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Generation of Enterococci Bacteria in a Coastal Saltwater Marsh and Its Impact on Surf Zone Water Quality

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Elevated levels of enterococci bacteria, an indicator of fecal pollution, are routinely detected in the surf zone at Huntington State and City Beaches in southern California. A multidisciplinary study was carried out to identify sources of enterococci bacteria landward of the coastline. We find that enterococci bacteria are present at high concentrations in urban runoff, bird feces, marsh sediments, and on marine vegetation. Surprisingly, urban runoff appears to have relatively little impact on surf zone water quality because of the long time required for this water to travel from its source to the ocean. On the other hand, enterococci bacteria generated in a tidal saltwater marsh located near the beach significantly impact surf zone water quality. This study identifies a potential tradeoff between restoring coastal wetlands and protecting beach water quality and calls into question the use of ocean bathing water standards based on enterococci at locations near coastal wetlands.

Introduction

Beaches are an important part of the culture and economy in California. An estimated 550 million people visit California's public beaches annually for a total economic benefit to the state of over 27 billion dollars (*1*). To protect beach-goers from exposure to waterborne disease, a new state law mandates the implementation of recreational water quality monitoring programs at public beaches with 50 000 or more annual visitors. Specifically, the law requires monitoring for total coliform (TC), fecal coliform (FC), and the enterococcus (ENT) groups of bacteria, all of which may indicate the presence of fecal contamination. The state also enforces a set of uniform standards for TC, FC, and ENT bacteria including single-sample standards (10 000, 400, and 104 most probable number (MPN) or colony forming units (CFU)/100 mL) and 30 day geometric mean standards (1000, 200, and 35 MPN or CFU/100 mL); a lower single-sample standard for TC of 1000 MPN or CFU/100 mL also applies when the TC/FC ratio falls below 10. The enterococci standard conforms closely to the national guidelines for marine water quality criteria published by the U.S. Environmental Protection Agency (*2*). If indicator bacteria levels in the ocean exceed any of the above standards, the local health officer is required to either post signs that warn against swimming in the water or close the ocean to the public if a sewage spill is suspected. The state standards and U.S. Environmental Protection Agency guidelines are based on a series of epidemiological studies that link gastrointestinal illness and exposure to ocean water containing high levels of indicator bacteria, particularly ENT (*3*−*11*). The origin of ENT in these epidemiological studies was presumed to be anthropogenic sources of fecal pollution, such as sewage, agricultural runoff, and urban runoff.

Huntington State and City Beaches in southern California have been heavily impacted by the passage of the new regulations. According to data provided by the Orange County Health Care Agency, there have been a total of 99 postings at Huntington State and City Beaches between July 26, 1999, when the bill went into effect, and September 5, 2000, approximately 72% and 25% of which were triggered by violations of the ENT single-sample and geometric mean standards, respectively. Persistently high levels of indicator bacteria in the surf zone at Huntington State and City Beaches in the summer of 1999 led to an extensive survey of the local sewage infrastructure (*12*). No significant sewage leaks were discovered, prompting speculation that urban runoff from the nearby Talbert Watershed was a source of fecal pollution (*12*). The present study was designed to test this hypothesis and, more broadly, to characterize the sources and transport of ENT in tidally influenced flood control channels and a saltwater marsh. ENT was the focus of this study because this particular group of indicator bacteria is responsible for the vast majority (97%) of beach advisories issued at Huntington State and City Beaches.

Field Site

The Talbert Watershed encompasses 3400 hectares in the cities of Huntington Beach and Fountain Valley. The watershed drains an urbanized area consisting of residential developments, commercial districts, plant nurseries, and light industry. This area of southern California has separate stormwater and sanitary sewer systems, so dry and wet weather runoff flows to the ocean without treatment.

Runoff from the Talbert Watershed is conveyed along street gutters to inlets that connect to underground storm-water pipelines. These pipelines connect to a network of three flood control channels (Fountain Valley, Talbert, and

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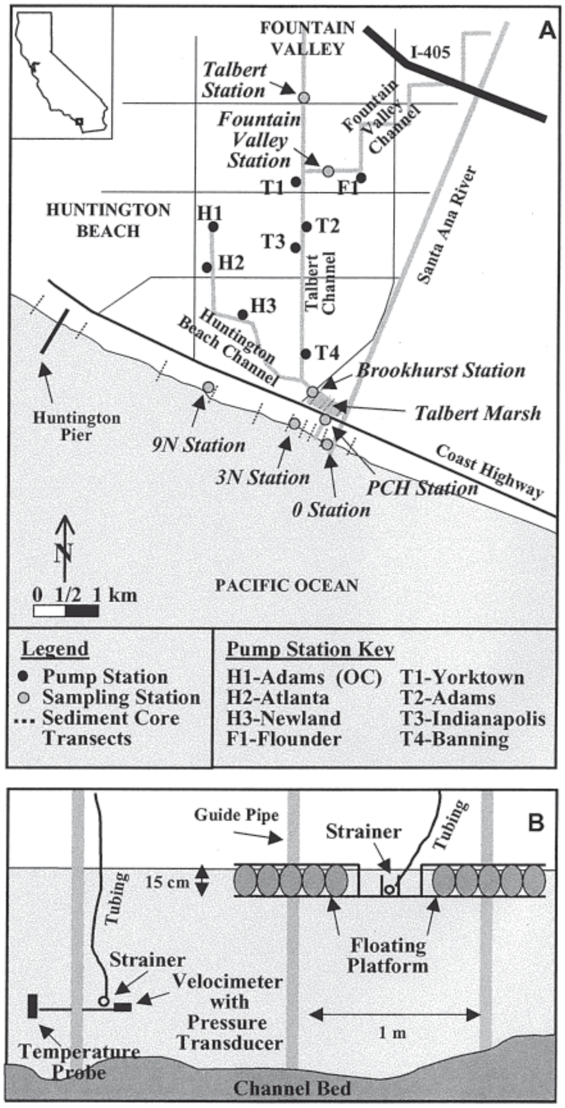

FIGURE 1. (A) A map of the Talbert Watershed showing the location of drainage channels, pump stations, water sampling stations, and sediment core transects in the marsh and surf zone. (B) A schematic cross section of the two marsh stations, showing the configuration of the surface and bottom sampling system, the velocimeter and pressure transducer, and the temperature sonde.

Huntington Beach) that converge near the ocean at a constructed wetland known as the Talbert Marsh (Figure 1A). Ocean water floods both the Talbert Marsh and the lower reaches of the open channels during rising tides (flood tides) and a brackish mixture of ocean water and runoff drains from the system during falling tides (ebb tides).

The Talbert Watershed is nearly flat and only a few feet above sea level. This geographical setting hinders drainage by gravity alone, so a system of transfer stations is used in the lower reaches of the Talbert Watershed to pump runoff into the open channels from stormwater pipelines. Each transfer station, or pump station, consists of a forebay, where runoff can be stored, and several pumps. Pumping of runoff to the channels occurs intermittently during dry weather periods and continuously during storms.

