

Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland

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Abstract

Recreational water quality at beaches in California and elsewhere is often poor near the outlets of rivers, estuaries, and lagoons. This condition has prompted interest in the role of wetlands in modulating surface water concentrations of fecal indicator bacteria (FIB), the basis of water quality standards internationally. A model was developed and applied to predict the dry-weather tidal cycling of FIB in Talbert Marsh, an estuarine, intertidal wetland in Huntington Beach, California, in response to loads from urban runoff, bird feces, and resuspended sediments. The model predicts the advection, dispersion and die-off of total coliform, *Escherichia coli*, and enterococci using a depth-integrated formulation. We find that urban runoff and resuspension of contaminated wetland sediments are responsible for surface water concentrations of FIB in the wetland. Model predictions show that urban runoff controls surface water concentrations at inland sites and sediment resuspension controls surface water concentrations near the mouth. Direct wash-off of bird feces into the surface water is not a significant contributor, although bird feces can contribute to the sediment bacteria load. The key parameters needed to accurately predict FIB concentrations, using a validated hydrodynamic model, are: the load due to urban runoff, sediment erodibility parameters, and sediment concentrations and surface water die-off rates of enteric bacteria. In the present study, literature values for sediment erodibility and water column die-off rates are used and average concentrations of FIB are predicted within 1/2 log unit of measurements. Total coliform are predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. Since wetland-dependent animals are natural sources of FIB, and FIB survive for long periods of time and may multiply in wetland sediments, these results highlight limitations of FIB as indicators of human fecal pollution in and near wetlands.



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Final Report: Identification and Control of Non-Point Sources of Microbial Pollution in a Coastal Watershed

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Description:

Objective:

The objectives of this study were to: (1) characterize the magnitude and variability of fecal indicator bacteria (FIB) loads in the watershed along an inland to coastal gradient that includes street gutters, storm channels, tidal channels, and the surf-zone at Huntington Beach; (2) examine linkages between FIB and other indicators of human pathogens; (3) develop strategies to control FIB loads during nonstorm periods; and (4) aid decisionmaking by examining the perspectives of stakeholders, including beachgoers, environmentalists, local businesses, public health officials, and wastewater utility managers on various aspects of beach pollution problems, such as the causes, health risks, and responsibility to pay.

California beaches are a critical component of the culture and economy of California and are threatened by coastal pollution. Beach recreation in California accounts for \$5.5 billion of the Gross State Product (King and Symes, 2003). Nowhere has there been greater attention on beach pollution than at Huntington Beach in southern California.

Huntington Beach, consisting of Huntington State Beach and Huntington City Beach, is located along a northwest to southeast striking section of the Pacific coastline between Los Angeles and San Diego, in Orange County, California. Several areas of Huntington State Beach have suffered chronic beach postings and closures over the past several years as a result of elevated concentrations of FIB in the surf zone (Kim and Grant, 2004). This beach is very popular (more than 5 million visitors per year), and the combination of surf zone pollution and significant beach usage implies that a large number of people (perhaps as many as 50,000) may acquire highly credible gastroenteritis from swimming and surfing in this area each year (Turbow, et al., 2003). FIB pollution at Huntington State Beach is thought to be caused by a combination of sources, including dry and wet weather runoff from the

surrounding community, bird droppings deposited in the Talbert Marsh, and regrowth of bacteria on vegetation and marsh sediments (Grant, et al., 2001; Reeves, et al., 2004). Additional potential sources of FIB include the offshore discharge of partially treated sewage effluent (Boehm, et al., 2002a), the offshore discharge of power plant cooling water that contains FIB from plant wash-down and other activities (Boehm, et al., 2002b), resuspension of contaminated sediments (Sanders, et al., 2004), bather shedding, the accumulation of bird droppings along the shoreline and offshore, the exfiltration of sewage-contaminated groundwater, and contributions from watershed outlets located north and south of the study area, including the Los Angeles River, the San Gabriel River, and outlets for Huntington Harbor and Newport Bay (Kim, et al., 2004).

This project focuses on the Talbert Watershed in Huntington Beach and Fountain Valley, California, which drains to Huntington Beach and is a significant stressor of Huntington Beach water quality. The Talbert Watershed encompasses 3,400 hectares in the cities of Huntington Beach and Fountain Valley. The watershed is urbanized and consists of residential developments, commercial districts, plant nurseries, and light industry. This area of southern California has separate storm water and sanitary sewer systems, therefore, 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 Huntington Beach) that converge near the ocean at a constructed wetland known as the Talbert Marsh. 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 storm water 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 (1,200 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 the 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 (Grant, et al., 2001). 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 Belding's savannah sparrow.

Summary/Accomplishments (Outputs/Outcomes):

To achieve the objectives, extensive monitoring of Talbert Watershed surface waters was conducted to measure the spatio-temporal variability of FIB loads (total coliform, *Escherichia coli*, and *Enterococcus*) and analysis was performed to examine the factors that control fate and transport. Monitoring also was performed to examine the association between FIB and other indicators of fecal pollution. Both one-dimensional and two-dimensional hydrodynamic models were developed to analyze the FIB loads in tidal channels and into the surf-zone and to develop a predictive tool that can be used to examine how bacteria loads would be altered by operational changes to the infrastructure. Surveys were performed to measure stakeholder preferences in the context of multi-

stakeholder, multi-objective beach pollution problems and to support decisionmaking analysis.

Closure and posting of Huntington Beach, California, during the study period was the source of widespread media attention. In response, members of the research team redirected efforts and/or engaged in a number of additional studies to better understand the factors controlling surface water quality in the Huntington Beach surf zone, as well as the response of stakeholders to the unfolding pollution problem. For example, co-principal investigator (PI) Keller focused attention on the decisionmaking of beachgoers (to swim or not to swim) in response to warning signs posted on the beach. Co-PI Keller also focused attention on the decisionmaking of public agencies, who were under great public pressure to remedy the pollution problem but had little understanding of its cause. To better understand the pollution problem, co-PI Grant analyzed short- and long-term FIB monitoring data to identify trends in Huntington Beach bathing water quality. The observed variability was examined in the context of historical management measures, such as passage of the Clean Water Act, construction of a new ocean outfall, and efforts to prevent urban runoff from draining directly to the beach. Co-PI Grant also developed a method to identify and rank the sources of pollution to the surf-zone using high-frequency monitoring data collected along the beach. PI Sanders teamed with University of California (UC) Irvine and UC San Diego researchers to examine the potential for Orange County Sanitation District effluent, discharged roughly 7 km offshore of Huntington Beach, to be transported onshore by internal tides. After the Talbert Marsh was identified as a contributor of FIB to the Huntington Beach surf zone, co-PI Sobsey focused attention on potential health risks associated with water contaminated with bird feces. In particular, marsh bird feces and surface water was examined for *Campylobacter*, *Salmonella*, and male-specific coliphages.

During dry weather, concentrations of FIB were highest in inland urban runoff, intermediate in tidal channels harboring variable mixtures of urban runoff and ocean water, and lowest in ocean water at the base of the watershed. This inland-to-coastal gradient is consistent with the hypothesis that urban runoff from the watershed contributes to coastal pollution. On a year-round basis, the vast majority (> 99%) of FIB loading occurs during storm events when runoff diversions, the management approach of choice, are not operating. During storms, the load of FIB in runoff follows a power law of the form $L \sim Q^n$, where L is the loading rate (in units of FIB per time), Q is the volumetric flow rate (in units of volume per time), and the exponent n ranges from 1 to 1.5. This power law and the observed range of exponent values are consistent with the predictions of a mathematical model that assumes FIB in storm runoff originate from the erosion of contaminated sediments in drainage channels or storm sewers. (Reeves, et al., 2004)

During dry weather periods, urban runoff controls surface water concentrations of FIB in channels where flushing is weak, and resuspension of FIB from the sediment/water interface controls surface water concentrations near the mouth where flushing by ocean water occurs once per day. The reservoir of FIB at the sediment/water interface is probably linked to settling of bacteria from both dry and wet weather urban runoff, deposition of animal feces, decaying vegetation, and bacterial regrowth. It is not clear whether the FIB are primarily attached to sediments, suspended in pore water, or incorporated into microbial biofilms. Nevertheless, surface water concentrations of FIB are rapidly amplified as turbulence in water column increases. A result is that dry weather urban runoff has little direct impact on surf zone water quality, but significant indirect impact given FIB loads from runoff accumulate at the sediment/water interface and are subsequently resuspended and exported to the surf-zone by tidal currents (Grant, et al., 2001; Arega and Sanders, 2004; Sanders, et al., 2004).

During the project period, dry-weather diversions of urban runoff to the sanitary sewer system were implemented to mitigate impacts to the surf-zone, at a cost of at least \$6 million to the County of

Orange and City of Huntington Beach. The efficacy of this approach is unclear, because the vast majority of watershed loads are shed during wet weather, whereas during dry weather, the tidal channels and marsh serve to dissipate loads by promoting die-off and settling. On the other hand, diversions presumably serve to reduce loads of other contaminants, including oil, grease, heavy metals, and so forth and, therefore, may be justified on these grounds. To evaluate whether the diversions are justified on the basis of FIB control, a better understanding of the cycling of FIB in sediments is needed. The alternative is to focus management efforts on wet weather controls. For example, if erosion of sediments is driving the loading of FIB, then regular removal of contaminated sediments accumulating in the storm sewer system might be an appropriate management strategy. The creation of distributed wetland treatment systems, in which contaminants in urban runoff are removed near their source, might also prove useful for reducing downstream impacts (Reeves, et al., 2004).

Research lead by PI Sanders shows that numerical modeling can be performed to predict FIB loads in tidal wetlands, analytes that are notoriously difficult to model because of poorly characterized non-conservative processes. The key parameters needed for accurate predictions of FIB loads, using a validated hydrodynamic model, are: (1) the load as a result of urban runoff; (2) sediment erodibility parameters; and (3) sediment concentrations and surface water die-off rates of enteric bacteria. For channels in the Talbert Watershed, literature values for sediment erodibility and water column die-off rates were used and average concentrations of indicator bacteria were predicted within one-half log unit of measurements. Total coliform were predicted more accurately than *E. coli* or enterococci, both in terms of magnitude and tidal variability. This work is important because it represents the first case where first-principle models were successfully applied to predict FIB in an estuarine setting with significant nonpoint sources. The approach adopted here is highly transferable and could benefit both wetland restoration and water quality compliance efforts on a widespread basis (Sanders, et al., 2004).

Plume tracking studies conducted by UC Irvine and UC San Diego researchers, including PI Sanders, show that Orange County Sanitation Department (OCSD) effluent occasionally moves shoreward toward Huntington Beach into water less than 20 m deep. Analyses of current and temperature observations indicate cold water is regularly advected crossshelf, into and out of the nearshore, at both semi-diurnal and diurnal frequencies. Isotherms typically associated with the wastefield near the outfall are observed just outside the Huntington Beach surf zone, where the total depth is less than 6 m, highlighting the extent of the cross-shelf transport. This advection is attributed to a mode 1 internal motion, or internal tide. Based on this analysis, it is not possible to rule out the possibility that the OCSD plume contributes to poor bathing-water quality at Huntington Beach (Boehm, et al., 2002a). Concerned over potential shoreline impacts, OCSD began a disinfection program in 2002 and initiated a roughly \$300 million program to build the necessary infrastructure for full secondary treatment.

Analysis of Huntington Beach monitoring data lead by co-PI Grant shows that the concentration of FIB varies over time scales that span at least seven orders of magnitude, from minutes to decades. Sources of this variability include historical changes in the treatment and disposal of wastewater and dry weather runoff, El Niño events, seasonal variations in rainfall, spring-neap tidal cycles, sunlight-induced mortality of bacteria, and nearshore mixing. On average, total coliform concentrations have decreased over the past 43 years, although point sources of shoreline contamination (storm drains, river outlets, and submarine outfalls) continue to cause transiently poor water quality. These transient point sources typically persist for 5 to 8 years and are modulated by the phase of the moon, reflecting the influence of tides on the sourcing and transport of pollutants in the coastal ocean. Indicator bacteria are very sensitive to sunlight; therefore, the time of day when samples are

collected can influence the outcome of water quality testing. These results demonstrate that coastal water quality is forced by a complex combination of local and external processes and raise questions about the efficacy of existing marine bathing water monitoring and reporting programs (Boehm, et al., 2002b). Further analysis led by co-PI Grant reveals that protocols used to decide whether to post a sign are prone to error. Errors in public notification (referred to here as posting errors) originate from the variable character of pollutant concentrations in the ocean, the relatively infrequent sampling schedule adopted by most monitoring programs (daily to weekly), and the intrinsic error associated with binary advisories in which the public is either warned or not. We derived a probabilistic framework for estimating posting error rates, which at Huntington Beach range from 0 to 41 percent, and show that relatively high sample-to-sample correlations (> 0.4) are required to significantly reduce binary advisory posting errors. Public misnotification of coastal water quality can be reduced by utilizing probabilistic approaches for predicting current coastal water quality, and adopting analog, instead of binary, warning systems (Kim and Grant, 2004).

Research lead by co-PI Sobsey on the potential health risks of bathing water contaminated by bird feces has lead to only preliminary findings. Specifically, *Campylobacter* and male specific coliphages were identified in Talbert Marsh bird feces and in marsh surface waters near the marsh. *Salmonella* was found only in bird feces samples and not water samples. Analysis continues to understand the relationship between microbes in bird feces and surrounding surface waters, and potential health impacts.

Research lead by co-PI Keller indicates that stakeholders share diverse opinions about the causes of beach pollution, the risks to beachgoers, and the responsibility to pay. In the context of a multi-objective decision model, stakeholders disagree on the appropriate weights of objectives. For example, local businesses heavily weigh economics whereas beachgoers heavily weigh health risks. Stakeholders also disagree on the severity of pollution problems. For example, environmentalists believe the probability of an environmental health problem is high when beaches are posted, but beachgoers do not. Relative to beachgoers' perceptions of potential health risks, surveys showed a peer effect: decisions to enter the water at posted beaches were strongly affected by whether or not others were in the water (Biswas and Keller, 2004; Biswas, et al., 2004).

Conclusions:

The vast majority of FIB loads in runoff from the Talbert Watershed are shed during storms and are associated with particles that appear to be scoured from the water collection system, including street gutters, storm pipes, and storm channels. Loads in runoff during dry weather periods account for roughly 1 percent of the annual runoff load and dissipate within the tidal channels by a combination of die-off and settling.

Loads exported from the watershed to the surf zone during dry weather period are deflected along the shoreline by wave driven currents and can cause exceedances of water contact recreation standards. Model predictions show the origin of such loads is the scouring by tidal currents of FIB at the sediment/water interface of tidal channels and Talbert Marsh. FIB at the sediment/water interface are linked to urban runoff FIB loads during both dry and wet weather periods, bird droppings, decaying vegetation, and bacterial regrowth. Because intertidal wetlands are to some extent natural generators of FIB, these results call into question the exclusive use of FIB as the basis of water contact recreation standards at beaches near the outlet of these water bodies.

On the basis of FIB control, the efficacy of dry weather diversions in Talbert Watershed is unclear,

although diversions presumably serve to mitigate other types of pollution as well. A better understanding of the cycling of FIB between the water column and sediments is needed to evaluate the linkages between wet weather and dry weather loads in relation to sediment interactions.

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Type	Citation	Project	Document Sources
Journal Article	Arega F, Sanders BF. Dispersion model for tidal wetlands. <i>Journal of Hydraulic Engineering</i> 2004;130(8):739-754.	R828011 (Final)	<i>not available</i>
Journal Article	Boehm AB, Sanders BF, Winant CD. Cross-shelf transport at Huntington Beach. Implications for the fate of sewage discharged through an offshore ocean outfall. <i>Environmental Science & Technology</i> 2002;36(9):1899-1906	R828011 (2001) R828011 (Final)	<ul style="list-style-type: none"> • Full-text: ACS Publications Full Text <small>EXIT Disclaimer</small> • Other: ACS Publications PDF <small>EXIT Disclaimer</small>
Journal Article	Grant SB, Sanders BF, Boehm AB, Redman JA, et al. Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surf zone water quality. <i>Environmental Science and Technology</i> 2001;35(12):2407-2416.	R828011 (2000) R828011 (2001) R828011 (Final)	<i>not available</i>
Journal Article	Reeves RL, Grant SB, Mrse RD, Copil-Oancea CM. Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California. <i>Environmental Science & Technology</i> 2004;38(9):2637-2648.	R828011 (Final)	<ul style="list-style-type: none"> • Full-text: ACS Full Text <small>EXIT Disclaimer</small> • Other: ACS PDF <small>EXIT Disclaimer</small>
Journal Article	Sanders BF, Arega F, Sutula M. Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. <i>Water Research</i> . 2005;39(14):3394-3408.	R828011 (Final)	<i>not available</i>

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Enviro Science & Technology

Bird Droppings Are Blamed for Bacteria

By Stanley Allison

June 02, 2001 in print edition B-9

A team of UC Irvine researchers has concluded that waterfowl and other animal droppings from a saltwater marsh and the Santa Ana River are a significant source of bacteria contaminating the ocean waters off Huntington Beach.

In a report that will be published in the June 15 issue of Environmental Science and Technology, the researchers point to inherent flaws in the design of the man-made saltwater Talbert Marsh.

Stanley Grant, the UCI professor who led the 18-month study of the ocean contamination problem at Huntington Beach, said water containing fecal bacteria, pesticides, nutrients and other materials filters through the marsh and then flows into the ocean in about 40 minutes—which is too fast.

For the marsh to act as a natural cleanser and remove contaminants, the water must spend at least a week filtering through the wildlife preserve, Grant said.

Even though other sources such as urban runoff from the Santa Ana River may have contributed to the contamination that resulted in four miles of beach closures for most of the summer of 1999, the levels of bacteria from the marsh were hundreds of times more than the state limits, the researchers said.

The team's conclusions contradict the accepted environmental theory that wetlands purify contaminated water flowing into the ocean.

The findings suggest that approximately 4.6 million saltwater marshes in the U.S. could be similarly affected, Grant said.

Mark Gold, a spokesman for the conservation group Heal the Bay, said that finding animal droppings in a nature preserve is nothing new, and insists that marshes still serve as a cleanser for other, more hazardous, contaminants.

"It's not surprising that wetlands are sources of fecal bacteria," Gold said. "What wetlands are great at doing is removing nutrients and metals."

The 25-acre wetlands preserve is on the inland side of Pacific Coast Highway at Brookhurst Street. Part of the Talbert watershed that encompasses 12 square miles in Huntington Beach and Fountain Valley, it attracts thousands of migratory birds and other wildlife each year.

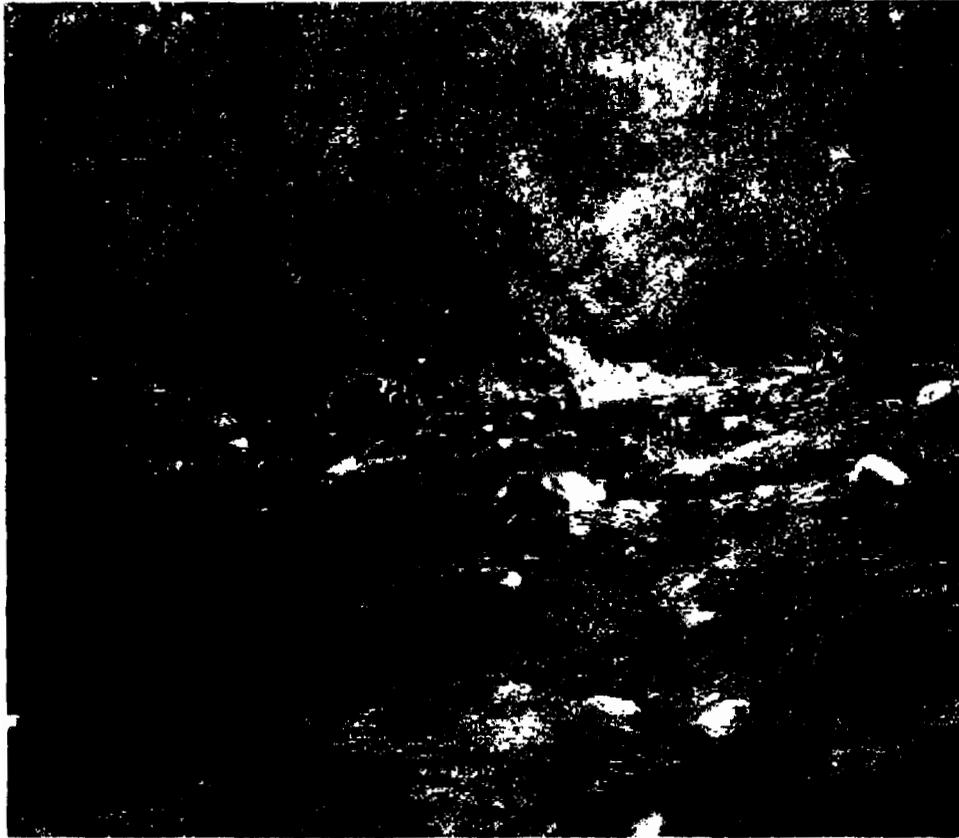
The UCI researchers also say that the nearby AES power plant contributes to the shore's contamination. The study suggests that partly treated sewage released four miles offshore from the Orange County Sanitation District treatment plant is being pulled back to the shore by tides and the plant as it draws water to cool its towers.

Fecal Indicator Bacteria (FIB)
Levels During Dry Weather
from Southern California
Reference Streams

Liesl L. Tiefenthaler

Eric D. Stein

Gregory S. Lyon



Southern California Coastal Water Research Project

Technical Report 542 - January 2008

Fecal Indicator Bacteria (FIB) Levels During Dry Weather from Southern California Reference Streams

Liesl L. Tiefenthaler, Eric D. Stein and Gregory S. Lyon

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ABSTRACT

High levels of fecal indicator bacteria (FIB) in surface waters is a common problem in urban areas that often leads to impairment of beneficial uses such as swimming or other contact recreation. Once impaired, common management and regulatory solutions include development of Total Maximum Daily Loads (TMDLs) and other water quality management plans. A critical element of these plans is establishment of a "reference" level of exceedances against which to assess management goals and TMDL compliance. Unfortunately, existing "background" or reference data on contributions of FIB from undeveloped catchments during dry weather is limited to a small number of locations measured at few time points. The goal of this study was to provide information on indicator bacteria contributions from natural streams in undeveloped catchments throughout southern California during dry weather, non-storm conditions. Specific questions addressed were: a) What are the "background" ranges of concentrations of FIB associated with dry weather flow from reference areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas? To help establish a regional reference data set, bacteria levels (i.e. *Escherichia coli* (*E. coli*), enterococci and total coliforms)) were measured from 15 unimpaired streams in 11 southern California watersheds weekly for one full year. A total of 590 water samples were collected from spring 2006 through spring 2007. Results were compared with data from the developed Ballona Creek watershed and to established State of California bacteria standards. Concentrations measured from reference areas were typically between one to two orders of magnitude lower than levels found in developed watersheds. The absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin. Nearly 82% of the time, samples did not exceed daily and monthly bacterial indicator thresholds, demonstrating good bacteriological water quality in natural streams throughout southern California. *E. coli* had the lowest daily percent exceedance (1.5%). A total of 13.7% of enterococci exceeded daily thresholds. The average measured enterococci levels of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml and a minimum of 160 MPN/100 ml. Indicator bacteria levels fluctuated seasonally with an average of 79% of both enterococci and total coliforms exceedances occurring during summer months (June-August). Temperature, at all sites, explained about one-half the variation in total coliforms density suggesting that stream temperatures regulated bacterial populations. Studies of human health risk associated with natural bacteria levels have not been conducted, but the levels observed in this study are below those reported to cause risk in freshwater systems with known human sources of FIB. Accounting for natural background levels will allow for management targets that are more reflective of the contributions from natural sources. Additional monitoring during wet weather is warranted to further characterize background bacterial contamination in southern California reference waterbodies.

Keywords: Dry Weather Water Quality, Indicator Bacteria, Reference Condition, Background Water Quality, TMDL

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TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
METHODS	2
Sampling Sites	2
Sampling	2
Laboratory Analysis.....	3
Data Analysis	3
RESULTS	5
Background Bacteria Concentrations and Fluxes.....	5
Frequency of Exceedance of Bacteria Standards at Natural Sites	5
Temporal and Spatial Patterns in FIB Levels	6
Perennial vs. Non-perennial Streams.....	6
Relationship of Bacteria Levels to Environmental Variables.....	6
DISCUSSION	8
Conclusion and Future Research	10
REFERENCES	12
APPENDIX A - SUMMARY BACTERIA DATA FOR ALL NATURAL STREAM SITES....	30
APPENDIX B - SUMMARY OF PHYSICAL PARAMETERS AT ALL NATURAL STREAM SITES	39
APPENDIX C - INTERLABORATORY CALIBRATION RESULTS	41

LIST OF TABLES

Table 1. List of natural stream sampling sites, characteristics and their median monthly fecal indicator bacteria densities (MPN/100 ml).....	16
Table 2. State of California marine water quality standards for fecal indicator bacteria as established in Assembly Bill 411. Currently a freshwater quality standard for total coliforms does not exist.	17
Table 3. Assessment of percent exceedances between counties in southern California during the present study. A ¹ represents those counties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes.	18
Table 4. Percent single-sample exceedance of fecal indicator bacteria (FIB) levels in natural streams during dry weather from May 2006-May 2007. Numbers in bold are significantly different ($p < 0.01$)......	19
Table 5. Correlation table (r^2 values) between water quality variables and fecal indicator bacteria (FIB) during dry weather in natural streams in southern California between May 2006-May 2007. Significant correlations ($p < 0.04$) are shown in bold, while significant correlations ($p < 0.001$) are both bolded and in italics.	20
Table A1. List of natural stream sampling sites, characteristics and their daily fecal indicator bacteria densities (MPN/100 ml).	31
Table A2. Monthly <i>E. coli</i> geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	32
Table A3. Monthly enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	33
Table A4. Monthly total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	34
Table A5. Dry season <i>E. coli</i> geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	35
Table A6. Dry season enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	36
Table A7. Dry season total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	37
Table A8. Annual dry season fecal indicator bacteria geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.	38
Table B1. Annual dry season averages of measured physical parameters in natural streams during May 2006-May 2007 in southern California, USA.	40
Table C1. List of participating laboratories and counties involved in the reference bacteria/watershed interlaboratory calibration.....	43
Table C2. List of the six common samples and their representative sewage dilutions in (ml) which each laboratory received for the interlaboratory calibration.	43

LIST OF FIGURES

Figure 1. Map of natural stream sampling sites and their respective catchments within southern California.	21
Figure 2. Comparison of dry weather log ₁₀ fecal indicator bacteria (FIB) densities (\pm standard deviations) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006-May 2007 in southern California, USA.	22
Figure 3. Dry season fecal indicator bacteria cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California, USA.	23
Figure 4. Mean monthly temperature ($^{\circ}$ C) and dissolved oxygen (mg/L) comparison (a) and geomean total coliform densities in natural streams in southern California (b) between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons ($p < 0.01$). <i>E. coli</i> and enterococci exhibited similar results. The dotted line indicates the 30-d geomean for total coliforms equal to 1,000 MPN/100 ml. All points above the line represent bacteria water quality exceedances.	24
Figure 5. <i>E. coli</i> a) and enterococci b) geomean densities in natural streams in southern California between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons. The dashed line indicates the monthly water quality standard equal to 235 MPN/100 ml and 104 MPN/100 ml for <i>E. coli</i> and enterococci respectively. All points above the line represent bacteria water quality exceedances.	25
Figure 6. Perennial and non-perennial stream comparison of log ₁₀ fecal indicator bacteria densities (MPN/100 ml) in southern California during the present study. The dotted line indicates the State single-sample bacterial water quality criterion. Significant differences in indicator densities existed between streams but ranges generally overlapped ($p < 0.05$). Boxplots show mean, median, 25th and 75th percentiles.	26
Figure 7. Natural stream temperatures in southern California versus total coliform densities (MPN/100 ml) during dry weather for an entire year. Solid line indicates the exponential trend line ($r^2 = 0.48$).	27
Figure 8. Distribution of log <i>E. coli</i> a); enterococci b); and total coliforms c) concentrations in natural streams, streams with minor perturbations, and in developed Ballona Creek watershed in southern California, USA. Natural streams were significantly lower than all other streams ($p < 0.001$). Minor perturbation streams were significantly lower than developed Ballona Creek ($p < 0.001$).	28
Figure 9. Distribution of log <i>E. coli</i> a); enterococci b); and total coliforms c) concentrations in natural streams during dry weather (present study) compared to wet weather (Natural Loadings; 2003-2005 and Los Angeles River watershed; 2001-2005) studies in southern California, USA. Dry weather bacteria concentrations were significantly lower than wet weather concentrations ($p < 0.001$).	29
Figure C1. Laboratory comparison results for log transformed total coliform data at Santiago Creek, Orange County. The dotted red line represents the median log criteria, while the solid blue lines are ± 0.5 median log count.	44

Figure C2. Laboratory comparison results for *E. coli* using a 3 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count..... 45

Figure C3. Laboratory comparison results for Enterococcus using a 1 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count..... 46

INTRODUCTION

The presence of fecal indicator bacteria (FIB) in surface waters is a prevalent concern for many municipalities, health departments, and regulatory agencies. Persistent or excessive bacteria levels often result in reduced opportunities for beneficial uses such as swimming, and may lead to waterbodies being listed as impaired under Section 303(d) of the Clean Water Act. Approximately 280 waterbodies are listed as impaired in the Los Angeles, Santa Ana, and San Diego regions (http://www.swrcb.ca.gov/tmdl/303d_lists.html). Management of impaired water bodies may involve development of Total Maximum Daily Loads (TMDLs), issuance of National Pollutant Discharge Elimination System (NPDES) permits, or development of water quality plans that are intended to reduce bacteria levels to a point where water quality standards are met and beneficial uses are protected. An important step in the development of TMDLs and other water quality management plans is to identify all sources of the constituent(s) of concern in order to accurately quantify loads and set appropriate management or regulatory targets. One of the challenges in developing appropriate targets is accounting for biogenic inputs, or the natural contribution from undeveloped catchments.

Most watersheds consist of both developed and undeveloped areas, both of which can contribute bacteria to streams via surface runoff. Bacteria associated with runoff from urban surfaces are well documented (Gore & Storrie Ltd. and Proctor & Redfern Ltd. 1981, USEPA 1993). For example, (Stein *et al.* 2007) observed that recreational (horse) and agricultural land uses in Los Angeles, CA contributed substantially higher storm fluxes for *Escherichia coli* (*E. coli*). Additional investigations by Bay and Schiff (1998), Noble *et al.* (2000) and Stein and Tiefenthaler (2005) found freshwater outlets such as storm drains to be especially high contributors of dry-weather FIB contamination.

Natural areas can also be a source of bacteria originating from wildlife, including birds and mammals, pets, and livestock (Griffith *et al.* 2006). Grant *et al.* (2001) found that enterococci bacteria generated in a restored wetland had greater effect on coastal water quality than dry season urban runoff. The presumed sources of these bacteria were birds that used the tidal salt marsh as habitat. (Ahn *et al.* 2005) also recognized that natural sources could be significant contributors to total bacteria levels in urban storm water in southern California. However, most previous studies have focused on either short measurements during or immediately following storm water runoff or on bacteria in coastal waters (beaches). Few studies have attempted to quantify naturally occurring background levels of bacteria in streams during baseflow (i.e. non-storm) conditions over an extended period of time. This data gap is critical because the non-storm period is when streams and the coastal waters they drain to receive the most human use and thus the potential risk is highest.

The goal of this study is to establish a "reference" level of bacteria that can be used to set appropriate water quality management targets. More specifically, we address the following questions: a) What are the "background" ranges of concentrations of FIB associated with dry-weather runoff from natural areas? b) What is the frequency with which reference FIB levels exceed relevant water quality standards? c) How does seasonality influence stream FIB levels associated with reference areas? and d) How do the ranges of FIB concentrations associated with reference areas compare with those associated with urban (developed) areas?

METHODS

The overall approach to the study was to characterize dry weather bacteria levels at a set of sites that is representative of existing natural conditions in southern California. The specific study design consisted of an intensive sampling regime with collection of weekly dry weather bacteria data for an entire year.

Sampling Sites

Fifteen sites were selected for inclusion in the study based on criteria developed by Stein and Yoon (2007, Stein and Yoon In press). Criteria were designed to ensure that sampling would capture natural conditions without influence from any land-based anthropogenic input. The criteria included: 1) contributing drainage area should be at least 95% undeveloped. 2) sites should be in a relatively homogenous setting in terms of underlying geology and landcover, 3) sites should have either year-round or prolonged dry-weather flow to allow sampling during at least a portion of the dry season, and 4) sites should not be within watersheds that have burned during the previous three years. Although fire can be a natural occurrence, inclusion of sites in burned catchments would have added a confounding factor and, therefore, were excluded. Catchment land use was determined by plotting watershed boundaries over (year 2003) land cover maps from the (National Oceanographic Administration (NOAA) 2003) Coastal Change Analysis Program (CCAP) - <http://www.csc.noaa.gov/crs/lca/ccap.html>. The 15 selected sites are located across five counties (Los Angeles, Orange, Riverside, San Bernardino and San Diego) and ten different watersheds: Los Angeles River, Los Alisos Canyon, Malibu Creek, Soltice Canyon, San Juan Creek, Santa Ana River, San Jacinto, Cucamonga, Santa Margarita, and San Dieguito (Figure 1, Table 1, and Appendix A).

Sampling

Weekly dry-season sampling was conducted at all 15 sites from May 15, 2006 through May 31, 2007. A site was eligible for sampling if it had not received measurable rainfall for at least 24 h and flow was no more than 20% above baseflow. Weekly sampling continued as long as there was measurable stream flow. For intermittent streams, sampling was suspended once the stream was too low to sample. Based on these criteria, the duration of sampling ranged from 9 to 55 weeks (Table 1). Water samples were collected as composite grab samples, with equivalent volumes collected from three different points across the stream (approximately 10, 50, and 90% distance across). These samples were taken from the flowing portion of the streams at a depth sufficient to exclude surface scum without introducing bottom sediment. A replicate water sample was collected in the same way after completion of the initial water sample for approximately 25% of the samples. A field blank sample was also collected at each site once a month. All water samples were collected in presterilized 125 ml high-density polyethylene (HDPE) sample bottles. Collected water samples were immediately placed on ice and transported to the laboratories within 6 h of sample collection for subsequent analyses.

At each sampling location and during each round of sample collection, water quality readings (i.e. temperature (°C), dissolved oxygen (DO) mg/L, pH, turbidity, and conductivity (µS/cm)) were measured using hand held field probes (i.e. Orion 125, YSI 63 and Horiba U-10). Measurements were taken in triplicate at each transect. In addition, physical and biological

parameters of the site and general climatic conditions were recorded and documented (using both data forms and photo documentation). Stream discharge was measured as the product of the channel cross-sectional area and flow velocity. Channel cross sectional area was measured in the field. At each sampling event, velocity was measured using a Marsh-McBirney Model 2000 flow meter (Frederick, MD). The velocity, width, and depth were measured at three points along each transect. Flow for each transect subsection was computed and summed for a total flow for the transect. Values from three transects were averaged to estimate overall flow at each site (Rantz 1982).

Laboratory Analysis

Water quality samples were analyzed for four bacteria indicators; *E. coli*, enterococci, total coliforms and *Bacteroides thetaiotaomicron*. Enterococci, total coliforms and *E. coli* were measured by the chromogenic substrate method using Enterolert® for enterococci and Colilert® for *E. Coli* and total coliforms (Idexx 24 h, Inc.). This commercially available product uses a Multiple Tube Fermentation (MTF) type format with defined substrate technology to detect the presence or absence of bacteria indicator density in a water sample. In this medium, the detection of coliform densities is based upon a color change caused by the reaction of a fluorogen with a bacterial enzyme. This assay is read within 24 hours and coliform densities are reported as most probable number (MPN)/100 milliliters (ml). Given the large geography covered by the study and the short holding time required for bacterial analysis, eight laboratories cooperated on sample analysis. Laboratory intercalibration studies were completed to ensure consistent methodology, data quality, and repeatability between laboratories. All laboratories had had good repeatability for all three bacterial indicators and all results fell within the median log comparability criteria. The low variability between labs indicated that interlab differences should not be a confounding factor in interpreting the results of the study. Details of the laboratory intercalibration study are provided in Appendix C.

Bacteroides thetaiotaomicron are anaerobic bacteria that comprise the majority of microorganisms that inhabit the human digestive tract. As such, they may be a more reliable measure of human fecal matter or pathogens than *E. coli* (Bernhard and Field 2000a,b). Samples were analyzed for either presence or absence of *B. thetaiotaomicron* as a negative control for human bacteria sources. This analysis was initiated at a sampling site when the State of CA single-sample water quality thresholds for both *E. coli* and enterococci were exceeded for two consecutive weeks. The presence of *B. thetaiotaomicron* would suggest that bacteria observed in the surface waters were predominantly of human origin. *B. thetaiotaomicron* was measured by DNA extraction followed by polymerase chain reaction (PCR) as described by (Brinkman *et al.* 2003).

Data Analysis

Three analyses were used to characterize FIB levels from natural streams. First the 30-d geomeans, variances, and ranges of concentrations, and fluxes were calculated to provide an estimate of expected baseline bacterial levels. Flux estimates facilitated region wide comparisons among watersheds of varying sizes. Flux was calculated as the ratio of the 30-d geomean or mean yearly bacterial concentration (MPN/100 ml) and contributing watershed area (km²) at a specific site. Second, dry weather FIB concentrations were compared with the state of CA standards for single-sample and 30-d geomean maximum allowable densities (Table 2).

Cumulative density frequency plots (CDFs) were produced to compare observed bacterial concentrations to the CA quantitative standards and to calculate accumulated relative exceedance percentages. Third, water quality statistics from natural sites were compared with previous data collected from watercourses draining developed areas of the greater Los Angeles basin to determine if significant differences existed between natural and developed areas (Stein *et al.* 2007, Stein and Yoon 2007).

Bacteria data were analyzed for differences between perennial vs. intermittent streams, between developed and undeveloped watersheds, and to assess temporal patterns. Differences in concentration or flux were tested using a one-way analysis of variance (ANOVA), with a significance level $p < 0.05$ (Sokal and Rohlf 1995). Differences based on flow regime were assessed using a Tukey-Kramer post-hoc test for multiple comparisons; differences between developed and undeveloped sites were investigated by comparing median values using a Kruskal-Wallis one-way ANOVA on ranks.

Spatial and temporal patterns were also investigated using Pearson's r correlation coefficient to determine if there were strong associations between FIB concentrations and continuous variables (i.e. temperature and flow; Helsel and Hirsch 2002); the null hypothesis, in this case, is that the correlation coefficient is zero.

RESULTS

Background Bacteria Concentrations and Fluxes

Annual median bacteria fluxes from the natural sites were 2 ± 1.4 MPN/100 ml/km², 3 ± 1.7 MPN/100 ml/km², and 106 ± 61.4 MPN/100 ml/km² for *E. coli*, enterococci, and total coliforms, respectively. *E. coli* and enterococci, median density values at the natural sites (based on single-sample measurements) were 10 MPN/100 ml and 20 MPN/100 ml respectively, while median density values in Ballona Creek are typically in the 10³ range. Densities and fluxes were significantly lower for all indicator bacteria at the natural sites relative to data from developed areas ($p < 0.001$, Figure 2).

Only two sites exceeded State water quality standards for both *E. coli* and enterococci for two or more weeks during the yearlong study. During the period of exceedance, *E. coli* levels ranged from 327 to 9804 MPN/100 ml while enterococci ranged from 388 to 7270 MPN/100 ml. Repeat exceedances were seen most commonly for enterococci. In both cases, the *thetaiotaomicron* samples were negative, suggesting that the bacterial populations represented by the FIB were probably derived from non-human sources.

Frequency of Exceedance of Bacteria Standards at Natural Sites

A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 14% of enterococci exceeded the daily threshold of 104 MPN/100 ml (Figure 3). The average enterococci level of these exceedances was 292 MPN/100 ml, with a maximum of 2098 MPN/100 ml (Orange County) and a minimum of 160 MPN/100 ml (San Bernardino County). For *E. coli*, 1.5% of the measurements exceeded the single sample standard of 235 MPN/100 ml with a maximum and a minimum of 5500 MPN/100 ml and 241 MPN/100 ml, respectively (Orange County). For total coliforms, 3% exceeded the single sample standard of 10,000 MPN/100 ml.

A total of 39% of enterococci samples from the natural sites exceeded the 30-d geomean water quality standard of 33 MPN/100 ml. The average enterococci level of these exceedances was 47 MPN/100 ml, with a maximum of 744 MPN/100 ml and a minimum of 3 MPN/100 ml. For *E. coli*, approximately 1% exceeded the 30-d geomean threshold of 126 MPN/100 ml with a maximum and a minimum of 146 MPN/100 ml and 1 MPN/100 ml, respectively (Orange County). For total coliforms, 45% exceeded the 30-d geomean of 1000 MPN/100 ml with a maximum and a minimum of 5040 MPN/100 ml and 23 MPN/100 ml, respectively.

Seventy-five percent of enterococci and 83% of total coliforms exceedances occurred during the summer months (June-August, Table 4). In August all indicator thresholds were exceeded with 12.5%, 62.5% and 75% of *E. coli*, enterococci and total coliforms samples exceeding monthly thresholds, respectively (Table 4).

Temporal and Spatial Patterns in FIB Levels

Bacteria levels for all three indicators were significantly higher during the summer than during all other seasons (Table 4, $p < 0.01$). For example, 30-d geomeans for total coliforms were near the water quality standard in May 2006 with levels approximately 878 MPN/100 ml \pm 3.2 SD, increased substantially during the summer, exceeding the criterion, peaking in July at 2586 MPN/100 ml \pm 3.1 SD (Figure 4b). Total coliform geomeans decreased gradually throughout the winter nearing zero in February, 2007 (289 MPN/100 ml \pm 4.2 SD), as stream temperatures fell below 10°C, before gradually returning to baseline geomeans throughout spring, 2007 (Figure 4a and b). Similar seasonal patterns were observed for *E. coli* and enterococci (Figure 5a and b).

Orange County had the highest daily and monthly water quality exceedances for both *E. coli* and total coliforms (12.9%; 25% and 3.2%; 100%, respectively, Table 3). For enterococci, approximately 47% of the San Diego County samples exceeded the daily threshold and 100% exceeded the monthly standard (Table 3). However, the Orange County and San Diego County streams had no flow in winter due to an unusually low 2006-2007 rainfall season, so the results are from only the spring and early summer months and do not represent annual averages that may occur in perennially flowing streams.

Perennial vs. Non-perennial Streams

Background bacteria levels differed based on the duration of stream flow (Table 1, Appendix A). *E. coli* and enterococci densities were significantly different in perennial vs. intermittent streams ($p < 0.05$, Figure 6). Mean \log_{10} concentrations for *E. coli* and enterococci at perennial streams were 1.0 ± 0.4 and 1.3 ± 0.5 , respectively. Intermittent streams had higher mean \log_{10} concentrations for *E. coli* and enterococci (1.6 ± 0.5 and 1.8 ± 0.6 , respectively). There were no statistical differences between stream types for total coliform densities (mean 2.7 ± 0.6 vs. 3.3 ± 0.4).

Relationship of Bacteria Levels to Environmental Variables

Of the five environmental variables measured (temperature, conductivity, dissolved oxygen, pH, turbidity), only stream temperature exhibited a significant correlation with seasonal FIB levels. Water temperature varied by about 5-10°C at each of the sites, reaching a maximum of 28°C on warm sunny afternoons. Streams located in the foothills (Mill Creek, San Bernardino Co.) or where the creek was significantly shaded had the lowest average temperatures (Table 1, Appendix B). For example streams in San Bernardino County ranged from 650 m to 1200 m in elevation and averaged 12.7°C. The highest monthly average water temperatures (20.4 °C) were recorded in Orange County where streams were approximately 200 m in altitude. Stream temperature and total coliforms were significantly positively correlated (Table 5, $p < 0.001$, $r^2 = 0.48$). A weaker, but still significant, positive correlation existed between stream temperature and *E. coli* or enterococci ($p < 0.04$, $r^2 = 0.20$ and $p < 0.04$, $r^2 = 0.26$, respectively). The Pearson's r for these two correlations was between 0.2 and 0.3 suggesting that similar processes may have controlled the relationship between stream temperature and FIB. A strong negative correlation existed between dissolved oxygen and both conductivity or stream temperature (Table 5, $p < 0.05$, $r^2 = -0.5$; $p < 0.001$, $r^2 = -0.84$, respectively). However, few statistically significant relationships existed among the other physical variables.

Total coliform densities increased exponentially at temperatures above 10°C (Figure 7, $r^2 = 0.48$). Dissolved oxygen concentrations varied inversely with stream temperatures throughout the study (Figure 4a). Monthly mean DO concentrations decreased sharply to approximately 8 mg/L at stream temperatures above 15°C, and concentrations increased to approximately 11 mg/L at stream temperatures below 10°C.

DISCUSSION

Enterococci, *E. coli* and total coliforms (FIB) are commonly used indicators of the possible presence of pathogenic (disease-causing) microorganisms in streams and the ocean. As shown in this study, these FIB can be found in natural streams, with populations increasing during warm summer months and persisting through winter. However, the densities observed in natural streams were usually below State water quality objectives, which are set below levels typically thought to impair beneficial uses (Geldreich 1978, Toranzos 2007). Furthermore, the absence of *B. thetaiotaomicron* indicated that the FIB in reference streams were likely of non-human origin (Carson *et al.* 2005). There are three possible sources of FIB observed in natural streams: External inputs from sources such as waterfowl, animals, or soil erosion; internal sources of bacterial growth and colonization within the stream associated with decomposition of organic matter; or a combination of the two (Byappanahalli *et al.* 2003, Toranzos 2007).

Higher bacteria levels observed during the summer suggest that factors existed which promote bacteria growth and regrowth in streams. The positive relationship between temperature and bacteria levels suggests that heat induced growth may be a contributing factor to seasonally high bacteria levels. In addition, warmer temperatures influence the dissolved oxygen content of the water. Decreased oxygen solubility associated with higher temperature may combine with lower dissolved oxygen levels producing algal blooms, which have been shown in previous studies to support growth of *E. coli* and enterococci in freshwater (Byappanahalli *et al.* 2003, Byappanahalli *et al.* 2007). These conditions may in turn accelerate death and decomposition of organic matter in the stream, further enhancing in situ bacterial growth. Increases in organic decomposition have been shown to increase survival and regrowth of enteric bacteria and viruses (Novotny and Olem 1994). This hypothesis is further supported by the negative correlation observed between conductivity and dissolved oxygen. Conductivity is closely correlated with total dissolved solids, which are typically comprised of inorganic and organic substances, a potential source of biological oxygen demand (BOD).

Higher FIB densities and incidence of water quality standard exceedences during the summer is consistent with the observations of others such as Noble *et al.* (2000) and Sieracki (1980). Nuzzi and Burhans (1998) compared the responses among indicator bacteria at 143 New York beach sites and found that survival was longer in the summer, but that the duration could be mediated by exposure to UV radiation from sunlight. More recently, growth or regrowth of fecal indicator bacteria in tropical and temperate soils during the summer months has also been reported (USEPA 2000, Ishii *et al.* 2006). Whitman *et al.* (1999) attributed a gradual increase of *E. coli* bacteria in water and sand at beaches during summer to higher survival and growth at warmer temperatures.

Another explanation for higher FIB levels during the summer could be higher external sources due to different patterns of use by wildlife and birds. A number of studies have shown that wildlife and other animals can be sources of bacteria in run-off (Baxter-Potter and Gilliland 1988, Bagshaw 2002, Stein *et al.* 2007). Previous studies have quantified that wildlife and bird feces contain high levels of FIB. Cox *et al.* (2005) measured fecal coliform levels of $10^3 - 10^5$ CFU/g from native wildlife in Australian watersheds. Ricca and Cooney (1998) reported that droppings from feral populations of pigeons, geese and herring gulls from the environment

around Boston Harbor, MA, USA contained up to 10^8 CFU/100 ml of enterococci. Bacteria from wildlife and birds can be associated with FIB levels in streams used by these animals. Noblet *et al.* (2004) found that birds were a likely source of intermittently high levels of FIB observed in the lower Santa Ana River watershed and the nearby surf zone in southern California. Similarly, Harwood *et al.* (2000) reported that animals were the dominant sources of indicator bacteria at Florida sample sites with relatively low anthropogenic impact. Bacterial source tracking studies conducted in Michigan suggested that feces from pets and raccoons were important contributors to FIB levels in streams and storm sewers (Ram *et al.* 2007). Moreover, levels increased in the late summer and fall coincident with increased raccoon den mobility following breeding.

Decreased stream flow may have also contributed to higher bacteria levels during the summer months. Although there was no statistically significant relationship between flow and bacterial densities, in all cases densities increased exponentially when stream flow decreased below approximately $0.5 \text{ m}^3/\text{s}$ (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially. Therefore, the combined range of bacteria levels for perennial and intermittent streams observed in this study should reflect expected levels in natural streams throughout southern CA.

Relatively minor perturbations in the contributing watershed can cause sites to quickly deviate from background conditions. Four sites originally considered, but later rejected from the study had bacteria levels 2-3 log units greater than the natural sites retained, but significantly lower than levels observed in the developed Ballona Creek watershed (Figure 8). The watersheds of these four sites were almost entirely natural open space, but had small portions subject to agricultural or transportation related runoff. In one instance, a portion of the contributing watershed was affected by a recent fire. These small perturbations in the watershed led to dramatic changes in bacteria levels that moved sites away from reference conditions. Although these sites were not included in the analysis of background conditions, they provide valuable insight into the sensitivity of natural watersheds to small increases in anthropogenic sources.

Although this study focused on background FIB levels during dry weather (non-storm) conditions, comparison of these results to background levels in storm water is important because FIB are major constituents of concern in storm water runoff that can result in impairment of receiving waters (Noble *et al.* 2003, Schiff *et al.* 2003, Stein and Tiefenthaler 2005). Stein and Yoon (2007) reported geometric mean FIB levels from natural streams during storms of 125, 140, and 4,460 MPN/100 ml for *E. coli*, enterococci and total coliforms, respectively. These levels are generally 1.5 - 2 log units higher than geomean levels observed in this study during dry weather conditions (Figure 9). As is the case in urban areas, bacteria levels in natural systems are significantly lower during dry weather conditions than during storms, although the higher levels observed during storms are much more transient in nature. Griffith *et al.* (2006) reported that one-fifth of all samples collected within three days of rainfall from beaches at the bottom of natural catchments exceeded water quality thresholds for at least one bacterial indicator.

Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize “background” bacterial contamination in southern California reference waters following storms.

The results of this study indicated that streams in undeveloped watersheds contain low levels of FIB of non-human origin. An important management question is whether the levels observed pose a potential health risk. Wade *et al.* (2003) reviewed 27 studies and concluded that *E. coli* levels between 45 and 170 CFU/100 ml in freshwater pose a relative human health risk level of 1.22 (i.e. low level risk). We observed 30-day geometric mean *E. coli* levels ranging from 2 – 138 MPN/100 ml, with an overall 30-day geometric mean of 41 ± 20 MPN/100 ml. Because the mean levels observed in this study were below the “low risk” range reported by Wade *et al.* (2003), it could be concluded that background levels in natural streams have a low likelihood of posing a human health risk. However, this conclusion should be made with caution because previous exposure and risk studies were conducted in areas known to receive wastewater or storm water discharges containing human fecal sources. In contrast, the FIB levels observed in this study were of non-human origin, so the actual risk is unknown.

Conclusion and Future Research

This study yielded the following conclusions about FIB levels in natural streams during dry weather conditions:

1. ***Fecal indicator bacteria typically occur in natural streams during dry weather conditions at levels below State water quality standards.*** Annual mean concentrations (both single sample and 30-day geometric mean) were below established water quality criteria for all three indicators. A total of 18.2% of the indicator bacteria samples (for all three indicators) from the natural sites exceeded daily (single sample) water quality standards. Approximately 1.5%, 14%, and 3% of *E. coli*, enterococci, and total coliforms, respectively, exceeded single sample water quality criteria.
2. ***Fecal indicator bacteria in natural streams are most likely of non-human origin.*** All samples tested for the presence of *B. thetaiotaomicron* were negative, indicating non-human sources in natural streams. FIB levels in natural streams likely result from a combination of natural inputs, such as wildlife, birds, and soil erosion and instream bacterial growth facilitated by high summer temperatures and presence of decaying organic matter.
3. ***Dry weather fecal indicator bacteria in natural streams are typically two orders of magnitude lower than those observed in streams draining developed watersheds.*** Data from the developed Ballona Creek watershed were typically in the 10^3 MPN/100 ml range for *E. coli* and enterococci. Even slight watershed modifications appear to result in a relatively rapid departure from background FIB levels.
4. ***Fecal indicator bacteria levels exhibit seasonal patterns.*** Mean bacteria levels and frequency of exceedance of water quality standards were higher during the warmer summer months for all three bacteria indicators. This suggests that summer is a critical period for assessing background bacteria levels. Past studies indicate that fecal indicator

bacteria levels in natural streams during storms are one to two orders of magnitude higher than those observed during dry weather conditions; however, the duration of these elevated levels is unknown. Studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of “background” bacterial contamination in southern California reference waters following storms.

5. ***Bacteria levels in natural streams were generally higher during lower flow conditions.*** For all three indicators, densities increased exponentially when stream flow decreased below approximately 0.5 m³/s (2 cubic feet/sec). In addition, median annual bacterial densities were higher in intermittent streams than in perennial, with the differences being mainly due to high levels in the period immediately prior to streams drying up. Despite the differences between perennial and intermittent streams, the annual ranges of observed bacteria levels overlapped substantially.
6. ***Dry weather fecal indicator bacteria levels were one to two orders of magnitude lower than those observed in natural streams during storm conditions.*** Past studies of water quality at beaches at the bottom of natural watersheds indicate that high bacteria levels may persist for up to three days following storms. Analogous measurements collected three days following recorded rainfall in natural streams is warranted to further characterize the persistence of “background” bacterial contamination in southern California reference waters following storms.
7. ***Fecal indicator bacteria in natural streams occurred at levels below those reported to pose health risks due to freshwater contact recreation.*** However, past risk assessments have all occurred in waters that are known to receive bacteria inputs of human origin. No epidemiology studies have been conducted on FIB of non-human origin, so the precise risk is unknown.

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Table 1. List of natural stream sampling sites, characteristics and their median monthly fecal indicator bacteria densities (MPN/100 ml).

Site Name	Watershed	County	Catchment Size (km ²)	Number Sampling Weeks/Yr	Mean Flow (m ³ /sec)	<i>E. coli</i>		Geomean (30-d)		Total coliforms (MPN/100 ml)	SD
						(MPN/100 ml)	SD	(MPN/100 ml)	SD		
Arroyo Seco	LA River		41.50	47	0.04	15.24	2.22	20.48	2.45	1291.90	2.85
Cold Creek	Malibu Creek		1.43	49	0.00	13.59	1.89	15.33	2.42	443.30	4.33
Lachusa Canyon	Los Alisos Canyon	Los Angeles	3.86	49	0.01	16.08	2.24	20.55	2.26	1486.50	2.14
Solstice Canyon	Solstice Canyon		8.74	49	0.01	16.97	2.28	20.64	2.43	1109.21	2.68
Chesebro Creek	Malibu Creek		7.55	49	0.00	90.30	5.49	68.25	4.24	2940.41	2.88
Bell Creek	San Juan		17.97	12 ^a	0.02	80.45	4.30	164.60	5.48	2008.67	3.16
San Juan Creek	San Juan	Orange	99.94	9 ^a	0.03	74.66	2.46	25.25	3.29	2848.15	1.66
Santiago Creek	Santa Ana		17.02	10 ^a	0.02	22.99	2.84	34.75	3.06	1869.15	1.98
Hurkey Creek	San Jacinto	Riverside	29.73	29	0.01	18.89	4.38	36.92	4.75	688.57	3.33
Mill Creek	Santa Ana		15.21	55	0.08	2.06	2.68	12.74	3.32	75.00	2.98
Cucamonga Creek	Cucamonga	San Bernardino	24.10	52	0.14	11.14	1.66	26.35	3.33	399.64	2.39
Day Creek	Santa Ana		11.70	55	0.32	11.02	1.58	25.18	2.87	545.71	2.41
Cajon Creek	Santa Ana		82.82	52	0.08	54.98	3.18	159.21	2.49	4794.47	2.04
Stone Creek	Santa Margarita	San Diego	7.00	50	0.00	138.18	3.86	52.72	3.58	1728.44	3.21
Boden Creek	San Dieguito		19.81	18 ^a	0.01	45.33	6.14	98.26	2.86	1658.46	2.54
		Mean	25.89	39	0.05	40.79	3.15	52.08	3.26	1592.51	2.70
		SD	14.54	9	0.04	19.84	0.71	25.32	0.47	622.94	0.34

^aIntermittent stream

Table 2. State of California marine water quality standards for fecal indicator bacteria as established in Assembly Bill 411. Currently a freshwater quality standard for total coliforms does not exist.

Fecal Indicator Bacteria	CA Maximum Allowable Density (MPN/100 ml)	
	single-sample	30-day geometric mean
Enterococci	104	33
<i>E. coli</i>	235	126
Total coliforms	10,000	1000

Additional Indicator

Bacteroides thetaiotaomicron Presence / absence of a human source

Table 3. Assessment of percent exceedances between counties in southern California during the present study. A ¹ represents those counties in which samples were collected only during spring and/or summer due to intermittent streams with less stable flow regimes.

	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total Coliforms
Daily			
Los Angeles County	0.0	6.3	0.0
Orange County ¹	12.9	38.7	3.2
San Bernardino	0.0	13.1	0.0
San Diego ¹	5.3	47.4	0.0
Monthly			
Los Angeles County	0.0	7.7	46.2
Orange County ¹	25.0	75.0	100.0
San Bernardino	0.0	23.1	0.0
San Diego ¹	0.0	100.0	80.0

Table 4. Percent single-sample exceedance of fecal indicator bacteria (FIB) levels in natural streams during dry weather from May 2006-May 2007. Numbers in bold are significantly different ($p < 0.01$).

	Exceedance (%)		
	<i>E. coli</i>	Enterococci	Total coliforms
Season			
Spring 06	0.0	41.7	75.0
Summer	12.5	75.0	83.3
Fall	0.0	0.0	28.6
Winter	0.0	0.0	11.1
Spring 07	0.0	22.2	44.4
Month			
May 2006	0.0	27.3	45.5
June 2006	0.0	66.7	75.0
July 2006	0.0	72.7	90.9
August 2006	12.5	62.5	75.0
September 2006	0.0	42.9	57.1
October 2006	0.0	0.0	14.3
November 2006	0.0	0.0	28.6
December 2006	0.0	0.0	14.3
January 2007	0.0	0.0	0.0
February 2007	0.0	12.5	25.0
March 2007	0.0	22.2	11.1
April 2007	0.0	11.1	44.4
May 2007	0.0	25.0	62.5
Annual	1.0	26.4	41.8

Table 5. Correlation table (r^2 values) between water quality variables and fecal indicator bacteria (FIB) during dry weather in natural streams in southern California between May 2006-May 2007. Significant correlations ($p<0.04$) are shown in bold, while significant correlations ($p<0.001$) are both bolded and in italics.

Parameter	Pearson r^2 -values				
	DO (mg/L)	Flow (m ³ /s)	<i>E. coli</i>	Enterococci (MPN/100 ml)	Total Coliform
Conductivity	-0.50	0.48	0.22	0.01	0.19
Dissolved Oxygen	-	0.12	0.18	0.21	0.16
pH	0.32	0.09	0.11	0.02	0.04
Flow	0.12	-	-0.06	-0.02	-0.08
Temperature (°C)	-0.84	0.02	0.20	0.26	0.48
Turbidity	0.19	0.00	0.02	1.44	0.07

Bolded values = $p<0.05$

Bolded italic values = $p<0.001$

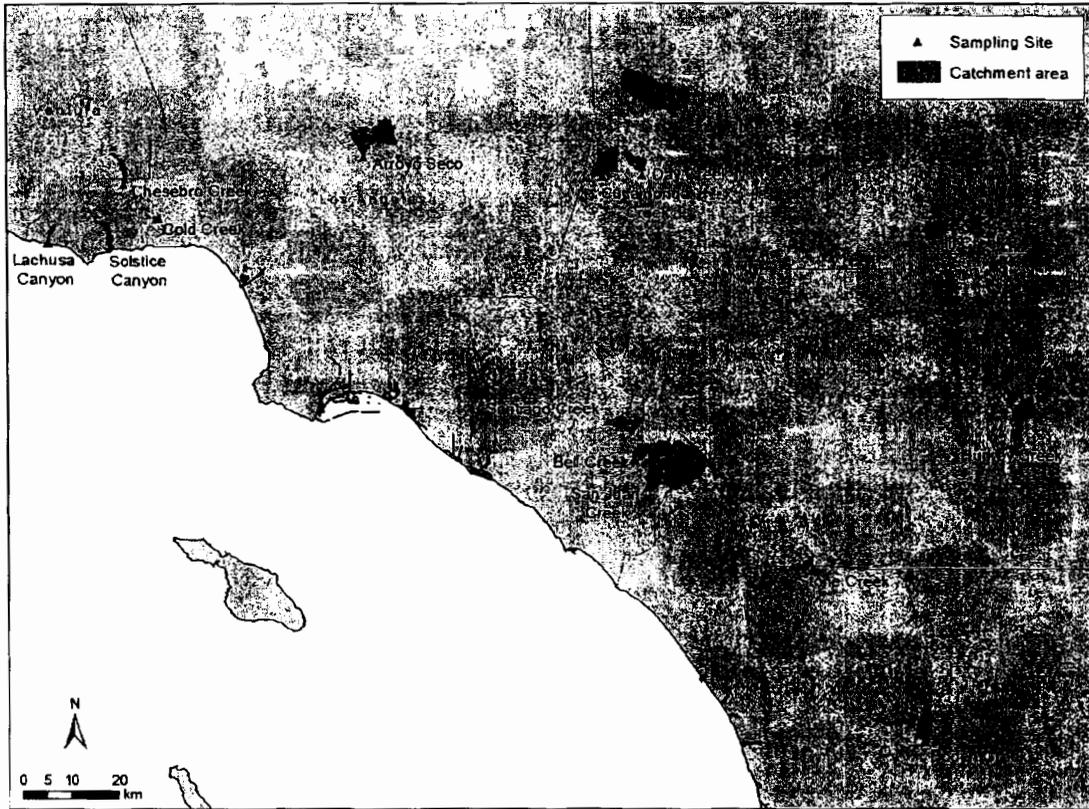


Figure 1. Map of natural stream sampling sites and their respective catchments within southern California.

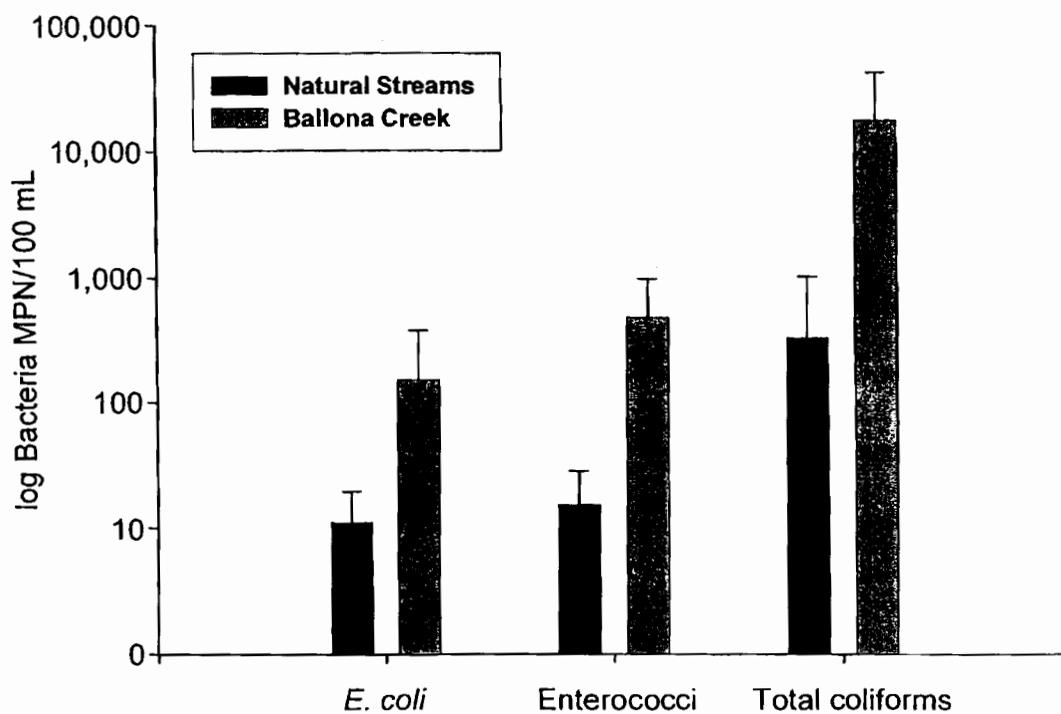


Figure 2. Comparison of dry weather log₁₀ fecal indicator bacteria (FIB) densities (\pm standard deviations) between natural streams in undeveloped watersheds and developed Ballona creek watershed from May 2006-May 2007 in southern California, USA.

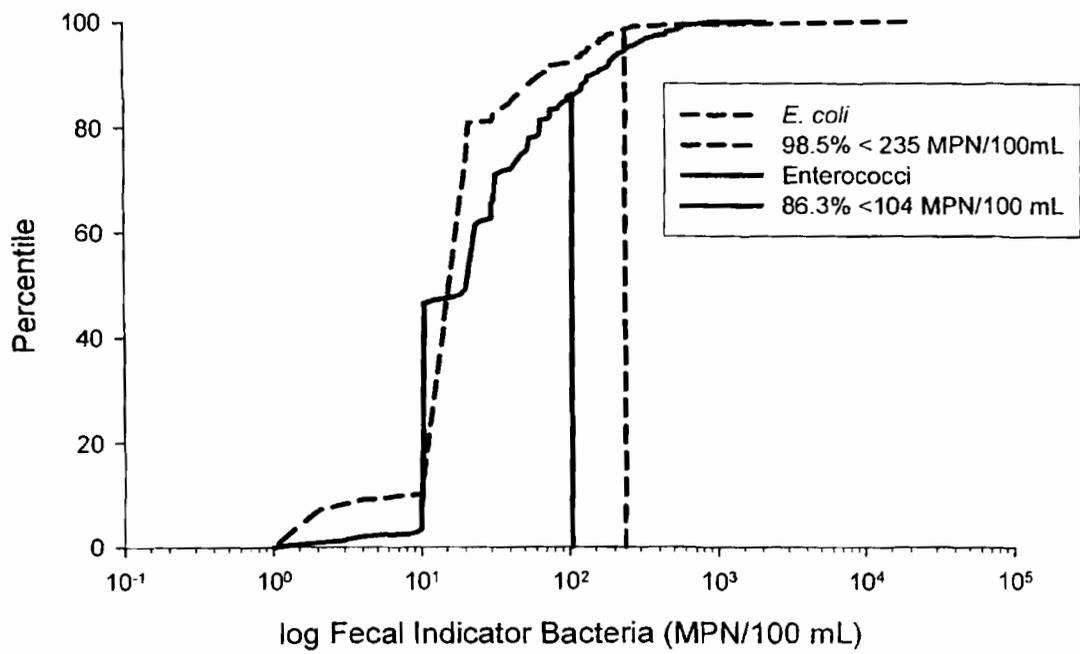


Figure 3. Dry season fecal indicator bacteria cumulative density frequency plots (CDFs) of natural streams relative to freshwater quality standards from May 2006 to May 2007 in southern California, USA.

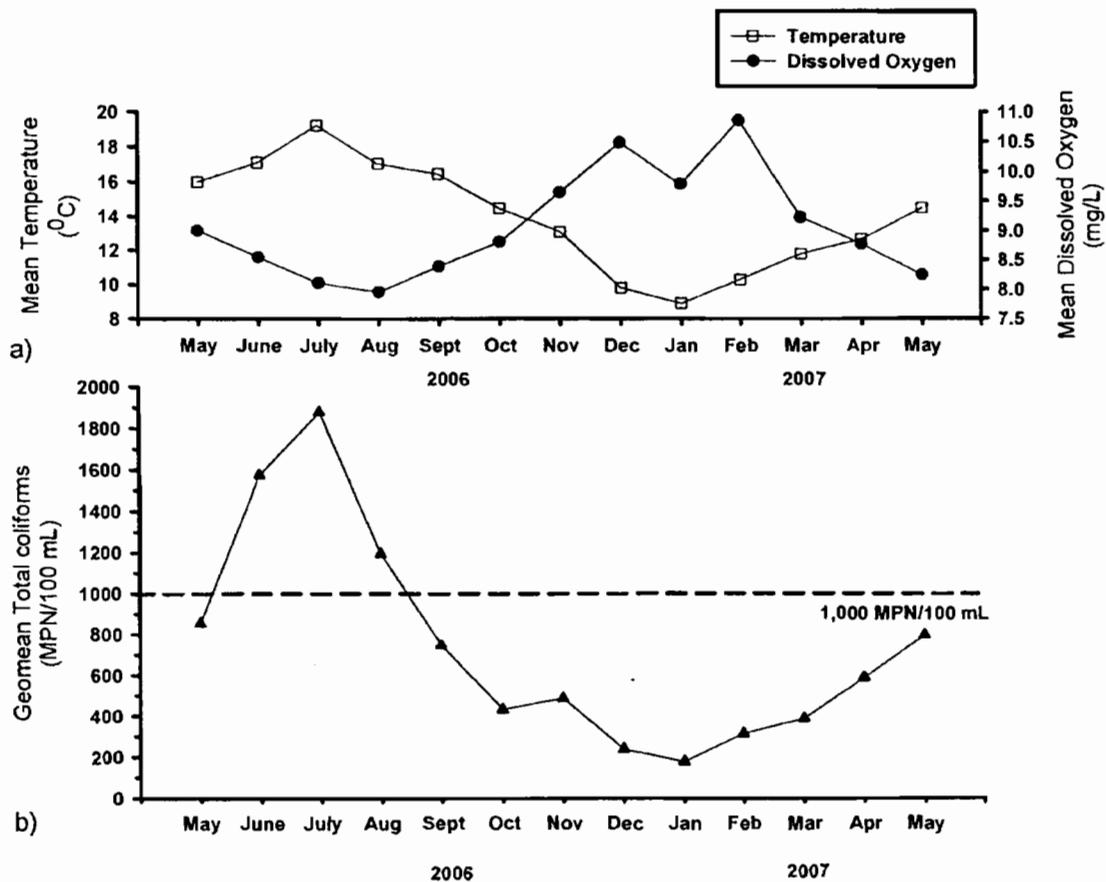


Figure 4. Mean monthly temperature (°C) and dissolved oxygen (mg/L) comparison (a) and geomean total coliform densities in natural streams in southern California (b) between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons ($p < 0.01$). *E. coli* and enterococci exhibited similar results. The dotted line indicates the 30-d geomean for total coliforms equal to 1,000 MPN/100 ml. All points above the line represent bacteria water quality exceedances.

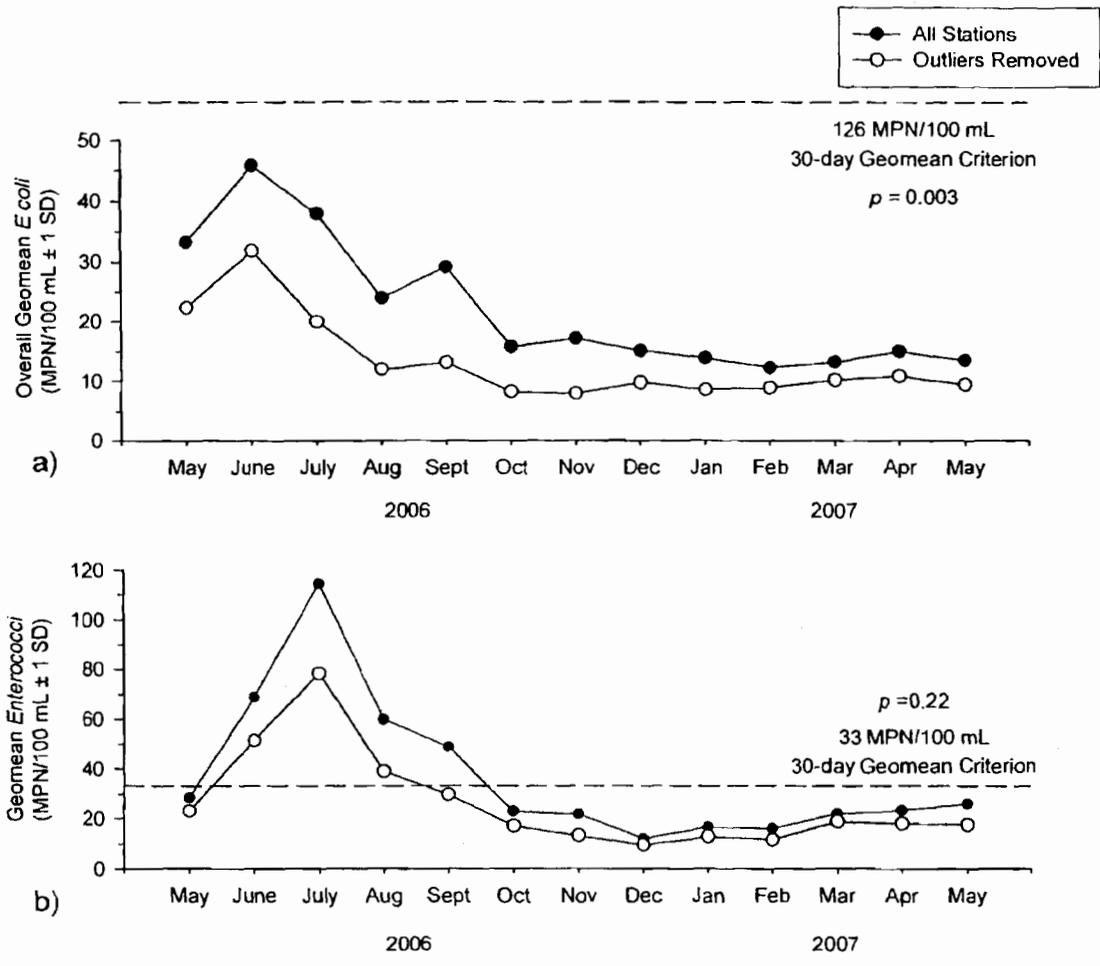


Figure 5. *E. coli* a) and enterococci b) geomean densities in natural streams in southern California between May 2006 and May 2007. Summer months (June-August) were substantially higher than all other seasons. The dashed line indicates the monthly water quality standard equal to 235 MPN/100 ml and 104 MPN/100 ml for *E. coli* and enterococci respectively. All points above the line represent bacteria water quality exceedances.

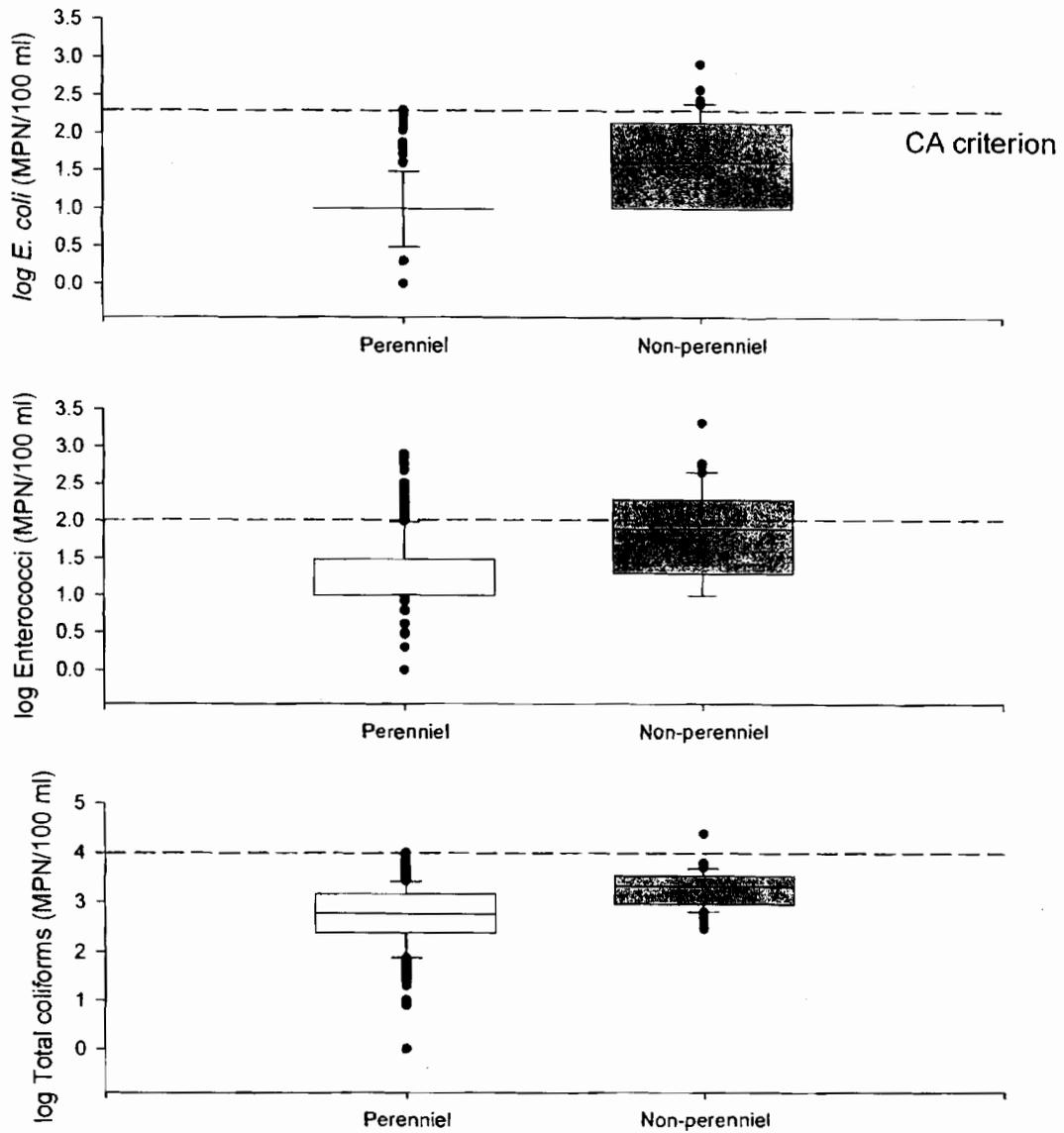


Figure 6. Perennial and non-perennial stream comparison of log₁₀ fecal indicator bacteria densities (MPN/100 ml) in southern California during the present study. The dotted line indicates the State single-sample bacterial water quality criterion. Significant differences in indicator densities existed between streams but ranges generally overlapped ($p < 0.05$). Boxplots show mean, median, 25th and 75th percentiles.

