Technical Report #448

Microbiological Water Quality at Reference Beaches in Southern California During Wet Weather

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ABSTRACT

Although wet weather discharges from urban watersheds may have elevated concentrations of fecal indicator bacteria that impact water quality at swimming beaches, not all of these bacteria may arise from human sources. In this study, the contribution of non-human sources of bacteria was quantified at coastal reference beaches in southern California. Operationally, reference beaches were defined as open beaches with breaking waves that receive runoff from undeveloped (>97% open space) watersheds and were selected to represent a range of geographical conditions and a range of watershed sizes. Four reference beaches were sampled during four storm events during the 2004-2005 wet season. Samples were analyzed for total coliform, E. coli, and enterococcus in the discharge from the undeveloped watershed and in the wave wash where the discharge and surf zone initially mix. Samples collected during wet weather exceeded water quality thresholds established by the State of California greater than 10 times more frequently during wet weather than during recent dry weather in summer or winter, although the frequency differed by beach. These exceedences were greatest < 24 hrs following recorded rainfall, then steadily declined for the following three days. Early season storms exceeded water quality thresholds more than twice as frequently as late season storms. In addition, over half of these early season storms exceeded by multiple bacterial indicators, while the vast majority of late season storms only exceeded thresholds for a single bacterial indicator. Large storms exceeded water quality thresholds three times more frequently than smaller-sized storms. This was partly due to the breaching of sand berms during large storm events; small storms could not breach these berms and this restricted watershed discharges from entering the surf zone. When watershed discharges did enter the surf zone, bacterial concentrations in the wave wash were correlated with salinity (which acts as a conservative tracer of freshwater inputs) and watershed bacterial flux.

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INTRODUCTION

Beaches in Southern California are a valuable recreational resource for swimming, surfing, and other body contact activities. For example, greater than 175 million beach-goers visit southern California beaches annually, more than all other parts of the country combined (Schiff et al. 2001). This year-round activity results in tremendous economic revenue estimated at more than $9 billion annually in ocean related activities for the region (NRC 1990).

The fecal indicator bacteria enterococcus, E. coli/fecal coliform, and total coliform are used to monitor the water quality of marine beaches because these measures have been shown to correlate with swimming related illness. For example, Cabelli (1982) demonstrated that increases in enterococcus concentrations correlated with an increase in the risk of highly credible gastrointestinal illness among swimmers on beaches in New Jersey. In Santa Monica Bay, California, Haile et al. (1999) observed an increase in the relative risk for diarrhea with blood and highly credible gastrointestinal illness in swimmers exposed to higher concentrations of enterococcus.

While the water quality at most beaches in southern California meets water quality thresholds established by the State during dry weather, several beaches have impaired water quality based on routine fecal indicator bacteria monitoring. Noble et al. (2000) conducted a regional study of all southern California beaches and found that approximately 5% of the shoreline exceeded water quality thresholds for fecal indicator bacteria during the summer of 1998. This level of exceedence was not randomly distributed. More than half of the exceedences occurred near storm drains that discharge across the beach. A retrospective analysis of fecal indicator bacteria based on five years of daily beach monitoring during dry weather in Santa Monica Bay found similar results, with over half of the water quality exceedences occurring in front of storm drains (Schiff et al. 2002).

The microbial water quality of beaches in southern California drastically changes following rainstorms. Noble et al. (2003) repeated their 1998 summer study, but sampled following a significant rainfall event during the winter of 1998-1999. In this case, over half of all beaches exceeded fecal indicator bacteria water quality thresholds. This frequency of impaired water quality jumped to nearly 90% when these beaches were located in front of storm drains. Similarly, Schiff et al. (2002) observed a doubling of microbial water quality exceedences between dry and wet weather, even though wet weather represented less than 10% of the year.

There are many potential sources of bacteria that could be in storm drains that discharge to beaches. Some of these sources may be of human origin including sewage spills, leaking sanitary sewage systems, faulty septic systems, or illicit discharges and illegal dumping (Geldrich 1978). However, many sources may not be of human origin and may actually arise from natural sources. Fecal indicator bacteria such as enterococcus, E. coli/fecal coliform and total coliform are known to come from any warm-blooded animal including domesticated dogs and cats as well as wild animals such as birds and mammals (Grant et al. 2001, Fujioka 1995). Furthermore, fecal indicator bacteria may have extended survival or even regrow in beach sediments and wrack (Weiskel et al. 1996, City of San Diego/MEC Weston 2004). Therefore, the reference condition for bacterial water quality, including those beaches that are located at the mouth of undeveloped watersheds, is likely not zero. In fact, some shoreline managers use the level of contributions from undeveloped watersheds as the benchmark for water quality from developed watersheds in the Los Angeles region (LARWQCB 2002). Unfortunately, the contributions of fecal indicator bacteria from undeveloped watersheds to reference beaches are largely unknown, which complicates this approach for assessing public health risk or beach management.

The goal of this study was to assess the microbial water quality at reference beaches following wet weather events in southern California. Reference beaches were defined as those beaches located at the mouth of undeveloped watersheds and whose bacterial contributions are not influenced by human activities. These data can then be used by public health agencies and beach managers for making informed decisions about the reference condition of microbial water quality during wet weather. A series of secondary objectives were also addressed during this study to enhance our ability to decipher processes that can influence reference beach water quality during wet weather. These objectives included assessments of: 1) beach water quality over time following rainfall to determine how long elevated concentrations of fecal indicator bacteria persist; 2) the influence of storm size and seasonality on beach water quality; and 3) the relationship between inputs and microbial water quality at reference beaches.

METHODS

Site Selection

Four coastal reference beaches in southern California were selected for assessment of water quality during wet weather. Reference beaches were selected based on four criteria: 1) each reference beach must be an open beach with breaking waves; 2) each reference beach must have a freshwater input; 3) the freshwater input must come from a watershed of similar size to nearby beaches that receive wet weather inputs from urban watersheds; and 4) the watershed discharging to the reference beach must be $\geq$ 95% undeveloped.

The four reference beaches were: 1) Deer Creek Beach located at the mouth of Deer Creek in Ventura County; 2) Leo Carrillo State Beach located at the mouth of Arroyo Sequit in Los Angeles County; 3) Dan Blocker Beach located at the mouth of Solstice Creek in Los Angeles County; and 4) San Onofre State Beach located at the mouth of San Onofre Creek in San Diego County (Table 1, Figure 1). All four reference beaches are open with breaking waves and have freshwater inputs. The four watersheds that discharge to these reference beaches range from 3 to 110 km^2, which is within the 25^{th} and 75^{th} interquartile range of watershed area for all of the watersheds that drain to impacted, urbanized beaches in southern California. All four of the watersheds that drained to the reference beaches were between 97% and 100% undeveloped based on land use data compiled by the U.S. Geological Survey and University of California Santa Barbara (Davis et al. 1998). Deer Creek was the smallest watershed and had the least amount of human activity, while San Onofre Creek was the largest watershed and had the greatest amount of human activity.