Talbert Marsh is a 10 hectare remnant of what used to be an extensive (1200 hectare) saltwater wetland and dune system in coastal Orange County. The majority of this wetland system was drained and filled over the past century for agricultural reclamation and urban development. Most of what remained of the historical wetland, including Talbert Marsh, was cut off from tidal flushing by the construction of Pacific Coast Highway and channelization of the surrounding area for flood control. As part of a habitat restoration effort, tidal flushing in the Talbert Marsh was restored in 1990 when a new tidal inlet was constructed. Since its restoration, Talbert Marsh has become a typical southern California tidal saltwater marsh with open water, wetland, and upland habitats (*13*−*15*). Pickle weed (*Salicornia virginica*) is the dominant macrophytic vegetation, and the marsh is utilized by several special-status bird species including the California Least Tern, Brown Pelican, and Beldings Savannah Sparrow.

At the outset of this study it was not clear what effect the Talbert Marsh had on surf zone water quality at Huntington State and City Beaches. On one hand, wetlands, particularly freshwater wetlands, are natural treatment systems that remove chemical and biological pollutants from domestic and agricultural wastewater and urban runoff (*19, 20*). On the other hand, coastal marshes are an important bird habitat, and bird feces are a potential source of ENT (*21, 22*), as is the environmental growth of these organisms in the sediments and on vegetation (*23*−*26*).

Methods and Materials

A series of investigations were carried out to (1) quantify the flow of water and ENT into the ocean from the Talbert Marsh and Talbert Watershed, (2) assess the impact of ENT from the marsh and watershed on local surf zone water quality, and (3) identify potential sources of indicator bacteria within these two systems (runoff, birds, vegetation, and sediment). These three different investigations are referred to throughout the paper as the Marsh Study, the Surf Zone Study, and the Source Study, respectively. The methods employed in these investigations are described below.

Marsh Study. The goal of the Marsh Study was to measure the flow of water and ENT from the Talbert watershed into the Talbert Marsh and from the Talbert Marsh into the ocean. Measurements were carried out for 15 days starting on May 2, 2000. During the 15 day study, pump stations in the Talbert Watershed were operated in two different modes: during the first 8 days the pump stations were offline, and for the following 7 days the pump stations were online. When the pump stations were offline, runoff that would normally be discharged into the drainage channels was either diverted into the regional sanitary sewer system or stored in the pump station forebays. When the pump stations were online, runoff was intermittently discharged into the drainage channels following normal operating procedures. The impact of these operational changes was monitored at two locations: (i) the junction of the drainage channel network and the marsh at the Brookhurst street bridge (Brookhurst Station) and (ii) the junction of the marsh and the ocean at the Pacific Coast Highway bridge (PCH Station) (see Figure 1A). Two additional sites (Talbert Station and Fountain Valley Station, see Figure 1A) were monitored to characterize the flow of runoff into the drainage channels from the upper reaches of the watershed where there are no pump stations. Methods for monitoring the flow of water and ENT concentrations at these four sites are described below.

Flow Measurements. The velocity and level of water at the Brookhurst Station and the PCH Station were measured using acoustic Doppler velocimeters outfitted with pressure transducers (4250 Area Velocity Flow Meter, Isco, Lincoln, NE). The velocimeters were suspended approximately 5 cm above the sediment bed (Figure 1B) and positioned so that the Doppler cone, or area over which the velocity is averaged, was pointing upward and in an inland direction. Data from the velocimeters was electronically logged every five minutes and downloaded onto a laptop computer. The velocity and water level data were used to calibrate a hydrodynamic model for the marsh and channel network (*27*). The calibrated model was then used to compute hourly average values of the volumetric flow rate at both the Brookhurst and PCH Stations over the study period. Water temperature at the two sites was recorded by a sonde (YSI, Yellow Springs, OH) positioned so that the probe was located approximately 5 cm above the sediment bed (Figure 1B).

The flow of urban runoff into the upstream reaches of the Talbert and Fountain Valley channels was too low to measure using acoustic Doppler technology. Consequently, flow rates at the Talbert and Fountain Valley Stations were estimated by recording the time 10 different pieces of submerged debris took to travel a fixed distance. Volumetric flow rates were then obtained by multiplying this average velocity by the estimated cross sectional area of the flowing water.

No water was discharged from the pump station forebays during the first 8 days of the Marsh and Surf Zone Studies. The volume of water discharged during the last 7 days of the study was estimated from City of Huntington Beach records of water volumes diverted into the sanitary sewer during the first 8 days of the study. The conductivity of forebay water at several pump stations was elevated (30 mS/cm), reflecting the fact that some fraction of the forebay water is ocean water that traveled up the channels during flood tides and spilled into the forebays through leaking flap gates. We computed the fraction of water discharged from the pump stations that was runoff (i.e., not ocean water) as follows

$$F = 1 - (C - C_R)/(C_O - C_R) \quad (1)$$

where C_O and C_R are the conductivity of ocean water and runoff (taken as 53.5 and 3 mS/cm, respectively) and C is the measured conductivity of samples from the pump stations.

The volume of runoff exiting the channel network through the outlet to the ocean was quantified from the magnitude of the conductivity depressions and the volumetric flow rate at the PCH Station by numerically evaluating the following integral

$$\int F(t)Q(t)\mathrm{d}t \quad (2)$$

where $F(t)$ represents the fraction of freshwater computed by applying eq 1 to the conductivity signal measured at the PCH Station and $Q(t)$ is the volumetric flow rate at the PCH Station computed using the calibrated hydrodynamic model (see above). The integral was taken separately over the first 8 days and last 7 days of the study.

ENT Measurements. At both the Brookhurst Station and the PCH Station, hourly water samples were collected from the surface and bottom of the water column using programmable sampling units (Isco models 3700 and 6700, Lincoln, Nebraska) (Figure 1B). Surface samples were obtained by drawing water over the lip of an acrylic box that was submerged approximately 1 cm below the water surface and supported by a floating platform (Figure 1B). Bottom samples were drawn through a strainer suspended approximately 5 cm above the sediment bed by a pole attached to the bridge. To obtain an average measure of water quality over each hour-long sampling interval, the automated samplers were programmed to collect 200 mL of water every 15 min for a total sampling volume of 800 mL per bottle per hour. Sample bottles consisted of a disposable plastic liner (Isco ProPak sample bags) supported by a plastic cage (Isco ProPak holder); the liners were used once and then discarded. A purge cycle was executed before and after each sampling event, and the sampling units were filled with ice to reduce bacterial die-off. Samples were retrieved from the Brookhurst and PCH Stations every 6 h and transported to a laboratory at the Orange County Sanitation District (Fountain Valley, CA) where 10 mL was immediately analyzed for ENT using a defined substrate test (IDEXX Enterolert test implemented in a 97 well Quanti-tray format), pH, turbidity, and conductivity (temperature-corrected to 20 °C). A total of 1416 samples were collected using the automated samplers. Automated samplers were employed here because they allowed us to collect hourly water samples in a reproducible manner from precisely the same locations in the water column, 24 h per day, 7 days per week. One potential disadvantage of the automated systems is that the tubing and sampling system (e.g., strainers) are not sterilized between sampling events, so there is a possibility that sample-to-sample cross-contamination might occur. A recent study of sources of *E. coli* in an estuarine system in Florida (*26*) found that automated samplers did not cause significant cross-contamination when a purge step was executed between sampling events, as was done here.