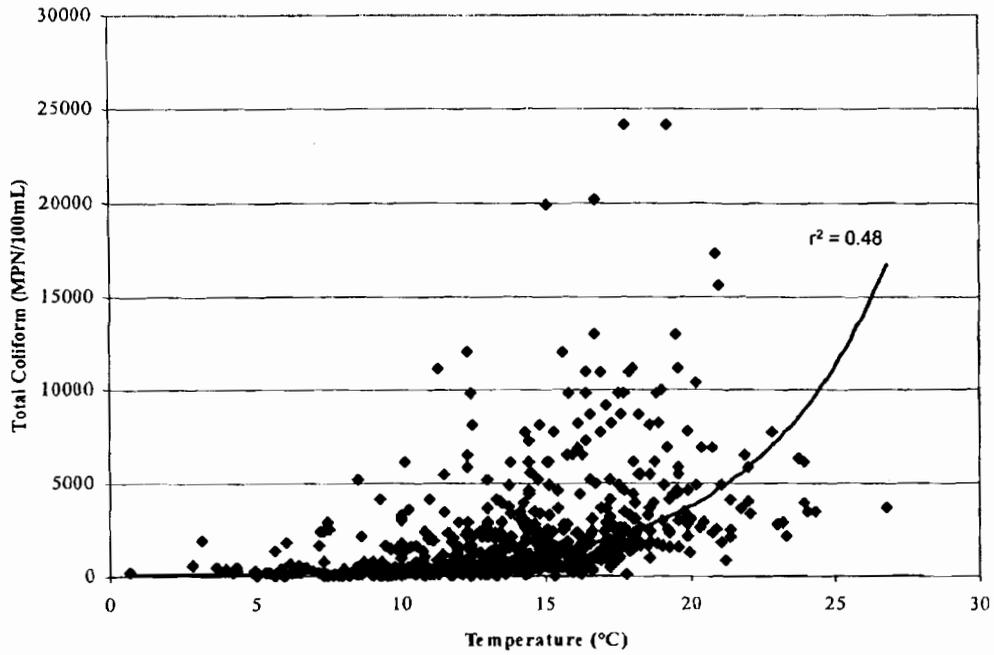


Figure 7. Natural stream temperatures in southern California versus total coliform densities (MPN/100 ml) during dry weather for an entire year. Solid line indicates the exponential trend line ($r^2 = 0.48$).

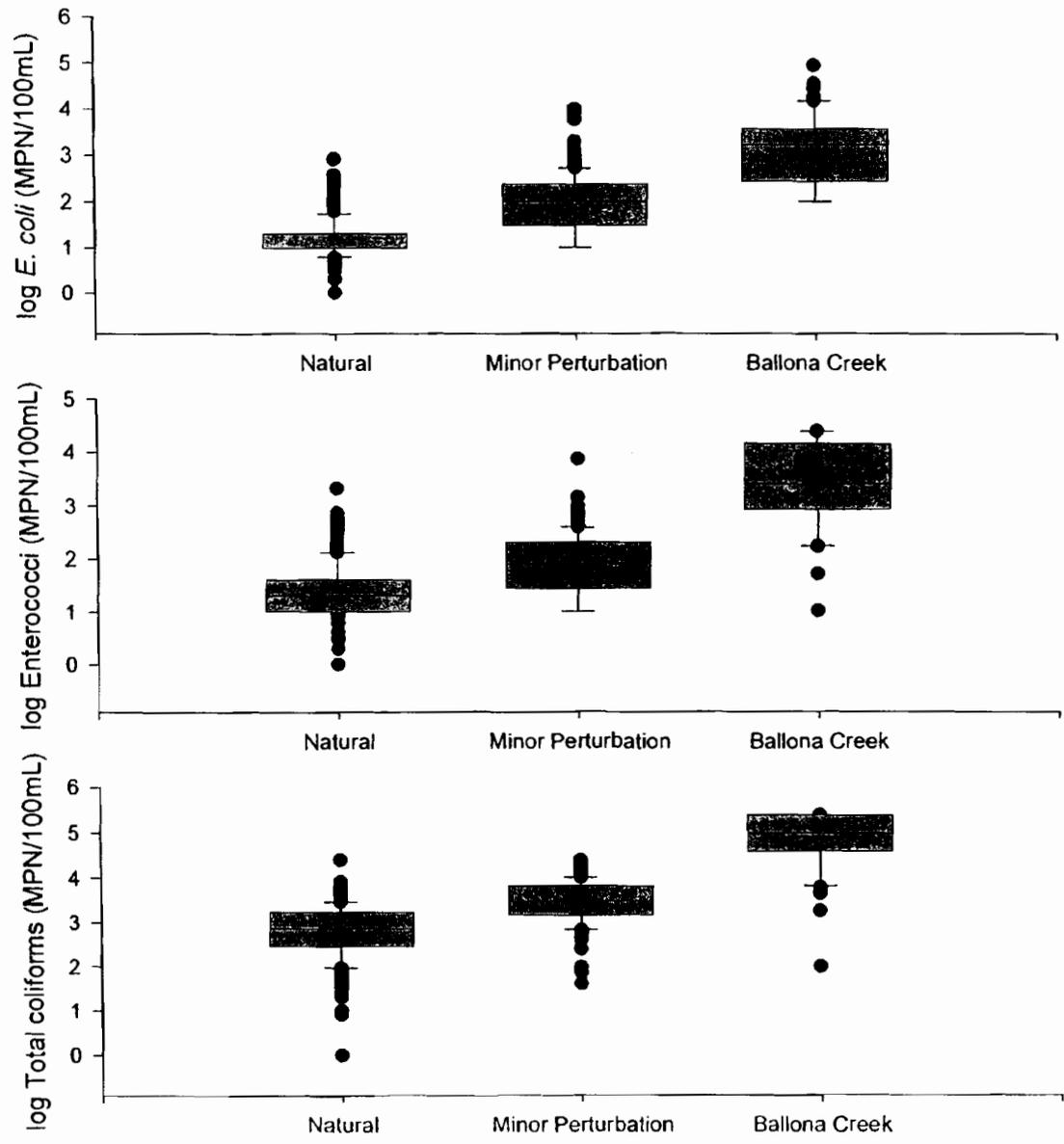


Figure 8. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams, streams with minor perturbations, and in developed Ballona Creek watershed in southern California, USA. Natural streams were significantly lower than all other streams ($p < 0.001$). Minor perturbation streams were significantly lower than developed Ballona Creek ($p < 0.001$).

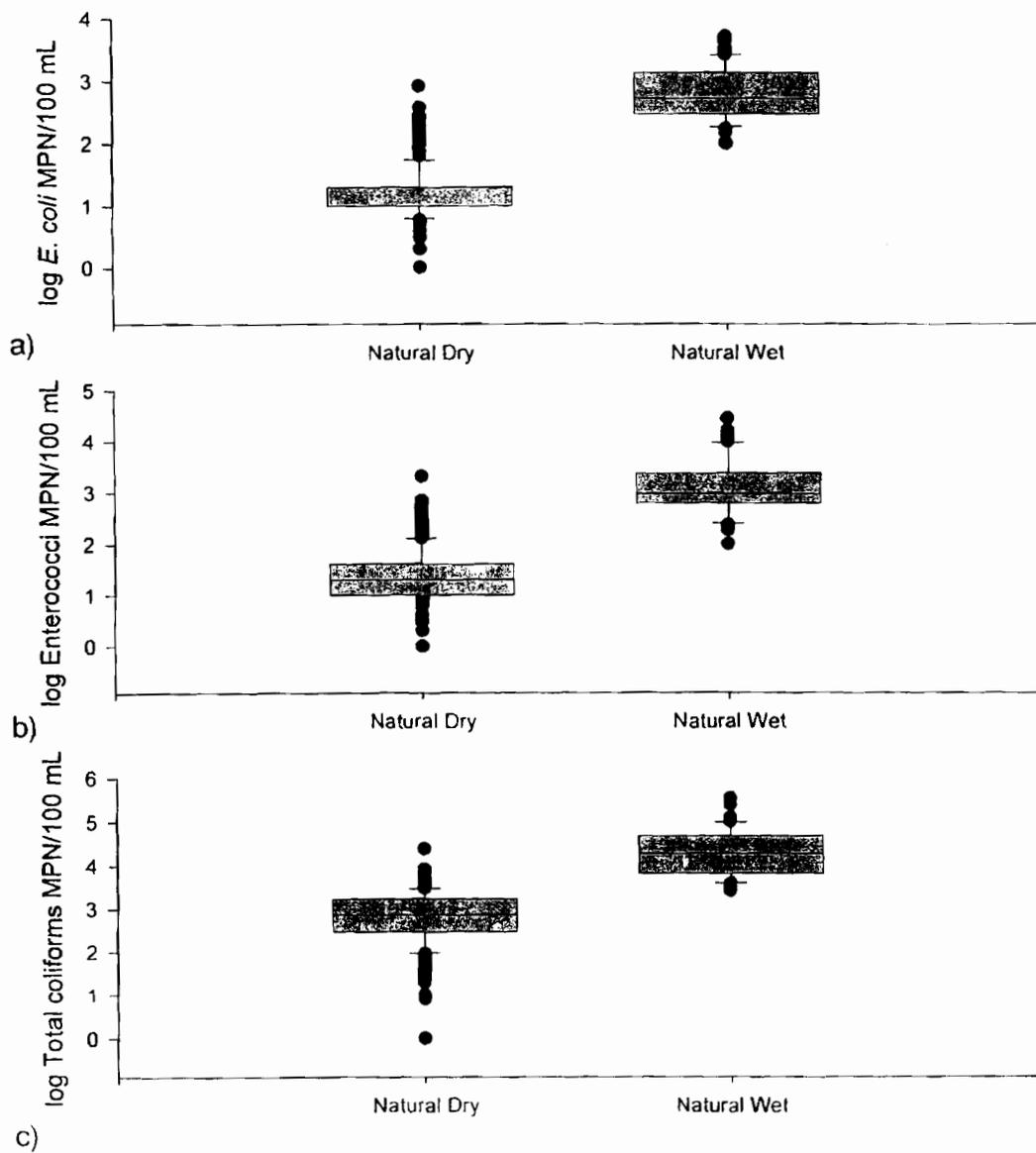


Figure 9. Distribution of log *E. coli* a); enterococci b); and total coliforms c) concentrations in natural streams during dry weather (present study) compared to wet weather (Natural Loadings; 2003-2005 and Los Angeles River watershed; 2001-2005) studies in southern California, USA. Dry weather bacteria concentrations were significantly lower than wet weather concentrations ($p < 0.001$).

**APPENDIX A - SUMMARY BACTERIA DATA FOR ALL NATURAL
STREAM SITES**

Table A1. List of natural stream sampling sites, characteristics and their daily fecal indicator bacteria densities (MPN/100 ml).

Sampling site	Watershed	Concentration (MPN/100 ml)											
		E. coli			Enterococci			Total coliforms					
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max			
Arroyo Seco	LA River	10	15.2	148	10	20.5	250	10	1291.9	6867			
Cold Creek	Malibu Creek	10	13.6	108	10	15.3	480	10	443.3	6131			
Lachusa Canyon	Los Alisos Canyon	10	16.1	161	10	20.6	197	146	1486.5	8164			
Solstice Canyon	Solstice Canyon	10	17.0	200	10	20.6	262	10	1109.2	5475			
Chesebro Creek	Malibu Creek	10	90.3	9804	10	68.2	7270	96	2940.4	24192			
Bell Creek	San Juan	10	80.5	820	10	164.6	2098	292	2008.7	24196			
San Juan Creek	San Juan	20	74.7	259	10	25.2	299	1664	2848.2	6294			
Santiago Creek	Santa Ana	10	23.0	134	10	34.7	228	469	1869.1	3873			
Hurkey Creek	San Jacinto	10	18.9	5500	10	36.9	780	210	688.6	7700			
Mill Creek	Santa Ana	1	2.1	20.9	1	12.7	190	1	75.0	435			
Cucamonga Creek	Cucamonga	6	11.1	180	10	26.3	580	10	399.6	2000			
Day Creek	Santa Ana	4	11.0	160	10	25.2	240	31	545.7	9800			
Cajon Creek	Santa Ana	10	55.0	520	20	159.2	960	730	4794.5	13000			
Stone Creek	Santa Margarita	10	138.2	5830	10	52.7	1408	40	1728.4	15530			
Boden Creek	San Dieguito	10	45.3	18600	10	98.3	563	388	1658.5	20140			
Mean		9.40	40.79	2829.66	10.07	52.08	1053.67	273.80	1592.51	10253.13			
StDev		2.04	19.84	2662.11	1.82	25.32	911.35	222.68	622.94	3837.71			

Table A2. Monthly *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	<i>E. coli</i> Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	10.0	37.5	56.1	11.5	26.0	12.5	10.0	10.0	10.0	10.0	10.0	16.5	10.0
Lachusa Canyon	82.8	30.7	20.0	12.5	28.5	10.0	10.0	14.1	10.0	10.0	16.0	10.0	25.1
Cold Creek	14.4	42.1	10.0	27.6	10.0	14.2	10.0	10.0	10.0	10.0	10.0	10.0	20.0
Solstice Canyon	32.2	59.6	11.9	15.2	29.9	10.0	10.0	40.0	12.6	10.0	15.2	10.0	20.0
Chesebro Creek	150.3	276.0	444.2	233.5	1336.8	111.3	27.1	58.7	11.9	25.3	65.8	28.9	10.0
Bell Creek	25.9	125.6	104.0	146.0									
San Juan Creek	36.0	121.6	84.2										
Santiago Creek	10.0	22.8	53.6										
Hurkey Creek	5500.0	18.9	14.1						22.6	10.0	10.0	10.0	
Cucamonga Creek	10.0	10.0	10.0	12.4	10.0	10.0	10.0	20.6	10.0	13.2	10.0	10.0	10.0
Mill Creek	10.0	10.0	5.0	2.6	2.8	1.4	1.0	1.0	1.1	2.0	1.0	1.0	1.0
Day Creek	10.0	20.0	10.0	11.0	10.0	13.2	10.0	10.0	10.0	11.9	10.0	10.0	10.0
Capon Creek	38.3	180.1	146.9	104.1	225.3	96.6	76.1	42.4	35.3	12.6	12.6	10.0	10.0
Stone Creek	65.7	129.2	269.5	134.6		156.1	441.1	57.8	240.1	82.8	20.2	99.4	112.1
Boden Creek	1082.5	26.1									21.5	63.5	30.7

Table A3. Monthly enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Enterococci Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	41.0	63.0	105.7	23.9	54.6	18.1	11.9	10.0	10.0	10.0	10.0	10.0	14.1
Lachusa Canyon	20.2	13.2	15.1	17.4	21.6	20.1	11.5	14.1	14.6	34.0	82.3	24.7	17.6
Cold Creek	12.6	18.8	115.6	16.6	16.5	10.0	10.0	10.0	10.0	10.0	11.5	17.6	10.0
Solstice Canyon	25.1	23.8	39.8	61.0	47.5	16.9	13.2	10.0	12.6	10.0	12.5	10.0	35.1
Chesebro Creek	59.0	200.3	563.1	146.5	252.2	31.1	41.1	24.9	11.9	29.0	51.5	26.8	62.0
Bell Creek	12.6	402.1	467.8	158.0									
San Juan Creek	20.2	47.5	10.0										
Santiago Creek	14.6	59.0	40.8										
Hurkey Creek	380.0	121.6	744.2						18.9	10.0	10.0	19.5	
Cucamonga Creek	33.9	90.2	241.1	85.8	31.2	14.3	10.0	14.1	10.0	11.9	12.6	18.4	10.0
Mill Creek	10.0	10.0	20.2	35.8	16.5	23.2	14.8	4.0	16.1	3.2	22.6	25.8	3.9
Day Creek	21.5	43.3	125.8	92.1	42.4	18.8	24.6	11.9	11.5	21.4	10.0	11.9	14.1
Cajon Creek	87.1	307.1	486.6	367.7	253.0	157.0	217.8	56.6	66.9	100.3	74.1	95.2	200.0
Stone Creek	53.6	163.0	192.3	133.8	79.0	31.8	53.4	12.6	46.1	18.5	11.9	45.9	74.2
Boden Creek	143.4	208.4									69.4	44.9	98.4

Table A4. Monthly total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Total coliforms Geomeans												
	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07
Arroyo Seco	708.0	1854.8	4200.8	1859.1	2506.6	1480.0	1155.9	534.0	134.9	588.4	1547.1	1843.3	2926.5
Lachusa Canyon	1611.2	1825.6	2724.7	3350.6	2074.7	998.6	1139.4	1206.9	725.0	1655.0	807.2	1009.0	2176.8
Cold Creek	997.6	1743.3	3567.4	1312.3	1347.5	488.0	250.7	109.2	70.7	78.3	123.9	218.3	277.4
Solstice Canyon	1064.2	1404.8	2278.4	2998.4	1048.4	499.8	550.8	654.2	761.3	1218.5	529.5	1783.9	2549.3
Chesebro Creek	2546.1	4655.0	9044.6	8141.9	8332.1	4770.4	2142.9	1017.2	789.6	1085.4	1515.9	1722.6	2540.4
Bell Creek	518.6	4780.6	2513.8	1483.0									
San Juan Creek	1748.1	3406.8	4139.9										
Santiago Creek	1189.6	1846.4	2985.1										
Hurkey Creek	6500.0	2102.0	5040.8										
Cucamonga Creek	419.1	688.2	1334.1	650.0	740.5	362.5	364.9	122.4	348.1	224.5	326.7	347.1	
Mill Creek	170.6	224.0	126.4	139.1	35.3	91.9	151.7	27.5	155.4	253.2	318.7	434.9	720.0
Day Creek	311.1	746.5	1146.1	1320.5	668.1	267.3	417.4	374.0	30.8	24.0	48.3	52.0	115.7
Cajon Creek	5915.7	8730.8	7512.4	3300.6	7335.3	9693.4	2667.5	3993.7	2747.3	2242.1	2946.6	5451.8	8200.0
Stone Creek	347.3	3493.6	4887.8	5727.9	7310.9	2482.6	1959.5	321.2	734.3	617.5	673.7	1610.4	1151.6
Boden Creek	7229.3	3207.2									603.5	1295.2	1302.1

Table A5. Dry season *E. coli* geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	<i>E. coli</i> Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	25.0	22.4	13.7	10.0	13.3
Lachusa Canyon	Los Alisos Canyon	45.9	15.9	13.4	12.0	13.7
Cold Creek	Malibu Creek	24.8	16.5	11.2	10.0	11.9
Solstice Canyon	Solstice Canyon	49.7	19.7	12.6	12.6	13.0
Chesebro Creek	Malibu Creek	213.3	531.7	56.0	21.5	32.8
Bell Creek	San Juan	48.3	115.8			
San Juan Creek	San Juan	74.2	75.2			
Santiago Creek	Santa Ana	16.8	31.4			
Hurkey Creek	San Jacinto	119.5	16.9		15.3	10.0
Cucamonga Creek	Cucamonga	10.0	10.8	12.7	11.1	10.0
Mill Creek	Santa Ana	10.0	4.2	1.2	1.3	1.0
Day Creek	Santa Ana	17.4	10.3	10.9	10.6	10.0
Cajon Creek	Santa Ana	84.7	126.2	102.7	20.1	11.2
Stone Creek	Santa Margarita	95.1	181.5	292.4	80.7	82.9
Boden Creek	San Dieguito	148.1	14.1		10.0	43.1

Table A6. Dry season enterococci geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Enterococci Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	54.6	49.4	15.6	10.0	11.0
Lachusa Canyon	Los Alisos Canyon	15.9	17.1	14.2	30.6	35.0
Cold Creek	Malibu Creek	15.2	35.0	10.0	10.0	14.5
Solstice Canyon	Solstice Canyon	22.1	52.9	14.7	12.2	13.7
Chesebro Creek	Malibu Creek	118.8	365.1	33.7	21.1	46.1
Bell Creek	San Juan	60.1	338.0			
San Juan Creek	San Juan	26.8	23.4			
Santiago Creek	Santa Ana	24.2	49.9			
Hurkey Creek	San Jacinto	127.2	386.5		14.2	14.9
Cucamonga Creek	Cucamonga	47.0	138.9	14.1	11.3	16.5
Mill Creek	Santa Ana	10.0	20.6	11.4	12.4	8.0
Day Creek	Santa Ana	28.7	77.4	20.5	13.7	11.9
Cajon Creek	Santa Ana	140.7	383.2	145.4	89.6	96.7
Stone Creek	Santa Margarita	83.1	151.8	40.0	22.0	51.2
Boden Creek	San Dieguito	154.0	305.7		28.6	81.6

Table A7. Dry season total coliforms geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Total coliforms Dry Season Geomeans				
		Spring 06	Summer 06	Fall 06	Winter 06-07	Spring 07
Arroyo Seco	LA River	1066.8	2610.9	1230.6	422.2	2163.9
Lachusa Canyon	Los Alisos Canyon	1663.9	2899.9	1092.8	1034.7	1099.7
Cold Creek	Mailbu Creek	1069.4	2133.9	295.8	97.2	180.3
Solstice Canyon	Solstice Canyon	1278.2	2165.1	543.3	616.5	1900.3
Chesebro Creek	Mailbu Creek	3776.6	8814.0	2535.0	889.0	2281.4
Bell Creek	San Juan	1169.5	2955.9			
San Juan Creek	San Juan	2001.5	4426.6			
Santiago Creek	Santa Ana	1417.1	2465.3			
Hurkey Creek	San Jacinto	2952.6	3345.5		326.8	310.5
Cucamonga Creek	Cucamonga	508.0	958.8	254.1	216.2	500.4
Mill Creek	Santa Ana	185.3	104.5	82.8	31.6	79.5
Day Creek	Santa Ana	425.2	1001.3	374.8	348.5	795.6
Cajon Creek	Santa Ana	6926.3	5634.3	5220.2	2595.1	5267.6
Stone Creek	Santa Margarita	1343.4	5682.0	2193.0	516.7	1361.0
Boden Creek	San Dieguito	5146.1	2216.8		514.8	1163.2

Table A8. Annual dry season fecal indicator bacteria geomeans (MPN/100 ml) in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Watershed	Annual Dry Season Geomeans		
		<i>E. coli</i>	Enterococci	Total Coliforms
Arroyo Seco	LA River	15.2	20.5	1291.9
Lachusa Canyon	Los Alisos Canyon	16.1	20.6	1486.5
Cold Creek	Malibu Creek	13.6	15.3	443.3
Solstice Canyon	Solstice Canyon	17.0	20.6	1109.2
Chesebro Creek	Malibu Creek	90.3	68.2	2940.4
Bell Creek	San Juan	80.5	164.6	2008.7
San Juan Creek	San Juan	74.7	25.2	2848.2
Santiago Creek	Santa Ana	23.0	34.7	1869.1
Hurkey Creek	San Jacinto	18.9	36.9	688.6
Cucamonga Creek	Cucamonga	11.1	26.3	399.6
Mill Creek	Santa Ana	2.1	12.7	75.0
Day Creek	Santa Ana	11.0	25.2	545.7
Cajon Creek	Santa Ana	55.0	159.2	4794.5
Stone Creek	Santa Margarita	138.2	52.7	1728.4
Boden Creek	San Dieguito	45.3	98.3	1658.5

**APPENDIX B - SUMMARY OF PHYSICAL PARAMETERS AT ALL
NATURAL STREAM SITES**

Table B1. Annual dry season averages of measured physical parameters in natural streams during May 2006-May 2007 in southern California, USA.

Sampling site	Physical Parameter Averages					
	Conductivity µs	DO mg/L	Flow Rate m ³ /s	pH	Temperature °C	Turbidity
Arroyo Seco	411.9	na	0.038	na	13.8	na
Lachusa Canyon	1431.1	na	0.006	na	16.2	na
Cold Creek	604.0	na	0.005	na	13.8	na
Solstice Canyon	1051.6	na	0.011	na	15.4	na
Chesebro Creek	3089.0	na	0.005	na	11.9	na
Bell Creek	738.8	8.7	0.018	8.0	18.7	1.1
San Juan Creek	518.8	10.4	0.028	8.2	21.1	0.7
Santiago Creek	636.9	9.6	0.017	8.1	22.2	0.5
Hurkey Creek	129.9	na	0.006	7.8	11.6	na
Cucamonga Creek	9.8	9.8	0.138	8.0	12.3	na
Mill Creek	0.7	9.4	0.080	8.0	10.6	12.3
Day Creek	13.7	9.9	0.317	8.0	12.6	1.8
Cajon Creek	37.7	8.7	0.082	7.9	15.7	8.0
Stone Creek	1171.6	7.2	0.002	7.5	16.4	16.1
Boden Creek	1012.0	7.5	0.005	7.8	15.3	6.1

APPENDIX C - INTERLABORATORY CALIBRATION RESULTS

RESULTS

SCCWRP is currently coordinating an investigation of bacteria levels in reference drainages throughout southern California. This is a cooperative study involving multiple jurisdictions that are each contributing to the project through combinations of in-kind services and direct funding. Because numerous analytical labs will be participating in analysis of fecal indicator bacteria, it was necessary to conduct a laboratory intercalibration study to ensure that comparable results could be achieved from all participating laboratories. This memo summarizes the results of this intercalibration study.

Eight laboratories from five counties participated in the calibration exercise, a performance-based approach used to evaluate analytical accuracy, reproducibility of results and to ensure that data from participating laboratories were comparable (Table C1). The calibration exercise occurred on March 22th, 2006 and consisted of each lab receiving six common samples for analysis (Table C2). Necessary dilutions or aliquot volumes were processed to insure that reportable values could be determined. Bacterial results were reported for total coliform, *Escherichia coli* (*E. coli*), and enterococcus as organism type per 100 ml of sample. Precision was examined by assessing repeatability variance (based on intralaboratory data) and reproducibility variance (based on interlaboratory data). All participating labs were required to fall within a +/- 0.5 median log count comparability goal (Noble *et al.* 2000).

All laboratories had low repeatability variability for all three constituents and all results fell within the median log comparability criteria. Based on all results there were not large variations between the laboratories (i.e. neither of the laboratories were consistently higher or lower for any parameters) in a given sample or for all samples. However, one lab (CSD) reported higher values than the rest, but this can be explained by their inadvertent double diluting of the sample. Also, both Truesdail and Weck laboratories tended to report lower values than the rest. These laboratories should be extra cautious and invest extra efforts in data interpretation in order to not bias the results of bacterial concentrations on the low side.

Figures C1-3 are an example of how the laboratories compared with the different analyses and how well the laboratories were able to reproduce results. These plots are representative of all the data and illustrated good comparability between the analytical labs. As a result of this study we conclude that there should be no bias introduced into the dataset due to sample analysis by different laboratories. All the data and plots are available from SCCWRP upon request.

Table C1. List of participating laboratories and counties involved in the reference bacteria/watershed interlaboratory calibration.

Laboratory Name	County
E. S. Babcock, & Sons, Inc.	Riverside
City of San Diego	San Diego
CRG Marine Laboratories, Inc.	Los Angeles
HCA Public Health Laboratory	Orange
Truesdale Laboratories, Inc.	Orange
Weck Laboratories	Los Angeles
Aquatic Bioassay & Consulting Laboratory (ABC)	Ventura
Weston Solutions, Inc.	San Diego

Table C2. List of the six common samples and their representative sewage dilutions in (ml) which each laboratory received for the interlaboratory calibration.

Media	Dilution (ml)
DI Water	-
Santiago Creek	-
Sewage Dilution 1	3
Sewage Dilution 2	1.5
Sewage Dilution 3	1
Sewage Dilution 4	0.5

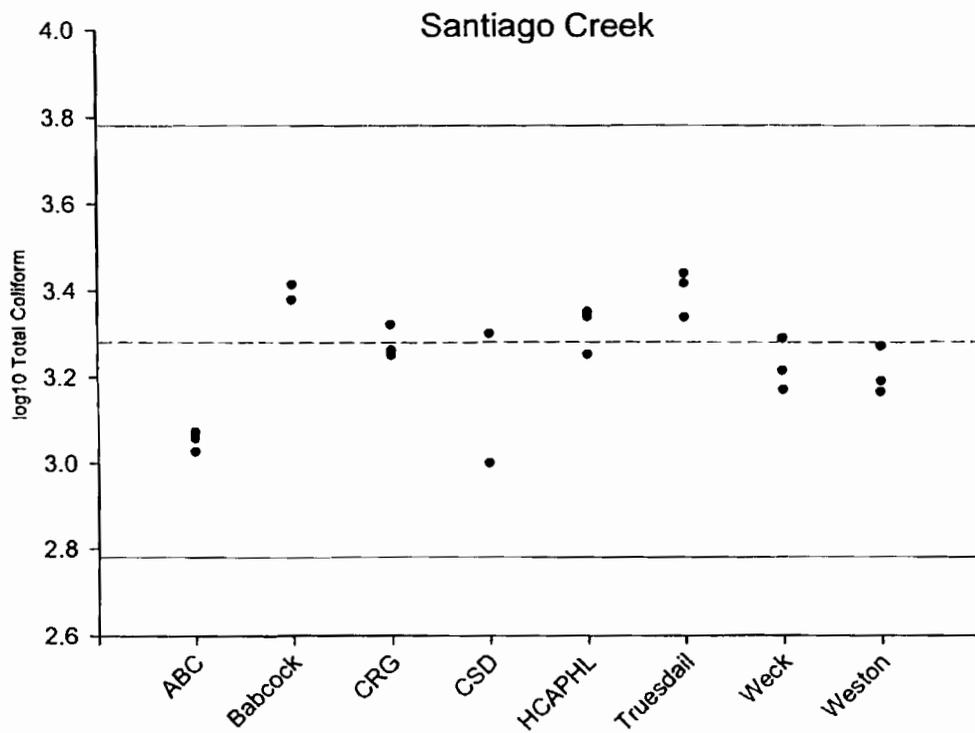


Figure C1. Laboratory comparison results for log transformed total coliform data at Santiago Creek, Orange County. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

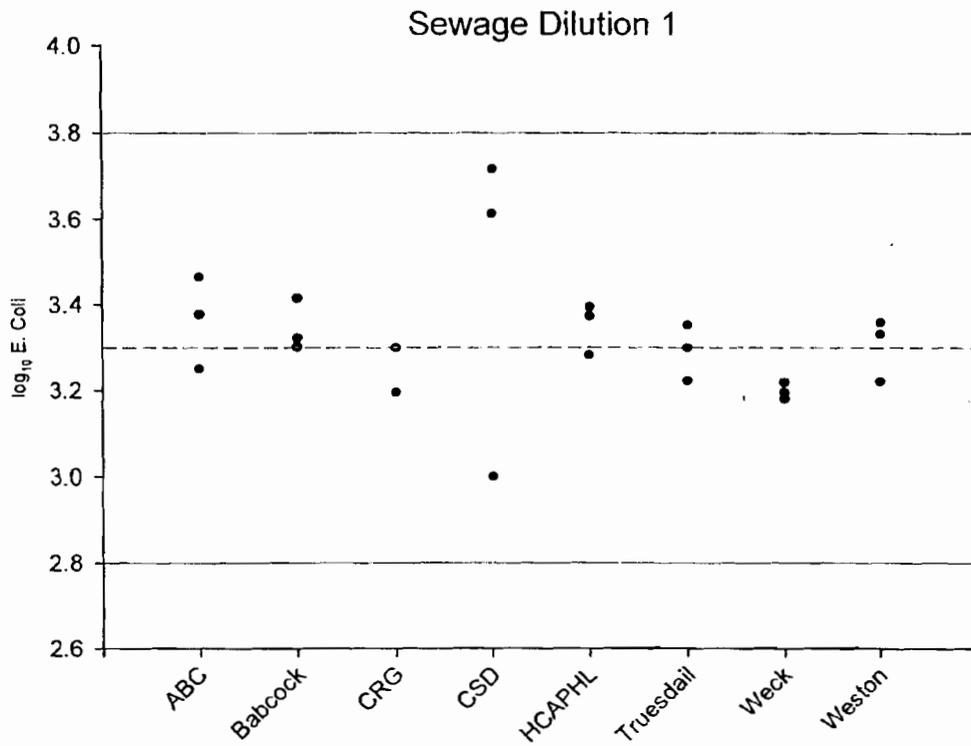


Figure C2. Laboratory comparison results for *E. coli* using a 3 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.

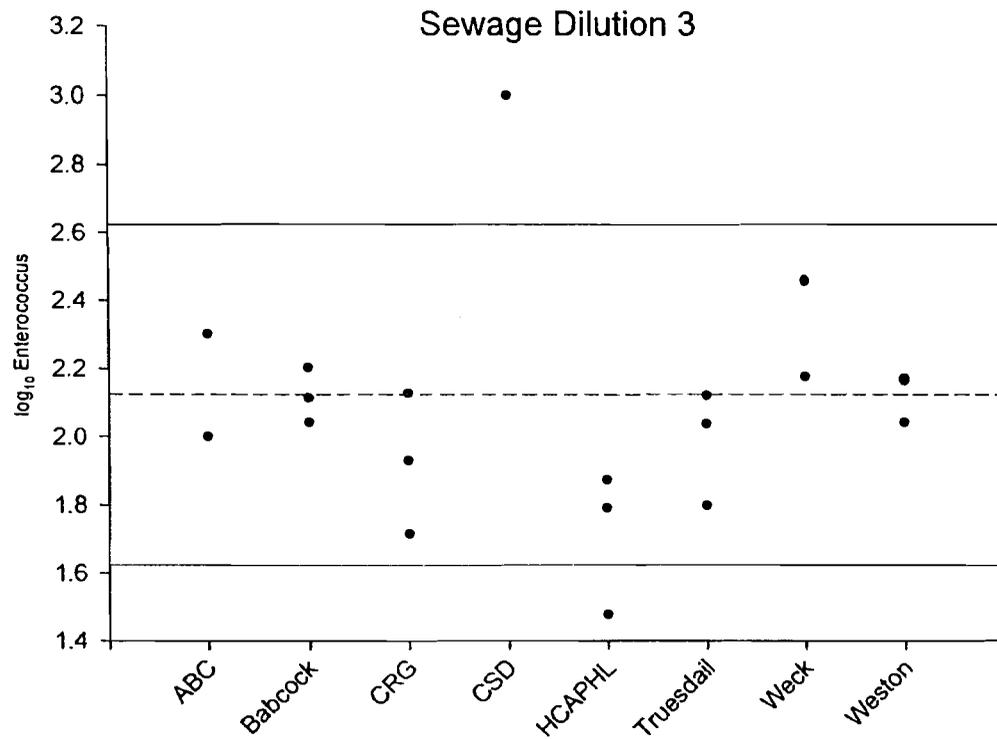


Figure C3. Laboratory comparison results for Enterococcus using a 1 ml sewage dilution. The dotted red line represents the median log criteria, while the solid blue lines are +/- 0.5 median log count.



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Coastal groundwater dynamics off Santa Barbara, California: Combining geochemical tracers, electromagnetic seepmeters, and electrical resistivity

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ABSTRACT

This paper presents repeat field measurements of ^{222}Rn and $^{223,224,226,228}\text{Ra}$, electromagnetic seepage meter-derived advective fluxes, and multi-electrode, stationary and continuous marine resistivity surveys collected between November 2005 and April 2007 to study coastal groundwater dynamics within a marine beach in Santa Barbara, California. The study provides insight into magnitude and dynamics of submarine groundwater discharge (SGD) and associated nutrient loadings into near-shore coastal waters, where the predominant SGD drivers can be both spatially and temporally separated. Rn-222 and $^{223,224,226,228}\text{Ra}$ were utilized to quantify the total and saline contribution, respectively, of SGD. The two short-lived $^{224,223}\text{Ra}$ isotopes provided an estimate of apparent near-shore water mass age, as well as an estimate of the Ra-derived eddy diffusion coefficient, K_h ($^{224}\text{Ra} = 2.86 \pm 0.7 \text{ m}^2 \text{ s}^{-1}$; $^{223}\text{Ra} = 1.32 \pm 0.5 \text{ m}^2 \text{ s}^{-1}$). Because ^{222}Rn ($t_{1/2} = 3.8$ day) and ^{224}Ra ($t_{1/2} = 3.66$ day) have comparable half-lives and production terms, they were used in concert to examine respective water column removal rates. Electromagnetic seepage meters recorded the physical, bi-directional exchange across the sediment/water interface, which ranged from -6.7 to 14.5 cm day^{-1} , depending on the sampling period and position relative to the low tide line. Multi-day time-series ^{222}Rn measurements in the near-shore water column yielded total (saline + fresh) SGD rates that ranged from 3.1 ± 2.6 to $9.2 \pm 0.8 \text{ cm day}^{-1}$, depending on the sampling season. Offshore ^{226}Ra ($t_{1/2} = 1600$ year) and ^{222}Rn gradients were used with the calculated K_h values to determine seabed flux estimates ($\text{dpm m}^{-2} \text{ day}^{-1}$), which were then converted into SGD rates (7.1 and 7.9 cm day^{-1} , respectively). Lastly, SGD rates were used to calculate associated nutrient loads for the near-shore coastal waters off Santa Barbara. Depending on both the season and the SGD method utilized, the following SGD-derived nutrient inputs were computed (mol per day per meter of shoreline): $\text{NH}_4^+ = 0.06\text{--}0.29 \text{ mol day}^{-1} \text{ m}^{-1}$; $\text{SiO}_4 = 0.22\text{--}0.29 \text{ mol day}^{-1} \text{ m}^{-1}$; $\text{PO}_4^{3-} = 0.04\text{--}0.17 \text{ mol day}^{-1} \text{ m}^{-1}$; $[\text{NO}_2 + \text{NO}_3] = 0\text{--}0.52 \text{ mol day}^{-1} \text{ m}^{-1}$; dissolved inorganic nitrogen (DIN) = $0.01\text{--}0.17 \text{ mol day}^{-1} \text{ m}^{-1}$, and dissolved organic nitrogen (DON) = $0.08\text{--}0.09 \text{ mol day}^{-1} \text{ m}^{-1}$. Compared to the ephemeral nature of fluvial and marine inputs into this region, such SGD-derived loadings can provide a sustained source of select nutrients to the coastal waters off Santa Barbara, California that should be accounted for in mass balance estimates.

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1. Introduction

It is now well accepted that submarine groundwater discharge (SGD), an almost ubiquitous coastal process, may substantially impact certain near-shore material budgets (Capone and Bautista, 1985; Moore, 2006; Burnett et al., 2006; Swarzenski, 2007; Charette et al., 2008). While the contribution of SGD-derived nutrients, bacteria, carbon, and select trace elements such as Ba or U (Charette and Sholkovitz, 2006; Swarzenski and Baskaran, 2007) can vary

widely depending on both local hydrogeologic conditions and anthropogenic perturbations, accurate assessments of the spatial and temporal distribution of SGD along a particular coastline remain rare (Burnett et al., 2002; Dulaiova et al., 2006b). This paucity of reliable data stems in large part in that SGD remains the 'hidden' vector in water and material transport from land to the sea, and that the physical drivers of SGD are complex, often inter-related, and still poorly constrained (Taniguchi, 2002; Michael et al., 2005; Robinson et al., 2007a,b). Furthermore, the discharge of submarine groundwater is usually expressed not through well-defined marine springs (Swarzenski et al., 2001), but rather through diffuse discharge that is often ephemeral and patchy in nature (Burnett et al., 2002; Taniguchi et al., 2003).

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Nonetheless, significant advances have recently been made in the application of select U/Th-series radionuclides as quantitative tracers of SGD (Moore, 1996, 2000a,b; Moore and de Oliveira, 2008; Burnett et al., 2001, 2002, 2003; Charette et al., 2001, 2008; Dulaiova and Burnett, 2004, 2006; Dulaiova et al., 2005, 2006a,b). Such tracer techniques can yield unprecedented information on: (i) SGD 'hotspots'; (ii) the source waters for SGD; (iii) the magnitude and dynamics of SGD rates; and (iv) the relative composition of SGD (i.e., fresh versus saline contributions). As proxies for fluid exchange these tracers are limited by how well they move with a mixed-salinity water parcel, and local quantification of fluid exchange is still most directly measured by some seepage meter device (Mulligan and Charette, 2006). **Seepmeters**, outfitted with autonomous salinity, temperature, and pressure sensors, can measure the bi-directional exchange of fluid across the sediment/water interface with high resolution (Taniguchi and Fukuo, 1993; Taniguchi et al., 2003, 2007). Such datasets can provide useful

constraints on the geochemical tracer-derived SGD results (Burnett et al., 2006; Swarzenski et al., 2007a). An additional complementary tool to study the movement of the fresh water/salt water interface and to map the geographic extent of a coastal SGD zone is stationary (land-based) electrical resistivity (Swarzenski et al., 2006a, 2007a,b; Taniguchi et al., 2007).

In environments that are fresh water limited or have low hydraulic gradients, the submarine exchange of groundwater often contains a large component of recycled sea water (Colbert and Hammond, 2007a,b; Colbert et al., 2008a,b; Weinstein et al., 2007). In such systems, even though the net discharge may be small or even negative (i.e., sea water infiltration), this continuous cycle of recharge and discharge, driven by waves and tides, may still significantly impact the flow of nutrients from land to the sea. In this paper, we address the exchange of groundwater with sea water at West Beach in Santa Barbara, California (Fig. 1), using a suite of geochemical tracers and electrical geophysical techniques. From

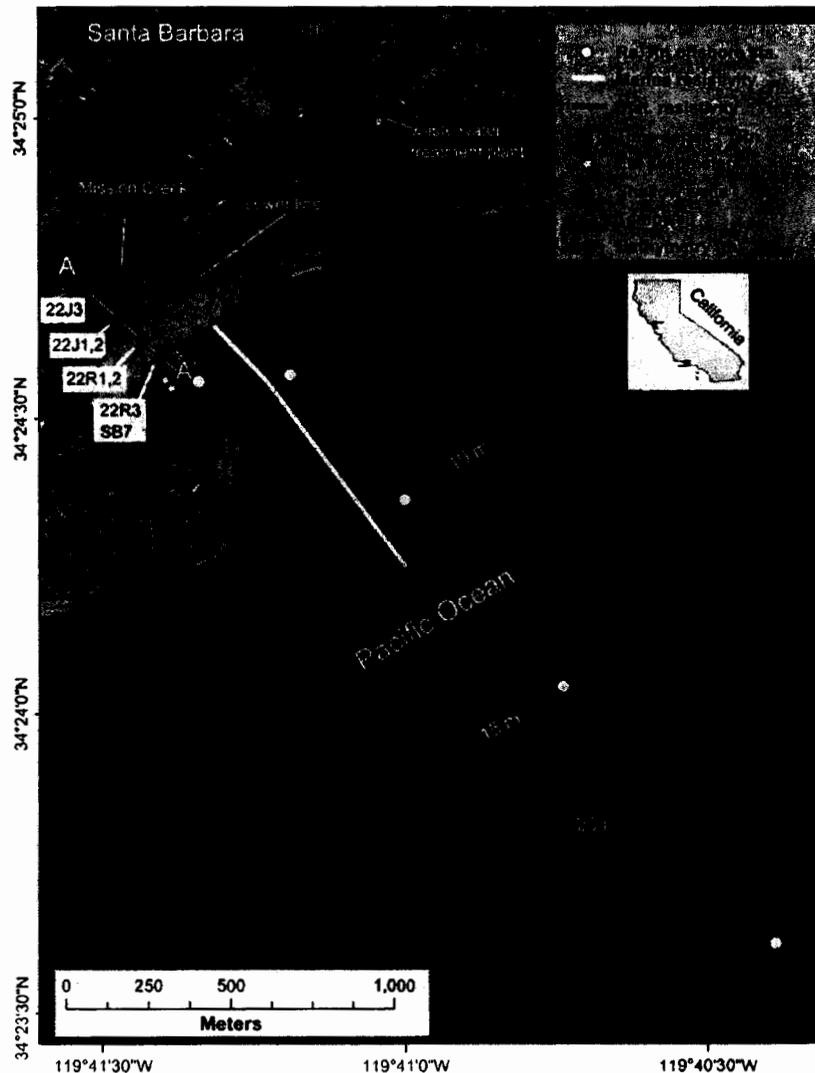


Fig. 1. West Beach, Santa Barbara, California showing the position of the electromagnetic seepmeters, time-series Rn deployments, offshore transect sites (Rn and Ra) and the streamer location for land-based, multi-electrode and the marine continuous resistivity profile. Also shown is the shore-parallel running sewer line (which may be a source of excess nutrients and bacteria to the beach), the nearby waste water treatment plant, and the position of the hydrogeologic transect (A–A') shown in Fig. 3.

calculated fluid exchange rates per season, we are able to derive SGD nutrient loading estimates. On the basis of our results, we have determined that the wave/tide-driven exchange of shallow groundwater with coastal sea water can convey a sustained load of select nutrients and trace elements to the near-shore waters off Santa Barbara, California, even without a net flow of fresh groundwater towards the sea.

2. Study site

Field data were collected at a meso-tidal, sandy beach ('West Beach') adjacent to Santa Barbara, California (Fig. 1), where fecal indicator bacteria (FIB) in the surf zone are occasionally present at high enough concentrations to necessitate beach warnings or closures (Izbicki et al., in review). Three beach and water column sampling campaigns targeted spring tide (November 2005, April

2007) and neap tide (May/June 2006) cycles, as well as seasonal variations (Fig. 2). Mission Creek, which discharges adjacent to West Beach, is an obvious potential source for FIB, as are subaerial and submarine groundwater discharge (Boehm et al., 2004; Paytan et al., 2004; Boehm and Weisberg, 2005; Boehm, 2007; Yamahara et al., 2007) and any possible leakage from the shore-parallel municipal sewer line that lies buried beneath the landward edge of West Beach. While a companion report addresses the various sources of FIB and their hydrologic forcing in coastal Santa Barbara (Izbicki et al., in review), here we examine the marine effects (i.e., tides) on groundwater exchange and associated nutrient loading to the coastal waters of Santa Barbara Harbor. Details on the hydro-geologic setting of this study site can be found in Muir (1968), Martin (1984), and Freckleton et al. (1998).

Santa Barbara, located about 150 km northwest of Los Angeles along the Pacific coast of the United States, has a Mediterranean-

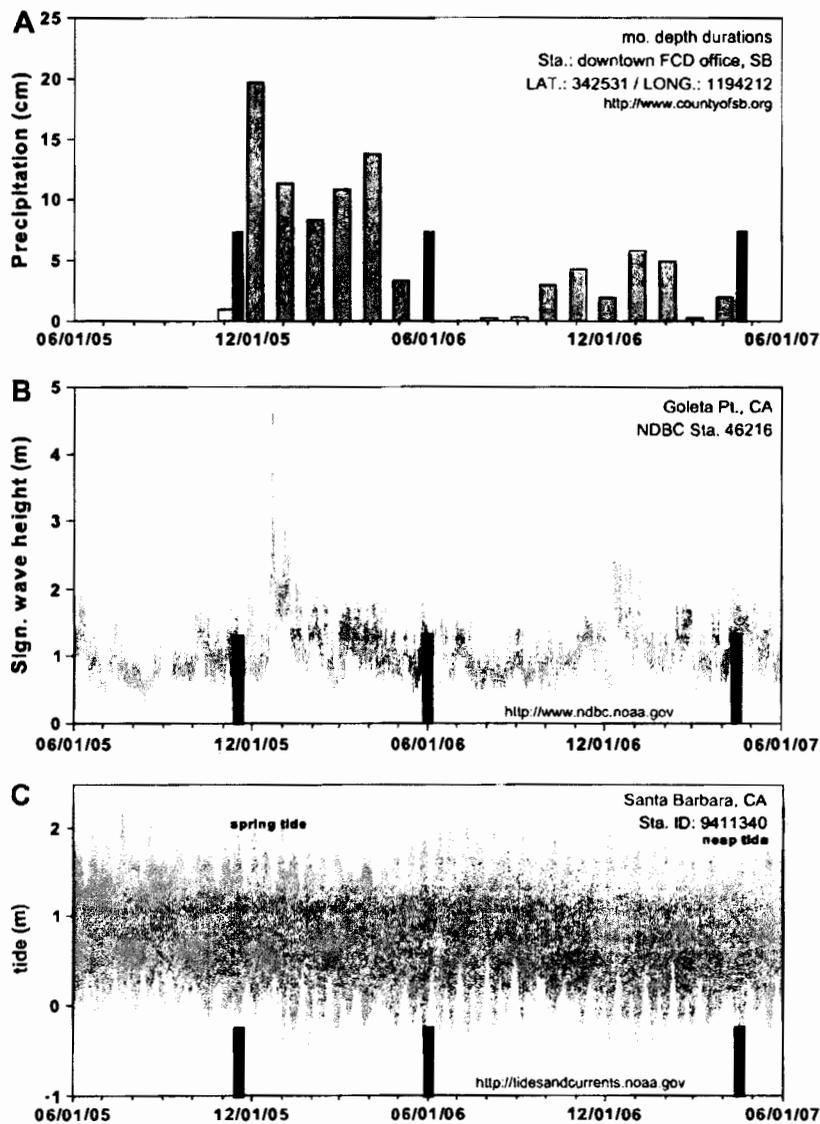


Fig. 2. Black bars denote sampling events as a function of: (A) the monthly precipitation record for down-town Santa Barbara, California; (B) significant wave height (m); and (C) the verified tide record (m) from 06/2005 to 06/2007. Sampling events were coordinated around maximum tides and seasons

type climate characterized by relatively dry and mild summers and winter months that can be periodically cool and wet. Temperatures are moderated by the sea; mean winter temperatures are $\sim 13^\circ\text{C}$, while summer temperatures average 18°C . The population of Santa Barbara exceeded 90,000 in 2004 (<http://www.santabarbaraca.gov>) and is confined along a narrow (~ 5 km wide) but highly developed coastal strip that is bounded to the north by the Santa Ynez coastal mountains. Average annual rainfall in Santa Barbara is ~ 45 cm and about 95% falls between November and March (Fig. 2). The Santa Barbara watershed is drained by several streams that are mostly intermittent along their lower reaches, including Mission Creek, which flows through the town's center and discharges into the Pacific Ocean within the study site (MacFadden et al., 1991).

Nearly all groundwater recharge and surface water flow is derived from precipitation within the region (Martin, 1984). Principal groundwater-bearing deposits of the regional aquifer system include the alluvium (terrace deposits, poorly-sorted sands, gravel, silt and clay) and the Santa Barbara Formation (marine origin, fine to coarse sands, gravel, silt and clays) (Freckleton et al., 1998). Historically, some groundwater has been locally artesian. Under sustained and heavy groundwater pumping along the coast, salt water intrusion is likely to occur wherever the water table of a coastal aquifer approaches sea level. In Santa Barbara, since the early 1960s, the groundwater levels at the coast have been below sea level, and salt water has locally intruded the shallow deposits as water levels have declined. In the late 1970's, groundwater levels declined by more than 30 m in response to increased municipal pumping, and salt water subsequently intruded deeper water-bearing deposits close to the coast. Presently, salt water intrusion along coastal Santa Barbara is carefully monitored (Martin, 1984).

Winter precipitation events can deliver substantial amounts of dissolved and particulate nitrogen, phosphate, and carbon to the coastal ocean, particularly from watersheds heavily influenced by agriculture and urban development (Beighley et al., 2008). During winter months, marine nitrogen inputs tend to be low, which contributes to a strong seasonality in both physical and geochemical signals in the coastal waters off Santa Barbara (Warrick et al., 2005; McFee-Shaw et al., 2007).

3. Field and analytical methods

3.1. Groundwater

A set of shallow monitoring wells, located either along a shore-perpendicular beach transect (Fig. 3), or within close proximity of the beach, were sampled in mid-November 2005, late May/early June 2006, and in mid-April 2007 for groundwater ^{222}Rn , $^{223,224,226,228}\text{Ra}$, nutrients (NH_4^+ , SiO_4 , PO_4^{3-} , $[\text{NO}_2^- + \text{NO}_3^-]$, DIN, TDN, and DON). For the duration of the study, groundwater levels in these monitoring wells were continuously recorded with pressure transducers and manually confirmed using a hydro-tape (Izbicki et al., in review). During each of the three sampling efforts, the monitoring wells were sampled following standard USGS protocols that included purging at least three well volumes before sample collection. In April 2007, a temporary well ('tempwell') was installed just landward of the high tide line by excavating sand to a depth of 1.5 m and installing a 10 cm diameter slotted irrigation pipe that was also screened to exclude larger-sized particulates. This tempwell was instrumented with a Solinst CTD DIVER to monitor salinity, temperature, and water levels (pressure), and also continuously (30 min updates) sampled for ^{222}Rn using one RAD7 ^{222}Rn monitor. The tempwell was sampled for a suite of nutrients and trace metals, before, during, and after a low tide event. The tempwell groundwater time-series was also complemented with simultaneous water column grab samples collected in the adjacent swash zone ('surface water'), as well as with a suite of time-series samples collected in the adjacent shallow groundwater well SB7 (see Fig. 3 for locations of SB7, tempwell, and surface water sites). Nutrients were immediately preserved in the field and analyzed at the Woods Hole Oceanographic Institution (WHOI) nutrient facility as per methods described in Charette and Buesseler (2004).

3.2. Surface water column

The near-shore coastal waters adjacent to Santa Barbara harbor and beach were sampled to achieve the following three objectives: (1) continuous ^{222}Rn surveys were first used to identify potential SGD 'hotspots' where elevated ^{222}Rn might reveal enhanced

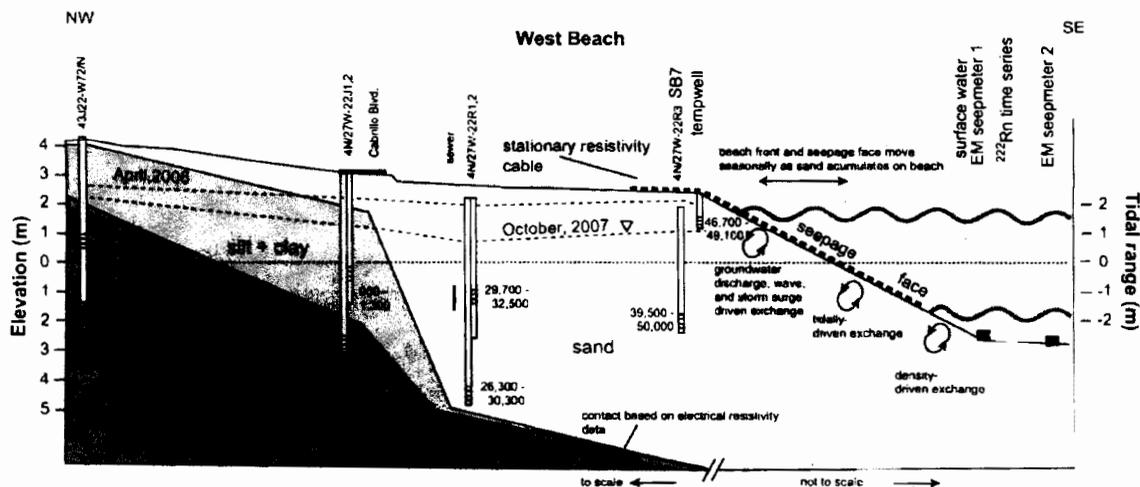


Fig. 3. Idealized shore-perpendicular cross-section, showing approximate configuration of clay, silt + clay, and sand layers relative to the land/sea interface. The position of the stationary, shore-perpendicular resistivity line, shown in Fig. 5, is illustrated, as are the relative positions of the two EM seepmeters (Fig. 6) and the 2007 time-series sampling sites for the tempwell, surface water sampling sites, and SB07 (Figs. 7 and 10). Observed groundwater/surface water exchange within this beach front can be expressed as density-, tidal-, or groundwater/wave-driven exchange, or a combination thereof. Ranges in specific conductivity per well reported in mS cm^{-1} from 2006 and 2007 sampling data.

advective exchange across the sediment/water interface; (2) ^{222}Rn time-series measurements just seaward of the low tide line off the beach were used to derive total (fresh + saline) SGD rates from a mass balance model; and (3) ~3 km long offshore transects of ^{222}Rn and $^{223,224,226,228}\text{Ra}$ were used to examine offshore constituent gradients, water mass mixing coefficients (K_h), apparent Ra ages, and ultimately, a more regional assessment of SGD.

The surface water column was sampled during November 2005, May/June 2006, and April 2007 using a submersible pump that was either attached to a weight placed on the seafloor (near-shore ^{222}Rn time-series) or suspended 0.5 m from the water surface off a small boat's gunwale (^{222}Rn surveys and offshore transects). During the first field campaign (November 2005), multi-day radon surveys were conducted prior to the installation of time-series stations and were used as a guide in their placement relative to shore. For the ^{222}Rn time-series measurements, a dedicated tender anchored just seaward of the low tide line housed two RAD7 ^{222}Rn detectors plumbed in parallel into a single air-water exchanger (Burnett et al., 2001, 2003, 2006; Dulaiova et al., 2005) that was fed by an instrumented (YSI-multi-probe) bottom water stream via a submersible bilge pump. Air from the exchanger was routed to one or more RAD7 detectors that quantified ^{222}Rn from the decay of its two alpha-emitting daughter isotopes, ^{214}Po ($t_{1/2} = 3.03$ min) and ^{218}Po ($t_{1/2} = 1.6 \times 10^{-4}$ s). During each sampling event, ^{222}Rn was also measured in ambient air using one dedicated RAD7 detector. For Ra isotope analyses, large volumes (20–100 L) of water were passed through individual MnO_2 impregnated acrylic fiber cartridges (Moore, 2000a; Moore and de Oliveira, 2008). Ra-223,224 activities were quantified from partially dried fibers using delayed coincidence counters (Moore and Arnold, 1996; Moore, 2000b). An ultra-low background HPGc well gamma counter was subsequently used to quantify $^{226,228}\text{Ra}$ (351.42, 609.31, 338.42, and 911.16 keV, respectively) from the same fiber sample after a chemical elution and BaSO_4 co-precipitation (Swarzenski et al., 2007c). Reported errors for Ra and Rn are typically <10%. Water column nutrient and trace element samples were collected following clean sampling procedures, including in-line filtration (individual 0.4 μm cartridge filters) and cold storage.

3.3. Electrical resistivity

The use of electrical resistivity to examine the fresh water/salt water interface in coastal groundwater is well established (cf. Mannheim et al., 2004) and has been enhanced by recent improvements in streamer configuration, as well as data acquisition and processing firmware and software (Swarzenski et al., 2006a,b, 2007a,b). In Santa Barbara, both land-based, stationary resistivity (56 electrodes, spaced 2 m apart) and marine continuous resistivity profiling (two current, nine potential electrodes, spaced 10 m apart) surveys were conducted during each field

campaign. To help define the shallow hydrogeology at the site, only one land-based time-series survey (high and low tide comparison) and one adjacent continuous resistivity profile are presented here.

In stationary mode, an Advanced Geosciences Inc. (AGI) external switching box connected to an R8 SuperSting multi-channel receiver controlled the current flow along the 56-electrode cable (Swarzenski et al., 2006a, 2007b). The 112 m stationary cable was oriented either shore-parallel or shore-perpendicular, and each electrode was pinned to the underlying sediment by a stainless steel 35 cm spike. The relative elevation of each electrode was measured using a laser level and the topography/bathymetry data were then incorporated into inverse modeling routines (AGI EarthImager). In continuous marine mode, the 120 m cable was pulled at a speed of ~3–4 kts on the surface of the water column (Swarzenski et al., 2006b). Real-time GPS data was simultaneously logged into the SuperSting receiver. Polyethylene floats attached to the streamer cable between each electrode were used to keep the cable buoyant on the water surface. Real-time continuous water column salinity/temperature measurements were recorded on an YSI multi-meter, while water depth and the ship's position were recorded on a separate GPS-enabled fathometer system.

3.4. Electromagnetic seepage meters

Electromagnetic (EM) seepage meters (Rosenberry and Morin, 2004; Swarzenski et al., 2004) were also deployed continuously for 4–5 days during each field season in the shallow coastal waters off West Beach to physically measure water exchange rates across the sediment/water interface. These meters were pushed into the sandy sediments >15 cm to assure a complete seal around the base of the meter and were generally positioned in a shore-perpendicular configuration such that 1 m (EMS1) was just seaward of the low tide line, and the other meter (EMS2) was positioned slightly further offshore (Fig. 3) in deeper water. The meter display/control panels and power supplies (12 VDC or 120 VAC) were placed on the offshore ^{222}Rn time-series tender. The EM seepage meters were outfitted with internal- and external-mounted Solinst CTD DIVERS to continuously monitor the salinity, pressure, and temperature both inside and outside the seepage meter domes. Each meter recorded the bi-directional (+ or –) flow rate once every minute.

4. Results and discussion

A compilation of the range in specific conductivity, and $^{223,224,226,228}\text{Ra}$ and ^{222}Rn activities, from a suite of monitoring well samples (November 2005, May/June 2006, April 2007), the 2007 tempwell samples, and from the adjacent sea water column are listed in Table 1. Groundwater collected from the wells exhibited a broad range (0.8–47.9 mS cm^{-1}) in specific conductivities

Table 1
Compilation of specific conductivity (mS cm^{-1}) and ^{222}Rn and $^{223,224,226,228}\text{Ra}$ activities (dpm m^{-3}) from select monitoring wells, a temporary well (tempwell), and near-shore surface sea water. The reported error for Ra and Rn is <10%. (x) denotes number of samples reported.

	Spec. Cond. (mS cm^{-1})	^{222}Rn (dpm m^{-3})	^{228}Ra	^{226}Ra	^{223}Ra	^{224}Ra
Groundwater						
Wells*	0.8–47.9	15,000–134,000 (30)	190–650 (9)	400–3640 (9)	170–430 (9)	360–14100 (9)
April 2007 tempwell	46.7–49.1	257,000–570,500 (90)	260–360 (3)	900–1350 (3)	200–310 (3)	420–8200 (3)
Sea water						
November 2005	40.5–43.6	700–8150 (135)	–	–	–	–
May/June 2006	50.0–54.5	580–11,300 (587)	55–120 (8)	23–150 (8)	3–37 (8)	30–530 (8)
April 2007	39.1–51.4	1190–7380 (414)	81–130 (8)	11–210 (8)	17–120 (6)	12–140 (6)

* All samples (November 2005, May/June 2006, and April 2007).

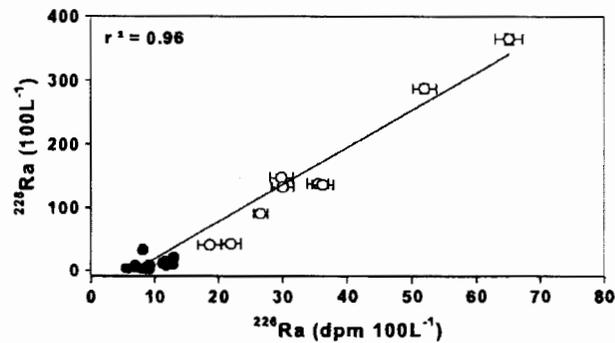


Fig. 4. Plot of combined (November 2005, May/June 2006, and April 2007) ^{226}Ra versus ^{228}Ra activities ($\text{dpm } 100\text{L}^{-1}$) in coastal surface (\bullet) and ground water (\circ) samples at Santa Barbara, California.

depending on distance from the shoreline and screen depths, and approached the upper limit of observed sea water values ($39.1\text{--}54.4\text{ mS cm}^{-1}$). Specific conductivities and water level data from wells along the shore-perpendicular transect (Fig. 3) revealed no significant net discharge of shallow, fresh groundwater to the coastal ocean during the dry season. During winter months, enhanced runoff and precipitation can collect along the upper beach in surface pools that eventually discharge along the beach seepage face as brackish fluxes (Izbicki et al., in review).

Fig. 4 shows a plot of all near-shore surface water and groundwater $^{228,226}\text{Ra}$ activities. Surface waters were typically elevated in the two Th-series Ra isotopes ($^{228,224}\text{Ra}$) relative to the two U-series Ra isotopes ($^{226,223}\text{Ra}$). There is a strong linear trend in both sets of $^{226,228}\text{Ra}$ samples, suggesting that Ra is diluted in surface waters from a common groundwater source. The four Ra isotope activities ($^{223,224,226,228}\text{Ra}$) were generally much higher in the surrounding monitoring well samples than in the shallow tempwell samples. This attribute illustrates the hydrogeologic controls on groundwater radionuclide activity, including changes in the subsurface salinity and residence (flushing) times. In contrast, ^{222}Rn activities were highest in the tempwell (mean = $463 \pm 61\text{ dpm L}^{-1}$) and attained values up to two orders of magnitude greater than near-shore surface waters (mean = 4 dpm L^{-1}). Nonetheless, these near-shore surface waters adjacent to West Beach were enriched in the two short-lived $^{223,224}\text{Ra}$ and ^{222}Rn relative to more distal sea water.

Groundwater and surface water nutrient (NH_4^+ , SiO_4 , PO_4^{3-} , $[\text{NO}_2^- + \text{NO}_3^-]$, dissolved inorganic nitrogen ($\text{DIN} = [\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-]$), dissolved organic nitrogen (DON), and total dissolved nitrogen (TDN)) concentrations per sampling campaign are summarized in Table 2. In general, there is little inter-

Table 3

Summary of mean submarine groundwater discharge rates (in cm day^{-1}), as calculated from stationary ^{222}Rn time-series (ts) deployments in the near-shore surface water column, electromagnetic seepmeters (EMS1 was always positioned closer to the low tide line than EMS2), and from excess ^{226}Ra and ^{222}Rn transect (trans.) measurements (see Section 4.3 for discussion).

	^{222}Rn (ts)	EMS1	EMS2	^{226}Ra (trans.)	^{222}Rn (trans.)
	cm day^{-1}				
November 2005	3.1 ± 2.8	-0.3 ± 1.0	6.0 ± 4.2	-	-
May/June 2006	0.2 ± 0.8	-6.7 ± 3.1	10.0 ± 5.2	-	-
April 2007	8.5 ± 9.8	-	14.5 ± 15.4	9.6	7.8

annual variation in groundwater nutrient concentrations, although in April 2007 the shallow beach well (SB07) and the tempwell exhibited distinctly different nutrient concentrations from those found in the monitoring wells. As expected, near-shore surface waters contained much lower nutrient concentrations than the adjacent groundwater, although this trend did not hold for DON, which was slightly greater in the surface water.

Electromagnetic seepmeter results yielded rates that were both positive (net upward flow = submarine groundwater discharge) and negative (net downward flow = sea water infiltration), depending on the meter's location relative to shore, the tidal stage, time of year (frequency and intensity of precipitation), and occurrence of antecedent storms. The observed range (-6.7 to 14.5 cm day^{-1}) in advective rates, while low in comparison to other coastal sites (Swarzenski et al., 2007a), did reveal inter-annual variations (Table 3).

4.1. Electrical resistivity

Only two examples of electrical resistivity surveys are presented here; one (Fig. 5A,B) consists of a land-based time-series (low tide/high tide) comparison across the beach face (see Figs. 1 and 3 for approximate orientation of the land-based streamer), while the other (Fig. 5C) is an adjacent marine continuous resistivity profile (CRP). The CRP data was collected by placing the streamer end at the shoreline and then running a shore-perpendicular 853 m transect offshore (see Fig. 1 for CRP transect location). Resistivity values are reported in Ohm-m.

Multi-electrode resistivity provides a useful means of examining the subsurface salinity structure to depths $>20\text{ m}$. Because the position of the land-based streamer is fixed during the high tide/low tide time-series and the data collection/inversion parameters remain constant, the observed change in resistivity must be due only to tidally modulated pore-fluid exchange. From Fig. 5A, the highest resistivity values (i.e., freshest groundwater) appear focused under the high tide water line, at a depth of about

Table 2

Mean groundwater and near-shore surface water nutrient concentrations (μM). Location of groundwater well sites described in Izbicki et al. (in review). Relative position of the surface water, tempwell, and SB07 time-series* samples relative to the beach face provided in Fig. 3.

	NH_4^+ (μM)	SiO_4	PO_4^{3-}	$[\text{NO}_2^- + \text{NO}_3^-]$	DIN	DON	TDN	n
Groundwater								
November 2005	64.7 ± 8.4	145.6 ± 15.6	36 ± 2.4	0.1 ± 0.2	64.8 ± 8.5	-	-	9
May/June 2006	62.1 ± 2.8	200.5 ± 5.1	37.5 ± 3.6	0.1 ± 0	62.1 ± 2.8	20.5 ± 2.1	82.6 ± 4.1	11
April 2007 tempwell*	17.4 ± 2.4	122.7 ± 35.5	11.6 ± 3.3	158.6 ± 42.9	176 ± 41.3	24.2 ± 24.9	280.2 ± 61.4	14
April 2007 SB07*	39.5 ± 2.4	272.2 ± 31.8	54.9 ± 6.8	0.1 ± 0	39.5 ± 2.5	28.2 ± 4.1	67.7 ± 3.6	14
Mean	45.9 ± 4	185.2 ± 22	35 ± 4	39.7 ± 10.8	85.6 ± 13.8	24.3 ± 10.3	116.8 ± 23	
Sea water								
November 2005	8.7 ± 7.7	15 ± 4.1	2.2 ± 1.5	0.3 ± 0.2	9 ± 7.7	-	-	9
April 2007*	13 ± 13.7	30.3 ± 9.2	3.2 ± 2.8	17.4 ± 7.8	30.4 ± 14.1	33.3 ± 60.5	63.7 ± 69.1	14
Mean	10.9 ± 10.7	22.6 ± 6.6	2.7 ± 2.2	8.9 ± 4	19.7 ± 10.9	-	-	

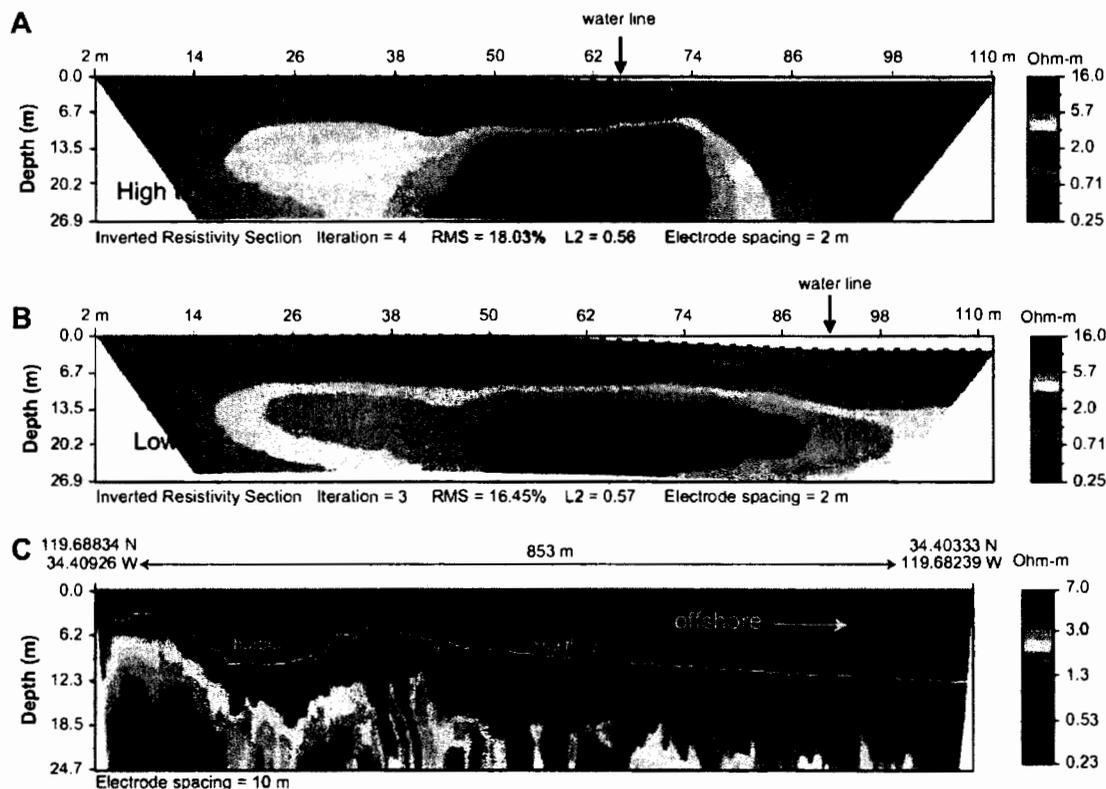


Fig. 5. Inverted resistivity image of the beach face during high (A); and low (B) tide. The tidal range was ~2 m. Approximate position of the shore-perpendicular oriented land-based streamer cable relative to the beach face is shown in Fig. 3. (C) Shows a marine continuous resistivity profile (CRP) survey of an 853 m line adjacent to the A-A' primary transect. Location of this transect line is illustrated in Fig. 1.

15 m. In contrast, at low tide (Fig. 5B), the zone of higher resistivity is stretched much further offshore, past the low tide water line (~50 m). The resistivity images show a shallow saline water wedge positioned on top of the water table and provide further evidence that there is likely no significant discharge of freshened groundwater along the beach face within the depths studied. Instead, it appears that the saline pore fluids respond more to tidal forcing; during high tide, incoming sea water pushes against the groundwater lens which consequently gets backed up close to the high tide line. At low tide, this pressure is released by a falling water table and the groundwater lens can migrate further seaward, inducing mixing within a zone of enhanced SGD that extends 50 m or so from shore. This tidally driven exchange likely discharges some SGD into the water column during every low tide event that is captured by the ^{222}Rn time-series and the EM seepmeters.

Marine continuous resistivity profiling (CRP) surveys were conducted in November 2005 and May/June 2006 in concert with the Rn surveys, and allow large datasets of subsurface resistivity to be collected (boat speed ~3–4 kts). However, without additional groundtruthing measures (i.e., core logs, pore water salinities, sediment resistivities), it is often difficult to extract more than qualitative information from such data. The modeled resistivity image shown in Fig. 9C shows an expected 'freshening' closest to shore at a depth >15 m. The CRP resistivity range is comparable to values derived from the land-based surveys (Fig. 5A,B), and confirms that no obvious discharge of reduced-salinity groundwater occurs beyond ~50 m from shore.

4.2. Time-series

To develop a mass balance model for submarine groundwater discharge, a time-series of excess ^{222}Rn in near-shore surface water was utilized (Burnett and Dulaiova, 2003; Burnett et al., 2003, 2006, 2007, 2008; Dulaiova et al., 2005, 2006a,b; Swarzenski et al., 2006b, 2007a; Weinstein et al., 2007). The premise of this technique relies on converting near-continuous excess ^{222}Rn (accounting for a mean parent ^{226}Ra water column activity) measurements (dpm m^{-3}) into inventories (dpm m^{-2}) using real-time water-level data. Rn-222 inventories are subsequently converted into hourly fluxes ($\text{dpm m}^{-2} \text{h}^{-1}$) and then corrected for both tidal radon exchanges (positive as offshore ^{222}Rn brought in by an inflowing tide and negative as ^{222}Rn lost during an outflowing tide) and atmospheric ^{222}Rn losses.

Fig. 6D shows a representative example of a multi-day ^{222}Rn time-series in November 2005, as a function of (Fig. 6A) tidal water level variations and specific conductivity. During this time-series, each low tide event was marked with a pulse of heightened SGD, as recorded also by the EM seepage meters (Fig. 6B,C) as well as the specific conductivity record that revealed that the salinity of the shallow groundwater was actually higher than the near-shore waters. Interestingly, EM seepage meter 1 showed distinct low tide-coincident pulses in spite of mostly net negative SGD rates and that slightly more saline water escaped the sediment/water interface at low tide relative to the surface water salinities. While the overall pattern of SGD, as quantified by the two EM seepage meters, shows some similar features, the mean rates (EM seepage meter 1 = $-0.3 \pm 1.0 \text{ cm day}^{-1}$; EM seepage meter 2 = $6.0 \pm 4.2 \text{ cm day}^{-1}$) reflect the different placement of the two seepmeters

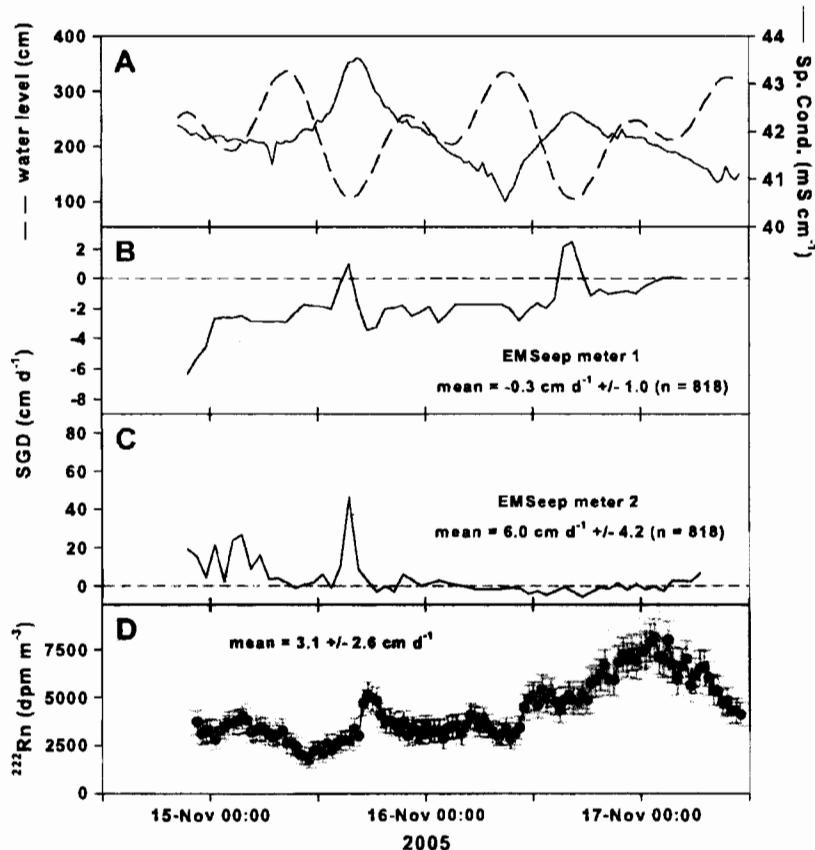


Fig. 6. Water column time-series (November, 2005), showing: (A) water level (tide), specific conductivity (mS cm^{-1}); (B, C) advective SGD rates derived from electromagnetic (EM) seepmeters (cm day^{-1}); and (D) ^{222}Rn activities (dpm m^{-3}). Note low tide coincident pulses in specific conductivities, EM seepmeter derived advective rates, and ^{222}Rn .

relative to the shoreline, and thus water depth. EMS1 was positioned further inshore, just seaward of the low tide line, than EMS2. From both EM seepage meter records, one can infer subtle nuances in SGD. For example, it is likely that EMS1 responded more to tidally-modulated shallow exchange processes that occurred within the beach face as shallow saline circulation cells (Robinson et al., 2007b) (see Fig. 3). Such discharge could have both physical and hydrologic controls. The passage of a high-energy storm just prior to sampling could push sea water much further up the beach face where recharge, subsurface flow, and eventual offshore discharge would influence the salinity and flow rates of SGD.

To be able to convert near-shore total ^{222}Rn fluxes ($\text{dpm m}^{-2} \text{h}^{-1}$) into advective exchange rates ($\text{cm}^3 \text{cm}^{-2} \text{day}^{-1}$), a representative groundwater endmember activity must be quantified. A groundwater ^{222}Rn time-series was collected within the high tide line tempwell, which was also monitored for continuous water levels and specific conductivities (Fig. 7). Fig. 7A shows tidally-driven, water level variations of near-shore surface waters, while a record of tempwell water levels and specific conductivities is shown in Fig. 7B. A slight increase in the salinity in the tempwell appears coincident with the first low tide event, but sustained pumping ran the tempwell temporarily dry, and the interruption in the water flow was recorded as a break in ^{222}Rn and specific conductivity values. Accounting for this interruption, the groundwater ^{222}Rn within the tempwell ranged (Fig. 7C) from ~ 360 to 570 dpm L^{-1} , with a mean of $463 \pm 61 \text{ dpm L}^{-1}$ ($n = 90$). This value is considerably higher than the range in ^{222}Rn observed (15 –

134 dpm L^{-1}) within the suite of groundwater monitoring wells adjacent to West Beach and does reflect more closely the water that is actually involved in the near-shore exchange. Dividing the total ^{222}Rn fluxes by this groundwater ^{222}Rn value results in a mean SGD rate of $3.1 \pm 2.6 \text{ cm day}^{-1}$. Such SGD rates were also calculated for the 2006 and 2007 ^{222}Rn time-series deployments and are reported in Table 3. If we estimate from land-based resistivity data a narrow seepage face of $\sim 50 \text{ m}$, then these advective rates would correspond to 1.5 – 4.6 m^3 of groundwater exchanged per day, per meter of shoreline. These results compare favorably to similar values obtained from the EM seepmeters, which yield a flux of 0.5 – $7.3 \text{ m}^3 \text{ m}^{-1} \text{ day}^{-1}$.