Sampling

There were two sampling locations at each reference beach. The primary sampling location was in the ocean immediately in front of the freshwater input at the so-called "wave wash" where the watershed discharge initially mixes with the ocean waves. All samples were collected between ankle and knee depth on an incoming wave. The secondary sampling location was in the watershed discharge as it crossed the beach at the closest sampleable location prior to mixing with the ocean.

Samples at the primary sampling sites were measured for fecal indicator bacteria and salinity. Samples at the secondary sampling sites were measured for fecal indicator bacteria, salinity and flow. A subset of samples (25% or once per storm per beach) at secondary sites was collected for analysis of human enteric virus to identify the presence of human contributions of fecal pollution. Samples were collected in sterile 250 mL polyethylene bottles (bacterial analysis, salinity analysis) or 4 L polyethylene carboys (enterovirus analysis) following Standard Methods 1060 protocol for aseptic sampling techniques (APHA 1995). Samples were transported on ice to the laboratory for analysis. Flow was measured using a hand held velocity meter (Marsh-McBirney, Inc. Frederick, MD) and estimates of wetted cross-sectional area.

Sampling focused on wet weather. Wet weather sampling criteria included three or more days of antecedent dry period and predicted minimum rainfall estimates of 0.10 in. Four samples were collected per site corresponding to the day of the storm (defined as within 24 hrs of recorded rainfall) and the three days following recorded rainfall (four days of sampling in total). Four storms were targeted based on two factors; size of storm and seasonality. Size of storm was stratified into small storm events (less than median daily rainfall) and large storm events (greater than median daily rainfall) based on historical rainfall at the nearest rain gage. Seasonality was stratified into early season (before December 31st) and late season (after January 1st) storm events. Storm season in southern California is defined as October 15th to April 15th. To summarize, four reference beaches were sampled over the course of four days during four different storm events for a total of 64 sampling events.

Laboratory Analysis

Concentrations of total coliforms, E. coli, and enterococci spp. were measured using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME). Coliforms and E. coli were measured together using the Colilert-18 reagents, while enterococci were measured using Enterolert reagents. Both tests used the Quanti-Tray/2000 for enumeration of cells. Samples were incubated overnight per the manufacturer's instructions and inspected for positive wells. Conversion of positive wells from these tests to a most probable number (MPN) was done following Hurley and Roscoe (1983). Samples taken at Deer Creek Beach, Dan Blocker Beach and Leo Carrillo State Beach were analyzed at the City of Los Angeles laboratory facilities (El Segundo, CA). Samples collected from San Onofre State Beach were analyzed at Weston Solutions Laboratories (Carlsbad, CA).

One-quarter of all discharge samples were analyzed for enterovirus, a human specific pathogen, at the University of Southern California (Los Angeles, CA). Samples were filtered to concentrate viruses. Then, filters were extracted using the RNeasy mini kit (Qiagen Cat. No. 74106) and QIAvac 24 vacuum manifold (Qiagen Cat. No. 19403). The extraction protocol was modified from the manufacturer's instructions as follows: 1ml lysis buffer RLT (with 10μl β-mercaptoethanol) was added directly into each Whirl-Pak bag, allowed to soak the filter for ten minutes, and the resulting extracts (lysates) were carefully removed by pipet into 2 ml microcentrifuge tubes (droplets hanging in the bag and water clinging to the filter were first squeezed to the bottom corner of the bag by manually applying pressure to the outside of the bag). If there was visible filter or sample debris, the particulate matter was removed by brief centrifugation. Then one volume of 70% ethanol (usually 1 ml) was added to the extract and mixed by pipetting. Samples were transferred to the RNeasy spin columns filtered through with the QIAvac at approx. 500 mm Hg vacuum, and were washed on the manifold once with 700 μl RW1 solution, and twice with 500 μl RPE solution to remove contaminants. The columns were cleared of remaining droplets of buffer by centrifugation into a 2 ml collection tube (14,000 rpm, Eppendorf 5415 microfuge, 2 minutes), and the buffer discarded. The RNA was eluted from the columns into a 1.5 ml collection tube with 50 μl volumes of RNase free water by centrifugation (Eppendorf 10,000 rpm, 2 min.), after allowing the water to stay in the column 1 min.

For each PCR reaction, 5 µl of the 50 µl RNA was analyzed by RTPCR on a Mx3000P Thermal Cycler (Stratagene, Inc.). A GenBank BLAST search done on 3 June 2004 revealed that only human (not other animal) enteroviruses matched all three primer and probe sequences. Each PCR reaction contained 5 µl RNA extract and 20 µl master mix, each 20 µl master mix contained: 1X Taq gold buffer (ABI), 5.5mM $MgCl_2$ (ABI), 500uM dNTPs (ABI), 6% glycerol (Sigma Chemical Co.), 2% PVP 40 (polyvinylpyrrolidone, av. MW 40,000, Sigma Chemical Co.), 500nM Ev1-primer, 400nM Ev2-primer, 120nM Ev-probe (Fuhrman et al. 2005), 1.5ug T4 gene 32 protein (Ambion), 10 units of RNAsin (ABI), 2.5 units of AmpliTaq gold (ABI) and 5 units MULV reverse transcriptase (ABI). Each RNA extract was analyzed in duplicate. Enterovirus RNA was transcribed into cDNA at 50°C for 45 minutes, the cDNA was amplified by PCR, after a 95°C 10 minute hot start, for 50 cycles at 94°C for 15 sec and 60°C for 1min. Fluorescence measurements were made during the extension step, every cycle at 60°C.

Data Analysis

Data analysis focused on five comparisons. The first compared the frequency of water quality threshold exceedences during wet weather to the frequency of exceedences during winter dry weather and summer dry weather. Wet weather was defined as the day of recorded rainfall plus the next three days. Dry weather was defined as any day greater than three days since recorded rainfall. Winter was defined as November 1 to March 31. and summer was defined as April 1 to October 31. Wet weather data were collected as part of this study. Winter and summer dry weather data (April 2004 to March 2005) for San Onofre State Beach were supplied by the City and County of San Diego, respectively. Winter and summer dry weather data (April 2004 to March 2005) for Leo Carrillo State Beach was supplied by the City of Los Angeles. Winter dry weather data (October 2004 to March 2005) for Dan Blocker Beach was also supplied by the City of Los Angeles, but no summer dry weather data were available. Winter and summer dry weather data (October 2003 through October 2004) for Deer Creek Beach was supplied by the Ventura County Department of Environmental Health (winter dry weather data from 2004-2005 at Deer Creek Beach were not collected by the Ventura County Health Dept.) Water quality thresholds were based on single samples compared to the State of California's public health standards for marine bathing beaches: 1) >104 enterococci / 100 mL; 2) > 400 fecal coliform / 100 mL (we substituted E. coli for fecal coliform); 3) > 10,000 total coliform / 100 mL; and 4) > 1,000 total coliform / 100 mL when the total coliform to fecal coliform (or E. coli) ratio < 10.