Solar Radiation. To assess possible relationships between sunlight and bacterial levels in the marsh, hourly measurements of solar radiation were recorded during the 15 day study period using a thermopile radiometer (Kipp & Zonen, CM3 Thermopile Radiometer, Netherlands) located at the San Joaquin Marsh, which is approximately 6 km west of the Talbert Marsh.

Surf Zone Study. Dye experiments and intensive surf zone water quality monitoring were carried out to quantify the impact of ENT from the Talbert marsh and watershed on surf zone water quality at Huntington State Beach. The methods employed for this element of the study are described below.

Dye Study. During ebb tides, water from the Talbert Watershed flows into the drainage channels (Huntington Beach, Talbert, and Fountain Valley), through the Talbert Marsh, and into the ocean. To determine how ebb flow from the Talbert marsh and watershed interacts with the surf zone, separate dye experiments were conducted on May 1 and May 10, 2000, as follows. Rhodamine WT dye (Keystone, Santa Fe Springs, California) was added for approximately 30 min to effluent from the Talbert Marsh during an ebb tide. The spatial distribution of the dye was recorded at a series of times post release by a four channel radiometer (DMSV MK-1 SpecTerra Sys., Nedlands, Australia) flown at approximately 1500 m above sea level. The dye field in these images was visualized by forming the ratio of emission and absorption maxima (570 and 550 nm, respectively) of Rhodamine WT.

Surf Zone Monitoring. To assess the impact of ENT from the marsh and watershed on surf zone water quality, hourly samples were collected at the PCH Station (to characterize the concentration of ENT entering and leaving the marsh) and at three locations in the surf zone (stations 0, 3N, and 9N, see Figure 1A). The Surf Zone Study was carried out during the same period of time (May 2−16, 2000) as the Marsh Study (see above). However, the methods used to collect and analyze samples in the Surf Zone Study differed from those described above for the Marsh Study. For the Surf Zone Study, hourly grab samples (total volume of approximately 1L) were collected in sterile Nalgene bottles at the PCH and the surf zone stations 24 h per day, 7 days per week, for 2 weeks. Within 6 h of collection, samples were transported to Sierra Laboratories, Inc. (Laguna Hills, California) on ice where 10 mL of each sample was immediately analyzed for ENT using multiple tube fermentation (MTF) (EPA Method 9230B). To characterize cross-shore variability of the ENT signal, separate samples were collected from ankle and waist depths at each surf zone station. A total of 2021 grab samples were collected for this element of the study.

ENT Source Study. Additional studies were carried out to identify specific sources of ENT in the marsh and watershed. Specific sources examined included urban runoff, bird feces in the marsh, marine vegetation, and marsh and surf zone sediments, as described below.

Bird Feces. To assess the amount of ENT present in bird feces, bird feces were collected, along with any attached sediment from mud flats, in the Talbert Marsh where birds congregate. The nature of the feces (wet or dry) was noted at the time of collection. Sediment that appeared to contain no bird feces was also collected to determine background levels of ENT. The sediment and feces samples were weighed and placed in acid washed Nalgene bottles with 500 mL of marsh water. The suspensions were shaken vigorously to disperse the feces and sediment and then allowed to settle for 15 min. Depending on the experiment, between 0.1 and 10 mL of supernatant was tested for ENT using the Enterolert protocol described in the Marsh Study. Control experiments were conducted to rule out the possibility that chemicals present in the feces and/or sediment might interfere with the Enterolert system. Specifically, Enterolert analyses were conducted on autoclaved suspensions of sediment and bird feces.

Bird Census. To quantify the input of ENT into the marsh from birds, a bird census was carried out as follows. Digital cameras (Kodak Model DC-290, Rochester, New York) were installed at three different locations along the northeastern margin of the marsh. These cameras were positioned so that, together, they provided a complete picture of the upland, wetland, and open water habitat areas. Images were shot hourly at a resolution of 2240 × 1500 pixels in 256 colors, 24 h per day, over the same period of time when samples were being collected in the marsh and in the surf zone (May 2−16, 2000). The images were uploaded to a desktop PC where they were analyzed with Adobe Photoshop (Adobe, San Jose, California). The birds in each image were enumerated manually to obtain an estimate for the total number of birds present in the marsh each hour of the 2-week study.

Urban Runoff. To characterize the concentration of ENT in urban runoff, daily grab samples were collected from all 11 pump stations in the Talbert Watershed and from the upstream reaches of the watershed at the Talbert and Fountain Valley Channel Stations (Figure 1A). Runoff sampling occurred over the same period of time that the Marsh and Surf Zone Studies were carried out (May 2−17, 2000). Prior to sampling the pump station forebays, water in the forebay was mixed by cycling the station pumps on and off. Sterile Nalgene bottles were lowered into the underground forebays, and approximately 1 L of water was collected. Five hundred mL samples of runoff at the Talbert and Fountain Valley Channel Stations were collected by manually placing a sterile Nalgene bottle directly in the flowing stream. All samples were stored on ice immediately after collection and transported to the Orange County Sanitation District where they were analyzed for pH, turbidity, conductivity, and ENT using the Enterolert protocol described in the Marsh Study.

Sediment and Vegetation. To assess the levels of ENT present in sediments, cores were collected from the marsh and surf zone with a Brandford 5024 Pneumatic Vibrator (Brandford Co., New Britain, CT) outfitted with a 1.52 m barrel (OD 4.4 cm) and Butyrate plastic liners (AMS Inc., American Falls, ID). Each core was cut into three 15 cm segments which were sealed at the ends with Teflon lined caps and transported to Sierra Laboratories, Inc. (Laguna Hills, CA) for bacterial analysis. Upon arrival at the laboratory, 50 g of each core section was suspended in 450 mL of phosphate buffered saline (PBS) (0.3 mM KH_2PO_4, 2 mM $MgCl_2$) in accordance with Standard Method 9221 A-3 (*28*). The clarified supernatant was analyzed for ENT using MTF following the protocol outlined in the Surf Zone Study. Seaweed samples were collected from the marsh, stored in disposable plastic bags, and transported on ice to Sierra Laboratories, Inc. Upon arrival at the lab, 50 g of vegetation was placed in a sterile container to which 450 mL of PBS was added. The solution was shaken vigorously and allowed to settle for 15 min and then reshaken. A 100 mL sample of the supernatant was analyzed for ENT using the MTF method described in the Surf Zone Study.