4.3. Offshore Ra and Rn transects

In coastal waters where water mass mixing is more a function of eddy diffusion than advection processes, it has been shown (Moore, 2000a) that the two short-lived Ra isotopes ($^{223}\text{Ra} = 11.3 \text{ day}$; $^{224}\text{Ra} = 3.66 \text{ day}$) can be used effectively to obtain Ra-derived apparent water mass ages (Fig. 8A) and eddy diffusion coefficients, K_h ($\text{m}^2 \text{ s}^{-1}$). The premise of this method is that all Ra inputs occur only at the shoreline (i.e., surface water column is stratified) and Ra losses occur only as a function of radioactive decay. Water mass mixing rates can then be derived from the slope of a natural logarithm of either Ra isotope as a function of distance from shore. During the April 2007 sampling, the near-shore surface water column was stratified due to thermal heating (<http://www.lternet>.

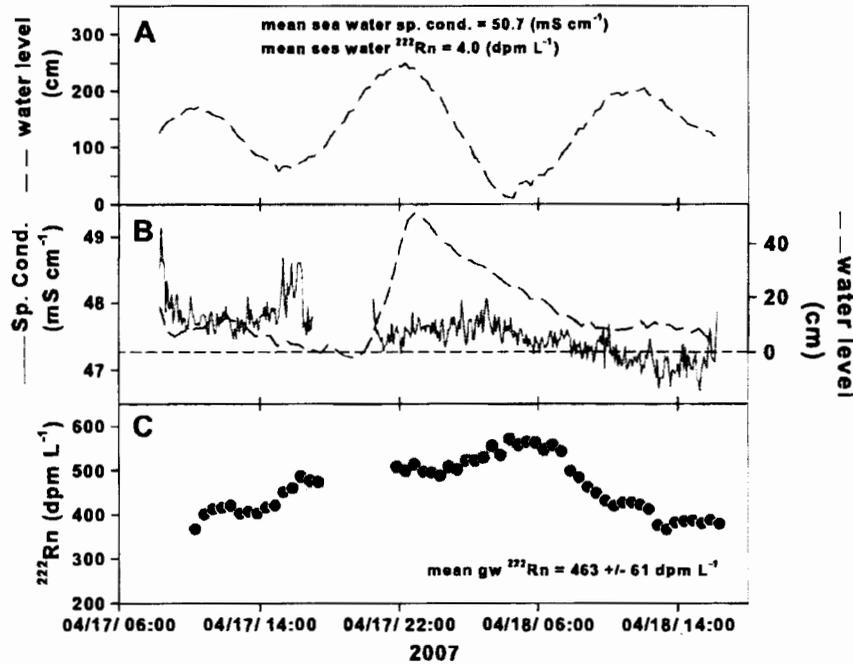


Fig. 7. Groundwater ²²²Rn time-series (C) from a temporary 1.5 m deep well ('tempwell'), positioned just landward of the high tide line (see Fig. 3 for relative location). The tempwell water level decline (B) is reflected in both Rn and specific conductivity (mS cm⁻¹). A mean groundwater ²²²Rn activity (●) of 463 ± 61 dpm L⁻¹ is used for water column time-series and offshore transect ²²²Rn-derived SGD derivations. Note mean near-shore surface water ²²²Rn activity of 4.0 dpm L⁻¹ and near-shore tidal fluctuation (A).

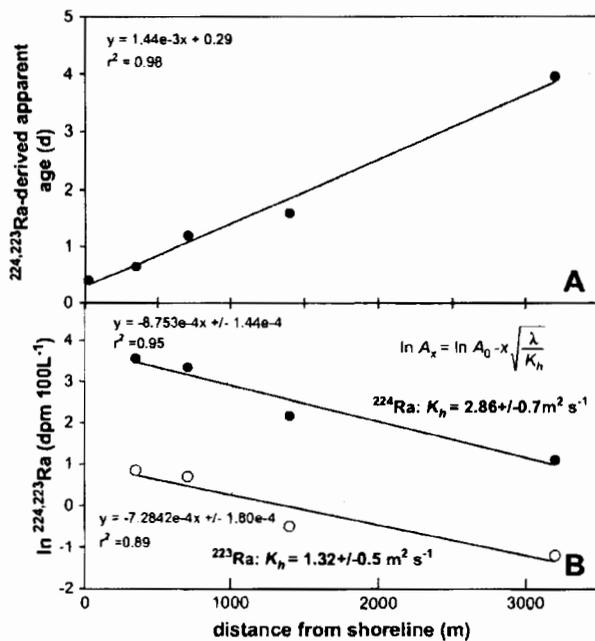


Fig. 8. (A) Plot of ^{223,224}Ra-derived apparent water mass ages ('Ra-ages') as a function of distance offshore (m); and (B) ln ^{223,224}Ra activity as a function of distance from shore used to derive eddy diffusion coefficients, K_h . The offshore transect was conducted in June 2006. At 3200 m (the distal station), the water depth was 34.2 m and the surface water column was stratified due to thermal heating and wind mixing (<http://sbc.lternet.edu/data>).

edu/sites/sbc/). Fig. 8B shows a plot of ln ^{223,224}Ra versus distance from the West Beach shoreline collected during the April 2007 field season. The ln ²²³Ra regression had a slope of $-7.28e-1 \pm 1.80e-1 \text{ km}^{-1}$ from which a mixing coefficient K_h of $1.32 \pm 0.5 \text{ m}^2 \text{ s}^{-1}$ was calculated using Eq. (1):

$$\text{slope} = \sqrt{\frac{\lambda}{K_h}} \quad (1)$$

A K_h of $2.86 \pm 0.7 \text{ m}^2 \text{ s}^{-1}$ can be similarly calculated from ln ²²⁴Ra. Differences in these two K_h values reflect the difference in the half-lives of ^{223,224}Ra. Nonetheless, these K_h values are comparable to other such estimates, as calculated for example off close-by Huntington Beach, California (Boehm et al., 2004; Colbert and Hammond, 2007a,b).

Once these eddy diffusion coefficients have been calculated, one can re-examine the offshore ²²⁶Ra distribution to derive another measure of SGD (e.g., Moore and de Oliveira, 2008; Burnett et al., 2008). The flux of ²²⁶Ra away from our study site at West Beach can be expressed as a product of ²²³Ra-derived K_h multiplied by the offshore ²²⁶Ra gradient and the thickness of the mixed layer. A linear ²²⁶Ra gradient of $-13.79 \pm 5.6 \text{ dpm m}^{-3} \text{ per km}$ was calculated along a 3.2 km transect using a mean near-shore surface water ²²⁶Ra activity of $125.48 \pm 6.5 \text{ dpm m}^{-3}$ ($n = 3$) and an offshore value of $82 \pm 0.5 \text{ dpm m}^{-3}$ ($n = 3$). In the absence of rivers or streams that could introduce 'new' Ra into the coastal waters, the calculated offshore ²²⁶Ra flux of $1.6 \pm 0.8 \times 10^6 \text{ dpm km}^{-1} \text{ day}^{-1}$ using the ²²³Ra-derived K_h and a water parcel depth of 1 m must be balanced by an equal groundwater exchange term. Three groundwater ²²⁶Ra values (mean ²²⁶Ra = $328.1 \pm 53.5 \text{ dpm m}^{-3}$) were obtained from the tempwell. Dividing the offshore ²²⁶Ra flux by this groundwater term yields a groundwater exchange rate of $4.8 \pm 3 \text{ m}^3 \text{ m}^{-1} \text{ day}^{-1}$, which corresponds to 9.6 cm day^{-1} , if we

assume again that Ra enters the water column within a 50 m wide band, as defined earlier by land-based resistivity. This SGD rate is very close to the similarly calculated SGD rate ($\sim 6\text{--}13\text{ m}^3\text{ m}^{-1}\text{ day}^{-1}$) obtained at Huntington Beach, California by Boehm et al. (2006).

During the offshore transect Ra sampling, the surface waters were also continuously analyzed for ^{222}Rn using four RAD7s. As each Ra sampling event lasted over 1 h, the RAD7s simultaneously recorded the ^{222}Rn concentration at each Ra station. The distribution of ^{222}Rn along the same shore-perpendicular transect can be similarly examined to obtain further SGD and mixing rate information, including combined atmospheric loss and radioactive decay terms. Based on an average Rn value obtained from multiple readings observed at each offshore station, a linear ^{222}Rn activity gradient of -1.17 dpm m^{-3} per m was obtained using the April 2007 near-shore ^{222}Rn time-series data (mean = $4040 \pm 1171\text{ dpm m}^{-3}$) and a mean offshore ^{222}Rn activity of 330 dpm m^{-3} . Atmospheric loss terms were negligible relative to the radon inventories. Multiplying this linear ^{222}Rn gradient by the ^{223}Ra -derived K_h yields a total offshore flux of $1.80 \times 10^6\text{ dpm m}^{-1}\text{ day}^{-1}$, or $1503\text{ dpm m}^{-2}\text{ h}^{-1}$ if one integrates this flux across the 50 m wide SGD zone. This ^{222}Rn flux rate compares well to the mean mixing loss term ($1290\text{ dpm m}^{-2}\text{ h}^{-1}$) calculated from the corresponding surface water column ^{222}Rn time-series data. Dividing this flux rate by the mean groundwater ^{222}Rn activity observed in the tempwell (Fig. 7: $463,000\text{ dpm m}^{-3}$) results in a groundwater exchange rate of $3.9\text{ m}^3\text{ m}^{-1}\text{ day}^{-1}$ (i.e., 7.8 cm day^{-1}) – a value very similar to the rate obtained using ^{226}Ra . The similarity of these two independent SGD estimates across a single shore-perpendicular transect confirms the utility of these two tracers of SGD, and that in these coastal waters off Santa Barbara, SGD consists of mostly of recycled sea water without a significant fresh water component.

As the half-lives of ^{224}Ra (3.66 day) and ^{222}Rn (3.8 day) are so similar, and their respective production rates in sediment are constant and quantifiable, one can use their unique geochemical characteristics in coastal waters to define differential mixing loss terms (Dulaiova and Burnett, 2006). While both ^{224}Ra and ^{222}Rn are conveyed into coastal waters by SGD (Ra may have a small additional seasonal riverine source), their estuarine fate is tied directly to water mass mixing and radioactive decay. Additionally, ^{222}Rn , as a noble gas, can escape the air/sea interface. Because of its strong concentration gradient across this interface in coastal waters, this isotope has been used frequently to study air–water gas exchange. Atmospheric evasion of ^{222}Rn is usually modeled as a function of molecular diffusion, taking into account Ostwald's solubility coefficient (α) and an estimate of the gas transfer velocity, k , which has been shown to vary complexly as a function of wind speed, temperature, water currents, and water depth (Macintyre et al., 1995). Dulaiova and Burnett (2006) elegantly developed an approach that quantifies the atmospheric ^{222}Rn loss term independently from a combined $^{222}\text{Rn}/^{224}\text{Ra}$ isotope method. The premise of this approach is that the $^{222}\text{Rn}/^{224}\text{Ra}$ ratio should change only as a function of gas evasion terms, including wind speed, currents, water depth, and temperature.

Observed $^{224}\text{Ra}/^{223}\text{Ra}$ activity ratios along a shore-perpendicular surface water transect and in representative groundwater can be used to estimate apparent ('Ra') water mass ages (Moore 2000). Fig. 8A shows a plot of these 'Ra ages' as a function of distance traveled away from shore. Following the Dulaiova and Burnett's (2006) approach, we decay-normalized the ^{224}Ra data to the slightly longer-lived ^{222}Rn and then plotted both isotopes against the $^{224}\text{Ra}/^{223}\text{Ra}$ -derived apparent ages (Fig. 9). As expected, the ^{222}Rn slope is much steeper than the ^{224}Ra slope, indicating preferential radon losses across the air/water interface. To quantify this loss, the ^{224}Ra line equation is multiplied by a ratio of the two y-intercepts

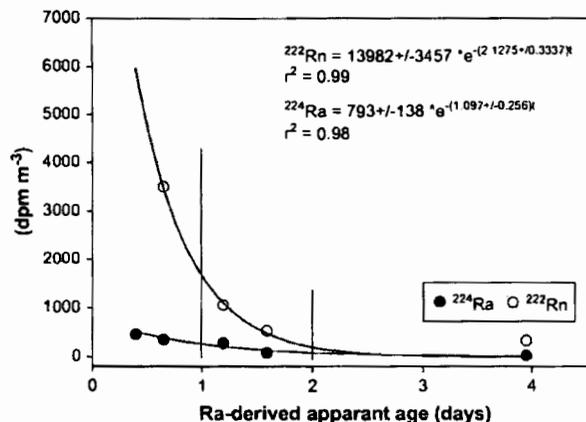


Fig. 9. A plot of decay-normalized ^{222}Rn (○) and ^{224}Ra (●) (dpm m^{-3}) as a function of apparent radium ages along an offshore transect (June, 2006) used to estimate the preferential rate of ^{222}Rn loss from the water column over time ($\text{dpm m}^{-3}\text{ day}^{-1}$) due to atmospheric evasion. Since the two nuclides have similar sources and half-lives, the radon air–water exchange rate can be estimated from the difference in the slopes of the ^{222}Rn and ^{224}Ra horizontal distributions.

(13,982/793) and the slope of each line thus represents a total loss rate per time ($\text{dpm m}^{-3}\text{ day}^{-1}$) along the transect. The difference in the ^{224}Ra and ^{222}Rn slopes yields an estimate of the ^{222}Rn evasion rate, which ranged up to $1400\text{ dpm m}^{-3}\text{ day}^{-1}$ closest to shore, and compared well theoretical model (Macintyre et al., 1995) results.

4.4. SGD-derived nutrient loading

While the geochemical tracers and the electrical resistivity images provide insight into the scales and patterns of total SGD off West Beach, the lack of a significant fresh water component in this SGD signal does not necessarily rule out the possibility that SGD-derived nutrient loadings to these coastal waters must be insignificant. Table 2 provides a summary of the mean nutrient concentrations in a suite of groundwater samples and adjacent surface water from our study site. The tempwell, which was located close to the high tide line (see Fig. 3 for time-series sampling locations along the beach face), had vastly different nutrient concentrations than the suite of surrounding monitoring wells, including nearby SB07 (Fig. 1). For example, mean PO_4^{3-} , SiO_4 and NH_4^+ concentrations from the tempwell time-series were all considerably lower than what was observed in the monitoring wells. In contrast, mean $[\text{NO}_2^- + \text{NO}_3^-]$ concentrations approached $0\text{ }\mu\text{M}$ in the monitoring well samples (i.e., strongly reducing nature of the regional aquifer deposits), while in the tempwell $[\text{NO}_2^- + \text{NO}_3^-]$ exceeded a mean value of $150\text{ }\mu\text{M}$. Time-series nutrient data (Fig. 10) show tidally modulated NH_4^+ , PO_4^{3-} and SiO_4 pulses in the surface waters that appear coincident with a low tide event. The absence of a corresponding $[\text{NO}_2^- + \text{NO}_3^-]$ peak suggests saline groundwater discharge. Thus, such nutrient profiles provide information as to redox state and residence times of these coastal groundwaters, as well as possible source terms. The position of the tempwell close to the high tide line suggests that $[\text{NO}_2^- + \text{NO}_3^-]$ inputs may also be locally derived. For example, just above the high tide line a heavy kelp line was actively scavenged by shoreline birds.

Table 2 also summarizes the adjacent sea water nutrient concentrations per season; all are substantially lower than mean near-shore groundwater nutrient concentrations. Such a scenario

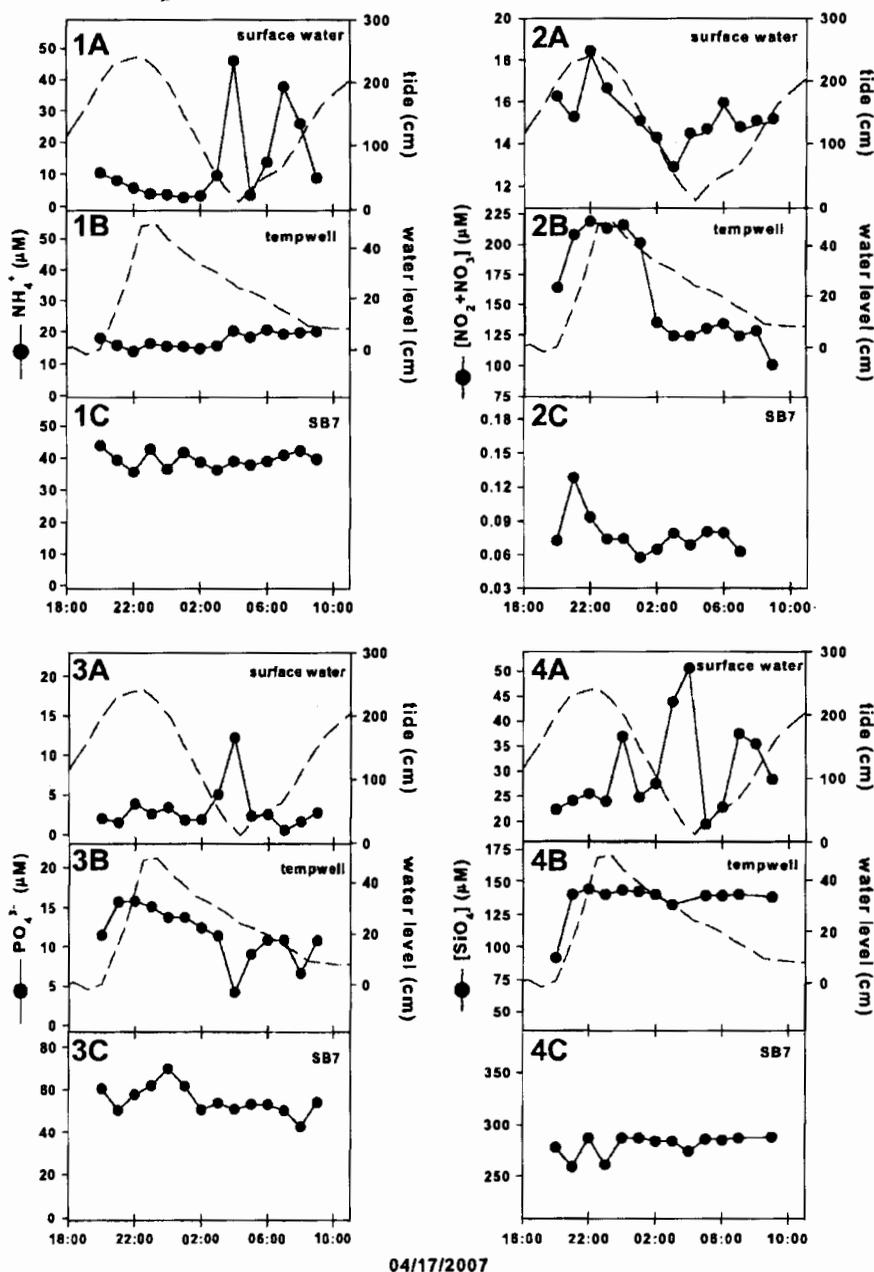


Fig. 10. Time-series of dissolved nutrient concentrations (μM) (1A–C: NH_4^+ , 2A–C: $[\text{NO}_2^- + \text{NO}_3^-]$, 3A–C: PO_4^{3-} , and 4A–C: SiO_4) collected concurrently in near-shore surface water, tempwell, and well SB07 as a function of water level (dashed line). Note scales held constant wherever feasible. Location of tempwell and SB07 relative to high tide line shown in Fig. 3.

sets up a positive concentration gradient that can drive nutrients into near-shore coastal waters with SGD. A summary of calculated SGD rates (cm day^{-1}) is shown in Table 3. Acknowledging conservative nutrient behavior during beach-face transport, one can multiply above SGD rates by the mean groundwater nutrient concentrations to yield the following SGD-derived nutrient loading estimates: $0.06\text{--}0.29 \text{ mol day}^{-1} \text{ m}^{-1}$ (NH_4^+), $0.22\text{--}0.92 \text{ mol day}^{-1} \text{ m}^{-1}$ (SiO_4), $0.04\text{--}0.17 \text{ mol day}^{-1} \text{ m}^{-1}$ (PO_4^{3-}), $0.10\text{--}0.57 \text{ mol day}^{-1} \text{ m}^{-1}$ (DIN), and $0.08\text{--}0.09 \text{ mol day}^{-1} \text{ m}^{-1}$ (DON). Such loadings (Table 4) are expectedly lower than similarly computed

estimates for sub-tropical Tampa Bay, Florida (Swarzenski et al., 2007c) or temperate Hood Canal, Washington (Swarzenski et al., 2007a), but similar in magnitude to SGD-derived DIN ($0.12 \text{ moles day}^{-1} \text{ m}^{-1}$) and SRP ($0.005 \text{ moles day}^{-1} \text{ m}^{-1}$) loadings calculated for the coastal waters off Huntington Beach (Boehm et al., 2006). Such results also suggest that a sustained flux of SGD-derived nutrients can be delivered to the near-shore coastal waters off Santa Barbara, California, where riverine nutrient loads are highly seasonal (Warrick et al., 2005; Beighley et al., 2008) and generally much less than the marine-derived nutrient contributions

Table 4

Combined SGD-derived nutrient loading estimates (mol day^{-1} per meter of shoreline) for the near-shore coastal waters off Santa Barbara. Cumulative uncertainty includes range in SGD rates and seasonality.

	NH_4^+	SiO_4	PO_4^{3-}	$[\text{NO}_2^- + \text{NO}_3^-]$	DIN	DON	TDN
	$(\text{mol day}^{-1} \text{ m}^{-1})$						
Range	0.06–0.29	0.22–0.92	0.04–0.17	0–0.52	0.19–0.57	0.08–0.09	0.38–0.65
Mean	0.13	0.51	0.09	0.17	0.33	0.08	0.52
\pm	0.10	0.30	0.06	0.24	0.01	0.08	0.14

(McFee-Shaw et al., 2007), but where there is no large fresh water SGD component.

4.5. Summary

Repeat geochemical and geophysical measurements have been made on a marine beach and the coastal waters of Santa Barbara, California, to study coastal exchange processes across the land/sea margin, including submarine groundwater discharge (SGD) and associated nutrient loading estimates. The following findings have resulted from this research:

- 1) the geochemical tracers ^{222}Rn and $^{223,224,226,228}\text{Ra}$ are present at much greater activities in the shallow groundwater of our study site than in adjacent near-shore sea water. This attribute allows us to use these radionuclides as quantitative tracers of coastal groundwater discharge. Calculated SGD rates ranged from 3.1 ± 2.6 to $9.2 \pm 0.8 \text{ cm day}^{-1}$, depending on which tracer approach was used and on the sampling season. There was very little difference between Ra- and Rn-derived SGD rates, suggesting that the saline component of SGD obtained from Ra accounts for most of total SGD signal;
- 2) within the constraints of a first-order model, the exponential distribution of $^{223,224}\text{Ra}$ and ^{222}Rn in surface waters along a shore-perpendicular transect from the marine beach to $>3 \text{ km}$ offshore (water depth = 34.2 m) was used to estimate an eddy diffusivity coefficient, K_h (^{223}Ra : $K_h = 1.32 \pm 0.5 \text{ m}^3 \text{ sec}^{-1}$; ^{224}Ra : $K_h = 2.86 \pm 0.7 \text{ m}^3 \text{ s}^{-1}$). Since ^{224}Ra and ^{222}Rn have similar sources and half-lives, the radon air–water exchange rate can also be estimated from the difference in the slopes of the ^{222}Rn and ^{224}Ra horizontal distributions. Combined $^{222}\text{Rn}/^{224}\text{Ra}$ water column removal terms yield observed air/sea evasion rates that are very similar to theoretical model (Macintyre et al., 1995) results;
- 3) electromagnetic seepage meter deployments in the near-shore waters recorded both discharge (positive) and recharge (negative) events that are tidally modulated, and ranged from -0.3 ± 1.0 to $14.5 \pm 55.4 \text{ cm day}^{-1}$, depending on the position of the meter relative to shoreline and the sampling season. Low tide coincident pulses in EM seepmeter data also showed a spike in salinity. Such discharge is likely also driven by past sea water intrusion events during storms and subsequent seepage of saline groundwater;
- 4) nutrients (NH_4^+ , SiO_4 , PO_4^{3-} , $[\text{NO}_2^- + \text{NO}_3^-]$, DIN, TDN, and DON) were also quantified in shallow groundwater and the near-shore coastal waters and used to calculate SGD-derived nutrient loading estimates. Depending on the sampling season and on the particular the SGD tracer used, the following nutrient loads were calculated: $\text{NH}_4^+ = 0.06\text{--}0.29 \text{ mol day}^{-1} \text{ m}^{-1}$; $\text{SiO}_4 = 0.22\text{--}0.29 \text{ mol day}^{-1} \text{ m}^{-1}$; $\text{PO}_4^{3-} = 0.04\text{--}0.17 \text{ mol day}^{-1} \text{ m}^{-1}$; $[\text{NO}_2^- + \text{NO}_3^-] = 0\text{--}0.52 \text{ mol day}^{-1} \text{ m}^{-1}$; dissolved inorganic nitrogen (DIN) = $0.01\text{--}0.17 \text{ mol day}^{-1} \text{ m}^{-1}$, and dissolved organic nitrogen (DON) = $0.08\text{--}0.09 \text{ mol day}^{-1} \text{ m}^{-1}$;

- 5) electrical resistivity surveys (marine CRP and land-based, stationary) were used to examine the movement of the fresh water/salt water interface in response to the lunar tide, as well as to map the offshore extent of the SGD zone. The land-based, tidal resistivity surveys were used to delineate the zone of active SGD (50 m).

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SOURCES OF FECAL INDICATOR BACTERIA IN URBAN STREAMS AND OCEAN BEACHES, SANTA BARBARA, CALIFORNIA

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ABSTRACT

Fecal indicator bacteria (FIB) indicative of fecal contamination in urban streams and recreational ocean beaches in Santa Barbara, California often exceed recreational water-quality standards. During low flow, FIB and human-specific *Bacteroides* concentrations in urban streams were associated with point discharges. FIB concentrations varied three-fold during diurnal sampling as a result of small variations in these discharges. During stormflow, FIB concentrations were higher than during low flow and varied over three orders of magnitude. FIB in stormflow were associated with non-point sources, and concentrations decreased as fecal contamination was washed from the urban watershed. Sources of fecal contamination to near-shore ocean water included surface discharges

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from urban streams, and fecal material from birds associated with sand, and to a lesser degree kelp, along the beachfront. FIB concentrations varied over three orders of magnitude during daily tidal cycles. Concentrations were higher during ebb tides and decreased to less than the detection limit during low tide when seepmeter and ²²²Rn data show groundwater discharge to the ocean was greatest. Groundwater discharge and leakage from a sewer line buried in the sand were not large sources of FIB contamination to near-shore waters. Interpretations of the sources of FIB from Principal Component Analysis (PCA) of genetic (Terminal-Restriction Fragment Length Polymorphism, T-RFLP, data), molecular (PhosphoLipid Fatty Acid, PLFA, data), and chemical data (such as caffeine, fecal sterols, and detergent metabolites) were similar and consistent with interpretations supported by physical measures of water flow. The most robust PCA results were from PLFA data which explained 97 percent of the total variance within the first and second principal components. In contrast PCA analysis of chemical and T-RFLP data, explained 34 and 32 percent of the total variance, respectively. However, T-RFLP and chemical tracers captured relations not apparent in PLFA data, and certain compounds, especially the fecal sterols, lent themselves to specific interpretations of the origin of fecal contamination.

Keywords: fecal indicator bacteria, submarine groundwater discharge (SDG), surface water, groundwater, bacterial source-tracking

1. INTRODUCTION

Direct measurement of human pathogens in recreational water is not commonly made because these assays are time consuming and expensive. In addition, assays for many human pathogens are not available for routine application. Instead, fecal indicator bacteria (FIB) are used as a surrogate for pathogens to determine fecal contamination and potential health hazards associated with recreational waters. FIB are used as a surrogate because they are 1) easily and relatively rapidly measured using standardized tests, 2) they are present at high concentrations in human waste, and 3) epidemiological studies have linked high FIB concentrations to gastrointestinal and respiratory illness in humans [1-6]. Some of the most commonly used FIB are fecal coliform, *Escherichia coli* (*E. coli*), and enterococci. Although not necessarily fecal in origin, total coliform bacteria also are commonly used

with FIB to assess microbial contamination of recreational waters.

The use of FIB to determine health hazards associated with recreational waters is complicated by their presence in warm-blooded animals other than humans, including seabirds living along shorelines, farm animals, pets, rodents and other animals common in urban and recreational areas. In addition to human and animal feces, growth or extended survival of FIB can occur in streambed sediments [7-10], in biofilms along stream channels and urban drains [11], and in beach sands [12-16]. The source of fecal contamination is important since it is widely believed that human feces pose a greater human-health risk than animal feces. This is because fecal contamination from non-human sources does not contain human-specific viral pathogens [17].

Fecal contamination to urban streams and recreational beaches from urban drains as incidental discharges during baseflow, and from larger discharges during stormflow, has long been known and has been the focus of much research in recent years [11,18-21]. Streamflow and other surface discharges to the ocean have been shown to be a source of fecal contamination to near-shore ocean water extending as much as 5,000 m along the shoreline from the discharge point - with dilution rather than death, predation, or other bacterial inactivation processes being the primary attenuation mechanism [22]. Recent studies have implicated groundwater discharge as a possible source of fecal contamination to recreational ocean beaches [23-25].

In recent years, a wide range of genetic, molecular, and chemical tracer techniques have become available to supplement traditional measurements of FIB concentrations in water and to aid in determining the source of fecal contamination [26,27]. These tracer techniques include, but are not limited to, 1) direct measurement of fecal microorganisms such as *Bacteroides* or enteroviruses [28-35], 2) genetic and molecular characterization of microbial populations associated with different fecal sources [31,36-40], and 3) measurement of low-concentrations of chemicals commonly associated with human wastewater [34,41-43], including fecal sterols [44-47].

The use of tracer techniques, especially genetically-based tracers, to determine the source of fecal contamination in recreational waters has expanded rapidly in recent years. However, only a few studies have attempted to constrain assessment of fecal contamination sources by integrating FIB data with multiple tracer techniques [30,32,36]. Even fewer studies have integrated FIB and tracer techniques with hydrologic data that quantify the movement of water

from different sources [11,36] or with groundwater exchange in beach settings over tidal cycles [32,48]. The combined use of FIB and multiple alternative tracers of fecal contamination constrained by an understanding of the movement of water may be a powerful approach for identifying FIB contributions from human and nonhuman sources.

The Santa Barbara area, 150 km northwest of Los Angeles, California (Figure 1) was selected to test the use of FIB data with multiple tracers of fecal contamination, constrained by an understanding of the physical hydrology, to determine the source of fecal contamination in an urban setting along the Pacific Ocean. The study area includes urban streams and ocean beaches. The urban streams are potentially subject to point and non-point FIB contamination from leaking sewer lines and laterals, discharges from urban baseflow, and stormflow runoff. Although not used as recreational waters, urban streams in the area are subject to incidental recreational use by the local population, and possible contamination by a transient homeless population. Ocean beaches in Santa Barbara are used for recreation and are potentially subject to fecal contamination from point and non-point sources similar to those affecting urban streams. Of particular concern was the potential for leakage from a sewer line underlying West Beach, less than 100 m inland from the high tide line. In addition, the ocean beaches also are potentially subject to fecal contamination from 1) shorebirds and other marine wildlife, 2) human use, including bathing and boating, and 3) discharges from streams and coastal estuaries known to contain high concentrations of FIB.

1.1. Purpose and Scope

The purpose of this study was to determine the source of fecal contamination to urban streams and to near-shore ocean water in Santa Barbara, California between April 2005 and April 2007. The scope of the study included: 1) measurement of stream discharge and sample collection along an urban stream during baseflow and stormflow, 2) measurement of water levels and sample collection from water-table wells installed at selected locations in the urban area, near the stream, and at the beachfront, and 3) measurement of water exchange and FIB concentrations at the beachfront during selected tidal cycles. Water exchange at the beachfront was evaluated on the basis of changes in water levels in wells, seepmeter data, and isotopic data. Potential sources of fecal contamination were evaluated using genetic, molecular, and chemical data from surface water, groundwater, near-

shore ocean water, wastewater, and from kelp and sand. Interpretations of the sources of fecal contamination based on these data, constrained by physical measures of water flow, were compared and contrasted.

1.2. Hydrologic Setting

The study area is in Santa Barbara, California, about 150 km northwest of Los Angeles (Figure 1). The city is located on a narrow coastal strip about 5 km wide,

flanked by mountains more than 1,300 m in altitude. In 2000, the population of Santa Barbara was 95,300, and the area is highly developed and urbanized. The study area has a Mediterranean climate with warm, dry summers and cool, wet winters. Most precipitation on the coastal strip, about 430 mm annually, falls during the rainy season from November to March.

The study area is drained primarily by Mission Creek and its tributaries. Mission Creek originates in the Santa Ynez Mountains to the north and discharges to the Pacific Ocean (Figure 1).

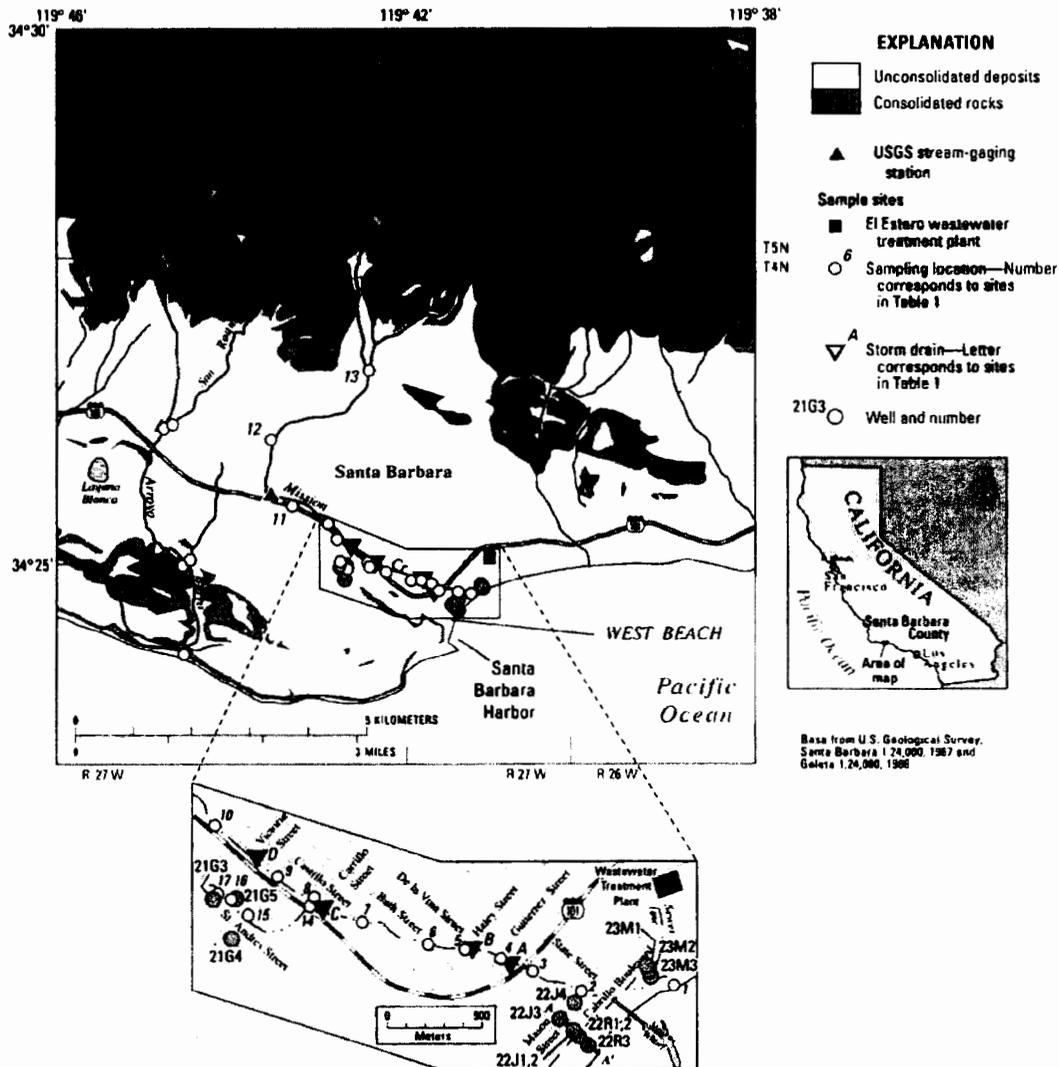


Figure 1 Location of study area

Mission Creek is perennial along its lower reaches, where groundwater discharge sustains flow during the dry season [49]. In addition, base flow in the perennial downstream reach is sustained by flow from urban drains and dewatering wells used to lower water levels near highway underpasses and larger buildings. Much of the city was built in the early part of the 20th century. Although the sewer infrastructure has been updated, laterals connecting individual homes to the sewer may date from the time of original construction. The potential for sewage from leaking sewer lines or laterals to enter shallow groundwater and discharge to streams is increased by the high water table underlying much of the city.

Discharge from Mission Creek to the ocean is not continuous. During the dry season the mouth of the creek is impounded by a sand berm built by wave action along the beachfront to form a coastal estuary or "lagoon". Water from the lagoon either discharges as small flows across the berm, or as infiltration through the berm. In recent years, water has been diverted from the lagoon to the El Estero wastewater treatment plant (WWTP). Occasionally the berm breaches, rapidly releasing a large amount of water to the ocean. These breaches commonly occur as a result of increased streamflow from precipitation and subsequent runoff into Mission Creek.

West Beach is a south facing ocean beach west of the mouth of Mission Creek. Discharges from Mission Creek may be a source of fecal contamination to West Beach. In addition, a sewer line runs the length of the beach about 100 m from the high tide line. There is concern that leakage from the sewer may contaminate shallow groundwater that subsequently discharges to the ocean. In addition, stormwater runoff from city streets discharged to the beach sand and direct discharges from commercial and recreational boats in the nearby harbor are other potential sources of fecal contamination to West Beach. West Beach is relatively protected from wave action because of its south facing position along the Santa Barbara Channel and the nearby harbor. Kelp and sand along protected beach areas may harbor FIB [14] and contribute to fecal contamination of near-shore ocean water [48].

2. METHODS

2.1. Field Methods

Grab samples were collected from streams and urban drains in the center of flow during April 2005 and August 2005 (Figure 1 and Table 1). Flow measure-

ments were made at the time of collection using current meters, flumes, or calibrated containers depending on site conditions. Automated samplers, equipped with Teflon sample lines were used to collect time-series data on Mission Creek at Gutierrez Street (site 4 in Figure 1) for diurnal sampling in August 2005, and stormflow sampling during January 2006. Intakes for water samplers were located in the center of flow and sample lines were rinsed three times prior to collection of each sample. Stream stage was measured using pressure transducers placed near the sample intake. For samples collected during August 2005, changes in stage were converted to flow from concurrent discharge measurements. Discharge measurements needed to convert stream stage to stream flow were not collected during stormflow.

Thirteen, 2-inch diameter PVC wells (Figure 1 and Table 1) installed using an auger drill rig were sampled in November 2005, May-June 2006, and April 2007. Wells were assigned numbers according to their position in the Public Land Survey System. In Tables and Figure titles, the complete well number including township, range, section, and sequence number is provided (for example 4N/27W-22R3). In the text a shortened form of the well number including only the section and sequence number (22R3) is used. Pressure transducers were installed on selected wells and data are available in the National Water Information System (NWIS-Web) and an online computer database operated by the U.S. Geological Survey. Prior to sample collection, wells were purged using portable pumps. Pumps were cleaned using Liquinox and distilled water between wells to minimize cross contamination. After purging, water samples from the wells were collected using peristaltic pumps. New nylon tubing (with a short length of Tygon tubing near the pump head) was used for each well and then discarded after use. Samples for trace organic and fecal sterol analysis were collected from wells using new glass bailers after the pumped samples were collected. Bailers were discarded after use. Most wells were sampled three times during the study. Well, 22R3, along the West Beach cross-section at the high-tide line (Figure 1) was sampled hourly during selected ebb tides in November 2005, May-June 2006, and April 2007. An additional well was installed and sampled at the beachfront during April 2007 to supplement data from Well 22R3, which because of sand accumulation on the beach was no longer located at the high tide line. Well 21G3 (Figure 1), was destroyed during the study and was only sampled twice.

Grab samples of near-shore ocean water were

collected in the "swash zone", approximately between ankle and mid-calf in depth, so that the sample depth remained approximately constant but the location varied with the ebb and flow of the tide. Boehm, [22] showed little difference in FIB concentrations in samples collected at ankle and waist depth for at beach near Avalon, California. Grab samples of influent to the El Estero WWTP (Figure 1) were collected using sampling equipment available on site.

Kelp and sand from the upper 0.5 cm were collected from near the high tide line along West Beach. Samples were collected with stainless steel implements and placed in stainless steel buckets. Implements and buckets were cleaned and baked at 800°C prior to use. The buckets were discarded after use and sample implements were thoroughly cleaned and rinsed with organic-free water between sample collection. The mass of the sample was determined in the field by subtracting the weight of the bucket from the weight of the sample plus the bucket. Samples of kelp and sand were washed with organic-free water adjusted to seawater salinity using organic-free NaCl. Organic-free NaCl was prepared by baking reagent grade NaCl at 800°C for 24 hours. The baked NaCl was stored in baked glass containers and added to the organic-free water immediately before use in the field. The supernatant was decanted from the buckets and stored in appropriate bottles using sample handling and preservation procedures described below.

pH and specific conductance were measured in the field using portable meters. Dissolved oxygen also was measured in the field using the indigo-carmin method (CHEMetrics, Inc., Calverton, VA). Water samples for selected anions, cations, and nutrients were filtered in the field through 0.45 µm pore sized filters, placed in plastic bottles and chilled. Samples for cation analysis were preserved in the field using nitric acid. Samples for FIB were unfiltered, placed in sterile bottles, and chilled. Samples for human-specific *Bacteroides* and enteroviruses, T-RFLP, PLFA, were unfiltered, placed in 1 L glass bottles, and chilled. Samples for trace organic compounds were unfiltered, placed in 1-L glass bottles, preserved in the field using 10 mL of dichloromethane, and chilled. Aluminum foil lined caps were used to seal sample bottles intended for trace organic analysis. All 1-L glass bottles were baked at 800°C prior to use.

Most FIB samples and samples for *Bacteroides*, enteroviruses, and T-RFLP were delivered to respective labs for analysis within 8 hours of collection. Stormflow samples and samples from diurnal studies that were collected after 2:00 PM were delivered to the lab the next morning. All other samples were

shipped on the day of collection by overnight delivery to their respective laboratories for analysis.

2.2. Analytical Methods

Total coliform and *E. coli* were analyzed by Colilert and enterococci were analyzed using Enterolert (IDEXX, Westbrook MN) at the City of Santa Barbara Water Resources Laboratory. A range of dilutions was used to ensure proper quantification of samples in accordance with the manufacturers' specifications.

Samples for human-specific *Bacteroides* [50], and enteroviruses were analyzed at the University of Southern California in Los Angeles, California. These samples were filtered in the laboratory within 8 hours of collection onto 47-mm 0.2-µm pore size Durapore filters. For surface-water and groundwater samples, the volume filtered ranged from 120 to 1,000 mL depending on the suspended sediment in the sample. Samples from the wastewater treatment plant and water rinses from kelp were difficult to filter and volumes ranged from 12 to 80 mL. Filters were frozen after filtration and thawed prior to extraction and analysis. DNA was extracted from all samples using the MoBio Ultraclean Fecal DNA kit and eluted in 50µL. The extracted DNA was quantified using the Molecular Probes dsDNA Quantitation Kit. *Bacteroides* levels were determined by SYBR Green-based quantitative Polymerase Chain Reaction (qPCR) [51]. For samples with DNA levels >0.2 ng/L, 2 ng of DNA were used as the template for the qPCR reaction. For samples with less than 0.2 ng DNA, 4 µL of eluted DNA was used. All samples were run in duplicate with a standard curve having a range of 10² to 10⁸ copies from a plasmid containing the target gene fragment. Samples for enteroviruses were filtered in the laboratory within 8 hours of collection and the frozen. The volume filtered for each sample ranged from 25 to 1,000 mL depending on the ease of filtration. RNA was extracted using the Qiagen RNeasy Mini Kit (tissue protocol) with the QIAvac Manifold. Reverse Transcription and the qPCR were done in a single reaction [50]. All samples were run in duplicate with additional duplicate samples spiked with vaccine-type poliovirus to test for inhibition. A standard curve was run simultaneously with a range 3.3 x 10¹ to 3.3 x 10⁵ poliovirus particles per assay.

Samples for Terminal Restriction Fragment Length Polymorphism (T-RFLP) measurements were analyzed by the University of California at Santa Barbara. Samples were filtered in the lab within 8 hours of collection, and microbial cells were separated from particulate material using methods described by

LaMontagne and Holden [52]. Cells were concentrated by centrifugation and DNA within the cells was extracted and purified using commercially available kits (UltraClean DNA; MoBio Laboratories Inc., Solana Beach, California) as specified by the manufacturer. After extraction and purification, the DNA was stored at -80°C until analysis [52]. 16rRNA genes from the purified DNA were amplified using Polymerase Chain Reaction (PCR) with eubacterial primers 8F hex (fluorescently labeled forward primer) [53] and 1389R [54]. PCR reaction mixtures were processed on a PCR Sprint thermal cycler (Hybaid US, Franklin, Mass.) using analytical and quality control procedures described by LaMontagne et al. [55]. PCR products were purified with the High Pure Kit (Boehringer Mannheim, Indianapolis, IN) and digested with H-hal and MspI restriction enzymes. The restriction enzymes were inactivated by heating (65°C for 10 min) and the length of the fluorescently labeled fragments was determined with an Applied Biosystems Instruments Model 373A automated sequencer (ABI; Foster City, California).

Samples for phospholipid fatty acids (PLFAs) were analyzed by Microbial Insights in Rockford, Tennessee. Samples were chilled and shipped in coolers on the day of collection for overnight delivery. Upon arrival at the lab, lipids were recovered using a modified Bligh and Dyer method [56]. Extractions were performed using one-phase chloroform-methanol-buffer extractant. Lipids were recovered, dissolved in chloroform, and fractionated on disposable silicic acid columns into neutral-, glyco-, and polar-lipid fractions. The polar lipid fraction was transesterified with mild alkali to recover phospholipid fatty acids (PLFA) as methyl esters in hexane. PLFA were then analyzed by gas chromatography with peak confirmation performed by electron impact mass spectrometry (GC/MS).

Samples for selected wastewater indicators were analyzed by the U.S. Geological Survey National Water Quality Laboratory (NWQL) in Denver, Colorado. Samples were preserved in the field using 10 mL of reagent grade dichloromethane (DCM), chilled, and shipped in coolers on the day of collection for overnight delivery to the lab. The DCM inhibited microbiological degradation and began the extraction of nonpolar organic compounds within the sample. Analysis was by Continuous Liquid-Liquid Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry [57]. Samples for nutrient analysis were field filtered, chilled and shipped to the U.S. Geological Survey National Water Quality Laboratory in Denver, Colorado for analysis by various methods

described by Fishman et al. [58].

2.3. Methods to Measure Exchange of Ocean Water with Groundwater

Seepmeter, radon-222 (^{222}Rn), and direct-current marine-resistivity data were collected to assess the magnitude, variability, and timing of exchange of near-shore ocean water with shallow groundwater. Direct-current resistivity data were collected from a boat along West Beach, near the mouth of Mission Creek, and along the beach to the east to determine the representativeness of seepmeter and ^{222}Rn data collected in more limited areas along the beachfront.

The seepmeters used in this study focus water through a 2.5-cm-diameter orifice at the top of a 1.2-m-diameter dome emplaced in the beach sand just below the low-tide line [59]. An electromagnetic (EM) flowmeter embedded within the orifice measures velocity according to Faraday's Law, where the voltage generated by movement of water through an induced magnetic field is proportional to the velocity of water flowing through the field [60]. The small diameter of the orifice constricts flow, thereby increasing the velocity of the water and increasing the sensitivity of the seepmeter. Positive values reflect discharge of water from the beach to the ocean; negative values reflect movement of water from the ocean into the beach deposits. Seepmeters must be deployed in a relatively calm environment as waves and currents may dislodge the meter and produce inaccurate results [61,62]. For this reason it is often difficult to operate seepmeters for extended periods. Seepmeter data are point measurements, and measurements can vary spatially and with depth [63].

^{222}Rn is produced by the decay of radium-226 (^{226}Ra) in the uranium-238 decay series and has a half-life of 3.8 days. Radon is the heaviest of the noble gases, does not react chemically with aquifer surfaces, and is highly mobile in groundwater [64]. ^{222}Rn concentrations in groundwater are commonly several orders of magnitude higher than in ocean water. Diffusion of ^{222}Rn from sediments is small [65], and increasing ^{222}Rn activities in near-shore ocean water reflect discharge of shallow groundwater [64,66] and exchange of water between the ocean and beach deposits [64,67]. ^{222}Rn was measured on an almost continuous basis using a water/air exchanger and a radon-in-air monitor [67,68]. In addition, ^{222}Rn data average groundwater discharge over larger volumes than the point measurements obtained from seepmeters and are often a better indicator of exchange between groundwater and the ocean [68].

Direct-current marine-resistivity data were collected using a 112-m cable containing a 56-electrode array [68,69]. For marine applications, GPS data were logged, while water depth and the ship's position were recorded on a separate GPS-enabled fathometer. Continuous salinity and temperature data also were recorded.

3. RESULTS

3.1. Fecal Indicator Bacteria, *Bacteroides*, and Enteroviruses in Urban Streams

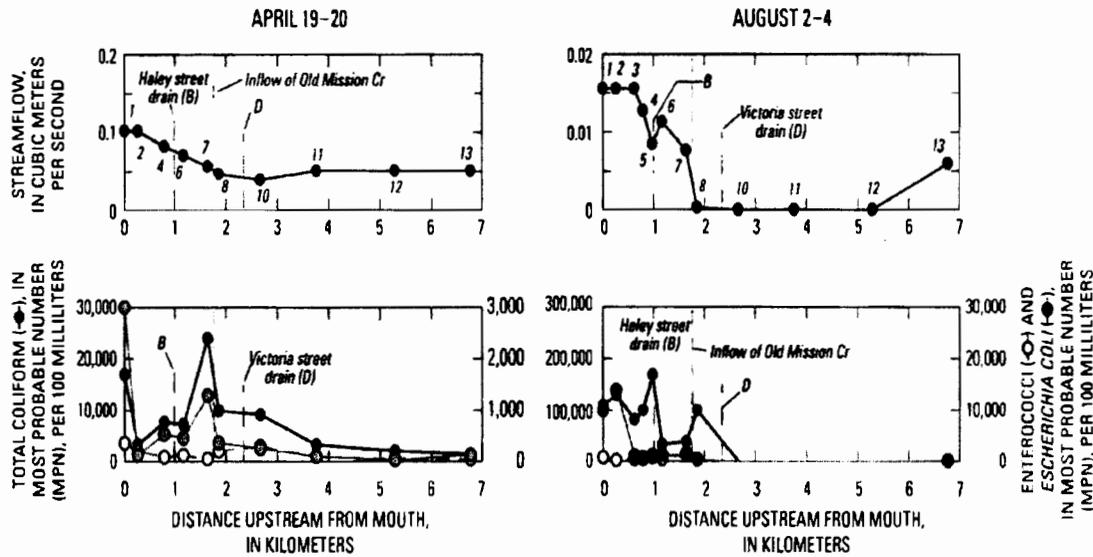
Streamflow and FIB concentrations were measured during baseflow in Mission Creek (including its tributary, Old Mission Creek) and Arroyo Burro, another urban stream, on April 19-21, 2005 and August 2-4, 2005 (Figure 1). On the basis of those data, 24-hour sample collection was done August 3-4, 2005 at Mission Creek at Gutierrez Street (Site 4, Figure 1) to determine temporal variability in FIB concentrations in the downstream urbanized reach of Mission Creek. Stormflow samples also were collected at Mission Creek at Gutierrez Street during

January 1-3, 2006 to determine FIB concentrations and sources during stormflow.

3.1.1 Seasonal and Spatial Distribution of Fecal Indicator Bacteria, *Bacteroides*, and Enteroviruses

Streamflow and FIB concentrations measured in Mission Creek during April 19-20 and August 2-4, 2005 provide a synoptic (snap-shot in time) view of streamflow and FIB concentrations under base flow conditions during spring and late summer (Figure 2 and Table 1).

Flow near the mouth of Mission Creek during April was as high as 0.1 m³/s. Streamflow during August was almost an order of magnitude less than flow during April. Discharge from Old Mission Creek, groundwater, storm drains, and dewatering wells used to lower the water table near highway underpasses contributed to flow along the downstream reaches of Mission Creek during both April and August (Figure 2). The upstream reach of Mission Creek where groundwater discharge and other sources of water were not present was dry during August, 2005 (Figure 2).



Numbers and letters in italics correspond to sites on figure 1 and in table 1; note scale change for total coliform on primary Y-axis and scale change for enterococci and *E. coli* on secondary Y-axis

Figure 2 Streamflow and fecal indicator bacteria (FIB) concentrations at selected sites along Mission Creek, Santa Barbara, California April 19-20 and August 2-4, 2005.

Table 1 Selected surface water sample sites, including stormdrains tributary to Mission Creek, Santa Barbara, California. [Number or letter corresponds to number or letter on Figure 1. Distance, in kilometers, corresponds to data shown on Figure 2. Additional sites shown on Figure 1 that are not specifically discussed in the paper are not listed.]

Number	Site name	Distance upstream from mouth (kilometers)
1	Mission Creek at mouth	0
2	Mission Creek at Mason Street	0.26
3	Mission Creek at Montecito Street	0.63
4	Mission Creek at Gutierrez Street	0.79
5	Mission Creek at Haley Street	0.99
6	Mission Creek at Bath Street	1.16
7	Mission Creek at Cannon Perdido	1.64
8	Mission Creek upstream from mouth of Old Mission Creek	1.85
9	Mission Creek at Anapamu Street	2.13
10	Mission Creek at Michel Torina Street	2.66
11	Mission Creek at West Mission Street	3.76
12	Mission Creek at Dela Vina Street	5.29
13	Mission Creek at Rocky Nook Park	6.77
14	Old Mission Creek at mouth	1.80
15	Old Mission Creek at Anapamu Street	2.32
16	Old Mission Creek at Bohnett Park	2.40
17	Old Mission Creek at West Victoria Street	2.50
A	discharge from drain at Highway 101	0.70
B	discharge from drain at Haley Street	0.99
C	discharge from drain at Carrillo Street	1.81
D	discharge from drain at Victoria Street	2.28
E	inflow to drain at Cabrillo Boulevard	--

During April 19-20, 2005, total coliform bacterial concentrations at selected sites along Mission Creek ranged from 1,500 to >24,000 MPN per 100 mL. At the same time, *E. coli*, and enterococci concentrations ranged from 31 to 3,000 and 41 to 360 MPN per 100 mL, respectively (Figure 2). During August 2-4, 2005, total coliform concentrations were higher than the April measurements, and ranged from 1,500 to 170,000 MPN per 100 mL (Figure 2). Similarly, *E. coli* and enterococci concentrations also were higher in August and ranged from 200 to 14,000 and 31 to 1,600 MPN per 100 mL, respectively. During both the April and August, 2005 sample collection periods, the lowest FIB concentrations were near the mountain front upstream from the urbanized area (site 13). Although FIB concentrations generally increased downstream, these increases were not monotonic and

FIB concentrations varied along the stream reach.

FIB concentrations measured in the discharge from four sampled drains tributary to Mission Creek (Figure 1, sites A, B, C, and D) were generally higher than those in the creek during both the April and August, 2005 sample periods. Total coliform concentrations in these four drains were as high as >240,000 MPN per 100 mL. *E. coli* and enterococci concentrations ranged from 540 to 29,000 and <10 to >240,000 MPN per 100 mL, respectively (data not shown on Figure 2). FIB concentrations from all sampled drains were higher during August than April. FIB concentrations were highest from the drain near Victoria Street (Site D), although the FIB loads to Mission Creek were higher from the Haley Street drain (Site B) because discharge was greater. FIB concentrations were lowest from the drain at Highway 101 (site A)

that contains a high fraction of shallow groundwater from dewatering wells.

Human-specific *Bacteroides* was detected in samples from the mouth of Mission Creek (site 1) and from three drains tributary to Mission Creek (sites B,C, and D) (Table 2). Although most concentrations were low, concentrations in the Haley drain on June 2, 2006 (Site B) were four orders of magnitude higher than other detections and were within 2-orders of magnitude of the concentrations in wastewater influent (Table 2). Enterovirus also were detected in samples from the Haley Drain (site B) (Table 2) and, with the exception of the El Estero WWTP, Haley drain was the only site where enterovirus was detected.

3.1.2 Diurnal Variations in Fecal Indicator Bacteria

Data were collected from Mission Creek at Gutierrez Street (Site 4) over a 24-hour period during August 3-4, 2005, after an extended period of baseflow, to measure diurnal variations in FIB concentrations.

Streamflow in Mission Creek at Gutierrez Street during August 3-4, 2005 ranged from 0.009 to 0.01 m³/s (Figure 3). If streamflow was maintained only by groundwater discharge, there should be only small diurnal variations from transpiration by riparian vegetation. These variations would produce a sinusoidal variation in flow, with lower flows during the day and higher flows during the night. However, streamflow abruptly increased at about 6 AM - lagging early morning increases in municipal water deliveries by less than an hour (Figure 3). The increased streamflow preceded increased inflow into the WWTP by only about 0.5 hour. However, streamflow did not continue to increase over the next 3 hours in the same manner as WWTP inflow (Figure 3). It is possible that the increase in early morning streamflow is the result of increased urban flow through lawn watering or other outdoor uses rather than leaking sewer lines. Urban contributions to Mission Creek at Gutierrez Street continued throughout the day and include discharge from storm drains, discharge from dewatering wells, leaking pipes or sewer lines, and runoff from lawn watering and other outdoor uses.

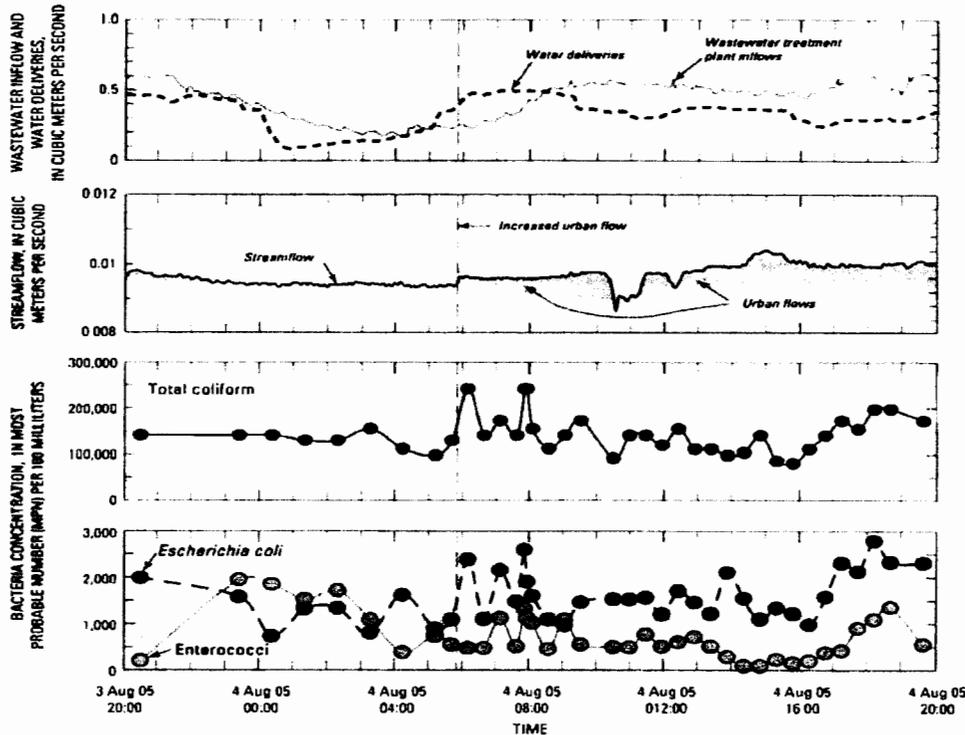


Figure 3 Streamflow and fecal indicator bacteria (FIB) concentrations in Mission Creek at Gutierrez Street, Santa Barbara, California, August 3-4, 2005.

Table 2 Human-specific *Bacteroides* and *Enterovirus* results for selected samples from urban stream and recreational beaches, Santa Barbara, California [\pm , plus or minus 1 standard deviation, for the purposes of this paper ± 2 standard deviations are considered as statistically significant result]

Site ID*	Station name	Date	Time	<i>Bacteroides</i> , in target copy number per liter	enteroviruses, in viral particles per liter
Stream sites					
I	Mission Creek at Mouth	6/1/06	13:00	47.1 \pm 11.8	<0.1 \pm 0.1
		4/17/07	12:30	<0.1 \pm 0.1	<0.1 \pm 0.1
4	Mission Creek at Gutierrez	8/4/05	07:45	<0.1 \pm 0.1	<0.1 \pm 0.1
14	Old Mission Creek at Mouth	8/4/05	09:00	<0.1 \pm 0.1	<0.1 \pm 0.1
					<0.1 \pm 0.1
--	Arroyo Burro Creek at mouth	8/4/05	07:45	<0.1 \pm 0.1	<0.1 \pm 0.1
					<0.1 \pm 0.1
Drain sample sites					
B	Haley drain discharge at Mission Creek	8/4/05	08:30	^{2/} --	89 \pm 0.1
				^{2/} --	22 \pm 0.1
		6/2/06	08:00	7.1 \pm 1.5 x 10 ⁴	<0.1 \pm 0.1
D	Victoria drain discharge at Mission Creek	6/6/06	11:30	294 \pm 139	<0.1 \pm 0.1
--	Cabrillo Street drain inflow	4/20/07	06:00	9.3 \pm 0.8	<0.1 \pm 0.1
Near-shore ocean sample sites					
--	West Beach at cross-section	6/1/06	02:00	38.3 \pm 11.0	<0.1 \pm 0.1
		6/1/06	10:00	557 \pm 515	<0.1 \pm 0.1
		4/17/07	21:00	20 \pm 0.8	0.11 \pm 0.11
		4/18/07	00:00	4.2 \pm 2.7	<0.1 \pm 0.1
		4/18/07	04:00	6.7 \pm 6.7	<0.1 \pm 0.1
		4/20/07	10:00	23 \pm 10	<0.1 \pm 0.1
		4/20/07	13:00	86 \pm 40	<0.1 \pm 0.1
--	^{1/} West Beach at Mission Creek	8/4/05	--	<0.1 \pm 0.1	<0.1 \pm 0.1
					<0.1 \pm 0.1
--	^{1/} Arroyo Burro Beach	8/4/05	--	<0.1 \pm 0.1	<0.1 \pm 0.1
					<0.1 \pm 0.1
Well sample sites					
21G3	4N/27W-21G3	6/2/06	09:00	0.2 \pm 0.2	<0.1 \pm 0.1
21G4	4N/27W-21G4	4/20/07	11:00	<0.1 \pm 0.1	<0.1 \pm 0.1
22R2	4N/27W-22R2	6/1/06	11:2	<0.1 \pm 0.1	0.3 \pm 0.3
		4/18/07	11:45	0.43 \pm 0.21	0.19 \pm 0.19
22R3	4N/27W-22R3	6/1/06	02:00	<0.1 \pm 0.1	<0.1 \pm 0.1
		6/1/06	10:00	6.7 \pm 6.7	<0.1 \pm 0.1
		4/17/07	21:00	1.2 \pm 1.2	<0.1 \pm 0.1
		4/18/07	00:00	0.11 \pm 0.004	<0.1 \pm 0.1
		4/18/07	04:00	<0.1 \pm 0.1	<0.1 \pm 0.1
22J2	4N/27W-22J2	6/2/06	13:30	0.5 \pm 0.5	<0.1 \pm 0.1
		4/17/07	09:30	<0.1 \pm 0.1	<0.1 \pm 0.1
22J3	4N/27W-22J3	6/2/06	13:20	<0.1 \pm 0.1	<0.1 \pm 0.1

*Site identification (Figure 1, Table 1); ^{1/} Sample collected by Heal the Ocean, Hillary Houser, written communication, August, 2005; ^{2/} Poor recovery from samples spiked with *Bacteroides* DNA suggesting that interference may have masked detection of *Bacteroides*

Table 2 (continued) Human-specific *Bacteroides* and *Enterovirus* results for selected samples from urban stream and recreational beaches, Santa Barbara, California [\pm , plus or minus 1 standard deviation, for the purposes of this paper ± 2 standard deviations are considered as statistically significant result]

Site ID	Station name	Date	Time	<i>Bacteroides</i> , in target copy number per liter	enteroviruses, in viral particles per liter
23M1	4N/27W-23M1	5/31/06	09:30	$<0.1 \pm 0.1$	$<0.1 \pm 0.1$
		4/19/07	12:30	$<0.1 \pm 0.1$	0.26 ± 0.26
23M2	4N/27W-23M2	5/31/06	14:10	24 ± 24.0	$<0.1 \pm 0.1$
		4/19/07	10:45	$<0.1 \pm 0.1$	$<0.1 \pm 0.1$
		4/19/07	10:46	$<0.1 \pm 0.1$	$<0.1 \pm 0.1$
23M3	4N/27W-23M3	5/31/06	12:30	4.7 ± 4.7	$<0.1 \pm 0.1$
Special source					
--	El Estero WWTP	6/2/06	10:00	$1.73 \pm 0.25 \times 10^6$	28.4 ± 26
		4/17/07	10:00	$3.35 \pm 3.7 \times 10^6$	682 ± 40
--	Kelp extract from West Beach	4/16/07	12:30	$<0.1 \pm 0.1$	$<0.1 \pm 0.1$
		4/19/07	13:00	63 ± 63	$<0.1 \pm 0.1$
--	Sand extract from West Beach	4/17/07	13:00	1.1 ± 1.1	$<0.1 \pm 0.1$

*Site identification (Figure 1, Table 1); ^{1/} Sample collected by Heal the Ocean, Hillary Houser, written communication, August, 2005; ^{2/} Poor recovery from samples spiked with *Bacteroides* DNA suggesting that interference may have masked detection of *Bacteroides*

Total coliform bacteria concentrations in Mission Creek at Gutierrez Street measured during August 3-4, 2005 ranged from about 81,000 to greater than 240,000 MPN per 100 mL. *E. coli* and enterococci concentrations ranged from 730 to 2,800, and 100 to 2,000 MPN per 100 mL, respectively. FIB concentrations were higher and more variable during the day, especially in the early morning when runoff from lawn watering and other outdoor uses contributed to streamflow (Figure 3). After the early morning measurements, the magnitude and variability of FIB concentrations decreased during the day presumably as fecal material that accumulated on streets was washed into the stream. An increase in FIB concentrations occurred in the evening beginning at about 1700 hrs. This increase occurred at about the same time as evening increase inflows to the wastewater treatment plant (Figure 3). Direct leakage of sewer lines into the stream was not observed during this study and did not appear to occur in the morning hours, but unpermitted discharge to urban drains could cause changes in FIB concentrations measured in Mission Creek and would be consistent with the presence of human-specific *Bacteroides* in these drains.

Total coliform and *E. coli* concentrations were positively correlated ($r = 0.64$), while the correlation

was less for total coliform and enterococci ($r = 0.37$). The correlation between *E. coli* and enterococci ($r = 0.06$) was not statistically significant. The lack of correlation between *E. coli* and enterococci concentrations suggests that these bacteria may be contributed to the stream from different sources in the watershed, each having different environmental and hydrologic histories that contribute to differential survival of FIB.

3.1.3 Fecal Indicator Bacteria in Stormflow

FIB concentrations were measured in Mission Creek at Gutierrez Street during a series of stormflows between January 1-3, 2006 (Figure 4). Total precipitation during this period was about 95 mm. Stormflow from a preceding storm that produced 44 mm of precipitation on December 31, 2005 was not sampled. The December 31st stormflow probably washed much of the highly mobile FIB and other material from the watershed that had accumulated in streets, storm-drains, and stream channels since the previous storm in September 2005. As a consequence, contributions from sanitary sewer lines, which could pressurize and leak as a result of increased flow during storms, were thought to be more easily detected during the sampled stormflow.

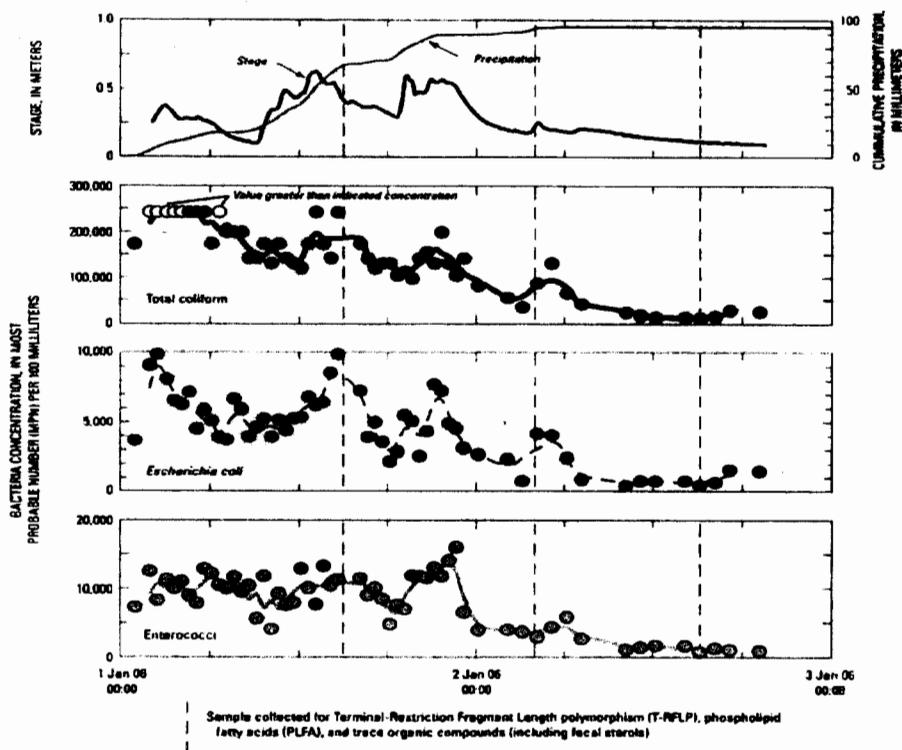


Figure 4 Precipitation, stream stage, and fecal indicator bacteria (FIB) from stormflow in Mission Creek at Gutierrez Street, Santa Barbara, California, January 1-2, 2006.

Stream stage increased in distinct peaks as a result of precipitation during January 1-3, 2006 (Figure 4). Total coliform, *E. coli*, and enterococci concentrations were as high as >242,000, 9,870 and 16,100 MPN per 100 mL, respectively. FIB concentrations generally decreased during the sample period although FIB concentrations increased during stormflow peaks. This decrease in concentrations during the storm is more consistent with successive stormflows washing material from the watershed than with repeated leaking from sanitary sewers during successive stormflow. FIB concentrations in stormflow samples were highly correlated with each other, having correlation coefficients ranging from 0.81 to 0.71 and suggesting a more uniform source and environmental history for FIB during stormflow than for diurnal variations discussed previously.

3.2. Fecal indicator Bacteria, *Bacteroides*, and Enteroviruses in Shallow Groundwater

Depth to water in sampled wells ranged from less than

1 to 5.3 m below land surface. Depths to water were greater inland in the upland residential areas, and less along Old Mission Creek and near the ocean.

Total coliform was detected at least once in every well installed as part of this study. The median concentration was 295 MPN per 100 mL, for samples having detections (data not shown). The highest total coliform concentration (>240,000 MPN per 100 mL) was measured in a sample collected from well 23M2 near the mouth of Mission Creek in November 2005. *E. coli* and enterococci were detected in 7 and 8 of the 13 sampled wells, respectively. The highest *E. coli* and enterococci values were 1,300 and 13,000 MPN per 100 mL in water from wells 23M1 and 23M3, respectively, near the mouth of Mission Creek.

E. coli and enterococci concentrations were lowest in water from wells in the inland residential areas (21G3-5). *E. coli* was not detected in any of these wells and enterococci was detected once in water from well 21G5 adjacent to Old Mission Creek. Low FIB occurrence in shallow groundwater in this area suggests that leakage from lateral lines

connecting older residential development to the sewer has not resulted in extensive FIB contamination of shallow groundwater in this part of the city. This is consistent with streamflow and FIB data from Mission Creek that shows diffuse sources of FIB contamination associated with discharging groundwater were less important than point sources associated with urban drains.

Human-specific *Bacteroides* were detected at low levels in water from wells 22R2 and 22R3 during the April 2007 sample collection (Table 2). Well 22R2, adjacent to the sewer line along West Beach well, is closer to the beachfront. However, neither of these detections were associated with high FIB concentrations. Recent work has shown *Bacteroides* to be poorly correlated with detections of traditional FIB [70].

3.3. Fecal Indicator Bacteria, *Bacteroides*, and Enteroviruses in Near-Shore Ocean Water

FIB data were collected from near-shore ocean-water at West Beach over three tidal cycles during November 14-18, 2005, May 30-June 3, 2006, and April 16-22, 2007 (Figures 5-7). The November 2005 and April 2007 sample periods bracketed a "spring" tide (highest high and lowest low monthly tides). The June 2006 sample period bracketed a neap tide (the lowest monthly tidal fluctuation). FIB data were collected hourly in near-shore ocean water and from well 22R3 during ebb of the spring and neap tides. The three data sets reflect dry, non-precipitation periods, with the exception of after April 20, 2007 when about 25 mm of precipitation fell during a late-season storm.

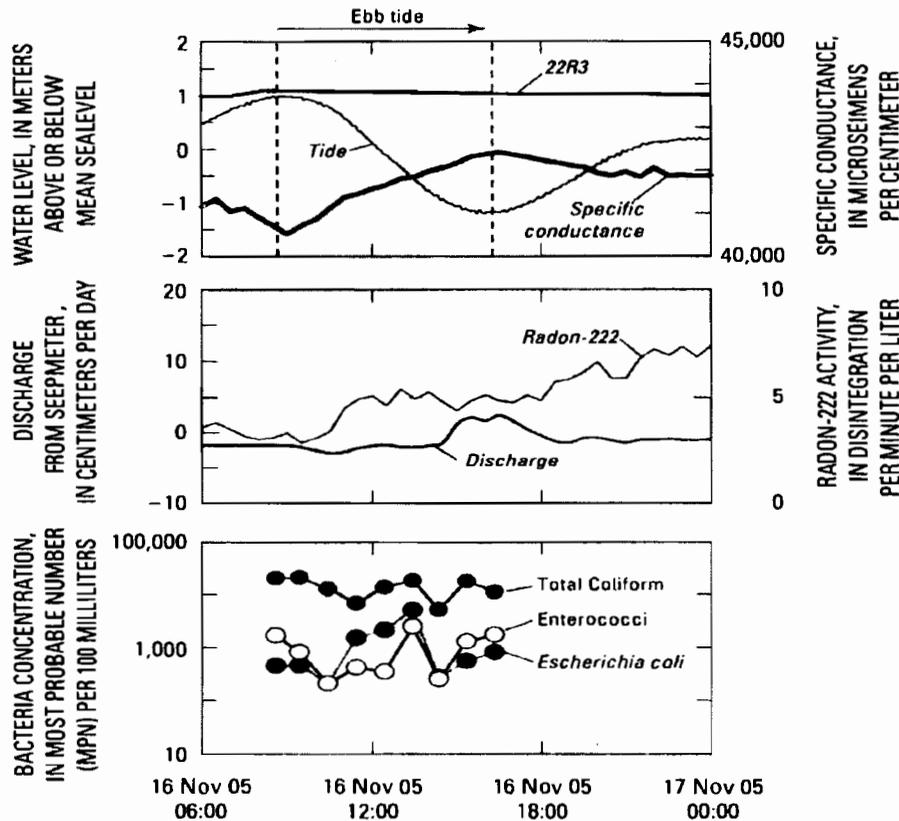


Figure 5 Groundwater levels at well 4N/27W-22R3, tides, groundwater discharge, specific conductance of groundwater discharge, radon-222 concentrations, and fecal indicator bacteria concentrations in near-shore ocean water, West Beach, Santa Barbara, California, during ebb-tide November 16-17, 2005.

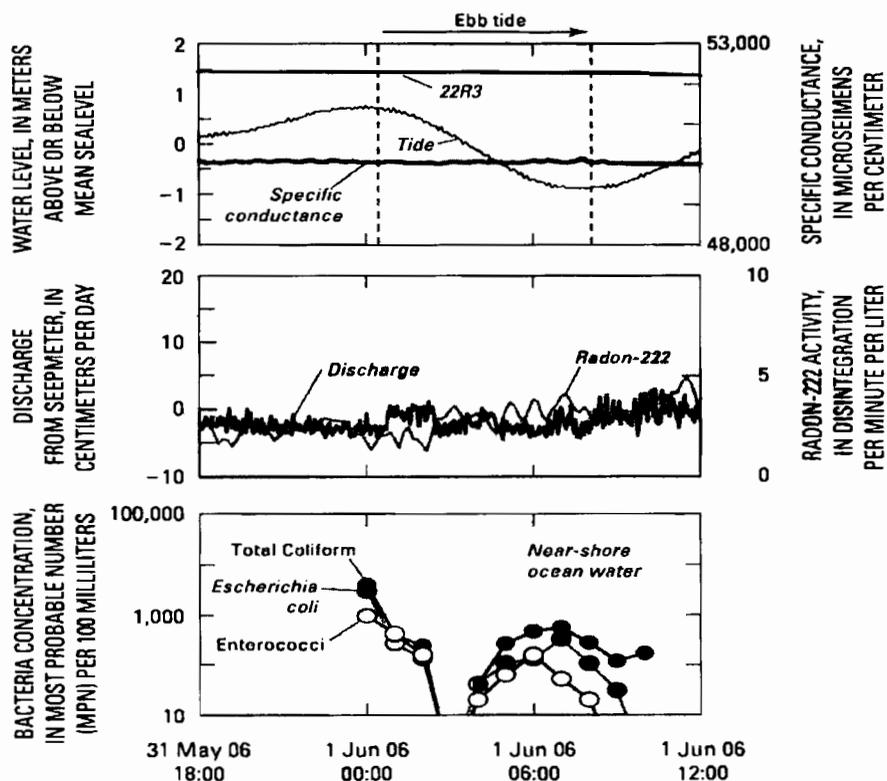


Figure 6 Groundwater levels at well 4N/27W-22R3, tides, groundwater discharge, specific conductance of groundwater discharge, radon-222 concentrations, ammonia, and fecal indicator bacteria concentrations in near-shore ocean water, West Beach, Santa Barbara, California, during ebb-tide May 31-June 1, 2006.

Total coliform concentrations in near-shore ocean water during the three sampled tides ranged from less than the detection limit of 10 to 21,000 MPN per 100 mL. *E. coli* and enterococci concentrations in near-shore ocean water ranged from less than 10 to 5,200 and less than 10 to 2,500 MPN per 100 mL. About 45 percent of enterococci samples exceeded the California state marine recreational contact single sample standard of 104 MPN per 100 mL [71]. Similar large variations in FIB concentrations over short time intervals were observed at other sites in California [72]. Samples for regulatory purposes are collected once daily without regard for hydrologic conditions such as tides. FIB data collected during this study suggest that samples collected without reference to ambient conditions, such as tides, may be inadequate to characterize FIB concentrations at recreational ocean beaches.