The second data analysis element compared the frequency of water quality exceedences among the four days that comprised wet weather. Concentrations of fecal indicator bacteria for all beaches combined were compared to the state's water quality thresholds within 24 hrs of rainfall and the three days following recorded rainfall.

The third data analysis element focused on comparisons among the four reference beaches. The first comparison examined the relative frequency of exceedence of the state's water quality thresholds for fecal indicator bacteria for all storms combined. The second comparison examined the magnitude of enterococcus concentrations between the four days that comprised wet weather. Mean concentrations and standard deviations were plotted against results within 24 hrs of rainfall and up to three days following recorded rainfall. Enterococcus was chosen as the example indicator for this analysis.

The fourth data analysis element compared the frequency of water quality exceedences between small and large storms and between early and late season storms. Concentrations of fecal indicator bacteria for all beaches combined were compared to the state's water quality thresholds for large and small storms as well as early and late season storms. A subsidiary data analysis examining storm bias quantified the frequency of water quality threshold exceedences when storm flows generated watershed discharges that did not cross the beach sand berm, when storm flows were large enough to breach the sand berm, and for those watersheds that always breached the sand berm regardless of storm size. Deer Creek and Solstice Creek were two reference watersheds that always breached the sand berm during this study. Arroyo Sequit and San Onofre Creeks had watershed discharges that intermittently breached the sand berm.

The fifth data analysis element compared bacterial concentrations at each reference beach to salinity measurements and flux of bacteria into the surfzone to evaluate the impact of watershed discharges on reference beach water quality. In this case, we assumed that salinity acted as a conservative tracer of freshwater inputs. Flux was calculated as the product of bacterial concentration and flow. For this analysis, we only examined data when watershed discharges were entering the wave wash. Once again, we chose enterococcus for this analysis.

RESULTS

A total of four storm events were sampled during this study (Table 2). Two of the storms were collected early in the season (Oct 27-30 and Dec 5-8) and two late in the season (Jan 29-Feb 1 and Feb 12-15). Two of the storms were larger (1.31 - 3.07 in) and two were smaller (0.16 – 0.44 in). Antecedent dry periods ranged from 2 to 17 days, depending upon the site and storm event.

Only a single discharge sample detected the presence of human enteric virus. This sample was collected from the discharge across San Onofre State Beach on February 12, 2005. The source of the virus particle(s) was unknown. Therefore, all samples from this storm event (February 12-15) at this site (wave wash and discharge) were excluded from the following data analysis.

Frequency and Magnitude of Wet Weather Exceedences

The prevalence of water quality exceedences at the four reference beach sites was greater during wet weather than during winter dry weather or summer dry weather, regardless of fecal indicator bacteria (Table 3). Eighteen percent of all samples exceeded water quality thresholds for at least one indicator during wet weather. This was greater than 10 times the frequency of water quality threshold exceedences during dry weather. Although the frequency of water quality threshold exceedences in wet weather was always greater than winter dry weather or summer dry weather, the discrepancy between time periods varied among the individual fecal indicator bacteria. For example, 13% of enterococcus samples exceeded water quality thresholds during wet weather compared to 1% of the samples collected during winter dry weather and 0% of the samples collected during summer dry weather. Comparatively, 10% of the samples analyzed for E. coli during wet weather exceeded water quality thresholds compared to 1% of samples during winter dry weather and none during summer dry weather. Water quality thresholds for total coliforms and total coliform to fecal coliform ratio only exceeded water quality thresholds during wet weather.

San Onofre State Beach had the greatest frequency of water quality threshold exceedences during wet weather compared to the other three beaches sampled during this study (Table 3). One-third (33%) of the samples at San Onofre State Beach exceeded water quality thresholds for at least one indicator during wet weather. In contrast, 19% and 25% of the samples exceeded water quality thresholds at Dan Blocker and Leo Carrillo Beaches for at least one indicator during wet weather, respectively, while Deer Creek Beach had no water quality threshold exceedences during wet weather.

The greatest frequency of water quality threshold exceedences occurred within 24 hrs of rainfall and steadily decreased for the next three days (Figure 2). Twenty-seven percent of all samples collected < 24 hrs of rainfall exceeded water quality thresholds for at least one indicator. This frequency of water quality threshold exceedences decreased to 20% of samples collected either 1 or 2 days following recorded rainfall and ultimately declined to 7% of samples 3 days following recorded rainfall. This pattern of water quality threshold exceedences was repeated by virtually every bacterial indicator, but at varying levels of frequency. For example, the frequency of water quality threshold exceedences for total coliform, E. coli, enterococcus, and total coliform:fecal coliform ratio < 24 hrs of recorded rainfall was 13%, 20%, 20%, and 7% of all samples, respectively.

San Onofre State Beach and Dan Blocker Beach had the highest average enterococcus concentrations < 24 hrs after recorded rainfall (Figure 3). Mean enterococcus concentrations were greater than 10^2 < 24 hrs after recorded rainfall at both reference beaches. In contrast, enterococcus concentrations at Deer Creek Beach and Leo Carrillo State Beach averaged 10^1 < 24 hrs after recorded rainfall. In nearly all cases, mean enterococcus concentrations were highest < 24 hrs after recorded rainfall, steadily declining on days 1 through 3 following rainfall.

Factors that Influence the Frequency and Magnitude of Exceedences

Early season storms resulted in a greater number of water quality threshold exceedences than late season storms (Figure 4). After combining all wet weather samples at all creeks, 25% of the samples from early season storms exceeded water quality thresholds for at least one indicator, while only 11% of the samples from late season storms exceeded water quality thresholds for at least one indicator. Furthermore, early season storms had a greater frequency of exceedence of more than one threshold compared to late season storms. In fact, 63% of the samples that exceeded water quality thresholds during early season storms exceeded more than one threshold (i.e. E. coli and enterococcus). In contrast, 67% of the samples that exceeded water quality thresholds during late season storms exceeded only one threshold (i.e. just enterococcus).

Larger storms resulted in a greater number of water quality threshold exceedences than small storms (Figure 4A). After combining all wet weather samples at all creeks, 29% exceeded water quality thresholds for at least one indicator during large storms, compared to 9% of wet weather samples in small storms. This discrepancy between large and small storms was similar, or greater, for each of the fecal indicator bacteria thresholds except the total coliform-to-fecal coliform ratio. For example, 21% of the enterococcus samples exceeded water quality thresholds following large-sized rainfall events compared to 6% during smaller-sized rainfall events.