Results and Discussion

Marsh Study: Dynamics. The Talbert Marsh is a highly dynamic system, primarily because the flow of water through the marsh is dominated by the tides (Figure 2). Because Southern California has semidiurnal unequal tides (*29, 30*), there are four different tidal extrema each day including high−high, low−high, high−low, and low−low tide levels. Furthermore, the tide range, which is the difference between the high−high and low−low levels, oscillates over a 14−15 day period. The Marsh and Surf-Zone Studies were carried out over a 15 day period that began shortly before a spring tide when the tide range is maximal, passed through a neap tide when the tide range is minimal, and returned back to a spring tide again. The four daily tide stages and the spring-neap-spring transition are evident in the water levels measured at the Brookhurst and PCH Stations (top panel in Figure 2).

During flood tides (indicated by negative velocities in the second panel of Figure 2), the water levels at the Brookhurst and PCH Stations increase as water flows from the ocean, through the marsh, and inland along the channel network. During ebb tides (indicated by positive velocities) the water levels at the two stations decrease as water flows out of the channel network, through the marsh, and into the ocean. When ebb tides occur during daylight hours, solar heating of water flowing out of the channel network causes a significant increase in the temperature of the marsh water (compare first, third, and fourth panels). The conductivity measured at the Brookhurst and PCH Stations (fifth panel) corresponds to pure ocean water during flood tides (53.5 mS/cm) and a brackish mixture of ocean water and urban runoff at the end of the ebb tides (conductivity depressions).

The next panel in Figure 2 is a plot of the ENT concentrations measured at the Brookhurst and PCH Stations. ENT concentrations in the marsh varied from below the detection limit (10 MPN/100 mL) to a high of 2142 MPN/100 mL. A total of 218 (15%) and 655 (46%) of the marsh samples exceeded the single-sample and geometric mean standards for ENT (104 MPN/100 mL and 35 MPN/100 mL, shown as dark and light blue lines in the plot), respectively. A total of 247 (17%) of the marsh samples fell below the detection limit of 10 MPN/100 mL; all values falling below the detection limit were arbitrarily assigned the detection limit value. The log-transformed ENT concentrations at the top and bottom of the water column in the marsh are correlated ($r = 0.7$and $r = 0.72$ at the Brookhurst and PCH Stations, respectively). Comparing the conductivity and ENT curves in Figure 2, we find that elevated ENT values frequently occur in the marsh during periods of time when runoff from the drainage channels, as indicated by the conductivity depressions, is not present.

The last panel in Figure 2 is a plot of the total number of birds that visited the Talbert Marsh during the course of our study. The birds followed a daily routine in which their numbers started out low in the morning, peaked in the afternoon, and tapered off in the evening. Gulls and Elegant Terns constituted the majority (80%) of birds visible in the images. The largest congregation of birds, 1180 individuals, occurred at 2:00 in the afternoon on May 5.

Marsh Study: ENT Source or Sink? A primary objective of this study was to determine if the marsh functions as a net source or sink of ENT as water flows out of the Talbert Watershed drainage channels, through the marsh, and into the ocean during ebb tides. To this end, we segregated all of the marsh ENT data into two groups based on whether the samples were collected during ebb tides (Figure 3A,B) or

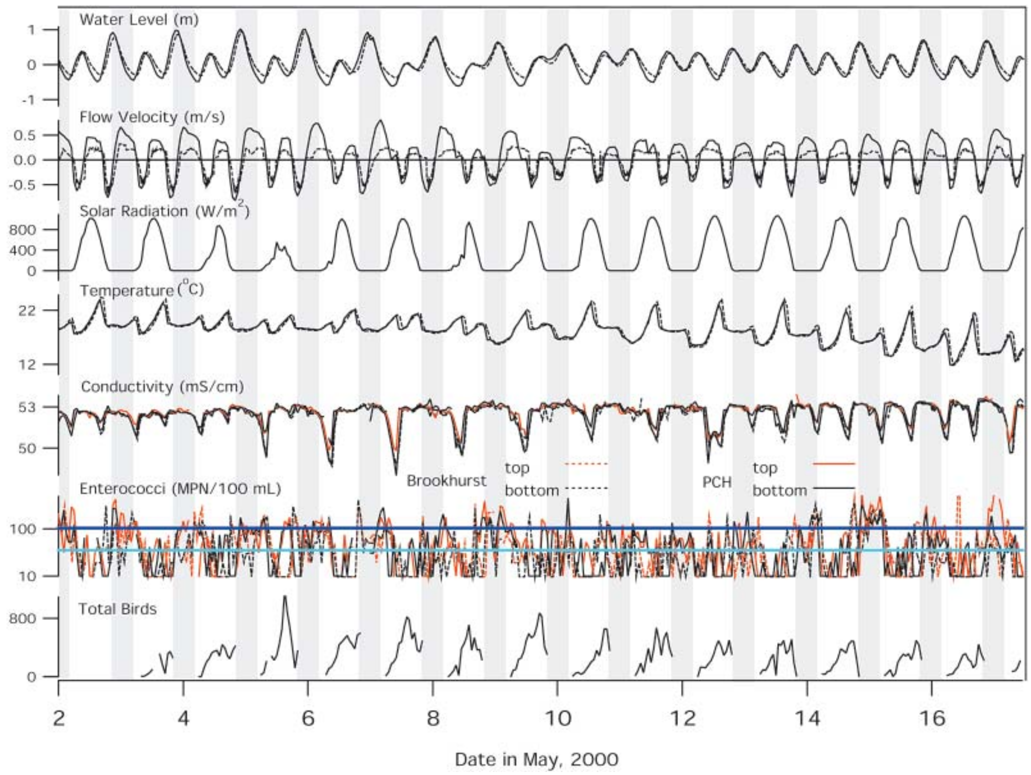

FIGURE 2. The dynamics of marsh parameters measured during the 15 day study period. The solid and dashed lines (water level, flow velocity, temperature panels) correspond to the PCH and Brookhurst stations, respectively. The key for conductivity and ENT traces is indicated in the figure. The dark and light blue lines denote the single sample and geometric mean standards for ENT. Water level is referenced to mean sea level. Positive and negative velocities correspond to shoreward and landward flow, respectively. The gray vertical stripes represent night-time conditions.

flood tides (Figure 3C,D). These data were further segregated based on whether the samples were collected during the first 8 days of the study (when the pump stations were offline) or the last 7 days of the study (when the pump stations were online) and based on the vertical location of samples in the water column (top or bottom). For each subgroup of data we computed a geometric mean and tabulated the percentage of samples that exceeded the single-sample standard for ENT. The results of this analysis identify the marsh, not urban runoff from the Talbert Watershed, as the primary source of ENT in the water flowing into the ocean. During ebb tides, the geometric mean of ENT (Figure 3A) and the percentage of samples exceeding the single-sample standard (Figure 3B) approximately double as the water flows through the marsh from the Brookhurst to PCH Station. The trend is reversed during flood tides when the geometric mean of ENT (Figure 3C) and percentage of single-sample exceedences (Figure 3D) increase as water flows through the marsh from the PCH to Brookhurst Station. With the exception of two flood-tide cases, water enters the marsh below the geometric mean standard for ENT (35 MPN/100 mL, dashed line in the figure) and exits the marsh in exceedence of the standard. In several cases, the ENT concentrations measured at the top of the water column are higher than the ENT concentrations measured at the bottom of the water column.

