits (http://www.idexx.com) based on enzyme substrate test methods (APHA *et al.* 1998: Standard Methods Section 9223 B.). During dry weather conditions, 10 ml of sample water was added to 90 ml of sterile deionized water mixed with either Enterolert media (for enterococci) or Colilert-18 (for total co-

Fig. 1. Map of the Ballona Wetlands in relation to Marina Del Rey, California **STA 5** Sampling stations are shown on the aerial photograph (USGS images from: http://edcsns17.cr.usgs.gov).

liforms and *E. coli*). During wet weather, and additional dilution of 1 ml sample in 99 ml of sterile water/media was added allowing for higher FIB densities. The inoculated 100 ml samples then were poured into 97-well QuantiTray-2000 containers, sealed, and transferred to the incubators. The total coliform/*E. coli* trays were incubated for 18-22 hrs at 35°C, and the enterococci trays for 24 hrs at 41°C. Tray-cells showing color changes (yellow for total coliforms, fluorescent

* Wet weather runoff event.

blue for *E. coli* and enterococci) were counted, and MPN/100 ml were determined for each indicator group.

Results

Water Quality Measurements

A summary of the water quality measurements is presented in Table 2. In general, water temperatures were cooler for water flooding into the wetlands from the Ballona Creek estuary with station averages ranging from 13.7–15.2°C. After residing in the wetlands for several hours, mean temperatures were elevated by

2.5 to 4.8°C in water ebbing back into Ballona Creek. Overall temperatures ranged from 7.8-22.4°C with the warmest occurring during spring low-tide ebb flows.

Salinities at the wetland Stations 2–4 generally were brackish with means ranging from 11.6–19.3 ppt. Upper salinity ranges at these sites were measured at about 23–33 ppt during both flood and ebb flows. Within the Ballona Creek estuary (Station 5) and at Station 1, mean salinities were slightly higher ranging from 18.9–22.0 ppt. During ebb flows, the highest values of 22.0 ppt were measured at both these stations. During the three wet weather runoff events (Table 1), salinities ranged from 0.5–2.2 ppt on the flood tides, and 7.5–8.9 on the ebbs, reflecting the influence of runoff from both Ballona Creek and the marsh flats and surrounding bluffs.

Concentrations of dissolved oxygen generally were lower during morning flood tide conditions compared with measurements taken later each day during the ebb flow. Mean concentrations during flood flows ranged from 4.47–7.64 mg/L, rising to 8.18–12.98 mg/L during ebb flows, presumably due to photosynthetic action from plants living in the tidal channels. Some of the lowest values for dissolved oxygen occurred during the flood tidal flow, the lowest occurring at Station 3 (1.88 mg/L on 2-21-03, and 1.13 mg/L on 10-26-03).

Bacterial Densities

Histograms of mean FIB densities for each sampling day for the tidal flows are presented in Figures 2 through 4. These data are summarized in Table 3 where mean densities for each fecal indicator bacteria (FIB) among the five stations during flood and ebb tidal flows are presented. In this table, dry- and wet-weather days were separated, yielding a mean FIB density for each weather situation.

In general, FIB densities in flood flows tended to be greater than those in ebb flows, especially for *E. coli* and enterococci (Table 3). However, this trend was only statistically significant on a few occasions for each FIB group, probably owing to the variability within these data. Densities of FIB groups were tested between flood and ebb flows at each station for the three sampling dates (March 6, 16 and 28, 2003) where replicate samples $(n = 3)$ were collected. Of the 15 tests performed, only six yielded significant differences ($p < 0.05$) with densities being greater during flood flows four of the six times (Table 4). During wet weather, all FIB densities increased by one to several orders of magnitude over dry weather densities. During dry weather, FIB densities tended to diminish with increasing salinity (Fig. 5). Overall, correlations were weak, although this relationship was significant for at least one FIB group at each of the wetland stations.

The ratio of bacterial density between flood and ebb flows was determined for each FIB group at each station, and regressed with the tidal ranges over the study period. This approach was used to more closely examine changes in FIB densities over varying tidal ranges, so a FIB Flood: Ebb ratio of ≥ 1 would indicate higher densities of FIB on the incoming flood tide. Using only dry weather data, regressions were performed for each FIB group at the five stations. The ratio of Flood:Ebb densities generally diminished with increasing tidal ranges suggesting that FIB densities were nearly equal as the tidal flows moved into spring tide conditions. This relationship was not significant at any of the stations, and was best demonstrated at Station 1 (Fig. 6).