One factor that accounted for the differences in water quality threshold exceedences observed between large- and small-sized rainfall events is whether the creeks at Leo Carrillo or San Onofre were able to breach the sand berm and discharge into the ocean (Figure 5). Water quality thresholds were exceeded more frequently when the creek breached the sand berm compared to when it had not breached the sand berm. For example, 55% of the wet weather samples exceeded water quality thresholds for at least one indicator when the sand berm was breached compared to 12% of the samples when it had not breached. For Leo Carrillo Beach, 37.5% of samples exceeded at least one indicator when the sand berm was breached compared to 13% when it had not breached. For San Onofre Beach, 100% of samples exceeded at least one indicator when the sand berm was breached compared to 5% when it had not breached. This was most evident for enterococcus where 46% of the wet weather samples exceeded water quality thresholds when the berm was breached compared to only 6% when it was not breached. Similar patterns of threshold exceedence frequency were observed for E. coli and total coliforms.

Although Deer Creek and Solstice Creek had breached the sand berm during every wet weather sampling event, the frequency of water quality threshold exceedences at these reference beaches was low and similar to when the sand berm had not been breached at the more intermittent Leo Carrillo or San Onofre State Beaches (Figure 5). For example, 6% of the enterococcus samples exceeded water quality thresholds at the sites where the berm was always breached (i.e. Deer Creek and Dan Blocker Beaches) and 6% of the enterococcus samples exceeded water quality thresholds at the sites when the berm was not breached at Leo Carrillo and San Onofre State Beaches. Leo Carrillo and San Onofre State Beaches differ from Dan Blocker and Deer Creek Beaches in that they have lagoonal systems at the mouth of their watersheds prior to discharge into the ocean. These lagoon systems can attract fecal sources (i.e. birds) and concentrate fecal indicator bacterial prior to discharge across the beach. In contrast, when these lagoons systems are cut off from the ocean, wave wash samples tended to be lower.

The concentration of enterococcus in the wave wash was inversely proportional to the salinity of the water at reference beaches (Figure 6A). As salinity at the reference beach decreased, enterococcus concentrations increased. Variations in salinity, which is a measure of freshwater mixing, could explain roughly 35% of the variation observed in enterococcus concentrations in the wave wash ($r^2 = 0.35$). Additionally, wave wash concentrations were positively correlated to the flux of enterococcus from undeveloped watersheds (Figure 6B). Flux of enterococcus from undeveloped watersheds could explain roughly 66% of the variation observed in enterococcus concentrations in the wave wash ($r^2 = 0.66$).

The relationship between concentrations of fecal indicator bacteria in the discharge across San Onofre State Beach and in San Onofre Creek was near unity (Figure 7). Concentrations in the creek could explain between 93% and 99% of the variability for each of the three fecal indicator bacteria in the discharge ($r^2 = 0.99$, 0.99, and 0.93 for enterococcus, E. coli, and total coliform, respectively). The relationship between concentrations of fecal indicator bacteria in the discharge across San Onofre State Beach and in the lagoon above the beach was also near unity (Figure 7). Concentrations in the lagoon could explain between 96% and 99% of the variability for each of the three fecal indicator bacteria in the discharge ($r^2 = 0.98$, 0.99, and 0.96 for enterococcus, E. coli, and total coliform, respectively). The similarity of fecal indicator bacteria concentrations between the creek, the lagoon, and the discharge demonstrated that the lagoon had little influence on inputs to the ocean and was essentially a conduit for the creek during wet weather. Attempts to examine similar relationships during dry weather were foiled due to lack of flow across the beach and by low concentrations of fecal indicator bacteria in the creek, lagoon, and discharge samples.

DISCUSSION

Based on the results from this study, natural contributions of nonhuman fecal indicator bacteria were sufficient to generate exceedences of the State of California water quality thresholds during wet weather. On average, one-fifth of all samples collected within three days of rainfall exceeded water quality thresholds for at least one bacterial indicator and these exceedences were observed at three-quarters of the reference beaches sampled. Nonhuman sources of enterococcus and E. coli led to exceedences of water quality thresholds most frequently, while total coliform and total coliform to fecal coliform ratios led to the least number of exceedences.

Wet weather discharges from undeveloped watersheds generally contributed to higher concentrations of nonhuman fecal indicator bacteria along reference beaches relative to other times of the year. A greater frequency of exceedence of water quality thresholds was observed during wet weather compared to winter or summer dry weather at reference beaches examined during this study. Furthermore, the enterococcus concentration at the reference beaches was correlated with salinity, demonstrating a link to freshwater discharges. Finally, the concentration of enterococcus at the reference beach was positively correlated with enterococcus flux in the discharge from the undeveloped watershed draining to that beach.

Storm size affected reference beach microbial water quality. Large storms had a greater frequency of water quality exceedences compared to smaller storms at reference beaches. We also observed a significant increase in the frequency of water quality exceedences when stable beach berms were breached. This occurred consistently during large storm events. However, when rainstorms were insufficiently large to break through stable beach berms, the frequency of water quality exceedences was reduced.

Seasonality also affected reference beach microbial water quality. Early season storms had a greater frequency of water quality exceedences compared to late season storm events. Additionally, early season storms had a greater magnitude of water quality threshold exceedences, exceeding by more than one indicator in the majority of the wet weather samples collected. In contrast, the vast majority of water quality exceedences in late season storms exceeded for only a single indicator.

San Onofre State Beach had the greatest concentrations of bacteria and the greatest frequency of water quality threshold exceedences. This may have been the result of several factors that we cannot disentangle. First, San Onofre Creek was the largest watershed we sampled, which may have led to a greater number of nonhuman sources of fecal indicator bacteria upstream. Schiff and Kinney (2001) found a large quantity of fecal indicator bacteria in wet weather discharges in similar-sized, almost entirely undeveloped watersheds from inland San Diego County, with no human activity. Second, San Onofre Creek had the largest and most mature lagoon of any site sampled, which was located at the beach interface and may have attracted nonhuman fecal sources (i.e. birds). While our limited sampling did not indicate significantly increased concentrations in the lagoon relative to the creek upstream, we cannot discount this potential confounding factor at either San Onofre State Beach or any other beach with a substantially-sized terminal lagoon. For example, Grant et al. (2001) found that lagoons contributed excess fecal indicator bacteria to beaches in Orange County. Third, San Onofre Creek was the only discharge where we found human enteric virus. While virus was detected during a single event and this event was excluded from our calculations, it cast a shadow of doubt regarding human contributions over the remaining storms at this site. The San Onofre Creek watershed had the greatest fraction of developed land use (3%) of any of the other watershed systems and human activities are known to occur in the lower part of this watershed. Ultimately, we had insufficient data to eliminate all results from San Onofre Creek, but the effects of lagoon systems, watershed size, and potential human contributions should be addressed before a complete understanding of natural source contributions will be known.