Human-specific *Bacteroides* was present in low

concentrations in the near-shore ocean water at West Beach in 6 of 7 samples (Table 2). Enteroviruses were not detected in any of those samples. *Bacteroides* samples were not collected at the same frequency as FIB, but the high frequency of detection suggests that low-levels of human fecal material were consistently present and could be at least partly responsible for at least some of the FIB detected on West Beach.

3.4. Possible Sources of Fecal Indicator Bacteria

Possible sources of FIB to near-shore ocean water at West Beach include 1) groundwater discharge contaminated with sewage from the nearby sewer line, 2) sewage from commercial and recreational boats in the nearby harbor, 3) guano contaminated sand, kelp, and debris on the beach, and 4) discharge from Mission Creek.

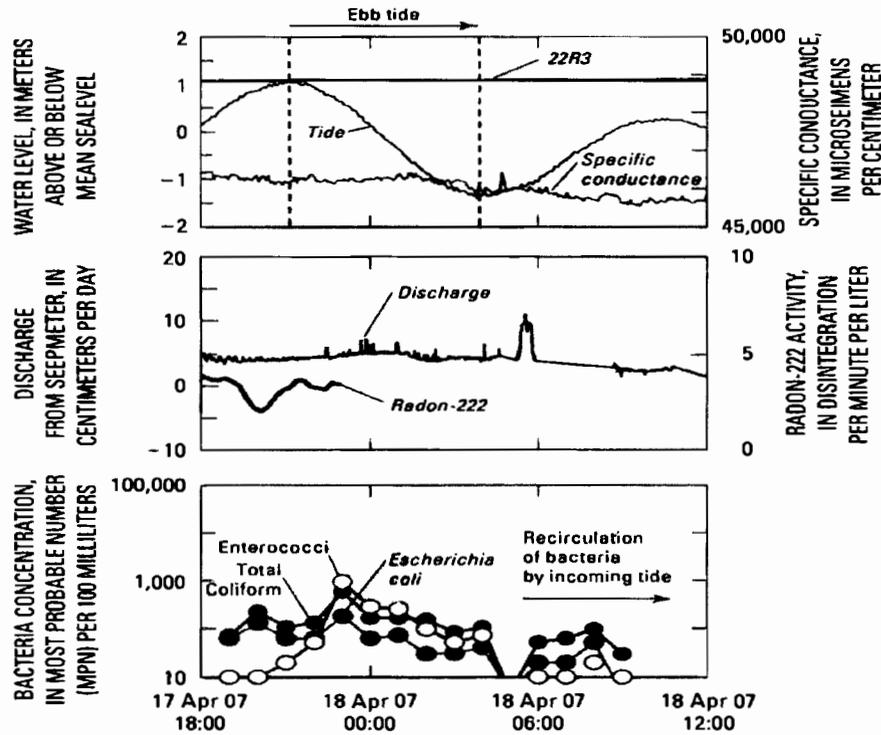


Figure 7 Groundwater levels at well 4N/27W-22R3, tides, groundwater discharge, specific conductance of groundwater discharge, radon-222 concentrations, and fecal indicator bacteria concentrations in near-shore ocean water, West Beach, Santa Barbara, California, during ebb-tide April 17-18, 2007.

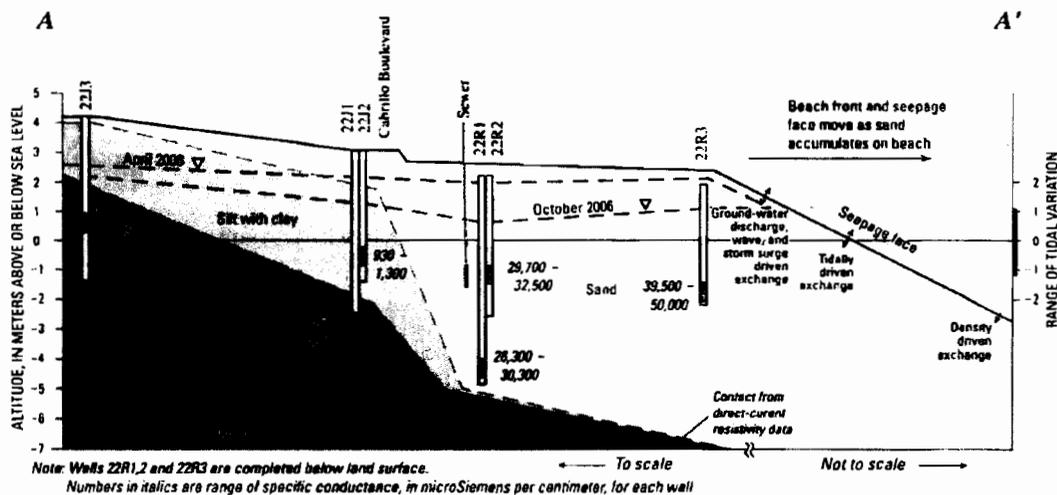


Figure 8 Section A-A' perpendicular to West Beach, Santa Barbara, California.

Groundwater discharge to West Beach near well 22R2 along Cabrillo Boulevard (Figure 8) was a considered a possible FIB source because of the proximity of the sewer line to the beachfront. Recent work at similar sites has suggested that groundwater discharge contaminated with sewage, especially at low tide, may be a source of FIB to ocean beaches [23,24,48]. Total coliform, enterococci, and *E. coli* concentrations in water from well 22R3 were generally less than the detection limit, and maximum FIB concentrations from this well (630, 85, and 63 MPN per 100 mL, respectively) were lower than concentrations in near-shore ocean water. Low levels of human-specific *Bacteroides* were detected once in water from well 22R3, during the April 2007 sample collection, after waves associated with a south swell drove water into the beach.

FIB concentrations in the nearby harbor (Figure 1) have been monitored at seven locations by the City of Santa Barbara since 2001 (City of Santa Barbara, written commun., 2007). Data show low-levels of FIB that do not approach standards for recreational water or concentrations measured at West Beach. In addition to monitoring within the harbor, sewer lines on the wharf near the harbor are routinely inspected to ensure their integrity. Monitoring within the harbor and

inspection of infrastructure does not exclude the possibility of discharges from commercial or recreational boats outside the harbor. If these discharges occur they would be expected to contain human-specific *Bacteroides* and other indicators of human fecal contamination consistent with sewage.

Kelp present at the high spring tide line and guano contaminated beach sands had high concentrations of FIB (Table 3). These materials may be a source of FIB at the ocean-beach interface especially during the spring tide when high tides wash material that has accumulated on the beach during the past month. Kelp and beach sands did not contain detectable levels of human-specific *Bacteroides* or enteroviruses (Table 2). These materials contain unique molecular and trace organic assemblages that will be discussed later in this paper.

Samples collected for this study indicate that the mouth of Mission Creek contains high concentrations of FIB (Figure 2) and occasionally detections of human-specific *Bacteroides* (Table 2). Mission Creek was discharging to the ocean during the ebb tides sampled in November 16-17, 2005 and May 31-June 1, 2006 and was a potential source of FIB at those times. Mission Creek was not discharging during the April 17-18, 2007 ebb tide.

Table 3 Fecal indicator bacteria concentrations in water extractions from kelp, and guano contaminated beach sands, West Beach, Santa Barbara, California April 17-19, 2007 [MPN per 100 ml, Most Probable Number per 100 milliliters; kg, kilogram; <, less than; >, greater than]

Sample	Date	Mass of sample, in kg	^a Mass of extractant, in kg			Fecal indicator bacteria (FIB), in MPN per 100 ml	Phospholipid fatty acids (PLFA), in picomoles per liter
				Total coliform	<i>Escherichia coli</i>		
Kelp	4/16/07	12	7.0	12,000	8,660	>24,200	17,600,000
				9,800	7,270	>24,200	--
^b Kelp	4/19/07	12	11.4	15,500	7,270	3,450	14,300,000
				17,300	8,160	2,480	--
Sand	4/16/07	0.5	7.0	15,500	3,450	9,800	65,000
Blank	4/16/07	--	--	<10	<10	<10	<100
Trip blank	4/16/07	--	--	<10	<10	<10	<100

^aExtractant was organic free water adjusted in the field to a salinity of 35 grams per kilogram with reagent-grade sodium chloride. The sodium chloride was baked at 200°C for 24 hours to volatilize organic material; ^bSample material was very dry and recovery of extract was poor.

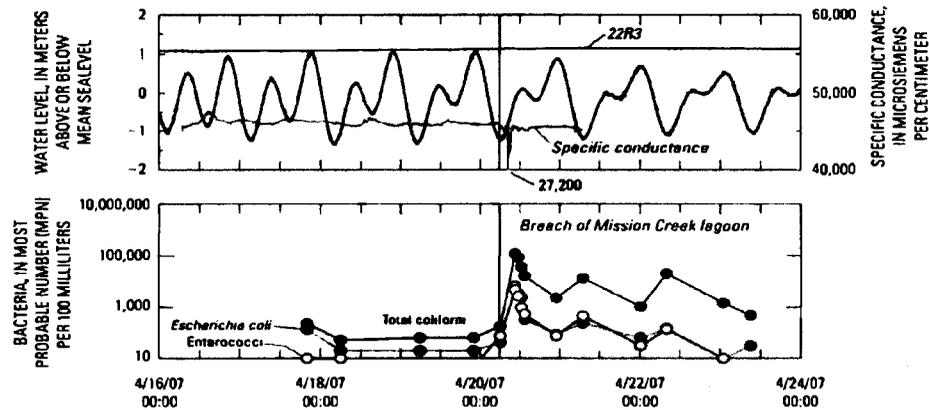


Figure 9 Tides, specific conductance, and fecal indicator bacteria (FIB) concentrations in near-shore ocean water, West Beach, Santa Barbara, California, April 16-23, 2007.

However, Mission Creek discharged rapidly beginning about 07:30 on April 20 after runoff from the storm breached the berm at the mouth of the creek. After the breach, FIB concentrations at West Beach increased rapidly as water discharged to the ocean (Figure 9). Total coliform, *E. coli*, and enterococci concentrations at West Beach, 3 hours after the breach of the berm, were 120,000, 6,500, and 4,600 MPN per 100 mL, respectively, and human-specific *Bacteroides* was present in near-shore ocean water (Table 2). At this time, specific conductance was 27,200 $\mu\text{S}/\text{cm}$, about half that of seawater - consistent with a large influx of fresh water from Mission Creek. FIB concentrations gradually declined to more normal values and specific conductance increased to near seawater values during the 3 days following the breach of the berm (Figure 9).

3.5. Physical and Isotopic Measures of the Exchange of Shallow Groundwater and Ocean Water

Water-level data from shallow wells in the cross-section perpendicular to West Beach, direct measures of groundwater discharge from seepmeters, and indirect measures of groundwater discharge from naturally occurring radioactive isotopes were used to evaluate the exchange of shallow groundwater and near-shore ocean water along West Beach. These data were supplemented with direct-current resistivity data collected along West Beach to extend interpretations from data collected at West Beach to other locations along the beachfront.

3.5.1 Groundwater Levels

Water-level data in wells perpendicular to West Beach show an oceanward gradient from the farthest inland well 22J3 toward well 22R2, adjacent to the sewer line (Figure 10). However, because the shallow deposits inland from the sewer line are predominately low permeability silt and clay (Figure 5), the groundwater flux toward the sewer line from inland areas was small. Water-level data show an inland gradient from well 22R3 near the beachfront toward well 22R2 adjacent to the sewer line (Figure 10).

Increases in water levels measured in wells 22J1 and 22J3 in early 2006 corresponded to precipitation events (Figure 10) and the water-level rises were consistent with the precipitation amount and the expected porosity of the deposits. When examined closely, increases in water levels measured in well 22R2 during the same periods were greater than expected solely from precipitation, and probably result from the discharge of stormflow runoff from adjacent streets to the beach. Runoff from streets adjacent to West Beach may be a potential source of FIB to shallow groundwater underlying the beach that was not considered at the onset of this study. Despite the water-level rise measured in well 22R2 in response to the precipitation and runoff, water levels in well 22R2 did not exceed water levels in well 22R3 at the beachfront, and the water-level gradient between wells 22R2 and 22R3 was inland toward the sewer line even during the rainy season (Figure 10).

Long-term net-infiltration of water from the ocean into the beach is indicated by the near-seawater

specific conductance of water from wells 22R2 and 22R3 on the ocean side of the sewer, which ranged from 29,700 to 50,000 $\mu\text{S}/\text{cm}$ (Figure 8). In contrast, the specific conductance of water from wells 22J1 and 22J2 on the inland side of the sewer line ranged from 930 to 3,530 $\mu\text{S}/\text{cm}$. Video logs show groundwater seepage into the sewer during dry periods, confirming water level and specific conductance data that show the sewer is a drain for shallow groundwater in the beach sands (City of Santa Barbara, Rebecca Bjork, written commun, 2006). As a consequence of the

direction of water movement, FIB bacteria associated with the sewer line or infiltrated from stormflow runoff into beach sands could not discharge to the ocean. However, exchange of water between the ocean and beach sands (Figure 8) could contribute FIB to near-shore ocean water during daily and longer tidal cycles. This exchange includes both discharge and recharge components, and is often referred to as submarine groundwater discharge (SGD) [62,68,73-75].

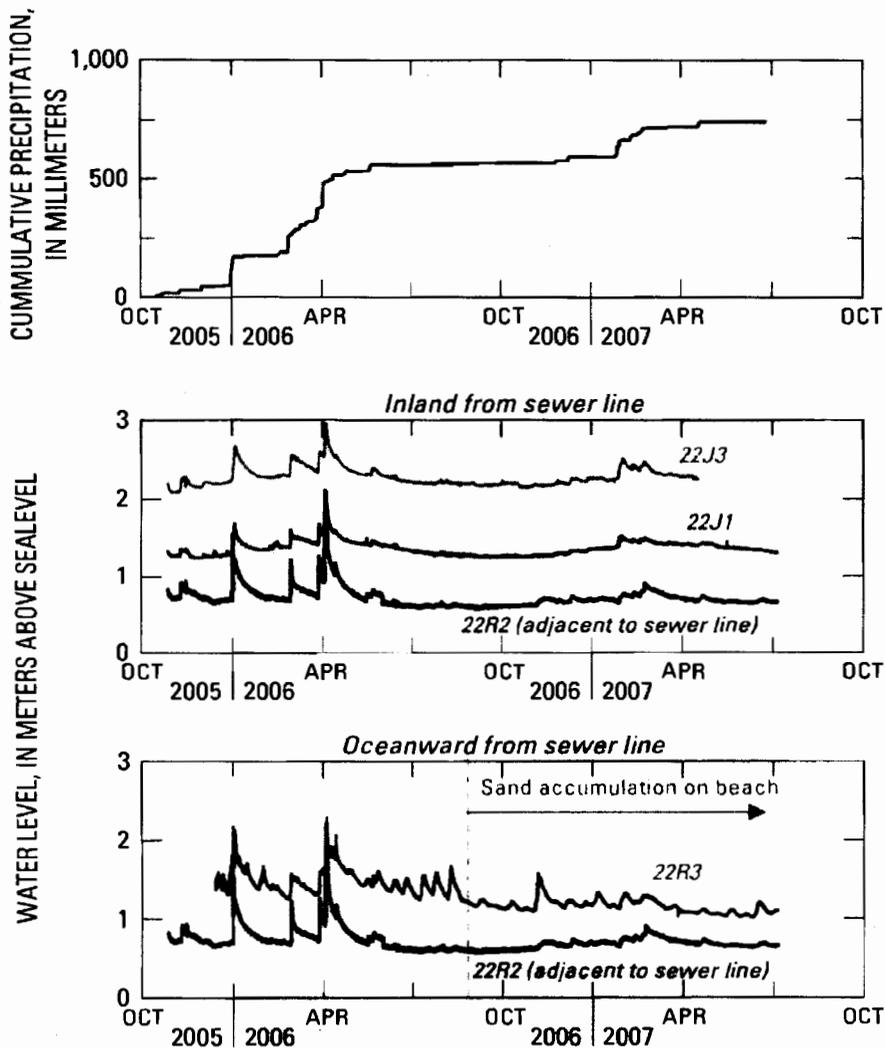


Figure 10 Water-level data in selected wells along section A-A' perpendicular to West Beach, Santa Barbara, California November 2005 to July, 2007.

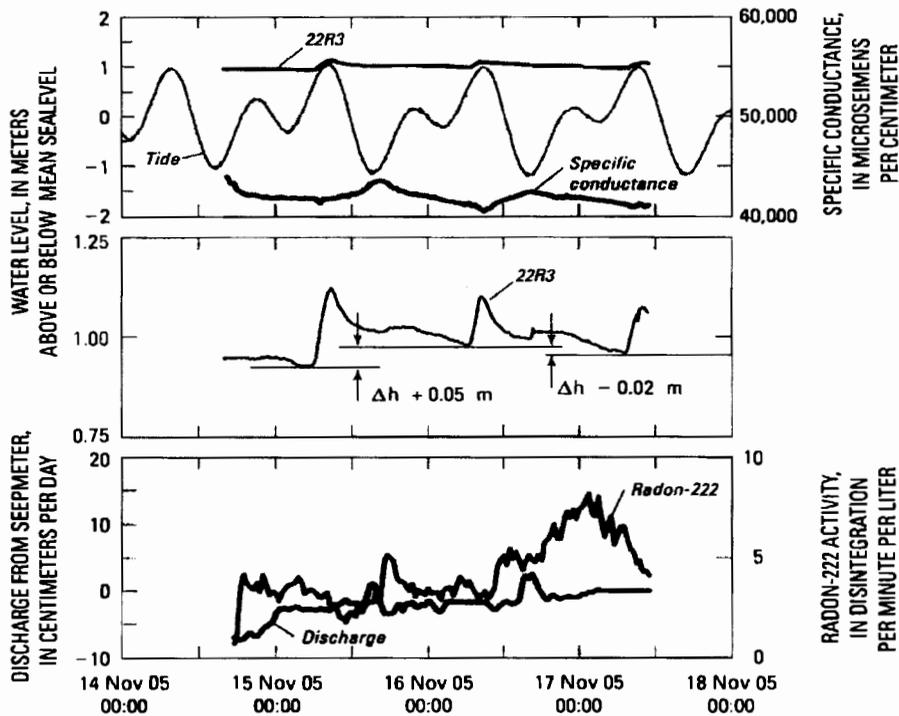


Figure 11 Groundwater levels at well 4N/27W-22R3, tides, groundwater discharge, and specific conductance of groundwater discharge, and radon-222 concentrations in near-shore ocean water, West Beach, Santa Barbara, California, November 14-18, 2005.

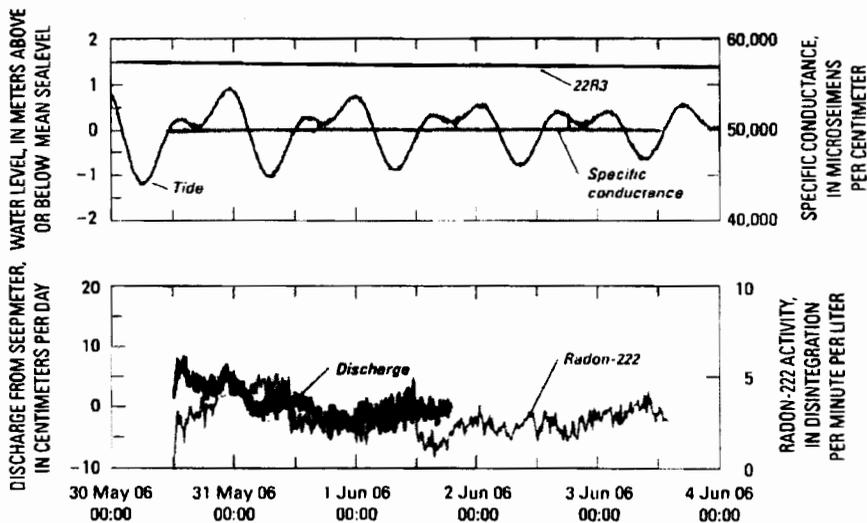


Figure 12 Groundwater levels at well 4N/27W-22R3, tides, quantity, direction, and specific conductance of groundwater discharge, and radon-222 concentrations in near-shore ocean water, West Beach, Santa Barbara, California, May 30-June 4, 2006.

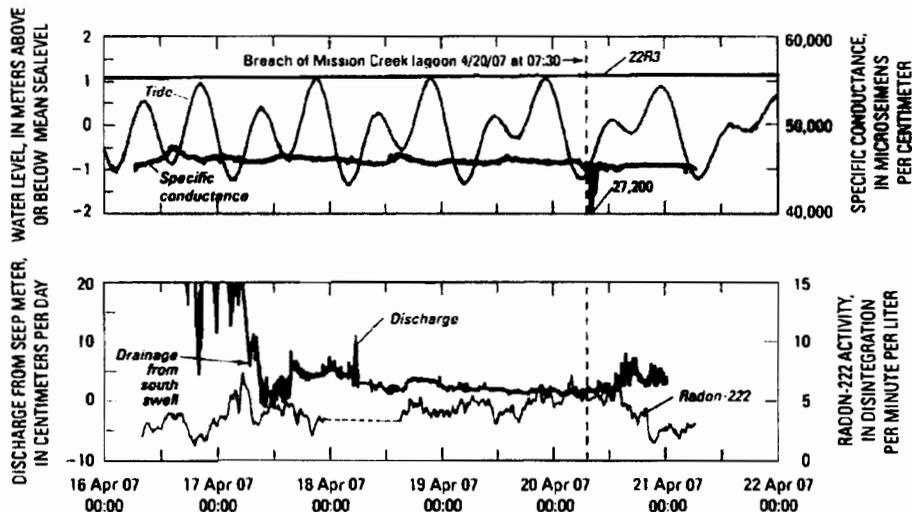


Figure 13 Groundwater levels at well 4N/27W-22R3, tides, quantity, direction, and specific conductance of groundwater discharge, and radon-222 concentrations in near-shore ocean water, West Beach, Santa Barbara, California, April 16-22, 2007.

3.5.2 Seepmeter and Radon-222 Data

Seepmeter and radon-222 (^{222}Rn) data were collected during two spring tides, and during a neap tide to assess the magnitude, variability, and timing of the exchange of shallow groundwater with near-shore ocean water. ^{222}Rn data were used in conjunction with seepmeter data to address spatial variability and to cover potential data gaps resulting from disturbance of the meters during deployment. Specific conductance was measured in water from the seepmeter to assess changes in salinity as shallow groundwater exchanged with ocean water. Although the water-level gradient from the ocean to the sewer line was inland, water levels measured in well 22R3 during the three measurement periods were always higher than the high tide, indicating the potential for groundwater discharge at the beachfront (Figures 11-13). Groundwater discharge and nutrient fluxes to near-shore ocean water along West Beach estimated from seepmeter data and ^{222}Rn data collected as part of this study are discussed in detail by Swarzenski and Izbicki [76].

Discharge from EM seepmeter data measured during November 14-17, 2005 reflect net infiltration of water from the ocean into the beach prior to the spring tide followed by net discharge of water to the ocean after the spring tide (Figure 11). These values

correspond with increasing and decreasing water levels in well 22R3 (Δh , on Figure 11). The largest magnitude positive values were measured during low tide, reflect the largest discharge of water from the beach to the ocean (Figure 11).

During the November, 2005 measurement period, discharge data were collected using seepmeters at two different depths to determine if there was a difference in groundwater flow with depth. The shallowest seepmeter (data shown on Figure 11) was placed at the low tide line and the second meter was placed about 1 m below the low tide line. The deeper meter recorded negative values throughout the period, indicating movement of water from the ocean into the beach (data not shown). This difference in water movement with depth is believed to be the result of density-driven flow driving circulation between ocean and beach deposits, even as groundwater discharges from beach sands to the ocean at shallower depths [77]. The data suggest that exchange of shallow groundwater with near-shore ocean water driven by tidal forces extends from the high tide line to a depth of less than 1 meter below the low tide line (Figure 5).

Discharge data collected during neap tide, May 31 to June 31, 2006 (Figure 12) show smaller magnitude discharges from the beach to the near-shore ocean throughout the daily tidal cycle. These discharges cease as the neap tide approached and the monthly

tidal cycle changed toward higher amplitude tides.

During the April 16-22, 2007 period, seepmeter data showed water moving from the beach sands into the ocean (positive values) throughout almost the entire measurement period irrespective of the daily tidal cycle (Figure 13). The greatest discharge, exceeding 300 cm/d, was measured on April 16, 2007 (not shown on Figure 13). These high values result from drainage of water driven into the beach sands by waves during a south swell prior to the measurement period.

^{222}Rn activities in near-shore ocean water along West Beach ranged from 0.6 to 8 dpm/L (disintegrations per minute per liter) (Figure 11-13). In contrast, ^{222}Rn activities in wells along West Beach were as high as 1,300 dpm/L with a median activity of 610 dpm/L. Low ^{222}Rn activities in near-shore ocean water at West Beach are consistent with water-level and seepmeter data that show little net groundwater discharge to the ocean. ^{222}Rn activities measured in near-shore ocean water along West Beach are almost an order of magnitude lower than values in areas where groundwater is actively discharging to the ocean [72], and activities were similar to values measured in areas where beach deposits are underlain by impermeable crystalline rock that conduct only small amounts of groundwater to the ocean [62].

Despite the low values, ^{222}Rn activities were positively correlated with groundwater discharge data from seepmeters, and ^{222}Rn activities in the near-shore ocean increased during the lowest daily tide (Figures 11 and 12). However, the maximum ^{222}Rn activity lagged the peak discharge measured by the seepmeter by several hours, possibly as water having longer contact with beach sediments and therefore higher ^{222}Rn activities discharged to the ocean (Figure 11). Similar lags between groundwater discharges and peak ^{222}Rn activities are apparent in data from the Florida, Mediterranean and Brazilian coasts [62,78]. Abrupt decreases in ^{222}Rn activity were measured on the turning tide as near-shore ocean water containing a high fraction of discharging groundwater was displaced by ocean water on the incoming flood tide (Figure 12). Over the monthly tidal cycle, ^{222}Rn activities in near-shore ocean water increased after the spring tide as groundwater having longer contact time with beach sediments discharged to the ocean (Figure 11).

Low ^{222}Rn activities were associated with the high groundwater discharges measured after a south swell on April 16-17, 2007 (Figure 10). These low ^{222}Rn activities probably result from drainage of ocean water only recently infiltrated into beach sands by

wave action. This water had not been in contact with beach sand long enough to equilibrate with ^{222}Rn derived from radioactive decay of ^{226}Ra sorbed on the sands. Increased specific conductance during low tides during this period is consistent with the discharge of recently infiltrated ocean-water (Figure 13). Similar increases in specific conductance of near-shore ocean water, measured during low tide on November 14-18, 2005 (Figure 11), suggest that this type of wave-driven exchange occurs frequently.

3.5.3 Exchange of Water at the Beachfront and Fecal Indicator Bacteria Concentrations

Measurements of FIB concentrations during the ebb of the spring and neap tides, coupled with physical and isotopic data collected at the ocean-beach interface, were used to understand the variation, timing and sources of FIB to near-shore ocean water. If groundwater were a source of FIB, on the basis of seepmeter and ^{222}Rn data the highest FIB concentrations would be expected on a daily basis shortly after low tide, with monthly maxima at neap tide when discharge from the beach sand to the ocean is greater.

FIB concentrations varied by as much as 2-orders of magnitude during the sampled ebb tides and the timing of the measured increases in FIB concentrations were consistent with contributions from the beach (Figures 5-7). However, groundwater has low FIB concentrations. Another source of FIB, capable of delivering high concentrations to near-shore ocean water during the ebb tide and turning tides, must be present along West Beach.

Kelp, and guano contaminated sands on West Beach near the high tide line contain high concentrations of FIB (Table 3). Drainage from these materials after the high tide may be a potential source of FIB. However FIB from these sources cannot explain low levels of human-specific *Bacterioides* that were consistently present in near-shore ocean water at West Beach. Because *Bacterioides* were not detected in kelp or guano contaminated beach sand other human-derived sources also contribute to FIB concentrations at West Beach. The potential for FIB from these sources is discussed in greater detail in the following section.

3.5.4 Direct-current Resistivity Data

Direct-current resistivity data were collected from a boat along West Beach, near the mouth of Mission Creek, and along the beach to the east. The data were

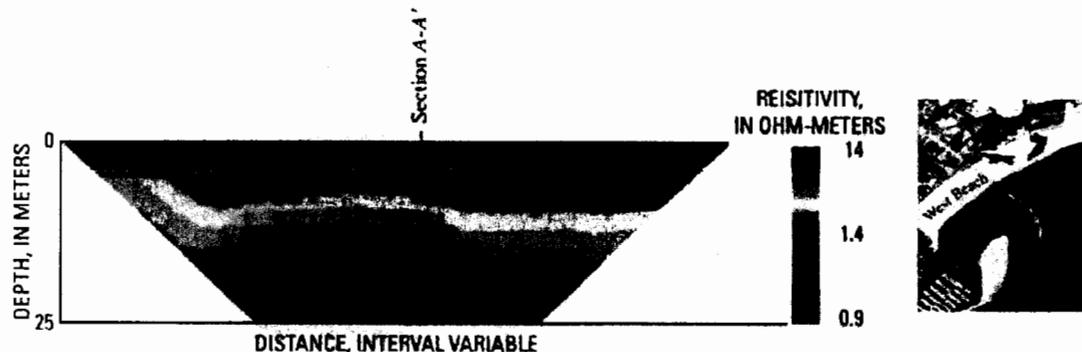


Figure 14 Shore parallel direct-current resistivity data, offshore from West Beach, Santa Barbara, California, November 15, 2005.

used to assess the variability of subsurface lithology and pore fluid resistivity off West Beach, and to evaluate the representativeness of data collected at Section A-A' to other areas along the beachfront.

Shore parallel direct-current resistivity data collected offshore from West Beach show lower resistivity (high conductance) material at shallow depths beneath the ocean, and higher resistivity (low conductance) material at depths greater than about 10 m (Figure 14). These data agree with results of test drilling, well installation, and sample collection along section A-A' (Figure 8) that show sand containing saline water overlying clay containing fresh water.

The direct-current resistivity data show that the sands thin toward the harbor, but that subsurface conditions are otherwise relatively uniform along West Beach. These data are consistent with diffuse exchange of groundwater and near-shore ocean water and do not show evidence of focused discharge from submarine springs. In contrast, shore parallel direct-current resistivity data collected near the mouth of Mission Creek (data not shown) suggest complex, highly-focused exchange of saline and fresh water through beach sands at the mouth of the stream. Focused discharge of fresh water also was observed near the mouths of streams to the east of Mission Creek.

3.6. Tracers of Fecal Indicator Bacteria Sources

Genetic, molecular, and trace organic tracers were used to evaluate potential sources of FIB collected from different hydrologic settings throughout the study area. Thirty-six samples from surface water (including Mission Creek and its estuary, urban

drains, and stormflow), wells, and near-shore ocean water at West Beach were compared and contrasted. Six additional samples from special sources including the influent to El Estero WWTP, and from kelp and sand collected near the high tide line also were included in this analysis.

Principal Component Analysis (PCA) was used to analyze the tracer data. PCA is a multivariate statistical technique that transforms a set of intercorrelated variables into a new coordinate system. The transformed variables, known as principal components, are uncorrelated linear combinations of the original variables. They have a mean of zero and the same variance as the original data set [79,80]. The values of the principal components are known as scores, and the scores are calculated on the basis of the contribution of each variable to the principal component [81]. The magnitude and direction (plus or minus) of the contribution of each variable to the principal-component score is described by an eigenvector. PCA presents differences in the tracer assemblage that is reflective of differences in the microbial community structure, and allows for a comparison and contrast of different samples [53].

Comparison of results from different tracers is intended to confirm, refine, or refute interpretations derived from individual tracers - thereby producing a more robust interpretation of the sources of FIB in the study area. PCA results were compared to and contrasted with the physical hydrology in the study area to ensure interpretations from tracer data are plausible. Detailed analysis of the contributions of individual eigenvectors to the principal component scores often yields increased understanding of the distribution of these tracers in the environment [11].

However, this level of analysis and subsequent discussion would preclude the comparisons between tracers and to the physical hydrology and were beyond the scope of this paper.

3.6.1 Terminal-Restriction Fragment Length Polymorphism Data

Genetic diversity in microbial populations was assessed using Terminal-Restriction Fragment Length Polymorphism (T-RFLP). T-RFLP uses restriction enzymes to break genetic material within the hypervariable region of mitochondrial DNA into smaller fragments known as amplicons. Amplicons

having different numbers of base pairs (amplicon length) represent different microorganisms. However, the sequence of base pairs within amplicons of the same length may be greatly different, and more than one type of microorganism may be represented. Two restriction enzymes, H-ha1 and M-spl1, were used in this study. Each breaks the mitochondrial DNA at different locations, and produces a different assemblage of amplicons (Figure 15). Quantitative Polymerase Chain Reaction (qPCR) was used to amplify the DNA to measurable concentrations, and the peak area is a measure of the abundance of an amplicon and the microorganism(s) it originated from.

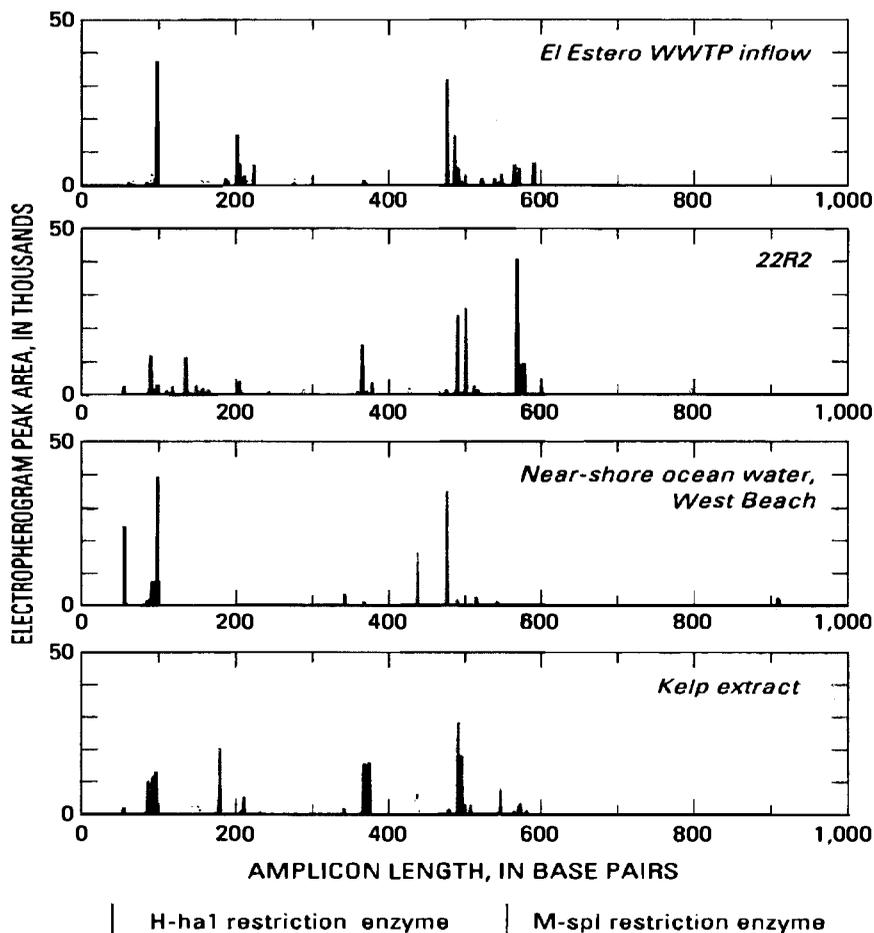


Figure 15 Representative Terminal-Restriction Fragment Length Polymorphism (T-RFLP) amplicons produced using H-ha1 and M-spl1 restriction enzymes from selected samples, Santa Barbara, California, August 2005-April 2007.

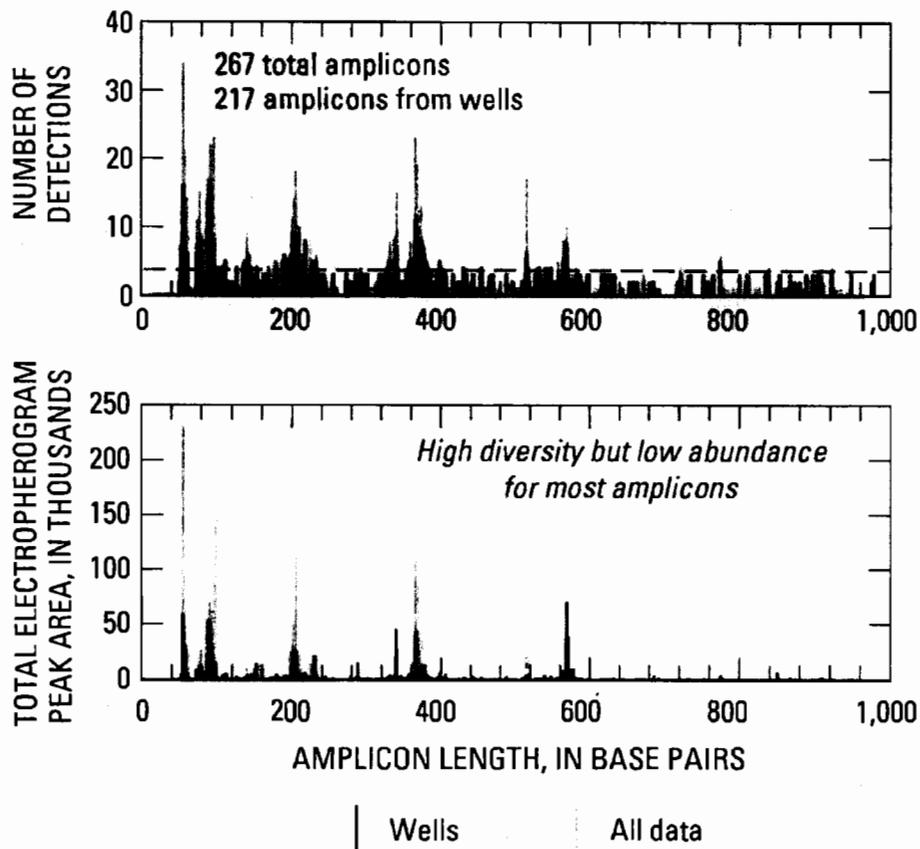


Figure 16 Terminal-Restriction Fragment Length Polymorphism (T-RFLP) H-hal amplicons in samples surface water (including storm drains), near-shore ocean water, water from wells and from selected sources, Santa Barbara, California, August 2005 to April 2007.

Amplicons that appear in more than one sample are common to those samples. Amplicons that appear in only one sample are unique. For example, only H-hal amplicons having 91, 94, and 367 base pairs and M-spl amplicons having 86 and 490 base pairs, respectively, are common to all four samples shown in Figure 15. In contrast, for samples shown in Figure 15, more than 20 amplicons from H-hal and M-spl enzymes were unique to water from the inflow to El Estero WWTP - and presumably represent at least 20 microorganisms not found in the other samples. Comparison, either visually or statistically, of the occurrence and distribution of amplicons from different sources is used to identify similarities and differences in microbial populations from those sources and to infer relations between those sources. As the numbers of samples and the number of

amplicons increases, the problem becomes increasingly complex and a statistical approach such as PCA is needed to analyze the data.

At least 267 amplicons were isolated using the H-hal restriction enzyme and 676 amplicons were isolated using M-spl restriction enzyme in samples collected as part of this study (Figure 16). More than 217 H-hal and 634 M-spl amplicons were present in water from wells, with 21 and 230 amplicons, respectively, unique to water from wells. Although most of these amplicons are present only at low abundances, this result is surprising because groundwater is not normally considered to be rich in its diversity of microorganisms. Examination of the data shows that most of the amplicons (178 amplicons) were detected in water from four wells: 22R2, and 23M1-3. The shallow groundwater in these

areas is recharged by surface runoff (22R2) or infiltration from Mission Creek (23MI-3). These wells also are near the main sewer line beneath West Beach serving that part of the city and may have a small component of sewage. These four wells have higher FIB concentrations than other wells sampled as part of this study.

The large numbers of amplicons detected in water from wells exert a high influence on the PCA results. This is especially true given the large number of unique amplicons in the four wells discussed above. These amplicons caused large magnitude differences

in the principal component scores for T-RFLP data from these wells that differentiated these wells from all other data. Groundwater moves slowly and organisms in groundwater have probably been there for a considerable period of time. The lack of similarity between the data from the four wells and sources of FIB such as stormflow, inflow to the El Estero WWTP, or from kelp and beach sands may result from changes in the microbial community through death and regrowth of different organisms as the microbial community adapts to the groundwater environment.

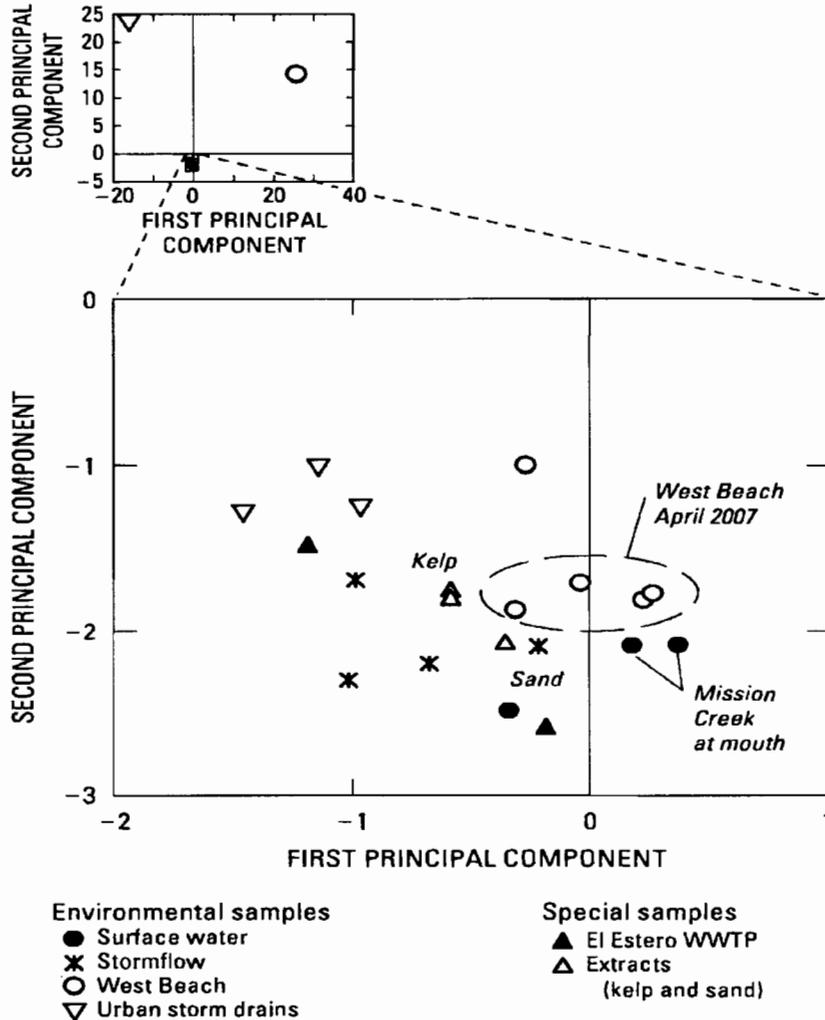


Figure 17 Results of Principal Component Analysis (PCA) for Terminal-Restriction Fragment Length Polymorphism (T-RFLP) data from surface water, near-shore ocean water, inflow to El Estero WWTP, and from selected sources, Santa Barbara, California, August 2005 to April 2007.

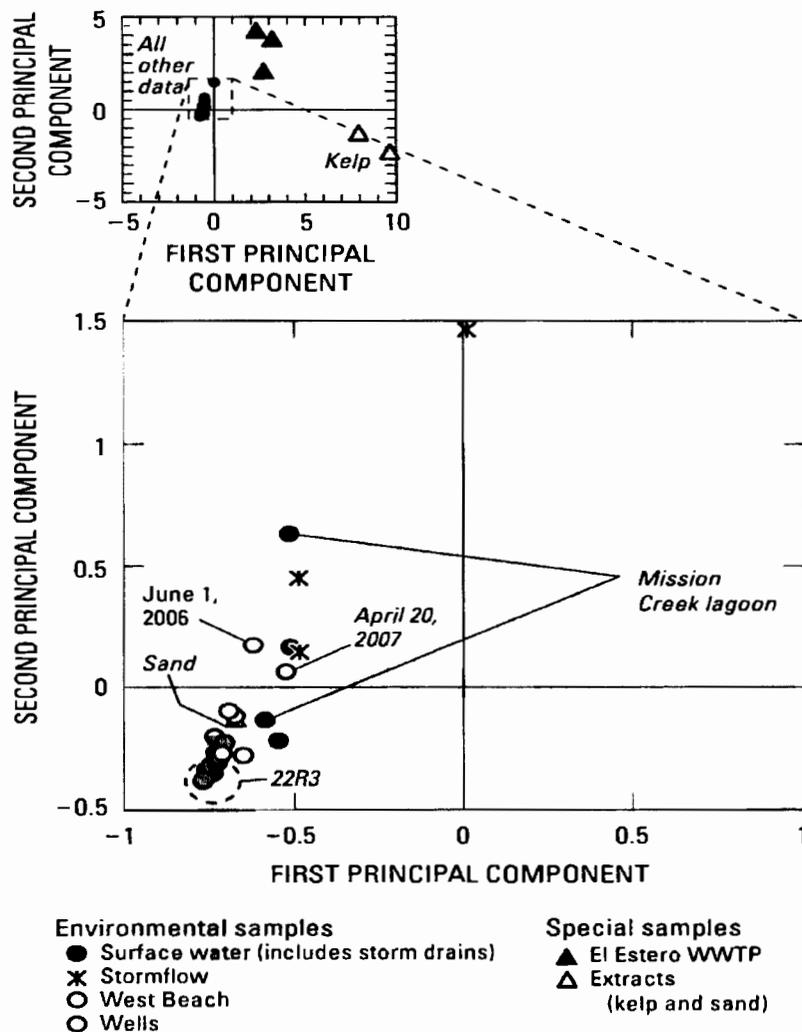


Figure 18 Results of Principal Component Analysis (PCA) for phospholipid fatty acid (PLFA) structural groups in surface water, water from wells, near-shore ocean water, influent to El Estero WWTP, and from selected sources, Santa Barbara, California, August 2005 to April, 2007.

If water from wells is excluded, the first and second principal components for the remaining 23 samples explain 32 percent of the variability in H-hal digested T-RFLP data (Figure 17). The first and second principal components in this smaller data set are dominated by large magnitude scores for samples collected from the Haley Drain and from near-shore ocean water at West Beach. These samples have the highest human-specific *Bacteroides* values sampled as part of this study (Table 2). Although these samples

do not closely resemble sewage influent to the El Estero WWTP, these samples appear to have been impacted by human fecal material.

The remaining samples plot within a comparatively small range on Figure 17. However, PCA preserves the variability of the original data set and differences in principal component scores within this range reflect real differences within the data. Within this small range there was considerable variability PCA scores for samples from influent to the El Estero

WWTP, and those samples span the range in scores for samples from stormdrains and surface water, including stormflow.

The PCA scores for near-shore ocean water at West Beach in April 2007 and from kelp and sand and water from Mission Creek lagoon are similar (Figure 17). Compared to influent to the El Estero WWTP, these samples fall within a small range and are similar to samples collected from the near-shore ocean water at West Beach during the ebb tide and after the breach of the lagoon.

Although not discussed specifically in this paper, results of PCA analysis of M-spl enzyme-digested T-RFLP data were similar to results of the H-hal digested data.

3.6.2 Phospholipid Fatty Acid Data

Fatty acids are components of all living cells. At the cellular level, they may be used for energy storage or they may be part of cellular organelles and structures where they participate in metabolic activity [82]. Individual phospholipid fatty acids (PLFA's) are associated with metabolic activities by a wide-range of microorganisms rather than indicators of specific organisms [83-87]. Because PLFA's contain phosphorus, they are rapidly degraded in the environment and are typically associated with living (or recently living) organisms [56,88].

PLFA concentrations and composition were analyzed on the same samples as T-RFLP data. Total PLFA concentrations ranged from 314 to 17.6×10^6 picomoles per liter (pmole/L) (Figure 18) and 38 individual fatty acids were identified. Higher concentrations reflect higher microbiological biomass. Concentrations were highest in kelp and lowest in water from wells. Total PLFA concentrations in samples from the El Estero WWTP ranged from 2.6×10^6 to 3.2×10^6 pmole/L. Concentrations in surface-water samples from Mission Creek and its tributaries ranged from 8,200 to 320,000 pmole/L, with the highest concentrations measured during stormflow. Concentrations in excess of 100,000 pmole/L also were measured in the lagoon near the mouth of Mission Creek. Concentrations in near-shore ocean water at West Beach ranged from 22,700 to 226,000 pmole/L, with the highest concentrations measured April 20, 2007 after the lagoon at the mouth of Mission Creek breached and discharged to the ocean.

Total PLFA concentrations in water from wells ranged from 314 to 28,000 pmole/L, although concentrations in water from most wells were less than 880 pmole/L. This is consistent with the low T-

RFLP abundance in water from wells. Over the very broad range of concentrations sampled, PLFA concentrations were positively correlated with FIB concentrations ($r = 0.43$). PCA analysis of the PLFA concentrations was simplified by grouping the fatty acids according to their structure into saturated, monounsaturated, branched saturated fatty acids, terminally branched saturated fatty acids, mid-chain branched fatty acids, and polyunsaturated fatty acids [86]. The concentration of each structural group was used to calculate the principal component scores and eigenvectors to provide a simplified analysis of the changes in PLFA concentration and composition of microbial communities from different sources. These structural groups, and the fatty acids within those groups, are commonly associated with metabolic functions common to a wide range of organisms sharing similar environments (for example anaerobic versus aerobic) or metabolizing similar substrates rather than specific species. Use of structural groups for PCA analysis was very robust and the first and second principal components explained 97 percent of the total variance within the data set (Figure 18). Unlike the PCA analysis of T-RFLP data, it was not necessary to exclude water from wells to obtain interpretable results.

Kelp and samples of influent to the El Estero WWTP had highly positive first principal component scores but differed in magnitude in their second principal component scores - reflecting the different microbial communities residing in the two sources (Figure 19). Unlike T-RFLP data, principle component scores show little variability in samples from influent to the El Estero WWTP (Figures 17-18) suggesting that although the specific microorganisms within sewage may vary greatly, the metabolic processes they carry out in this environment are relatively constant and therefore sewage contamination would be traceable on the basis of PLFA compositions. Also unlike T-RFLP data, principle component scores show little similarity between kelp and near-shore ocean water at West Beach (Figures 17 and 18). This result suggests that although kelp on West Beach contains high concentrations of FIB, it is not the primary source of FIB to near-shore ocean water. In contrast, the composition of PLFAs in sand was very similar to PLFAs in near-shore ocean water, suggesting FIB in sand is a likely source of fecal bacteria to near-shore ocean water.

Principal component scores of stormflow samples tend toward the PLFA composition in samples from influent to the El Estero WWTP. This similarity decreased later in the stormflow, suggesting the

possible presence of wastewater in initial stormflow runoff. However, wastewater indicator data, discussed later in this paper, show that El Estero WWTP influent was not present in the stormflow samples. Animal or human wastes (from leaking laterals or the homeless populations) released directly to the environment and washed into the stream during stormflow may be the cause of PLFA composition in stormwater samples collected early in the storm.

The PLFA composition of samples of near-shore ocean water collected from West Beach varied with discharge from Mission Creek. PLFA scores from West Beach samples collected when Mission Creek was discharging to the ocean (June 1, 2006 and April 20, 2007) were more similar to water sampled from the estuary at the mouth of the creek (Mission Creek lagoon). In contrast, the PLFA composition of samples from West Beach collected when Mission Creek lagoon was not discharging to the ocean were more similar to the PLFA composition of guano

contaminated beach sand and less similar to water from the creek. As previously discussed, the PLFA composition of near-shore ocean water showed little similarity to the PLFA composition of kelp (Figure 18). However, it is important to remember that the kelp is not the source of FIB, but rather fecal material deposited on the kelp by birds feeding along the shoreline or other sources are the source of FIB. The PLFA signature of the bird droppings may be overwhelmed by the large microbial populations residing on the kelp.

PCA also was conducted using the concentrations of the 38 individual fatty acids identified in samples from this study (results not shown). This analysis also was very robust and the first, second, and third principal components explained 91 percent of the variability within the data set. Interpretations derived from PCA of the individual fatty acids were similar to interpretations derived from analysis of the structural groups.

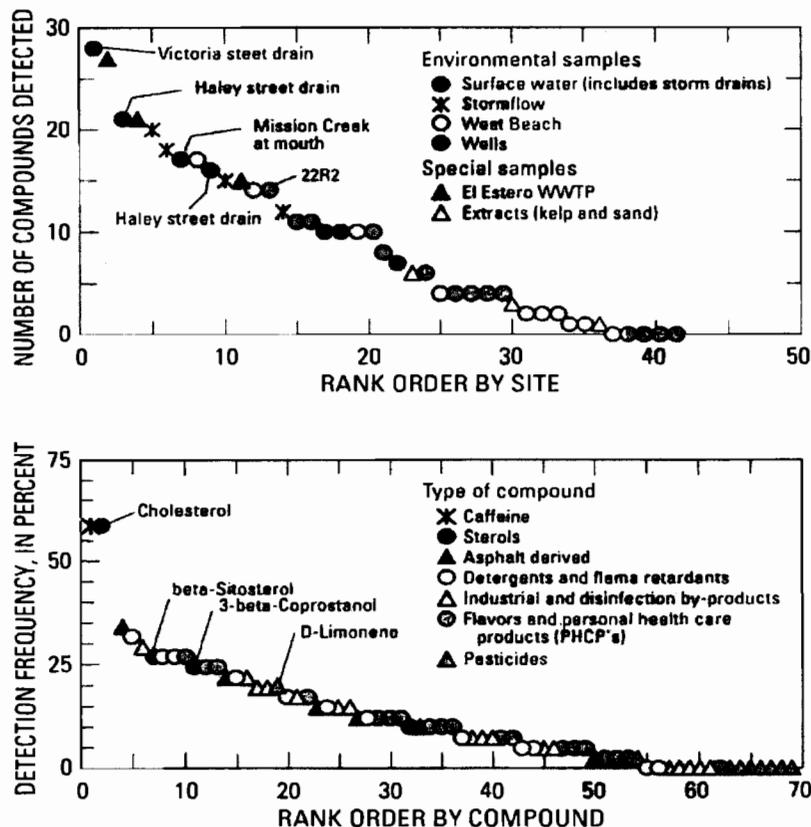


Figure 19 Trace organic compound abundance in surface water, water from shallow wells, near-shore ocean water, and from selected sources, Santa Barbara, California, August 2005 to April 2007.

3.6.3 Trace Organic Compounds

A suite of 69 organic compounds was measured as part of this study to help identify the source or sources of FIB in the near-shore ocean water. The compounds can be divided into a number of categories on the basis of their use and origin (Table 4). Reporting limits for most analyzed compounds were within the part per trillion range, and concentrations were below thresholds for public health or environmental concerns. Compounds analyzed as part of this study are anthropogenic and do not occur naturally. Data are available on the U.S. Geological Survey on-line data base NWIS-Web.

At least one trace organic compound was detected in 88 percent of all samples. Not surprisingly, compounds were detected more frequently and at the higher concentrations in samples of wastewater influent to the El Estero WWTP (Figure 19). Large numbers of compounds also were detected in urban stormdrains and stormflow samples, and in near-shore ocean water at West Beach following the April 20, 2007 stormflow and subsequent discharge from the lagoon at the mouth of Mission Creek. Smaller numbers of compounds were detected in kelp and guano contaminated sands. Almost two-thirds of samples from wells had two or fewer compounds detected, and no compounds were detected in almost 25 percent of sampled wells. However, more than ten compounds were present in water from wells 22R2 and 23M2. These wells were discussed previously because of their high FIB concentrations, high diversity of microorganisms, and connection with surface sources of contamination.

Caffeine and cholesterol were the most commonly detected compounds and were present in almost 60 percent of the environmental samples (Figure 19). Caffeine and the various sterols were positively correlated, and these compounds were positively correlated with many personal-care products, flame retardants, flavors/fragrances, and d-limonene. In contrast, caffeine and sterols were poorly correlated with most industrial, and asphalt-derived compounds. Caffeine is associated with human use and consumption. Caffeine concentrations in samples from the El Estero WWTP influent ranged from 18 to 84 $\mu\text{g/L}$. High concentrations, 8 and 4 $\mu\text{g/L}$, also were measured in the Cabrillo and Haley Street storm drains tributary to Mission Creek, respectively. As previously discussed, both sites had measurable human-specific *Bacteroides*. Caffeine concentrations in samples from kelp were as high as 67 $\mu\text{g/L}$. This caffeine concentration was surprisingly high, although

these samples contained high concentrations of FIB, neither sample contained human-specific *Bacteroides*.

Sterols tend to degrade under aerobic conditions and have been interpreted as evidence of recent fecal contamination [89]. Four sterols (listed in order of abundance) were measured as part of this study, cholesterol, beta-sitosterol, 3-beta-coprostanol, and stigmastanol. Cholesterol is associated with wide range of sources, including human dietary cholesterol, but is not necessarily fecal in origin. 3-beta-coprostanol is a fecal sterol produced in the gut of some mammals (including humans, pigs, and cats) by the microbially mediated reduction of cholesterol under anaerobic conditions. Although the sterol content of bird feces can be highly variable, they do not generally contain the proper bacteria to reduce cholesterol to 3-beta-coprostanol [90]. The absence of 3-beta-coprostanol in kelp and guano contaminated sand from West Beach is consistent with fecal contamination from birds rather than human or other mammalian fecal material (Table 5). Although 3-beta-coprostanol was absent, cholesterol and beta-sitosterol were present in samples of kelp- and guano-contaminated beach sands. Beta-sitosterol occurs in some plants and, as a consequence, in human dietary cholesterol and in the gut of birds.

At least one personal health-care product, detergent metabolite, flame-retardant, or asphalt-derived compound was present in 75 percent of environmental samples, although many compounds within these groups were detected only infrequently or not at all.

Most pesticides were notably absent compared to their abundance in urban surface water and stormflow in other parts of California [91]. However, d-limonene, an "environmentally-friendly" substitute for traditional pesticides used for termite control, was detected in 20 percent of environmental samples. Low pesticide detections may reflect local restrictions on pesticide use and sales.

There was concern that high concentrations of FIB present during stormflow in Mission Creek could result from discharge of wastewater from sewer lines near the stream if those lines flow under pressure during stormflow. This discharge might not occur during low-flow conditions. PCA analysis of PLFA data suggest there may be such a connection. However, stormflow samples collected from Mission Creek at Gutierrez Street did not contain detectable levels of the sterols 3-beta-coprostanol, beta-sitosterol, and beta-stigmastanol, but all of these sterols are present at high concentrations in wastewater. In contrast, stormflow samples contained high concentra-

tions of the detergent metabolites NPEO-1 and NPEO-2. These compounds were not present in wastewater influent to the El Estero WWTP. Furthermore, the detergent metabolite 4-nonylphenol, which is present

at high concentrations in wastewater, was absent in stormflow from Mission Creek. These data are not consistent with direct discharge of sewage to Mission Creek from leaking sewer pipes during stormflow.

Table 4 Detections of trace organic compounds in surface water (including storm drains), water from wells, near-shore ocean water, wastewater treatment plant influent, and other sources, Santa Barbara, California August 2005-April 2007. [Concentrations are in micrograms per liter. WWTP, wastewater treatment plant; MC, Mission Creek; (sf), stormflow sample. Environmental samples are the sum of surface water, wells and West Beach samples.]

Compound	Report ing level	Surface water (11)	Water- table wells (17)	West Beach (7)	Environ- mental samples (35)	El Estero WWTP influent (3)	Extract samples (3)	Maximum concentrat- ion	Source
Caffeine	1	8	6	4	18	3	3	83.3	El Estero WWTP
Sterols									
3-beta-Coprostanol	2	3	3	1	7	3	0	308	El Estero WWTP
Beta-Sitosterol	2	3	1	1	5	3	3	42.8	El Estero WWTP
Beta-Stigmastanol	2	1	6	0	7	3	3	6.0	El Estero WWTP
Cholesterol	2	7	6	5	18	3	3	244	El Estero WWTP
Flavors and fragrances									
Acetophenone	0.5	2	2	0	4	0	0	1.46	Cabrillo drain inflow
Benzophenone	0.5	0	1	1	2	3	0	0.793	El Estero WWTP
Galaxoide (HHCB)	0.5	0	0	0	0	2	0	3.6	El Estero WWTP
Indole	0.5	1	0	0	1	1	3	10.6	El Estero WWTP
Isoborneol	0.5	0	0	0	0	1	0	2.4	El Estero WWTP
Menthol	0.5	2	1	0	3	2	0	22.5	El Estero WWTP
Menthyl-1H-indol	1	0	1	0	1	2	0	4	El Estero WWTP
Triethyl citrate	0.5	0	0	0	0	1	0	0.9	El Estero WWTP
Tonalide		1	0	0	1	1	0	0.9	El Estero WWTP
Personal health-care products									
Camphor	0.5	6	0	3	9	1	0	1.7	El Estero WWTP
3,4-Dichlorophenyl isocyanate	0.5	2	1	1	4	0	0	10.5	4N/27W-22R2
1,4-Dichlorobenzene	0.5	0	0	1	1	1	0	0.7	El Estero WWTP
Carbazole	0.5	1	0	0	1	0	0	0.074	MC at Gutierrez (sf)
DEET	0.5	3	5	0	8	2	0	1.01	El Estero WWTP
Naphthalene	0.5	2	4	0	6	2	0	1.29	4N/27W-21G4
Pentachlorophenol	2	2	0	2	4	0	0	0.62	West Beach
Tricolsan	1	1	0	0	1	2	0	4	El Estero WWTP
p-Cresol	1	5	1	1	4	3	1	70.6	El Estero WWTP
Detergent metabolites									
4-n-Octylphenol	1	1	0	0	1	1	0	0.75	El Estero WWTP
4-tert-Octylphenol	1	0	0	0	0	1	1	0.75	El Estero WWTP
Diethoxynonyl- phenol NPEO-2	5	9	1	0	10	1	0	77.5	Victoria drain
Diethoxynonyl- phenol OPEO-2	1	3	0	1	4	0	0	0.8	MC at Gutierrez (sf)

Table 4 (continued) Detections of trace organic compounds in surface water (including storm drains), water from wells, near-shore ocean water, wastewater treatment plant influent, and other sources, Santa Barbara, California, August 2005-April 2007. [Concentrations are in micrograms per liter. WWTP, wastewater treatment plant; MC, Mission Creek; (sf), stormflow sample. Environmental samples are the sum of surface water, wells and West Beach samples.]

Compound	Reporting level	Surface water (11)	Water-table wells (17)	West Beach (7)	Environmental samples (35)	El Estero WWTP influent (3)	Extract samples (3)	Maximum concentration	Source
Ethoxynonylphenol NPOE-1	2	6	0	0	6	1	0	17.6	Victoria drain
Ethoxyoctylphenol OPEO-1	1	2	0	0	2	1	0	3	El Estero WWTP
4-Nonylphenol	5	3	4	0	7	3	1	24.8	El Estero WWTP
Flame retardants									
Tris-2-butoxyethylphosphate	0.5	5	3	3	11	2	0	6.38	El Estero WWTP
Tris-2-chloroethylphosphate	0.5	5	1	1	7	2	0	0.201	El Estero WWTP
Tris-dichloroisopropylphosphate	0.5	2	2	0	4	2	0	0.49	Victoria drain
Asphalt derived compounds									
Methylnaphthalene	0.5	2	1	0	3	1	0	0.186	4N/27W-21G4
2,6-Dimethylnaphthalene	0.5	0	1	0	1	0	0	0.115	4N/27W-21G4
2-Methylnaphthalene	0.5	3	1	0	4	1	0	0.31	4N/27W-21G4
Benzo(a)pyrene	0.5	1	1	1	3	2	0	0.17	Haley drain
Fluoranthene	0.5	9	1	2	12	2	0	0.9	Haley drain
Phenanthrene	0.5	6	1	0	7	2	0	0.31	El Estero WWTP
Pyren	0.5	9	1	2	12	1	0	0.5	El Estero WWTP
Pesticides, insecticides, and herbicides									
Carbaryl	1	1	0	0	1	0	0	0.32	Victoria drain
d-Limonene	0.5	4	0	1	5	2	0	18	El Estero WWTP
Industrial compounds									
Anthraquinone	0.5	3	0	0	3	0	0	0.37	MC at Gutierrez
Bisphenol-A	0.1	3	5	2	10	1	0	69.3	MC at mouth
Bis-2-ethylhexylphthalate	2	2	3	3	8	1	0	49.9	Victoria drain
Diethylphthalate	0.5	1	3	0	4	3	0	15	El Estero WWTP
Isophorone	0.5	1	1	0	2	0	0	0.14	Cabrillo drain inflow
Methyl salicylate	0.5	2	0	0	2	1	0	1.6	El Estero WWTP
Phenol	0.5	1	1	0	2	2	2	38	El Estero WWTP
Triphenyl phosphate	0.5	2	0	0	2	1	0	0.14	El Estero WWTP
5-Methyl-1H-benzotriazole	2	0	0	1	1	1	0	30	West Beach
Tetrachloroethene	0.5	3	1	2	6	2	0	0.48	MC at mouth
Tribromomethane	0.5	1	0	5	6	0	2	0.588	Kelp extract

Table 5 Summary of caffeine and fecal sterol data for surface water, wells, near-shore ocean water, El Estero wastewater treatment plant, and extracts from kelp and sand, Santa Barbara, Calif. August 2005 to April 2007. [All concentrations in micrograms per liter. Number is maximum concentration, number in parenthesis is number of detections. If constituent detected in all samples minimum and maximum values are given. --, not detected.]

	Number of samples	Caffeine	Cholesterol	3-beta-Coprostanol	beta-Sitosterol	beta-Stigmasterol
Environmental samples						
Wells	17	0.93 (6)	1.6 (6)	0.95 (3)	0.82 (1)	--
Near-shore ocean water, West Beach	7	0.48 (4)	2.0 (5)	1.0 (1)	--	--
Surface water, includes stormflow and urban drains	11	8.0 (8)	4.3 (6)	4.1 (3)	2.9 (3)	1.2 (1)
Other samples						
El Estero WWTP	3	18-84	43-244	20-309	2.9-43	1.2 (1)
Kelp extracts	2	67-68	3.6-7.2	--	1.8-2.0	0.86 (1)
Sand extracts	1	0.28	28	--	5.2	0.81
^a Reporting limit	41	0.2	0.8	0.8	0.8	0.8
^a Lowest detection	41	0.03	0.2	0.62	0.82	0.81

^aReporting limit is the lowest defensible, quantification of an analyte concentration. The value is usually set near the lowest calibration standard during method development for Environmental Laboratory Approval Program (ELAP) certification. The detection limit is the lowest reasonable estimate of the presence of an analyte. The detection limit is variable depending on instrument setting and sample matrix effects and is greater than 10 percent of the spiked concentration in a sample run. (California Department of Health Services, <http://www.dhs.ca.gov/ps/ls/elap/pdf/MethodDetectionLimits.pdf>, accessed December 4, 2007)

However, the results are consistent with FIB contributions from non-point sources indicated by changing FIB concentrations during successive stormflows (Figure 4).

The distribution of trace organic compounds was analyzed using PCA in the same manner as T-RFLP and PLFA. The first principal component explained 20 percent of the variability in the data, while the second principal component explained 14 percent. Unsurprisingly, influent to the El Estero WWTP had highly positive first principal component scores and large magnitude (positive and negative) second principal component scores (Figure 20). The range in second principal component scores suggests that concentrations of some trace organic compounds varied widely in influent to the WWTP. As a group, the personal health care products and industrial compounds (Table 4) had the largest magnitude second principal component eigenvectors, suggesting that their use and discharge to the sewers may be highly variable.

Examination of the distribution of the first and

second PCA scores (Figure 20) shows that stormflow samples plot on a mixing line with water from the Haley Street drain just upstream from the Gutierrez Street collection site. In contrast, runoff along Cabrillo Street exerts a strong influence on the trace organic composition of water from shallow water-table wells along Cabrillo Street (22J1) in the beach where this water is discharged (22R2). With the exception of samples collected after the breach of Mission Creek, the trace organic compounds in shallow groundwater from well 22R3 were nearly indistinguishable from near-shore ocean water along West Beach.

4. DISCUSSION

This study addressed fecal contamination from a variety of different sources to urban streams and near-shore ocean water near Santa Barbara, California. Streamflow measurements, water-level data, samples from wells, seepmeter and radon-22 measurements of groundwater discharge were effective at evaluating the

movement of water and consequently important for determining the sources of fecal contamination. These data were supplemented with genetic (T-RFLP), molecular (PLFA), and chemical tracers (including caffeine, fecal sterols, and detergent metabolites) to identify similarities and differences between samples

collected from different sources during this study.

At the beginning of this study, leaking sewer lines and laterals connecting homes to sewer lines were believed to be an important source of FIB in much of the older residential area underlying the City of Santa Barbara.

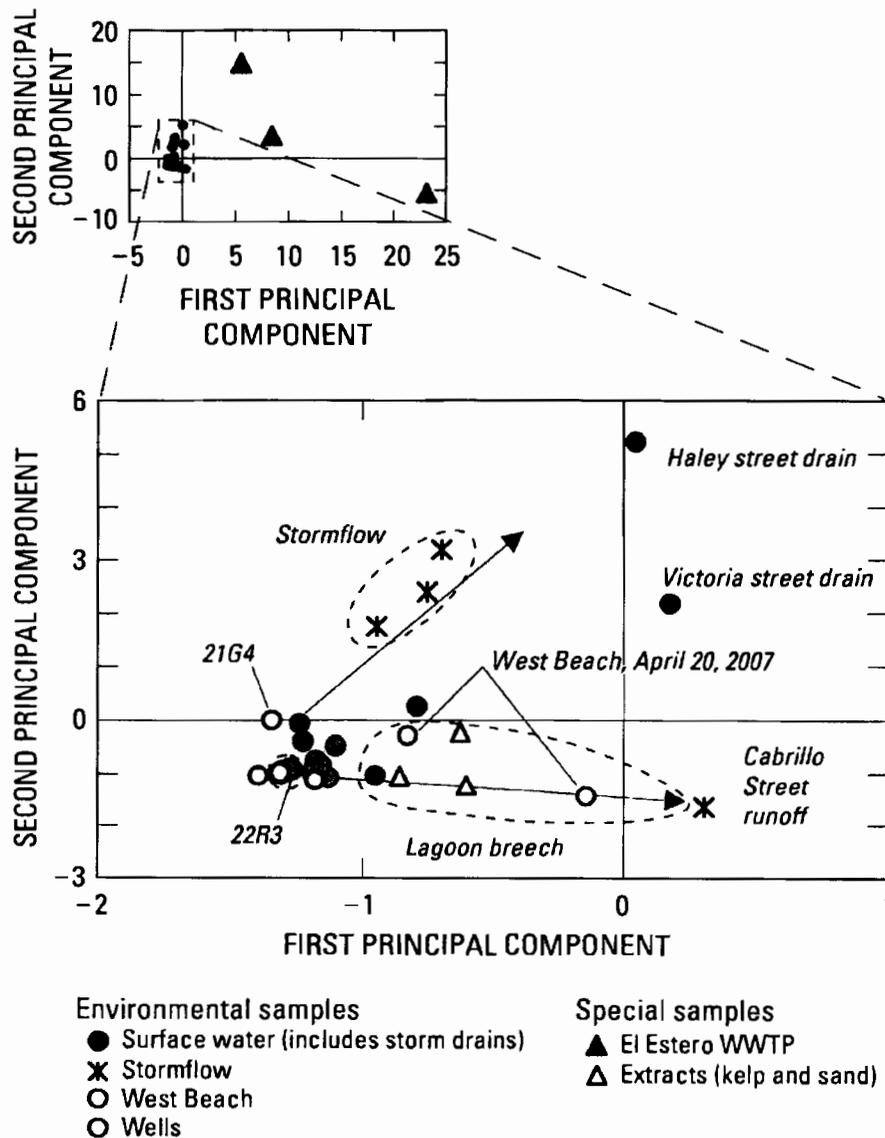


Figure 20 Results of Principal Component Analysis (PCA) for selected trace organic compounds in surface water (including urban stormdrains), water from wells, near-shore ocean water, influent to El Estero WWTP, and from selected sources, Santa Barbara, California, August 2005 to April 2007.

The absence of FIB in water-table wells installed in the urban area suggests that while leaking laterals may be locally important, they have not resulted in areally extensive FIB contamination of groundwater. Consistent with this result, synoptic measurements of streamflow and FIB concentrations along Mission Creek showed urban drains tributary to Mission Creek contributed more to the FIB concentrations than groundwater discharge during dry periods. The presence of human-specific *Bacteroides* in some urban drains, especially the Haley Street drain, is indicative of human fecal contamination. This is consistent with recent results showing dry weather flows can contribute fecal contamination in urban areas [92].

Comparison of FIB concentrations in streamflow over a diurnal cycle indicated that FIB concentrations varied in a manner consistent with runoff from lawn watering and other urban flows. The lack of correlation in streamflow and FIB concentrations with WWTP inflows suggests that direct leakage from sewer lines into the stream is not the source of FIB concentrations during baseflow. FIB concentrations collected during successive stormflows decreased with runoff, consistent with contributions from non-point sources within the urban watershed.

The highest FIB concentrations in near-shore ocean water at West Beach were associated with stormflow discharges from nearby Mission Creek. High FIB concentrations persisted in near-shore ocean water for several days after the stormflow discharges to the ocean. This is consistent with a wide range of studies that show stream discharges can contaminate near-shore ocean water for considerable distances from the discharge point [11, 18-22]. However, during dry periods FIB concentrations in near-shore ocean water increased consistently during the ebb of sampled spring and neap tides. Groundwater discharge measured by seepmeters and ^{222}Rn data was small [76] and because groundwater in wells on the beach did not contain high concentrations of FIB, discharging groundwater cannot explain near-shore ocean beach FIB concentrations. This is different from a number of recent studies that suggest groundwater is a possible source of fecal contamination to near-shore ocean water [23-25]. The timing of high FIB concentrations in the near-shore ocean water can be explained, in part, as drainage from kelp and guano-contaminated sand having high FIB concentrations at the high-tide line, consistent with work showing kelp and sand along protected beach areas may harbor FIB [14,48]. However, high FIB concentrations in kelp and sand cannot explain the consistent low-levels of human-specific *Bacteroides* detected in near-shore ocean

water at West Beach and additional sources of FIB contamination also must be present. Human-specific *Bacteroides* in near-shore ocean water at West Beach may be associated with discharges from urban streams [92].

Genetic, molecular, and chemical data provided additional information on the sources of FIB and support interpretations derived from traditional hydrologic data. Principal Component Analysis (PCA) was used to interpret these data and identify similarities and differences between samples from different sources. All three types of data showed a high degree of similarity between samples from Mission Creek and samples collected in near-shore ocean water at West Beach, and suggest a common source of FIB at West Beach when Mission Creek is discharging to the ocean. The most robust PCA results were from molecular (PLFA) data, which captured 97 percent of the total variance in the data set within the first and second principal components. In contrast, the PCA analysis for T-RLFP data explained only 32 percent of the total variance within the first and second principal components. Neither genetic, molecular, nor chemical tracers show a strong similarity between samples from influent to the El Estero WWTP and samples collected in near-shore ocean water along West Beach. This is consistent with water-level data and specific conductance data from wells along the beachfront that show that the predominant direction of groundwater movement in the beach sands is from the beach towards the sewer line and that sewage contaminated groundwater does not discharge at the beachfront.

PCA analysis of the three types of tracer data did not always produce the same interpretation of the FIB sources in the near-shore ocean water. For example, PCA analysis of genetic and chemical data showed a similarity between samples collected from near-shore ocean water and kelp and guano-contaminated sand. PCA analysis of PLFA data suggest only a similarity between near-shore ocean water and sand and that PLFA contributions from kelp were greatly different. These data suggest that guano-contaminated sand is a more important source of FIB to near shore ocean water than FIB in kelp - despite the very high FIB concentrations in kelp. Differing interpretations of FIB sources from tracer data illustrate the need to use multiple tracers in conjunction with hydrologic data collected at appropriate spatial and temporal scales to identify sources of FIB.

In addition to the results from PCA analysis, a wide range of other information can be extracted from the tracer data collected as part of this study. For

example, although FIB were not present in water from wells, genetic and molecular data indicate a unexpectedly large diversity of organisms present at low concentrations in groundwater where surface runoff was discharged to beach sands. Chemical tracer data also are consistent with street runoff in water from some wells. Similarly, assemblages of waste water indicator compounds in stormflow, especially the presence of certain detergent metabolites and the absence of personal-health-care products (PHCP), flame retardants, and other compounds commonly present in waste water influent to the El Estero WWTP suggest that leakage of sewer lines is not an important source of FIB to Mission Creek during stormflow.

5. CONCLUSIONS

Point sources dominated FIB contamination to streams during baseflow and non-point sources dominated FIB contamination to stream during stormflow. In most areas FIB concentrations in shallow groundwater were low, suggesting that leakage from sewer lines and laterals connecting sewer lines to residences, although locally important, were not a regional source of FIB contamination. Groundwater flow at West Beach was toward a regional sewer line, which acted as a drain. Sewage from the sewer could not move toward the beachfront and groundwater discharge at the beachfront was small. The timing of FIB concentrations during the ebb of the spring and neap tides is consistent with FIB from guano contaminated kelp and beach sand. Discharge from nearby streams also contributed FIB to West Beach, especially after stormflow. Results of this study show the combined use of FIB and multiple tracers of fecal contamination, constrained by an understanding of the movement of water, is a powerful approach for identifying FIB sources to streams and near-shore ocean water.

1. FIB concentrations in the environment are highly variable and range over three-fold in streams during baseflow, over three-orders of magnitude during stormflow, and over 2-orders of magnitude in near-shore ocean water over tidal cycles.
2. Increases in FIB concentrations in near-shore ocean water after stormflow were large and concentrations remained high for at least three days after the cessation of stormflow.
3. Traditional hydrologic data, collected at appropriate timescales, were the most valuable information for guiding interpretations on the sources of FIB to streams, shallow groundwater,

and near-shore ocean-water.

4. Water-level, seepmeter and isotopic data captured the magnitude and direction of groundwater exchange with near-shore ocean water and were valuable in explaining FIB variations at the beachfront.
5. Tracer data captured aspects of FIB contamination sources that could not be obtained from hydrologic data alone.
6. The most robust PCA results were from PLFA data which explained 97 percent of the total variance within the first and second principal components. Smaller fractions of the total variance were explained by genetic (T-RFLP) and chemical data, with 32 and 34 percent of the total variance, respectively, explained in the first and second principal components.
7. Certain compounds used in this study as tracers of FIB sources, especially the fecal sterols, lent themselves to specific interpretations of the origins of FIB. The presence or absence of individual compounds were more easily interpreted than the presence or absence of amplicons and fatty acids also used as tracers.

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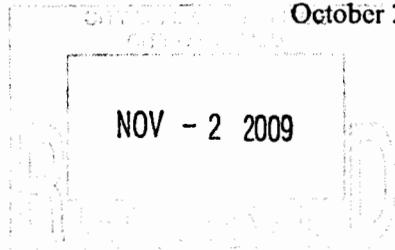
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October 29, 2009

Mr. James Thorsen,
City Manager,
City of Malibu,
23815 Stuart Ranch Road,
Malibu, California 90265



Dear Mr. Thorsen;

This letter summarizes preliminary results of our cooperative water-resources study to identify the source of fecal indicator bacteria in the Malibu Lagoon and ocean beaches near Malibu, California. The study was cooperatively funded by the City of Malibu and the U.S. Geological Survey. The study was done under the direction of Dr. John Izbicki in our San Diego Projects Office.