The idea that the marsh is a net source of ENT is also supported by Figure 3E, where we plot the hour-by-hour difference between the ENT concentrations measured at the Brookhurst and PCH Stations (ΔENT). On average, the ENT concentration is higher at the PCH Station during ebb tides (mean $\Delta ENT = -29 \pm 7$ MPN/100 mL) and higher at the Brookhurst Station during flood tides (mean $\Delta ENT = 27 \pm 6$ MPN/100 mL). A direct comparison of the ENT concentrations at the Brookhurst and PCH Stations is valid only if the residence time of water in the marsh is less than our sampling interval of 1 h. This condition appears to be satisfied based on a dye study conducted on the morning of May 19, 2000, which found that the residence time of water in the marsh during a weak spring tide is less than 40 min (*27*).

Surf Zone Study: Dye Experiment. The above analysis demonstrates that the Talbert Marsh is a net source of ENT, but it is not clear that ENT generated by the marsh negatively impact surf zone water quality. To characterize how ebb flow from the Talbert Marsh interacts with the ocean, a set of experiments were conducted in which dye (Rhodamine WT) was injected into the outlet of the Talbert Marsh during two separate ebb tides, one on May 1 and the other on May 10, 2000. The spatial pattern of dye released from the Talbert Marsh during the May 1 experiment is displayed in Figure 4. The dye pulse split into two plumes as it flowed into the ocean. One plume was entrained in the surf zone where it rapidly advected upcoast at velocities exceeding 0.2 m/s; a portion of this plume was subsequently taken offshore by a rip current. The second plume was carried directly offshore by a momentum jet located at the mouth of the marsh. The portion of the dye entrained in the surf zone on May 1 was advected in an upcoast direction because, on that day, ocean waves with average significant heights of 0.7 m were from the south (*31*). During the second release on May 10, ocean waves with significant heights of 1.4 m were from the west, and the portion of the dye entrained in the surf zone was advected rapidly (0.3 m/s) in a down coast direction (data not shown). Hence, water flowing out of the marsh during ebb tides can impact surf zone water quality at Huntington State and City Beaches directly upcoast of the Talbert Marsh outlet, provided that ocean waves strike the beach in an upcoast direction. Interestingly, wave conditions similar to those observed during the May 1 experiment were also present during the summer of 1999 when large stretches of Huntington State and City Beaches were closed to the public

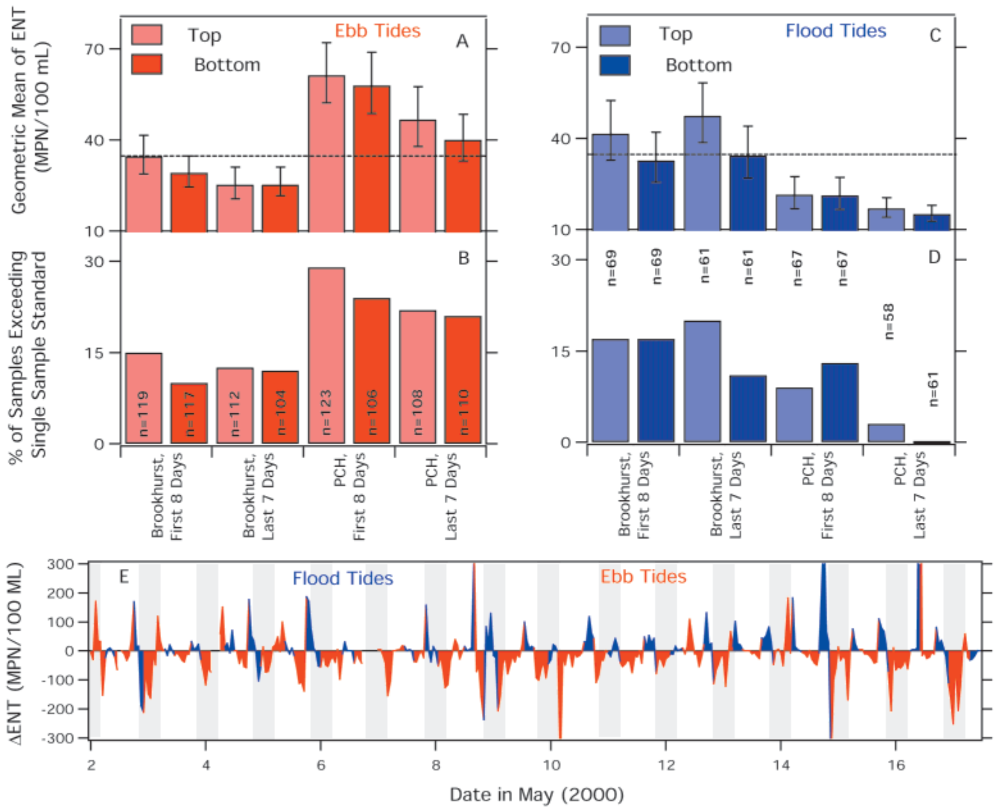

FIGURE 3. Geometric means of ENT in samples collected during ebb tides (A) or during flood tides (C). The dashed line in these figures represents the geometric mean standard for ENT (35 MPN/100 mL). Also shown are the percentage of samples collected during ebb tides (B) or flood tides (D) that exceeded the single sample standard for ENT (104 MPN/100 mL) and the difference in ENT concentrations at Brookhurst and PCH (E). Error bars represent 95% confidence intervals. The number of samples used to calculate geometric mean values are indicated in the figure.

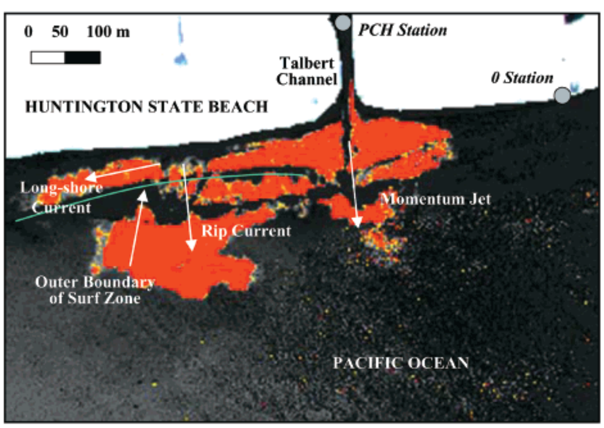

FIGURE 4. An areal image showing the near shore distribution of Rhodamine WT dye at 11:51 PDT, approximately 25 min into a release from the Talbert outlet during ebb tide on May 1, 2000.

(personal communication City of Huntington Beach lifeguards, 2000).