The risk associated with wet weather discharges of nonhuman sources of fecal indicator bacteria is uncertain. Several epidemiology studies have examined the effect of increased fecal indicator bacteria on the risk of swimming-related illnesses (See Wade et al. 2003 for a review). Cabelli et al. (1982) found a relationship between enterococcus and health effects at a marine bathing beach in New Jersey, but this beach was impacted by known human point sources of fecal pollution. Haile et al. (1999) found a relationship between indicator bacteria concentrations and health effects after swimming near storm drains in Santa Monica Bay, but these drains were also known to contain human sources of fecal pollution. Colford et al. (2005) found no relationship between largely nonhuman sources of fecal indicator bacteria and health effects in Mission Bay, a marine bathing beach in San Diego, but they only examined dry weather. In this study, we quantified nonhuman sources of bacteria from nonpoint sources during wet weather, but we did not examine health risks. Epidemiological studies during wet weather need to be conducted in order to estimate the risk of swimming related illnesses at reference beaches like those examined in this study.

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Table 1. Reference beach and watershed characteristics. See text for data sources.

Reference Beach	Watershed	Latitude (NAD 83)	Longitude (NAD 83)	Watershed Size (km^2)	Open Space (%)	Beach Direction	Beach Substrate	Lagoonal System
Deer Creek	Deer Creek	34° 03.724' N	118° 59.164' W	3.1	100	SW	Sand	No
Leo Carrillo	Arroyo Sequit	34° 2.671' N	118° 55.950' W	28.1	100	SW	Sand	Yes
Dan Blocker	Solstice Canyon	34° 01.970' N	118° 44.539' W	11.5	99	SW	Sand and Cobble	No
San Onofre	San Onofre Creek	33° 22.842' N	117° 34.719' W	110	97	W	Sand and Cobble	Yes

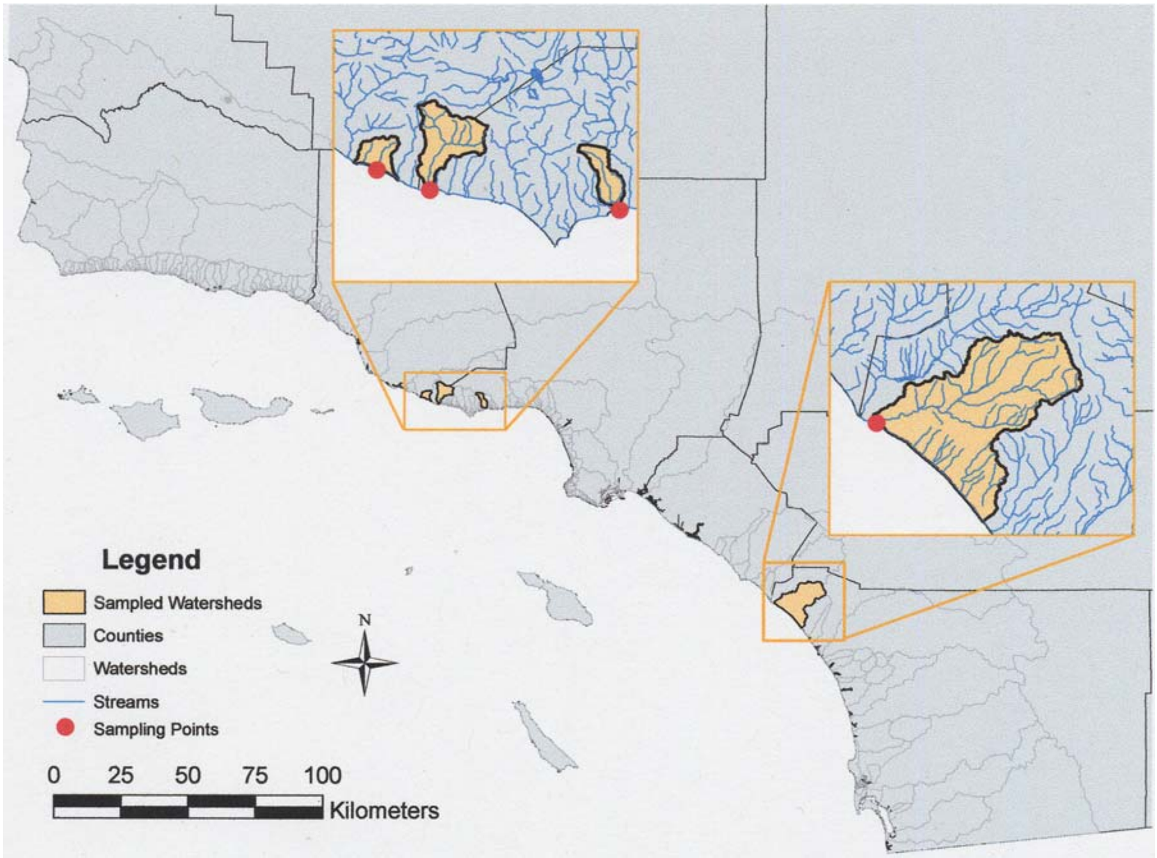


Figure 1. Map of reference beaches and watersheds.

Table 2. Inches of precipitation (antecedent dry days) during the four storm events at the four reference beaches during the 2004/05 wet season

	Storm 1	Storm 2	Storm 3	Storm 4
Storm Date	10/27-10/30	12/5-12/8	1/29-2/1	2/12 - 2/15
Deer Ck, Leo Carrillo, Dan Blocker[1]	1.31 (6)	0.41 (6)	0.44 (17)	2.04 (13)
San Onofre[2]	3.07 (6)	0.39 (9)	0.16 (2)	2.44 (4)

[1] Malibu Big Rock rain gage
[2] San Onofre Rain gage

Table 3. Frequency of water quality threshold exceedences for fecal indicator bacteria (total coliform, *E. coli*, enterococcus, total coliform:*E. coli* ratio, and any indicator) during wet weather ($\leq$ 3 days after rainfall, this study), winter dry weather (> 3 days after rainfall, November to March) and summer dry weather (> 3 days after rainfall, April to October) at the reference beaches targeted during this study. See text for dry weather data sources.

		Total	E.coli	Entero	TC:FC	Any Indicator
Deer Creek Beach	Wet	0	0	0	0	0
	Winter Dry	0	0	0	0	0
	Summer Dry	0	0	0	0	0
Leo Carrillo State Beach	Wet	0	6.3	12.5	18.8	25.0
	Winter Dry	0	0	0	0	0
	Summer Dry	0	0	0	0	0
Dan Blocker Beach	Wet	6.3	12.5	12.5	0	18.8
	Winter Dry	0	0	0	0	0
	Summer Dry	-[1]	-	-	-	-
San Onofre State Beach	Wet	25.0	25.0	33.3	0	33.3
	Winter Dry	0	6.7	6.7	0	6.7
	Summer Dry	0	0	0	0	0
All Beaches	Wet	6.7	10.0	13.3	5.0	18.3
	Winter Dry	0	1.4	1.4	0	1.4
	Summer Dry	0	0	0	0	0