Previous work has shown that fecal indicator bacteria (FIB), indicative of fecal contamination, are present in Malibu Lagoon and at ocean beaches near Malibu, California at concentrations that exceed recreational water-quality standards. The source, or combination of sources, of fecal material to the lagoon and near-shore ocean water is not precisely known but may include: (1) groundwater containing residential or commercial septage; (2) natural sources either directly deposited by birds and other wildlife, or indirectly mobilized as tides and waves wash beach sands and material accumulated at the high-tide line (rack line) along the beach; and/or (3) surface flow into the Malibu Lagoon. FIB present in the lagoon could be a source of contamination to the near-shore ocean by surface flow from the lagoon to the ocean or by groundwater flow from the lagoon through the berm separating the lagoon from the ocean. Data were collected during a falling monthly tidal cycle during the dry summer season from July 21-27, 2009 and reflect conditions present at that time.

The purpose of this letter is to provide a summary of preliminary results from the July 21-27, 2009 sample period. Data collected during the sampling period included: (1) groundwater-level data; (2) Radon-222 (^{222}Rn) data and direct-current (DC) resistivity data to estimate groundwater discharge to Malibu Lagoon and the near-shore ocean; (3) fecal indicator bacteria concentrations in groundwater, Malibu Lagoon, and near-shore ocean water; and (4) bacterial source tracking data (including genetic, molecular, and chemical data). Most data collected as part of the study are publically available in the U.S. Geological Survey's on-line data base NWIS-Web other data are on-file at the U.S.

Geological Survey office in San Diego and are publically available on request. The information presented in this letter is for internal planning and program development purposes. Interpretations presented in this letter have not been reviewed within the U.S. Geological Survey, and as such are preliminary and are not intended for public release.

Although results of this study are preliminary, and reflect the conditions during the sample period, FIB were present at only low concentrations, in 10 of 11 sampled water-table wells. In contrast, high concentrations of FIB were present in Malibu Lagoon. Given the general absence of FIB in groundwater, measured rates of groundwater discharge to the lagoon, and other hydrologic conditions at the time of sample collection groundwater discharge was not a likely source of FIB to the lagoon. Enterococcus concentrations in excess of the U.S. Environmental Protection Agency single sample standard for recreational water (104 MPN per 100 ml) in near-shore ocean water near the lagoon berm were related to movement of water through the berm at the mouth of the lagoon during low tide. FIB concentrations in near-shore ocean water at three sampled beaches were higher at high tide and are more consistent with FIB associated with wave run-up washing fecal material from beach sands and the rack line at high tide, than with discharge of groundwater contaminated with septic wastewater which would be expected to be greater at low tide. Enterococcus concentrations occasionally exceeded the U.S. Environmental Protection Agency single sample standard for recreational water at the three beaches during the sample period.

As stated previously, data collected as part of this study reflect conditions present during sample collection and may not reflect conditions at other times. The results of this initial study are preliminary and will be used to develop a more detailed proposal for future work to address these issues. If you have any questions concerning the study results, do not hesitate to contact me at (619) 225-6127 or Dr. John Izbicki at (619) 225-6131. The U.S. Geological Survey looks forward to working with the City of Malibu on future water-resource investigations.

Sincerely,

A handwritten signature in black ink, appearing to read "Peter Martin", with a long horizontal flourish extending to the right.

Peter Martin
Program Chief

Introduction

Previous work has shown that fecal indicator bacteria (FIB), indicative of fecal contamination, are present in Malibu Lagoon and at ocean beaches near Malibu, California at concentrations that exceed recreational water-quality standards (Ambrose and Orme, 2000; Stone Environmental, 2004). The source, or combination of sources, of fecal material to the lagoon and near-shore ocean water is not precisely known but may include: (1) groundwater containing residential or commercial septage; (2) natural sources either directly deposited by birds and other wildlife, or indirectly mobilized as tides and waves wash beach sands and material accumulated at the high-tide line (rack line) along the beach; and/or (3) surface flow into the Malibu Lagoon. In addition, FIB present in the lagoon could be a source of contamination to the near-shore ocean by surface flow from the lagoon to the ocean, or by groundwater flow from the lagoon through the berm separating the lagoon from the ocean.

Data were collected in the Malibu area during a falling monthly tidal cycle during the dry summer season from July 21-27, 2009. Previous investigations of microbial contamination at beaches near Santa Barbara, California showed that groundwater discharge to the near-shore ocean is greater during the low tides of the falling monthly tidal cycle (Swarzenski and Izbicki, 2009; Izbicki et al., 2009). Data collected during the sampling period included: (1) groundwater-level data; (2) Radon-222 (^{222}Rn) data and direct-current (DC) resistivity data to estimate groundwater discharge to Malibu Lagoon and the near-shore ocean; (3) fecal indicator bacteria concentrations in groundwater, Malibu Lagoon, and near-shore ocean water; and (4) bacterial source tracking data (including genetic, molecular, and chemical data. Sample site locations are shown on figures 1 and 2. Not all data collected during the study period were available for inclusion in this letter.

Groundwater levels

As part of this study, groundwater levels were continuously measured at four wells (SMBRP-12, SMBRP-13, C-1, and P-9) to help determine the interaction between groundwater and the near-shore ocean. Tide, ocean swell, and groundwater-level data are shown in (fig. 3).

Water-levels in wells SMBRP-13 and SMBRP-12 (fig. 3c) in Malibu Colony respond to tidal fluctuations in the ocean. The tidal efficiency (amplitude of tidally affected water levels in the well divided by the tidal amplitude in the ocean) of well SMBRP-13 on the east side of Malibu Colony was 0.13; whereas, the tidal efficiency of well SMBRP-12 near the center of Malibu Colony was about 0.005. Higher tidal efficiency indicates that tides have greater influence on water levels in affected wells. Malibu Colony is protected by a seawall consisting of wooden pilings driven into the sand to a depth of about 15 ft. (fig. 4). The pilings act as a barrier to the interaction between the ocean and groundwater inland of the seawall. Well SMBRP-13 is closer to the eastern edge of the seawall; therefore, the seawall has less effect on the water levels than in well SMBRP-12.

In addition to tidal effects, the water levels in wells SMBRP-12 and SMBRP-13 also were affected by a south swell during the measurement period. The swell produced wave heights of about 5.5 ft between July 25-26, 2009 (fig. 3b) at the Santa Monica Basin bouy (46025), about 20 miles offshore. Wave heights on the beach at Malibu were greater and were reported in excess of 12 feet during this period. Water levels in well SMBRP-13 increased during the swell and reached their peak during the swell (fig. 3c). The maximum water-level response in well SMBRP-12 (fig. 3c) occurred about 1 day after the peak swell intensity and the effect of the swell persisted longer than in well SMBRP-13.

Water levels in wells C-1 and P-9 respond to water-level fluctuations in Malibu Lagoon in addition to tidal fluctuations in the ocean (fig. 3d). The combination of tides and swells caused the ocean to overtop the berm separating the lagoon from the ocean between July 22-25. This allowed ocean water to enter the lagoon at high tide, increasing water levels in the lagoon and in wells C-1 and P-9 during the sample period (fig. 3d). The ocean did not overtop the berm after July 25 and water levels in the lagoon and wells C-1 and P-9 began to decline at that time (fig. 3d).

Groundwater discharge to Malibu Lagoon

^{222}Rn data were collected to estimate groundwater discharge to Malibu Lagoon. Radon is a noble gas, and consequently it is non-reactive and highly mobile in groundwater. ^{222}Rn is radioactive, produced by the decay of radium-226 as part of the uranium-238 decay series, and has a half-life of 3.8 days. ^{222}Rn is present at concentrations several orders of magnitude higher in groundwater than in surface water or ocean water (Swarzenski and Izbicki, 2009). If the average ^{222}Rn concentration in groundwater discharging to a surface water body is known, and the ^{222}Rn concentration of the surface water is known, then the groundwater discharge rate can be calculated. Calculations account for exchange with atmospheric ^{222}Rn and mixing with seawater having low ^{222}Rn concentrations.

^{222}Rn concentrations ranged from 650 to 1,370 dpm/L (disintegrations per minute per liter) in the eight wells sampled for this study (Table 1). In addition to groundwater samples, ^{222}Rn samples were collected continuously from two locations in the lagoon (ML-Upper and ML-Lower, fig. 2) by equilibrating water pumped from the lagoon with air in an enclosed chamber. The radioactive decay of ^{222}Rn in the chamber was measured and the water concentration was calculated according to Henry's law (Swarzenski and Izbicki, 2009). ^{222}Rn concentrations in lagoon water ranged from 8 to 62 dpm/L during the sample period (fig. 5c). ^{222}Rn concentrations in the lagoon were higher at the beginning of the sample period and decreased as seawater (having low ^{222}Rn concentrations) entered the lagoon during high tides.

Preliminary analysis of ^{222}Rn data show groundwater discharge to the upstream part of Malibu Lagoon averaged 2.8 cm/d between July 21-26. Groundwater discharge rates to the upper lagoon decreased during the sample period from about 15 cm/d (6-hour

moving average) on July 21-22, to 2.3 cm/d (6-hour moving average) on July 24-25 (fig. 5d). Groundwater discharge rates to the lagoon increased after July 26 after seawater no longer overtopped the berm during high tide (fig. 5d). Groundwater discharge to the downstream part of the lagoon (ML-Lower) was less than discharge in the upstream part of the lagoon and averaged 0.8 cm/d on July 22-23 (not shown in figure 5).

Groundwater discharge to the near-shore ocean

The original study plan included collecting ^{222}Rn data with seepage meter data to measure groundwater discharge along the Malibu Colony beach. However, high surf conditions during the sample period prevented the collection of these data. It was possible to collect DC-resistivity data along the Malibu Colony beachfront near well SMBRP-12 (fig. 1) to determine the location of groundwater discharge to the ocean (fig. 6). The resistivity data shows a thin lens of resistive material, presumably sand containing fresh groundwater, at a depth of about 15 ft below land surface. As stated previously, the seawall pilings extend to a depth of about 15 ft below land surface. Therefore, groundwater must flow beneath these pilings to discharge to the ocean. The lens of resistive material is overlain and underlain by more conductive material, presumably sand containing saline groundwater. The shallower saline groundwater is probably ocean water emplaced in the sand at the base of the seawall during high tides and swells. The deeper saline groundwater probably results from the density contrast between seawater and fresh water, which creates a wedge of seawater extending beneath fresh groundwater within the alluvial deposits.

Occurrence of fecal indicator bacteria

Fecal indicator bacteria (FIB) measured as part of this study included *Escherichia coli* (*E. coli*) and enterococci. Although not necessarily fecal in origin, total coliform bacteria also were measured to assess microbial contamination of sampled water. Total coliform and *E. coli* were analyzed by Colilert and enterococci were analyzed using Enterolert (IDEXX, Westbrook MN). Samples were analyzed within 6 hours after collection in a temporary laboratory established in the study area. A range of dilutions were used to ensure proper quantification of samples in accordance with the manufacturers' specifications.

Fecal indicator bacteria in groundwater

FIB samples were collected from 11 shallow wells in the study area (fig. 1 and Table 1). Total coliform, *E. coli*, and enterococcus bacteria were less than the detection limit or were present at low concentrations in water from 10 of the 11 wells sampled (Table 1). FIB were detected in water from well CCPE (Table 1), which is in a commercial area adjacent to Malibu Lagoon (fig. 1). The sample from the well was saline (specific conductance of 10,800 $\mu\text{S}/\text{cm}$), which is similar to water in the lagoon at the time of sample collection. FIB present in the lagoon during the sampling period could be the source of the FIB in well CCPE. However, FIB were not detected in well C-1, adjacent to Malibu Lagoon, which also has saline water that may have originated from

the lagoon. FIB also were not detected in well P-9, adjacent to the lagoon. Water from well P-9 is fresh (specific conductance of 2,000 $\mu\text{S}/\text{cm}$) and more similar to groundwater than to water from Malibu Lagoon. FIB also were not detected in water from wells SMBRP-12 and SMBRP-13 in Malibu Colony near the ocean.

Fecal indicator bacteria in Malibu Creek

Malibu Creek was not flowing and as a consequence was not an important source of FIB to the lagoon during the sample period. However, pools of water were present in the stream channel upstream from the lagoon (fig. 2). Total coliform, *E. coli*, and enterococcus concentrations in a sample collected on July 24 from one of these pools were 14,100, 10, and 280 MPN per 100 ml, respectively.

Fecal indicator bacterial in Malibu Lagoon and the adjacent near-shore ocean

Samples from the Malibu Lagoon were analyzed for FIB during the high and low tide at the downstream site near the berm of the lagoon (ML-Berm). FIB concentrations in the lagoon at that site were higher than concentrations in samples from wells or surface water collected during the study period. Total coliform concentrations in Malibu Lagoon ranged from <1,000 to 650,000 MPN per 100 ml (Most Probable Number per 100 milliliters) (fig. 7). *E. coli* concentrations ranged from <10 to 130,000 MPN per 100 ml (fig. 7d). Total coliform and *E. coli* concentrations generally decreased during the sample period as a result of dilution as ocean water overtopped the berm and entered the lagoon during high tide (fig. 7d). The decrease in total coliform and *E. coli* concentrations was accompanied by an increase in water level and specific conductance of water in the lagoon (figs. 7b and 7c). Total coliform and *E. coli* concentrations increased during the later part of the sample period when ocean water was no longer entering the lagoon during high tide. Enterococcus concentrations ranged from <10 to 3,400 MPN per 100 ml (fig. 7d). Enterococcus concentrations in Malibu Lagoon commonly exceeded the U.S. Environmental Protection Agency single sample standard for (marine) recreational water of 104 MPN per 100 ml (U.S. Environmental Protection Agency, 2003). Unlike total coliform and *E. coli* concentrations, enterococcus concentrations did not consistently decrease during the sample period. Instead, enterococcus concentrations show a diurnal pattern with the lowest concentrations in samples collected later in the day (fig. 7d), possibly as a result of inactivation of bacteria by UltraViolet (UV) radiation in sunlight.

Ocean water entering Malibu Lagoon during high tide has a higher salinity than lagoon water. As a consequence, ocean water is denser and will tend to sink to the bottom of the lagoon stratifying water in the lagoon by density (fig. 8). Initially it was expected that saline water at the bottom of the lagoon, which originated from the ocean, would have low bacteria concentrations. However, data collected from this study indicate that the deeper saline water had higher bacteria concentrations than near surface water (fig. 8). A possible explanation is that ocean water enters the lagoon during high tide and the denser ocean water sinks to the bottom of the lagoon. As the dense ocean water moves to the lagoon bottom sediment and associated bacteria are resuspended into the water column.

Surface discharge from Malibu Lagoon to the ocean did not occur during the sample period. To determine if water and associated FIB from the lagoon can flow through the sand berm to the near-shore ocean at low tide, data were collected during a 24-hour tidal cycle on July 23-24 from the lagoon, the sand berm separating the lagoon from the ocean, and in the near-shore ocean (fig. 9). The specific conductances of (1) near-shore ocean water adjacent to the berm and at several nearby beaches, (2) Malibu Lagoon, and (3) water from piezometers and seepage samplers in the berm are shown in figure 9b. The enterococcus concentrations of near-shore ocean water and water from Malibu Lagoon are shown in figure 9c. The enterococcus concentrations of water from piezometers and seepage samplers in the berm are shown in figure 9d.

During the falling tidal cycle specific conductance of near-shore ocean water adjacent to the berm (ML-Berm-OF, fig. 9b) decreased and reached a minimum about one hour after low tide. This decrease is the result of water from the lagoon discharging through the berm. As a consequence of this discharge, the specific conductance of near-shore ocean water adjacent to the berm (ML-Berm-OF, fig. 9b) was lower than near-shore ocean water at nearby beaches sampled as part of this study (OF-A, OF-B, and OF-C, fig. 9b). As water from the lagoon discharged to the ocean, enterococcus concentrations increased in near-shore ocean water adjacent to the berm (ML-Berm-OF, fig. 9c). Enterococcus concentrations were highest about 1 hour after low tide when the specific conductance of near-shore ocean water was lowest. Enterococcus concentrations exceeded the U.S. Environmental Protection Agency (2003) single sample standard for recreational water at that time. A similar pattern was observed in total coliform and *E. coli* concentrations (not shown on fig. 9).

Increases in enterococcus concentration also were observed during the falling tide in the piezometer driven into the berm adjacent to Malibu Lagoon at a depth of 5 ft below land surface (Pz 5ft, fig. 9d). However, enterococcus concentrations were low in water from samplers emplaced in the seepage face along the berm near the secondary high-tide line (Seepage-Shallow, fig. 9d) and the secondary low-tide line (Seepage-Deep, fig. 9d). The specific conductance of water from the sampler near the secondary high-tide line (Seepage-Shallow, fig. 9b) was consistent with ocean water emplaced in the berm during the previous high tide. The specific conductance of water from the sampler near the secondary low-tide line was consistent with water from Malibu lagoon (Seepage-Shallow, fig. 9b).

DC-resistivity sections were collected across the berm, perpendicular to the ocean, on July 24 at low tide and at the secondary high tide (fig. 10a and 10b). The DC-resistivity data show water from the lagoon (delineated as less saline on fig. 10) in a thin layer within the sand berm discharging to the ocean near the low tide line (fig. 10). This layer is overlain by more saline water emplaced in the berm during high tide when ocean water flowed over the top of the berm into the lagoon. The less saline water is underlain by water from deeper parts of the lagoon containing moderately saline water originating from the ocean during previous high tides. The DC-resistivity data suggest that most of the water from the lagoon is moving through the berm slightly below the deepest sampler

on the seepage face (Seepage-Deep, fig. 10). This result suggests that the enterococcus bacteria present in the near-shore ocean probably moved through the berm with the lagoon water near the altitude of the low tide line, at a depth below the deepest seepage sampler.

Fecal indicator bacteria in the near-shore ocean

FIB concentrations were measured in near-shore ocean water at the Malibu Colony beach (OF-B), a beach about 3 miles to the west Malibu Colony (OF-A), and a beach about 0.25 miles east of the Malibu Lagoon (OF-C) between July 12-26, 2009 (fig. 11). Samples were collected at the high, secondary-high, low, and secondary-low tidal stands. Figure 11 shows the tidal range (11a), ocean swell height (11b), specific conductance of near-shore ocean water (11c), and total coliform, *E. coli*, and enterococcus concentrations (11d, 11e, and 11f, respectively) at the sampled beaches.

Total coliform and *E. coli* concentrations were generally lower at all tidal stands at the Malibu Colony beach (OF-B) than at the other sampled beaches (fig. 11d, and 11e). Enterococcus concentrations exceeded the U.S. Environmental Protection Agency (2003) single sample standard for recreational water at all three sampled beaches during the sample period, with the most exceedances at the OF-A site to the west of the Malibu Colony beach (fig. 11f). A health advisory, associated high FIB concentrations, was posted for the beach east of OF-C near the beginning of the sample period.

Prior to the high surf associated with the south swell that occurred from July 24-26, total coliform and *E. coli* concentrations were higher during high tide and lower during low tide (fig. 11d and 11e). This pattern is consistent with bacterial contributions from wave run-up on the beach during high tide. The pattern is not consistent with bacteria from groundwater discharge containing septage because groundwater discharge to the near-shore ocean is greatest at low tide. Tidally associated changes in bacteria concentrations also were present for enterococcus but were less pronounced (fig. 11f).

The specific conductance of near-shore ocean water was the lowest at the onset of high surf associated with the south swell beginning July 24 (fig. 11c). Presumably the high surf disturbed the discharge of groundwater to the ocean along the beachfront, although the high surf did not affect tidally associated FIB concentrations in near-shore ocean water until after the swell subsided. After the swell subsided, total coliform, *E. coli*, and enterococcus concentrations were higher during low tide than at high tide (fig. 11d, 11e, and 11f). This may represent drainage of ocean water emplaced in the beach sand during the swell rather than discharge of groundwater from onshore alluvial deposits.

Fecal indicator bacteria in septage water and other sources

FIB concentrations were sampled in the discharge from a traditional septic system (MC-OLD-Septic, fig. 2) and an advanced septic system (MC-ADV-Septic, fig. 2) containing (1) biological treatment media, (2) an aeration tank, and (3) UV disinfection.

Samples were collected October 1, 2009 and bacteria were analyzed within 24 hours of collection at the U.S. Geological Survey office in San Diego. Advanced septic systems in the Malibu area differ in their construction and consequently the quality of water discharged from these systems also may differ. The data show a 2-log-order reduction in FIB concentrations in water discharged from advanced systems compared to more traditional systems (Table 2). FIB concentrations in the discharge from the advanced septic system were generally lower than FIB concentrations in Malibu Lagoon during the July sample period.

FIB were extracted from about 0.5 kg of sand collected on the berm at Malibu Lagoon on October 1, 2009 using about 4 L of water adjusted to seawater salinity. The samples were collected from recently wetted beach sand along the rack line about 1 hour after high tide. Although birds were actively feeding in the area, the sample was not obviously contaminated by guano. Total coliform and *E. coli* concentrations in the extract were low, 10 MPN per 100 ml, while enterococcus concentrations were high, 230 MPN per 100 ml.

Bacterial Source-Tracking

Genetic, molecular, and chemical data were collected to determine the source of FIB to groundwater, Malibu Lagoon, and near-shore ocean water. Genetic material from bacterial cells were analyzed by Terminal-Restriction Fragment Length Polymorphism (T-RFLP) at University of California Santa Barbara (UCSB). Human-specific *Bacteroides* also were analyzed by UCSB. Molecular data consisted of phospholipid fatty acids (PLFA) from bacterial cells. PLFA are associated with specific metabolic activities by a range of organisms rather than specific organisms. PLFA data were analyzed by a contract laboratory (Microbial Insights, Rockford, Tenn.). Chemical data included a suite of 69 organic compounds including caffeine, fecal sterols, detergent metabolites and other compounds collectively known as wastewater indicators. Chemical data were analyzed by the U.S. Geological Survey National Water Quality Laboratory (NWQL) in Denver, Colo. Samples were delivered to the UCSB laboratory by courier on the day of collection. Samples were shipped on the day of collection to the contract laboratory and the NWQL by overnight delivery. Only PLFA results were available at the time this letter was prepared.

The distribution of PLFA structural groups was analyzed using principal component analysis (PCA). PCA is a multivariate statistical technique that transforms a set of intercorrelated variables into a set of uncorrelated variables having a mean of zero and the same variance as the original data set. The new uncorrelated variables are known as principal components and the value of the principal component are known as scores. Analysis of the transformed principal component scores, rather than the original data, allows for statistically unbiased comparison and contrast of samples from different sources.

The first three principal components explain 91 percent of the total variability in the PLFA data. PCA results show differences in the PLFA composition of microbial

communities in samples from water-table wells and from near-shore ocean water (fig. 11). Samples from piezometers and seepage samplers in beach sands are intermediate in composition, and samples from Malibu Lagoon are similar to samples from the near-shore ocean. The first and second principle components for sample collected from near-shore ocean water near Malibu Lagoon at low tide (ML-Berm-OF) are almost identical in PLFA composition to water from the lagoon (ML_Berm), consistent with seepage from the lagoon as a likely source of the enterococcus bacteria in the near-shore ocean water near the lagoon at low tide.

Additional interpretation of PLFA data, with genetic (T-RFLP) and wastewater indicator data is warranted before final conclusions can be drawn.

Summary

Groundwater level, radon-222, direct current resistivity, FIB, and bacterial-source tracking data were collected during a falling monthly tidal cycle during the dry summer season from July 21-27, 2009 near Malibu, California. These data reflect conditions present during the study period and may not reflect conditions at other times. Preliminary results of the study are:

1. FIB concentrations were less than the detection limit or were present at low concentrations in samples from 10 of the 11 water-table wells sampled. FIB from septic-tank discharge was not a major source of FIB contamination to groundwater sampled by the wells during the study period.
2. Shallow groundwater was discharging to Malibu Lagoon during high tidal stands at an average rate of 2.8 cm/d during the sample period. Discharge rates as high as 15 cm/d (6 hour average) were measured during high tidal stands at the beginning of the sample period. Discharge to the lagoon declined during the sample period as a result of increased water levels in the lagoon resulting from ocean water overtopping the berm of the lagoon during high tide.
3. High concentrations of fecal indicator bacteria were present in Malibu Lagoon during the sample period. Total coliform and *E. coli* concentrations decreased during the sample period as a result of dilution by ocean water entering the lagoon at high tide. Enterococcus concentrations showed a daily variation consistent with inactivation by UV radiation during the day. Enterococcus concentrations rebounded to high concentrations during the night.
4. Water movement through the berm of Malibu Lagoon was a source FIB, especially enterococcus, to the near-shore ocean at the mouth of the lagoon during low tide. Enterococcus concentrations exceeded the U.S. Environmental Protection Agency single sample standard for recreational water (104 MPN per 100 ml) in near-shore ocean water near the lagoon berm during low tide.

5. FIB concentrations increased during high tide at three sampled beaches. These increases were consistent with wave run-up on the beach washing FIB from the rack line and beach sands. FIB concentrations did not increase in near-shore ocean water during low tide when groundwater discharge to the ocean would be the greatest. As a result, groundwater discharge did not appear to be a source of FIB concentrations to the near-shore ocean at three sampled beaches. Enterococcus concentrations occasionally exceeded the U.S. Environmental Protection Agency single sample standard for recreational water at the three beaches during the sample period.

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List of Figures

Figure 1.—Location of sampled wells and direct-current (DC) resistivity lines, Malibu, California, July 21-27, 2009

Figure 2.—Location of surface water sample sites, hand-driven piezometers, seepage samplers, and other samples, Malibu, California, July 21-27, 2009

Figure 3.—Predicted tides, surface swell, and water-level data for selected wells, Malibu Calif., July 6 to August 5, 2009

Figure 4.—Photograph showing seawall at Malibu Colony, Malibu, California.

Figure 5.—Radon-222 (^{222}Rn) concentrations and calculated groundwater discharge to Malibu Lagoon (ML-Upper), Malibu, California, July 21-27, 2009.

Figure 6.—Direct current (DC) resistivity section along Malibu Colony beachfront, Malibu California, July 26, 2009. (Location of section shown on figure 1)

Figure 7.—Water level, specific conductance, and fecal indicator bacteria of water in Malibu Lagoon (ML-Berm), Malibu, California, July 21-26, 2009

Figure 8.—Specific conductance and fecal indicator bacteria concentrations with depth in Malibu Lagoon, July 23, 2009

Figure 9.—Specific conductance and fecal indicator bacteria concentrations in water from Malibu Lagoon, piezometers and seepage samplers in the berm separating Malibu Lagoon from the ocean, and in adjacent near-shore ocean water, Malibu, California, July 23-24, 2009.

Figure 10.—Direct current (DC) resistivity section through the berm separating Malibu Lagoon from the ocean, July 24, 2009. (Location of section shown on figure 1)

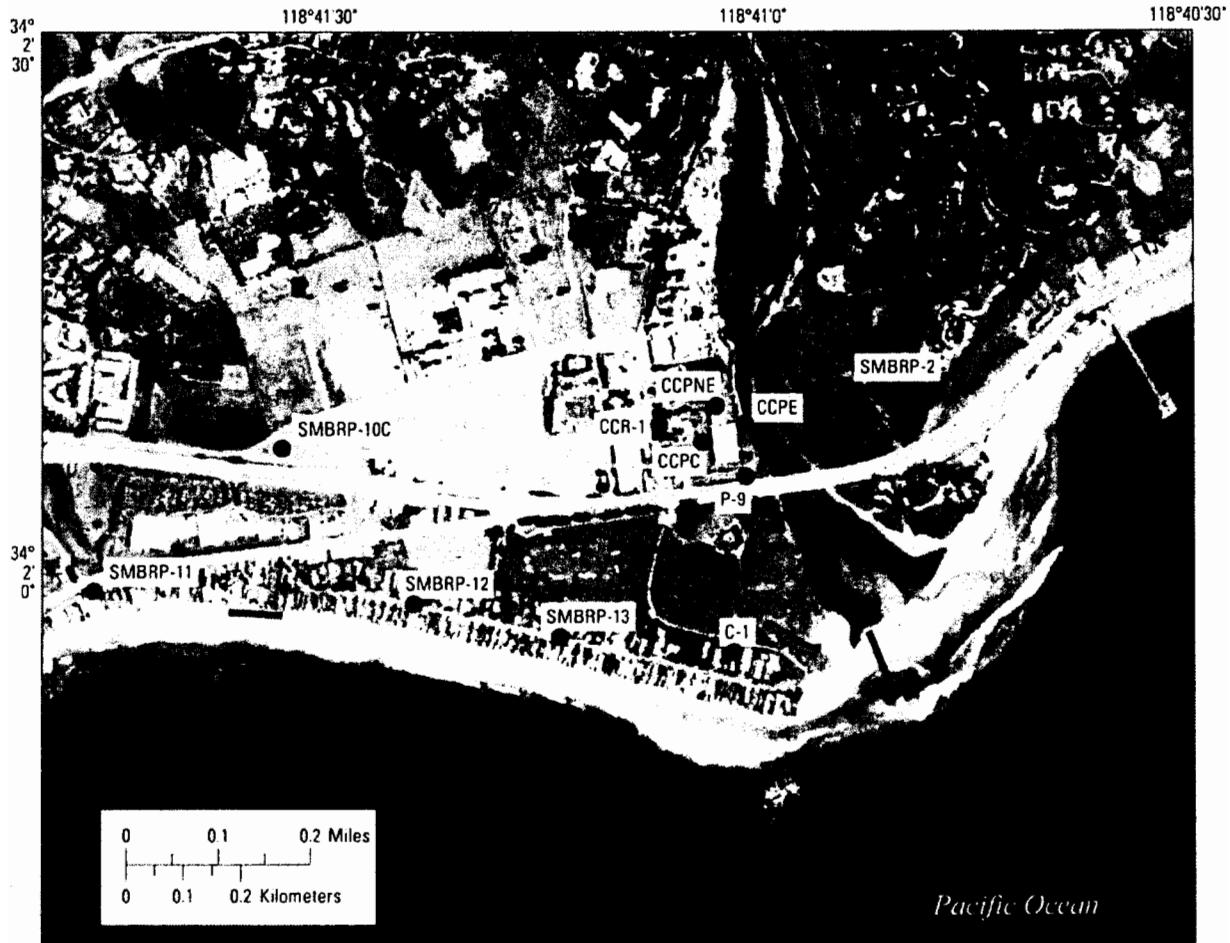
Figure 11.—Specific conductance and fecal indicator bacteria concentrations in near-shore ocean water at selected beaches near Malibu, California, July 21-26, 2009

Figure 12.—Results of Principal Component Analysis (PCA) for phospholipid fatty acid (PLFA) structural groups in water from wells, Malibu Lagoon, and near-shore ocean water, Malibu, California, July 21-26, 2009

List of Tables

Table 1.—Fecal indicator bacteria (FIB) concentrations in water from selected water-table wells, Malibu, California, July 21-26, 2009

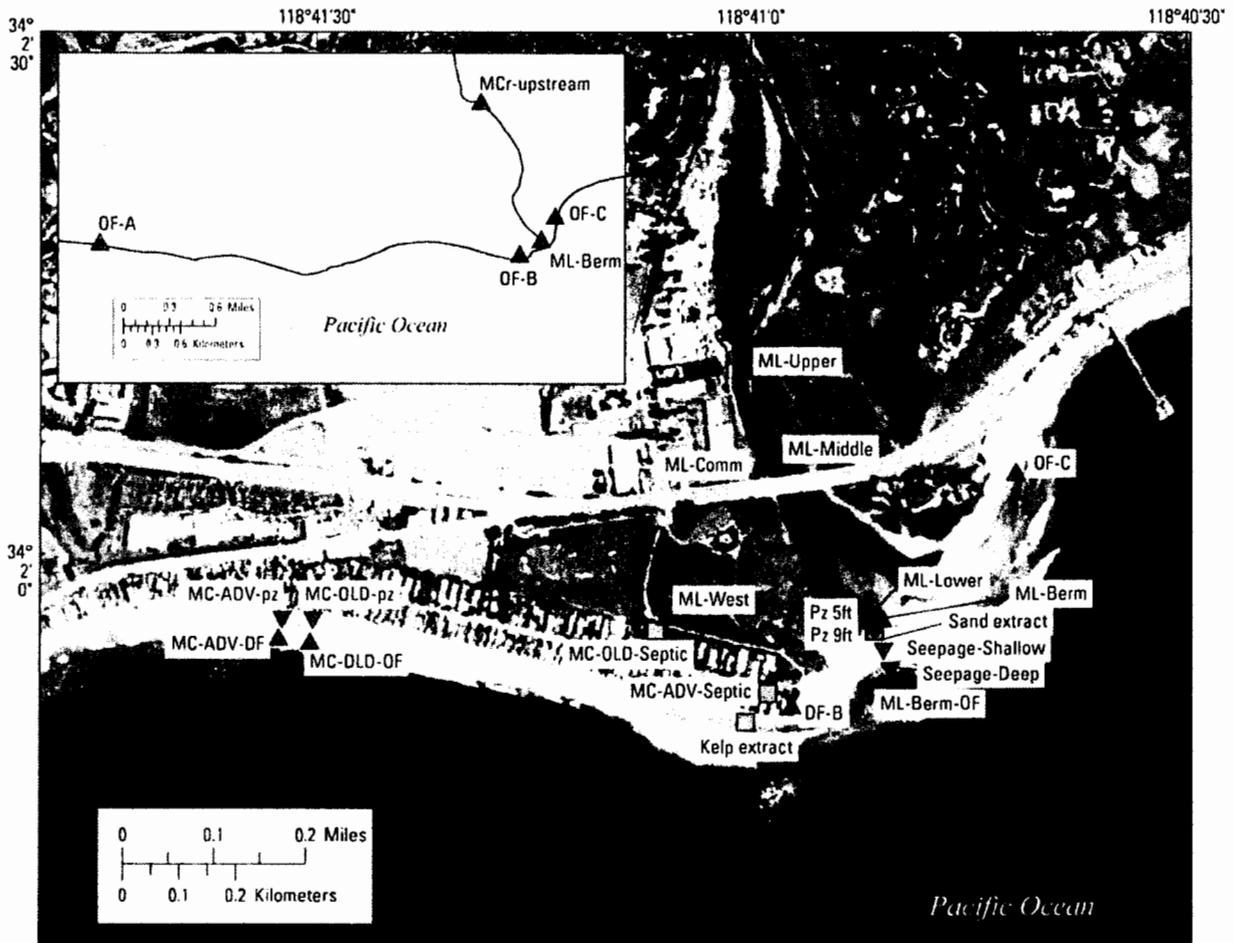
Table 2.—Fecal indicator bacteria (FIB) concentration in discharge water from a traditional septic system and from an advanced septic system, Malibu, California, October 1, 2009.



EXPLANATION

- Resistivity line
- Sampled wells and identifier—
- C-1 ●

Figure 01



EXPLANATION

Sample sites and identifier—

Surface-water

▲ ML-middle

Hand-driven piezometers
or seepage samplers

▼ ML-Berm-9ft

Other

□ Kelp extract, sand extract,
or septic sample

Figure 02

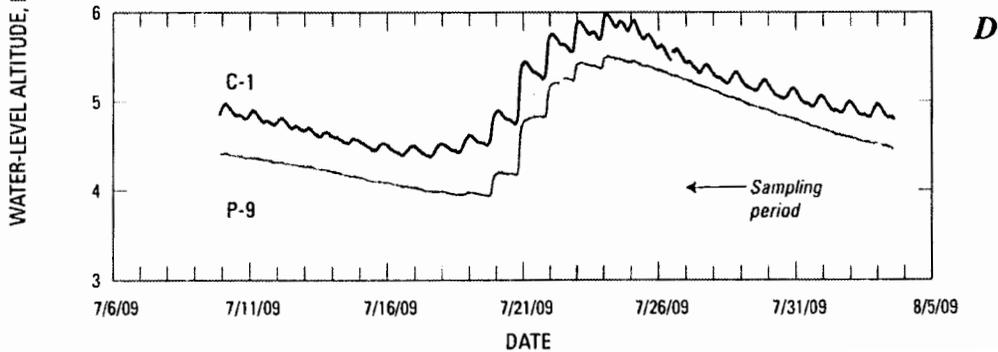
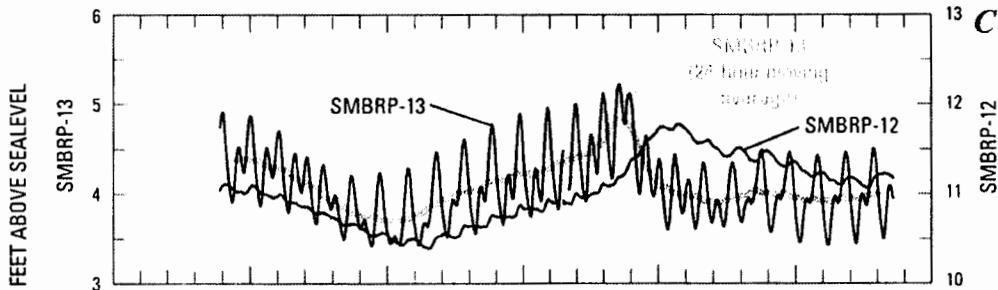
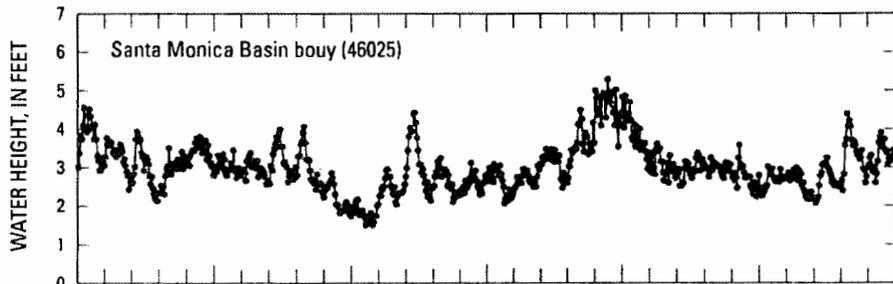
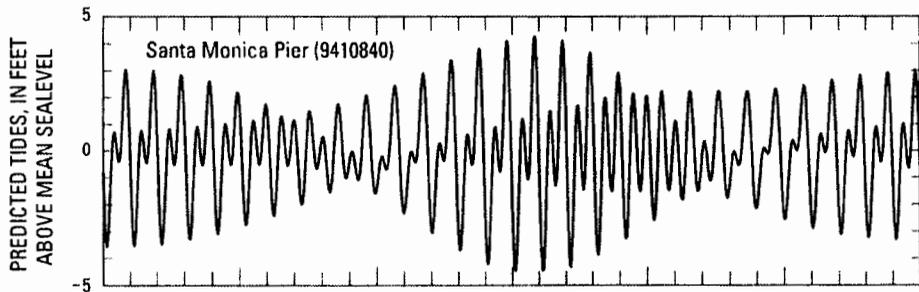




Figure 04

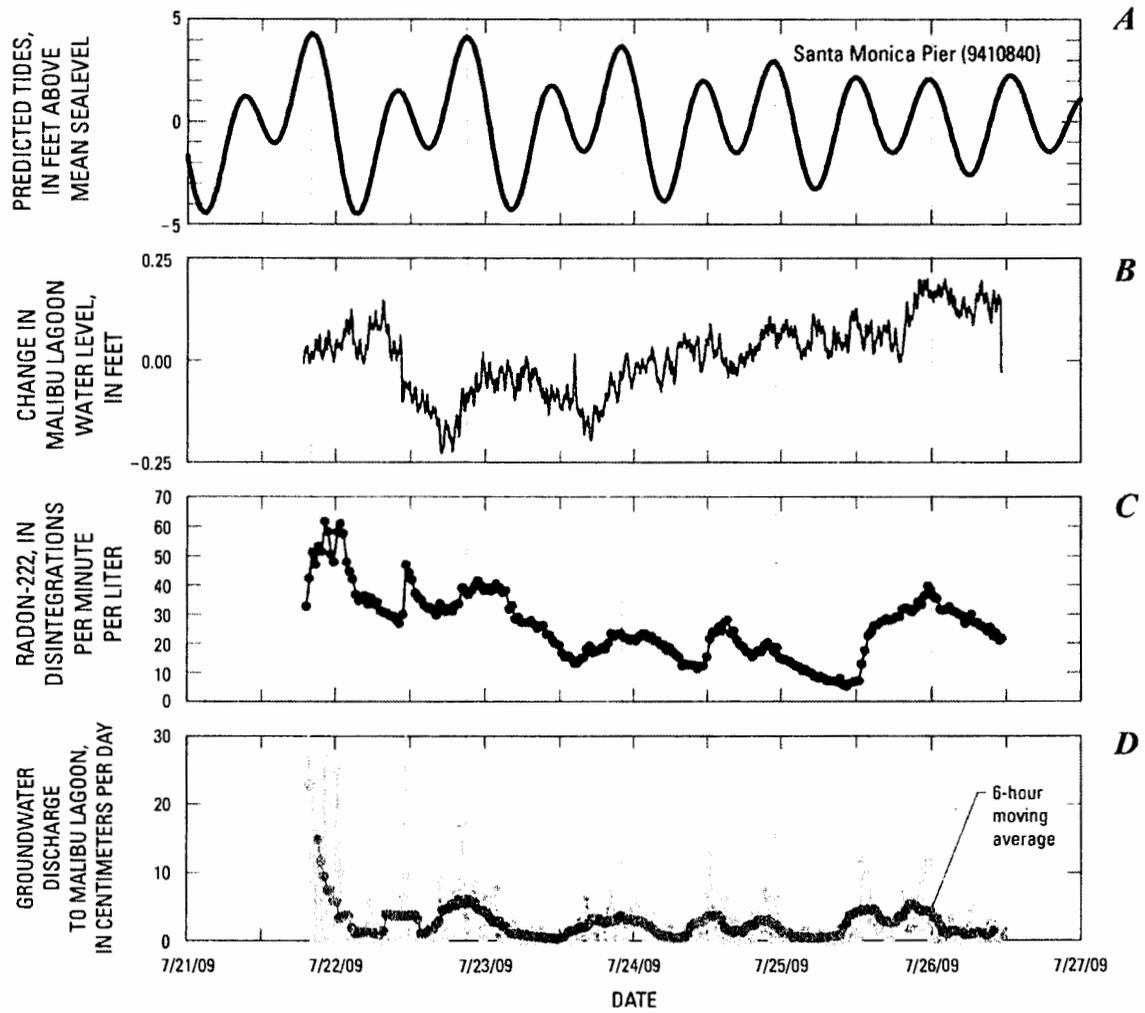


Figure 05

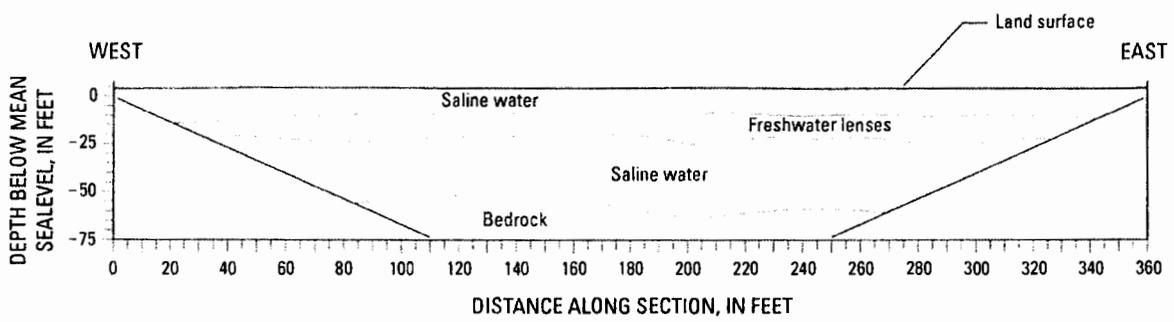
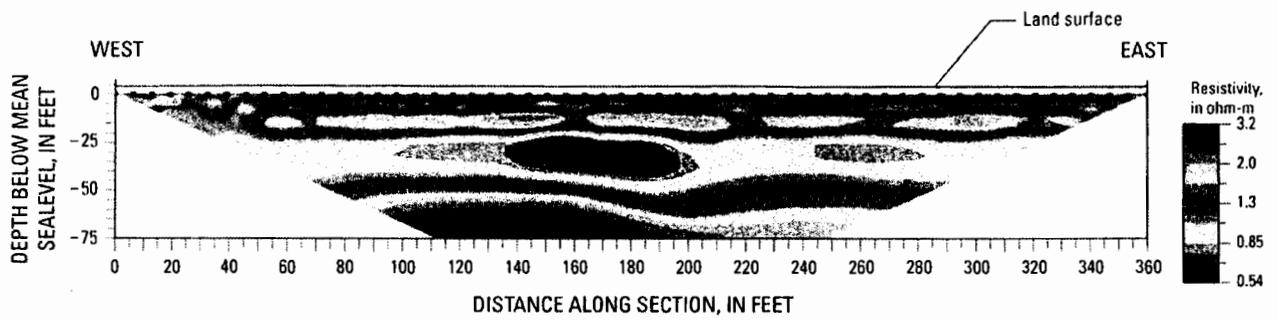


Figure 06

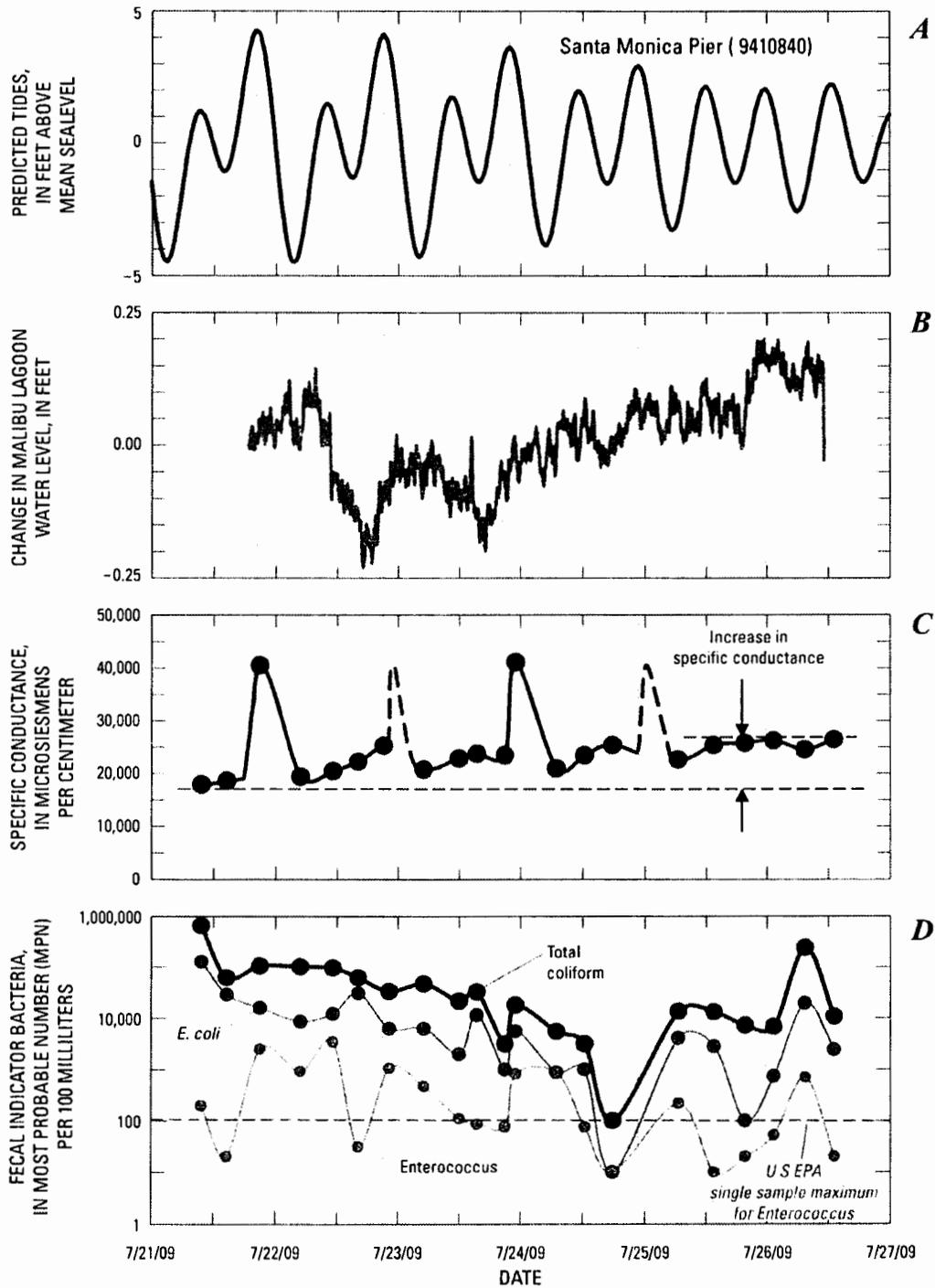


Figure 07

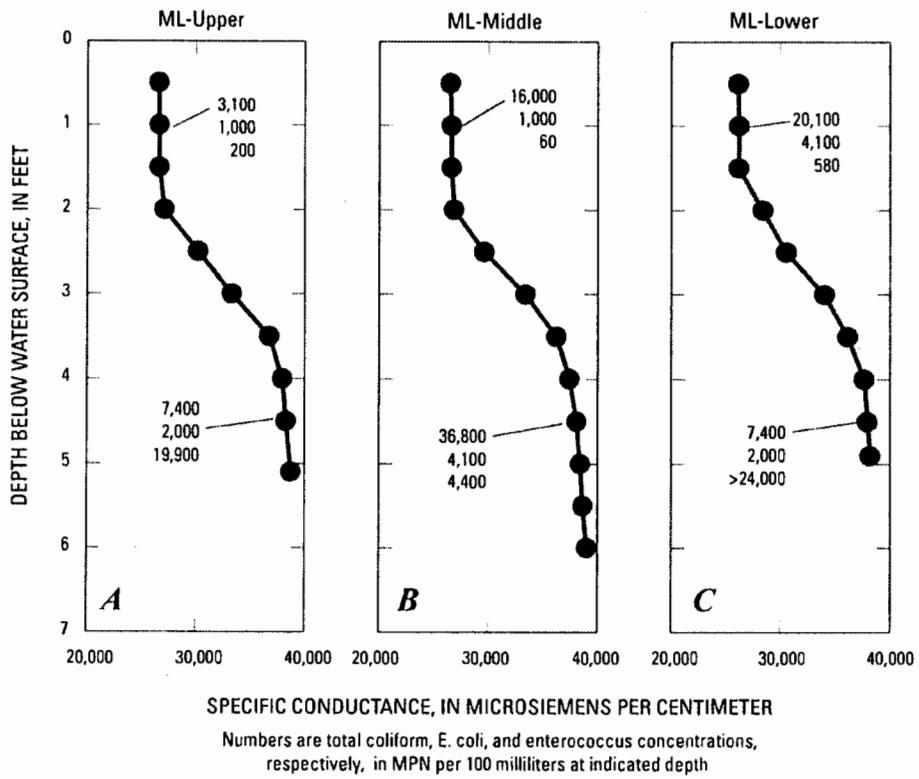


Figure 08

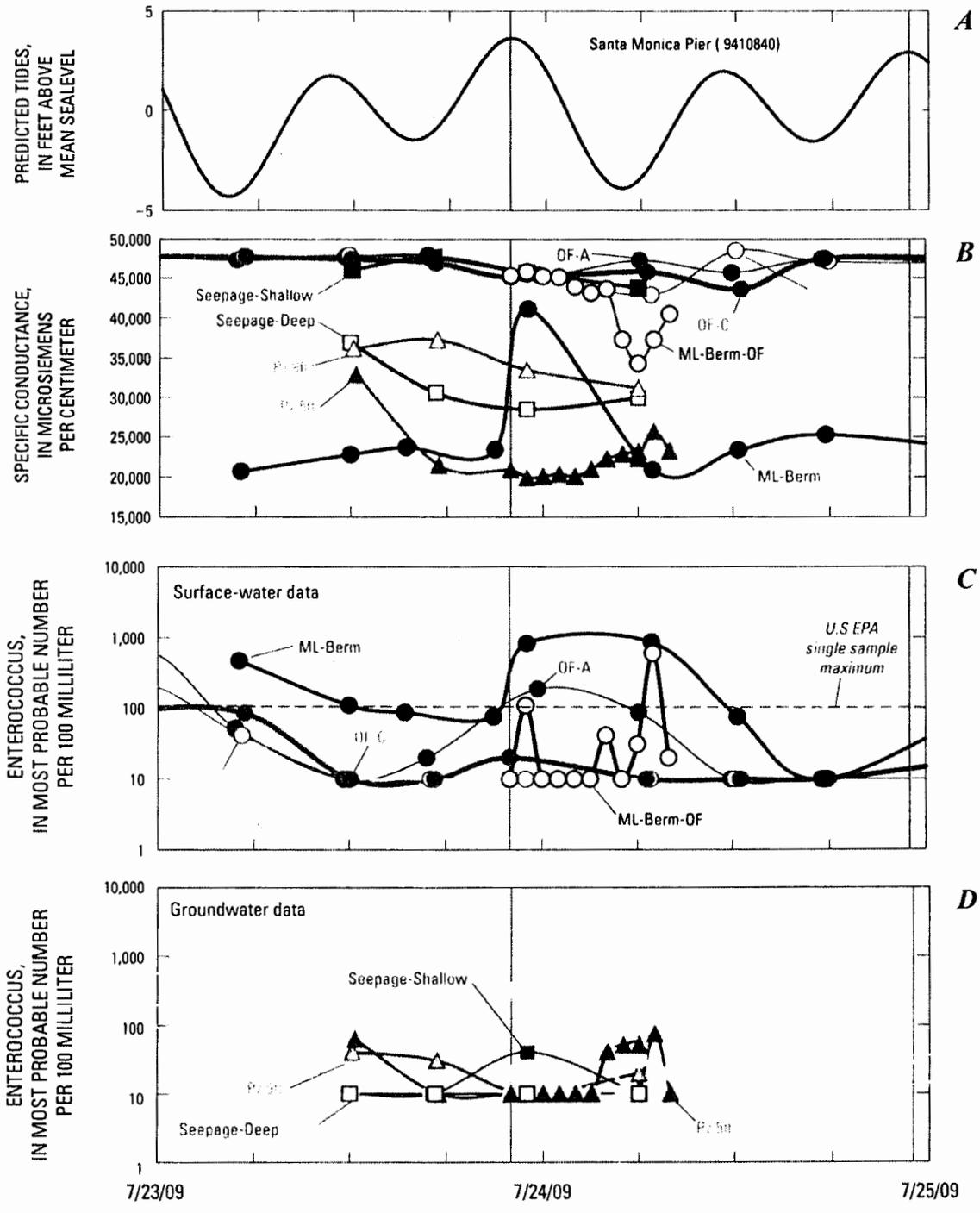
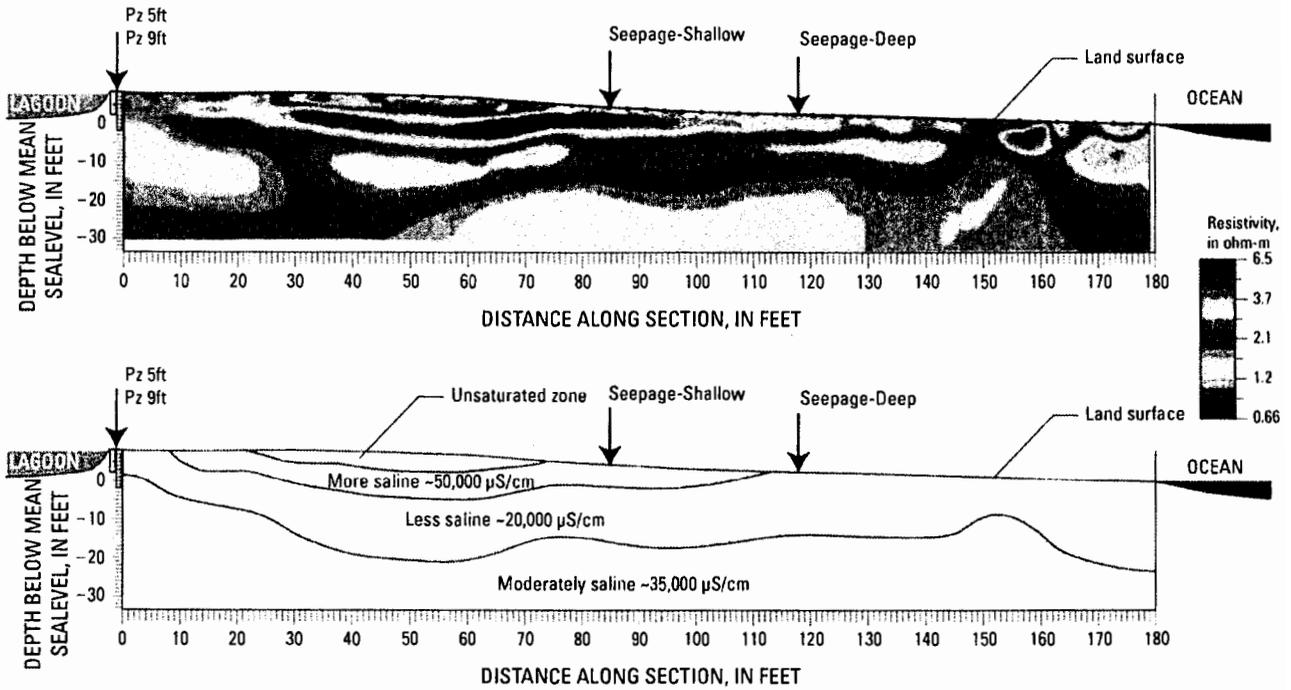


Figure 09

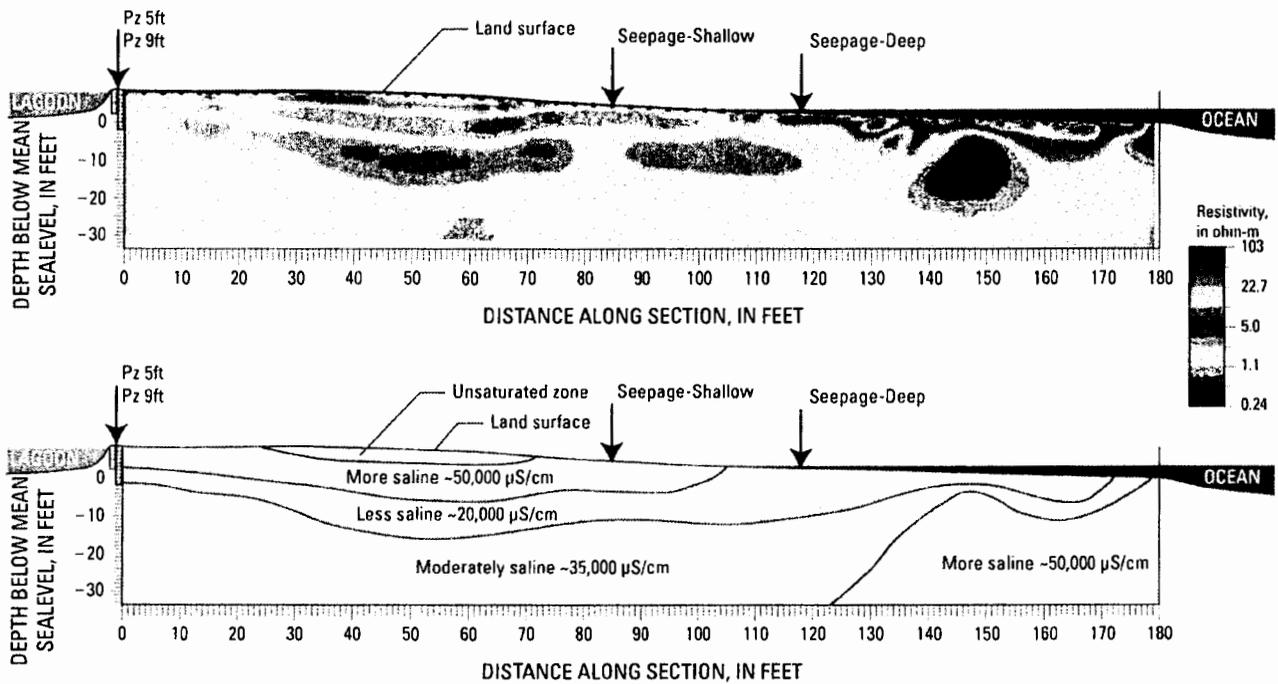
RESISTIVITY ACROSS MALIBU LAGOON BERM — LOW TIDE
8:00 am - 7/24/09

A



RESISTIVITY ACROSS MALIBU LAGOON BERM — SECONDARY HIGH TIDE
11:00 am - 7/24/09

B



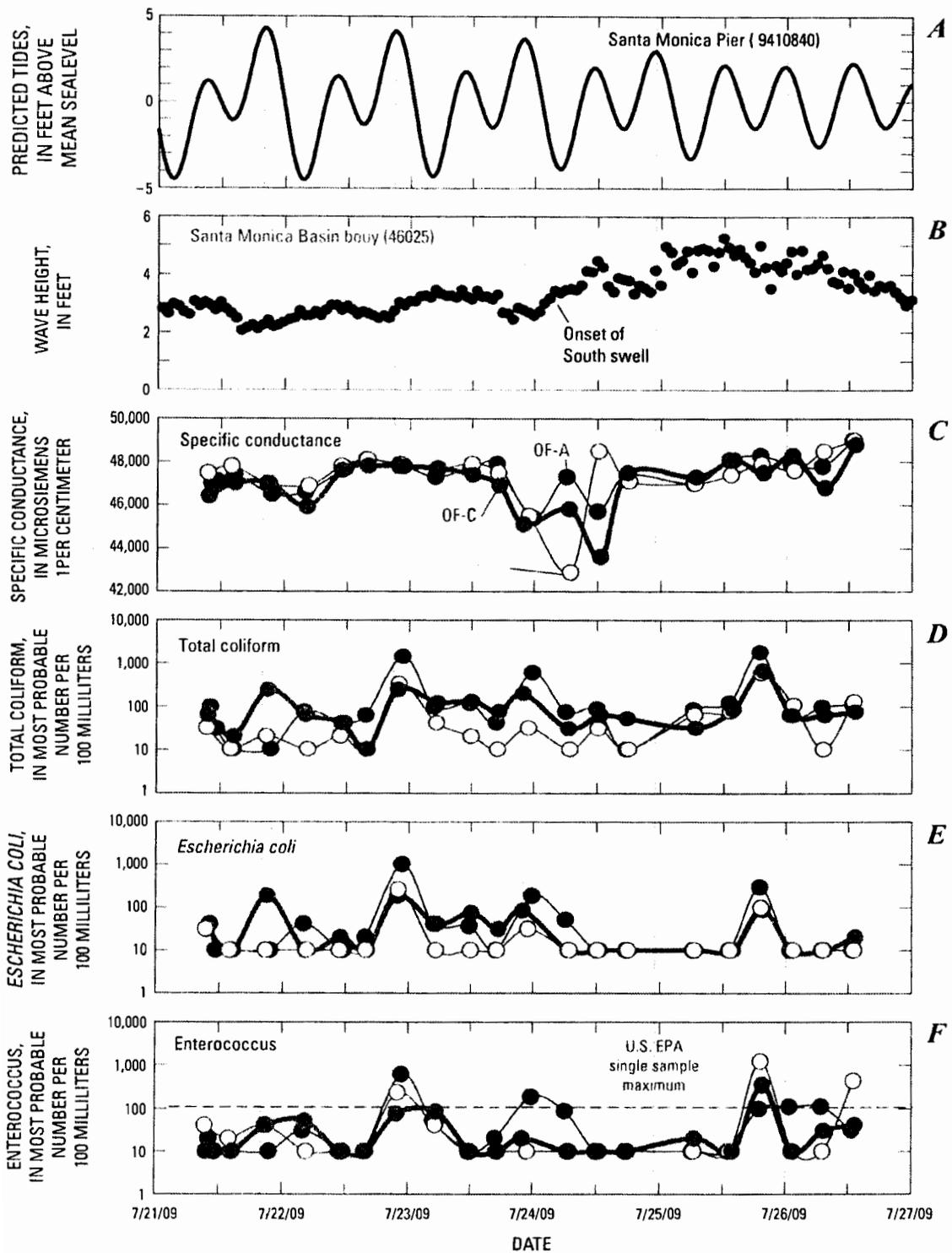


Figure 11

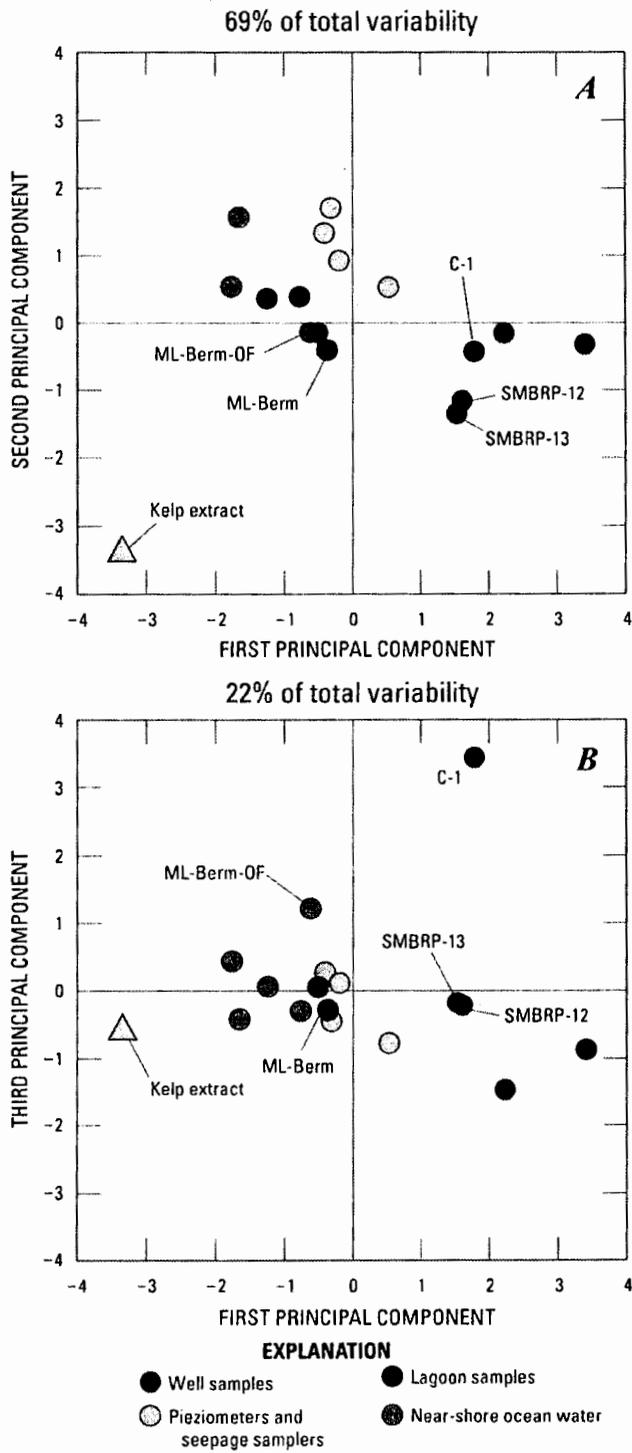


Figure 12

Table 1. Fecal indicator bacteria (FIB) concentration in water from selected water-table wells, Malibu, California, July 21-26, 2009.

[The five-digit parameter code below the constituent name is used by the U.S. Geological Survey to uniquely identify a specific constituent or property. C, Celsius; dpm/L, disintegrations per minute per liter; ft, feet; LSD, land surface datum; mg/L, milligrams per liter; mL, milliliters; MPN, most probable number; nc, not collected; μ S/cm, microsiemens per centimeter; <, less than;]

Well Identification No.	Date (m/dd/yyyy)	Time (24 hour)	Water level (ft below LSD)	Well depth (feet)	Dissolved oxygen, (mg/L) (00300)	pH (standard units) (00400)
SMBRP-10C	7/21/2009	14:45	6.12	25	2.9	7.2
SMBRP-11	7/21/2009	11:45	8.40	20	1	6.4
SMBRP-2	7/22/2009	13:15	5.34	11	0.4	7.1
SMBRP-12	7/22/2009	10:30	6.97	25	0.2	7.1
SMBRP-13	7/22/2009	14:30	7.47	20	1.7	7.3
P-9	7/22/2009	10:00	nc	12	0.3	7.1
CCR-1	7/24/2009	9:00	5.69	19	0.1	7.4
CCPE	7/23/2009	14:30	4.97	53	0.2	NR
CCPNE	7/23/2009	9:00	6.03	25	0.2	NR
CCPC	7/23/2009	10:25	5.76	22	0.2	NR
C-1	7/26/2009	11:45	4.47	14	0.1	7.3

Well Identification No.	Specific conductance (μ S/cm at 25°C) (00095)	Total coliforms (MPN/100 mL) (50569)	<i>Escherichia coli</i> (MPN/100 mL) (50468)	<i>Enterococci</i> (MPN/100 mL) (99601)	Radon-222 (dpm/L)
SMBRP-10C	12,700	< 10	< 10	< 10	nc
SMBRP-11	2,960	< 10	< 10	< 10	nc
SMBRP-2	3,360	< 1	< 1	< 1	1,220 \pm 189
SMBRP-12	3,820	< 1	< 1	< 1	650 \pm 141
SMBRP-13	2,450	< 1	< 1	< 1	850 \pm 158
P-9	2,000	< 1	< 1	< 1	1,340 \pm 198
CCR-1	2,080	2	< 1	2	1660 \pm 163
CCPE	10,800	11	65	1,600	1,050 \pm 139
CCPNE	1,960	1	< 1	7.5	1,370 \pm 160
CCPC	2,020	< 1	< 1	< 1	950 \pm 134
C-1	22,300	< 10	< 10	< 10	nc

Table 2. Fecal indicator bacteria (FIB) concentration in discharge water from a traditional septic system (OLD) and from an advanced septic system (ADV), Malibu, California, October 1, 2009.

[The five-digit parameter code below the constituent name is used by the U.S. Geological Survey to uniquely identify a specific constituent or property. C, Celsius; mL, milliliters; MPN, most probable number; $\mu\text{S/cm}$, microsiemens per centimeter]

Site Identification No.	Date (mm/dd/yyyy)	Time (24 hour)	pH (standard units) (00400)	Specific conductance ($\mu\text{S/cm}$ at 25°C) (00095)
MC-OLD-Septic	10/1/2009	12:30	6.9	1160
MC-ADV-Septic	10/2/2009	11:00	7.5	990

Site Identification No.	Total coliforms (MPN/100 mL) (50569)	<i>Escherichia coli</i> (MPN/100 mL) (50468)	Enterococcus (MPN/100 mL) (50569)
MC-OLD-Septic	610,000	220,000	7,300
MC-ADV-Septic	16,000	1,400	52

Title: Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu, California

Cooperating Agency: City of Malibu

Project Chief: John A. Izbicki

Period of Project: 2010-2011

Problem: Malibu Lagoon and near-shore ocean water in Malibu, Calif. have concentrations of fecal indicator bacteria (FIB) that occasionally exceed public health standards for recreational water. Discharge of water from commercial and residential septic systems, and subsequent transport through shallow groundwater to the lagoon or near-shore ocean has been proposed as a possible source of FIB to the lagoon and the near-shore ocean. Other possible sources include direct deposition of fecal material and FIB from birds and other wildlife. The problem is complicated by the possibility of sustained survival or regrowth of FIB in the lagoon, especially during the summer months when water temperatures are warm.

Objective: The purpose of this study is to evaluate the occurrence, distribution, and sources of FIB and nutrients in shallow groundwater, Malibu Lagoon, and near-shore ocean water near Malibu, Calif.

Benefits: The study will determine the source of FIB and nutrients in a hydrologically complex coastal setting. Results of this study will have significant transfer value to recreational ocean beaches impacted by FIB contamination in California and elsewhere. This study addresses issues 1, 2, and 8 from the Strategic Directions for the Water Resources Division, 1998-2008. Specifically, this study will address the effects of urbanization and suburbanization on water resources (issue 1), the effects of land use and population increases on water resources in the coastal zone (issue 2), and surface-water and ground-water interactions as related to water-resource management (issue 8). The study will facilitate integration of physical and isotopic hydrologic data with genetic, molecular, and chemical tracers of fecal contamination to determine the source of FIB in coastal areas.

Approach: The scope of the study includes detailed synoptic sample collection and time-series data collection in shallow groundwater, Malibu Lagoon, and the near-shore ocean. The study uses a combination of physical and isotopic hydrologic techniques coupled with state-of-the-art genetic, molecular, chemical, and optical tracers to determine the source of fecal contamination. Preliminary data collected during the summer and fall of 2009 were used to determine which techniques and tracers were suitable for use in this study.

On the basis of the preliminary data, synoptic and time-series data collection strategies were developed. The synoptic data collection will include collection of traditional physical and isotopic hydrologic data coupled with genetic, molecular, and chemical tracers of fecal contamination. Data will be collected from shallow groundwater, Malibu Lagoon, and the near-shore ocean shortly after the rainy season to contrast with data collected during the dry summer season (summer 2009 data). Time-series data will be collected from selected wells, Malibu Lagoon, and the near-shore ocean at approximately bimonthly intervals for one year to provide information of groundwater quality and FIB concentrations under hydrologic conditions not sampled during synoptic data collection.

Isotopic data are proposed to trace the source of water ($\delta^{18}\text{O}$ and δD) and discharge of groundwater to Malibu Lagoon and the near-shore ocean (^{222}Rn). Genetic (Terminal-Restriction Fragment Length Polymorphism, and human-specific *Bacteroidales* data), molecular (Phospholipid fatty acid data), and chemical data (wastewater indicator compounds) are proposed to trace the movement of bacteria and fecal contamination through the hydrologic flow system. Other tracers are proposed evaluate changes in the chemical composition of nutrients ($\delta^{15}\text{N}$ of nitrate and ammonia, and $\delta^{18}\text{O}$ of nitrate) and dissolved organic carbon (Ultraviolet absorbance and Excitation Emission Spectroscopy) as water flows through the system. No single hydrologic or bacteriological source tracking technique provides a truly unique identification of the source or hydrologic history of water sample or bacteria. As a consequence, interpretations from tracer data, used in both the synoptic and time-series data collection, are constrained by interpretations derived from traditional hydrologic and microbiological data.

Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu, California

By: John A. Izbicki

PROBLEM AND STUDY AREA

Malibu Lagoon and near-shore ocean water near Malibu, Calif. (fig. 1) have concentrations of fecal indicator bacteria (FIB) that occasionally exceed public health standards for recreational water. Discharge of water from commercial and residential septic systems and subsequent transport through shallow groundwater to the lagoon or to the near-shore ocean is a possible source of FIB. Concern over septic discharges has prompted regulatory agencies to impose a ban on septic discharges to shallow groundwater in the area (Los Angeles RWQCB, 2009). As part of this ban, no new septic systems are permitted and existing commercial and residential discharges are to be sewered and treated prior to discharge outside the Civic Center area. Historical data show FIB concentrations in shallow wells in the Malibu area are highly variable (Stone Environmental Inc., 2004). Recent data collected during July 2009 as part of this work show FIB concentrations in water from most shallow wells to be less than method detection limits for total coliform, *Escherichia coli* (*E. coli*), and enterococcus. These results suggest other sources may contribute FIB to Malibu Lagoon and the near-shore ocean.

In addition to septic discharges, other possible sources of FIB to Malibu Lagoon and near-shore ocean water include direct deposition of fecal material from birds and other wildlife to the lagoon, beach, and near-shore ocean. Surface discharges from Malibu Lagoon also have been shown to be a source of FIB contamination to the near-shore ocean, and groundwater movement through the berm of the lagoon at low-tide also may contribute FIB to the near-shore ocean. Uncertainty concerning the source of FIB to the lagoon and near-shore ocean is complicated by the possibility of sustained survival or regrowth of FIB, especially during the summer months when water temperatures are warm (Ferguson and others, 2005).

Study area—The study area is the Civic Center area of Malibu, Calif., including Malibu Lagoon and the near-shore ocean (fig. 1). The area contains unsewered residential and commercial development adjacent to Malibu Lagoon and the near-shore ocean. The area is underlain by alluvial deposits in places more than 150 ft thick. Depth to water is less than 10 feet in some areas underlying commercial and residential development. Groundwater in the alluvial deposits discharges to Malibu Lagoon or to the ocean. Groundwater in the area is not pumped for public supply.

Malibu Lagoon is open to the ocean during wet periods, especially after stormflows in Malibu Creek. Surface flow in Malibu Creek is not perennial and flow ceases shortly after permitted seasonal discharges from upstream wastewater treatment plants cease in mid-April. While open to the ocean, water-levels in the lagoon vary with ocean tides and have near-ocean water salinities (fig. 2). During dry periods a sand berm develops at the mouth of the lagoon, separating the lagoon from the ocean. After closure of the sand berm, water-levels in the lagoon rise as a result of surface inflow from Malibu Creek and from groundwater discharge to the lagoon. During the summer months, high tides occasionally overtop the berm allowing seawater to enter the lagoon. The influx of seawater increases the water levels and salinity in the lagoon. After the

high tidal stands, water levels and salinity decrease as saline water drains to the near-shore ocean through the berm and is replaced by fresh groundwater discharging to the lagoon.

Data from the Los Angeles RWQCB show FIB concentrations in Malibu Lagoon during 2009 were highly variable and enterococcus concentrations in the lagoon occasionally exceed the U.S. Environmental Protection Agency single sample standard for marine recreational water of 104 colonies (or equivalent) per 100 mL (U.S. Environmental Protection Agency 2003; Federal Register, 2004). Enterococcus concentrations in Malibu Lagoon were generally less than the detection limit of 10 Most Probable Number per 100 milliliters (MPN per 100 mL) when the berm at the mouth of the lagoon was open to the ocean and seawater could readily exchange with water in the lagoon during daily tidal cycles. Enterococcus concentrations as high as 6,100 MPN per 100 mL were present during stormflows, and concentrations were as high as 2,900 MPN per 100 ml during the dry season when the berm of the lagoon was closed to the ocean. Dry season enterococcus concentrations decreased to below the recreational water standard as a result of the influx of seawater into the lagoon during high tidal stands. After the influx of seawater, enterococcus concentrations in the lagoon increased to higher concentrations until diluted by seawater during the next high tidal stand. Between high tides, low enterococcus concentrations persist for several weeks if water temperatures in the lagoon remain low. Enterococcus concentrations were higher during summer when lagoon water temperatures were higher, and concentrations decreased during late summer through early fall as lagoon water temperatures declined. This pattern suggests strong hydrologic controls on FIB concentrations in Malibu Lagoon, coupled with possible regrowth of FIB in the warm water of the lagoon during the summer months.

Previous work, show FIB concentrations in near-shore ocean water also are highly variable. Concentrations in near-shore ocean water are affected by surface discharges from streams and rivers (Boehm et al., 2005), and also can depend on solar and tidal cycles (Boehm, 2007). High tides can wash FIB from beach sand and from debris accumulated along the high tide (wrack) line (Yamahara and others, 2007; Boehm and Weisberg, 2005; Izbicki and others, 2009). Groundwater discharge has been implicated as a source of FIB to the near-shore ocean during low tide (Paytan and others, 2004; Boehm and others, 2004 and 2006). If groundwater discharge is a source of FIB, concentrations in the near-shore ocean may increase during low tide when groundwater discharge is greater. These increases may be greater during falling monthly tidal cycles (Izbicki and others, 2009).