In addition to providing qualitative information about the fate of marsh effluent as it enters the ocean, the dye experiments can also be used to estimate the dilution that occurs as ebb flow from the marsh becomes entrained in the surf zone. Concentrated dye was released into the Talbert Marsh outlet at a rate of $Q_{dye} = 8 \times 10^{-6}$ m^3/s. From the calibrated hydrodynamic model, we estimate that the volumetric flow of water out of the marsh during the dye study on May 1 was relatively steady and equal to $Q_{effluent} = 11.6$ m^3/s. Photographs of the dye release indicate that the dye plume mixed over approximately one-half of the channel cross section before reaching the surf zone (*31*). Taking this observation into account, we estimate that the initial dilution of the dye plume into the marsh effluent stream was approximately 7.0×10^5 (($Q_{effluent}/2)/Q_{dye}$). The volume of the dye field at 11:51 PDT (the time at which the DMSV image in Figure 4 was shot) was approximately 7×10^4 m^3 assuming a 1.5 m mixing depth. Therefore, the dilution of the plume at 11:51 PDT, which includes both the initial and the surf zone dilution, is the volume of the dye field (7×10^4 m^3) divided by the volume of the dye released (6.51×10^{-2} m^3) or 1.1×10^6. Taking the ratio of this total dilution (1.1×10^6) and the initial dilution (7.0×10^5) indicates that the marsh effluent stream was diluted by a factor of 1.6 as it became entrained in the surf zone. Hence, effluent leaving the Talbert Marsh during ebb tides suffers approximately a factor two dilution as it is entrained in the surf zone.

Surf Zone Study: Bacterial Monitoring. To measure the actual impact of ebb flow from the marsh on surf zone water quality, an intensive surf zone monitoring program was carried out in parallel with the 15 day Marsh Study described above. ENT measurements in the surf zone varied from below detection limit (10 MPN/100 mL) to a high of 5700 MPN/100 mL. A total of 69 (3%) and 298 (15%) surf zone samples exceeded the single-sample and geometric mean standard for ENT, respectively. A total of 1067 (53%) of the surf zone samples fell below the detection limit. As with the data collected in the Marsh Study, samples falling below the detection limit were arbitrarily assigned the detection limit value.

Figure 5 displays the geometric mean and 95% confidence intervals of ENT measured at surf zone stations (9N, 3N, and 0, see Figure 1A) and at the PCH Station during either rising or falling tides. These data are also segregated based on whether samples were collected in the first 8 days of the study or the last 7 days of the study (indicated in the figure

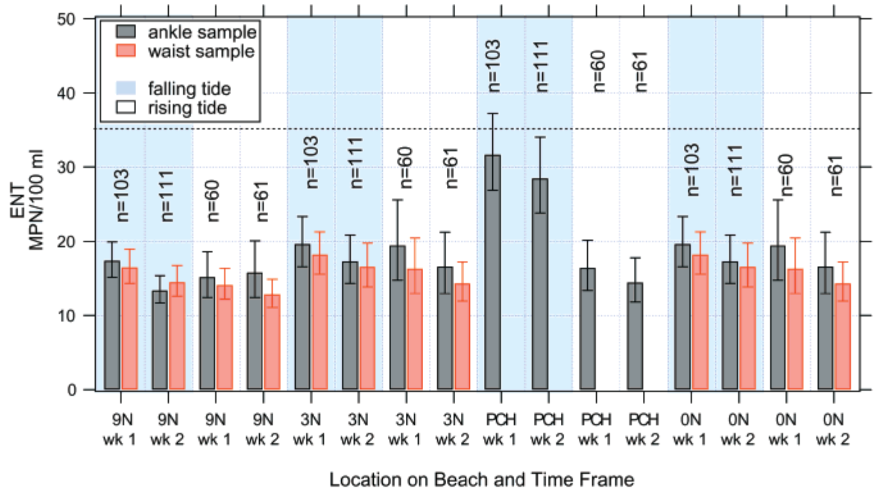

FIGURE 5. Geometric means and 95% confidence intervals of ENT concentrations (MPN/100 mL) at the PCH and surf zone stations measured during falling (blue background) and rising (white background) tides. The stations are displayed from north (left) to south (right): 9N, 3N, PCH, and 0 (see map in Figure 1). At each station, the geometric means are shown for the first 8 days and last 7 days (denoted wk 1 and wk 2, respectively). For the surf zone stations, geometric means for samples taken at ankle and waist depth are indicated. At the PCH site, only a surface sample was analyzed. The sample sizes are shown above the bars. The dotted line represents the geometric mean standard for ENT (35 MPN/100 mL).

as "wk 1" and "wk 2", respectively), whether the samples were collected at ankle or waist depth, and whether the samples were collected during rising or falling tides. As described in more detail in the Methods and Materials section, all of the ENT data plotted in Figure 5 were obtained by performing MTF analysis on grab samples, while the ENT data collected for the Marsh Study were obtained by performing an Enterolert analysis on samples collected with an automated sampling system. Comparing the PCH Station data in Figure 3A with the PCH Station data in Figure 5, we find that during ebb tides the geometric mean of ENT estimated using the Enterolert/automated sampling system is approximately 60 MPN/100 mL, compared to 30 MPN/100 mL using MTF/grab samples. ENT values estimated by the two approaches are weakly correlated ($r = 0.5$), but the magnitude of the ENT values estimated by the MTF/grab sample method appear to be lower. This difference could arise due to differences in the analytical technique employed (MTF versus Enterolert) and/or the sampling methodology employed (grab versus automated). A strong correlation between Enterolert and MTF measurements of ENT in marine samples ($r = 0.927$) has been previously reported (*32*). Hence, the differences reported here are probably due to the differences in the sample collection protocols employed in the Marsh and the Surf Zone Studies.

Because all of the data presented in Figure 5 were collected and analyzed using the same procedure (MTF on grab samples), we can directly compare the ENT signal leaving the marsh during ebb tides with the ENT signal measured in the surf zone over the same period of time. Figure 5 reveals that during falling tides, when ebb flow from the marsh enters the ocean, the geometric mean of ENT at the PCH Station is approximately two times higher compared to the geometric mean of ENT measured at the surf zone stations. With one exception, the geometric means of surf zone samples collected at waist depth are slightly lower than the geometric mean of samples collected at ankle depth. Based on these data, the ENT signal at stations 0, 3N, and 9N could have been caused by ebb flow from the Talbert Marsh provided that the following conditions were met: (1) near complete surf zone entrainment of the marsh effluent as it flows over the beach and into the ocean during falling tides, (2) no more than a factor of 2 dilution as effluent from the marsh is entrained in the surf zone, and (3) littoral flow in the surf zone directed in an upcoast direction. The first two conditions appear to be met based on the results of the dye study described above. Based on wave azimuth data recorded at Huntington Beach during the 15 day study (*31*), wave-induced flow in the surf zone was directed in an upcoast direction 60% of the time, including long stretches of time between May 4 and 8 and again between May 12 and May 16. Hence, ENT generated in the marsh appear to have at least a localized impact on surf zone water quality at Huntington State Beach.