[1] No data available

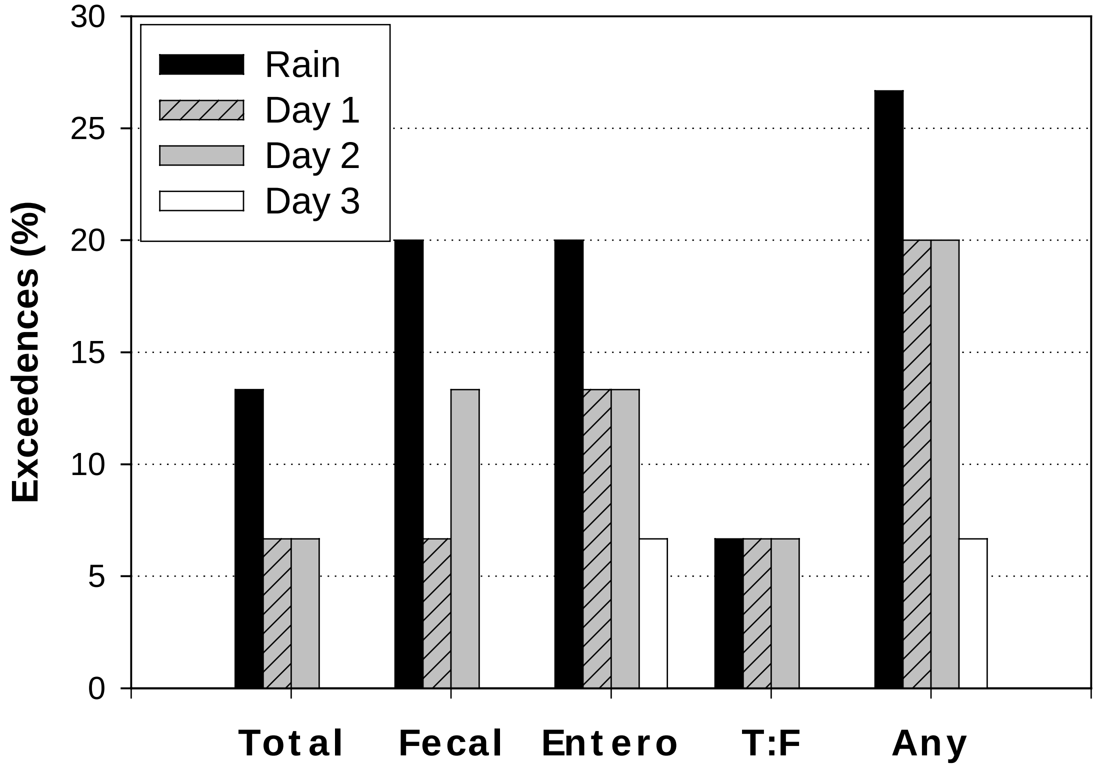


Figure 2. Frequency of water quality threshold exceedences for fecal indicator bacteria (total coliform, *E. coli*, enterococcus, total coliform:*E. coli* ratio, and any threshold) within 24 hrs of rainfall, and three subsequent days, at four reference beaches used in this study during the 2004/05 storm season.

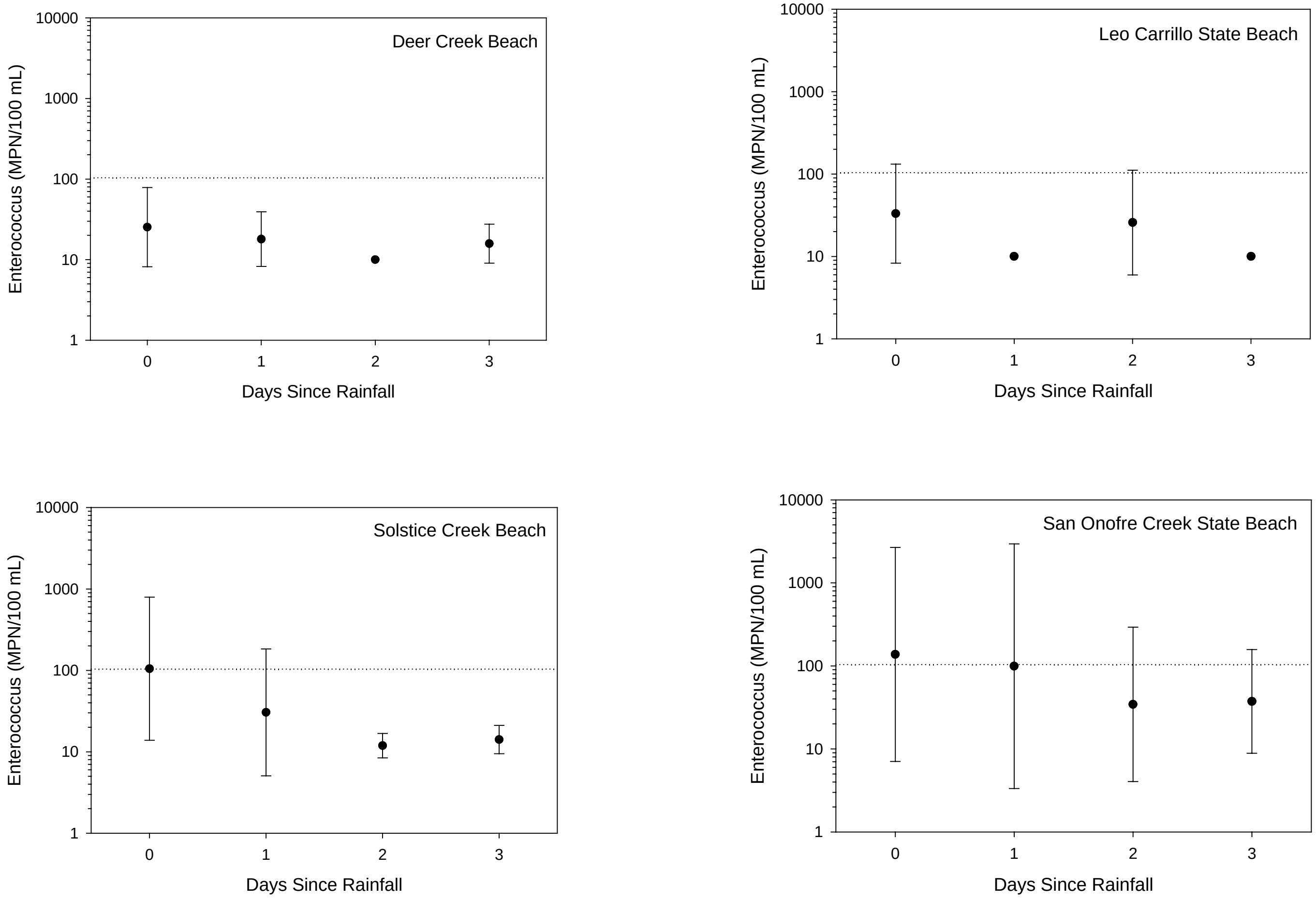
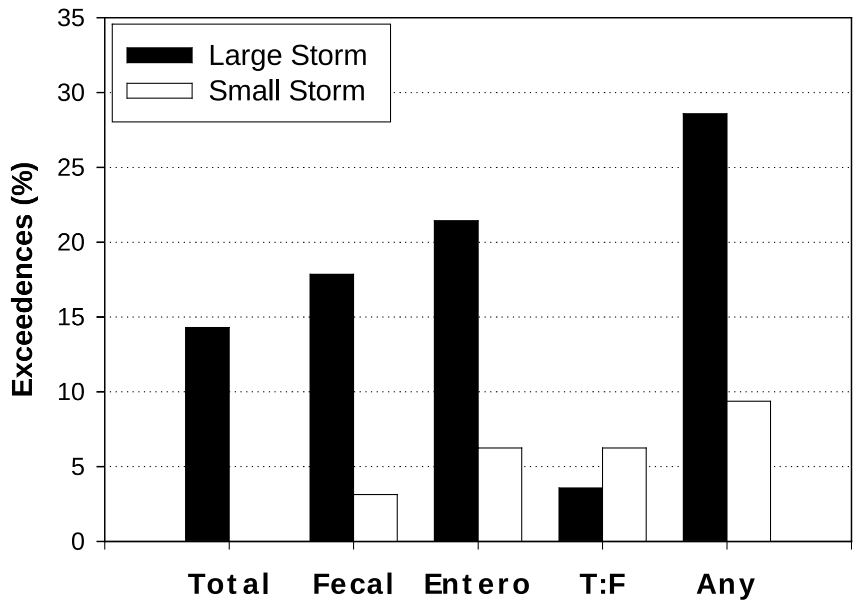