PURPOSE AND SCOPE

The purpose of this study is to evaluate the occurrence, distribution, and possible sources of FIB and nutrients in shallow groundwater, Malibu Lagoon, and near-shore ocean water near Malibu, Calif. The scope of the study includes detailed synoptic sample collection and time-series data collection in shallow groundwater, Malibu Lagoon, and the near-shore ocean. The study uses a combination of physical and isotopic hydrologic techniques coupled with state-of-the-art genetic, molecular, chemical, and optical tracers to determine the source of fecal contamination.

RELEVANCE AND BENEFITS

The study addresses the highly emotional issue of fecal indicator bacteria contamination in surface water and near shore ocean water. Contamination and closure of recreational beaches has lowered the perceived quality of life for southern California residents and has cost local economies millions of dollars in lost tourist revenue over the last several years. Results of this study are expected to have transfer value to recreational ocean beaches impacted by FIB contamination elsewhere in California and in other parts of the United States.

This study addresses issues 1, 2, and 8 from the Strategic Directions for the Water Resources Division, 1998-2008. Specifically, this study will address the effects of urbanization and suburbanization on water resources (issue 1), the effects of land use and population increases on water resources in the coastal zone (issue 2), and surface-water and ground-water interactions as related to water-resource management (issue 8).

The study uses a combination of physical and isotopic hydrologic data coupled with genetic, molecular, and chemical tracers of fecal contamination to determine the source of fecal contamination. The genetic, molecular, and chemical data collected as part of this study have been widely applied as research tools, and are beginning to be used by resource managers and regulators responsible for the management of recreational water and for the control fecal contamination. This study will provide an important link between the research community developing new genetic and molecular techniques and water-resource managers responsible for applying those techniques.

APPROACH

Preliminary data were collected during the summer of 2009 to: 1) evaluate temporal and spatial sampling strategies that would be effective in this hydrologic setting (including the high-energy surf zone), 2) evaluate ancillary hydrologic and isotopic data useful to the understanding the movement of water, FIB, and nutrients in the study area, and 3) evaluate genetic, molecular, and chemical tracers useful to understand the occurrence, distribution, and sources of FIB. Data collection and analysis described in this proposal were developed on the basis of preliminary data collected during the summer of 2009 and include coupled synoptic and time-series data collection.

Preliminary Data Collection

Groundwater level, radon-222 (^{222}Rn), direct-current resistivity, FIB, and bacterial-source tracking data were collected during a falling monthly tidal cycle in the dry summer season from July 21-27, 2009 near Malibu, California. Data collection was coordinated with an epidemiological study of FIB occurrence and human health coordinated by the Southern California Coastal Waters Research Program (SCCWRP) and University of California Berkeley. Additional data were collected from septic systems and the near-shore ocean in the fall of 2009 to provide end-members for interpretation of the July data, and to verify the ability to collect ^{222}Rn data in the high-energy surf of the near-shore ocean near Malibu. Preliminary data provided a snap-shot in time of the occurrence, distribution and movement of FIB, nutrients, and other constituents in shallow groundwater, Malibu Lagoon (including its tributary Malibu Creek) and near-shore ocean water. In addition, preliminary data collection provided an opportunity to

asses: 1) which data collection strategies (including the spatial and temporal distribution of samples) would be successful, and 2) which ancillary data would be useful in this complex hydrologic setting. Data collection issues were of special concern given the high surf conditions commonly present in the near-shore ocean near Malibu. Groundwater and surface water samples sites sampled are shown in figures 3 and 4, respectively.

Preliminary data collection showed:

1. Groundwater levels were affected by tides, ocean swells, and water levels in Malibu Lagoon (fig. 5). The seawall along Malibu Colony (fig. 6), which consists of wooden pilings driven to a depth of about 18 feet below land surface, may have damped tidal effects on water levels in well SMBRP-12 (fig. 5).

2. FIB concentrations (total coliform, *E. coli*, and enterococcus) were less than the detection limit, or were present at only low concentrations, in samples from the 10 of 11 water-table wells sampled (Table 1). The highest concentrations were in water from well CCPE in the commercial district near Malibu Lagoon. Water from well CCPE was saline and possibly impacted by water from Malibu Lagoon rather than septic systems. Nitrate and ammonia concentrations in shallow groundwater also were low with average concentrations of 1.5 and 1.7 mg/L as nitrogen, respectively. The highest nitrate concentration of 6.4 mg/L as nitrogen in water from well SMBRP-11 in unsewered residential development near Malibu Colony is less than the Maximum Contaminant Level (MCL) for nitrate in drinking water of 10 mg/L as nitrogen. The highest ammonia concentration was 12.2 mg/L as nitrogen in water from well SMBRP-12 in Malibu Colony. Ammonia was the primary form of nitrate in 3 sampled wells.

3. On the basis of ^{222}Rn data, shallow groundwater was discharging to Malibu Lagoon at an average rate of 2.8 cm/d during the July 2009 sample period (fig. 7). Discharge rates as high as 15 cm/d (6-hour average) were measured during high tidal stands at the beginning of the sample period. Discharge to the lagoon declined during the sample period because of increased water levels in the lagoon resulting from ocean water overtopping the berm separating the lagoon from the ocean during high tide.

4. High concentrations of FIB were present in Malibu Lagoon during the sample period (fig. 8). Total coliform and *E. coli* concentrations decreased during the sample period as a result of dilution by ocean water entering the lagoon at high tide. Enterococcus concentrations decreased during the day (consistent with inactivation by UV radiation in sunlight) and rebounded to higher concentrations at the night (fig. 8).

5. Water movement through the berm of Mailbu Lagoon was a source of FIB, especially enterococcus, to the near-shore ocean near the mouth of the lagoon during low tide (fig. 9). Enterococcus concentrations exceeded the U.S. Environmental Protection Agency single sample standard for (marine) recreational water (104 MPN per 100 ml) in near-shore ocean water near the lagoon berm at this time.

6. FIB concentrations increased during high tide at three sampled beaches (fig. 10). These increases were consistent with wave run-up on the beach washing FIB from kelp and other debris in the wrack line and from beach sands. FIB concentrations did not increase in near-shore ocean water during low tide when groundwater discharge to the ocean, measured on the basis of ^{222}Rn

data, was greatest. Detailed sample collection during a falling tidal cycle in November 2009 confirmed this observation.

Proposed data collection

Data from July 2009 show low FIB concentrations in groundwater, high FIB concentrations in Malibu Lagoon, high FIB in the near-shore adjacent to Malibu Lagoon at low tide, and high FIB concentrations in the near-shore ocean at other sampled beaches during high tide. Additional data collection is intended to confirm results from July 2009. Additional data collected also is intended to determine how the distribution and sources of FIB, nutrients, and other constituents change under different hydrologic conditions. The proposed data collection includes both synoptic and time-series data collection. The proposed data collection also includes detailed collection of field parameters, FIB, and nutrients from selected wells during purging to evaluate the performance and representativeness of data from those wells. Understanding of the processes controlling FIB occurrence obtained from these data will be used to interpret regulatory data having longer periods of record, but less analytical, temporal and spatial detail.

Synoptic data collection from groundwater, Malibu Lagoon (including its tributary Malibu Creek), and near-shore ocean water will be done during a falling monthly tidal cycle under wet conditions prior to closure of the berm at the mouth of the lagoon. These data will be contrasted with preliminary data collected in July 2009 under dry conditions when the lagoon was not discharging to the ocean. Time-series data will be collected from selected wells and surface water sites in the lagoon and near-shore ocean. Time-series data will allow assessment of the range in variability for measured constituents for hydrologic conditions not specifically addressed during synoptic sample collection. Samples collected as part of synoptic and time-series data collection will be analyzed for a range of constituents including: FIB, major-ions and nutrients, and a suite of tracers including 1) selected isotopes, 2) genetic, molecular, and chemical tracers of wastewater, and 3) dissolved organic carbon and optical property data.

Task 1: *Synoptic sample collection:* Synoptic data collection from groundwater, Malibu Lagoon (including its tributary Malibu Creek), and near-shore ocean water will be done during a falling monthly tidal cycle under wet conditions prior to closure of the berm at the mouth of the lagoon. The purpose of synoptic sample collection is to provide a snap-shot in time of the occurrence and distribution of FIB, nutrients, and other constituents in shallow groundwater, Malibu Lagoon (including its tributary Malibu Creek) and near-shore ocean water. Hydrologic, isotopic, and geophysical data collected during synoptic sample collection are intended to evaluate the movement of water between groundwater, lagoonal, and near-shore ocean environments. These data will be supplemented with genetic, molecular, and chemical data used to determine the source of FIB in these environments. The design of synoptic sample collection is similar to the design used for preliminary data collection during the summer of 2009, and the two synoptic data sets are expected to be comparable. Groundwater and surface water samples sites sampled during summer 2009 are shown in figures 2 and 3, respectively. On the basis of previous experience, data collection will require about 1 week.

Shallow groundwater—Five selected wells will be instrumented with pressure transducers to determine changes in water levels several weeks prior to, during, and for several weeks after the sample period. Water level data from these wells will be compared to and contrasted with tidal data, ocean swell data, and water-level data from Malibu Lagoon (fig. 5) to determine the effects

of tides, ocean swells, and changes in lagoon water levels on the discharge of shallow groundwater to Malibu Lagoon and the near-shore ocean. Fifteen wells will be sampled. This represents an increase in the number of wells compared to the July 2009 synoptic sample collection. The increase is intended to allow additional wells to be sampled in the commercial area near Malibu Lagoon and in the Sierra Retreat area. Wells will be sampled for field parameters (pH, specific conductance, and dissolved oxygen), FIB (total coliform, *E. coli*, and enterococcus), major-ion and nutrient concentrations (Table 2), the stable isotopes of oxygen and hydrogen (oxygen-18 and deuterium), ^{222}Rn activity, and dissolved organic carbon and optical property data (including full-spectrum ultraviolet absorbance and excitation emission spectroscopy). Ten sampled wells will be analyzed for a more complete list of constituents including $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate (or the $\delta^{15}\text{N}$ of ammonia where appropriate), and bacterial source-tracking data including genetic (Terminal-Restriction Fragment Length Polymorphism, and human-specific *Bacteoidales*), molecular (Phospholipid fatty Acids), and wastewater indicator (Table 3) data. Observation wells will be purged and sampled using peristaltic pumps. As many as four peristaltic pumps will be used to purge observation wells thereby minimizing purge times. Tubing and hoses used during purging and sample collection will be discarded after each use to prevent cross-contamination between sampled wells.

Malibu Lagoon—Samples from Malibu Lagoon will be collected from three sites in the lagoon (ML-West, ML-Comm, and ML-Berm, fig. 4) to evaluate the spatial distribution of field parameters, FIB, chemistry and nutrients (including nitrogen isotopes), dissolved organic carbon (including optical properties), and genetic, molecular, and chemical bacterial source-tracking data. Additional samples will be collected at different depths from other three sites (ML-Upper, ML-Middle, and ML-Lower, fig. 4) within the lagoon to determine the vertical distribution of field parameters, FIB, nutrients, and dissolved organic carbon (including optical properties) with depth (fig. 11). Continuous monitoring of lagoon water levels, field parameters, and ^{222}Rn activity will be done at two of these three sites (ML-Upper and ML-Lower) to evaluate groundwater discharge to the lagoon. ^{222}Rn samples from observation wells will be used to evaluate the groundwater radon activity for calculation of groundwater discharge to the lagoon. Atmospheric ^{222}Rn and relevant meteorological parameters will be measured continuously during this period to evaluate atmospheric boundary conditions that constrain these calculations. Lagoon water levels, ^{222}Rn activity, and calculated groundwater discharge will be compared to water-level data from wells, tidal data, and ocean swell data to evaluate changes in discharge to the lagoon during the sample period (fig. 7). These data will be used to estimate FIB and nutrient fluxes from groundwater to the lagoon.

Samples also will be collected near the berm of the lagoon (ML-Berm, fig. 4) at high and low tidal stands (approximately four times each day) to determine variations in field parameters, and FIB during the synoptic sample collection period, about 1 week. These data will be evaluated to determine hydrologic processes that control FIB concentrations, and if there are diurnal variations in FIB concentrations (especially enterococcus) resulting from inactivation by UV radiation in sunlight (fig. 8). The data also will be compared and contrasted with similar data collected from the near-shore ocean (fig. 10).

Near-Shore Ocean Water—Near-shore ocean water will be sampled at high and low tidal stands (approximately four times each day) at three beach locations to determine how field parameters and FIB concentrations change with tidal fluctuations. The three sample sites (OF-A, AF-B, and OF-C) are located to the west of the unsewered residential development in Malibu Colony, near

the berm of Malibu Lagoon, and the east of Malibu Lagoon, respectively (fig. 4). The data will be compared and contrasted with tidal and swell data and with similar data collected from Malibu Lagoon (fig. 10).

Near-shore ocean water also will be sampled during a falling tidal cycle (from high tide to low tide) at the berm at the mouth of Malibu Lagoon (ML-Berm-OF, fig. 4), adjacent to unsewered residential development where septage is discharged to shallow groundwater (MC-ADV-OF and MC-OLD-OF, fig. 4). Temporary piezometers will be installed at selected locations and depths each site (PZ 5ft, PZ 9ft, MC-ADV pz and MC-OLD pz, fig 4) to permit collection of water levels and water samples from shallow groundwater in the beach sands. Seepage samplers (specially designed for this study) also will be installed at the mid low and low tide line (Seepage Shallow and Seepage Deep, respectively, fig. 4) to collect samples discharging from the seepage face to the ocean at low tidal stands. Malibu Lagoon, near-shore ocean water, piezometers, and seepage samplers will be sampled hourly during a falling tidal cycle for field parameters, FIB, and optical property data. Water from near-shore ocean water, selected piezometers, and selected seepage samplers will be analyzed for additional parameters including: chemistry and nutrients, dissolved organic carbon and optical properties, and genetic, molecular, and chemical source tracking data at high tide and low tide. ^{222}Rn will be measured continuously in near-shore ocean water and in water from selected piezometers and seepage samplers during several tidal cycles prior to sample collection to estimate groundwater discharge to the ocean (fig. 12). For data collected near Malibu lagoon, discharge through the berm to the ocean also will be estimated from measured water-level data assuming reasonable hydraulic property values for the berm material. Estimated discharge from the lagoon through the berm to the near-shore ocean will be compared to groundwater discharge into the lagoon calculated from ^{222}Rn data to evaluate the lagoon water budget. If ocean conditions permit, electromagnetic seepage meters will be placed below the low tide line to provide point measurements of groundwater discharge to the near-shore ocean. Land-based direct-current resistivity data will be collected in shore-parallel and shore perpendicular configurations (depending on the site) to assess the distribution of fresh and saline water at high and low tidal stands (figs 13 and 14).

Other samples—Water from within a conventional and an advanced residential septic system and from within a commercial septic system will be sampled and analyzed for field parameters, FIB, chemistry and nutrients, dissolved organic carbon and optical properties, the stable isotopes of oxygen and hydrogen, and genetic, molecular, and chemical source tracking data. It may not be possible to resample the same septic systems that were sampled in 2009. Water extracts from kelp and beach sands (2 samples each) will be prepared in the field according to methods described by Izbicki et al. (2009). Extract water will be analyzed for FIB, nutrients, dissolved organic carbon and optical properties, and genetic, molecular, and chemical source-tracking data. Septic samples and water-extract data are important end-members for process oriented and statistical analysis of data collected in the study area.

TASK 2: Time-series data collection—Data will be collected from wells, Malibu Lagoon, and the near-shore ocean to assess variation in FIB, nutrients, and other constituents over an annual cycle.

Water from five selected wells will be sampled bimonthly (every other month) and analyzed for field parameters, FIB, chemistry and nutrients, and the stable isotopes of oxygen and hydrogen, dissolved organic carbon and optical property data. These wells will be in areas of unsewered

residential development (including Malibu Colony and the Sierra Retreat area), unsewered commercial development, and near Malibu Lagoon. Samples also will be analyzed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate (or $\delta^{15}\text{N}$ of ammonia will be measured where appropriate), and tracer data. Water samples also will be collected bimonthly from Malibu Creek and Malibu Lagoon at the berm near the mouth of the lagoon. These samples will be analyzed for the same constituents as the groundwater time series. Additional samples will be collected from bed sediments at the bottom of the lagoon. Bed material will be analyzed for FIB, nutrient, dissolved organic carbon and optical property data, and bacterial source tracking data.

^{222}Rn data and associated FIB and nutrient data will be collected from the near-shore ocean at Malibu Lagoon and near Malibu Colony two additional times during the course of the study. These data will be collected at tidal stands not represented during synoptic data collection

TASK 3: Microcosm experiments—There is increasing evidence for extended survival and possible regrowth of FIB in environmental settings (Von Donsel, 1971; Matson and others, 1978; Myers and others, 1998; and Byappanahalli and others, 2003). Regrowth of FIB may be a possibility in Malibu Lagoon, given 2009 summer water temperatures as high as 31°C (fig. 2)—human body temperature is 36.7°C . To address the possibility of regrowth of FIB a series of microcosm experiments will be done at the USGS laboratory in San Diego. Microcosms will contain lagoon water or lagoon water with sediment, and will be incubated at ambient temperature, 27°C , 31°C , and 35°C for a total of 8 microcosms. Two negative controls prepared using sterilized lagoon water and sterilized sediment will be incubated at 35°C . If necessary, dissolved organic carbon and phosphorous will be added to the microcosms to maintain nutrients within an optimal range for bacterial growth (Toothman and others, 2009; Haller and others, 2009; Surbeck and others, 2010). Microcosms will be aerated, but other factors such as pH, salinity will not be controlled but will be monitored during the study.

Microcosms will be incubated in the dark for seven days and sampled at 12 hour intervals for the first 2 days and at 24 hours intervals for the remaining 5 days. High-frequency sampling will be done at 2 hour intervals for 12 hours after the first day and after the sixth day, to assess the effect of predation and development of periodic steady-state oscillations within the microcosms (Surbeck and others, 2010). The volume of the microcosm will be sufficiently large that subsampling does not appreciably affect the experiment. Samples will be run in duplicate and analyzed for *E. coli* and enterococcus bacteria using membrane filtration techniques—modified mTEC (*E. Coli*), and mEI medium (enterococcus).

Results of this experiment will be compared to published decay and regrowth rates from similar experiments. In general, FIB decay rates in water are rapid if regrowth does not occur (McFeter and others, 1974), and growth rates are significant for microcosm experiments that exhibit regrowth (Toothman and others, 2009; Haller and others, 2009; Surbeck and others, 2010). Published data suggest that measureable differences in bacterial decay or growth can be obtained from this experiment. Published results also suggest that FIB decay will be less and regrowth will be greater in the presence of sediment (Toothman and others, 2009; Haller and others, 2009; Surbeck and others, 2010).

Water-quality sample handling and analysis

Synoptic and time-series sample collection will generate a large number of chemical and microbiological samples that have specific sample handling requirements with strict holding times prior to analysis.

Sample collection and analyses—FIB samples collected during synoptic sample collection will be analyzed for total coliform, *E. coli*, and enterococcus using Colert-18 (for total coliform and *E. coli* in saline water) and Enterolert (for enterococcus). Samples will be collected in sterile, disposable bottles, placed in coolers, and chilled immediately after collection. Samples will be analyzed in a temporary field laboratory set up in an office building available in the study area. Use of a field laboratory will enable most samples to be analyzed within 6 hours of collection—the recommended holding time for FIB analysis to be used for regulatory purposes. Holding times for samples collected late at night will be slightly longer but are not expected to exceed 12 hours. Previous data collection suggests that analyzing the samples at 3 dilutions (10 to 1, 100 to 1, and 1000 to 1) will produce results within an acceptable range, although samples from Malibu Lagoon may require additional dilutions to obtain results in an acceptable range. Samples for Colert-18 and Enterolert will be incubated at the field laboratory in laboratory incubators for 18 to 20 hours and 22 to 26 hours, respectively. Sample quanti-trays will be counted in the field laboratory after incubation. A smaller number of samples will be analyzed using membrane filtration techniques m-ENDO (total coliform), modified mTEC (*E. Coli*), and mEI medium (enterococcus). The plated cultures will be incubated and counted in the field laboratory. Results of membrane filtration techniques will be compared with results from Colert-18 and Enterolert analysis. Laboratory process blanks will be run daily and replicate analysis will be done on 5 percent of the samples.

It will not be possible to incubate FIB samples collected as part of time-series sample collection in a field laboratory. These samples will be either delivered to the USGS laboratory in San Diego California on the day of collection for analysis, or will be shipped overnight to the USGS laboratory in San Diego for analysis. Holding times for these samples are not expected to exceed 24 hours—the recommended holding time for FIB analysis to be used for scientific purposes.

Samples to be analyzed for Terminal-Restriction Fragment Length Polymorphism (T-RFLP), and human-specific *Bacteroidales* will be analyzed at the University of California Santa Barbara. These samples will be collected in 1-L baked amber glass bottles. Sample bottles will be stored in coolers and chilled immediately after collection. Samples will be delivered by courier or USGS personnel to the UCSB laboratory for filtration and extraction of DNA on the day of collection. Sample for T-RFLP will be analyzed by qPCR using methods described by LaMontagne and Holden (2003), Samples for human-specific *Bacteroidales* will be analyzed using methods described by Kildare and others (2007), using the Taqman qPCR assay to determine the lowest template dilution without inhibition (Haugland and others, 2005; Morrison and others, 2008).

Samples to be analyzed for Phospholipid Fatty Acids will be collected in 1-L baked amber-glass bottles. Sample bottles will be stored in coolers and chilled immediately after collection. Samples will be shipped overnight to a contract laboratory for analysis using a modified Bligh and Dyer method (White and others, 1979).

Samples for nutrients (NWQL Schedule 1034) will be filtered at the time of collection and placed in 125 mL amber plastic bottles. Samples for wastewater indicators (NWQL Schedule 4433) will be collected in 1-L baked amber-glass containers. Nutrient and wastewater indicator samples will be stored in coolers, chilled immediately after collection, and shipped overnight to the U.S. Geological Survey National Water Quality Laboratory (NWQL) for analysis. Nutrients will be analyzed using various methods described by Patton and Truitt (1992 and 2000) and Fishman and others (1993). Wastewater indicator samples will be analyzed by continuous liquid-liquid extraction and capillary-column gas chromatography/mass spectrometry (Zaugg and others, 2006). Extracts from selected wastewater indicator samples also will be analyzed for tentatively identified compounds (NWQL Laboratory Code 2753). Samples for major-ion, minor-ion, and trace element chemistry (NWQL Schedule 1261) and the stable isotopes of oxygen and hydrogen (NWQL schedule 1142) will be field filtered and preserved (as needed) at the time of collection, stored in coolers, and shipped to the NWQL (and the U.S. Geological Survey Isotope Laboratory in Reston, Va.) at the end of the field trip. Selected samples for $\delta^{15}\text{N}$ of nitrate and ammonia and $\delta^{18}\text{O}$ of nitrate will be field filtered through 0.2 mm pore-sized filter at the time of collection. Samples for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate and frozen prior to shipment to the U.S. Geological Survey Isotope Laboratory for analysis. Samples for $\delta^{15}\text{N}$ of ammonia will be preserved with reagent grade 4.5 N H_2SO_4 to pH < 2 at the time of collection. Samples for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate and $\delta^{15}\text{N}$ of ammonia will not be shipped until nitrate and ammonia results are available from the NWQL (U.S. Geological Survey Office of Water Quality Technical Memorandum 2008.04, June 26, 2008).

Samples for dissolved organic carbon and optical properties including full-spectrum ultraviolet (UV) absorbance and excitation-emission (EEM's) spectroscopy will be chilled, filtered as soon as practical after collection, and shipped to the USGS laboratory in Sacramento for analysis. DOC will be analyzed using UV-promoted persulfate oxidation (Brenton and Arnett, 1993). UV absorbance at wavelengths from 190 to 310 nm will be measured spectrometrically (American Public Health Association, 1992). Excitation Emission Spectroscopy data will be collected using a Jobin Horiba Fluoromax 4 spectrofluorometer with excitation wavelengths from 240-440 nm and emission wavelengths collected from 290-600 nm.

Quality Assurance Data—Approximately 10 percent of the laboratory analytical budget is reserved for quality assurance data. Quality assurance procedures for field microbiological data are discussed in the sample collection and analysis section. For other chemical and bacteriological analysis, quality assurance data will be distributed evenly between field blanks and replicate data. Field blank data will be used to assess the possibility of low-level contamination interfering with results. This is of special concern for many of the wastewater indicator analysis that can be easily contaminated during sample collection and handling. Replicate analysis will be used to assess the precision of sample collection and handling procedures. These data will be used to determine the precision of individual measurements and to ultimately constrain interpretation of the data. For many genetic and isotopic analyses, collection and analysis of field blank data does not make sense. Quality assurance for these data will be assessed on the basis of replicate analysis. If problems in field blank or replicate data are discovered during the course of this study sample collection or handling procedures will be altered if needed or the data will be censored to appropriate levels prior to interpretation.

Quality assurance data for the two non-USGS laboratories used as part of this study will be compared with quality assurance data collected as part of previous work (Izbicki and others,

2009). Internal laboratory practices within those laboratories will be assessed on the basis of standard operating protocols provided by the laboratories.

One unexpected result from the preliminary data collection in July 2009 was the general absence of FIB in shallow groundwater. Historical data from sampled wells (Stone Environmental, 2004) suggests that FIB are present in water from some wells but that concentrations in wells are variable. The variation appears random although there may be some seasonality to the variability in some wells. To quality assure FIB data from wells, FIB and nutrient concentrations will be monitored in water from selected wells during well purging. The data will be used to determine the minimum purge volume required to collect a representative FIB sample and assess the effect of inadequate purging on FIB and nutrient concentrations.

Tracer Data

Isotopic, genetic, molecular, wastewater indicator data are proposed for this study to trace the movement of water and bacteria through the hydrologic flow system. Other tracers used in this study evaluate changes in the chemical composition of nutrients and dissolved organic carbon as water flows through the system. No single hydrologic or bacteriological source tracking technique provides truly unique identification of the source or hydrologic history of a water sample or of bacteria. As a consequence, tracer data, used in both the synoptic and time-series data collection, are intended to constrain interpretations derived from traditional hydrologic or microbiological data.

The following discussion provides information of the theoretical basis of each proposed tracer and examples of the application of these data from the preliminary 2009 data collection. Examples of applications of tracer data provided in this proposal are intended for illustrative purposes, and are not intended to be definitive interpretations of those data.

Isotopic data—Isotopes are atoms of the same element (atoms having the same number of protons) but different number of neutrons. Isotopes may be stable and their abundance does not change with time, or radioactive and their abundance changes through radioactive decay with time. The difference in atomic mass caused by the difference in the number of neutrons causes slight but measurable differences in the physical, chemical, and biological reactions of different isotopes. Radioactive decay, measured as the time it take for half of the isotope to change into another element (half-life), produces an atomic clock that can be used to measure physical, chemical, or biological reaction rates. Isotopic data collected and analyzed as part of this study will be used to determine the source and hydrologic history of water (oxygen-18 and deuterium), groundwater discharge rates (^{222}Rn), and processes effecting nitrate and ammonia concentrations ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate and $\delta^{15}\text{N}$ of ammonia).

Oxygen-18 and deuterium—Oxygen-18 and deuterium are naturally occurring stable (non-radioactive) isotopes of oxygen and hydrogen, respectively. Oxygen-18 and deuterium abundances are reported as ratios of the heavier isotope (oxygen-18 or deuterium) to the more common lighter isotope (oxygen-16 or hydrogen) using delta (δ) notation in per mil (parts per thousand) differences relative to the standard known as Vienna Standard Mean Ocean Water (VSMOW) (Gonfiantini, 1978). By convention the value of VSMOW is 0 per mil. $\delta^{18}\text{O}$ and δD ratios can be measured more accurately than absolute abundances, with precisions of about ± 1

per mil and ± 0.1 per mil, respectively. When using this notation samples having less-negative δ values contain more of the heavier isotope than samples having more-negative δ values.

Most of the world's precipitation originates as evaporation of seawater. As a result, the $\delta^{18}\text{O}$ and δD composition of precipitation throughout the world is linearly correlated and distributed along a line known as the meteoric water line (Craig, 1961). Atmospheric and hydrologic processes combine to produce broad global and regional differences in the $\delta^{18}\text{O}$ and δD composition of water. These processes provide a record of the source and hydrologic history of the water.

Water that condensed from precipitation in cooler environments at higher altitudes or higher latitudes is isotopically lighter, or more negative, than water that condensed in warmer environments or lower latitudes (International Atomic Energy Agency, 1981). In the study area, water imported for public supply from northern California or from the Colorado River is isotopically lighter than water derived from local precipitation (shown as the composition at Santa Maria, Calif.) (fig. 15). The more negative values in water from wells SMBRP-11 and SMBRP-12 suggest a higher fraction of imported water in those samples compared to water from other wells. Water from wells SMBRP-11 and SMBRP-12 also had the highest nitrate and ammonia concentrations of all sampled wells, respectively. Water from these wells was presumably used for public supply and discharged through septic systems to groundwater. In contrast, near zero values were measured in near-shore ocean water, and a less negative value was measured in saline water from well CCPE adjacent to Malibu Lagoon (fig. 15). Well CCPE is the only sample well that had significant concentrations of FIB (Table 1). Assuming the only source of salinity in water from well CCPE is ocean water (that entered shallow groundwater through Malibu Lagoon) water from well CCPE is about 0.18 seawater. Native water in this sample would have had an initial $\delta^{18}\text{O}$ and δD composition of about -5.4 and -40 per mil, respectively—similar to water from other wells in the commercial area near the lagoon. The $\delta^{18}\text{O}$ and δD composition of water from well CCPE and other wells in the commercial area is similar to the expected composition of groundwater recharged from local precipitation and heavier than the composition of imported water. These data suggests the presence of only a small fraction of imported water discharged from septic systems in the commercial area near the lagoon.

Additional $\delta^{18}\text{O}$ and δD will be used to establish the hydrologic link between imported water, discharged from septic systems or other sources, and FIB and nutrient concentrations. $\delta^{18}\text{O}$ and δD data collection, in the commercial area near Malibu Lagoon will help resolve the apparent absence of imported water having a septic history in this area.

Radon-222—Radon-222 (^{222}Rn) is a naturally-occurring radioactive isotope produced by the decay of radium-226 (^{226}Ra) in the uranium-238 (^{238}U) decay series. ^{222}Rn has a half-life of 3.8 days. Radon, the heaviest of the noble gases, does not react chemically and is highly mobile in groundwater (Swarzenski, 2007). ^{222}Rn concentrations in groundwater are commonly several orders of magnitude higher than concentrations in surface water or ocean water. Diffusion of ^{222}Rn from sediments is small and ^{222}Rn activities in surface water and near-shore ocean water reflect the discharge of shallow groundwater (Burnett and Dulaiova, 2003; Swarzenski and Izbicki, 2009). ^{222}Rn in surface water and near-shore ocean water will be measured on a near-continuous basis using an air/water exchanger and a radon-in-air monitor. ^{222}Rn data average groundwater discharge over larger volumes than point measurements (such as those obtained

from seepmeters) and are often a better indicator of groundwater discharge than point measurements (Swarzenski and Izbicki, 2009).

In July 2009, ^{222}Rn activities in water from 8 wells ranged from 650 to 1,370 dpm/L (disintegrations per minute per liter). ^{222}Rn activities in Malibu Lagoon ranged from 8 to 62 dpm/L. Preliminary analysis of ^{222}Rn data show groundwater discharge to the upstream part of Malibu Lagoon (ML-Upper, fig. 3) averaged 2.8 cm/d between July 21-26, 2009 (fig. 6). This value agreed well with a model derived average groundwater discharge rate to Malibu Lagoon of 3.2 cm/d. A lower groundwater discharge rate of 0.8 cm/d was obtained in the downstream part of the lagoon between July 24-25, 2009. ^{222}Rn activity data collected in the near-shore ocean adjacent to Malibu lagoon and adjacent to unsewered residential development near Malibu Colony in November 2009 showed increased groundwater discharge was associated with low tidal stands at both locations (fig. 12). FIB concentrations in the near-shore ocean adjacent to Malibu Lagoon increase during low tide (fig. 9 and 12) but did not increase during low tide adjacent to unsewered residential development in Malibu Colony.

Additional ^{222}Rn data, coupled with FIB and nutrient data, will be collected during falling tidal cycles to extend results obtained during 2009 sample collection to different hydrologic conditions. Groundwater discharge rates to Malibu Lagoon and to the near-shore ocean calculated from ^{222}Rn data will be used to determine the timing of groundwater discharge, and to calculate the flux of FIB and nutrients from groundwater to these environments using method described by Swarzenski and Izbicki (2009) and Izbicki and others (2009). Additional data collected in Malibu Lagoon will be used to bracket the range in average and maximum groundwater discharge rates under different hydrologic conditions.

Delta nitrogen-15 and delta oxygen-18 of nitrate, and delta nitrogen-15 of ammonia—There are two stable isotopes of nitrogen: nitrogen-14 (^{14}N) and nitrogen-15 (^{15}N). The average $^{15}\text{N}/^{14}\text{N}$ ratio in atmospheric air (1/272) is constant. Nitrogen isotope abundances are reported as ratios using delta (δ) notation in per mil differences relative to the $^{15}\text{N}/^{14}\text{N}$ ratio of nitrogen gas in atmospheric air (Coplen and others, 2001). By convention the $\delta^{15}\text{N}$ value of nitrogen gas in atmospheric air is 0 per mil. $\delta^{15}\text{N}$ isotope ratios can be measured with a precision of about ± 0.2 per mil. Positive $\delta^{15}\text{N}$ values contain more of the heavier isotope, and negative $\delta^{15}\text{N}$ values contain less of the heavier isotope than atmospheric nitrogen gas.

The biological reactivity and the wide range of oxidation states in nitrogen compounds results in a wide range in $\delta^{15}\text{N}$ isotopic compositions spanning more than 200 per mil relative to standard atmospheric nitrogen gas (Coplen et al., 2002). Despite this wide range, the $\delta^{15}\text{N}$ isotopic composition of ammonia in septage is relatively constant averaging 4.9 ± 0.4 per mil (Hinkle and others, 2008). The $\delta^{15}\text{N}$ isotopic composition of nitrate derived from septage varies more widely, averaging 7.2 ± 2.6 per mil (Hinkle and others, 2008). The range in $\delta^{15}\text{N}$ composition of nitrate derived from septic tank discharges is not random, instead higher $\delta^{15}\text{N}$ values of nitrate result from more extensive loss of nitrogen through biological processes during the conversion of ammonia in septic waste to nitrate and lower $\delta^{15}\text{N}$ values result from less loss of nitrogen. In the environment, the isotopic composition of nitrate from septic discharges can be further altered as a result of processes such as denitrification. As nitrate is converted to nitrogen gas during denitrification, nitrate is lost from the system and the $\delta^{15}\text{N}$ composition of residual nitrate increases as nitrate concentrations decrease. Interpretation of nitrogen isotope data from septic

discharges is complicated by loss on nitrogen through the volatilization of ammonia, addition of nitrogen from other sources including fertilizer and animal wastes, and by the number and complexity of biological reactions that can alter the $\delta^{15}\text{N}$ isotopic composition of nitrate and ammonia. The $\delta^{18}\text{O}$ composition of oxygen in the nitrate molecule can be used to understand some of the complexity and in some cases to distinguish nitrate from septic and fertilizer sources (Kendall, 1998). The combination of chemical and isotopic data will be used to understand environmental processes controlling ammonia and nitrate concentrations in groundwater, Malibu Lagoon and near-shore ocean water.

In July 2009, the nitrogen concentrations (ammonia plus nitrate as nitrogen) in water sampled from a traditional and an advanced septic tank were 42.9 and 3.4 mg/L as nitrogen, respectively. The nitrogen was primarily in the form of ammonia and the $\delta^{15}\text{N}$ composition of ammonia was consistent with literature values and ranged from 5.3 to 5.4 per mil. At that time, the $\delta^{15}\text{N}$ range for ammonia in groundwater was between 19 to 23 per mil, the $\delta^{15}\text{N}$ composition of nitrate ranged from 15 to 102 per mil, and the $\delta^{18}\text{O}$ composition of nitrate ranged from 0.9 to 21 per mil. The average ammonia and nitrate concentrations in sampled wells were 1.7 and 1.5 mg/L as nitrogen. Decreases in ammonia and nitrate concentrations and changes in isotopic compositions in groundwater are consistent with nitrogen losses from septic discharges through denitrification or other processes.

Interpretation of chemical and nitrogen isotopic data is complicated by the wide, often overlapping range in concentration and isotopic composition of nitrate from different sources, and by the changing isotopic composition of nitrate as reactions proceed (Xue et al., 2009). Additional nitrogen isotope data will be used to evaluate nitrogen contributions from other sources, and to determine if denitrification or other processes are occurring in the groundwater system. If these processes are occurring, additional data will help determine the extent these processes act to reduce the nutrient discharges to Malibu Lagoon and the near-shore ocean.

Genetic, molecular, and wastewater indicator data—Genetic (Terminal-Restriction Fragment Length Polymorphism and human-specific *Bacteroidales*), molecular (phospholipid fatty acids), and wastewater indicator data will be used to evaluate to source of bacteria of the hydrologic history of a water sample with respect to septic discharges. No single hydrologic or bacteriological source tracking technique provides truly unique identification of the source or hydrologic history of a water sample or bacteria. The combination of genetic, molecular, and chemical tracers used in conjunction with the isotopic data discussed previously is intended to confirm, refine, or refute possible interpretations on the movement of water and bacteria in this complex hydrologic setting. Additional data collection will extend results from the 2009 preliminary sample collection to a wider range of hydrologic conditions.

Genetic data—Genetic diversity in microbial populations is assessed using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) data. T-RFLP uses restriction enzymes to break genetic material within the hypervariable region of mitochondrial DNA into smaller fragments known as amplicons. Amplicons having different number of base pairs (amplicon length) represent different microorganisms. However, the sequence of base pairs within amplicons of the same length may be greatly different, and more than one microorganism may be represented within a single amplicon. Quantitative polymerase chain reaction (qPCR) is used to amplify DNA in a water sample to measurable concentrations, and the electropherogram peak area is a measure of the abundance of an amplicon and the microorganism(s) from which it originated.

Amplicons that appear in more than one sample are common to those samples, and potentially represent the same organism. Amplicons that appear in only one sample are unique to that sample, and represent unique microorganisms. Comparison of common and unique amplicons in samples from different settings allows understanding of the similarities and differences in the microbial community in those settings. This approach is known as microbial community structure analysis (CSA).

Comparison of T-RFLP amplicons from Malibu Lagoon (ML-Berm), a piezometer driven into the berm of the lagoon to a depth of 5 feet (ML-Berm-Pz5), and the near-shore ocean at low tide shows a number of different amplicons representing the microbial communities in each of these samples (fig. 16). Malibu Lagoon is the least diverse of the three communities and the microbial population is dominated by 2 amplicons having lengths of 520 and 690 base pairs (M-spl and H-hal restriction enzymes, respectively). These amplicons also are present in water sampled from the piezometer and from the near-shore ocean at low tide. Common amplicons indicate the potential presence of the same organisms in each sample. This result is consistent with bacterial transport from the lagoon to in the near-shore ocean as seepage through the berm at low tide. In contrast, little similarity was observed in the amplicons representing microbial communities in septic tanks, shallow groundwater and the near-shore ocean at high and low tide near Malibu Colony. Those data suggest large differences in the microbial community between these samples. Although water may flow from the septic tanks to discharge at the near-shore ocean the microbial community is greatly altered and transport of individual bacteria is probably limited from septic tanks to the near-shore ocean is probably.

As the number of amplicons within individual samples and the number of samples being compared increases, microbial CSA becomes increasingly complex. Statistical approaches such as Principal Component Analysis (discussed later in this proposal) are used for these more complex microbial CSA.

Human-specific *Bacteroidales* is a tracer of the origin of fecal material and FIB in a water sample. Although fecal material from other mammals, birds, and in some cases even fish also may produce low positive detections of *Bacteroidales*; and dilution, sorption, or other processes may cause *Bacteroidales* concentrations to be below the detection limit even when small amounts of human fecal material are present—human-specific *Bacteroidales* is considered to be one of the most robust indicators of human fecal contamination.

Human-specific *Bacteroidales* were quantifiable in samples collected within the two septic systems sampled in Malibu Colony (MC-OLD-Septic and MC-ADV-Septic) (Table 4). Human-specific *Bacteroidales* concentrations were higher in the sample collected from within the traditional septic system (MC-OLD-Septic) than the sample collected within the advanced septic system (MC-ADV-Septic). High concentrations of human-specific *Bacteroidales* in samples from septic system are not unexpected. Human-specific *Bacteroidales* were present but not quantifiable from one well (P-9) near commercial septic discharges adjacent to Malibu Lagoon, and in water extracts from kelp and sand (Kelp extract and Sand extract). Well CCPE which had the highest FIB concentrations was not analyzed for *Bacteroidales*. Human-specific *Bacteroidales* were not detected in other groundwater samples, samples from Malibu Lagoon, and samples from near-shore ocean water. The absence of human-specific *Bacteroidales* in water from Malibu Lagoon is consistent with results by Ambrose and Orme (2000).

Molecular data—Fatty acids are components of all living cells. Because phospholipid fatty acids (PLFAs) contain phosphorus, they are rapidly degraded in the environment and are typically associated with living (or recently living) organisms. At the cellular level, they may be used for energy storage, or they may be part of cellular organelles and structures where they participate in metabolic activities (Tunlid and White, 1992). Individual PLFAs are associated with specific metabolic activities by a wide-range of microorganisms (Haack and others, 1994). In contrast to genetic data which identify different microorganisms, PLFA data identify what the microorganisms are doing. PLFA data are highly robust and are often able to explain more of the variability in microbial community structure than genetic data (Izbicki, and others, 2009).

The distribution of PLFA structural groups in groundwater, Malibu Lagoon, and the near-shore ocean during July 2009 was analyzed using principal component analysis (PCA). The first three principal components explain 91 percent of the total variability in the PLFA data. PCA results show differences in the PLFA composition of microbial communities in samples from water-table wells and from near-shore ocean water (fig. 11). Samples from piezometers and seepage samplers in beach sands are intermediate in composition, and samples from Malibu Lagoon are similar to samples from the near-shore ocean. The first and second principle components for samples collected from near-shore ocean water near Malibu Lagoon at low tide (ML-Berm-OF) are almost identical in PLFA composition to water from the lagoon (ML-Berm), consistent with seepage from the lagoon as a possible source of bacteria in the near-shore ocean water near the lagoon at low tide.

Wastewater indicator data—A suite of 69 organic compounds will be measured as part of this study. The compounds can be divided into a number of categories on the basis of their use and origin and include specific indicators of human septic contamination (Glassmeyer and others, 2005). Reporting limits for most analyzed compounds are within the part per trillion range, and detectable concentrations are generally below thresholds for public health or environmental concerns. Compounds analyzed as part of this study are anthropogenic and do not occur naturally. Many of these compounds, such as caffeine, fecal sterols, detergent metabolites, personal care products (PCPs), and pharmaceuticals lend themselves to specific interpretations of the origin of fecal contamination (Glassmeyer and others, 2005; Izbicki, and others, 2009).

Data collected during 2009 show the highest concentrations of wastewater indicator samples in the two sampled septic systems (MC-OLD Septic, and MC-ADV Septic), where more than 20 of these compounds were detected including caffeine, fecal sterols associated with human waste (such as 3-beta-coprostanol), several detergent metabolites, and a number of common PCPs and pharmaceuticals (such as DEET and triclosan). Fewer of these compounds were detected in piezometers driven into the beach adjacent to the ocean and near the bottom of the seawall in Malibu Colony. However, the presence of caffeine, several detergent metabolites, and a wide range of PCPs suggest a possible wastewater origin to some of the sampled water—even though other indicators of septic contamination such as FIB and human-specific *Bacteroidales* were absent. Wastewater indicator compounds were almost completely absent in the near-shore ocean adjacent to Malibu Colony although one detergent metabolite and several PCPs associated with sunscreen use (such as DEET) were present at low tide. In contrast, wastewater compounds including caffeine, detergent metabolites, PCPs and pharmaceuticals were less than detection in Malibu Lagoon and in the near-shore ocean adjacent to the lagoon, and consequently do not suggest a wastewater origin—even though FIB concentrations in the lagoon exceed U.S. EPA single sample standards for recreational water.

Dissolved organic carbon and optical property data—Optical property data, including Ultraviolet (UV) absorbance, and Excitation Emission Spectroscopy, (EEM's) are used to evaluate the source of organic carbon in sample water (Leenheer, 2009; Izbicki and others, 2007).

Dissolved organic carbon data are important because of the high organic load associated with septic discharges. For example, water from the two sampled septic systems had DOC concentrations ranging from 19 to 23 mg/L. DOC concentrations of water from wells in unsewered residential areas near Malibu Colony (SMBPR-11, SMBRP-12, and SMBRP-13) ranged from 7.6 to 3.3 mg/L. These samples have been previously identified on the basis of their $\delta^{18}\text{O}$ and δD composition as containing a high fraction of imported water likely discharged to the groundwater through septic systems. Consistent with septic discharges, water from wells SMBRP-12 had the highest nitrate concentration of all sampled wells, 6.4 mg/L as nitrogen, and water from well SMBRP-13 had the highest ammonia concentration 12 mg/L as nitrogen. In contrast, water from wells in the commercial district near Malibu Lagoon that did not have $\delta^{18}\text{O}$ and δD compositions consistent with imported water had low dissolved organic carbon concentrations typical of native groundwater of about 1.8 mg/L. DOC concentrations in Malibu Lagoon were higher than those in shallow groundwater and ranged from 8.7 to 3 mg/L. Optical property data are intended to evaluate changes in DOC composition as concentrations decrease and to be used as tracers of DOC from different sources.

Statistical Analysis of Data—Principal Component Analysis (PCA) will be used to analyze tracer data collected as part of this study. PCA is a multivariate statistical technique that transforms a set of intercorrelated variables into a new coordinate system. The transformed variables, known as principal components, are uncorrelated linear combinations of the original variables. They have a mean of zero and the same variance as the original data set. The values of the principal components are known as scores, and the scores are calculated on the basis of the contribution of each variable to the principal component. The magnitude and direction (plus or minus) of the contribution of each variable to the principal-component score is described by an eigenvector. PCA presents differences in the tracer assemblage that is reflective of differences in the microbial community structure, and allows for a comparison and contrast of different samples. Comparison of results from different tracers is intended to confirm, refine, or refute interpretations derived from individual tracers—thereby producing a more robust interpretation of the sources of FIB and the hydrologic processes that control the occurrence of high concentrations of FIB in the study area.

Understanding of the processes controlling FIB occurrence will be used to interpret regulatory data having longer periods of record, but less analytical, temporal and spatial detail. Logistic regression will be used to attempt develop explanatory relationships between hydrologic processes such as tidal conditions and lagoon water levels. Many regulatory FIB data and supporting ancillary data sets in the area have sufficiently long periods of record to permit development of the regression equations with part of the data and subsequent testing the predictive power of the equations with the remainder of the data.

REPORTS

A journal article describing the results of preliminary data collection will be prepared during FY-10. A final report from this project will be prepared during FY-11. The final paper will compare and contrast the distribution and source of FIB in shallow groundwater, Malibu Lagoon, and near-shore ocean water near Malibu, California. Both papers will use processes identified as part of this study to interpret regulatory data having longer periods of record, but less temporal and spatial detail. The final paper will be submitted to an appropriate journal by September 2011.

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LIST OF FIGURES

- Figure 1.**—Study area location
- Figure 2.**—Ocean tides, Malibu lagoon water levels, salinity and temperature, streamflow in Malibu Creek, and precipitation data, near Malibu, California, 2009
- Figure 3.**—Groundwater sample sites, Malibu, California, 2009
- Figure 4.**—Surface water sample sites, Malibu California, 2009
- Figure 5.**—Ocean tides, surface swell, and water level data for selected wells, Malibu California, July 6 to August 5, 2009
- Figure 6.**—Photograph showing seawall at Malibu Colony, Malibu California, July 23, 2009
- Figure 7.**—Radon-222 (^{222}Rn) concentrations and calculated groundwater discharge to Malibu Lagoon (ML-Upper, figure 3), Malibu California, July 21-27-2009
- Figure 8.**—Water level, specific conductance, and fecal indicator bacteria (FIB) concentrations in water from Malibu Lagoon (ML-Berm, figure 3), Malibu California, July 21-26, 2009.
- Figure 9.**—Specific conductance and fecal indicator bacteria (FIB) concentrations in water from Malibu Lagoon (ML-Berm), piezometers and seepage samplers in the berm separating Malibu Lagoon from the ocean, and in adjacent near-shore ocean water (ML-Berm-OF).
- Figure 10.**—Specific conductance and fecal indicator bacteria (FIB) concentrations in Malibu Lagoon and near-shore ocean water at selected beaches, Malibu Calif., July 21-26, 2009.
- Figure 11.**—Specific conductance and fecal indicator bacteria concentrations with depth in Malibu Lagoon, July 23, 2009
- Figure 12.**—Ocean tides, radon-222, specific conductance, and enterococcus data in the near-shore ocean adjacent to Malibu Lagoon, Malibu, Calif., November 9-11, 2009
- Figure 13.**—Shore perpendicular direct-current (DC) resistivity section through the berm separating Malibu Lagoon from the ocean, July 24, 2009 (location of section shown on figure 3)
- Figure 14.**—Shore parallel direct-current (DC) resistivity section along Malibu Colony beachfront, Malibu California, July 26, 2009 (Location of section shown of figure 3)
- Figure 15.**—delta Deuterium and a function of delta Oxygen-18 in water from selected wells and septic systems, Malibu California, 2009.
- Figure 16.**—Terminal-Restriction Fragment Length Polymorphism (T-RFLP) amplicons from selected sites in Malibu Lagoon, the berm separating the lagoon from the ocean, and the near-shore ocean adjacent to the lagoon, Malibu, Calif., July 25, 2009

LIST OF TABLES

- Table 1.**—Fecal indicator bacteria (FIB) concentrations in water from selected wells, Malibu, California, July 21-26, 2009
- Table 2.**—Major-ions, selected minor and trace elements, and nutrient concentrations to be determined as part of this study.
- Table 3.**—Wastewater indicators to be determined as part of this study.
- Table 4.**—Human-specific *Bacteroidales* concentrations near Malibu, California, July 2009

Figures

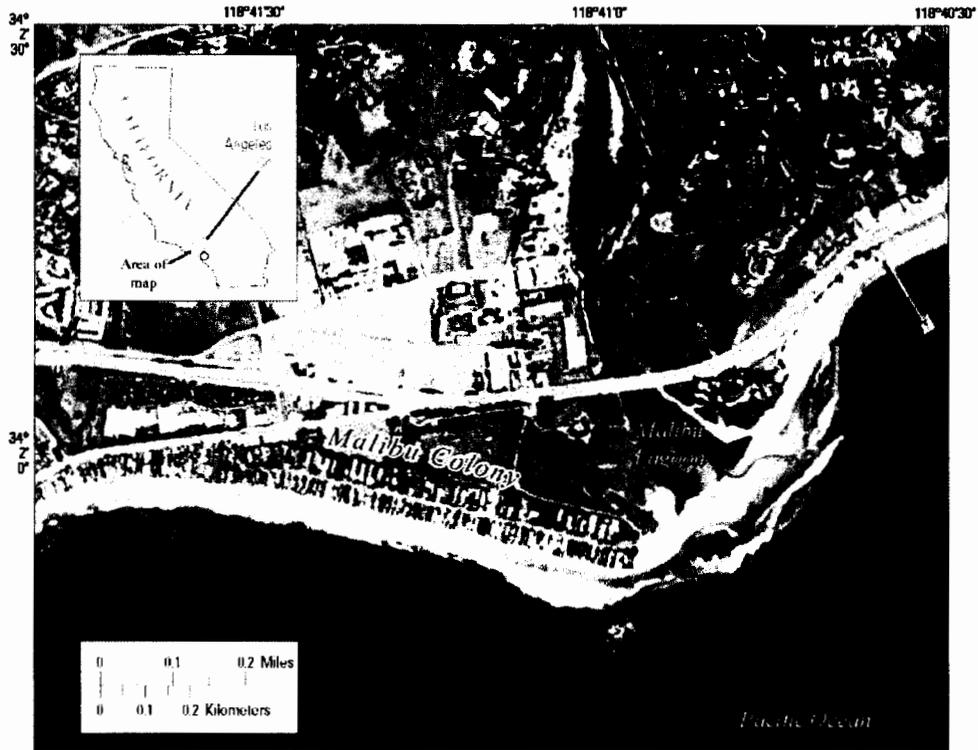


Figure 1.—Study area location

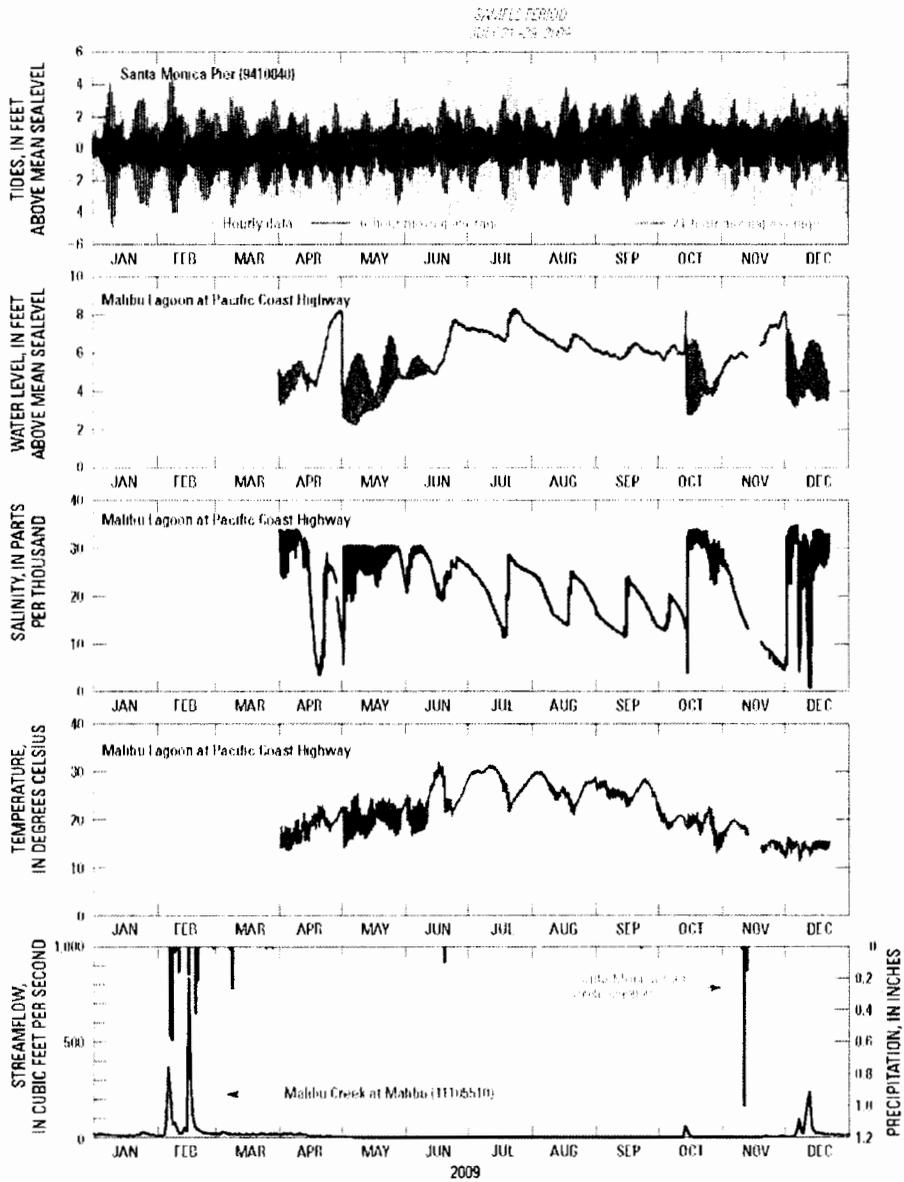


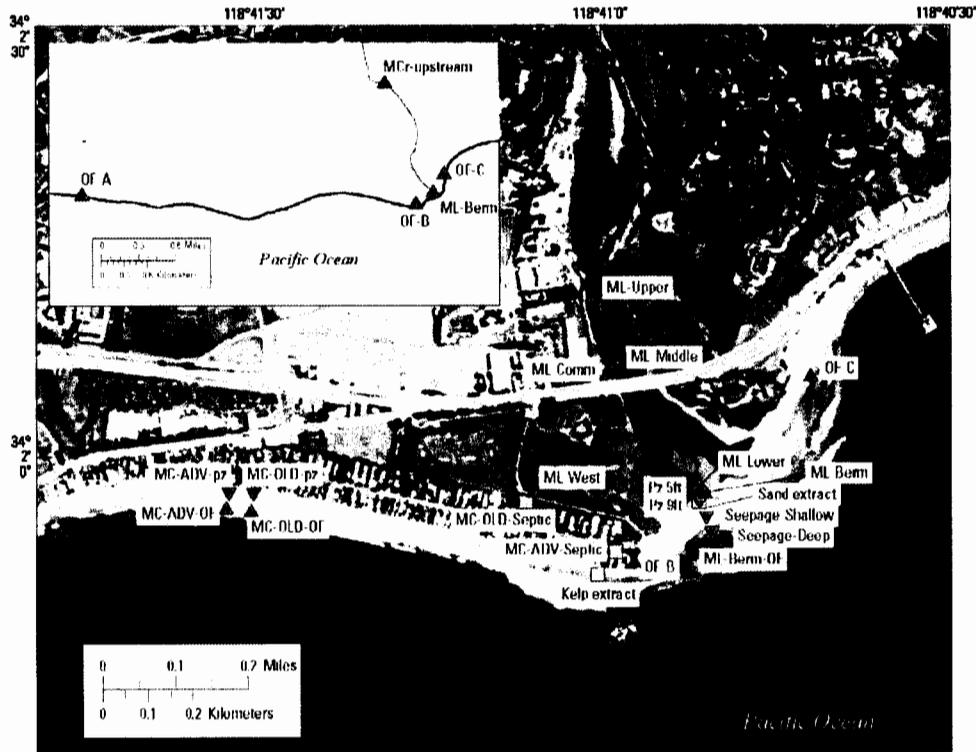
Figure 2.—Ocean tides, Malibu lagoon water levels, salinity and temperature, streamflow in Malibu Creek, and precipitation data, near Malibu, California, 2009



EXPLANATION

- Resistivity line
- Sampled wells and identifier—
C-1 ●

Figure 3—Groundwater sample sites, Malibu, California, 2009



EXPLANATION

Sample sites and identifier—

Surface water
▲ ML middle

Hand driven piezometers
or seepage samplers
▼ ML Berm 9ft

Other
□ Kelp extract, sand extract,
or septic sample

Figure 4.—Surface water sample sites, Malibu California, 2009

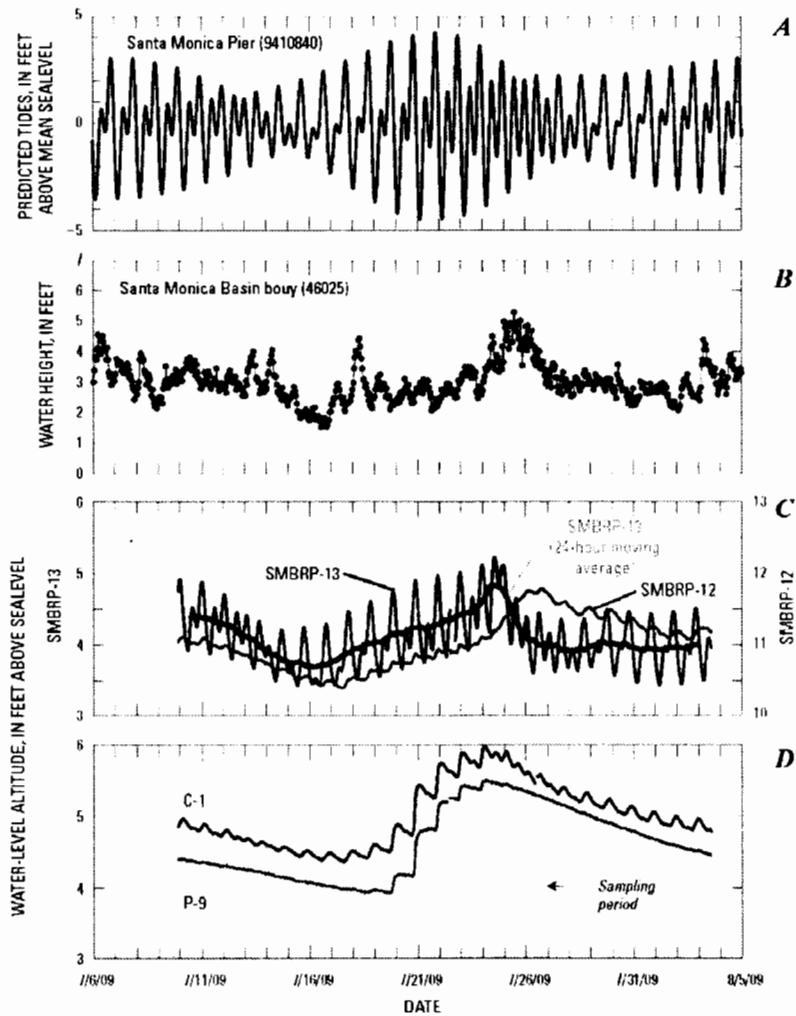


Figure 5.—Ocean tides, surface swell, and water level data for selected wells, Malibu California, July 6 to August 5, 2009



Figure 6.—Photograph showing seawall at Malibu Colony, Malibu California, July23, 2009

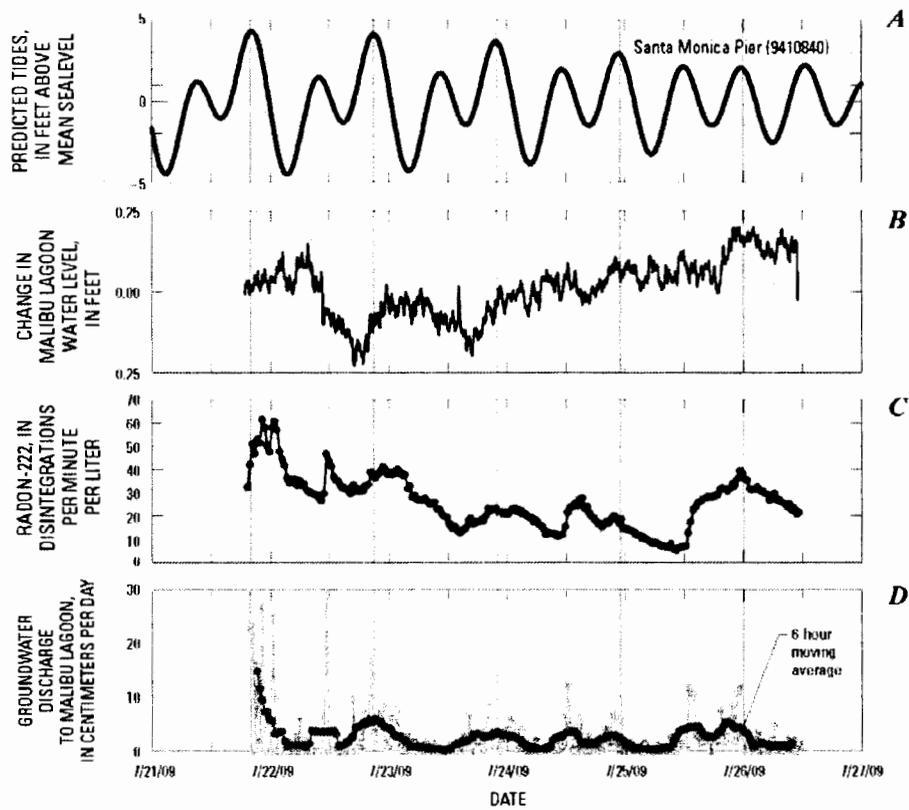


Figure 7.—Radon-222 (^{222}Rn) concentrations and calculated groundwater discharge to Malibu Lagoon (ML-Upper, figure 3), Malibu California, July 21-27-2009

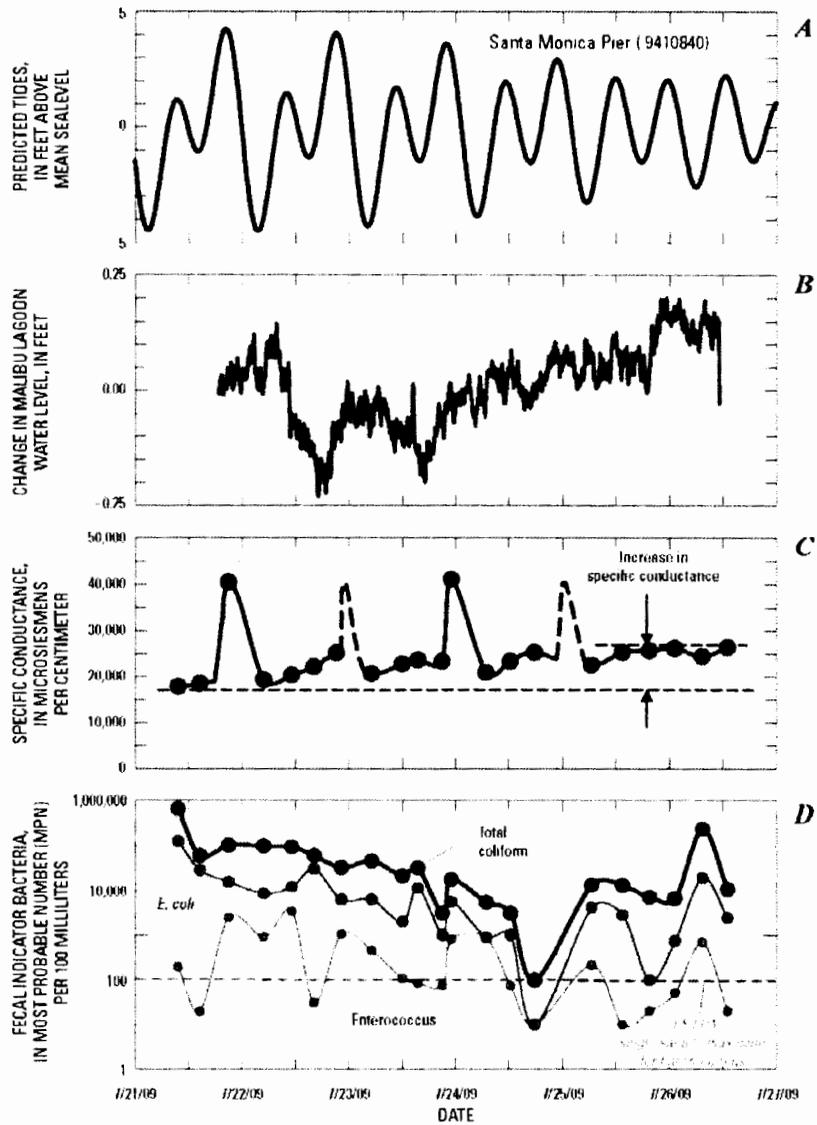


Figure 8.—Water level, specific conductance, and fecal indicator bacteria (FIB) concentrations in water from Malibu Lagoon (ML-Berm, figure 3), Malibu California, July 21–26, 2009.

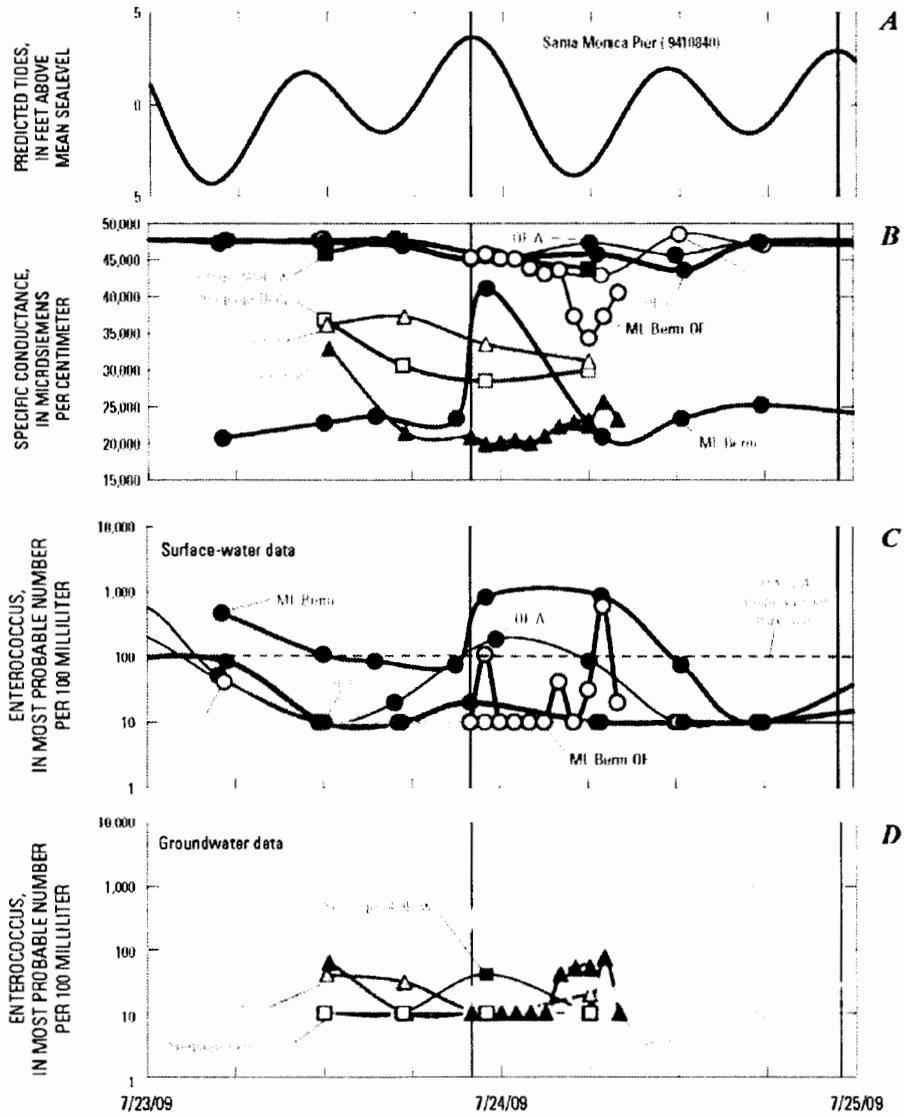


Figure 9.—Specific conductance and fecal indicator bacteria (FIB) concentrations in water from Malibu Lagoon (ML-Berm), pieziometers and seepage samplers in the berm separating Malibu Lagoon form the ocean, and in adjacent near-shore ocean water (ML-Berm-OF).

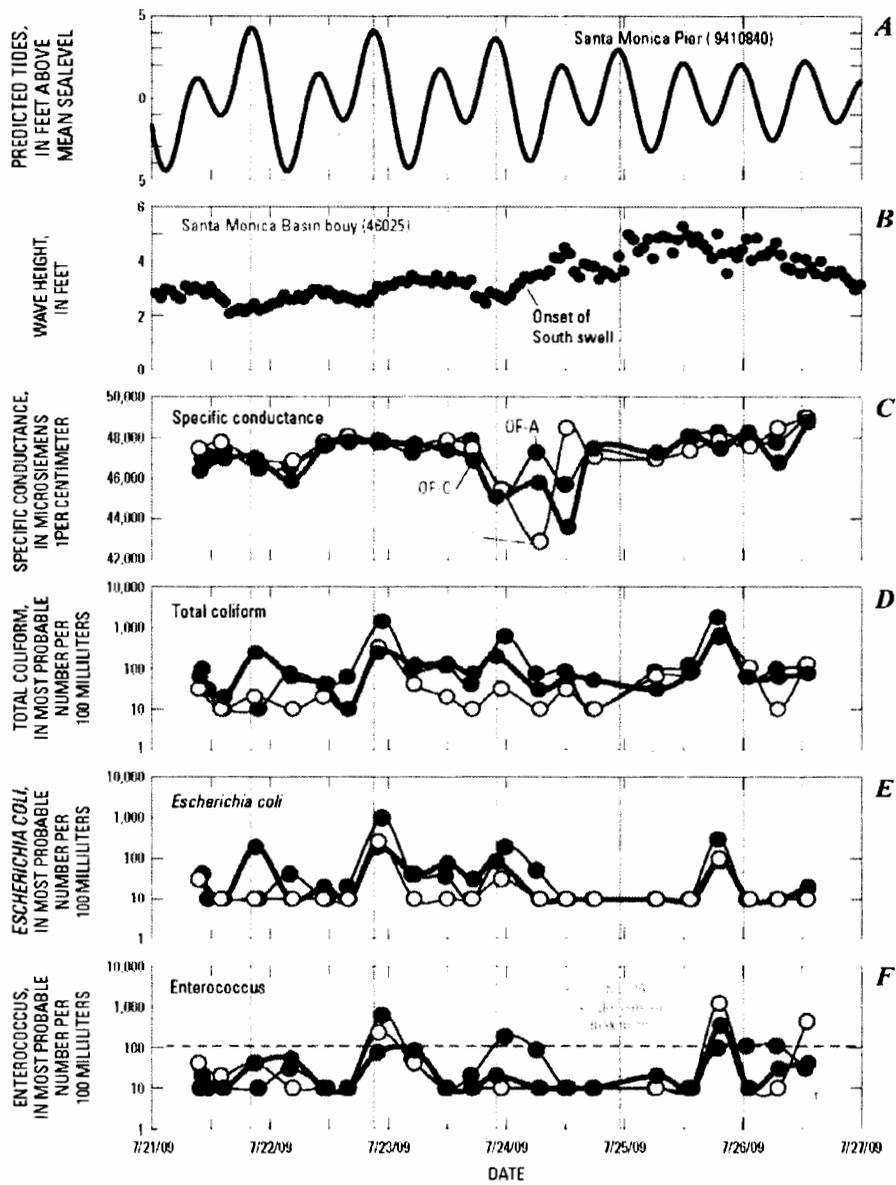


Figure 10.—Specific conductance and fecal indicator bacteria (FIB) concentrations in Malibu Lagoon and near-shore ocean water at selected beaches, Malibu Calif., July 21-26, 2009.