ENT Source Study: Urban Runoff. No more than trace levels of rainfall were measured in Huntington Beach either during, or 14 days prior to, our 15 day study. Therefore, all runoff generated by the Talbert Watershed during this period was from dry weather sources, including landscape irrigation, street cleaning, car washing, and other activities that lead to surface water flow. To determine if the Talbert Watershed might be a significant source of ENT, samples of runoff were collected from pump station forebays and upstream at the Talbert and Fountain Valley Channel Stations (Figure 1A) and then analyzed for ENT using the Enterolert system (Table 1). The largest concentration of ENT (61 310 MPN/100 mL) was detected in a sample collected from the Flounder pump station on 5/8/00 (data not shown). The geometric mean of ENT in the runoff ranged from 23.1 MPN/100 mL at the Indianapolis pump station to 3477 MPN/100 mL in the upstream reaches of the Fountain Valley Channel (Table 1). Despite the high concentration of ENT measured in most urban runoff samples, the activation of pump stations during the last 7 days of our study did not appear to negatively impact downstreamwater quality. Indeed, the geometric means of ENT at the Brookhurst and PCH Stations during ebb tides (Figure 3A) actually *decreased* when pump stations came online. Likewise, the geometric means of ENT at all surf zone stations (Figure 5) were either unchanged when the pump stations went from offline to online or declined slightly.

There are several possible reasons why the discharge of pump station water did not lead to higher ENT concentrations in the marsh and surf zone. Mathematical modeling of tidal flow in the channel network reveals that water discharged from a particular pump station may or may not be flushed to the ocean in a single tide cycle, depending on the tidal range, when in the tide cycle the discharge occurred, and the pump station's inland distance from the shore. Specifically, the model predicts that at least 50% of runoff discharged during the last 7 days of our study was temporarily trapped

TABLE 1. Quality of Water That Enters the Channel Network from Either Uncontrolled Sources of Runoff (Talbert (T.) and Fountain Valley (F.V.) Channels) or from Pump Stations (p.s.)[a]

source	conduct. [mS/cm]	pH [−]	turbidity [NTU]	ENT (×10³) geometric mean [MPN/100 mL]	ENT (×10³) mean [MPN/100 mL]
Adams p.s.	4.5 (±1.3)	7.7 (±0.3)	10.2 (±5.1)	1.6 (+1.7/−0.8))	3.6 (±6.0)
Atlanta p.s.	32.3 (±6.9)	7.3 (±0.3)	22.1 (±4.8)	1.6 (+0.75/−0.51)	2.0 (±1.3)
Banning p.s.	36.3 (±3.8)	7.4 (±0.3)	9.3 (±2.0)	0.7 (+0.7/−0.3)	1.8 (±3.2)
OC Adams p.s.	3.0 (±0.8)	7.6 (±0.2)	24.7 (±11)	2.87 (+2.8/−1.4)	5.2 (±6.1)
Flounder p.s.	3.5 (±2.4)	7.4 (±0.4)	13.8 (±19.8)	1.9 (+6.1/−1.5)	12.5 (±17)
Indianapolis p.s.	11.1 (±1.9)	7.6 (±0.4)	11.5 (±5.3)	0.023 (+0.06/−0.02))	0.012 (±0.02)
Yorktown p.s.	8.0 (±2.6)	7.4 (±0.4)	27.2 (±9.9)	2.2 (+5.1/−1.6)	9.7 (±11)
Newland p.s.	19.7 (±4.5)	7.5 (±0.3)	10.4 (±4.9)	1.2 (+1.1/−0.6)	2.1 (±2.4)
F. V. channel	3.1 (±4.8)	9.0 (±0.5)	2.1 (±0.8)	3.5 (+2.0/−1.3)	5.2 (±6.3)
T. channel	2.5 (±4.9)	8.8 (±0.5)	3.22 (±2.0)	0.5 (+0.4/−0.2))	0.9 (±1.1)

[a] Standard deviations and 95% confidence intervals are given in parentheses for mean and geometric mean values, respectively.

in the channel network due to the tidally driven oscillation of water flow in the drainage channels.

By integrating the conductivity depressions evident in Figure 2 (see Methods and Materials), we estimate that the volume of runoff flowing into the ocean at the PCH Station during the first 8 days and last 7 days was 5000 m^3 and 4000 m^3, respectively. Furthermore, we estimate the amount of flow entering the upper reaches of the channels at the Fountain Valley and Talbert Stations to be approximately 8000 m^3 (first 8 days) and 7000 m^3 (last 7 days), and we estimate the amount of runoff discharged from pump stations the last 7 days of the study to be 16 000 m^3. Hence, the net inflow and outflow of runoff roughly balance during the first 8 days (8000 and 5000 m^3, respectively), while the net inflow and outflow of runoff do not balance during the last 7 days (22 000 and 4000 m^3, respectively). These volume estimates support the conclusion that the majority of the pump station water discharged in the last 7 days of the study was trapped in the channel network. Importantly, the 7000 m^3 per week of runoff continuously entering the drainage channels from the upper reaches of the Talbert Watershed had relatively little impact on downstreamwater quality, at least compared to the ENT signal generated by the Talbert Marsh. Die-off of ENT and the relatively long residence time (~1 week) of runoff in the drainage channels may limit the downstream impact of urban runoff (*33−35*). The fate and transport of bacterial pollutants in the drainage system at Huntington Beach is a subject of ongoing investigations.

ENT Source Study: Sediment and Vegetation. Sediment cores were collected from May 22 to June 6, 2000 along a set of transects (dotted lines in Figure 1A) located both in the marsh and surf zone. ENT levels in the sediment cores are consistent with the marsh being a significant source of these bacteria. Nineteen percent of sediment samples from the marsh ($n = 96$) were positive for ENT, compared to 2% of the sediment samples from the surf zone ($n = 121$). A total of 65% of the surface sediment samples in the marsh were positive for ENT. Vertical profiles of ENT in the marsh sediments indicate that the bacteria are concentrated in the top 1 cm of the cores (Figure 6). The largest concentration of ENT in the sediment cores (50 000 MPN/100 g) was from a surface sample collected from the northeast corner of the marsh. Of the sediment collected from the surf zone, only one sample had significant levels of ENT (800 MPN/100 mL), and this was a surface sample collected directly upcoast of the Talbert Marsh outlet.

High levels of ENT, ranging from 18 to 450 000 MPN/100 g (geometric mean of 2284 MPN/100 g, $n = 9$), were also found on seaweed collected from the marsh. The fact that sediments and vegetation are enriched in ENT suggests that these organisms are surviving, and perhaps even growing, in the marsh environment. Marine vegetation supports the growth of certain strains of ENT in New Zealand, and estuarine sediments can apparently support the growth of ENT in tropical settings such as Hawaii and Guam (*21, 22*), although there are no published reports of this occurring in Mediterranean climates such as southern California.

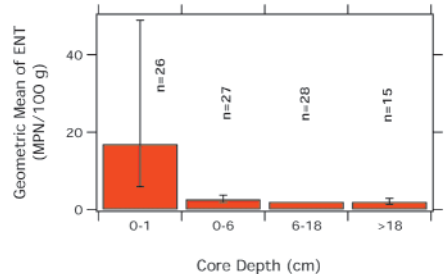

FIGURE 6. The vertical distribution of ENT in marsh sediments. Error bars represent 95% confidence intervals. The number of cores used to calculate the geometric mean values are indicated.