Figure 3. Comparison of mean (<u>+</u> SD) log enterococcus concentration within 24 hrs of rainfall and three days following storms at four different reference beaches during the 2004/05 storm season. Horizontal reference line represents the State of California water quality threshold (104 cells/ 100 mL).

A)



B)

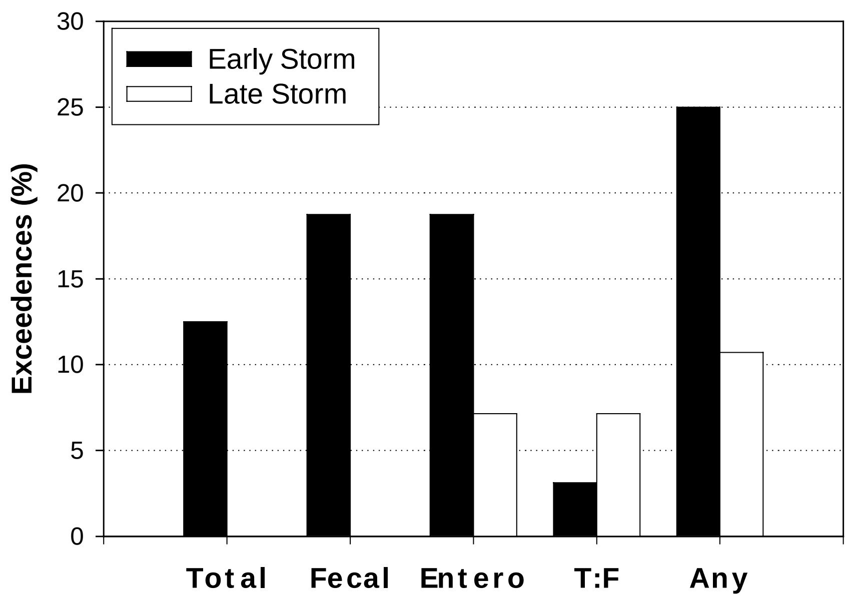


Figure 4. Comparison of water quality threshold exceedences ≤ 3 days of rainfall at four reference beaches used in this study for fecal indicator bacteria (total coliform, *E. coli*, enterococcus, total coliform:*E. coli* ratio, and any threshold) during: A) large and small storm events during the 2004/05 storm season; and B) early and late season storms during the 2004/05 storm season.

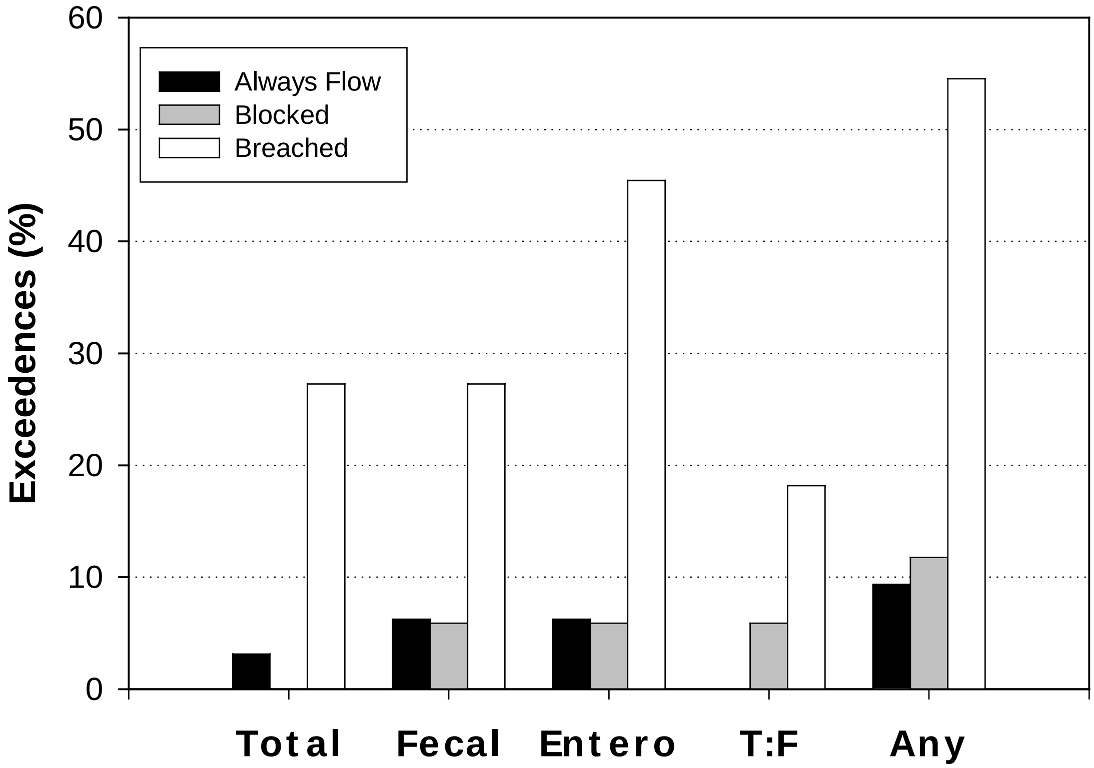
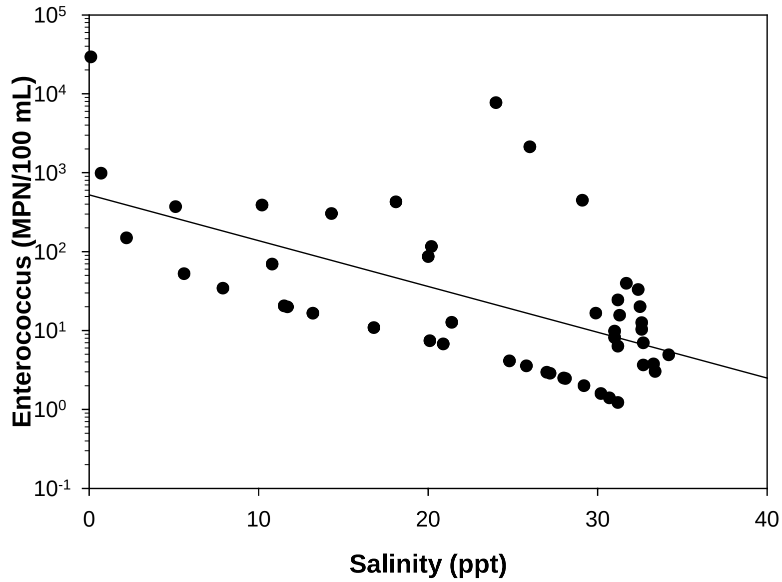