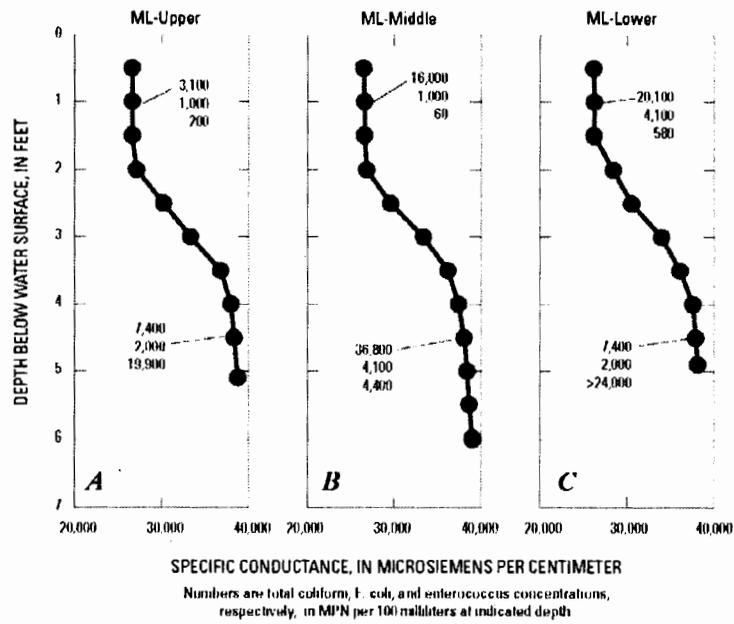


Figure 11.—Specific conductance and fecal indicator bacteria concentrations with depth in Malibu Lagoon, July 23, 2009

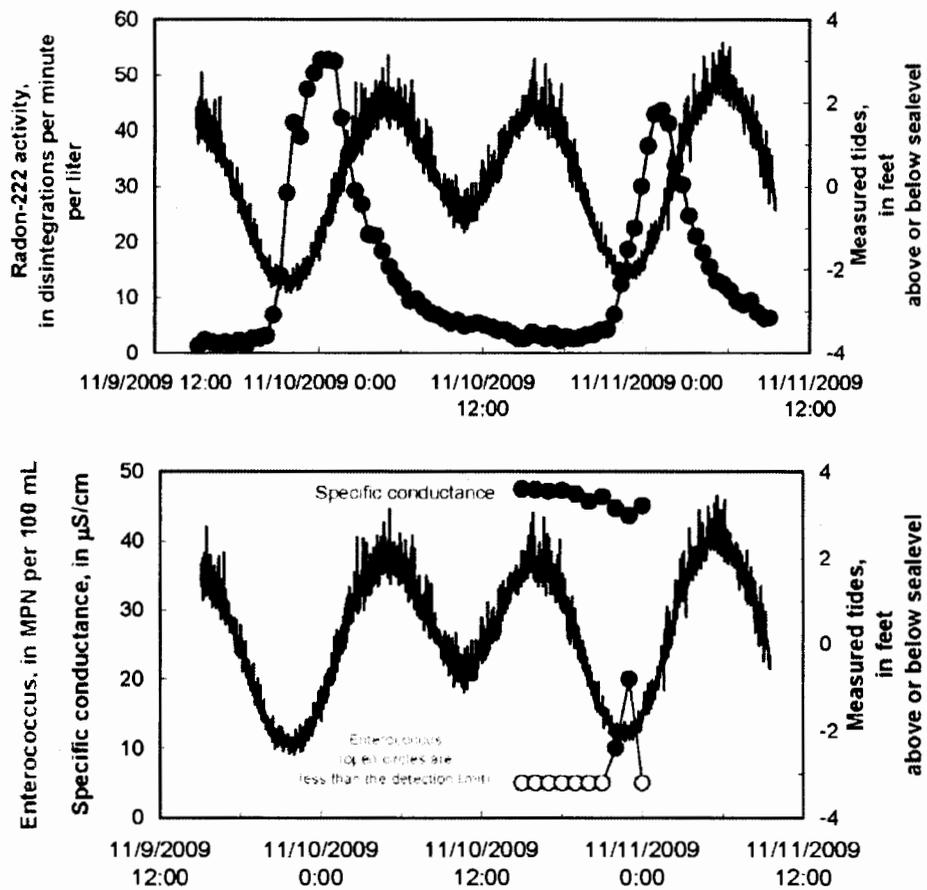


Figure 12.—Ocean tides, radon-222, specific conductance, and enterococcus data in the near-shore ocean adjacent to Malibu Lagoon, Malibu, Calif., November 9-11, 2009

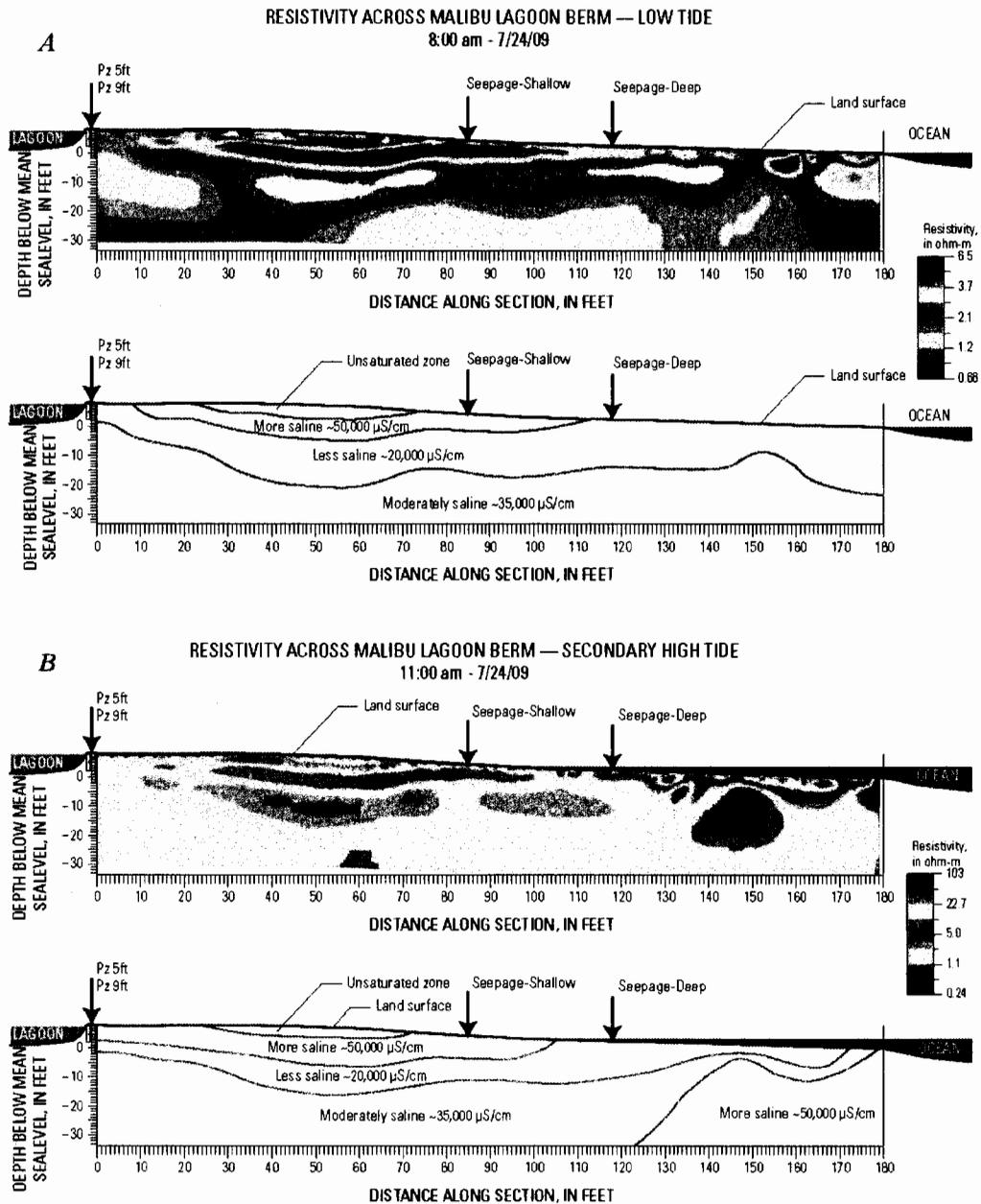


Figure 13.—Shore perpendicular direct-current (DC) resistivity section through the berm separating Malibu Lagoon from the ocean, July 24, 2009 (location of section shown on figure 3)

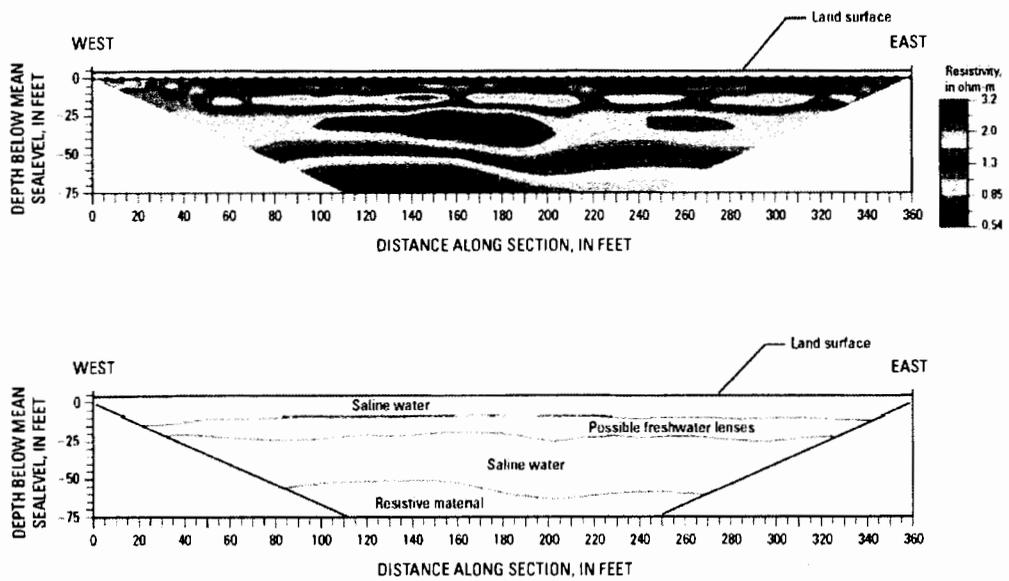


Figure 14.—Shore parallel direct-current (DC) resistivity section along Malibu Colony beachfront, Malibu California, July 26, 2009 (Location of section shown of figure 3)

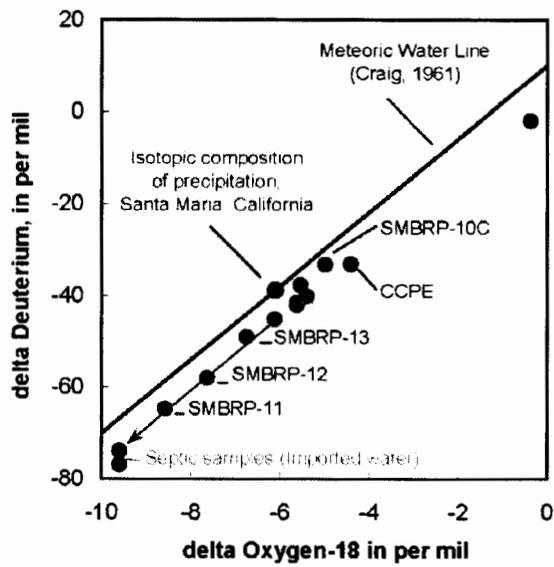


Figure 15.—delta Deuterium and a function of delta Oxygen-18 in water from selected wells and septic systems, Malibu California, 2009.

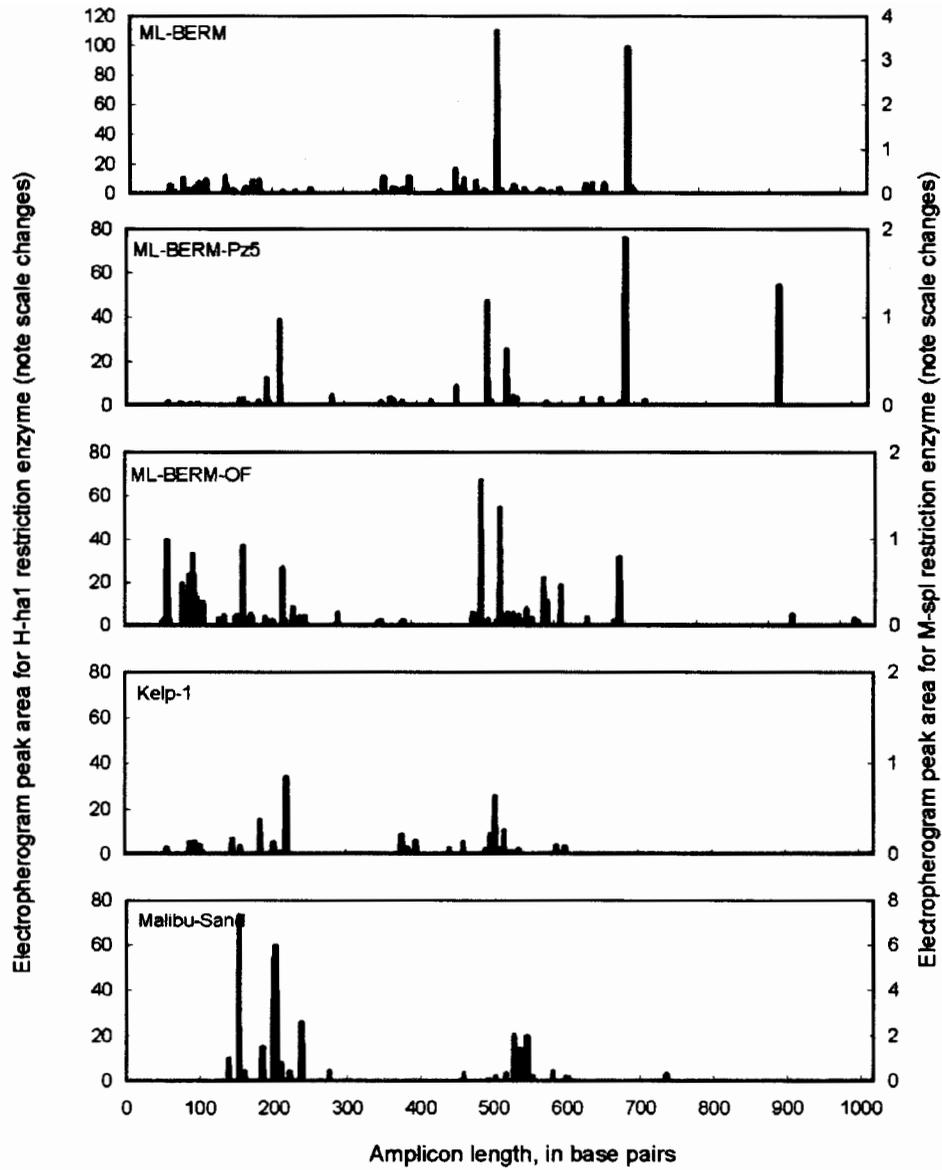


Figure 16.—Terminal-Restriction Fragment Length Polymorphism (T-RFLP) amplicons from selected sites in Malibu Lagoon, the berm separating the lagoon from the ocean, and the near-shore ocean adjacent to the lagoon, Malibu, Calif., July 25, 2009

Tables

Table 1.—Fecal indicator bacteria (FIB) concentrations in water from selected wells, Malibu, California, July 21-26, 2009

Table 1. Fecal indicator bacteria (FIB) concentration in water from selected water-table wells, Malibu, California, July 21-26, 2009.

[The five-digit parameter code below the constituent name is used by the U.S. Geological Survey to uniquely identify a specific constituent or property. C, Celsius; dpm/L, disintegrations per minute per liter; ft, feet; LSD, land surface datum; mg/L, milligrams per liter; mL, milliliters; MPN, most probable number; nc, not collected; μ S/cm microsiemens per centimeter; <, less than;]

Well Identification No.	Date (m/dd/yyyy)	Time (24 hour)	Water level (ft below LSD)	Well depth (feet)	Dissolved oxygen, (mg/L) (00300)	pH (standard units) (00400)
SMBRP-10C	7/21/2009	14:45	6.12	25	2.9	7.2
SMBRP-11	7/21/2009	11:45	8.40	20	1	6.4
SMBRP-2	7/22/2009	13:15	5.34	11	0.4	7.1
SMBRP-12	7/22/2009	10:30	6.97	25	0.2	7.1
SMBRP-13	7/22/2009	14:30	7.47	20	1.7	7.3
P-9	7/22/2009	10:00	nc	12	0.3	7.1
CCR-1	7/24/2009	9:00	5.69	19	0.1	7.4
CCPE	7/23/2009	14:30	4.97	53	0.2	NR
CCPNE	7/23/2009	9:00	6.03	25	0.2	NR
CCPC	7/23/2009	10:25	5.76	22	0.2	NR
C-1	7/26/2009	11:45	4.47	14	0.1	7.3

Well Identification No.	Specific conductance (μ S/cm at 25°C) (00095)	Total coliforms (MPN/100 mL) (50569)	<i>Escherichia coli</i> (MPN/100 mL) (50468)	<i>Enterococci</i> (MPN/100 mL) (99601)	Radon-222 (dpm/L)
SMBRP-10C	12,700	< 10	< 10	< 10	nc
SMBRP-11	2,960	< 10	< 10	< 10	nc
SMBRP-2	3,360	< 1	< 1	< 1	1,220 \pm 189
SMBRP-12	3,820	< 1	< 1	< 1	650 \pm 141
SMBRP-13	2,450	< 1	< 1	< 1	850 \pm 158
P-9	2,000	< 1	< 1	< 1	1,340 \pm 198
CCR-1	2,080	2	< 1	2	1660 \pm 163
CCPE	10,800	11	65	1,600	1,050 \pm 139
CCPNE	1,960	1	< 1	7.5	1,370 \pm 160
CCPC	2,020	< 1	< 1	< 1	950 \pm 134
C-1	22,300	< 10	< 10	< 10	nc

Table 2.—Major-ions, selected minor and trace elements, and nutrient concentrations to be determined as part of this study.

[CAS, Chemical Abstract Service; mg/L, milligrams per liter; µg/L, micrograms per liter]

Constituent	USGS Parameter Code	CAS Registry Number	Reporting Level	Units
Major ions, minor ions, and trace elements				
Alkalinity, laboratory	29801	471-34-1	8	mg/L
Aluminum	01106	7429-90-5	3.4	µg/L
Arsenic	01000	7440-38-2	0.044	µg/L
Barium	01005	7440-39-3	0.6	µg/L
Boron	01020	7440-42-8	2	µg/L
Bromide	71870	24959-67-9	0.02	mg/L
Calcium	00915	7440-70-2	0.044	mg/L
Chloride	00940	16887-00-6	0.12	mg/L
Fluoride	00950	16984-48-8	0.08	mg/L
Iodide	71865	7553-56-2	0.002	mg/L
Iron	01046	7439-89-6	6	µg/L
Lithium	01130	7439-93-2	0.06	µg/L
Magnesium	00925	7439-95-4	0.016	mg/L
Manganese	01056	7439-96-5	0.2	µg/L
pH, laboratory	00403		0.1	pH
Potassium	00935	7440-09-7	0.064	mg/L
Residue, 180 degrees Celsius (TDS)	70300		10	mg/L
Silica	00955	7631-86-9	0.2	mg/L
Sodium	00930	7440-23-5	0.10	mg/L
specific conductance, laboratory	90095		5	µS/cm
Strontium	01080	7440-24-6	0.4	µg/L
Sulfate	00945	14808-79-8	0.18	mg/L

Table 2 (cont.).—Major-ions, selected minor and trace elements, and nutrient concentrations to be determined as part of this study.

[CAS, Chemical Abstract Service; mg/L, milligrams per liter; µg/L, micrograms per liter]

Constituent	USGS Parameter Code	CAS Registry Number	Reporting Level	Units
Nutrients				
Nitrogen, ammonia as N	00608	7664-41-7	0.02	mg/L
nitrogen, ammonia + organic nitrogen	00623	17778-88-0	0.10	mg/L
nitrogen, nitrite	00613	14797-65-0	0.002	mg/L
nitrogen, nitrite + nitrate	00631		0.04	mg/L
Phosphorus	00666	7723-14-0	0.04	mg/L
phosphorus, phosphate, ortho	00671	14265-44-2	0.008	mg/L

Table 3.—Wastewater indicators to be determined as part of this study.
 [CAS, Chemical Abstract Service; µg/L, micrograms per liter]

Compound	USGS Parameter code	CAS Registry Number	Reporting Level	Units
Cotinine	61945	486-56-6	0.8	µg/L
3,4-Dichlorophenyl isocyanate	63145	102-36-3	1.6	µg/L
4-Nonylphenol monoethoxylate, (sum of all isomers) aka NP1EO	61704		1.6	µg/L
4-tert-Octylphenol diethoxylate, aka OP2EO	62486		0.5	µg/L
4-tert-Octylphenol monoethoxylate, aka OP1EO	62485		1	µg/L
5-Methyl-1H-benzotriazole	61944	136-85-6	1.6	µg/L
Anthraquinone	62813	84-65-1	0.2	µg/L
Acetophenone	62811	98-86-2	0.4	µg/L
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	62812	21145-77-7	0.2	µg/L
Anthracene	34220	120-12-7	0.2	µg/L
Atrazine	39630	1912-24-9	0.2	µg/L
1,4-Dichlorobenzene	34571	106-46-7	0.2	µg/L
Benzo[a]pyrene	34247	50-32-8	0.2	µg/L
Benzophenone	62814	119-61-9	0.2	µg/L
Bromacil	30234	314-40-9	0.8	µg/L
Bromoform	32104	75-25-2	0.2	µg/L
3-tert-Butyl-4-hydroxy anisole (BHA)	61702	25013-16-5	0.2	µg/L
Caffeine	81436	58-08-2	0.2	µg/L
Camphor	62817	76-22-2	0.2	µg/L
Carbaryl	39750	63-25-2	0.2	µg/L
Carbazole	77571	86-74-8	0.2	µg/L
Chlorpyrifos	38932	2921-88-2	0.2	µg/L
Cholesterol	62818	57-88-5	1.6	µg/L

Table 3 (cont.).—Wastewater indicators to be determined as part of this study.
[CAS, Chemical Abstract Service; µg/L, micrograms per liter]

3-beta-Coprostanol	62806	360-68-9	1.6	µg/L
Isopropylbenzene	77223	98-82-8	0.2	µg/L
N,N-diethyl-meta-toluamide (DEET)	61947	134-62-3	0.2	µg/L
Diazinon	39570	333-41-5	0.2	µg/L
Dichlorvos	30218	62-73-7	0.2	µg/L
Bisphenol A	62816	80-05-7	0.4	µg/L
Triethyl citrate (ethyl citrate)	62833	77-93-0	0.2	µg/L
Tetrachloroethylene	34475	127-18-4	0.4	µg/L
Fluoranthene	34376	206-44-0	0.2	µg/L
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	62823	1222-05-5	0.2	µg/L
Indole	62824	120-72-9	0.2	µg/L
Isoborneol	62825	124-76-5	0.2	µg/L
Isophorone	34408	78-59-1	0.2	µg/L
Isoquinoline	62826	119-65-3	0.2	µg/L
d-Limonene	62819	5989-27-5	0.2	µg/L
Menthol	62827	89-78-1	0.2	µg/L
Metalaxyl	04254	57837-19-1	0.2	µg/L
Metolachlor	82612	51218-45-2	0.2	µg/L
Naphthalene	34696	91-20-3	0.2	µg/L
1-Methylnaphthalene	81696	90-12-0	0.2	µg/L
2,6-Dimethylnaphthalene	62805	581-42-0	0.2	µg/L
2-Methylnaphthalene	30194	91-57-6	0.2	µg/L
4-Nonylphenol diethoxylate, (sum of all isomers) aka NP2EO	61703		3.2	µg/L
p-Cresol	77146	106-44-5	0.2	µg/L

Table 3 (cont.).—Wastewater indicators to be determined as part of this study.
[CAS, Chemical Abstract Service; µg/L, micrograms per liter]

4-Cumylphenol	62808	599-64-4	0.2	µg/L
para-Nonylphenol (total) (branched)	62829	84852-15-3	1.6	µg/L
4-n-Octylphenol	62809	1806-26-4	0.2	µg/L
4-tert-Octylphenol	62810	140-66-9	0.4	µg/L
2,2',4,4'-Tetrabromodiphenylether (PBDE 47)	63147	5436-43-1	0.3	µg/L
Phenanthrene	34461	85-01-8	0.2	µg/L
Phenol	34694	108-95-2	0.2	µg/L
Pentachlorophenol	39032	87-86-5	0.8	µg/L
Tributyl phosphate	62832	126-73-8	0.2	µg/L
Triphenyl phosphate	62834	115-86-6	0.2	µg/L
Tris(2-butoxyethyl)phosphate	62830	78-51-3	0.2	µg/L
Tris(2-chloroethyl)phosphate	62831	115-96-8	0.2	µg/L
bis(2-Ethylhexyl) phthalate	39100	117-81-7	2	µg/L
Diethyl phthalate	34336	84-66-2	0.2	µg/L
Prometon	39056	1610-18-0	0.2	µg/L
Pyrene	34469	129-00-0	0.2	µg/L
Methyl salicylate	62828	119-36-8	0.2	µg/L
Sample volume	99963			mL
3-Methyl-1(H)-indole (Skatole)	62807	83-34-1	0.2	µg/L
beta-Sitosterol	62815	83-46-5	1.6	µg/L
beta-Stigmastanol	61948	19466-47-8	1.7	µg/L
Triclosan	61708	3380-34-5	0.2	µg/L
Tris(dichlorisopropyl)phosphate	61707	13674-87-8	0.2	µg/L

Table 4. Human-specific *Bacteroidales* (HBM) concentrations near Malibu, California, July 2009

[ND = not detected. DNQ = detected but not quantifiable.]

Site identification (figs. 1 and 2)	Date	Time	qPCR Dilution (1:x)	HBM Copies per liter	Standard error
Samples from wells and piezometers					
P-9	7/22/2009	10:00	5	DNQ	-
C-1	7/26/2009	11:45	5	ND	-
SMBRP-13	7/22/2009	14:30	10	ND	-
SMBRP-12	7/22/2009	10:30	5	ND	-
SMBRP-2	7/23/2009	13:15	5	ND	-
ML-BERM-Pz5'	7/23/2009	21:00	5	ND	-
Seepage-Deep	7/24/2009	6:00	5	ND	-
MC-ADV-Pz	7/25/2009	6:00	5	ND	-
MC-OLD-Pz	7/25/2009	6:00	10	ND	-
Samples from the near-shore ocean					
ML-BERM-OF (low tide)	7/24/2009	6:00	5	ND	-
MC-ADV-OF (low tide)	7/25/2009	6:00	10	ND	-
MC-OLD-OF (low tide)	7/25/2009	6:00	10	ND	-
MC-OLD-OF (high tide)	7/25/2009	13:00	10	ND	-
Samples from Malibu Lagoon					
ML-BERM	7/23/2009	21:00	10	ND	-
ML-Comm	7/24/2009	11:20	10	ND	-
ML-W	7/26/2009	12:45	5	ND	-
Samples from septic systems and special sources					
MC-OLD-Septic	10/1/2009	12:30	10	7.6E+07	1.3E+06
MC-ADV-Septic	10/1/2009	11:00	10/5*	4.2E+04	3.7E+03
Kelp extract	7/24/2009	17:00	10	DNQ	-
Sand extract	10/1/2009	8:00	5	DNQ	-

*Malibu Adv Septic, when run at 10 fold dilution was not within quantifiable range of the HBM qPCR assay. When run at 5 fold dilution, the sample results were within quantifiable range despite inhibition in this dilution in salmon testes qPCR.

DNQ—Detected but not quantifiable. Human-specific *Bacteroidales* is present in the sample but the concentration was less than the quantification limit obtainable from the laboratory standards. The DNQ concentration is dependent on sample volume, the amount of DNA extracted, and the dilution required to eliminate sample inhibition during the PCR reaction. The DNQ varied from sample to sample but the quantification limit would commonly be about 10^3 copies per liter.

ND—Not detected. Human-specific *Bacteroidales* was not detected in the sample. The ND concentration is dependent on sample volume, the amount of DNA extracted, and the dilution required to eliminate sample inhibition. The ND varied from sample to sample. Assuming 1 copy of *Bacteroidales* DNA per sample tray, a 5 to 1 dilution to eliminate inhibition during the PCR reaction, the addition of 2.5 μ l of reagents, a 1-L sample containing 50 μ g of DNA, and 100 percent efficiency in the PCR reaction—the limit of detection would be about 25 copies per liter. For a 10 to 1 dilution, the limit of detection would be about 50 copies per liter.

Summary of 2009 UCLA Study in Malibu Lagoon

Richard F. Ambrose

Jenny Jay

Vanessa Thulsiraj

Steven Estes

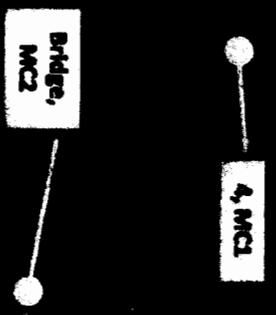
University of California, Los Angeles

Objectives

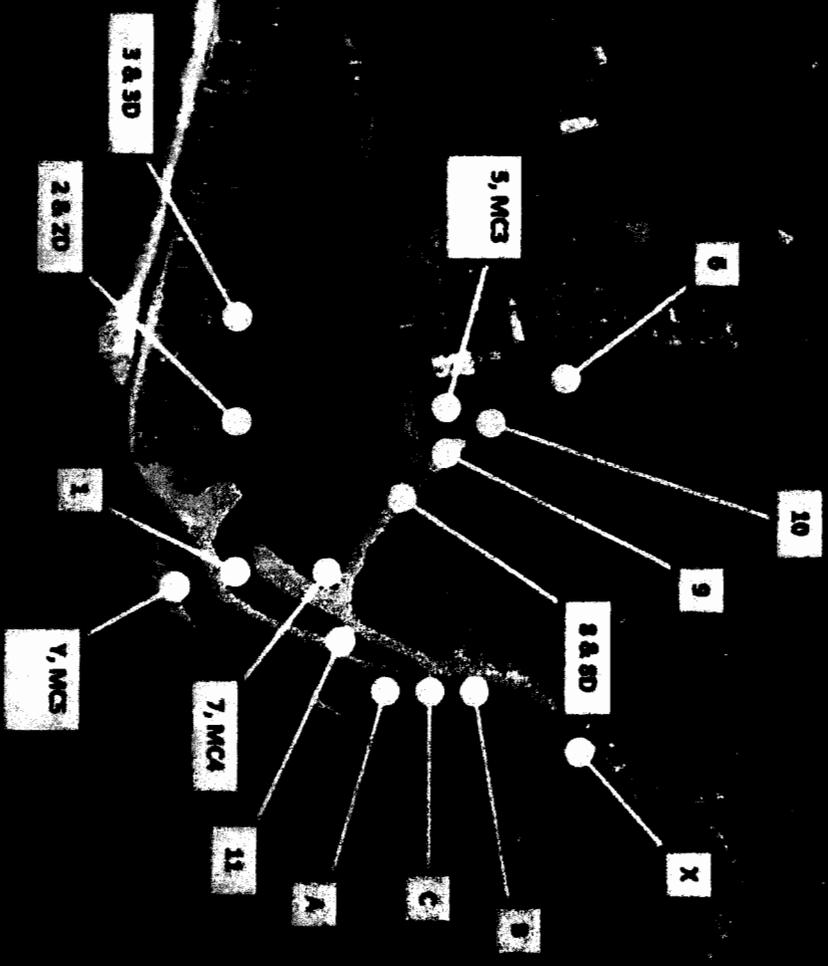
- 1) Determine Fecal Indicator Bacteria (FIB) and Human-specific Bacteriodes Marker (HBM) concentrations for the following environmental conditions
 - a) Wet weather, open lagoon
 - b) Dry weather, open lagoon
 - c) Dry weather, closed lagoon
- 2) Compare traditional FIB concentrations to HBM concentrations

Map of Sample Sites

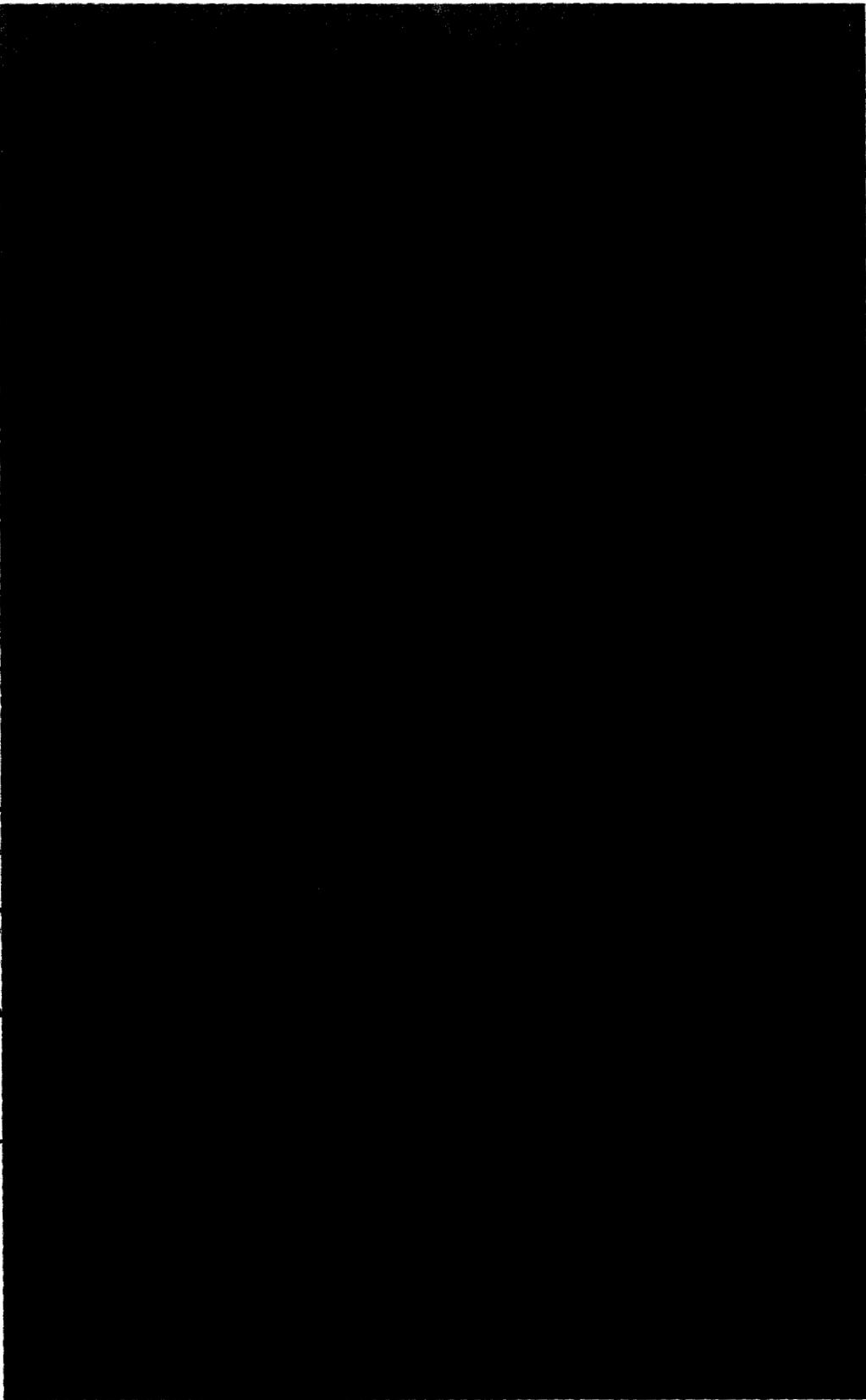
20 sites total



- 2 sites in Malibu Creek (site 4 & Bridge)
- 5 sites in Surfrider Beach (sites A, B, C, X, Y)
- 10 sites in Malibu Lagoon (sites 1-3, 5-11)
- 3 storm drains in Malibu Lagoon (2D, 3D, 8D)
- storm drains discharged during in wet weather



Study Design



analyzed for HBM (1 sample had interference)

Additional City of Malibu Study

- **2 week study conducted in Malibu Creek and Lagoon**
- **5 sample locations (MC1 – MC5) were further analyzed for presence of HBM**
 - **MC1 – MC4 sampled on 4/29**
 - **MC5 samples on 4/29, 4/30, 5/5, 5/7**
 - **No detection of HBM in any samples taken during this time (April/May)**

Conductivity (ms)

Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed

1.00
1.38
2.10
2.21

-
1.34
0.99
2.19

1.0
1.28
3.29
16.20

0.44
1.91
0.95
10.01

-
18.8
37.0
20.30

1.40
14.1
32.0
20.29

1.57
14.6
47.8
18.45

1.28
2.28
11.7
16.02

1.00
3.12
27.2
10.38

1.29
2.81
-
10.14

4.90
3.08
31.1
10.24

1.28
3.04
38.1
10.00

-
>20
48.3
00.2

0.57
13.4
41.5
00.57

2.12
0.09
39.5
40.00

34.3
21.3
24.9
40.00

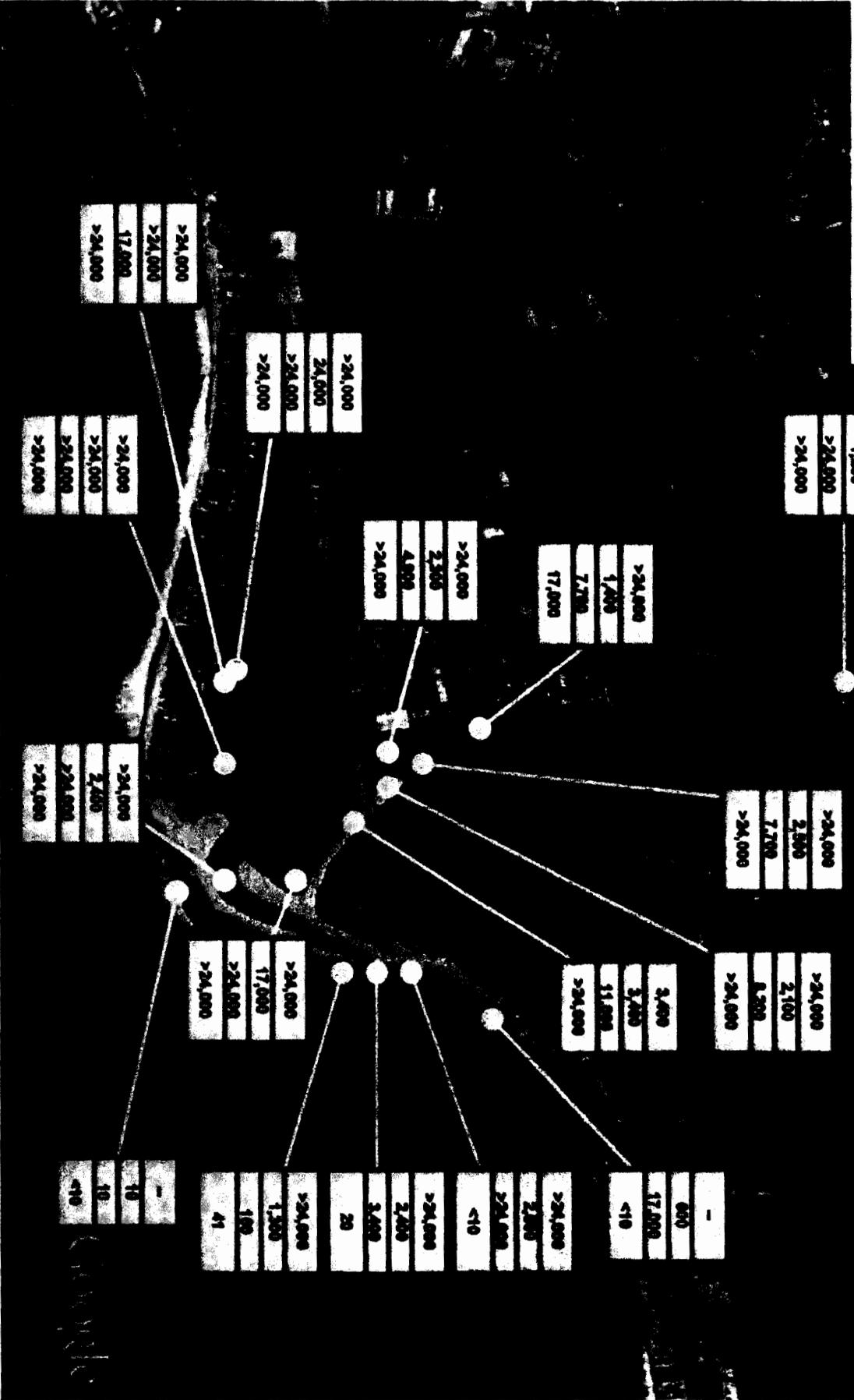
-
40.1
40.0
00.2

000000

Total Coliforms (MPN/100mL)

Water Quality Limit: 10,000 CFUs/100mL

Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed



>24,000
1,300
2,100
24,000

-
1,200
>24,000
>24,000

>24,000
1,700
7,700
17,000

>24,000
2,300
4,800
>24,000

>24,000
2,800
7,700
>24,000

>24,000
2,100
8,200
>24,000

3,400
5,200
11,000
>24,000

>24,000
17,000
>24,000
>24,000

>24,000
1,300
100
41

-
600
17,000
<10

>24,000
2,200
>24,000
<10

>24,000
2,400
2,600
20

>24,000
>24,000
17,000
>24,000

>24,000
>24,000
>24,000
>24,000

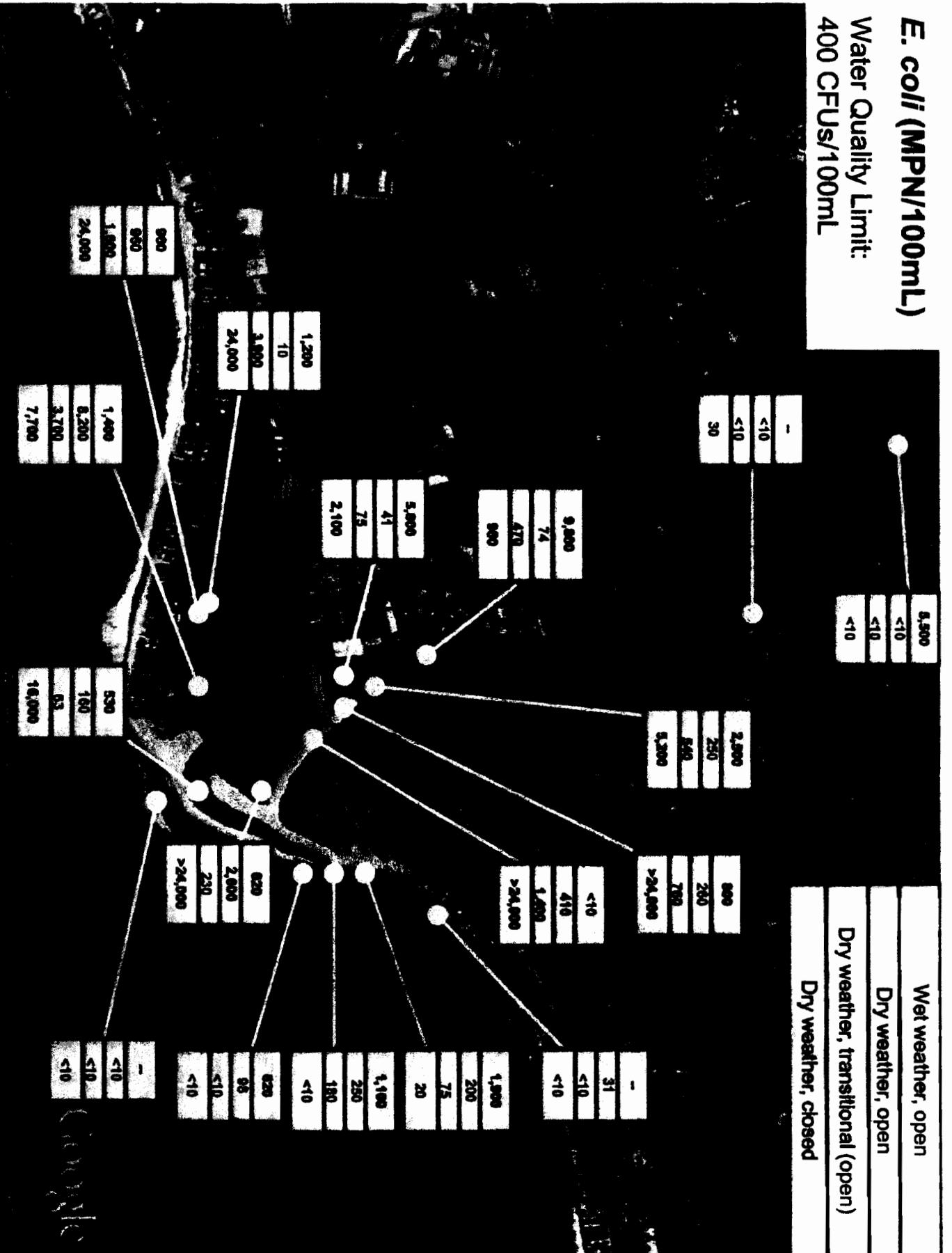
>24,000
2,200
>24,000
>24,000

-
18
10
<10

0.00010

E. coli (MPN/100mL)

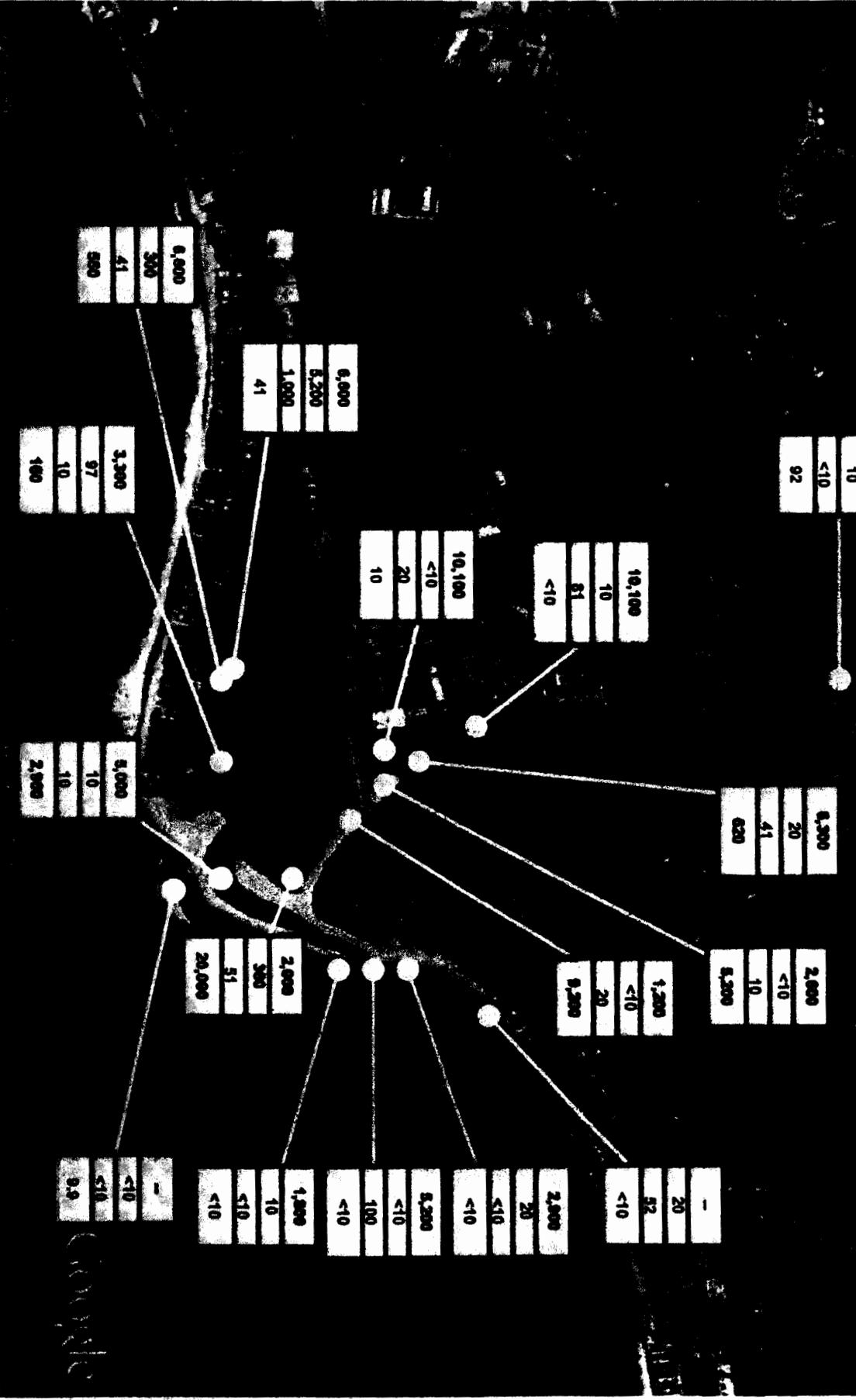
Water Quality Limit:
400 CFUs/100mL



Enterococcus (MPN/100mL)

Water Quality Limit:
104 CFUs/100mL

Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed

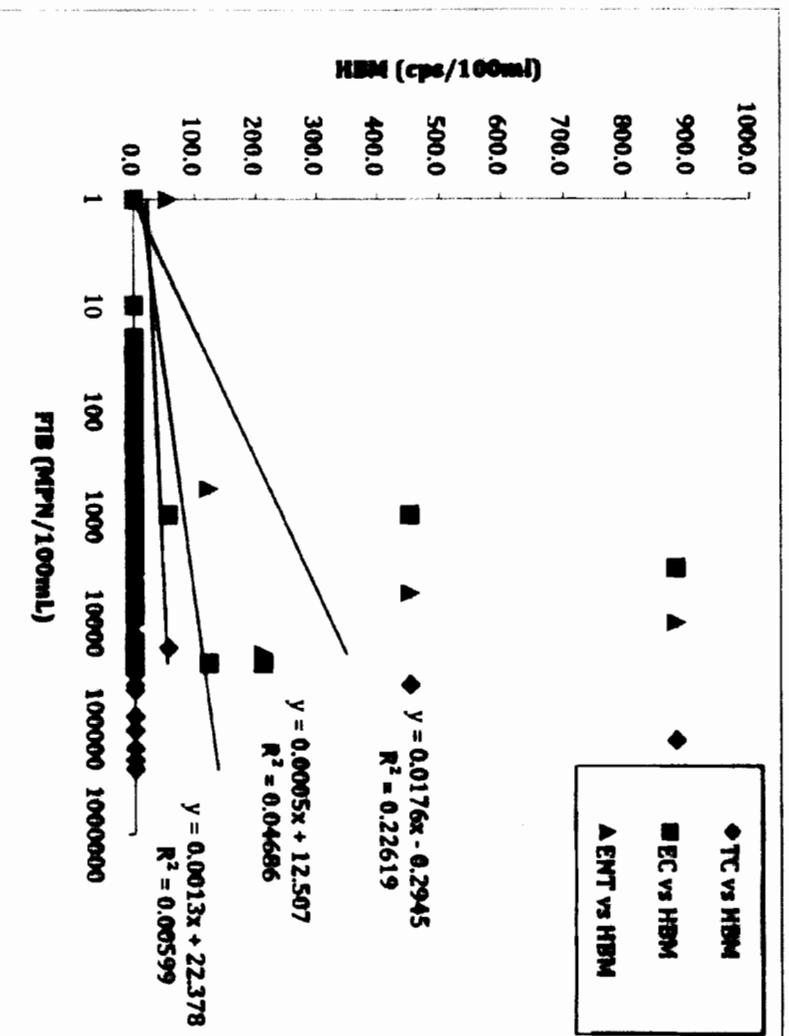


Malibu Creek, Malibu Lagoon and Surf Rider Beach 2009 Human Specific Bacterioidales

Site	2/16/09 Wet- Open	3/20/09 Dry-Open	4/29 & 4/30/09 Dry - Open	5/5 & 5/7/09 Dry-Open	5/21/09 Dry- Transition al Open	7/18/09 Dry- Closed	10/30/09 Dry- Closed	Total Samples
1	N	N	-	-	N	N	N	5
2	N	N	-	-	N	N	-	4
2D ¹	N	-	-	-	-	N	-	2
3	Y	N	-	-	N	Y	1	4
3D ¹	N	N	-	-	N	N	-	4
4 (MC1)	-	N	N	-	N	N	-	4
5 (MC3)	Y	N	N	-	N	N	N	6
6	N	N	-	-	N	Y	N	5
7 (MC4)	N	N	N	-	N	Y	N	6
8	N	N	-	-	N	N	-	4
8D ¹	N	N	-	-	-	-	-	2
9	N	N	-	-	N	N	-	4
10	N	N	-	-	N	N	-	4
11	-	-	-	-	N	N	-	2
A	1	N	-	-	N	N	-	3
B	N	N	-	-	N	N	-	4
C	N	N	-	-	N	N	-	4
Y (MC5)	-	N	NN	NN	N	N	-	7
X	-	N	-	-	N	N	-	3
Bridge (MC2)	-	N	N	-	N	1	-	3
	2 of 14	0 of 18	0 of 6	0 of 2	0 of 18	3 of 18	0 of 4	5 of 80

¹ No samples taken in dry weather from drains at 2 D, 3D or 8 D because there were – no dry weather discharges from drains. (Although Samples at these sites were taken from where drains would overflow within the Lagoon if there had been discharge, no discharge was collected. Therefore these samples could be counted as duplicate samples of 3, 2 and 8 during dry weather. Samples were collected at 3D and 8D in March; 3D in May; and 2D and 3D in July.

Relationship between FIB and HBM

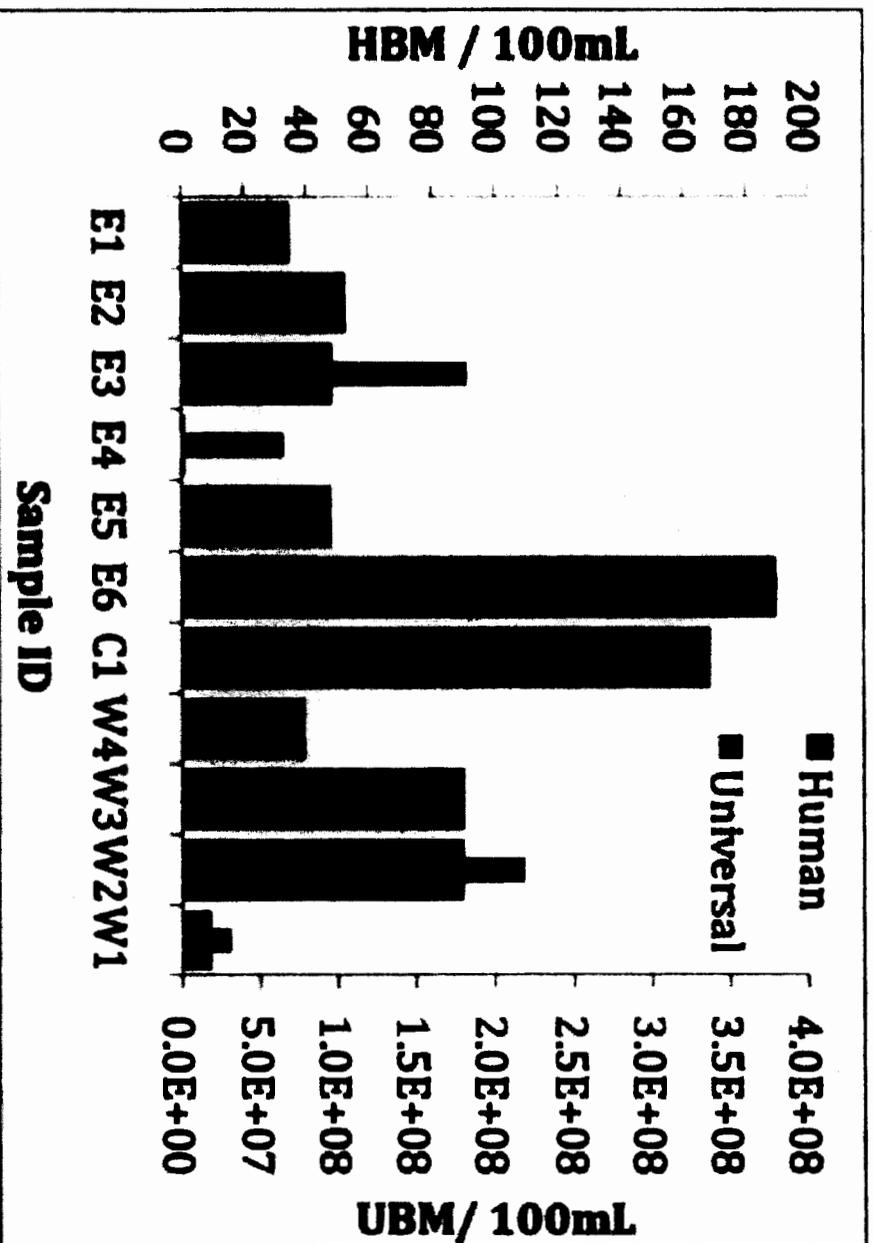


- Samples positive for HBM occurred only when FIB was high
- High FIB observed in absence of HBM

Other work by Jay Lab in Santa Monica Canyon (SMC)

- SMC carries runoff from residential areas and natural mountain watersheds to the ocean.
- In this watershed, HBM was quantifiable in more than 60% of samples in the channel draining the less urbanized subwatershed, and 35% of samples in the channel draining the more urbanized subwatershed.
- After rain events, FIB levels would increase by a factor of 2-18, while *Bacteroidales* was not observed to increase more than a factor of 2 at any location.
- Very little correlation was observed between human-specific or universal *Bacteroidales* and traditional FIB in the Santa Monica Canyon watershed ($R^2 < 0.05$).

Universal (UBM) and human-specific Bacteroidales (HBM) along Santa Monica Canyon



Samples W1-W4 are from the less urbanized channel. A storm drain located at E6 flowed regularly during sampling events.

Conclusions

- FIB concentrations were high throughout stream, lagoon and ocean after rainfall
 - Only time of high FIB upstream and in ocean
- FIB concentrations generally low when lagoon was open
 - Specific hot spot areas
- FIB concentrations were high throughout lagoon when lagoon closed
- Human-specific bacteroides marker (HBM) detected in 5 out of 80 samples
 - Only detected in Lagoon samples
 - Very weak relationship between FIB and HBM

Malibu Lagoon Bacteria Study
Synopsis with Preliminary Results
4-25-09

Richard F. Ambrose
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Monique Myers
Steve Estes
University of California, Los Angeles

1. Introduction

The purpose of the current UCLA study in Malibu Lagoon is generally to increase our understanding of the dynamics of bacteria in the Lagoon and adjacent ocean waters. We are interested in spatial and temporal patterns of bacteria concentrations as well as the bacteria sources. For this study, we are focusing on water samples only (not sediments).

2. Methods

To understand the spatial pattern of bacteria concentrations, samples are being collected from 18 sites in and around the Lagoon, including one site upstream of all development in Serra Retreat, four sites in the restored salt marsh, and five sites along the beach in or near the mouth of the Lagoon (Figure 1). The furthest upstream site provides information about bacteria entering the Lagoon from the upstream Malibu Creek watershed. The Lagoon samples include samples on the east and west banks of the main lagoon as well as samples from the restored wetland area (taken near outlets draining into that area). The beach samples include a sample where the lagoon water meets the ocean (when the lagoon is open) and two samples east and two samples west of this location. These samples represent exposure to ocean users. In addition to collecting water samples for bacteria, we are measuring other water quality parameters, particularly conductivity (salinity), which provides a useful indicator of how much mixing there has been between freshwater and ocean water at each site when we sample it.

To understand the temporal pattern of bacteria concentrations, samples are being collected at times that represent different important phases of Lagoon dynamics. Our first sample was during a major rainstorm (February 16, 2009) when the Lagoon was open to the ocean and dominated by fluvial (stream) processes. More than 1.5" of rain had fallen in the upper watershed when sampling occurred, with a total of about 2.3" for the storm. Although we call the February 16 sample a "wet weather" sample, it really represents storm conditions in the lagoon with significant runoff from upstream and the areas around the lagoon.

Our second sample (March 20, 2009) occurred while the lagoon was still open, but more than three weeks after a significant rain. Although the lagoon was open to the ocean, the

outlet was near the downcoast (closer to the pier) limit of its normal migration as the lagoon closes. Interestingly, this was also the outlet location during the February 16 sampling despite the heavy rainfall.

We plan on sampling at least one more time, when the Lagoon is closed.

To understand the sources of bacteria in the Lagoon, we are analyzing our samples for human-specific and universal bacteroides as well as the traditional fecal indicator bacteria. The results of these analyses will provide an indication of how much of the indicator bacteria may be due to human sources. We will also look at markers for other sources (e.g. birds, dogs) if feasible. Although bacteroides samples were taken February 16 and March 20, those data are not yet available.

3. Preliminary Results

No attempt is made to provide a detailed description of the results in this preliminary report, but a general summary of results is given below. The presentation of the results follows a consistent approach for each parameter: First we present a map with parameter values for the February 16 sampling event, then we present a map with parameter values for the March 20 sampling event, and finally we present a map with values from both sampling periods on the same map.

The data for total coliform bacteria are shown in Figure 2, Figure 3 and Figure 4.

The data for *E. coli* are shown in Figure 5, Figure 6 and Figure 7.

The data for enterococcus are shown in Figure 8, Figure 9 and Figure 10.

For all indicator bacteria, nearly all stations had concentrations above water quality standards during the wet weather sampling event. The samples were taken after 1.5" of rain had fallen in the upper watershed and water flow rates were very high. The uppermost sample, taken a day later by Heal the Bay, still had high FIB concentrations. The one exception to sample exceedances was the station on the east side of the lagoon near the Pacific Coast Highway, which had concentrations of total coliforms and *E. coli* that were below the water quality standard. Interestingly, the enterococcus concentration at this station did exceed the water quality standard. All other stations exceeded the standards, but some stations had exceptionally high values. Three stations north of the PCH bridge and the westernmost station in the restored salt marsh (where the sample was taken from a running drain) had total coliform values >100,000 MPN/100 ml. The same three stations north of PCH had *E. coli* concentrations >2,300 MPN/100 ml. Two stations in the restored salt marsh and two ocean stations near the lagoon mouth had *E. coli* concentrations >1,000 MPN/100 ml. The same three stations north of PCH and two stations in the westernmost area of the restored salt marsh had enterococcus concentrations >6,000 MPN/100 ml.

The February 16 values reflected runoff with high FIB concentrations entering the lagoon from a number of different locations, including upstream from Malibu Creek, outlets draining into the restored marsh, and perhaps inputs into the main lagoon north of PCH. Runoff into the lagoon resulted in high FIB values throughout the lagoon and in the adjacent coastal water.

In contrast to the February 16 storm sampling event, when the lagoon was open during dry weather nearly all stations had FIB concentrations below the water quality standards. There were two exceptions. (1) Two or three stations in the restored salt marsh exceeded the standards for all three indicators. These samples were taken near culverts that drain into the marsh. (2) In addition, the station in the southeast portion of the main lagoon exceeded water quality standards for all three indicators. This station is adjacent to the Adamson house property, near the exposed shoals that routinely serve as a roosting area for hundreds of birds, and is isolated from the main water flow in and out of the lagoon because of the configuration of the lagoon outlet.

The March 20 sampling event identified two "hotspots" of high FIB concentrations in the lagoon. In the restored salt marsh, it seems likely that the sources of FIB are the pipes that drain into the sampled tidal creek. However, we do not know if the high values are due to continued input or FIB regrowth or survival in that area of the lagoon. The high FIB concentrations in the SE corner of the main lagoon may be due to the high concentrations of birds in that area coupled with relatively poor circulation, but we cannot conclude this definitively. The bacteroides results may help clarify the possible FIB sources in these two hotspots.

The conductivity data (Figure 11, Figure 12, and Figure 13) provide an indication of the amount of freshwater influence at the different stations. During the February 16 sampling event, the entire lagoon system was dominated by freshwater runoff. The only station that showed even moderate salinity was an ocean station by the lagoon mouth (although the other two ocean stations were largely freshwater). During the March 20 sampling event, most stations were still dominated by freshwater even though the lagoon was open to the ocean. This is not too surprising because we sampled around low tide, when the lagoon would be least influenced by ocean water. The ocean stations, of course, had higher salinities, although the stations near the outlet were much lower than full seawater and even the station near Malibu Pier was much lower than full seawater. The stations in the restored wetland, in contrast to all other lagoon stations, showed significant influence of seawater.



Figure 1. Location of Malibu Lagoon sampling sites.

The two farthest upstream sites (one upstream of Serra Retreat and one at Cross Creek Road) and the easternmost and westernmost beach samples were not included in the first sampling period.

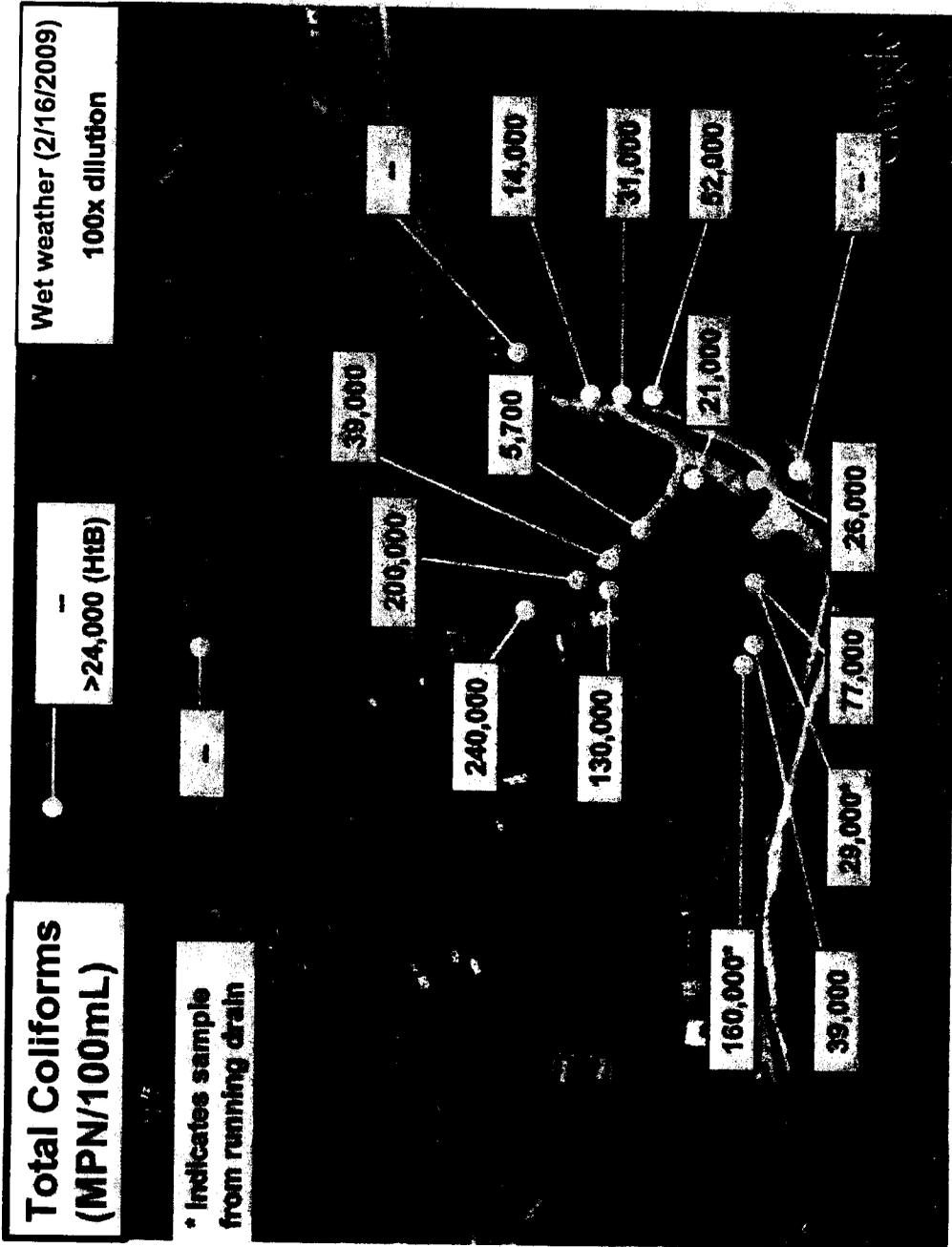


Figure 2. Total coliform concentrations during wet weather sampling period. The single-sample water quality standard is 10,000 CFU/100 ml. The two upstream sites were not sampled, but data collected nearby by Heal the Bay one day after our samples were collected are shown.

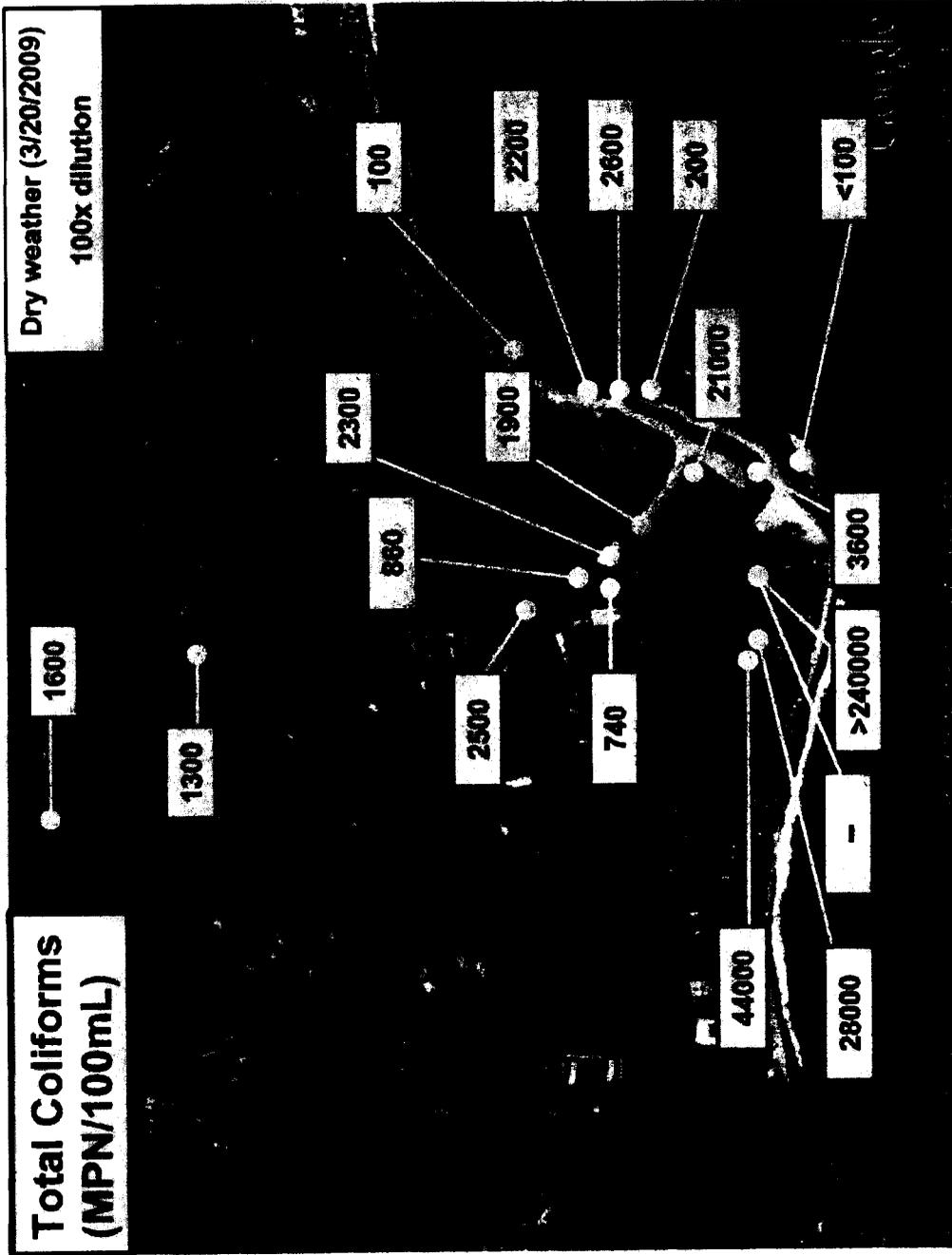


Figure 3. Total coliform concentrations during dry weather (lagoon open) sampling period. The single-sample water quality standard is 10,000 CFU/100 ml.

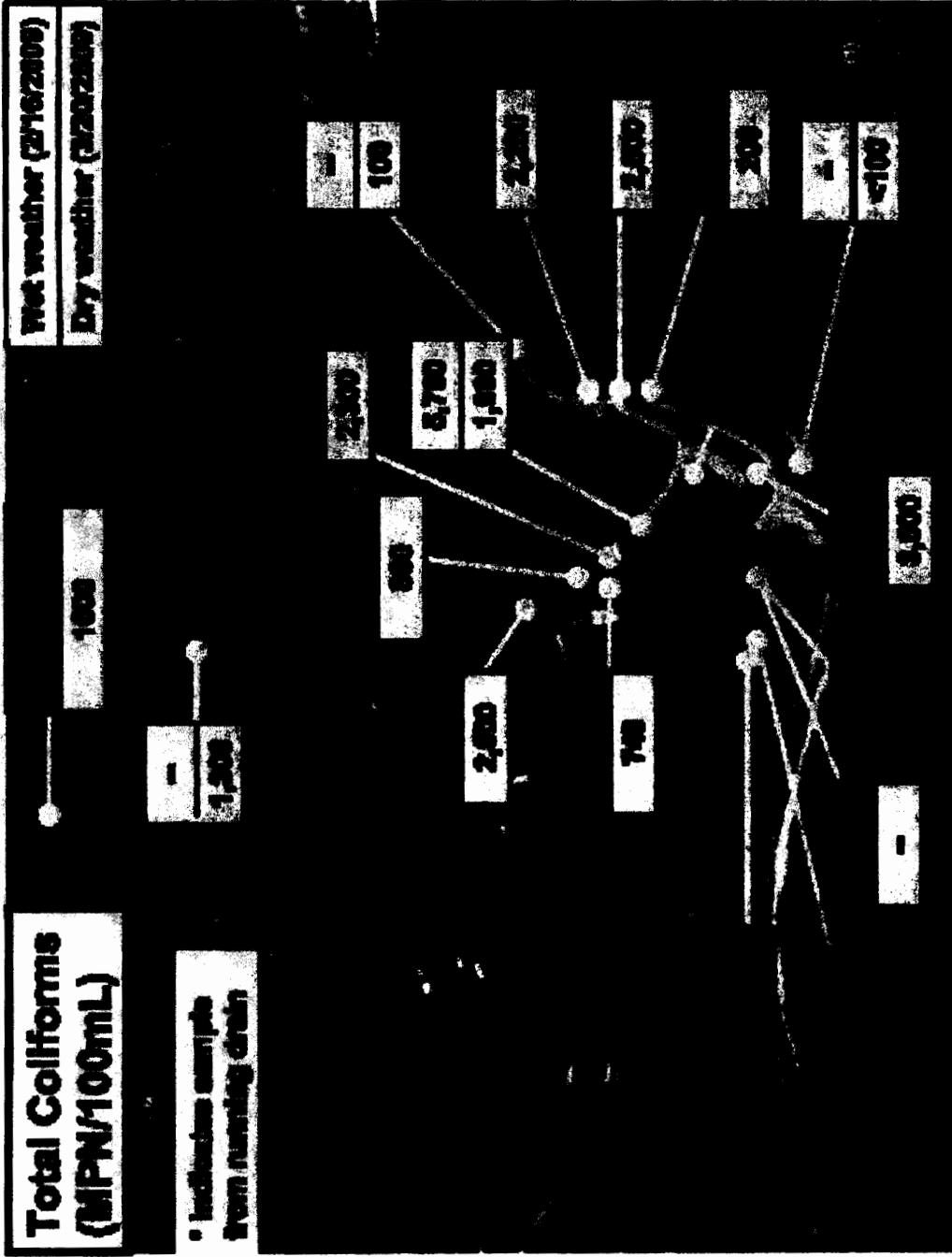


Figure 4. Comparison of total coliform concentrations during wet weather and dry weather (lagoon open) conditions. The single-sample water quality standard is 10,000 CFU/100 ml. Red shading indicates values exceeding water quality standard.

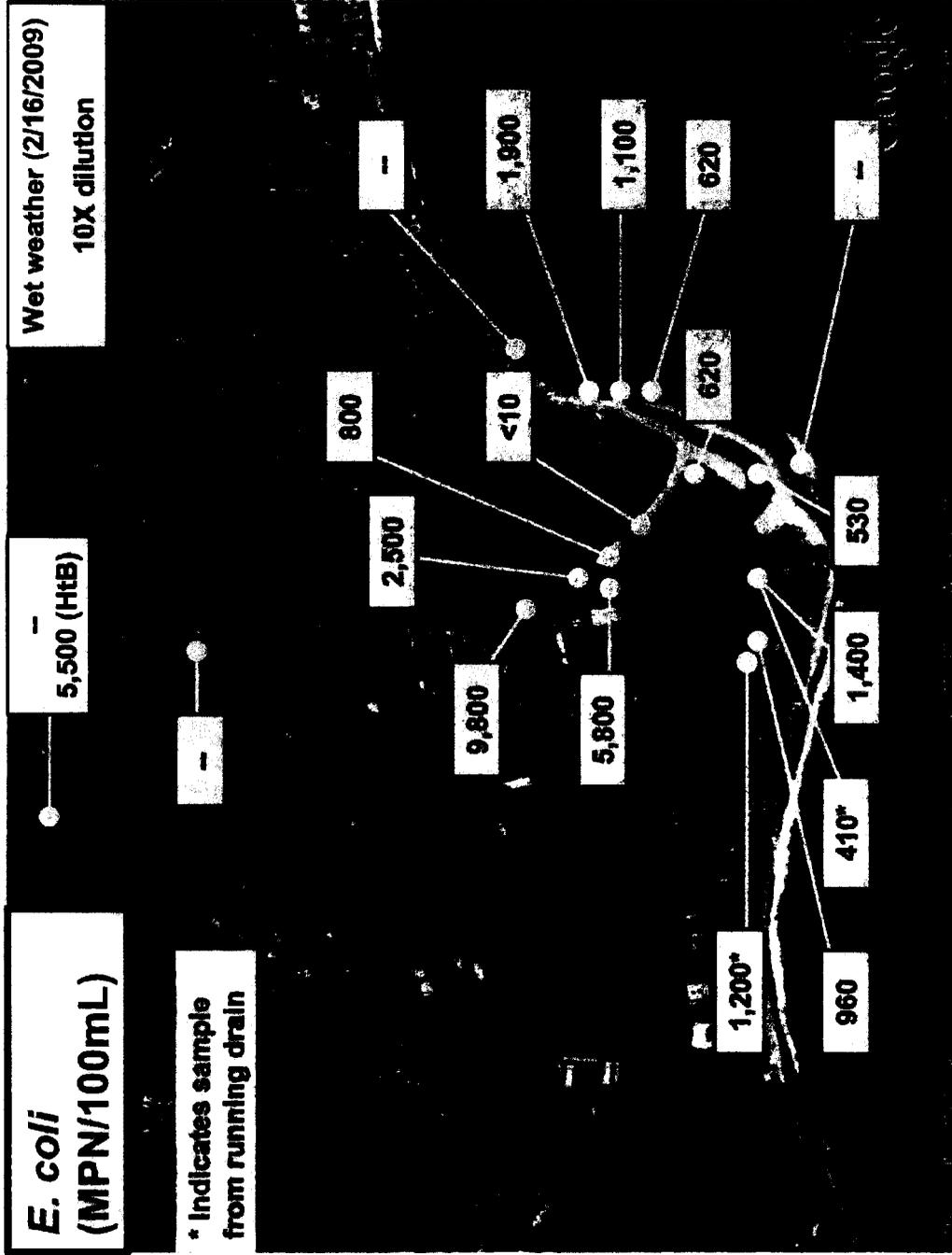


Figure 5. *E. coli* concentrations during wet weather sampling period. The single-sample water quality standard is 400 CFU/100 ml. The two upstream sites were not sampled, but data collected nearby by Heal the Bay one day after our samples were collected are shown.

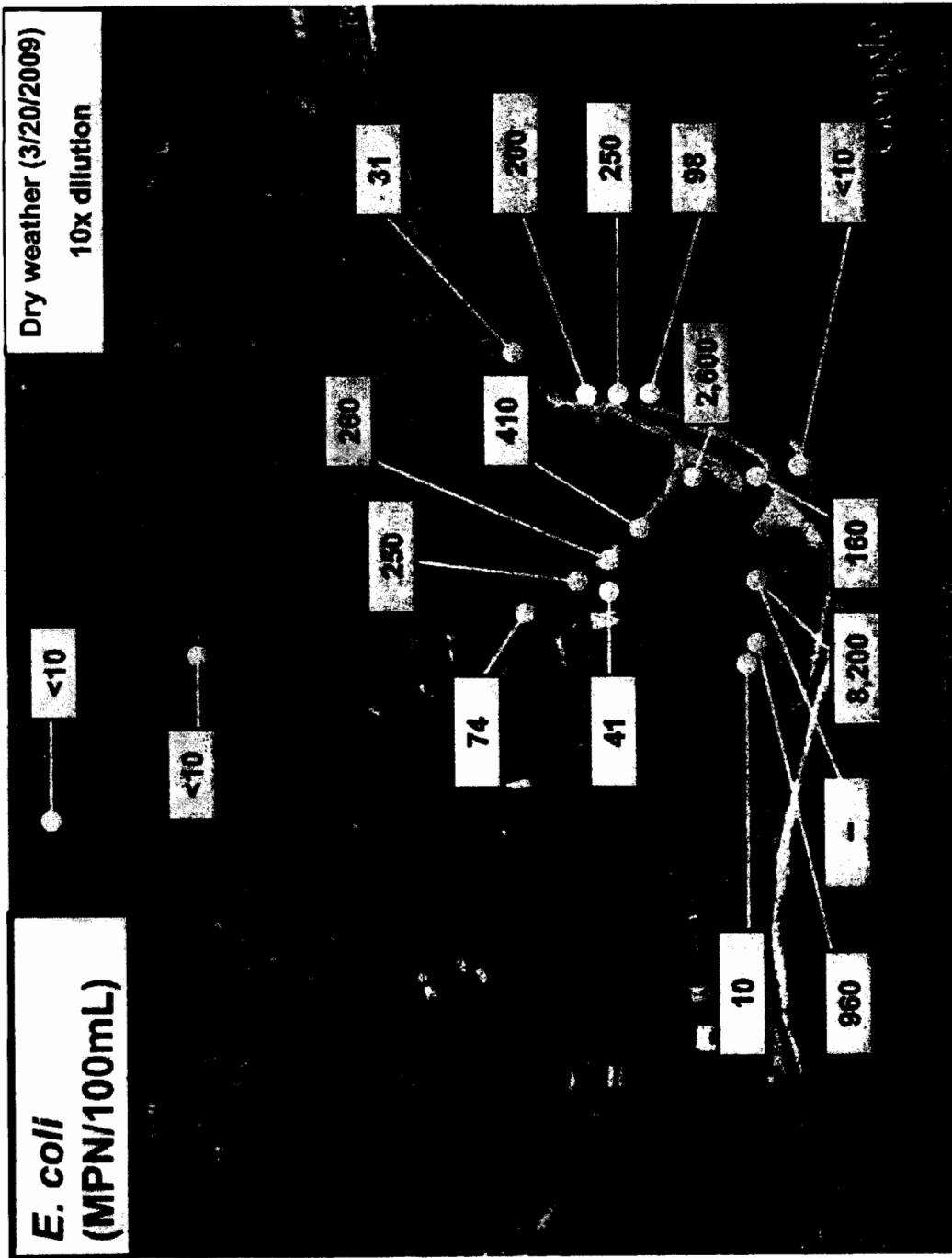


Figure 6. *E. coli* concentrations during dry weather (lagoon open) sampling period. The single-sample water quality standard is 400 CFU/100 ml.