Fig. 2. Densities of total coliforms (MPN/100 ml) during flood and ebb tidal flows, February 2003–2004. The "greater than" symbol $(>)$ indicates densities greater than the method detection level for the dilution used.

Total coliforms. Total coliforms generally ranged from $10³-10⁵$ with higher values occurring during the two wet weather runoff events (Table 3). The highest value recorded (1.4 \times 10⁶ MPN/100 ml) occurred during wet weather at Station 1 during the ebb flow. Histograms (Fig. 2) among the five stations over the entire sampling period indicated that densities of total coliforms at Stations 5 and 1, positioned in and adjacent to Ballona Creek, respectively, tended to be greater during flood flows (Table 3). This pattern was most consistent at Station 1 where flood densities exceeded ebb densities 90% of the time (Table 5) followed by Station 5 at 70%. This pattern, however, was not statistically significant on the three occasions where replicate samples were taken. Results of t-tests for replicated samples (Table 4) indicated that total coliforms were significantly greater during flood flows only at Station 4 on March 16, 2003 during a runoff event. On this same day, the ebb flow had significantly greater densities of total coliforms at Station 5 ($p = 0.004$), demonstrating the impacts of wetland runoff. Differences in densities of this FIB group during the flood and ebb flows tended to be more similar further into the wetlands at Stations 2–4 where the percentage of samples with flood densities \ge ebb densities ranged from 40–50% (Table 5).

E. coli. Mean densities of *E. coli* generally were greater during flood than ebb

Fig. 3. Densities of *E. coli* (MPN/100 ml) during flood and ebb tidal flows, February 2003–2004. The "less than" symbol (\le) indicates densities below the method detection level for the dilution used.

flows at all stations, ranging from $10²$ to $10³$ MPN/100 ml with the greater densities occurring during runoff events (Tables 3 and 4). However, the greatest density of this FIB group (41,060 MPN/100 ml) was measured at Station 4 on September 30, 2003 during dry weather. When looking at individual station histograms for *E. coli* (Fig. 3), flood densities exceeded ebb densities more than 50% of the time (Table 5) with the greatest percentages (80%) at Stations 1–3.

Enterococci. Mean Enterococci densities (Table 3) were slightly greater than those of *E. coli,* ranging from $10³$ to $10⁴$; the greatest density occurred during wet weather at Station 4 on March 16, 2003, where the count was measured at 141,360 MPN/100 ml. During dry weather, enterococci densities in flood flows tended to be greater on average than ebb flows, but this trend was not statistically significant (Table 3). Tests among replicated events did show a significant increase in Enterococci during the ebb flow at Station 2 during the March $16th$ wet weather event (Table 4). When individual stations histograms are examined, flood densities exceeded ebb densities greater than 50% of the time at all stations (Table 5).

Discussion

The central question in this study is whether or not the Ballona Wetlands act as a sink or source for FIB groups of bacteria. If acting as a sink, densities of

Fig. 4. Densities of Enterococci (MPN/100 ml) during flood and ebb tidal flows, February 2003– 2004. The "less than" symbol (\le) indicates densities below the method detection level for the dilution used.

FIB would be greater in flood tidal flows entering the marsh system from the Ballona Creek estuary. After entering the wetlands, FIB densities would be reduced through natural processes associated with sedimentation within vegetated areas (e.g. Wong and Geiger 1997, Dixon *et al.* 2003, Karathanasis *et al.* 2003) and deactivation by sunlight (e.g. Mayo 1995, Stinton 2002). Conversely, if the wetlands were acting as a source, then densities would be greater during ebb flows, and be most apparent in water out welling from the tidal gates into the Ballona Creek estuary. This situation has been found in Huntington Beach, California where enterococci out welling from the Talbert Marsh impacted adjacent coastal beaches (Grant *et al.* 2001). In Talbert Marsh, enterococci probably originated from bird feces and contaminated sediments suspended through tidal action.

Results presented herein suggest that the wetlands may act as a sink in that FIB densities tended to be greater during flood flows into the wetlands, but less in water draining out of the system during ebb flows. However, this condition was not consistently met, especially at stations farthest from the tide gates. These sites could be reflecting increased FIB densities through regrowth within sediments and other unidentified sources.