Figure 5. Comparison of water quality threshold exceedences for fecal indicator bacteria (total coliform, *E. coli*, enterococcus, total coliform:*E. coli* ratio, and any threshold) at reference beaches when creeks from undeveloped watersheds behind the beach are always flowing, blocked behind a sand berm, and when the sand berm is temporarily breached.

A)



B)

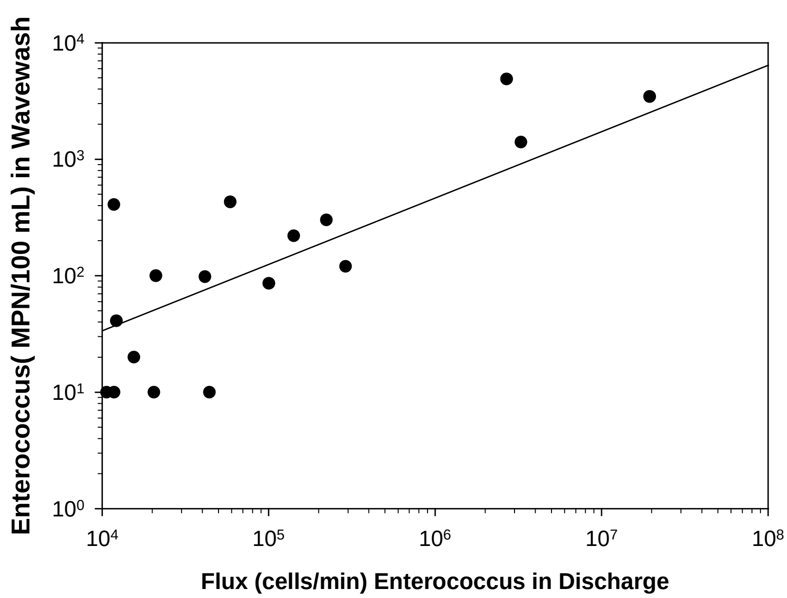


Figure 6. Comparison of enterococcus concentration in the wave wash at reference beaches to: A) salinity in the wave wash; and B) flux from the undeveloped watershed discharging to the reference beach.

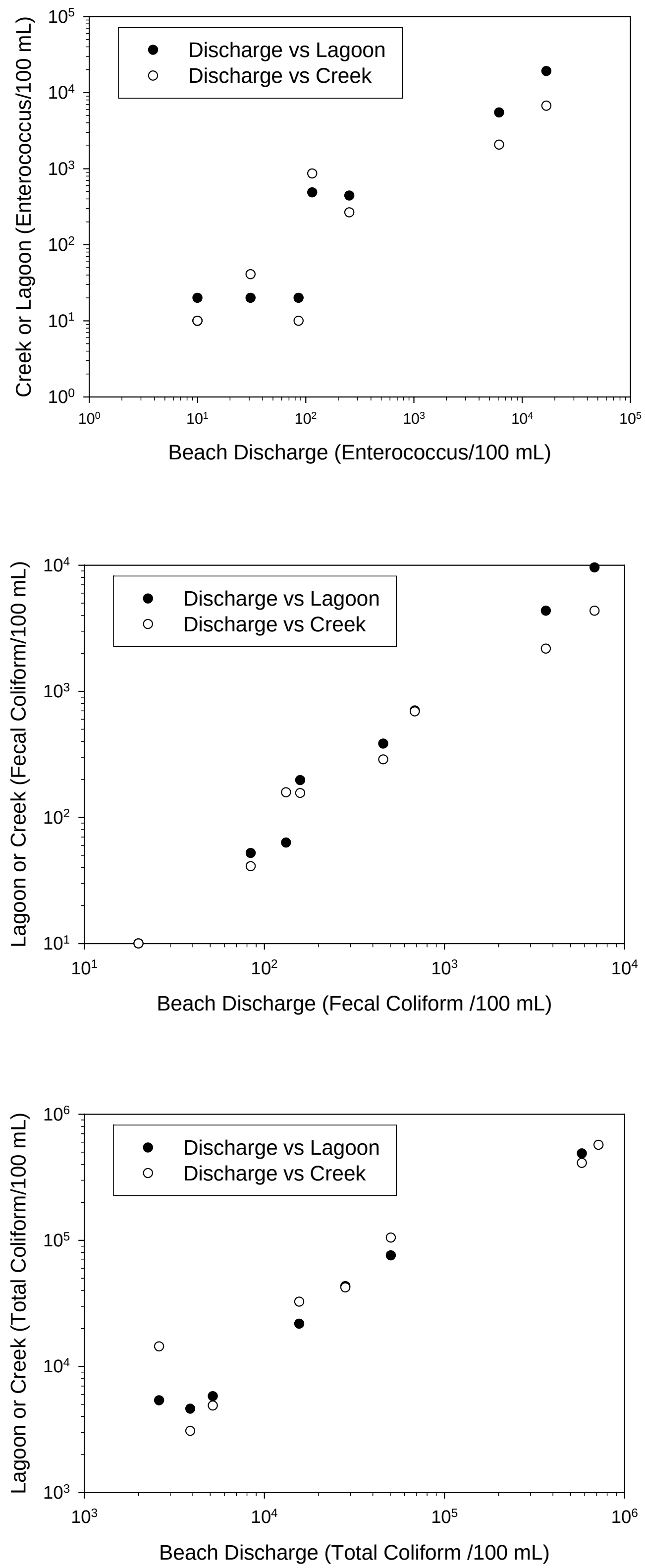


Figure 7. Comparison of fecal indicator bacteria concentration in the discharge across San Onofre Beach to concentrations in San Onofre Creek and in the San Onofre Lagoon.