ENT Source Study: Bird Feces. Bird feces are a significant source of ENT in the marsh environment. This conclusion was arrived at by measuring the ENT levels in the following: (1) marsh water alone, (2) 500 mL of marsh water after addition of approximately 10 g of marsh sediment, and (3) 500 mL of marsh water after addition of approximately 10 g of marsh sediment containing bird feces that were either wet or dry at the time of collection. The concentration of ENT was below the detection limit (100 MPN/100 mL) in samples of pure marsh water and in marsh water containing feces-free sediment. However, when marsh water was exposed to sediment containing feces that were wet at the time of collection, the ENT concentrations ranged from 9090 to 24 192 000 MPN/100 mL ($n = 10$). Likewise, marsh water exposed to sediment containing feces that were dry at the time of collection had ENT concentrations ranging from 100 to 241 920 MPN/100 mL ($n = 10$). The geometric mean and 95% confidence intervals of the ENT measured in marsh water exposed to wet and dry feces were $1.8 \times 10^5 + 6.2 \times 10^5/-1.4 \times 10^5$ and $6.8 \times 10^2 + 3.3 \times 10^3/-5.6 \times 10^2$ MPN/100 mL, respectively. Expressing these geometric means and confidence intervals on a per feces basis, we obtain $8.9 \times 10^5 + 3.1 \times 10^6/-6.9 \times 10^5$ and $3.4 \times 10^4 + 1.6 \times 10^4/-2.8 \times 10^3$ MPN/feces for wet and dry feces, respectively.

The majority of the bird feces are deposited on low-lying mud flats in the marsh which become submerged to varying degrees during high tides. To determine if bird feces deposited in the marsh can account for the observed increase of ENT in water as it flows through the marsh, we performed a simple mass balance calculation as follows:

$$G = C_{out}Q_{out} - C_{in}Q_{in} \quad (3)$$

Here G is the rate of generation of bacteria in the marsh with units of [MPN/T], C_{out} and C_{in} are the concentrations of ENT at the outlet and inlet of Talbert Marsh, respectively, with units of [MPN/L^3], and Q_{out} and Q_{in} are the volumetric flow rates of water at the outlet and inlet of Talbert Marsh with units [L^3/T], where L and T represent length and time scales, respectively.

During ebb tides, in-situ measurements of flow velocity and water elevation at Brookhurst and PCH Stations indicate that the flow in and out of Talbert Marsh roughly balance so that $Q_{out} \approx Q_{in}$ and eq 3 simplifies as follows:

$$G = Q(\Delta C) \tag{4}$$

The parameter ΔC is the increase in ENT measured in water as it flows through Talbert Marsh.

Using average ebb tide values of $\Delta C = 29$ MPN/100 mL (see Figure 3E) and $Q = 8.37$ m^3/s from the calibrated hydrodynamic model, we estimate a generation rate for ENT in the marsh to be $G \approx 10^{10}$ MPN/h. Assuming each bird dropping has 10^6 MPN/feces (the geometric mean for wet bird feces), then 10^4 wet feces/h would be needed to account for the estimated generation rate. Our bird census indicates that, at most, 10^3 birds are present in the marsh, which corresponds to a deposition rate of more than 1 feces per bird every six minutes. If instead we use the maximum number of ENT liberated from the wet bird feces (10^8 MPN/feces) and the average number of birds present in the marsh during the day (228 birds), the deposition rate required decreases to approximately 1 feces per bird every 3 h. This latter deposition rate is comparable to rates observed for the same bird species in captivity, typically one dropping every 3 h (personal communication, J. Pavlat, Wildlife Care Facility, Huntington Beach, CA).

The above analysis does not consider the potential contributions of older, dried, bird feces, which were also found to contain significant levels of ENT. Portions of the mud flats in Talbert Marsh may remain exposed over many tide cycles, allowing the quantity of bird feces deposited there to increase. During a spring tide, when higher than average high tides occur, these older feces may become suspended in the marsh water and thereby increase the concentration of ENT in the water column. This idea is consistent with the fact that the highest level of ENT recorded at the Brookhurst and PCH Stations occurred during spring tides when the mud flats are most likely to be washed by tidal action (see Figure 2). Vegetation in the Talbert Marsh may also contribute to the levels of ENT in the water column, as could the growth of these organisms at the sediment/water interface. Indeed, growth at the sediment/water interface is supported by the distribution of ENT in cores taken from Talbert Marsh (see Figure 6). While bird droppings are clearly a significant source of ENT in the marsh, other sources may also contribute to the generation of ENT in the marsh including urban runoff, sediment, and vegetation.

Implications. ENT generated in the Talbert Marsh appear to be at least partially responsible for the frequency with which surf zone samples in Huntington State and City Beaches exceed state bathing water standards. This conclusion is based on two findings from our study: (i) ENT concentrations are increased above ENT standards (both single-sample and geometric mean) as water passes through the marsh and (ii) water flowing out of the marsh can be transported by littoral currents to the region of Huntington State and City Beaches where ENT standards are routinely exceeded. The ENT appear to enter the marsh from birds and runoff, and once there these organisms accumulate, and perhaps even grow, on marsh vegetation and sediments.

While ENT flowing into the surf zone during ebb tides may be responsible for beach postings that occur near the marsh outlet, the marsh is probably not the only source of ENT at Huntington State and City Beaches. During the summers of 1999 and 2000, for example, surf zone station 9N (see Figure 1) was frequently posted or closed (total of 70 days) due to elevated levels of ENT, even during periods of time when the concentration of ENT at stations near the Talbert Marsh outlet were relatively low (*31*). Given this spatial distribution of ENT, it is unlikely that the bacteria at 9N are coming solely from the Talbert Marsh, and their exact source is a matter of ongoing investigation. Indeed, we anticipate that the impact of marsh effluent on surf zone water quality will be relatively localized, given the factor two dilution that occurs as the marsh water mixes into the surf zone, and the fact that ENT die-off in ocean water (*34, 35*).

Based on the results presented in this paper, there may be a tradeoff between the restoration of coastal wetlands and compliance with marine water contact standards. This tradeoff could be ameliorated by specifically designing wetlands to remove bacteria from the water column. For example, freshwater wetlands remove bacterial pollutants most efficiently when the flow velocities are slow (<0.7 m/s) and the residence time of water is long (10 h) (*36, 37*). While the flow velocities in the Talbert Marsh are within the recommended range, the residence time of water (<1 h) is not. On the other hand, if there are no human health risks associated with ENT from wetland effluent, then marine water contact standards may need to be modified to account for the existence of both benign and nonbenign sources of these bacteria. An epidemiological study could help to define the human health risks associated with human exposure to nonanthropogenic sources of ENT such as marsh effluent. These issues are especially timely, as a Federal law has recently been enacted that mandates national monitoring and reporting of coastal water quality (*38*).

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