Tidal amplitudes appear to be linked to FIB densities during ebb flows as

Table 3. Mean densities $(\pm 1$ S.D.) of indicator bacteria (MPN/100 ml) at each station during flood and ebb tidal flows over the entire sampling period.

¹ Values of *p* were derived from a t-test using log_{10} transformed data and assuming equal variances; mean FIB densities tested between flood and ebb flows at each station during dry- and wet-weather periods.

evident from the FIB Flood:Ebb ratios (Fig. 6), and may be caused by bank washing during spring tides as hypothesized in Fig. 7. During neap tides the tidal range is relatively small, so little water washes the banks in the wetlands. Bacteria are carried into the system on the flood tides from Ballona Creek where they subsequently are reduced in numbers, probably from sunlight and other natural wetland process. Subsequently, densities are lower in the ebb tide flows. As the

		Ebb			
$\mathbf n$	Mean	\pm SD	Range	\mathfrak{p}^1	
12	6,548	2,669	1,490-10,462	0.007	$\frac{1}{2}$
11	13,423	6,308	2,770-24,192	0.130	NS
12	30,790	46,424	3,094-173,290	0.025	\approx
12	86,601	77,948	4,245-198,630	0.068	NS
11	4,188	3,463	$960 - 12,033$	0.005	$\frac{1}{2}$
4	731,066	589,950	10,462-1,413,600	0.280	NS
4	577,258	385,239	38,730-920,800	0.382	NS
4	503,128	317,977	46,110-770,100	0.245	NS
$\overline{4}$	295,995	298,661	30,760-613,100	0.066	×.
$\overline{4}$	577,955	233,024	241,920-770,100	0.083	NS
12	163	282	$20 - 1,053$	0.494	NS
12	209	346	$10 - 1,243$	0.410	NS
12	202	392	$10 - 1,434$	0.165	NS
12	242	441	$20 - 1,625$	0.363	NS
11	292	515	$52 - 1,816$	0.320	NS
4	1,078	674	$12 - 1,610$	0.093	NS
4	1,631	894	$354 - 2,400$	0.006	$\frac{1}{2}$
4	1,340	639	$399 - 1,750$	0.050	$\frac{1}{2}$
4	1,106	558	$272 - 1,460$	0.072	NS
$\overline{4}$	6,350	7,360	1,990-17,329	0.118	NS
12	89	80	$10 - 259$	0.064	NS
12	141	265	$10 - 940$	0.050	\ast
12	84	50	$10 - 152$	0.210	NS
12	103	81	$10 - 223$	0.110	NS
11	107	83	$10 - 272$	0.082	NS
4	12,281	8,204	733-19,863	0.206	NS
4	56,136	37,421	1,014-81,640	0.379	NS
$\overline{4}$	47,557	32,591	987-77,010	0.472	NS
$\overline{4}$	39,766	28,176	933-62,940	0.374	NS
$\overline{4}$	12,929	5,626	4,721-17,329	0.144	NS

Table 3. Extended.

tidal cycle shifts into spring tide conditions, the tidal range increases. During high tide, contaminated Ballona Creek water is able to flood some areas in the wetlands (see Fig. 7). When the tide turns and flows back out into Ballona Creek, sediment particles with attached FIB species can be resuspended, thus adding to the ebb flows. Recent investigations by Ferguson *et al.* (2005) showed that densities of enterococci were much greater in sediments impacted by contaminated runoff, indicating retention and regrowth. They pointed out that resuspension of these sediments into the overlying water column was of concern since the attached densities of enterococci could be sufficient to exceed bathing water standards.

Table 4. Results of t-tests between mean FIB densities during flood and ebb flows during sampling events where replicate $(n = 3)$ samples were collected at each station. For testing, variances were assumed to be unequal; only significant results ($p \le 0.05$) are presented in this table.