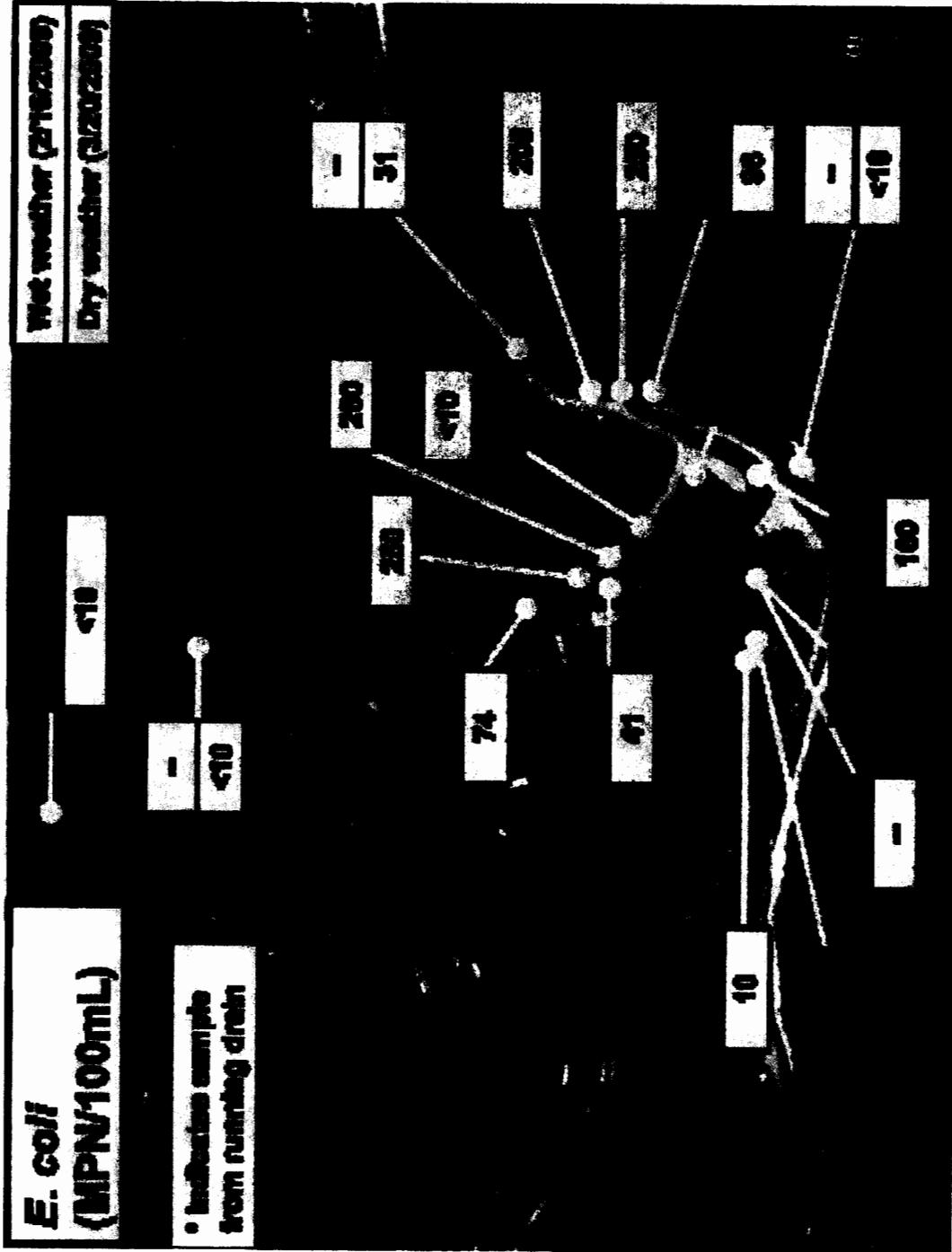


Figure 7. Comparison of *E. coli* concentrations during wet weather and dry weather (lagoon open) sampling periods. The single-sample water quality standard is 400 CFU/100 ml. Red shading indicates values exceeding water quality standard.

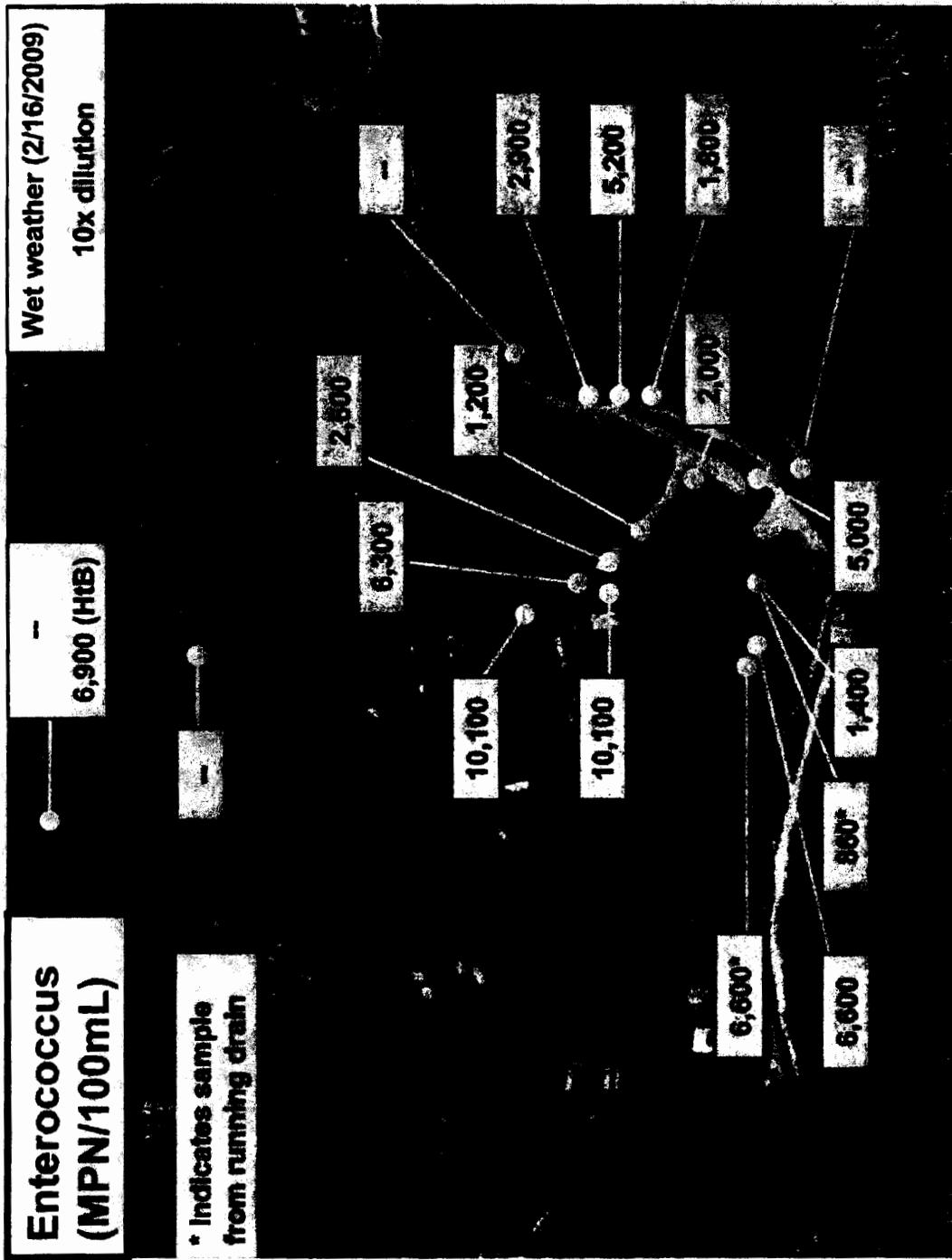


Figure 8. Enterococcus concentrations during wet weather sampling period. The single-sample water quality standard is 104 CFU/100 ml. The two upstream sites were not sampled, but data collected nearby by Heal the Bay one day after our samples were collected are shown.

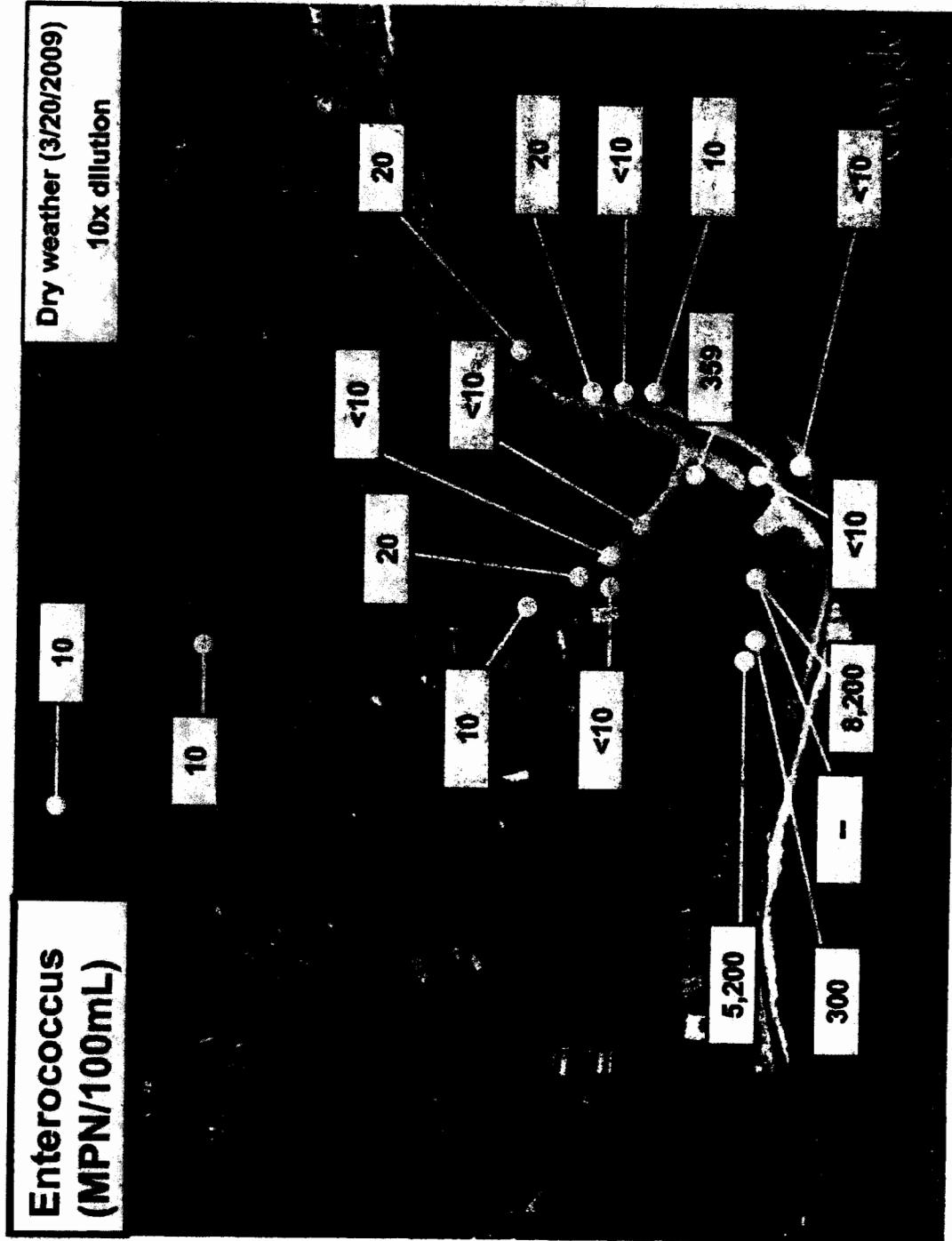


Figure 9. Enterococcus concentrations during dry weather (lagoon open) sampling period. The single-sample water quality standard is 104 CFU/100 ml.

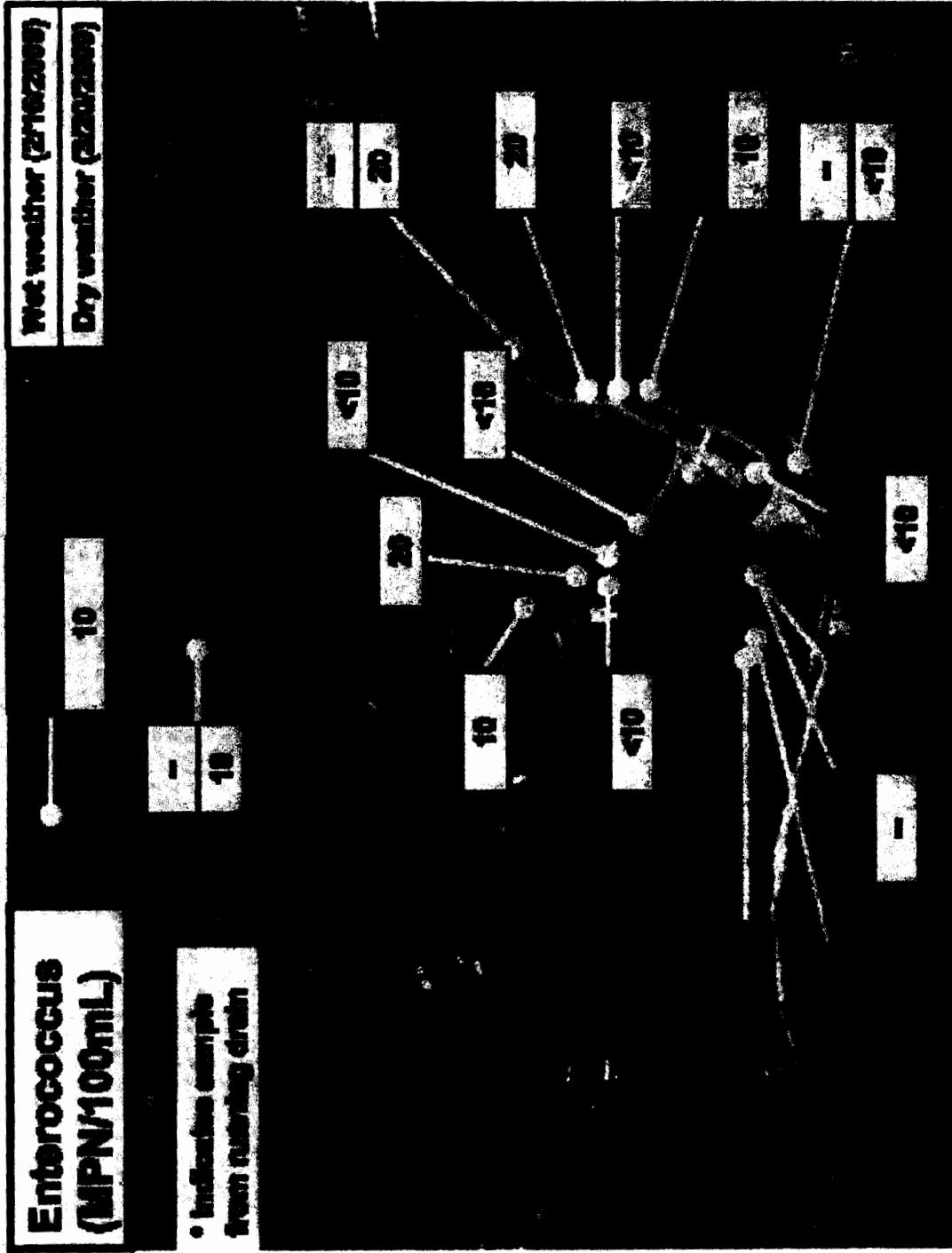


Figure 10. Comparison of Enterococcus concentrations during wet weather and dry weather (lagoon open) sampling periods. The single-sample water quality standard is 104 CFU/100 ml. Red shading indicates values exceeding water quality standard.

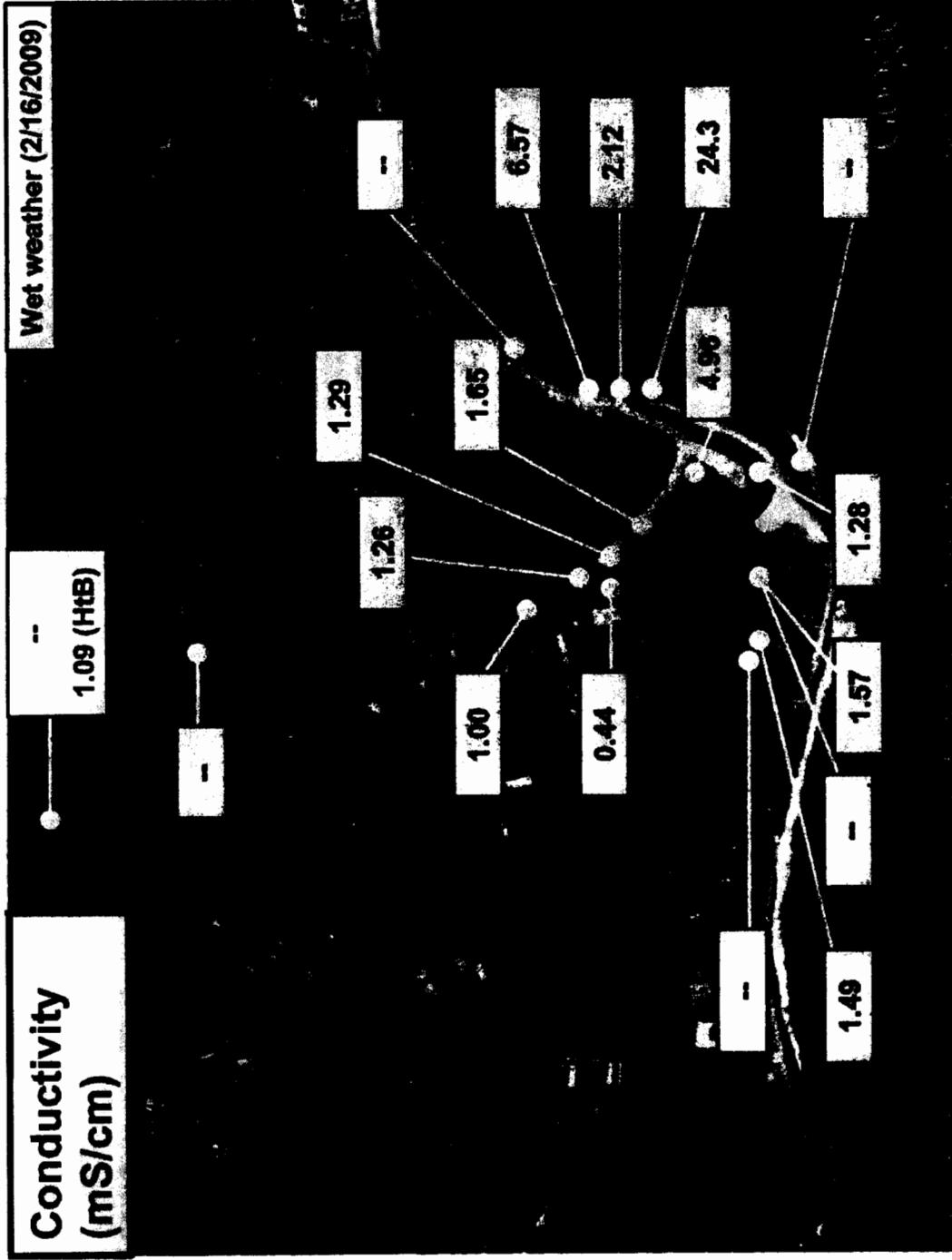


Figure 11. Conductivity values during wet weather sampling period. The two upstream sites were not sampled, but data collected nearby by Heal the Bay one day after our samples were collected are shown.

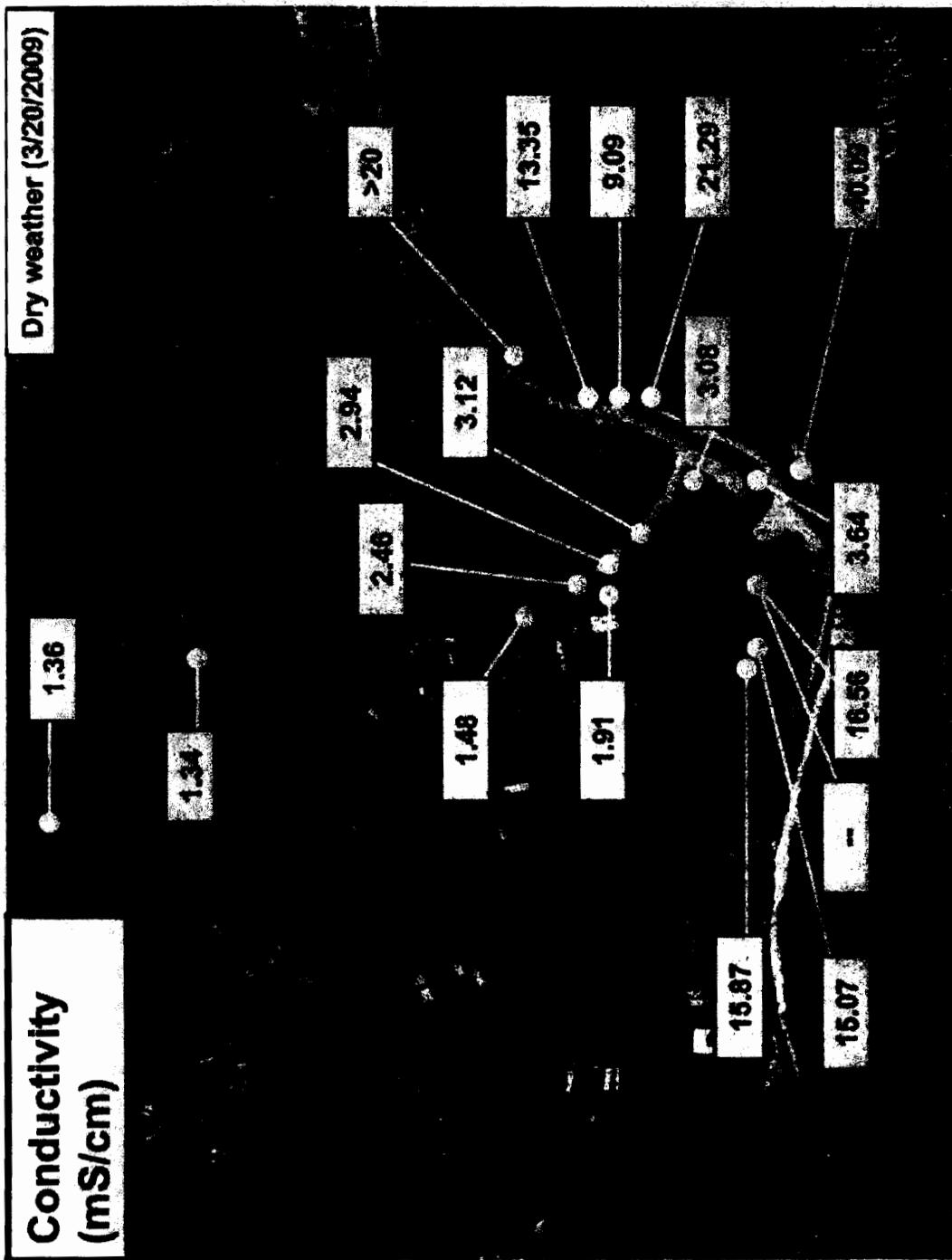


Figure 12. Conductivity values during dry weather (lagoon open) sampling period.

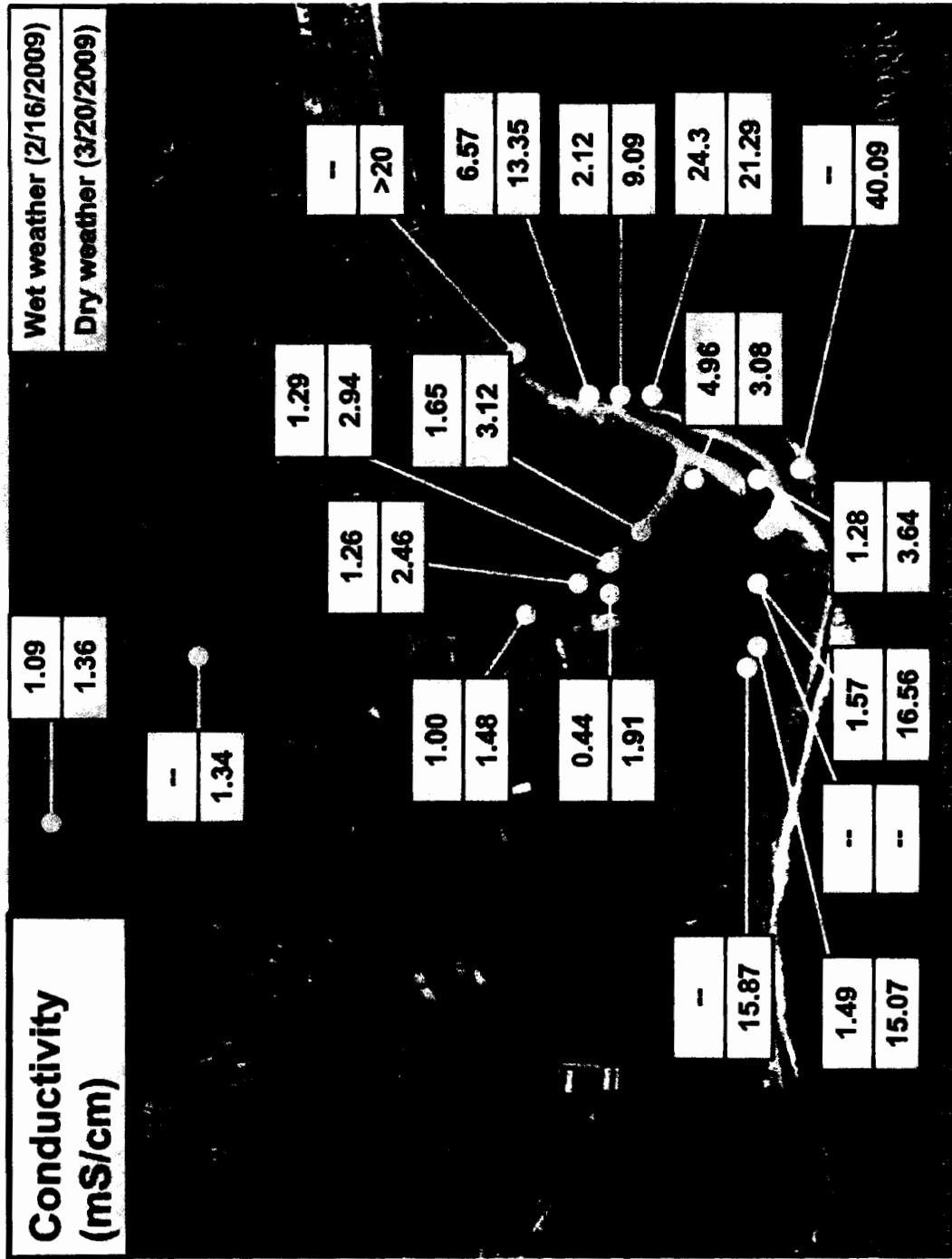


Figure 13. Conductivity values during wet weather and dry weather (lagoon open) sampling periods.



United States Department of the Interior

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June 25, 2010

Mr. James Thorsen,
City Manager,
City of Malibu,
23815 Stuart Ranch Road,
Malibu, California 90265

JUN 28 2010

Dear Mr. Thorsen;

This letter summarizes preliminary results from Task 1 (Synoptic sample collection) of our cooperatively funded water resources program to identify sources of fecal indicator bacteria (FIB) and nutrients to Malibu Lagoon and near-shore ocean water, Malibu, California. The purpose of the synoptic sample collection was to provide a snap-shot in time of the occurrence and distribution of FIB nutrients, and other constituents in shallow groundwater, Malibu Lagoon (including its tributary Malibu Creek) and near-shore ocean water. The study was done under the direction of Dr. John Izbicki in our San Diego Projects Office.

Data were collected as part of Task 1 from April 17-22, 2010. The sample period was selected to reflect conditions near the end of the rainy season for comparison and contrast with data previously collected during the dry summer season between July 21-27, 2009. The primary hydrologic differences between the July 2009 and April 2010 sample periods were:

1. During the July sample period, the sand berm at the mouth of Malibu Lagoon was closed and seawater water did not exchange freely with lagoon water during tidal cycles. However, during July, seawater overtopped the berm and entered the lagoon during high tide, and lagoon water moved through the sand berm at the mouth of the lagoon to discharge to the near-shore ocean during low tide. During the April sample period, the berm of the lagoon was open to the ocean and seawater flowed into the lagoon during high tide and water from the lagoon flowed into the ocean during low tide. Water levels in the lagoon were lower during the April sample period than during the July sample period.
2. Malibu Creek was not flowing during the July sample period, whereas, the creek was flowing during the April sample period. Streamflow measured at the U.S. Geological Survey streamgage upstream from the lagoon (Malibu Creek at Malibu, Calif 11105510) during April 17-19 varied daily from about 11 to 20 cubic feet per second (cfs) as a result of upstream discharges. Precipitation in the Malibu Creek

watershed, beginning late in the day on April 19, increased streamflow at the gage to a maximum of 30 cfs on April 20.

3. Groundwater levels in many of the sampled wells, especially wells near Malibu Lagoon, were lower in April 2010 than in July 2009.

More than 230 samples were collected during the April 2010 sample period. Samples were collected once from selected wells in the study area, from Malibu Creek upstream from residential and commercial development in the Malibu Civic Center area, and from selected sites in Malibu Lagoon. Samples also were collected daily during the sample period at high, low, mid-high, and mid-low tide from Malibu Lagoon, the discharge of the lagoon to the near-shore ocean, and from three selected recreational beaches. Additional samples were collected hourly during a falling tidal cycle from high to low tide from piezometers and seepage samplers installed in the sand berm at the mouth of Malibu Lagoon and near Malibu Colony, and from associated sites in the lagoon and the near-shore ocean. Groundwater sample sites are shown in figure 1 and surface-water sites are shown in figure 2. On-site wastewater treatment systems sampled previously were not sampled during the April 2010 sample period, but will be sampled later as part of Task 1.

USGS staff set up a temporary laboratory in the Malibu Civic Center area and FIB were analyzed on site using Colilert and Enterolert. On most days, samples were analyzed within 6 hours after collection, the recommended holding time for sample collected for regulatory purposes. On some days, samples were held for slightly longer prior to analysis. All samples were analyzed within 24 hours of collection, the recommended holding time for samples collected for scientific purposes. In addition to routine laboratory Quality Assurance data, selected FIB samples analyzed within 6 hours after collection were reanalyzed 24 hours after collection to determine how differences in sample holding time may affect results, and selected samples analyzed for FIB using Colilert and Enterolert also were analyzed using membrane filtration to determine how different analytical methods may affect results. FIB data for water from wells, Malibu Lagoon, and the near-shore ocean are summarized in the following paragraphs.

Total coliform were detected at concentrations greater than the detection limit of 1 Most Probable Number (MPN) per 100 milliliters (ml) in water from 10 of 15 sampled wells, and concentrations were as high as 2,400 MPN per 100 ml in three wells during the April sample period. The frequency of detection and concentration of total coliform was greater during April 2010 than during July 2009; however, total coliform occur naturally in the environment and are not necessarily associated with fecal sources. Enterococci was detected in only four sampled wells at concentrations generally near the detection limit of 1 MPN per 100 ml. Well SMBRP-2, in the undeveloped riparian area east of the lagoon, had an enterococci concentration of 96 MPN per 100 ml. *Escherichia coli* concentrations were less than the detection limit of 1 MPN per 100 ml in samples from all wells. In general, Enterococcus and *E. coli* concentrations in water from wells sampled in April 2010 were similar to concentrations measured during July 2009.

During the April 2010 sample period, Total coliform, *E. coli*, and enterococcus concentrations in Malibu Lagoon were as high as 105,000, 8,400, and 19,900 MPN per

100 ml, respectively. In general, FIB concentrations were lower in the lagoon during April 2010 than during July 2009. FIB concentrations in the lagoon during both sample periods were several orders of magnitude greater than concentrations in water from sampled wells. During April 2010, FIB concentrations varied widely between low and high tide. Concentrations in the lagoon were low (often near the detection limit of 10 MPN per 100 ml for *E. coli* and enterococcus) during high tide when seawater having low FIB concentrations entered the lagoon. FIB concentrations increased to high values by the next low tide when water from the lagoon discharged to the ocean.

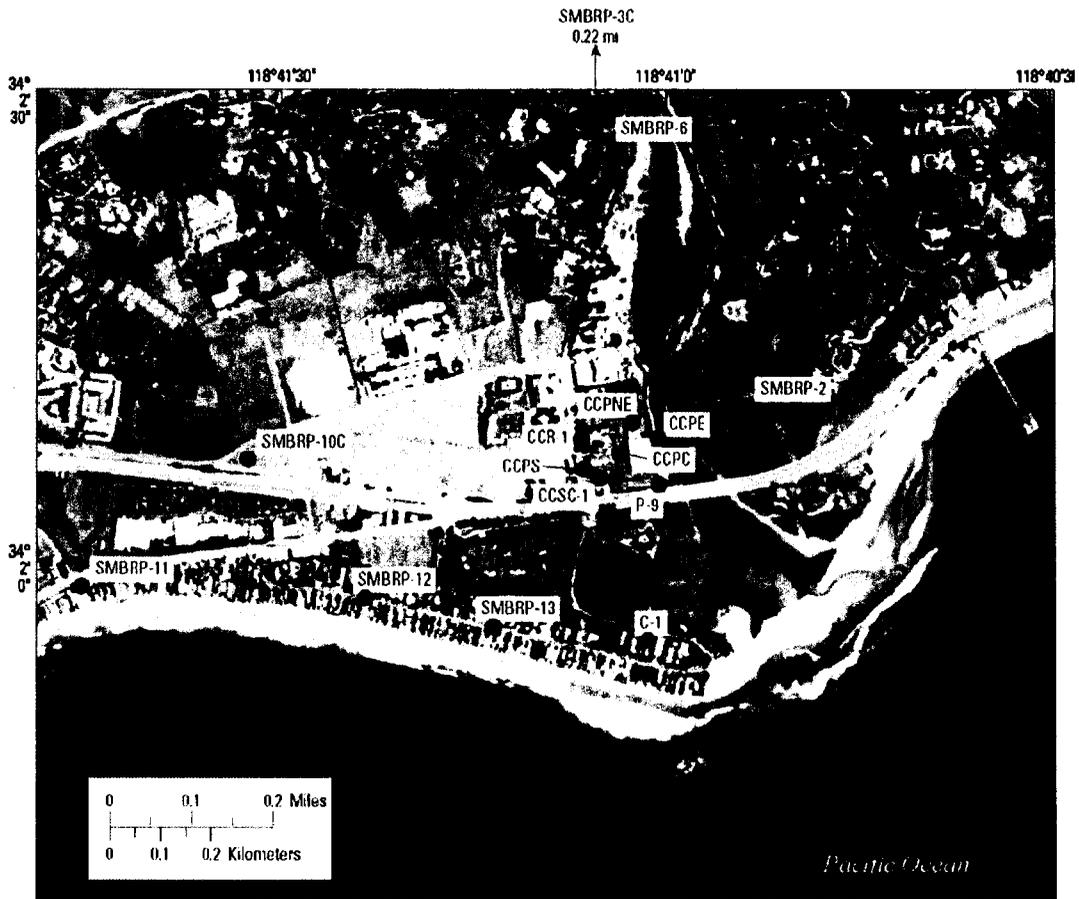
FIB concentrations in samples from the near-shore ocean at Puerco Beach (OF-A, fig. 2) and at Malibu Colony (AF-B, fig. 2) generally were less than the detection limit during low tide when radon-222 data collected as part of this study show that groundwater discharge to the ocean was occurring. FIB concentrations increased slightly during high tide when groundwater discharge to the ocean was not occurring. A similar increase in FIB at high tide also was observed in July 2009 and was attributed to wave run-up on the beach washing FIB from kelp accumulated at the wrack line. High FIB concentrations were measured in water extracts from kelp during both the July 2009 and April 2010 sample periods. Enterococcus concentrations in near-shore ocean samples collected at Puerco Beach or Malibu Colony during the April 2010 sample period did not exceed the U.S. Environmental Protection Agency (USEPA) single sample standard of 104 MPN per 100 ml. In contrast, enterococcus concentrations in near-shore ocean samples collected at at Surfrider Beach (site OF-C, fig. 2) west of Malibu Lagoon commonly exceeded the USEPA single sample standard for enterococcus in marine recreational water at low tide when the lagoon was discharging to the ocean. The occurrence and concentrations of FIB at Surfrider Beach closely parallel the occurrence and concentrations of FIB data collected from the discharge of the lagoon. Of the three beach sites sampled, FIB concentrations were lowest in the near-shore ocean adjacent to unsewered residential development in the Malibu Colony area in both the July 2009 and April 2010 sample periods.

FIB data collected during both July 2009 and April 2010 have been entered into the U.S. Geological Survey's computerized data base NWIS (National Water Information System) and are publicly available on line at <http://waterdata.usgs.gov/nwis>. Chemical, isotopic, and genetic analyses of samples collected during the April 2010 sample period have not yet been completed. Please contact me at 619-225-6127 or John Izbicki at 619-225- 6131 if you have any questions concerning these preliminary results. We look forward to working with you and your staff on the completion on this important study.

Sincerely,



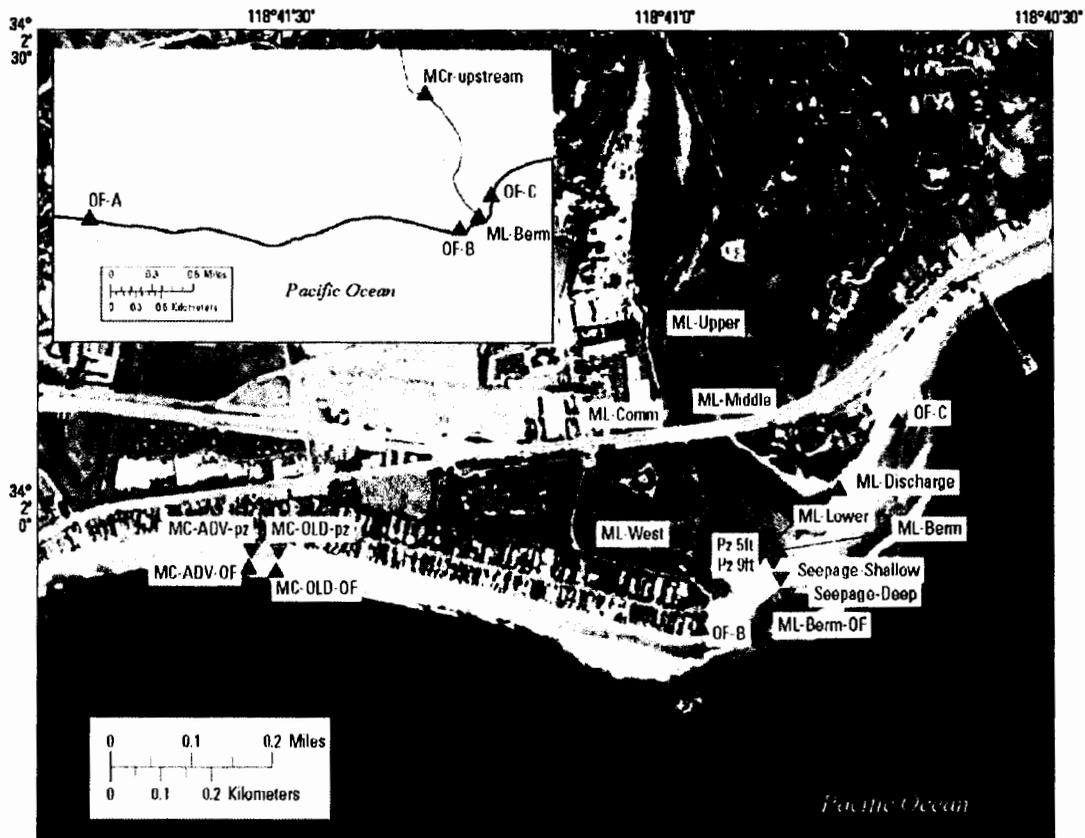
for
Peter Martin
Program Chief



EXPLANATION

Sampled wells and identifier—
 C-1 ●

Figure 1.—Sampled wells, July 2009 to April 2010, Malibu, California



EXPLANATION

- Sample sites and identifier—
- | | |
|---------------|--|
| Surface water | Hand-driven piezometers
or seepage samplers |
| ▲ ML-middle | ▼ ML-Berm-9ft |

Figure 2.—Surface water sample sites, July 2009 to April 2010, Malibu, Calif.

2009 Investigation of Spatial and Temporal Distribution of Human-specific *Bacteroidales* marker in Malibu Creek, Lagoon and Surfrider Beach

University of California, Los Angeles Study

Principal Investigators: Jennifer Jay and Richard F. Ambrose

Graduate Student Researchers: Vanessa Thulsiraj and Steven Estes

Introduction

WQ impairment near urbanization & Malibu as an important site

Fecal pollution, human and non-human, is a major cause of water impairment in coastal areas. However, our understanding of fecal pollution in coastal ecosystems, as well as our ability to identify and mitigate its sources, is greatly limited by the uncertainties surrounding its behavior in two major reservoirs: wetlands and beach sediments. Fecal indicator bacteria (FIB) and pathogens can enter coastal creeks and rivers from upland sources, but near-shore beach sources are also significant reservoirs (Desmarais et al., 2002; Davies et al., 1995; Craig et al., 2004; Gerba and McLeod 1976), and coastal wetlands have been shown to both increase (Ferguson et al., 2005; Grant et al., 2001; Gersberg et al., 1995; Sanders et al., 2005) and decrease (Evanson and Ambrose 2006) the levels of FIB in water.

Understanding whether there is a relationship between populations of FIB and human-specific *Bacteroidales* (HSB) in wetlands is important for determining impact that these environments pose to human health in coastal bathing waters. Recent work in Southern California has shown that coastal wetlands can be a source of FIB due to wildlife congregation (Lu et al., 2008), regrowth of FIB in sediment (Lee et al., 2006; Davies et al., 1995), and scouring of sediment. Although contrasting conclusions exist it is important to obtain a better understanding of how coastal wetlands influence FIB. Malibu is an important study site and serves as a template to investigate how wetlands specifically influence human-specific factors. This information can assist managers and policymakers to better understand how wetland-related decisions impact beach closure days and consequently local economies and human health.

Our understanding of the fate of fecal pollution in coastal ecosystems, as well as our ability to identify and mitigate its sources, is greatly hindered by limitations in the detection of microbial contamination (in terms of analysis times and host-specificity) and by uncertainties surrounding its behavior in watershed and beach sediments. Both FIB and pathogens appear to have greater persistence in sediments than they do in water, and have been shown to grow in this environment (Lee et al., 2006; Desmarais et al., 2002). Present efforts to identify fecal contamination sources employ a tiered approach in which traditional fecal indicator bacteria (FIB) levels are used to inform advanced host-specific investigation. However, a persistent lack of correlation between FIB and human-specific *Bacteroidales* markers (HSM), high temporal variability in water quality parameters, and long analysis times obscure these endeavors. Rapid detection methods and alternative, host-specific fecal indicators are at the forefront of current coastal water quality protection efforts.

Use of Bacteroidales for fecal source identification

Rapid detection methods and alternative indicators are clearly needed for the development of effective recreational water initiatives (Gregory et al., 2006). Current techniques are primarily culture-based, requiring a minimum of 18 hours for analysis. This delay could result in swimmers being exposed to poor water quality or an unnecessary beach closure, thereby impacting their personal health or the local economy. Among these explored technologies are quantitative polymerase chain reaction (qPCR) (Khan et al., 2007; McDaniels et al., 2005; Shanks et al., 2008; Siefring et al., 2008), fluorescent in situ hybridization (Lee and Deininger 2004; Field and Samadpour 2007), enzymatic methods (Scott et al., 2002), flow cytometry (Griffith et al., 2003; Paster et al., 1994) and immunomagnetic separation/ATP quantification (Gerba 2000). Quantitative PCR is advantageous because it is one of the mentioned rapid processes (3-5 hour processing time), and host-specific.

Microbial source tracking (MST) is an actively growing and important area of research, as information on host-specific sources of fecal contamination can be the key to successful remediation efforts (Bernhard and Field 2000b; Dick et al., 2005a). Griffith et al (2003) compared twelve MST techniques and found detection of *Bacteroidales* to be the most effective method to detect human fecal pollution in various mixtures of fecal sources. Members of the order *Bacteroidales* are found exclusively in endothermic organisms, and reside within feces, the digestive tract, and other body cavities (Dick et al., 2005b). *Bacteroidales* levels in human sewage are orders of magnitude higher than levels of fecal coliform bacteria (Dick and Field 2004). These organisms are obligate anaerobes, and thus do not have the potential for regrowth in the environment that confounds the use of *E. coli* and enterococci as indicators (Seurinck et al., 2005). Most importantly, *Bacteroidales* organisms from different fecal sources exhibit distinct genetic sequences, thus allowing the development of host-specific nucleic acid-based assays. Significant research has been directed toward the development of conventional and quantitative PCR methods for *Bacteroidales* markers specific to human, bovine, pig, horse and dog as well as to universal *Bacteroidales* (Kildare et al., 2007; Shanks et al., 2008; Carson et al., 2005; Nobel et al., 2006; Santoro and Boehm 2006). These assays have been applied to fecal source tracking in many environments: urban watersheds coastal beaches (Boehm 2007; Boehm et al., 2002), freshwater lakes and rivers (Lund 1996), groundwater (Reischer et al., 2008), and agriculturally impacted estuaries and bays (Shanks et al., 2006; Gourmelon et al., 2007).

This study examines the spatial and temporal distribution of human-specific *Bacteroidales* marker (HBM) in lower Malibu Creek, Lagoon and Surfrider Beach under specific hydrologic conditions. Specifically, this work identifies the distribution of fecal indicator bacteria and HBM during wet and dry weather, when the lagoon is open and closed. We investigate whether detectable concentrations of HBM are present in the Lagoon, and if concentrations of HBM correlate with fecal indicator bacteria.

Materials and Methods

Site description of Surfrider Beach and Malibu Creek

The Malibu Creek watershed (109 mi²) is partially developed (mixed use 17%, 83% open), with 90,000 residents in five cities and unincorporated Los Angeles County. Reaches of the creek are

impaired for bacterial contamination, and Surfrider Beach, with over 10,000 visitors on a typical summer weekend day, has frequent postings for impaired water quality. Fecal sources include non point sources and wildlife. Possible human sources of fecal contamination include septic systems and disinfected discharge from Tapia Wastewater Reclamation Facility into Malibu Creek (discharging only in the winter). Malibu Creek ends in a 13-acre lagoon, so birds are likely an important fecal source at Surfrider Beach as well. The beach is currently sampled daily for FIB. Additionally, Heal the Bay's Stream Team has gathered long-term nutrient and FIB data at 17 locations throughout this watershed, showing increasing nutrient and FIB levels with distance downstream in each of four subwatersheds.

Sample design and collection

For this study, a snapshot of bacteria concentrations was measured from the lower Malibu Creek watershed. Surface water samples were collected from a total of 20 sample locations throughout Malibu Creek, Lagoon and Surfrider Beach (Fig 1). Water samples were collected with sterile 500mL Nalgene bottles attached to a sampling pole, or by submerging a sterile 2L Nalgene bottle. Samples taken from flowing storm drains collected end-of-pipe discharges. Samples were stored on ice and transported to the laboratory within 6 hours for immediate analysis. Samples were taken during wet and dry weather, while Malibu Lagoon was open and discharging to Surfrider Beach. Additional sampling occurred during a transitional phase where the Lagoon had been previously closed, but opened overnight and was open during time of sampling. The third hydrologic condition investigated was during dry weather while the Lagoon was closed due to the formation of a sand berm, which prevented flow from the Lagoon to the beach.

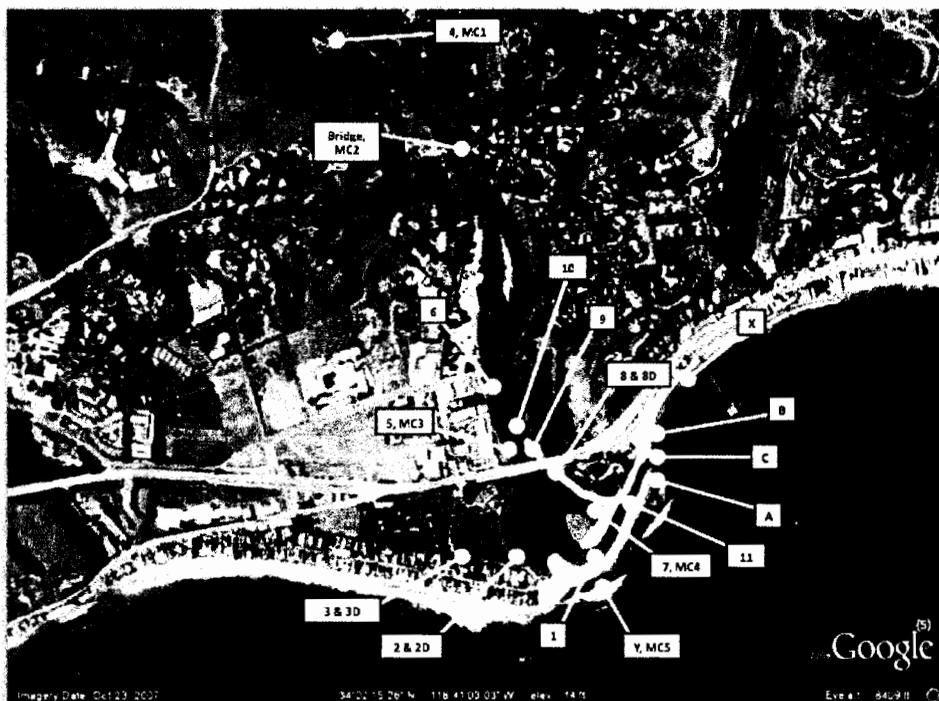


Fig. 1. Sampling locations within the Malibu Watershed. Samples were taken from Malibu Creek (Site 4, Bridge), Lagoon (Site 1-4, 5-11) Surfrider Beach (Site A, B, C, X, Y). Sample sites 2D, 3D, and 8D represent storm drain discharges.

FIB analysis by traditional methods

Water samples were measured for three types of fecal indicator bacteria including total coliform (TC), *Escherichia coli* (EC), and enterococci (ENT) using IDEXX Laboratories, Inc. (Westbrook, ME) defined substrate tests commercially known as Colilert-18 and Enterolert in a 97 well quantitray format. Ten fold and 100-fold dilutions of water samples were used as recommended by the manufacturer.

Geochemical analysis of water samples

Water samples were analyzed for nitrate and ammonia using the Hach Spectrophotometer (model DR 280). Combo by Hanna (model H 18130), a multi-parameter probe, was used to measure total dissolved solids (TDS), water temperature, electrical conductivity and pH. Dissolved oxygen concentrations were measured by the YSI 55 DO probe (model 55/12 FT). Water samples were also analyzed for total suspended solids (TSS) in the lab using Environmental Sciences Section Method 340.2.

Extraction of DNA

DNA was extracted from filters using the Mobio UltraClean Fecal DNA Kit. DNA was extracted according to manufacturer's protocol with the addition of 90 seconds of bead-beating in lieu of 10 minutes vortexing, as listed in the protocol. DNA extracts were stored at -20°C until they could be processed for human-specific *Bacteroidales*. The concentration of extracted DNA from each sample was measured fluorometrically (Stratagene, La Jolla, CA (Santoro and Boehm 2007)) using the Quant-It PicoGreen double-stranded DNA reagent kit (Invitrogen, Carlsbad, CA). Each sample was measured and total DNA(ng) was found by reference to Lambda DNA standards (n=8 ranging from 0.1 to 25 ng μ l⁻¹). Prior work in Jay lab has shown that superior DNA amount and quality are obtained with no further purification steps.

Quantitative PCR analysis of Bacteroidales

Detection of 16S rRNA gene markers for human-specific *Bacteroidales* were performed using qPCR (Stratagene, Inc., Mx3000P), by the SYBR Green-based method, with DNA primers HF183F and HF183R (Dick and Field 2005; Seurinck et al., 2005). Each qPCR mixture (total volume 25 μ l) contained approximately 1-2 ng DNA (diluted in known volume of RNase-free water) and ~13 μ l master mix (2 \times SYBR Green, Stratagene, Inc.; 140 μ M each primer, Operon Biotechnologies, Huntsville, AL). Samples were run in duplicate and converted to concentration by reference to *Bacteroidales* standards (a *Bacteroidales* plasmid DNA kindly provided from the Furman laboratory (n=8 ranging from 2 \times 10⁰ to 2 \times 10⁶ copies μ l⁻¹). Samples "spiked" (i.e., positive controls) with 1- μ l (2 \times 10⁵ copies) *Bacteroidales* standard are used to estimate low rates of recovery and possible inhibition by contaminants in DNA extracts. In the case of interference, samples were diluted two-fold and reprocessed (Nobel et al., 2006). Negative controls were run with every reaction, and consist of all elements except target DNA.

Results

Fecal Indicator Bacteria Results

Over the entire study period (February to July 2009), a total of 70 water samples were taken from up to 20 different sample locations within lower Malibu Creek, Lagoon and Surfrider Beach. During this time, fecal indicator bacteria were above the water quality single sample standard in 50%, 54% and 39% for TC, EC and ENT. The greatest number of exceedances occurred at site 3 and 3D for total coliforms, however the single highest level (241957 MPN/100ml) for TC was measured from site 6 (lagoon) during wet weather on February 16, 2009. For *E. coli*, sites 2 and 3 showed the greatest number of exceedances, with 4/4 samples above the threshold for both sample locations. Several sites had maximum EC concentrations above the detection limit (>24196 MPN/100ml), including sites 7, 8 and 2D. The greatest number of exceedances for enterococci was measured at site 3D, however highest concentrations of ENT (19863MPN/100ml) was measured during wet weather from sample site 7, during dry weather when the lagoon was closed.

Fecal indicator bacteria concentrations were found to be high throughout the Malibu Creek, Lagoon and Surfrider Beach during a storm event on February 16, 2009. This was the only time that elevated fecal indicator bacteria concentrations were measured upstream in Creek waters or in ocean water samples in this study. The mean concentration during wet weather was measured as 69280 MPN/100ml for TC, 1328 MPN/100ml for EC and 3755 MPN/100ml for ENT. Highest percent exceedance was measured during wet weather. Samples exceeded water quality standards 87% of the time for both TC and EC and 100% for ENT. All samples collected exceeded standards for all three indicators except for two samples. Water samples exceeded water quality standards 87% (13/15) and 100% (15/15) of the time for EC and ENT. Samples collected from site 8, taken within the lagoon, and site 2D, stormwater runoff did not exceed standards for total coliforms and *E. coli*.

In March 2009, field sampling occurred during dry weather while the lagoon was breached. FIB concentrations were typically below the health standard of 400MPN/100mL and 104MPN/100mL for *E. coli* and enterococci. However, specific hot spots were found within Malibu Lagoon under this hydrologic condition. Exceedances for FIB occurred at four sample locations (2, 3, 7, 8) within the lagoon and adjacent to two storm drains (3D and 8D) in March. FIB levels were above health limits in 22% (TC and ENT) and 28% (EC) of samples collected. Mean concentrations for this sampling field campaign were 19924 MPN/100ml TC, 699 MPN/100ml EC, and 371MPN/100ml ENT. However, median concentrations were all below the recreational water quality threshold (TC 2006, EC 168 and ENT 10 MPN/100ml).

During dry weather, transitional open lagoon conditions, elevated levels of FIB occurred in 10 sample locations resulting in 22%, 44% and 6% exceedance for TC, EC and ENT respectively. Slightly larger number of exceedances were observed for *E. coli*, however exceedances were much lower for enterococci in the May sampling event. Samples were above the health standard for *E. coli* at sites 2, 3, 6, 8-11, and 3D. Highest FIB concentrations were measured from site 3 (EC 1552 MPN/100ml) and site 3D (EC 2307MPN/100ml, ENT 1004 MPN/100ml). The largest number of exceedances occurred within Malibu Lagoon, although one ocean water sample (site X) and one upstream sample (site Bridge) were above the water quality limit for total coliforms. Both of these samples did not exceed the single sample standard for any other indicator organism. Mean sample concentrations were 12861 MPN/100ml TC, 531 MPN/100ml EC and 6

MPN/100ml ENT. Median concentrations were again lower than mean values, with values of 3189 MPN/100ml TC, 206 MPN/100ml ENT, and 20 MPN/100ml ENT.

After closure of the lagoon, fecal indicator bacteria concentrations increased, and higher level of exceedences were observed for all three indicators (74% for TC, 63% for EC, and 37% for ENT). Despite exceedences in the lagoon throughout the study period, FIB levels did not exceed health standards for samples collected in Malibu Creek and at Surfrider Beach. The lowest mean concentrations for Surfrider Beach ocean water samples were observed during the closed lagoon, dry weather condition in July 2009 (60 MPN/100ml TC, 20 MPN/100ml EC, and 2 MPN/100ml ENT). And although concentrations were intermediate to high (10^3 to $10^{4.7}$ MPN/100ml) for total coliforms in upstream locations, site 4 and Bridge, these sites never exceeded standards for either *E. coli* or enterococci throughout the sampling period. Mean concentrations for the closed lagoon, dry weather condition were 17096 MPN/100ml TC, 10297 MPN/100ml EC, and 2096 MPN/100ml ENT. Median concentrations were still high, with both TC and EC above the single standard sample threshold (24196 MPN/100ml TC, 5172 MPN/100ml EC and 74 MPN/100ml ENT).

Human-specific HF183 Bacteroidales Results

The human-specific HF183 *Bacteroidales* marker (HBM) concentrations were measured from water samples taken within the Malibu Creek, Lagoon and Surfrider Beach. The human-specific HF183 *Bacteroidales* marker was analyzed during open and closed lagoon, dry and wet weather conditions. Duplicate concentrations were averaged and reported as the value for positive samples. A total of 80 water samples were analyzed for the HBM. Forty-four samples were taken during dry weather while the lagoon was breached (open and transitional). Human-specific *Bacteroidales* marker was not detected in any of the samples taken during this condition. During wet weather open lagoon conditions in February 14.3% (2/14) of samples were positive for HBM. Concentrations of the HBM measured at sites 3 and 5 were 452 copies/100ml and 880 copies/100ml. In July, during dry weather closed lagoon conditions 3 of 22 (13.6%) samples were positive for the HBM. Sample locations 3, 6 and 7 all located within the Malibu lagoon had HBM concentrations of 121 copies/100ml, 55c opies/100ml and 210 copies/100ml.

In addition to the four field sampling days at the 20 locations throughout the watershed, 5 sample sites were measured during a two-week sampling campaign in the months of April and May. Samples were taken at sites 4, 5, 7, Y and Bridge on April 29 2009. Samples taken from sites 4, 5, 7, and the Bridge did not have detectable concentrations of the human-specific *Bacteroidales* marker. Sample site Y, an ocean water sample taken from Surfrider Beach at the mouth of the lagoon, was sampled on April 29 and 30th as well as May 5th and 7th. Site Y did not have detectable concentrations of HBM in the additional four samples taken during the two-week period.

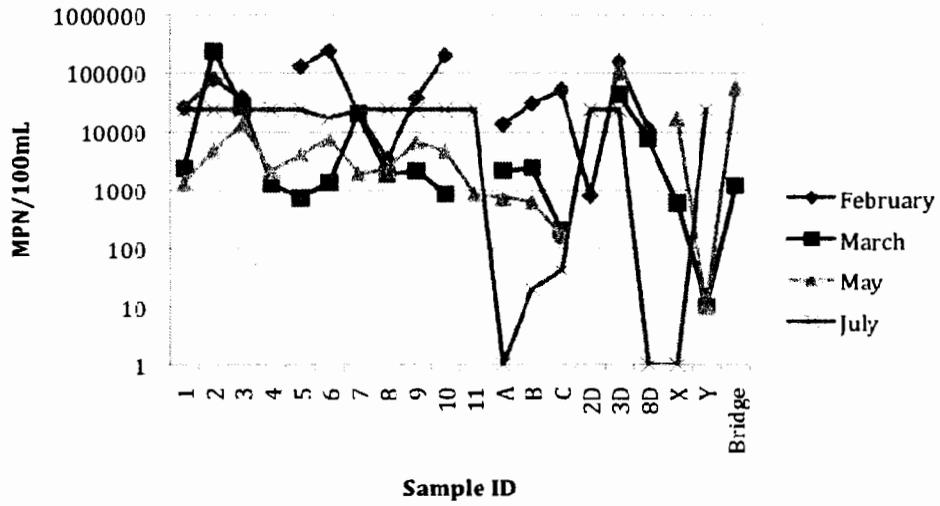
Conclusion

Of the 80 water samples analyzed within the Malibu watershed, five samples were positive for the human-specific HF183 *Bacteroidales* marker. The five positive samples were measured from 4 sample locations, all located within the Malibu lagoon. Site 3 was positive for the HBM during wet and dry weather. Concentrations at this site ranged between 121 – 452 copies/100ml. Other sites that were positive for Malibu Lagoon included sites 5, 6, and 7. Concentrations at these

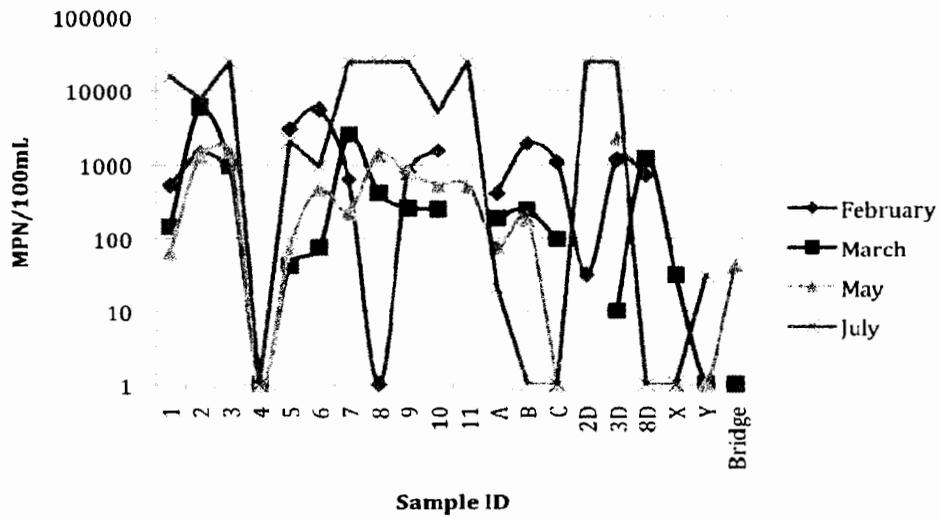
sites ranged between 55 – 880 copies/100ml, which is equivalent to 0.00005 - .0009% sewage. The highest percent exceedance of FIB and HBM concentrations were measured during wet weather. During the study, 93.8% of the samples did not have detectable concentrations of HBM. These data do not rule out any particular potential sources of human fecal contamination. The human-specific *Bacteroidales* marker was not measured in any of the lower Malibu Creek samples and ocean water samples taken from Surfrider Beach.

Figures

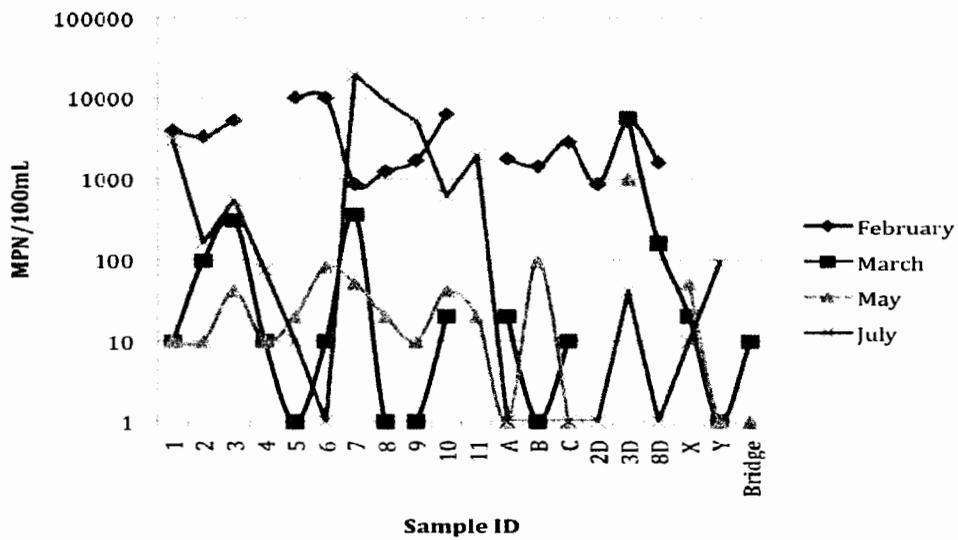
Total Coliforms



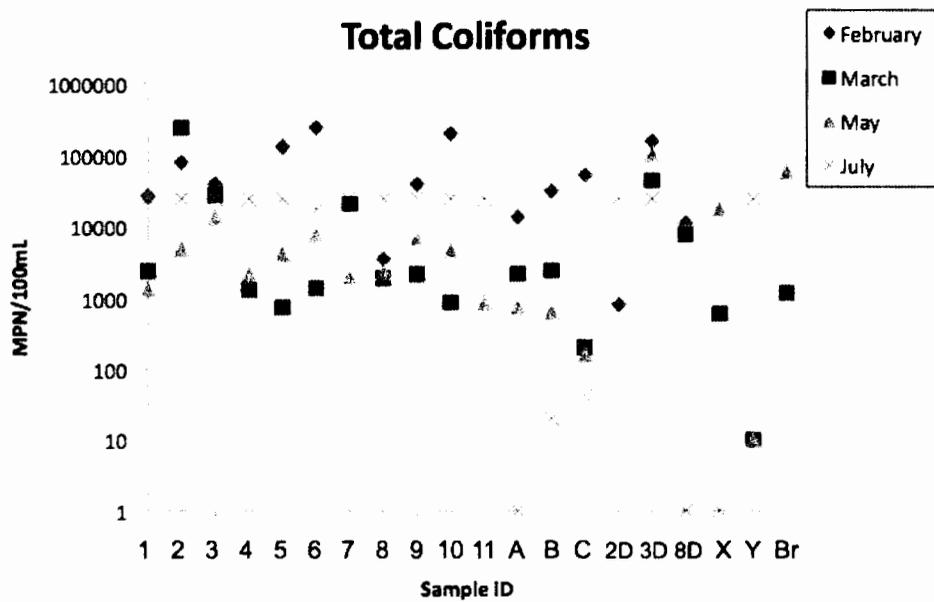
Escherichia coli

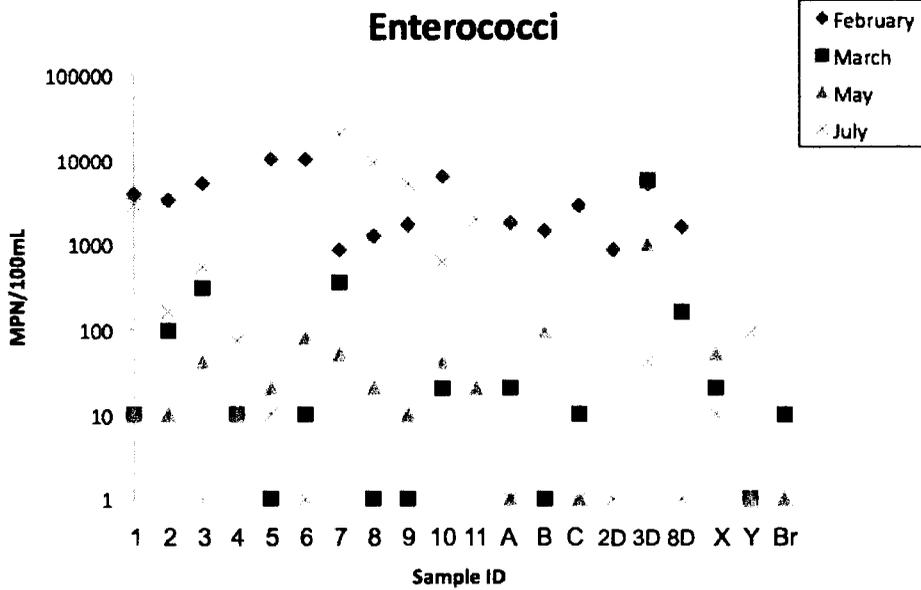
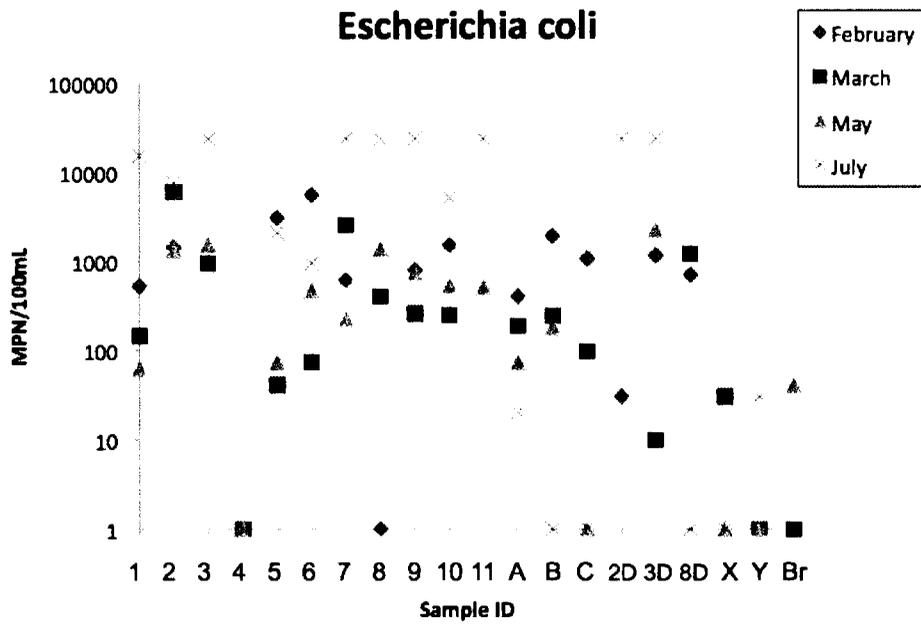


Enterococci

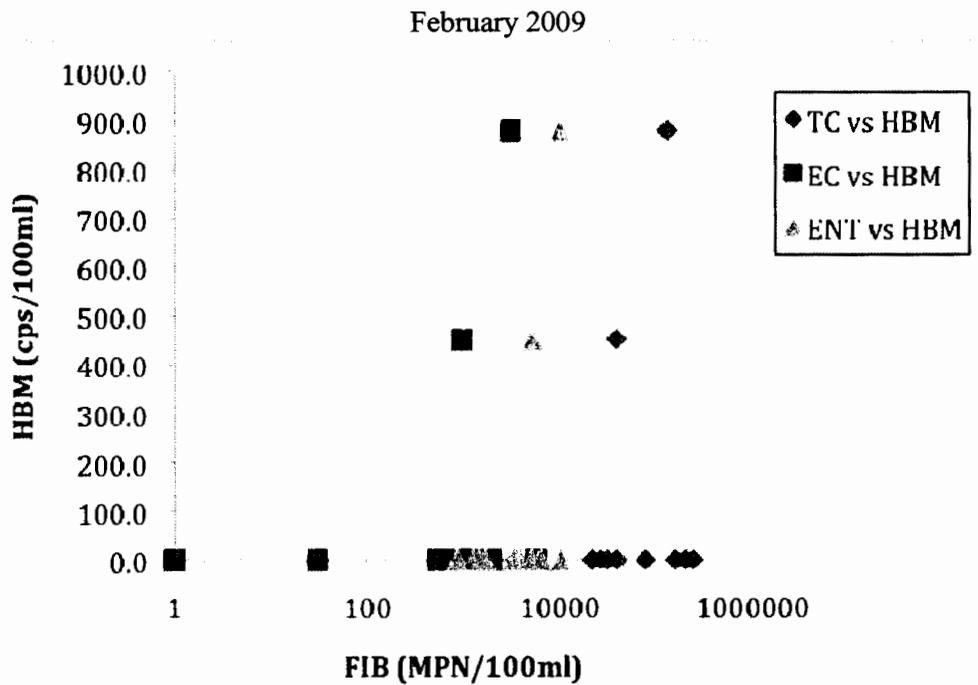
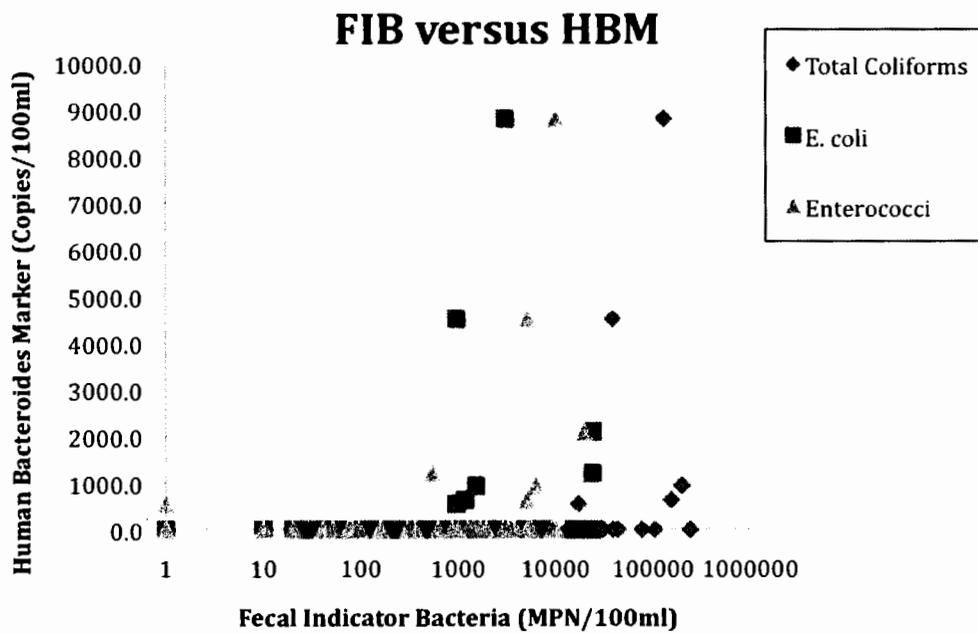


Total Coliforms

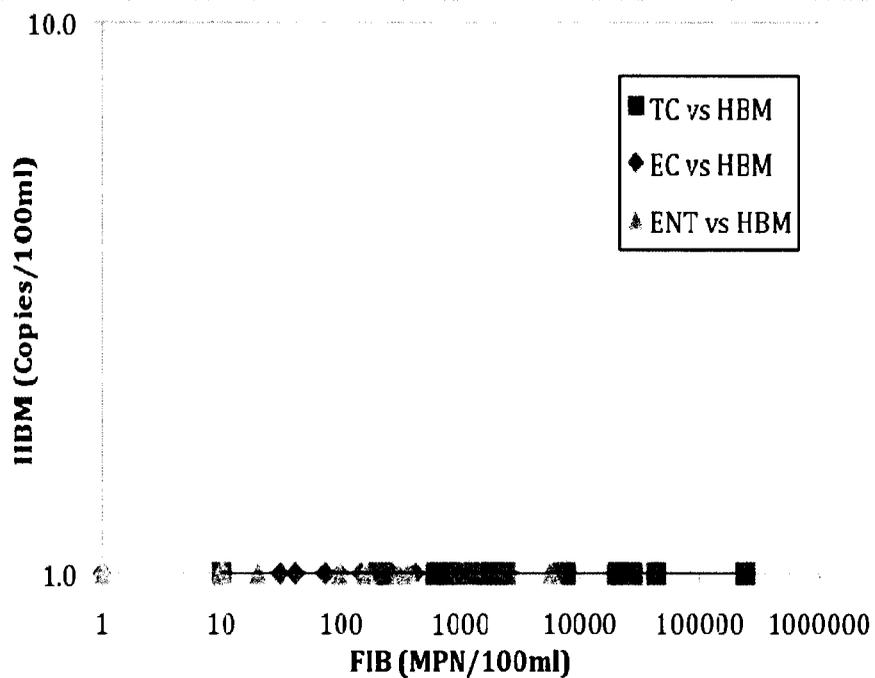




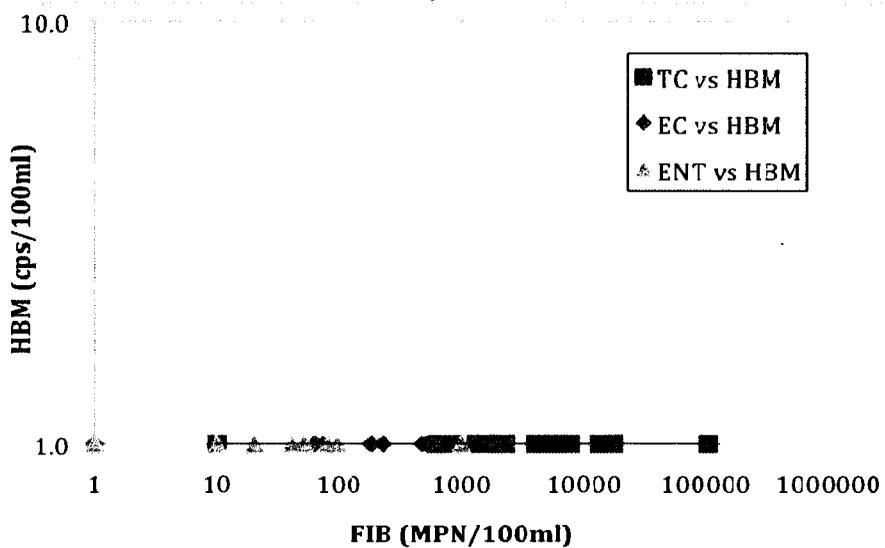
Fecal Indicator Bacteria Versus Human-specific *Bacteroidales* for entire study period

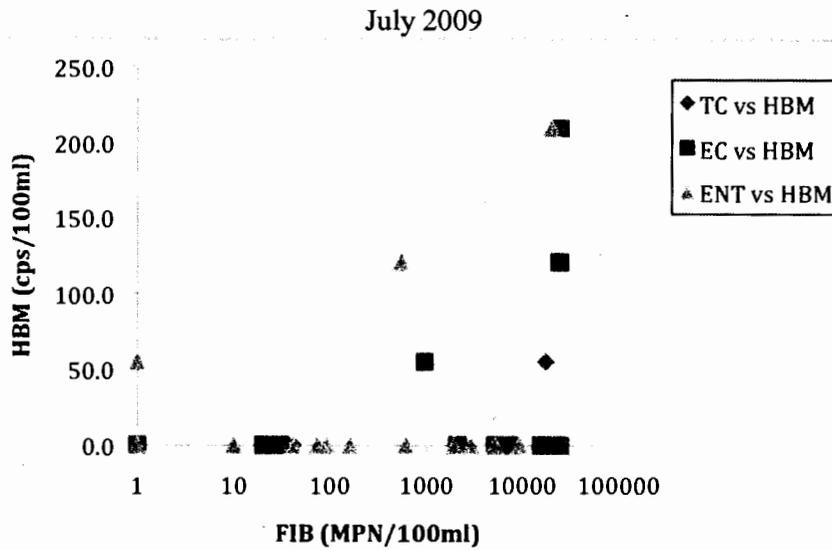


March 2009

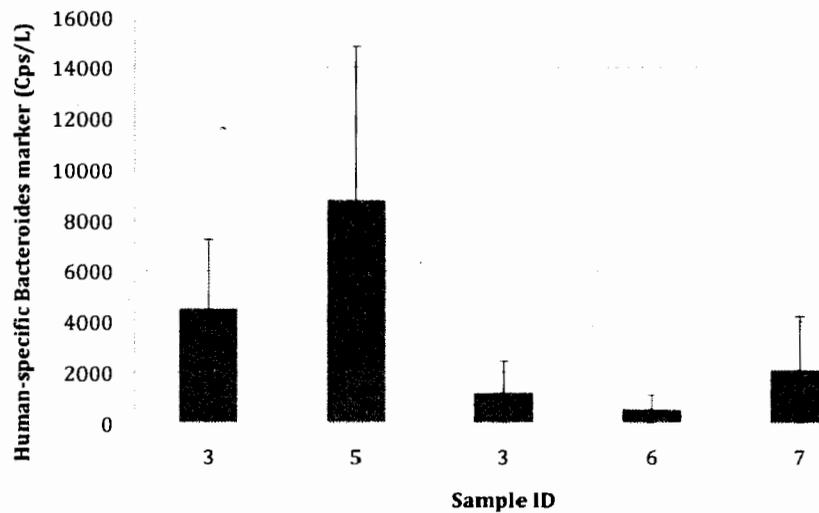


May 2009





Human-specific Bacteroides marker



Average HBM concentrations from duplicates are graphed above. The error bars represent the minimum and maximum concentrations measured from each sample processed. (Sample 3 and 5 on the left were taken in February, and samples 3, 6 and 7 on the right were taken in July).

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