			Mean density (MPN/100 ml)		
Date	Sta	FIB Grp	Flood	Ebb	р
3/16/03	$\overline{4}$	Totals	876,100	384,407	0.039
3/16/03	5	Totals	372,967	689,967	0.004
3/16/03	$\mathcal{D}_{\mathcal{L}}$	E. coli	7.327	2.057	0.001
3/16/03	3	E. coli	2,087	1,653	0.011
3/16/03	5	E. coli	7.970	2.690	0.017
3/16/03	っ	Enterococci	18,552	74.510	0.001

Stronger tidal flows associated with spring tide conditions may resuspend sediments, thus adding to FIB concentrations in the water column. Steets and Holden (2003) considered hydraulic resuspension of FIB contaminated sediments to be an important component in a model they developed to predict out welling of fecal coliforms from a small coastal lagoon in Santa Barbara, California.

If this ''bank washing'' model is operating in the wetlands, then increased tidal flows through restoration actions could have mixed outcomes with regard to FIB densities. As the tidal flow is increased within the wetland system, the mud banks of the tidal channels could broaden, and more vegetative banks would be submerged during high tides. More extensive mud bank exposure to tidal flows could resuspend greater loads of sediments, thus FIB. Conversely, during daylight hours at higher tidal levels, greater quantities of water contaminated with FIB would be exposed to sterilizing UV light as it lies over the submerged bank, thus reducing FIB densities.

Ballona Creek may be a significant source of FIB entering the Ballona Wetlands. Densities of FIB groups in flood tide flows entering the wetlands at Station 1 were within the ranges measured by others in Ballona Creek. Stein and Tiefenthaler (2004) measured dry weather densities of total coliforms, *E. coli,* and enterococci along the entire 12.7 km length of Ballona Creek. Highest densities were measured at sites immediately upstream of the tidal prism, and at the head of the open channel where it daylights, with geometric mean densities (MPN/100 ml) ranging from 10^2-10^3 for *E. coli* and enterococci, and 10^4 for total coliforms. Along the lower reaches of Ballona Creek and the upper portions of the Estuary,

Table 5. Percent of sampling events ($n = 10$) where FIB densities during flood flows were \geq ebb flows. Percentages based on histograms in Figures 2–4.

SALINITY vs. FIBs AT STATIONS 1-5

Fig. 5. Regression analyses of dry-weather salinity against each FIB group at Stations 1 through 4 in the wetlands, and Station 5 in Ballona Creek.

Dorsey and Lindaman (2004) measured similar FIB densities with total coliforms averaging10⁴–10⁵ and *E. coli* and enterococci ranging from 10²–10³ MPN/100 ml.

During wet weather events, densities of the FIB groups in Ballona Creek increase several orders of magnitude as contaminated water enters the Creek system from storm drains throughout the watershed (Los Angeles County Department of Public Works 2002 and http://ladpw.org/wmd/NPDES/2001-02_report; Dorsey

Ratio of Flood: Ebb FIB Density vs. Tidal Range

Fig. 6. Regression of tidal ranges versus the ratios of flood:ebb FIB densities for each indicator at Station 1, Ballona Wetlands. The wet-weather outlier from 2-3-04 was removed from the analyses.

and Lindaman 2004). Subsequently, this contaminated water enters the wetland system, lowering salinity to freshwater levels (Table 2) and increasing FIB densities by several orders of magnitude. Additional FIB sources would include bacteria attached to sediments carried by runoff from the salt marsh flats and the bluffs bordering the wetlands to the south.

Although data presented herein suggest that the wetlands may be acting as a

Fig. 7. Hypothesized bank-washing model (see text for explanation). Photographs are from Station 1 where the bank is covered with water during spring high tides.

sink for FIB, and that Ballona Creek may be a significant source for these bacteria, these premises were based on only 16 days of sampling at five stations over a one year period. Future studies need to focus on establishing overall hydrodynamics and tidal flushing of the wetlands, and determining mass balance of FIB entering and leaving this system during varying tidal cycles. Twenty-four hour studies employing frequent sampling will better determine the flux of FIB, especially during daylight hours when UV light can presumably reduce densities. More research is needed to determine the fate and transport of FIB within this system, retention and regrowth in wetland sediments, and purifying process within the tidal channels and on tidally submerged vegetation. As restoration actions are implemented, changes may occur in the dynamics of the wetland habitat and ecosystem conducive to water purification and FIB removal. At the same time, populations of vertebrates, especially birds, may significantly increase, thus increasing FIB densities. Therefore, we need to track these changing conditions and populations to better understand the consequences of restoration actions as this wetland returns to a more natural condition.

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